Psychopharmacology (2011) 214:891–899
DOI 10.1007/s00213-010-2099-x

ORIGINAL INVESTIGATION

Amphetamine-induced enhancement of responding for conditioned reward in rats: interactions with repeated testing

Todor V. Gerdjikov · Tyson W. Baker · Richard J. Beninger

Received: 5 October 2009 / Accepted: 8 November 2010 / Published online: 24 November 2010

Abstract
Rationale The mesolimbic dopamine system underlies the ability of reward-related stimuli to control operant behavior. Previous work has shown that amphetamine potentiates operant responding for conditioned rewards (CRs).
Objectives Here, we asked whether the profile of this amphetamine-produced potentiation changes with repeated CR presentation, i.e., as the CR is being extinguished.
Methods Amphetamine (0–1.0 mg/kg, i.p.), administered over four daily sessions using a Latin square design, dose-dependently increased lever pressing for a 'lights-off' stimulus previously paired with food in rats.
Results The amphetamine-produced enhancement of responding for CR was significantly modulated with repeated CR exposure: it was strongest on day 1 and became less pronounced in subsequent sessions whereas the CR effect persisted. In further experiments, rats receiving LiCl devaluation of the primary reward failed to show a significant reduction in the amphetamine-produced enhancement of responding for CR.
Conclusions The nature of the dissociable effects of amphetamine on responding for CR versus the CR effect itself remains to be elucidated.

T. V. Gerdjikov · T. W. Baker · R. J. Beninger
Department of Psychology, Queen's University,
Kingston, ON K7L 3N6, Canada

R. J. Beninger
Department of Psychiatry, Queen's University,
Kingston, ON K7L 3N6, Canada

Present Address:
T. V. Gerdjikov (✉)
School of Psychology, University of Leicester,
Leicester LE1 9HN, UK
e-mail: tvg3@le.ac.uk

Keywords Amphetamine · Conditioned reward · Devaluation · Stimulus-outcome learning

Introduction

Reward-related learning depends on mesolimbic dopamine (Beninger 1983; Carr and White 1986; Sutton and Beninger 1999; Everitt et al. 1999; Baldwin et al. 2002; Wise 2004; Schultz 2006). Neutral stimuli repeatedly paired with primary rewards such as food or with psychostimulant drugs acquire incentive motivational properties, i.e., the ability to elicit approach responses similar to those elicited by the unconditioned stimuli (Bindra 1974; Robbins 1978; Taylor and Robbins 1984; Sutton and Beninger 1999). Such conditioned incentive stimuli can be shown to act as conditioned rewards (CRs), for example animals learn to press a lever that produces a stimulus previously paired with reward (Mackintosh 1974).

Responding for CRs and the role of the mesolimbic system are focal area of research because of their relevance to drug addiction (Robbins 1978; Robbins and Koob 1978; Ranaldi and Beninger 1993; Di Ciano et al. 2008; Wise 2009). Psychostimulant drug addiction may be acquired through the sensitization of reward processes mediated by the mesolimbic dopamine system (Kelley 2004; Hyman et al. 2006; Robinson and Berridge 2008). Thus psychostimulants potentiate instrumental responding for natural rewards depending on the reinforcement schedule (Slawecki and Samson 1996), for brain stimulation reward (Gilliss et al. 2002) and for reward-predicting stimuli (Sutton and Beninger 1999). Amphetamine selectively enhances nucleus accumbens neuronal firing to a conditioned stimulus (CS) paired with sucrose in a conditioned approach task (Wan and Peoples 2008). On the other hand, CS extinction and

unexpected reward omission are associated with a suppression in dopamine cell firing (Schultz et al. 1997; Pan et al. 2008) and dopamine antagonism results in extinction-like behavior (Wise et al. 1978).

Augmentation of dopaminergic neurotransmission during extinction should result in enhanced operant responding. Consistent with this, amphetamine selectively enhances responding for a reward-paired CS during extinction and this effect is mediated by nucleus accumbens dopamine (Beninger 1983; Taylor and Robbins 1986; Cador et al. 1991; Sutton and Beninger 1999). Responding for reward may be subserved by multiple striatal regions and could in part be independent of mesolimbic dopamine (Yin et al. 2008), suggesting that responding for CR may in part have a mesolimbic dopamine-independent component. For example, dorsal striatum which is implicated in stimulus–response (S-R) learning is also implicated in conditioned avoidance, a behavior mediated by conditioned reinforcers, but appears not to play a role in stimulant-produced enhancement of CR responding (Koob et al. 1984; Kelley and Delfs 1991). The mesolimbic dopamine-independent component of CR may be revealed by independently assessing the timecourse of responding for CR and of its enhancement by psychostimulants. In one of the original demonstration of the psychostimulant enhancement effect, pipradrol-induced CR enhancement was shown to vary depending on test day (Robbins 1978).

In the current study we characterized the temporal course of the amphetamine-produced enhancement of responding for CR in the expectation that the effect of amphetamine would change over the four testing days. We found that the amphetamine enhancement showed a time course markedly different from the time course of responding for CR itself. In a second experiment, we found no evidence of a reduction of the amphetamine-produced enhancement of responding for CR by LiCl devaluation of the food reward.

Method

Subjects

Male Wistar rats (N=87), obtained from Charles River, St. Constant, QC, weighing between 200–250 g on arrival were initially housed in pairs on a 12-h reversed light–dark cycle (lights on at 1900 hour) at an average temperature of 21°C and humidity of 40–70%. Water and food (LabDiet 5001, PMI Nutrition Intl, Brentwood, MO) were freely available. Rats were handled for about 1 min on each of five consecutive days after arrival, after which they were housed singly and daily food access was restricted to maintain body weight at 85% of free-feeding levels. The experimental protocol was approved by the Animal Care Committee at Queen's University. The work followed the "Principles of laboratory animal care" (http://www.nap.edu/readingroom/books/labrats/). All rats were treated in full compliance with the Animals for Research Act and relevant guidelines set by the Canadian Council on Animal Care.

Apparatus

Initial lever training was conducted in four identical operant chambers measuring 29×23 cm in floor space and 19 cm in height, each placed in a dimly lit, sound-attenuated, and ventilated wooden box. The walls of each operant chamber were made of Plexiglas and the floor was parallel stainless-steel bars (diameter 3 mm, spaced 1.0 cm apart). There was a recessed food magazine in the center of the 29 cm wall and a single operant lever (1.5×5.0×1.0 cm) 2.0 cm to the right of the magazine. Both were elevated 6.0 cm above the floor. Illumination was provided by two 2 W incandescent lights elevated 10 cm above the floor. Subsequent Pavlovian conditioning and CR testing was conducted in a different set of four operant chambers, which were similar to the ones described above except that the floor was a wire grid with openings of 1.0 cm^2 and the chamber was equipped with two levers each measuring 3.5 wide×0.5 cm thick and extending 2.5 cm into the chamber at a height of 2.5 cm above the floor, centered in each side wall. Dustless precision food pellets (45 mg) from Bio-serv (Frenchtown, NJ; product number: F0021) were used as rewards. Experimental events were controlled and recorded by a 6809 microcontroller using custom made software and transferred to a Macintosh computer for analyses.

Procedure

Behavioral testing was conducted daily between 1000 and 1800 hours during the dark phase of the circadian cycle. The behavioral paradigm consisted of thee phases:

Operant training Food-restricted rats were habituated for ~20 min to the operant chambers with food pellets freely available in the food magazine. On the next day, rats were trained to lever press for food in the same operant chambers using standard shaping techniques. Food was available on a fixed ratio 1 schedule, and all subjects were trained until each rat achieved at least 30 lever presses in 30 min. This typically took 1–2 days. Subsequent sessions were 30 min in duration. On the next day, rats were trained on a variable interval 15 s, followed by 3 days of variable interval 30-s training. Rats in all the groups responded reliably.

Pavlovian conditioning During the 4-day Pavlovian conditioning phase, rats were moved to the CR chamber where in

each 60-min session they received 80 pairings of a 3-s lights-off stimulus with food at a variable intertrial interval of 45 s (range, 5–90 s). During the first conditioning session each lights-off stimulus was terminated with the delivery of a single food pellet. During the remaining three sessions, food delivery occurred following a random 33% of the lights-off stimulus. This procedure was employed because partial pairing results in greater conditioned reward than continuous pairing (Knott and Clayton 1966). The two levers were retracted during the classical conditioning phase.

CR testing During the four test days, the two levers were extended into the CR chamber. One lever (CR) produced a 3-s lights-off stimulus and the other (NCR) had no effect. Responding was recorded over 30 min. Amphetamine sulfate (USP; Rockvill, MD) was dissolved in saline daily before each testing session and injected i.p. (0.0, 0.05, 0.5, or 1.0 mg/kg; injection volume: 1 ml/kg) immediately before testing. This procedure was repeated three times in three independent groups of rats in each case using a different partial Latin square procedure (Table 1).

Two control groups received 80 presentations of the food US or the lights-off CS alone (n=12 per group) during the Pavlovian conditioning phase to test whether pre-exposure to each of these cues alone may account for subsequent lever pressing for the lights-off CS.

Reward devaluation Reward devaluation training took place in the home cage immediately following Pavlovian conditioning and was carried out in a subset of 27 rats.

Table 1 Design of the three Latin squares used to administer amphetamine prior to CR testing sessions

Latin square	Group[a]	Amphetamine dose (mg/kg)			
		0	0.05	0.5	1.0
1	1	Day 1	Day 2	Day 3	Day 4
	2	Day 2	Day 3	Day 4	Day 1
	3	Day 3	Day 4	Day 1	Day 2
	4	Day 4	Day 1	Day 2	Day 3
2	5	Day 1	Day 3	Day 2	Day 4
	6	Day 2	Day 4	Day 3	Day 1
	7	Day 3	Day 1	Day 4	Day 2
	8	Day 4	Day 2	Day 1	Day 3
3	9	Day 1	Day 4	Day 3	Day 2
	10	Day 2	Day 1	Day 4	Day 3
	11	Day 3	Day 2	Day 1	Day 4
	12	Day 4	Day 3	Day 2	Day 1

[a] The group factor was nested within Latin squares. Rat number per group=3

Firstly, after each Pavlovian conditioning session, rats were pre-exposed to an empty clay bowl (height, 6.5 cm; diameter, 13 cm) for 1 h. The devaluation protocol itself lasted 4 days: all rats received i.p. LiCl (20 ml/kg of 0.15 M LiCl; Sigma-Aldrich, Oakville, ON) on days 1 and 3 and saline on days 2 and 4. In the 15 min prior to injection, the bowl was placed in the homecage. For rats in the devaluation group, the bowl was filled with reward pellets on LiCl days; for control animals, the bowl was filled with pellets on saline days. Pellet consumption was measured for each exposure. Devaluation training was followed by a day off (day 5) and a single amphetamine (0.0 vs. 0.5 mg/kg) CR test day (day 6).

Data analysis

The effect of amphetamine on CR and NCR lever presses was analyzed with a two-way (dose×lever) repeated measures analysis of variance (ANOVA). To correct for violations of sphericity of the variance–covariance matrix, degrees of freedom were adjusted using the Greenhouse-Geisser procedure (Stevens 2002). Reward devaluation was analyzed using a devaluation×amphetamine treatment×lever mixed ANOVA.

Significant main effects were followed up by paired samples t tests or planned between subjects comparisons where appropriate. To assess the combined effect of amphetamine and testing day on CR and NCR lever pressing, a dose×day×lever ANOVA was used. Since day-dose combinations were assigned using Latin squares, the standard repeated measures ANOVA approach was not appropriate. Lever presses on each lever could be grouped by either day or dose; however, dose-day combinations were unique to each rat, even though upon completion of the experiment it had received all dose treatments. This corresponds to a three-way within-subjects design with dose and day but not lever balanced by means of Latin squares. Sums of squares for this analysis were calculated using formulas given in Winer et al. (1991) and proceeded in two steps. In the first step, data were collapsed across day and a three-way mixed ANOVA was calculated using dose as the within-subjects variable. The between subject variables were three different Latin squares used in three different replications of the experiment and a grouping variable nested in Latin squares. The grouping variable refers to the treatment combinations in each row of the Latin squares (see Table 1). In the second step, the remaining sums of squares were calculated as described by Winer et al. (1991) and combined yielding F statistics for the effects of interest. Sums of squares used for this analysis as well as all other statistics were calculated using SPSS version 14.0 (Chicago, IL).

Results

Ampehetamine-potentiated responding for CR

Animals pressed the CR lever more than the NCR lever. In addition, as previously reported by a number of groups, amphetamine had an inverse U-shaped effect on the number of lever presses on the CR lever—lever presses were augmented by the 0.5 mg/kg but not by the 0.05 or 1.0 mg/kg doses (Fig. 1). These observations were confirmed by a lever×dose (2×4) repeated measures ANOVA with a Greenhouse-Geisser sphericity adjustment, which revealed a significant lever×dose interaction [$F(3, 105)=3.20$; $p<.05$]. There were also significant effects of lever [$F(1, 35)=50.52$; $p<.001$] and dose [$F(3, 105)=3.82$; $p<.05$]. One-way repeated measures ANOVA of dose for each lever revealed a significant effect of dose on the CR lever [$F(3, 105)=3.82$; $p<.05$]. Follow-up paired samples t tests revealed that when animals were injected with 0.5 mg/kg amphetamine CR lever pressing was significantly higher than when animals were injected with either vehicle [$t(35)=2.73$; $p<.01$] or 0.05 mg/kg amphetamine [$t(35)=2.02$; $p<.05$]. The effect of dose on the NCR lever was not significant.

The effect of dose on lever pressing for individual days is shown in Fig. 2. The effect of amphetamine was most pronounced on day 1, when 0.5 mg/kg produced the highest levels of lever pressing. On days 2, 3, and 4, the effect of amphetamine was progressively less apparent. These observations were confirmed by a dose×day×lever (4×4×2) ANOVA (Winer et al. 1991) which revealed a significant dose×day×lever interaction [$F(6, 224)=2.23$; $p<.05$]. Looking at each day individually collapsing across replication, the dose×lever interaction was significant on day 1 [$F(3, 32)=3.25$; $p<.05$] but not on days 2, 3, or 4. The effect of lever was significant on each day [$F(1, 32)=27.48$; $p<.001$on day 1; $F(1, 32)=50.60$; $p<.001$ on day 2; $F(1, 32)=23.55$; $p<.001$, on day 3; $F(1, 32)=51.73$, $p<.001$ on day 4].

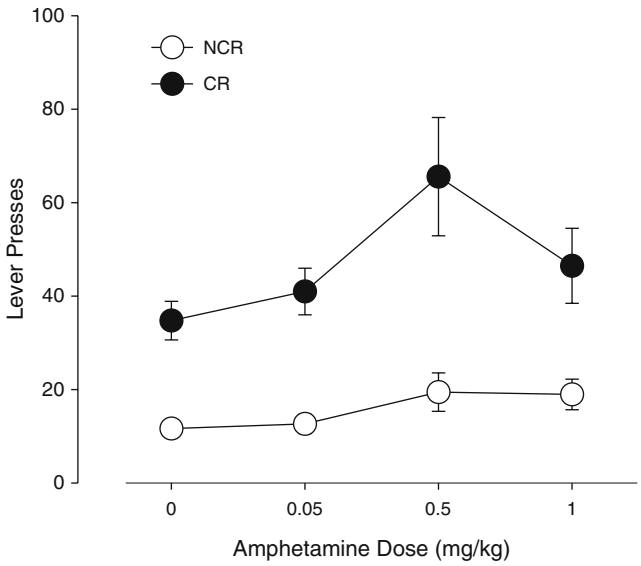

Fig. 1 Mean number of lever presses (±SEM) for a 'lights-off' conditioned stimulus previously paired with food reward. Amphetamine (0–1 mg/kg, i.p.) was administered immediately before testing sessions. *CR* lever that produces the conditioned reward ('lights-off' stimulus), *NCR* inactive lever

To assess whether some of the decrease in amphetamine's ability to enhance CR responding may be due to tolerance to amphetamine with repeated drug injections, we broke down day-2 CR responding in rats receiving 0.5 mg/kg amphetamine on that day by their day-1 amphetamine history—0.0, 0.05, or 1.0 mg/kg. We found no systematic relationship between day-1 amphetamine history and day-2 CR responding (0.0 mg/kg, 36.33±16.70; 0.05 mg/kg, 53.67±29.87; 1.0 mg/kg, 33.00±15.52 (mean±SEM)). Thus drug history is unlikely to account for the marked decrease in amphetamine's ability to enhance CR responding with repeated testing.

Control experiments

Pre-exposure to the CS or the US alone did not differentially affect pressing on the CR and NCR lever (Fig. 3). These effects were confirmed by paired samples t tests comparing responding on the CR and NCR lever for each group, which revealed that neither the US [$t(11)=0.035$; $p=0.973$] nor the CS [$t(11)=1.75$; $p=.11$] pre-exposed animals pressed the CR lever significantly more. This contrasts with the significantly higher number of lever presses on the CR lever on the first day of testing for saline-treated rats pooled from the experimental groups [$t(8)=2.75$; $p=0.025$].

Reward devaluation

Stimulus-outcome (S-O) representations are sensitive to outcome devaluation (Balleine 2001). To determine whether an S-O representation plays a role in the amphetamine-produced enhancement of CR, we tested whether primary reward devaluation impairs the amphetamine enhancement. LiCl injections markedly reduced number of pellets consumed over the three devaluation days (Fig. 4a). This impression was confirmed by a devaluation×exposure (2×3) mixed ANOVA which revealed significant main effects of exposure [$F(2, 50)=7.41$; $p<.01$], devaluation [$F(1, 25)=66.20$; $p<.01$] and an exposure×devaluation interaction [$F(2, 50)=86.84$; $p<.01$]. Next, we assessed the effect of devaluation on the amphetamine enhancement of responding for CR (Fig. 4b). The devaluation×amphetamine treatment×lever mixed ANOVA revealed significant main effects of lever [$F(1, 23)=60.54$; $p<.001$] and amphetamine treatment [$F(1, 23)=17.91$; $p<.01$]. The devaluation main effect failed

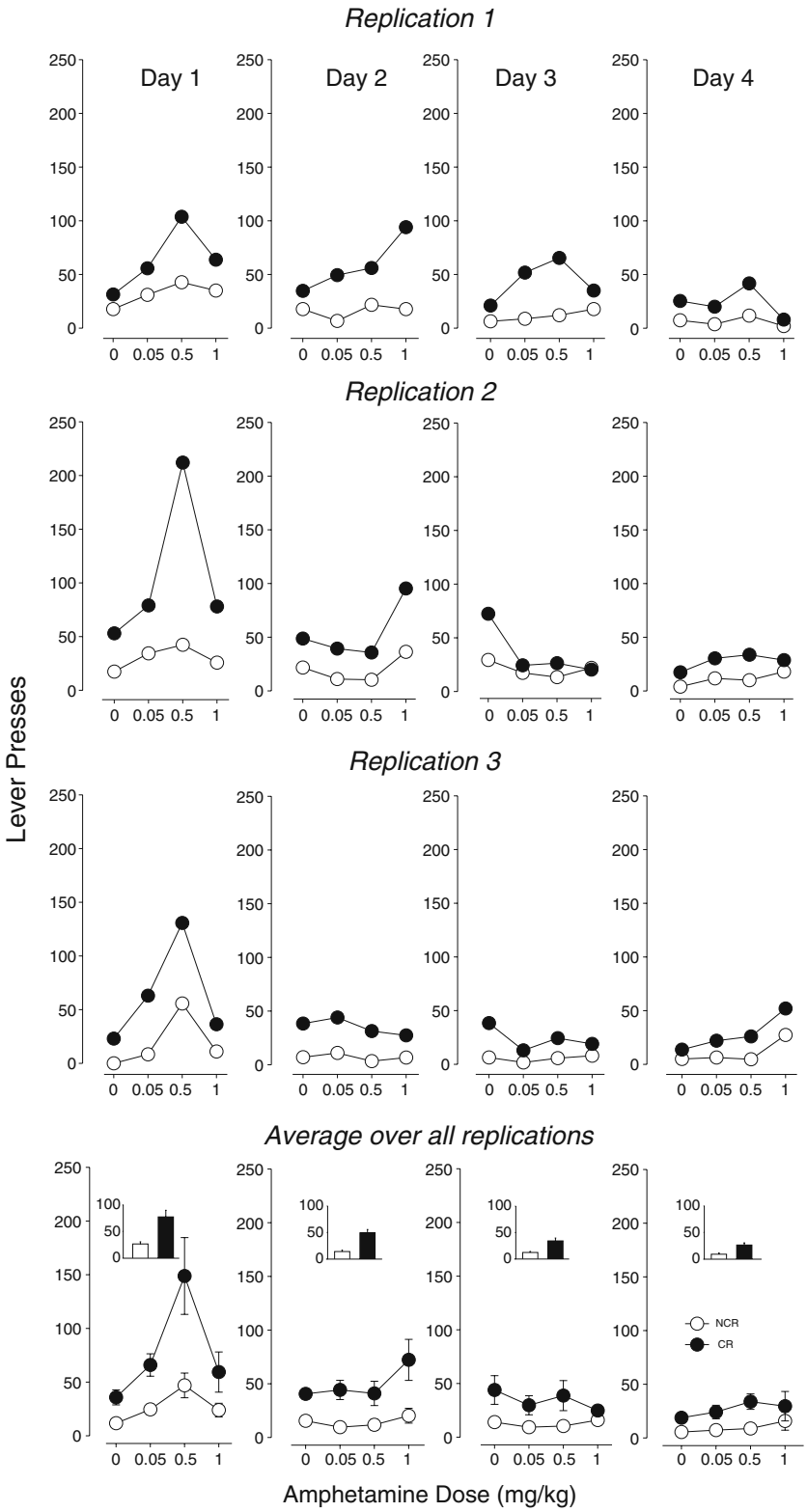

Fig. 2 Mean number of lever presses for a conditioned reward on four consecutive testing days during which the conditioned reward was being extinguished. See Fig. 1 for lever presses averaged across days. Amphetamine (0–1 mg/kg, i.p.) was administered immediately before testing sessions. Each of the *top three panels* shows an independent replication of the experiment (n=9). The *bottom panel* shows lever presses averaged across the three replications. *Insets in the bottom panel* show lever pressing on each lever collapsed across amphetamine dose. *Error bars* in the bottom panel refer to SEM. *CR* lever that produces the conditioned reward ('lights-off' stimulus), *NCR* inactive lever

to reach statistical significance [$F(1, 23)=2.77$; $p=0.11$]. We also noted a significant lever×amphetamine treatment interaction [$F(1, 23)=13.02$; $p<.01$]. The devaluation×amphetamine treatment×lever interaction was not significant.

Discussion

Pairing a lights-off stimulus with reward resulted in a selective increase in responding for the lights-off stimulus.

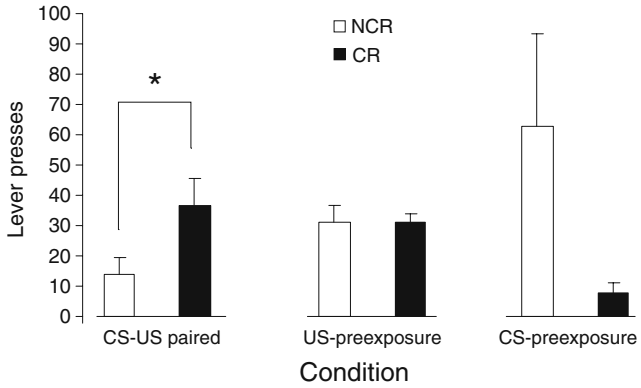

Fig. 3 Mean number of lever presses (+SEM) on a lever producing the lights-off-conditioned stimulus (*CS*) for animals which had received pairings of the CS with food and for two control groups. *Left panel*, CS-US paired animals (n=9) had previously undergone pairings of the CS with food. These data were obtained by averaging responses on day 1 of testing from saline-treated animals and are provided for comparison. *Middle panel*, US-preexposed animals (n=12) had previously undergone presentations of the food unconditioned stimulus (*US*) in the absence of the CS. *Right panel*, CS-preexposed animals (n=12) had previously undergone presentations of the CS without the food US. *CR* lever that produces the 'lights-off' stimulus, *NCR* inactive lever. *p<0.05

Neither CS nor US presentations alone differentially affected pressing on the CR vs. NCR lever. CS pre-exposure appeared to decrease lever pressing on the CR lever, however this effect was nonsignificant and furthermore opposite in direction to what would be expected if the CR effect was due to CS pre-exposure. In previous experiments, negatively correlated presentations of the lights-off and US stimuli also did not differentially affect CR vs. NCR responding (Beninger and Phillips 1980; Hoffman and Beninger 1985). These results confirm that the lights-off stimulus had become a conditioned reward.

A novel aspect of the study, implemented to enhance the learning effect, was that rats were pretrained to lever press before conditioning. In a somewhat related procedure in earlier work cited by Mackintosh (1974; p. 234), rats were pretrained to press a lever for food and food reward was signaled by a click. On a test of extinction where no food was presented, a group that was reinforced with a click showed higher responding than a group that was not reinforced with a click. Since the latter group underwent extinction in an environment more different from the original training environment, lower responding on test could be due to stimulus generalization decrement rather than to CR in the group reinforced with a click in extinction (Mackintosh 1974). In the current study however the CS was not presented during the acquisition of lever pressing; therefore an animal pressing the CR lever is faced with a stimulus configuration that is more different from the initial lever pressing acquisition than an animal pressing the NCR lever. Thus, if anything, the foregoing arguments would

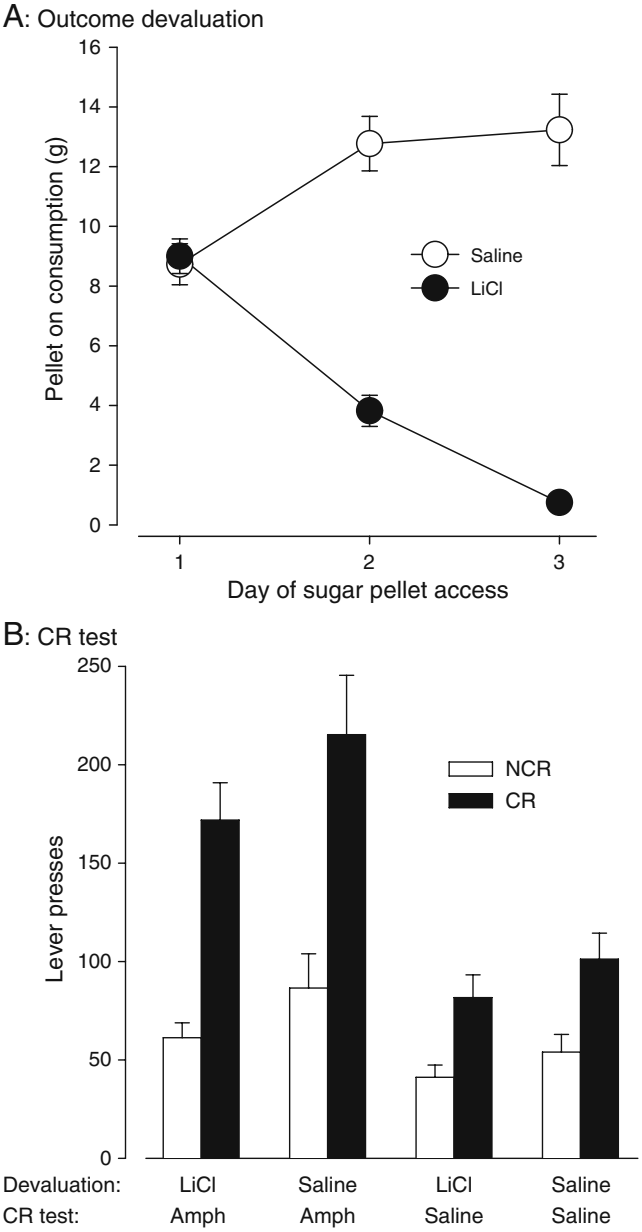

Fig. 4 Effect of reward devaluation with LiCl on responding for conditioned reward and its enhancement by amphetamine (Amph). **a** Outcome devaluation. Two LiCl pairings reduced the mean (±SEM) amount of sugar pellets consumed. Access to sugar pellets was either on days 1 and 3 of the devaluation protocol and was followed by an injection of 20 mL/kg of 0.15 M LiCl or on days 2 and 4 of the devaluation protocol and was followed by an injection of saline. See text for details. No injection was given on the third day of sugar pellet access (day 5 of the devaluation protocol). **b**. CR test: mean (+SEM) number of lever presses for a conditioned reward for animals that had received reward devaluation with LiCl ('Devaluation'). To assess the effect of devaluation on amphetamine-produced enhancement of responding for conditioned reward, 0.5 mg/kg of amphetamine was injected immediately prior to test in some animals ('CR test'). *CR* lever that produces the 'lights-off' stimulus, *NCR* inactive lever

suggest decreased lever pressing on the CR lever—something that we did not observe. Therefore we conclude that in the current work, stimulus generalization decrement may not account for the enhanced lever pressing on the CR lever on extinction.

Amphetamine dose-dependently enhanced responding for the CR (Fig. 1) as shown previously (Robbins et al. 1983; Mazurski and Beninger 1986; Ranaldi and Beninger 1993). When animals were re-tested with different doses of amphetamine over several days they showed higher rates of responding on the CR lever, however, amphetamine potentiated lever-pressing on the CR reward lever on day 1 but it showed no significant effect on subsequent days (Fig. 2). A general motor stimulant effect of amphetamine cannot account for the effect for the following reasons: (1) lever pressing on the NCR lever was not affected significantly, and (2) the effect of amphetamine was only significant on the first day.

Previous work has shown that the enhancement of responding for CR by amphetamine is dopamine-specific. Thus dopamine but not norepinephrine depleting lesions in nucleus accumbens disrupted amphetamine-produced enhancement of responding for CR (Cador et al. 1991).

Amphetamine could potentially increase CR responding by enhancing attentional mechanisms; for example, more focused attention to the CR lever may result in increased responding. The CR vs. NCR lever discrimination on test likely engages selective attentional mechanisms, whereby attending to the relevant stimulus cues (e.g., left vs. right lever) is a prerequisite for stimulus-outcome learning to take place (Sutherland and Mackintosh 1971). Since both prefrontal and nucleus accumbens dopamine receptor activation with SKF 38393 enhances attentional processes, the discrimination component of learning may be enhanced by amphetamine in the CR paradigm (Chudasama and Robbins 2004; Pezze et al. 2007). In fact, the hypothesized inverted U-shaped relationship between dopaminergic activation and attention fits well with the dose–response curve obtained for the amphetamine-produced CR enhancement in this and in previous studies (Ranaldi and Beninger 1993; Arnsten 1997; Zahrt et al. 1997; Granon et al. 2000) (Figs. 1 and 2). However, higher nucleus accumbens SKF 38393 doses result in more impulsive responding in an attentional task, which in the current paradigm might have translated into more presses on either the NCR, the CR, or both levers—an effect which we did not observe. Moreover, lesions of the nucleus basalis, a structure implicated in attention, exaggerated rather than reduced amphetamine enhancement of CR (Olmstead et al. 1998). Finally, it is not obvious why repeated exposure may compromise attentional processes whose gain is increased by amphetamine. It appears therefore unlikely that the observed effect is due to enhanced attentional mechanisms. Another alternative is that the diminishing effects of amphetamine over days of testing may be linked to the role of mesolimbic dopamine in reward-related learning.

The psychological processes mediating conditioned reward and its enhancement may be dissociable. The putative dissociation may result from the concurrent operation of differing reward processes driving CR responding, which may extinguish at different rates and are differentially affected by amphetamine. The S-O component may extinguish quickly as the animal is initially deprived of an expected reward. Once the outcome representation is extinguished, responding may be driven by a S-R process that is independent of mesolimbic dopamine, explaining why the enhancement disappears with repeated testing. A hallmark of S-O responding is its sensitivity to outcome devaluation (Ostlund and Balleine 2007). We carried out a second experiment to test whether a S-O representation may play a role in the amphetamine enhancement of CR using LiCl devaluation of the unconditioned reward. While we did not expect the CR response to be affected significantly by devaluation based on previous work (Parkinson et al. 2005), we reasoned that if the amphetamine enhancement works on an S-O process, LiCl devaluation may act to specifically reduce the amphetamine enhancement. Consistent with Parkinson et al. (2005) devaluation had no significant effect on responding for CR when tested in amphetamine-free rats but contrary to our expectation it also did not have a significant effect in amphetamine-treated animals. Thus, the devaluation paradigm failed to provide support for the idea that amphetamine may preferentially act on an S-O representation and further work is clearly necessary to elucidate the nature of the representation specifically enhanced by amphetamine.

In a previous work, responding on the CR lever was found to be insensitive to outcome devaluation with LiCl (Parkinson et al. 2005). Thus, an S-R association may be *sufficient* to maintain CR responding as we observed with repeated non-reinforced CR exposures. These authors suggest that the CS may elicit responding by evoking not only an US representation but also a general appetitive representation. However, even though conditioned approach is enhanced by amphetamine (Wyvell and Berridge 2000; Wan and Peoples 2008), in the present study the CR response became less amphetamine-sensitive with repeated testing. Thus, our results extend and qualify these conclusions and suggest that a generalized appetitive representation is unlikely to maintain CR responding as extinction progresses.

Devaluation studies have revealed that operant behavior may be mediated by multiple representations of the US. Limited CS-US pairings result in the CS activating perceptual processes normally activated by the food; extended training on the other hand results in a loss of this specific US representation and activates computations that are independent of the perceptual representations and

evaluative processes dependent on that perceptual representation (Holland 2008). Our results may reflect a shift from one type of processing to the other across testing sessions as the CS is represented repeatedly without the US (i.e., being extinguished). However, our attempt to test this explicitly produced inconclusive results.

A closer examination of the amphetamine dose–response curve appears to support the interpretation that one reward process extinguishes with repeated non-rewarded presentation of the CS. Figure 2 gives some indication that repeated testing shifted the peak of the amphetamine dose–response function to the right. On day 1, 0.5 mg/kg was the most effective amphetamine dose whereas on day 2 the most effective dose appeared to be 1.0 mg/kg. This is similar to previous results where the D2 antagonist pimozide shifted the peak of the amphetamine dose–response curve to the right, suggesting decreased reward with repeated non-reinforced exposures; a higher dopamine activation was necessary to maintain one aspect of the reward representation (Ranaldi and Beninger 1993). A second, S-R component is potentially more resistant to extinction (the perseverance of the CR effect across days), but less susceptible to amphetamine enhancement because it may not be under the control of mesolimbic dopamine (Balleine and Ostlund 2007). Further support for the idea that CR may involve multiple reward systems comes from work showing that nucleus accumbens amphetamine-produced enhancement of responding for CR was disrupted by 6-OHDA lesions of the nucleus accumbens, even though the CR effect itself was not disrupted (Taylor and Robbins 1986). Consistent with this idea 6-OHDA lesions of both nucleus accumbens and dorsal striatum were necessary for disruption of conditioned avoidance, a behavior mediated by conditioned reinforcers (Koob et al. 1984). These anatomical observations to some extent parallel the proposed roles of dorsolateral striatum vs. dorsomedial striatum and nucleus accumbens in S-R and S-O learning (Balleine and Killcross 2006; Balleine and Ostlund 2007); however in a devaluation experiment, we were unable to find evidence that a S-O representation drives the amphetamine-produced enhancement of CR.

Responding for CR has implications for drug-seeking behavior as the ability of psychostimulants to enhance the motivational effects of reward-related stimuli may mediate their incentive motivational properties (Taylor and Horger 1999; Kelley 2004; Hyman et al. 2006; Robinson and Berridge 2008). Specifically, lever pressing can be maintained by a drug-paired CR and the same mesolimbic structures have been implicated (Taylor and Robbins 1986; Kelley and Delfs 1991; Di Ciano et al. 2008). Consistent with this idea, psychostimulant sensitization enhanced both responding for CR and its potentiation by intra-accumbens amphetamine (Taylor and Horger 1999). However, parallels between behaviors driven by food- and drug-paired stimuli may not always hold. For example, lever pressing for cocaine may be reinstated up to a year after a single session of access to cocaine and the same is not true of a highly palatable food, although in that study the CS (white noise) was continuously available during an initial cocaine self-administration session (Ciccocioppo et al. 2004). Further work assessing the temporal profile of responding for CR supported by drug-paired stimuli is awaited.

Conclusions

This is the first report of amphetamine effects systematically investigated at distinct time points during the extinction of responding for CR. We found that the amphetamine-produced enhancement became less pronounced with repeated testing whereas the CR effect itself persisted longer. Furthermore, we found no evidence of a reduction in the amphetamine enhancement of responding for CR by outcome devaluation. These findings suggest that dissociable reward processes may drive responding for CR, on the one hand, and the effects of amphetamine on that responding, on the other. Further work is needed to identify the nature of those dissociable reward processes.

Acknowledgment Funded by a grant from the Natural Sciences and Engineering Research Council of Canada to RJB.

References

Arnsten AF (1997) Catecholamine regulation of the prefrontal cortex. J Psychopharmacol 11:151–162

Baldwin AE, Sadeghian K, Holahan MR, Kelley AE (2002) Appetitive instrumental learning is impaired by inhibition of cAMP-dependent protein kinase within the nucleus accumbens. Neurobiol Learn Mem 77:44–62

Balleine BW (2001) Incentive processes in instrumental conditioning. In: Mowrer RR, Klein SB (eds) Handbook of contemporary learning theories. Lawrence Erlbaum, London, pp 307–365

Balleine BW, Killcross S (2006) Parallel incentive processing: an integrated view of amygdala function. Trends Neurosci 29:272–279

Balleine BW, Ostlund SB (2007) Still at the choice-point: action selection and initiation in instrumental conditioning. Ann NY Acad Sci 1104:147–171

Beninger RJ (1983) The role of dopamine in locomotor activity and learning. Brain Res 287:173–196

Beninger RJ, Phillips AG (1980) The effect of pimozide on the establishment of conditioned reinforcement. Psychopharmacol Berl 68:147–153

Bindra D (1974) A motivational view of learning, performance, and behavior modification. Psychol Rev 81:199–213

Cador M, Taylor JR, Robbins TW (1991) Potentiation of the effects of reward-related stimuli by dopaminergic-dependent mechanisms in the nucleus accumbens. Psychopharmacol Berl 104:377–385

Carr GD, White NM (1986) Anatomical disassociation of amphetamine's rewarding and aversive effects: an intracranial microinjection study. Psychopharmacol Berl 89:340–346

Chudasama Y, Robbins TW (2004) Dopaminergic modulation of visual attention and working memory in the rodent prefrontal cortex. Neuropsychopharmacology 29:1628–1636

Ciccocioppo R, Martin-Fardon R, Weiss F (2004) Stimuli associated with a single cocaine experience elicit long-lasting cocaine-seeking. Nat Neurosci 7:495–496

Di Ciano P, Robbins TW, Everitt BJ (2008) Differential effects of nucleus accumbens core, shell, or dorsal striatal inactivations on the persistence, reacquisition, or reinstatement of responding for a drug-paired conditioned reinforcer. Neuropsychopharmacology 33:1413–1425

Everitt BJ, Parkinson JA, Olmstead MC, Arroyo M, Robledo P, Robbins TW (1999) Associative processes in addiction and reward. The role of amygdala-ventral striatal subsystems. Ann NY Acad Sci 877:412–438

Gilliss B, Malanga CJ, Pieper JO, Carlezon WA Jr (2002) Cocaine and SKF-82958 potentiate brain stimulation reward in Swiss-Webster mice. Psychopharmacol Berl 163:238–248

Granon S, Passetti F, Thomas KL, Dalley JW, Everitt BJ, Robbins TW (2000) Enhanced and impaired attentional performance after infusion of D1 dopaminergic receptor agents into rat prefrontal cortex. J Neurosci 20:1208–1215

Hoffman DC, Beninger RJ (1985) The effects of pimozide on the establishment of conditioned reinforcement as a function of the amount of conditioning. Psychopharmacol Berl 87:454–460

Holland PC (2008) Cognitive versus stimulus-response theories of learning. Learn Behav 36:227–241

Hyman SE, Malenka RC, Nestler EJ (2006) Neural mechanisms of addiction: the role of reward-related learning and memory. Annu Rev Neurosci 29:565–598

Kelley AE (2004) Memory and addiction: shared neural circuitry and molecular mechanisms. Neuron 44:161–179

Kelley AE, Delfs JM (1991) Dopamine and conditioned reinforcement. I. Differential effects of amphetamine microinjections into striatal subregions. Psychopharmacol Berl 103:187–196

Knott PD, Clayton KN (1966) Durable secondary reinforcement using brain stimulation as the primary reinforcer. J Comp Physiol Psychol 61:151–153

Koob GF, Simon H, Herman JP, Le MM (1984) Neuroleptic-like disruption of the conditioned avoidance response requires destruction of both the mesolimbic and nigrostriatal dopamine systems. Brain Res 303:319–329

Mackintosh NM (1974) The psychology of animal learning. Academic, New York

Mazurski EJ, Beninger RJ (1986) The effects of (+)-amphetamine and apomorphine on responding for a conditioned reinforcer. Psychopharmacol Berl 90:239–243

Olmstead MC, Robbins TW, Everitt BJ (1998) Basal forebrain cholinergic lesions enhance conditioned approach responses to stimuli predictive of food. Behav Neurosci 112:611–629

Ostlund SB, Balleine BW (2007) The contribution of orbitofrontal cortex to action selection. Ann NY Acad Sci 1121:174–192

Pan WX, Schmidt R, Wickens JR, Hyland BI (2008) Tripartite mechanism of extinction suggested by dopamine neuron activity and temporal difference model. J Neurosci 28:9619–9631

Parkinson JA, Roberts AC, Everitt BJ, Di CP (2005) Acquisition of instrumental conditioned reinforcement is resistant to the devaluation of the unconditioned stimulus. Q J Exp Psychol B 58:19–30

Pezze MA, Dalley JW, Robbins TW (2007) Differential roles of dopamine D1 and D2 receptors in the nucleus accumbens in attentional performance on the five-choice serial reaction time task. Neuropsychopharmacology 32:273–283

Ranaldi R, Beninger RJ (1993) Dopamine D1 and D2 antagonists attenuate amphetamine-produced enhancement of responding for conditioned reward in rats. Psychopharmacol Berl 113:110–118

Robbins TW (1978) The acquisition of responding with conditioned reinforcement: effects of pipradrol, methylphenidate, d-amphetamine, and nomifensine. Psychopharmacol Berl 58:79–87

Robbins TW, Koob GF (1978) Pipradrol enhances reinforcing properties of stimuli paired with brain stimulation. Pharmacol Biochem Behav 8:219–222

Robbins TW, Watson BA, Gaskin M, Ennis C (1983) Contrasting interactions of pipradrol, d-amphetamine, cocaine, cocaine analogues, apomorphine and other drugs with conditioned reinforcement. Psychopharmacol Berl 80:113–119

Robinson TE, Berridge KC (2008) Review. The incentive sensitization theory of addiction: some current issues. Philos Trans R Soc Lond B Biol Sci 363:3137–3146

Schultz W (2006) Behavioral theories and the neurophysiology of reward. Annu Rev Psychol 57:87–115

Schultz W, Dayan P, Montague PR (1997) A neural substrate of prediction and reward. Science 275:1593–1599

Slawecki CJ, Samson HH (1996) Effect of amphetamine on behavior maintained by sucrose: interaction of reinforcement schedule and food restriction. Pharmacol Biochem Behav 54:595–600

Stevens JP (2002) Applied Multivariate Statistics for the Social Sciences. Lawrence Erlbaum, London

Sutherland MA, Mackintosh NM (1971) Mechanisms of animal discrimination learning. Academic, New York

Sutton MA, Beninger RJ (1999) Psychopharmacology of conditioned reward: evidence for a rewarding signal at D1-like dopamine receptors. Psychopharmacol Berl 144:95–110

Taylor JR, Horger BA (1999) Enhanced responding for conditioned reward produced by intra-accumbens amphetamine is potentiated after cocaine sensitization. Psychopharmacol Berl 142:31–40

Taylor JR, Robbins TW (1984) Enhanced behavioural control by conditioned reinforcers following microinjections of d-amphetamine into the nucleus accumbens. Psychopharmacol Berl 84:405–412

Taylor JR, Robbins TW (1986) 6-Hydroxydopamine lesions of the nucleus accumbens, but not of the caudate nucleus, attenuate enhanced responding with reward-related stimuli produced by intra-accumbens d-amphetamine. Psychopharmacol Berl 90:390–397

Wan X, Peoples LL (2008) Amphetamine exposure enhances accumbal responses to reward-predictive stimuli in a pavlovian conditioned approach task. J Neurosci 28:7501–7512

Winer BJ, Brown DR, Michels KM (1991) Statistical principles in experimental design. McGraw-Hill, Boston

Wise RA (2004) Dopamine, learning and motivation. Nat Rev Neurosci 5:483–494

Wise RA (2009) Ventral tegmental glutamate: a role in stress-, cue-, and cocaine-induced reinstatement of cocaine-seeking. Neuropharmacology 56(Suppl 1):174–176

Wise RA, Spindler J, deWit H, Gerberg GJ (1978) Neuroleptic-induced "anhedonia" in rats: pimozide blocks reward quality of food. Science 201:262–264

Wyvell CL, Berridge KC (2000) Intra-accumbens amphetamine increases the conditioned incentive salience of sucrose reward: enhancement of reward "wanting" without enhanced "liking" or response reinforcement. J Neurosci 20:8122–8130

Yin HH, Ostlund SB, Balleine BW (2008) Reward-guided learning beyond dopamine in the nucleus accumbens: the integrative functions of cortico-basal ganglia networks. Eur J Neurosci 28:1437–1448

Zahrt J, Taylor JR, Mathew RG, Arnsten AF (1997) Supranormal stimulation of D1 dopamine receptors in the rodent prefrontal cortex impairs spatial working memory performance. J Neurosci 17:8528–8535