AMPHETAMINE SENSITIZATION DISRUPTS CERTAIN ASPECTS OF ASSOCIATIVE LEARNING ABOUT NATURAL REWARDS

by

Anna Cantor
B.A., Concordia University, 2008

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF ARTS

in

The Faculty of Graduate Studies

(Psychology)

THE UNIVERSITY OF BRITISH COLUMBIA

(Vancouver)

August 2010

© Anna Cantor, 2010
ABSTRACT
Repeated exposure to psychostimulant drugs induces numerous behavioral, and neuronal changes, which in animals is thought to model certain neural adaptations that may contribute to drug addiction. Chronic AMPH has repeatedly been shown to alter the acquisition and expression of associations between a conditioned stimulus (CS) and natural rewards. Although repeated psychostimulant exposure can interfere with associative learning about natural food rewards, the manner in which these treatments affect acquisition and expression of these associations remains unclear. The current study investigated how repeated AMPH exposure (5 x 2 mg/kg over 10 days) affects learning, extinction and cue-induced reinstatement of instrumental responding of food-seeking behavior. Rats were trained over 7 days to press one of two levers for food and a tone/light CS. During subsequent extinction conducted over 3-6 days, responding delivered neither food nor the CS. On reinstatement tests, active lever presses produced the CS, but not food. Rats received repeated AMPH or saline prior to training (exp. 1A), after instrumental training (exp. 1B), or after training and extinction (exp. 1C). In experiment 1A, cue-induced reinstatement was blunted significantly in AMPH-treated rats. In contrast, AMPH-treatment after initial training (experiment 1B) significantly retarded extinction relative to controls, but did not affect cue-induced reinstatement. In experiment 1C, AMPH exposed rats displayed enhanced cue-induced reinstatement. Experiment 2 was conducted to clarify the results of experiment 1A. Rats were trained to nosepoke for food following a CS, and were then tested in the presence of two novel levers, responding on one delivered the food-associated CS. AMPH treatment impaired the acquisition of a new response with conditioned reinforcement. These findings suggest that repeated AMPH exposure prior to formation of response-CS associations selectively disrupts the ability of food-related stimuli to influence instrumental responding. Exposure after initial associative learning impedes extinction. AMPH administration after training and extinction enhance responding. Collectively, these findings suggest that AMPH sensitization can perturb certain aspects of amygdala-mediated associative learning related to natural, food rewards, and this impairment seems to reflect a weakened CS-reward association as opposed to a reduced preference for the food.
# TABLE OF CONTENTS

Abstract .................................................................................................................................................. ii

Table of Contents ................................................................................................................................. iii

List of Figures .......................................................................................................................................... iv

Acknowledgments .................................................................................................................................... v

Dedication ............................................................................................................................................... vi

Introduction ........................................................................................................................................... 1

Methods .................................................................................................................................................. 6

Results .................................................................................................................................................... 12

- Acquisition and extinction (exp. 1A) ............................................................................................... 12
- Cue-induced reinstatement drug-free (exp. 1A) ............................................................................... 14
- Challenge tests (exp. 1A) ............................................................................................................... 14
- Extinction (exp. 1B) ....................................................................................................................... 16
- Cue-induced reinstatement drug-free (exp. 1B) ............................................................................. 16
- Challenge tests (exp. 1B) ............................................................................................................... 17
- Cue-induced reinstatement drug-free (exp. 1C) ............................................................................. 19
- Challenge tests (exp. 1C) ............................................................................................................... 19
- Expression of Pavlovian approach (exp. 2) .................................................................................... 21
- Acquisition of a new response (exp. 2) ......................................................................................... 22

Discussion ............................................................................................................................................. 24

- Impairments in cue-induced reinstatement induced by pre-training AMPH exposure ................ 25
- Delayed extinction induced by post-training AMPH exposure ..................................................... 27
- Impairments in cue-induced reinstatement induced by post-training and extinction AMPH exposure .................................................................................................................. 28
- Sensitization and tolerance to the different aspects of acute AMPH following repeated AMPH exposure .................................................................................................................. 30
- Neural circuity underlying the effects of repeated AMPH exposure on reward-related learning ................................................................................................................................. 31
- Conclusion ......................................................................................................................................... 33

Bibliography ......................................................................................................................................... 35

Appendix ............................................................................................................................................... 42
LIST OF FIGURES

Figure 1  Experimental design for Experiment.........................................................19
Figure 2A  Acquisition of instrumental responding (exp. 1A) ..............................12
Figure 2B  Extinction of responding (exp. 1A) ......................................................... 12
Figure 2C  Drug-free cue-induced reinstatement (exp. 1A) .................................12
Figure 2D  Drug-free cue-induced reinstatement (epochs; exp. 1A) ...................12
Figure 2E  Challenges cue-induced reinstatement (exp. 1A) ..............................12
Figure 2F  AMPH challenge locomotion (exp. 1A) .............................................12
Figure 3A  Extinction of responding (exp. 1B) .......................................................17
Figure 3B  Drug-free cue-induced reinstatement (exp. 1B) ....................................17
Figure 3C  Challenges cue-induced reinstatement (exp. 1B) .............................. 17
Figure 3D  AMPH challenge locomotion (exp. 1B) .............................................17
Figure 4A  Drug-free cue-induced reinstatement (exp. 1C) .................................19
Figure 4B  Drug-free cue-induced reinstatement (epochs; exp. 1C) ...................19
Figure 4C  Challenges cue-induced reinstatement (exp. 1C) ..............................19
Figure 4D  AMPH challenge locomotion (exp. 1C) .............................................19
Figure 5  Acquisition of a new response with conditioned reinforcement (exp. 2) ....22
ACKNOWLEDGEMENTS

I would like to extend my gratitude to all those who have supported me throughout the past couple of years, specifically the Floresco lab members and all those in Vancouver who have made this experience memorable. In particular, I would like to thank Dr. Floresco for all his advice, words of wisdom and encouragement that have guided and enlightened me. Special thanks are owed to my family and all my Montreal support, whose love, continuous strength and undying belief in my ability has helped persevere and achieve my goals. Finally, I am especially grateful to David, who stood by me during these past two years and believed in us to know that I needed to fulfill my personal dreams.
DEDICATION

To Bubbie
INTRODUCTION

Repeated exposure to psychostimulant drugs induces numerous behavioral, and neuronal changes, which in animals is thought to model certain neural adaptations that may contribute to drug addiction (Robinson and Berridge, 1993; O’Brien, Childress, Ehrman, & Robbins, 1998; Kalivas, Peters, & Knackstedt, 2006). These changes include enhanced behavioral activation (Kalivas and Stewart, 1991; Robinson and Berridge, 1993; Taylor and Horger, 1999), potentiated dopaminergic response within corticolimbic structures (Robinson, Jurson, Bennett, & Bentgen, 1988, Kalivas and Duffy, 1990; Kalivas, Pierce, & Cornish, 1998), and alterations in learning and memory (O’Brien, Childress, Ehrman, & Robbins, 1998; Harmer and Phillips, 1998). It is particularly well established that repeated administration of psychostimulants produce enduring sensitization to the locomotor response induced by these drugs after a challenge dose (Kalivas and Stewart, 1991; Robinson and Berridge, 1993; Stewart and Badiani, 1993; Pierce and Kalivas, 1997). This phenomenon is the result of alterations within corticolimbic dopamine pathways, which produce long-lasting enhancements of function within this system (Robinson and Berridge, 1993; Pierce and Kalivas, 1997). Specifically, repeated exposure to psychostimulants produces structural changes in the morphology of neurons in the nucleus accumbens (NAc) and prefrontal cortex (PFC) increasing dendritic length on medium spiny neurons in the NAc and on pyramidal neurons in the PFC (Robinson, Mitton, Gorney, & Kolb, 1999; Robinson and Kolb, 1997; 1999; Robinson, Jurson, Bennett, & Bentgen, 1988). In addition, these treatments also augment dopamine (DA) overflow in the NAc (Robinson and Becker, 1986; Kalivas and Duffy, 1990; Kalivas and Stewart, 1991; Hamamura and Fibiger, 1993; Sorg, Davison, Kalivas, & Prasad, 1997). Consequently, the study of behavioral and neuroadaptations underlying
behavioral sensitization may provide important insights into the addiction process (Kalivas and Stewart, 1991; Robinson and Berridge, 1993; Pierce and Kalivas, 1997).

In addition to enhancing locomotor activity, acute or chronic AMPH has repeatedly been shown to alter the acquisition and expression of associations between a conditioned stimulus (CS) and natural rewards. For instance, systemic administration of d-AMPH enhances responding for a conditioned reinforcer associated with food or sucrose (Robbins 1975; 1976; Beninger and Phillips, 1980; Robbins, Watson, Gaskin, & Ennis, 1983). Repeated AMPH exposure can also cause long-lasting increases in behavior directed toward non-drug rewards, such as sex (Fiorino and Phillips, 1999) and food (Wyvell and Berridge, 2001; Harmer and Phillips, 1998) that persist when animals are tested drug free. Specifically, Fiorino and Phillips (1999) found that a history of d-AMPH treatment augmented the attribution of incentive value to a CS associated with the expectation of a receptive female and also facilitated sexual behavior. In a similar vein, acquisition of a conditioned approach towards a location associated with sucrose reward was facilitated by repeated d-AMPH (Harmer and Phillips, 1998). Likewise, in a study conducted by Wyvell and Berridge (2001), rats initially learned to press a lever for sucrose reward, before being conditioned in a Pavlovian manner to associate a CS with the passive delivery of sucrose. When subsequently tested in a drug-free state, rats with a history of repeated AMPH displayed enhanced lever pressing in response to the food-associated CS during tests of conditioned incentive salience. Furthermore, repeated AMPH also potentiated the effects of acute intra-accumbens AMPH on responding. Similar findings were reported by Taylor and Horger (1999), where rats pretreated with cocaine showed enhanced instrumental responding for a conditioned reinforcer following an intra-NAc infusion of AMPH. Taken together, these
findings show that chronic psychostimulant exposure can, in many cases, augment behaviors induced by stimuli associated with natural rewards.

Despite the above-mentioned findings, there have been other studies showing that repeated AMPH can impair associative learning about food rewards. In a recent study by Simon and colleagues (2009), rats were trained on a discriminative Pavlovian autoshaping task, in which presentation of a visual CS was immediately followed by delivery of a food reward. Repeated AMPH exposure impaired acquisition of a Pavlovian approach response towards the CS+. Similarly, repeated manual administration of AMPH in mice, or self-administration of cocaine in rats, impairs acquisition of a conditioned cue preference for food (Kantak et al., 2001; Ito and Canseliet, 2010). These findings indicate that repeated psychostimulant exposure can in some instances exert detrimental effects on reward-related associative learning about natural, food rewards.

The findings reviewed above highlight that repeated psychostimulant exposure can interfere with associative learning about natural food rewards, but the manner in which these treatments affect acquisition and expression of these associations remains unclear. One possible reason for the inconsistencies in the literature may be related to when AMPH treatments have been administered relative to different phases of learning. For instance, in the abovementioned studies that have shown impairments in reward-related learning, rats received repeated drug treatments prior to behavioral training. In comparison, in some studies reporting enhanced responding for food-related cues (e.g., Taylor and Horger, 1999; Wyvell and Berridge, 2001), sensitizing treatments were administered after initial stimulus/response-reward associations had been formed. Thus, it is possible that systematically assessing the effects repeated AMPH administered at distinct phases of learning may resolve some of the inconsistencies between
these studies and clarify how these treatments interfere with different aspects of associative learning about food rewards.

Alterations in learning and behavior induced by repeated AMPH have traditionally been associated with changes in NAc and PFC functioning. However, it is notable that the acquisition and expression of reward-related learning are also critically dependent on the amygdala, particularly the basolateral nucleus (BLA). It is well established that lesions of the BLA impair acquisition of stimulus-reward associations linked to food rewards, such as conditioned cue preference for food (Everitt, Morris, O’Brien, & Robbins, 1991; McDonald and White, 1993; Kantak, et al., 2001; Ito and Canseliet, 2010). BLA lesions also impair the acquisition of a novel instrumental response for conditioned reinforcement associated with sucrose (Cador, Robbins, & Everitt, 1989; Burns, Robbins, & Everitt, 1993; Everitt, et al., 1999). Moreover, this nucleus also appears to play a key role in monitoring changes in the reinforcing value of Pavlovian conditioned stimuli linked to action-outcome associations once these associations have been formed. Lesions or inactivation of the BLA after initial learning delays extinction of instrumental responding for conditioned reinforcement when a CS is no longer paired for the delivery of food (Burns, Everitt, & Robbins, 1999; Lindgren, Gallagher, & Holland, 2003; McLaughlin and Floresco, 2007). Furthermore, research in our laboratory has shown that inactivation of the BLA after initial learning and extinction potentiates responding for food-related cues in the absence of primary food reward during tests of cue-induced reinstatement (McLaughlin and Floresco, 2007). Thus, BLA appears to play distinct roles in the acquisition, extinction and reinstatement of behavior driven by conditioned stimuli associated with food rewards.

As described above, disruption in BLA functioning can induce distinct effects on reward-related learning depending on when these disruptions occur relative to different phases of
learning. These findings resemble the effects of repeated AMPH administration, which may also induce differential effects on acquisition and expression of reward-related learning. Therefore, it is possible that the effects of repeated AMPH exposure on associative learning about food rewards may be mediated in part by perturbations in BLA functioning. With these issues in mind, the goal of the current experiments was to investigate how repeated exposure to AMPH affects learning, extinction and reinstatement of instrumental responding for natural food rewards. In so doing, we employed instrumental procedures we have used previously to examine the effects of BLA inactivation on these processes (McLaughlin and Floresco, 2007). We assessed the effects of repeated AMPH administered at different phases of learning (i.e.; prior to acquisition, extinction or reinstatement). The use of these procedures enabled us to compare how alterations in learning induced by repeated AMPH exposure may resemble those caused by disruption of BLA function, providing insight into the neural mechanisms that may underlie these effects.
METHODS

Animals

Male Long-Evans rats (Charles River Laboratories, Montreal, QC, Canada) weighing 250-275 g were used. For one week following arrival, rats were group housed in plastic cages in a temperature-controlled colony room on a 12-h light/dark cycle. During this time, rats were given free access to rat chow and water. One week later and for the remainder of the experiment, rats were individually housed and food restricted to 85% of their free feeding body weight. Ad libitum access to water was maintained throughout the experiment. One day prior to training, rats were given several sucrose pellets in their home cages to acclimatize them to the food. All testing was in accordance of the Canadian Council of Animal Care and the Animal Care Committee of the University of British Columbia.

Apparatus

Experimental sessions were conducted in four operant chambers (30.5x24x21 cm; Med-Associates, St. Albans, VT, USA) enclosed in sound-attenuating boxes equipped with a ventilation fan. Each operant chamber was outfitted with two retractable levers on either side of a food receptacle, where sucrose reinforcement (45 mg; Bioserv, Frenchtown, NJ, USA) was delivered via a pellet dispenser. Chambers contained two identical 100-mA stimulus lights, 2.5 cm in diameter above each lever and were illuminated by a house light situated in the top-center of the wall opposite the levers. Auditory stimuli were delivered by a speaker connected to a programmable audio generator (ANL-926, Med Associates) located in the top-left corner of the wall opposite the levers. Four infrared photobeams were located on the sides of each chamber and another was positioned in the food receptacle. Locomotor activity was assessed by the
number of photobeam breaks that occurred during a session. Data were recorded by a computer connected to the chambers via an interface.

**Experiment 1**

**Acquisition of instrumental responding**

The protocol used in the current experiments was modeled after that used by McLaughlin and Floresco (2007), which was originally modified from that described by McLaughlin and See (2003). Behavioral training commenced after 7 days of food restriction. The first two days consisted of 30-min sessions where rats were acclimatized to the operant chambers and sucrose pellets were dispensed on a variable-interval-60 schedule of reinforcement with no conditioned stimulus (CS) paired with food presentation. Over the next 7 days, rats received 20-min training sessions, in which both levers were inserted into the chamber, with one designated as the active lever and the other as the inactive lever (the side counterbalanced across animals). Prior to placing rats in the chamber on the first day of instrumental training, two to three pellets were placed in the food receptacle and crushed on the active lever to assist instrumental learning. Pressing on the active lever delivered a 5 s light-tone CS (illumination of the stimulus light above the active lever and presentation of an 80 dB, 3 kHz tone), followed by one sucrose pellet, and then a 10 s time-out period, where lever presses did not result in food/CS delivery. Pressing on the inactive lever had no programmed consequences. On the first day of training, active lever presses delivered food and the CS on a fixed-ratio-1 schedule of reinforcement. On the second day, rats were trained on a fixed-ratio-2 schedule. On the remaining five days of training, food and CS were delivered on a variable-ratio-5 schedule.
Extinction

Following five days of training on a VR5 schedule, rats received daily, 20-min extinction sessions, where responding resulted in neither food nor CS delivery. Extinction sessions continued until presses on the active lever were less than 10% of presses relative to the last day of VR5 training compared with its own and the group’s mean number of presses. For instance, if on the last day of VR5 training for food, a rat pressed the active lever 300 times, extinction criterion was achieved when it made fewer than 30 presses during a session. Generally, most rats took 2-6 days to reach this criterion. However, in each experiment, there were 1-2 rats that did not achieve this extinction criterion even after 15-30 days of training, which in each instance was greater than 2 standard deviations of the group mean. Their data were excluded from the analyses.

Cue-induced reinstatement

Once a rat reached its extinction criterion, it received the first of three 20-min reinstatement tests. During these tests, pressing the active lever produced the light/tone CS on a VR5 schedule, but no food. No injections were given prior to the first reinstatement test to ensure that any changes in behavior could not be attributed to conditioned effects of IP injections. Following this first test, rats received at least two more extinction sessions to re-establish baseline responding prior to undergoing subsequent reinstatement sessions. For the next two tests, rats received an IP injection of either saline or AMPH (0.5 mg/kg) in a counterbalanced order 10 min prior to the start of the test session.

AMPH sensitization and general experimental design

In each experiment, rats were matched for baseline levels of locomotor activity and/or lever pressing and assigned to AMPH or saline treatment groups. They then received five
injections of either AMPH (2 mg/kg) or saline, every second day over 10 days, followed by a 7-day drug washout period.

In Experiment 1A, rats received drug/saline treatment prior to any instrumental training. In Experiment 1B, rats received injections after acquisition of instrumental responding, but prior to extinction. In Experiment 1C, rats received injections after extinction, but prior to any of the reinstatement tests (see Figure 1 for experimental design). Thus, these procedures allowed us to examine the effects of repeated AMPH on acquisition of instrumental responding, extinction and reinstatement when these manipulations were given at different points in training.

**Figure 1** Experimental design for experiment 1. In A, rats were repeatedly exposed to AMPH prior to instrumental training (exp. 1A). Rats in B were repeatedly injected with AMPH after initial training, but prior to extinction (exp. 1B). In C, rats were exposed to AMPH after initial training and extinction, but prior to reinstatement tests (exp. 1C).

**Experiment 2**

**Conditioned Reinforcement**

**Pavlovian training**

In a second experiment, we examined the effects of repeated AMPH treatment on the acquisition of a novel instrumental response with conditioned reinforcement. The experimental paradigm was adapted from Robbins (1978) and Parkinson et al. (1999). Rats initially received 20 daily Pavlovian training sessions. During these sessions, rats were trained to nosepoke for two
sucrose pellets when a tone/light CS was presented on a VI 30 schedule of reinforcement. Each session consisted of 30 CS-food pairings, and took approximately 18 min to complete. During this training period, both levers were retracted. Discriminative Pavlovian approach was assessed by calculating the proportion of nosepokes that occurred during the 5 s CS and 3 sec of food delivery, divided by the total number of nosepokes during the session.

**AMPH sensitization**

After 20 days of Pavlovian training, rats were matched for levels of locomotor activity, total nosepokes and mean discrimination ratios during the last two Pavlovian training sessions, and assigned to AMPH or saline groups. Rats then received five injections of either AMPH (2 mg/kg) or saline over 10 days, followed by a 7-day washout period.

**Acquisition of a new response**

Seven days after the last injection, rats received two days of reminder Pavlovian training sessions. The next day, rats were exposed to the levers for the first time for a 30 min session. Here, a press on the active resulted in a brief presentation of the CS (1 s) on a random ratio 2 schedule, but, importantly, no food was delivered during these tests. Responding on the inactive lever had no programmed consequences. Acquisition of a new response with conditioned reinforcement was assessed by comparing the total number of presses on the active versus inactive lever during this session.

**Statistical analyses**

For Experiment 1, the main dependent variable of interest was number of lever presses on the active and inactive levers. For the first two phases of the experiment, these data were analyzed with between/within subjects factorial ANOVAs with Treatment Group as the between-subjects factor and Training Day as the within-subjects factor. Lever pressing data from the
extinction phase were taken from the first four days of extinction training. These data were
transformed to a percentage of responses relative to the last day VR5 training, when food and
tone/light stimuli were delivered. For the reinstatement tests and the preceding extinction trials,
the number of active and inactive lever presses was divided into five 4-min epochs. Separate
ANOVAs were conducted for the first reinstatement test, and the subsequent saline/AMPH
challenge tests. These data were analyzed with four-way, between/within subjects factorial
ANOVAs. For the first reinstatement test, the ANOVA held Group as the between-subjects
factor and Test Day (extinction or reinstatement; two levels), Lever (active or inactive; two
levels) and Epoch (five levels) as three within subjects factors. Data from the challenge tests
were analyzed in a similar manner, with the Test Day factor consisting of three levels
(extinction, saline and AMPH reinstatement days). For this analysis, lever pressing on the
extinction days preceding both challenge test days were averaged. We also analyzed locomotor
data obtained during these challenge tests, using two-way ANOVAs.
The data from Experiment 2 were analyzed in a similar manner, using mixed factorial ANOVAs.
RESULTS

Experiment 1A

Acquisition and extinction of lever pressing

Rats were randomly assigned to either repeated saline (n=8) or AMPH (n=8) treatment conditions. After treatment and a 7-day washout period, they commenced instrumental training. Over the seven days of lever pressing training, no significant differences were found between groups in the acquisition of lever pressing, as analysis of these data did not reveal any significant main effect or interactions with the factor of Group (all Fs <1.0, n.s.; see Figure 2a).

Subsequently, rats underwent extinction training. Lever pressing data were converted to percentage of responding relative to the last day of VR5 training. Analysis of the lever pressing data over the first 4 days of extinction again, revealed no differences in their rate of extinction between the two groups (all Fs <1.0, n.s. see Figure 2b). Presses on the inactive lever remained consistently low throughout lever press training and extinction sessions and no significant between- or within-group differences were found (all Fs <0.9, n.s.). Hence, repeated AMPH exposure prior to initial instrumental training did not disrupt the acquisition or extinction of lever pressing for food.
**Figure 2** Experiment 1A: (A) number of active and inactive (inset) lever presses made during the 7 days of lever training and (B) 4 days of extinction in AMPH exposed and control rats. (C) Active lever presses during drug-free test and (D) active lever presses across epochs during drug-free test as compared to extinction day. (E) Active lever presses during challenge reinstatement tests as compared to extinction day. (F) Mean locomotion during the AMPH challenge. AMPH treated rats are represented by filled symbols and controls are represented by open symbols. Stars denote $p < .05$ versus controls.
Cue-induced reinstatement of food-seeking behavior

Following extinction, rats were subjected to their first 20 min test of cue-induced reinstatement. Lever pressing data were analyzed using a four-way ANOVA, with Group as a between subjects factor, and Test day (extinction vs reinstatement), lever and epoch as three within-subjects factors. Analyses of these data revealed significant Test Day x Lever interaction ($F_{1,14}=22.87, p < .01$; Figure 2c), indicating that rats in both groups increased responding on the active lever during the reinstatement test relative to the prior day of extinction. Most pertinently, the analysis also revealed a significant Day x Lever x Epoch x Group interaction ($F_{4,56}=2.96, p < .05$; Figure 2d), although the Day x Lever x Group interaction only approached significance ($F_{1,14}=2.88, p=0.11$). Simple main effects analysis confirmed that AMPH-treated rats emitted significantly (p<0.05) fewer responses on the active lever compared to controls, with this effect being most prominent during the first 8 min of the session. However, there were no differences between groups in terms of inactive lever presses. Therefore, the ability of the food-related cues to reinstate instrumental responding was blunted in AMPH-treated rats compared to controls.

Challenge tests

Rats subsequently received two more reinstatement tests under saline or AMPH (0.5 mg/kg) challenge, each of which was interspersed with 1-2 days of extinction training. It is well established that acute treatment with AMPH can potentiate instrumental responding for conditioned stimuli (Robbins, 1978, Everitt and Robbins, 1992; Robbins and Everitt, 1992; Fibiger et al., 1992). Analysis of the data obtained from these sessions again revealed a significant Day x Lever interaction ($F_{2,28}=2.15, p < .01$), indicating that all animals responded significantly more on the active versus inactive lever on reinstatement days when compared to extinction. More importantly, the analysis also confirmed a significant main effect of Group


\( F_{1,14}=6.34, p < .05 \), a significant Day x Group interaction \( F_{2,28}=8.56, p < .01 \), and, in particular, a significant Day x Lever x Epoch x Group interaction \( F_{8,112}=2.54, p < .05 \). Subsequent simple main effects analyses of this four-way interaction with two, three-way ANOVAs confirmed both groups made a comparable number of inactive lever presses during extinction, saline and AMPH challenge test days (all Fs<2.0, n.s., Fig 2e, inset). In contrast, analysis of the active lever presses revealed a significant Day X Group interaction \( F_{2,28}=5.36, p < .05 \). As is shown in Figure 2d, this interaction was driven by the fact that, relative to saline challenge, acute AMPH significantly potentiated reinstatement in controls rats. In contrast, acute AMPH failed to potentiate responding in rats that had received repeated AMPH treatment previously. However, even though acute AMPH failed to enhance lever pressing in AMPH-treated animals, these rats did show a sensitized locomotor response during this challenge compared to controls, as analysis of these data indicated a significant Day x Group \( F_{2,28}=20.84, p < .01 \); see Figure 2f) interaction.

To summarize, the results from Experiment 1A revealed that repeated AMPH exposure prior to instrumental learning did not impair the acquisition or extinction of lever-pressing behavior for food. However, the ability of food-related cues to induce reinstatement of responding was blunted in AMPH-treated animals. Furthermore, the challenge data show repeated AMPH exposure blocked the ability of acute AMPH to enhance cue-induced reinstatement of food-seeking behavior, while at the same time sensitizing animals to the psychomotor effects of the drug.
**Experiment 1B**

**Extinction**

Prior to drug or saline treatment, all animals were trained for seven days to press a lever for a light/tone CS and food. Animals were then matched for lever pressing and locomotor activity prior to being assigned to repeated AMPH or saline treatment conditions. Following treatment and a 7-day “washout” period, rats received two reminder lever pressing sessions and then underwent at least four days of extinction, where lever presses no longer delivered food or the CS. Analysis of the data obtained over the first four days of extinction training revealed a significant Day x Group interaction ($F_{3,36}=4.64, p<.01$). As opposed to what was observed in Experiment 1A, AMPH treated rats ($n=8$) were significantly slower to extinguish their lever pressing compared to controls ($n=6$). As shown in Figure 3a, AMPH-treated rats made significantly more active lever presses on days two and three of extinction relative to saline treated rats. No significant differences were apparent between groups in terms of days to reach extinction criterion, days between reinstatement tests or inactive lever presses (all $Fs<3.8$, n.s.). Hence, repeated AMPH-treatment after initial learning retarded extinction when responding no longer delivered food and the associated CS.

**Cue-induced reinstatement of food-seeking behavior**

As in Experiment 1A, once rats achieved extinction criterion, they received their first cue-induced reinstatement test under drug-free conditions. Both groups reinstated lever pressing, as indicated by a significant Day x Lever interaction ($F_{1,12}=59.37, p<0.001$). However, in contrast to Experiment 1A, the analysis did not reveal any significant main effect of Group, or any interactions with this factor (all $Fs<1.0$, n.s.; Figure 3b). Thus, AMPH treatment after initial
learning but prior to extinction did not affect the ability of food-related cues to reinstate instrumental responding.

**Challenge tests**

Rats subsequently received counterbalanced injections of AMPH and saline on separate reinstatement test days. Analyses of the challenge data again revealed a Day x Lever interaction ($F_{2,24}=15.18, p < .001$), indicating that rats in both groups increased responding during reinstatement sessions relative to extinction, and made significantly more responses on the active lever during the AMPH challenge compared to the saline challenge (Figure 3c). In this experiment, rats in both groups made a similar number of active lever presses after saline injection. However, AMPH-treated rats tended to make fewer active-lever responses compared to controls after acute AMPH challenge. Despite this apparent trend, the overall analysis did not reveal any significant main effect of Lever x Day x Group interaction ($F_{2,24}=1.61$, n.s.) and the four-way interaction only approached significance ($F_{8,96}=1.91, p = 0.068$). With respect to locomotor activity during these challenges, acute AMPH injections tended to increase locomotion to a greater degree in rats that had received repeated AMPH treatments relative to controls, with analysis of these data producing a trend towards a significant Group x Day interaction ($F_{1,12}=3.92, p=0.071$; Figure 3d). However, a simple t-test revealed that AMPH exposed rats did display significantly greater locomotor activity on the AMPH challenge compared to controls ($t_{12}=5.40, p < .05$).
Figure 3 Experiment 1B: (A) Mean ± S.E.M. number of active lever presses on the last day of training using a VR 5 schedule of food+CS reinforcement, and number of presses on the active lever across the 4 days of extinction. (B) Active and inactive lever presses made by rats during the drug-free reinstatement compared to extinction. (C) Active (and inactive, inset) lever presses on challenge sessions compared to extinction. (D) Mean locomotion during AMPH challenge. AMPH exposed rats are represented by filled symbols and controls are represented by open symbols. Stars denote $p < .05$ differences between treatment groups (in C, stars denote $p < .05$ differences across days).
**Experiment 1C**

**Cue-induced reinstatement of food-seeking behavior**

After initial lever pressing training and subsequent extinction, rats were matched for rates of lever pressing, locomotion and rate of extinction, and were subsequently allocated into repeated AMPH (n=8) or saline (n=6) treatment groups. After the drug “washout” period and two reminder extinction sessions, they received their first reinstatement test. Analyses of the lever pressing data revealed a significant Day x Epoch x Group interaction ($F_{4,48}=3.25, p <.05$). Simple main effects analysis revealed that rats that had received repeated AMPH treatments made significantly more lever presses during the first, third and fourth epochs compared to saline controls. This increase in responding was directed primarily at the active lever, although the four-way interaction only approached significance ($F_{4,48}=2.15, p =0.089$; Figure 4a and b). Thus, in contrast to what was observed in Experiments 1A and B, repeated AMPH exposure after instrumental training and extinction enhanced reinstatement of responding induced by food-related cues.

**Challenge tests**

Rats subsequently received counterbalanced injections of acute AMPH and saline on separate reinstatement test days. Analyses of these data revealed a significant Day x Lever x Group interaction ($F_{2,23}= 3.43, p < .05$; Figure 4b). Simple main effects analysis once again confirmed that after acute saline injection, both groups displayed a comparable increase in responding on the active (but not inactive) lever relative to extinction. However, as observed in Experiment 1A, acute AMPH selectively potentiated responding on the active lever in controls, but not in AMPH-treated animals, relative to responding displayed after saline challenge.

Analysis of the locomotor data unveiled a significant main effect of Test Day ($F_{1,12}=13.30, p$
<.01), indicating that rats in both groups displayed greater activity after AMPH versus saline challenge. This analysis failed to produce a significant Test Day x Group interaction ($F_{1,12}=0.00$, n.s.). However this lack of effect was attributable to the fact that AMPH-treated rats displayed greater levels of locomotor activity compared to controls after both acute saline and AMPH challenges, which in the ANOVA manifested itself as a main effect of Group ($F_{1,12}=6.17$, $p < .05$; Figure 4c). Thus, as was observed in Experiment 1A, repeated AMPH treatment after initial learning and extinction blocked the ability of acute AMPH challenge to potentiate instrumental responding induced by food-related cues.

**Figure 4** Experiment 1C: (A) Active and inactive lever presses made by rats during the drug-free reinstatement compared to extinction. (B) Total lever presses across epochs on drug-free test as compared to extinction. (C) Active (and inactive, inset) lever presses on challenge sessions compared to extinction. (D) Mean locomotion during AMPH challenge. AMPH exposed rats are represented by filled symbols and controls are represented by open symbols. Stars denote $p < .05$ group effect.
**Experiment 2**

A key finding of Experiment 1A was that repeated AMPH exposure prior to initial instrumental learning led to reduced reinstatement of responding induced by food-related cues. In that experiment, animals initially associated lever pressing with the delivery of food and the CS, whereas during reinstatement tests responding only delivered the food-associated CS. Thus, the reduced reinstatement displayed by AMPH-treated animals may reflect a weakened association between the food and the CS that attenuated the impact that food-related cues exert to invigorate instrumental responding. Alternatively the deficits in Experiment 1A may have been due to a more general disruption in the formation of response-CS+food associations. To clarify this issue, Experiment 2 tested the effects of repeated AMPH exposure on the acquisition of a new instrumental response for conditioned reinforcement. In this classical task (Robbins, 1978), rats are first trained to associate a CS with passive food delivery, and subsequently are permitted to press a lever for the food-associated CS alone. AMPH or saline treatments were administered after initial Pavlovian training, but prior to the tests of conditioned reinforcement. As animals never associate the lever pressing response with the delivery of primary food reward, this measure provides a more unbiased assessment of how repeated AMPH may interfere with the ability of food-related cues to support instrumental responding.

**Expression of Pavlovian approach**

Rats underwent 20 sessions of Pavlovian approach training before being matched for discrimination ratio, locomotion and overall number of approaches towards the food alcove (nosepokes). After receiving repeated AMPH (n=10) or saline (n=10) treatments and a subsequent “washout” period, rats were retrained to nosepoke for food following the presence of
the CS. There were no differences between the two groups in terms of total nosepokes or the discrimination ratio during these sessions (all Fs < 1.0, n.s.).

**Acquisition of a new response with conditioned reinforcement**

During this critical stage, two novel levers were introduced into the chamber. Responding on the active lever produced only the CS previously associated with food delivery (inactive lever responses had no programmed consequences). As displayed in Fig 5, control rats successfully acquired a novel instrumental response for conditioned reinforcement, making substantially more responses on the active lever versus the inactive lever. However, learning was substantially impaired in rats that had received repeated AMPH, which made substantially fewer lever presses during this test session. Analysis of these data confirmed this observation, revealing a significant main effect of Group ($F_{1,18}=11.61, p<.01$). Although the reduction in responding by AMPH-treated rats was proportionally greater when comparing active versus inactive lever presses, we did not observe a significant Lever x Group interaction ($F_{1,18}=2.10$, n.s.). However, exploratory analyses confirmed that control rats made significantly more presses on the active vs inactive lever ($t_{9}=3.08, p<0.05$), whereas AMPH-treated rats did not ($t_{9}=1.51$, n.s.). These data suggest that repeated AMPH treatment disrupt the ability of food-related stimuli to support instrumental responding.
Figure 5 Experiment 2: number of active and inactive lever presses emitted by rats on acquisition of a new response with conditioned reinforcement test. AMPH exposed rats are represented by filled symbols and controls are represented by open symbols. Star denotes $p < .05$ difference between treatment groups.
DISCUSSION

The objective of the current studies was to determine how repeated AMPH exposure may lead to long-lasting alterations in the learning, extinction and cue-induced reinstatement of instrumental responding of food and food associated cues. In Experiment 1A, repeated AMPH exposure prior to formation of any response-CS-food associations did not affect learning to lever press for food, nor did it alter rates of extinction. However, these treatments blunted the ability to food-related cues to induce reinstatement of responding. These findings suggest that repeated AMPH exposure prior to formation of response-food-CS associations selectively disrupts the ability of food-related stimuli (but not primary food reward) to reinforce instrumental responding. A similar result was obtained in Experiment 2, where similar treatments prior to being introduced to levers disrupted the acquisition of a novel instrumental response with conditioned reinforcement. Conversely, AMPH sensitization after instrumental training, significantly delayed extinction, indicating that following the initial formation of instrumental associations for food, repeated AMPH treatment can impede the ability to reduce responding when reinforcement contingencies change. In contrast, repeated AMPH treatment after instrumental learning and extinction training resulted in enhanced cue-induced reinstatement. Collectively, these findings suggest that AMPH sensitization can alter different aspects of associative learning about food-related stimuli, in a manner that is critically dependent on when drug exposure occurs relative to different phases of learning. Moreover, it appears that these impairments are attributable more to a disruption in the ability to form or modify novel response-CS associations, rather than reduced motivation for primary food reward.
Impairments in cue-induced reinstatement induced by pre-training AMPH exposure

Repeated AMPH exposure prior to initial instrumental training did not impair the acquisition of lever pressing for food, suggesting that the ability of primary reward to support instrumental learning was intact. Similarly, extinction of lever pressing when both the food and CS were withheld was also unaffected by pre-training AMPH treatment. It is of interest to note that Mendez and colleagues (2009) found that rats pretreated with AMPH exhibited a robust increase in instrumental responding for food. However, it should be taken into account that rats in their study were trained on a fixed ratio schedule of reinforcement one month after their last treatment, and the effects of repeated AMPH were only observed using ratios substantially higher than those used here (FR 20-40). Despite these differences, both the results of Mendez et al. (2009) and those of the present study show that repeated AMPH does not reduce responding for food reward. Importantly, however, AMPH treatments did disrupt the ability food-related stimuli to increase instrumental responding during tests of cue-induced reinstatement. This suggests that a history of AMPH exposure prior to instrumental training weakened stimulus-reward associations, whereby a previously-reinforced CS was less effective at invigorating responding. Our findings are comparable to those reported by Simon and colleagues (2009), where rats were trained to associate the presentation of a CS with the receipt of a sucrose pellet during a discriminative autoshaping Pavlovian approach task. Rats with a history of AMPH exposure showed reduced contact with the CS compared to controls. Similarly, repeated AMPH treatment has also been reported to impair the acquisition of a conditioned cue preference for food (Ito and Canseliet, 2010). Conversely, using a simpler Pavlovian approach task, Harmer and Phillips (1998) found that approach to the food alcove, but not the cue, was facilitated in rats that were pre-exposed to AMPH. Collectively, these findings suggest that repeated AMPH exposure
shifts the direction of approach behavior towards the location of food reward delivery, but at the same time, shifts approach away from cues predictive of those rewards. More generally, these findings are in keeping with the present results in that AMPH exposure prior to acquisition of CS-food associations weakens these associations, so that food-related cues exert a reduced impact over behavior.

In Experiment 2, we sought to clarify further how repeated AMPH exposure may disrupt the ability of food-associated cues to influence instrumental responding. Specifically, we assessed how these treatments affected the acquisition of a new response with condition reinforcement, a classical assay of the ability of a conditioned reinforcer to support new learning. In this task, rats are trained to nosepoke for food following the presentation of a CS. After repeated AMPH administration, animals are tested on acquisition of a new response with conditioned reinforcement, where they are exposed to the levers for the first time. Here, pressing on the active lever results in the presentation of the CS (but not food). Using this procedure, animals were allowed to form CS-reward associations intact before receiving AMPH treatment. Acquisition of a new response with conditioned reinforcement was disrupted as a result of repeated AMPH exposure, illustrating a diminished impact that food-related cues exert over learning a novel instrumental response. These findings suggest that the blunted reinstatement in AMPH pretreated rats in Experiment 1A reflects specifically a reduced impact of cues on behavior, but not motivation for the primary food reward. This impairment in responding for conditioned reinforcement is somewhat surprising given that it is well established that acute treatment with psychostimulants such as AMPH enhances responding for conditioned reinforcement, using similar procedures (Robbins, 1976; Robbins, Watson, Gaskin, & Ennis, 1983; Beninger and Ranaldi, 1992; Everitt and Robbins, 1992; Robbins and Everitt, 1992). The
present results would suggest that although acute AMPH may potentiate responding for food-related cues, repeated exposure to AMPH results in long-lasting impairments in these processes.

In contrast to the present findings, another study reported that repeated exposure to cocaine produced opposite effects to those reported here, potentiating instrumental responding for conditioned reinforcement under both drug–free conditions, and following intra-NAc infusions of AMPH (Taylor and Horger, 1999). One possibility for the difference between the present findings and these previous results may be related to differences between the long-lasting effects of cocaine versus AMPH. It has been suggested that differences between chronic AMPH and cocaine may be due to their different mechanisms of action (Carboni, Imperato, Perezzani, & Di Chiara, 1989; Cadoni, et al., 1995). There appears to be different mechanisms by which AMPH increases the release of DA and that of other psychostimulants (Braestrup, 1977; McMillen, 1983), such that the effect of AMPH is firing and calcium-independent, however, stimulants such as cocaine are dependent upon intact neuronal firing activity and increase extracellular DA concentrations by blocking the reuptake of DA by nerve impulses (McMillen, 1983). Furthermore, while cocaine does not reduce firing of mesoprefrontal neurons, AMPH inhibits these neurons (Chiodo, et al., 1984; White, Wachtel, Johansen, & Einhorn, 1987), and both drugs inhibit firing of mesoaccumbens neurons (White, Wachtel, Johansen, & Einhorn, 1987; Einhorn, Johansen, & White, 1988). Therefore, dissimilar results between experiments using cocaine and AMPH may simply reflect the pharmacological differences of these drugs.

Delayed extinction induced by post-training AMPH exposure

In Experiment 1B, animals were permitted to normally acquire response-CS-food associations in an intact state before receiving repeated AMPH or saline treatment. Under these conditions, subsequent extinction of instrumental responding was perturbed. Thus, over the first
four days of extinction, AMPH treated animals were slower to reduce their responding in the absence of the CS or food, although these animals did eventually achieve levels of responding comparable to controls. Others have also observed that rats with previous cocaine self-administration experience showed higher rates of responding in the absence of reinforcement (Sutton, Karanian, & Self, 2000). Interestingly, even an acute dose of AMPH has been shown to retard extinction using a T-maze task (Shoblock et al., 2003). Taken together, these findings suggest that once CS-reward associations are formed, subsequent modifications in behavior in response to changes in the motivational significance of these associations are impaired by repeated AMPH. Note that in this experiment, cue-induced reinstatement was not altered by repeated AMPH treatment. Our inability to find group differences suggests that if CS-food associations are formed intact, subsequent treatment with repeated AMPH does not appear to disrupt with the ability of food-related cues to instigate responding.

Impairments in cue-induced reinstatement induced by post-training and extinction AMPH exposure

In contrast to Experiments 1 A and B, Experiment 1C revealed that repeated AMPH after initial learning and subsequent extinction enhanced the ability of food-associated cues to reinstate responding relative to controls. In this experiment, responding on both the active and inactive lever was increased, although the increase in active lever presses was more prominent. Our findings of enhanced responding following repeated AMPH is in keeping with other reports that these treatments can augment the ability of food-related cues to increase instrumental responding, even when tested in a drug free state (Wyvell and Berridge, 2001). Thus, it is apparent that repeated AMPH exposure can lead to persistent enhancements in the impact of food-related cues, but that this effect manifests itself if treatments are administered after initial
formation and subsequent extinction of CS-reward associations. Collectively, the findings of Experiment 1 show that repeated AMPH can have differential effects on aspects of associative learning about food rewards in a manner critically dependent on when in the learning process drugs are administered. Specifically, AMPH administration prior to instrumental learning impairs reinstatement of responding; AMPH treatment after learning impairs extinction; and AMPH administration after both learning and extinction enhances reinstatement.

Further insight into how repeated AMPH may potentiate responding for food-related stimuli comes from an assessment of the different phases of learning rats experience during training, extinction and reinstatement. During training, rats learn to associate a lever press and the CS with food delivery. Rats then undergo extinction in which they learn that lever pressing no longer results in food/CS delivery, and so pressing on the lever diminishes. Subsequent reinstatement tests introduce a novel condition, where responding delivers the food-associated CS, but no food. Under normal circumstances, the CS produces an initial enhancement in responding, and in Experiment 1C, this effect was enhanced in rats with a history of AMPH treatment. A parsimonious explanation of these findings is that these treatments may have enhanced the incentive salience of these cues, leading to an increase in responding for them (Wyvell and Berridge, 2001). Note however that over the course of a reinstatement session, control animals eventually decrease responding, as they learn that the CS no longer predicts food delivery. AMPH treated rats were slower to reduce responding during these tests, resulting in an apparent enhancement in responding. As such, an alternative explanation for the results of Experiment 1C is that these findings reflect a special type of extinction deficit. Repeated AMPH treatment may have impaired the ability to modify behavior over the course of a reinstatement session when it became apparent that the CS no longer predicted food delivery. This notion is
consistent with the findings of Experiment 1B, where treatments administered after initial learning retarded extinction of lever pressing when both food and CS were withheld.

Sensitization and tolerance to different effects of acute AMPH following repeated AMPH exposure

Data from the acute AMPH challenge sessions provided particularly interesting insight into how repeated AMPH exposure may induce dissociable alterations of different behaviors induced by acute drug treatment. Heightened behavioral activation induced by acute drug challenge has long been considered a key index of plasticity that occurs with repeated psychostimulant exposure, which may in turn be linked to changes that contribute to the addiction process (Kalivas and Stewart, 1991; Robinson and Berridge, 1993). In the current experiments, acute AMPH challenge during the reinstatement tests also yielded a sensitized locomotor response in rats with a history of AMPH exposure. However, at the same time, we found that the ability of acute AMPH to enhance responding for food-related cues was blunted compared to controls. In essence, repeated AMPH exposure led to an apparent tolerance to the effects of acute AMPH on responding for conditioned stimuli. Although this latter effect was somewhat surprising, there have been other examples where repeated psychostimulant exposure may induce tolerance to other, non-psychomotor effects of the drug. For example, Dalley et al., (2005) trained animals on a 5-choice serial reaction time task (5CSRTT), before undergoing AMPH self-administration. In these animals, the ability of acute AMPH challenge to increase impulsive action on the task was attenuated. Similarly, Winstanley et al. (2007) trained rats on either the 5CSRTT, or a delay-discounting task. Following chronic cocaine administration, animals were given an acute cocaine challenge prior to being tested on their respective tasks. Rats that received repeated cocaine showed a tolerance to some of the cognitive impairments
following an acute dose of cocaine. Thus, the current findings add to a growing literature
highlighting the differential forms of behavioral plasticity that may occur after repeated
psychostimulant exposure. Some of the effects of acute treatment (i.e., increased locomotion)
sensitize upon repeated exposure, whereas other effects (enhanced responding for food-related
cues, alterations in impulsive choice or action) develop tolerance.

**Neural circuitry underlying the effects of repeated AMPH exposure on reward-related learning**

The NAc has long been implicated as a key structure in mediating the effects of both acute and repeated psychostimulant exposure on reward-related learning (Robbins, Cador, Taylor, & Everitt, 1989; Koob, 1992; Everitt and Robbins, 1992; Robbins and Everitt, 1996). DA release in the NAc may increase Pavlovian and instrumental conditioned responding by enhancing approaches to the CS and increasing control over behavior by reward-related stimuli (Everitt and Robbins, 1992; Robbins and Everitt, 1992). Furthermore, it is well established that chronic psychostimulant exposure can enhance basal and evoked mesoaccumbens DA activity (Robinson, Jurson, Bennett, & Bentgen, 1988; Carboni, Imperato, Perezzani, & Di Chiara, 1989; Paulson and Robinson, 1995; Cadoni, Solinas, & Di Chiara, 2000). Given the importance of NAc DA in reward-related learning, one would expect that repeated AMPH treatments (as administered in the present study) would induce a uniform enhancement in responding for food-related stimuli. However, this was not observed in the current experiments, suggesting that the alterations in learning induced by repeated AMPH exposure may not be mediated by changes in NAc DA transmission.

When reviewing the present data, it is of particular interest to note that the profile of behavioral effects induced by repeated AMPH exposure at different phases in training resemble those observed after lesions or inactivations of the BLA. For instance, like repeated AMPH
treatment (Experiment 1A), BLA lesions prior to training impairs associative learning about cues linked to natural rewards (Gaffan and Harrison, 1987; Gaffan, Gaffan, & Harrison, 1988; Ito and Canseliet, 2010). Likewise, AMPH exposure after initial Pavlovian conditioning impaired acquisition of a new response with conditioned reinforcement (Experiment 2) in a manner similar to BLA lesions (Cador, Robbins, & Everitt, 1989; Burns, Robbins, & Everitt, 1993; Everitt, et al., 1999). Conversely, BLA inactivations (McLaughlin and Floresco, 2007) or AMPH treatment (Experiment 1B) administered after initial associative learning impair instrumental extinction. Furthermore, inactivation of the BLA prior to reinstatement enhances subsequent responding, (McLaughlin and Floresco, 2007), in a manner similar to that induced by repeated AMPH treatments given after learning and extinction (Experiment 1C). Viewed collectively, these findings provide indirect evidence that repeated exposure to AMPH interferes with different aspects of associative learning about food rewards by disrupting amygdala functioning.

The notion that repeated psychostimulant exposure may lead to perturbations in amygdala functioning is further supported by findings from studies of human stimulant abusers as well as work with animal models. Negative blood oxygen level dependent (BOLD) contrast in the amygdala has been found in rats given an AMPH challenge (Dixon, et al., 2005; Easton, Marshall, Fone, & Marsden, 2007). Repeated psychostimulant exposure has also been associated with a dramatic reduction in GABA\textsubscript{\text{B}} receptor binding in the amygdala (Frankouwska et al., 2008), an enhanced dopaminergic response in this region to an acute dose of \textit{d}-AMPH and presentation of a CS+ (Harmer, Hitchott, Morutto, & Phillips, 1997; Harmer and Phillips, 1999), as well as elevated expression of brain derived neurotrophic factor mRNA in the BLA (Meredith, Callen, & Scheuer, 2002). Electrophysiological work in our laboratory has shown that while ventral tegmental area (VTA) stimulation reduces cell firing rate in some BLA cells, VTA
stimulation in AMPH exposed rats reduces the firing in the majority of neurons (Tse and Floresco, 2009). Furthermore, after an acute AMPH dose, half of the BLA cells recorded in control rats showed reduced spike firing, whereas the majority of the cells in AMPH treated rats exhibited suppressed cell activity. Functional magnetic resonance imaging (fMRI) studies have shown reduced amygdala activation during cocaine intoxication in humans (Breiter et al., 1997). Interestingly, a study by Makris and colleagues (2004) found that the amygdala of cocaine-dependent subjects was significantly reduced in volume in comparison to healthy controls. Moreover, the regions of the amygdala that showed the reduction in volume were the subnuclei of the amygdala, particularly the BLA. These findings, in light of the present data, render it plausible that prior stimulant exposure produces alterations in amygdala functioning, which subsequently interferes with the acquisition, extinction and reinstatement of conditioned responding.

**Conclusion**

The current studies sought to examine how repeated AMPH exposure may induce long-term modifications in learning, extinction and cue-induced reinstatement of responding for food and food related cues. We found that the changes induced by repeated AMPH administration depend on when AMPH is administered, such that AMPH exposure prior to instrumental training impairs reinstatement for food-associated cues, AMPH treatment after training impairs extinction, and AMPH exposure after training and extinction enhances reinstatement of responding.

The effect of repeated AMPH exposure on reducing the impact of food-associated cues over behavior is particularly interesting, when viewed in light of how stimulant abuse may perturb motivational processes related to natural rewards. Research with human stimulant addicts
reveals that stimulant abusers often report reduced motivation for natural rewards without a corresponding reduction in the pleasure associated with these rewards (Berridge and Robinson, 1995; Goldstein and Volkow, 2002; Janowsky, Pucilowski, & Buyinza, 2003; Koob and Le Moal, 2007). In fact, research has shown that DA-related manipulations affect the motivational value of food rewards without changing the palatability of these rewards (Berridge, Vernier, & Robinson, 1989; Berridge, 1996). Consistent with this literature, the findings of the present experiments suggest that repeated psychostimulant exposure may blunt the impact of cues associated with natural rewards to invigorate instrumental behaviors to obtain those rewards. The data presented here would suggest that the reduced motivation for natural rewards reported by stimulant abusers may not be due to reduced pleasure they may receive when consuming these rewards. Rather, this amotivational syndrome for rewards such as food or sex may be attributable to a reduced influence that cues predictive of these natural rewards have over behavior, so that they are less likely to seek out and obtain these rewards.
BIBLIOGRAPHY


Appendix

UBC Animal Care Certificate
ANIMAL CARE CERTIFICATE

Application Number: A06-0300

Investigator or Course Director: Stan Floresco

Department: Psychology, Department of

Animals:

<table>
<thead>
<tr>
<th>Rats Sprague Dawley 80</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rats Long Evans 160</td>
</tr>
</tbody>
</table>

Start Date: July 1, 2006

Approval Date: June 25, 2008

Funding Sources:

Funding Agency: Canadian Institutes of Health Research (CIHR)
Funding Title: Alternations in amygdala-prefrontal cortex circuitry by repeated psychostimulants: Electrophysiological and behavioural analyses

Funding Agency: Parkinson Society Canada
Funding Title: Dopaminergic mechanisms underlying risky decision-making

Funding Agency: Canadian Institutes of Health Research (CIHR)
Funding Title: Functional interactions between basolateral amygdala and mesocortical dopamine inputs to the medial prefrontal cortex: electrophysiological and behavioral analyses

Funding Agency: Dainippon Sumitomo Pharma Co., Ltd.
Funding Title: Animal models of cognitive deficits in schizophrenia

Unfunded title: n/a

The Animal Care Committee has examined and approved the use of animals for the above experimental project.

This certificate is valid for one year from the above start or approval date (whichever is later) provided there is no change in the experimental procedures. Annual review is required by the CCAC and some granting agencies.

A copy of this certificate must be displayed in your animal facility.

Office of Research Services and Administration
102, 6190 Agronomy Road, Vancouver, BC V6T 1Z3
Phone: 604-827-5111 Fax: 604-822-5093