Available online at www.sciencedirect.com

SCIENCE DIRECT

Pharmacology, Biochemistry and Behavior 81 (2005) 1–8

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

www.elsevier.com/locate/pharmbiochembeh

# Withdrawal, tolerance, and sensitization to dopamine mediated interoceptive cues in rats trained on a three-lever drug-discrimination task

Robert J. Barrett[a,*], William F. Caul[b], Randy Smith[c]

[a]*Veterans Administration Medical Center, Departments of Psychology and Pharmacology, Vanderbilt University, 1310 24th Avenue South, Nashville, TN 37212-2637, USA*
[b]*Department of Psychology, Wilson Hall, Vanderbilt University, Nashville, TN 37240, USA*
[c]*Department of Psychiatry, Vanderbilt School of Medicine Nashville, TN 37203, USA*

Received 11 October 2004; received in revised form 16 December 2004; accepted 30 December 2004
Available online 22 April 2005

**Abstract**

In the present experiment rats were trained on a three-lever, drug-discrimination task to discriminate the cues associated with 0.30 mg/kg of the indirect dopamine (DA) agonist, amphetamine (AMPH), saline (SAL), and 0.03 mg/kg of the DA, $D_2$ receptor antagonist, haloperidol (HAL). Choice behavior determined from tests on 0.30 and 0.15 mg/kg AMPH, SAL 0.03 and 0.015 mg/kg HAL provided a behavioral baseline presumed to represent changes along a continuum of DA mediated, interoceptive cues. Results from separate groups tested on 0.30 and 0.15 mg/kg AMPH, SAL, 0.03 and 0.015 mg/kg HAL, 24 h post-treatment with an acute 7.5 mg/kg dose of AMPH, showed rapid tolerance and withdrawal to the AMPH cue and sensitization to the HAL cue. The same tests 24 h following treatment with 1.0 mg/kg HAL showed rapid tolerance to the HAL cue, sensitization to the AMPH cue, but not AMPH-like withdrawal cues. Analysis of the results showed that tolerance to the AMPH and HAL cues reflected neuroadaptive baseline shifts and not weaker cue properties. These findings are consistent with predictions from opponent process theory of motivation and provide an animal model to study the motivational consequences that aversive symptoms of AMPH withdrawal such as dysphoria and anhedonia can have on drug-taking behavior.
Published by Elsevier Inc.

*Keywords:* Three-lever drug-discrimination; Withdrawal; Sensitization; Rapid tolerance; Amphetamine; Haloperidol; Animal model

## 1. Introduction

Opponent process theory of motivation (Solomon and Corbit, 1973; Solomon, 1980; Barrett, 1985; Koob et al., 1989, 1997) postulates that following use of a mood altering drug, there is an initial period of mood enhancement followed by a rebound period during which the mood state is opposite that first experienced, i.e., withdrawal. The rebound period is thought to reflect neuroadaptive processes that oppose the drug's primary action in an attempt to maintain homeostasis. Symptoms of withdrawal observed following termination of drug use reflect a temporary period during which the opponent processes are no longer opposed by the drug's primary action. In the absence of further drug use, homeostasis is gradually recovered.

The importance of aversive mood-related symptoms of withdrawal, as opposed to physical signs of withdrawal in motivating compulsive drug use is emphasized by the fact that physical signs of withdrawal are not prominently associated with withdrawal from drugs like cocaine and amphetamine (Gawin, 1991) yet have a high abuse potential. The opponent process theory of motivation (Markou et al., 1993) proposes that initially, the mood enhancement (Fischman et al., 1976; Smith and Beecher, 1960) associated with drugs like amphetamine (AMPH) and cocaine motivate their use. Continued use is theoretically maintained by both the motivation to escape the aversive

* Corresponding author. Tel.: +1 615 3274 751x5475; fax: +1 615 3216 305.
*E-mail address:* robert.j.barrett@vanderbilt.edu (R.J. Barrett).

0091-3057/$ - see front matter. Published by Elsevier Inc.
doi:10.1016/j.pbb.2004.12.012

mood states associated with withdrawal (negative reinforcing properties), and the motivation to reinstate the mood enhancement initially experienced (positive reinforcing properties).

The main objective of the present experiment was to test for the development of aversive symptoms of withdrawal following treatment with amphetamine, using an animal behavior thought to parallel the mood altering properties of drug's in humans. The challenge in developing animal models relevant to studying drug abuse in humans is that the response of interest is drug-induced, subjective changes in hedonic state. On the basis of an extensive literature, it has been suggested that drug-induced interoceptive cues in animals parallel the mood altering properties of drugs in humans, and theoretically, are mediated by common physiological mechanisms (Balster, 1991; Preston and Bigelow, 1991; Kamien et al., 1993; Colpaert, 1999, 1996, 1978). More specifically, Colpaert (1999) states that results from early animal drug-discrimination studies characterizing the cue properties of opiates (Colpaert, 1978) have provided evidence that drug-produced interoceptive stimuli in animals are homologous with the subjective effects opiates produce in humans (Preston and Bigelow, 1991; Altman et al., 1977). Whereas, previous drug-discrimination studies have comprehensively detailed characteristics of the cues associated with amphetamine's primary effect, the present study was designed to detect and characterize the cues associated with the neuroadaptive processes that develop following treatment with amphetamine and to understand the relationship between these processes and the development of tolerance.

An earlier drug-discrimination experiment (Barrett and Leith, 1981) designed to study tolerance, trained rats to discriminate 0.50 mg/kg AMPH from SAL, following which training was suspended while rats were injected with increasing doses of AMPH over a four-day period. Tolerance was observed when rats were challenged with 0.35 mg/kg AMPH post-chronic AMPH. The 0.35 mg/kg dose of AMPH produced only 10% responding on the AMPH lever compared to 79% when tested prior to chronic treatment. Because the rats challenged with 0.35 mg/kg AMPH responded as though tested with SAL, this finding was described as demonstrating complete tolerance. One finding of interest in that study was the observation that when the same rats were tested on SAL following chronic AMPH, they made a greater percent of their responses on the SAL lever than at any other time during the experiment. Observation of this small increase (from 90% to 95%) in SAL lever responding was possible because the rats were trained on a reinforcement schedule (VI-30 s, TO-15 s) that yielded graded rather than quantal measures of discrimination (Barrett et al., 1994). Since it is known that animals will respond on the SAL lever when challenged with "third state" cues, this small increase in SAL lever responding post chronic AMPH suggested the presence of cues qualitatively different from SAL cues. The objective of a follow up study by Haenlein et al. (1985) was to pursue this possibility and develop a drug-discrimination procedure that would be sensitive to detecting bidirectional changes in cue state following treatment with AMPH. Since it is known that dopamine (DA) plays a central role in mediating AMPH's cue properties (Van Groll and Appel, 1992; Exner and Clark, 1992; Callahan et al., 1991; Dworkin and Bimle, 1989; Nielsen et al., 1989; Woolverton and Cervo, 1986; Nielsen and Jepsen, 1985), rats were trained to discriminate between AMPH, an indirect DA agonist and HAL, a $D_2$ antagonist. The results from the Haenlein et al. (1985) study showed that rats could learn to discriminate changes along a continuum of presumed DA mediated cues, and that when tested on SAL 24 h following chronic AMPH treatment, responded primarily on the HAL lever before the pretreatment baseline was recovered. These results confirm the presence of withdrawal cues following AMPH, but considered alone do not prove that they are HAL-like. An alternative explanation is that because the rats are forced to respond on either the AMPH or HAL lever, it is possible that responding occurred on the HAL lever because the withdrawal cues were more similar to HAL than AMPH. However, results from recent three-lever studies (Caul et al., 1996; Stadler et al., 1999) that involved training rats to discriminate among the cues associated with AMPH, SAL and HAL show this not to be the case. Those studies also reported that rats responded on the HAL lever during withdrawal from AMPH, a finding that provides strong support for the interpretation that the cues associated with AMPH withdrawal are HAL-like. If the cues were different from HAL, rats trained on the three-lever task would have responded on the SAL lever.

The purpose of the present experiment was to train rats on a three-lever AMPH–SAL–HAL discrimination prior to determining AMPH and HAL dose–response functions. Rats were then treated with a single large dose of AMPH and HAL to determine if the respective treatments would shift the dose–response functions in the predicted directions. On the basis of opponent process theory it was predicted that rats tested 24 h after treatment with a large single dose of AMPH would show baseline shifts opposite those observed 24 h following treatment with a large single dose HAL. Of special interest was the extent to which the baseline shifts predicted diminished (tolerance) and enhanced choice (sensitization) of the AMPH and HAL levers.

## 2. Materials and methods

### 2.1. Apparatus

Six commercially available operant chambers (BRS/LVE model No. RTC-022) each housed in a sound-attenuating chamber were used for training rats on the discrimination task. The front panel of each box was

divided into thirds by two plastic dividers that extended from the ceiling to the grid floor and extended 6.0 cm into the chambers. In the center of each of the three divisions a response lever requiring a force of 28 g to activate was mounted 4.92 cm above the floor. Responding on the levers was reinforced with food pellets (45 mg: P.J. Noyes ) delivered by a pellet dispenser mounted in the center of the opposite back panel. A house light in each chamber was turned on and off to signal the start and end of a session. Experimental sessions were controlled and data recorded by a computer and interface equipment located outside the experimental room.

### 2.2. Animals

Fifty male Sprague–Dawley rats obtained from Harlan Laboratories, Indianapolis, IN and weighing approximately 250–300 g at the start of the experiment, were housed in individual cages and food deprived to 85% of their expected free-feeding weight. The rats were maintained on a 12-h light–dark cycle (lights on at 0600 h) and given enough food (Purina Lab Chow) immediately following each training session and on weekends to maintain their control weight throughout the experiments. The animals had free access to water in their home cage. All experimental procedures were carried out in accord with the NIH Guide for the Care and Use of Laboratory Animals (1996 edition).

## 3. Procedures

### 3.1. Training on the three-lever amphetamine–saline–haloperidol discrimination

After the lever press response was acquired, the reinforcement contingency was changed to a variable interval 10 s (VI-10 s) and discrimination training was initiated. Rats were initially injected subcutaneously (sc) 15 min prior to the start of daily 20-min training sessions with either 0.25 mg/kg amphetamine (AMPH), saline (SAL) or 0.0125 mg/kg haloperidol (HAL). The position of the correct lever associated with each of the training cues was counterbalanced across rats and operant chambers so that the number of rats trained to respond on the left, center and right lever after injections of AMPH, SAL and HAL was nearly equal in each chamber. Over a two-week period, the VI schedule was gradually increased to a VI-30 s and a 10-s time out (TO-10 s) from reinforcement was introduced for incorrect lever responses on the 17th training session and increased to 15 s over several additional sessions. Reinforcement on the VI-30 s schedule for correct lever responses was reinstated after a 15-s period of error free responding elapsed. The concurrent VI-30 s, TO-15 s reinforcement schedule remained in effect for the duration of the experiment. Periodically during training, acquisition of the discrimination was monitored during 2.5 min extinction sessions scheduled at the beginning of the 20-min training sessions. This allowed for monitoring acquisition of the discrimination unconfounded by reinforcement. During the remaining 17.5 min of the session, correct responding was reinforced on the VI-30 s, TO-15 s schedule. In order to improve discrimination, starting with the 30th training session the training dose of AMPH was increased to 0.30 mg/kg and the training dose of HAL was increased to 0.03 mg/kg and remained at these concentrations for the duration of the experiment. Discrimination training continued until no further improvement was observed in choice of the correct lever for the three training cues.

### 3.2. Determination of amphetamine and haloperidol dose–response functions

In order to determine AMPH and HAL dose–response functions, the fifty rats were matched on their acquisition data and then assigned to one of five groups ($n$=10). Subjects in the five groups were then given 5-min extinction tests on either 0.30 mg/kg AMPH, 0.15 mg/kg AMPH, SAL, 0.015 mg/kg HAL or 0.03 mg/kg HAL.

### 3.3. Determination of amphetamine and haloperidol dose–response functions 24 h following treatment with a single injection of 7.5 mg/kg AMPH

After five days of retraining, in order to study the effects of a single large dose of AMPH on the AMPH and HAL dose–response functions, the same five groups described above were injected with 7.5 mg/kg AMPH 24 h prior to being given 5-min extinction tests on either 0.30 mg/kg AMPH, 0.15 mg/kg AMPH, SAL, 0.015 mg/kg HAL or 0.03 mg/kg HAL.

### 3.4. Determination of amphetamine and haloperidol dose–response functions 24 h following treatment with a single injection of 1.0 mg/kg HAL

After 10 days of retraining, in order to study the effects of a large single dose of HAL on the AMPH and HAL dose–response functions, the same five groups described above were injected with 1.0 mg/kg HAL 24 h prior to being given 5-min extinction tests on either 0.30 mg/kg AMPH, 0.15 mg/kg AMPH, SAL, 0.015 mg/kg HAL or 0.03 mg/kg HAL.

### 3.5. Drugs

D-amphetamine sulphate (Sigma Chemical St. Louis, MO, USA) and haloperidol (McNeil Laboratories in solution of 5 mg/ml) were dissolved or diluted in isotonic saline and injected in volumes of 1 ml/kg. The doses of D-amphetamine were calculated as those of the salt.

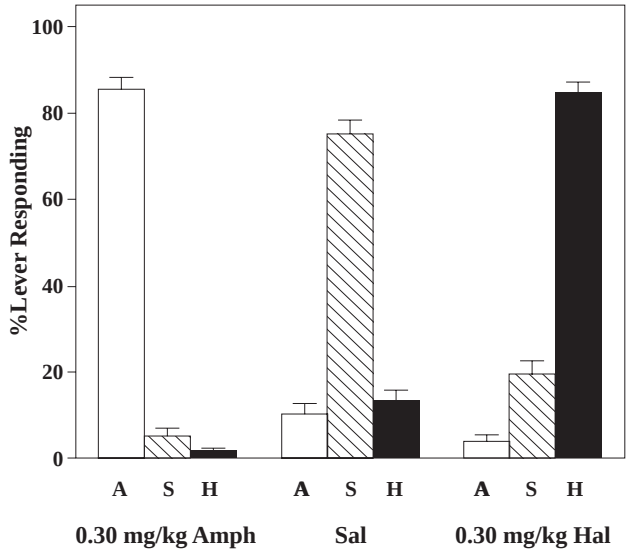


Fig. 1. Percent responding on the amphetamine (A), saline (S) and haloperidol (H) levers when rats were given 5-min extinction tests following injections of 0.30 mg/kg AMPH, SAL, or 0.03 mg/kg HAL. Each bar represents the mean±SEM of 50 subjects.

### 3.6. Data analysis

The data of primary interest were percent choice of the AMPH, SAL and HAL levers during the 5-min extinction test sessions. Because, percent lever responding by rats trained on a concurrent VI=30 s, TO-15 s schedule of reinforcement is normally distributed (Barrett et al., 1994), parametric statistics including one- and two-way repeated measures analyses of variance (ANOVAs), were used to evaluate the results from the present experiments. Following significant findings in the ANOVA tests, the Neuman–Keuls test was used to make post hoc paired comparisons.

## 4. Results

### 4.1. Acquisition of the three-lever amphetamine–saline–haloperidol lever discrimination

A total of 66 training sessions were required for the rats to attain criterion discrimination as defined by the observation of asymptotic discrimination for each of the three levers. Fig. 1 shows percent choice of the AMPH, SAL and HAL levers when rats were given 5-min extinction tests on 0.30 mg/kg AMPH, SAL and 0.03 mg/kg HAL, following 22 AMPH, 23 HAL and 21 SAL training sessions. As can be seen, the 50 rats made an average of 86% of their responses on the AMPH lever when tested on the 0.30 mg/kg training dose of AMPH, 76% on the SAL lever when tested on SAL, and 84% on the HAL lever when tested on the 0.03 mg/kg training dose of HAL.

### 4.2. Determination of amphetamine and haloperidol dose–response functions

In Fig. 2A, lever choice is plotted for the five groups tested on either 0.30 mg/kg AMPH, 0.15 mg/kg AMPH, SAL, 0.015 mg/kg HAL, or 0.03 mg/kg HAL. Separate repeated measures ANOVAs comparing percent choice of the AMPH lever following injections of 0.30 and 0.15 mg/kg AMPH and SAL, and percent choice of the HAL lever following injections of 0.03 and 0.015 mg/kg HAL and SAL, showed that in both cases lever choice varied significantly as a function of Test Dose ($p<.001$). Newman–Keuls tests used to compare individual means indicated that percent AMPH lever responding was significantly ($p<.01$) greater following 0.30 mg/kg AMPH (84%) than 0.15 mg/kg AMPH (44%) or SAL (7%) and significantly greater following 0.15 mg/kg AMPH than SAL ($p<.01$). Comparable tests showed that percent HAL lever responding was significantly greater when tested on 0.03 mg/kg

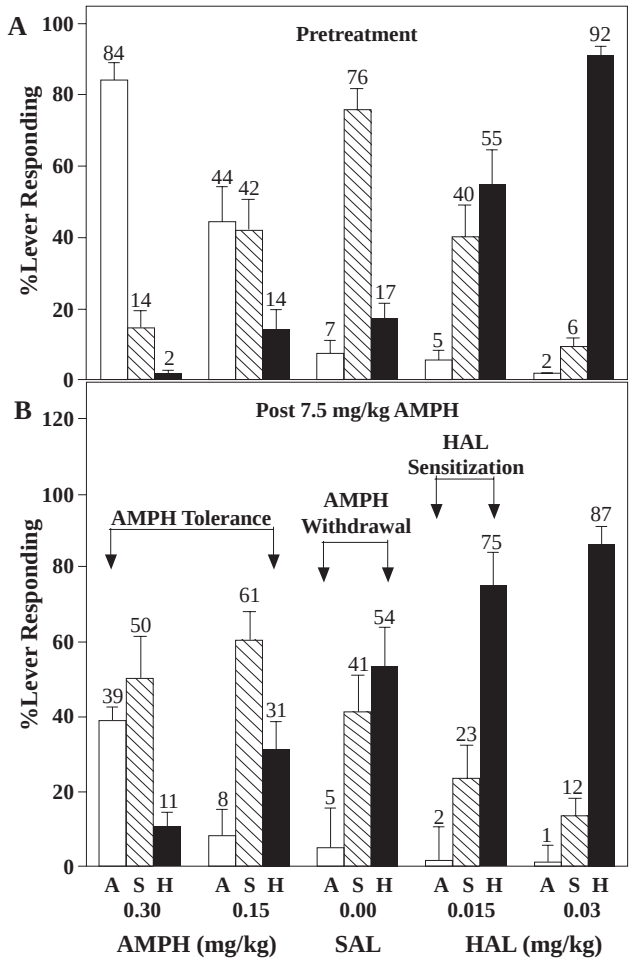


Fig. 2. (A) Lever choice from 5-min extinction tests is plotted for the five groups ($n$=10) tested on either 0.30 mg/kg AMPH, 0.15 mg/kg AMPH, SAL, 0.015 mg/kg HAL, or 0.03 mg/kg HAL prior to treatment with 7.5 mg/kg AMPH. (B) Lever choice is plotted for the same tests given 24 h following treatment with 7 mg/kg AMPH. Each bar represents the mean±SEM of 10 subjects.

(92%) HAL than when tested on 0.015 mg/kg HAL (55%) ($p<.01$) or SAL (17%), ($p<.01$) and significantly greater when tested on 0.015 mg/kg HAL than on SAL ($p<.01$).

*4.3. Determination of amphetamine and haloperidol dose–response functions 24 h following treatment with a single dose of 7.5 mg/kg AMPH*

Fig. 2B shows percent responding on the AMPH, SAL and HAL levers for the groups tested on either 0.30 mg/kg AMPH, 0.15 mg/kg AMPH, SAL, 0.015 mg/kg HAL, or 0.03 HAL mg/kg, 24 h following treatment with 7.5 mg/kg AMPH. Tolerance to the AMPH cue can be seen by comparing AMPH lever choice (see Fig. 2A vs. B) between the groups tested on 0.30 mg/kg AMPH and 0.15 mg/kg AMPH pre- and post-treatment with 7.5 mg/kg AMPH. Pre-AMPH treatment (Fig. 2) A, these two groups made 84% and 44% of their responses on the AMPH lever, compared to 39% and 8% post-AMPH (Fig. 2B). A 2 (Pre-Post) ×3 (AMPH Test Dose) repeated measures ANOVA computed on these data indicated significant Pre-Post ($p<.001$) and AMPH Test Dose ($p<.001$) main effects as well as a significant Pre-Post × AMPH Test Dose interaction ($p<.05$). Post-hoc Newman–Keuls tests showed that rats tested on 0.30 mg/kg AMPH ($p<.05$) and 0.15 mg/kg AMPH ($p<.05$), but not SAL, made significantly fewer responses on the AMPH lever post-AMPH treatment.

In Fig. 2B it can also be seen that choice of the HAL lever increased when rats were tested on 0.30 mg/kg AMPH (2–11%), 0.15 mg/kg AMPH (14–31%) and SAL (17–54%) following treatment with 7.5 mg/kg AMPH. A 2 (Pre-Post) ×3 (AMPH Test Dose) repeated measures ANOVA computed on these data indicated that the overall increase in choice of the HAL lever was significant ($p<.001$).

A 2 (Pre-Post) ×3 (HAL Test Dose) repeated measures ANOVA on percent choice of the HAL lever when the groups were tested on 0.03 mg/kg HAL, 0.015 mg/kg HAL and SAL, showed choice of the HAL lever increased significantly as a function of treatment with 7.5 mg/kg AMPH 24 h prior to testing ($p<.001$). There was also a significant HAL Test Dose ($p<.001$) and Pre-Post × Hal Test Dose interaction ($p<.001$). Newman–Keuls tests comparing HAL lever pre- and post-AMPH treatment in the group tested on 0.015 mg/kg HAL showed that the increase (from 55% to 75%) observed post-AMPH was significant ($p<.05$, one-tailed test). In the group tested on 0.03 mg/kg HAL, the near ceiling level of responding on the HAL lever (92%) prior to treatment with AMPH, precluded the observation of enhanced HAL choice at this dose.

*4.4. Determination of amphetamine and haloperidol dose–response functions 24 h following treatment with a single injection of 1.0 mg/kg HAL*

The data presented in Fig. 3B show that when rats were tested 24 h following treatment with 1.0 mg/kg HAL,

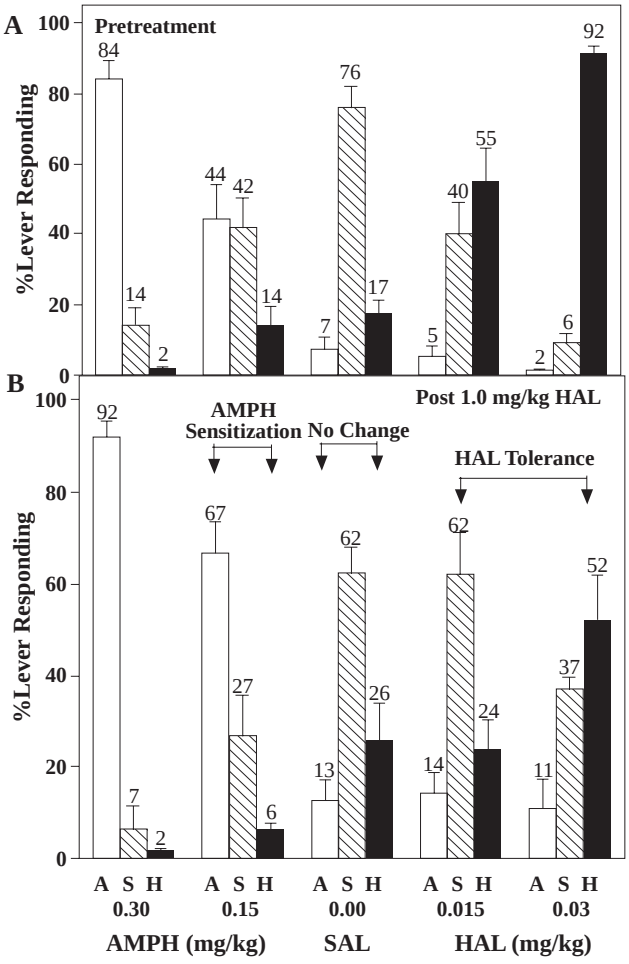


Fig. 3. (A) Lever choice from 5-min extinction tests is plotted for the five groups ($n$=10) tested on either 0.30 mg/kg AMPH, 0.15 mg/kg AMPH, SAL, 0.015 mg/kg HAL, or 0.03 mg/kg HAL prior to treatment with 1.0 mg/kg HAL. (B) Lever choice is plotted for the same tests given 24 h following treatment with 1.0 mg/kg HAL. Each bar represents the mean±SEM of 10 subjects.

tolerance was observed to the HAL cue. A 2 (Pre-Post) ×3 (Hal Test Dose) repeated measures ANOVA comparing responding on the HAL lever when rats were tested on 0.03 HAL, 0.015 mg/kg HAL and SAL, indicated there was a significant overall decrease ($p<.001$) in choice of the HAL lever following treatment with HAL, and a significant Pre-Post × Hal Test Dose interaction ($p<.001$). Newman–Keuls paired-comparison tests showed (compare Fig. 3A to B) choice of the HAL lever was significantly decreased ($p<.01$) for the groups tested on 0.03 mg/kg HAL (92–52%) and 0.015 mg/kg HAL (55–24%). The significant interaction was a result of the group tested on SAL showing no change (17–26%) pre- to post-HAL.

## 5. Discussion

The results from the present study show that rats can learn to discriminate among cues associated with AMPH, an

indirect DA agonist, HAL, a DA, $D_2$ receptor antagonist, and SAL. By testing rats on two doses of AMPH, SAL and two doses of HAL, it was possible to show orderly dose-related changes in choice of the three levers that presumably reflect changes along a continuum of DA-mediated interoceptive cues. This baseline provided a sensitive index to assess adaptive changes in an animal behavior thought to be relevant to understanding the motivational role aversive symptoms of withdrawal play in motivating drug use in people.

With regard to AMPH withdrawal, the results showed that when the rats were tested on SAL 24 h following treatment with 7.5 mg/kg AMPH, there was a rebound shift to responding primarily on the HAL lever, i.e., withdrawal. Furthermore, the data indicate that the interoceptive cues characterizing this withdrawal period were equivalent to those observed after an acute injection of 0.015 mg/kg HAL. This conclusion is supported by the finding that when rats were tested on SAL, 24 h post-AMPH, (see Fig. 2B) the distribution of responses on the AMPH, SAL and HAL levers was almost identical to that normally observed when the rats were tested on an acute dose of 0.015 mg/kg HAL (see Fig. 2A). Specifically, the rats tested on SAL post-AMPH increased responding on the HAL lever from 17% to 54%, that was virtually identical to the 55% HAL lever responding observed when rats were tested on 0.015 mg/kg HAL prior to AMPH treatment. This finding is consistent with previous results from two-lever, drug-drug discrimination studies (Haenlein et al., 1985) where rats trained to discriminate between AMPH and HAL responded on the HAL lever during withdrawal from AMPH. In the Barrett et al. study, choice of the AMPH and HAL levers following treatment with 3 mg/kg AMPH changed in a bi-directional manner as a function of time since drug administration. When tested at short, post-AMPH intervals (6–8 h), rats responded primarily on the AMPH lever with responding gradually shifting to the HAL lever at intermediate intervals (16–30 h) before returning to pretreatment levels by 48–72 h. Although those data provided convincing evidence for a withdrawal cue state following treatment with AMPH, it was less clear whether rats responded on the HAL lever because the withdrawal cues were qualitatively similar to the HAL training cue, or simply because the rats were forced to choose between responding on the AMPH and HAL lever, and the withdrawal cues were more similar to HAL. In the three-lever experiment reported here, if the cues associated with withdrawal were qualitatively different from HAL, rats would have responded on the SAL lever. Instead they responded as though administered an acute dose of 0.015 mg/kg HAL.

Although pretreatment with 7.5 mg/kg AMPH resulted in tolerance to the AMPH cue, it had the opposite effect on the HAL cue. For example, sensitization to the HAL cue can be seen by comparing choice of the HAL lever when rats were tested on 0.015 mg/kg HAL pre- and post-treatment with 7.5 mg/kg AMPH. By comparing the data in Fig 2A and B, it can be seen that choice of the HAL lever increased from 55% pre-AMPH to 75% post-AMPH. Thus, for the same reason that tolerance to the AMPH cue does not reflect a weaker cue, sensitization to the HAL cue does not imply a stronger or more salient cue. In both cases, what changed was the baseline (compare SAL results pre- and post-AMPH) at the time the drugs were tested.

Treating rats with 1.0 mg/kg HAL 24 h prior to tests on 0.30 mg/kg AMPH, 0.15 mg/kg AMPH, SAL, 0.03 mg/kg HAL and 0.015 mg/kg HAL produced changes in choice of the three levers, that for the most part, were exactly opposite (see Fig. 3B) to those observed following treatment with 7.5 mg/kg AMPH. Tolerance to the HAL cue, as defined by reduced choice of the HAL lever, was observed when rats were tested on 0.015 HAL and 0.03 mg/kg HAL, 24 h-post 1.0 mg/kg HAL. Also observed was sensitization to the AMPH cue, as defined by an increase from 44% to 67% AMPH lever choice when rats were tested on 0.15 mg/kg AMPH 24-h post HAL treatment. There was one significant difference between the adaptive changes observed following AMPH and HAL. In contrast to the shift in responding from the SAL to the HAL lever when rats were tested on SAL following treatment with 7.5 mg/kg AMPH, no shift in SAL lever choice was observed 24 h following treatment with 1.0 mg/kg HAL. This finding was informative because previous (Barrett et al., 1992; Barrett and Steranka, 1983) two-lever studies reported AMPH-like rebound cues following HAL treatment. In the Barrett and Steranka (1983) study, rats significantly increased responding on the AMPH lever when tested 24 h following chronic treatment with 1.0 mg/kg HAL. Failure to observe a similar shift in the present three-lever experiment simply means that the HAL withdrawal cue does not substitute for the AMPH cue when rats are given the additional choice of responding on a SAL lever. It does not question the presence of post-HAL withdrawal cues, but rather illustrates the importance of using the two-lever, agonist–antagonist discrimination as an initial generic screen for detecting withdrawal cues. It remains for future three-lever studies to further characterize the HAL rebound cue. Although the cues associated with HAL withdrawal do not substitute for AMPH, the present results indicate they are additive with the AMPH cue and competitive with the HAL cue. For example, when tested on 0.30 mg/kg AMPH and 0.15 mg/kg AMPH, 24 h after treatment with 1.0 mg/kg HAL, rats increased choice of the AMPH lever from 84% to 92% and from 44% to 67%, for the two doses, respectively. By contrast, testing rats on 0.03 mg/kg HAL, post-1.0 mg/kg HAL reduced choice of the HAL lever from 92% to 52%. The 52% was virtually identical to the 55% responding on the HAL lever typically seen in rats following an acute dose of 0.015 mg/kg HAL (see Fig. 3A). Tests with 0.015 mg/kg HAL, 24 h post-treatment with 1.0 mg/kg HAL, showed that choice of the HAL lever was reduced from 55% to 24% and was not different from the 17% HAL lever responding normally observed when rats were tested on SAL. Thus, the

rebound cues present 24 h following treatment with 1.0 mg/kg HAL completely blocked the cues associated with 0.015 mg/kg HAL.

The current findings illustrating the pronounced shift in baseline also seem relevant to interpreting data from cocaine and heroin drug self-administration studies (Ahmed and Koob, 1999) reporting escalation of drug intake. In these studies, when rats have limited access to the drug each day (1 h), they develop stable levels of intake that remain relatively constant over time. Changing the dose per injection results in rats adjusting their rate of intake to achieve a desired pharmacological effect. When access time was increased to 6 h for cocaine (Ahmed and Koob, 1999) or 11 h for heroin a gradual increase in intake during the daily sessions was observed for all doses tested (vertical shift in dose–response function). Drug self-administration procedures have no way to directly assess rebound changes in the non-drug baseline, i.e., shift in hedonic starting point, that might develop when subjects are given extended access to the drug each day. In a recent study, rats trained to self-administer cocaine were also implanted with stimulating electrodes in the lateral hypothalamus and trained on an intracranial self-stimulation (ICSS) reward threshold procedure. Changes in ICSS reward thresholds were used as an operational measure of brain reward function. The results from this study showed that the same conditions that resulted in an escalation of cocaine self-administration produced a baseline increase in ICSS reward thresholds. More importantly, similar to what we observed, the authors state that tolerance observed to the reinforcing properties of cocaine did not result from a diminished cocaine effect on basal reward thresholds, per se. Rather, tolerance reflected a baseline increase in reward thresholds that prevented the thresholds from reaching the same absolute level as observed prior to prolonged exposure to cocaine. The tolerance observed in the present three-lever, drug-discrimination study appeared to exactly parallel the report in that a post AMPH baseline shift accounted for tolerance to the AMPH cue, not a diminished or less discriminable cue.

Finally, the results from the present experiment provide insight into understanding why tolerance to a drug's cue properties is observed when discrimination training is suspended during chronic drug treatment (see reviews by Young, 1990, 1991; Young and Sannerud, 1989) but not when training is continued (Colpaert, 1995). The explanation generally given for this apparent discrepancy (Sannerud and Griffiths, 1993; Hirschhorn and Rosecrans, 1974) is that continuing training during chronic treatment allows subjects to gradually transfer the discrimination to a weaker cue such that when rats are tested following a chronic regimen, no change in discrimination is observed. This explanation is supported by studies demonstrating that doses of a training drug, too low to support acquisition of a discrimination, will maintain an already acquired discrimination, if training is continued while the dose is gradually reduced (Overton, 1979). When training is suspended there is no opportunity for subjects to transfer the discrimination to the weaker cue presumably associated with the training drug. Thus, when subjects are tested for tolerance following chronic drug treatment, higher doses of the drug are required to obtain the level of discrimination previously observed i.e., rightward shift of dose–response function. Our results suggest that the reason tolerance is not observed when training is continued during chronic drug treatment is that rats learn to transfer the discrimination to qualitatively different, not less salient cues associated with the response levers. Thus, for example, the cue associated with the SAL lever would now include withdrawal cues and the cues associated with the Drug lever would be altered by withdrawal cues present during the drug training sessions. However, our results indicate that the discriminable difference between the cues associated with the SAL and Drug levers would remain unchanged. This explanation is also consistent with the fact that subjects can continue receiving drug training for months and even years without a breakdown in discrimination.

## Acknowledgments

The authors would like to express their appreciation for the expertise and meticulous attention to detail provided by Ms. Barbara Rowland in conducting this study. This research was supported by the Veterans Administration.

## References

Ahmed SH, Koob GF. Long-lasting increases in the set point for cocaine self-administration after escalation in rats. Psychopharmacology 1999;146:303–12.

Altman JL, Albert J-M, Milstein SL, Greenberg I. Drugs as discriminable events in humans. In: Lal H, editor. Discriminative stimulus properties of drugs. New York: Plenum; 1977. p. 187–206.

Balster RL. Drug abuse potential evaluation in animals. Br J Addict 1991;86:1549–58.

Barrett RJ. Behavioral approaches to individual differences in substance abuse. In: Galizio M, Maisto S, editors. Determinants of substance abuse. New York: Plenum Press; 1985. p. 125–75.

Barrett RJ, Leith NJ. Tolerance to the discriminative stimulus properties of D-amphetamine. Neuropharmacology 1981;20:251–5.

Barrett RJ, Steranka LR. Drug discrimination in rats: evidence for amphetamine-like cue state following chronic haloperidol. Pharmacol Biochem Behav 1983;18:611–7.

Barrett RJ, White DK, Caul WF. Tolerance, withdrawal and supersensitivity to dopamine mediated cues in a drug–drug discrimination. Psychopharmacology 1992;109:63–7.

Barrett RJ, Caul WF, Huffman EM, Smith RL. Drug discrimination is a continuous rather than quantal process following training on a VI–TO schedule of reinforcement. Psychopharmacology 1994;113:289–96.

Callahan PM, Appel JB, Cunningham KA. Dopamine D1 and D2 mediation of the discriminative stimulus properties of D-amphetamine and cocaine. Psychopharmacology 1991;103:50–5.

Caul WF, Barrett RJ, Huffman EM, Stadler JR. Rebound responding following a single dose of drug using an amphetamine–vehicle–haloperidol drug discrimination. Psychopharmacology 1996;128:274–9.

Colpaert FC. Discriminative stimulus properties of narcotic analgesic drugs. Pharmacol Biochem Behav 1978;9:863–87.
Colpaert FC. Drug discrimination: no evidence for tolerance to opiates. Pharmacol Rev 1995;47:605–29.
Colpaert FC. System theory of pain and of opiate analgesia: no tolerance to opiates. Pharmacol Rev 1996;8:355–402.
Colpaert FC. Drug discrimination in neurobiology. Pharmacol Biochem Behav 1999;64:337–45.
Dworkin SI, Bimle C. 6-Hydroxydopamine lesions of the nucleus accumbens attenuate the discriminative stimulus effects of D-amphetamine. Drug Dev Res 1989;16:435–41.
Exner M, Clark D. Agonist and antagonist activity of low efficacy D2 dopamine receptor agonists in rats discriminating D-amphetamine from saline. Behav Pharmacol 1992;3:609–19.
Fischman MW, Schuster CR, Resenekov L, Shick JF, Krasnegor NA, Fennell W, et al. Cardiovascular and subjective effects of intravenous cocaine administration in humans. Arch Gen Psychiatry 1976;33:983–9.
Gawin FH. Cocaine addiction: psychology and neurophysiology. Science 1991;29:1580–6.
Haenlein M, Caul WF, Barrett RJ. Amphetamine–haloperidol discrimination: effects of chronic drug treatment. Pharmacol Biochem Behav 1985;23:949–52.
Hirschhorn ID, Rosecrans JA. Morphine and delta-9-tetrahydrocannabinol: tolerance to the stimulus effects. Psychopharmacologia 1974;36: 243–53.
Kamien JB, Bickel WK, Hughes JR, Higgins ST, Smith BJ. Drug discrimination by humans compared to nonhumans: current status and future directions. Psychopharmacology 1993;111:259–70.
Koob GF, Stinus L, Le Moal M, Bloom FE. Opponent-process theory of motivation: neurobiological evidence from studies of opiate dependence. Neurosci Biobehav Rev 1989;13:135–40.
Koob GF, Caine SB, Parsons L, Markou A, Weiss F. Opponent process model of addiction. Pharmacol Biochem Behav 1997;57:513–21.
Markou A, Weiss F, Gold LH, Caine SB, Schulties G, Koob GK. Animal models of drug craving. Psychopharmacology 1993;112:163–82.
Nielsen EB, Jepsen SA. Antagonism of the amphetamine cue by both classical and atypical antipsychotic drugs. Eur J Pharmacol 1985; 111:167–76.
Nielsen EB, Randrup K, Andersen PH. Amphetamine discrimination: effects of dopamine receptor agonists. Eur J Pharmacol 1989;160: 253–62.
Overton DA. Drug discrimination training with progressively lowered doses. Science 1979;205:720–1.
Preston KL, Bigelow GE. Subjective and discriminative effects of drugs. Behav Pharmacol 1991;2:293–313.
Sannerud CA, Griffiths RR. Tolerance to the discriminative stimulus effects of midazolam: evidence for environmental modification and dose fading. Behav Pharmacol 1993;4:125–33.
Smith GM, Beecher HK. Amphetamine, secobarbital and athletic performance: II. Subjective evaluations of performance, mood and physical states. JAMA 1960;172:1502–14.
Solomon RL. The opponent-process theory of acquired motivation: the costs of pleasure and the benefits of pain. Am Psychol 1980;35: 691–712.
Solomon RL, Corbit JD. An opponent-process theory of motivation: II. Cigarette addiction. J Abnorm Psychology 1973;81:158–71.
Stadler JR, Caul WF, Barrett RJ. Characterizing withdrawal in rats following repeated drug administration using an amphetamine–vehicle–haloperidol drug discrimination. Psychopharmacology 1999; 143:219–26.
Van Groll BJ, Appel JB. Stimulus effects of D-amphetamine 1: DA mechanisms. Pharmacol Biochem Behav 1992;43:967–73.
Woolverton WL, Cervo L. Effects of central dopamine depletion on D-amphetamine discriminative stimulus in rats. Psychopharmacology 1986;88:196–200.
Young AM. Tolerance to drugs acting as discriminative stimuli. Psychopharmacology 1990;101:S77.
Young AM. Tolerance to drugs acting as discriminative stimuli. In: Glennon RA, Jarbe TUC, Frankenheim J, editors. Drug discrimination: applications to drug abuse research NIDA research monograph. 116 Washington, DC: U.S. Government Printing Office; 1991. p. 197–212.
Young AM, Sannerud CA. Tolerance to drug discriminative stimuli. In: Goudie AJ, Emmett-Oglesby MW, editors. Tolerance and sensitization. Clifton, (NJ): Humana Press; 1989. p. 221–78.