Available online at www.sciencedirect.com

SCIENCE DIRECT®

Pharmacology, Biochemistry and Behavior 79 (2004) 761–771

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

www.elsevier.com/locate/pharmbiochembeh

Evidence for bidirectional cues as a function of time following treatment with amphetamine: implications for understanding tolerance and withdrawal

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

[a]*Veterans Administration Medical Center, Departments of Psychology and Pharmacology, Vanderbilt University, Research Service (151), 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 3 7203, USA*

Received 9 October 2004; accepted 14 October 2004
Available online 14 November 2004

Abstract

Rationale: Previous drug-discrimination studies have focused on characterizing the cue properties associated with amphetamine's (AMPH) primary effect. Results from recent experiments indicate that equally prominent cues are associated with AMPH withdrawal.
Objectives: The purpose of the present study was to investigate the extent to which AMPH-induced withdrawal cues, opposite to those associated with AMPH's primary effect are observed.
Methods: Since dopamine (DA) has been implicated in mediating the AMPH cue, rats were trained to discriminate between 0.25 mg/kg AMPH, an indirect DA agonist, and 0.033 mg/kg haloperidol (HAL), a DA antagonist at the D2 receptor site. Training doses were chosen so that rats responded about equally on both levers when tested on saline (SAL) providing a behavioral baseline sensitive to assessing AMPH-related bidirectional changes in cue state. Following acquisition of the discrimination, rats were tested for choice of responding on the AMPH and HAL levers at intervals from 6 to 72 h following treatment with a single dose of 3.0 mg/AMPH. Also, in order to investigate the relationship between withdrawal and tolerance to AMPH's cue properties, AMPH dose–response curves were determined 24 h following treatment with SAL, 1.5 and 3.0 mg/kg AMPH.
Results: At short intervals after treatment with 3.0 mg/kg AMPH, rats responded primarily on the AMPH lever followed by a shift to predominant responding on the HAL lever 16–30 h post-treatment, before returning to predrug levels. Treatment with 1.5 and 3.0 mg/kg AMPH produced parallel dose–response curve shifts to the right.
Conclusions: Following a single dose of AMPH, robust cues associated with AMPH withdrawal were observed that lasted approximately three times longer than the cues associated with the drug's primary effects. Furthermore, results from the tolerance tests indicate that tolerance reflects a baseline shift rather than a loss in drug efficacy.

Keywords: Drug-discrimination; Tolerance; Withdrawal; Amphetamine; Haloperidol

1. Introduction

The drug-discrimination procedure has proven to be a valuable tool in characterizing the interoceptive cue properties of drugs in both animals and people. In animals, the procedure has been used to identify drugs with similar cue properties, characterize the neurotransmitter–receptor interactions responsible for mediating a drug's cue properties and to help identify the neuroanatomical sites where these interactions are thought to occur. A drug that has received considerable attention in the drug-discrimination literature is the CNS stimulant amphetamine (AMPH), a drug with a long history documenting its potential for abuse (Ellinwood, 1973). Drug-discrimination studies have comprehensively

* Corresponding author. Tel.: +1 615 327 4751x5475; fax: +1 615 321 6305.
E-mail address: r2k2@comcast.net (R.J. Barrett).

0091-3057/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2004.10.005

investigated the mechanisms mediating amphetamine's primary cue properties and the results converge on an important role for the neurotransmitter dopamine (DA). For example, other drugs thought to activate DA function such as cocaine (D'Mello and Stolerman, 1977), methylphenidate (Rush and Pazzaglia, 1997) and L-cathinone (Huang and Wilson, 1986) generalize to the amphetamine cue. More specifically, results from studies that have evaluated DA agonists have shown that D_2 agonists including quinpirole, pergolide and piribedil all generalize to amphetamine (Evans and Johanson, 1987; Arnt, 1988; Nielsen et al., 1989; Callahan et al., 1991). Likewise, D_2 antagonists including haloperidol (HAL), pimozide, raclopride, sulpiride, spiroperidol and sulpiride have been shown to block the amphetamine cue (Jarbe, 1982; Nielsen and Jepsen, 1985; Arnt, 1988; Nielsen et al., 1989; Callahan et al., 1991). Evidence that the specific DA reuptake inhibitor GBR 12909 completely generalizes to amphetamine (Van Groll and Appel, 1992) is also consistent with a role for DA. Results from experiments with D_1 agonists and antagonists are less clear. Several studies reported that neither the full D_1 agonist SKF 81297 nor the partial D_1 agonist SKF 38393 generalize to amphetamine (Arnt, 1988; Kamien and Woolverton, 1989; Nielsen et al., 1989; Callahan et al., 1991; Reavill et al., 1993), although a number of experiments found that the D_1 antagonists SCH 23390 and SCH 39166 block the amphetamine cue (Nielsen and Jepsen, 1985; Arnt, 1988; Kamien and Woolverton, 1989; Van Groll and Appel, 1992). Taken together, the literature clearly suggests that DA is involved in mediating the amphetamine cue at D_2 receptors although a role for D_1 receptors is less clear. Studies aimed at identifying the neuroanatomical locus of the amphetamine cue have found that rats trained to discriminate systemically administered amphetamine will generalize to amphetamine injected directly into the nucleus accumbens (Nielsen and Scheel-Kruger, 1986), while 6-OHDA lesions of the nucleus accumbens disrupt amphetamine generalization (Dworkin and Bimle, 1989). Studies have described the conditions that produce chronic tolerance to the amphetamine cue (Barrett and Leith, 1981; Young et al., 1992) and have characterized its temporal properties (Jones et al., 1976; Silverman and Ho, 1980). In all of the studies cited above, rats were trained on a conventional, saline (SAL)–amphetamine discrimination, which provides a unidirectional measure of the interoceptive cues associated with amphetamine's primary effects. Results from several recent studies (Barrett et al., 1992; Barrett and Smith, 1988; Michaelis et al., 1988; Smith et al., 1995), specifically designed to provide a behavioral measure sensitive to bidirectional changes in cue state, have discovered that the gradual dissipation and eventual disappearance of a drug's primary cue is often accompanied by the gradual development and eventual appearance of robust and long lasting withdrawal cues that previously had gone undetected. Initial findings suggest that the withdrawal cues are qualitatively opposite to those associated with the primary cue and account for a significant percent of the overall change in cue state induced by drug administration (Barrett et al., 1992; Barrett and Smith, 1988; Barrett and Steranka, 1983; Smith et al., 1995). The purpose of the present experiment was to use the bidirectional measure previously described (Barrett et al., 1992) to characterize the intensity and duration of the rebound cues as a function of treatment dose of amphetamine and to investigate the relationship between withdrawal and the observation of rapid tolerance. Rapid tolerance is defined here as the diminished response to a drug upon administration of a second dose 24 h after the first dose (Silveri and Spear, 2001).

2. Materials and methods

2.1. Subjects and apparatus

Thirty-six male Sprague–Dawley rats obtained from Harlan Laboratories, Indianapolis, IN 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. 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. On the front panel of each chamber, two response levers were mounted 4.92 cm above the floor and required a 28-g force to activate. Responding on the levers was reinforced with food pellets (45 mg: P.J. Noyes) delivered by a pellet dispenser to a cup centered on the opposite back panel of the chamber. Experimental sessions were controlled and data recorded by a computer and interface equipment located outside the experimental room. 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 a two-lever task to discriminate between amphetamine and haloperidol

The rats in the present experiment had received training on a three-lever, amphetamine–saline–haloperidol discrimination prior to being switched to the two-lever, amphetamine–haloperidol task used in this experiment. The three-lever training occurred during daily 20-min training sessions in the operant chambers described above with the exception that three levers were positioned on the front panel of each chamber. Reinforcement during the three-lever training was programmed on a concurrent, variable interval 30 s, time-

out 15 s (VI-30″, TO-15″) schedule of reinforcement. Rats were injected subcutaneously (s.c.) with either 0.30 mg/kg AMPH, SAL or 0.035 mg/kg HAL 15 min before the training sessions. A total of 123 three-lever training sessions consisting of 42, 41 and 40 with SAL, HAL and AMPH, respectively, was given before the rats were switched to a two-lever, AMPH-HAL discrimination. Periodically during the three-lever training, acquisition of the discrimination was monitored during 2.5-min extinction periods scheduled at the beginning of the 20-min training sessions as described below. Training was changed to the two-lever task when it became apparent that it was better suited to establishing a discrimination baseline that was not only sensitive to bidirectional cue changes, but could be made equally sensitive in both directions. This equality of bidirectional sensitivity can be assured in the two-lever procedure by adjusting the training doses of AMPH and HAL such that, when rats are tested on SAL following criterion discrimination, they make about 50% of their responses on each lever. Changing from three-lever to two-lever training involved removing the middle lever. For 12 rats, the middle lever had been associated with SAL so for these subjects the levers associated with AMPH and HAL remained the same. For 12 rats, the AMPH lever remained the same and the lever previously associated with SAL was now paired with HAL. For the remaining 12 rats, the HAL lever remained the same and the lever previously associated with SAL was now paired with AMPH. The preliminary training three-lever training appeared not to affect in any way the results obtained from the two-lever discrimination task. Two observations support this conclusion. First, by the time all rats had reached criterion discrimination and dose–response functions had been determined in the two-lever study, no differences were apparent among the three groups of 12 rats that experienced different specific changes in drug-appropriate levers when the saline lever was withdrawn. Second, portions of this experiment replicated exactly portions of previous two-lever studies where rats were trained exclusively on the same AMPH-HAL discrimination.

Training continued on the two-lever discrimination with responding on the correct levers reinforced on the concurrent VI-30″, TO-15″ as described above. Periodically, during training, acquisition of the discrimination was monitored during 2.5-min extinction periods scheduled at the beginning of the 20-min training session. 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 concurrent schedule. Discrimination training continued until choice of the correct lever during the 2.5-min extinction periods reached a criterion of 85% correct or greater following injections of both AMPH and HAL. A second criterion was that rats responded about equally (50%) on both the AMPH and HAL levers when tested on saline following attaining 85% correct lever choice. This required adjustments in the AMPH and HAL training doses such that by the end of acquisition the training doses for AMPH and HAL were 0.25 and 0.035 mg/kg, respectively.

3.2. General testing procedures

Throughout this experiment, certain precautions were taken to limit or at least control for possible withdrawal effects influencing the outcome of the treatments being investigated. First, because there are some rebound effects associated with training doses as small as those used in the present experiment, the AMPH and HAL training trials were given on alternate days. This procedure attempts to maintain constancy in the cues associated with the two levers during training. Also, throughout the experiment in order to preclude withdrawal cues associated with the drugs given on training sessions from influencing test results, 3 drug-free days always preceded a test session. Thus, the following 3-week sequence was followed. Following a test on Tuesday, training resumed Wednesday through Friday of the first week and continued Monday through Friday of the second week before another test was scheduled on Tuesday of the third week. The test sessions consisted of 5-min extinction periods during which responding on both levers was recorded. Following the 5-min tests, rats were returned to their home cages where they received their daily allotment of food.

3.3. Determination of dose–response functions for amphetamine and haloperidol

Following criterion discrimination performance, dose–response functions for AMPH and HAL were determined by testing groups of rats (n=9) on four doses of AMPH (0.25, 0.125, 0.062 and 0.031 mg/kg) and three doses of HAL (0.035, 0.018 and 0.004 mg/kg) during 5-min extinction test sessions.

3.4. Time course of the primary and subsequent withdrawal cues associated with a single dose of amphetamine

The rats were assigned to one of four groups (n=9) matched on their SAL test scores. Over a period of 3 weeks, the four groups were treated with AMPH and given 5-min extinction tests at two of eight retest intervals (6, 8, 16, 20, 24, 30, 48 and 72 h). On Tuesday of the first week, the four groups were tested either 6, 20, 24 or 48 h following treatment with 3 mg/kg AMPH. Training sessions were resumed on Thursday through Friday of the first week and continued Monday through Friday of the second week. On Tuesday of the third week, the same four groups were tested either 16, 8, 30 or 72 h, respectively, following the same 3 mg/kg dose of AMPH.

3.5. Tests to determine the relationship between rapid tolerance and withdrawal

Using the same four groups described above, rats were tested on one of three doses of AMPH (0.25, 0.125 and

0.062 mg/kg) or SAL 24 h following treatment with either 3.0 mg/kg AMPH or SAL. Following these tests, the rats were given a 5-min SAL test session to assure that the SAL baseline still represented a point midway between the training drugs, i.e., 50% responding on the AMPH and HAL levers. Four new matched groups were formed using the saline test scores. These groups were treated with 1.5 mg/kg AMPH and 24 h later tested on the same three doses of AMPH (0.25, 0.125 and 0.062 mg/kg) and SAL used to determine the AMPH dose–response curves 24 h following treatment with SAL and 3 mg/kg AMPH.

3.6. The effect of acute treatment with haloperidol on the amphetamine dose–response function

A final dose–response function was determined by treating the same four groups of rats with 0.025 mg/kg HAL, 15 min prior to being injected with either 0.25, 0.125, 0.062 mg/kg AMPH or SAL. Animals were tested, as always, 15 min after being injected with the challenge dose of AMPH.

3.7. Drugs

D-Amphetamine sulphate (Sigma, St. Louis, MO, USA) and haloperidol (McNeil Laboratories in solution of 5 mg/ml) were dissolved or diluted in 0.9% saline and injected in volumes of 1 ml/kg. The doses of D-amphetamine were calculated as those of the salt.

3.8. Data analysis

The data of primary interest were percent responding on AMPH and HAL levers during 5-min extinction test sessions. Because percent lever responding by rats trained on a concurrent VI=30″, TO-15″ 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.

4. Results

4.1. Acquisition of the amphetamine–haloperidol discrimination

From the time the rats were switched from training on the three-lever procedure to training on the two-lever, AMPH-HAL task, 10 sessions with AMPH and 10 with HAL were required for the animals to reach criterion discrimination. By the conclusion of acquisition and throughout the duration of the experiment, the rats consistently averaged over 90% correct lever choice when tested on the training doses of both AMPH (0.25 mg/kg) and HAL (0.035 mg/kg). When all rats were tested on SAL following criterion acquisition, percent choice of the two levers was 51.8% and 48.2% for the AMPH and HAL levers, respectively. Periodically, throughout the experiment, rats were tested on SAL in order to assure that the behavioral baseline remained equally sensitive to detecting primary and withdrawal cues associated with amphetamine. In Fig. 1, a frequency distribution is plotted of the 100 SAL test scores that resulted from these tests. Each rat was tested an average of three times on SAL over the course of the experiment. The scores plotted represent percent responding on the AMPH lever. As can be seen, the mean of the distribution was 51.6%, the median 52.9% and the standard deviation 20.3%.

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

Fig. 2 shows the results when rats were tested with four doses of AMPH and three doses of HAL. Separate one-way ANOVAs computed on results from the four doses of AMPH and three doses of HAL showed that choice of the AMPH [$F(3,26)=4.5$, $P<0.01$] and HAL [$F(2,20)=12.4$, $P<0.01$] levers varied in an orderly manner as a function of dose. The SAL data point in Fig. 2 represents the average percent responding on the two levers when all 36 rats were tested on SAL immediately prior to determination of the dose–response functions.

The rats injected with 0.25, 0.125, 0.06 and 0.03 mg/kg AMPH averaged 35, 27, 36 and 39 responses, respectively, during the 5-min extinction sessions. The groups injected with 0.035, 0.015 and 0.008 mg/kg HAL averaged 21, 23 and 30 responses, respectively. Separate ANOVAs used to test for differences among the four AMPH groups [$F(3,25)=1,5$, $P=0.34$] and the three HAL groups [$F(2,20)=1.3$, $P=0.29$] were not significant.

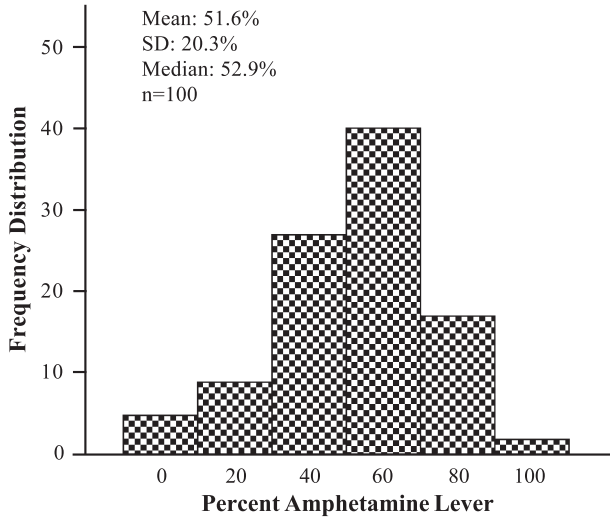

Fig. 1. The data represent the distribution of % AMPH lever responding resulting from 5-min SAL extinction tests given during the course of the experiment. A chi-square test indicated that the distribution did not differ significantly from a normal distribution ($\chi^2=2.90$, $df=5$, $p=0.716$).

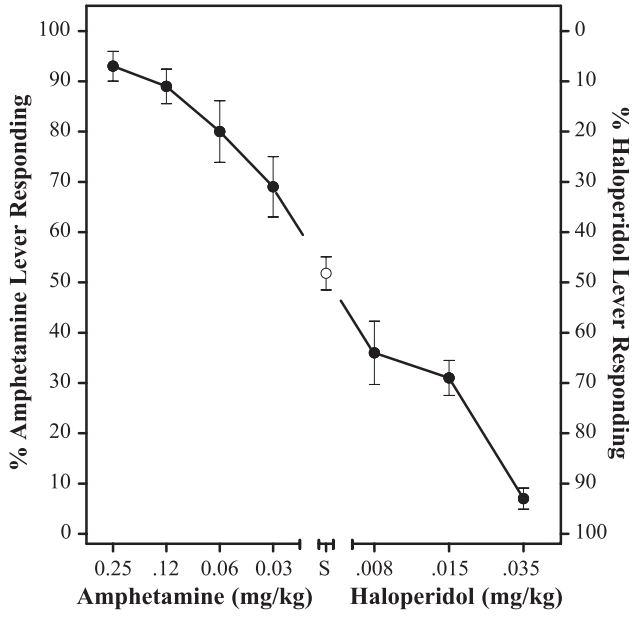

Fig. 2. Percent choice of the AMPH and HAL levers when rats were tested during 5-min extinction test sessions administered 15 min following injections with four doses of AMPH, SAL and three doses of HAL. Data represent means±S.E.M. of nine rats per group. The SAL data point represents mean lever choice when all 36 rats were tested on SAL following acquisition.

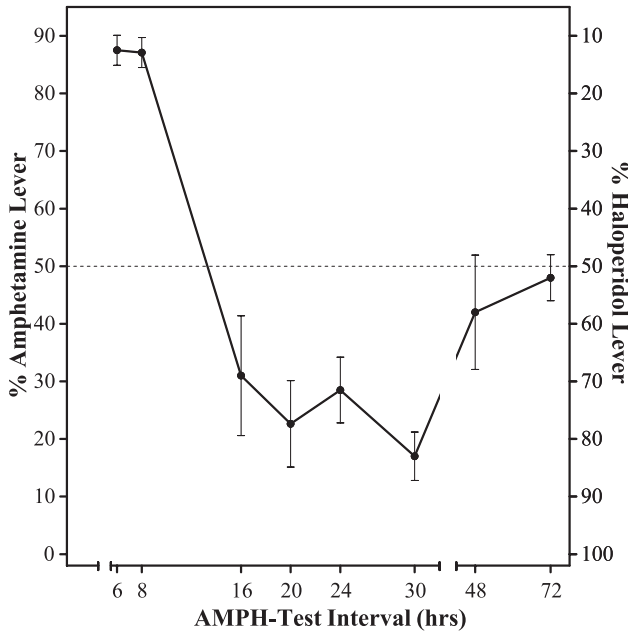

Fig. 3. Percent choice of the AMPH and HAL levers during 5-min extinction test sessions as a function of time following treatment with 3 mg/kg AMPH. All groups were injected with SAL 15 min prior to testing. Data represent means±S.E.M. of nine rats per group. The dotted horizontal line represents mean percent lever choice when all animals were tested on saline prior to treatment.

4.3. Time course of the primary and subsequent withdrawal cues associated with a single dose of amphetamine

In Fig. 3, responding on the AMPH and HAL levers is shown as a function of time since treatment with 3 mg/kg AMPH. As can be seen, rats responded primarily on the AMPH lever (87–88%) at the short intervals (6–8 h), shifted to responding primarily on the HAL lever (70–83%) at intermediate intervals (16–30 h) and returned to baseline responding by the 72-h interval. Separate ANOVAs found that lever choice varied significantly as a function of time across the 6-, 20-, 24- and 48-h intervals [$F(3,21)=15.5$, $P<0.001$] as well as the 8-, 16-, 30- and 72-h intervals [$F(3,25)=18.8$, $P<0.001$].

Total number of responses for the 8-, 16-, 30- and 72-h retest groups during the extinction test sessions averaged 36, 18, 13 and 24 responses, respectively. A one-way ANOVA indicated the differences were not significant [$F(3,25)=2.7$, $P=0.07$]. Average total number of responses for the 6-, 20-, 24- and 48-h retest groups was 34, 13, 13 and 13, respectively. An ANOVA computed on these totals showed the differences were significant [$F(3,19)=6.6$, $P<0.03$].

4.4. Tests to determine the relationship between rapid tolerance and withdrawal

In Fig. 4, it can be seen that rapid tolerance, defined as a rightward shift in the dose–response function, was observed when rats were tested 24 h following treatment with 1.5 and 3.0 mg/kg AMPH. A 2 treatment (SAL vs. 3.0 AMPH)×4 test dose (0.00, 0.062, 0.125 or 0.25 mg/kg AMPH) repeated measures ANOVA was used to test for the effect of treatment with 3.0 mg/kg AMPH. The results showed that the shift in the dose–response curve to the right was

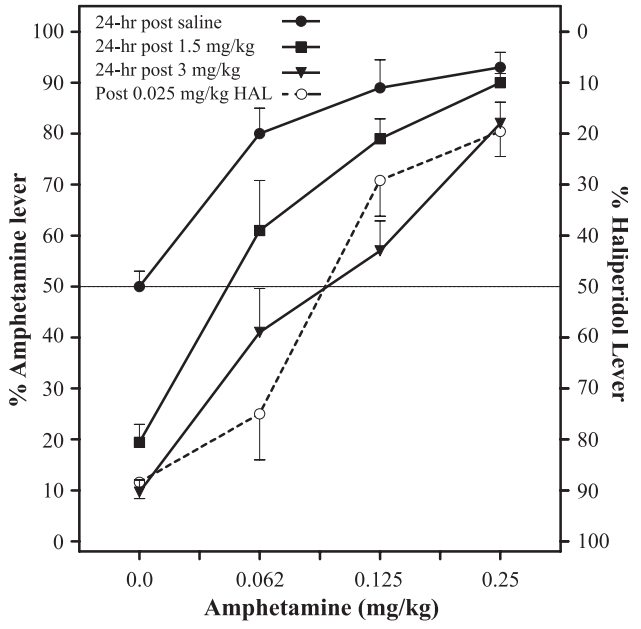

Fig. 4. Percent choice of the AMPH and HAL levers when different groups of rats were tested on SAL, 0.062, 0.125 or 0.25 mg/kg AMPH, 24 h after treatment with either SAL (filled circle), 1.5 (open triangle), 3.0 (filled square) mg/kg AMPH or 15 min after an acute dose of 0.025 mg/kg HAL (open circle). The data represent the means of nine rats±S.E.M.

significant [$F(1,50)=13.1$, $P<0.001$] and that treatment did not interact with test dose [$F(3,50)=1.9$, $P=0.12$]. Statistical analysis examining the effects of treatment with 1.5 mg/kg AMPH employed a 2 treatment (SAL or 1.5 AMPH)×4 test dose (0.00, 0.062, 0.125 or 0.25 mg/kg AMPH) factorial ANOVA. As observed for 3.0 mg/kg AMPH, the shift observed following 1.5 mg/kg AMPH was also significant [$F(1,50)=12.0$, $P<0.01$] and similar to the results observed with 3.0 mg/kg AMPH, no significant treatment×test dose interaction [$F(3,50)=1.5$, $P=0.20$] was found indicating parallel shifts. Since the AMPH dose–response functions in this experiment extend from 50% (SAL) to 100% (AMPH training dose), the ED_{75} rather than the ED_{50} was computed to compare the dose representing the midpoint of the function. Linear regression equations were computed for each of the three dose–response functions shown in Fig. 4. Substituting 75% for Y and solving for X (dose) in each equation, yielded ED_{75} doses of 0.064, 0.13 and 0.20 mg/kg AMPH for the groups pretreated with SAL, 1.50 and 3.0 mg/kg AMPH, respectively. Thus, treatment with 1.5 mg/kg AMPH caused a 2-fold increase in the ED_{75} dose, while treatment with 3.0 mg/kg AMPH increased the ED_{75} dose by a factor of 3.12.

The average total number of responses made during the test sessions by the group pre-injected with SAL 24 h before being tested on SAL, 0.062, 0.125 or 0.25 mg/kg AMPH, were 18, 26, 20 and 22, respectively. The same data for the groups pre-injected with 3 mg/kg 24 h before being tested on SAL, 0.062, 0.125 or 0.25 mg/kg AMPH, were 20, 18, 29 and 18 responses, respectively. For the groups pre-injected with 1.5 mg/kg AMPH 24 h before being tested on SAL, 0.062, 0.125 or 0.25 mg/kg AMPH, the average total number of responses were 27, 17, 28 and 33, respectively. One-way ANOVAs computed separately on the dose response data for the above groups showed that in no case did total number of responses differ as a function of test dose.

4.5. The effect of acute treatment with haloperidol on the amphetamine dose–response function

In Fig. 4, it can be seen that injecting rats with 0.025 mg/kg HAL prior to determining the AMPH dose response function produced a rightward shift. Between groups and repeated measures ANOVAs showed that the shift was significant when compared to the dose–response curve observed for rats treated with SAL [$F(1,50)=25.6$, $P<0.001$] or 1.5 mg/kg AMPH [$F(1,51)=6.4$, $P<0.01$]. The dose–response curve following treatment with 0.025 mg/kg HAL was not different from the dose–response curve determined 24 h following treatment with 3.0 mg/kg AMPH [$F(1,50)=1.1$, $P<0.29$]. Means for the groups tested on 0.25, 0.125 and 0.062 mg/kg AMPH and SAL were 85%, 74%, 32% and 12% for the four groups, respectively. The ED_{75} for these groups was 0.17 mg/kg AMPH, a dose 2.65-fold higher than the ED_{75} dose (0.064 mg/kg AMPH) observed for rats treated with SAL 24 h prior to testing.

The average total number of responses made by the groups tested on SAL, 0.062, 0.125 or 0.25 mg/kg AMPH were 19, 8, 8 and 19, respectively, and were significantly different as tested by a one-way ANOVA [$F(3,26)=3.9$, $P<0.02$].

5. Discussion

The results from the present study show that rats can learn to discriminate differences along a continuum of presumed DA-mediated cues. By adjusting the training doses of the indirect DA agonist AMPH and the DA, D2 receptor antagonist HAL, it was possible to obtain a discrimination baseline that was equally sensitive to detecting increases and decreases in DA mediated cues. Of particular interest is the normal distribution of the SAL test scores that were acquired throughout the experiment that is plotted in Fig. 1. These data reflect the graded and normally distributed nature of the response measure generated by the concurrent VI-30″, TO-15″ schedule of reinforcement used to train rats in this experiment. As can be seen, on average rats made 51.6% of their responses on the AMPH lever and 48.4% on the HAL lever when tested on SAL. In previous AMPH-HAL discrimination studies (Barrett et al., 1992), we observed that the distribution of responding on the two levers during SAL tests is precisely controlled by the training doses of AMPH and HAL. Thus, small changes in the training dose of either drug shifts the mean of the distribution.

The findings from the present experiment replicate and extend the results from previous studies examining the effects of neuroadaptive processes (Barrett et al., 1992; Barrett and Smith, 1988; Caul et al., 1996; Smith et al., 1995) on drug-induced cues and are consistent with predictions made from an opponent process theory of motivation (Solomon and Corbit, 1973; Solomon, 1980; Barrett, 1985; Koob et al., 1989, 1997). Of particular significance was the observation that the cues associated with AMPH withdrawal accounted for a far greater percentage of the total variance associated with AMPH's cue properties than did the primary cues. This can be seen in Fig. 3, where following treatment with 3.0 mg/kg AMPH, the primary cues are dominant during the first 8 h. By interpolating between the 8- and 16-h test intervals, it can be seen that at about 12 h following treatment with 3.0 mg/kg AMPH, a brief period of equilibrium occurs between the decreasing intensity of the drug's primary cues and the increasing intensity of the withdrawal cues that oppose the primary cues. By 16 h after treatment with 3.0 mg/kg AMPH, rats were making 70% of their responses on the HAL lever indicating the presence of withdrawal cues qualitatively opposite to the primary cues initially observed. Considered alone, the finding that rats responded on the HAL lever during AMPH withdrawal does not confirm that the AMPH withdrawal cues 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 which provides strong support for the interpretation that the cues associated with AMPH withdrawal are HAL-like, because if they were different from HAL, rats trained on the three-lever task would have responded on the SAL lever. In the present study, the HAL-like cue state continued to increase in intensity such that by 30 h after treatment with 3.0 mg/kg AMPH, the rats were making 84% of their responses on the HAL lever. By referencing the HAL dose–response function plotted in Fig. 2, it can be seen that 84% HAL lever choice would be equivalent to the %HAL lever responding expected following acute treatment with approximately 0.025 mg/kg HAL. Not until the 48-h test interval did choice of the two levers approach the 50% non-drug baseline level.

Also of interest in the present experiment was the finding that the reduced responding on the AMPH lever (rapid tolerance) observed when rats were challenged with AMPH during AMPH withdrawal, was completely accounted for by the baseline shift and did not reflect a weaker cue. This can be seen by comparing the dose–response functions for rats pretreated with SAL and 3.0 mg/kg AMPH. The rats pretreated with 3.0 mg/kg AMPH made 10% of their responses on the AMPH lever (90% on HAL lever) when tested on SAL 24 h later compared to 50% AMPH lever responding for rats pretreated with SAL. When both groups were tested on 0.062 mg/kg AMPH, the first dose in the dose–response curve, the AMPH-pretreated group made significantly fewer AMPH lever responses (41%) than the group pretreated with SAL (80%). This difference satisfies the operational definition of tolerance. However, it can also be seen that the test dose of 0.062 mg/kg AMPH produced nearly identical increases from baseline in %AMPH lever choice for the SAL-pretreated rats (from 50% to 80%) and the 3.0 mg/kg AMPH-pretreated rats (from 10% to 41%). Although the difference in %AMPH lever responding between the two groups is less at the 0.25 mg/kg AMPH test dose, this is due to the ceiling of 100% inherent in the measure. The finding that tolerance reflects a baseline shift is consistent with results from other studies (Barrett et al., 1992; Smith and Barrett, 1997; Smith et al., 1995) showing that tolerance often, perhaps usually, reflects an adaptive shift in baseline. Because many behavioral tasks are not sensitive to measuring baseline shifts, they have often gone undetected and, as a result, their relevance to understanding tolerance has not been apparent. Tolerance and withdrawal in the present experiment were further characterized by demonstrating that when an AMPH dose–response curve was determined 30 min following administration of 0.025 mg/kg HAL, the curve showed a parallel shift to the right, virtually identical to the rightward shift observed when the same doses were tested 24 h after treatment with 3.0 mg/kg AMPH.

The present results also contribute to understanding the variables that determine whether or not tolerance will be observed to a drug's cue properties. It has long been known that demonstrating tolerance in drug-discrimination studies requires suspending discrimination training while daily supplemental doses of the training drug are administered (Shannon and Holtzman, 1976). Using this procedure tolerance has been demonstrated to the cue properties of a broad cross section of drugs including amphetamine (Barrett and Leith, 1981; Young et al., 1992; Caul et al., 1989), cocaine (McKenna and Ho, 1977; Woods and Emmett-Oglesby, 1986), midazolam (Sannerud and Griffiths, 1993), L-cathinone (Schechter, 1986), delta-9-tetrahydrocannabinol (Semjonow and Binder, 1985; Wiley et al., 1993) and ethanol (Emmett-Oglesby, 1990). On the other hand, others (Colpaert, 1995) have failed to show tolerance in studies where training was continued during chronic administration of supplemental doses of the training drug. The failure to show tolerance when training is continued during chronic drug treatment has generally been explained (Sannerud and Griffiths, 1993; Hirschhorn and Rosecrans, 1974) by proposing that continued training affords subjects an opportunity to gradually transfer stimulus control from the original cue to a weaker stimulus such that no deficit in discrimination is observed when subjects are subsequently tested for tolerance. This explanation is supported by studies (Beardsley et al., 1987; Overton, 1979; Rosen et al., 1986) showing that if the dose of a training drug is gradually reduced after a discrimination is acquired, doses too low to support acquisition of the discrimination can maintain a discrimination already acquired. Colpaert (1995) suggests that if subjects are gradually transferring stimulus control to a weaker stimulus, a leftward rather than a rightward shift in the dose–response function should be observed following continued training during chronic drug treatment, similar to the leftward shifts observed when training doses are systematically lowered (Colpaert and Janssen, 1986). Colpaert (1995) also argued that, if tolerance develops to a drug's cue property, it eventually should not make any difference if training is continued or suspended during chronic treatment. The implication being that at some point the cue would be too weak to support discrimination of any kind.

Although the two views discussed above differ with respect to predictions about tolerance, they are similar in one respect. Both explanations implicitly assume that an inherent characteristic of tolerance to a drug's cue property is that tolerance involves a loss in drug efficacy. For example, the notion that rats learn to transfer cues to a weaker stimulus (Sannerud and Griffiths, 1993; Young and Sannerud, 1989) when training is continued during chronic treatment, conveys the notion of reduced efficacy. Similarly, Colpaert's prediction that if tolerance occurs to a drug's cue

properties, dose–response functions should shift to the left when training is continued during chronic treatment, likewise assumes that tolerance involves a loss in efficacy. The results from the present experiment as well as those from previous experiments suggest that tolerance to a drug's cue property seldom reflects a loss of efficacy and when it does, it is the exception rather than the rule. Conceptually, the cues associated with the two levers in a drug-saline discrimination procedure can be thought of as representing equidistant points on a continuum ranging from the primary cues initially associated with the drug lever at one end, drug neutral cues initially associated with the SAL lever representing the midpoint of the continuum, and withdrawal cues opposite to the primary cues at the opposite end. It appears that chronic drug shifts the position of the two cues toward the withdrawal end of the continuum but that the distance between them, and therefore their discriminable difference, remains relatively constant. Application of this conceptual framework helps clarify issues related to tolerance. First, with regard to why tolerance is not observed when training is continued during chronic drug treatment. The data in Fig. 4 demonstrate what would occur, if after learning to discriminate between a 0.25 mg/kg dose of AMPH and SAL, rats were given daily supplemental doses of 3.0 mg/kg AMPH while discrimination training was continued. As can be seen, when rats were tested on 0.25 mg/kg AMPH, 24 h following treatment with 3.0 mg/kg AMPH, they made 82% of their responses on the AMPH lever which is less than the 93% the SAL-treated rats made when tested on the same 0.25 mg/kg dose, but nearly identical to the 80% the SAL-treated rats made when tested on 0.062 mg/kg AMPH. Taken alone, these data not only provide strong support for the notion that stimulus control is being transferred to a weaker cue, but in addition suggest that the cue is weaker by a factor of four, i.e., rats treated with 3.0 mg/kg AMPH respond to 0.25 mg/kg AMPH as though it were 0.062 mg/kg AMPH. An implicit assumption in this reasoning is that the cues associated with the SAL lever remain unchanged. The most important new finding from the present experiment is that this assumption is incorrect and that the cues associated with the SAL lever change to the same extent as the cues associated with the drug lever. This is evident in Fig. 4 where it can be seen that the group tested on SAL 24 h following 3.0 mg/kg AMPH, made 90% of its responses on the HAL lever which is almost identical to the 93% responding observed when rats were tested on 0.035 mg/kg HAL while determining the HAL dose–response function (Fig. 2). This observation makes it clear that stimulus control is not being transferred to a weaker cue, but rather to a different set of cues. The new cues associated with the SAL lever would be cues similar to those normally associated with an acute dose of 0.035 mg/kg HAL. The new cues associated with the AMPH lever would be cues normally associated with 0.062 mg/kg AMPH. Thus, continued training during chronic drug treatment would provide subjects the opportunity to transfer old responses (SAL and AMPH lever responding) to new cues (responding on the SAL lever in the presence HAL-like cues and responding on the AMPH lever in the presence of 0.062 mg/kg AMPH-like cues). Our analysis of the data indicates that the new cues associated with the SAL and drug lever are no less discriminable from each other than the original cues. This can be assessed by comparing the % change from baseline normally observed following a challenge dose of AMPH with that observed following treatment with 3 mg/kg AMPH. In Fig. 4, inspection of the dose–response data for rats pretreated with SAL shows that 0.25 mg/kg AMPH normally produces a 43% increase in choice of the AMPH lever (from 50% to 93%). When the same dose is given to rats pretreated with 3.0 mg/kg AMPH, it produces a 72% increase in %AMPH lever choice (from 10% to 82%). This would seem to suggest that the AMPH cue was stronger, not weaker, after treatment with 3.0 mg/kg AMPH. However, examination of the dose–response function for the rats treated with SAL shows that 43% underestimates the change from baseline produced by 0.25 mg/kg AMPH because the ceiling restriction inherent in the measure imposes an upper limit of 100% in this group. This was not a factor in the AMPH-pretreated rats since the mean representing 0.25 mg/kg AMPH fell on the linear portion of the dose–response curve. Thus, a more valid method of assessing whether a given dose of AMPH was more or less salient following treatment with 3.0 mg/kg AMPH would be to compare the percent increase in %AMPH lever responding observed between two doses representing the linear portion of the dose–response curves for both groups. In Fig. 4, it can be seen that SAL and 0.062 mg/kg AMPH satisfy this criterion. This comparison showed that 0.062 mg/kg AMPH resulted in a 31% increase in %AMPH lever choice (from 10% to 41%) for the rats pretreated with 3.0 mg/kg AMPH and a 30% increase in %AMPH lever choice (from 50% to 80%) for rats treated with SAL 24 h prior to testing. This finding illustrates that tolerance occurs in the absence of any apparent loss in drug efficacy.

Evidence that tolerance involves a loss in drug efficacy would require demonstrating nonparallel, dose–response curve shifts to the right. More specifically, the slopes of the dose–response functions should become flatter with increasing loss of efficacy. Pharmacologically, efficacy is related to a drug's intrinsic activity or magnitude of response per drug–receptor interaction (Ariens, 1954; Furchgott, 1955). Drugs with high intrinsic activity require fewer drug–receptor interactions than drugs with low intrinsic activity to produce a maximal effect leading to the concept of spare receptors (Furchgott, 1966). A loss in efficacy would be observed when receptor-down regulation caused by chronic drug treatment reduced the population of available receptors below that required to produce a maximal response. With opiates Holtzman (1997) has used the irreversible ligand β-funlatrexamine (B-FNA) to reduce the available population of μ-opioid receptors, the receptor responsible for mediating most of the effects of opioid drugs. He found that even

reducing the μ-receptor population by 90%, although causing a dose–response curve shift to the right, did not reduce morphine's maximal cue property. In two additional studies, morphine infusion (0.8 mg/kg/h) for 7 days resulted in a loss of efficacy to the antinociceptive effect of buprenorphine (Paronis and Holtzman, 1994) but not to buprenorphine's maximal cue property. To our knowledge, a paper by Young et al., 1991 contains the only report of loss in cue efficacy as a function of chronic drug treatment. In that study, it was shown that following twice daily injections of 10 mg/kg morphine for 14–18 days, cross-tolerance to the low efficacy agonist nalbuphine was not fully surmounted. From a pharmacological perspective, tolerance to the drug cues occurs irrespective of whether training is continued or suspended during chronic treatment. Thus, the role that continuing or suspending training during chronic treatment plays is simply determining the extent to which the pharmacological tolerance will be manifested behaviorally. For reasons stated above, tolerance from chronic drug administration given while training is continued will generally not be manifested behaviorally. However, when training is suspended, for reasons illustrated in the data presented in Fig. 4, tolerance in the form of a parallel rightward shift in the dose–response function will be manifested behaviorally.

If tolerance to a drug's cue property generally reflects a baseline shift and not a loss in drug efficacy, it questions how the results from cross-tolerance tests should be interpreted. The results from the present study indicate that failure to show cross-tolerance would require a significantly greater change from baseline than normally observed. For example, in the present study, for cross-tolerance not to be observed would require a 65% increase in %AMPH lever choice (from 10% to 75%) compared to the 25% increase (from 50% to 75%) normally observed for an ED_{75} dose. Clearly, not finding cross-tolerance would be the finding of greater theoretical significance. The above explanation of tolerance also helps conceptualize why it would be highly unusual and unexpected if the dose of a training drug had to be increased during the course of an experiment and predicts the often reported finding (Colpaert et al., 1978a,b) that dose–response functions remain relatively constant over extended time periods.

It is interesting to speculate about the implications the present results have for understanding chronic drug use. On the basis of an extensive drug-discrimination 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; Colpaert et al., 1978a,b). To the extent that drug-induced interoceptive cues in animals and drug-induced hedonic change in humans represent homologous physiological processes, the present results identify a potentially important source of motivation for continued drug use. Some investigators (Wise and Bozarth, 1987; Stewart et al., 1984) have questioned whether symptoms of withdrawal play an important role in understanding drug abuse, especially abuse of stimulant drugs. For example, in a recent article, Robinson and Berridge (2000) state that psychostimulants like amphetamine and cocaine that do not produce strong withdrawal syndromes can be highly addictive. Although psychostimulants do not produce strong physical signs of withdrawal, the present results suggest that rather profound changes in hedonic state, opposite those initially observed, occur following exposure to AMPH. Furthermore, the finding that HAL-like cues are associated with AMPH withdrawal suggests that withdrawal reflects a neuroadaptive down-regulation in DA activity. These changes would seem to parallel the well known symptoms of dysphoria humans experience during AMPH withdrawal (Watson et al., 1972). Within the context of an animal model of drug abuse, the results presented in Fig. 4 illustrate why a person experiencing AMPH withdrawal would likely be highly motivated to continue using the drug. First, using the linear regression equation computed for the rats pretreated with 3.0 mg/kg, a predicted dose of 0.08 mg/kg AMPH would be required to effectively block the withdrawal cues and reinstate normal 50% responding on both levers. The ability of a drug to relieve aversive symptoms of withdrawal is an example of the drug's negative reinforcing properties. In control rats (pretreated with SAL), a dose of 0.08 mg/kg would be expected to produce close to 80% AMPH lever responding. Following treatment with 3.0 mg/kg AMPH, the expected dose that would produce 80% AMPH lever choice would be three-fold higher, i.e., 0.25 mg/kg AMPH, a dose that would now have both negative and positive reinforcing properties. In the present study, the original level of AMPH lever responding observed following challenge with 0.25 mg/kg was approximately 93% which theoretically could be reinstated by increasing the dose of AMPH to approximately 0.50 mg/kg.

The current findings illustrating the pronounced shift in baseline also seem relevant to interpreting data from drug-self administration studies (Ahmed and Koob, 1999; Ahmed et al., 2000) 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 self-injection to achieve a desired pharmacological effect. When access time was increased to 6 h for cocaine (Ahmed and Koob, 1997) or 11 h for heroin (Ahmed and Koob, 1998), 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 assess changes in the non-drug baseline, i.e., shift in hedonic starting point, that might result after subjects are given extended access to the drug each day. Our drug discrimination data suggest that the increase in drug self-administration reflects a baseline shift, which requires a higher drug dose to achieve the original desired pharmacological effect.

Finally, even if a drug's interoceptive cues are unrelated to the mood elevating properties of drugs in humans, the present results nevertheless serve to illustrate the role adaptive processes can play in determining the acute and chronic effects of drugs on behavior.

In summary, results from the present experiment demonstrate how training rats to discriminate between a drug agonist and antagonist can be used to provide a behavioral measure sensitive to detecting bidirectional changes in cue state. Furthermore, the results presented here for AMPH and similar findings previously reported for the anxiolytic agent, diazepam (Barrett and Smith, 1988), document the presence of robust and long lasting withdrawal cues associated with drugs of abuse that might be relevant to understanding the motivation to use drugs chronically.

Acknowledgments

The authors would like to express their appreciation for the expertise and meticulous attention to detail provided by Ms. Barbara Gilreath 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.

Ahmed SH, Walker JR, Koob GF. Persistent increase in the motivation to take heroin in rats with a history of drug escalation. Neuropsychopharmacology 2000;22:413–21.

Ariens EJ. Affinity and intrinsic activity in the theory of competitive inhibition. Arch Int Pharmacodyn Ther 1954;99:32–49.

Arnt J. The discriminative stimulus properties of the D1 agonist SKF 38393 and the D2 agonist (−)-NPA are mediated by separate mechanisms. Life Sci 1988;42:565–74.

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, Smith RL. Time dependent pentylenetetrazol-like cues subsequent to diazepam administration. Psychopharmacology 1988; 96:169–73.

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.

Beardsley PM, Balster RL, Salay JM. Separation of the response rate and discriminative stimulus effects of phencyclidine: training dose as a factor in phencyclidine–saline discrimination. J Pharmacol Exp Ther 1987;241:159–65.

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, Burgin K, Barrett RJ. Drug discrimination training during chronic drug treatment affects the development of tolerance. Behav Neurosci 1989;103:373–7.

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. 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;48:355–402.

Colpaert FC. Drug discrimination in neurobiology. Pharmacol Biochem Behav 1999;64:337–45.

Colpaert FC, Janssen PAJ. Agonist and antagonist effects of prototype opiate drugs in fentanyl dose–dose discrimination. Psychopharmacology 1986;90:222–8.

Colpaert FC, Niemegeers CJ, Janssen PA. Studies on the regulation of sensitivity to the narcotic cue. Neuropharmacology 1978a;17:705–13.

Colpaert FC, Niemegeers CJ, Janssen PA. Changes of sensitivity to the cuing properties of narcotic drugs as evidenced by generalization and cross-generalization experiments. Psychopharmacology (Berl) 1978b; 58:257–62.

D'Mello GD, Stolerman IP. Comparison of the discriminative stimulus properties of cocaine and amphetamine in rats. Br J Pharmacol 1977; 61:415–22.

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.

Ellinwood Jr EH. Amphetamine and stimulant drugs. Drug use in America: problem in perspective. Second report of the National Commission on Marijuana and Drug Abuse, vol. I. Washington (DC): U.S. Gov't. Printing Office; 1973. p. 140–57.

Emmett-Oglesby MW. Tolerance to the discriminative stimulus effects of ethanol. Behav Pharmacol 1990;1:497–503.

Evans SM, Johanson C-E. Amphetamine like effects of anorectics and related compounds in pigeons. J Pharmacol Exp Ther 1987;241:817–25.

Furchgott RF. The pharmacology of vascular smooth muscle. Pharmacol Rev 1955;7:183–265.

Furchgott RF. The use of B-haloalkylamines in the differentiation of receptors and the determination of dissociation constants of receptor agonist complex. Adv Drug Res 1966;3:21–56.

Hirschhorn ID, Rosecrans JA. Morphine and delta-9-tetrahydrocannabinol: tolerance to the stimulus effects. Psychopharmacologia 1974;36: 243–53.

Holtzman SG. Antagonism of morphine-like discriminative effects by beta-funaltrexamine. Pharmacol Biochem Behav 1997;57:771–7.

Huang D, Wilson MC. Comparative discriminative stimulus properties of DL-cathinone, D-amphetamine, and cocaine in rats. Pharmacol Biochem Behav 1986;24:205–10.

Jarbe TUC. Discriminative stimulus properties of D-amphetamine in pigeons. Pharmacol Biochem Behav 1982;17:671–5.

Jones CN, Grant LD, Vospalek DM. Temporal parameters of D-amphetamine as a discriminative stimulus in the rat. Psychopharmacologia 1976;46:59–64.

Kamien JB, Woolverton WL. A pharmacological analysis of the discriminative stimulus properties of D-amphetamine in rhesus monkeys. J Pharmacol Exp Ther 1989;248:938–46.

Kamien JB, Bickel WK, Hughes JR, Higgins ST, Smith B. Drug discrimination by humans: a comprehensive comparison with nonhumans. In: Harris LS, editor. Problems of drug dependence 1992. NIDA Research Monograph, vol. 132. Rockville (MD): U.S. Department of Health and Human Services; 1992. p. 280.

Koob GF, Stinus L, 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.

McKenna ML, Ho BT. Induced tolerance to the discriminative stimulus properties of cocaine. Pharmacol Biochem Behav 1977;7:273–6.

Michaelis RC, Holohean AM, Criado JR, Harland RD, Hunter EA, Holloway FA. The chlordiazepoxide/pentylenetetrazol discrimination characterization of drug interactions and homeostatic responses to drug challenges. Psychopharmacology 1988;96:15–20.

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, Scheel-Kruger J. Cueing effects of amphetamine and LSD: elicitation by direct microinjections of the drugs into the nucleus accumbens. Eur J Pharmacol 1986;125:85–92.

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.

Paronis CA, Holtzman SG. Sensitization and tolerance to the discriminative stimulus effects of mu-opioid agonists. Psychopharmacology 1994;114: 601–10.

Preston KL, Bigelow GE. Subjective and discriminative effects of drugs. Behav Pharmacol 1991;2:293–313.

Reavill C, Bond B, Overend P, Hunter AJ. Pharmacological characterization of the discriminative stimulus properties of the dopamine D1 agonist, SKF 81297. Behav Pharmacol 1993;4:135–46.

Robinson TE, Berridge KC. The psychology and neurobiology of addiction: an incentive-sensitization view. Addiction 2000;95:S91–S117.

Rosen JB, Young AM, Beuthin FC, Louis-Ferdinand RT. Discriminative stimulus properties of amphetamine and other stimulants in lead-exposed and normal rats. Pharmacol Biochem Behav 1986;24:211–5.

Rush CR, Pazzaglia PJ. Discriminative stimulus effects of d-amphetamine, bupropion, methylphenidate and triazolam in D-amphetamine-trained humans. In: Harris LS, editor. Problems of drug dependence 1996. NIDA Research Monograph, vol. 174. Rockville (MD): U.S. Department of Health and Human Services; 1997. p. 182.

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.

Schechter MD. Dopaminergic mediation of a behavioral effect of L-cathinone. Pharmacol Biochem Behav 1986;25:337–40.

Semjonow A, Binder M. Generalization of the discriminative stimulus properties of delta-9-THC to delta-9(11)-THC in rats. Psychopharmacology 1985;85:178–83.

Shannon HE, Holtzman SG. Evaluation of the discriminative effects of morphine in the rat. J Pharmacol Exp Ther 1976;198:54–65.

Silveri MM, Spear LP. Acute, rapid, and chronic tolerance during ontogeny: observations when equating ethanol perturbation across age. Alcohol, Clin Exp Res 2001;25:1301–8.

Silverman PB, Ho BT. Amphetamine discrimination: onset of the stimulus. Pharmacol Biochem Behav 1980;12:303–4.

Smith RL, Barrett RJ. Tolerance to the anticonflict effects of diazepam: importance of methodological considerations. Pharmacol Biochem Behav 1997;58:61–6.

Smith RL, Barrett RJ, Sanders-Bush E. Neurochemical and behavioural evidence that quipazine-ketanserin discrimination is mediated by serotonin$_{2A}$ receptor. J Pharmacol Exp Ther 1995;275:1050–7.

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 Psychol 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.

Stewart J, DeWit H, Eikelboom R. Role of conditioned and unconditioned drug effects in the self-administration of opiates and stimulants. Psychol Rev 1984;91:251–68.

Van Groll BJ, Appel JB. Stimulus effects of d-amphetamine 1: DA mechanisms. Pharmacol Biochem Behav 1992;43:967–73.

Watson R, Hartman E, Schildkraut JJ. Amphetamine withdrawal: affective state, sleep patterns and MHPG excretion. Am J Psychiatr 1972;129: 263–9.

Wiley JL, Barrett RL, Balster RL, Martin BR. Tolerance to the discriminative stimulus effect of delta9-tetrahydrocannabinol. Behav Pharmacol 1993;4:581–5.

Wise RA, Bozarth MA. A psychomotor stimulant theory of addiction. Psychol Rev 1987;94:469–92.

Woods DM, Emmett-Oglesby MW. Characteristics of tolerance, recovery from tolerance and cross-tolerance for cocaine used as a discriminative stimulus. J Pharmacol Exp Ther 1986;237:120–30.

Young AM, Sannerud CA. Tolerance to drug discriminative stimuli. In: Goudie AJ, Emmett-Oglesby MW, editors. Tolerance and sensitization Clifton: Humana Press; 1989. p. 221–78.

Young AM, Kapitsopoulos G, Makhay MM. Tolerance to morphine-like stimulus effects of mu opioid agonists. J Pharmacol Exp Ther 1991;257: 795–805.

Young AM, Walton MA, Carter TL. Selective tolerance to discriminative stimulus effects of morphine or D-amphetamine. Behav Pharmacol 1992;3:201–9.