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Attenuation of the amphetamine discriminative cue in rats with the atypical antipsychotic olanzapine

Jordan A. Mechanic*, Jill A. Wasielewski, Kathy L. Carl, Frank A. Holloway

Psychobiology Laboratories, Department of Psychiatry and Behavioral Sciences, University of Oklahoma Health Sciences Center, Room 302-R, 800 Northeast 13th Street, Oklahoma City, OK 73190-3000, USA

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Abstract

Sixteen male Sprague–Dawley rats were trained to discriminate between saline and amphetamine injections (1.0 mg/kg ip) using a standard two-lever (FR10) drug discrimination paradigm. A baseline dose–effect curve was generated for amphetamine administration alone, using doses both above and below the training dose (0.0–2.2 mg/kg ip). Once completed, a single dose of olanzapine (OLZ; 1.5 mg/kg sc) was tested for its ability to attenuate the amphetamine cue. OLZ pretreatment (60 min) successfully interfered with an animal's ability to discriminate amphetamine injections across various doses. The percentage of correct responding on the amphetamine lever and rate of responding were both significantly decreased across some but not all of the amphetamine doses. Therefore, we believe that this preliminary investigation has successfully shown that an OLZ dose of 1.5 mg/kg sc at 60 min can interfere with an animal's ability to detect some subjective cue(s) associated with amphetamine administration.

Keywords: Amphetamine; Stimulant abuse; Olanzapine; Atypical antipsychotic; Drug discrimination

1. Introduction

Amphetamine and methamphetamine abuse and dependence represents a large and growing problem in the United States. Collecting emergency room data from metropolitan hospital throughout the 1990s, DAWN (Drug Abuse Warning Network) reported a doubling (10,447 mentions for methamphetamine in 1999) and tripling (11,954 mentions for amphetamine in 1999) in stimulant-related emergency room visits over the previous decade, with corresponding increases in deaths attributed to overdose (Substance Abuse Mental Health Service Administration). In some western states (California, Washington, Hawaii), methamphetamine was the leading drug of abuse cited by clients in treatment (Heischober and Miller , 1991). There is currently no accepted pharmacotherapy for the treatment of amphetamine/methamphetamine abuse.

Reduction of drug-induced euphoria has been postulated to lead to increased abstinence. Due to its binding profile (and anecdotal clinical reports), the atypical antipsychotic, olanzapine (OLZ) may possess properties that facilitate abstinence from stimulants (see Arnt and Skarsfeldt, 1998, for review). In the present study, the drug discrimination paradigm was used to investigate the interactive effects between amphetamine and OLZ. In this animal model of subjective drug effects, animals are trained to recognize the subjective, interoceptive effects of a drug as a discriminative cue. Once the discrimination has been established, pretreatment with an experimental drug can be used to investigate this drug's ability to interfere with such cues. In the case of self-administered (abused) drugs, this cue may be what human subjects report as euphoria (Arnt, 1996; Brauer et al., 1997).

Thorough investigations of the amphetamine discrimination have established the critical involvement of dopamine (DA) in the development and expression of an amphetamine cue. The amphetamine discrimination depends on increased DA levels in limbic regions (Arnt, 1996; Brauer et al., 1997; Carr and White, 1986; Nielsen and Scheel-Kruger, 1986). Additionally, in human subjects such increased limbic DA levels are correlated with self-reports of euphoria (Arnt, 1996; Brauer et al., 1997). Likewise, human subjects performing an amphetamine discrimination report using the subjective feelings of euphoria as a primary discriminative

* Corresponding author. Tel.: +1-405-271-2011; fax: +1-405-271-2356.
E-mail address: jordan-mechanic@ouhsc.edu (J.A. Mechanic).

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cue (Arnt, 1996; Jonsson, 1972). As such, animals performing amphetamine discrimination may use euphoria (or the animal equivalent) as part or as the entire discriminative cue (see Brauer et al., 1997, for review). Therefore, we hypothesized that if the established amphetamine discrimination can be disrupted by OLZ pretreatment in this animals model, OLZ may be able to block amphetamine-induced euphoria in humans. These results, however, would still remain tentative until human trials using subjective reporting are initiated, as the subjective and the discriminative effects of a drug are not always the same (Chait et al., 1985; Kollins and Rush, 1999; Porter and Strong, 1996) (see Chait et al., 1986; Preston and Bigelow, 1998, for examples of this dissociation).

Several characteristics of the atypical antipsychotic OLZ suggest it may have potential as a therapeutic candidate. Pharmacological antagonism can be achieved at the receptor subtypes believed to be responsible for: (a) stimulus reward: D1/D2 in the nucleus accumbens (Arnt, 1996; Arnt and Skarsfeldt, 1998; Carr and White, 1986; Moore et al., 1992), (b) the discriminative cue: DA in the striatum and nucleus accumbens and possibly norepinephrine in the median forebrain bundle (Arnt and Skarsfeldt, 1998; Stein and Wise, 1969), and (c) anxiety: 5-$HT_{2a/c}$ (conflict responding) and possibly D4 in the striatum (conditioned freezing) (Arnt and Skarsfeldt, 1998; Inoue et al., 1996; Wiley et al., 1993).

Finally, one previous study reported that OLZ pretreatment (0.44–2.8 mg/kg sc, 120 min) successfully inhibited a trained amphetamine discrimination (1.0 mg/kg ip, 15 min) in rats (Arnt, 1996). An OLZ dose of 1.84 mg/kg reduced amphetamine appropriate responding to 50%, while maximal inhibitory effects (59% reduction) were obtained at 2.48 mg/kg (ibid.). The present study was designed to partially replicate these findings using, a shorter OLZ pretreatment interval (60 min) and food-reinforced animals (instead of the water-restricted animals used previously). Food reinforcement was chosen to reflect the majority of previous amphetamine discrimination studies. Of the 145 amphetamine drug discrimination studies conducted in the last 50 years, 60 were conducted using food as the primary reinforcer, while only 15 used water as the reinforcer (the remainder did not specify choice of reinforcer in the abstract) (on-line Drug Discrimination Datebase; Stolerman, 2001). While the anorexic effects of amphetamine are well recognized in the clinic, their effects in the drug discriminating (i.e., food deprived) animal appear to be negligible (see D'Mello and Stolerman, 1977, for an example). Additionally, given a plasma half-life for OLZ of 3–4 h in the rat (Chiu and Franklin, 1996) and pilot data suggesting a greater efficacy at shorter intervals, we believed a more focused study of OLZ's effects was warranted.

The earlier study was conducted as a part of a large investigation of classical and atypical antipsychotics to validate the amphetamine discrimination as a screen for antipsychotic activity, not to specifically investigate the effects of any particular antipsychotic (Arnt, 1996). Therefore, the present study was undertaken to further expand on these early results using a more standard and focused design, using Sprague–Dawley instead of Wistar rats, a larger number of subjects, food instead of water-deprived animals, and a shorter OLZ pretreatment interval.

2. Methods

2.1. Subjects

Sixteen experimentally naïve male Sprague–Dawley rats were purchased from Harlan (Indianapolis, IN). Housing was maintained in a colony room at the University of Oklahoma Health Sciences Center. Each animal was individually housed in suspended stainless steel cages and allowed to acclimate to the new environment for 2 weeks. Food and water were available continuously during the acclimation period. At the conclusion of the acclimation period, food was restricted until animals reached 85% of their free-feeding weight (approximately 2–3 weeks). The colony room was maintained on a 12-h light/dark cycle (06:00–18:00 h) and at a stable temperature (22–24 °C). Care and maintenance were administered by an AAALAC-accredited team of technicians (University of Oklahoma). Animals were run at the same time of day, 5 days a week. All experimental procedures had the prior approval of the University's Institutional Animal Care and Use Committee.

2.2. Apparatus

Four standard operant chambers (Lehigh Valley Electronics, Lehigh Valley, PA) were used to train and test the animals. These chambers were equipped with two levers, stimulus lamps, a house lamp, a fan, a pellet dispenser (45-mg pellets, Noyes Standard Formula), and a food trough. Each chamber was enclosed in a sound-attenuated box, equipped with a speaker for the delivery of white noise. Contingency control and data monitoring/collecting were achieved using Med Associates hardware and software (St. Albans, VT). The two-lever chambers were chosen over alternative drug discrimination techniques (i.e., T-maze) as the two-lever approach allows for high rates of responding and limited stress to the animal, thereby allowing lower training doses (Colpaert, 1987).

2.3. Training

Animals were first trained to receive food reinforcement by pressing either lever once (FR1) using the technique of successive approximations. Training sessions at this stage lasted for 30 min. The start/finish of each session was signaled by the stimulus and house lights turning on/off. Once animals had acquired the association of lever pressing and pellet dispensation, responding on only one of the levers was reinforced for a given day. Additionally, the reinforced lever was alternated each day. To control for the possibility

of a position preference (or olfactory cue), half of the animals were trained with lever assignments reversed (between odd and even groups, and counterbalanced within groups). Once the animals were correctly switching between levers, the number of presses required for delivery of a pellet was steadily increased while the session length was decreased. During training sessions, consecutive correct responses were required, such that a response on the wrong lever reset the count. Once the parameters reached a session length of 10 min with an FR5, discrimination training began.

On each day, animals in a given group received either a saline injection or an amphetamine injection (1.0 mg/kg ip) 15 min prior to being placed in the chambers. Injections for each squad alternated between saline and amphetamine. This injection schedule was continued until the animals were responding at an FR10 over a 10-min session. The FR schedule was chosen over alternative schedules due to its tendency to produce highly accurate responding with relatively few training sessions (Colpaert, 1987). The pretreatment interval and dose of amphetamine were chosen to produce a peak effect and to ensure that the cue trained was central and DA mediated (and not peripheral and/or norepinephrine-mediated) (Arnold et al., 1977; Creese and Iversen, 1975; Dingell et al., 1967; Smith et al., 1989). Numerous studies have demonstrated reliable data using these parameters (see Arnt, 1996; Colpaert et al., 1978a; Jones et al., 1976, for examples).

2.4. Testing criteria

During test days (unlike during training days), 10 consecutive responses on either the amphetamine- or the saline-appropriate lever resulted in the delivery of a reinforcer (assuming all 10 responses were on the same lever). Therefore, animals were capable of receiving a reinforcer for 10 consecutive responses on the ''incorrect'' lever, otherwise conditions during testing were identical to those described for training sessions (see above). As such, two criteria were used to determine if animals had acquired the drug discrimination (both of which had to be met for the animal's performance to be scored a ''pass''). First, animals had to make less than 20 responses before receiving their first reinforcer. Second, at least 90% of these responses (as well as of the total responses for the entire session) had to be made on the drug appropriate lever (see Data Analysis for the discussion of these variables). After animals had successfully met both of these criteria for five consecutive days, animals were put through a double alternation schedule.

In this phase, contingencies for a given group remained the same for 2 days then switched for the next two (saline, saline, amphetamine, amphetamine). Both of the above criteria had to be met for all four test sessions before animals were advanced into the next phase. The double alternation tests provided a means to insure that the animals were differentiating the two drug states, and were not simply switching back and forth between levers each day. Test sessions were conducted twice weekly with training sessions interspersed on the remaining days to maintain/confirm the discrimination. Finally, at the start of each week, animals had to meet both criteria for each condition (amphetamine and saline) to demonstrate that stimulus control was maintained. Between tests, animals had to meet both criteria for one of the conditions (amphetamine or saline), for the same purpose.

2.5. Experimental procedures

2.5.1. Amphetamine temporal parameters

The training dose of amphetamine (1.0 mg/kg ip) was administered at longer pretreatment intervals (30, 45, 60, 90, 120, and again at 15 min) in order to determine the temporal parameters of the amphetamine cue. The 15-min training interval was tested at the end of this phase to determine if testing had any impact on the stability of the cue properties (a control analysis).

2.5.2. Amphetamine dose–effect curve

Doses both higher and lower than the amphetamine training dose were tested (15 min) according to the following randomized order: 1.0, 0.0, 1.8, 0.56, 2.2, 0.32, and 0.25, with second doses of 1.0 and 0.0 mg/kg ip. Although not of primary concern, these second tests were administered at the end of the dose–effect curve (as controls) to determine if the injection schedule had any effect on the stability of the cue (referred to as ''control analyses'' below). Numerous studies have established the feasibility and the properties of the described discrimination (Arnt, 1996; Callahan et al., 1991; Colpaert et al., 1978a,b; Druhan et al., 1991; Jones et al., 1976). The pretreatment interval and training dose were chosen to reflect the bulk of this literature. The remaining doses represent one-fourth log increments, with two exceptions. The 3.2-mg/kg dose was found to produce marked locomotor depression in preliminary trials. Therefore, this dose was dropped down to 2.2 mg/kg in order to remain below 2.4 mg/kg, a dose previously shown to disrupt operant responding (Jones et al., 1976). Similarly, once it was realized that a majority of the animals responded to the 0.32-mg/kg dose as if it were the training dose, the 0.25-mg/kg dose was added in an effort to capture intermediate levels of drug-appropriate responding.

2.5.3. Amphetamine+OLZ dose–effect curve

A second amphetamine dose–effect curve was generated in which OLZ (1.5 mg/kg sc) was administered 45 min before the amphetamine injections. The full range of amphetamine doses was tested, only this time they were preceded by OLZ treatment. Likewise, the training doses (1.0 mg/kg amphetamine or saline) were again retested (as above) at the completion of the dose–effect curve to determine if the injection schedule had any effect on the stability of the cue. Previous studies with OLZ have reported near complete response suppression when doses of 2.7 mg/kg sc or higher were used (Porter and Strong, 1996). Therefore, the dose of

OLZ used in this study was intentionally kept below such levels. The only previous amphetamine drug discrimination study conducted with OLZ found that a 1.8-mg/kg dose of OLZ significantly reduced drug appropriate responding (Arnt,1996). Such a dose is high enough to obtain antiserotonergic effects (and possibly anxiolytic properties) (Moore et al., 1992), which may be desirable, as 5-HT_2 antagonists have been shown to block some amphetamine-induced behaviors (Arnt, 1995).

2.6. Drugs

OLZ (LY170053, 2-methyl-4-(4-methyl-1-piperazinyl)-1OH-thieno[2,3-*b*][1,5] benzodiazepine) was donated by Eli Lilly & Company (Indianapolis, IL). D-Amphetamine (expressed as the salt) was purchased from Sigma (St. Louis, MO). Drugs were dissolved in physiological (0.9%) saline. As OLZ was relatively insoluble in saline, minute amounts of dilute hydrochloric acid (0.1 N) were added to dissolve the drug into solution. The solution then was back-titrated with dilute sodium hydroxide (0.1 N) to bring the pH back toward neutral (pH≅6), according to the standard procedures recommended by Eli Lilly & Company (Indianapolis, IL).

2.7. Data analysis

Discriminative performance for a given test was recorded using two variables: percentage of total session responses emitted on the drug-appropriate lever and total rate of responding (responses per second). Total rate of responding was obtained as a measure of gross locomotor activity in order to detect interference due to nonspecific drug effects (Colpaert, 1987, Gauvin et al., 1992).

There is large disagreement in the field concerning the choice of overall (entire session) vs. first reinforcer (responding up to the delivery of the first reinforcer) data in drug discrimination studies. There are benefits and drawbacks to both, a discussion of which is beyond this paper. Our laboratory does not use an extinction or ''lock-out'' procedure; as such, throughout the entire test session, 10 responses on either lever will result in delivery of a pellet (as long as all 10 are made consecutively on the same lever) (see Nencini and Woolverton, 1988; Smith et al., 1989, for examples of this methodology). Therefore, we commonly use data from the entire 10-min test session when conducting drug discrimination (especially antagonist) studies (see below for discussion of the benefits).

In an attempt to normalize the data for rate of responding, they were converted to a percentage; expressed as the rate of responding compared to a baseline level of responding. The baseline was determined for each animal by averaging its overall rate of responding from 10 saline training days. The same days were used for each animal and were selected from days just prior to, including, and just after the generation of the first dose–effect curve. Each animal's rate data were then divided by its own saline baseline rate and multiplied by 100 to produce the ''standardized'' measure that was subjected to analysis (rate as a percentage of saline baseline).

The standard method used to analyze drug discrimination data, the ANOVA, was not employed for a variety of reasons, explained in depth elsewhere (see Clark et al., 1995; Colpaert, 1987; Exner et al., 1989; Furmidge et al., 1991). Examination of the distribution and variance in the data suggested that the assumptions that are required for even the simplest of the parametric statistics (i.e., normality, heterogeneity of variance, etc.) could not be met. Consistent with previous investigators' conclusions, we decided that nonparametric techniques were the more appropriate analytic approach (see Colpaert, 1987, for discussion). On the few occasions when the number of observations was too small to allow for meaningful interpretations using nonparametric tests (i.e., $n \leq 4$ making tabular significance impossible—as in the overall rate analysis for the amphetamine time course), standard parametric approaches were employed.

Basically, the primary experimental question can be addressed with two pairwise tests, one for the percentage of correct responding variable and one for the rate-of-responding variable (i.e., ED50s from amphetamine dose–effect curve vs. amphetamine + OLZ dose–effect curve). Wilcoxon Matched-Pairs Signed-Ranks test (ED50 data) and Friedman ANOVA by ranks (dose–effect curve comparisons) were used to determine significance (Siegel and Castellan, 1988). All tests were conducted as two-tailed tests with the probability level for rejection of the null hypothesis set at .05. Additionally, levels of antagonism were predefined to represent the bulk of the literature (Appel et al., 1991; Gauvin et al., 1992): (a) complete antagonism: reductions in drug lever responding of 80% or more; (b) partial antagonism: reductions in drug lever responding between 21% and 79%; and (c) no antagonisms: reductions in drug lever responding of 20% or less.

2.7.1. Amphetamine temporal parameters

Data collected in this phase of the experiment were used to determine the temporal parameters of the amphetamine cue. Pretreatment intervals longer than 15 min were analyzed for significant effects using a Friedman's ANOVA by ranks for each dependent variable. Additionally, two control analyses (one Wilcoxon for each dependent variable) were conducted to determine if successive amphetamine tests had any effect on the stability of the cue; for each dependent variable the results of the amphetamine training dose (1.0 mg/kg ip, 15 min) from the beginning of this phase were compared to the same dose/interval at the completion of the phase.

2.7.2. Amphetamine vs. amphetamine+OLZ dose–effect curves

Data collected in this phase of the experiment were used to generate a baseline level of responding (amphetamine dose–effect curve) and a post-treatment level of responding (amphetamine + OLZ dose–effect curve) for the spectrum of amphetamine doses. These levels of responding can be

conveniently represented by a single statistic, the ED50; the dose of amphetamine or amphetamine in combination with a dose of OLZ that elicits 50% amphetamine-appropriate responding (or 50% of maximum rate). Individual ED50s were extrapolated via an equation produced by linear regression using a least-squares procedure on the ascending portion of the dose–effect curve ($m = \Delta Y/\Delta X$; slope–intercept: $y = -mx + b$). Individual ED50s were averaged for each of the dependent variables from each of the dose–effect curves. In addition, the average slopes were similarly calculated and a test for parallelism conducted (see Tallarida and Murray, 1987, for methodology).

The question of a therapeutic window (i.e., over what range of amphetamine doses is 1.5 mg/kg OLZ effective?) was addressed using change scores. By subtracting the data collected in generating the amphetamine dose–effect curve (no treatment) from the data collected in generating the amphetamine + OLZ dose–effect curve (treatment), a change score was created (pre − post). The change scores for each animal were used to conduct two-tailed, multiple comparison tests for Friedman's rank sums for each dependent variable (Siegel and Castellan, 1988).

The effect of OLZ treatment alone (no amphetamine present) was also addressed by a single pairwise comparison, one Wilcoxon for each dependent variable comparing the 0.0-mg/kg amphetamine dose from the amphetamine dose–effect curve to the 0.0-mg/kg amphetamine dose from the amphetamine + OLZ dose–effect curve.

Control analyses for the effects of repeated amphetamine administration on the discriminative cue's stability were also analyzed. Two Wilcoxons for each dependent variable were conducted on data collected in the amphetamine dose–effect curve; each comparing the training dose (1.0 mg/kg amphetamine or volumetric equivalent of saline) tested at both the beginning and at the end of the generation of the curve.

Finally, the effects of low doses of amphetamine (0.25 and 0.32 mg/kg) on overall rate were analyzed by comparing response rates (from the amphetamine dose–effect curve) at these two doses of amphetamine to rates when no amphetamine was present (0.0 mg/kg) using a single Wilcoxon for each amphetamine dose.

3. Results

Of the 16 animals that began training, 10 reached criteria performance within 75 days. The remaining six animals either died during the training period ($n = 4$) or were unable to learn the discrimination ($n = 2$).

3.1. Amphetamine temporal parameters

Analysis of the effects of the amphetamine pretreatment interval revealed significant main effects of the pretreatment interval on both dependent variables: percentage of drug-appropriate responding [$F(5,35) = 6.063$, $P < .001$, $n = 8$] and overall rate [$F(5,35) = 23.4$, $P < .001$, $n = 8$]. Follow-up comparisons for the percentage drug-appropriate responding revealed that all time points were significantly different from the 120-min interval but not from one another (differences in averaged ranks ≥ 10.4, $P < .05$). Similarly, comparisons for overall rate of responding revealed that the 15-, 30-, and 45-min intervals were significantly different from the 60-, 90-, and 120-min intervals, while the 60- and 90-min intervals were also significantly different from one another (differences in averaged ranks ≥ 7.82, $P < .05$).

The control analyses concerning the stability of the cue over the course of this phase of the experiment were to be addressed using a single Wilcoxon for each dependent variable. Due to ceiling effects on the percentage of the drug-appropriate responding variable, the number of observations ($n = 4$) was too small to obtain meaningful tabular results using the Wilcoxon (see above for discussion). However, even the t test did not detect any significant differences [$t(7) =$ n.s., $\alpha < .05$]. As the rate data could be analyzed with an $n = 8$, these comparisons were conducted using the two-tailed Wilcoxon. Overall rate was not significantly altered by the repeated administrations of amphetamine during the time–response experiment [Wilcoxon(7) = n.s., $\alpha < .05$].

3.2. Amphetamine vs. amphetamine + OLZ dose–effect curves

Full dose–effect curves (dose–effect curves) for amphetamine treatment and for amphetamine + OLZ treatments are represented graphically, as well as in tabular form, for both dependent variables (see Table 1, Fig. 1 — overall percentage and Fig. 2 — overall rate). Points represent overall averages for each dependent variable at each dose of amphetamine.

The ED50 values (amphetamine vs. amphetamine + OLZ) for the percentage of drug-appropriate responding were significantly increased after OLZ treatment (see Table 1 and Fig. 3). The mean ED50 value for the percentage of drug-appropriate responding for the amphetamine + OLZ dose–effect curve (0.98 ± 0.23 mg/kg, mean ± S.E.M.) was significantly elevated [Wilcoxon(7), $P = .008$] from that for the amphetamine dose–effect curve (0.26 ± 0.047 mg/kg). The ED50 values (amphetamine vs. amphetamine + OLZ) for rate of responding were not significantly different ($\alpha \leq .05$) (see Table 1 and Figs. 2 and 3). The mean ED50 value for rate of responding for the amphetamine + OLZ dose–effect curve (1.5 ± 0.071 mg/kg) was not significantly elevated [Wilcoxon(7), $P = .461$] from that for the amphetamine dose–effect curve (1.3 ± 0.124 mg/kg). Additionally, the slopes calculated from the two regression analyses (amphetamine vs. amphetamine + OLZ) were significantly different [$t(14)$, $P < .05$] regardless, of which dependent variables (percentage or rate) were analyzed, indicative of nonparallel shifts.

A more thorough analysis of the data was conducted using a Friedman's ANOVA by ranks. Unlike in the analyses of the ED50s, both dependent variables (percentage and

Table 1
Effect of OLZ treatment on the amphetamine (AMPH) discrimination

Pretreatment × Drug	*n*	Test drug	% AMPH response	Rate	% Baseline level rate
None	8/9	0.0 AMPH	1.0 ± 0.37	1.3 ± 0.19	92.2 ± 8.1
None	8/9	0.0 AMPH (2nd)	0.19 ± 0.11	1.6 ± 0.14	123 ± 6.8
None	8/9	sham injection	0.21 ± 0.14	1.7 ± 0.11	128 ± 9.3
OLZ (1.5)	8/9	0.0 AMPH	4.0 ± 2.7	0.076 ± 0.02	6.49 ± 1.6
None	8	0.25 AMPH	37.7 ± 18	1.6 ± 0.16	116 ± 6.2
OLZ (1.5)	8	0.25 AMPH	17.9 ± 12	0.56 ± 0.12	47.2 ± 9.0
None	10	0.32 AMPH	66.7 ± 14	1.4 ± 0.17	104 ± 12
OLZ (1.5)	10	0.32 AMPH	13.3 ± 9.0	0.57 ± 0.12	41.3 ± 7.3
None	10	0.56 AMPH	99.3 ± 0.37	0.96 ± 0.12	74.7 ± 10
OLZ (1.5)	10	0.56 AMPH	38.4 ± 14	0.63 ± 0.14	47.0 ± 8.3
None	9/8	1.0 AMPH	98.9 ± 0.49	0.77 ± 0.16	46.8 ± 6.7
None	9/8	1.0 AMPH (2nd)	96.6 ± 1.6	0.54 ± 0.09	90.8 ± 18
OLZ (1.5)	9/8	1.0 AMPH	37.4 ± 14	0.41 ± 0.07	36.0 ± 7.8
None	8/9	1.8 AMPH	98.8 ± 0.69	0.54 ± 0.08	45.8 ± 8.6
OLZ (1.5)	8/9	1.8 AMPH	47.9 ± 18	0.23 ± 0.06	19.0 ± 4.4
None	7	2.2 AMPH	99.8 ± 0.11	0.44 ± 0.13	31.8 ± 9.2
OLZ (1.5)	7	2.2 AMPH	67.3 ± 16	0.17 ± 0.05	11.6 ± 2.5

ED50s	*n*	% ED50	Interpolated (%)	Rate ED50	Rate ED50 (% baseline level)
None	8/10	AMPH≅0.26 ± 0.047	50	AMPH≅1.4 ± 0.11	AMPH≅1.3 ± 0.12
OLZ (1.5)	8/10	AMPH≅0.98 ± 0.23	50	AMPH≅1.5 ± 0.17	AMPH≅1.5 ± 0.07

Each value represents the mean ± S.E. of the number of animals (*n*) indicated. Where two numbers are given the numerator represents the number of animals used to calculate the percentage of drug-appropriate responding and the denominator represents the number of animals used to calculate rate-of responding. The rate of responding is given as both the raw data (in responses per second) and the transformed data (as percentage of saline baseline responding). Test drugs (amphetamine and saline) were administered intraperitoneally 15 min prior to testing while pretreatment drugs (OLZ) were administered subcutaneously 60 min prior to testing.

overall rate) were significantly decreased by OLZ treatment: percentage of drug-appropriate responding [$F(6,48) = 3.44$, $P = .007$] (see Fig. 1) and overall rate [$F(6,48) = 3.93$, $P = .003$] (see Fig. 2).

OLZ treatment resulted in decreases in the percentage of drug-appropriate responding that were greater than the decreases at the 0.0-mg/kg amphetamine dose (differences in averaged ranks ≥ 16.5, $P < .05$) (see Fig. 1). A similar

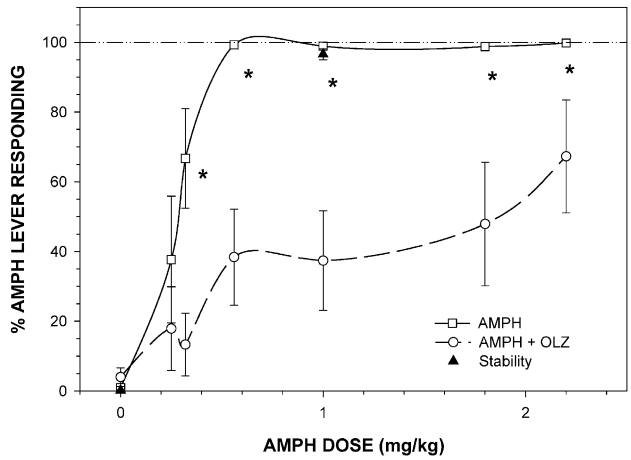

Fig. 1. Effect of OLZ treatment on the percentage of drug-appropriate responding. A significant main effect of OLZ treatment was returned for the percentage of drug-appropriate responding ($P = .007$, $n = 9$). Follow-up comparisons revealed that the change scores for all but the lowest dose of amphetamine (0.25 mg/kg AMPH) were significantly greater than the change score for OLZ alone ($\alpha < .05$, $n = 9$). * Change scores that are significantly different from the "control" change score at an $\alpha < .05$. Error bars indicate ± 1 S.E.

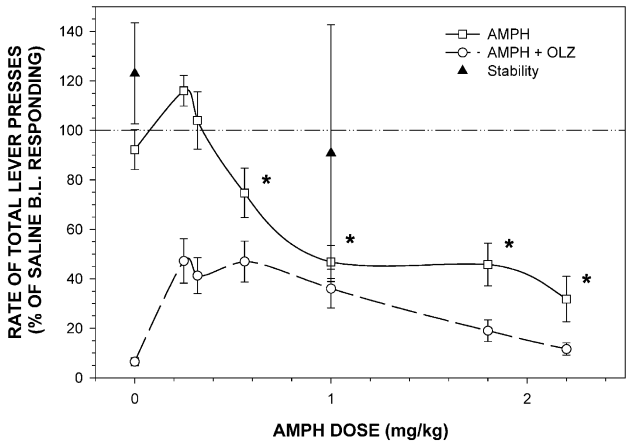

Fig. 2. Effect of OLZ treatment on the total rate of responding. A significant main effect of OLZ treatment was returned for overall rate of responding ($P = .003$, $n = 9$). Follow-up comparisons revealed that the change scores for all but the two lowest doses of amphetamine (0.25 and 0.32 mg/kg AMPH) were significantly smaller than the change score for OLZ alone ($\alpha < .05$, $n = 9$). Additionally, 0.25 mg/kg amphetamine (but not 0.32 mg/kg AMPH) significantly increased overall rate of responding from baseline levels (Wilcoxon: $P = .05$, $n = 7$). * Change scores that are significantly different from the "control" change score at an $\alpha < .05$. Error bars indicate ± 1 S.E.

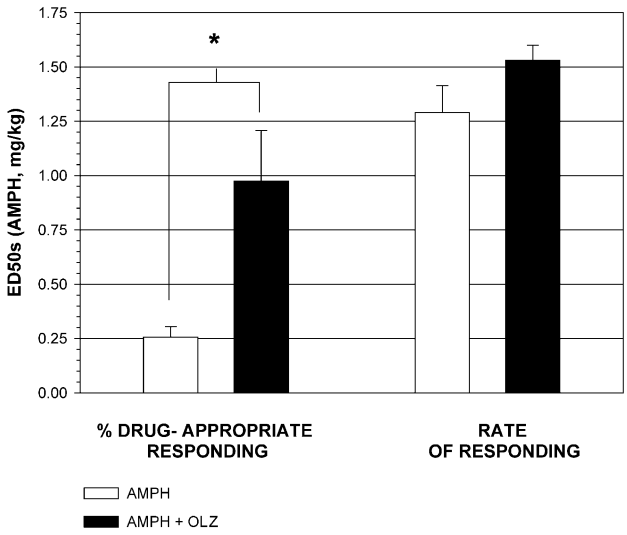

Fig. 3. Effect of OLZ treatment on the amphetamine discrimination: ED50s. The amphetamine ED50 for the percentage of drug-appropriate responding was significantly increased following OLZ (1.5 mg/kg) treatment (Wilcoxon: $P=.008$, $n=8$). However, the amphetamine (AMPH) ED50 for overall rate was not significantly altered by OLZ treatment (Wilcoxon: n.s., $\alpha<.05$, $n=8$). *ED50s that are significantly different at an $\alpha<.05$. Error bars indicate ± 1 S.E.

trend was observed in the analysis of the overall rate data. OLZ treatment resulted in decreases in rates of responding that were significantly smaller than the decreases in rates at the 0.0-mg/kg amphetamine dose, for all but the lowest doses of amphetamine (0.25 and 0.32 mg/kg) (differences in averaged ranks ≥ 16.1, $P<.05$) (see Fig. 2). When OLZ was given without amphetamine present (the 0.0-mg/kg amphetamine dose), the percentage of drug-appropriate responding was not significantly affected [Wilcoxon(5), n.s.] (see Table 1 and Fig. 1). However, the overall rate of responding was significantly reduced [Wilcoxon(8), $P=.004$] (see Table 1 and Fig. 2).

The control analyses concerning the stability of the cue over the course of these experiments were addressed using a two-tailed Wilcoxon for each dependent variable. The percentage of drug-appropriate responding at the 1.0-mg/kg amphetamine training dose was not significantly altered by the repeated administration of amphetamine or by the passage of time [Wilcoxon(8), n.s.] (see Table 1 and Fig. 1). Likewise, overall rate of responding was not significantly altered [Wilcoxon(7), n.s.], although this appears due to an extremely high degree of variance at this point (see Table 1 and Fig. 2).

When the saline training dose (0.0 mg/kg amphetamine) was again tested at the end of the dose–effect curve, the percentage of drug-appropriate responding was significantly reduced (more saline-like responding) compared to when it was first tested [Wilcoxon(6), $P=.0156$] (see Table 1 and Fig. 1). Likewise, overall rate of responding was significantly increased after the generation of the dose–effect curve [Wilcoxon(8), $P=.039$] (see Table 1 and Fig. 2).

Finally, the lowest dose of amphetamine (0.25 mg/kg) produced significant increases in rate of responding over baseline (0.0 mg/kg amphetamine) rates that were no longer present at the next lowest dose (0.32 mg/kg): 0.25 mg/kg amphetamine [Wilcoxon(6), $P=.047$] and 0.32 mg/kg amphetamine [Wilcoxon(8), n.s.] (see Table 1 and Fig. 2).

4. Discussion

There is large disagreement in the field concerning the choice of overall (entire session) vs. first reinforcer data in drug discrimination studies. There are benefits and drawbacks to both (see Colpaert, 1987, for brief discussion). Our laboratory does not use an extinction or "lock-out" procedure; throughout the entire test session, 10 responses on either lever will result in delivery of a pellet, as long as all 10 are made consecutively on the same lever. By the theory of "corrupted responding," there should be no reason for animals to sample a different contingency (the nonselected lever) after receiving a reinforcer on the selected lever. However, we frequently observe animals during test days (with pretreatment antagonists) that switch responding and begin receiving reinforcement for responding on the alternate lever after the first reinforcement. Animals appear capable of altering their discriminative choice within a given session and appear unaffected (uncorrupted) by choice/reinforcement during the first reinforcer portion of the session. We believe that such responding represents individual differences in drug kinetics.

In extinction or "lock-out" procedures, the test session (or the decision that sets the "correct" lever) occurs over a very brief period of time, resulting in a very narrow window to detect the activity of the therapeutic (or set the "correct" lever). If antagonist effects have not reached a threshold value by this point, animals' subsequent responding throughout the remainder of the session may be "corrupted." There is no longer the potential to receive a reinforcer by responding on the "incorrect" level; animals cannot express later developments in the cue state without negative consequences (i.e., failure to receive a reinforcer, as the lever is now considered "incorrect"). By comparison, use of the entire 10-min session, with either lever providing a reinforcer, yields a greatly expanded opportunity (at least 10–20 times longer on average) to detect such effects.

Despite the questionable utility of the first reinforcer variable, its wide use in other drug discrimination methodologies makes it a more widely recognized variable. Therefore, although not analyzed for statistical significance, we compared the graphic representations of both the first reinforcer and total measures for drug-appropriate responding and rate of responding. Dose–effect curves were virtually identical excepting for larger degrees of variance within the first reinforcer data.

The loss of several animals during drug discrimination training (or failure of animals to attain criteria level perform-

ance) has been reported in previous amphetamine discrimination studies. Using a slightly higher training dose (1.25 mg/kg), one study reported loosing 4 of 10 animals during training to either death or failure to learn the discrimination (Colpaert et al., 1978b). Deaths in this, and the current study, may have resulted from repeated and/or errant drug injections or a heightened sensitivity to the toxic effects of amphetamine in these animals. The two animals that failed to learn the discrimination are more difficult to explain. Simple individual differences in drug sensitivity or learning abilities may have resulted in these failures. However, it may be that a heightened or developed sensitivity to some of the drug's side effects (e.g., stereotypies) may have interfered with learning or prevented the execution of this learning. We suspect the lack of such reports in other amphetamine studies may be due more to a difference in procedure (drawing subjects from a pretrained pool) or failure to report such losses, rather than to such effects being unique or rare.

4.1. Amphetamine temporal parameters

Effects of amphetamine reached a maximal level for both cue generalization and rate reduction within 15 min of injection (the earliest pretreatment interval tested). As expected, longer amphetamine pretreatment intervals resulted in the eventual dissipation of any amphetamine effect. The majority of the decrease in overall cue generalization occurred between 60 and 120 min, while the majority of the decrease in rate reduction occurred earlier, between 45 and 60 min. However, the dissipation of both effects began within 60 min of injection. Overall, these results correspond well with the published data and the known kinetics of amphetamine (Jones et al., 1976; Kuhn and Schanberg, 1978). Additionally, the cue remained stable throughout the generation of the time–response curve.

4.2. Amphetamine vs. amphetamine+OLZ dose–effect curves

After treatment with OLZ (1.5 mg/kg), higher doses of amphetamine were required to achieve the same level of cue generalization. However, the ED50s for animals' overall rates of responding were unaffected after OLZ treatment. While graphically OLZ treatment appears to have a rate-decreasing effect, it did not reach statistical significance in the ED50 analysis. This may have been due to a floor effect, as amphetamine administration alone was rate suppressing at all but the lowest doses (see Figs. 2 and 3).

Overall, these ED50 values are in agreement with the bulk of the drug discrimination literature (Arnt, 1996; Cunningham and Appel, 1982; D'Mello and Stolerman, 1977; Meert, 1991; Nielsen and Jepsen, 1985; Overton, 1987). Generally, drug doses as low as 30–50% of the original training dose are still readily discriminated (Colpaert, 1977; Overton, 1987). ED50 values are most sensitive to the level of the training doses, with higher training doses resulting in higher ED50s (between three to eight times lower than the training dose) (Colpaert et al., 1978b). The earlier experiments using OLZ to block the amphetamine cue reported a maximal inhibition of 59% using 2.48 mg/kg OLZ (Arnt, 1996). We observed a slightly higher level of inhibition (65%) with a lower dose (1.5 mg/kg) of OLZ at a shorter pretreatment interval (60 vs. 120 min).

The effect of OLZ treatment on the percentage of drug-appropriate responding was greater at most doses of amphetamine (except 0.25 mg/kg) than it was at the "control" dose (0.0 mg/kg), a hallmark of a clinically useful therapeutic. Across the majority of the amphetamine+OLZ dose–effect curve (except the lowest amphetamine dose, which was poorly discriminated in the amphetamine dose–effect curve), OLZ treatment was significantly more effective than when given with no amphetamine present. Therefore, OLZ treatment greatly reduced the discriminability of amphetamine, as reflected by significant increases in the percentage of responding on the saline-appropriate lever after treatment.

Similarly, the rate data reveal that at low doses of amphetamine (0.25 and 0.32 mg/kg), OLZ treatment decreases rate of responding as much as it does with no amphetamine present. Such rate-reducing effects of OLZ at the low doses of amphetamine are a common distinguishing feature of atypical antipsychotics (Arnt and Skarsfeldt, 1998). However, at the lower doses of amphetamine (including the 0.0-mg/kg dose), the rate-decreasing effects of OLZ were greater than at the higher doses of amphetamine. This difference is likely due to an additive effect of OLZ's rate-depressing effects and those of the higher doses of amphetamine (which alone represent the majority of the reduction at these points).

Because the rate of responding is severely depressed at the extremes of the amphetamine+OLZ dose–effect curve, depressed locomotor activity may be responsible (in part) for poorer performance (cue generalization) at these doses. However, across the range of amphetamine doses where OLZ treatment appears to interfere with the discrimination, the rate of responding is no more decreased in the amphetamine+OLZ dose–effect curve than in the amphetamine dose–effect curve. Additionally, despite the rate-reducing effects of OLZ, animals were still capable of emitting a sufficient number of responses to perform the discrimination. Therefore, taken together, the rate and cue generalization results suggest that interference due to nonspecific drug effects (i.e., rate inhibition) cannot be the sole cause of the lower generalization observed after OLZ administration.

OLZ treatment did not significantly alter generalization of the control (saline) dose, suggesting that OLZ alone was not discriminated as drug-like. Again, the weakening of the discriminative stimulus effects of amphetamine by OLZ does not appear to be due to nonspecific drug effects, as appropriate saline generalization continued after OLZ pretreatment. However, overall rate of responding was significantly decreased when OLZ was given alone, exposing the

generalized rate depressant effect of OLZ treatment. Therefore, OLZ too possesses a generalized inhibitory effect on rate of responding (even with no amphetamine present), despite having no effect on the cue generalization at this "control" dose. Importantly, when given alone, OLZ produced a rate-decreasing effect that did not interfere with the animal's ability to perform the appropriate discrimination (saline lever responding).

Partial generalization is said to occur when levels of discrimination are observed (as in the present study) that are intermediate to the trained extremes (Appel et al., 1991; Barrett and Appel, 1989; Colpaert, 1987; Meert, 1991; Nencini and Woolverton, 1988; Picker et al., 1993). Much controversy surrounds the analysis and interpretation of such results (see Colpaert, 1977; Colpaert et al., 1978a; Holloway and Gauvin, 1989, for discussion). Depending on whether data are conceptualized as quantal or continuous, various interpretations are offered for partial generalization (see Mathis et al., 1987; Gauvin and Young, 1987, for discussion, respectively). We believe that partial generalization reflects a lesser degree of generalization (the cue is somewhat drug-like), rather than random/disorganized responding or chance performance (Holloway and Gauvin, 1989). Further, the parallel line tests returned significant differences between the slopes for each regression line (amphetamine vs. amphetamine + OLZ) indicative of a nonparallel shift; parallel shifts indicating competitive antagonism.

Regarding cue stability, there was no evidence of any shifts in stability of the amphetamine cue due to repeated administration of the 1.0-mg/kg amphetamine training dose. However, it appears that animals continued to improve on the saline portion of this discrimination over time. Additionally, animals responded more rapidly in subsequent sessions, compared to when the saline dose (0.0 mg/kg amphetamine) was first tested. Taken together, these results suggest that animals continue to improve their performance on the saline portion of this discrimination throughout the generation of the dose–effect curve.

Although the pharmacological profile of OLZ is predictive of competitive antagonism, the lack of parallelism in the current study suggests that other mechanisms may be responsible. Most obviously, nonspecific effects (notably locomotor) could be responsible for the reduced drug lever responding. However, the current results do not support such a conclusion. Alternatively, if one supports the existence of perceptual masking in drug discriminations, such effects could explain reduced drug lever responding. However, masking has not yet been determined a reliable phenomenon, nor to exert a considerable/frequent influence, in drug discrimination paradigms (Overton, 1983, 1987; Witkin et al., 1980). To our knowledge, masking has never been reported in an amphetamine discrimination study. In fact, in a search of the drug discrimination literature for the last 50 years, only five matches were found for the term "masking" (on-line Drug Discrimination Datebase; Stolerman, 2001).

However, if masking were responsible for the current results, several additional effects should have been observed. First, drugs from pharmacological classes other than DA should be able to interfere with the amphetamine discrimination. While not directly examined in the current study, to our knowledge no study has demonstrated inhibition with such agents. In fact, several have failed to find effects, including those using cholinergic, adrenergic, and serotonergic antagonists (Ho and Huang, 1975; Moser, 1992; Przegalinski and Filip, 1997), opiate antagonists (Schechter, 1978), and 5-HT reuptake inhibitors (Schechter, 1980). Therefore, none of the pharmacological classes studied (with drugs at doses know to be effective against training drugs within the same class) have any effect on the amphetamine discrimination, excepting DA antagonists. Therefore, these drugs (at these doses) have both physiological and discriminative effects yet appear unable to mask the amphetamine cue.

Additionally, in the present study, when OLZ was administered without amphetamine, animals made almost exclusively saline-appropriate responses. If the two-lever drug discrimination is viewed as a "presence" vs. "absence" of any interoceptive effects (as may be the case in drug–saline discriminations), then failure of OLZ alone to produce any drug lever responding may suggest a lack of interoceptive effects; the OLZ state produced no cue.

By more traditional interpretations ("presence" vs. "absence" of class-specific cues), such results could be viewed as "default" responding; OLZ produced a discriminative cue, just one that was not amphetamine-like (Gauvin et al., 1992). Even accepting this quantitative interpretation, the near complete lack of drug lever responding (when OLZ was given alone) suggests that if such a cue exists, it is almost exclusively saline-like (Browne, 1981). Therefore, the dose of OLZ used in the present study appeared to have no discriminative effect (at least without prior OLZ training as the discriminandum; see below) and hence could not mask the amphetamine cue. However, lower doses of OLZ have been trained in drug discriminations, suggesting that OLZ may produce a significant discriminative cue (Porter and Strong, 1996) or simply that almost any centrally active compound can eventually be trained (Overton, 1984).

Final, one frequently overlooked requirement for masking is that the treatment drug belongs to a different pharmacological class (Gauvin et al., 1994). OLZ is primarily a DA/5-HT antagonist and the 5-HT effects (at least in drug discriminations) have been discounted by previous studies (see above). Therefore, as a DA antagonist, OLZ fails to meet one of the primary characteristics required for masking.

While not completely ruled-out, we do not believe that stimulus masking by OLZ is an appropriate explanation for the current results. Based on the lack of support for nonspecific interference or masking, and the binding profile of OLZ, we believe the current results can only be explained by pharmacological antagonism at the DA receptor. The

failure to recapture the same level of maximal efficacy after OLZ treatment (and hence the lack of parallelism) likely resulted from the extreme depression of locomotor activity that would be caused by any amphetamine dose large enough to override this antagonism. If higher doses of amphetamine were tested, 100% drug-appropriate lever responding may have been recaptured (there was an upward trend at the highest doses of amphetamine, see Fig. 1). Alternatively, the dramatic rate-reducing effects of high dose amphetamine (and contributions from OLZ) may have completely abolished lever responding.

In the current study, a 1.5-mg/kg dose of OLZ successfully disrupted the cueing effects of amphetamine, reducing both the efficacy and the potency at which amphetamine exerts discriminative control. We believe these experiments have bolstered the conclusion that OLZ prevents (or interferes with) the amphetamine cue, supporting its potential as a pharmacotherapy for stimulant abusers. We plan to further investigate the potential dose/temporal parameters of OLZ in future experiments. Additionally, we believe that our findings with amphetamine will generalize to methamphetamine (due to their similar structure and shared metabolic pathway) and may generalize to cocaine, as the cue properties of both drugs are fairly similar (Colpaert et al., 1978a,b; D'Mello and Stolerman, 1977), and are currently exploring this hypothesis. However, the two drug states are distinct enough to allow training of the amphetamine–cocaine discrimination (using well over 100 training sessions), likely involving the local anesthetic properties of the latter (Goudie and Reid, 1988).

Clinical treatment with OLZ should approximate the laboratory condition of extinction by nonreinforcement, especially if the individual continues to engage in drug taking behaviors while treated with OLZ. However, there remains the risk of accidental overdose if the individual increases stimulant intake attempting to override the antagonistic effects. After further experimentation using additional animal behavioral paradigms (conditioned place preference and self-administration), we plan to examine clinical efficacy in a small population of human stimulant abusers.

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