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Oxazepam does not modulate the behavioral effects of *d*-amphetamine in humans

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Abstract

Benzodiazepines, which are γ-aminobutyric acid-A ($GABA_A$) receptor positive modulators, can block the behavioral effects of psychomotor stimulants. In the present study, the ability of oxazepam, which may have less abuse potential compared to some other benzodiazepines, to attenuate the discriminative-stimulus, subject-rated and psychomotor performance effects of *d*-amphetamine in humans was determined. Six healthy participants (2 female, 4 male) learned to discriminate 15 mg oral *d*-amphetamine. After acquiring the discrimination (i.e., ≥80% correct responding on 4 consecutive days), the effects of *d*-amphetamine (0, 2.5, 5, 10 and 15 mg), alone and in combination with acutely administered oxazepam (0 and 20 mg) were assessed. *d*-Amphetamine alone functioned as a discriminative stimulus, produced stimulant-like subject-rated effects (e.g., increased ratings of Stimulated on a Drug–Effect Questionnaire) and enhanced psychomotor performance. Oxazepam alone increased subject ratings of sedation (e.g., increased ratings of Sluggish, Fatigued and Lazy on a Drug–Effect Questionnaire) and impaired psychomotor performance. Oxazepam alone did not occasion *d*-amphetamine-like discriminative-stimulus effects, and had no effect on the discriminative-stimulus or subject-rated effects of *d*-amphetamine when given in combination. The results of this experiment are discordant with previous research and suggest that benzodiazepines differ in their ability to modulate the behavioral effects of *d*-amphetamine.

Keywords: *d*-Amphetamine; Oxazepam; Drug discrimination; Subjective effects; Human; Pharmacotherapy; Dependence; DSST

1. Introduction

The abuse of psychomotor stimulants remains a significant public health concern. In particular, amphetamine abuse and dependence is escalating at an alarming rate. Methamphetamine is the primary form of amphetamine used recreationally in the United States (Substance Abuse and Mental Health Services Administration (SAMHSA), 2004). Between 1996 and 2002, the number of Americans that reported methamphetamine use increased by 250% (4.8 million in 1996, 12 million in 2002; SAMHSA, 2003). Consistent with those findings, data from the Arrestee Drug Abuse Monitoring Program indicated that methamphetamine use has grown considerably from 1991 to 2001, especially in the Western and Midwestern parts of the country (Yacoubian and Peters, 2004). Between 1992 and 2002, per capita rates of admissions to treatment programs for amphetamine increased by over 500%, and methamphetamine made up 90% of all these admissions (SAMHSA, 2004). The escalating rates of methamphetamine use are consistent with greater drug availability. From 1991 to 2001, increasingly larger amounts of methamphetamine and its precursors have been seized worldwide, and the number of clandestine manufacturing laboratories that have been discovered has risen (United Nations Office on Drugs and Crime, 2003).

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Amphetamines act as substrates for dopamine (DA), serotonin and norepinephrine transporters and are transported into the nerve terminal where they promote the release of these monoamines into the synapse by preventing the accumulation of neurotransmitter in storage vesicles, and also by carrier-mediated exchange (Rothman et al., 2001). Preclinical research has implicated the elevation of synaptic DA in the mesocorticolimbic system as the primary mediator of the abuse-related effects of stimulants (Everitt et al., 1999). Because of the predominant role of DA, one strategy in the development of a pharmacotherapy for stimulant addiction has been to evaluate drugs that interact with the mesocorticolimbic DA system. Neurochemical studies have demonstrated that non-selective pharmacological stimulation of γ-aminobutyric acid (GABA) receptors inhibits stimulant-induced increases in extracellular DA (Gerasimov et al., 2000; Morgan and Dewey, 1998). Because of the accumulating evidence that DA is under the inhibitory control of GABA, our laboratory has conducted a series of studies to determine whether GABAergic drugs modify the behavioral effects of stimulants (Haga et al., 2003; Lile et al., 2004a,b; Rush et al., 2004).

Benzodiazepines are $GABA_A$ receptor positive modulators. They produce their effects by binding at a site on the $GABA_A$ receptor where they act in an allosteric manner to enhance the activity of the receptor when concomitantly bound by GABA. Laboratory research in animals and humans has shown that benzodiazepines can attenuate the behavioral effects of stimulants. For example, diazepam blocked the development (Leri and Franklin, 2000; Meririnne et al., 1999) and expression (Leri and Franklin, 2000) of a conditioned place preference induced by *d*-amphetamine in rats, and oxazepam blocked the expression of a conditioned place preference induced by methamphetamine in rats (Goeders and Goeders, 2004). Similarly, pretreatment with alprazolam and chlordiazepoxide decreased cocaine self-administration in rats (Goeders et al., 1989, 1993). In monkeys trained to discriminate injections of cocaine, administration of triazolam and imidazenil attenuated cocaine-appropriate responding (Negus et al., 2000). A drug-discrimination study in humans with alprazolam produced comparable results to the monkey data (Rush et al., 2004). In that study, healthy participants learned to discriminate *d*-amphetamine. Concurrent administration of alprazolam attenuated the discriminative-stimulus and some of the positive subject-rated effects of *d*-amphetamine. Together, the results from these studies suggest that benzodiazepines may be viable treatments for stimulant dependence.

Unfortunately, benzodiazepines possess abuse liability of their own, and amphetamine users have been reported to regularly use benzodiazepines as well (Darke et al., 1994). However, certain benzodiazepines, such as alprazolam, are preferred over others (Griffiths and Wolf, 1990; Malcolm et al., 1993; Rush et al., 1993). Oxazepam appears to have less abuse potential compared to some of the other benzodiazepines (Griffiths et al., 1984a,b; Griffiths and Wolf, 1990), and may therefore be a more appropriate option as a pharmacotherapy. Based on our positive findings from the previous study with alprazolam (Rush et al., 2004), we conducted the present study to determine if acutely administered oxazepam would also modify the behavioral effects of *d*-amphetamine. In this study, six healthy participants learned to discriminate 15 mg *d*-amphetamine from placebo. Next, the ability of concurrent administration of 20 mg oxazepam to attenuate the discriminative-stimulus, subject-rated and performance effects of a range of doses of *d*-amphetamine (0, 2.5, 5, 10 and 15 mg) was determined. We hypothesized that, like alprazolam, oxazepam would attenuate the discriminative-stimulus and some of the positive-subjective effects of *d*-amphetamine.

2. Methods

2.1. Participants

Nine healthy adults were recruited via newspaper ads, flyers and word-of-mouth to participate in this experiment. Participants were paid $40/session to participate in this experiment and also received a performance-based completion payment as outlined below. One participant was unable to accurately discriminate 15 mg *d*-amphetamine, while another participant withdrew for reasons unrelated to the study protocol. A final participant was released from the study due to sensitivity to the cardiovascular effects of *d*-amphetamine. This individual's blood pressure exceeded our safety criteria during the Sampling Phase of the experiment, in which participants initially receive the training dose of *d*-amphetamine (i.e., 15 mg). Data from these participants were not included in the analyses. Six participants (1 Hispanic female, 1 Caucasian female, 4 Caucasian males) completed this experiment. These participants ranged in age from 21 to 25 years (mean=23), in education from 13 to 18 years (mean=15) and in weight from 59.5 to 131.8 kg (mean=87.7). Prior illicit drug use included marijuana (4 participants), opiates (two participants), amphetamines (1 participant) and hallucinogens (1 participant). These participants reported consuming 10 to 380 mg caffeine/day (mean=112.6). Three participants were current smokers of tobacco cigarettes (mean=7 cigarettes/day).

Participants completed questionnaires assessing drug use, physical and psychiatric histories and provided written informed consent prior to participating. Individuals with current or past histories of serious psychiatric disorder, including substance dependence disorders (except nicotine), were excluded from participating. All participants were in good health with no apparent contraindications to amphetamines or benzodiazepines. Drug urine screens conducted during the initial screening were negative for amphetamine, barbiturates, benzodiazepines, cocaine and opioids (OnTrak TESTSTIK, Varian, Inc., Lake Forest, CA). One participant tested positive for tetrahydrocannibinol (THC). In the female participants, urine pregnancy tests taken before and during study participation were negative. Both female participants used a hormonal contraceptive for the duration of the study. The Institutional Review Board of the University of Kentucky Medical Center approved this study and the informed consent document.

2.2. General procedures

Participants enrolled as outpatients at the Laboratory of Human Behavioral Pharmacology at the University of Kentucky Medical Center Monday through Friday for 22 to 26 (mean = 24) experimental sessions. Participants were informed that during their participation they would receive various drugs and that these could include placebo and medications indicated for the treatment of attention deficit and hyperactivity disorder and anxiety disorders. Participants were told that the purpose of the study was to see if they could detect the presence of a drug, and how drugs affect mood and behavior. Other than receiving this general information participants were blind to the type of drug administered, and were given no instructions regarding what they were "supposed" to do or what outcomes might be expected.

Prior to initiating drug testing, participants completed two "practice" sessions. These "practice" sessions were used to familiarize participants with the drug-discrimination task, self-reported drug–effect questionnaires, performance measure and daily laboratory routine. No drugs were administered on these sessions.

Throughout the study, participants were required to abstain from using all illicit psychoactive drugs. In addition, participants were required to abstain from ingesting caffeine and solid food for 4 h prior to a scheduled experimental session, licit psychoactive substances (e.g., antihistamines, excluding nicotine) for 12 h prior to a scheduled experimental session, and alcohol for 12 h prior to, and following, a scheduled experimental session. On each experimental-session day, participants arrived at the laboratory and provided a urine sample before drug administration, which was immediately screened for the presence of amphetamines, barbiturates, benzodiazepines, cocaine, opioids and THC. These urine samples were occasionally positive for amphetamine, which coincided with experimental administration. One participant's urine specimen was positive for opioids at the beginning of a single experimental session; the session for this participant was rescheduled. Another participant's urine specimen was positive for THC eleven times during the experimental protocol, the majority of which occurred at the beginning of that individual's participation. Because of the extensive length of time necessary for THC to undergo total body clearance, this individual was allowed to participate in those sessions. Participants also provided an expired air specimen, which was assayed for the presence of alcohol using a hand-held breathalyzer (Intoximeters, Inc., St. Louis, MO). Two participants were sent home, each on one occasion, for having a positive breath-alcohol level. Otherwise, all expired air samples were negative.

On experimental-session days, participants completed the self-reported drug–effect questionnaires and performance task approximately 30 min before drug administration, and then completed the drug-discrimination task, self-reported drug–effect questionnaires and performance task 1, 2, 3, 4 and 5 h after drug administration. When not completing the drug-discrimination task, self-reported questionnaires and performance task, participants were allowed to engage in recreational activities (e.g., watch television, play cards, read or socialize). Participants were provided with a fat-free breakfast when they arrived at the laboratory and lunch after the 3-h observation.

2.3. Drug-discrimination procedures

This experiment consisted of three phases, which were completed in fixed order: 1) sampling phase, 2) acquisition phase and 3) test phase.

2.3.1. Sampling phase

All participants completed two sampling sessions to acquaint them with the drug effects. During each sampling session, participants ingested four capsules that contained a total of 15 mg *d*-amphetamine. *d*-Amphetamine was identified by letter code (e.g., DRUG A), but the participants were not explicitly informed of the capsules' contents. *d*-Amphetamine (15 mg) is identified as DRUG A for illustrative purposes only; a unique letter code was used for each participant. An instruction set was given to each participant during the sampling phase. Participants were asked to carefully read the instructions before each sampling session, and a research assistant also read the instructions aloud. Briefly, the instructions explained that they were receiving DRUG A, but in the future they would be asked to decide whether they had received DRUG A or NOT DRUG A (for the exact instructions, see Rush et al., 2003).

2.3.2. Acquisition phase

Following the sampling phase, an acquisition phase was conducted to determine if participants could discriminate 15 mg *d*-amphetamine. During this phase, participants ingested capsules under double-blind conditions, but were not told whether the capsules contained 15 mg *d*-amphetamine (e.g., DRUG A) or placebo (e.g., NOT DRUG A). Participants were not explicitly instructed that they would be attempting to acquire a drug versus placebo discrimination (for the exact instructions, see Rush et al., 2003). After capsule administration, participants completed the drug-discrimination task, self-reported drug–effect questionnaires and performance measure periodically for five hours. Participants were instructed that they could change their responses on the drug-discrimination task between hours 1, 2, 3, 4 and 5 based on what they believed at the time. After completing the drug-discrimination task, self-reported drug–effect questionnaires and performance task at the five-hour observation, participants opened a sealed envelope that informed the participant and the research assistant of the identity of the drug administered (i.e., DRUG A or NOT DRUG A). The criterion for having acquired the discrimination was $\geq$80% correct responding on four consecutive sessions on the drug-discrimination task described below. The order of drug administration was random except that each participant received each training condition, 15 mg *d*-amphetamine and placebo, at least twice.

2.3.3. Test phase

Following the acquisition phase, participants entered a test phase. The test phase consisted of test sessions interspersed with acquisition sessions. Approximately 63% of these sessions were test sessions, and the remainder were acquisition sessions. As noted above, participants were instructed that there would be sessions for which they would not be given any feedback concerning the accuracy of their drug-discrimination performance, and that on these sessions they would be credited with the greater number of points allocated to the DRUG A or NOT DRUG A option. Thus, these sessions were similar to the acquisition sessions except that participants did not receive any feedback concerning their drug-discrimination performance and they earned the bonus money allocated to DRUG A or NOT DRUG A, whichever was greater. Participants were not told the purpose of these "test" sessions, nor did they know when they were scheduled until after they opened the sealed envelope.

To ensure that participants continued to reliably discriminate 15 mg *d*-amphetamine throughout the test phase, acquisition sessions were intermixed among the test sessions. These acquisition sessions were identical to those in the acquisition phase (i.e., participants received 15 mg *d*-amphetamine or placebo, completed the drug-discrimination task periodically for five hours after drug administration, were informed whether they had received DRUG A or NOT DRUG A and received bonus money contingent upon the accuracy of their drug-discrimination performance). If a participant responded incorrectly on an acquisition day (i.e., $\leq$80% correct), additional acquisition sessions were scheduled. These additional acquisition sessions continued until the participant correctly identified both conditions once (i.e., 15 mg *d*-amphetamine and placebo).

Ten *d*-amphetamine–oxazepam conditions were studied during the test phase: 1) 0 mg *d*-amphetamine plus 0 mg oxazepam; 2) 2.5 mg *d*-amphetamine plus 0 mg oxazepam; 3) 5 mg *d*-amphetamine plus 0 mg oxazepam; 4) 10 mg *d*-amphetamine plus 0 mg oxazepam; 5) 15 mg *d*-amphetamine plus 0 mg oxazepam; 6) 0 mg *d*-amphetamine plus 20 mg oxazepam; 7) 2.5 *d*-amphetamine plus 20 mg oxazepam; 8) 5 mg *d*-amphetamine plus 20 mg oxazepam; 9) 10 *d*-amphetamine plus 20 mg oxazepam; and 10) 15 mg *d*-amphetamine plus 20 mg oxazepam. The order of drug administration during this phase of the experiment was random with the exception that an active drug dose was never administered on more than three consecutive sessions.

2.4. Drug-discrimination measure

A point-distribution drug-discrimination task (Rush et al., 2003, 2004) was completed 1, 2, 3, 4 and 5 h after oral drug administration on an Apple Macintosh computer (Apple Computer, Inc., Cupertino, CA). In this procedure, the participant distributed 100 points between two options (i.e., DRUG A or NOT DRUG A). During acquisition sessions, points accumulated on the correct option were exchangeable for money at a rate of $0.08/point. During test sessions, participants were credited with the greater number of points allocated to the DRUG A or NOT DRUG A option, which were exchangeable at the same rate. Thus, participants were able to earn a maximum of $40.00/session on this task. The dependent measure in this procedure was percent *d*-amphetamine-appropriate responding.

2.5. Self-report questionnaires, performance task, cardiovascular measures

Self-reported drug–effect questionnaires were administered on an Apple Macintosh computer and were completed in fixed order. These questionnaires were completed approximately 30 min before drug administration, and 1, 2, 3, 4 and 5 h after drug administration.

2.5.1. Addiction Research Center Inventory (ARCI)

The short form of the ARCI consisted of 49 true/false questions and contained five major subscales: the morphine–benzedrine group (MBG; a measure of euphoria), the pentobarbital, chlorpromazine, alcohol group (PCAG; a measure of sedation), the lysergic acid diethylamide (LSD; a measure of dysphoria) and the benzedrine group and amphetamine scales (BG and A, respectively; stimulant-sensitive scales) (Jasinski, 1977; Martin et al., 1971).

2.5.2. Adjective-Rating Scale

The Adjective-Rating Scale consisted of 32 items and contained two subscales: Sedative and Stimulant (Oliveto et al., 1992). The Adjective-Rating Scale was comprised of the following items: Active; Agitated; Clumsy; Alert; Dizzy; Confused; Energetic; Good Mood; Dazed; Excited; Sleepy; Depressed; Euphoric; Irregular Heartbeat; Difficulty Walking; Talkative; Muscles Twitching; Drowsy; Nausea; Drunk; Nervous; Fatigued; Heart Racing; Irritable; Restless; Lazy; Shaky; Relaxed; Tired; Sluggish; Sweaty; and Spaced Out. Participants rated each item using the computer mouse to point to and select among one of five response options: Not at All, A Little Bit, Moderately, Quite a Bit and Extremely (scored numerically from 0 to 4, respectively).

2.5.3. Drug–Effect Questionnaire

The Drug–Effect Questionnaire consisted of 20 items, including: Any Effect; Bad Effects; Good Effects; High; Rush; Like Drug; Stimulated; Performance Impaired; Performance Improved; Take Again; Pay For This Drug; Active, Alert or Energetic; Euphoric; Irregular or Racing Heartbeat; Talkative or Friendly; Nauseated, Queasy or Sick to Stomach; Shaky or Jittery; Nervous or Anxious; Restless; and Sluggish, Fatigued or Lazy. Each item was presented on the video screen, one at a time. Participants rated each adjective with a 5-point scale similar to the one described above.

2.5.4. Stimulant-Sensitive Adjective-Rating Scale

The Stimulant-Sensitive Adjective-Rating Scale consisted of 21 items (Di Marino et al., 1998), including: Crave Cocaine; Dizzy or Lightheaded; Drug Effect; Dry Mouth; Excited; Fearful; Powerful; Feel a Thrill; Fidgety; Seeing or Hearing Anything Unusual; Irritable; Jittery; Nauseous; Nervous; Sleepy; Stimulated; Suspicious; Sweaty; Thirsty; Tingling;

and Tremor. Participants rated each item using a 5-point scale identical to the one described above. Note that the item Sleepy is reverse scored. Responses to individual items are summed to create a composite score, with a maximum total score of 84.

2.5.5. Digit-Symbol-Substitution Test (DSST)

A computerized version of the DSST, which has been described previously, was used in this experiment (McLeod et al., 1982). Briefly, participants used a numeric keypad to enter a geometric pattern associated with one of nine digits displayed on a video screen. Participants had 90 s to enter as many geometric patterns as possible. The dependent measure was the number of patterns the participant entered correctly (i.e., trials correct).

2.5.6. Heart rate and blood pressure

Heart rate and blood pressure were recorded using an automated blood-pressure monitor (DINAMAP, Johnson and Johnson, Alexandria, TX). Heart rate and blood pressure were monitored for approximately 30 min before drug administration and at hourly intervals for five hours afterwards. Heart rate and blood pressure were recorded immediately before participants completed the drug-discrimination, self-reported drug–effect questionnaires and performance task.

2.6. Drug administration

d-Amphetamine doses were prepared by over-encapsulating 2.5 or 5 mg of the commercially available generic formulation (Barr Laboratories, Inc., Pomona, NY) in a size 0 blue/white capsule. Oxazepam doses were prepared by over-encapsulating 20 mg of the commercially available generic formulation (IVAX Pharmaceuticals, Inc., Miami, FL) in identical capsules. Corn starch was used to fill the remainder of all the capsules. Placebo capsules contained only corn starch.

During each experimental session participants ingested four capsules (i.e., three *d*-amphetamine- or placebo-containing capsules, and one oxazepam or placebo-containing capsule). Administering the appropriate number of drug- or placebo-containing capsules varied dose. Capsules were taken orally with approximately 150 ml of water. Drug administration procedures were designed to ensure that participants swallowed the capsules. To accomplish this, the research assistant: a) watched the participant to ensure that he/she swallowed the capsules and did not remove them from his/her mouth, b) conducted a brief oral examination to ensure that the participant was not hiding the capsules under his/her tongue, and c) spoke with the participant to determine if he/she had anything in his/her mouth.

d-Amphetamine doses were based on the results from previous human behavioral pharmacology research (Kollins and Rush, 1999; Rush et al., 1998, 2003, 2004). The active oxazepam dose was chosen based on two considerations. First, a pilot study was conducted to determine the dose of oxazepam at which sedative-like effects and impairment of psychomotor performance occurred. The 20 mg dose of oxazepam appeared to be the threshold dose at which sedation and performance impairment occurred under those conditions (data not shown). Second, the potency relationship between oxazepam and alprazolam was calculated based on the clinical efficacy of these drugs to determine the dose of oxazepam that was comparable to the dose of alprazolam (0.5 mg) used in our prior study (Rimon et al., 1991; Rush et al., 2004; Vaisanen and Jalkanen, 1987). The behavioral effects of *d*-amphetamine peak approximately two to three hours after oral administration (Chait et al., 1985, Rush et al., 1998). Peak oxazepam plasma concentrations occur approximately three to four hours after oral administration (Greenblatt et al., 1980), but in the pilot study noted above, the behavioral effects of oxazepam peaked approximately two to three hours after administration (Lile et al., unpublished observations). Therefore, *d*-amphetamine and oxazepam were administered simultaneously. In addition, both drugs were administered together to avoid any potential confusion that may have resulted from asking the volunteers to discriminate the effects of only one of two sets of capsules that they would have received if the drug administration had been staggered.

References below to placebo pertain to sessions in which placebo doses of both *d*-amphetamine and oxazepam were administered. References to *d*-amphetamine alone pertain to sessions in which an active dose of *d*-amphetamine was administered in combination with 0 mg oxazepam. References to oxazepam alone pertain to sessions in which the active dose of oxazepam was administered in combination with 0 mg *d*-amphetamine.

2.7. Data analysis

Statistical analyses of group data were conducted to examine drug effects on the drug-discrimination task, self-reported drug–effect questionnaires and performance measure. Effects were considered significant for $p \leq 0.05$. For the 15 mg *d*-amphet-

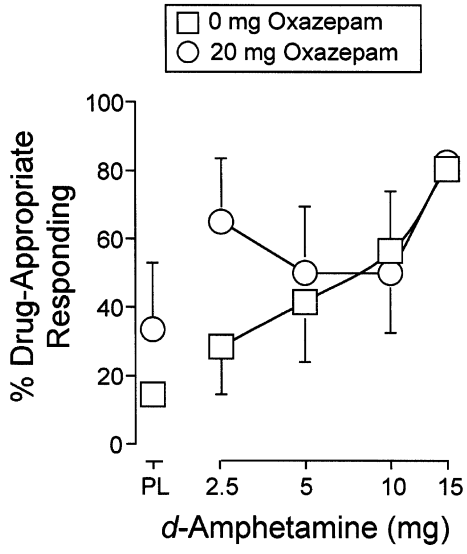

Fig. 1. Percent drug-appropriate appropriate responding maintained by *d*-amphetamine alone, oxazepam alone, *d*-amphetamine–oxazepam combinations and placebo. *X*-axes: *d*-Amphetamine dose. Data points above PL represent values when the doses of oxazepam were administered in combination with 0 mg *d*-amphetamine. Data points above 2.5, 5, 10 and 15 represent the effects of the *d*-amphetamine dose administered in combination with 0 mg (squares) or 20 mg (circles) oxazepam. Data points show means of 6 participants. Unidirectional brackets indicate one S.E.M.

amine alone and placebo conditions, data were averaged across the four sessions of the acquisition phase in which the participant met the discrimination criterion as well as all exposures to these conditions in the test phase. Drug-discrimination data were analyzed statistically as the total percent of points allocated to the drug option across the five-hour session (i.e., percent drug-appropriate responding). Self-reported drug–effect questionnaire and performance data were analyzed statistically as peak effect (i.e., maximum or minimum effect across the 5 h session). Data were analyzed by two-factor repeated-measures analysis of variance (ANOVA) with *d*-amphetamine (0, 2.5, 5, 10 and 15 mg) and oxazepam (0 and 20 mg) as factors (StatView 5.0.1, SAS Institute Inc., Cary, NC). All statistically significant main effects of *d*-amphetamine and oxazepam, or significant interaction of these factors, are reported for each measure.

3. Results

3.1. Drug-discrimination performance

The six participants met the discrimination criterion in an average of 6.2 sessions (range=4–9). ANOVA revealed a significant main effect of *d*-amphetamine on the percentage of *d*-amphetamine-appropriate responding ($F_{4,20}=6.2$, $p<0.01$). *d*-Amphetamine increased drug-appropriate responding as a function of dose regardless of oxazepam administration (Fig. 1).

3.2. ARCI

There were no statistically significant effects of *d*-amphetamine or oxazepam on any of the scales of the ARCI.

3.3. Adjective-Rating Scale

ANOVA revealed a significant main effect of *d*-amphetamine on the Stimulant subscale of the Adjective-Rating Scale ($F_{4,20}=4.6$, $p<0.01$). *d*-Amphetamine increased ratings on this scale as a function of dose regardless of oxazepam administration (data not shown). ANOVA also revealed a significant main effect of oxazepam on the Sedative subscale of the Adjective-Rating Scale ($F_{1,5}=10.5$, $p<0.05$). The active oxazepam increased ratings on this scale compared to placebo (data not shown).

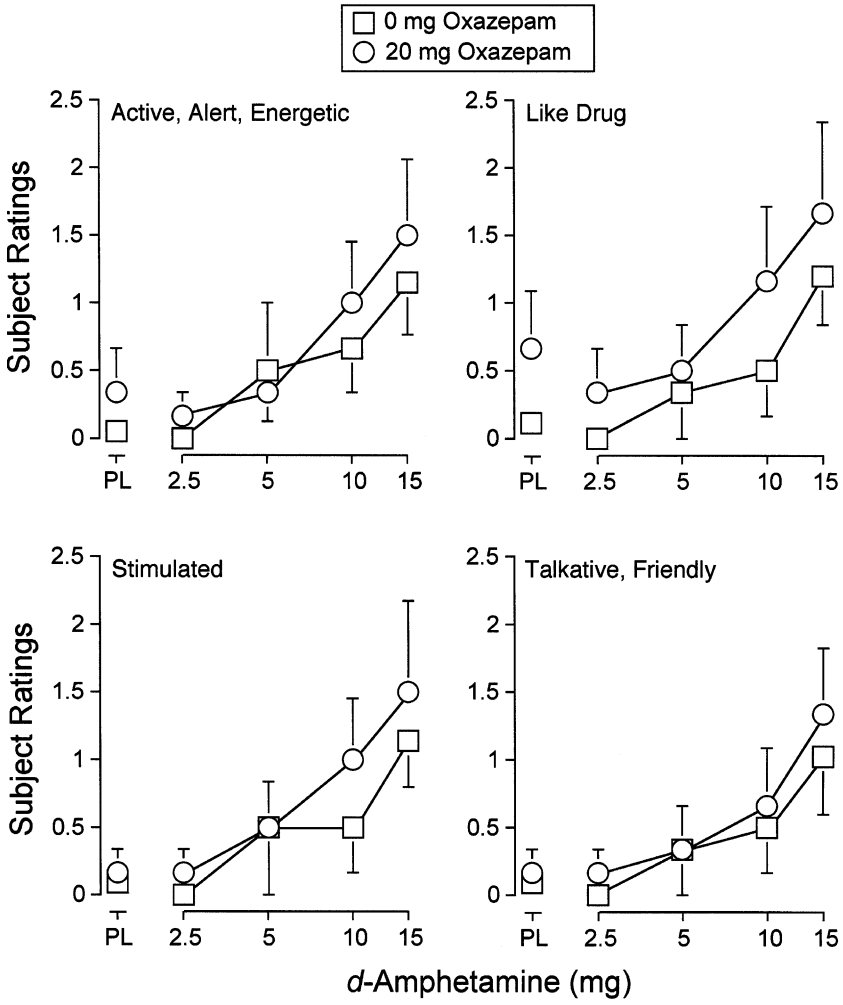

Fig. 2. Effects of *d*-amphetamine alone, oxazepam alone, *d*-amphetamine–oxazepam combinations and placebo on peak ratings of Active, Alert, Energetic; Like Drug; Stimulated; and Talkative, Friendly from the Drug–Effect Questionnaire. All other details are as in Fig. 1.

3.4. Stimulant-Sensitive Adjective-Rating Scale

ANOVA revealed a significant main effect of *d*-amphetamine on the composite score of the Stimulant-Sensitive Adjective-Rating Scale ($F_{4,20}=4.7$, $p<0.01$). *d*-Amphetamine increased ratings on this scale as a function of dose regardless of oxazepam administration (data not shown).

3.5. Drug–Effect Questionnaire

ANOVA revealed a significant main effect of *d*-amphetamine on nine items of the Drug–Effect Questionnaire: Active, Alert, Energetic; Any Effect; Good Effects; Like Drug; Performance Improved; Restless; Rush; Stimulated; and Talkative, Friendly ($F_{4,20}>3.4$, $p<0.05$). *d*-Amphetamine increased ratings on these items as a function of dose regardless of oxazepam administration. Fig. 2 shows the effects of *d*-amphetamine alone, and in combination with oxazepam, for four of these items: Active, Alert, Energetic; Like Drug; Stimulated; and Talkative, Friendly. ANOVA also revealed a significant main effect of oxazepam on ratings of Performance Impaired; and Sluggish, Fatigued and Lazy from the Drug–Effect Questionnaire ($F_{1,5}=>3.7$, $p<0.05$). The active oxazepam dose increased ratings on these items compared to placebo (data not shown).

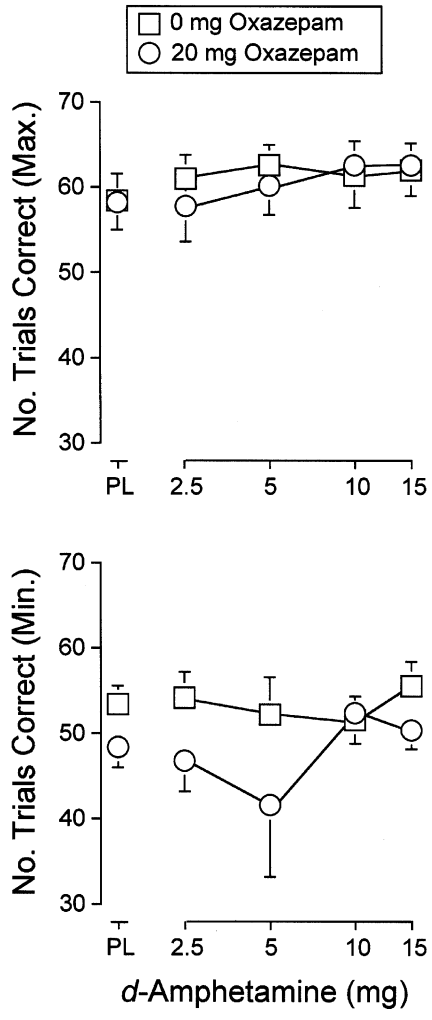

Fig. 3. Effects of *d*-amphetamine alone, oxazepam alone, *d*-amphetamine–oxazepam combinations and placebo on the maximum (top panel) and minimum (bottom panel) number of trials correct on the DSST. All other details are as in Fig. 1.

3.6. DSST

ANOVA revealed a significant main effect of *d*-amphetamine ($F_{4,20}=6.7$, $p=0.001$) on the maximum number of trials correct on the DSST (Fig. 3). In addition, a trend towards a significant main effect of oxazepam was found ($F_{1,5}=5.6$, $p=0.06$) on the minimum number of trials correct on the DSST (Fig. 3).

3.7. Heart rate and blood pressure

ANOVA revealed significant main effects of *d*-amphetamine ($F_{4,20}=2.9$, $p=0.05$) and oxazepam ($F_{1,5}=7.2$, $p<0.05$) on systolic pressure. *d*-Amphetamine increased systolic pressure as a general function of dose and oxazepam appeared to have attenuated this effect (data not shown). A significant interaction between *d*-amphetamine and oxazepam was found for heart rate ($F_{4,20}=5.8$, $p<0.01$). *d*-Amphetamine increased heart rate as a general function of dose and oxazepam appeared to have attenuated this effect (data not shown). Finally, ANOVA revealed a trend towards a main effect of *d*-amphetamine on diastolic pressure ($F_{4,20}=2.6$, $p=0.06$).

4. Discussion

In the present experiment, the discriminative-stimulus, self-reported and performance effects of *d*-amphetamine were assessed alone and in combination with acutely administered oxazepam in women and men who had learned to discriminate *d*-amphetamine. *d*-Amphetamine alone functioned as a discriminative stimulus and dose dependently increased drug-appropriate responding. *d*-Amphetamine alone also produced stimulant-like self-reported drug effects (e.g., increased ratings of Alert, Active and Energetic on the Drug–Effect Questionnaire) and enhanced psychomotor performance, generally in a dose-dependent manner. These findings are concordant with the results of several previous reports in which the discriminative-stimulus, self-reported and psychomotor performance effects of *d*-amphetamine were assessed in humans (e.g., Heishman and Henningfield, 1991; Kollins and Rush, 1999; Lamb and Henningfield, 1994; Rush et al., 1998, 2003, 2004).

Oxazepam alone did not engender *d*-amphetamine-appropriate responding, consistent with previous drug-discrimination experiments in which benzodiazepines did not occasion significant levels of drug-appropriate responding in laboratory animals and humans trained to discriminate a stimulant (Druhan et al., 1991; Negus et al., 2000; Rush and Baker, 2001; Rush et al., 1998, 2002; Stoops et al., 2005). Oxazepam administration did, however, result in increased subject ratings of sedation (i.e., scores on the Sedative subscale of the Adjective-Rating Scale and ratings on the item Sluggish,

Fatigued and Lazy from the Drug–Effect Questionnaire), consistent with the well-documented sedative–hypnotic effects of benzodiazepines. These data indicate that oxazepam produced a discernable interoceptive cue, but that this cue was dissimilar to that produced by *d*-amphetamine. These results provide further support for the pharmacological specificity of the drug-discrimination procedure.

The active dose of oxazepam (20 mg) also appeared to impair performance on a computerized version of the DSST. In agreement with the decrements in performance on the DSST, oxazepam increased subject ratings on the Performance Impaired item from the Drug–Effect Questionnnaire, indicating that participants perceived their impairment in performance on the DSST. These findings are consistent with previous research that assessed the effects of oxazepam on psychomotor performance measures, including the DSST (Griffiths et al., 1984b). In that study, a dose range of 30–480 mg oxazepam was tested, and doses above 60 mg appeared to decrease the number of correct trials completed on the DSST. Because the participants in the study by Griffiths and colleagues had a history of sedative use, tolerance to the effects of benzodiazepines may explain potency differences between that study and the present study in the ability of oxazepam to impair performance.

Oxazepam, administered alone and in combination with *d*-amphetamine, was well tolerated and no adverse events occurred. Acute oxazepam was ineffective at modulating the discriminative-stimulus or subject-rated effects of *d*-amphetamine. While there appeared to be an enhancement of the effects of the lowest dose of *d*-amphetamine when administered in combination with oxazepam on the drug-discrimination task (Fig. 1) and the item Any Effect from the Drug–Effect Questionnaire (data not shown), a statistically significant interaction between these factors was not revealed. One limitation of the present study is that only a single dose of oxazepam was tested. A higher dose of oxazepam may have been required to impact the discriminative-stimulus and subject-rated effects of *d*-amphetamine, although the use of a higher dose may have resulted in a functional, as opposed to a receptor-mediated, attenuation of these behavioral effects. For example, a significant main effect of oxazepam was detected for the number of correct trials completed on the DSST. Oxazepam appeared to attenuate the elevations in psychomotor performance resulting from *d*-amphetamine administration, but also decreased the number of correct trials completed when given alone. Ideally, a pharmacotherapy should antagonize the behavioral effects of the stimulant, but be relatively devoid of effects itself. Because of the potential for more intense sedative-like side effects, higher doses of oxazepam would likely be unacceptable to stimulant-dependent patients.

As described above, in a previous study from our laboratory, the benzodiazepine alprazolam significantly attenuated the discriminative-stimulus and some of the positive subject-rated effects of *d*-amphetamine in humans (Rush et al., 2004). Those results are consistent with other studies that have demonstrated that the effects of stimulants are attenuated by benzodiazepines under a variety of behavioral arrangements (Druhan et al., 1991; Goeders and Goeders, 2004; Goeders et al., 1989, 1993; Leri and Franklin, 2000; Meririnne et al., 1999; Negus et al., 2000). Worth noting, however, is that not all studies that have attempted to block the behavioral effects of a stimulant with a benzodiazepine have been successful. In another study from our laboratory, we evaluated the ability of triazolam to attenuate the subject-rated effects of cocaine (Haga et al., 2003). In that study, cocaine produced prototypical stimulant-like effects in participants with a history of cocaine use as assessed using a battery of self-report questionnaires. Triazolam alone increased subject ratings associated with sedation, but did not modify the subject-rated effects of cocaine when given in combination. These data suggest that benzodiazepines differ in their ability to modulate the behavioral effects of stimulants.

One potential explanation for the discrepancies between the present study and our previous study with alprazolam is that the mechanism of action for these drugs differs in some way. The $GABA_A$ receptor is an oligomeric protein complex that consists of five subunits. These subunits belong to one of seven families, each with multiple isoforms, but most mammalian CNS $GABA_A$ receptors are thought to be typically composed of isoforms from the α, β and γ families. The configuration of these isoforms determines the sensitivity of the receptor to ligands at the benzodiazepine binding site (reviewed in Möhler et al., 2002). In addition, the various isoforms, particularly those of the α family, appear to be linked to the different physiological and behavioral effects of benzodiazepines (reviewed in Möhler et al., 2002). While speculative, perhaps oxazepam and alprazolam bind to distinct populations of $GABA_A$ receptors, which could confer the ability to modulate the behavioral effects of *d*-amphetamine to alprazolam only. Another possibility is that one or both of these drugs has a significant affinity for targets from neurotransmitter systems other than GABA that could be influencing their behavioral effects.

One caveat of this study is the small number of participants that were enrolled. However, it is important to note that a sample size of six participants is consistent with prior drug-discrimination studies in humans in which drug pretreatments or combinations were administered in an attempt to modify the discriminative-stimulus effects of a stimulant (e.g., Hart et al., 2002; Oliveto et al., 1997; Perkins et al., 1999; Rush et al., 2004). In our previous study with alprazolam, six participants were also enrolled and nearly identical experimental procedures were used (Rush et al., 2004). In that study, a significant attenuation of the discriminative-stimulus and some of the positive subject-rated effects of the higher doses of *d*-amphetamine was observed. Similarly, in six participants who had learned to discriminate caffeine, triazolam shifted the dose–effect curve for percent drug-appropriate responding rightward (Oliveto et al., 1997). In another study, the cholinergic antagonist mecamylamine attenuated the discriminative-stimulus effects and some of the subject-rated effects of nicotine in the same number of participants (Perkins et al., 1999). The positive results from those studies suggest that this sample size provides sufficient power to detect antagonism of the behavioral effects of a stimulant.

One possible means by which a putative medication for stimulant dependence could produce a therapeutic effect is by blocking the acute effects of the stimulant, which may result in extinction of drug-seeking and drug-taking behaviors over time. Oxazepam did not attenuate the abuse-related behavioral effects of *d*-amphetamine, and therefore may not be useful as a pharmacotherapy for stimulant dependence in this manner. Other human laboratory studies have also found that GABAergic drugs are not effective, or only partially effective, at modifying the acute behavioral effects of stimulants (Haga et al., 2003; Hart et al., 2004; Lile et al., 2004a,b). For example, the $GABA_B$ agonist baclofen did not block the reinforcing or subject-rated effects of intranasal cocaine in participants with a history of cocaine use (Lile et al., 2004a). Likewise, the GABA reuptake inhibitor tiagabine did not block the discriminative-stimulus, reinforcing or subject-rated effects of oral cocaine in cocaine users (Lile et al., 2004b). Worth noting is that, with the exception of the study by Hart and colleagues (2004), the GABAergic drugs were administered acutely in the present and previous studies. That these drugs were administered acutely is a limitation of these laboratory studies, as chronic dosing will likely be required for the treatment of stimulant dependence. Maintenance on baclofen or tiagabine has shown initial promise in controlled clinical trials at decreasing cocaine-positive urines in dependent patients (Gonzalez et al., 2003; Shoptaw et al., 2003; Winhusen et al., 2005). Together, these findings suggest that, while GABAergic drugs may have utility as pharamcotherapies for stimulant dependence, these drugs may not produce their therapeutic effects by blocking the acute effects of stimulants and will likely require chronic administration for their efficacy to become apparent (see Lile et al., 2004a,b for further discussion). Future laboratory research should therefore adopt different strategies for evaluating GABAergic potential pharmacotherapies, such as examining the ability of chronic administration of GABAergic drugs to prevent the reinitiation of drug use (i.e., relapse).

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