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TITLE PAGE

METABOLIC TRANSFORMATION PLAYS A PRIMARY ROLE IN THE PSYCHOSTIMULANT-LIKE DISCRIMINATIVE-STIMULUS EFFECTS OF SELEGILINE ((R)-(-)-DEPRENYL)

Sevil Yasar, Zuzana Justinova, Sun-Hee Lee, Roman Stefanski, Steven R. Goldberg and Gianluigi Tanda

Division of Geriatric Medicine and Gerontology, Johns Hopkins University School of Medicine, Baltimore, MD 21224 (SY), Preclinical Pharmacology Section, Behavioral Neuroscience Research Branch (ZJ, RS, SHL, SRG) and Psychobiology Section, Medications Development Research Branch (GT), Intramural Research Program, National Institute on Drug Abuse, National Institutes of Health, DHHS, Baltimore, MD 21224

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b) Corresponding Author:
Sevil Yasar, M.D.
Johns Hopkins University School of Medicine
5505 Johns Hopkins Bayview Circle
Baltimore, MD 21224
Phone : (410) 550-2668
Fax : (410) 550-2513
E-mail: syasar@jhmi.edu

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ABSTRACT

l-Deprenyl (selegiline, (R)-(-)-deprenyl) is a selective inhibitor of monoamine-oxidase B (MAO-B) used in the treatment of Parkinson's disease and proposed as an antidepressant and an aid for cigarette-smoking cessation and treatment of psychostimulant abuse. Beneficial therapeutic effects of (R)-(-)-deprenyl may also result from indirect actions. Brain levels of dopamine and ß-phenylethylamine (ß-PEA), a behaviorally-active endogenous trace amine, increase after (R)-(-)-deprenyl treatment due to MAO-B blockade and (R)-(-)-deprenyl is metabolized to (R)-(-)-methamphetamine and (R)-(-)-amphetamine, suggesting that (R)-(-)-deprenyl may have psychostimulant-like behavioral effects. Indeed, (R)-(-)-deprenyl produces psychostimulant-like discriminative-stimulus effects in experimental animals. Here we tested the hypothesis that psychostimulant-like behavioral effects of (R)-(-)-deprenyl are mainly mediated by its metabolites. Male Fisher F344 rats were trained to discriminate intra-peritoneal (i.p.) injection of 1.0 mg/kg (S)-(+)-methamphetamine or 10.0 mg/kg cocaine from injection of saline using two-lever choice schedules of food delivery or stimulus-shock termination. When (R)-(-)-deprenyl was tested by substitution, it had (S)-(+)-methamphetamine- and cocaine-like discriminative-stimulus effects, but only at doses of 10-30 mg/kg, doses 10- to 20-times higher than those selective for MAO-B inhibition. Ro 16-1649, a selective inhibitor of MAO-B enzyme activity without psychoactive metabolites, had no psychostimulant-like discriminative effects. Also, blockade of (R)-(-)-deprenyl's metabolism with SKF 525A (50 mg/kg, i.p.) reduced or eliminated (R)-(-)-deprenyl's psychostimulant-like discriminative effects. When ß-PEA synthesis was blocked by NSD 1015 (30 mg/kg, i.p.), there was a modest reversal of (R)-(-)-deprenyl's psychostimulant-like discriminative effects under some conditions, indicating a facilitatory

modulation of the psychostimulant-like discriminative effects of (R)-(-)-deprenyl metabolites by elevated levels of ß-PEA under certain conditions.

INTRODUCTION

l-Deprenyl (selegiline, (R)-(-)-deprenyl), a selective inhibitor of monoamine-oxidase, type-B enzyme (MAO-B), is best known for its proven clinical efficacy in the treatment of Parkinson's disease (PD), but it has been clinically evaluated for treatment of Alzheimer's disease (Sano et al., 1997; Tariot et al., 1998) and as a transdermal patch for treatment of major depressive disorders (Amsterdam, 2003). (R)-(-)-deprenyl has also been proposed as a cognitive enhancer (Gelowitz et al., 1994), as a treatment for attention deficit hyperactivity disorder (Akhondzadeh et al., 2003), as a treatment for drug dependence (Houtsmuller et al., 2004)(Grasing and Ghosh, 1998) and as an aid for smoking cessation (George et al., 2003).

Blockade of MAO-B enzyme activity by MAO-B selective doses of (R)-(-)-deprenyl (0.5 to 4.0 mg/kg, i.p., in rats; Paterson et al., 1991) can alter circulating and brain levels of catecholamines like dopamine and norepinephrine, and of their metabolites (Okuda et al., 1992), potentiate neuronal responses to dopamine agonists (Paterson et al., 1991) and reduce the formation of highly reactive oxygen species (Ebadi et al., 2002; Magyar et al., 2004) which may lead to cell damage and/or cell death. These actions might contribute to (R)-(-)-deprenyl's beneficial therapeutic effects. (R)-(-)-deprenyl treatment alone, in the absence of *l*-DOPA treatment, slows the progression of PD indicating neuroprotective effects in humans (see Magyar and Haberle, 1999, for review). These effects are consistent with numerous studies demonstrating neuroprotective effects of (R)-(-)-deprenyl in *in-vitro* models of neurotoxicity in cell lines (Ebadi et al., 2002; Magyar and Szende, 2004), which do not appear to be related to its ability to bind to and inactivate the MAO-B enzyme (Magyar and Haberle, 1999; Ebadi et al., 2002).

Among the many psychoactive substances thought to play a role in the effects of (R)-(-)-deprenyl, the most important are ß-phenylethylamine (ß-PEA), brain levels of which increase dramatically after (R)-(-)-deprenyl treatment because of MAO-B blockade (Paterson et al., 1991; Paterson et al., 1995), and (R)-(-)-methamphetamine and (R)-(-)-amphetamine, which are two of the main metabolites of (R)-(-)-deprenyl (Heinonen et al., 1994; Lajtha et al., 1996). (R)-(-)-Methamphetamine, (R)-(-)-amphetamine, and ß-PEA have distinct pharmacological properties, including potentially undesirable effects, such as the ability to reinforce drug-seeking behavior in animal models of drug abuse (Yokel and Pickens, 1973; Shannon and Degregorio, 1982; Winger et al., 1994; Sannerud et al., 1996; Bergman et al., 2001).

However, (R)-(-)-deprenyl has not been reported to induce any drug-dependence syndrome related to its non-medical, recreational, use or abuse. Nonetheless, the increased levels of psychostimulant-like substances produced after its administration has been viewed as potentially harmful, and the abuse liability of (R)-(-)-deprenyl has been a matter of debate (Yasar et al., 1996; Wu and Zhu, 1999; Yasar et al., 2005a).

Although (R)-(-)-deprenyl is not self-administered above vehicle placebo levels by monkeys (Winger et al., 1994; Yasar et al., 2005b) under fixed-ratio or second-order schedules of intravenous drug injection, it can potentiate the reinforcing effects of intravenously administered ß-PEA (Bergman et al., 2001) and can induce conditioned place preferences in rats (Wu and Zhu, 1999). In addition, at high non-therapeutic doses, (R)-(-)-deprenyl can substitute for (S)-(+)-amphetamine, (S)-(+)-methamphetamine and cocaine in rats and monkeys when studied using drug-discrimination procedures (Yasar et al., 1993; Yasar and Bergman, 1994; Yasar et al.,

1994). The purpose of the present study was to test the hypothesis that the cocaine- and (S)-(+)-methamphetamine-like discriminative-stimulus effects of (R)-(-)-deprenyl are the result of active (R)-(-)-deprenyl metabolites and not the result of MAO-B blockade or increased levels of endogenous psychoactive compounds, such as ß-PEA, that are neurochemical substrates for the MAO-B enzyme, or of other non-MAO-B actions of (R)-(-)-deprenyl itself.

We first compared the discriminative-stimulus effects of (R)-(-)-deprenyl with those of Ro 16-6491, a selective and reversible inhibitor of MAO-B enzyme activity without psychoactive metabolites (Da Prada et al., 1987). Stimulus-shock termination and food-presentation schedules were used in rats trained to discriminate (S)-(+)-methamphetamine or cocaine from a saline placebo in order to allow direct comparisons with previous studies. The metabolic enzyme inhibitor SKF 525A was then tested in combination with (R)-(-)-deprenyl, to determine whether blockade of the CYP-450 enzyme, which converts (R)-(-)-deprenyl to (R)-(-)-methamphetamine and (R)-(-)-amphetamine metabolites (Yoshida et al., 1987), reduces the ability of (R)-(-)-deprenyl to produce (S)-(+)-methamphetamine - or cocaine-like discriminative effects. In addition, (R)-(-)-deprenyl was tested in combination with NSD 1015, a dopamine-decarboxylase enzyme inhibitor (Carlsson et al., 1972), to determine whether blockade of the accumulation of ß-PEA, a substrate for the MAO-B enzyme produced by decarboxylation of the amino acid *l*-phenylalanine, reduced the ability of (R)-(-)-deprenyl to produce psychostimulant-like discriminative-stimulus effects.

METHODS

Subjects. Fifteen male Fisher F344 rats (Harlan Sprague Dawley, Inc., Indianapolis, IN), weighing 275 to 325 g at the start of the experiments, were trained under a stimulus-shock termination schedule. Between experimental sessions, rats were housed in groups of three per cage with free access to water and food. Sixteen additional male Fisher F344 rats, weighing around 350 g at the start of the experiments, were trained under a food-presentation schedule and were housed individually with free access to water. These rats had food availability restricted such that their weights were maintained at approximately 80% of initial free- feeding weights throughout the experiments. All rats were housed in a temperature- and humidity-controlled room that was illuminated from 6:00 a.m. to 6:00 p.m. and experiments were conducted during the light phase. The rats were experimentally naive at the start of this study.

Animals used in this study were maintained in facilities fully accredited by the American Association for the Accreditation of Laboratory Animal Care (AAALAC) and all experimentation was conducted in accordance with the guidelines of the Institutional Care and Use Committee of the Intramural Research Program, NIDA, NIH, and the Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research (National Research Council 2003).

Apparatus. For the stimulus-shock termination procedure, six identical operant-conditioning chambers, enclosed in light- and sound- attenuating, fan-ventilated enclosures (model 1101-L, Grason-Stadler, Bolton, MA) were used. Each chamber was equipped with two response levers separated by a clear Plexiglas partition which extended 5 cm into the cage, a white house light

and a red light. Scrambled electric shocks could be delivered to the grid floor of the chamber by a constant-current electric shock generator (model 700, Grason-Stadler). Six additional two-lever, operant-conditioning chambers (model no. E10-10, Coulbourn Instruments, Allentown, PA) were used for the food-presentation procedure, in which the levers were separated by a recessed tray into which a pellet dispenser could deliver 45-mg food pellets (F0021, Bio- Serv, Frenchtown, NJ). The control of experimental contingencies and data collection were accomplished with an IBM PC compatible computer using MED-PC software and an associated interface (Med Associates, East Fairfield, VT).

Discrimination performance with (S)-(+)-methamphetamine as the training drug.

Both stimulus-shock termination and food-presentation schedules were used in order to allow direct comparisons with previous studies (Yasar et al., 1993; 1994). The food-presentation baseline provided a measure of behaviorally active disruptive effects that can appear as dose of test drugs are increased, while the stimulus-shock termination baseline allowed testing of higher doses of drugs, which might otherwise eliminate behavior and preclude measurement of discriminative effects. Rats were trained under a 5-response fixed-ratio (FR5) schedule of stimulus-shock termination or under a 10-response fixed-ratio (FR10) schedule of food-pellet delivery to respond on one lever after an injection of a training dose of 1.0 mg/kg of (S)-(+)-methamphetamine and on the other lever after either an injection of 1.0 mg/ml of saline vehicle or no injection (frequent no-injection conditions were interspersed with infrequent saline injections to minimize frequency of injection during training). Injections of methamphetamine or saline were given i.p. 15 min before the start of the session. Under the schedule of stimulus-shock termination, a white house light was turned on at the start of the session; in the presence of

the white light an electric shock (approximately 1.0 mA) was delivered through the grid floor in 1.0-sec pulses every 4.0-sec (4-sec intershock interval). Completion of 5 consecutive responses on the lever appropriate to the pre-session treatment terminated the white house light and shock presentations and initiated a 45-sec timeout during which lever-pressing responses had no specified consequences and a dim red light was present. Under the schedule of food presentation, the completion of 10 consecutive responses on the lever appropriate to the pre-session treatment produced delivery of a 45-mg food pellet and initiated a 45-sec timeout during which lever-pressing responses had no specified consequences and the chamber was dark. Under both the stimulus-shock termination and the food-presentation procedures, responses on the incorrect lever had no programmed consequences other than to reset the response requirement to 5 or 10 on the correct lever. After each timeout the next trial began. Each session ended after completion of 20 trials or after 30 min, whichever occurred first.

Discrimination-training sessions were initially conducted 5 days per week under a double alternation schedule *(i.e.*, D, D, N, N, D, D, etc.; D = drug, N = saline injection or no injection). Training continued until there were eight consecutive sessions during which rats completed at least 90% of their responses during the session on the correct lever and no more than 4 responses occurred on the incorrect lever during the first trial. Once this level of performance was maintained for at least eight consecutive sessions, an additional two sessions were conducted, one drug session and one saline vehicle session (in that order), in which 5 or 10 consecutive responses on either lever terminated the stimulus-shock sequence or delivered food; if the level of accuracy remained as high as during the previous eight sessions, testing with other doses and other drugs was initiated. An average of 70 or 81 sessions was required for rats to reach the

required levels of discrimination accuracy under the stimulus-shock termination and food-presentation schedules, respectively.

Discrimination performance with cocaine as the training drug. Although there is usually relatively symmetrical cross generalization between cocaine and amphetamine-like drugs (D'Mello and Stolerman, 1977; Colpaert et al., 1979), we previously found that high doses of (R)-(-)-deprenyl produced complete generalization to a (S)-(+)-amphetamine training stimulus but not to a cocaine training stimulus under stimulus-shock termination schedules (Yasar et al., 1993; 1994). Consequently, an additional group of rats was trained under a FR5 schedule of stimulus-shock termination to respond on one lever after an injection of a training dose of 10 mg/kg of cocaine and on the other lever after an injection of 1.0 mg/ml of saline vehicle. Training conditions were identical to those described above for (S)-(+)-methamphetamine discrimination. An average of 87 sessions was required for rats to reach the required level of discrimination accuracy under the stimulus-shock termination schedule with cocaine as the training drug.

Discrimination tests with other drugs and drug doses. When a rat met the above criterion for consistent (S)-(+)-methamphetamine or cocaine discrimination performance, test sessions were started. During test sessions, different doses of the training drugs ((S)-(+)-methamphetamine or cocaine) or a range of doses of (R)-(-)-deprenyl and Ro 16-6491 were substituted for the training drug dose. Thus, dose-effect curves were obtained for all drugs tested. Different drugs and drug doses were tested in a mixed order. During some test sessions, SKF 525A (50 mg/kg) or NSD 1015 (30 mg/kg) was injected i.p. 30 min before different doses of (R)-(-)-deprenyl in order to

explore possible drug interactions. Test sessions were identical to training sessions with the exception that five or ten consecutive responses (for shock termination or food presentation schedules, respectively) on either lever ended the trial; each test session ended, as before, after completion of 20 trials or after 30 min elapsed, whichever occurred first. When test sessions were started, a single alternation schedule was introduced and tests were conducted on Tuesdays and Fridays. Thus, a 2-week sequence starting on Monday was: D, T, N, D, T, N, T, D, N, T (T = test). In this way, test sessions occurred with equal probability after no-injection or saline-injection sessions as after drug sessions. Test sessions were conducted only if a rat had completed both previous training sessions at an accuracy level of at least 90%.

Drugs. The drugs used were (R)-(-)-deprenyl hydrochloride, *l*-deprenyl, (selegiline) (Chinoin Pharmaceutical and Chemical Works, Budapest, Hungary - now Sanofi-Synthelabo), Ro 16-6491 (N-(2-Aminoethyl)-4-chlorobenzamide hydrochloride), SKF 525A (beta-diethylaminoethyl-diphenylpropylacetate hydrochloride; proadifen), NSD 1015 (m-hydroxy-benzyl-hydrazine dihydrochloride), (S)-(+)-methamphetamine (*d*-methylamphetamine) (Research Biochemicals Inc., Natick, MA), and cocaine hydrochloride (National Institute on Drug Abuse, Rockville, MD). Drugs were dissolved in 0.9% NaCl and injected i.p. in a volume of 1.0 ml/kg. (R)-(-)-deprenyl was injected at different time intervals ranging from 0 min to 180 min before the session to establish time of peak effect, which was found to be 30 min. In the remaining experiments, (R)-(-)-deprenyl was injected 30 min before the session. Ro 16-6491 was injected 30 min before the session, and cocaine and methamphetamine were injected 15 min before the session. SKF 525A and NSD 1015 were injected 30 min before (R)-(-)-deprenyl (60 min before the start of the session). Pretreatment times for SKF 525A and NSD 1015 were based on

previous reports (Berry et al., 1994; Magyar et al., 2004). Each drug dose or combination was usually tested twice. On some no-drug sessions the saline injection was omitted; although results were similar to sessions when saline was injected, only those no-drug sessions with saline injections were used for data analysis. All drug doses and calculations are based on the salts.

Data analysis. Drug-discrimination data are expressed as the percentage of the total responses emitted on the drug-associated lever. Complete generalization is defined as 90% or more of responses on the drug-associated lever. Response-rate data are expressed as responses per second averaged over the session, with responding during timeout periods not being included in calculations. Each data point shows the group average ± S.E.M. Statistical analysis of generalization test data was done using one-way analysis of variance (ANOVA) for repeated measures. Significant main effects were analyzed further by subsequent paired comparisons with vehicle control (responding after vehicle injections in generalization tests) using post-hoc Dunnett's test. Shifts in (R)-(-)-deprenyl dose-response curves after pretreatment with SKF 525A and NSD 1015 were evaluated by using two-way ANOVA for repeated measures. Changes were considered significant when $p < 0.05$. The SigmaStat program (Jandel Scientific, USA) was used. ED_{50} values (± 95% confidence intervals) for each drug were calculated by linear interpolation of dose-effect curves to calculate the dose that produced 50% responding on the training-drug appropriate lever. Differences in ED_{50} values were considered statistically significant if the 95% confidence intervals for mean values did not overlap.

RESULTS

Drug-discrimination studies in (S)-(+)-methamphetamine-trained rats under the food-presentation schedule.

Increasing doses of (S)-(+)-methamphetamine produced dose-dependent increases in drug-lever responding, with complete generalization occurring at both the 1.0-mg/kg training dose and, also, at the higher 3.0 mg/kg dose of (S)-(+)-methamphetamine ($F(3,21)=34.82$, $p < 0.001$) which markedly depressed rates of responding (Figure 2). In one group of eight rats, a dose of 10 mg/kg (R)-(-)-deprenyl was given at times, ranging from 0 min to 180 min before the session. Peak generalization to the (S)-(+)-methamphetamine training stimulus occurred at the 30-min pretreatment time ($F(6,42)=16.32$, $p < 0.001$) but (S)-(+)-methamphetamine-like discriminative effects of the 10 mg/kg dose of (R)-(-)-deprenyl decreased markedly at shorter or longer pretreatment times and saline-like discriminative effects prevailed (Figure 1, top panel). At none of the time points did the dose of 10 mg/kg (R)-(-)-deprenyl produce significant changes in the rate of responding ($F(6,42)=1.55$, $p = 0.19$) (Figure 1, bottom panel). When different doses of (R)-(-)-deprenyl (3-30 mg/kg) were tested at the 30 min pretreatment time in another group of rats, (R)-(-)-deprenyl produced partial generalization to the (S)-(+)-methamphetamine-training stimulus at a dose of 10 mg/kg, and complete generalization only at a very high 30.0 mg/kg dose (Figure 2, top panel) ($F(3,21)=29.12$, $p < 0.001$). In contrast, Ro 16-6491 (1-100 mg/kg) did not generalize to the (S)-(+)-methamphetamine-training stimulus at any dose tested in these rats (Figure 2, top panel), producing only saline-like effects ($F(4,28)=2.33$, $p = 0.08$). Pretreatment with 50 mg/kg SKF 525A, administered 30 min before (R)-(-)-deprenyl (3-30 mg/kg), almost completely blocked, the ability of (R)-(-)-deprenyl to generalize to the (S)-(+)-methamphetamine-training stimulus (Figure 2, top panel) ($F(1,8)=7.05$, $p = 0.03$), but alone 50

mg/kg of SKF 525A produced only saline-like discriminative effects and did not alter rates of responding.

Over the range of doses studied, none of the drugs significantly affected rates of lever-press responding under the food- presentation schedule (Figure 2, bottom panel), with the exception of the high 3.0 mg/kg dose of (S)-(+)-methamphetamine that markedly depressed food-reinforced responding ($F(3,21)=11.82$, $p < 0.001$) and a 100 mg/kg dose of Ro 16-6491 that completely suppressed food-maintained responding ($F(5,35)=8.46$, $p < 0.001$).

Drug-discrimination studies in (S)-(+)-methamphetamine-trained rats under the stimulus-shock termination schedule.

Increasing doses of (S)-(+)-methamphetamine produced dose-dependent increases in drug-lever responding with complete generalization to the (S)-(+)-methamphetamine-training stimulus ($F(3,27)=23.84$, $p < 0.001$) occurring at the 1.0-mg/kg training dose of (S)-(+)-methamphetamine and at the higher dose of 3.0 mg/kg of (S)-(+)-methamphetamine (Figure 3A, top panel). As under the food- presentation schedule, (R)-(-)-deprenyl (3-30 mg/kg) produced partial generalization to the (S)-(+)-methamphetamine-training stimulus at a dose of 10 mg/kg, and complete generalization only at the very high 30.0 mg/kg dose ($F(3,27)=22.79$, $p < 0.001$). Also, Ro 16-6491 did not generalize to the (S)-(+)-methamphetamine-training stimulus at any dose tested, even when the dose was increased to 100 mg/kg (Figure 3A, top panel) ($F(4,36)=0.43$, $p = 0.786$), which completely suppressed responding under the food-presentation schedule (Figure 2, bottom panel). Finally, pretreatment with 50 mg/kg SKF 525A, administered 30 min before (R)-(-)-deprenyl (3-30 mg/kg), significantly reduced the ability of (R)-(-)-deprenyl

to generalize to the (S)-(+)-methamphetamine-training stimulus ($F(1,18)=17.52$, $p = 0.002$) under the shock termination schedule as it did under the food- presentation schedule (Figure 4A, top panel). A similar significant reduction in the ability of (R)-(-)-deprenyl to generalize to the (S)-(+)-methamphetamine-training stimulus was produced by pretreatment with 30 mg/kg NSD 1015 ($F(1,18)=19.13$, $p = 0.002$), administered 30 min before (R)-(-)-deprenyl (3-30 mg/kg) (Figure 4A, top panel). Both shifts in (R)-(-)-deprenyl dose-response curve were also significant according to non-overlapping 95% CIs of the ED_{50} values (Table 1). Over the range of doses studied, none of these drugs or drug combinations significantly affected rates of lever-press responding under the stimulus-shock termination schedule with (S)-(+)-methamphetamine as the training stimulus (Figures 3A, and 4A, bottom panels).

Drug-discrimination studies in cocaine-trained rats under the stimulus-shock termination schedule.

Increasing doses of cocaine produced dose-dependent increases in drug-lever responding with complete generalization to the cocaine-training stimulus occurring at the 10.0-mg/kg training dose of cocaine (Figure 3B, top panel) ($F(3,12)=27.60$, $p < 0.001$). (R)-(-)-deprenyl (3-30 mg/kg) produced partial generalization to the cocaine-training stimulus at a dose of 3 mg/kg, and complete generalization at both the intermediate 10.0 mg/kg and high 30.0 mg/kg doses ($F(3,12)=20.34$, $p < 0.001$). As with (S)-(+)-methamphetamine-trained rats, Ro 16-6491 did not generalize to the cocaine-training stimulus at any dose tested, even when the dose was increased to 100 mg/kg (Figure 3B, top panel) ($F(5,20)=1.48$, $p = 0.241$). Pretreatment with 50 mg/kg SKF 525A completely blocked the ability of (R)-(-)-deprenyl to generalize to the cocaine training stimulus ($F(1,8)=277.12$, $p < 0.001$) under the shock termination schedule (Figure 4B, top

panel). Pretreatment with 30 mg/kg NSD 1015 produced a partial reduction in the ability of (R)-(-)-deprenyl to generalize to the cocaine-training stimulus (Figure 4B, top panel), but the effect did not reach statistical significance as shown by two-way ANOVA for repeated measures ($F(1,8)=6.23$, $p = 0.067$) and by marginally overlapping 95% CIs of the ED_{50} values (Table 1). Over the range of doses studied, none of the drugs significantly affected rates of lever-press responding under the stimulus-shock termination schedule with cocaine as the training stimulus (Figures 3B and 4B, bottom panels).

DISCUSSION

In agreement with our previous studies, increasing doses of (R)-(-)-deprenyl produced dose-related increases in generalization to cocaine- and (S)-(+)-methamphetamine-training stimuli in rats trained to discriminate cocaine or (S)-(+)-methamphetamine injection from saline injection under schedules of food delivery or stimulus-shock termination. In all cases, the psychostimulant-like discriminative-stimulus effects of (R)-(-)-deprenyl only occurred at doses 10 to 30 fold greater than MAO-B selective doses of 0.5-4 mg/kg i.p. in rats (Paterson et al., 1991) and the MAO-B selective doses in clinical use for Parkinson's disease and in clinical trial for the treatment of Alzheimer's disease, tobacco smoking cessation and psychostimulant abuse. Thus, the present experiments extend the range of conditions under which high doses of (R)-(-)-deprenyl have clear and pronounced psychostimulant-like discriminative-stimulus effects.

Since (R)-(-)-deprenyl is a selective blocker of the MAO-B enzyme, endogenous substrates of this enzyme, such as ß-PEA, might accumulate after MAO-B blockade to produce neurobiological effects (Paterson et al., 1991; Paterson et al., 1995), which are discriminated by rats as similar to those of psychostimulant drugs. However, Ro 16-6491 (Da Prada et al., 1987), a drug that shares with (R)-(-)-deprenyl the ability to selectively block MAO-B enzyme activity but does not share with (R)-(-)-deprenyl the metabolic conversion to methamphetamine and amphetamine metabolites, did not produce any significant generalization to (S)-(+)-methamphetamine or cocaine training stimuli. Ro 16-6491 is roughly equipotent to, or two-fold more potent than, (R)-(-)-deprenyl in its ability to bind to and inactivate the MAO-B enzyme, depending on the assay used (Cesura et al., 1988). Also, the range of i.p. doses of Ro 16-6491 used in the present experiments (1 to 100 mg/kg) were well above the range of oral doses (2.4 to

24.0 mg/kg) able to inhibit the activity of the MAO-B enzyme 30 min after oral administration (Da Prada et al., 1987). However, Ro 16-6491 produced only saline-like discriminative effects under the stimulus-shock termination schedule in the present experiments, even at a three-fold higher dose (100 mg/kg) than the 30 mg/kg dose of (R)-(-)-deprenyl that produced complete generalization to the (S)-(+)-methamphetamine- and cocaine-training stimuli. This high 100 mg/kg dose of Ro 16-6491 completely suppressed food-maintained behavior in the rats. The lack of psychostimulant-like discriminative effects in the behavioral profile of Ro 16-6491 indicates that MAO-B blockade does not mediate the psychostimulant-like discriminative effects of (R)-(-)-deprenyl. Also, if accumulation of endogenous substrates of MAO-B mediated (R)-(-)-deprenyl's psychostimulant-like discriminative effects, similar effects would be expected from a drug like Ro 16-6491 that inhibits activity of the same MAO-B enzyme inhibited by (R)-(-)-deprenyl.

After systemic administration (R)-(-)-deprenyl is converted primarily to (R)-(-)-methamphetamine and (R)-(-)-amphetamine (and, as well, to desmethyl-deprenyl) (e.g., Lajtha et al., 1996; Magyar et al., 2004). (R)-(-)-methamphetamine and (R)-(-)-amphetamine have been studied with drug-discrimination and self-administration procedures and have been shown to fully generalize to (S)-(+)-methamphetamine-, (S)-(+)-amphetamine- or cocaine-training stimuli in rats (Colpaert et al., 1979; Glennon et al., 1984; Yasar et al., 1993; Yasar et al., 1994; Bondareva et al., 2002) and monkeys (Yasar and Bergman, 1994) and to be actively self-administered by rats (Yokel and Pickens, 1973) and monkeys (Winger et al., 1994). We previously found that a dose of 2.0 mg/kg (R)-(-)-amphetamine produced complete generalization to a 1.0 mg/kg training dose of (S)-(+)-amphetamine or a 10.0 mg/kg training dose

of cocaine (Yasar et al., 1993, 1994). Similarly, Bondareva et al. (2002) found that a dose of 2.0 mg/kg (R)-(-)-methamphetamine produced complete generalization to a 1.0 mg/kg training dose of *d*(S)-(+)-methamphetamine. In contrast, 10 to 30 mg/kg doses of (R)-(-)-deprenyl were required to produce complete generalization to training doses of 1.0 mg/kg (S)-(+)-amphetamine or (R)-(-)-methamphetamine or 10.0 mg/kg cocaine in our present experiments and in our previous studies (Yasar et al., 1993; 1994) Thus, (R)-(-)-methamphetamine and (R)-(-)-amphetamine are about two-fold less potent than their respective (S)-(+)-stereoisomers, but they are equally effective in producing these discriminative-stimulus effects and are five- to fifteen-fold more potent than (R)-(-)-deprenyl itself (Winger et al., 1994; Yasar and Bergman, 1994).

Doses of (R)-(-)-methamphetamine and (R)-(-)-amphetamine which had psychostimulant-like discriminative effects in previous studies (0.3 to 2.0 mg/kg; Colpaert et al., 1979; Glennon et al., 1984; Yasar et al., 1993; Yasar et al., 1994; Bondareva et al., 2002) fall in the range of doses obtained by metabolic transformation of (R)-(-)-deprenyl after systemic administration. For example, Melega et al. (1999) showed that brain levels of metabolically generated (R)-(-)-amphetamine after a 10 mg/kg subcutaneous dose of (R)-(-)-deprenyl in rats corresponded to those that would be obtained after a 0.4 mg/kg dose of (R)-(-)-methamphetamine. Thus, levels of metabolically generated (R)-(-)-methamphetamine after a 30 mg/kg dose of (R)-(-)-deprenyl would correspond to levels after a 1.2 mg/kg dose of (R)-(-)-methamphetamine. Also, these estimates are somewhat undervalued, since they consider only metabolically generated (R)-(-)-methamphetamine, and not the smaller amounts of metabolically generated (R)-(-)-amphetamine that would be produced after administration of (R)-(-)-deprenyl. Finally, Melega et al. (1999) observed peak brain levels of (R)-(-)-methamphetamine 30 min after administration in rats,

which corresponds to the time of peak (S)-(+)-methamphetamine-like discriminative effects of (R)-(-)-deprenyl in the present experiments (Figure 1).

SKF 525A blocks the CYP450 enzyme that transforms (R)-(-)-deprenyl into (R)-(-)-methamphetamine and (R)-(-)-amphetamine (Yoshida et al., 1987). Administration of a 50 mg/kg dose of SKF 525A, which is fully effective in blocking the CYP450 enzyme responsible for the metabolism of (R)-(-)-deprenyl (Engberg et al., 1991; Haberle et al., 2002; Magyar et al., 2004; Magyar and Szende, 2004), completely blocked, or significantly reduced, the ability of (R)-(-)-deprenyl to produce generalization to (S)-(+)-methamphetamine- and cocaine-training stimuli under both the food-reinforcement and stimulus-shock termination schedules. In previous experiments with rats (Engberg et al., 1991), (R)-(-)-methamphetamine, in doses relevant to the formation of (R)-(-)-methamphetamine metabolites from (R)-(-)-deprenyl (Melega et al., 1999), produced locomotor activation effects similar to those of (R)-(-)-deprenyl and the effects of (R)-(-)-deprenyl, but not those of (R)-(-)-methamphetamine, were blocked by pretreatment with 50 mg/kg of SKF 525A. The present results are consistent with these previous findings and show for the first time that when (R)-(-)-deprenyl metabolism is blocked, and there is no transformation of (R)-(-)-deprenyl into its amphetamine-like metabolic products, it looses its ability to produce psychostimulant-like discriminative effects. Thus, transformation to active metabolites appears to primarily mediate the psychostimulant-like discriminative effects of (R)-(-)-deprenyl in this study and in numerous previous studies.

Although accumulation of active endogenous substrates of the MAO-B enzyme after blockade by (R)-(-)-deprenyl or Ro 16-1649 did not appear sufficient to produce psychostimulant-like

discriminative-stimulus effects of (R)-(-)-deprenyl under the present experimental conditions, there were indications that, under some conditions, accumulation of such active endogenous substrates might modulate the psychostimulant-like discriminative-stimulus effects of (R)-(-)-deprenyl. The amino-acid ß-phenylethylamine (ß-PEA) is usually present in the brain in very small amounts, and it is usually rapidly metabolized by the MAO-B enzyme after its synthesis from ß-phenyl-alanine. ß-PEA appears to play several neurobiological roles in drug reinforcement processes in the brain. It has been shown to inhibit the neuronal membrane transporter protein and to increase extracellular brain levels of dopamine, epinephrine and serotonin, with potencies comparable to that of amphetamines (Dyck, 1984; Sotnikova et al., 2004). Moreover, ß-PEA has amphetamine-like, behavioral effects that include stimulation of locomotor activity and production of stereotyped movements (Timar and Knoll, 1986; Barroso and Rodriguez, 1996) and can maintain intravenous self-administration behavior in different animal species under some conditions (Shannon and Degregorio, 1982; Sannerud et al., 1996; Bergman et al., 2001). Note that all of these biochemical, behavioral, and reinforcing effects of ß-PEA were obtained after exogenous administration of large amounts of the drug, which likely produced brain levels at least 100 fold higher than normal physiological levels. Although MAO-B inhibition may increase brain levels of ß-PEA to such high concentrations (Paterson et al., 1991; Paterson et al., 1995), acute doses of MAO-B blockers such as (R)-(-)-deprenyl usually have limited effects and chronic dosing is usually necessary to produce near complete inhibition (Lamensdorf and Finberg, 1997). However, acute blockade of MAO-B activity by (R)-(-)-deprenyl has been shown to potentiate locomotor and stereotypic effects (Timar and Knoll, 1986; Barroso and Rodriguez, 1996), as well as reinforcing effects (Bergman et al., 2001), of exogenously administered ß-PEA.

The drug NSD 1015 blocks the dopamine-decarboxylase enzyme that converts the amino-acid ß-phenylalanine into ß-PEA (Carlsson et al., 1972). NSD 1015 pretreatment, at a dose that selectively blocks dopamine decarboxylase activity (30.0 mg/kg administered 30 min before (R)-(-)-deprenyl) (Lamensdorf and Finberg, 1997; Treseder et al., 2003), significantly reduced the ability of (R)-(-)-deprenyl to generalize to the methamphetamine-discriminative stimulus in rats under the stimulus-shock termination schedule, but not under the food-reinforcement schedule. NSD1015, also, partially reduced the ability of (R)-(-)-deprenyl to generalize to the cocaine-discriminative stimulus under the stimulus-shock termination schedule. These results suggest that increased levels of ß-PEA after administration of large doses of (R)-(-)-deprenyl may play a facilitatory role in the psychostimulant-like discriminative effects of (R)-(-)-deprenyl. The limited ability of (R)-(-)-deprenyl to markedly depress MAO-B activity when given acutely may result in the need for very high doses of (R)-(-)-deprenyl in order to produce blockade of MAO-B activity rapidly enough to elevate ß-PEA levels within the one-hr time frame of measured effects (30-min (R)-(-)-deprenyl pretreatment followed by 30-min session) to levels sufficient to facilitate the psychostimulant-like discriminative effects of the (R)-(-)-methamphetamine and (R)-(-)-amphetamine metabolites.

In conclusion, the delayed appearance of psychostimulant-like discriminative effects of (R)-(-)-deprenyl, the failure to find psychostimulant-like discriminative effects with Ro 16-1649 and the reversal of (R)-(-)-deprenyl's psychostimulant-like discriminative effects by blockade of its metabolism with SKF 525A demonstrate that the psychostimulant-like discriminative effects of (R)-(-)-deprenyl are mediated by transformation to active amphetamine-like metabolites and are

not the direct result of MAO-B blockade or increased levels of psychoactive neurochemical substrates of the MAO-B enzyme. The modest reversal of (R)-(-)-deprenyl's psychostimulant-like discriminative effects seen when ß-PEA synthesis was blocked by NSD 1015 pretreatment indicates a facilitatory modulation of the psychostimulant-like discriminative effects of the (R)-(-)-deprenyl metabolites by elevated levels of ß-PEA.

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FOOTNOTES

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Reprints requests: Sevil Yasar, Johns Hopkins University School of Medicine, 5505 Johns Hopkins Bayview Circle, Baltimore, MD 21224, E-mail: syasar@jhmi.edu

Sun-Hee Lee, present address: Drug Evaluation Department, Narcotic and Neuropharmacological Drug Division, Korea Food and Drug Administration, 5 Nokbun-dong, Eulnpyung-gu, Seoul 138-227, South Korea

Roman Stefanski, present address: Department of Pharmacology, Institute of Psychiatry and Neurology, Al. Sobieskiego 1/9, 02-957 Warsaw, Poland

LEGENDS FOR FIGURES

Figure 1. Generalization tests with (R)-(-)-deprenyl, given at different times before the experimental session, in methamphetamine-trained rats under a food-delivery schedule. Rats were trained to discriminate 1.0 mg/kg i.p. (S)-(+)-methamphetamine from saline under a fixed ratio, food-delivery schedule. The percentage of (S)-(+)-methamphetamine-appropriate responding is shown as a function of time after the injection of (R)-(-)-deprenyl (top panel) and response rates are expressed as responses per second averaged over the session (bottom panel). Each point represents the mean ± SEM from eight rats. $^{**}p<0.01$, post hoc comparison with the vehicle (VEH) pretreatment after significant ANOVA for repeated measures main effect, Dunnett's test.

Figure 2. Generalization tests with (S)-(+)-methamphetamine, (R)-(-)-deprenyl and Ro 16-6491 and effects of pretreatment with vehicle, SKF 525A or NSD 1015 on the (R)-(-)-deprenyl dose-response curve in (S)-(+)-methamphetamine-trained rats under a food-delivery schedule. Rats were trained to discriminate 1.0 mg/kg i.p. (S)-(+)-methamphetamine from saline under a fixed ratio, food-delivery schedule. The percentage of responses on the lever associated with (S)-(+)-methamphetamine (top panel) administration is shown as a function of dose (mg/kg) and response rates are expressed as responses per second averaged over the session (bottom panel). Numbers in parentheses at the 100.0 mg/kg dose of Ro 16-6491 indicate that lever-press responding was completely eliminated in all of the eight rats at this dose. Each point represents the mean ± SEM from eight rats. $^{*}p<0.05$, $^{**}p<0.01$, post hoc comparison with the vehicle (VEH) pretreatment after significant ANOVA for repeated measures main effect, Dunnett's test.

Figure 3. Generalization tests with (S)-(+)-methamphetamine, cocaine, (R)-(-)-deprenyl and Ro 16-6491 in (S)-(+)-methamphetamine- and cocaine-trained rats under a stimulus-shock termination schedule. Rats were trained to discriminate either 1.0 mg/kg i.p. of (S)-(+)-methamphetamine or 10.0 mg/kg i.p. of cocaine from saline under a fixed ratio, stimulus-shock termination schedule. The percentage of responses on the lever associated with (S)-(+)-methamphetamine (left top panel) or cocaine (right top panel) administration is shown as a function of dose (mg/kg) and response rates are expressed as responses per second averaged over the session (bottom panels). Each point represents the mean ± SEM from ten (S)-(+)-methamphetamine-trained and five cocaine-trained rats. *p<0.05, **p<0.01, post hoc comparison with the vehicle (VEH) pretreatment after significant ANOVA for repeated measures main effect, Dunnett's test.

Figure 4. Effects of pretreatment with vehicle, SKF 525A or NSD 1015 on the (R)-(-)-deprenyl dose-response curve in (S)-(+)-methamphetamine- and cocaine-trained rats under a stimulus-shock termination schedule. The percentage of responses on the lever associated with (S)-(+)-methamphetamine (left top panel) or cocaine (right top panel) administration is shown as a function of dose (mg/kg) and response rates are expressed as responses per second averaged over the session (bottom panels). Rats were trained to discriminate either 1.0 mg/kg i.p. of (S)-(+)-methamphetamine or 10.0 mg/kg i.p. of cocaine from saline under a fixed ratio, stimulus-shock termination schedule. Each point represents the mean ± SEM from ten (S)-(+)-methamphetamine-trained and five cocaine-trained rats. VEH – vehicle.

Table 1

ED_{50} values (95% CIs) of (R)-(-)-deprenyl for percentage of drug-lever selection when (R)-(-)-deprenyl was administered with vehicle and with 50 mg/kg of SKF 525A and 30 mg/kg of NSD 1015

	ED_{50} (95% CI)
	mg/kg
(S)-(+)-Methamphetamine-trained rats under the food-presentation schedule	
(R)-(-)-deprenyl + vehicle	12.78 (9.77-15.79)
(R)-(-)-deprenyl + SKF 525A	no value
(R)-(-)-deprenyl + NSD 1015	10.33 (7.91-12.74)
(S)-(+)-Methamphetamine-trained rats under the stimulus-shock termination schedule	
(R)-(-)-deprenyl + vehicle	13.49 (10.56-16.42)
(R)-(-)-deprenyl + SKF 525A	37.36 (28.21-46.52) [a]
(R)-(-)-deprenyl + NSD 1015	162.98 (16.76-309.20) [a]
(S)-(+)-Cocaine-trained rats under the stimulus-shock termination schedule	
(R)-(-)-deprenyl + vehicle	5.71 (0.75-10.67)
(R)-(-)-deprenyl + SKF 525A	no value
(R)-(-)-deprenyl + NSD 1015	22.80 (7.98-37.62)

[a] Non-overlapping 95% CI compared with the dose-response curves after vehicle pretreatment.

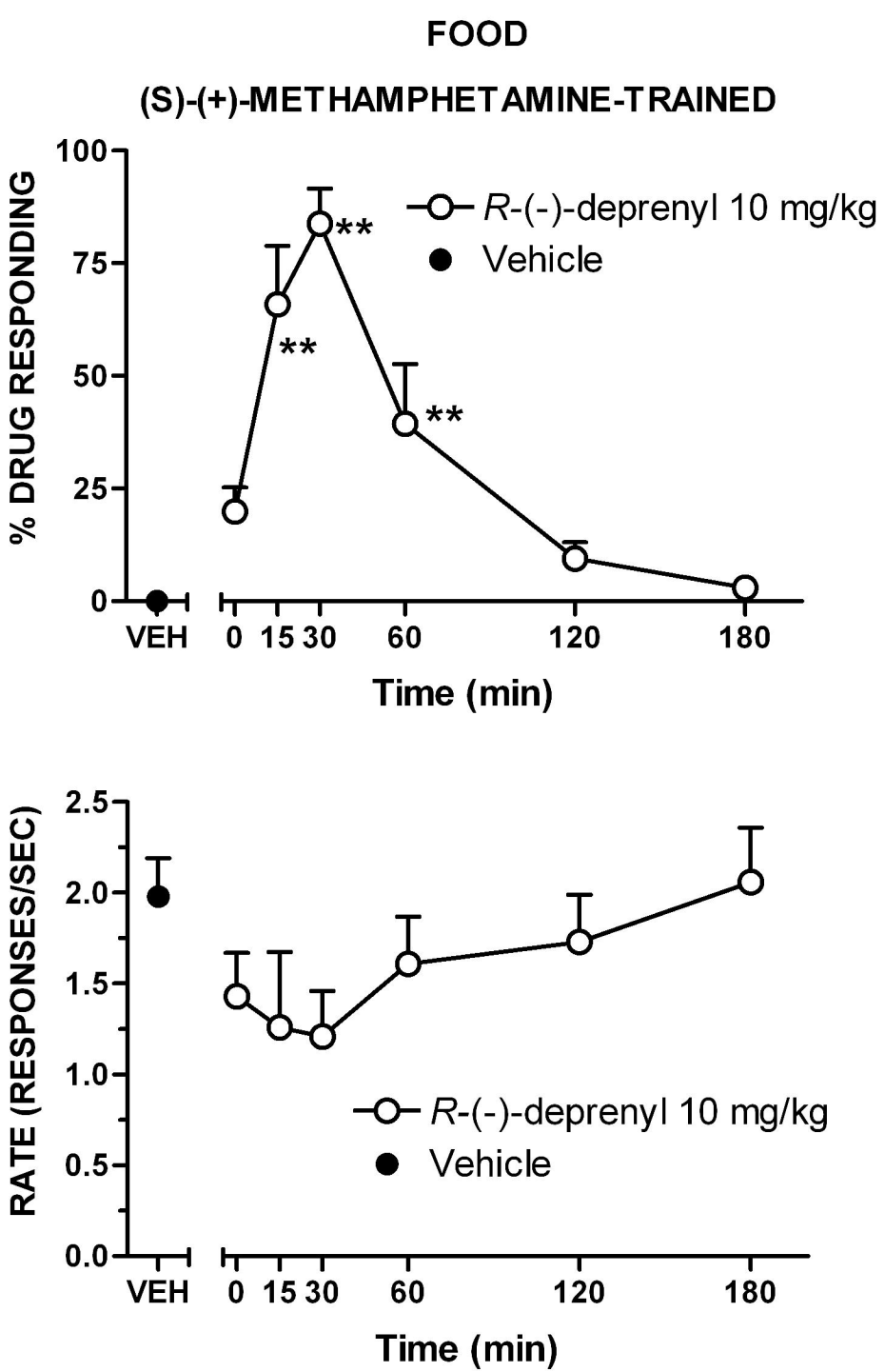
FOOD
(S)-(+)-METHAMPHETAMINE-TRAINED
% DRUG RESPONDING
100
75
50
25
0
R-(-)-deprenyl 10 mg/kg
Vehicle
**
**
**
VEH
0 15 30 60 120 180
Time (min)
RATE (RESPONSES/SEC)
2.5
2.0
1.5
1.0
0.5
0.0
R-(-)-deprenyl 10 mg/kg
Vehicle
VEH
0 15 30 60 120 180
Time (min)

Fig. 1

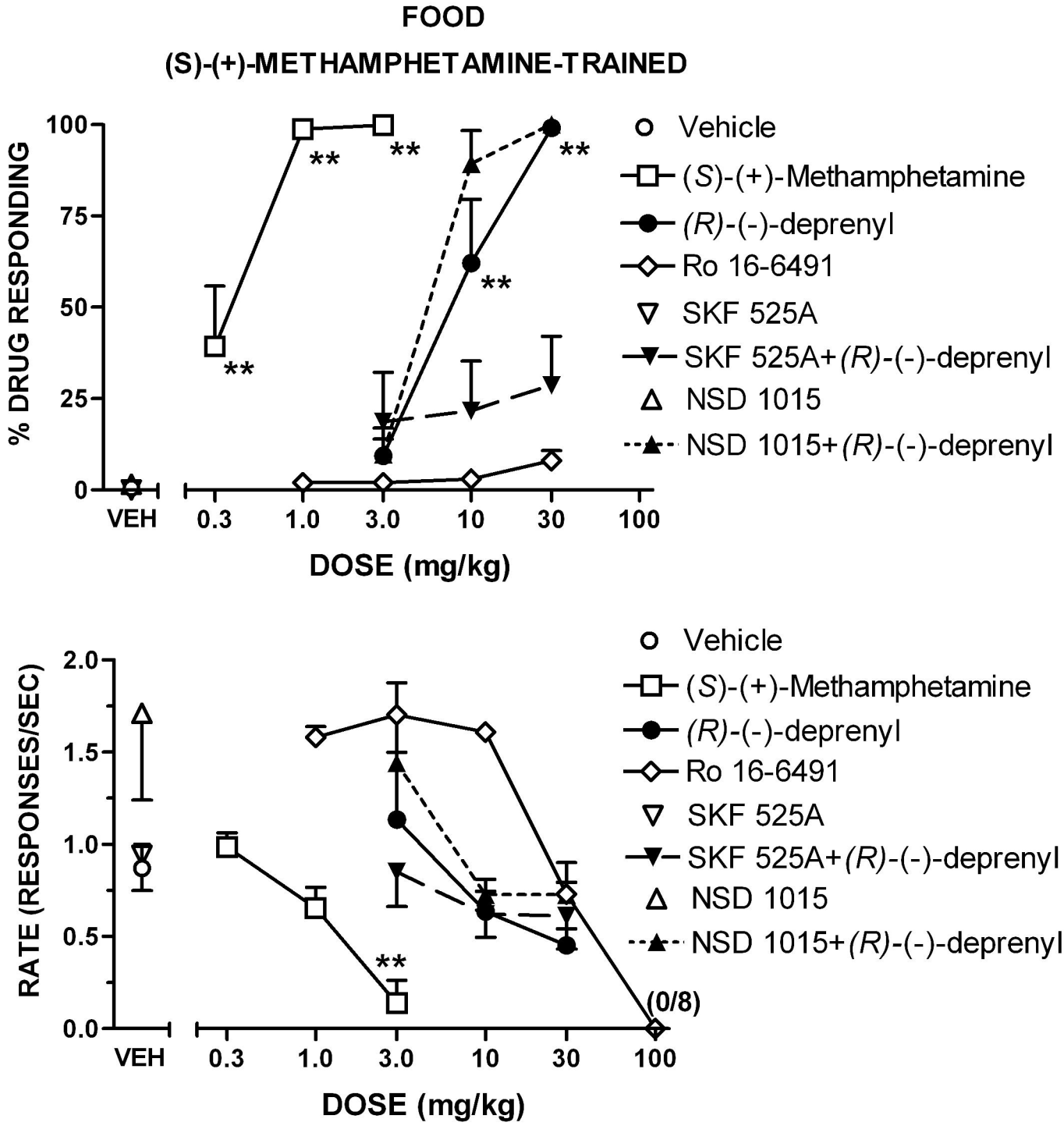
FOOD
(S)-(+)-METHAMPHETAMINE-TRAINED
% DRUG RESPONDING
100
75
50
25
0
VEH
0.3
1.0
3.0
10
30
100
DOSE (mg/kg)
**
Vehicle
(S)-(+)-Methamphetamine
(R)-(-)-deprenyl
Ro 16-6491
SKF 525A
SKF 525A+(R)-(-)-deprenyl
NSD 1015
NSD 1015+(R)-(-)-deprenyl
RATE (RESPONSES/SEC)
2.0
1.5
1.0
0.5
0.0
(0/8)

Fig. 2

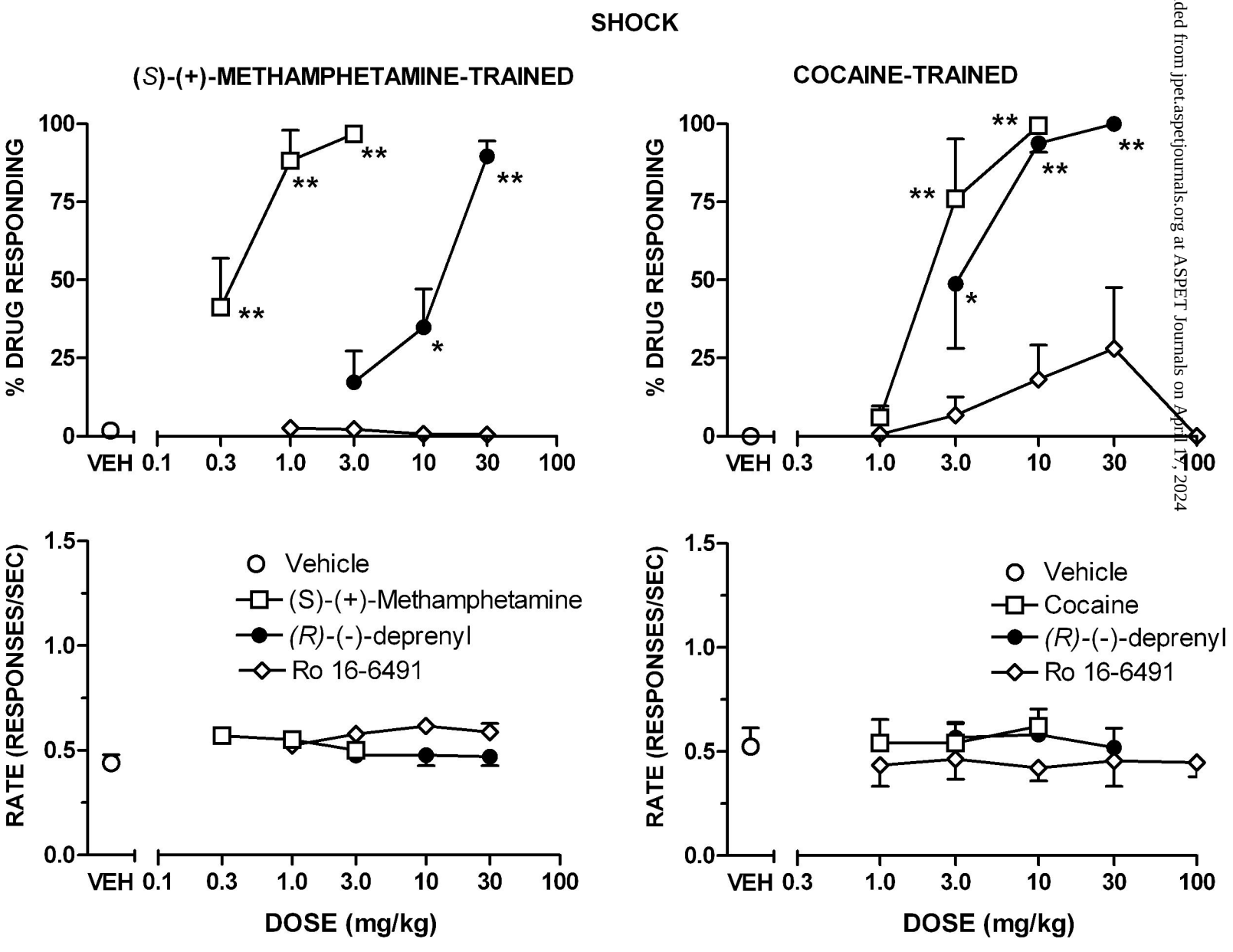

Fig. 3

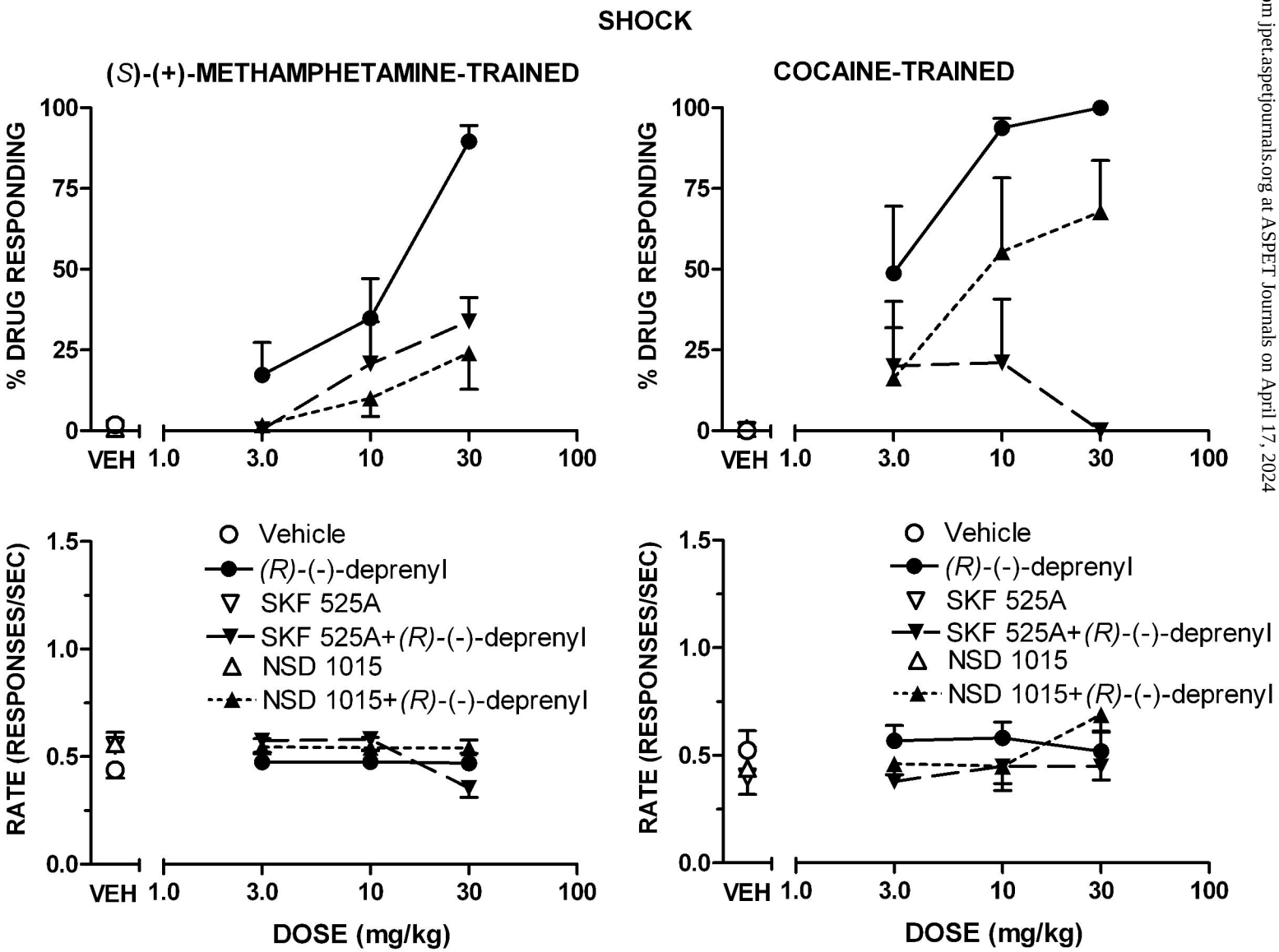

Fig. 4