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A comparison of drug-seeking behavior maintained by *d*-amphetamine, *l*-deprenyl (selegiline) and *d*-deprenyl under a second-order schedule in squirrel monkeys

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Abstract

Rationale: *l*-Deprenyl (selegiline) is used in the treatment of Parkinson disease and has been proposed as an aid for cigarette smoking cessation and a treatment for psychostimulant abuse. *l*-Deprenyl is metabolized in the body to *l*-methamphetamine and *l*-amphetamine, suggesting that it may have abuse potential. *Objectives:* The current study assessed whether drug-seeking behavior for *l*-deprenyl or its isomer would be maintained on a second-order schedule and whether *l*-deprenyl would alter drug-seeking behavior maintained by *d*-amphetamine if given as a pretreatment. *Methods:* Squirrel monkeys learned to respond on a second-order schedule of reinforcement, where every tenth response was followed by a brief light flash and the first brief light flash after 30 min was paired with intravenous (i.v.) injection of *d*-amphetamine (0.56 mg/kg), administered over a two-minute period at the end of the session. When responding was stable, saline or different i.v. doses of *d*-amphetamine (0.3-1.0 mg/kg), *l*-deprenyl (0.1-10.0 mg/kg) and *d*-deprenyl (0.1-3.0 mg/kg) were substituted for 10 days each. Subsequently, monkeys were pretreated with 0.3 or 1.0 mg/kg *l*-deprenyl i.m. 30-min prior to *d*-amphetamine baseline sessions. *Results:* Drug-seeking behavior for *d*-amphetamine was well maintained on the second-order schedule. *d*-Deprenyl maintained high rates of drug-seeking behavior similar to *d*-amphetamine. *l*-Deprenyl maintained lower rates of responding that were not significantly above saline substitution levels. Pretreatment with *l*-deprenyl failed to alter drug-seeking behavior maintained by *d*-amphetamine. *Conclusions:* These results indicate that *d*-deprenyl, but not *l*-deprenyl, may have abuse potential. Under conditions where drug-seeking and drug-taking behavior is actively maintained by *d*-amphetamine, *l*-deprenyl, at doses that specifically inhibit MAO-B, may not be effective as a treatment.

Keywords

Amphetamine; Deprenyl; Drug self-administration; Second-order schedule; Selegiline; Squirrel monkeys

l-Deprenyl (selegiline) is a selective irreversible inhibitor of type B mono-amine oxidase (MAO-B) that is used primarily as an adjunct to *l*-DOPA in the treatment of Parkinson's disease. Because of its ability to inhibit MAO-B, thereby augmenting dopamine levels in the human brain (Youdim and Finberg 1994), *l*-deprenyl has also been proposed as a treatment for both

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cocaine and amphetamine abuse (Bartzokis et al. 1999; Koston et al. 2002; Newton et al. 1999) and as a “safe and efficacious adjunctive treatment to behavioral counseling for smoking cessation” (George et al. 2003; George and O'Malley 2004). *l*-Deprenyl's ability to inhibit MAO-B also reduces the metabolism of β-phenylethylamine (β-PEA), which leads to increased levels of this trace amine in brain (Elsworth et al. 1998; Riederer and Youdim 1986; Paterson et al. 1996). Enhanced psychomotor stimulant effects of exogenously administered β-PEA can be seen following pretreatment with *l*-deprenyl (Bergman et al. 2001; Ortmann et al. 1984; Timar and Knoll 1986). In addition to its ability to inhibit MAO-B activity and, thus, increase brain levels of dopamine and β-PEA, *l*-deprenyl is also metabolized to *l*-methamphetamine and *l*-amphetamine (Heinonen et al. 1994; Szökö et al. 1999). Plasma levels of *l*-methamphetamine equivalent to a dose of over 0.1 mg/kg can be achieved following chronic treatment with 1 mg/kg *l*-deprenyl (Schindler et al., 2003).

The ability to increase dopamine and β-PEA levels and the production of these active metabolites suggests that *l*-deprenyl may have abuse potential on its own (Yasar et al. 1996). The mechanism of action for most drugs of abuse is thought to be related to their ability to increase extracellular dopamine levels in the brain (Wise 1998). While the *l*-isomers of methamphetamine and amphetamine are about two-fold less potent than the *d*-isomers, both isomers have the ability to release dopamine from nerve terminals (Heikkila et al. 1975) and both isomers have similar behavioral effects (Yasar et al. 1996), including the ability to support self-administration behavior (Yokel and Pickens 1973).

Despite the fact that there is little evidence of abuse of *l*-deprenyl in the human Parkinson's disease population, it has been abused as a “smart drug” to increase cognitive functioning by humans (Schneider et al. 1994) and there is some evidence from the animal literature suggesting the possibility of abuse potential. For example, *l*-deprenyl generalized to both amphetamine and cocaine training stimuli in studies of drug discrimination in rats (Yasar and Bergman 1994; Yasar et al. 1993, 1994) and pigeons (Johanson and Barrett 1993) and to a methamphetamine training stimulus in squirrel monkeys (Yasar and Bergman 1994). Similarly, *d*-deprenyl generalized to both *d*-amphetamine (Yasar et al. 1993) and cocaine (Yasar et al. 1994) training stimuli in rats and was approximately two-fold more potent than *l*-deprenyl. In mice, *l*-deprenyl has also been reported to produce positive conditioned place preferences (Wu and Shu 1999), and in squirrel and rhesus monkeys it has been reported to potentiate the intravenous (i.v.) self administration of β–phenylethylamine (β-PEA), a behaviorally active endogenous trace amine that is rapidly metabolized by MAO-B (Bergman et al. 2001). However, in a study of drug self-administration in rhesus monkeys, *l*-deprenyl did not support intravenous (i.v.) self-administration behavior when substituted for cocaine under a simple fixed-ratio (FR) schedule nor did it alter the self-administration of either cocaine or methamphetamine when given before the session (Winger et al. 1994).

While *l*-deprenyl did not support self-administration behavior in rhesus monkeys, those studies were performed using a simple FR schedule of cocaine self-administration baseline, in which 30 lever presses were required to produce each intravenous injection and multiple injections were available during each session (Winger et al. 1994). Since many pharmacological effects of *l*-deprenyl, particularly its behavioral effects, appear to be due to generation of active methamphetamine and amphetamine metabolites after its administration (Yasar et al. 1996; Yasar and Bergman 1994), self-administration procedures sensitive to delayed drug reinforcement processes and insensitive to cumulative response-depressant effects of repeated injections within a session might be more suitable for studying deprenyl's reinforcing effects.

Second-order schedules have proven useful in demonstrating self-administration of a number of compounds that are not readily self-administered on ratio schedules (Goldberg et al. 1990; Schindler et al. 2002). They have been shown to be particularly useful in demonstrating

reinforcing effects of drugs under conditions where the onset of drug effect might be delayed (e.g., intramuscular injection; Goldberg et al. 1976; Katz 1979) or where repeated injections within a session might have cumulative effects that depress responding (Goldberg 1973; Goldberg et al. 1990; Schindler et al. 2002). On a second-order schedule, animals respond on one schedule for the presentation of a brief stimulus (e.g., a light), and the brief stimulus is then intermittently paired with a primary reinforcer (e.g., amphetamine) according to a second schedule. Through these repeated pairings with the primary reinforcer, the brief stimulus becomes a secondary reinforcer. For example, animals might respond on an FR schedule for the presentation of a brief light flash and then be reinforced with an intravenous injection of amphetamine paired with the light flash according to a long fixed-interval (FI) schedule. Second-order schedules of drug injection allow for the measurement of drug-seeking behavior in the absence of the direct effects of a drug (Goldberg et al. 1977, 1978, 1981; Schindler et al. 2002) and may allow detection of treatment effects missed with procedures that allow repetitive drug reinforcement within each experimental session (e.g., FR schedules).

In the current experiments, squirrel monkeys learned to self-administer *d*-amphetamine on a second-order schedule of i.v. drug injection with drug injected at the end of each session. After testing different doses of *d*-amphetamine and saline vehicle, various doses of *l*-deprenyl were substituted for amphetamine to assess their ability to sustain drug-seeking behavior.

Squirrel monkeys were chosen for this study because of the similarity between monkeys and humans in MAO-B levels and dopamine metabolism. Monkeys, like humans, have higher brain levels of MAO-B than MAO-A, while rodents have approximately equal levels (Murphy et al. 1979), and metabolism of dopamine occurs preferentially via MAO-B in some brain areas in monkeys and humans (e.g., Garrick and Murphy 1980; Lakshmana et al. 1998). Since the MAO-B selective dose range for *l*-deprenyl in monkeys is 0.1 to 0.3 mg/kg, which corresponds to the therapeutic MAO-B selective dose range in humans (Mahmood 1997; Yasar and Bergman 1994), and since food-maintained operant responding is markedly disrupted or eliminated by a subcutaneous dose of 3.0 mg/kg *l*-deprenyl (Winger et al. 1994), intravenous doses of 0.1 to 10.0 mg/kg *l*-deprenyl were chosen for the present study. *l*-Deprenyl was also studied as a pretreatment, with doses of 0.3 and 1.0 mg/kg given intramuscularly before *d*-amphetamine self-administration sessions.

For comparison with the effects of *l*-deprenyl, *d*-deprenyl (0.1 to 3.0 mg/kg) was also studied. It is important to note that the metabolites *l*-methamphetamine and *l*-amphetamine are produced following *l*-deprenyl injection while the metabolites *d*-methamphetamine and *d*-amphetamine are produced following *d*-deprenyl injection; metabolism is stereoselective with no racemic transformation (Heinonen et al. 1994; Szökö et al. 1999). Given the approximately two-fold greater potency of the *d*-isomers of the amphetamines in behavioral studies (e.g., Winger et al., 1994; Yasar and Bergman 1994; Yokel and Pickens 1973), *d*-deprenyl would be more likely to be self-administered at lower doses than *l*-deprenyl if reinforcing efficacy is a function of the metabolites' potency.

Materials and Methods

Subjects

Seven adult male squirrel monkeys (*Saimiri sciureus*) housed in individual cages in rooms in which temperature and humidity were controlled were used as subjects. Room lights were on a 12:12 hour cycle with lights on at 0700. Fresh water was continuously available. Four monkeys that were studied under a schedule of intravenous drug self-administration were fed a daily food ration consisting of five biscuits of high protein monkey diet (Lab Diet 5045, PMI Nutrition International, Richmond, Indiana) and two pieces of banana-flavored food treats (Banana Softies, Bio-Serv, Frenchtown, NJ) that maintained their body weight throughout the

course of the experiment (800-1000 g). Fresh fruits, vegetables and environmental enrichment were provided daily. Three monkeys that were studied under a schedule of food delivery were fed (approximately two hours after the session) a daily food ration consisting of four biscuits of high protein monkey diet and one piece of banana-flavored food treat that maintained their body weights at a constant level throughout the study (750 – 950 g). Monkeys were implanted with a venous catheter for the delivery of drug. The general surgical procedure has been described in detail elsewhere (Goldberg 1973). During an initial surgery, a polyvinyl chloride catheter (inside diameter, 0.38 mm; outside diameter, 0.76 mm) was implanted in a femoral or jugular vein. If the catheter failed during the experiment, it was removed and another catheter was implanted in an alternate vein. The distal end of the catheter was passed subcutaneously (s.c.) out through the skin in the middle of the back. Monkeys wore nylon jackets (Lomir Biomedical, Canada) at all times to protect the catheters. Catheters were flushed with saline daily and sealed with stainless steel obturators when not in use. Following a 2-week recovery period, experiments were begun.

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

Apparatus

During experimental sessions, the monkeys sat in Plexiglas chairs and were loosely restrained in the seated position by a waist lock (see Justinova et al. 2005). The chairs were enclosed in ventilated sound-attenuating chambers (model AC-3; Industrial Acoustics Co., Bronx NY) that were provided with continuous white noise to further mask extraneous sounds. The monkeys were fully adapted to the chair and acoustic chamber prior to surgery. The chair contained a response lever (No. 121-05; BRS/LVE Corp., Laurel, Md., USA) mounted on a transparent front wall. Each press of the lever with a force greater than 0.2 N produced an audible click and was recorded as a response. Pairs of green and amber stimulus lights, mounted behind the transparent wall of the chair, could be illuminated and used as visual stimuli. A food trough was located in the bottom middle of the same panel as the levers. The monkey's catheter was connected to polyethylene tubing that passed out of the isolation chamber where it was attached to a motor-driven syringe pump (model No. 57-6496; Harvard Apparatus, South Natick, Mass., USA). A computer using the MED Associates MED-PC software package (East Fairfield, Vt., USA) controlled the operation of the experimental events and data collection.

Procedure

Daily sessions were conducted Monday through Friday. Sessions began with the illumination of the green stimulus lights. Four monkeys first learned to respond on a fixed-ratio (FR) schedule of i.v. *d*-amphetamine self-administration (see Goldberg 1973 for details), and then progressed to a second-order schedule. On this schedule, every 10 lever-press responses (FR 10) turned off the green light and turned on the amber light for 2 sec. The first amber light presented after 30 min (FI 30 min) was presented along with a series of 10, 0.2 ml i.v. injections of amphetamine (total dose 0.56 mg/kg). Each injection was accompanied by a 2-sec presentation of the amber light, followed by an 8-sec timeout. This injection procedure was used to minimize the possibility of toxic effects (e.g., convulsions) produced by high doses and also to maintain consistency with previous experiments (Goldberg and Tang 1977; Goldberg et al. 1979; Goldberg et al. 1981). Once the final injection was given, the session ended. There was a limited hold on the FI of 30 min; however, this contingency was not encountered by any of the monkeys on any training day. According to standard terminology

for second-order schedules (Kelleher 1966; Schindler et al. 2002) the monkeys were trained on a FI 30 min (FR 10: S) schedule.

Once the monkeys were stable on the terminal schedule, as judged by examination of cumulative-response records, drug testing began. All drug conditions were in effect for 10 consecutive days with 10 days of baseline training between each treatment. Various doses of *d*-amphetamine (0.3 and 1.0 mg/kg i.v.), *l*-deprenyl (0.1, 0.3, 1.0, 3.0 and l0.0 mg/kg i.v.), *d*-deprenyl (0.1, 0.3, 1.0 and 3.0 mg/kg i.v.) and saline were substituted for amphetamine, each for a 10-day period. Monkeys were also pretreated with *l*-deprenyl (0.3 mg/kg or 1.0 mg/kg, given intramuscularly 30 min before the session) for 10 consecutive sessions. Not all monkeys were tested under every condition.

Three monkeys were trained to respond on an FR 10 schedule of food delivery. On the FR 10 schedule, every 10^{th} lever-press response was reinforced by delivery of a 190 mg banana flavored pellet. There was a 60-sec timeout following each reinforcement delivery. Sessions were 60 min in length. Once responding stabilized on this schedule, the animals were treated with 1.0 or 10 mg/kg *l*-deprenyl i.v. (10 injections of 0.1 or 1.0 mg/kg *l*-deprenyl, respectively, given as described above) following the session for 10 consecutive days. Monkeys remained in the chair for 15 min following the injection.

Data Analysis

Response rates were calculated for each session based on the time the green stimulus light was on and the number of responses emitted during the green light. Response rates from the last 5 sessions of any condition were averaged for analysis. These data were subjected to multi-level analysis with maximum likelihood estimation (PROC MIXED; SAS Institute, Cary, NC; see Singer 1998), which has the capability to analyze repeated-measures data for which some subjects have not received every level of treatment. Four analyses were performed, one for self-administration of *d*-amphetamine, one for self-administration of *l*- and *d*-deprenyl, one for the effects of *l*-deprenyl on self-administration of *d*-amphetamine, and one for the effects of *l*-deprenyl on food-reinforced responding. Paired comparisons to saline were performed using the Duncan-Hsu procedure, maintaining a 0.05 significance level for each set of tests.

Drugs

d-Amphetamine sulfate, *l*-deprenyl hydrochloride and *d*-deprenyl hydrochloride were obtained from Sigma Chemical Company (St. Louis). All drugs were dissolved in sterile saline and doses are expressed as the salt. Drug concentration was adjusted for the monkey's body weight. Pretreatment injections were administered intramuscularly (i.m.) 30 min before the start of the session in a volume of 0.3 ml/kg.

Results

All three doses of *d*-amphetamine maintained reliable drug-seeking behavior (Figure 1), with overall rates of responding averaging 0.6 responses per sec at the 0.56 mg/kg dose, significantly above the mean rates of responding of 0.04 responses per sec maintained during saline substitution. Injections of *d*-amphetamine maintained self-administration responding in a dose-dependent fashion. Responding rapidly dropped to a very low level when saline was substituted for *d*-amphetamine and rapidly recovered when *d*-amphetamine injections were again made available. Figure 2 shows a representative cumulative-response record for one monkey (subject 2390) when responding was maintained by intravenous injection of *d*-amphetamine at the end of the session. Response rates were very low at the start of the single 30-min fixed interval during each session, but increased to a high rate by the end of the session.

When *l*-deprenyl was substituted for *d*-amphetamine in doses ranging from 0.1 to 10.0 mg/kg, moderate levels of responding were maintained by two of the intermediate doses (1.0 and 3.0 mg/kg), with responding decreasing at the highest 10.0 mg/kg dose (Figure 3). When saline was substituted for *d*-amphetamine, all monkeys responded at rates less than 0.1 responses/sec. Although individual monkeys responded at rates higher than 0.1 responses/sec when tested with either 1.0 or 3.0 mg/kg *l*-deprenyl, mean response rates for the four monkeys at the 1.0 or 3.0 mg/kg doses of *l*-deprenyl were not significantly higher than mean response rates for saline (p's > 0.6), and were well below the rates maintained by *d*-amphetamine. In contrast, *d*-deprenyl dose-dependently maintained higher levels of drug-seeking behavior, with the 1.0 and 3.0 mg/kg doses of *d*-deprenyl maintaining mean response rates in the four monkeys significantly above saline substitution levels and the 3.0 mg/kg dose of *d*-deprenyl maintaining mean response rates comparable to those maintained by *d*-amphetamine.

Representative cumulative records for subject 2390, when responding was maintained by either *l*-deprenyl or *d*-deprenyl injection, are shown in Figure 2. As described above with *d*-amphetamine, rates of responding during *l*-deprenyl or *d*-deprenyl substitution were near zero early in the interval, but response rates progressively increased over the 30-min FI. In contrast, saline maintained virtually no responding. The 3.0 mg/kg dose of *l*-deprenyl maintained response rates well below those of *d*-amphetamine, but rates of responding for this dose of *l*-deprenyl did show a typical FI scallop and were above those observed during saline substitution. A ten-fold lower dose of *d*-deprenyl (0.3 mg/kg) maintained higher rates of responding in this monkey, with response rates and patterns that were comparable to those maintained by *d*-amphetamine. The 3.0 mg/kg dose of *d*-deprenyl maintained very high rates of responding similar to the peak rates of responding maintained by *d*-amphetamine and patterns comparable to those maintained by *d*-amphetamine.

Under the second-order self-administration schedule, response rates were lower for the highest dose of *l*-deprenyl (10 mg/kg) than for intermediate doses. A possible explanation for this downturn at the highest dose is that non-selective depressant effects of this dose may have carried over from one session to the next. To evaluate this possibility, the effects of the 10 mg/kg *l*-deprenyl dose given post-session for 10 consecutive sessions were evaluated in monkeys trained to respond for food reinforcement. This dose of *l*-deprenyl, given after the daily session, did lead to decreased responding the following day, and this effect persisted throughout the 10 days of post-session treatment and for up to 10 sessions following termination of treatment. Average rate of responding for food during the 1-hour session was reduced by 58.6% during the last 5 sessions of treatment, by 54.5% during the first 5 sessions following termination of treatment and by 42.9% during the second 5 sessions following termination of treatment, compared to rates during the 5 day baseline period before treatment (1.68 ± 0.5 responses/sec). In contrast, the lower 1.0 mg/kg *l*-deprenyl dose given post-session for 10 consecutive sessions produced no significant decrease in rates of responding for food (13.4% decrease during the last 5 sessions of treatment). Statistical analysis confirmed that the overall effect of *l*-deprenyl on food responding was significant, $F(5,10) = 7.75$, $p < 0.01$, with response rates during and after treatment with 10.0 mg/kg *l*-deprenyl significantly lower than rates during the 5 day baseline period before treatment (p's <0.05), but with response rates during treatment with the 1.0 mg/kg *l*-deprenyl dose not different than rates during the 5 day baseline period before treatment, ($p > 0.96$).

Because *l*-deprenyl has been proposed as a treatment for psychostimulant abuse, responding maintained by *d*-amphetamine was examined while monkeys were pretreated for 10 days with 2 different doses of *l*-deprenyl (0.3 and 1.0 mg/kg) administered i.m. 30-min before the experimental session. These doses were chosen to have selective effects on MAO-B relative to MAO-A, and to not decrease food-reinforced responding. Figure 4 shows the results for these pretreatments. Neither dose of *l*-deprenyl altered amphetamine-maintained responding.

Response rates following *l*-deprenyl pretreatment were always within the variability of the *d*-amphetamine rates, even when response rates were averaged over either the first or second 5 days of substitution.

Discussion

The present finding, that *d*-amphetamine was clearly and significantly self-administered above saline-vehicle levels by squirrel monkeys under a second-order schedule with drug injection at the end of each session, is similar to previous findings with cocaine (Goldberg et al. 1976; Goldberg et al. 1981; Katz, 1979) and morphine (Goldberg et al. 1976; Goldberg and Tang 1977) under this type of second-order schedule in squirrel and rhesus monkeys. Response rates were very low at the start of the single 30-min fixed interval, but increased to a high rate by the end of the fixed interval. Mean rates of responding for *d*-amphetamine were significantly above saline-vehicle levels and comparable to mean rates of responding maintained by i.v. injections of cocaine (Goldberg et al. 1981) or morphine (Goldberg and Tang 1977) in previous experiments with squirrel monkeys. Responding was also clearly controlled by the brief stimulus, with a pause following each presentation of the brief stimulus followed by a run of responding until the next brief stimulus presentation.

Also like *d*-amphetamine, *d*-deprenyl maintained drug-seeking behavior in a dose-dependent manner, with rates and patterns of responding similar those maintained by the most effective dose of *d*-amphetamine (0.56 mg/kg). In contrast, mean response rates maintained by *l*-deprenyl were much lower than those maintained by either *d*-amphetamine or *d*-deprenyl, showing only a hint of an inverted U-shaped dose-effect function**,** with a peak in mean response rates at 1.0 mg/kg and a decline in response rates at the high 10 mg/kg dose. Although *l*-deprenyl maintained more responding than saline in some monkeys at the two intermediate doses, this difference was not statistically significant at any dose. The decrease in both self-administration responding and food responding 24 hr following administration of the highest dose of *l*-deprenyl most likely reflects the depressant effects of non-selective, irreversible inhibition of both MAO-B and MAO-A by *l*-deprenyl (Batke and Gaal 1993), rather than direct effects of *l*-deprenyl or its metabolites. These depressant effects on responding for food persisted for up to 10 days after treatment ceased, by which time *l*-deprenyl and its metabolites would have been cleared but MAO would still have been inhibited due to the protracted recovery time of brain MAO in primates (half-time of about 30 days in monkeys and 40 days in humans; Arnett et al. 1987 and Fowler et al. 1994, respectively).

Since *d*-deprenyl is much less potent than *l*-deprenyl at inhibiting MAO-B and MAO-A (Magyar and Knoll 1977; Heinonen and Lammintausta 1991), MAO inhibition is clearly not responsible for the self-administration of these drugs. The metabolism of deprenyl is stereospecific (Szökö et al. 1999), with *d*-deprenyl being metabolized to *d*-methamphetamine and *d*-amphetamine and *l*-deprenyl metabolized to *l*-methamphetamine to *l*-amphetamine. The *d*-isomers of amphetamine and methamphetamine are about two-fold more potent than the *l*-isomers in a variety of behavioral tests, including drug self-administration (Yokel and Pickens 1973) and drug discrimination (Yasar and Bergman 1994), with the two isomers being equally effective when dose is adjusted for differences in potency. Therefore, in the present study, *l*-deprenyl was tested at doses up to a log unit higher than those tested with *d*-deprenyl. This range of doses appears to have covered the ascending limb, the peak, and the descending limb of the *l*-deprenyl dose-effect function, and it also encompassed doses (3.0 and 10.0 mg/kg) known to drastically reduce or totally suppress food-reinforced responding in monkeys (Winger at al. 1994). Nonetheless, *d*-deprenyl maintained high rates of responding, similar to those maintained by *d*-amphetamine and significantly higher than those maintained by saline, while *l*-deprenyl maintained only low rates of responding that were not significantly greater than those maintained by saline.

There are two possible explanations for this failure of *l*-deprenyl to maintain self-administration comparable to that maintained by *d*-deprenyl. First, *l*-deprenyl might limit its own self-administration. At doses of *l*-deprenyl that produce levels of metabolites (*l*-methamphetamine and *l*-amphetamine) high enough to have reinforcing effects, *l*-deprenyl's effects on MAO-B and MAO-B may produce a nonspecific suppression of behavior. As described above, a high dose of *l*-deprenyl produced a long-lasting depression of food-reinforced responding that was probably due to effects specific to *l*-deprenyl, such as non-selective MAO-B and MAO-A inhibition. Second, the higher rates of self-administration for *d*-deprenyl than *l*-deprenyl may be the result of factors other than the production of active metabolites. One possible factor is inhibition of dopamine uptake. *d*-Deprenyl has been shown to be a potent inhibitor of dopamine uptake in the rat striatum, while *l*-deprenyl only weakly inhibits dopamine uptake (Fang and Yu 1994; Magyar et al. 2004). Thus, inhibition of dopamine uptake could also account for the observed differences in self-administration between *d*-deprenyl and *l*-deprenyl.

As noted, the present findings, that *d*-deprenyl was significantly self-administered above saline-vehicle levels by squirrel monkeys, and that *l*-deprenyl showed a non-significant trend toward self-administration above saline-vehicle levels, are in contrast to the results obtained by Winger et al. (1994) in rhesus monkeys. In that study, neither *l*-deprenyl nor *d*-deprenyl maintained self-administration behavior above saline control levels. One clear difference between the procedures is the schedule of reinforcement used. In the Winger et al. study, rhesus monkeys repeatedly responded on a simple FR schedule with multiple reinforcements per day, while in the current study squirrel monkeys responded on a second-order schedule, with only a single reinforcement per day. We have recently shown similar differences in the reinforcing effects of intravenous injections of the para-flouro analog of *l*-deprenyl under fixed-ratio and second-order schedules. In this study, responding was maintained by para-flouro-*l*-deprenyl at rates significantly greater than those of vehicle under a second-order schedule but not under a fixed-ratio schedule (Yasar et al., 2005).

Another difference between the Winger et al. (1994) study and the present study is that Winger et al. did not increase the injection dose of *d*-deprenyl above 0.03 mg/kg because of a tendency for monkeys to show reduced rates of responding for cocaine for several sessions after *d*-deprenyl substitution. With the second-order schedule used in the present study, there was no tendency for reduced rates of responding when doses of *d*-deprenyl or *l*-deprenyl as high as 3.0 mg/kg, i.v. were substituted for *d*-amphetamine, or when doses of *l*-deprenyl as high as 1.0 mg/kg, i.m., were administered prior to *d*-amphetamine sessions.

l-Deprenyl has been proposed as a treatment for psychostimulant abuse (Bartzokis et al. 1999; Newton et al. 1999; Schindler et al. 2003) and smoking cessation (George et al. 2003; George and O'Malley 2004). These proposals are based in part on *l*-deprenyl's ability to increase extracellular levels of dopamine in the brain similarly to other drugs of abuse (Wise 1998). The present finding, that even at i.v. doses about 10 to 100 fold higher than oral doses proposed for treatment in humans (about 0.1 mg/kg in a 70 kg subject) - *l*-deprenyl supported only moderate levels of drug-seeking behavior that were not significantly above saline substitution levels - suggest that it would have low abuse potential if used in clinical treatment. However, when given as a pretreatment at doses that would be specific for MAO-B inhibition, 0.3 and 1.0 mg/kg, *l*-deprenyl failed to alter drug-seeking behavior maintained by *d*-amphetamine on the second-order schedule. Similarly, Winger et al. (1994) reported that pre-treating rhesus monkeys with 1.0 mg/kg of *l*-deprenyl failed to alter either methamphetamine or cocaine self-administration. Higher doses of *l*-deprenyl (e.g., 10 mg/kg) would be expected to decrease self-administration, but in a non-specific manner, as these doses also produce long-lasting decreases in food-reinforced behavior. These findings, together, do not support the use of *l*-deprenyl as a treatment for ongoing psychostimulant abuse. More research will be needed to evaluate other aspects of psychostimulant abuse. For example, reinstatement procedures (Shaham et al.

2003) could be used to determine whether *l*-deprenyl may be more effective as a treatment for the prevention of relapse than for ongoing psychostimulant abuse, or whether it might actually prime drug seeking if given during abstinence.

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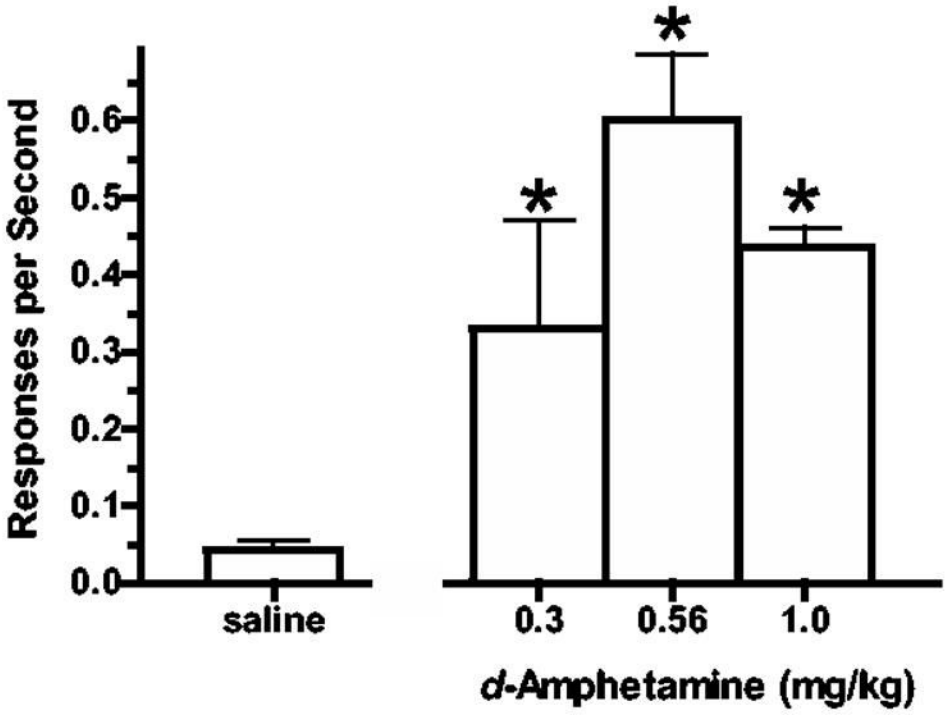

Figure 1.
Mean response rates (± SEM) at three different doses of *d*-amphetamine under the second-order schedule of i.v. drug injection. Bars show the results from the last 5 days under each dose. Also shown are the results for saline substitution (far left bar), where response rates dropped to near zero. Responding was dose-dependent, $F(3,6) = 11.96$, $P < 0.01$. Asterisks indicate that each dose of *d*-amphetamine maintained responding at rates significantly above saline substitution levels, $p < 0.05$.

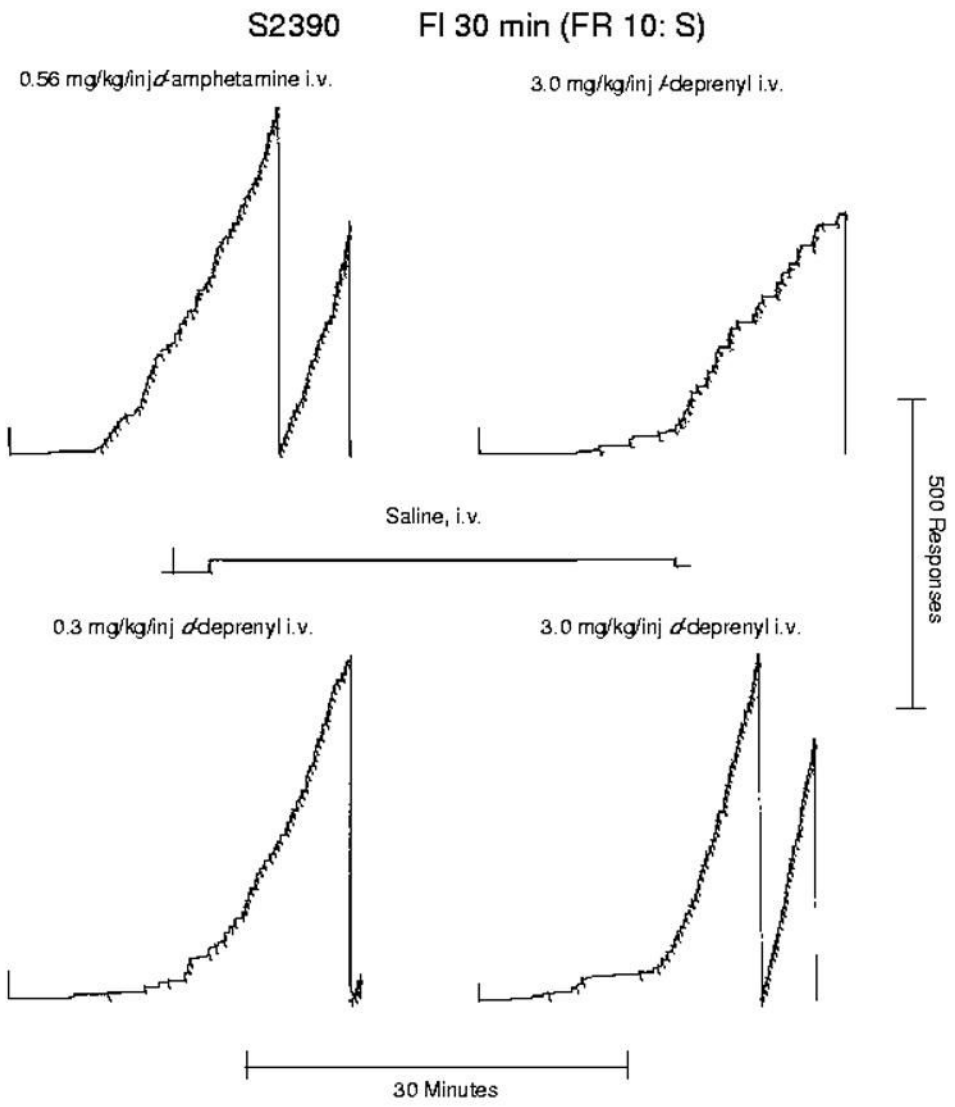

Figure 2.

Representative cumulative-response records for subject S2390. Baseline responding maintained by *d*-amphetamine (upper left record) was typical for this type of second-order schedule, with rates near zero at the beginning of the session and increasing toward the end of the session to a high rate. At the highest dose of *l*-deprenyl, responding was maintained above saline levels (middle record), but was still well below the baseline levels of responding maintained by *d*-amphetamine. In contrast, at the two doses of *d*-deprenyl shown (0.3 and 3.0 mg/kg, lower records), higher levels of responding were maintained that were comparable to baseline responding maintained by *d*-amphetamine.

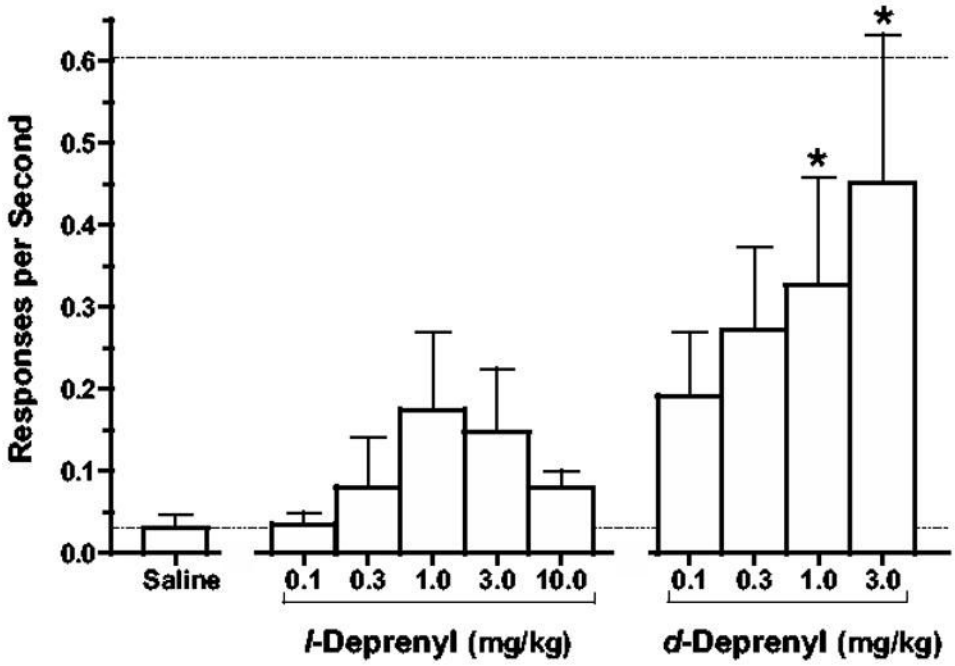

Figure 3.

Mean response rates when different doses of the *l*- and *d*-isomers of deprenyl were substituted for *d*-amphetamine under the second-order schedule of drug injection. Bars show the means (± SEM) of results from the last 5 days at each of five doses of *l*-deprenyl (middle set of bars) and four doses of *d*-deprenyl (right-hand set of bars). The dotted horizontal lines show mean response rates for saline substitution (lower line) and for baseline response rates maintained by 0.56 mg/kg *d*-amphetamine. During saline substitution (far left bar), response rates dropped to near zero. Overall, these results were statistically significant, $F(9,17) = 3.72$, $p < 0.01$, and asterisks indicate points that differed from saline, $p < 0.05$.

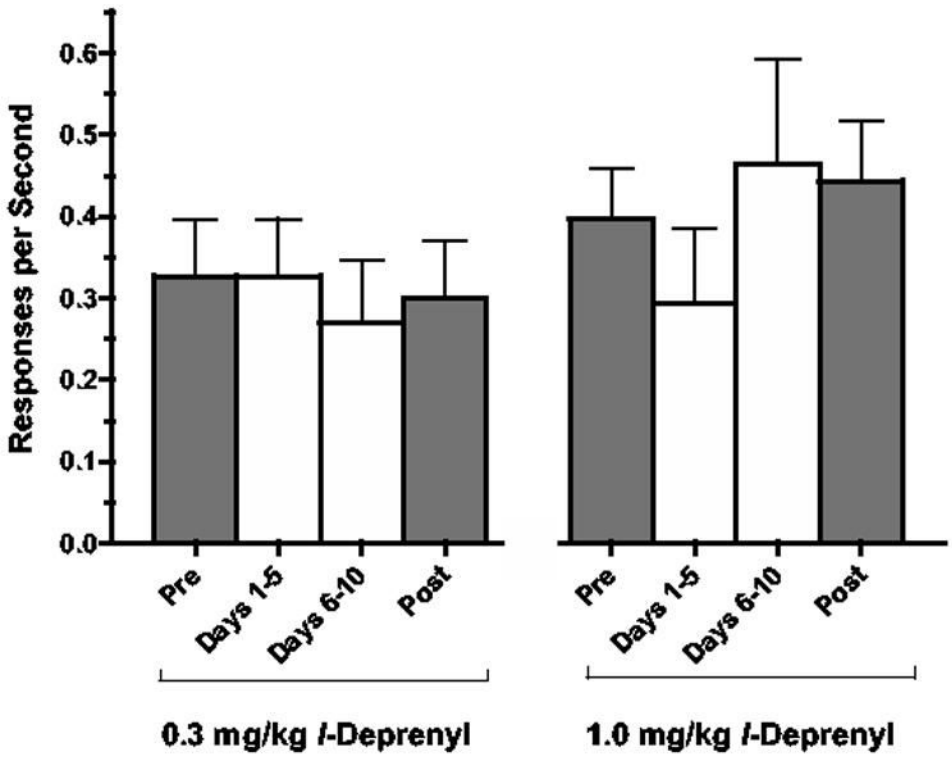

Figure 4.
Mean response rates (± SEM) maintained by 0.56 mg/kg *d*-amphetamine following pretreatment with i.m. doses of 0.3 and 1.0 mg/kg *l*-deprenyl. Bars shows the results from the last 5 days of baseline before pretreatment, the first 5 days and the second 5 days of treatment, and the first 5 days following treatment.