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L-Methamphetamine and selective MAO inhibitors decrease morphine-reinforced and non-reinforced behavior in rats; Insights towards selegiline's mechanism of action

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Abstract

Selegiline is an inhibitor of type B monoamine oxidase (MAO) with psychostimulant effects that can decrease morphine-reinforced and non-reinforced responding. The present study was undertaken to compare the effects of MAO inhibition and treatment with L-methamphetamine, the major psychostimulant metabolite of selegiline, on these behaviors. After rats acquired a stable pattern of morphine self-administration under a progressive ratio schedule, chronic treatment was initiated with vehicle, L-methamphetamine, clorgyline (a selective inhibitor of MAO-A), or rasagiline (a selective inhibitor of MAO-B); with both MAO inhibitors administered at a dose selective for one MAO isoform and a higher dose that inhibited both isoforms. Rats were evaluated for up to four cycles of opiate dependence maintained by morphine self-administration and withdrawal during which extinction responding was recorded. Most behavioral measures (92.4%) did not differ in animals evaluated during an initial and subsequent cycles of dependence and withdrawal. All active treatments attenuated non-reinforced responding during extinction. Morphine reinforcement was also decreased by each of the three active treatments, but greater and more prolonged effects were observed following inhibition of MAO-B with rasagiline. Responding during either cue- or morphine-induced reinstatement was attenuated by either clorgyline or rasagiline administered at nonselective doses, but not by either compound administered at selective dose levels. Treatment with L-methamphetamine did not produce significant effects on cue-induced reinstatement, but decreased non-reinforced responding during morphine-induced reinstatement. These findings indicate that morphine reinforcement and different non-reinforced behaviors differ greatly in their susceptibility to modification by psychostimulant treatment or MAO inhibition.
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1. Introduction

Selegiline (L-deprenyl) is a selective irreversible inhibitor of type B monoamine oxidase (MAO) (Youdim et al., 2001) that is used clinically as an adjunct to L-DOPA in Parkinson's Disease. When administered at relatively high doses or chronically, selegiline can also inhibit MAO-A (Waldmeier and Felner, 1978). Selegiline forms active psychostimulant metabolites that include L-methamphetamine, the less-active enantiomer of methamphetamine (Melega et al., 1999), and can also block reuptake of dopamine and other amines (Gerlach et al., 1996). Because of its ability to augment dopamine transmission, selegiline is being evaluated as an aid for smoking cessation (George et al., 2003) and as a potential treatment for abuse of cocaine (Kosten et al., 2002).

Changes in dopamine neurotransmission may be a common substrate that explains the reinforcing actions of psychostimulants, opiates, and alcohol (Spanagel and Heilig, 2005). Each of these classes of abused substances can cause acute increases in dopamine transmission in animal models, with reduced

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transmission associated with withdrawal (Rossetti et al., 1992). Basal levels of dopamine in the nucleus accumbens shell have been shown to be decreased in rats prior to the start of sessions for daily self-administration of heroin or cocaine (Gerrits et al., 2002). By preventing inactivation of dopamine through MAO and producing small amounts of psychostimulant metabolites, selegiline may serve as a form of substitution therapy for augmentation of dopamine transmission caused by abused substances.

For opiates, the aversiveness of a well-characterized withdrawal syndrome may contribute to subsequent drug-seeking behavior (Hutcheson et al., 2001). After opiate dependence has been established, withdrawal can also be precipitated by treatment with an opiate antagonist (such as naloxone). Physical signs of opiate withdrawal resolve spontaneously over time, typically within one week for withdrawal of morphine treatment in the rat. Levels of the dopamine D2 receptor are decreased in the striatum of opiate-dependent rodents (Navarro et al., 1992) and humans (Wang et al., 1997). Systemic treatment with dopamine agonists (Ary et al., 1977) or activation of dopamine D2 receptors within the accumbens (Walters et al., 2000) prevents somatic signs of withdrawal induced by blockade of opiate receptors.

We have previously shown that chronic treatment with selegiline can attenuate morphine reinforcement and non-reinforced responding during extinction of morphine self-administration (Grasing et al., 2005; Grasing and He, 2005). However, selegiline treatment failed to modify non-reinforced responding during cue-induced reinstatement. In these studies selegiline also decreased the occurrence of ptosis during naloxone-precipitated withdrawal, but did not alter other signs of opiate withdrawal.

If selegiline's actions on opiate-reinforced and opiate-seeking behaviors could be explained by a single mechanism, this could lead to development of more selective therapy, perhaps improving the effectiveness of treatment while diminishing the potential for adverse effects. Therefore, the present study was undertaken to characterize the mechanisms through which selegiline modifies morphine-reinforced and non-reinforced behaviors. To examine the role of these mechanisms, rats received chronic treatment with inhibitors of MAO-A or -B, delivered at either low and relatively selective doses, or at higher dose levels chosen to inhibit both MAO isoforms. Because the formation of psychostimulant metabolites may also contribute to actions of selegiline, some rats received chronic treatment with L-methamphetamine, the major psychostimulant metabolite of selegiline (Melega et al., 1999).

2. Materials and methods

2.1. Animals

Nine-week old, male Wistar rats ($n=59$, Charles River Laboratories, Raleigh, NC) were housed individually and maintained on a reversed light–dark cycle (14 h of darkness beginning at 9:00 AM, and 10 lighted hours), without restriction of drinking water and food restriction as outlined below. Animals were maintained according to standards outlined in the NIH Guide for Care and Use of Laboratory Animals (NIH publication no. 86-23, 1996) and experimental procedures approved by the local Animal Care and Use Committee.

2.2. Apparatus

Experiments were controlled with a commercially available interface and software (DIG-700P1 and Med PC software, version 1.15, Med Associates, Inc., Georgia, VT). Self-administration sessions were conducted in clear Plexiglas chambers (24 cm wide, 25 cm deep, by 26 cm tall) housed within sound attenuating boxes equipped with a transparent Plexiglas door that allowed penetration of ambient lighting. White noise was provided for each chamber by a ventilating fan. Chambers for self-administration had a floor made of stainless steel bars and were equipped with two response levers (model ENV-110 M) and a white cue light that was 2.5 cm in diameter (model ENV-221 M, Med Associates, Inc.). Morphine reinforcement was supplied by a pneumatic syringe (model 100, IITC, Inc., Woodland Hills, CA) set to deliver a volume of 0.030 ml. Activation of pneumatic syringes was paired with an audible tone (model 273 060B operated at 28 V with a 206 kΩ resistor, Radio Shack, Fort Worth, Texas). At the start of self-administration sessions, availability of morphine reinforcement was signaled by flashing the cue light which was alternately illuminated for 0.75 s and turned off for 3.0 s. When a ratio was completed, an injection of morphine was delivered over approximately 1 s, the tone sounded, and the cue light was continuously illuminated. Two seconds after completing the ratio, the tone and cue light were turned off, with neither signal presented during a 5-s time out period. Responses had no consequence during either the injection or time out periods. Following the time out period, the cue light was flashed, and lever presses are again counted towards completion of ratios. Each chamber was equipped with a liquid swivel (model 375/22, Instech, Plymouth Meeting, PA) to allow drug delivery with free movement of the animal in the chamber. Connections to swivels were made via steel-spring tethers attached to a skull cap.

2.3. Initial food self-administration

To facilitate acquisition of drug self-administration, rats were trained to self-administer food pellets prior to surgery and morphine self-administration (Caine et al., 1993). Food reinforcement was supplied by a pellet dispenser (model ENV-203, Med Associates, Inc.), using the same presentation of a tone and cue light described above with a 5-s time out period. To differentiate environments associated with different reinforcers, food was always self-administered in aluminum chambers with a plastic floor arranged in a pattern 0.5″ squares, equipped with a single response lever (model ENV-110 M, Med Associates, Inc.). In both food and morphine self-administration contexts, levers were placed at a height of 5 cm over grid floors.

Initially, 45 mg food pellets (Bio-Serve, Frenchtown, NJ) were self-administered under a fixed ratio 1 (FR-1) schedule during daily 20 min sessions. Before the initial food self-

administration session, animals were maintained without food for 48 h. After the initial session, food intake was limited to 14 g of standard rat chow daily for the duration of experiments. Rats maintain stable body weights on this level of food intake, but have more consistent self-administration of drug or food reinforcers. After successively self-administering at least 50 food pellets during a single session under FR-1, rats were advanced to self-administration of food pellets under the more difficult PR 9-4 schedule for two additional sessions (see below).

2.4. Catheter placement

Rats were anesthetized with 50 mg/kg of intraperitoneal pentobarbital, and the internal jugular vein was then exposed and dissected free of surrounding connective tissue. A small incision was made in the vein, and a commercially available silastic catheter (model S25, IITC, Inc., Woodland Hills, CA) inserted and fastened in place by silk suture and cyanoacrylic cement. The catheter was then passed subcutaneously to a connector embedded within a skull cap made of dental acrylic cement.

2.5. Morphine pretreatment

Prior to morphine self-administration, animals were pretreated with morphine to establish opiate dependence. Morphine sulfate was donated by the National Institute on Drug Abuse (Bethesda, MD) and dissolved in 0.9% saline. Three days after catheter placement, animals received one week of a continuous intravenous morphine infusion at an initial dose of 16.8 mg/kg-day. Over this 7 day period, the initial dose was increased by 20% every 24 h, which corresponded to doses of 16.8, 20.1, 24.1, 28.9, 34.7, 41.7, and 50.0 mg/kg-day administered over days 1 through 7, respectively. Increases in morphine dose were accomplished through changes in the infusion rate, and by adjusting the concentration of morphine delivered. Morphine infusions were administered while animals were maintained in 'shoe box' style Plexiglas cages. Each home cage was equipped with an infusion pump (Razel, model A, Stanford, CT), liquid swivel, counter-balanced arm, and steel-spring tether to allow chronic drug infusions.

2.6. Progressive ratio schedules for morphine and food reinforcement

Because responding under a progressive ratio schedule may be more sensitive to factors that modify natural or drug reinforcers (Stafford et al., 1998), effects of L-methamphetamine and MAO inhibitors were evaluated as morphine was self-administered under a progressive ratio schedule (Li et al., 2003). For progressive ratio responding, the number of lever pressing responses required to receive a reinforcer (response requirement) was set to one at the start of each session and increased throughout the session according to the number of reinforcers administered. This schedule was described as PR 9-4, because the 9th response requirement was 4 lever presses (Grasing et al., 2003). Under PR 9-4, response requirement was calculated according to the following equation:

$$\text{Response requirement} = \text{Round}\ (C1 * e^{[C2 * (\text{Step Number} - C3)]} - C1 + C4).$$

Where results are rounded to the nearest integer value, and C1, C2, C3, and C4 are constants with values of 10, 0.0350, 1, and 0.5, respectively. This equation caused response requirement to be incremented according to the following progression: 1, 1, 1, 2, 2, 2, 3, 3, 4, 4, 5, 5, 6, 6, 7, 7, 8, 9, 9, 10, 11, 11, 12, 13, ... (Grasing et al., 2003).

2.7. Behavioral procedure

In order to promote acquisition of morphine self-administration, all rats received initial food self-administration training and morphine pretreatment. Morphine was always self-administered in cages that differed in appearance from those used for food self-administration as outlined above. To maintain high levels of opiate dependence, self-administration of morphine was initiated immediately following termination of chronic morphine infusions and was conducted seven days per week using 17-h sessions that were initiated at 4:00 PM each day and terminated on the following morning at 9:00 AM. In addition, a break point criterion procedure was not used (sessions were continued for 17 h, irrespective of how often ratios were completed). As an additional step to maintain opiate dependence, morphine was always self-administered at the same relatively high dose of 3.2 mg/kg per injection.

Rats were initially allowed to self-administer 3.2 mg/kg per injection of morphine under FR-1 over four sessions. On the following day, animals were advanced to self-administer morphine under PR 9-4 for three or more additional sessions. During morphine self-administration, each ratio completed on the active lever was followed by an injection of morphine. Animals that self-administered an average of at least 12 morphine infusions per day over three consecutive baseline sessions were then advanced to the different phases of study outlined in Table 1.

In the event of morphine self-administration that failed to meet expected levels, catheter patency was tested by observing rats for rapid onset of ataxia or sleep after injection of 5 mg of pentobarbital intravenously. All data from subjects that developed non-patent catheters was excluded from analysis. Overall, self-administration data was not available because of technical problems other than catheter patency for approximately 3% of self-administration sessions conducted. This included data that was missing because of experimenter error, computer malfunction, or catheters that became temporarily disconnected during a self-administration session.

2.8. Drug treatments

Secondary treatments (saline, L-methamphetamine [administered at 3.2 mg/kg-day], clorgyline [1.0 or 10 mg/kg-day], or rasagiline [1.0 or 10 mg/kg-day]) were each administered as a

Table 1
Procedures for measuring morphine reinforcement and non-reinforced responding during extinction and reinstatement

Phase (session #)	Procedures
Baseline (three daily sessions)	Morphine was self-administered under PR 9-4 with drug availability and completion of ratios signaled by the cue light and tone during daily 17-h sessions; Intravenous infusions to deliver secondary treatments were initiated immediately following completion of the final baseline session.
Extinction (five daily sessions during the first five days of a seven day period)	Secondary treatments were administered as a continuous intravenous infusion, 24 h per day, over seven days; Non-reinforced responding under PR 9-4 was recorded during daily 4-h sessions for the first five days of extinction; During extinction sessions, the cue light and tone were not activated at any time, and completed ratios were recorded but had no consequence.
Cue- and morphine-induced reinstatement (one session)	Secondary treatments continued to be administered as a continuous intravenous infusion, over one additional 24 h period; Throughout a single 17-h session, the cue light and tone were again enabled to signal availability of drug and completion of ratios under PR 9-4, but no morphine was administered contingently; The number of ratios completed during the initial 2 h of the session was recorded as a measure of cue-induced reinstatement; After 2 h of responding, the response requirement was reset back to one, and 7.5 mg/kg of morphine was injected intravenously; The number of ratios completed during the final 15 h of the session was recorded as a measure of morphine-induced reinstatement.
Reacquisition (three daily sessions)	Prior to the first of three reacquisition sessions, secondary treatments were administered as a continuous infusion during the extinction and reinstatement sessions; For the second and third reacquisition sessions, secondary treatments were administered at the same daily dose as a continuous infusion over the 7 h prior to morphine self-administration sessions; Morphine was again self-administered under PR 9-4 with the cue light and tone enabled normally.
Post-treatment (three daily sessions)	Procedures were identical to reacquisition, except that secondary treatments were not administered prior to morphine self-administration sessions.

Rats responded under PR 9-4 for all phases of study. Morphine was self-administered at a dose of 3.2 mg/kg per injection during the baseline, reacquisition, and post-treatment phases. Secondary treatments (L-methamphetamine, clorgyline, rasagiline, or vehicle) were first administered as a 24-h-per day infusion, initiated immediately following completion of the final baseline session and continued for eight days during the extinction and reinstatement phases of study. Prior to the second and third reacquisition sessions, secondary treatments were administered at the same daily dose levels as a 7-h-per day infusion. No secondary treatments were administered during the post-treatment phase of study.

chronic intravenous infusion through the same catheter used for morphine self-administration. Treatments were delivered by Razel Model A infusion pump equipped with a 0.008 RPM motor and a 10 ml syringe. Prior to the second and third days of reacquisition, secondary treatments were infused over 7 h preceding self-administration sessions, in a volume of 1.17 ml per day. Otherwise, during days in which extinction and reinstatement sessions were run, treatments were administered at the same daily dose using a 24-h-per day infusion at the rate of 4.0 ml per day.

L-methamphetamine hydrochloride was purchased from Research Biochemicals International (Natick, MA), clorgyline hydrochloride purchased from Sigma Chemical Company (St. Louis, MO), and rasagiline mesylate donated by Teva Pharmaceutical Industries Ltd.

A total of 59 rats evaluated in this study, with 32 animals studied during a single cycle of morphine self-administration followed by extinction, reinstatement, and reacquisition of morphine self-administration. For 27 rats that maintained patent catheters and successfully reacquired morphine self-administration, one to three additional cycles of extinction, reinstatement, and reacquisition of morphine self-administration were conducted as different secondary treatments were administered. Successful reacquisition of morphine self-administration was defined as self-administering an average of at least 12 morphine infusions per day over three consecutive days. For animals that received additional secondary treatments, treatments were assigned in a random pattern and behavior was monitored during the same sequence of phases and duration of treatment as is outlined in Table 1.

2.9. Data analysis

Statistical tests were performed by Systat Inc. software (version 5, Evanston, IL) with comparisons made by analysis of variance (ANOVA), using repeated measures ANOVA when appropriate. Post hoc comparisons were made by Bonferroni *t*-tests. Based on evaluation of five secondary treatments, $p<0.01$ (0.05 divided by five) was used as a criteria for statistical significance of post hoc comparisons. Inactive lever responding was included as a measure of non-reinforced, nonspecific behavior. The numbers of ratios completed on active and inactive levers were chosen as the primary statistical measures. In order to more concisely describe results, the term 'responding' is used interchangeably with the phrase 'number of ratios completed' when describing results for inactive levers during morphine self-administration, and for either active or inactive levers during extinction and reinstatement sessions. In addition, the term 'reinforcement' is used to describe the number of ratios completed on active levers during morphine self-administration sessions, which were always followed by morphine injections.

Responding on inactive levers and during extinction was evaluated by calculating the number of ratios that would have been completed, excluding responses which would have occurred during delivery of reinforcers or time out periods. To facilitate comparisons with previous reports and between the different phases evaluated in the present study, extinction responding is also reported as the number of ratios completed. In general, use of ratios completed rather than the number of lever pressing responses decreased variability in extinction responding. For example, variance for the number of lever presses made by saline-treated rats was 124 and 109% during the second and third days of extinction, respectively; but variance for ratios completed over the same time points was 66.8 and 56.1%, respectively.

Secondary measures were calculated for data averaged over all sessions obtained for each phase of study. These included final response requirement, defined as the number of lever presses needed to complete the last ratio of a session; duration of responding, defined as the time interval between completion of the initial and final ratios for a session; and latency, calculated as the average time interval required to finish each of the ratios completed during a session. For the number of ratios completed during morphine self-administration, variance differed between the different treatment groups, especially during the reacquisition phase of study. For example, variance for animals receiving saline or 1.0 mg/kg of rasagiline during reacquisition was 26.7 and 56.7%, respectively. Because of unequal variance between treatment groups, values for the ratios completed and final ratio were log transformed prior to statistical analysis.

3. Results

3.1. Multiple cycles of morphine dependence and withdrawal

The design for the present study was based on the use of multiple cycles of morphine self-administration separated by seven day periods of withdrawal during which extinction responding and changes in body weight were recorded. Although only the first cycle was preceded by noncontingent pretreatment with morphine, the dose level for morphine pretreatment was selected to match amounts of morphine self-administered when 3.2 mg/kg per injection was available under PR 9-4 during 17-h sessions. Our hypothesis was that primary measures would be similar in vehicle-treated animals during initial and subsequent cycles of dependence and withdrawal.

3.1.1. Body weight and extinction responding

Absolute values for body weight at the end of the final baseline morphine self-administration session were 291.2±3.6, 291.4±5.6, 290.8±5.3, and 286.6±8.5, for saline-treated animals during their first, second, third, and fourth dependence-withdrawal cycles, respectively. ANOVA failed to show a significant effect of dependence-withdrawal cycle on absolute body weight [$F(3,94)=0.09$, p not significant].

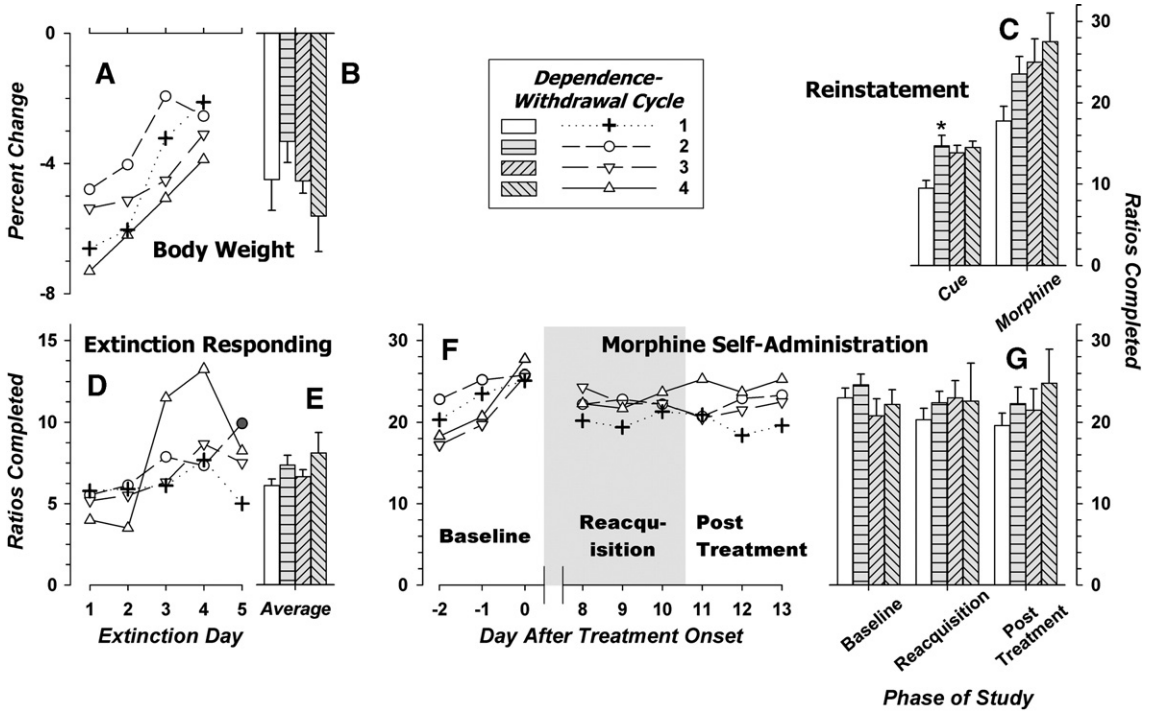

Fig. 1. Effects of dependence-withdrawal cycle on changes in body weight and behavioral measures. Group means for daily measures of body weight and non-reinforced responding during extinction are shown in panels A and D, respectively, with corresponding averages and standard error shown in panels B and E. Results for cue- and morphine-induced reinstatement are shown in panel C. Panel F shows group means for ratios completed during morphine self-administration, with data averaged by phase shown in panel G. For panels C and D, * and gray-filled symbols correspond to $P<0.01$ for post hoc comparisons with the first dependence-withdrawal cycle, respectively.

Fig. 1 shows change in body weight and active lever responding for rats that received saline treatment during their first, second, third, or fourth exposure to morphine dependence and withdrawal. During previous or subsequent cycles, animals received either saline or active secondary treatments in a random pattern. Extinction and body weight data is shown for 18, 15, 6, and 4 animals that received saline during a first, second, third, and fourth dependence-withdrawal cycle, respectively. For body weight (panel A), repeated measures ANOVA showed a significant effect for day of withdrawal [$F(3,117)=14.1$, $p<0.001$] but not for dependence-withdrawal cycle [F (3,39)= 0.69, p not significant] or the interaction of withdrawal day and cycle [$F(9,117)=1.22$, p not significant]. ANOVA also failed to show a significant effect of cycle on body weight averaged over the initial four days of withdrawal [panel B, $F(3,39)=0.69$, p not significant].

For ratios completed during the five extinction sessions (panel D of Fig. 1), repeated measures ANOVA failed to show a significant effect of cycle [$F(3,39)=0.63$, p not significant], but showed significant effects for day of withdrawal [$F(4,156)=9.37$, $p<0.001$] and the interaction of day and cycle [$F(12,156)=2.42$, $p<0.01$]. Post hoc comparisons indicated that rats undergoing a second dependence-withdrawal cycle had increased responding during the fifth extinction session. Otherwise, ratios completed during extinction sessions did not differ in animals evaluated during different dependence-withdrawal cycles. In addition, extinction responding averaged over five sessions did not differ with dependence-withdrawal cycle [panel E, $F(3,39)=1.63$, p not significant].

3.1.2. Reinstatement and morphine self-administration

Ratios completed during reinstatement are shown in panel C of Fig. 1 for 12, 13, 6, and 4 animals that received saline during a first, second, third, and fourth dependence-withdrawal cycle, respectively. For non-reinforced responding during reinstatement, ANOVA showed a significant effect of dependence-withdrawal cycle on responding induced by exposure to cues [F (3,31)=5.31, $p<0.01$] but not morphine [$F(3,31)=2.80$, $p<$not significant]. Post hoc comparisons indicated that rats undergoing a second dependence-withdrawal cycle had increased responding during cue-induced reinstatement.

Effects of dependence-withdrawal cycle on morphine self-administration are shown in panel F of Fig. 1 with results for data averaged across phase shown in panel G. Morphine self-administration data is shown for 18, 13, 6, and 3 animals that received saline during a first, second, third, and fourth dependence-withdrawal cycle, respectively. For ratios completed (morphine injections), repeated measures ANOVA showed a significant effect of session number [$F(8,288)=2.65$, $p<0.01$] but not dependence-withdrawal cycle [$F(3,36)=0.53$, p not significant] or the interaction of session and cycle [$F(24,288)=0.77$, p not significant]. For data averaged across three sessions during baseline, reacquisition, and post-treatment phases, no significant effects of dependence-withdrawal cycle [$F(3,36)=0.55$, p not significant], phase [$F(2,72)=0.42$, p not significant], or the interaction of phase and cycle [$F(6,72)=0.77$, p not significant] were observed.

Taken together, the data presented in Fig. 1 indicate that rats undergoing a second dependence-withdrawal cycle had greater levels of non-reinforced responding during the final extinction- and cue-induced reinstatement sessions, relative to values obtained during an initial cycle, but these measures did not differ significantly for animals evaluated during a third and fourth cycle. The number of times the rats underwent dependence and withdrawal did not alter behavioral measures recorded at 14 other time points or withdrawal induced changes in body weight. Excluding averaged values, results obtained on the second, third, or fourth cycle did not differ from data obtained during the first cycle for 92.4% of the behavioral measures presented in Fig. 1. Based on this finding, results were collapsed across different dependence-withdrawal cycles to evaluate effects of secondary treatments in the following sections.

3.2. Effects of secondary treatments on changes in body weight and extinction responding

3.2.1. Body weight

Absolute values for body weight at the end of the final baseline morphine self-administration session are shown in Table 2. For this measure, ANOVA showed a significant effect of secondary treatment [$F(5,92)=2.37$, $p<0.05$], but post hoc comparisons failed to show significant differences between animals receiving saline- and treatment with L-methamphetamine, clorgyline, or rasagiline.

Effects of different treatments on measures obtained during the extinction phase of study are shown in Fig. 2. Body weight declined by approximately 6.0% over the first 24 h after the onset of morphine withdrawal, and afterwards, gradually increased towards baseline (panel A). This pattern indicates that rats established a high level of opiate dependence during the

Table 2
Group means with standard error for secondary measures of non-reinforced responding during extinction

Treatment	Dose [subject number]	Body weight (g)	Final ratio	Duration of responding (h)	Latency (h)
Saline	– [43]	285.79 (4.65)	3.29 (0.15)	1.98 (0.08)	0.342 (0.017)
L-Methamphetamine	3.2 [13]	290.38 (5.32)	2.02 (0.12) *	1.71 (0.09)	0.508 (0.038) *
Clorgyline	1.0 [9]	279.33 (4.02)	1.91 (0.12) *	1.45 (0.16) *	0.469 (0.057) *
	10 [10]	312.30 (6.92)	1.94 (0.26) *	1.33 (0.16) *	0.436 (0.062)
Rasagiline	1.0 [12]	292.25 (4.68)	2.00 (0.14) *	1.77 (0.13)	0.555 (0.054) *
	10 [11]	298.45 (6.18)	1.75 (0.13) *	1.47 (0.15) *	0.683 (0.116) *

Values for body weight were recorded immediately after completion of the final baseline morphine self-administration session. Each value is the average of responding over five sessions. *Indicates $p<0.01$ for post hoc comparisons with saline-treated animals.

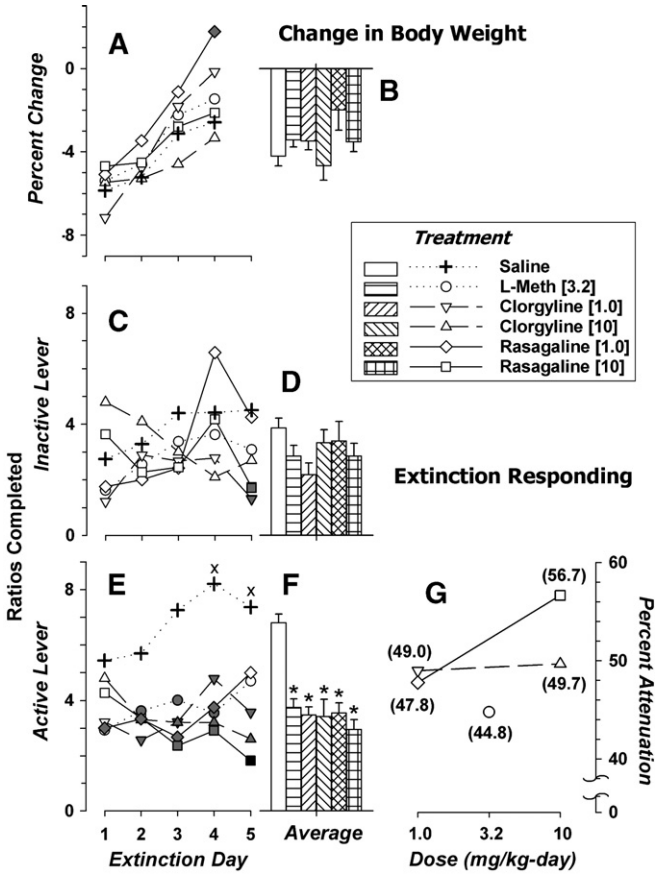

Fig. 2. Effects of secondary treatments on changes in body weight and non-reinforced responding during extinction–withdrawal. Changes in body weight, expressed as a percentage of values recorded immediately following the final baseline morphine self-administration session are shown for the first four days of extinction–withdrawal in panel A, with the average of all four values and standard error shown in panel B. Group means of ratios completed during each of five sessions for inactive and active levers are shown by panels C and E, respectively. Group means of ratios completed during each of five sessions for inactive and active levers are shown by panels C and E, respectively. Panels D and F show group means with standard error for the average number of ratios completed over all five sessions, for inactive and active levers, respectively. Panel G shows the degree to which different treatments decreased average levels of active lever responding, expressed as a percent of responding in saline-treated animals. For panels A, C, and E, gray-filled symbols correspond to $P<0.01$ for post hoc comparisons with values for saline-treated animals at the same time point. For panel E, ^{X} indicates significant post hoc comparisons with ratios completed by saline-treated animals during the first extinction session, and the black-filled symbol indicates significantly different than treatment with L-methamphetamine or 1.0 mg/kg-day of rasagiline.

baseline phase of morphine self-administration. Repeated measures ANOVA failed to show a significant effect of treatment [$F(5,92)=1.56$, p not significant], but showed significant effects for day of withdrawal [$F(3,276)=83.2$, $p<0.001$] as well as the interaction of treatment by withdrawal day [$F(15,276)=3.01$, $p<0.001$]. There was no significant effect of treatment on body weight averaged over the four days of withdrawal [panel B, $F(5,92)=1.56$, p not significant]. Post hoc comparisons showed that treatment with 1.0 mg/kg-day of rasagiline increased body weight on the fourth day of morphine withdrawal, relative to treatment with saline.

3.3. Ratios completed

For inactive lever responding (Fig. 2, panel C), repeated measures ANOVA failed to show a significant effect of secondary treatment [$F(5,92)=1.67$, p not significant], but did show significant effects for day of extinction [$F(4,368)=2.47$, $p<0.05$] and the interaction of extinction day and treatment [$F(20,368)=1.92$, $p<0.05$]. One-way ANOVA performed on the average of inactive lever responding across all extinction sessions also failed to show a significant treatment effect [panel D, $F(5,92)=1.27$, p not significant]. Post hoc comparisons showed that treatment with either 1.0 mg/kg-day of clorgyline or 10 mg/kg-day or rasagiline decreased inactive lever responding during the fifth extinction session.

For active lever responding, repeated measures ANOVA failed to show a significant effect for day of extinction [$F(4,368)=1.05$, p not significant], but did show significant effects of secondary treatment [$F(5,92)=19.8$, $p<0.001$] and the interaction of extinction day and treatment [$F(20,368)=2.18$, $p<0.01$]. ANOVA also showed a significant effect for active lever responding averaged over the entire extinction phase [panel F, $F(5,92)=20.5$, $p<0.001$]. Post hoc comparisons showed that all five active treatments attenuated active lever responding during the third and fourth extinction sessions, as well as responding averaged throughout extinction. In addition, active lever responding was decreased by treatment with 1.0 mg/kg-day of rasagiline on the first extinction session; 1.0 mg/kg-day of clorgyline, 1.0 mg/kg-day of rasagiline, or L-methamphetamine on the second extinction session; and either dose level of clorgyline or 10 mg/kg-day of rasagiline on the final extinction session. On day 5 of extinction, treatment with 10 mg/kg-day of rasagiline decreased active lever responding significantly more than what was observed after treatment with L-methamphetamine or 1.0 mg/kg-day of rasagiline. Otherwise, none of the active treatments differed from each other in the degree to which responding was attenuated.

Compared to the number of ratios completed during the first extinction session, saline-treated rats responded at higher levels during the fourth and fifth extinction sessions. Ratios completed on active levers did not change significantly over time for any of the other treatment groups.

When expressed as a percentage of the average of active lever responding across all five extinction sessions in saline-treated animals (Fig. 2, panel G), all of the treatments evaluated decreased extinction responding by more than 40%, with the greatest effect observed for 10 mg/kg-day of rasagiline which attenuated responding by more than 55%.

3.3.1. Secondary measures

For data averaged across five extinction sessions, ANOVA showed significant effects of treatment on final ratio, duration of responding, and latency [Table 2, $F(5,92)=18.5$, 4.93, and 6.69, respectively, $p<0.001$ for all values]. Post hoc comparisons showed that all treatments decreased final ratio values. Either clorgyline, administered at 1.0 or 10 mg/kg-day, or rasagiline administered at 10 mg/kg-day decreased the duration of responding. With the exception of clorgyline administered at 10 mg/kg-day, all treatments prolonged latency.

3.4. Effects of secondary treatments on reinstatement responding

3.4.1. Ratios completed

To determine if procedures for cue- and morphine-induced reinstatement increased active lever responding relative to levels that occurred at the end of the extinction phase of study, we compared the number of ratios completed during the initial 2 h of the fifth extinction session with values obtained during reinstatement. For saline-treated rats, the number of ratios completed on active levers over the first 2 h of the final extinction session, the first 2 h of reinstatement (cue-induced responding), and the initial 2 h following injection of noncontingent morphine (morphine-induced responding) were 5.84±0.49, 12.7±0.72, and 12.2±1.08, respectively. One-way ANOVA showed a significant effect for phase of study [$F(2,110)=21.0$, $p<0.001$], with post hoc comparisons indicating that procedures for either cue- or morphine-induced reinstatement increased active lever responding, relative to the final extinction session ($p<0.001$ for either measure).

The number of ratios completed on inactive and active levers over 2 h after the cue light and tone were reinstated and over 15 h following noncontingent administration of morphine are shown in Fig. 3. For inactive lever responding, ANOVA showed significant effects of treatment for both cue- and morphine-induced responding [$F(5,81)=4.10$ and 2.60, respectively, $p<0.05$ for either value]. Post hoc comparisons showed that treatment with 10 mg/kg-day of rasagiline decreased inactive lever responding during cue-induced reinstatement, but none of the other treatments produced significant effects, and no treatment produced significant effects on inactive lever responding during morphine-induced reinstatement.

For active lever responding, ANOVA showed significant effects of treatment for both cue- and morphine-induced responding [$F(5,81)=12.5$ and 6.33, respectively, $p<0.001$ for either value]. Post hoc comparisons showed that treatment with 10 mg/kg-day of either clorgyline or rasagiline decreased active lever responding during both cue- and morphine-induced reinstatement. In addition, L-methamphetamine decreased active lever responding during morphine-induced but not cue-induced reinstatement. Neither clorgyline nor rasagiline administered at 1.0 mg/kg-day modified either cue- or morphine-induced reinstatement.

When expressed as a percentage of the average number of ratios completed on active levers during cue-induced reinstatement in saline-treated animals, treatment with 10 mg/kg-day of clorgyline or rasagiline decreased responding by approximately 40 and 70%, respectively (Fig. 3, panel B). For morphine-induced reinstatement, treatment with L-methamphetamine or 10 mg/kg-day of clorgyline decreased responding by approximately 30%, with a greater than 50% reduction produced by 10 mg/kg-day of rasagiline (Fig. 3, panels B and D).

3.4.2. Secondary measures

Data obtained for secondary measures of responding during reinstatement is shown in Table 3. For cue-induced reinstatement, ANOVA showed significant effects of treatment on final ratio [$F(5,81)=10.9$, $p<0.001$], duration of responding [$F(5,81)=3.78$, $p<0.01$], and latency [$F(5,78)=3.68$, $p<0.05$]. Post hoc comparisons showed that treatment with 10 mg/kg-day of either clorgyline or rasagiline decreased final ratio while increasing latency. Rasagiline administered at 10 mg/kg-day also decreased the duration of responding.

For morphine-induced reinstatement, ANOVA showed significant effects of treatment on final ratio [$F(5,81)=6.32$, $p<0.001$] and latency [$F(5,80)=3.17$, $p<0.05$], but not duration of responding [$F(5,81)=1.63$, p not significant]. Post hoc comparisons showed that treatment with L-methamphetamine or 10 mg/kg-day of either clorgyline or rasagiline decreased final ratio. Rasagiline administered at 10 mg/kg-day also decreased latency.

3.5. Effects of secondary treatments on morphine self-administration

3.5.1. Ratios completed

Fig. 4 shows the effects of different treatments on morphine self-administration during baseline, reacquisition, and post-treatment phases of study. For inactive lever responding, repeated measures ANOVA showed a significant effect for day of treatment [$F(8,640)=7.01$ $p<0.001$], but not for either treatment [$F(5,80)=0.497$, p not significant] or the interaction of day and treatment [$F(40,640)=0.897$, p not significant]. Panel B of Fig. 4 shows inactive lever responding averaged over three days during baseline, reacquisition, and post-treatment phases. For these values repeated measures ANOVA showed a significant effect for

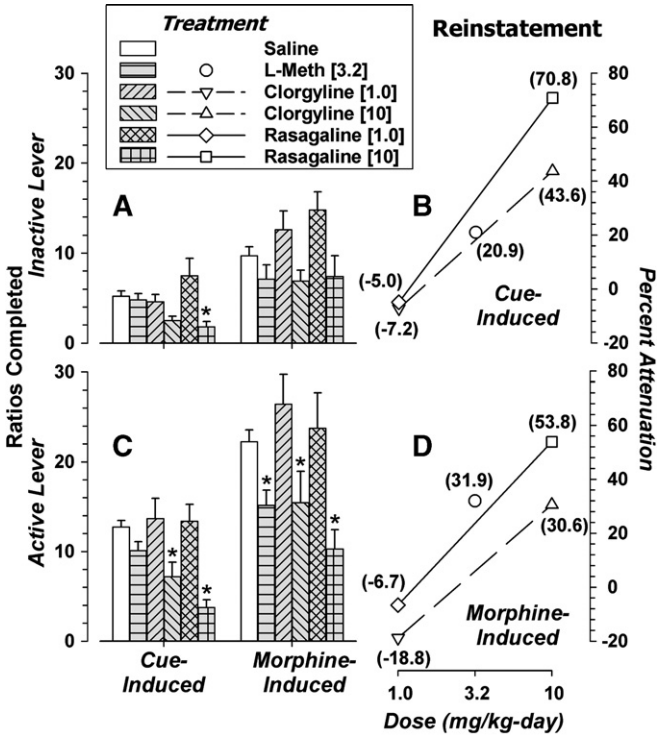

Fig. 3. Effects of secondary treatments on non-reinforced responding during reinstatement. Group means with standard error for ratios completed during cue- and morphine-induced reinstatement for inactive and active levers are shown by panels A and C, respectively. Panels B and D show the degree to which different treatments decreased active lever responding, expressed as a percent of values for saline-treated animals. For panels A and C, * corresponds to $P<0.01$ for post hoc comparisons with the number of ratios completed by saline-treated animals.

Table 3
Group means with standard error for secondary measures of non-reinforced responding during cue- and morphine-induced reinstatement

Type of reinstatement	Treatment	Dose (mg/kg-day)	Final ratio	Duration of responding (h)	Latency (h)
Cue-induced	Saline	– [35]	6.29 (0.42)	0.83 (0.04)	0.073 (0.006)
	L-Methamphetamine	3.2 [13]	4.85 (0.59)	0.72 (0.04)	0.082 (0.012)
	Clorgyline	1.0 [9]	7.22 (1.51)	0.78 (0.09)	0.062 (0.007)
		10 [11]	3.73 (0.76) *	0.61 (0.11)	0.177 (0.061) *
	Rasagiline	1.0 [8]	6.88 (1.08)	0.72 (0.04)	0.061 (0.006)
		10 [11]	2.09 (0.30) *	0.47 (0.10) *	0.217 (0.084) *
Morphine-induced	Saline	– [35]	13.03 (1.12)	11.06 (0.57)	0.563 (0.047)
	L-Methamphetamine	3.2 [13]	7.85 (0.99) *	9.07 (1.25)	0.637 (0.126)
	Clorgyline	1.0 [9]	17.44 (3.84)	10.85 (1.09)	0.444 (0.051)
		10 [11]	9.18 (2.24) *	9.25 (1.49)	1.065 (0.311)
	Rasagiline	1.0 [8]	15.25 (3.27)	11.58 (0.77)	0.698 (0.183)
		10 [11]	5.36 (1.23) *	7.98 (1.22)	1.168 (0.255) *

*Indicates $p<0.01$ for post hoc comparisons with saline-treated animals.

phase of study [$F(2,160)=9.87$, $p<0.001$], but failed to show significant effects of treatment [$F(5,80)=0.64$, p not significant] or the interaction of phase by treatment [$F(10,160)=0.875$, p not significant].

Post hoc comparisons showed that inactive lever responding collapsed across all of the six treatment groups was lower during each of the three days of the reacquisition phase of study (days 8, 9, and 10 after treatment onset), relative to values obtained during the baseline phase of study. Inactive lever responding averaged over the reacquisition phase was also lower than baseline responding.

For active lever responding, repeated measures ANOVA showed significant effects for treatment [$F(5,80)=2.39$, $p<0.05$], day after treatment onset [$F(8,640)=12.8$ $p<0.001$], and the interaction of day and treatment [$F(40,640)=2.73$, $p<0.001$]. Panel D of Fig. 4 shows active lever responding averaged over three days during baseline, reacquisition, and post-treatment phases. For these values, repeated measures ANOVA failed to show a significant effect of treatment [$F(5,80)=2.31$, p not significant], but showed significant effects for phase of study, [$F(2,160)=30.5$, $p<0.001$] and the interaction of treatment and phase [$F(10,160)=3.10$, $p<0.01$].

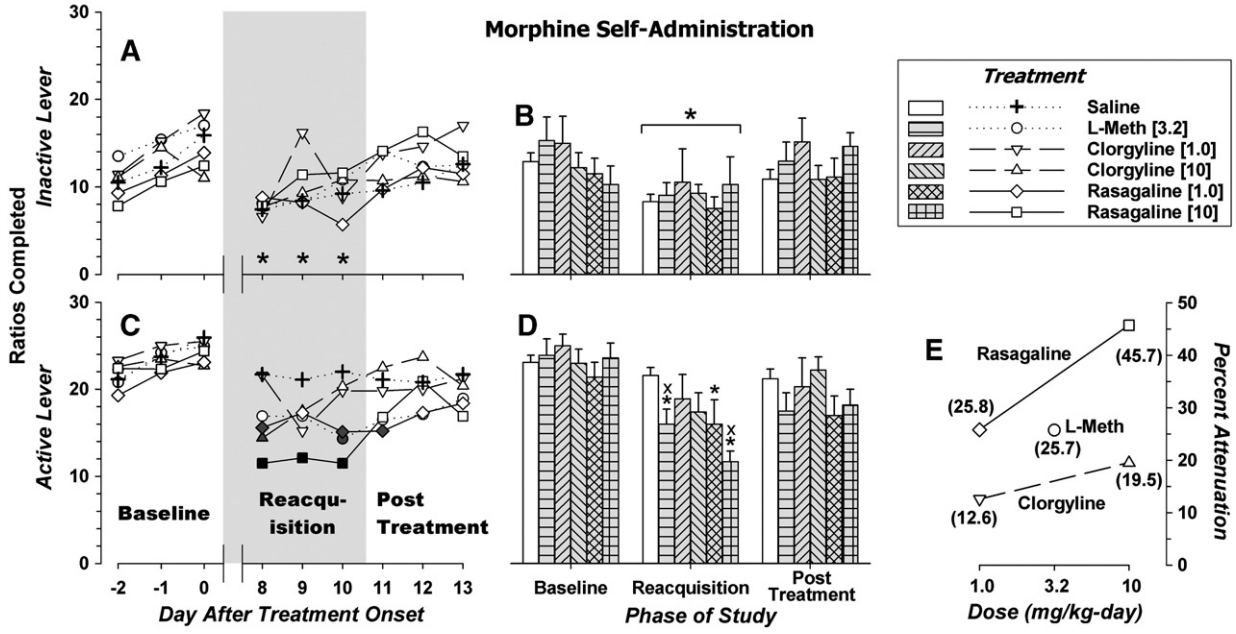

Fig. 4. Effects of secondary treatments on morphine self-administration. During morphine self-administration, each ratio completed on the active lever was followed by an injection of morphine. Group means of ratios completed for each session during baseline, reacquisition, and post-treatment phases of study are shown for inactive and active levers by panels A and C, respectively. Drug treatments were administered during the gray-shaded portion of these panels. Group means with standard error for the average number of ratios completed over three sessions during the baseline, reacquisition, and post-treatment phases of study are shown for inactive and active levers in panels B and D, respectively. Panel E shows the degree to which different treatments decreased morphine reinforcement during the reacquisition phase of study, expressed as a percent of values for saline-treated animals. For panel A, * indicates significant post hoc comparisons for data collapsed across all treatment groups, compared with the average of baseline responding. For panel C, gray-filled symbols correspond to $P<0.01$ for post hoc comparisons with data for saline-treated animals at the same time point, and black-filled symbols correspond to $P<0.01$ for comparisons with values averaged over baseline and for saline-treated animals at the same time point. For panel D, ^{X} and * indicate significant post hoc comparisons with baseline or saline-treated animals during the same phase, respectively.

Table 4
Group means with standard error shown in parentheses for secondary measures of morphine self-administration

Phase of study	Treatment	Dose [subject number]	Final ratio	Duration of responding (h)	Latency (h)
Baseline	Saline	– [40]	13.5 (0.6)	12.5 (0.6)	0.617 (0.042)
	L-Methamphetamine	3.2 [10]	14.3 (1.3)	12.7 (1.0)	0.593 (0.064)
	Clorgyline	1.0 [5]	14.8 (1.1)	12.4 (1.4)	0.566 (0.088)
		10 [11]	13.8 (1.2)	10.7 (1.4)	0.694 (0.165)
	Rasagiline	1.0 [12]	12.3 (1.3)	14.6 (1.0)	0.786 (0.105)
		10 [8]	13.8 (1.3)	15.0 (0.6)	0.704 (0.081)
Reacquisition	Saline	– [40]	12.3 (0.7)	10.9 (0.4)	0.560 (0.034)
	L-Methamphetamine	3.2 [10]	8.5 (1.1) *,x	10.7 (0.8)	0.745 (0.060)
	Clorgyline	1.0 [5]	10.6 (1.7)	12.4 (1.1)	0.976 (0.258)
		10 [11]	10.0 (1.5)	9.0 (1.0)	1.080 (0.388)
	Rasagiline	1.0 [12]	9.7 (1.6)	10.9 (1.1)	0.743 (0.075)
		10 [8]	5.9 (0.7) *,x	11.4 (0.8)	1.145 (0.066)
Post-treatment	Saline	– [40]	12.3 (0.8)	12.2 (0.6)	0.735 (0.079)
	L-Methamphetamine	3.2 [10]	9.6 (1.4)	11.8 (1.0)	0.843 (0.136)
	Clorgyline	1.0 [5]	11.7 (2.3)	14.2 (0.7)	0.846 (0.164)
		10 [11]	12.8 (1.1)	11.7 (1.3)	0.850 (0.284)
	Rasagiline	1.0 [12]	10.4 (1.6)	13.2 (1.0)	0.920 (0.143)
		10 [8]	9.9 (1.2)	13.9 (1.1)	0.860 (0.093)

[x] and * indicate significant post hoc comparisons with baseline values or saline-treated animals during the same phase, respectively.

Saline-treated animals completed approximately 20 ratios per session during baseline, reacquisition, and post-treatment phases of study, which corresponded to a daily morphine dose of 64 mg/kg-day. No significant differences were observed for morphine reinforcement in saline-treated animals during these three phases of study.

Post hoc comparisons showed that treatment with 10 mg/kg-day of rasagiline attenuated morphine reinforcement during all three days of the reacquisition phase of study (when animals were receiving active treatment), while the 1.0 mg/kg-day of dose level of rasagiline attenuated responding on the first, and third days of reacquisition as well as the initial post-treatment session (Fig. 4, panel C). Treatment with 10 mg/kg-day of clorgyline decreased morphine self-administration on the initial reacquisition session, and treatment with L-methamphetamine decreased self-administration on the third day of reacquisition. For data averaged over the reacquisition phase, treatment with L-methamphetamine or either dose of rasagiline attenuated morphine self-administration (panel D). None of the active treatments differed from each other in the degree to which responding was attenuated.

When expressed as a percentage of responding averaged over the reacquisition phase of study for saline-treated animals, neither dose level of clorgyline attenuated morphine reinforcement by more than 20% (Fig. 4, panel E, dashed line). L-methamphetamine administered at 3.2 mg/kg-day attenuated morphine reinforcement by approximately 25%. Treatment with rasagiline produced the greatest reductions in morphine reinforcement, with decreases of 25.8 and 45.7% for dose levels of 1.0 and 10 mg/kg-day, respectively (panel E, solid line).

3.5.2. Secondary measures

Effects of different treatments on secondary measures of morphine reinforcement are shown in Table 4. For final ratio, ANOVA failed to show a significant main effect of treatment [$F(5,80)=1.67$, p not significant, but showed significant effects for phase [$F(2,160)=30.9$, $p<0.001$] and the interaction of treatment and phase [$F(10,160)=2.88$, $p<0.01$]. Post hoc comparisons indicated that treatment with L-methamphetamine or 10 mg/kg-day of rasagiline decreased final ratio values during reacquisition, but did not modify values during the post-treatment phase. Significant main effects of treatment or interactions between treatment and phase were not observed for either duration or latency.

4. Discussion

In summary, for vehicle-treated animals undergoing a second cycle of morphine self-administration followed by extinction–withdrawal and reinstatement, non-reinforced responding was increased during a final extinction session and cue-induced reinstatement. Otherwise, no additional differences were observed for changes in body weight or behavior in saline-treated animals evaluated after one to four cycles of morphine self-administration and withdrawal–extinction. It should be noted that relatively few subjects were evaluated during a third and fourth cycle, decreasing our ability to detect statistically significant changes. Even so, our findings show that studies in which different treatments are administered during repeated cycles of morphine dependence and withdrawal are feasible.

Each of the active treatments administered in the present study attenuated non-reinforced responding during extinction. With the exception of clorgyline administered at 1.0 mg/kg-day, all active treatments attenuated morphine self-administration during reacquisition at one or more time points. Effects on morphine self-administration were more prolonged after treatment with rasagiline at either dose level, and treatment with rasagiline also attenuated morphine self-administration to a greater extent than clorgyline. Responding during either cue- or morphine-induced reinstatement was attenuated by either clorgyline or rasagiline administered at nonselective doses, but not by either compound administered at selective dose

levels. Treatment with L-methamphetamine did not produce significant effects on cue-induced reinstatement, but decreased non-reinforced responding during morphine-induced reinstatement. These findings show that psychostimulant treatment and MAO inhibition have complex effects on morphine reinforcement and different non-reinforced behaviors.

If administered chronically by subcutaneous injection to rats, clorgyline dose levels of 1.0 and 10 mg/kg-day inhibit MAO-A by nearly 100%, and MAO-B by approximately 30 and 75%, respectively (Felner and Waldmeier, 1979). The higher 10 mg/kg-day of clorgyline can be viewed as nonselective in that it inhibits either form of MAO by more than 50%. Effects of rasagiline administered to rats by a chronic parenteral route have not been reported. However, when administered as single oral doses in rats, rasagiline dose levels of 1.0 and 10 mg/kg provide nearly complete inhibition of MAO-B, with inhibition of MAO-A by approximately 20 and 60%, respectively (Youdim et al., 2001). The higher 10 mg/kg-day dose of rasagiline is also nonselective in that inhibition of both MAO-A and -B exceeds 50%.

Although less potent than the corresponding D-enantiomer, L-methamphetamine can stimulate release of dopamine from striatal tissue (Heikkila et al., 1975) and supports intravenous self-administration (Winger et al., 1994). Melega et al. (1999) have estimated that L-methamphetamine should be administered at a dose of approximately 4.0% of a given selegiline dose level to provide comparable striatal levels of L-methamphetamine (i.e., striatal levels after 10 mg/kg of selegiline corresponds to an L-methamphetamine dose of 0.4 mg/kg). However, when formed through metabolism of selegiline, L-methamphetamine and selegiline are likely to augment concentrations of monoamines through various mechanisms, i.e. MAO inhibition and inhibition of DA reuptake. When compared for their abilities to augment striatal dopamine, L-methamphetamine administered alone has approximately 40% of the potency of selegiline (Melega et al., 1999). That is, 4.2 mg/kg of L-methamphetamine produces an equivalent increase in striatal dopamine to 10 mg/kg of selegiline. Based on these considerations, the L-methamphetamine dose level chosen for this study is expected to produce a similar increase in striatal dopamine to that occurring after the 6.4 mg/kg-day dose level of selegiline, which has been shown to attenuate morphine reinforcement (Grasing and He, 2005).

As previously reported (Grasing et al., 2003), rats evaluated in the present study maintained stable levels of morphine self-administration under baseline conditions in which 3.2 mg/kg per injection of morphine was self-administered using a progressive ratio schedule. For saline-treated rats during extinction of morphine reinforcement, a significantly greater number of ratios were completed on active levers during the fourth and fifth daily sessions after withdrawal of morphine in the present study. Increases in responding at these time points may have been motivated by a greater severity of symptoms in the later stages of opiate withdrawal. We also found that re-exposing animals to contingent activation of the tone and cue light following the extinction period, with or without noncontingent administration of morphine, increased active lever non-reinforced responding. As observed previously (Li et al., 2003), saline-treated rats readily reacquired morphine self-administration at levels that did not differ statistically from baseline after extinction and reinstatement procedures in the present study.

Procedures in which noncontingent drug or drug-associated cues are used to produce non-reinforced responding are increasingly being utilized as animal models of substance abuse disorders (Shaham et al., 2002). Because relapse in patients typically involves conscious use of drug, reinstatement procedures have been criticized as lacking face validity (Pierce and Kumaresan, 2006). The present study along with our earlier evaluations of selegiline (Grasing et al., 2005; Grasing and He, 2005) are the only published reports we are aware of that have examined the effects of potential treatments for substance abuse disorders across different phases of study in which exposure to cues, noncontingent drug, and contingent drug are systematically varied. Our findings show that these different behaviors vary greatly in their susceptibility to modification through psychostimulant treatment or MAO inhibition.

In the present study, selective inhibition of either MAO-A or -B, as well as treatment with MAO inhibitors at nonselective doses, or treatment with L-methamphetamine, decreased non-reinforced responding during extinction of morphine reinforcement. With the exception of clorgyline administered at 1.0 mg/kg-day, exposure to any active treatment decreased reinforcement at one or more time points during reacquisition. Although we did not find statistical differences for reacquisition of morphine reinforcement by different active treatment groups, several findings are consistent with greater attenuation of morphine reinforcement following treatment with rasagiline or L-methamphetamine. Firstly, pretreatment with rasagiline produced the greatest declines in morphine reinforcement with an intermediate effect observed for L-methamphetamine (see Fig. 4, panel E). Secondly, attenuation of morphine reinforcement by either dose level of rasagiline was observed at three different time points, with effects of L-methamphetamine or 10 mg/kg-day of clorgyline observed at only one time point. Finally, of the active treatments used, only L-methamphetamine or 10 mg/kg-day of rasagiline decreased values for final ratio during reacquisition of morphine self-administration (see Table 4). Neither selective inhibition of MAO-A or -B decreased non-reinforced responding during cue- or morphine-induced reinstatement. However, either behavior was attenuated by administration of clorgyline or rasagiline at nonselective dose levels expected to produce more than 50% inhibition of both MAO isoforms. Pretreatment with L-methamphetamine attenuated non-reinforced responding during morphine-induced reinstatement, but failed to modify cue-induced responding.

It should be noted that responding during the extinction phase of study was attenuated by a wider range of treatments than was observed for reinstatement or reacquisition of morphine self-administration. Because animals were undergoing opiate withdrawal during this phase of study, responding that was motivated to avoid withdrawal symptoms may have contributed to a greater extent during extinction than for other phases of study. This difference may explain the effectiveness of a wider range of treatments in attenuating extinction responding.

Rather than an effect of one specific mechanism, results of the present study suggest that selegiline can attenuate morphine-

reinforced and non-reinforced behaviors through formation of L-methamphetamine, inhibition of MAO-B, or inhibition of MAO-A. If selegiline's actions on these behaviors occur through changes in levels of dopamine, it is likely that these mechanisms combine to have additive effects which lead to enhanced dopamine release, and additive effects on behavior. Based on studies of in striatal homogenates, it appears that dopamine is metabolized primarily by MAO-A in rats, but primarily by MAO-B in humans and other primates (Garrick and Murphy, 1980). Even so, selective inhibition of either MAO-A or -B in rats can augment both unstimulated and potassium-evoked levels of extracellular dopamine in striatum (Lamensdorf et al., 1996). Results of the present study also support an important role for MAO-B in rats for maintaining both morphine reinforcement and non-reinforced responding. Serotonin is a preferred substrate for MAO-A (Robinson, 2002). Acute treatment with morphine increases in extracellular serotonin in various brain regions, including the nucleus accumbens (Tao and Auerbach, 1995; Tao et al., 1998), and effects of MAO inhibitors on this neurotransmitter system may also have contributed to changes in behavior observed in the present study.

Combined inhibition of both MAO-A and -B with irreversible inhibitors has been shown to produce neurochemical and behavioral actions that differ from selective inhibition of either MAO isoform (Finberg and Youdim, 1983). These effects include prolongation of dopamine responses in midbrain dopaminergic neurons (Mercuri et al., 1997), greater and more sustained release of dopamine and serotonin (Celada and Artigas, 1993) and increases in locomotor activity (Green and Youdim, 1976). The ability of MAO inhibitors to suppress freezing behavior in rats, a putative measure of fear-conditioned anxiety, is much greater for either single compounds or drug combinations that inhibit both MAO-A and -B (Maki et al., 2000). Similarly, greater than two-fold attenuation of oral self-administration of ethanol can be achieved by compounds that inhibit both MAO-A and -B, or through administration of MAO inhibitors at relatively high doses that loose selectivity (Cohen et al., 1999). Combined inhibition of both MAO-A and -B is likely to have contributed to the ability of high doses of either clorgyline or rasagiline to attenuate non-reinforced responding during cue- or morphine-induced reinstatement, behaviors that were relatively resistant to modification by drug treatments.

Selegiline and related compounds irreversibly inactivate MAO through covalent binding to the flavin portion of the enzyme (Heinonen et al., 1994). Because recovery of MAO function must occur through synthesis of new enzyme, it occurs slowly with a half life of approximately 10 days in rat brain (Felner and Waldmeier, 1979). However, some behavioral effects in rats resolve within several hours or days following administration of MAO inhibitors (Maitre et al., 1976). In the present study, effects of different MAO inhibitors on morphine reinforcement did not persist for more than one day after discontinuation of treatment, indicating that behavioral effects of MAO inhibition resolve long before brain levels of enzyme have been fully restored.

Selegiline's active metabolites, L-methamphetamine and L-amphetamine, increase to peak values over the initial hour after selegiline administration and then slowly decline (Melega et al., 1999). When administered following behavioral sessions to minimize the effects of psychostimulant metabolites, 2.0 mg/kg-day of selegiline failed to modify morphine self-administration during reacquisition, but decreased non-reinforced responding during extinction (Grasing et al., 2005). In a subsequent study, morphine self-administration during reacquisition was attenuated by 6.4 mg/kg-day of selegiline administered daily prior to but not following behavioral sessions (Grasing and He, 2005). Extinction responding was decreased by selegiline administered through either schedule in this study. The lack of an effect on morphine reinforcement for selegiline administered following sessions may be explained by the time course of active metabolites or the limited duration of the behavioral effects of MAO inhibition (Maitre et al., 1976). Neither of our previous studies showed effects of selegiline on cue-induced reinstatement, again showing that this behavior is relatively resistant to MAO inhibition (Grasing et al., 2005; Grasing and He, 2005).

Inactive lever responding was decreased after treatment with either 1.0 mg/kg-day of clorgyline or 10 mg/kg-day or rasagiline during the fifth extinction session, and by treatment with 10 mg/kg-day of rasagiline during cue-induced reinstatement. None of the drug treatments administered modified inactive lever responding during the first four extinction sessions, morphine-induced reinstatement, or reacquisition of morphine self-administration. These results suggest that secondary treatments administered in the present study produced some degree of nonspecific behavioral suppression, but that this effect was not pronounced. In a previous study, 6.4 mg/kg-day of selegiline administered prior to sessions did not modify food self-administration under a progressive ratio schedule (Grasing and He, 2005), indicating that selegiline effects on morphine reinforcement occur at a dose that does not disrupt behavior maintained by a natural reinforcer.

None of the secondary measures of self-administration evaluated in the present study appeared to offer an advantage over our primary measures for identifying drug effects. With the exception of 1.0 mg/kg-day of rasagiline which did not modify final ratio during reacquisition of morphine self-administration, all treatments that decreased final ratio also decreased average values for ratios completed on active levers during a given phase. In several instances, drug treatments that attenuated active lever responding by large amounts during extinction or reinstatement also decreased either duration of responding or latency.

Declines in body weight during morphine withdrawal recovered more rapidly for animals that received 1.0 mg/kg-day of rasagiline. Aside from this effect, none of the different treatments administered in the present study modified changes in body weight during morphine withdrawal. Although treatment with dopamine agonists have been reported to attenuate the severity of opiate withdrawal (Ary et al., 1977; Walters et al., 2000), this effect was not observed for enhanced dopamine transmission produced by MAO inhibition or L-methamphetamine treatment in the present study. This finding is consistent with results from our previous study in which treatment with single 6.4 mg/kg doses of selegiline decreased

the occurrence of ptosis but did not modify other signs of precipitated withdrawal (Grasing and He, 2005).

To summarize, results of the present study show that treatment with L-methamphetamine and MAO inhibition have different effects on morphine-reinforced and non-reinforced behaviors. Non-reinforced responding during extinction can be decreased by treatment with L-methamphetamine or inhibitors for MAO-A and -B administered at either selective or nonselective dose levels. Morphine reinforcement can also be diminished by the same mechanisms, but greater and more prolonged effects are observed following treatment with rasagiline. Pretreatment with L-methamphetamine attenuated non-reinforced responding during morphine—but not cue-induced reinstatement. Neither selective inhibition of MAO-A or -B decreased non-reinforced responding during cue- or morphine-induced reinstatement, but both behaviors were attenuated by doses that inhibited both MAO isoforms. Further study is needed to determine how these preclinical measures are related to treatment response in human patients with substance abuse disorders.

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