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Comparison of some behavioral effects of *d*- and *l*-methamphetamine in adult male rats

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Abstract

Rationale—Both *l*- and *d*-methamphetamine (*l*- and *d*-MA) are more potent to release norepinephrine (NE) than dopamine, and the selectivity is greater for *l*-MA than *d*-MA. Little is known of the *in vivo* pharmacology of *l*-MA.

Objective—This study compared the effects of *l*-MA and *d*-MA in assays of nociception, behavioral disruption, and impulsivity.

Methods—Antinociceptive effects of *d*- and *l*-MA were examined in two pain assays: the warm water tail withdrawal test for acute nociception and the von Frey test in complete Freund's adjuvant (CFA)-treated rats for chronic inflammatory pain. Food-maintained operant responding and locomotion tests were used to assess generalized behavioral disruption. The 5-choice serial reaction time test (5-CSRTT) was used to assess drug-induced effects on impulse control. A delay discounting procedure was used to determine drug-induced changes in sensitivity to reinforcer delay (impulsive choice).

Results—*l*-MA (3.2–10 mg/kg) produced dose-dependent antinociception in both pain assays, decreased the rate of food-maintained operant responding, and decreased locomotor activity at a higher dose (17.8 mg/kg). In contrast, *d*-MA (0.32–3.2 mg/kg) did not produce antinociception in either assay, produced biphasic effects on response rate, and increased locomotor activity. In the 5-CSRTT, *d*-MA but not *l*-MA produced significant increase in premature responses. In the delay discounting procedure, both drugs did not affect the delay function at doses that did not increase omissions.

Conclusions—These data suggest that *d*- and *l*-MA have different behavioral profiles. Consideration should be given to these differences in future studies when *l*-MA is proposed for potential therapies.

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Conflicts of interest

The authors declare that they have no competing interests.

Keywords

l-methamphetamine; antinociception; 5-CSRTT; operant responding; locomotion; delay discounting; rats

Introduction

The abuse of methamphetamine (MA) and other psychostimulants is a serious public health problem with no effective pharmacological therapies available (Vocci and Appel, 2007). Methamphetamine abusers suffer a variety of medical, psychological, socioeconomic, and legal consequences (Karila et al., 2010) and thus effective treatments are needed. One strategy that has been among the most promising is agonist replacement therapy (Stoops and Rush, 2013; Tiitonen et al., 2007). For this approach, a therapy drug with a similar mechanism of action but lower abuse liability and other adverse effects is used to decrease intake of the abused drug (Grabowski et al., 2004).

Although commonly known as a schedule II controlled substance and indirect dopamine (DA) receptor agonist, MA actually contains two isomers which exert different physiological actions. Both the *d*-isomer (*d*-MA) and the *l*-isomer (*l*-MA) are more potent to release NE than DA in rat brain synaptosomes, but the degree of this selectivity is greater for *l*-MA (~15-fold) than for *d*-MA (~2-fold) (Rothman, et al., 2001). In *in vivo* studies, at doses of *d*- and *l*-MA which produced equal stereotypy in rats (2 and 12 mg/kg, respectively), caudate dopamine release was higher in *d*-MA than *l*-MA-treated rats while hippocampus norepinephrine release was approximately 6.5-fold higher in *l*-MA- than *d*-MA-treated rats (Kuczenski et al., 1995). Caudate serotonin was equal following both treatments. *l*-MA is used by humans in over-the-counter nasal decongestant spray, and is also a metabolite of the monoamine oxidase B inhibitor *l*-deprenyl (Melega et al., 1999). Although *l*-MA is self-administered in monkeys (Winger et al., 1994) when considered with the finding that *l*-MA was rated less preferable than racemic MA or *d*-MA by human MA abusers (Mendelson et al., 2006), *l*-MA appears to have lower abuse liability than its *d*-enantiomer in addition to several known therapeutic effects.

Interestingly, in preclinical drug discrimination studies, *l*-MA substituted for the indirect DA receptor agonists cocaine and *d*-MA with approximately 5-fold lower potency than *d*-MA (Desai and Bergman, 2010; Kohut et al., 2016), which indicates that *l*-MA produces a discriminative cue similar to psychostimulants. However, pretreatments of *l*-MA in monkeys trained to self-administer cocaine dose-dependently decreased cocaine intake (Kohut et al., 2016), suggesting that *l*-MA may have therapeutic potential as an “agonist replacement therapy” for methamphetamine and psychostimulant abuse. Unfortunately, information about the preclinical behavioral effects of *l*-MA is surprisingly sparse in the literature and further behavioral characterization is needed to better understand this compound.

This study performed a pharmacological comparison of the behavioral effects of *d*- and *l*-MA in adult male rats. Since monoaminergic drugs, particularly those with serotonergic and/or noradrenergic components, often produce antinociceptive effects in animals (Iyengar et al., 2004; Leventhal et al., 2007; Shen et al., 2013) and analgesia in humans with chronic

pain (McCleane, 2008), the warm water tail withdrawal and von Frey filament tests were used to examine the antinociceptive effects of the two compounds in acute and persistent pain modalities, respectively. Food-maintained operant responding and locomotor activity assays were used to examine the effects of these compounds on generalized behavioral disruption. Lastly, the five-choice serial reaction time task (5-CSRTT) and delay discounting procedure were used to examine the pharmacological effects of *d*- and *l*-MA on different components of impulsivity (impulse control and impulsive choice, respectively) which are often affected by psychostimulants.

Methods

Animals

Adult male (n = 118) Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing 250–350 g at experiment onset were individually housed on a 12/12-hour light/dark cycle with behavioral experiments conducted during the light period. Subjects had free access to water except during test sessions. Rats used in food-reinforced operant responding (n = 14), the 5-CSRTT (n = 8), and delay discounting (n = 8) were given restricted access to standard rodent chow, such that their bodyweights were maintained at approximately 85% of their free-feeding counterparts. Rats used in the locomotion studies (n = 47) and in the nociception studies (n = 42) always had free access to rodent chow. Rats used for the warm-water tail withdrawal assay received complete Freund's adjuvant (CFA, see 'Mechanical Nociception' below) after tail withdrawal testing was finished and were then used for mechanical nociception tests; grouping was randomized between the two assays and at least three days were interspersed between tests. Animals were maintained and experiments were conducted in accordance with guidelines of the International Association for the Study of Pain (Zimmermann, 1983) and with the *2011 Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources on Life Sciences, National Research Council, National Academy of Sciences, Washington, DC), and were approved by the Institutional Animal Care and Use Committee, University at Buffalo, the State University of New York (Buffalo, NY).

Warm Water Tail Withdrawal Assay

The warm water tail withdrawal assay was conducted as described in detail previously (Thorn et al., 2011). Briefly, rats (n = 6–7 per group) were slightly restrained and the distal five to ten cm of the tail was immersed in the water baths with different temperatures (44°, 48°, and 52 °C). Testing with different temperatures varied nonsystematically among rats and across time points. When a subject failed to remove its tail within 20 s, the experimenter removed the tail from the water and a latency of 20 s was recorded. Test sessions began with control (no drug) withdrawal latency determinations for each temperature, followed immediately by a single injection of *d*-MA, *l*-MA, or saline. Tail withdrawal latencies were measured every subsequent 15 min for each of the three temperatures with ~ 1 min between determinations. Tests ended after 75 min.

Mechanical Nociception

Mechanical hypersensitivity was induced by CFA inoculation as previously described (Li et al., 2014). Briefly, 0.1 ml of CFA (Difco, Detroit, MI) containing approximately 0.05 mg of *Mycobacterium butyricum* dissolved in paraffin oil was injected in the right hind foot pad when rats were under isoflurane anesthesia (2% isoflurane mixed with 100% oxygen). Sufficient anesthesia was determined by the loss of righting and toe-pinch reflexes. Mechanical nociception tests were conducted 1 day after CFA inoculation, which is a time point used in previous studies (Li et al., 2014; Thorn et al., 2015).

Mechanical nociception was measured using von Frey filaments consisting of calibrated filaments (1.4–26 g; North Coast Medical, Morgan Hill, CA). Rats (n = 6 per group) were placed in elevated plastic chambers with a wire mesh floor (IITC Life Science Inc., Woodland Hills, CA) and allowed to habituate prior to testing. Filaments were applied perpendicularly to the medial plantar surface of the hind paw from below the mesh floor in an ascending order of filament force, beginning with the lowest filament (1.4 g). A filament was applied until buckling occurred and maintained for approximately two seconds. Mechanical thresholds correspond to the lowest force that elicited a behavioral response (withdrawal of the hind paw) in at least two out of three applications. Filament forces greater than 26 g were not tested because forces larger than 26 g would physically elevate the non-CFA-treated paw and did not reflect pain-like behavior. Rats received a single injection of *d*-MA, *l*-MA, or saline immediately following the t = 0 min measurement and were assessed every subsequent 15 min for 75 min.

Schedule-Controlled Responding

Food-maintained operant responding experiments were conducted as previously described (An et al., 2012) in commercially available chambers located within sound-attenuating, ventilated enclosures (Coulbourn Instruments Inc., Allentown, PA). Chambers contained two levers; responses on the inactive (right) lever were recorded and had no programmed consequence. Data were collected using Graphic State 3.03 software and an interface (Coulbourn Instruments Inc.). Two groups of rats (n = 7 per group) were trained to respond for food under a fixed ratio (FR) 5 schedule. Each cycle of the five cycle program began with a 10 min pretreatment period, during which the chamber was dark and responses had no programmed consequence, followed by a 5 min response period, during which a light above the active (left) lever was illuminated and rats could receive food pellets (45 mg dustless precision pellets; Bio Serv Inc., Frenchtown, NJ) by responding on the active lever. The light was terminated after the delivery of five food pellets or after 5 min had elapsed, whichever occurred first. This procedure allows for the determination of a time course of rate-altering pharmacological effects without the confounding factor of satiation. Daily sessions consisted of five cycles, and the response rates averaged across all five cycles within a session was required to not vary by more than 20% for two days prior to each test (An et al., 2012). During testing, rats received a single injection of *d*-MA, *l*-MA or saline immediately prior to being placed into the operant chamber and the program was started.

Locomotion

Locomotor activity was monitored by an infrared motor-sensor system (AccuScan Instruments, Columbus, OH) fitted outside clear acrylic chambers (40 × 40 × 30 cm) that were cleaned before each test, as previously described (Liu et al., 2017). Before tests began, rats were exposed to at least three days of handling by the experimenter. On test day, rats ($n = 5-6$ per group) received a 15 min pretreatment of *d*-MA, *l*-MA or saline before being placed in the chambers and the locomotor activity was recorded for 20 min, a time point after which the locomotor activity of rats decreases to a level where the rats move very little (Thorn et al., 2014).

Five-Choice Serial Reaction Time Task

Eight rats were trained under a 5-CSRTT procedure according to published protocols (Bari et al., 2008) for these experiments. Sessions were conducted in standard chambers designed for the 5-CSRTT (Med Associates, Inc., Georgia, VT) within sound-attenuating and ventilated enclosures. Session programs were controlled and data were collected through a PC-interface and Med-PC IV software (Med Associates, Inc.). Briefly, sessions began with illumination of the house light and food magazine and delivery of one food pellet (45 mg dustless precision pellets; Bio Serv Inc., Frenchtown, NJ). Once the food pellet was collected, the inter-trial interval (ITI) began and only the house light was illuminated. At the end of the ITI, one of the five response holes on the chamber wall opposing the food magazine was illuminated for a brief amount of time, the stimulus duration (SD). A correct response into this hole within the limited hold (LH) period turned off the target stimulus, turned on the food magazine light, and delivered one food pellet. Once the food pellet was collected, the next ITI began. The location of the target stimulus varied pseudorandomly between trials. A response into a non-target hole was considered an incorrect response, and a failure to respond was considered an omission; each caused a 5 s timeout period in which all chamber lights were extinguished, followed by an initiation of the next ITI. Responses during the ITI prior to target stimulus presentation also caused a timeout and were considered premature responses. Responses during the timeout period reset the timeout period and were considered timeout responses. Sessions lasted for 100 total trials or 60 min, whichever occurred first.

At the onset of training, the SD, LH, and ITI were 30, 30, and 5 s, respectively. Over the course of training, the SD and LH decreased according to each rat's performance (Bari et al., 2008). Once the SD and LH reached 2.5 and 5 s, respectively, the LH remained constant for the rest of the experiment. The SD was adjusted further by 0.25 s increments until performance was maintained at a stable level of >70% accuracy and <30 omissions for three consecutive sessions, between 1.0 and 2.0 s across the rats. During testing, rats received a single injection of saline, *d*-MA, or *l*-MA 15 min prior to being placed in the chamber, were allowed to habituate for 5 min, and then the program was started; doses of each drug were administered according to two Latin-square schedules, whether *d*- or *l*-MA was tested first was counterbalanced among the eight rats. Non-drug days were interspersed between tests; rats were required to stably perform at >70% accuracy and <30 omissions for at least two sessions before the next test.

Delay Discounting

Sessions were conducted using the same apparatuses and software as for the schedule-controlled responding experiments. The program details were previously described with minor modifications (Maguire et al., 2012). Rats ($n = 8$) were first trained to press either of two levers for food. At the beginning of the session, the houselight and both lever lights were illuminated. A response on either lever turned off both lever lights and delivered one food pellet immediately followed by a re-illumination of the lever lights and another opportunity to respond. After 3 sessions in which 50 pellets were earned, the number of food pellets delivered for responding on the non-preferred lever was increased to three. The delay for large reinforcer delivery after responding was gradually increased according to each rat's performance in training sessions in which the rat was trained to alternate its responding for the small and large reinforcer on each trial. Timeout periods, in which all lights were extinguished and lever presses had no programmed consequence, were also introduced during this training period after each reinforcer delivery.

After initial training, daily sessions were conducted 7 days per week and began with a 10 min timeout period. In the final program, the houselight was illuminated at the beginning of each block and remained illuminated until the end of the block. Each block began with two forced choice trials. In the first trial, the light above the small reinforcer lever was illuminated and rats were required to respond on that lever for an immediate reinforcer. On the second forced choice trial, the light above the opposite lever was illuminated and rats were required to respond on that lever for the large reinforcer delivered after a delay, which varied (0, 5, 10, 20, or 40 s, in an ascending order) according to the current block. Ten free choice trials then began in which both lever lights were illuminated and responses on each lever maintained the same delay contingencies as those established in the most recent forced choice trials. Trials began every 60 s. If rats failed to lever press within 10 s, lever lights were extinguished and the trial was scored as an omission. Following completion of the 10 free choice trials, a 100 s timeout period occurred until the next block began. For each rat, three days of stable responding in which the delay function (% choice of large reward) did not vary by more than 20% across all blocks of delay was required before drug testing began. During testing, saline, *d*-MA, or *l*-MA were administered immediately before rats were placed in the chambers and the program initiated; doses of each drug were administered according to two Latin-square schedules, whether *d*- or *l*-MA was tested first was counterbalanced among the eight rats. Two days of stable responding was interspersed between drug test days.

Data Analysis

All graphs and statistical analyses were performed using Prism 5.0 (GraphPad Software, Inc., San Diego, CA). Rate of schedule-controlled responding is expressed as a percentage of the saline control response rate. For each cycle of a drug test, the control response rate for an individual rat was the average response rate of the corresponding cycle from three saline sessions immediately prior to the test. Log (ED₅₀) values were calculated individually for each animal and averaged across the group to generate an ED₅₀ (95% confidence limit) for each drug. Warm water tail withdrawal data were quantified in each animal as % maximal possible effect (MPE) using the following formula: % MPE = [(test latency–control

latency) / (20 s–control latency)] $\times$ 100], where the control latency was defined as the latency determined in the absence of drug at the 48°C water bath. The other water temperatures were not included because the tail withdrawal latencies were always close to the cut-off time of 20 sec (44°C) or lower than 5 sec (52°C) and were unaffected by drug treatment in the current study. Mechanical nociception data were quantified in each animal as % maximal possible effect (MPE) using the following formula: % MPE = [(post-drug value for a behavioral response (gram)–pre-drug value for a behavioral response) / (pre-manipulation [CFA] value–pre-drug value for a behavioral response) $\times$ 100)]. For these assays, the data were averaged within each group ($\pm$ SEM) and plotted as a function of time. Repeated-measures two-way ANOVA (*d*- or *l*-MA dose $\times$ time), with dose (schedule-controlled responding) or time (nociception studies) as the within-subject factor, followed by Bonferroni's *post-hoc* test was used to determine statistical significance. Locomotion data are expressed as the total distance in cm ($\pm$ SEM). The data from the 30 min time point of the tail withdrawal, mechanical nociception, and operant responding assays and the total 20 min of the locomotion assay were used to construct dose-effect curves for *d*- and *l*-MA. Repeated measures (schedule-controlled responding) or normal (nociception assays) one-way ANOVA followed by Dunnett's *post-hoc* test was used to determine statistical significance. For the 5-CSRTT, the primary measures were response accuracy (correct responses divided by total responses multiplied by 100%), omissions, premature responses, and timeout responses). Dose-effect data for each measure were analyzed using one-way repeated-measures ANOVA with dose as the within-subject factor, or normal one-way ANOVA when data points were missing due to omissions, followed by Dunnett's *post-hoc* test. For delay discounting, the percent choice for the larger reinforcer was calculated by dividing the number of large rewards chosen by the total number of rewards made in each block and multiplying by 100%. Choice data were analyzed by two-way repeated measures ANOVA (*d*- or *l*-MA dose $\times$ delay), with treatment dose and time entered as the within-subject factor, followed by Bonferroni's *post-hoc* test. Total omissions were calculated by summing omissions that occurred in all blocks of a session, and were analyzed by one-way repeated measures ANOVA followed by Dunnett's *post-hoc* test. Indifference points (the delay at which the large reward was chosen 50% of the time) were calculated for each subject using a linear regression model to determine a group mean (95% CL) indifference point. $P < 0.05$ was considered significant for all statistical analyses.

Drugs

Drugs used in this study were *d*-MA hydrochloride (Sigma-Aldrich, St. Louis, Missouri, USA) and *l*-MA hydrochloride (provided by Dr. Bruce Blough, RTI International). Both drugs were dissolved in 0.9% saline and administered intraperitoneally. Drug doses are expressed as the salt form and injection volumes were 1–2 ml/kg.

Results

Under control conditions the average latency for rats to remove their tails from the 48°C water bath was 6.70 ± 0.28 s. When vehicle was administered, the latency did not significantly change over a 75 min period according to one-way ANOVA ($F(5, 30) = 1.84$, $p > 0.05$). When treated with *l*-MA, two-way repeated measures ANOVA revealed significant

main effects of *l*-MA dose $\times$ time interaction ($F(15, 115) = 2.35, p < 0.01$), *l*-MA dose ($F(3, 115) = 3.95, p < 0.05$), and time ($F(5, 115) = 6.90, p < 0.0001$). Bonferroni's *post hoc* tests revealed significant differences in withdrawal latency at time 30 min for the 5.6 and 10 mg/kg *l*-MA treatment groups as compared to vehicle (top left panel, Fig. 1). When treated with *d*-MA, two-way repeated measures ANOVA revealed no significant effects of *d*-MA treatment (top right panel, Fig. 1).

Under control conditions the average paw withdrawal threshold for CFA-treated rats was 4.33 ± 0.33 grams. When administered with vehicle, the withdrawal threshold did not change over the course of 75 min based on one-way ANOVA ($F(5, 30) = 0.65, p > 0.05$). When treated with *l*-MA, two-way repeated measures ANOVA revealed significant main effects of *l*-MA dose $\times$ time interaction ($F(15, 100) = 6.41, p < 0.0001$), *l*-MA dose ($F(3, 100) = 7.49, p < 0.01$), and time ($F(5, 100) = 23.76, p < 0.0001$). Bonferroni's *post hoc* tests revealed significant differences in withdrawal threshold at 30 and 45 min for the 5.6 mg/kg *l*-MA treatment group and at 15–45 min for the 10 mg/kg *l*-MA treatment group as compared to vehicle. When treated with *d*-MA, two-way repeated measures ANOVA revealed no significant effects of *d*-MA treatment (middle right panel, Fig. 1)

The average rate of operant responding for all cycles on the three days preceding the test was 0.53 ± 0.02 responses per second. When vehicle was administered the response rate for each rat, expressed as a percentage of its response rate on the three preceding control days, was $110.95 \pm 14.23\%$ for the *l*-MA group and $98.72 \pm 6.14\%$ for the *d*-MA group and did not significantly change over the five response periods (one-way ANOVA, *l*-MA group: $F(4, 30) = 0.30, p > 0.05$, *d*-MA group: $F(4, 30) = 0.64, p > 0.05$). Two-way repeated measures ANOVA revealed that *l*-MA dose-dependently reduced responding rate with significant main effects of *l*-MA dose $\times$ time interaction ($F(12, 90) = 2.52, p < 0.01$), *l*-MA dose ($F(3, 90) = 11.16, p < 0.0001$), and time ($F(4, 90) = 8.24, p < 0.001$). Bonferroni's *post hoc* test revealed significant differences compared to vehicle at 15–45 min for the 10 mg/kg *l*-MA treatment group (bottom left panel, Fig. 1). Two-way repeated measures ANOVA revealed that *d*-MA dose-dependently altered the responding rate with a significant main effect of *d*-MA treatment ($F(3, 90) = 26.38, p < 0.0001$) but not time or interaction. Bonferroni's *post hoc* test revealed significant differences compared to vehicle at 30 min for the 3.2 mg/kg *d*-MA treatment group (bottom right panel, Fig. 1). The ED₅₀ (95% CL) values for *l*-MA and *d*-MA to reduce response rate were 6.62 (5.08, 8.61) mg/kg and 2.59 (2.03, 3.32) mg/kg, respectively. Thus, *d*-MA was 2.6-fold more potent than *l*-MA.

The data from the warm water tail withdrawal, mechanical nociception, and response rate assays at time 30 min were used to construct dose-effect curves for *d*- and *l*-MA. For locomotion, the total locomotor activity in the 20 min test period was used to construct the dose-effect curve (Fig. 2). In the tail withdrawal assay, *l*-MA dose-dependently increased the withdrawal latency when one-way ANOVA was performed ($F(3, 23) = 3.28, p < 0.05$) whereas *d*-MA produced no significant effect. In the test of mechanical nociception, *l*-MA dose-dependently increased the paw withdrawal threshold ($F(3, 20) = 9.09, p < 0.001$) whereas *d*-MA produced no significant effects. In the schedule-controlled responding assay, one-way repeated measures ANOVA showed that *l*-MA dose-dependently decreased the response rate ($F(3, 24) = 7.24, p < 0.01$), and *d*-MA produced a dose-dependent biphasic

alteration of the response rate ($F(3, 24) = 24.51, p < 0.0001$). In the locomotion assay, *l*-MA dose-dependently decreased locomotor activity ($F(4, 24) = 4.96, p < 0.01$) whereas *d*-MA dose-dependently increased locomotor activity ($F(3, 20) = 12.55, p < 0.0001$). For all assays, results of Dunnett's *post hoc* test are shown as asterisks in Fig. 2.

In the 5-CSRTT (Fig.3), one way ANOVAs revealed that *l*-MA dose-dependently increased omissions ($F(3, 28) = 29.47, p < 0.0001$) but did not significantly affect response accuracy, premature responses, or timeout responses. *d*-MA dose-dependently increased both omissions ($F(4, 35) = 52.83, p < 0.0001$) and premature responses ($F(4, 35) = 8.65, p < 0.0001$) but did not significantly affect response accuracy or timeout responses. Results of Dunnett's *post hoc* test are shown as asterisks in Fig. 3.

In the delay discounting procedure (Fig. 4), rats chose the large reward in > 90% of free choice trials when rewards were delivered with no delay in the vehicle test established before each drug was tested (upper panels, circles above "0"). One-way repeated measures ANOVA revealed that percent choice of large reward decreased as a function of delay of large reward delivery ("V" test on *l*-MA graph: $F(4, 35) = 15.74, p < 0.0001$; "V" test on *d*-MA graph: $F(4, 35) = 30.06, p < 0.0001$). Two-way repeated measures ANOVA revealed that *l*-MA produced dose-dependent downward shifts of the delay function with main effects of *l*-MA treatment ($F(3, 105) = 6.50, p < 0.001$) and delay ($F(4, 105) = 17.08, p < 0.0001$). Bonferroni's *post hoc* test revealed significant differences between 10 mg/kg *l*-MA and vehicle in the 0 and 5 s delay blocks. However, indifference points did not significantly differ among different doses of *l*-MA treatment or between *l* and *d*-MA treatments. Indifference points (95% CL) were 14.5 (7.9, 26.4), 14.8 (10.6, 20.6), and 7.4 (3.8, 14.5) s for V, 3.2, and 5.6 mg/kg *l*-MA, respectively. The indifference point for 10 mg/kg *l*-MA was not calculated due to a high number of omissions. In contrast, two-way repeated measures ANOVA revealed no significant effects of *d*-MA treatment. Likewise, *d*-MA did not alter the indifference point. Indifference points (95% CL) were 11.2 (6.4, 19.7), 11.6 (6.5, 20.9), and 13.8 (9.6, 19.6) for V, 0.32, and 1.0 mg/kg *d*-MA, respectively. The indifference point for 1.0 mg/kg *d*-MA was not calculated due to a high number of omissions. One-way repeated measures ANOVA indicated that both compounds dose-dependently increased the total number of omissions that occurred across the session (*l*-MA: $F(3, 28) = 8.74, p < 0.001$; *d*-MA: $F(3, 28) = 3.55, p < 0.05$). Results of Dunnett's *post hoc* test are shown as asterisks in Fig. 4.

Discussion

The primary findings of the current study were that *l*-MA dose-dependently increased antinociception in both acute and chronic pain assays in rats. Within the same dose range as these assays of nociception, *l*-MA dose-dependently reduced the rate of food-maintained operant responding but did not alter locomotor activity. In contrast, *d*-MA did not produce significant antinociceptive effects in the same assays, but instead produced biphasic effects on operant responding rate and dose-dependently increased locomotor activity. When tested in the 5-CSRTT, *l*-MA and *d*-MA each dose-dependently increased omissions but only *d*-MA significantly increased premature responses, which are indicative of impulsivity. Finally,

when tested in the delay discounting procedure, both drugs dose-dependently increased omissions but did not affect the delay function at doses that did not increase omissions.

Methamphetamine abuse is a significant public health problem with no current pharmacotherapies, thus treatments are desperately needed. Among therapeutic strategies, agonist replacement therapy has delivered some of the most promising results (Grabowski et al., 2004; Stoops and Rush, 2013; Tiihonen et al., 2007). Interestingly, the *L*-enantiomer of MA has displayed potential utility as agonist replacement therapy in animal models (Desai and Bergman, 2010; Kohut et al., 2016). Although *L*-MA is already used in humans as a nasal decongestant, its behavioral effects have not been systematically characterized in the existing literature which currently limits its potential for further application in humans. Therefore, to better understand its effects, we first investigated the antinociceptive effects of *L*-MA in rats, since *L*-MA is more selective in releasing NE than DA as compared to *D*-MA and noradrenergic drugs often induce analgesia in chronic pain patients (Atkinson et al., 1999; O'Connor AB et al., 2007). In the acute and chronic pain assays, the effects of *L*-MA were similar to those of direct (e.g. clonidine) (Stone et al., 2014; Zhang et al., 2011) and indirect (e.g., desipramine) (Iyengar et al., 2004; Rojas-Corrales et al., 2003) NE agonists which, similar to other monoaminergic agents, often produce more robust effects in chronic than acute pain assays. Indirect 5-HT and DA agonists have also been reported to produce antinociception (Lin et al., 1989; Wang et al., 1999), and thus the effects of *L*-MA may be due to the efflux of more than one monoamine, based on the results of an *in vivo* microdialysis study (Kuczenski et al., 1995). Indeed, the combination reuptake inhibitors appear to produce more effective analgesia than the selective reuptake inhibitors (Shen et al., 2013), thus future investigations with selective receptor antagonists will clarify the relative neurotransmitter contributions to the antinociception found in the current study. It should be noted that the overall antinociceptive effects of *L*-MA was quite weak as compared to other analgesics such as oxycodone (Thorn, et al., 2015). Therefore, the potential clinical utility of *L*-MA for pain management would be limited. In contrast, no antinociceptive effects were observed following *D*-MA administration.

Since the ability of the animals to express pain-stimulated behaviors (e.g., tail or paw withdrawal) depends upon normal motoric function, it was possible that the observed antinociceptive effects were due to motor disruption. Additionally, *D*-MA produced known psychostimulant-like effects which include hyperactivity (Li et al., 2013; McGuire et al., 2011). Thus, we tested the ability of *D*- and *L*-MA to produce generalized behavioral disruption in two behavioral assays. First, we observed that *L*-MA produced a dose-dependent reduction on the rate of food-maintained operant responding, the time course of which appears to inversely correlate with the antinociceptive effects. In the locomotion assay, although the same doses of *L*-MA did not suppress the locomotor activity in rats, a larger dose (17.8 mg/kg) significantly reduced the locomotor activity. Combined, it seems that the doses for reducing antinociception and producing behavioral suppression are quite similar or slightly different (0.25-log unit dose). Therefore, the contribution of nonspecific behavioral suppression to the observed antinociceptive effects of *L*-MA cannot be completely ruled out.

Since DA plays a prominent role in the discriminative stimulus effects of indirect DA agonists (Kleven et al., 1990; Thorn et al., 2016), and since *l*-MA substituted for cocaine and *d*-MA in drug discrimination studies (Desai and Bergman, 2010; Kohut et al., 2016), it was possible that *l*-MA induces cocaine- or amphetamine-like effects on impulsivity. Impulsivity is a multifactorial phenomenon (Evenden, 1999; Winstanley et al., 2006), thus we used two behavioral tasks which measure distinct components. The 5-CSRTT assesses a subject's ability to withhold premature responses (impulse control) (Carli et al., 1983; Robbins, 2002), whereas delay discounting assesses a subject's preference for a small immediate reward over a large delayed reward (impulsive choice) (de Wit, 2009; Perry and Carroll, 2008). Indirect DA agonists such as *d*-amphetamine and cocaine produce well-described increases in premature responding in the 5-CSRTT, indicative of deficient impulse control (Baarendse and Vanderschuren, 2012; Fletcher et al., 2011; Terry et al., 2014; van Gaalen et al., 2006). Interestingly, these drugs often increase preference for the larger reward, thereby producing upward shifts of the delay function, in the delay discounting task (Baarendse and Vanderschuren, 2012; Maguire et al., 2014; Paterson et al., 2012). This is indicative of reduced impulsive choice although opposite results have also been found (Evenden and Ryan, 1996). Surprisingly, *d*-MA appears to not have been previously tested in rats in either of these behavioral assays. However, variations of the delay discounting task, in which reward size is progressively altered instead of reward delay, suggest that acute *d*-MA treatment also reduces impulsive choice (Leite-Almeida et al., 2013; Richards et al., 1999). In the present study, *d*-MA produced significant increase in premature responses while *l*-MA did not, consistent with the findings of previous studies investigating NE-selective compounds (Baarendse and Vanderschuren, 2012; Paterson et al., 2012). Since *d*-MA had not been well-characterized in the 5-CSRTT previously and since *d*-MA is a psychomotor stimulant, our data suggest that the effects of *d*-MA were specific to impulsive responding as response accuracy remained high and generalized increases in nose-poke behavior (timeout responses) did not occur. In the delay-discounting assay, both *l*-MA and *d*-MA did not significantly alter the discounting functions or the indifference points up to the largest dose that did not significantly increase omissions. Thus, in the present study both MA enantiomers did not increase impulsive choice. These results are consistent with previous delay discounting studies using NE-selective agents which found no change in the delay function (Baarendse and Vanderschuren, 2012; Paterson et al., 2012). In any case, *l*-MA produced results on 5-CSRTT distinct from *d*-MA. In both the 5-CSRTT and delay discounting procedures, each drug increased omissions at sufficiently large doses.

This study and others clearly showed different behavioral profiles between *d*-MA and *l*-MA. Similar stereo-selectivity profiles have been reported with other amphetamines. For example, the two enantiomers of amphetamine (*l*- and *d*-amphetamine) maintained intravenous self-administration behavior at the similar rate with *l*-amphetamine being ~6-fold less potent than *d*-amphetamine (Risner, 1975). In rats discriminating 1.0 mg/kg *d*-amphetamine, *l*-amphetamine fully substituted for *d*-amphetamine (Yasar et al., 1993). In the locomotion assay, *d*-amphetamine produced hyperactivity while *l*-amphetamine did not (Wiig et al., 2009). In this context, the observed effects in this study were consistent with existing literature on other amphetamines such as amphetamine. Importantly, both *d*-MA and *l*-MA reduced cocaine intake in monkeys, which did not show stereo-selectivity (Negus

et al., 2007; Kohut et al., 2016). Given that *l*-MA did not produce behavioral effects which could be considered as “adverse” (e.g., psychomotor stimulation, impulsivity) as an agonist replacement therapy drug for psychostimulant abuse, *l*-MA might be more beneficial than *d*-MA. It is unclear why *d*-MA and *l*-MA demonstrate similar behavioral effects in some (e.g., drug discrimination, attenuation of cocaine self-administration) but different effects in other (e.g., locomotion, 5-CSRTT) assays. One possibility could be due to the higher selectivity on NE release as compared to DA release for *l*-MA than for *d*-MA.

In summary, this study found that the *l*-MA produced dose-dependent antinociception in acute and persistent pain assays as well as reductions in food-maintained operant responding rate and locomotion. *l*-MA did not increase premature responses in the 5-CSRTT and did not alter the delay function at doses that did not increase omissions. In comparison, *d*-MA produced a drastically different behavioral pattern. *d*-MA did not produce antinociception in either assay but increased both food-maintained operant responding rate and locomotor activity. *d*-MA increased premature responses in the 5-CSRTT but did not affect the delay function. When considered with other preclinical and clinical information, these data suggest that future studies in the exploration of using racemic MA or its enantiomers for potential clinical therapy should consider these differences.

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Fig. 1. Effects of *l*-MA (*left*) or *d*-MA (*right*) in warm water tail withdrawal (*top*), von Frey (*middle*), and schedule-controlled responding (*bottom*) assays in rats. Ordinates are tail withdrawal latency in seconds, paw withdrawal threshold in grams, and percentage of control responding, respectively. Abscissa are the time after drug or vehicle administration.

Fig. 2.

Dose-effect functions for *l*-MA- or *d*-MA-induced changes in behavior. Each data point represents the data collected at the 30 min time point in Figure 1, except for locomotor activity, which represents the sum of the data from the total 20 min test period. Ordinates are tail withdrawal latency in seconds (*upper left*), paw withdrawal threshold in grams (*upper right*), percentage of control responding (*lower left*), and locomotor units in cm (*lower right*). Abscissa are the doses of *l*-MA; symbols above “V” represent data collected after vehicle administration. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ as compared to vehicle.

Fig. 3. *d*-MA (filled symbols) and *l*-MA (open symbols) dose-effect curves for accuracy (*upper left*), omissions (*upper right*), premature responses (*lower left*), and timeout responses (*lower right*) in the 5-CSRTT. Ordinates are percentage of response accuracy (*upper left*), correct response latency in seconds (*lower right*), or number of specified responses (*remaining panels*). Abcissa are the doses of drug; symbols above “V” represent data collected after vehicle administration. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ as compared to vehicle.

Fig. 4.

Effects of *l*-MA (*left*) and *d*-MA (*right*) on delay functions in the delay discounting procedure. Ordinates are percent choice of the larger reinforcer (*upper panels*) or number of total omissions per session (*bottom panels*). Abcissa are the delay to delivery of the larger reinforcer. * $P < 0.05$, *** $P < 0.001$ as compared to vehicle.