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S(+)- and *R*(−)*N*-Methyl-1-(3,4-methylenedioxyphenyl)-2-aminopropane (MDMA) as discriminative stimuli: Effect of cocaine

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Abstract

Racemic *N*-methyl-1-(3,4-methylenedioxyphenyl)-2-aminopropane (methylenedioxymethamphetamine, MDMA), a central stimulant and empathogenic agent, and cocaine are drugs of abuse that function as training drugs in drug discrimination studies. In tests of stimulus generalization (substitution), asymmetric generalization occurs between the two agents: a (±)MDMA stimulus generalized to cocaine, but a cocaine stimulus did not generalize to (±)MDMA. A possible explanation may be found, at least in part, in the stimulus effects of the optical isomers of MDMA. In the present study, groups of male Sprague–Dawley rats were trained to discriminate either *S*(+)MDMA (training dose = 1.5 mg/kg, i.p.; $n = 10$; $ED_{50} = 0.6$ mg/kg) or *R*(−)MDMA (training dose = 1.75 mg/kg, i.p.; $n = 7$; $ED_{50} = 0.4$ mg/kg) from saline vehicle using a VI-15s schedule of reinforcement. Tests of stimulus generalization with cocaine were conducted in each of the two groups. Cocaine only partially substituted for the *S*(+)MDMA stimulus (maximum = 39% drug-appropriate responding), and various doses of cocaine did not enhance the percent drug-appropriate responding produced by a low dose (0.5 mg/kg) of *S*(+)MDMA. In contrast, the *R*(−)MDMA stimulus generalized completely to cocaine ($ED_{50} = 1.3$ mg/kg). Taken together with an earlier report that a (±)MDMA stimulus generalizes to cocaine, it would seem that the stimulus actions of cocaine might share greater similarity with *R*(−)MDMA than with *S*(+)MDMA.

Keywords: MDMA; 3,4-Methylenedioxymethamphetamine; *N*-Methyl-3,4-methylenedioxyamphetamine; Cocaine; Drug discrimination; Drug abuse; MDMA isomers; MDMA synthesis

N-Methyl-1-(3,4-methylenedioxyphenyl)-2-aminopropane (3,4-methylenedioxymethamphetamine or MDMA; also known as "Ecstasy" or "XTC") is an acknowledged drug of abuse with central stimulant and empathogenic character that began gaining popularity in the early 1980s (Green et al., 2003). Structurally, MDMA possesses a phenylalkylamine chemical skeleton and bears compositional similarity to the phenylalkylamine hallucinogen DOM [1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane] and the phenylalkylamine stimulant amphetamine. On the basis of established structure–activity relationships (reviewed: Glennon, 1989), it was initially expected that MDMA would pharmacologically act more like a stimulant than a hallucinogenic phenylalkylamine. This forecast was realized in drug discrimination studies that showed a (+)amphetamine, but not a DOM, training stimulus generalized to MDMA in rats (Glennon et al., 1982; Glennon and Young, 1984). Similarly, rats trained to discriminate MDMA from saline vehicle recognized *S*(+)amphetamine, but not DOM (Glennon et al., 1986; Glennon, 1989; Oberlender and Nichols, 1988). *S*(+)Amphetamine stimulus generalization to MDMA also has been shown in other species such as pigeon and monkey (Evans and, Johanson 1986; Kamien et al., 1986). There are, however, some studies that report less than complete substitution of MDMA in amphetamine-trained animals (Oberlender and Nichols, 1988; Schechter, 1987). The apparent inconsistencies might reflect procedural differences in schedules of reinforcement, training doses and/or testing times. Finally, even though there appears to be an overlap in the stimulus properties of MDMA and (+)amphetamine regardless of which agent is used as training drug, there also must be a significant difference between their

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stimulus effects because animals can be trained to discriminate between MDMA, (+)amphetamine and saline in a three-choice task (Goodwin and Baker, 2000) demonstrating that their stimulus effects are distinguishable.

Apart from any other action(s) that MDMA might produce (e.g., 5-HT releasing action, empathogenic effect), it does seem to act, at least in part, as a central stimulant. With regard to amphetamine-like stimulant actions, *S*(+)isomers of stimulant phenylalkylamines are generally several times more potent than their *R*(−)enantiomers; for example, *S*(+)amphetamine (i.e., dextroamphetamine) is a more potent central stimulant than *R*(−)amphetamine (Nichols, 1994). Likewise, *S*(+)amphetamine is several-fold more potent than *R*(−)amphetamine as a discriminative stimulus (Young and Glennon, 1986). The optical isomers of MDMA have been examined in drug discrimination studies. A DOM stimulus did not generalize to either optical isomer of MDMA (Glennon et al., 1982; Glennon and Young, 1984); stimulus generalization occurred to *S*(+)MDMA but not to *R*(−)MDMA in (+)amphetamine-trained animals (Glennon et al., 1982; Glennon and Young, 1984). Another study, however, reported that neither isomer of MDMA fully substituted for a (+)amphetamine stimulus (Oberlender and Nichols, 1988). Both *S*(+)MDMA and *R*(−)MDMA substituted for an (±)MDMA stimulus (Glennon et al., 1986; Glennon, 1989; Oberlender and Nichols, 1988; Schechter, 1987) indicating some commonality of stimulus effects. Overall, then, the results of several drug discrimination studies suggest that similarities might exist between the stimulus effects of (+)amphetamine and MDMA even though, in some instances, substitution was asymmetrical. In animals trained to discriminate (+)amphetamine from either the hallucinogen mescaline or LSD from saline in a three-choice procedure, the MDMA isomers failed to substitute for either the stimulant or the hallucinogen stimulus (Baker and Taylor, 1997). However, given that specific drug-pairings in a three-lever choice procedure can occasionally influence lever selection and because results can be different from those obtained in a corresponding two-lever procedure, three-choice studies can sometimes be difficult to interpret (Appel et al., 1999). In any event, both MDMA isomers substitute for (±)MDMA, and *S*(+)MDMA has been consistently demonstrated to be several times more potent than its *R*(−)enantiomer.

MDMA and/or one, or both, of its optical isomers have been shown to produce, in a variety of other in vivo assays, behavioral effects commonly associated with central stimulants. Both MDMA isomers increased locomotor activity in mice, and the *S*(+)isomer was at least 10 times more potent than *R*(−)MDMA in doubling baseline locomotor activity (Glennon et al., 1988). More recent studies have also reported that *S*(+)MDMA produces hyperlocomotion in rodents (Herin et al., 2005; Russell and Laverty, 2001) or is more potent than its *R*(−)enantiomer in this regard (Fantegrossi et al., 2003). Other behavioral studies have demonstrated, too, the amphetamine-like nature (e.g., hyperthermia, self-administration and aggregated toxicity) of MDMA and its isomers in rats, mice, rabbits or monkeys (Anderson et al., 1978; Beardsley et al., 1986; Daniela et al., 2004; Fantegrossi et al., 2002, 2003, 2004, 2005; Glennon, 1992; Glennon et al., 1987; Green et al., 2003; Herin et al., 2005; Hiramatsu et al., 1989; Lamb and Griffiths, 1987; Lile et al., 2005). It should be noted that there might be more of a serotonergic component of action in the effects produced by MDMA than with psychostimulants (e.g., Fantegrossi et al., 2003, 2005), and this could account for observed differences. Nevertheless, the general overall conclusions that can be derived from these studies are that MDMA possesses some amphetaminergic character and that the *S*(+)isomer is, typically, at least several times more potent than *R*(−)MDMA. In humans, (±)MDMA has been shown to produce amphetamine-like subjective effects (Tancer and Johanson, 2001; Vollenweider et al., 1998). Moreover, Anderson et al. (1978) concluded on the basis of human studies that *S*(+)MDMA accounts for most of the sensory and interpretive actions (as well as toxic and other side effects) associated with racemic MDMA.

Despite any possible similarities, the behavioral actions of MDMA are clearly distinguishable from those of psychostimulants (Goodwin and Baker, 2000; Green et al., 2003; Tancer and Johanson, 2001; Vollenweider et al., 1998). Nevertheless, the discriminative stimulus effects of MDMA and cocaine have generated some distinctive laboratory results. For example, cocaine has been found to substitute for a (±)MDMA stimulus, but MDMA (and its isomers) failed to substitute for a cocaine stimulus (Khorana et al., 2004). In addition, the effects of cocaine have been evaluated in a study that used the optical isomers of MDMA as training stimuli (Baker et al., 1995). In rats trained to discriminate 1.25 mg/kg of *S*(+)MDMA or 3.5 mg/kg of *R*(−)MDMA from saline using an FR 20 schedule of reinforcement, cocaine engendered a maximum of about 40% *S*(+)MDMA-appropriate responding (at a cocaine dose of 22.5 mg/kg) and 61% *R*(−)MDMA-appropriate responding (at a dose of 20 mg/kg) (Baker et al., 1995). Because training dose and methodological differences have been shown to qualitatively and/or quantitatively influence the results of substitution studies when animals are trained to discriminate MDMA optical isomers (Baker et al., 1995, 1997), the purpose of the present study was to re-investigate the effects of cocaine in rats trained to discriminate either *S*(+)MDMA or *R*(−)MDMA from saline vehicle. The initial goal of this study was to train rats to discriminate isomer doses that would be reflective of what is encountered with the racemate. That is, the intent was not to train animals to discriminate the lowest possible doses of MDMA isomers; rather, it was to train rats to discriminate equivalent doses of *S*(+)MDMA and *R*(−)MDMA because the racemate is a mixture of equal amounts of the two isomers. Because (±)MDMA training doses of from 1.0 to 1.5 mg/kg have been most often employed previously, the studies were begun with training doses of 0.75 mg/kg for each MDMA isomer.

1. Materials and methods

Seventeen male Sprague–Dawley rats (Charles River Laboratories), weighing 250–300 g at the beginning of the study, were trained to discriminate (15-min presession injection interval) doses of *S*(+)MDMA ($n=10$) or *R*(−)MDMA ($n=7$)

from saline vehicle (sterile 0.9% saline) under a variable interval 15-s schedule of reinforcement for sweetened condensed milk reward using standard two-lever Coulbourn Instruments operant equipment as previously described for (±)MDMA (Glennon and Young, 2000). Animal studies were conducted under an approved Institutional Animal Care and Use Committee protocol.

In brief, animals were food-restricted to maintain body weights at approximately 80% of free-feeding weight but were allowed access to water ad lib in their individual home cages. Daily training sessions were conducted with the training dose of the training drugs or saline. For approximately half the animals, the right lever was designated as the drug-appropriate lever, whereas the situation was reversed for the remainder of the animals. Learning was assessed every fifth day during an initial 2.5-min non-reinforced (extinction) session followed by a 12.5-min training session. Data collected during the extinction session included response rate (i.e., responses per minute) and number of responses on the drug-appropriate lever (expressed as a percent of total responses). Animals were not used in the subsequent stimulus generalization studies until they consistently made $\geq$80% of their responses on the drug-appropriate lever after administration of training drug and $\leq$20% of their responses on the same drug-appropriate lever after administration of saline. During the testing (i.e., stimulus generalization) phase of the study, maintenance of the training-drug/saline discrimination was ensured by continuation of the training sessions on a daily basis (except on a generalization test day). On 1 of the 2 days before a generalization test, approximately half the animals would receive the training dose of training drug and the remainder would receive saline; after a 2.5-min extinction session, training was continued for 12.5 min. Animals not meeting the original training criteria during the extinction session were excluded from the subsequent generalization test session. During the investigations of stimulus generalization, test sessions were interposed among the training sessions. The animals were allowed 2.5 min to respond under non-reinforcement conditions. An odd number of training sessions (usually 5) separated any two generalization test sessions. Doses of test drugs were administered to the groups of rats in a random order using a 15-min presession injection interval. Stimulus generalization was considered to have occurred when the animals, after a given dose of drug, made $\geq$80% of their responses (group mean) on the training drug-appropriate lever. Animals making fewer than five total responses during the 2.5-min extinction session were considered to be behaviorally disrupted and were considered as having failed to meet the testing criteria. Percent drug-appropriate responding and response rate data refer only to animals making $\geq$5 responses during the extinction session (Young and Glennon, 1986) unless otherwise noted. If >50% of the animals were disrupted following administration of a given drug dose, data were not plotted. Where applicable, an ED_{50} dose was calculated by the method of Finney (1952). The ED_{50} doses represent the drug dose where animals would be expected to make 50% of their responses on the drug-appropriate lever.

1.1. Drugs

Racemic *N*-methyl-1-(3,4-methylenedioxyphenyl)-2-aminopropne HCl (MDMA) was obtained as a gift from NIDA. The isomers of MDMA were synthesized in our laboratory using a new procedure described below. All drugs were administered via the intraperitoneal (i.p.) route 15 min prior to testing unless otherwise noted. Doses refer to the weight of the salts. Solutions in sterile 0.9% saline were freshly prepared each day and administered in a constant volume of 1.0 ml/kg.

The isomers of MDMA were prepared through variants of several different procedures that had been earlier employed to synthesize other structurally related phenylalkylamines; although each of the synthetic intermediates has been previously reported in the literature (references provided below), the MDMA isomers have not been previously prepared by this method. Piperonyl acetone was prepared according to a general procedure by Monti et al. (1983) by treatment of known 5-(2-nitropropenyl)-1,3-benzodioxole (Karmarkar et al., 1985) with Raney nickel and sodium hypophosphite. Sodium triacetoxyborohydride (Abdel-Magid et al., 1996) was used to reductively aminate the resulting ketone using *R*(+)- or *S*(−)*N*,α-dimethylbenzylamine in 1,2-dichloroethane with a catalytic amount of AcOH at room temperature, followed by catalytic (10% Pd/C) debenzylation to obtain the corresponding *R*(−) and *S*(+) isomers of MDA (Anderson et al., 1978). Acylation with ethyl chloroformate provided the intermediate carbamates, which were subsequently reduced with $LiAlH_4$ to obtain the corresponding *R*(−)- and *S*(+)MDMA isomers as reported earlier (Glennon et al., 1987). The two MDMA isomers were isolated as their hydrochloride salts and recrystallized from absolute EtOH/anhydrous Et_2O. Both isomers analyzed within 0.4% of theory for C, H and N; melting points, spectral data and optical rotations were consistent with what has been previously reported for the two MDMA isomers (Anderson et al., 1978; Glennon et al., 1987).

2. Results

2.1. Training

The study began with *S*(+)- and *R*(−)MDMA training doses of 0.75 mg/kg versus saline vehicle. In the *S*(+)MDMA-training group, 2 months of training at that dose followed by 1 month of training at 1 mg/kg did not result in consistent responding under the drug (i.e., $\geq$80% drug-appropriate responding) or saline (i.e., $\leq$20% drug-appropriate responding) conditions. After 2 to 3 weeks of additional training at 1.5 mg/kg of *S*(+)MDMA, however, the animals reliably learned the discrimination (Fig. 1).

Fig. 2 shows that substantially more training sessions, at different training doses, were required to establish *R*(−)MDMA as a training drug. Specifically, 1 month of training at 0.75 mg/kg of *R*(−)MDMA versus saline did not produce much separation in percent *R*(−)MDMA-appropriate versus saline-appropriate responding. This was followed by

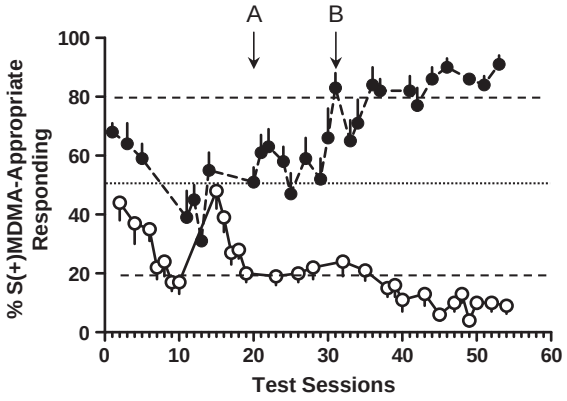

Fig. 1. Learning curve showing the training of rats to discriminate *S*(+)MDMA from saline vehicle. The study began with an *S*(+)MDMA training dose of 0.75 mg/kg. Over time, the training dose was increased to 1 mg/kg (A) and then to 1.5 mg/kg (B). Closed circles represent the effect (group mean and S.E.M.) of *S*(+)MDMA and open circle represent saline treatment.

about 6 weeks of training at 1.5 mg/kg of *R*(−)MDMA versus saline; performance was improved, but insufficiently consistent to meet training criteria (i.e., 3 consecutive weeks of ≥80% drug-appropriate responding following administration of *R*(−)MDMA and ≤20% responding on the same lever following administration of saline vehicle). Subsequently, 6 additional weeks of training at 2.5 mg/kg of *R*(−)MDMA versus saline resulted in the establishment of a clear and consistent discrimination (Fig. 2). A "fade-down" procedure was then initiated and consistent performance was obtained when the training dose of *R*(−)MDMA was reduced to 2 mg/kg versus saline for 1 month. The dose of *R*(−)MDMA was then lowered to 1.5 mg/kg, but discrimination performance deteriorated and was not consistent enough to meet the criteria of the study after 5 months of training. Finally, the training dose of *R*(−)MDMA was raised to 1.75 mg/kg and, after 6 weeks of training, the animals reliably learned the discrimination (Fig. 2). Approximately 1 year was devoted to the training of animals to discriminate *R*(−)MDMA from saline vehicle.

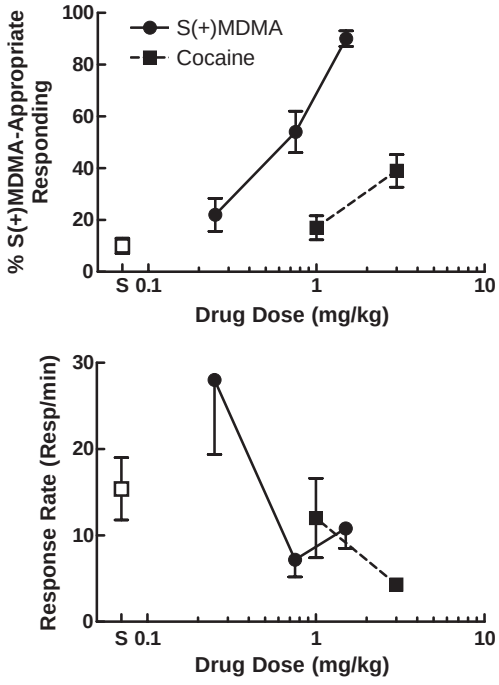

Fig. 3. Results of stimulus generalization studies in rats trained to discriminate 1.5 mg/kg of *S*(+)MDMA from saline vehicle (upper panel). Shown is the mean (±S.E.M.) percent drug-appropriate responding following administration of *S*(+)MDMA and cocaine doses; *S*=effect of saline (1 ml/kg). The animals' response rates are shown in the lower panel. Cocaine doses >3 mg/kg resulted in behavioral disruption.

Using a "fade-down" procedure, animals could perhaps have been trained to a lower dose of *S*(+)MDMA. However, our intent was to train animals to discriminate doses of the MDMA isomers that were as close to one another as possible, and the training dose of *S*(+)MDMA was already lower than that which could be established for *R*(−)MDMA.

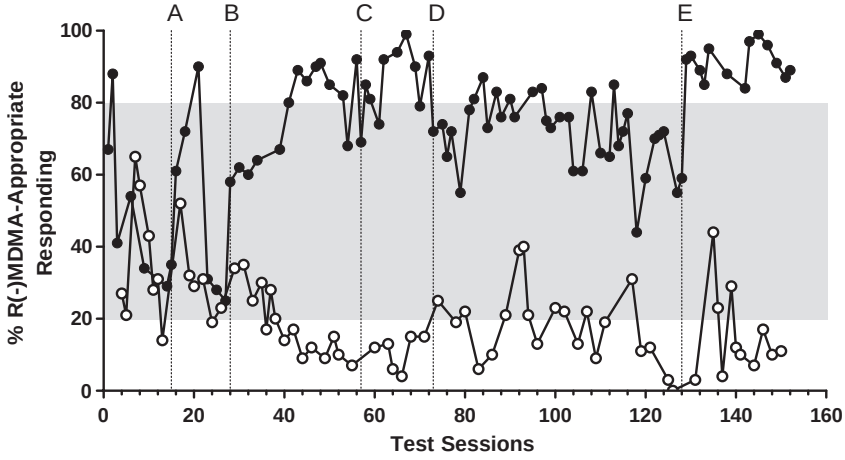

Fig. 2. Learning curve showing the training of rats to discriminate *R*(−)MDMA from saline vehicle. The study began with an *R*(−)MDMA training dose of 0.75 mg/kg. Over time, the training dose was incrementally increased to 1.5 mg/kg (A) and then to 2.5 mg/kg (B). Once responding was fairly consistent, a "fade-down" procedure was employed to decrease the training dose to 2 mg/kg (C) and later to 1.5 mg/kg (D). Due to the instability of the latter dose to reliably maintain drug-appropriate responding, the training dose was increased to 1.75 mg/kg (E). Closed circles represent the effect (group mean) of *R*(−)MDMA and open circle represent saline treatment. S.E.M. not shown for purpose of clarity.

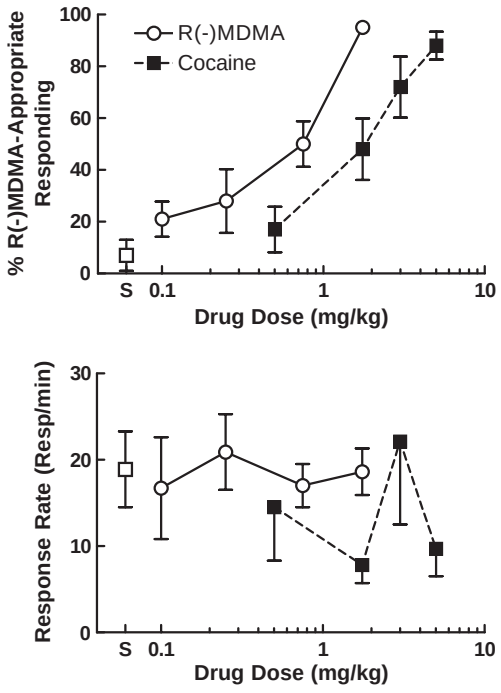

Fig. 4. Results of stimulus generalization studies in rats trained to discriminate 1.75 mg/kg of *R*(−)MDMA from saline vehicle (upper panel). Shown is the mean (±S.E.M.) percent drug-appropriate responding following administration of *R*(−)MDMA and cocaine doses; *S*=effect of saline (1 ml/kg). The animals' response rates are shown in the lower panel.

2.2. Substitution tests

Although the two groups consisted of different numbers of animals, comparisons can be made in the stimulus generalization studies so long as the animals employed in a given session meet the established training/testing criteria. *S*(+)MDMA at 1.5 mg/kg (Fig. 3) and *R*(−)MDMA at 1.75 mg/kg (Fig. 4) served as effective training stimuli. Administration of lower doses of training drug to the respective groups of animals showed an orderly, dose–responsive relationship; calculated ED_{50} doses for the MDMA isomers are 0.6 (95% CL 0.3–1.0) mg/kg for *S*(+)MDMA and 0.4 (95% CL 0.2–1.0) mg/kg for *R*(−)MDMA. The animals' response rates were fairly consistent except that the 0.25 mg/kg dose of *S*(+)MDMA produced a relatively high rate of responding (Fig. 3).

Administration of 1 mg/kg of cocaine to the *S*(+)MDMA-trained animals resulted in saline-appropriate responding (Fig. 3). Administration of 3 mg/kg elicited 39% *S*(+)MDMA-appropriate responding (Fig. 3) with 7/10 animals meeting the test criteria; at a cocaine dose of 5 mg/kg, the lever pressing behavior of 8/10 animals was disrupted (the two animals that responded made 13% of their responses on the drug-appropriate lever; data not shown). In contrast, administration of cocaine doses of 0.5 to 5 mg/kg to the *R*(−)MDMA-trained animals resulted in an orderly, dose-related substitution (Fig. 4), with the highest cocaine dose producing 88% drug-appropriate responding. The calculated ED_{50} dose for cocaine in the *R*(−)MDMA-trained animals was 1.3 (95% CL 0.7–2.7) mg/kg. Response rate data are provided in Figs. 3 and 4.

In an attempt to determine whether cocaine might influence the stimulus effects of *S*(+)MDMA, doses of cocaine were administered together with an *S*(+)MDMA dose of 0.5 mg/kg. By itself, this dose of *S*(+)MDMA produced 36% drug-appropriate responding. Administered in combination with cocaine doses ranging from 0.5 to 5 mg/kg, substitution failed to occur (Fig. 5). The animals' response rates were slightly suppressed (except for the MDMA+4 mg/kg cocaine combination) and fewer than all animals responded at each dose combination; with cocaine doses of 1, 3, 4 and 4.5 mg/kg, only 9, 8, 6 and 6 animals, respectively, met the test criteria. Only 5/10 animals responded when given *S*(+)MDMA in combination with 5 mg/kg of cocaine (Fig. 5).

3. Discussion

Racemic MDMA has been used as a training stimulus in numerous drug discrimination studies and a typical training dose is 1.5 mg/kg; hence, the racemate training dose consists of 0.75 mg/kg of *S*(+)MDMA and 0.75 mg/kg of *R*(−)MDMA. *S*(+)MDMA is thought to be the more potent enantiomer of MDMA and it was expected that 0.75 mg/kg of this isomer would function as a discriminative stimulus. This was found not to be the case and, at this time, a ready explanation is not apparent. In the present study, training doses were incrementally increased until responding was consistent. Baker et al. (1995, 1997) previously reported that 1.25 and 1.5 mg/kg of *S*(+)MDMA serve as effective discriminative stimuli in rats; at

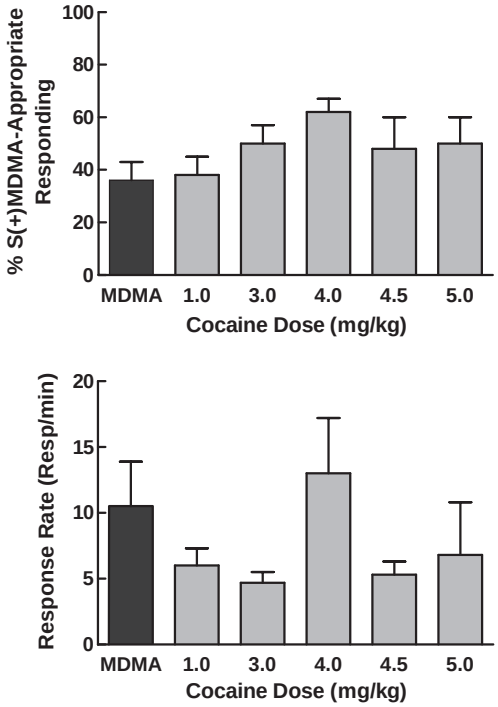

Fig. 5. Results of stimulus generalization studies in rats trained to discriminate 1.5 mg/kg of *S*(+)MDMA from saline vehicle (upper panel). Shown is the mean (±S.E.M.) percent drug-appropriate responding following administration of 0.5 mg/kg of *S*(+)MDMA in combination with various doses of cocaine; MDMA=0.5 mg/kg of *S*(+)MDMA. The animals' response rates are shown in the lower panel.

the higher training dose, the ED_{50} value for *S*(+)MDMA was 0.66 mg/kg. In the present study, rats also were found to effectively discriminate 1.5 mg/kg of *S*(+)MDMA (ED_{50}=0.6 mg/kg). Whereas Baker et al. (1995, 1997) trained rats to discriminate either 3.0 or 3.5 mg/kg of *R*(−)MDMA from vehicle, in the present study, we were able to train rats to discriminate 1.75 mg/kg of *R*(−)MDMA (ED_{50}=0.4 mg/kg). Attempts to train animals to a lower *R*(−)MDMA dose did not result in a reliable discriminative stimulus.

It is noteworthy that half the training dose of racemic MDMA failed to establish reliable stimulus control of behavior for either optical isomer of MDMA. That is, it was necessary to resort to training doses of MDMA isomers that were at least two-fold higher than the amount of either isomer found in the usual training dose of racemic MDMA. Baker et al. (1995) also noted difficulty in training animals to discriminate doses of *R*(−)MDMA lower than what they eventually used. This raises the idea that a combination of the two isomers of MDMA (as found in the racemate) might produce unique stimulus effects that are similar to, but perhaps somewhat different from, that produced by the individual MDMA isomers. That is, racemic MDMA might produce a compound stimulus reflecting the actions of its individual component isomers. This already has been demonstrated for the optical isomers of the structurally related *N-des*methyl counterpart of MDMA (i.e., MDA) (Young and Glennon, 1996). But, with MDA, the compound nature of the stimulus produced by racemic MDA was more apparent and (±)MDA required a much longer time (greater than 1 year of training) than normally required for (±)MDMA to establish reliable stimulus control of behavior. Another possibility is that one of the two MDMA optical isomers might potentiate or otherwise modulate the potency of the other MDMA isomer when given in combination (i.e., as with the racemate). Indeed, there is some evidence for this. For example, it has been reported that (±)MDMA produces a more pronounced locomotor stimulation in mice than either of its optical isomers and that (±)MDMA produced little head-twitch behavior in mice whereas both optical isomers were active (Fantegrossi et al., 2003, 2005). Furthermore, *S*(+)MDMA induces ipsilateral rotations in unilateral 6-OHDA-lesioned rats in a much more pronounced fashion than *R*(−)MDMA; however, additional treatment with *R*(−)MDMA after *S*(+)MDMA produced increased (i.e., potentiation of) rotational behavior (Lebsanft et al., 2005). Future studies should address the issue of whether one isomer of MDMA can influence the actions of its antipode.

Once the two groups were trained to consistently discriminate the MDMA optical isomers, tests of stimulus generalization were conducted with cocaine. Cocaine produced a maximum of 39% *S*(+)MDMA-appropriate responding at 3 mg/kg; administration of higher cocaine doses disrupted the animals' lever-pressing behavior. Furthermore, cocaine failed to modulate the stimulus effects of a low dose of *S*(+)MDMA in the *S*(+)MDMA-trained animals. In contrast, cocaine substituted for the *R*(−)MDMA stimulus in a dose-related fashion (ED_{50}=1.3 mg/kg) and was approximately three times less potent than *R*(−)MDMA. The partial generalization seen upon administration of cocaine to the *S*(+)MDMA-trained animals is consistent with the findings of Baker et al. (1995). However, whereas Baker et al. (1995) found that cocaine produced only 61% *R*(−)MDMA-appropriate responding, the present results indicate that cocaine fully substituted for an *R*(−)MDMA stimulus. The difference between the two studies might be related to the different training doses of *R*(−)MDMA.

That cocaine substitutes for the *R*(−)isomer of MDMA is consistent with a brief report that cocaine-trained (10 mg/kg) animals recognize *R*(−)MDMA but not *S*(+)MDMA (Emmett-Oglesby et al., 1990). But, such substitution might be a dose- and method-related phenomenon. Others have found that neither MDMA isomer substituted for cocaine training doses of 3.5 mg/kg (Broadbent et al., 1989), 8 mg/kg (Khorana et al., 2004) or 10 mg/kg (Broadbent et al., 1989). Although *R*(−)MDMA, but not *S*(+)MDMA, substituted for cocaine in animals trained to a high dose of cocaine (20 mg/kg) substitution did not occur in an orderly, dose-dependent manner (Broadbent et al., 1989). In light of differences that have been noted between the stimulus effects of MDMA and cocaine, further work is required to investigate factors already implicated as playing a role in their stimulus actions (e.g., training dose, temporal parameters). The results of the present investigation merely serve to further emphasize the complexity of the effects produced by MDMA and its optical isomers.

The major finding of this study is that cocaine substitutes for an *R*(−)MDMA, but not *S*(+)MDMA, stimulus. Given that it is commonly considered that *S*(+)MDMA is the more amphetamine-like isomer of MDMA, the present results were unexpected. That is, because *R*(−)MDMA possesses some stimulant character as described above, it is probably not surprising that stimulus generalization occurred. What was unanticipated was that substitution did not occur in the *S*(+)MDMA-trained animals. One explanation that can be offered is that cocaine might have substituted in the *S*(+)MDMA-trained animals had cocaine not been so behaviorally disruptive. That is, in the *S*(+)MDMA-trained animals, 3 mg/kg of cocaine disrupted several of the animals and the response rate for the animals that met testing criteria was substantially reduced. Because each of the two agents produces some behavioral disruption, we sought to determine if doses of cocaine might influence a low dose of *S*(+)MDMA when given in combination (Fig. 5); however, no effect was observed. Similar studies were not conducted with the *R*(−)MDMA-trained animals because in this group all animals responded at all dose levels (i.e., up to 5 mg/kg) of cocaine when cocaine was administered by itself. It might be noted that substantially more time was required to train animals to discriminate *R*(−)MDMA than *S*(+)MDMA; the extended training period for the former agent could have resulted in the animals becoming somewhat more tolerant to the behaviorally disruptive effects of *R*(−)MDMA and/or cocaine. Taken together, these results are difficult to reconcile but they might reflect differences or slight nuances in the stimulus mechanisms of *R*(−)MDMA and *S*(+)MDMA relative to cocaine.

Although the present study did not, nor was it meant to, focus on mechanisms underlying the stimulus actions common

to MDMA and cocaine, we have previously suggested that they are complex and probably involve differential actions of norepinephrine, serotonin and dopamine (Khorana et al., 2004). Rothman et al. (2001) have argued that the subjective effects of amphetamine-like stimulants are not likely mediated by a single neurotransmitter system nor brain region, that increased dopamine levels might be a necessary but insufficient condition to produce positive subjective effects, and that increased noradrenergic levels might play a hitherto unrecognized and important role in the action of these agents. Cocaine is nearly equieffective (i.e., <3-fold potency difference) in blocking the reuptake of norepinephrine, serotonin and dopamine; interestingly, (±)MDMA possesses a similar profile and potency (with about five-fold lower potency for dopamine uptake) (Rothman et al., 2001). Furthermore, unlike cocaine, MDMA can release all three neurotransmitters (but again with several-fold reduced potency for release of dopamine). The potency of *S*(+)MDMA to release these neurotransmitters is nearly identical with that of racemic MDMA; however, *R*(−)MDMA, although several-fold less potent than its *S*(+)enantiomer, is 7- to 10-fold less potent in releasing dopamine than either norepinephrine or serotonin, respectively (Setola et al., 2003). It just might be this different ratio of effects on synaptic neurotransmitter levels that accounts for the similarity or difference between the actions of cocaine and MDMA and, in particular, the individual optical isomers of MDMA.

Overall then, the present investigation confirms that MDMA optical isomers can be difficult to establish as training drugs; it also demonstrates that (a) curiously, half the training dose of the racemate was ineffective as a training dose for either MDMA isomer (even after exhaustive investigation); (b) animals learn to discriminate lower doses of *S*(+)MDMA more readily than *R*(−)MDMA; (c) a fading procedure can be successfully employed to lower the training dose of *R*(−)MDMA; (d) one MDMA isomer might functionally influence the stimulus effects of its antipode (e.g., as seen by the ability to use a training dose of racemic MDMA lower than that possible for either isomer); and that (e) cocaine more readily substitutes for an *R*(−)MDMA stimulus than an *S*(+)MDMA stimulus. It would be interesting in future studies to examine the actions of cocaine in tests of stimulus generalization using a three-lever drug discrimination procedure with animals trained to discriminate *S*(+)MDMA from *R*(−)MDMA from saline, or racemic MDMA from an optical isomer of MDMA from saline.

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