

## **Drug Discrimination in Methamphetamine-trained Monkeys: Effects of Monoamine Transporter Inhibitors**

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d) nonstandard abbreviations: DAT, dopamine transporter; ED<sub>50</sub>, dose calculated to produce 50% of the maximum effect; GBR 12909, 1-{2-(bis(4-fluorophenyl)-methoxy)ethyl}-4-(3-phenylpropyl)piperazine; NET, norepinephrine transporter; SERT, serotonin transporter.

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## ABSTRACT

The involvement of brain monoamine systems in the discriminative stimulus effects of methamphetamine (MA) was studied in squirrel monkeys by evaluating the effects of differentially-selective monoamine uptake inhibitors alone and in combination. In monkeys discriminating i.m. injections of 0.3 mg/kg MA from saline, methamphetamine (0.01–0.3 mg/kg) and dopamine transporter (DAT) inhibitors, including GBR 12909 (1.0–17.8mg/kg) and its analogs AM2502 (1.0–17.8 mg/kg), AM2506 (1.0–30.0 mg/kg), AM2515 (1.0–17.8 mg/kg) and AM2517 (1.0–5.6 mg/kg), produced dose-related increases in responding on the MA-associated lever and, at the highest doses, full substitution. The time course of MA-like effects was similar for equivalent (3.0 mg/kg) doses of GBR 12909 and its most potent analog, AM2517. Unlike the DAT blockers, the selective serotonin (5-HT) uptake inhibitor clomipramine (1.0-10.0 mg/kg) and the selective norepinephrine (NE) uptake inhibitor desipramine (1.0–10 mg/kg) produced responding primarily on the saline lever. The selective NE uptake inhibitor nisoxetine partially substituted at the highest dose tested (10.0 mg/kg). Pretreatment with GBR 12909 or AM2517 enhanced the discriminative stimulus effects of MA, shifting the dose-effect curve leftward. The NE uptake inhibitors desipramine or nisoxetine also enhanced the discriminative stimulus effects of MA, whereas clomipramine only attenuated them. These results support the view that dopaminergic mechanisms play a prominent role in the discriminative stimulus effects of MA in monkeys, whereas involvement of serotonergic and noradrenergic systems may be limited to a modulatory role.

Behavioral studies in humans and laboratory animals generally support the view that the discriminative stimulus effects of psychomotor stimulants such as cocaine and MA are closely related to their ability to increase extracellular dopamine (DA; Woolverton, 1996; Volkow et al., 1997). In nonhuman primates, for example, both indirect and direct DA D<sub>1</sub> and D<sub>2</sub> receptor agonists can reproduce discriminative stimulus effects of cocaine or MA, whereas D<sub>1</sub> and D<sub>2</sub> receptor blockers can attenuate such effects (Kleven et al., 1990; Spealman et al., 1991; Tidey and Bergman, 1998). Involvement of other monoamines in the discriminative stimulus effects of these stimulant drugs is less clearly understood, with some evidence supporting the involvement of serotonergic and noradrenergic mechanisms.

A full understanding of the role of monoaminergic systems has been complicated by a lack of concordant findings in rodents and primates. For example, although both low and high efficacy D<sub>1</sub> receptor agonists substitute at least partially for cocaine in drug discrimination experiments in rodents, lower efficacy D<sub>1</sub> agonists do not mimic cocaine in monkeys (Witkin et al., 1991; Terry et al., 1994; Katz et al., 1999). D<sub>2</sub> agonists also are more likely to fully substitute for cocaine in rodents than in monkeys (Spealman et al., 1991; Cunningham and Callahan, 1993; Acri et al., 1995; Spealman, 1996; Caine et al., 2000). Several studies have found that selective 5-HT transporter (SERT) inhibitors do not readily substitute for cocaine in either rats or monkeys (Cunningham and Callahan, 1991; Kleven et al., 1990; Spealman, 1993). Yet, they appear to reliably enhance cocaine's discriminative stimulus effects in rats, but not in monkeys (Callahan and Cunningham, 1991, Kleven and Koek, 1998; Spealman, 1993). In contrast, NE transporter (NET) inhibitors appear to substitute for low training doses of cocaine in monkeys. However, they fail to substitute for higher training doses of cocaine, indicating the pharmacological complexity of such findings (Colpaert et al., 1979; Kleven et al., 1990; Terry et al., 1994; Spealman, 1995b).

While the contribution of nondopaminergic mechanisms to the discriminative stimulus effects of cocaine has received attention, the involvement of SERT and NET inhibition in discriminative stimulus effects of MA are less studied. Like cocaine, MA produces increases in extracellular concentrations of all three monoamines (Kuczenski et al., 1995). Unlike cocaine, however, MA serves as a substrate for

monoamine transporters (Rudnick and Clark, 1993), and MA-induced increases in monoamine release may occur independently of neuronal activity. One intriguing outcome of such differences in mechanism of action is that MA-induced monoamine release may be less sensitive to autoreceptor-mediated negative feedback (Kuczenski et al., 1990; Gundlach et al., 1997). Consequently, MA may be more effective than cocaine in increasing extracellular levels of monoamines (e.g. Berger et al., 1992; Crespi et al., 1997). Such neurochemical distinctions between MA and cocaine notwithstanding, marked differences in the roles of monoaminergic systems in their discriminative stimulus effects are not documented.

The present studies were conducted to examine the involvement of DAT, SERT and NET inhibition in the discriminative stimulus effects of MA in monkeys. First, the ability of monoamine uptake inhibitors to substitute for MA was assessed by evaluating the effects of the DAT inhibitor GBR 12909 and four structural analogs, AM2502, AM2506, AM2515 and AM2517 (Ramanathan et al., submitted). Analogs' DAT binding affinity varies from approximately equal to that of GBR 12909 to 20 times less potent, with varying selectivity for the DAT versus SERT and NET (table 1). The ability of the selective SERT inhibitor clomipramine and the selective NET inhibitors desipramine and nisooxetine to produce MA-like subjective effects likewise was evaluated. Second, the time course of MA-like discriminative stimulus effects of GBR 12909 and AM2517, the most potent analog in discrimination studies, was assessed by administering equivalent doses (3.0 mg/kg) at varying pretreatment times. Finally, GBR 12909, AM2517, clomipramine, desipramine and nisooxetine were administered in combination with a range of doses of MA to compare their ability to modify the MA discriminative stimulus.

## METHODS

**Subjects.** Nine adult male squirrel monkeys (*Saimiri sciureus*) weighing 750 to 1000 g were individually housed in stainless steel cages in a climate-controlled vivarium with unlimited access to food (LabDiet high protein monkey diet, Brentwood, MA, with fresh fruit or vegetables daily and trail mix three times per week) and water. Animals were studied in daily experimental sessions (Monday-Friday). Four monkeys (s8, s12, s16, s21) had not previously received drugs, whereas five (s1, s91, s92, s125, s220) previously had been exposed to a variety of drugs including dopaminergic and opioid ligands.

**Apparatus.** During experimental sessions, monkeys sat in Plexiglas chairs (Kelleher and Morse, 1968) enclosed in ventilated, sound-attenuating chambers provided with white noise to mask extraneous sounds. While seated, monkeys faced a panel equipped with colored stimulus lights and two response levers 15 cm apart. Each press of a response lever produced an audible click and was recorded as a response. Before each session, a shaved portion of the monkey's tail was coated with electrode paste and placed under brass electrodes for the delivery of brief, low-intensity shock stimuli (200 msec, 3mA).

**Behavioral procedure.** Monkeys were trained to discriminate i.m. injections of MA from saline under a 10-response fixed ratio (FR10) schedule of stimulus termination. Under this schedule, shock stimuli occurred every 10 sec during the illumination of stimulus lights. Either completion of 10 consecutive presses of one of the two response levers within 10 sec (see below) or delivery of four shock stimuli turned off the stimulus lights for a 50-sec period (timeout, TO) during which responding had no scheduled consequences. Once responding was stable under the FR10 schedule, monkeys were trained to discriminate 0.3 mg/kg MA from saline. For each monkey, one lever was associated with MA injection and the other lever was associated with injection of vehicle/saline. In the present experiments, the left lever was associated with MA in six monkeys and the right lever was associated with MA in three monkeys. During all training sessions, responses on the incorrect lever, i.e., the lever not associated with the preceding injection, reset the response requirement. When discrimination performance was stable, daily training sessions were extended to comprise 1-4 components, each consisting of 10 presentations of the FR10;TO 50-sec schedule, with each component separated by a 10-min TO. The number of daily

training sessions varied on a pseudo-random basis, provided that MA was injected only before the final component of the day's session and that sessions with only saline injections occurred periodically to avoid invariant association between injection of MA and the final session component.

**Drug testing.** Drug testing was conducted once or twice per week, and training sessions were conducted on intervening days. Test sessions were conducted if >90% of responses were made on the injection-appropriate lever during the preceding training session and four of the last 5 training sessions. During test sessions, 10 consecutive responses on either lever extinguished stimulus lights and the associated schedule of shock delivery. The effects of MA, GBR 12909, AM2502, AM2506, AM2515, AM2517, clomipramine, desipramine and nisoxetine were determined using previously described procedures for cumulative dosing (Tidey and Bergman, 1998; Spealman et al., 1991). Briefly, incremental doses of the test drug were administered at the outset of the 10-min TO period preceding each component of the test session. This procedure permitted the determination of the effects of up to four cumulative doses during a single test session. The effects of five or more drug doses were determined by administering overlapping ranges of cumulative doses in separate sessions. The time course of effects of 3.0 mg/kg GBR 12909 and AM2517 were determined by administering single doses at various pretreatment times from 0 min to 8 hours. For pretreatment times longer than 10 min, monkeys remained in their home cages until immediately before the session, and an injection of saline was administered 10 min prior to the start of the session. Finally, studies were conducted to examine the effects of MA following pretreatment with selected doses of GBR 12909, AM2517, clomipramine, desipramine and nisoxetine. Pretreatment doses generally were below those that produced MA-like effects when administered alone (sub-threshold). In these experiments, pretreatment doses of each drug were given 10 min before the first component of a test session during which cumulative doses of MA were administered.

**Data analysis.** For each component of a test session, percent drug lever responding was calculated by dividing the number of responses on the injection lever by the total responses on both levers. Data from components in which average response rates were less than 0.2 responses/sec were excluded from further analysis. Full substitution with a test drug in individual monkeys was defined by

the occurrence of >90% responding on the MA-associated lever following at least one dose. When possible, interpolation of the linear portion of the dose-effect function for individual monkeys was used to determine the ED<sub>50</sub> value for MA discrimination, i.e., the dose of drug calculated to produce 50% responding on the drug-associated lever. Response rates were calculated by dividing the total number of responses in each component by the total duration of the component in seconds.

The effects of drugs for groups of monkeys are expressed in terms of averaged data ( $\pm$  S.E.M.). In studies involving drug interactions, enhancement or antagonism of the discriminative stimulus effects of MA by the pretreatment dose was analyzed by comparing ED<sub>50</sub> values for MA alone and following pretreatment using paired *t*-tests. One-way, repeated-measures analyses of variance (RM ANOVA) with *post-hoc* Dunnett's tests were used to evaluate the effects of drug administration on response rates in substitution studies. For pretreatment studies, response rates were compared to response rates observed after MA alone using two-way RM ANOVA with pretreatment dose (0.01-0.1 for GBR 12909 and AM2517 and 0.01-0.3 mg/kg for clomipramine, desipramine and nisooxetine) and MA dose as factors; *post-hoc* comparisons were made using Bonferroni's tests when significant main effects were observed. In all cases, significance was defined at the 95% level of confidence ( $p < 0.05$ ).

**Drugs.** Methamphetamine HCl, clomipramine, desipramine and nisooxetine were obtained from Sigma/RBI (Natick, MA) and were dissolved in 0.9% saline. GBR 12909, AM2502, AM2506, AM2515 and AM2517 were synthesized for the present experiments (Ramanathan et al., submitted). These phenylpiperazines were dissolved in a vehicle consisting of 95% ethanol (20%), Alkamuls (20%) and 0.9% saline (60%). Drug solutions were administered i.m. in the calf or thigh muscle in volumes of 0.4 ml/kg body weight or less. Control injections were similar amounts of saline.



## RESULTS

**Control performance.** All monkeys consistently discriminated injections of MA from saline throughout the present studies, without apparent development of tolerance or sensitization. During training sessions on days preceding test sessions, injections of the training dose of MA (0.3 mg/kg) produced >99% responding on the MA-associated lever, whereas saline injections produced an average of <1% MA-lever responding (table 2). The average response rate following MA administration ( $2.97 \pm 0.40$  responses/sec) was significantly higher than that observed after saline administration ( $2.16 \pm 0.20$  responses/sec,  $t_{(8)}=3.52$ ).

**Substitution with monoamine uptake inhibitors.** Cumulative doses of MA (0.01–0.3 mg/kg) engendered dose-related increases in the percentage of responding emitted on the MA-associated lever in all monkeys, as reported previously (Tidey and Bergman, 1998; Czoty et al., 2004). The training dose (0.3 mg/kg) produced full substitution in all monkeys, whereas 0.1 mg/kg produced varying degrees of MA-lever responding (range: 15–75%, not shown). The  $ED_{50}$  value for the discriminative stimulus effects of MA averaged 0.10 mg/kg in all monkeys (table 3; range 0.05–0.15 mg/kg). Response rates were significantly increased by 0.1 and 0.3 mg/kg MA ( $F_{(4,32)} = 5.66$ ) above values obtained following saline administration (not shown).

Cumulative doses of the DAT inhibitor GBR 12909 (1.0–17.8 mg/kg) engendered dose-related increases in responding on the MA-associated lever, producing full substitution in each monkey (fig. 1a). GBR 12909 also significantly decreased response rates ( $F_{(5,20)}=9.23$ ; fig 1b), with rates observed after 17.8 mg/kg GBR 12909 differing significantly from rates obtained following saline. The GBR 12909 analogs AM2502 (1.0–17.8 mg/kg), AM2506 (1.0–30.0 mg/kg), AM2515 (1.0–17.8 mg/kg) and AM2517 (1.0–5.6 mg/kg) similarly increased responding on the MA-associated lever, fully substituting for the MA training dose in all monkeys. However, only AM2502 significantly altered response rates ( $F_{(5,10)}=5.15$ ), producing increases at a dose of 10.0 mg/kg comparable to those observed with MA.

In contrast to GBR 12909 and its analogs, the SERT inhibitor clomipramine and the NET inhibitor desipramine (1.0–17.8 mg/kg) engendered responding almost exclusively on the saline-

associated lever (table 4). However, behavioral effects of the NET inhibitor nisoxetine differed from those of desipramine. Low to intermediate doses of nisoxetine engendered saline-lever responding, whereas the highest dose of nisoxetine, 10.0 mg/kg, fully substituted for MA in three of four monkeys. No dose of clomipramine, desipramine or nisoxetine significantly altered response rates. Higher doses of these drugs were not studied in the present experiments to avoid adverse physiological effects (*e.g.* emesis or convulsions, see Spealman, 1995).

**Time course assessments.** The time courses of an intermediate dose of GBR 12909 and its most potent analog, AM2517, were characterized by determining their effects at pretreatment times up to 8 hours. The rate of onset of MA-like discriminative stimulus effects was similar for GBR 12909 and AM2517, and each drug fully and consistently substituted for MA within 60 min (fig. 2). On average, both drugs continued to produce greater than 60% MA-lever responding 4 hours after administration, whereas responding occurred almost exclusively on the saline lever 8 hours following pretreatment. In control experiments, pretreatment with saline at various pretreatment times produced responding exclusively on the saline-associated lever (not shown). Response rates during the session were not appreciably influenced by changes in pretreatment time throughout the present experiments.

**Pretreatment with dopamine uptake inhibitors.** Pretreatment with GBR 12909 enhanced the discriminative stimulus effects of MA, shifting the position of the MA dose-effect curve upward and to the left in a dose-related manner (fig. 3a). As shown in table 3, ED<sub>50</sub> values for MA-discrimination were significantly decreased by 1.0 mg/kg ( $t_{(3)}=4.95$ ) and 3.0 mg/kg GBR 12909 ( $t_{(3)}=4.39$ ). Like GBR 12909, 3.0 mg/kg of its analog AM2517 significantly shifted the MA dose-effect curve upward and to the left ( $t_{(3)}=4.28$ ; fig. 3b). A lower dose of AM2517, 1.0 mg/kg, enhanced the discriminative stimulus effects of intermediate doses of MA and decreased ED<sub>50</sub> values in 3 of 4 monkeys outside the 95% confidence limits for MA alone.

With regard to FR performance, GBR 12909 had a significant effect on response rates ( $F_{(2,4)}=25.40$ , fig. 3c), with no significant interaction. However, *post-hoc* tests indicated that response rates after GBR 12909 pretreatment did not differ from response rates observed after MA alone. In contrast,

AM2517 did not have main effects on response rates (fig. 3d), although a significant interaction with MA dose was observed ( $F_{(4,12)}=8.84$ ). Thus, responding after the combination of 3.0 mg/kg AM2517 and 0.01 mg/kg MA differed from response rates following 0.01 mg/kg MA alone, and rates observed after both AM2517 doses in combination with 0.03 mg/kg MA differed from the effects of that dose of MA alone.

**Pretreatment with serotonin or norepinephrine uptake inhibitors.** In contrast to the DAT inhibitors, pretreatment with the selective SERT inhibitor clomipramine (3.0 and 10.0 mg/kg) generally attenuated the dose-related discriminative stimulus effects of MA, producing a downward and rightward movement of its dose-effect curve (fig. 4a), and increased  $ED_{50}$  values for MA discrimination in most cases (table 3). Although pretreatment with 3.0 or 10.0 mg/kg of clomipramine produced a 2- to 3-fold increase in  $ED_{50}$  values in three of four monkeys, the  $ED_{50}$  value for MA discrimination was decreased by clomipramine in the fourth monkey (s92), precluding statistical significance for grouped data. Unlike clomipramine, the selective NET inhibitor desipramine (3.0 and 10.0 mg/kg) enhanced the discriminative stimulus effects of the intermediate dose of MA (0.1 mg/kg), resulting in decreases in  $ED_{50}$  values for MA discrimination in three of four monkeys (table 3; fig. 4b). As with clomipramine, opposite effects were observed in the fourth monkey, precluding statistical significance for grouped data. Pretreatment with 3.0 mg/kg of nisoxetine, 0.5 log units less than the dose of nisoxetine that generally substituted for MA, also enhanced the discriminative stimulus effects of MA. However, these effects were reflected in a slight upward and leftward movement of the MA dose-effect curve (fig. 4c), and, despite some decreases in individual monkeys, the average  $ED_{50}$  value for the group of monkeys did not differ from that for MA alone (table 3). Response rates were not significantly affected by pretreatment with clomipramine, desipramine or nisoxetine.

## DISCUSSION

**Substitution with monoamine uptake inhibitors.** Consistent with the results of previous studies in monkeys and rats, GBR 12909 and its structural analogs produced dose-related increases in MA-lever responding and, at the highest doses, full substitution (Tidey and Bergman, 1998; Munzar and Goldberg, 2000). Of interest, despite the greater DAT/SERT selectivity of AM 2517 (20-fold) compared to GBR 12909 (9-fold), direct comparisons of GBR 12909 and AM 2517 revealed similar potency, maximum effect, and time course of action. The DAT/NET selectivity of GBR 12909 is similar to that of AM 2517, but other less behaviorally potent analogs are similarly selective. On the whole, an evaluation of affinities and selectivities at monoamine transporters suggests that relative behavioral potency (AM2515  $\approx$  AM2517  $\approx$  GBR 12909 > AM2506 > nisooxetine > AM2502  $\gg$  clomipramine  $\gg$  desipramine) was most closely related to relative affinity at DAT binding sites and was not predictable on the basis of either DAT/SERT or DAT/NET selectivity. Further highlighting these observations, DAT/SERT selectivity ratios for the two most behaviorally potent drugs are intermediate to those of the two least potent analogs. Thus, structural modifications that increase or decrease the selectivity with which GBR 12909 analogs bind DAT sites appear not to predictably enhance (or reduce) their ability to reproduce the subjective effects of MA. The view that comparable MA-like stimulus effects reflect comparable dopaminergic activity is consistent with microdialysis studies in monkeys in which 5.6 mg/kg AM2517, which produced full substitution in the present experiments, produced comparable peak increases in extracellular DA as the MA training dose (Czoty et al., 2004).

Unlike the DAT inhibitors, the SERT inhibitor clomipramine did not substitute for MA. This result agrees with data from previous studies of SERT inhibitors in MA- or cocaine-trained animals (Tidey and Bergman, 1998; Munzar et al., 1999; Cunningham and Callahan, 1991; Terry et al., 1994; Spealman, 1993; Schama et al., 1997). Moreover, like clomipramine, the NET inhibitor desipramine did not engender MA-like stimulus effects—again consistent with the results of previous studies in MA-trained animals (Tidey and Bergman, 1998; Munzar and Goldberg, 1999). Unlike desipramine, however, the highest dose of the NET inhibitor nisooxetine engendered responding exclusively on the MA-

associated lever in most monkeys. Previously, nisoxetine has been shown to substitute for amphetamines in monkeys, mice and pigeons (Snoddy and Tessel, 1985; Evans and Johanson, 1987; Kamien and Woolverton, 1989), but not rats (Schechter and Rosecrans, 1973; West et al., 1995; Munzar and Goldberg, 1999). Commonality in the discriminative stimulus effects of amphetamines and nisoxetine across studies could result from common noradrenergic actions. However, it is noteworthy that the DAT affinity of nisoxetine is within the range of values for the GBR 12909 analogs that substituted fully for MA in the present studies. These observations suggest that overlap in behavioral effects may be due instead to actions of nisoxetine at DAT binding sites. The MA-like behavioral effects of higher doses of nisoxetine further support the views that: a) DAT inhibition plays a more prominent role than NET inhibition in the discriminative stimulus effects of DA indirect agonists and b) affinity at DAT binding sites is a more powerful determinant than DAT selectivity in reproducing the stimulus effects of monoamine transport inhibitors.

**Pretreatment with monoamine transport inhibitors.** Pretreatment with sub-threshold doses of GBR 12909 or AM2517 enhanced the discriminative stimulus effects of MA, shifting the MA dose-effect curve leftward and upward in a dose-related manner and significantly decreasing ED<sub>50</sub> values for MA. Although the effects of DAT inhibitors in combination with MA in MA-trained monkeys have not been reported previously, the present results are consistent with the documented enhancement of cocaine's discriminative stimulus effects by GBR 12909 and by DA receptor agonists (Spealman, 1993; 1996). In conjunction with those and other findings (Kamien and Woolverton, 1989; Kleven et al., 1990; Katz et al., 1999; Tidey and Bergman, 1998), the present results further suggest a prominent role for DA in the discriminative stimulus effects of MA and, also, GBR 12909 and AM2517.

In contrast to GBR 12909 and AM2517, clomipramine attenuated the discriminative stimulus effects of MA. These results are comparable to those previously reported for the SERT inhibitor citalopram in cocaine-trained monkeys (Spealman, 1993; but see Schama et al., 1997). However, they differ markedly from previous results in rats showing that, with few exceptions, SERT inhibitors enhance the discriminative stimulus effects of cocaine (e.g., Simon and Appel, 1997; Callahan and Cunningham,

1997; Kleven and Koek, 1998; Munzar et al., 1999). Together, previous and present data raise the possibility that SERT inhibitors may differently modulate the discriminative stimulus effects of DA indirect agonists in rats and monkeys.

Unlike clomipramine, and in agreement with previous studies of NET inhibitors in drug discrimination studies with cocaine or amphetamines (Snoddy and Tessel, 1983; Spealman, 1995; Munzar and Goldberg, 1999), pretreatment with desipramine and nisoxetine moderately enhanced the discriminative stimulus effects of MA. However, the effects of the two drugs differed qualitatively. The limited effects of desipramine occurred primarily in combination with 0.1 mg/kg MA, a dose that engendered varying, but only intermediate levels of responding on the MA lever. These results may reflect a noradrenergic contribution of desipramine to the behavioral effects of MA and, as discussed below, also suggest that this contribution is most apparent with doses of MA below the present training dose. In contrast to the effects of desipramine, pretreatment with a sub-threshold dose of nisoxetine appeared to shift the dose-effect curve for MA leftward and upward. These results are comparable to the effects of GBR 12909 and AM 2517 and, thus, more consistent with supplemental dopaminergic actions of nisoxetine.

**The role of training dose.** Numerous pharmacological and methodological factors may contribute to inconsistencies in the effects of MA or cocaine across drug discrimination studies. Among these, differences in training dose can play a major role in the degree to which monoamine uptake inhibitors and other drugs substitute for cocaine or MA. The training dose of 0.3 mg/kg MA in the present studies likely is a moderate-to-high training dose, based on the ability of monkeys to achieve stable performance at lower MA training doses (0.56-1.0 mg/kg) in other, unrelated studies (unpublished data; also Stadler et al. 2001). In this regard, several previous studies suggest that NET and SERT inhibitors may more readily reproduce or modulate the discriminative stimulus effects of cocaine in animals trained to discriminate relatively low doses of cocaine. Thus, a variety of NET inhibitors appear to substitute for cocaine in monkeys and rats trained to discriminate doses of, respectively, 0.18 and 3.0 mg/kg of cocaine but not higher doses of, respectively, 0.3 and 10 mg/kg of cocaine (Spealman, 1995; Colpaert et al., 1979;

Cunningham and Callahan, 1991; Terry et al., 1994). Limited data suggest that differences in training dose may similarly influence results in experiments with SERT inhibitors. In the present and previous studies, for example, SERT inhibitors including clomipramine or fluoxetine did not reproduce, and even attenuated, the discriminative stimulus effects of dopaminergic psychomotor stimulants in monkeys trained with the relatively high doses of 0.3 mg/kg MA and 1.0 mg/kg cocaine (Spealman, 1993). Yet, the SERT inhibitor fluoxetine previously has been shown to accentuate the discriminative stimulus effects of cocaine in monkeys trained with a lower cocaine dose (0.3 mg/kg; Schama et al., 1997). While the role of training dose in such effects of SERT inhibitors needs to be documented more fully, these results suggest, overall, that NET and SERT mechanisms play limited, modulatory roles in the discriminative stimulus effects of cocaine or MA. Their roles may be prominent at lower doses of indirect DA agonists and, consequently, lesser levels of DA receptor activation, but diminish as dose and levels of DA receptor activation increase.

**Implications for medications development.** Despite a variety of approaches used to develop medications for psychostimulant abuse, a satisfactory pharmacotherapy for widespread clinical use has not yet emerged (Mendelson and Mello, 1996). One approach that remains promising is the development of DAT inhibitors as maintenance medications (Howell and Wilcox, 2001). GBR 12909 has been a lead compound in these efforts, having completed Phase-I clinical trials for the treatment of cocaine addiction (Preti et al., 2000). GBR 12909 has many characteristics of an effective maintenance medication, including a relatively slow onset and long duration of effects, and the ability to attenuate the reinforcing effects of cocaine (present results; Howell and Byrd, 1991; Glowa et al., 1995). This profile is shared by DAT inhibitors of other pharmacological classes, including PTT (Nader et al., 1997) and the 3-phenyltropane series of cocaine analogs (Carroll et al., 1999). However, GBR 12909, like other DAT inhibitors, has cocaine-like behavioral stimulant effects (Spealman, 1993; Howell et al., 1997), enhances the discriminative stimulus effects of cocaine (Kleven et al., 1990) and maintains self-administration with rates and patterns similar to cocaine under some conditions (Bergman et al., 1989; Howell and Byrd, 1991). Such cocaine-like effects conceivably might limit the use of DAT inhibitors as maintenance

medications, and it seems reasonable to examine modifications of this medications development strategy. Although enhancing the DAT selectivity of current candidate medications might appear to be an attractive approach, in the present study DAT selectivity did not appear to be an important determinant of the MA-like stimulus effects. Thus, it appears unlikely that altering monoaminergic selectivity without manipulating affinity will appreciably affect the degree to which behavioral effects of indirect monoamine agonists overlap with those of abused stimulants.



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## Footnotes.

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## Legends for Figures

**Fig 1.** MA-like discriminative stimulus effects of GBR 12909 (n=5), AM2502 (n=3), AM2506 (n=3), AM2515 (n=4) and AM2517 (n=4). Abscissae, cumulative dose, log scale; ordinates, (a) percent of responses on the methamphetamine-associated lever, (b) response rate. Dashed horizontal line indicates 90% drug-appropriate responding (full substitution). Points represent averages ( $\pm$  SEM). Points above S and MA indicate response rates following administration of saline or the training dose of methamphetamine, respectively. Some error bars have been omitted for clarity. \*,  $p > 0.05$  vs. saline.

**Fig 2.** Time course of effects of GBR 12909 (n=4) and AM2517 (n=5) on MA discrimination. Abscissae, pretreatment time, hours. Other details as in figure 1.

**Fig 3.** MA-appropriate responding (a,b) and response rates (c,d) after administration of methamphetamine alone or in combination with GBR 12909 (a,c; n=4) or AM2517 (b,d; n=4). \*,  $p > 0.05$  vs. respective dose of cocaine administered alone. Other details as in figure 1.

**Fig 4.** MA-appropriate responding and response rates (b) after administration of methamphetamine alone or in combination with the SERT inhibitor clomipramine (a; n=4) and the NET uptake inhibitors desipramine (b; n=4) or nisoxtetine (c; n=4). Other details as in figure 1.



**Table 1.** Binding affinities and selectivity ratios for standard and novel monoamine transporter inhibitors.

	<u>Affinity (K<sub>i</sub>), nM</u>			<u>Fold selectivity for DAT</u>	
	<u>DAT</u>	<u>SERT</u>	<u>NET</u>	<u>vs. SERT</u>	<u>vs. NET</u>
Cocaine	279	382	15409	1.4	55.2
GBR 12909	36.2	332	1573	9.2	43.5
Clomipramine	2190	0.3	38	< 0.0002	0.017
Desipramine	9231	82.6	4.5	0.009	< 0.001
Nisoxetine	429	124	8.7	0.3	0.02
AM 2502	705	265	438	0.4	0.6
AM 2506	147	5899	5250	40.1	35.7
AM 2515	19	510	1206	26.6	62.8
AM 2517	33	671	1475	20.2	44.4

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**Table 2.** Individual monkeys' average ( $\pm$  S.D.) percentage of responses emitted on the methamphetamine-associated lever and overall response rate after injections of saline or the training dose of methamphetamine during training sessions that preceded test sessions.

Monkey	Saline components			Methamphetamine components		
	n	% Responses	Responses/sec	n	% Responses	Responses/sec
s1	112	0.4 $\pm$ 2.2	2.8 $\pm$ 0.6	50	100.0 $\pm$ 0.0	4.5 $\pm$ 0.8
s8	53	0.6 $\pm$ 4.0	2.4 $\pm$ 0.5	23	99.9 $\pm$ 0.4	3.6 $\pm$ 0.5
s12	113	0.0 $\pm$ 0.1	2.4 $\pm$ 0.5	50	99.8 $\pm$ 1.4	3.7 $\pm$ 0.6
s16	61	0.0 $\pm$ 0.3	1.8 $\pm$ 0.2	32	99.5 $\pm$ 2.1	1.8 $\pm$ 0.4
s21	84	0.4 $\pm$ 2.6	2.7 $\pm$ 0.4	41	99.8 $\pm$ 0.7	4.4 $\pm$ 0.8
s91	17	0.0 $\pm$ 0.0	1.2 $\pm$ 0.2	11	100.0 $\pm$ 0.0	1.5 $\pm$ 0.1
s92	20	0.4 $\pm$ 2.2	1.3 $\pm$ 0.2	10	100.0 $\pm$ 0.0	1.3 $\pm$ 0.2
s125	20	0.3 $\pm$ 1.5	2.9 $\pm$ 0.4	10	100.0 $\pm$ 0.0	3.7 $\pm$ 0.6
s220	17	0.0 $\pm$ 0.0	1.9 $\pm$ 0.5	11	100.0 $\pm$ 0.0	2.2 $\pm$ 0.5

**Table 3.** Average ED<sub>50</sub> values (and 95% confidence intervals) for the discriminative stimulus effects of methamphetamine alone and after pretreatment with monoamine uptake inhibitors in individual monkeys.

Drug treatment	s1	s8	s12	s16	s21	s91	s92	s125	s220	average
MA alone	0.09	0.13	0.15	0.06	0.12	0.08	0.16	0.09	0.05	
	(0.06-0.12)	(0.09-0.19)	(0.11-0.19)	(0.04-0.09)	(0.07-0.20)	(0.06-0.11)	(0.15-0.16)	(0.08-0.10)	(0.05- 0.06)	
+1.0 mg/kg GBR 12909	0.06	<i>a</i>	0.06	<i>a</i>	0.03	0.01	<i>a</i>	<i>a</i>	<i>a</i>	0.04*
+3.0 mg/kg GBR 12909	0.04	<i>a</i>	0.01	<i>a</i>	0.04	0.01	<i>a</i>	<i>a</i>	<i>a</i>	0.02*
+1.0 mg/kg AM2517	0.04	0.03	0.05	0.11	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>	0.06
+3.0 mg/kg AM2517	< 0.01	< 0.01	< 0.01	0.02	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>	0.01*
+1.0 mg/kg clomipramine	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>	0.08	0.07	0.17	0.06	0.09
+3.0 mg/kg clomipramine	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>	0.17	0.09	0.17	0.17	0.15
+10.0 mg/kg clomipramine	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>	0.25	0.10	<i>b</i>	0.17	0.17
+1.0 mg/kg desipramine	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>	0.08	0.17	0.07	0.06	0.09
+3.0 mg/kg desipramine	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>	0.06	0.06	0.05	0.15	0.08
+10.0 mg/kg desipramine	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>	0.05	0.09	0.05	0.06	0.06
+3.0 mg/kg nisoxetine	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>	0.03	0.03	0.06	0.15	0.07

*a*, not tested

*b*, ED<sub>50</sub> value could not be determined

\*, significant difference ( $p < 0.05$ ) from average ED<sub>50</sub> for MA alone in respective monkeys

**Table 4.** Average ( $\pm$  SEM) percentage of responses emitted on the methamphetamine-associated lever and overall response rate after injections of the SERT inhibitor clomipramine, or the NET inhibitors desipramine or nisoxetine.

	Dose (mg/kg)	% MA-lever responses	Responses/sec
Clomipramine	1.0	0.3 $\pm$ 0.3	1.6 $\pm$ 0.5
	3.0	0.0 $\pm$ 0.0	1.6 $\pm$ 0.4
	10.0	2.3 $\pm$ 2.6	1.5 $\pm$ 0.4
	17.8	0.0 $\pm$ 0.0	1.6 $\pm$ 0.2
Desipramine	1.0	0.0 $\pm$ 0.0	2.4 $\pm$ 0.5
	3.0	0.0 $\pm$ 0.0	2.3 $\pm$ 0.4
	10.0	1.5 $\pm$ 1.7	2.5 $\pm$ 0.6
	17.8	0.0 $\pm$ 0.0	2.1 $\pm$ 0.3
Nisoxetine	0.3	0.0 $\pm$ 0.0	2.1 $\pm$ 0.4
	1.0	0.3 $\pm$ 0.3	1.9 $\pm$ 0.4
	3.0	2.5 $\pm$ 2.9	2.2 $\pm$ 0.3
	10.0	77.5 $\pm$ 26.0	1.9 $\pm$ 0.5

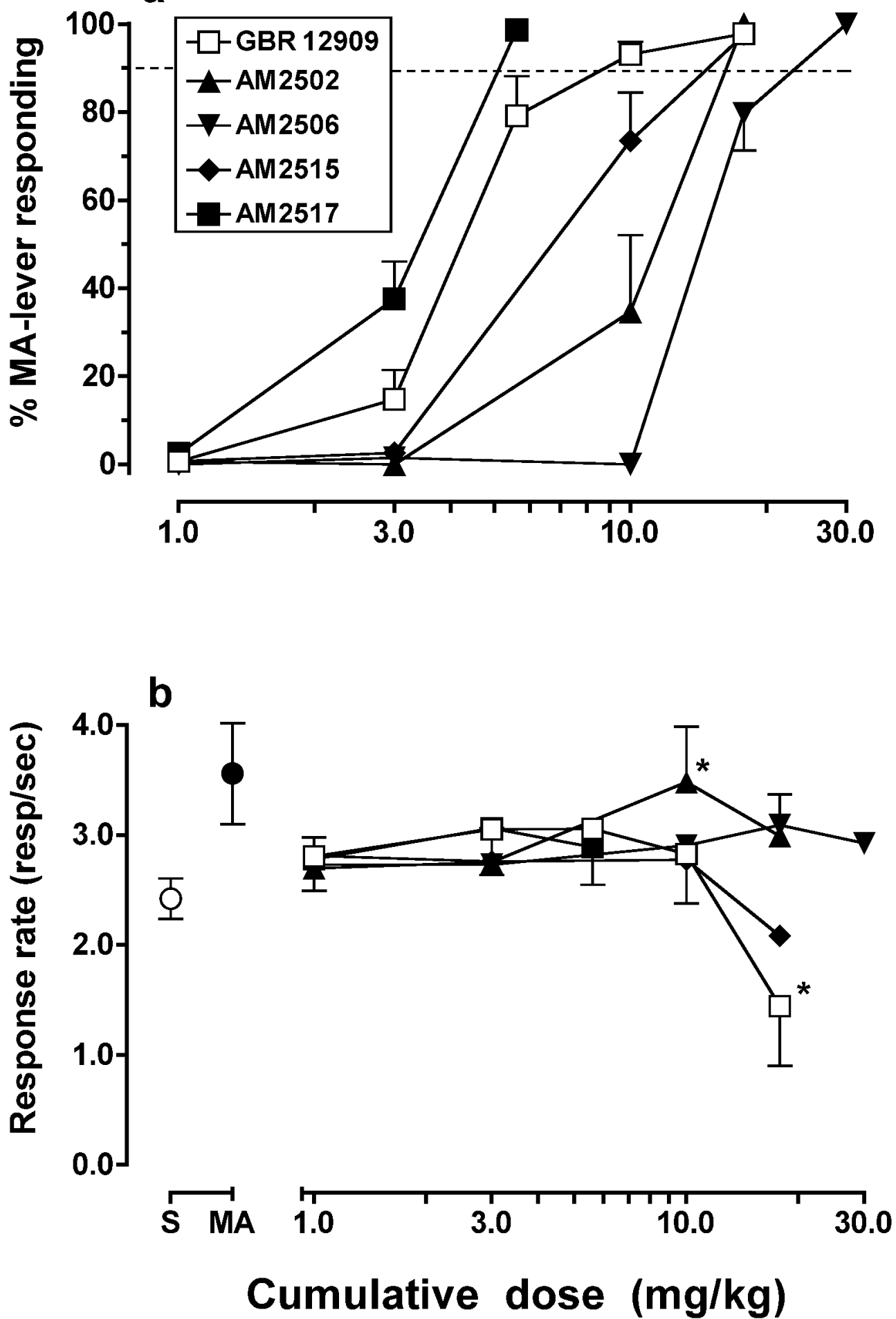


Figure 1.

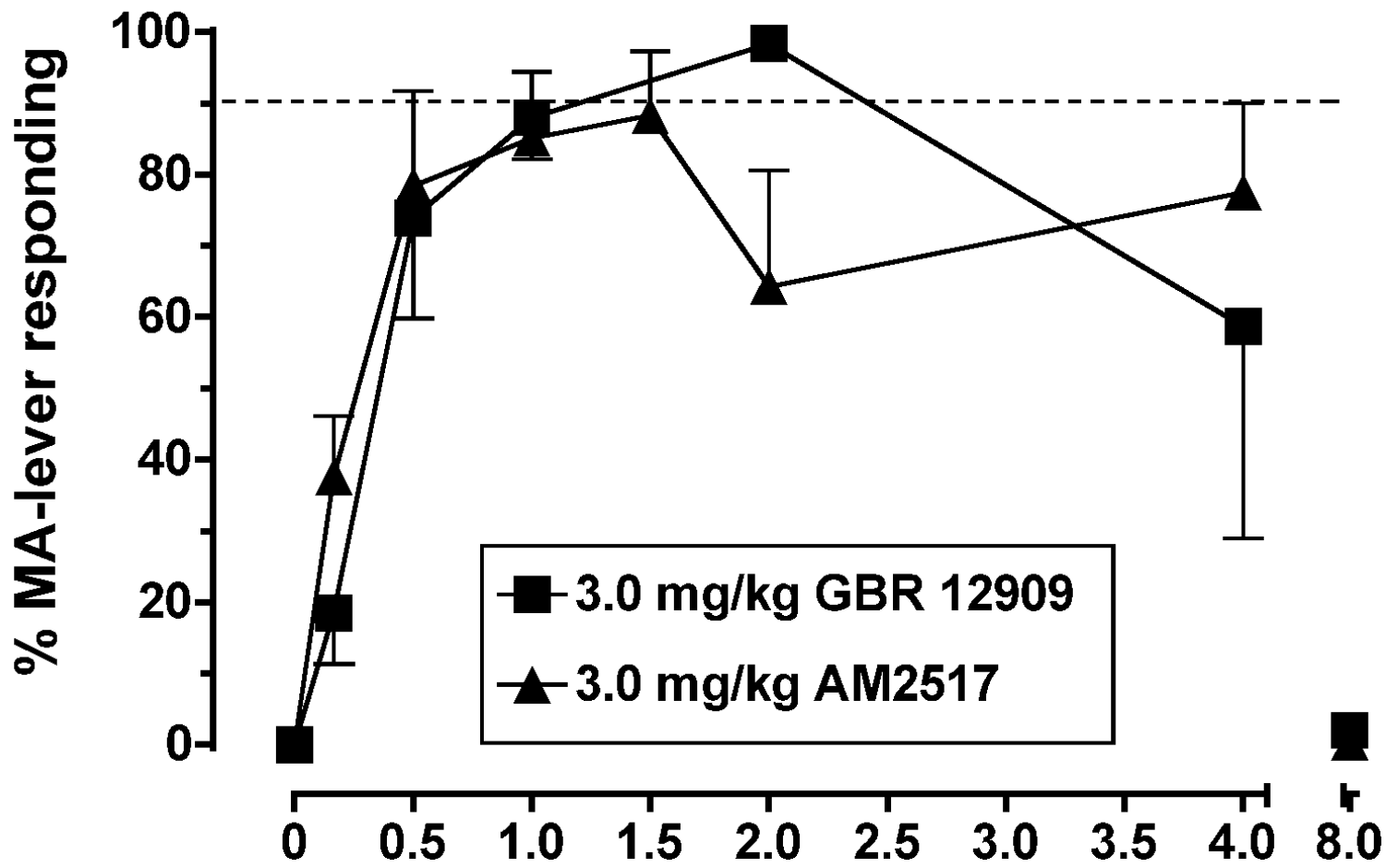


Figure 2.

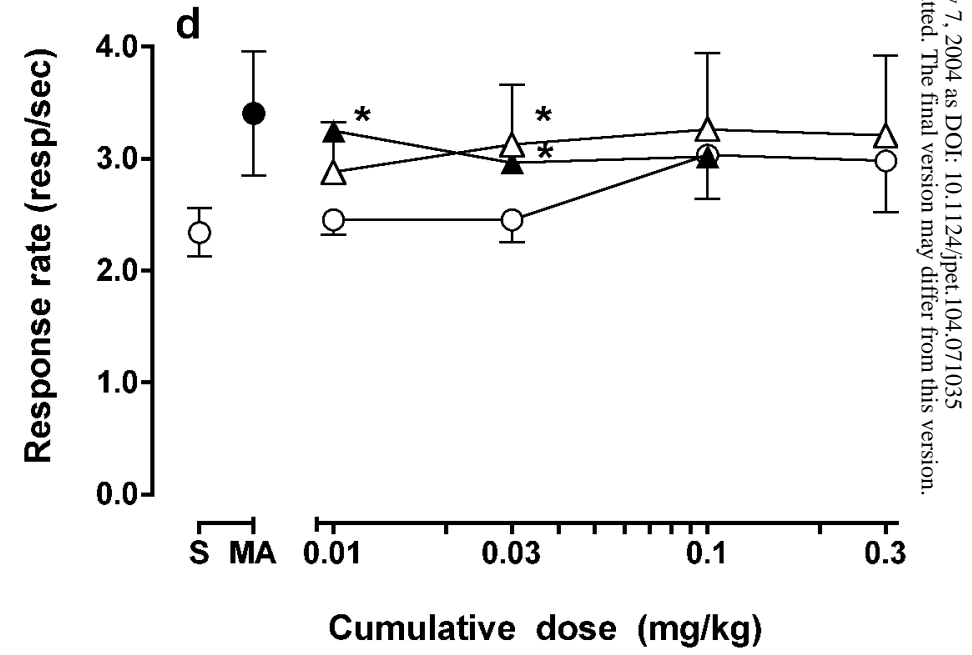
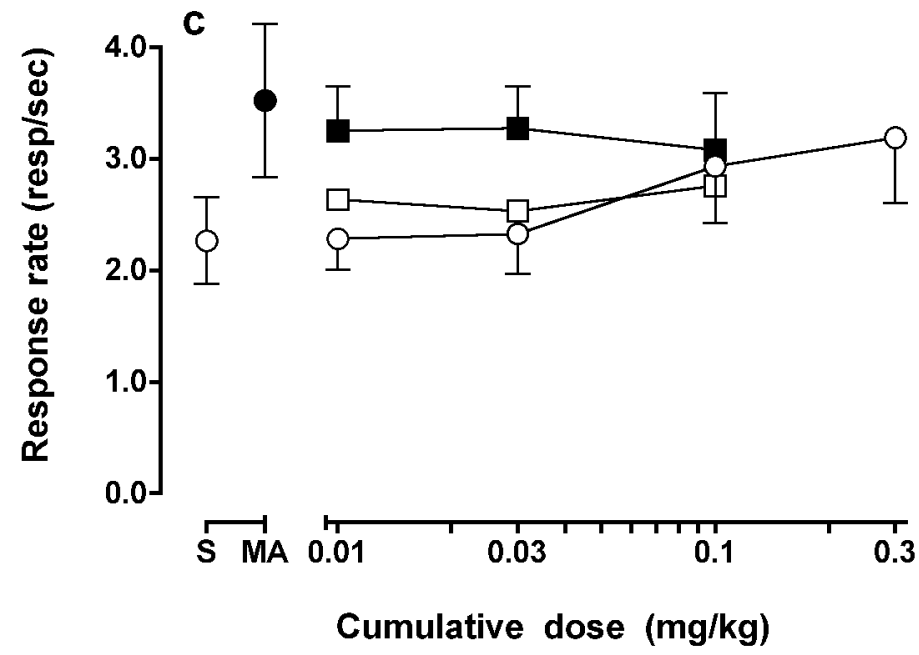
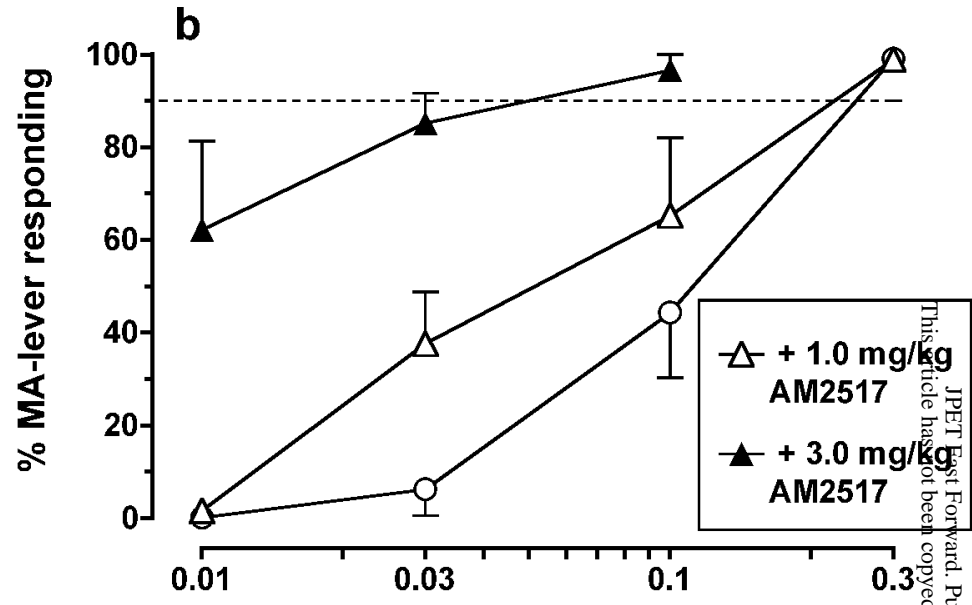
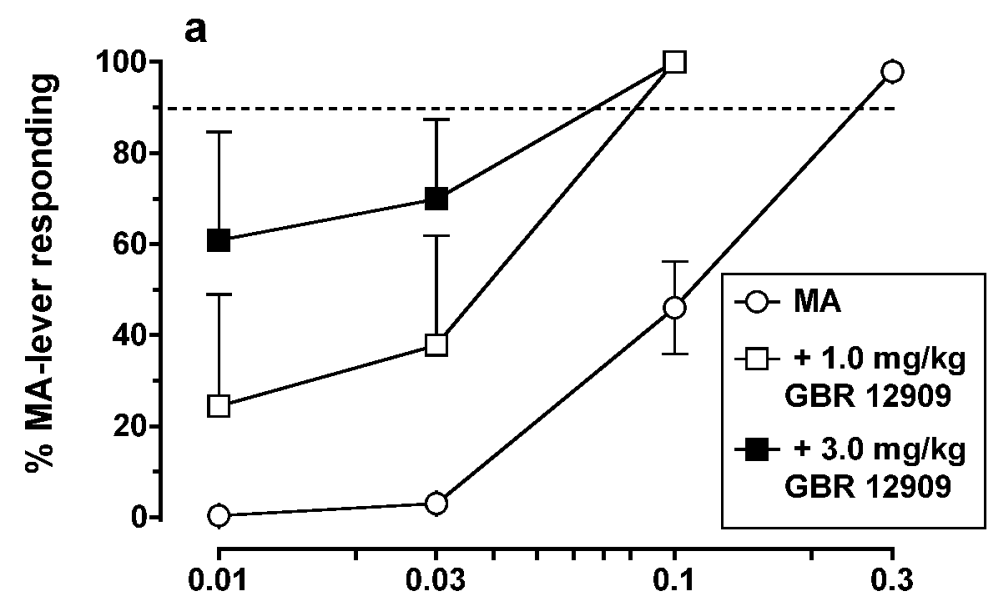


Figure 3.

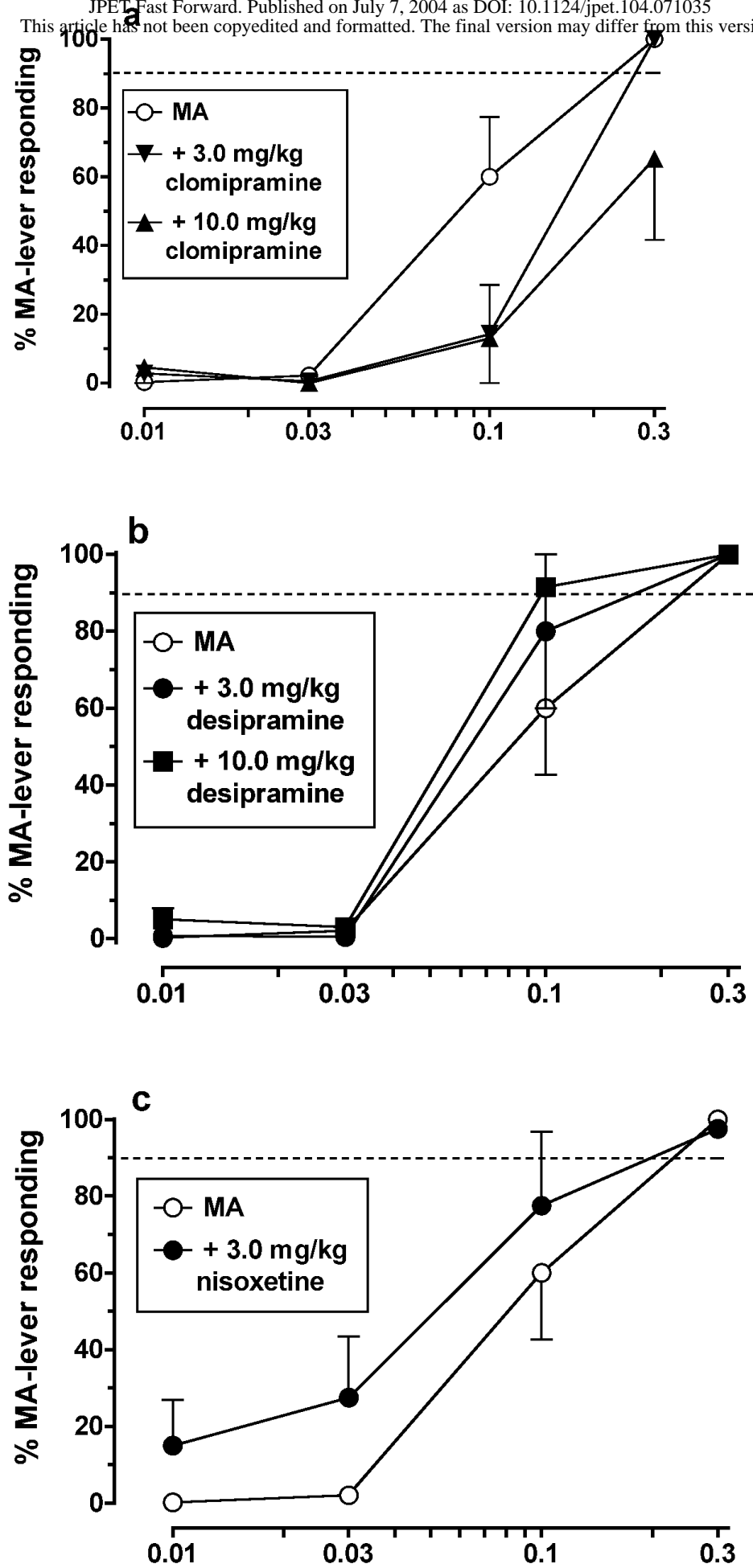


Figure 4.