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Discriminative stimulus properties of 1.25 and 5.0 mg/kg doses of clozapine in rats: examination of the role of dopamine, serotonin, and muscarinic receptor mechanisms

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Abstract

Clozapine (CLZ), an atypical antipsychotic drug (APD), produces minimal extrapyramidal side effects (EPS) and has significant advantages for treating both positive and negative symptoms in schizophrenic patients. CLZ has been established as a discriminative cue in the drug discrimination paradigm and in generalization tests the CLZ cue is more selective for atypical, rather than typical, APDs. However, greater selectivity for atypical antipsychotics has been demonstrated with a lower (1.25 mg/kg) CLZ training dose in rats [Psychopharmacology, 149 (2000) 189], rather than the traditional, higher training dose (5.0 mg/kg). It is therefore of interest to evaluate the properties mediating the 1.25 mg/kg CLZ discriminative cue. In the present study, rats were trained to discriminate either 1.25 mg/kg ($N=7$) or 5.0 mg/kg ($N=7$) CLZ from vehicle in a two-lever drug discrimination task. The typical antipsychotic haloperidol (0.1–0.4 mg/kg) did not substitute for either CLZ cue, whereas the atypical antipsychotic melperone (0.37–3.0 mg/kg) provided full substitution in both groups (>80% CLZ-appropriate responding). The 5-HT_{1A} receptor agonist (+)-8-OH-DPAT (0.04–0.16 mg/kg), and the selective 5-HT_{2A} receptor antagonist M100907 (0.03–1.0 mg/kg) did not produce substitution in either group. (+)-8-OH-DPAT combined with haloperidol (0.05 mg/kg) engendered only partial substitution (>60% CLZ-appropriate responding) for both CLZ cues, and M100907 combined with haloperidol (0.05 and 0.1 mg/kg doses) failed to provide substitution in either group. Trihexyphenidyl (0.18–6.0 mg/kg), a muscarinic M_1-preferring receptor antagonist, engendered full substitution for the 1.25 mg/kg CLZ cue, but only partial substitution for the 5.0 mg/kg CLZ cue. These results provide evidence that antagonism at the muscarinic M_1 receptor is sufficient to provide 1.25 mg/kg CLZ-like discriminative stimulus effects.
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1. Introduction

Clozapine (CLZ), one of the first atypical antipsychotic drugs (APDs), has been found to be superior to typical neuroleptics with regard to improvement in positive symptoms in neuroleptic-resistant patients (Kane et al., 1988; Chakos et al., 2001), cognition (Hagger et al., 1993; Meltzer and McGurk, 1999), suicidality (Meltzer et al., 2003), negative symptoms (Kane et al., 1988), and extrapyramidal side effects (EPS) (Kane et al., 1988; Chakos et al., 2001). Some, but not all, of these benefits are also shared by other atypical APDs including quetiapine, olanzapine, risperidone, and ziprasidone. The pharmacology of all of these agents is extremely complex, as they have relatively high affinities for a variety of serotonin (5-HT), adrenergic, and muscarinic receptors, as well as a moderate affinity for dopamine (DA) D_1, D_2, and D_4 receptors (Schotte et al., 1996). The most widely accepted theory for at least some of the key effects of CLZ and related APDs pertains to their relatively greater affinity for 5-HT_{2A} compared to D_2 receptors (Meltzer et al., 1989; Meltzer, 1999, 2001). This theory has led to the identification of many novel compounds that produce APD-like effects in animal models without the onset of catalepsy, an indicator of liability to produce EPS in humans (Meltzer, 1999).

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CLZ has been studied extensively in the drug discrimination procedure as a screening method to separate atypical from typical APDs. Typical APDs, such as chlorpromazine (Goas and Boston, 1978), haloperidol, amisulpride, and raclopride, which act primarily through DA D_2 receptor blockade, have all failed to provide substitution for the traditional 5.0 mg/kg CLZ training dose (Wiley and Porter, 1992; Goudie and Taylor, 1998; Millan et al., 1999) and a 1.25 mg/kg CLZ training dose in rats (Porter et al., 2000) as well as other CLZ training doses in pigeons and monkeys (Hoenicke et al., 1992; Carey and Bergman, 1997, respectively). Atypical APDs, which bind to multiple receptor subtypes, generally provide stimulus generalization in both 1.25 and 5.0 mg/kg CLZ-trained rats, as has been demonstrated with olanzapine (Moore et al., 1992; Millan et al., 1999; Porter et al., 2000), quetiapine (Goudie and Taylor, 1998; Millan et al., 1999), and the putative atypical APD S16924 (Millan et al., 1999). Other atypical APDs, such as sertindole (Goudie and Taylor, 1998) and risperidone (Goudie and Taylor, 1998) fail to substitute for a 5.0 mg/kg CLZ training dose, but do substitute for a 1.25 mg/kg CLZ dose (Porter et al., 2000). Therefore, CLZ has been shown to be selective to atypical APDs in rats, but generalization to these antipsychotics may be dependent on the CLZ training dose used. Based on these findings, it has been concluded that a 1.25 mg/kg CLZ training dose in rats, rather than the traditional 5.0 mg/kg CLZ training dose, is more representative of CLZ's atypical APD profile (Porter et al., 2000).

Receptor-selective ligands have been tested in both 1.25 and 5.0 mg/kg CLZ-trained rats in order to elucidate the basis of these particular cues. In generalization tests, 5-HT_{2A} (Millan et al., 1999), 5-HT_{2C} (Goudie et al., 1998; Millan et al., 1999), DA D_1, D_2, D_4 (Nielsen, 1988; Goudie et al., 1998; Porter et al., 1999), and α_1 adrenergic receptor antagonists (Nielsen, 1988; Kelley and Porter, 1997; Goudie et al., 1998, Millan et al., 1999) as well as 5-HT_{1A} agonists (Millan et al., 1999) have all failed to produce stimulus generalization in 5.0 mg/kg CLZ-trained rats. Similarly, *d*-amphetamine (Nielsen, 1988) and the 5-HT_{1A} antagonist WAY 100,635 have also failed to block the 5.0 mg/kg CLZ cue (Goudie et al., 1998). Full generalization from a 5.0 mg/kg CLZ cue has been demonstrated by the histamine H_1 receptor antagonists promethazine and cyproheptadine, although these compounds also serve as antagonists at multiple 5-HT and muscarinic receptors (Kelley and Porter, 1997). The muscarinic receptor antagonist scopolamine (Nielsen, 1988; Kelley and Porter, 1997; Goudie et al., 1998; Millan et al., 1999) and the muscarinic M_1-receptor-preferring antagonist trihexyphenidyl (Kelley and Porter, 1997) have provided substitution for a 5.0 mg/kg CLZ discriminative stimulus (DS), and additionally, the M_1 and M_4 preferring muscarinic receptor agonist oxotremorine fully blocks a 5.6 mg/kg CLZ cue (Nielsen, 1988). However, scopolamine has failed to substitute for a 1.25 mg/kg CLZ discriminative stimulus (Wise et al., 2001). This is contrary to evidence that muscarinic receptor antagonism is sufficient to provide a CLZ-like cue (Kelley and Porter, 1997). Ligands selective for DA D_2, D_4, 5-HT_{2A}, 5-HT_{2C}, and H_1 receptors tested in 1.25 mg/kg CLZ-trained rats also failed to produce stimulus generalization. However, full generalization to the α_1 adrenergic receptor antagonist prazosin and a single dose of the 5-HT_{2A} receptor antagonist M100907 was also observed (Wise et al., 2001).

The major purpose of the present study was to further investigate the effects mediating 1.25 and 5.0 mg/kg CLZ discriminative stimuli in rats using agents selective for D_2, $5HT_{2A}$, $5HT_{1A}$, and the M_1 muscarinic receptor in a two-choice drug discrimination task. Furthermore, the 5-HT_{2A} receptor antagonist M100907 and the 5-HT_{1A} receptor agonist (+)-8-OH-DPAT were each tested in combination with haloperidol to investigate if these interactions may produce a CLZ cue, as would be consistent with the serotonin–dopamine hypothesis of atypical APD action (Meltzer et al., 1989). In addition, the putative atypical APD melperone, which has not been reported in previous CLZ drug discrimination research, was also tested to verify that an atypical APD would produce generalization in these animals.

2. Method

2.1. Subjects

Sixteen male Sprague–Dawley rats (Charles River, Portage, MI) were obtained at 50–60 days old and delivered to the animal colony. Living facilities were maintained at a constant temperature (20–22 °C) under 12-h light/dark conditions (lights on 0700–1900 h). Animals were housed in standard plastic hanging cages with free access to water and were food deprived to 85% of free-feeding weights. Subjects were weighed before each session, and all subjects were maintained in full compliance with Institutional Animal Care and Use Committee protocols. After 1 week of habituation to housing conditions, 16 rats were randomly assigned to two groups of equal number. One group of eight rats was trained to discriminate 5.0 mg/kg CLZ from vehicle and the other group was trained to discriminate 1.25 mg/kg CLZ from vehicle. One subject in each group failed to maintain the drug discrimination criteria and was excluded from the study, leaving $N=7$ in each group. An additional subject in the CLZ 1.25 mg/kg group was euthanized before the completion of the study due to poor health.

2.2. Apparatus

Eight standard operant chambers equipped with food-delivery mechanisms were used for the drug discrimination procedure (MED Associates, Georgia, VT). Each chamber contained three retractable levers, two of which were

equidistant from either side of a food access, with the third lever located directly in between the two levers. The third (center) lever was only used for acquisition of the initial lever press response, and was retracted during all subsequent procedures. The chambers were controlled and data were collected using MED-PC for Windows software (version 1.15, MED Associates).

2.3. Drug

All drugs were administered with aseptic injection methods 30 min before each session with the exception of haloperidol, which was given 60 min before test sessions. All doses were made at 1 mg/ml volume. CLZ, M100907, and haloperidol doses refer to the free base form. CLZ (0.07–7.5 mg/kg ip; Novartis) and M100907 (0.03–1.0 mg/kg sc; Aventis) were dissolved in 0.1 N HCl and then adjusted to a pH ~ 5.0 with NaOH. Haloperidol (0.05–0.4 mg/kg ip; Sigma Chemical, St. Louis, MO) was dissolved in a few drops of lactic acid and diluted with deionized water to volume. (+)-8-OH-DPAT HCl (0.04 0.16 mg/kg sc; Sigma), melperone HCl (0.37–3.0 mg/kg sc; a gift from Lundbeck, Copenhagen, Denmark), and trihexyphenidyl HCl (0.18–6.0 mg/kg ip; Sigma) were each dissolved in deionized water (doses refer to salt form).

2.4. Training Procedures

Before each session, the levers were cleaned with isopropyl alcohol to decrease the likelihood of preference due to olfactory cues (Extance and Goudie, 1981). All subjects were initially exposed to a fixed-time, 60-s schedule of food delivery with no levers present. Following this procedure, all rats began on a fixed-ratio (FR) 1 schedule without the presence of drug and with only the center lever present. On four subsequent trials, errorless training was conducted after either CLZ or vehicle administration with only the condition-appropriate lever (left or right) present. Lever assignments for drug and vehicle were counterbalanced within each group. All subjects were given two errorless training sessions per condition. Once lever pressing was emitted consistently during the errorless training sessions, both levers were presented to subjects after CLZ or vehicle was administered. Reinforcers were delivered during 20-min training sessions for responses on the condition-appropriate lever beginning with an FR 1 schedule that was increased progressively until responding under an FR 20 reinforcement schedule was maintained. Responses made on the inappropriate lever reset the FR counter. The order of CLZ (C) and vehicle (V) training sessions were as follows: VCVVCVCC. Once responding on the FR 20 schedule was maintained under both CLZ and vehicle conditions, daily training sessions were conducted until all subjects met the drug discrimination criteria. The discrimination criteria consisted of at least 80% of condition-appropriate responses during the first FR 20 and for the remainder of the 20-min session. These criteria must have been met for 9 out of 10 consecutive sessions before testing could begin.

2.5. Stimulus generalization tests

Before a test session, each subject must have had both a CLZ and vehicle training session with at least 80% of condition-appropriate responses before the first reinforcer and for the remainder of each of these sessions. All test sessions ended without reinforcement after the first 20 consecutive responses on one lever, while responses made on the opposite lever caused the response counter to be reset. If 20 consecutive responses did not occur, the test session ended after 20 min. Percent CLZ-lever responding was recorded for subjects emitting at least 10 responses during the session, consistent with other drug discrimination procedures where response disruption is evident (Nielsen, 1988). The drugs were tested in the following order: CLZ, melperone, haloperidol, (+)-8-OH-DPAT, (+)-8-OH-DPAT + haloperidol, trihexyphenidyl, M100907, and M100907 + haloperidol. The first dose of each drug an animal was administered was balanced within the group so that every dose in the dose–response curve consisted of at least one animal's first exposure to that drug. Subsequent sessions tested the next dose in ascending order.

2.6. Data analysis

The percentage of CLZ-appropriate responses and the number of responses emitted per second (RPS) were calculated for each dose tested. Group means were calculated and graphed in dose–response curves. All data analyses and graphs were produced using Prism GraphPad version 3.0 (GraphPad Software, San Diego, CA). Full substitution for the CLZ stimulus was defined as 80% or greater CLZ-appropriate responding. CLZ-appropriate responding between 60% and 80% was considered partial substitution, while less than 20% was considered no substitution. ED_{50}s and 95% confidence intervals were assessed through a least squares method of linear regression analysis only on the linear portion of the curve for drugs providing full substitution (Goldstein, 1964). One-factor repeated measures analysis of variance (ANOVA) tests were conducted on response rate data to assess significant differences from vehicle controls, and Tukey HSD post hoc comparison tests were conducted after significant effects were found.

3. Results

Substitution results for CLZ are shown in Fig. 1, left panels. Subjects trained to discriminate 5.0 mg/kg CLZ from vehicle reached the discrimination criterion in 48.8 (standard error of the mean [S.E.M.] = 15.3) sessions

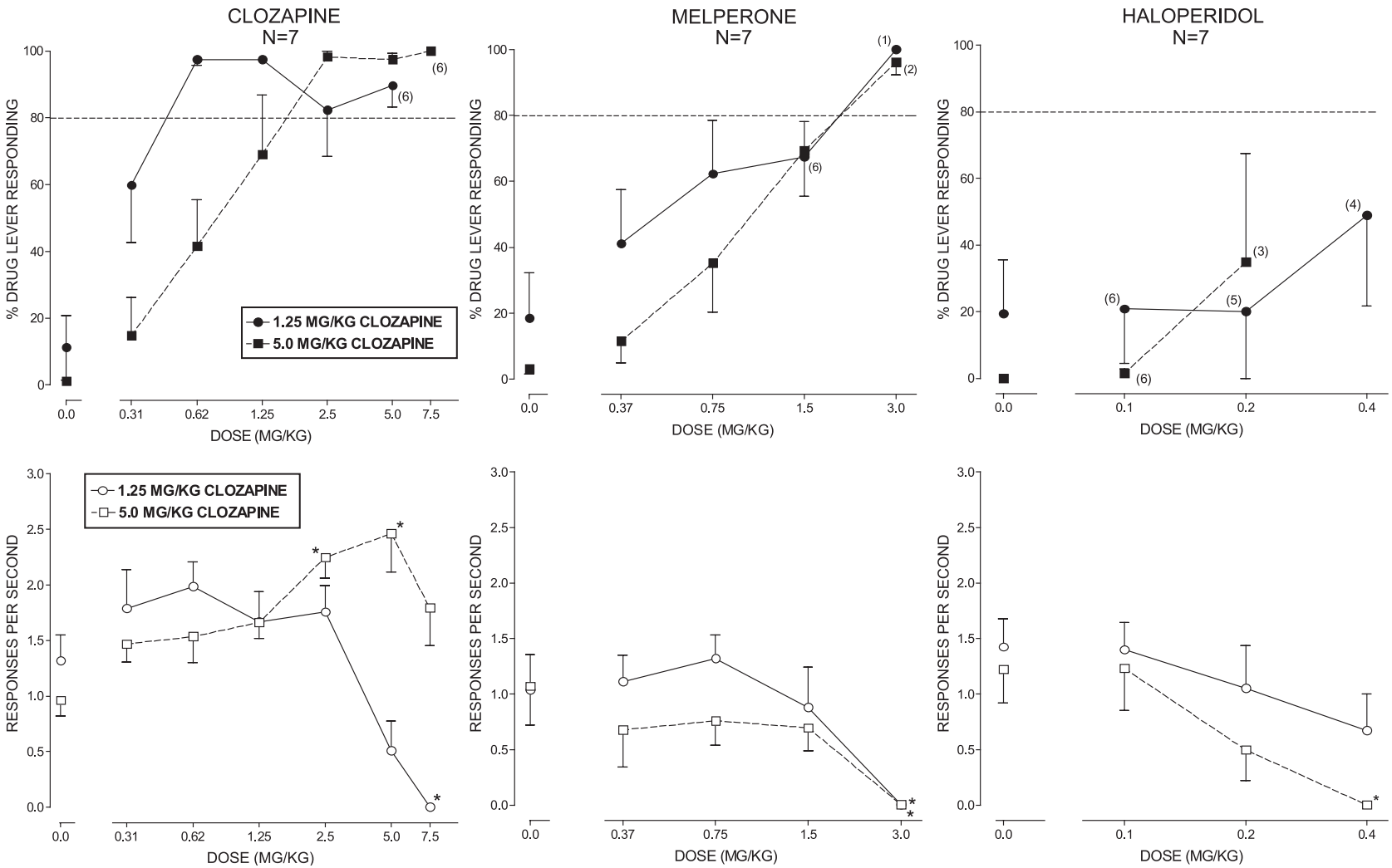

Fig. 1. The atypical APDs clozapine (left), melperone (center), and the typical antipsychotic haloperidol (right) were tested for stimulus generalization in rats trained to discriminate either 1.25 mg/kg (–●–) or 5.0 mg/kg (–■–) clozapine (ip) from vehicle ($N=7$ per group) in a two-choice drug discrimination task. Mean percent CLZ lever responding (± S.E.M.) (top) and mean responses per second (± S.E.M.) (empty symbols; bottom panels) are shown for each group. Numbers in parenthesis indicate the number of subjects that completed the test session; otherwise, the number of subjects is equal to N. The level of full stimulus generalization (≥ 80% drug lever responding) is indicated by a dashed line. * $P<.05$.

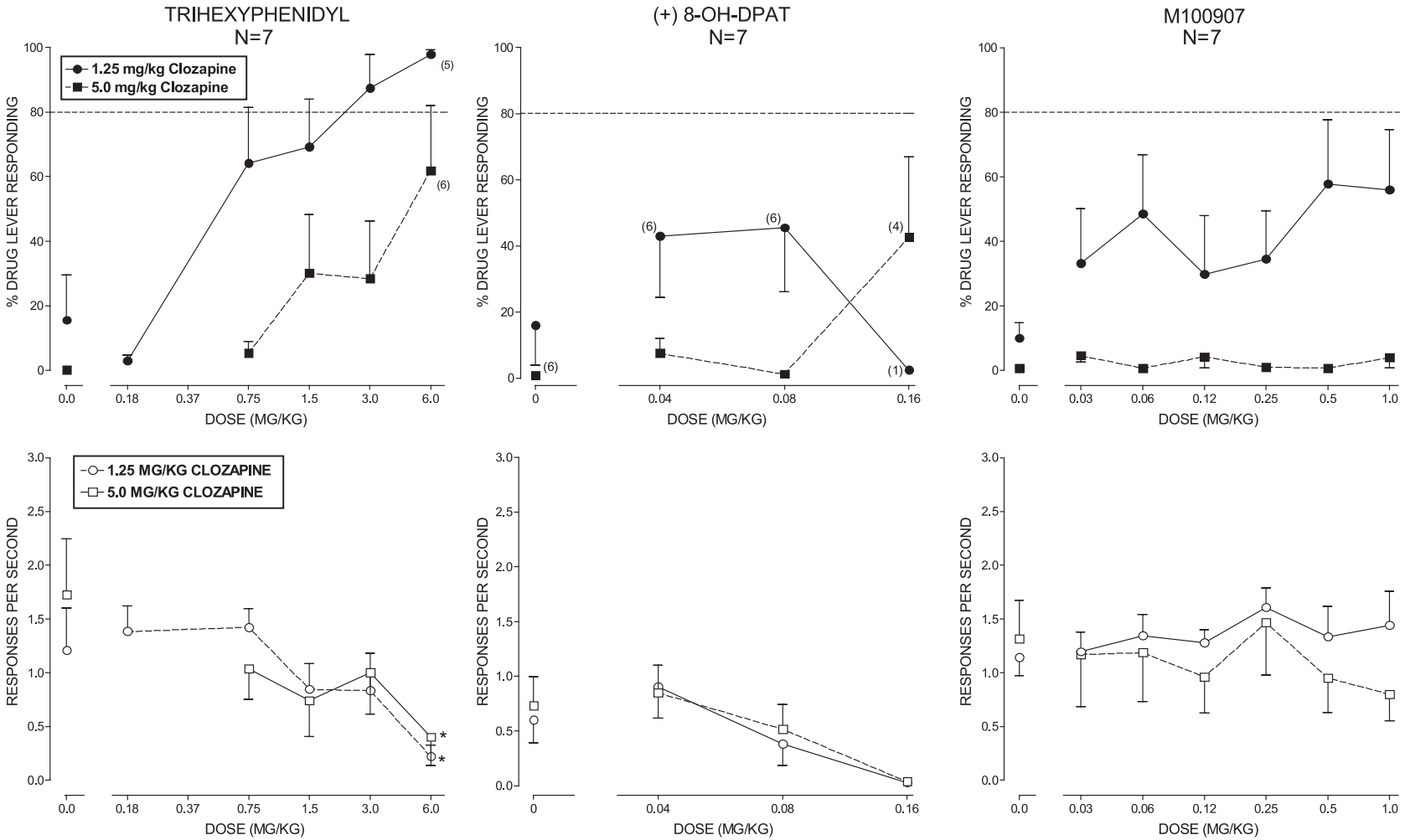

Fig. 2. The muscarinic M1 receptor antagonist trihexyphenidyl (right), the 5-HT1A receptor agonist (+)-8-OH-DPAT (center), and the 5-HT2A receptor antagonist M100907 (left) were tested for stimulus generalization in rats trained to discriminate either 1.25 mg/kg (–●–) or 5.0 mg/kg (–■–) CLZ from vehicle. Other details are the same as in Fig. 1. *$P < .05$.

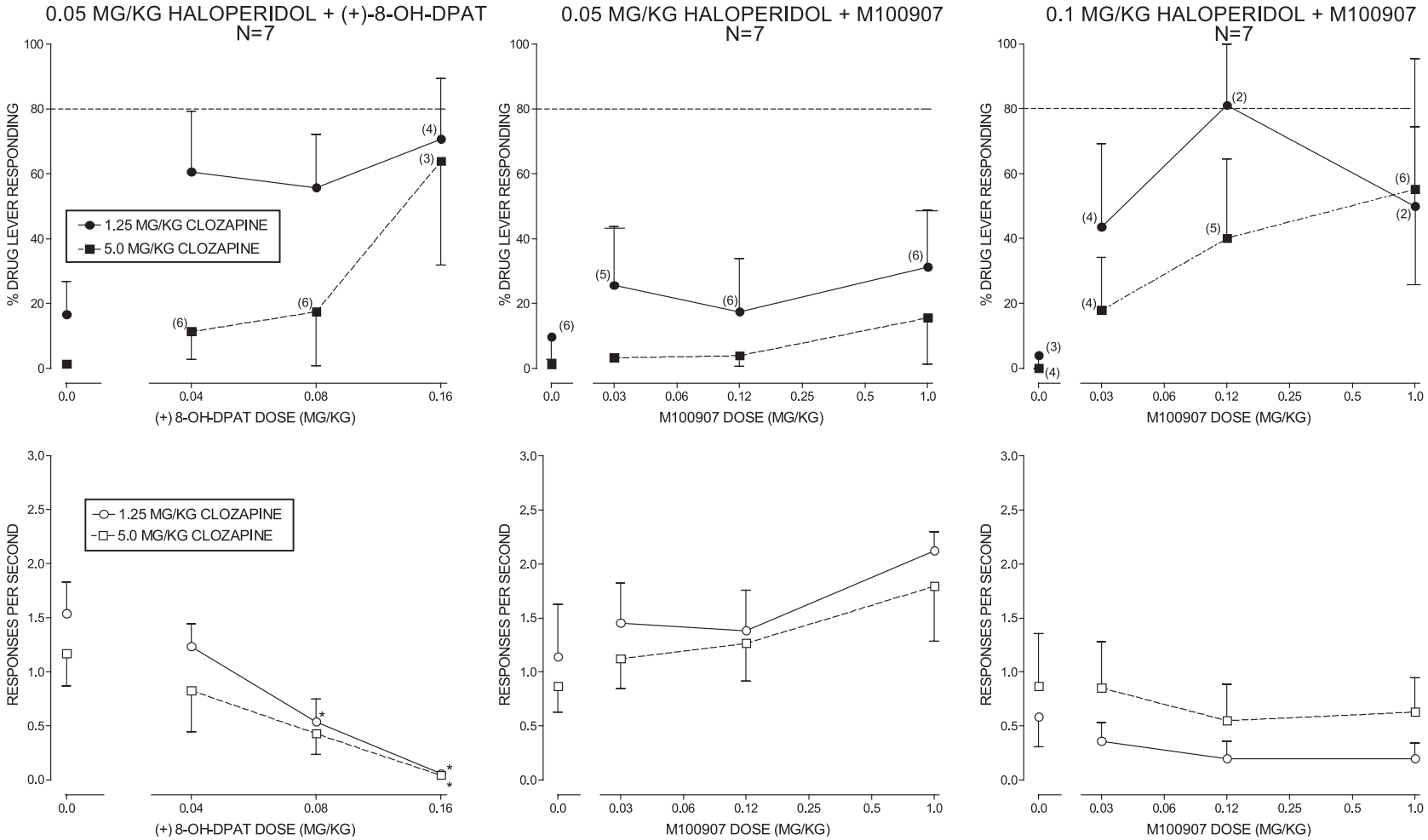

Fig. 3. Haloperidol (0.05 mg/kg) was combined with (+)-8-OH-DPAT doses (right) and haloperidol 0.05 mg/kg (center) and 0.1 mg/kg doses (right) combined with M100907 were tested for stimulus generalization in rats trained to discriminate either 1.25 mg/kg (–●–) or 5.0 mg/kg (–■–) CLZ from vehicle. Other details are the same as in Fig. 1. *$P < .05$.

(range = 30–70 sessions). The number of sessions to criterion for the subjects trained to discriminate 1.25 mg/kg CLZ from vehicle was not significantly different (54.7 ± S.E.M. 18.5) but slightly more variable (range = 27–79 sessions). Both groups exhibited dose-dependent responding when doses of CLZ (0.31–7.5 mg/kg) were administered (top left panel). The 1.25 mg/kg CLZ group achieved full generalization to 0.62 mg/kg CLZ with an ED_{50} = 0.24 mg/kg (95% confidence interval [CI] = 0.18–0.31 mg/kg). Lever pressing by 1.25 mg/kg CLZ-trained rats was completely disrupted [$F(6,36) = 16.74$, $P < .01$] following a 7.5 mg/kg CLZ dose. Full substitution in the 5.0 mg/kg CLZ group was produced by 2.5 mg/kg CLZ with an ED_{50} = 1.04 mg/kg (CI = 0.79–1.36 mg/kg), whereas response rates were not suppressed following 7.5 mg/kg CLZ. These ED_{50} values were subjected to a t test and were found to be significantly different [$t(54) = 6.84$, $P < .01$].

The results of substitution testing with the atypical APD melperone are shown in Fig. 1, center panels. Melperone (0.37–3.0 mg/kg) produced full substitution with 3.0 mg/kg in both the 1.25 mg/kg CLZ (ED_{50} = 0.53 mg/kg, CI = 0.27–1.03 mg/kg) and 5.0 mg/kg CLZ groups (ED_{50} = 1.04 mg/kg, CI = 0.79–1.36 mg/kg), and these curves were significantly different [$t(44) = 2.26$, $P < .05$]. The 3.0 mg/kg dose of melperone significantly reduced response rates in both the 1.25 mg/kg CLZ [$F(4,24) = 4.61$, $P < .05$] and 5.0 mg/kg CLZ [$F(4,24) = 4.71$, $P < .05$] groups.

Haloperidol (0.1–0.4 mg/kg) (Fig. 1, right panels) failed to produce substitution in both groups of animals. A 0.4 mg/kg dose of haloperidol significantly reduced lever press responding in the 5.0 mg/kg CLZ-trained rats [$F(3,18) = 6.16$, $P < .01$] to such a degree that percent drug lever responding could not be calculated for this dose. However, response rates were not reduced by haloperidol in the 1.25 mg/kg CLZ group [$F(3,18) = 1.61$, $P > .05$].

Substitution tests for the M_1-preferring receptor antagonist trihexyphenidyl are shown in Fig. 2, left panels. Trihexyphenidyl (0.75–6.0 mg/kg) produced dose-dependent increases in CLZ-appropriate responding in both groups, but full substitution was exhibited only in the 1.25 mg/kg CLZ-trained subjects to both 3.0 and 6.0 mg/kg doses (top left panel). An additional trihexyphenidyl dose, 0.18 mg/kg, was tested in the 1.25 mg/kg CLZ group to allow for a more representative ED_{50} calculation (ED_{50} = 0.62 mg/kg; CI = 0.24–1.62 mg/kg). The 5.0 mg/kg CLZ group exhibited only partial generalization (61.8 ± 20.0%) to the highest trihexyphenidyl dose (top left panel). Response rates in both 1.25 and 5.0 mg/kg CLZ groups were statistically lower than vehicle controls [$F(4,24) = 3.02$, $P < .05$; $F(4,24) = 3.29$, $P < .05$, respectively].

The 5-HT_{1A} agonist (+)-8-OH-DPAT (0.04–0.16 mg/kg) failed to substitute for either the 1.25 or 5.0 mg/kg CLZ cues (Fig. 2, top center panel). The highest (+)-8-OH-DPAT dose tested, 0.16 mg/kg, greatly reduced responding in both groups (bottom center panel) and precluded assessment of higher doses. Response rates decreased in a dose-dependent matter, but were not significantly different from vehicle controls in either group.

The 5-HT_{2A} antagonist M100907 (0.03–1.0 mg/kg) produced vehicle condition responding with relatively little variability in the 5.0 mg/kg CLZ-trained rats, while M100907 provided less than partial substitution for the 1.25 mg/kg CLZ cue (Fig. 2, right panels). In the 1.25 mg/kg CLZ group, M100907 provided full substitution in three to four different subjects at each M100907 dose, although this occurred in a non-dose-related fashion that alternated between extreme values. Given this pattern of responding for M100907 by 1.25 mg/kg CLZ subjects, substitution was not shown as a group. Despite a lack of response suppression by M100907 in either group (Fig. 2, bottom left panel), greater doses of M100907 were not tested given a decrease in selectivity for the 5-HT_{2A} receptor at higher doses (Gobert et al., 2000).

Results of substitution testing for haloperidol, M100907, and (+)-8-OH-DPAT dose combinations are shown in Fig. 3. A 0.1 mg/kg haloperidol dose was initially administered with several doses of (+)-8-OH-DPAT (0.04–0.16 mg/kg), but these combinations suppressed all responding in those rats tested (data not shown). Subsequently, a 0.05 mg/kg haloperidol dose was combined with a 0.16 mg/kg (+)-8-OH-DPAT dose and resulted in partial generalization for the 1.25 mg/kg CLZ (70.75 ± 18.73%) and 5.0 mg/kg CLZ (63.89 ± 32.04%) cues. Dose-dependent rate suppression was exhibited in both the 1.25 and 5.0 mg/kg CLZ groups [$F(3,18) = 14.70$, $P < .05$; $F(3,18) = 5.51$, $P < .05$, respectively; bottom left panel]. A 0.1 mg/kg haloperidol+ 0.12 mg/kg M100907 combination in 1.25 mg/kg CLZ-trained subjects engendered full substitution (ED_{50} = 0.04 mg/kg; CI = 0.006–0.28 mg/kg). 0.1 mg/kg haloperidol combined with M100907 provided less than partial substitution in the 5.0 mg/kg CLZ-trained rats (top right panel). Combinations of 0.05 mg/kg haloperidol with M100907 did not provide generalization in either group (top center panel). Response rates by M100907 and haloperidol combinations were not significantly reduced compared to 0.05 and 0.1 mg/kg haloperidol controls (bottom center and left panels).

4. Discussion

These results support previous findings that both CLZ 5.0 mg/kg (Moore et al., 1992; Goudie et al., 1998, 2001; Porter et al., 1999; Millan et al., 1999) and 1.25 mg/kg doses (Porter et al., 2000; Wise et al., 2001) readily establish DS control in rats with nonsignificant differences in acquisition. In addition, the typical APD haloperidol failed to substitute for both CLZ training doses, as is consistent with previous reports (Goas and Boston, 1978; Goudie et al., 1998; Goudie and Taylor, 1998; Millan et al., 1999; Porter et al., 1999, 2000).

Melperone, a butyrophenone that has been characterized as a putative atypical APD based on a limited ability to cause catalepsy (Wiesel et al., 1978), a lack of increased prolactin secretion (Bjerkenstedt et al., 1977), and a CLZ-like increase in DA release in the rat medial prefrontal cortex (mPFC) (Ichikawa et al., 2001), produced full substitution for both CLZ discriminative stimuli. Moreover, the melperone dose–response curve for the 1.25 mg/kg CLZ DS was shifted significantly further to the left compared to the 5.0 mg/kg CLZ DS. It is unlikely that substitution for these CLZ cues is due to muscarinic receptor antagonism, since melperone has a very weak affinity for these receptors (Bolden et al., 1992). Melperone also has a weak affinity for adrenergic, histamine, and 5-HT_{1A} receptors, although it has a relatively high affinity for 5-HT_{2A} compared to D_2 receptors (Richelson and Souder, 2000) thus providing a more likely mechanism for CLZ substitution.

The highly selective 5-HT_{2A} receptor antagonist M100907 in the present study, and in previous research, failed to substitute for a 5.0 mg/kg CLZ DS (Goudie et al., 1998; Millan et al., 1999). Full stimulus generalization was shown in individual 1.25 mg/kg CLZ-trained subjects to each M100907 dose, although in a non-dose-dependent manner that did not provide substitution as a group. This pattern of responding has also been reported elsewhere in rats trained to discriminate 1.25 mg/kg CLZ from vehicle, and in this study, enough animals provided CLZ-lever responding at a 1.0 mg/kg M100907 dose to provide full substitution (Wise et al., 2001). M100907 (0.16 mg/kg) has recently been established as a DS in rats (Dekeyne et al., 2002, 2003) and in generalization tests, a 2.5 mg/kg dose of CLZ produced full substitution in these animals (Dekeyne et al., 2003). Unfortunately, generalization tests with 1.25 and 5.0 mg/kg CLZ doses in this study were not conducted, thus precluding a more direct comparison with the present study.

Other 5-HT antagonists that have a much lower specificity than M100907 for 5-HT_{2A} compared to 5-HT_{2B} or 5-HT_{2C} receptor subtypes (e.g., fanaserin, ketanserin, and SR-46349) have failed to provide substitution for the 5.0 mg/kg CLZ discriminative stimulus in rats (Nielsen, 1988; Wiley and Porter, 1992; Kelley and Porter, 1997; Tang et al., 1997; Goudie et al., 1998; Millan et al., 1999). However, CLZ has been shown to fully block the discriminative stimulus effects produced by the 5-HT_{2A} receptor agonists DOM (Fiorella et al., 1995; Palumbo and Winter, 1994) and DOI (Schreiber et al., 1994), and partially block the LSD discriminative cue (Palumbo and Winter, 1994). In contrast, CLZ failed to block the DS properties elicited by the 5-HT_{2C} receptor agonist mCPP (Gommans et al., 1998). Therefore, CLZ displays 5-HT_{2A} receptor stimulus properties when tested as an antagonist at low to moderate doses, yet it is not clear to what degree these features are represented by CLZ as a DS.

The role of 5-HT_{1A} receptor agonism in the actions of CLZ and other atypical APDs has been suggested based on a moderate affinity of CLZ, quetiapine, and ziprasidone for these receptors, as well as in vivo animal studies, which show that the efficacy of CLZ, quetiapine, olanzapine, risperidone, and ziprasidone to increase extracellular DA concentrations in the rat mPFC is dependent, in part, on the availability of 5-HT_{1A} receptors (Ichikawa et al., 2001; Heidbreder et al., 2001). Despite this evidence, the 5-HT_{1A} agonist (+)-8-OH-DPAT failed to provide generalization at greater than chance levels in either group, and accordingly, (+)-8-OH-DPAT (Millan et al., 1999) and buspirone (Nielsen, 1988; Wiley and Porter, 1992) have also failed to substitute for a 5.0 mg/kg CLZ dose. Moreover, while the atypical antipsychotic and partial 5-HT_{1A} agonist, quetiapine, engenders full substitution for a 5.0 mg/kg CLZ DS (Goudie and Taylor, 1998; Carey and Bergman, 1997; Millan et al., 1999), but not for a 1.25 mg/kg CLZ DS (Porter et al., 2000), ziprasidone, which also shares these features, neither substitutes for a 1.25 mg/kg (Wise et al., 2001) nor a 5.0 mg/kg CLZ cue (Millan et al., 1999). Thus, it does not appear that 5-HT_{1A} receptor stimulation is an essential component of either low- or high-dose-mediated CLZ cues.

The typical APD and preferential DA D_2 receptor antagonist haloperidol was paired with (+)-8-OH-DPAT and M100907 to determine if these combinations may provide a more CLZ-like cue, as would be consistent with the hypothesis that dopamine and serotonin interactions are important for the unique therapeutic efficacy generated by atypical APDs (Meltzer et al., 1989). (+)-8-OH-DPAT, when combined with 0.05 mg/kg haloperidol, provided partial generalization for both the 1.25 and 5.0 mg/kg CLZ discriminative stimuli, although at a dose combination with marked rate-suppressant effects. Full substitution for the 1.25 mg/kg CLZ DS, though not for the 5.0 mg/kg CLZ DS, was produced in two subjects when M100907 was combined with haloperidol (Fig. 3). However, since this dose combination disrupted responding in the majority of 1.25 mg/kg CLZ subjects tested, these findings must be interpreted with caution until they are replicated. Partial generalization (>60% CLZ-lever responding) by the haloperidol and (+)-8-OH-DPAT combinations for both groups may indicate some similar, although limited, effects for these CLZ discriminative stimuli. However, some degree of drug lever responding was shown by each of these compounds alone, thus making an interpretation of these combined actions rather tenuous. Since neither combination of M100907 nor (+)-8-OH-DPAT with haloperidol produced convincing CLZ-like stimulus effects, it appears that the CLZ cue is mediated by more than the combination of dopamine and serotonin receptor blockade. Similar conclusions have been reached by Goudie and Taylor (1998) and Millan et al. (1999).

CLZ has a high affinity for muscarinic receptors and it has been suggested by Kelley and Porter (1997), based on substitution by the muscarinic receptor antagonist scopolamine and the M_1 receptor preferring antagonist trihexy-

phenidyl, that muscarinic receptor blockade is sufficient to provide a 5.0 mg/kg CLZ-like cue (Kelley and Porter, 1997). However, scopolamine has failed to substitute for a 1.25 mg/kg CLZ training dose (Wise et al., 2001). In the present study, trihexyphenidyl fully substituted for the 1.25 mg/kg CLZ DS but only engendered partial substitution for the 5.0 mg/kg CLZ DS. Trihexyphenidyl did produce an upward trend in 5.0 mg/kg CLZ-condition responding, but rate suppression at the 6.0 mg/kg dose precluded testing higher doses in these animals. These data indicate that muscarinic receptor antagonism is sufficient to provide substitution for the 1.25 mg/kg CLZ DS as well, although the 1.25 mg/kg CLZ cue may be more specific to M_1 muscarinic receptor antagonism. Blockade of these receptors, however, does not appear to be necessary for CLZ substitution to occur given full substitution for both 1.25 and 5.0 mg/kg CLZ cues by melperone in the present study as well as substitution by the atypical APD risperidone for a 1.25 mg/kg CLZ DS in a previous study (Porter et al., 2000). Both melperone and risperidone have very weak affinities for muscarinic receptors (Bolden et al., 1992; Bymaster et al., 1996, respectively), and this would suggest that the CLZ-like stimulus properties generated by these compounds are likely due to other mechanisms. It is likely that a number of common features between CLZ and other atypical APDs, which may or may not include muscarinic receptor antagonism, are important for CLZ stimulus generalization to occur. Thus, these results support previous conclusions that CLZ elicits a compound stimulus, which, with the notable exception of muscarinic receptor antagonism, requires multiple receptor actions to produce CLZ-like discriminative stimulus effects (Goudie and Taylor, 1998).

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