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**EVALUATION OF CLOZAPINE DISCRIMINATIVE STIMULUS PROPERTIES
AS A FUNCTION OF TRAINING DOSE**

by

Adam J. Prus

**A Thesis
Submitted to the
Faculty of The Graduate College
in partial fulfillment of the
requirements for the
Degree of Master of Arts
Department of Psychology**

**Western Michigan University
Kalamazoo, Michigan
April 2002**

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Adam J. Prus

EVALUATION OF CLOZAPINE DISCRIMINATIVE STIMULUS PROPERTIES AS A FUNCTION OF TRAINING DOSE

Adam J. Prus, M.A.

Western Michigan University, 2002

Clozapine (CLZ) is an atypical antipsychotic with negligible extrapyramidal side-effects. Unfortunately, CLZ drug discrimination (DD) research has yielded inconsistencies with CLZ's known pharmacological characteristics. Porter et al. (2000) have suggested that the standard 5.0 mg/kg CLZ training dose is too high, thus accounting for difficulty in assessing clozapine's discriminative stimulus (S^D) effects. Therefore, 16 male Sprague-Dawley rats were trained to discriminate either 1.25 (Group II) or 5.0 mg/kg (Group I) CLZ from vehicle in a two-choice DD task. The typical antipsychotic haloperidol (0.1-0.4 mg/kg) did not substitute for either CLZ S^D , with the exception of one Group I subject for a 0.4 mg/kg haloperidol dose. The muscarinic M_1 antagonist trihexyphenidyl engendered full substitution in Group II but not Group I subjects. The atypical antipsychotic melperone (0.375-3.0 mg/kg) also engendered full substitution in both groups, but at a dose (3.0 mg/kg) that severely disrupted responding. The 5-HT_{1A} agonist 8-OH-DPAT (0.04-0.16 mg/kg) and the 5-HT_{2A} antagonist MDL 100907 (0.03125-1.0 mg/kg) displayed only partial generalization in both groups. Haloperidol (0.05 mg/kg) plus 8-OH-DPAT and haloperidol (0.05-0.1 mg/kg) plus MDL 100907 combinations produced greater CLZ-appropriate responding than each drug tested alone, but only Group II subjects exhibited greater than 80% CLZ-appropriate responding to a 0.1 mg/kg haloperidol and 0.12 mg/kg MDL 100907 dose. Given similar data between groups, claims that a 1.25 mg/kg CLZ S^D is more indicative of clozapine's atypical profile are questionable.

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INTRODUCTION

The 1951 synthesis of chlorpromazine offered the first truly effective psychotherapeutic compound capable of alleviating schizophrenic symptoms and began the era of antipsychotics (Owens, 1999). However, these first antipsychotic drugs portrayed common adverse effects including extrapyramidal side effects (EPS). EPS exhibited by these compounds are characterized by loss of motor control that can range from spasms of facial musculature to the inability to walk (Kinon & Lieberman, 1996; Owens, 1999). Clozapine, a dibenzodiazepine initially synthesized in 1959, was screened in clinical trials in 1974 and did not induce EPS (Matz, Rick, Oh, Thompson, & Gershon, 1974). Clozapine was therefore considered an antipsychotic with atypical therapeutic characteristics. However, what was hailed as the ideal antipsychotic drug revealed severe consequences in 1975 when Finland hospitals reported several onsets of agranulocytosis that resulted in eight deaths (Owens, 1999). Yet, amid waning support for clozapine, Kane, Honigfeld, Singer, and Meltzer (1988) found that clozapine appeared effective in patients resistant to other antipsychotic treatments and in these patients, agranulocytosis was seldom shown. The search for clozapine-like antipsychotics effective in a broader patient population has led to a class of drugs termed atypical antipsychotics.

As opposed to “typical” antipsychotics, “atypical” antipsychotics drugs are classified by low occurrences of EPS (Seeman, Corbett, & Van Tol, 1997) and a greater therapeutic effect on negative symptoms (Kinon & Lieberman, 1996; Goudie & Smith, 1999; Owens, 1999). Further atypical characteristics include low prolactin elevation, antagonist actions at mesolimbic dopamine sites as opposed to striatal dopamine sites (Goudie & Smith, 1999), and high affinities for serotonin (5-HT)₂ receptor subtypes

versus dopamine D₂ receptors (Meltzer, Matsubara, & Lee, 1989; Kinon & Lieberman, 1996). Clozapine is considered an ideal atypical antipsychotic in patients resistant to other antipsychotic treatments (Kinon & Lieberman, 1996; Goudie & Smith, 1999; Owens, 1999), but due to severe side-effects in all but this specific patient population, the search for safer clozapine-like antipsychotics for use in a broader population continues to this day. Moreover, the pharmacological actions responsible for clozapine's therapeutic effectiveness and low EPS remain elusive after decades of study.

Binding assays have shown clozapine to act as an antagonist at histamine, serotonin (5-HT)_{2A}, muscarinic, dopamine D₂ and D₄ sites, and alpha adrenoceptors (Leysen, Janssen, Schotte, Luyten, & Megens, 1993; Goudie, Baker, Smith, Prus, Svensson, Cortes-Burgos, Wong, & Haadsma-Svensson, 2001), while also functioning as a 5-HT_{1A} agonist (Ichikawa & Meltzer, 2000). Various in-vitro and in-vivo experiments have been conducted to characterize the pharmacological profile responsible for clozapine's therapeutic value, but all have limitations. In-vitro experiments lack the multitude of variables associated with a living organism, while in-vivo experiments often lack the sensitivity achieved through in-vitro study. Moreover, as Goudie and Taylor (1998) argue, relying on binding assays alone may not yield results indicative of clozapine's pharmacological actions in-vivo, given that in-vitro and in-vivo binding assays produce dissimilar results and that data from functional behavioral assays are at times inconsistent with binding data.

A particularly useful tool for the in-vivo pre-clinical study of clozapine is the drug discrimination task. Drug discrimination is a well-established behavioral assay employed to assess the similarities of drug-induced stimulus effects that are correlated with the eliciting neurochemical actions (Stolerman, 1993; Poling & Byrne, 2000). The general notion is that drugs with similar stimulus effects also have similar pharmacological actions. In order to assess stimulus generalization in non-humans, a

two-or-three choice task is used with animals trained to respond on one operandum when the relevant drug effect is present and on another operandum when absent or noticeably different (Stolerman, 1993). It is in this way that researchers can determine the similarity of drug effects in non-humans.

Drug discrimination is a common procedure in clozapine pre-clinical research (Neilson, 1988; Goudie, Smith, Taylor, Taylor, & Tricklebank, 1998; Porter, Varvel, Vann, Philbin, & Wise, 2000), and has yielded a useful screening method for potential clozapine-like antipsychotics. Beyond antipsychotic screening, however, this assay's utility in isolating individual stimulus effects as components in the clozapine S^D has not led to definitive information into clozapine's *in vivo* effects.

Rats trained to respond in the presence of a clozapine discriminative stimulus (S^D) seldom generalize to ligands selective to receptors for which clozapine exhibits high binding affinities (Table 1) including 5-HT_{2A} (Millan, Schreiber, Monneyron, Donorme, Melon, Queriaux, & Dekeyne, 1999) 5-HT_{2C} (Goudie et al., 1998; Millan et al., 1999), dopamine (DA) D₁, D₂, D₄ (Nielson, 1988; Goudie et al., 1998; Porter, Villanueva, & Rosencrans, 1999), non M₁ muscarinic (Goas & Boston, 1978; Nielson, 1988, Kelly & Porter, 1997), alpha adrenoceptor α_1 (Nielson, 1988; Kelley & Porter, 1997; Goudie et al., 1998, Millan et al., 1999), and histamine H₁ receptors (Nielson, 1988, Kelley & Porter, 1997). However, H₁ antagonists have engendered full generalized responding in pigeons (Hoenicke, Vanecek, & Woods, 1992). In addition, atypical antipsychotics that have a 'polyvalent' receptor pharmacology similar to clozapine will often fully substitute for the clozapine S^D , including fluperlamine (Neilson, 1988), olanzapine (Moore, Tye, Axton, & Risius, 1992; Millan, et al, 1999; Porter et al., 2000, 1.25 mg/kg clozapine S^D), quetiapine (Goudie & Taylor, 1998; Millan et al., 1999), sertindole, and risperidone (Porter et al., 2000, 1.25 mg/kg clozapine

Table 1
Receptor Binding Affinities (K_i , nM)

Compound	Receptor Preference	$>$ = affinity ratio greater than 2 $>>$ = greater than 10, $>>>$ = greater than 100				
Clozapine	$H_1 >>$ $*M_4 >$ $D_3 >$	$5-HT_{2A} >$ $*M_3 >$ $D_1 >$	$5-HT_{2C} >$ $5-HT_3 >$ $5-HT_{1D}$	$\alpha_1-A >$ $5-HT_{1A} >$	$*M_5 >$ $D_2 >$	$*M_1; M_2 >$ $\alpha_2-A >$
Haloperidol	$\sigma,$	$D_2 >$	$\alpha_1-A >$	$D_3,$	$5-HT_{2A} >>>$	D_1
Melperone	$\sigma >>$	$5-HT_{2A} >$	$\alpha_1-A >$	$D_2 >$	$H_1 >$	D_3
MDL 100907**	$5HT_{2A} >>>$	$\sigma >$	$5-HT_{2C} >$	$\alpha_1-A >$	$D_4 >$	$D_2 >$
	$D_3 >$	D_5				
Trihexyphenidyl***	$M_1 >$	M_2				

Leysen et al. (1993); *Goudie et al. (2001); **Kehne et al. (1996); ***Kelley & Porter (1997)

S^D). This has led to the hypothesis that clozapine substitutable compounds must also be complex stimuli (Goudie & Smith, 1999) as opposed to compounds selective for a particular receptor subtype. However, this hypothesis does not account for the muscarinic antagonists that reliably engender full generalization (at least 80% clozapine-appropriate responding) such as scopolamine (Nielson, 1988; Kelley & Porter, 1997) and trihexyphenidyl (Kelley & Porter, 1997) as well as full stimulus blockade produced by the muscarinic agonist oxotremorine (Nielson, 1988). Some histamine antagonists have also fully substituted for clozapine both in rats (Kelley & Porter, 1997) and pigeons (Hoenicke et al, 1992).

Several studies have attributed drug discrimination findings that are inconsistent with clozapine's in-vitro characterization to the clozapine training dose used. Historically, rats have been trained to discriminate 5.0 mg/kg clozapine from vehicle with this training dose as the standard in clozapine S^D research. An investigation into a

nonstandard clozapine training dose was conducted with rats trained on 10.0 mg/kg clozapine with substitution tests across various typical and atypical antipsychotics yielding data similar to those produced by 5.0 mg/kg clozapine trained rats (Porter et al., 1999). However, 5.0 mg/kg, let alone 10.0 mg/kg, may be much higher than necessary, thus leading to a much higher degree of confounding stimulus effects that are responsible for the failure of relevant receptor-selective compounds to engender substitution for the clozapine S^D .

Systematic investigations into lower training doses were conducted by Porter et al. (2000) who found atypical antipsychotics (olanzapine, risperidone, and sertindole) that did not evoke generalization from a 5.0 mg/kg clozapine S^D , produced full substitution for a 1.25 mg/kg clozapine dose in rats. Moreover, the typical antipsychotics fluphenazine and loxapine, which exhibit pharmacological effects dissimilar to clozapine, did not produce substitution for the 1.25 mg/kg clozapine training doses. Porter et al. (2000) therefore suggested that a 1.25 mg/kg training dose of clozapine may be more indicative of clozapine's atypical profile. Goudie et al. (2001) also investigated a lower clozapine training dose and found dopamine D_3 antagonists ((+)-AJ76, nafadotride, PD 152255, (\pm)-S11566, and (+)-S14297) that failed to substitute for the 5.0 mg/kg dose also failed to substitute for a 2.0 mg/kg clozapine training dose while the D_3 antagonist PNU-99194A produced full substitution for both training doses. The Goudie et al. (2001) study remains the only one that tested receptor-selective compounds for generalization to a low clozapine training dose. Therefore, whether other selective ligands and non-antipsychotic compounds that failed to substitute for the 5.0 mg/kg clozapine training dose would yield substitution for a nonstandard clozapine training dose is unknown.

In order to further assess low versus standard clozapine training doses in the present study, two groups of rats were trained to discriminate either 5.0 mg/kg or 1.25

mg/kg clozapine from vehicle in a two-choice drug discrimination procedure. The typical antipsychotic haloperidol and the atypical antipsychotic melperone were administered to determine if they would serve as substitutes for either clozapine S^D. The muscarinic M₁ antagonist trihexyphenidyl was tested for generalization from both clozapine discriminative stimuli based upon reports that clozapine displays high affinities for these receptors and previous findings that trihexyphenidyl proved a full substitute for a 5.0 mg/kg clozapine S^D (Kelley & Porter, 1999). Clozapine also demonstrates a high affinity for 5-HT_{2A} as an antagonist and to assess this, MDL 100907 was tested for substitution. Despite clozapine's relatively low affinity in binding assays as a 5-HT_{1A} agonist (Leysen et al., 1993), recent research has alluded to this action as important in maintaining clozapine's low EPS (Ichikawa, Dai, & Meltzer, 2001) and was the rationale for testing the 5-HT_{1A} agonist (+)-8-OH-DPAT for substitution in the present study. Haloperidol was then tested in combination with (+)-8-OH-DPAT and with MDL 100907 to assess whether these combinations would exhibit pharmacological actions similar to clozapine (Homan, Copping, Elfström, van der Veen, Hallema, Mohell, Unelius, Johansson, Wikström, Grol, 1998; Ichikawa & Meltzer, 2000; Ichikawa et al., 2001) and in consideration of clozapine as a complex discriminative stimulus (Goudie & Taylor, 1998; Goudie & Smith, 1999; Millan, et al., 1999).

METHODS

Subjects

Sixteen male Sprague Dawley rats (Charles River, Portage, MI) were obtained at 50-60 days old and delivered to the animal colony at Haenicke Hall, Western Michigan University. Living facilities were maintained at a constant 20-22° and 20-24% humidity under 12 hour light/dark conditions. Animals were housed in standard plastic hanging cages with free access to water, but were food deprived to 85% of free feeding weight. Subjects were weighed prior to each session.

Materials

Apparatus

Eight standard operant chambers equipped with a food delivery mechanism and two levers equidistant from the food access were used for the drug discrimination procedure (MED Associates Inc., St. Albans, VT). Data were collected on a Dell OptiPlex Gxa computer with a Pentium II processor using the Windows 95 operating system and MED-PC (version 1.15) for Windows software (MED Associates Inc., St. Albans, VT).

Drugs

Monoject insulin syringes (Sherwood Medical, St. Louis, MO) were used for drug administration. The following drugs were used in this study: the atypical antipsychotics clozapine (0.078 – 7.5 mg/kg, i.p.) and melperone (0.75-3.0 mg/kg, s.c.), the 5-HT_{1A} agonist (+)-8-OH-DPAT (0.04 – 0.16 mg/kg, s.c.), the 5-HT_{2A} antagonist MDL 100907 (0.03125-1.0 mg/kg, s.c.) (gifts from Herbert Meltzer, Vanderbilt

University, Nashville, TN), the typical antipsychotic haloperidol (0.05-0.4 mg/kg, i.p.) (Sigma, St. Louis, MO), the M_1 antagonist trihexyphenidyl (0.1875-6.0 mg/kg, i.p.) (Sigma, St. Louis, MO), and the D_2 agonist amphetamine (1.0 mg/kg, i.p.) (NIDA, Bethesda, MD). All drugs were administered 30 minutes prior to each session with the exception of haloperidol, which was given 60 minutes prior. Clozapine and MDL 100907 were dissolved in 0.1 N HCl and then adjusted to pH~5.0 with NaOH. Haloperidol was dissolved in a few drops of Lactic Acid and adjusted to pH~4.5 with NaOH. Amphetamine was dissolved in sterile 0.9% physiological saline, and (+)-8-OH-DPAT and trihexyphenidyl were dissolved in sterile de-ionized water.

Training Procedures

After 1 week habituation to housing conditions, 16 rats were randomly divided into two groups of equal number. One group was trained to discriminate 5.0 mg/kg clozapine from vehicle (Group I) and the other group was trained to discriminate 1.25 mg/kg clozapine from vehicle (Group II). Prior to each session, levers were cleaned with isopropyl alcohol to decrease the likelihood of preference due to olfactory cues (Extance & Goudie, 1981). Subjects were weighed to determine injection volume. All subjects were exposed to a fixed-time 60 second 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 only the center lever present. On subsequent trials, errorless training was conducted after both clozapine and vehicle administrations consisting of only the condition-appropriate lever present. All subjects were exposed to two of these errorless training sessions per condition.

Once lever pressing was emitted consistently during the errorless training sessions, both levers were presented to subjects after clozapine or vehicle was administered. Clozapine and vehicle injections were administered intraperitoneally 30

minutes prior to the relevant training session. Reinforcement was provided during 20-minute training sessions for pressing the appropriate lever beginning on a FR 1 schedule that was increased progressively until responding under a FR 20 reinforcement schedule occurred. The order of drug (D) and vehicle (V) training sessions was set in this pattern: VDVVDVDDVD. Once FR 20 responding was established under both clozapine and vehicle conditions, condition-appropriate responding was assessed for reaching the drug discrimination criteria. The criteria for reaching accurate discrimination of either the clozapine S^D or vehicle S^D was at least 80% of condition-appropriate responses during the first FR 20 and for the remainder of the 20 minute session. This criterion must have been reached for 9 out of 10 consecutive sessions before testing could begin.

Testing Procedures

Before a test session, each subject must have been exposed to both a clozapine and vehicle training session with at least 80% of condition-appropriate responses prior to 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. Otherwise, the test session ended after 20 minutes. Percent responding was recorded for subjects emitting at least 10 responses during the session as is consistent with other drug discrimination procedures where response disruption from test compounds denies completion of 20 responses (Nielson, 1988). Data were collected in terms of percentage of clozapine-appropriate responding and responses emitted per second (RPS). Percentages were graphed in dose-response curves as average group percent responding for each dose.

Data Analysis

All data analyses and graphs were produced using GraphPad version 3.0 for Macintosh (GraphPad Software, Inc., San Diego, CA). Dose-response curves were generated for each drug tested, with the exception of a single dose of amphetamine. The group mean percent clozapine-appropriate responding and responses per second were plotted for each dose tested. Full stimulus generalization referred to 80% or greater clozapine S^D-appropriate responding with only subjects that emitted at least 10 responses included in these percentage calculations. Moreover, 20-80% clozapine S^D-appropriate responding was considered partial substitution, while less than 20% was referred to as no clozapine S^D-appropriate responding. The ED₅₀s were assessed through non-linear regression analyses only on dose-response curves reaching greater than 80% clozapine S^D-appropriate responding for three or more subjects included in that point. Therefore, ED₅₀s were calculated for dose-response curves that not only reached full generalization, but also contained sample sizes high enough to adequately represent the group. Both one-way and two-way analyses of variance (ANOVA) were used to determine statistical significance at the $p = 0.05$ level across response rate data for each drug tested, and curves with $p < 0.05$ were further assessed in Tukey post hoc comparison tests to determine which points were statistically different.

RESULTS

Discrimination Training

Subjects trained to discriminate 5.0 mg/kg clozapine (Group I) from vehicle reached the discrimination criterion in 48.83 ± 15.28 sessions (range 30-70 sessions), while the number of sessions for the subjects trained to discriminate 1.25 mg/kg clozapine (Group II) from vehicle was higher at 54.71 ± 18.47 (range 27-79 sessions). One subject in each group failed to maintain the drug discrimination criteria due to chamber malfunctions and was excluded from the study, leaving $N=7$ in each group. The difference between group means was not statistically significant in a two-tailed t test, $t(11) = 0.6184$, $p = 0.5489$.

The session numbers in Figure 1 refer to the n th exposure to the drug or vehicle condition for each subject after maintaining responding under the FR 20 schedule. Therefore, the fifth exposure to drug for each subject was plotted in the session 5 point. In Figure 1 percentages are based upon clozapine-appropriate responding assessed during the first FR 20 for each session, and therefore illustrates the development of stimulus control as slopes increased for clozapine sessions (Group I = 1.50; Group II = 1.49) and decreased for vehicle sessions (Group I = -5.26; Group II = -1.32) to beyond criterion levels.

Maintenance of condition-appropriate responding during the final clozapine and vehicle training sessions of this study is shown in Figure 2. These session numbers refer to the final exposures to drug and vehicle training sessions for each subject. Percentages are based upon clozapine-appropriate responding assessed during the first FR 20 for each session.

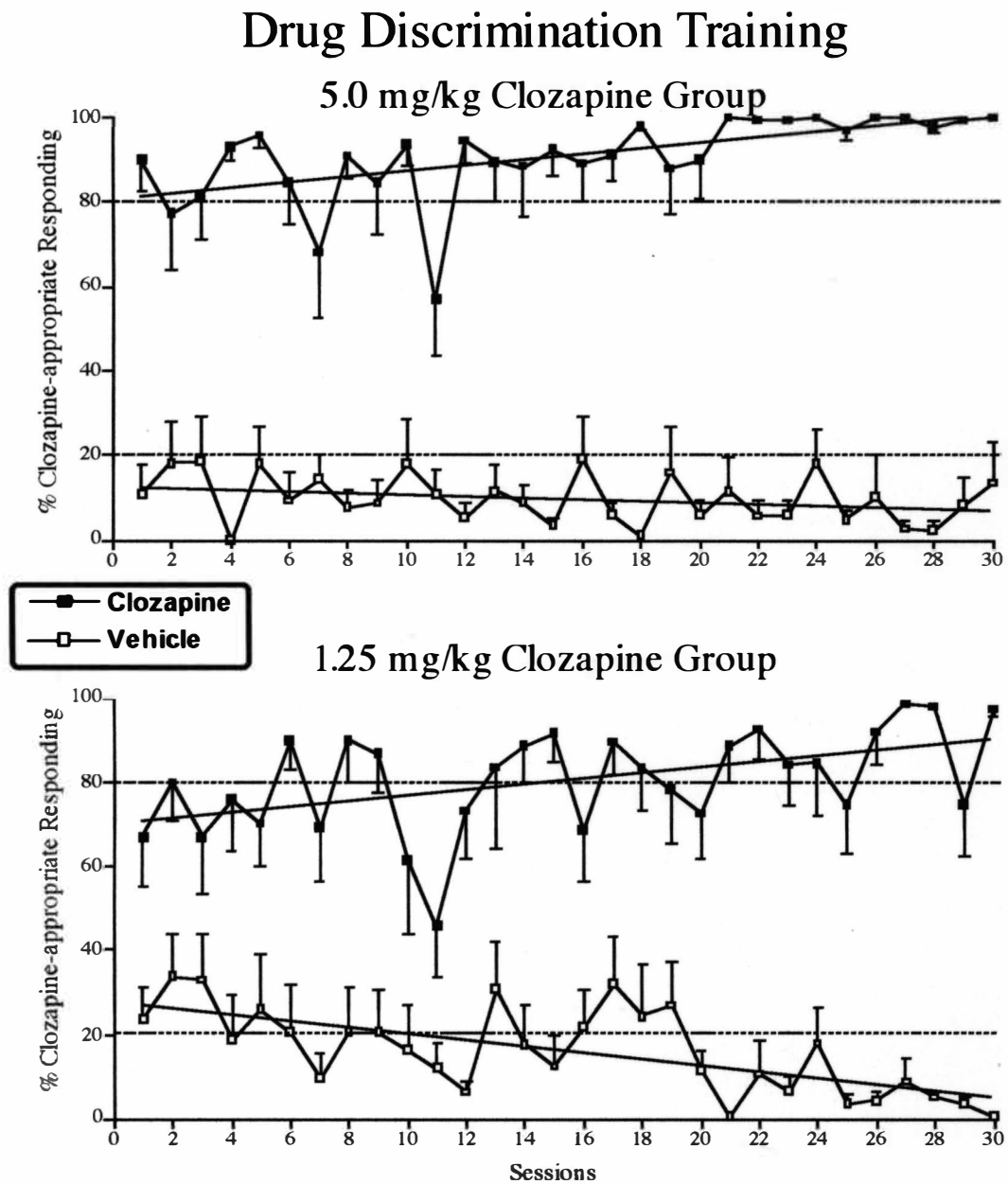


Figure 1. Drug Discrimination Training

Clozapine

Both groups exhibited dose-dependent responding when doses of clozapine (0.31-7.5 mg/kg) were administered. The 1.25 mg/kg clozapine group achieved full

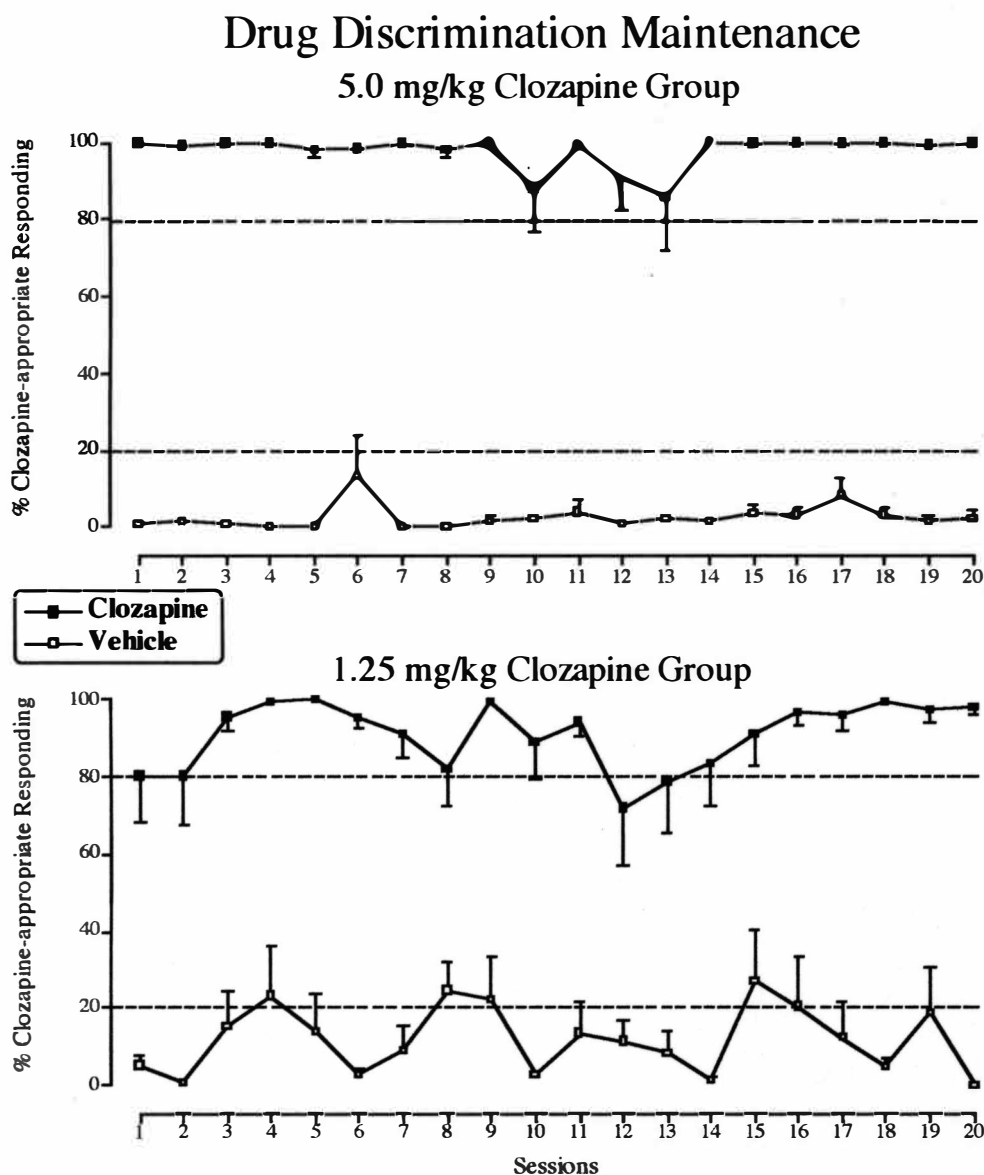
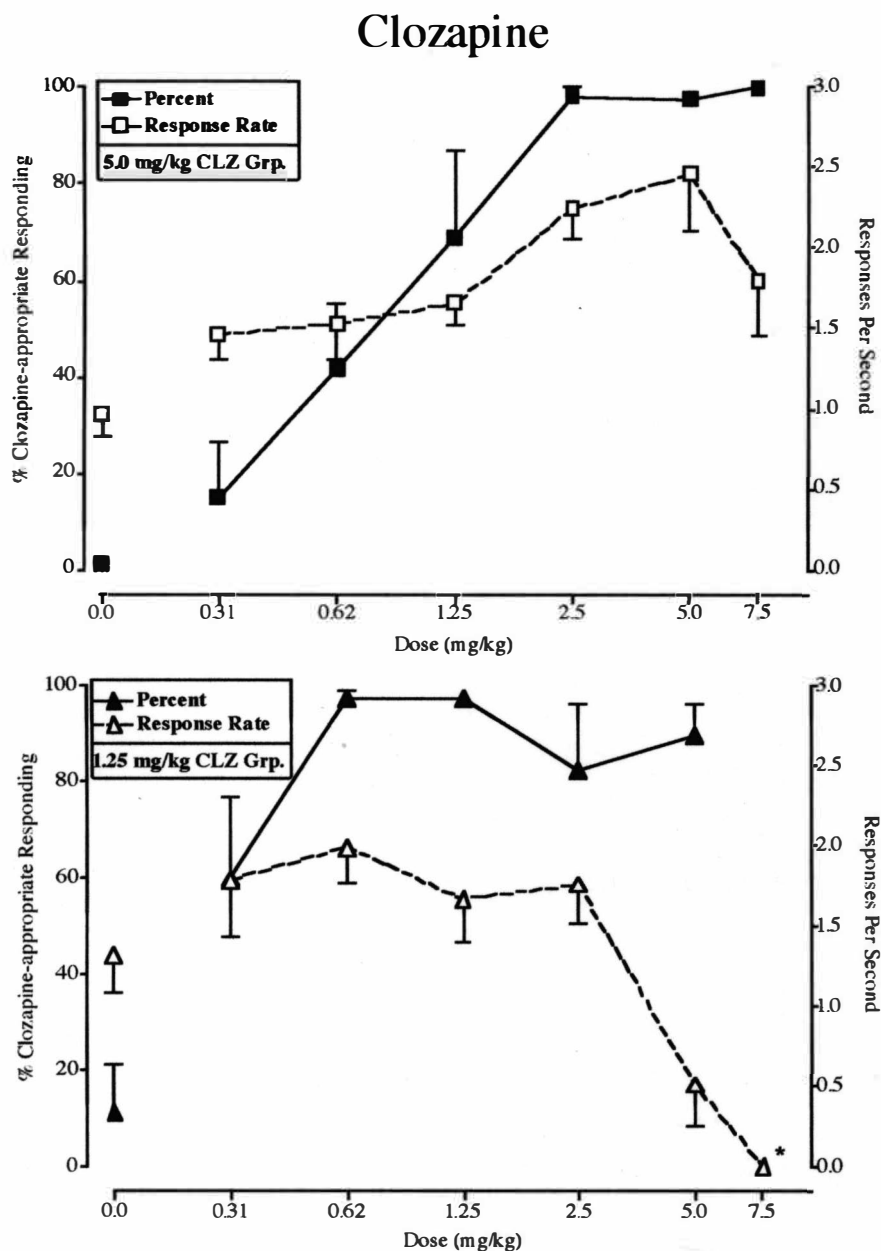


Figure 2: Drug Discrimination Maintenance

generalization with 0.625 mg/kg clozapine at $97.4 \pm 1.67\%$ clozapine-appropriate responding, but due to much greater than 20% clozapine-appropriate to a 0.31 mg/kg dose, an accurate ED_{50} could not be calculated. A 0.07 mg/kg dose was tested in Group II subjects (Data not shown), but even this dose engendered full generalization in some subjects, thus raising the mean well above 20% clozapine-appropriate responding.



*Fewer than half of the subjects emitted 10 or more responses

Figure 3. Clozapine Dose-Response Curves

Group II responding was completely disrupted (0.001 ± 0.001 responses per second [RPS], $F(6, 41)$, $p < 0.0001$) following the 7.5 mg/kg clozapine dose (Figure 3). Full substitution (greater than 80% clozapine-appropriate responding) did not occur in the 5.0 clozapine group until a 2.5 mg/kg clozapine dose was tested ($ED_{50} = 0.85$ mg/kg;

95% confidence interval (CI) 0.31-2.32 mg/kg), and responding was neither suppressed following a 7.5 mg/kg clozapine dose (1.577 ± 0.363 RPS) nor statistically different from vehicle in this group (Figure 3).

Amphetamine

A single dose of amphetamine (1.0 mg/kg) was tested as a negative control to ensure that a drug effect presumably not present in the clozapine cue would not engender clozapine-appropriate responding. When tested, the 5.0 mg/kg clozapine group produced 0.00 ± 0.00 (N=6) percent clozapine-appropriate responding while Group II subjects engendered $6.70 \pm 2.69\%$ (N=5). Sample sizes lower than the group size (N=7) were due to subjects not attaining the test session criteria prior to the amphetamine test, and due to one subject in each group emitting no responses to either condition during this session. The difference between groups was significant at the 0.05 level in a two-tailed t-test ($t(8) = 2.491$) with $p = 0.0221$, but neither group emitted full nor partial generalization to the amphetamine stimulus.

Trihexyphenidyl

Trihexyphenidyl (0.75-6.0 mg/kg), a selective M_1 antagonist, produced dose-dependent increases in clozapine-appropriate responding in both groups, but greater than 80% responding was exhibited only in the 1.25 mg/kg clozapine trained subjects (Group II) to both a 3.0 and 6.0 mg/kg dose (Figure 4). An additional trihexyphenidyl dose, 0.1875 mg/kg, was tested in Group II rats to allow for a more representative ED_{50} calculation ($ED_{50} = 0.62$ mg/kg; CI 0.24-1.62 mg/kg). Group I clozapine-appropriate responding increased to only $61.80 \pm 20.08\%$ at the highest trihexyphenidyl dose tested. Group I response rates were not statistically different from vehicle, although differences in Group II rates were statistically significant ($F(4, 34) = 2.766$, $p = 0.0454$). A Tukey

Trihexyphenidyl

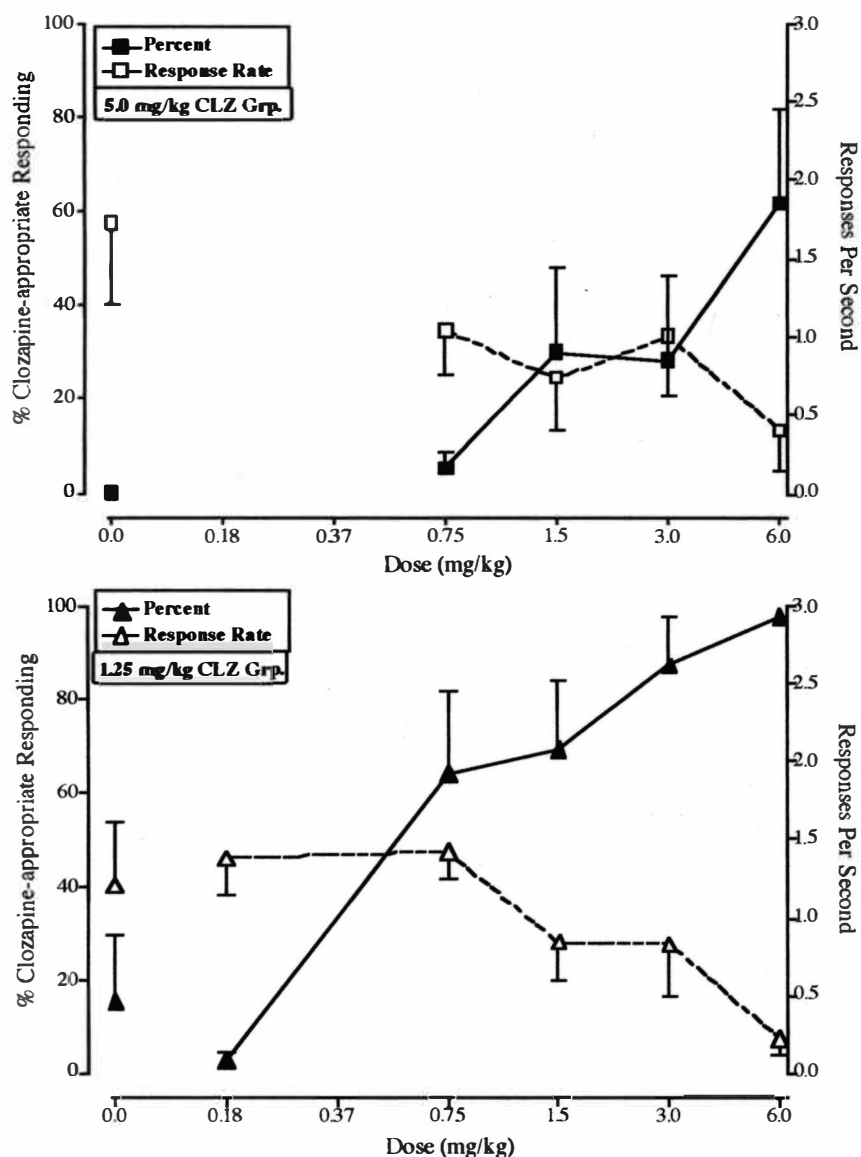


Figure 4. Trihexyphenidyl Dose-Response Curve s

post hoc test revealed a statistically significant difference only between 0.75 and 6.0 mg/kg doses ($p < 0.05$; CI 0.07-2.33).

Melperone

The atypical antipsychotic melperone (0.375-3.0 mg/kg) (Figure 5) produced incremental dose-dependent responding to levels reaching full substitution in both

groups, although at a dose (3.0 mg/kg) that markedly reduced response rates (Group I, 0.01 ± 0.00 RPS, $F(4, 34) = 2.67$, $p < 0.05$; Group II, 0.00 ± 0.00 RPS, $F(4, 34)$, $p < 0.05$). Less than half of the subjects completed at least 10 responses at a 3.0 mg/kg dose, with only $N=2$ in Group I and $N=1$ in Group II. This precluded calculation of ED_{50} s despite exceeding full substitution criteria. Group II response rates following the 3.0 mg/kg dose were significantly different from the 0.75 mg/kg dose ($p < 0.01$; CI 0.26-2.37), but not significantly different from vehicle in Tukey post hoc comparisons.

Haloperidol

Haloperidol (0.1-0.4 mg/kg) (Figure 6) produced full generalized responding in only one animal from Group I at a 0.4 mg/kg haloperidol dose. Other Group I subjects tested at this dose did not emit at least 10 responses (0.001 ± 0.001 RPS) and were not included in this percentage. Dose-dependent response suppression was exhibited in both groups following haloperidol administration, but 0.04 mg/kg exhibited higher rates of responding (0.671 ± 0.329 RPS) in Group II than in Group I. This difference in rate was not statistically significant.

8-OH-DPAT

The 5-HT_{1A} agonist 8-OH-DPAT (0.04-0.16 mg/kg) (Figure 6) engendered only partial substitution in both groups. Group II subjects emitted up to $45.4 \pm 19.29\%$ clozapine lever responses following a 0.08 mg/kg dose and Group I subjects emitted up to $42.65 \pm 24.33\%$ clozapine lever responses following a 0.16 mg/kg dose. The highest 8-OH-DPAT dose tested, 0.16 mg/kg, greatly reduced responding in both groups (Group I, 0.037 ± 0.028 RPS; Group II, 0.029 ± 0.020 RPS), precluding assessment of higher doses.

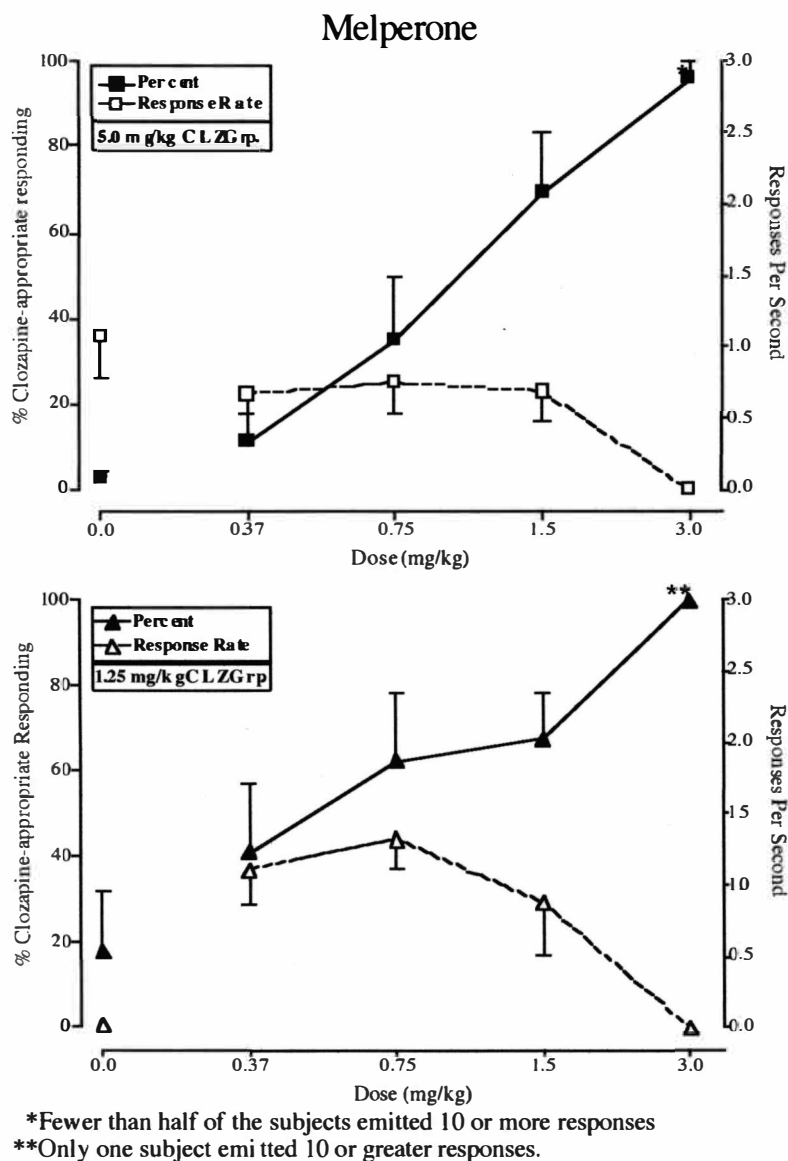


Figure 5. Melperone Dose-Response Curves

Haloperidol + 8-OH-DPAT

A 0.1 mg/kg haloperidol dose was initially combined with several doses of the 5-HT_{1A} agonist 8-OH-DPAT (0.04-0.16 mg/kg), but these combination doses suppressed all responding in the rats tested (Data not shown). Subsequently, a 0.05 mg/kg haloperidol dose was combined with the same 8-OH-DPAT doses (Figure 6) and these combination doses resulted in dose-dependent rate decreases that were

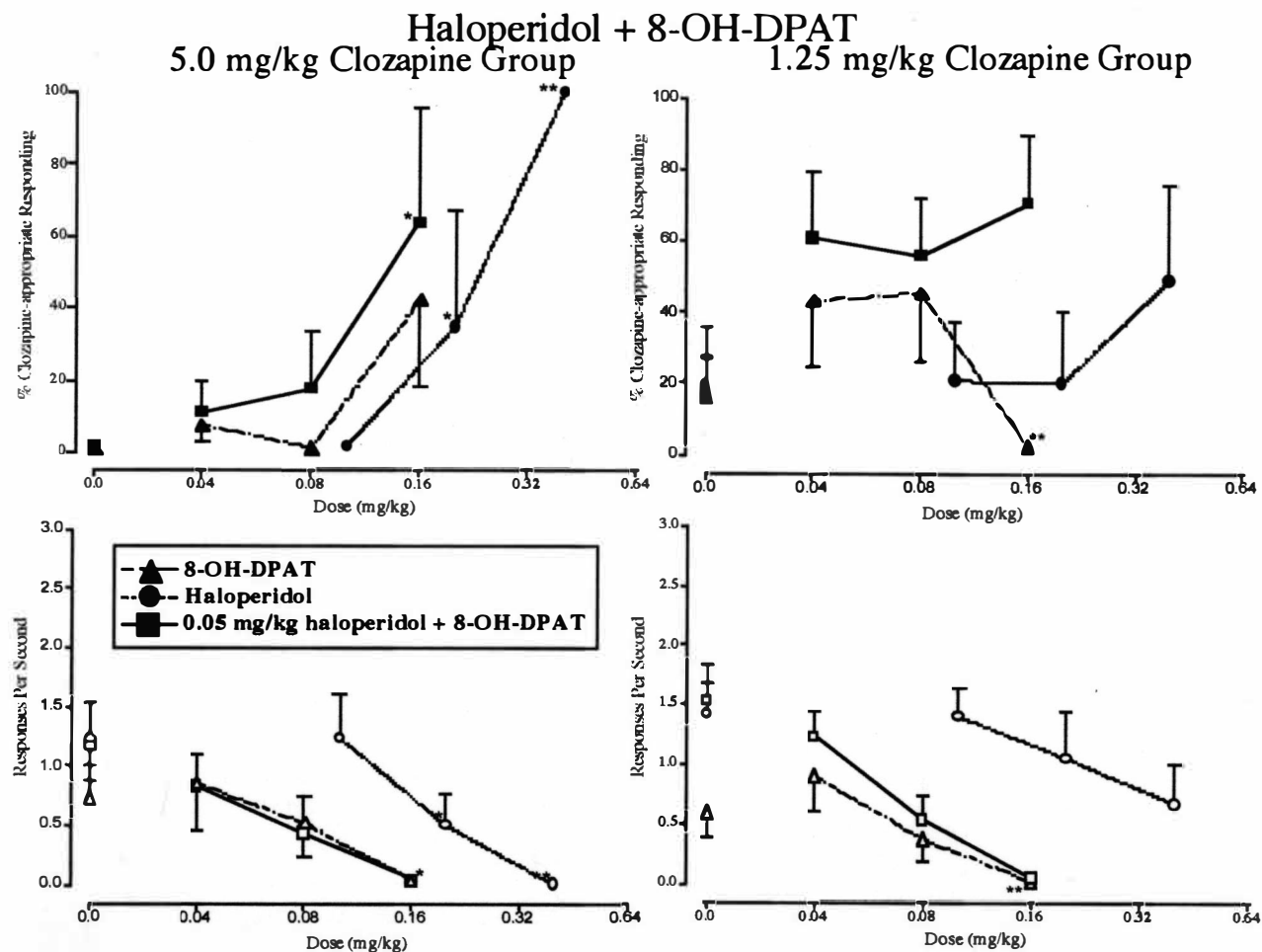
statistically different from vehicle in both groups (Group I, $F(3, 24)=3.50$, $p=0.0307$; Group II, $F(3,24)=10.25$, $p=0.0009$). Neither group demonstrated greater than partial generalized responding, but each exhibited greater clozapine-appropriate responding with the combination of haloperidol and 8-OH-DPAT. These curves were not statistically different from each other in two-way ANOVA's.

MDL 100907

Group I subjects completely failed to generalize to the 5-HT_{2A} antagonist MDL 100907 (0.03125-1.0 mg/kg), while Group II subjects emitted partial clozapine-appropriate responding with considerable variations in mean responding across the dose range (Figure 7). Individual data from Group II revealed that 6 out of 7 subjects exhibited full generalized responding, but in a “razor tooth” fashion precluding determination of MDL 100907 substitution in a dose dependent manner (Figure 8). In contrast, individual Group I subjects displayed no substitution for the 5.0 mg/kg clozapine S^D with negligible variability (Figure 9). Response rates for both groups were not statistically different in a one-way ANOVA.

Haloperidol + MDL 100907

A 0.1 mg/kg haloperidol dose in combination with MDL 100907 (0.03-1.0) produced incremental clozapine-appropriate responding in Group I subjects up to $55.16 \pm 19.35\%$ at a 0.1 mg/kg haloperidol and 1.0 mg/kg MDL 100907 combination. However, a 0.1 mg/kg haloperidol and 0.125 mg/kg MDL 100907 combination in Group II subjects engendered full substitution for the 1.25 mg/kg clozapine S^D ($81.00 \pm 19.00\%$, $n=2$). Full substitution did not occur when 0.1 mg/kg haloperidol and 1.0 mg/kg MDL 100907 doses were administered. A two-way ANOVA revealed that both Group I and Group II percent clozapine-appropriate responding was significantly

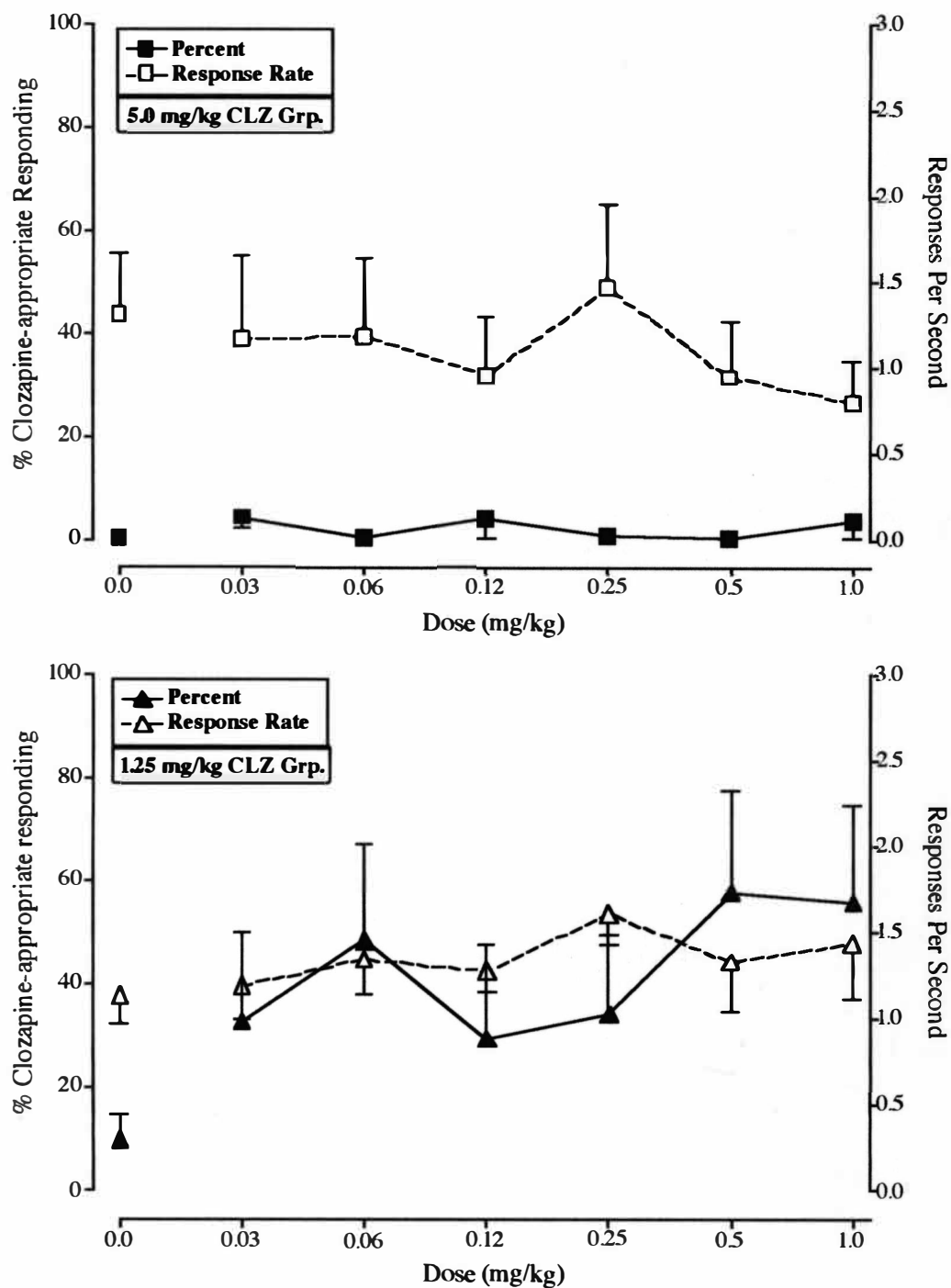


*Fewer than half of the subjects emitted 10 or more responses.
 **Only one subject emitted 10 or more responses.

Figure 6. Haloperidol + 8-OH-DPAT Dose-Response Curves

altered as the dose of haloperidol changed (Group I, $F(2, 63)$, $p=0.0022$; Group II, $F(2, 50)=3.19$, $p=0.0498$), although the interaction with MDL 100907 doses was not statistically significant. Response rates for either 0.05 or 0.1 mg/kg haloperidol and MDL 100907 were not statistically different from vehicle in either group. Haloperidol and MDL 100907 combination dose-response curves are shown in Figure 10.

MDL 100907

**Figure 7.** MDL 100907 Dose-Response Curves

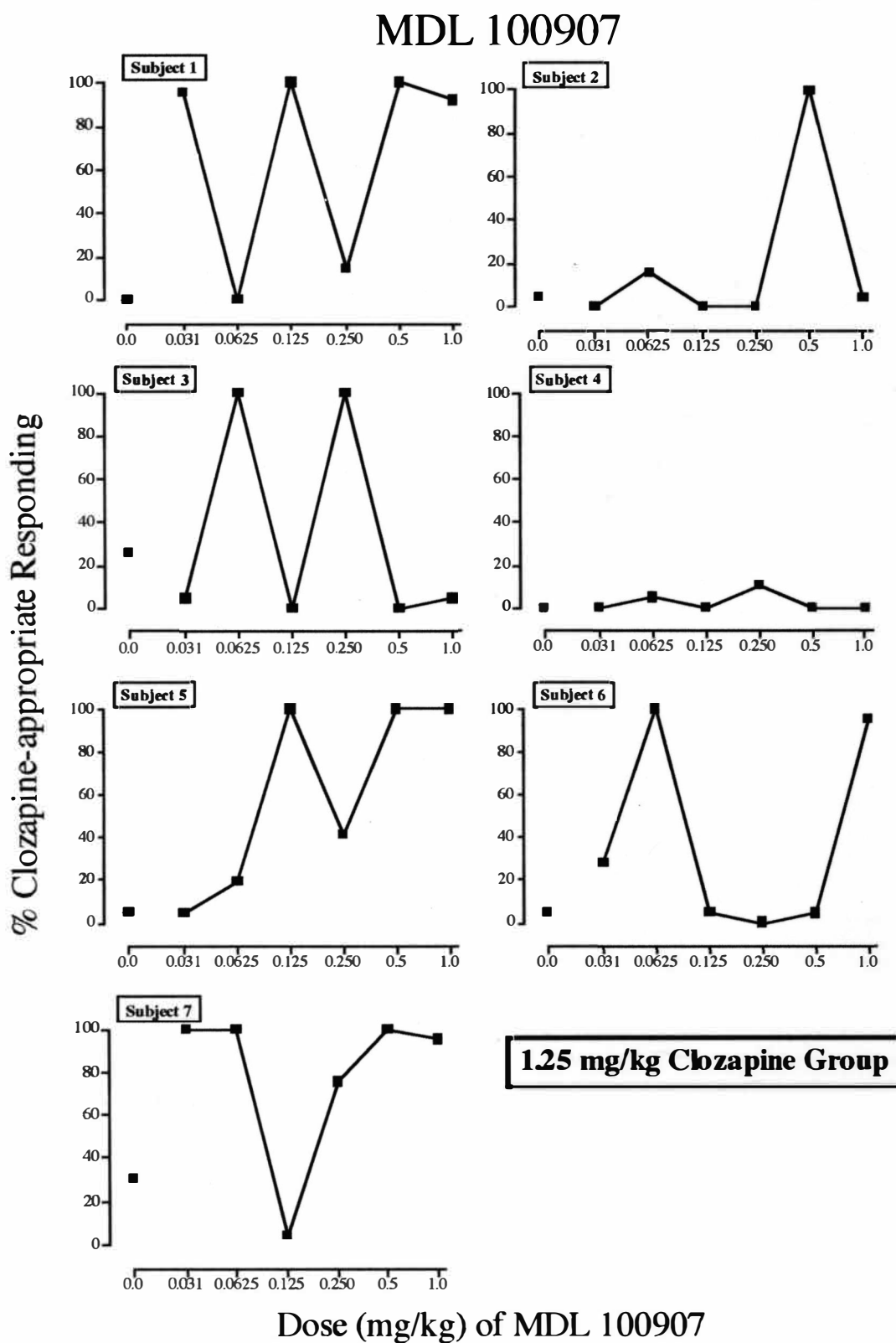


Figure 8. MDL 100907 Group II Individual Subject Dose-Response Curves

MDL 100907

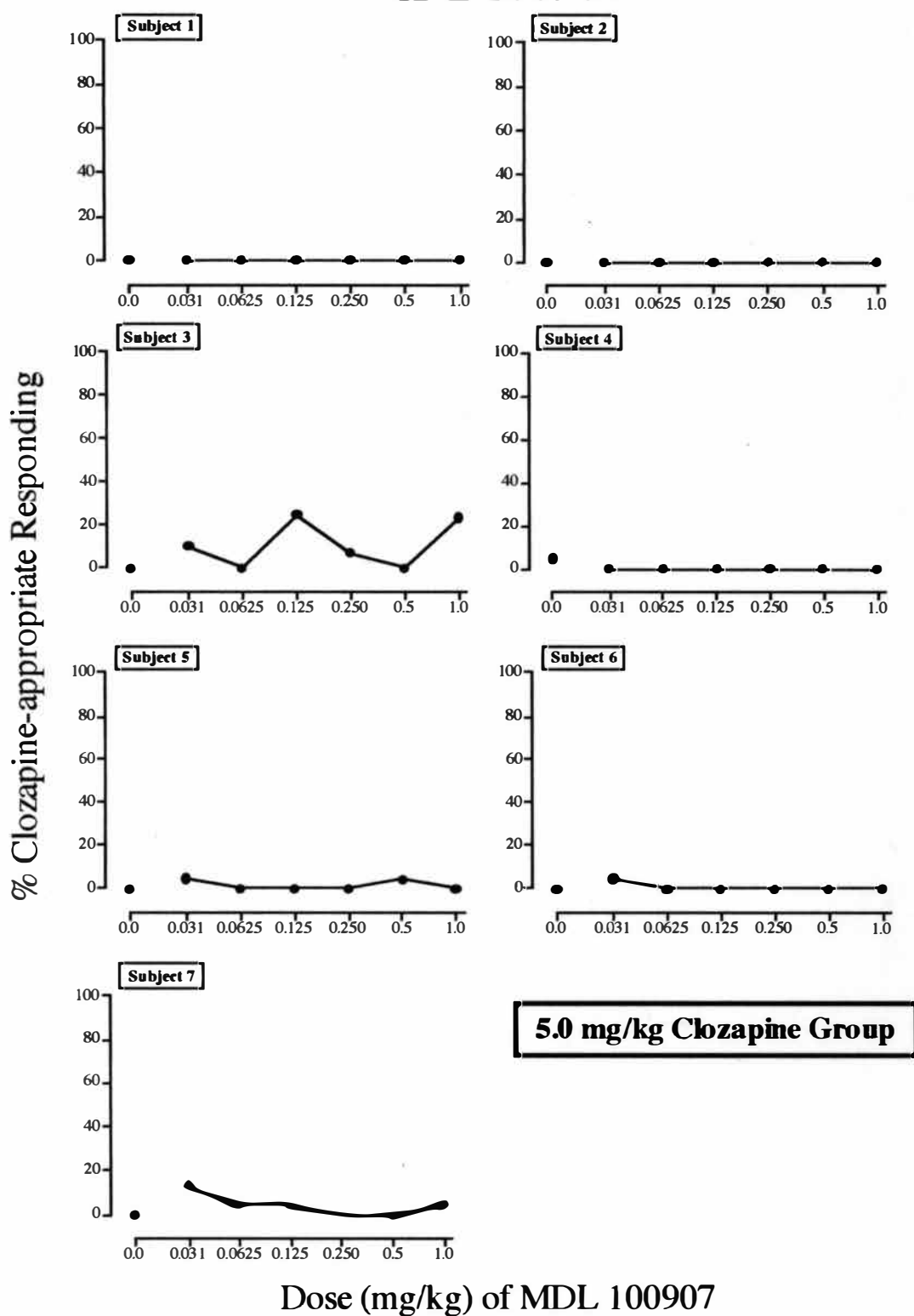


Figure 9. MDL 100907 Group I Individual Subject Dose-Response Curves

DISCUSSION

Both groups established accurate responding under clozapine and vehicle conditions with the number of sessions to criterion not statistically different between groups, despite high variability exhibited by the rats trained to discriminate 1.25 mg/kg clozapine from vehicle (Group II) relative to those discriminating 5.0 mg/kg clozapine from vehicle (Group I) (Figure 1). The number of sessions for Group II (54.71 ± 18.47) was much greater than those reported by Porter et al. (2000) at only 28.1 (range 21-32) mean sessions, but this differential of approximately 26 sessions is most likely due to differences in training procedures between studies.

The training criteria used by Porter et al. (2000) required only four out of five consecutive sessions of greater than 80% condition-appropriate responding whereas the current study required 9 out of 10 sessions. Most Group II subjects achieved 4 out of 5 sessions of 80% or greater clozapine-appropriate responding, but did not maintain such accuracy for 9 out of 10 sessions until much later, thus drawing question to the lower drug discrimination criteria previously that Porter et al. (2000) used.

Drug discrimination accuracy remained lower in Group II subjects than in Group I subjects throughout the study. The issue of response maintenance when using a low dose of drug as an S^D has been a concern in the drug discrimination literature, because according to Tomie, Shultz, Spicer, and Peoples (1995), subjects trained on low training doses will at times emit greater non-drug than drug-appropriate responding. Therefore, maintaining drug-appropriate responding during training sessions can be problematic. However, in the current experiments, Group II subjects displayed periods of inaccuracy to both clozapine and vehicle conditions, as was evident at the beginning (Figure 1) and end (Figure 2) of this study.

Both groups expectedly displayed full generalized dose-dependent responding to clozapine (0.312–7.5 mg/kg). Moreover, the Group I dose response curve appeared to be shifted further right ($ED_{50}=0.85$ mg/kg) than the Group II curve (ED_{50} N/A), and with the exception of haloperidol, Group II dose response curves displayed higher clozapine-appropriate responding to compounds tested throughout the study. Such evidence has led to the hypothesis that low clozapine dose S^D 's are more sensitive than higher training doses (Porter et al., 2000) and similarly may attribute for the high variability exhibited in the Group II dose response curves. In order to ensure that a presumably non-clozapine like effect would engender little clozapine-appropriate responding, a single dose of amphetamine (1.0 mg/kg) was tested and failed to act as a clozapine S^D substitute.

The typical antipsychotic and D_2 antagonist haloperidol also generally fails to substitute for the clozapine stimulus (Goas & Boston, 1978; Moore et al., 1992; Tang et al., 1997; Goudie et al., 1998; Goudie & Taylor, 1998; Millan et al., 1998; Porter et al., 1999; Porter et al., 2000), as was shown in this study with the exception of one Group I subject that generated full clozapine-appropriate responding to a 0.4 mg/kg haloperidol dose. All other subjects failed to make at least 10 responses at this dose, thus precluding analysis of other Group I subjects. Conversely, Group II subjects appeared less disrupted by all haloperidol doses with over half completing test sessions at a 0.4 mg/kg haloperidol dose, in comparison to this dose tested in Group I subjects. Moreover, in rats also trained to discriminate 1.25 mg/kg clozapine from vehicle, Porter et al. (2000) reported severe rate suppression (2.8 ± 1.1 responses per minute) at a 0.2 mg/kg haloperidol dose, which is a much greater response disruption than shown in Group II subjects at 0.2 (1.05 ± 0.38 responses per second) and 0.4 mg/kg (0.67 ± 0.33 responses per second) haloperidol doses.

The implication for a 1.25 mg/kg clozapine dose to exhibit greater actions at D₂ receptor sites seems unlikely given clozapine's relatively low preference at D₂ sites (Leysen, Janssen, Schotte, Luyten, and Megens, 1993; Bymaster, Calligaro, Falcone, Marsh, Moore, Tye, Seeman, and Wong, 1996) and lack of generalization to haloperidol in Group II rats. Other mechanistic parallels shown through receptor binding are also unlikely (Leysen et al., 1993) given that rats not trained on clozapine or clozapine-like compounds are also disrupted by comparable haloperidol doses (Baker, Riddle, Saunders, & Apple, 1993; Baker, Virden, Miller, & Sullivan, 1997). Recent microdialysis research indicates that 5-HT receptor binding generated by clozapine may act to facilitate dopamine release, therefore counteracting some dopamine inhibition engendered by clozapine's antagonist actions (Ichikawa, Ishii, Bonaccorso, O'Laughlin, Fowler, & Meltzer, 2001). Given chronic treatment on low dosage clozapine, this counter dopaminergic receptor function exhibited by 5-HT receptor effects may be less significant in the low dose cue. Therefore, this would possibly create an increased tolerance to dopamine inhibition than that demonstrated by chronic clozapine administration at a higher dose.

Through radio-ligand binding assays, clozapine displays a high preference for 5-HT_{2A} versus D₂ receptors (Leysen et al., 1993) and is a feature of most other atypical antipsychotics (Kinon & Lieberman, 1996). This is reinforced by full substitution in both groups by melperone, an atypical antipsychotic that primarily shares high preference 5-HT_{2A} characteristics with clozapine (Christensson, 1989; Leysen et al., 1993). Given clozapine's high preference for 5-HT_{2A} receptors, the surprising inability for selective 5-HT_{2A} antagonists to substitute for clozapine is one of the defining traits for clozapine's "elusive" discriminative stimulus properties (Wiley & Porter, 1992; Goudie et al., 1998; Goudie & Smith, 1999; Millan, et al., 1999), thus strengthening claims that clozapine functions as a complex discriminative stimulus consisting of

multiple components. This hypothesis has been used to account for the failure of many relevant subtype-selective compounds to engender clozapine substitution, although it has been established that antihistamines (Kelley & Porter, 1997) and antimuscarinic (Nielson, 1988; Kelley & Porter, 1997) compounds have in some studies produced generalization to the clozapine S^D .

The highly selective 5-HT_{2A} antagonist MDL 100907 (Kehne et al., 1996; Zhang & Bymaster, 1999) also completely failed to substitute for the clozapine S^D in Group I subjects and only partially substituted in Group II subjects. However, individually graphed subject data revealed full generalized responding exhibited by most Group II subjects (Figure 8), although a “razor-tooth” pattern that alternated between extreme values within these graphs preventing identification of a dose-dependent relationship. Similar findings were shown by Millan et al. (1999) who reported a biphasic dose response curve with MDL 100907 in 5.0 mg/kg clozapine trained rats with up to 67% mean clozapine-appropriate responding. Given that MDL 100907 was the first 5-HT_{2A} antagonist tested for substitution in rats trained to discriminate 1.25 mg/kg clozapine from vehicle, further testing should be conducted to determine whether this finding is resultant of increased 5-HT_{2A} sensitivity in the low-dose S^D or an effect unique to MDL 100907. The combination of haloperidol and MDL 100907 provided greater stability in Group II responding, and generated higher clozapine-appropriate responding with the haloperidol-MDL 100907 combination than with either compound tested alone in both groups. This was especially evident when a haloperidol 0.1 mg/kg and MDL 100907 0.125 mg/kg dose combination engendered full generalization in Group II subjects. Given that clozapine has marked affinities to both 5-HT_{2A} and D₂ receptors, the combination of these two effects in haloperidol and MDL 100907 combined may grant a more clozapine-like stimulus. Since full substitution resultant of haloperidol and MDL 100907 concomitant effects occurred only in Group II subjects

and at only one point, it is difficult to assess whether this was only due to a 1.25 mg/kg clozapine S^D's greater representation of clozapine's atypicality or to general instability in the maintenance of stimulus control in Group II.

Other serotonergic clozapine actions have been identified as possible keys to clozapine's therapeutic effectiveness, particularly recent evidence that clozapine functions as an agonist at 5-HT_{1A} receptors (Goudie & Taylor, 1998; Ichikawa & Meltzer, 2000), which may account for cortical dopamine release exhibited after clozapine administration (Ichikawa & Meltzer, 1999; Ichikawa & Meltzer, 2000; Ichikawa, Dai, & Meltzer, 2001). The 5-HT_{1A} agonist 8-OH-DPAT failed to engender clozapine-appropriate responding in Group I rats as was consistent with previous reports (Goudie & Taylor, 1998) and did not substitute in Group II subjects. However, the combination of haloperidol (0.05 mg/kg) with 8-OH-DPAT (0.16 mg/kg) resulted in high-partial substitution for clozapine in Group I rats completing the session, lending a greater clozapine-like stimulus effect from the haloperidol-8-OH-DPAT combination than with each compound tested alone. Haloperidol and 8-OH-DPAT concomitant effects may, in addition to reducing haloperidol D₂ antagonist effects in the striatum, potentiate dopamine antagonism in the nucleus accumbens, but also stimulate cortical dopamine release--all in a clozapine-like manner, (Ichikawa & Meltzer, 2000; Ichikawa et al., 2001) thus possibly accounting for high-partial substitution in the drug discrimination task.

Although 5-HT effects comprise the leading hypotheses for clozapine's therapeutic effectiveness, muscarinic receptors have also been considered in mediating clozapine function given clozapine's high muscarinic receptor affinity. As demonstrated previously, the muscarinic antagonists scopolamine (Nielson, 1988; Kelley & Porter, 1997) and trihexyphenidyl (M₁ selective) (Kelley & Porter, 1997) both engendered full clozapine-appropriate responding in rats trained to discriminate 5.0

mg/kg clozapine from vehicle while the non-subtype selective muscarinic agonist oxotremorine induced full clozapine stimulus blockade (Neilson, 1988).

Trihexyphenidyl administered in the current study produced full generalization in Group II subjects but only up to 61.8% substitution in the 5.0 mg/kg clozapine rats. Given trihexyphenidyl induced response reduction, it is unclear whether full generalization would have been attained in Group I subjects had higher doses been tested, despite an upward trend in the Group I dose response curve.

CONCLUSIONS

Although the current study has demonstrated differences between 1.25 and 5.0 mg/kg clozapine discriminative stimuli, difficulty in maintaining stimulus control with the 1.25 mg/kg dose and subsequent high variability in dose response curves, question the practicality of training rats to discriminate a 1.25 mg/kg clozapine dose from vehicle. Moreover, generalization to compounds tested in this study were generally closely related between groups, regardless of claims that a 1.25 mg/kg training dose is more representative of clozapine's atypical profile (Porter et al., 2000).

Given that appropriate receptor selective drugs often fail to substitute for the clozapine S^D , this study demonstrated that combining such drugs can yield higher percentages of clozapine-appropriate responding than each drug tested alone as demonstrated with the haloperidol + 8-OH-DPAT and haloperidol + MDL 100907 combinations. Therefore, locating a solitary receptor action responsible for mediating clozapine's discriminative stimulus effects may be impossible.

BIBLIOGRAPHY

- Baker, L.E., Riddle, E.E., Saunders, R.B., & Apel, J.B. (1993). The role of monoamine uptake in the discriminative stimulus effects of cocaine and related compounds. *Behavioural Pharmacology*, 4, 69-79.
- Baker, L.E., Virden, T.B., Miller, M.E., & Sullivan, C.L. (1997). Time course analysis of the discriminative stimulus effects of the optical isomers of 3,4-methylenedioxymethamphetamine (MDMA). *Pharmacology Biochemistry and Behavior*, 58, 505-516.
- Bymaster, F.P., Calligaro, D.O., Falcone, J.F., Marsh, R.D., Moore, N.A., Tye, N.C., Seeman, P., & Wong, D.T. (1996). Radioreceptor binding profile of the atypical antipsychotic olanzapine. *Neuropsychopharmacology*, 14, 87-96.
- Christensson, E.G. (1989). Pharmacological data of the atypical neuroleptic compound Melperone (Buronil®). In G. Sedvall (Ed.), Melperone – an atypical neuroleptic: Proceedings of a workshop in Malmo, Sweden, October 8, 1986. *Acta Psychiatrica Scandinavica*, 80, No. 352.
- Extance, K., & Goudie, A.J. (1981). Inter-animal olfactory cues in operant drug discrimination procedures in rats. *Psychopharmacology*, 73, 363-371.
- Gauvin, D.V. & Baird, T.J. (1999). Discriminative effects of compound drug stimuli: A focus on attention. *Pharmacology Biochemistry and Behavior*, 64, 229-235.
- Glennon, R.A. (1986). Discriminative stimulus properties of the 5-HT_{1A} agonist 8-hydroxy-2-(Di-*n*-propylamino)tetralin (8-OH-DPAT). *Pharmacology Biochemistry and Behavior*, 25, 135-139.
- Goas J.A. & Boston, J.E. (1978). Discriminative stimulus properties of clozapine and chlorpromazine. *Pharmacology Biochemistry and Behavior*, 8, 235-241.
- Goudie, A.J., Baker, L.E., Smith, J.A., Prus, A.J., Svensson, K.A., Cortes-Burgos, L.A., Wong, E.H.F., & Haadsma-Svensson, S. (2001). Common discriminative stimulus properties in rats of muscarinic antagonists, clozapine and the D₃ preferring antagonist PNU-99194A: an analysis of possible mechanisms. *Behavioural Pharmacology*, 12, 303-315.
- Goudie, A.J. & Smith, J.A. (1999). Discriminative stimulus properties of antipsychotics. *Pharmacology Biochemistry and Behavior*, 64, 193-201.
- Goudie, A., Smith, J.A., Taylor, A., Taylor, M.A.I., & Tricklebank, M.D. (1998). Discriminative stimulus properties of the atypical neuroleptic clozapine in rats: Tests with subtype selective ligands. *Behavioural Pharmacology*, 9, 699-710.

- Goudie, A., & Taylor, A. (1998). Comparative characterization of the discriminative stimulus properties of clozapine and other antipsychotics in rats. *Psychopharmacology*, 135, 392-400.
- Grant, K.A. (1999). Strategies for understanding the pharmacological effects of ethanol with drug discrimination procedures. *Pharmacology Biochemistry and Behavior*, 64, 261-267.
- Gray, A.M. & Connick, J.H. (1998). Clozapine-induced dopamine levels in the rat striatum and nucleus accumbens are not affected by muscarinic antagonism. *European Journal of Pharmacology*, 362, 127-136.
- Hoenicke, E.M., Vanecek, S.A., & Woods. (1992). The discriminative stimulus effects of clozapine in pigeons: Involvement of 5-hydroxytryptamine_{1C} and 5-hydroxytryptamine₂ receptors. *Journal of Pharmacology and Experimental Therapeutics*, 263, 276-284.
- Homan, E.J., Copinga, S., Elfström, L., van der Veen, T., Hallema, J.-P., Mohell, N., Unelius, L., Johansson, R., Wikström, H.V., & Grol, C.J. (1998). 2-aminotetralin-derived substituted benzamides with mixed dopamine D₂, D₃, and serotonin 5-HT_{1A} receptor binding properties: A novel class of potential atypical antipsychotic agents. *Bioorganic & Medicinal Chemistry*, 6, 2111-2126.
- Ichikawa, J., Dai, J., & Meltzer, H.Y. (2001). DOI, a 5-HT_{2A/2C} receptor agonist, attenuates clozapine-induced cortical dopamine release. *Brain Research*, 907, 151-155.
- Ichikawa, J., Ishii, H., Bonaccorso, S., O'Laughlin, I.A., Fowler, W.L., & Meltzer, H.Y. (2001). 5-HT_{2A} and D₂ receptor blockade increases cortical DA release via of 5-HT_{1A} receptor activation: A possible mechanism of atypical antipsychotic-induced cortical dopamine release. *Journal of Neurochemistry*, 76, 1521-1531.
- Ichikawa, J., & Meltzer, H.Y. (1999). Valproate and carbamazepine increase prefrontal dopamine release by 5-HT_{1A} receptor activation. *European Journal of Pharmacology*, 380, R1-R3.
- Ichikawa, J., & Meltzer, H.Y. (2000). The effect of serotonin_{1A} receptor agonism on antipsychotic drug-induced dopamine release in rat striatum and nucleus accumbens. *Brain Research*, 858, 252-263.
- Jann, M.W. (1991). Clozapine. *Pharmacotherapy*, 11, 179-195.
- Jarbe, T.U., Hiltunen, A.J., & Swedberg, M.D.B. (1989). Compound drug discrimination learning. *Drug Development Research*, 16, 111-122.
- Kane, J.M., Honigfeld, G., Singer, J., & Meltzer, H.Y. (1988). Clozapine and the treatment-resistant schizophrenic: A double-blind comparison with chlorpromazine. *Archives of General Psychiatry*, 45, 789-796.

- Kapur, S., & Remington, G. (2001). Dopamine D₂ receptors and their role in atypical antipsychotic action: Still necessary and may even be sufficient. *Biological Psychiatry*, 50, 873-883.
- Kehne, J.H., Baron, B.M., Carr, A.A., Chaney, S.F., Elands, J., Feldman, D.J., Frank, R.A., Van Giersbergen, P.L.M., McCloskey, T.C., Johnson, M.P., McCarty, D.R., Poirot, M., Senyah, Y., Siegel, B.W., & Widmaier, C. (1996). Preclinical characterization of the potential of the putative atypical antipsychotic MDL 100907 as a potent 5-HT_{2A} antagonist with a favorable CNS safety profile. *The Journal of Pharmacology and Experimental Therapeutics*, 277, 968-981.
- Kelley, B.M., & Porter, J.H. (1997). The role of muscarinic cholinergic receptors in the discriminative stimulus properties of clozapine in rats. *Pharmacology Biochemistry and Behavior*, 57, 707-719.
- Kinon, B.J., & Lieberman, J.A. (1996). Mechanisms of action of atypical antipsychotic drugs: a critical analysis. *Psychopharmacology*, 124, 2-34.
- Leysen, J.E., Janssen, P.M.F., Schotte, A., Luyten, W.H.M.L., & Megens, A.A.H.P. (1993). Interaction of antipsychotic drugs with neurotransmitter receptor sites in vitro and in vivo in relation to pharmacological and clinical effects: role of 5HT₂ receptors. *Psychopharmacology*, 112, S40-S54.
- Mariathasan, E.A., Stolerman, I.P., & White, J.-A.W. AND and AND-OR drug mixture discriminations in rats: generalization to single drugs and drug mixtures. *Psychopharmacology*, 143, 54-63.
- Matz, R., Rick, W., Oh, D., Thompson, H., & Gershon, S. (1974). Clozapine-a potential antipsychotic agent without extrapyramidal manifestations. *Current Therapeutic Research*, 16, 687-695.
- Meltzer, H.Y., Matsubara, S., & Lee, J.-C. (1989). Classification of typical and atypical antipsychotic drugs on the basis of D-1, D-2, and serotonin₂ pK_i values. *Journal of Pharmacology and Experimental Therapeutics*, 251, 238-246.
- Michal, P., Lysíková, M., El-Fakahany, E.E., & Tucek, S. (1999). Clozapine interaction with the M₂ and M₄ subtypes of muscarinic receptors. *European Journal of Pharmacology*, 376, 119-125.
- Millan, M.J., Schreiber, R., Dekeyne, A., Rivet, J.-M., Bervoets, K., Mavridis, M., Sebban, C., Maurel-Remy, S., Newman-Tancredi, A., Spedding, M., Muller, O., Lavielle, G., & Brocco, M. (1998). S 16924 ((R)-2-{1-[2-(2,3-dihydro-benzo[1,4] dioxin-5-yloxy)-ethyl]-pyrrolidin-3y}-1-(4-fluoro-phenyl)-ethanone), a novel, potential antipsychotic with marked serotonin (5-HT)_{1A} agonist properties: II. Functional profile in comparison to clozapine and haloperidol. *Journal of Pharmacology and Experimental Therapeutics*, 286, 1356-1373.
- Millan, M.J., Schreiber, R., Monneyron, S., Denorme, B., Melon, C., Queriaux, S., & Dekeyne, A. (1999). S-16924, a novel, potential antipsychotic with marked serotonin_{1A} agonist properties. IV. A drug discrimination comparison with

- clozapine. *Journal of Pharmacology and Experimental Therapeutics*, 289, 427-436.
- Moore, N.A., Tye, N.C., Axton, M.S., & Risius, F.C. (1992). The behavioural pharmacology of olanzapine, a novel "atypical" antipsychotic agent. *Journal of Pharmacology and Experimental Therapeutics*, 262, 545-551.
- Nielson, E.B. (1988). Cholinergic mediation of the discriminative stimulus properties of clozapine. *Psychopharmacology*, 94, 115-118.
- Owens, D.G.C. (1999). A guide to the extrapyramidal side-effects of antipsychotic drugs. Cambridge: Cambridge University Press.
- Poling, A., & Byrne, T. (2000). Assessing drug effects in nonhumans. In A. Poling & T. Byrne (Eds.), *Behavioral Pharmacology* (pp. 87-110). Reno, NV: Context Press.
- Porter, J.H., Varvel, S.A., Vann, R.E., Philbin, S.D., & Wise, L.E. (2000). Clozapine discrimination with a low training dose distinguishes atypical from typical antipsychotic drugs in rats. *Psychopharmacology*, 149, 189-193.
- Porter, J.H., Villanueva, H.F., & Rosecrans, J.A. (1999). Role of D₁ and D₂ dopamine receptors in the discriminative stimulus properties of the atypical antipsychotic clozapine in rats. *Drug Development Research*, 46, 139-147.
- Prinssen, E.P.M., Ellenbroek, B.A., & Cools, A.R. (1994). Combined antagonism of adrenoceptors and dopamine and 5-HT receptors underlies the atypical profile of clozapine. *European Journal of Pharmacology*, 262, 167-170.
- Seeman, P., Corbett, R., & Van Tol, H.H.M. (1997). Atypical neuroleptics have low affinity for dopamine D₂ receptors or are selective for D₄ receptors. *Neuropsychopharmacology*, 16, 93-110.
- Stolerman, I.P. (1993). Drug discrimination. In F. van Haaren (Ed.), *Methods in behavioral pharmacology* (pp. 217-243). London: Elsevier Science Publishers.
- Tang, A.H., Franklin, S.R., Himes, C.S., Smith, M.W., & Tenbrink, R.E. (1997). PNU-96415E, a potential antipsychotic agent with clozapine-like pharmacological properties. *Journal of Pharmacology and Experimental Therapeutics*, 281, 440-447.
- Tomie, A., Shultz, P.L., Spicer, M.S., & Peoples, L.L. (1995). Drug discrimination training with low doses: Maintenance of discriminative control. *Pharmacology Biochemistry and Behavior*, 50, 115-119.
- Wiley, J.L., & Porter, J.H. (1992). Serotonergic drugs do not substitute for clozapine in clozapine-trained rats in a two-lever drug discrimination procedure. *Pharmacology Biochemistry and Behavior*, 43, 961-965.

- Zhang, W., & Bymaster, F.P. (1999). The in vivo effects of olanzapine and other antipsychotic agents on receptor occupancy and antagonism of dopamine D₁, D₂, D₃, 5HT_{2A} and muscarinic receptors. *Psychopharmacology*, 141, 267-278.
- Zorn, S.H., Jones, S.B., Ward, K.M., & Liston, D.R. (1994). Clozapine is a potent and selective muscarinic M₄ receptor agonist. *European Journal of Pharmacology*, 269, R1-R2.

Appendix A.
Institutional Animal Care and Use Committee
Approval Forms

WESTERN MICHIGAN UNIVERSITY

Date: January 8, 2001
 To: Dr. Bruce Bejcek, IACUC Chairperson
 From: Dr. Lisa E. Baker
 Adam Prus
 Re: Amendment to Protocol # 99-10-01

We propose the following amendment to IACUC protocol # 99-10-01.

Sixteen male Sprague-Dawley rats will undergo drug discrimination training as outlined in the approved protocol with the exception that the training drug stimulus will be the atypical antipsychotic drug clozapine, rather than the dopamine D₁ receptor antagonist PNU-99194A. One group of eight animals will be trained to discriminate 1.25 mg/kg clozapine and the other group of eight animals will be trained to discriminate 5.0 mg/kg clozapine. Clozapine will be administered no more than three days a week during training as specified in the original protocol. Drugs to be tested for stimulus generalization will include other putative atypical antipsychotics, dopamine D₃ receptor antagonists, serotonin₂ receptor antagonists and agonists, and antimuscarinic agents listed below. Drugs will be administered either intraperitoneally or subcutaneously, using sterile techniques, as specified in the original protocol.

atypical antipsychotics	D ₁ antagonists	5-HT ₂ antagonists	5-HT _{2A} agonists	antimuscarinic agents
risperidone	PNU-99194A	ritanserine	(-) DOM	scopolamine
melperone	PD 152255			trihexyphenidyl
olanzapine	S-33084			
quetiapine	S-14297			

A thorough literature search on clozapine discrimination research has been conducted using MEDLINE, PsychInfo and the Drug Discrimination Database to assure that these experiments are not duplicative. A list of references is provided below.

Fiorella, D., Helsley, S., Rabin, R.A., and Winter, J.C., 1995. The interactions of typical and atypical antipsychotics with the (-)-2,5-dimethoxy-4-methamphetamine (DOM) discriminative stimulus. Neuropharmacology, 34, 1297-1303.

Goudie, A., and Taylor, A., 1998. Comparative characterisation on the discriminative stimulus properties of clozapine and other antipsychotics in rats. Psychopharmacology, 135, 392-400.

Kinon, B.J., and Lieberman, J.A., 1996. Mechanisms of action of atypical antipsychotic drugs: a critical analysis. Psychopharmacology, 124, 2-34.

Leysen, J.E., Janssen, P.M.F., Schotte, A., Luyten, W.H.M.L., and Megens, A.A.H.P., 1993. Interaction of antipsychotic drugs with neurotransmitter receptor sites in vitro and in vivo in relation to pharmacological and clinical effects: role of 5HT₂ receptors. Psychopharmacology, 112, S40-S54.

Nielson, E.B., 1988. Cholinergic mediation of the discriminative stimulus properties of clozapine. Psychopharmacology, 94, 115-118.

Peacock, L., and Gerlach, J., 1999. New and old antipsychotics versus clozapine in a monkey model: adverse effects and antiamphetamine effects. Psychopharmacology, 144, 189-197.

Porter, J.H., Varvel, S.A., Vann, R.E., Philbin, S.D., and Wise, L.E., 2000. Clozapine discrimination with a low training dose distinguishes atypical from typical antipsychotic drugs in rats. Psychopharmacology, 149, 189-193.

Porter, J.H., Villanueva, H.F., and Rosecrans, J.A., 1999. Role of D1 and D2 dopamine receptors in the discriminative stimulus properties of the atypical antipsychotic clozapine in rats. Drug Development Research, 46, 139-147.

Prinssen, E.P.M., Ellenbroek, B.A., Cools, A.R., 1994. Combined antagonism of adrenoceptors and dopamine and 5-HT receptors underlies the atypical profile of clozapine. European Journal of Pharmacology, 262, 167-170.

**WESTERN MICHIGAN UNIVERSITY
INVESTIGATOR IACUC CERTIFICATE**

Title of Project: Discriminative Effects of Dopamine D3 Receptor Ligands

The information included in this IACUC application is accurate to the best of my knowledge. All personnel listed recognize their responsibility in complying with university policies governing the care and use of animals.

I declare that all experiments involving live animals will be performed under my supervision or that of another qualified scientist. Technicians or students involved have been trained in proper procedures in animal handling, administration of anesthetics, analgesics, and euthanasia to be used in this project.

If this project is funded by an extramural source, I certify that this application accurately reflects all procedures involving laboratory animal subjects described in the proposal to the funding agency noted above.

☒ Any proposed revisions to or variations from the animal care and use data will be promptly forwarded to the IACUC for approval.

☐ Disapproved

☒ Approved

☐ Approved with the provisions listed below

Provisions or Explanations:

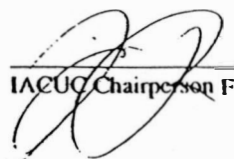
IACUC Chairperson

Date

Acceptance of Provisions


Signature: Principal Investigator/Instructor

12/1/99
Date


IACUC Chairperson Final Approval

11/17/99
Date