REFERENCES

Deneau, G.A.; Yanagita, T.; and Seevers, M.H. Self-administration of psychoactive substances by a monkey – a measure of psychological dependence. Psychopharmacologia 16: 40-48, 1969.

Fantegrossi, W.E.; Ullrich, T; Rice, K.C.; Woods, J.H. and Winger G. 3,4-methylenedioxymethamphetamine (MDMA, 'ecstasy') and its stereoisomers as reinforcers in rhesus monkeys: serotonergic involvement. Psychopharmacology 161: 356-364, 2002

Gerak, L.R. and France, C.P. Discriminative stimulus effects of flumazenil in diazepam-treated rhesus monkeys. Psychopharmacology 146:252-261, 1999.

Lelas, S.; Gerak, L.R.; and France, C.P. Discriminative stimulus effects of triazolam and midazolam in rhesus monkeys. Behavioural Pharmacology 10:39-50, 1999.

Woolverton, W.L.; Massey, B.W.; Winger, G.; Patrick, G.A.; and Harris, L.S. Evaluation of the abuse liability of aminorex. Drug Alcohol Depend 36: 187-192, 1994.

ACKNOWLEDGEMENTS

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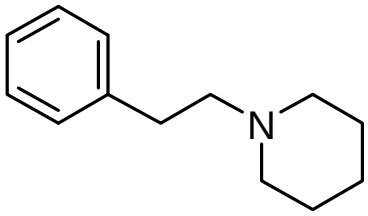
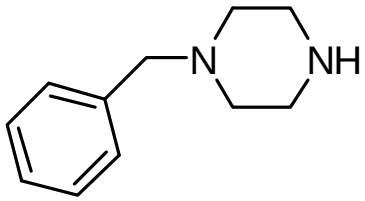
that markedly decreased rate of responding. In rats, CPDD 0064 generated (+)-MDMA appropriate responding, suggesting that this compound might have MDMA-like subjective effects in humans. This compound did not substitute for LSD in any rats tested at any dose, however, CPDD 0064 did partially generalize in (+)-MDMA trained rats up to doses that suppressed responding. CPDD 0064 induced a hypothermic response in mice at all doses tested. Further, CPDD 0064 suppressed locomotor activity at 100 mg/kg, although it should be notes that this dose was also lethal to a significant percentage of the population to which it was administered. Similarly, in rats, CPDD 0064 suppressed vertical locomotor activity at the highest dose tested.



CPDD 0065
N-Phenethylpipridine Oxalate

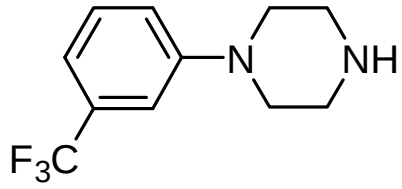
In self-administration studies, CPDD 0065 N-Phenethylpipridine Oxalate maintained responding at rates intermediate to those engendered by contingent saline and contingent cocaine in all monkeys tested. Responding for CPDD 0065 (at a dose of 0.03 mg/kg/inj) peaked at approximately 60% of cocaine control responding, suggesting that this compound may have moderate reinforcing effects under the presently employed conditions. CPDD 0065 lacks amphetamine-like discriminative stimulus effects up to a dose of 17 mg/kg. Doses higher than this were not tested in amphetamine-trained animals due to possible toxicity. These results predict a lack of amphetamine-like subjective effects of CPDD 0065 in humans. CPDD 0065 did not substitute for flumazenil in diazepam-treated monkeys or midazolam in untreated monkeys, suggesting that CPDD 0065 does not have benzodiazepine antagonist or agonist actions in rhesus monkeys.

CONCLUSIONS



CPDD 0063
1-Benzylpiperazine difumarate (BZP)

In self-administration studies, CPDD 0063 (1-Benzylpiperazine difumarate; BZP) maintained responding at rates equivalent to those engendered by contingent cocaine in all monkeys tested. Direct, long-lasting stimulant effects on behavior precluded self-administration studies on doses of CPDD 0063 larger than 0.3 mg/kg/injection. CPDD 0063 fully substituted for amphetamine in all monkeys tested, but failed to substitute for a flumazenil discriminative stimulus in diazepam-treated monkeys or for a midazolam discriminative stimulus in untreated monkeys, up to doses that decreased response rates. These results predict that CPDD 0063 might exhibit amphetamine-like subjective effects in humans, but would not possess benzodiazepine antagonist or agonist actions. CPDD 0063 did not fully substitute for LSD in any rats tested at any dose. CPDD 0063 induced a modest and transient hypothermia at the highest dose tested, and increased locomotor activity at all doses tested in mice. This compound did not significantly alter locomotor activity in rats up to the highest dose tested.



CPDD 0064
1-(3-Trifluoromethylphenyl)piperazine HCl (TFMPP)

In self-administration studies, CPDD 0064 1-(3-Trifluoromethylphenyl)piperazine HCl (TFMPP) failed to maintain responding at rates greater than those engendered by contingent saline in all monkeys tested. Direct, long-lasting suppressive effects on cocaine-maintained behavior were evident following a single non-contingent infusion of 1.0 mg/kg CPDD 0064. Self-administration studies on doses of CPDD 0064 larger than 1.0 mg/kg/injection were therefore not tested. CPDD 0064 lacked amphetamine-like discriminative stimulus effects in monkeys at doses up to 17 mg/kg, although observations indicated that these doses were indeed behaviorally active. It is interesting to note that 8515, the only female in the group, appeared to be more sensitive to the effects of CPDD 0064. These results predict a lack of amphetamine-like subjective effects of CPDD 0064 in humans. CPDD 0064 did not substitute for midazolam in otherwise untreated monkeys that discriminate between this prototypic benzodiazepine and vehicle, indicating that CPDD 0064 does not have benzodiazepine agonist actions in rhesus monkeys. CPDD 0064 substituted for the benzodiazepine antagonist flumazenil in one of three diazepam-treated (dependent) monkeys, suggesting that CPDD 0064 might have benzodiazepine antagonist-like activity in rhesus monkeys; however, the apparent flumazenil-like effects of CPDD 0064 in monkey JE occurred only at doses

Table 15. Discriminative stimulus effects of CPDD 0063 in diazepam (5.6 mg/kg/day) treated rhesus monkeys discriminating flumazenil: dose reponse

Subject	CPDD 0065 Dose (mg/kg) Veh	1.0	3.2
DA	0 / 0.90	0 / 0.83	* / 0
JI	1 / 1.35	4 / 0.97	* / 0

See Tables 2 and 3 for details.

Midazolam Discrimination. In monkeys discriminating between 0.32 mg/kg of midazolam and vehicle, midazolam dose-dependently increased responding on the drug (midazolam)-associated lever with a dose of 0.32 mg/kg occasioning greater than 80% drug-lever responding in each monkey (Table 16). The largest dose of midazolam (0.32 mg/kg) slightly decreased response rate.

Table 16. Discriminative stimulus effects of subcutaneous midazolam in midazolam-trained rhesus monkeys

Subject	Midazolam Dose (mg/kg) Veh	0.01	0.032	0.1	0.32
LI	0 /1.14	0 / 0.78	0 / 1.12	0 / 1.05	100 / 0.98
RO	0 / 2.75	0 / 2.75	0 / 2.97	0 / 2.31	100 / 1.60

See Tables 2 and 3 for details

CPDD 0065 did not substitute (i.e., did not occasion at least 80% drug-lever responding) for the midazolam discriminative stimulus (Table 17) up to a dose (17.8 mg/kg) that significantly decreased response rate and that produced emesis and uncooperativity in one monkey (RO). Data shown are from 15 min after administration of CPDD 0065 (peak onset for rate-decreasing effects).

Table 17. Discriminative stimulus effects of CPDD 0063 in rhesus monkeys discriminating midazolam: dose response

Subject	CPDD 0065 Dose (mg/kg) Veh	3.2	10.0	17.8
LI	0 /1.58	0 / 1.35	0 / 1.92	NS
RO	0 / 2.41	0 / 2.43	0 / 2.96	* / 0.03

See Tables 2 and 3 for details

Table 13. Discriminative stimulus effects of intragastric CPDD 0065 in amphetamine-trained rhesus monkeys

Subject	AMPH	Saline	CPDD 0065 Dose (mg/kg)			
			1	3	10	17
8515	100/1.4	1.5/1.8	0/1.2	0/1.3	6/1.4	0/1.0
M163	100/1.8	5/1.4	nt	0/2.5	0/1.5	0/2.0
Ou3	100/2.3	0/2.7	0/1.9	0/2.5	0/2.0	0/2.1

See Table 1 for details.

Discriminative Stimulus Effects in Rhesus Monkeys (flumazenil and midazolam discriminations): CPDD 0065

Flumazenil Discrimination. In monkeys receiving 5.6 mg/kg/day of diazepam p.o. and discriminating between 0.1 mg/kg (JI) or 0.32 mg/kg of flumazenil and vehicle, flumazenil dose-dependently increased responding on the drug (flumazenil)-associated lever with a dose of 0.1 mg/kg occasioning greater than 80% drug-lever responding in each monkey (Table 14). Over the doses studied, flumazenil did not reliably modify response rate.

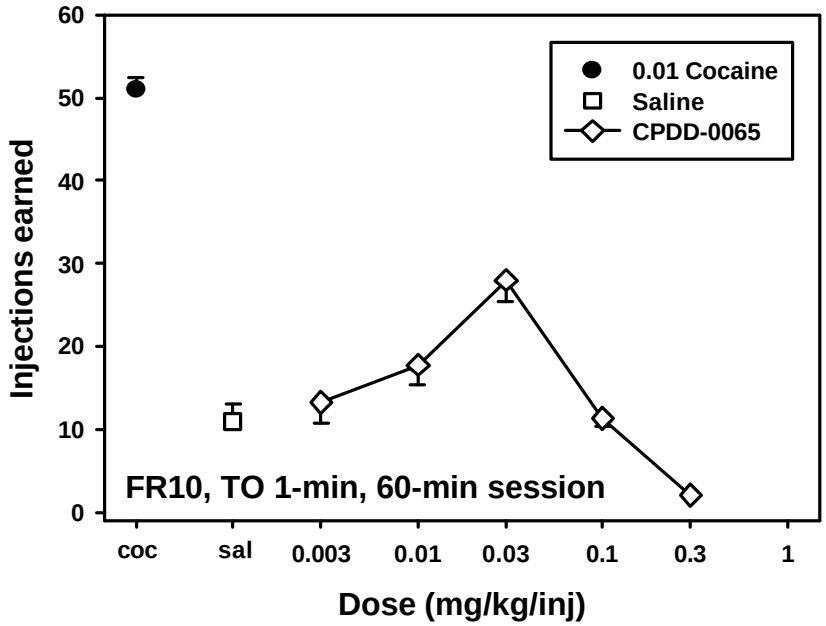
Table 14. Discriminative stimulus effects of oral flumazenil in flumazenil-trained rhesus monkeys

Subject	Flumazenil Dose (mg/kg)			
	Veh	0.01	0.032	0.1
DA	0 / 0.84	10 / 1.23	10 / 1.21	97 / 1.41
JI	0 / 1.45	0 / 1.75	18 / 1.79	85 / 1.26

See Tables 2 and 3 for details.

CPDD 0065 did not substitute (i.e., did not occasion at least 80% drug-lever responding) for the flumazenil discriminative stimulus (Table 15) up to a dose (3.2 mg/kg) that eliminated responding in both monkeys. Data shown are from 15 min after administration of CPDD 0065 (peak onset for rate-decreasing effects).

Figure 11. Self-administration of CPDD 0065 in monkeys experienced with cocaine and MDMA



CPDD-0065 generated an inverted U shaped function across the dose range tested. A dose of 0.03 mg/kg/inj engendered significantly more injections than saline, but no dose maintained as many injections as 0.01 mg/kg/inj cocaine. Responding for CPDD-0065 fell to saline-like levels when the available dose was increased beyond 0.03 mg/kg/inj.

Discriminative Stimulus Effects in Rhesus Monkeys (amphetamine discrimination): CPDD 0065

When given 60 minutes before the session, CPDD 0065 engendered essentially no responding on the amphetamine-appropriate lever in any monkey up to 17 mg/kg (Table 13). Any effects on response rate were small and unsystematic, and there were no obvious observable behavioral effects at any dose. CPDD 0065 was also tested in an untrained monkey at a dose of 30 mg/kg, i.g. After about half of the dose (approximately 8 ml) had been administered, labored breathing was observed and the infusion was aborted. Within five minutes the monkey became unconscious and attempts at resuscitation were unsuccessful. A post-mortem exam was conducted but was unrevealing in terms of cause of death. The primary possibilities were drug overdose and accidental drug administration into the lung. Since the monkey had previously been tested with 17 mg/kg with any untoward effects, and only half of the 30 mg/kg dose had been administered, it seems unlikely that the cause was drug overdose. Nevertheless, doses higher than 17 mg/kg of CPDD 0065 were not tested in trained monkeys.

Competition binding in rat brain: CPDD 0064

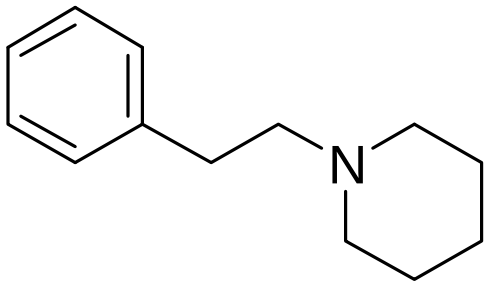
Binding affinity at 5-HT_{1A} and 5-HT_{2A} receptors were determined in homogenates harvested from three adult male CDF rats. Affinity of the various compounds at [^{3}H]8-OH-DPAT binding sites in hippocampus, and at [^{3}H]ketanserin binding sites in frontal cortex were measured as described in the Methods. Data are expressed in Table 12 as negative log of the equilibrium dissociation constant (pK_I) and are presented as mean $\pm$ SEM (N=3)

Table 12. Binding affinity of CPDD 0064 at 5-HT_{1A} and 5-HT_{2A} and 5-HT_{2C} receptors

Compound	pK_I [^{3}H]8-OH-DPAT	pK_I [^{3}H]ketanserin	pK_I [^{3}H] mesulergine
CPDD 0064	**6.84 ± 0.051**	**6.36 ± 0.152**	**7.01 $\pm$ 0.73**

CPDD 0065

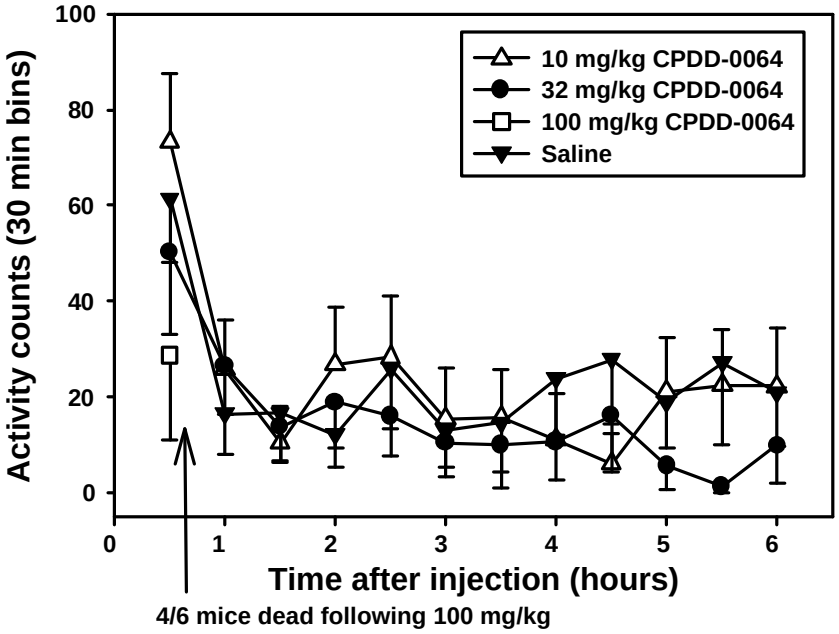
N-Phenethylpipridine Oxalate



Reinforcing Effects in Rhesus Monkeys: CPDD 0065

Five doses of CPDD-0065 were evaluated in four rhesus monkeys. Figure 11 shows the mean dose-effect curve (± SEM) for CPDD-0065 self-administration. All animals self-administered this compound, although responding was intermediate between that engendered by contingent saline and the maintenance dose of cocaine for all subjects.

Figure 9. Effects of CPDD 0064 on spontaneous locomotor activity in mice



Effects on Spontaneous Locomotor Activity in Rats: CPDD 0064

Six doses of CPDD 0064 (0.1, 0.2, 0.4, 0.8, 1.6 and 3.2 mg/kg) and an equivolume saline control injection were tested for effects on locomotor activity in two separate groups of rats (*n*=8 rats per group.) No significant effects on horizontal or vertical locomotor activity were noted (Figure 10).

Figure 10. Effects of CPDD 0064 on spontaneous locomotor activity in rats

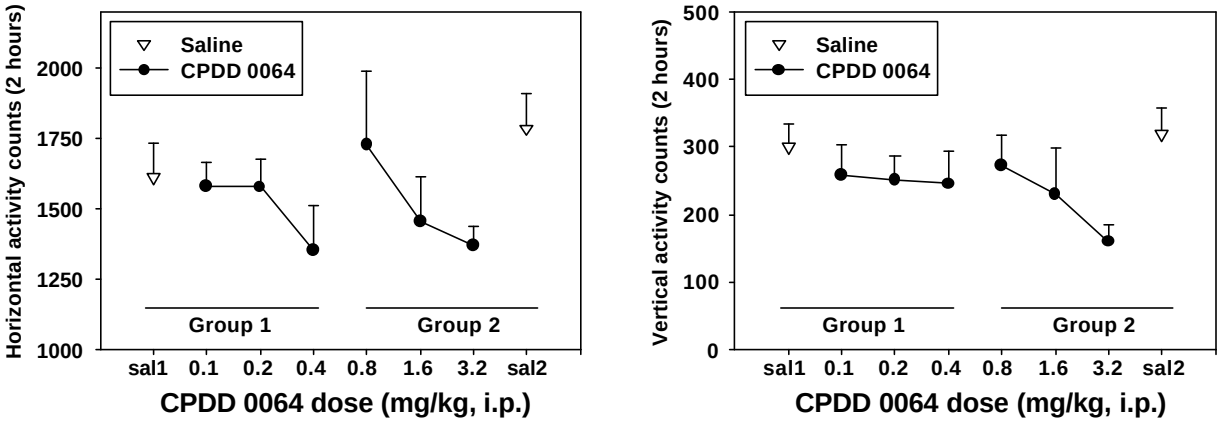
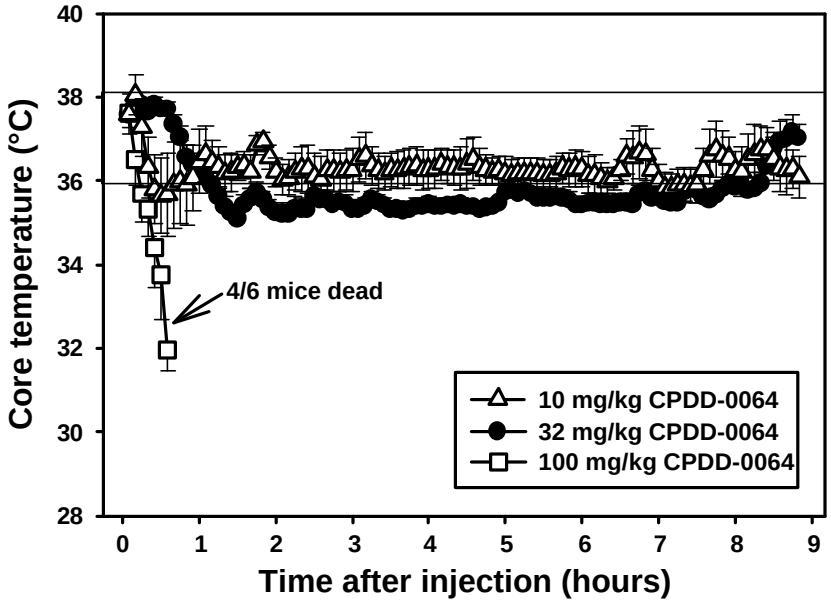


Figure 8. Effects of CPDD 0064 on core temperature in mice



The remaining two animals from the 100 mg/kg CPDD 0064 group survived up to 24 hours post-injection, despite large and long-lasting hypothermic reactions. Mouse 2 exhibited an enormous (10°C) hypothermic response that gradually returned to basal temperature over approximately 22 hours. Mouse 3 exhibited a lesser, but still pronounced, hypothermic response (4°C) that recovered over approximately 7 hours.

Figure 9 presents the effects of CPDD-0064 on locomotor activity. Neither 10 nor 32 mg/kg CPDD-0064 affected locomotor activity, however, motor behavior was almost completely suppressed immediately following injection of 100 mg/kg CPDD 0064. Since 4/6 animals expired within 30 minutes of dosing, data beyond this time point are not presented. The remaining two animals were largely immobile as their temperatures fell and then gradually returned to basal range.

Table 12. Discriminative stimulus and rate suppressant effects of CPDD 0064 in rats discriminating (+)-MDMA: dose response

Drug	n/N	Dose (mg/kg)	(+)-MDMA-responding ± SEM (%)	Response rate ± SEM (resp/min)
Saline	**16/16**	**N/A**	**2.23±1.22**	**31.21±2.94**
(+)-MDMA	**16/16**	**0.25**	**15.24±6.67**	**27.94±2.48**
(+)-MDMA	**16/16**	**0.375**	**34.06±10.8**	**27.93±4.25**
(+)-MDMA	**16/16**	**0.5**	**70.16±10.6**	**31.81±2.41**
(+)-MDMA	**16/16**	**0.75**	**86.61±8.46**	**27.97±3.82**
(+)-MDMA	**16/16**	**1**	**92.88±6.22**	**28.87±3.34**
CPDD 0064	**12/12**	**0.1**	**14.85±8.04**	**28.48±2.78**
CPDD 0064	**15/16**	**0.8**	**72.68±7.11**	**19.88±3.61**
CPDD 0064	**7/10**	**1**	**77.34±10.8**	**17.74±4.97**

Effects on Core Temperature and Spontaneous Locomotor Activity in Mice: CPDD 0064
Doses of CPDD 0064 were evaluated in separate groups of mice (n=6 mice per group). Figure 8 shows the group mean effects of CPDD 0064 (± SEM) on core temperature over a nine hour period. The outlined region between roughly 36°C and 38°C represents the normal range of temperature for a mouse following an equivolume saline injection. Temperatures significantly above this range would therefore be considered instances of hyperthermia, while temperatures significantly below this range would be considered instances of hypothermia. Injections of 10 and 32 mg/kg CPDD 0064 induced mild dose- and time-dependent hypothermic states. This effect was not significant with 10 mg/kg, but was significant for the 32 mg/kg dose condition from approximately 1 hour post-injection until approximately 5 hours post injection. The magnitude of the temperature effect, however, was relatively small (about 1°C.) At the highest dose tested, the mean temperature dropped precipitously and death occurred in of 4/6 mice by 30 minutes post-injection. Mean data after this point are therefore not presented.

Table 10. Discriminative stimulus effects of CPDD 0064 in rhesus monkeys discriminating midazolam: dose response

Subject	CPDD 0064 Dose (mg/kg) Veh	1.0	3.2	5.6
NI	0 / 3.06	0 / 1.36	0 / 2.11	* / 0.28
LI	0 /1.14	0 / 1.59	23 / 1.48	* / 0
SA	0 / 2.51	0 / 2.47	0 / 2.14	0 / 0.71

See Tables 2 and 3 for details

Discriminative Stimulus Effects in Rats (LSD discriminations): CPDD 0064

Rats trained to discriminate 0.1 mg/kg LSD were subsequently probed with two doses of CPDD 0064. This compound did not substitute (i.e., did not occasion at least 80% drug-lever responding) for the LSD discriminative stimulus at either tested dose, but completely suppressed responding at a dose of 1.0 mg/kg (Table 11).

Table 11. Discriminative stimulus effects of CPDD 0064 in LSD-trained rats

Dose CPDD 0064 mg/kg [15 min]	# Completing / # Tested	% LSD-Responding ± SEM	Mean Rate [Resp/min] ± SEM
0.1	6 / 6	30.83 ± 17.27	7.80 ± 1.47
1.0	2 / 6 [3X]	0.00 ± 0.00	1.00 ± 0.50

See Table 6 for details.

Discriminative Stimulus Effects in Rats (MDMA discriminations): CPDD 0064

Three doses of CPDD 0064 (0.1, 0.8, 1.0 mg/kg) have been tested for substitution (generalization). CPDD 0064 was administered 30 minutes prior to placement in the discrimination chambers. In 12/12 rats CPDD 0064 (0.1 mg/kg) elicited 14.85±8.04% drug-appropriate responding without affecting the response rate. In 15/16 rats, CPDD 0064 (0.8 mg/kg) elicited a partial substitution for (+)-MDMA (72.68±7.11% (+)-MDMA-appropriate responding); however, the response rate was significantly lower than the previous drug training session. In 7/10 rats, CPDD 0064 (1.0 mg/kg) partially substituted for (+)-MDMA engendering 77.24±10.8% (+)-MDMA-appropriate responding and a significantly reduced response rate as compared to the previous drug training session. From the data collected (Table 12), it appears that CPDD 0064 partially substitutes for the discriminative stimulus effects of (+)-MDMA (1.0 mg/kg).

Discriminative Stimulus Effects in Rhesus Monkeys (flumazenil and midazolam discriminations): CPDD 0064

Flumazenil Discrimination. In monkeys receiving 5.6 mg/kg/day of diazepam p.o. and discriminating between 0.1 mg/kg (JI) or 0.32 mg/kg of flumazenil and vehicle, flumazenil dose-dependently increased responding on the drug (flumazenil)-associated lever with a dose of 0.1 mg/kg occasioning greater than 80% drug-lever responding in each monkey (see Table 2). Over the doses studied, flumazenil did not reliably modify response rate.

CPDD 0064 substituted (i.e., occasioned at least 80% drug-lever responding) for the flumazenil discriminative stimulus (Table 9) in one of three monkeys (JE). In JE, a dose of 1.0 mg/kg occasioned 84% flumazenil-lever responding and a 3-fold larger dose occasioned responding exclusively on the flumazenil-associated lever. Doses of CPDD 0064 that substituted for flumazenil in monkey JE also markedly decreased rate of lever pressing in this monkey. In contrast, CPDD 0064 did not substitute for flumazenil in two other monkeys (RO and JI) up to doses (1.0-3.2 mg/kg) that significantly decreased response rate. Data shown are from 45 minutes after administration of CPDD 0064 (peak onset of flumazenil-like and rate-decreasing effects).

Table 9. Discriminative stimulus effects of CPDD 0064 in diazepam (5.6 mg/kg/day) treated rhesus monkeys discriminating flumazenil: dose reponse

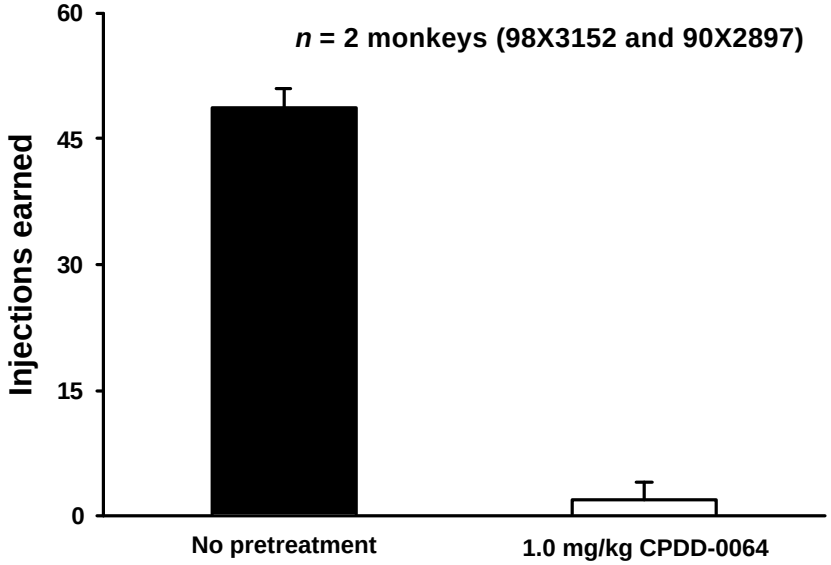
	CPDD 0064 Dose (mg/kg)			
Subject	**Veh**	**0.32**	**1.0**	**3.2**
JE	**0 / 0.87**	**0 / 0.57**	**84 / 0.2**	**100 / 0.25**
RO	**0 / 1.37**	**0 / 0.98**	*** / 0**	*** / 0.04**
JI	**1 / 1.35**	**0 / 1.17**	**0 / 1.23**	*** / 0**

See Tables 2 and 3 for details.

Midazolam Discrimination. In monkeys discriminating between 0.32 mg/kg of midazolam and vehicle, midazolam dose-dependently increased responding on the drug (midazolam)-associated lever with a dose of 0.32 mg/kg occasioning greater than 80% drug-lever responding in each monkey (see Table 3). The largest dose of midazolam (0.32 mg/kg) slightly decreased response rate in monkeys NI and SA.

CPDD 0064 did not substitute (i.e., did not occasion at least 80% drug-lever responding) for the midazolam discriminative stimulus (Table 10) up to a dose (5.6 mg/kg) that significantly decreased response rate in two monkeys (NI and LI). Data shown are from 45 minutes after administration of CPDD 0064 (peak onset for rate-decreasing effects).

Figure 7. Effects of non-contingent CPDD 0064 on subsequent cocaine self-administration



Discriminative Stimulus Effects in Rhesus Monkeys (amphetamine discrimination): CPDD 0064

When given 60 minutes before the session, CPDD 0064 did not generalize for amphetamine up to a dose of 17 mg/kg (Table 8). Partial substitution at 3.0 mg/kg in monkey 8515 was the result of averaging full substitution during the initial test session with no substitution when this dose was retested. This variable effect and the lack of amphetamine-like responding at any dose in this or the other monkeys makes it likely that the full substitution seen in the initial test in 8515 was a spurious result. CPDD 0064 decreased response rate in 8515 at 10 and 17 mg/kg, though rate decreases were not observed in the other monkeys. Following 17 mg/kg CPDD 0064, ataxia and locomotor slowing was observed in all monkeys. Similar effects were observed in monkey 8515 at 10 mg/kg.

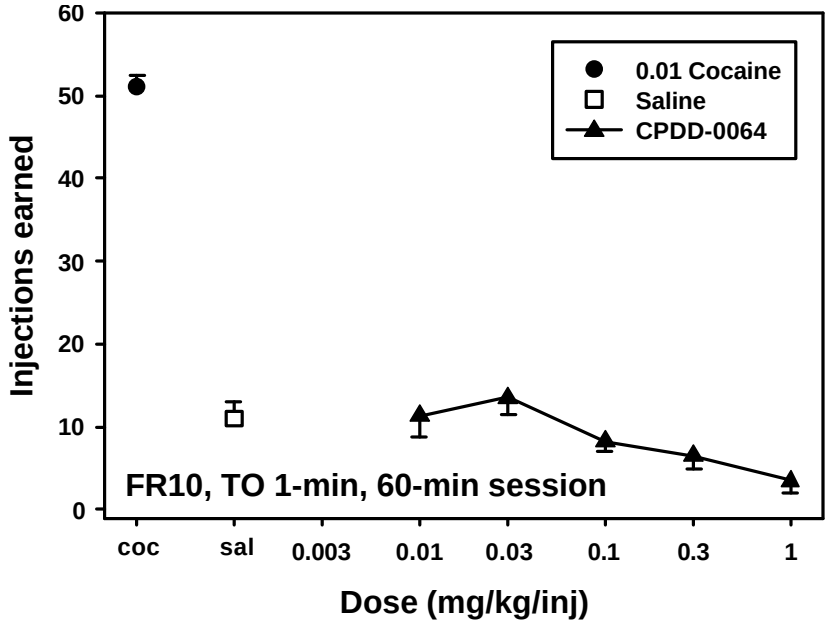
Table 8. Discriminative stimulus effects of intragastric CPDD 0064 in amphetamine-trained rhesus monkeys

			CPDD 0064 Dose (mg/kg)			
Subject	**AMPH**	**Saline**	**1**	**3**	**10**	**17**
8515	**100/1.4**	**1.5/1.8**	**0/1.2**	**46.5/1.3**	**0/0.8**	**0/0.4**
M163	**100/1.8**	**5/1.4**	**nt**	**0/2.3**	**0/1.9**	**0/2.4**
Ou3	**100/2.3**	**0/2.7**	**0/1.7**	**0/1.6**	**0/1.5**	**0/1.1**

See Table 1 for details.

approximately 50 injections per session. Response rates and number of injections earned were markedly reduced when contingent saline was presented for self-administration. Similarly, no dose of CPDD 0064 maintained significant responding in any animals tested.

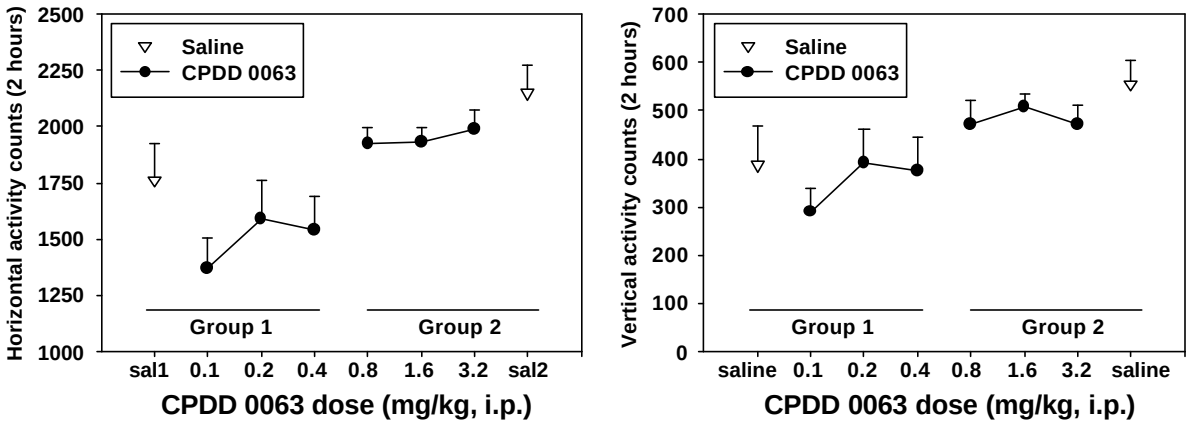
Figure 6. Self-administration of CPDD 0064 in monkeys experienced with cocaine and MDMA



CPDD 0064 engendered low rates of responding across the dose range tested, and the number of injections earned at each dose was never greater than that engendered by contingent saline. Following sessions where high doses (0.3 – 1.0 mg/kg/inj) were available, no overt signs of intoxication were observed, however, responding for the maintenance cocaine dose during subsequent afternoon sessions was usually disrupted. In this regard, two animals failed to emit a single response during sessions immediately following AM self-administration of high dose CPDD 0064, while the remaining two animals responded at saline-like levels.

To further investigate this effect on subsequent cocaine self-administration, two animals were administered 1.0 mg/kg CPDD 0064 non-contingently (iv) 20 minutes before sessions where the maintenance dose of cocaine was available. These pretreatments suppressed cocaine-maintained responding (Figure 7), indicating that this dose is behaviorally active when administered intravenously. Doses higher than 1.0 mg/kg/inj were not tested for self-administration due to these disruptive effects on behavior.

Figure 5. Effects of CPDD 0063 on spontaneous locomotor activity in rats



Competition binding in rat brain: CPDD 0063

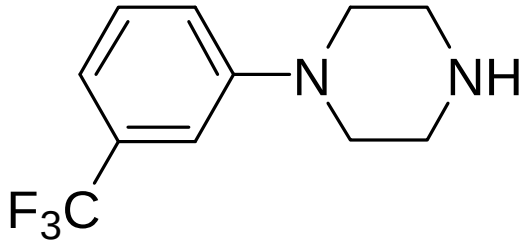
Binding affinity at 5-HT_{1A} and 5-HT_{2A} receptors was determined in homogenates harvested from three adult male CDF rats. Affinity of the various compounds at [^{3}H]8-OH-DPAT binding sites in hippocampus, and at [^{3}H]ketanserin binding sites in frontal cortex were measured as described in the Methods. Data are expressed in Table 7 as negative log of the equilibrium dissociation constant (pK_I) and are presented as mean $\pm$ SEM (N=3)

Table 7. Binding affinity of CPDD 0063 at 5-HT_{1A} and 5-HT_{2A} and 5-HT_{2C} receptors

Compound	pK_I [^{3}H]8-OH-DPAT	pK_I [^{3}H]ketanserin	pK_I [^{3}H] mesulergine
CPDD 0063	IC50 > 10mM	IC50 > 33mM	IC50> 10mM

CPDD 0064

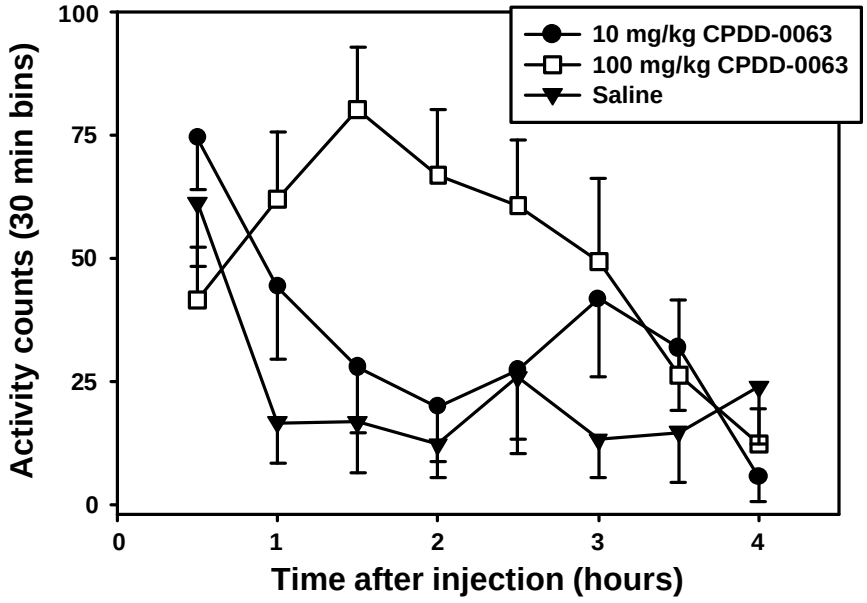
1-(3-Trifluoromethylphenyl)piperazine HCl (TFMPP)



Reinforcing Effects in Rhesus Monkeys: CPDD 0064

Five doses of CPDD 0064 were evaluated in four rhesus monkeys (except for the highest dose, which was tested in only two subjects.) Each animal was tested at least twice per dose. Figure 6 shows the mean dose-effect curve (± SEM) for cocaine, saline and CPDD 0064 self-administration. Cocaine consistently maintained high rates of behavior with all subjects earning

Figure 4. Effects of CPDD 0063 on spontaneous locomotor activity in mice



Effects on Spontaneous Locomotor Activity in Rats: CPDD 0063

Six doses of CPDD 0063 (0.1, 0.2, 0.4, 0.8, 1.65 and 3.2 mg/kg) and an equivolume saline control injection were tested for effects on locomotor activity in two separate groups of rats (*n*=8 rats per group.) No significant effects on horizontal or vertical locomotor activity were noted (Figure 5).

temperature did dip just slightly below the normal range approximately 20 minutes after injection. The effect was transient, however, and temperature returned to the normal range quickly. The lower dose had no significant effects on core temperature.

Figure 3. Effects of CPDD 0063 on core temperature in mice

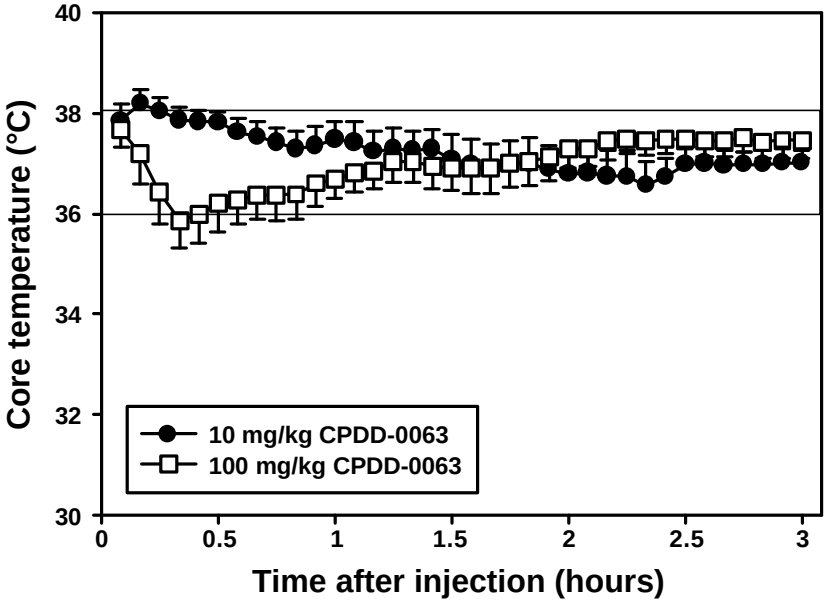


Figure 4 presents the effects of CPDD 0063 on locomotor activity in separate groups of mice (*n*=6 mice per group.) All groups show an initial burst of locomotor activity following injections, likely due to removal from the home cage, restraint, and injection rather than to a drug effect. With saline-treated animals, this initial increase in activity rapidly returns to basal levels (within 30-mins). Compared to the equivolume saline control condition, both doses of CPDD 0063 produced significant increases in locomotor activity. At 10 mg/kg, a transient increase in locomotor activity was observed at 1 hour post-injection, although this effect was less of an amplification in motor behavior and more of a delay in return to basal levels. In contrast, by 1 hour following administration of 100 mg/kg CPDD 0063, locomotor activity was significantly elevated above saline control, and continued to increase over the next 30-mins. Locomotor behavior did not return to saline levels until 3.5 hours post-injection.

Table 5. Discriminative stimulus effects of CPDD 0063 in rhesus monkeys discriminating midazolam

Subject	CPDD 0063 Dose (mg/kg) Veh	3.2	5.6	10.0
NI	0 / 3.06	0 / 2.26	0 / 3.01	0 / 2.31
LI	0 /1.14	0 / 1.91	0 / 1.50	0 / 1.85
SA	0 / 2.51	0 / 2.56	0 / 2.68	0 / 3.30

See Tables 2 and 3 for details.

CPDD 0063 did not substitute for flumazenil in diazepam-treated monkeys or for midazolam in untreated monkeys, suggesting that CPDD 0063, up to otherwise behaviorally active doses (e.g., rate-decreasing effects), does not have benzodiazepine antagonist or agonist actions in rhesus monkeys.

Discriminative Stimulus Effects in Rats (LSD discrimination): CPDD 0063

Rats trained to discriminate 0.1 mg/kg LSD were subsequently probed with five doses of CPDD 0063. This compound did not substitute (i.e., did not occasion at least 80% drug-lever responding) for the LSD discriminative stimulus or suppress responding at any doses tested (Table 6).

Table 6. Discriminative stimulus effects of CPDD 0063 in LSD-trained rats

Dose CPDD 0063 mg/kg [15 min]	# Completing / # Tested	% LSD-Responding ± SEM	Mean Rate [Resp/min] ± SEM
0.01	3 / 3	22.60 ± 18.35	11.30 ± 6.40
0.1	13 / 13 [3X]	33.69 ± 7.75	10.05 ± 1.19
0.3	4 / 4	0.00 ± 0.00	14.75 ± 4.15
1	4 / 5	4.50 ± 2.59	9.20 ± 2.77
3	11 / 11	12.82 ± 8.45	11.55 ± 2.35

CPDD 0063, LSD, and saline were injected as 15-minute pretreatments. Parenthetical numbers denote number of replications for a given dose.

Effects on Core Temperature and Spontaneous Locomotor Activity in Mice: CPDD 0063

Two doses of CPDD 0063 were evaluated in separate groups of mice (n=6 mice per group). Figure 3 shows the group mean effects of CPDD 0063 (± SEM) on core temperature over a 3-hour period. The outlined region between roughly 36°C and 38°C represents the normal range of temperature for a mouse following an equivolume saline injection. Temperatures significantly above this range would therefore be considered instances of hyperthermia, while temperatures significantly below this range would be considered instances of hypothermia. Injections of CPDD 0063 were relatively innocuous in terms of core temperature. At the highest dose tested, the mean

Table 3. Discriminative stimulus effects of CPDD 0063 in diazepam (5.6 mg/kg/day) treated rhesus monkeys discriminating flumazenil

Subject	CPDD 0063 Dose (mg/kg) Veh	1.0	3.2	5.6
JE	0 / 0.87	0 / 0.70	0 / 0.90	NS
RO	0 / 1.37	4 / 1.40	0 / 1.42	* / 0
JI	1 / 1.35	NS	0 / 1.34	* / 0

Data represent percent drug-appropriate responding / response rate (responses per second).
*Discrimination data are not presented when response rate was <20% of control response rate.
NS = not studied; see table 2 for other details

Midazolam Discrimination. In monkeys discriminating between 0.32 mg/kg of midazolam and vehicle, midazolam dose-dependently increased responding on the drug (midazolam)-associated lever with a dose of 0.32 mg/kg occasioning greater than 80% drug-lever responding in each monkey (Table 4). The largest dose of midazolam (0.32 mg/kg) slightly decreased response rate in monkeys NI and SA.

Table 4. Discriminative stimulus effects of SC midazolam in midazolam-trained rhesus monkeys

Subject	Midazolam Dose (mg/kg) Veh	0.01	0.032	0.1	0.32
NI	0 / 3.06	0 / 2.54	0 / 2.12	67 / 1.68	100 / 0.94
LI	0 /1.14	0 / 0.78	0 / 1.12	0 / 1.05	100 / 0.98
SA	0 / 2.51	0 / 2.54	0 / 2.49	0 / 2.66	89 / 2.05

See Tables 2 and 3 for details.

CPDD 0063 did not substitute for the midazolam discriminative stimulus (Table 5). Doses larger than 10.0 mg/kg of CPDD 0063 were not studied because at this dose of CPDD monkeys exhibited hyperventilation, piloerection and tremor and became uncooperative. Data shown are from 30 minutes after administration of CPDD 0063.

Table 1. Discriminative stimulus effects of intragastric CPDD 0063 in amphetamine-trained rhesus monkeys

			CPDD 0063 Dose (mg/kg)			
Subject	**AMPH**	**Saline**	**3**	**10**	**17**	**30**
8515	**100/1.4**	**1.5/1.8**	**0/1.3**	**43/1.6**	**10/1.7**	**96.5/1.2**
M163	**100/1.8**	**5/1.4**	**0/2.3**	**51.5/1.9**	**100/1.7**	**nt**
Ou3	**100/2.3**	**0/2.7**	**48/1.75**	**48/1.3**	**100/1.4**	**nt**

Monkeys were trained to discriminate 1.0 mg/kg (i.g.) amphetamine from saline in a discrete-trials paradigm to avoid shock (8515 and Ou3) or for food delivery (M163). The response requirement was FR 2 (monkey 8515) or FR5 (monkeys M163 and Ou3). Data represent the percent drug-appropriate trials/average response rate (resp/sec). CPDD 0063 was administered via nasogastric tube 60 minutes prior to testing.

Discriminative Stimulus Effects in Rhesus Monkeys (flumazenil and midazolam discriminations): CPDD 0063

Flumazenil Discrimination. In monkeys receiving 5.6 mg/kg/day of diazepam p.o. and discriminating between 0.1 mg/kg (JI) or 0.32 mg/kg of flumazenil and vehicle, flumazenil dose-dependently increased responding on the drug (flumazenil)-associated lever with a dose of 0.1 mg/kg occasioning greater than 80% drug-lever responding in each monkey (Table 2). Over the doses studied, flumazenil did not reliably modify response rate.

Table 2. Discriminative stimulus effects of subcutaneous flumazenil in flumazenil-trained rhesus monkeys

	Flumazenil Dose (mg/kg)			
Subject	**Veh**	**0.01**	**0.032**	**0.1**
JE	**8 / 1.1**	**0 / 0.87**	**2 / 0.75**	**82 / 1.04**
RO	**0 / 1.39**	**4 / 1.43**	**10 / 1.71**	**86 / 1.50**
JI	**0 / 1.45**	**0 / 1.75**	**18 / 1.79**	**85 / 1.26**

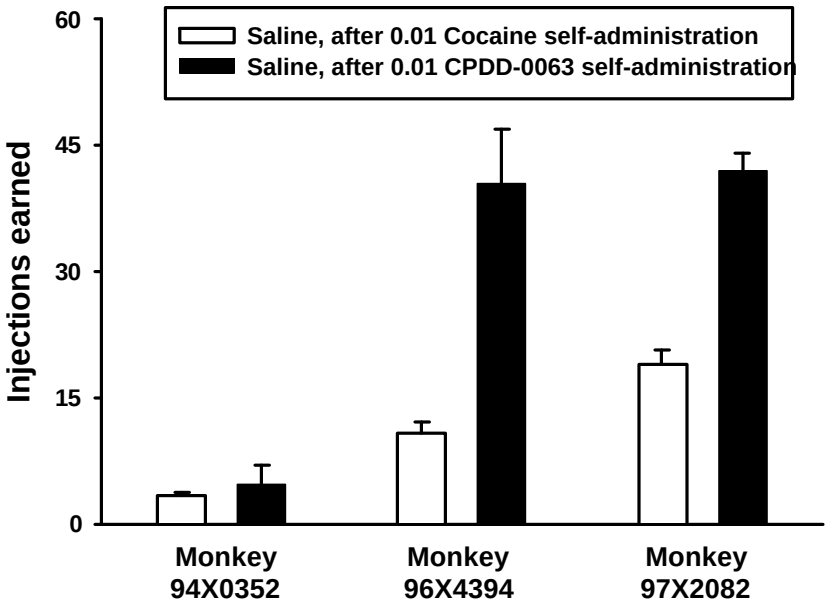
Data represent percent drug-appropriate responding / response rate (responses per second).
Veh =vehicle

CPDD 0063 did not substitute for the flumazenil discriminative stimulus (Table 3) up to a dose (5.6 mg/kg) that eliminated responding in two monkeys (RO and JI). Data shown are from 30 minutes after administration of CPDD 0063 (peak onset for rate-decreasing effects).

higher than 0.3 mg/kg/inj were not tested due to the intensity and duration of these behavioral effects.

A rough assessment of the effects of self-administered CPDD 0063 on locomotor activity was accomplished by comparing saline self-injection in PM sessions following AM sessions where either the maintenance dose of cocaine or 0.1 mg/kg/inj CPDD 0063 was available. For two out of three animals (96X4394 and 97X2082, Figure 2), significantly more saline was self-administered following CPDD 0063 self-injection, suggesting a fairly long-lasting locomotor stimulant effect.

Figure 2. Effects of self-administered CPDD 0063 on subsequent saline self-administration

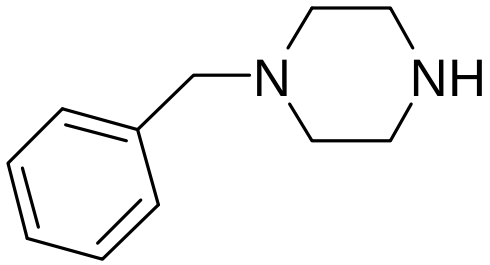


Discriminative Stimulus Effects in Rhesus Monkeys (amphetamine discrimination): CPDD 0063

When CPDD 0063 was administered 60 minutes before the session, there was a dose-related increase in amphetamine-lever responding that reached full substitution for amphetamine in all three monkeys (Table 1). For the two male monkeys (M163 and Ou3), 17 mg/kg substituted completely for amphetamine, while in the female monkey (8515) a dose of 30 mg/kg was required for full substitution. The ED_{50} of CPDD 0063 was, then, between 10 and 30 mg/kg. For comparison, the ED_{50} of amphetamine itself is approximately 0.2 mg/kg in this procedure. There was no obvious effect on response rate. There were no directly observable behavioral effects of CPDD 0063 at any dose in any of the three monkeys. These findings suggest that at high doses CPDD 0063 would have amphetamine -like subjective effects in humans.

RESULTS

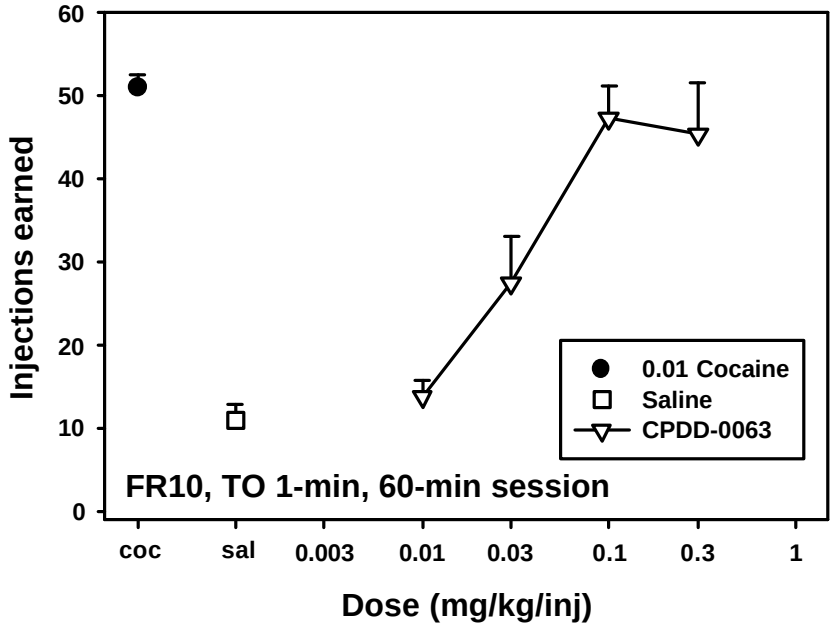
CPDD 0063: 1-Benzylpiperazine difumarate (BZP)



Reinforcing Effects in Rhesus Monkeys: CPDD 0063

Four doses of CPDD 0063 were evaluated in three rhesus monkeys. Each animal was tested at least twice per dose. There was no pre-session stimulus change or priming injection to predict which solution was available to the animal for self-injection, a small amount of "sampling" occurred at the beginning of each saline session but generally abated within 10 infusions. Figure 1 shows the mean dose-effect curve (± SEM) for CPDD 0063 self-administration. All animals self-administered this compound.

Figure 1. Self-administration of CPDD 0063 in monkeys experienced with cocaine and MDMA



CPDD 0063 engendered high rates of responding, and at doses of 0.1 and 0.3 mg/kg/inj, animals earned a similar amount of injections as they did when the maintenance dose of cocaine was available. Following these sessions where drug intake was high, animals exhibited several signs of intoxication, including stereotyped visual scanning around the room, head twitches (that became quite vigorous in one animal), jaw chattering, bizarre body postures, hyperactivity, and "fly catching" (fixating on an empty point in space and attempting to quickly grasp the area.) Doses

Institute on Drug Abuse, Rockville, MD], CPDD 0063 and CPDD 0064 were dissolved in 0.9% NaCl.

Competition binding in rat brain (SUNYB)

Subjects

Brain samples were harvested from three adult male CDF rats (Charles Rivers Laboratories).

Procedure

Frontal cortex (5-HT_{2A} receptors), hippocampus (5-HT_{1A} receptors) or brain stem (5-HT_{2C} receptors) from male CDF rats (Charles Rivers Laboratories) were homogenized (Dounce tissue grinder) in 50 mM Tris-HCl (pH 7.4). The homogenates were then centrifuged at 40,000 *g* for 15 min at 4°C. The resulting pellets were resuspended in the Tris buffer and stored at –80°C. On the day of the assays tissue samples were thawed and centrifuged at 40,000 *g* for 15 min at 4°C. The resulting pellets were resuspended in 30 ml warm 50 mM Tris –HCl (pH 7.4) and incubated for 10 min at 37°C to remove endogenous serotonin. Samples were then again centrifuged at 40,000 *g* for 15 min at 4°C. Final resuspension of the pellets (frontal cortex: 6.7 mg/ml; hippocampus: 5 mg/ml; brain stem 13.3 mg/ml) was in Tris assay buffer (50 mM Tris-HCl, pH 7.4, containing 4 mM $MgCl_2$, 10μM pargyline and 0.1% ascorbate). For [^{3}H]8-OH-DPAT binding assays were carried out for 30 min at 37°C in a final volume of 0.5 ml containing Tris assay buffer, 1 nM radioligand (129 Ci/mmole; Perkin-Elmer, Boston MA), appropriate drugs and (hippocampal membranes (2 mg wet weight/tube). For [^{3}H]ketanserin binding assays were carried out for 30 min at 30°C in a final volume of 0.5 ml containing Tris assay buffer, 1.5 nM radioligand (88 Ci/mmole; Perkin-Elmer, Boston MA), 100nM prazosin to prevent binding to α_1-adrenergic receptors, appropriate drugs and frontal cortical membranes (2 mg wet weight/tube). For [^{3}H]mesulergine binding assays were carried out for 45 min at 37°C in a final volume of 0.5 ml containing Tris assay buffer, 2 nM radioligand (77 Ci/mmole; Amersham Biosciences), 100nM spiperone to prevent binding to 5-HT_{2A} and dopamine D_2 receptors, appropriate drugs and membranes from the brain stem (4 mg wet weight/tube).. Reactions were terminated by rapid vacuum filtration (Brandel harvester) through GF/B glass fiber filters presoaked in 0.1% polyethylenimine. Filters were washed twice with cold 50 mM Tris-HCl (pH 7.4), and the amount of bound radioactivity measured by scintillation spectrophotometry. Nonspecific binding was defined as the difference in the amount of radioligand binding in the absence and presence of either 10 μM 5-HT ([^{3}H]8-OH-DPAT binding), 20 μM 5-HT ([^{3}H]mesulergine binding) or 100 μM cinanserin ([^{3}H]ketanserin binding). Data were analyzed by nonlinear regression using the program EBDA/LIGAND (Elsevier BIOSOFT).

Procedure

Following at least seven days of recovery after surgery, mice were weighed, injected i.p. with saline or various doses of test compounds, and returned to their individual cages. Temperature and locomotor activity data were collected for at least three hours post-injection.

Drugs

CPDD 0063 and CPDD 0064 were dissolved in physiological saline solution and injected i.p. in a constant volume of 0.01 ml/g bodyweight. The effects of CPDD 0063 on core temperature and locomotor activity were assessed at doses of 10 and 100 mg/kg, and the effects of CPDD 0064 on these same endpoints were evaluated at 10, 32 and 100 mg/kg.

Effects on Spontaneous Locomotor Activity in Rats (UTMB)

Subjects

Adult, male, Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing 250-350 g were used in the locomotor experiments. Rats were housed 4 per cage in standard plastic rodent cages in a colony room maintained at 21+2 C and at 40-50% humidity under a 12 h light/dark cycle (lights were on at 0700 h). Animals were provided with continuous access to tap water and rodent chow except during experimental sessions. All experiments were conducted during the light phase of the light/dark cycle (between 0800-1400 h), and were carried out according with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approval from the Institutional Animal Care and Use Committee.

Apparatus

All animals were handled and weighed for five days prior to locomotor experiments. Following injection, animals were directly placed into the locomotor chambers activity was monitored and quantified for 2 h using a modified open field activity system under low light conditions (San Diego Instruments, San Diego, CA). Each clear plexiglass chamber (40 cm x 40 cm x 40 cm) was housed in a sound attenuated enclosure and was surrounded with a 4 x 4 photobeam matrix located 4 cm from the floor of the chamber.

Procedure

Interruptions of the photobeams resulted in counts of activity in the peripheral and central fields of the chamber. Activity recorded in the inner 16 x 16 cm of the open field was counted as central activity while the field bounded by the outer 12 cm band registered as peripheral activity. Another horizontal row of photobeams, located 16 cm from the floor of the chamber, provided measurements of vertical activity (rearing). Separate counts of peripheral, central and vertical activity were made by the control software (Photobeam Activity Software, San Diego Instruments) and stored for subsequent evaluation. Video cameras positioned above the chambers permitted continuous observation of behavior without disruption.

Drugs

Doses of all drugs refer to the weight of the salt injected i.p. in a volume of 1 ml/kg. Pretreatment times represent the interval between drug injection and placement into the chamber and illumination of the house light. (+)-3,4-methylenedioxymethamphetamine [(+)-MDMA, National

responding and response rates during test sessions with the corresponding values for the previous (+)-MDMA or saline sessions.

Drugs

Doses of all drugs refer to the weight of the salt injected i.p. in a volume of 1 ml/kg. Pretreatment times represent the interval between drug injection and placement into the operant chamber and illumination of the house light. (+)-3,4-methylenedioxymethamphetamine [(+)-MDMA, National Institute on Drug Abuse, Rockville, MD] and CPDD 0064 were dissolved in 0.9% NaCl.

Effects on Core Temperature and Spontaneous Locomotor Activity in Mice (UM)

Subjects

Male NIH Swiss mice (Harlan Sprague Dawley Inc., Indianapolis, IN) weighing 20-30 g were housed 12 animals per 20.32 x 30.48 x 12.70 cm Plexiglas cage in a temperature-controlled room that was maintained at an ambient temperature of 22±2°C at 45-50% humidity. Lights were set to a 12-h light/dark cycle. Animals were fed Lab Diet rodent chow (Laboratory Rodent Diet #5001, PMI Feeds, Inc., St. Louis, MO) and water *ad libitum* until immediately before testing. Animals were not used in experiments until at least 2 days after arrival in the laboratory. Following appropriate anesthetization with ketamine (100 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.), the abdominal area of each mouse was shaved and sanitized with iodine swabs. A rostral-caudal cut approximately 1.5 cm in length was made with skin scissors, providing access to the intraperitoneal cavity. A cylindrical glass-encapsulated radiotelemetry probe (model ER-4000 E-Mitter, Mini Mitter, Bend, OR, USA) was then inserted, and the incision was closed using absorbable 5-0 chromic gut suture material. Immediately following surgery, mice were placed individually into 15.24 x 25.40 x 12.70-cm Plexiglas mouse cages where they remained for the duration of the experiments. Surgeries were carried out at least 7 days before initiation of experimental conditions, allowing time for incisions to heal and for the mice to recover normal body weights. Each animal was used only once, and was sacrificed immediately after use. Studies were carried out in accordance with the Declaration of Helsinki and with the Guide for Care and Use of Laboratory animals as adopted and promulgated by the National Institutes of Health. The experimental protocol was approved by the University of Michigan's University Committee on the Use and Care of Animals.

Apparatus

The implanted transmitters produced activity- and temperature-modulated signals which were sent to a receiver (model ER-4000 Receiver, Mini Mitter Co., Inc.) underneath each mouse cage. Temperature and locomotor activity data were thus collected from singly-housed mice at 5-min intervals and processed simultaneously by the Vital View data acquisition system (Mini Mitter Co., Inc.) installed on an IBM computer.

chambers to a PC computer running Med-PC for Windows software (MedAssociates) that controlled and recorded all experimental events.

Procedure

MDMA Discrimination. Rats were initially trained to discriminate an injection of (+)-3,4-methylenedioxymethamphetamine [(+)-MDMA] (1.25 mg/kg, i.p.) from saline (1 ml/kg, i.p.) administered 20 min before daily (Monday – Friday) 30 min sessions. Initial training began under a schedule of continuous water reinforcement with only the stimulus-appropriate (drug or saline) lever present ("errorless training"); the response requirement was then increased until all rats were responding reliably under a FR 20 schedule. For half of the rats, responses on the right lever were reinforced following drug administration; for the remaining rats, responses on the left lever were reinforced following drug administration. To control for the possible development of position cues based upon olfactory stimuli, a pseudo-random relationship was maintained between the lever programmed to deliver reinforcement for each consecutive rat run in the same experimental chamber (e.g. Extance & Goudie, 1981). During this phase of training, (+)-MDMA and saline were administered irregularly with the restriction that neither training condition prevailed for more than three consecutive sessions. After responding stabilized on an FR 20 schedule of reinforcement, both levers were presented simultaneously and rats were required to respond on the stimulus-appropriate (correct) lever in order to obtain water. The session time for the training sessions was shortened from 30 min to 20 min; there was no programmed consequence for responding on the incorrect lever ("discrimination" training). At the start of the seventh week of training, the training dose of (+)-MDMA used lowered to 1 mg/kg and remained at 1 mg/kg for the remainder of the study. After responding stabilized, training sessions were shortened from 20 min to 15 min and both levers were presented simultaneously during 15 min sessions. This phase of training continued until the performance of all rats reached criterion (individual mean accuracies of at least 80% correct prior to the first reinforcer for ten consecutive sessions). Establishment of stimulus control by (+)-MDMA varied across individual subjects within a range of 17 – 38 sessions. Test sessions were then initiated and were conducted once a week with training (maintenance) sessions intervening on other days. Only rats that met the 80% performance criterion during the preceding (+)-MDMA and saline sessions were tested. During test sessions, rats were placed in the chamber as during training sessions and upon completion of 20 responses on either lever or after the session time (15 min) had elapsed, a single (water) reinforcer was delivered, the house light was turned off and the rat was removed from the chamber. After being returned to the home cages, all rats were allowed 10 to 15 min of free access to water.

During training sessions, accuracy was defined as the percentage of correct responses before the delivery of the first reinforcer; during test sessions, performance was expressed as the percentage of (+)-MDMA-appropriate responses to the total responses prior to the first reinforcer. Response rates (responses per min) were also evaluated during training and test sessions as a measure of behavioral disruption. For the training sessions, the response rate was calculated as the total number of responses emitted on either lever before the completion of the first FR 20, divided by the number of minutes taken to complete the first ratio. During test sessions, the response rate was calculated as the total number of responses prior to completion of 20 responses on either lever, divided by the number of minutes taken to complete that FR 20. Only data from rats that completed the FR 20 during test sessions were included for analysis. For (+)-MDMA substitution tests, Student's *t*-test for repeated measures was used to compare the percentage of cocaine-lever

emitted on the drug-appropriate lever. Response rate was calculated for each session by dividing the total number of responses emitted on both levers by the elapsed time prior to 10 responses on either lever.

For purposes of discussion of these data, complete generalization of a training drug to a test drug is said to be present when (a) a mean of 80% or more of all test responses occurs on the drug-appropriate lever; (b) there is no statistically significant difference between the response distributions of the training drug and the test drug; and (c) there is a statistically significant difference between the response distributions of the test drug and saline control sessions. An intermediate degree of generalization is defined as being present when response distributions after a test drug are less than 80% drug-appropriate, and are significantly different from both training conditions. Finally, when the response distribution after a test drug is not statistically significantly different from that in saline control sessions, an absence of generalization of the training drug to the test drug is assumed. Similar criteria are applied to the definitions of full, partial, and no antagonism. Thus, full antagonism is assumed to be present when (a) less than 20% of all test responses are on the training drug-appropriate lever; (b) there is no significant difference between the response distributions in the test of antagonism and the saline control, and (c) there is a statistically significant difference between the response distributions of the test drug alone and in combination with the antagonist.

Drugs

(+)-LSD was supplied by the National Institute on Drug Abuse (Rockville, MD, USA). All drugs used in the behavioral experiments were dissolved in 0.9 % saline solution and intraperitoneally (i.p.) injected in a volume of 1.0 ml/kg bodyweight.

Discriminative Stimulus Effects in Rats (MDMA discriminations, UTMB)

Subjects

Sixteen experimentally naïve, male Sprague-Dawley rats (Harlan, Houston, TX) weighing between 300 and 350 g at the beginning of the study were used. The rats were housed in pairs in a colony room maintained at constant humidity (40-50%) and temperature (21-23 ° C); lighting was maintained on a 12 h light/dark cycle (lights on between 07:00– 19:00 h) and all experiments were conducted during the light phase. Food was always available in the home cage but not during the experimental sessions; however, the amount of water each animal received during the duration of the drug discrimination studies was restricted to that given during operant training sessions, after test sessions (10-15 minutes) and on weekends (36 h). Experiments were conducted Monday through Friday at 10:00 h.

Apparatus

Discrimination trials were conducted in 16 commercially available, two-lever operant chambers (Lafayette Instruments Model 80001, Lafayette, IN or MedAssociates Model ENV-001, St. Albans, VT) housed in sound attenuating chambers (Lafayette Instruments Model 80015 or MedAssociates Model ENV-015). The operant chambers contained two levers with a tap-water dispensing dipper centered between them. A 28-V house light provided illumination; ventilation and masking noise were supplied by a blower. An interface (Med Associates) connected the

shock schedule. CPDD 0063, CPDD 0064, and CPDD 0065 were studied up to 2 hours after administration in tests comprising eight cycles.

Drugs

Diazepam (Zenith Laboratories, Northvale, NJ) was suspended in 37-57 ml (depending on body weight) of fruit punch containing suspending Agent K to yield a dose of 5.6 mg/kg/daily drinking episode. Flumazenil (F. Hoffman LaRoche, LTD, Basel, Switzerland) was dissolved in a vehicle of 10% ethanol, 40% propylene glycol and 50% saline; midazolam hydrochloride (Roche Pharma, Inc., Manati PR) was purchased as a commercially-prepared solution. CPDD 0063, CPDD 0064 and CPDD 0065 were dissolved in saline and were studied up to doses of 10.0 mg/kg sc, 5.6 mg/kg sc, and 17.8 mg/kg sc respectively.

Discriminative Stimulus Effects in Rats (LSD discriminations, SUNYB)

Subjects

Male Fischer-344 rats were obtained from Harlan Sprague-Dawley Inc. (Indianapolis, IN, USA) at an age of approximately 6 weeks. They were housed in pairs with free access to food and water in a temperature-controlled room under a constant 12:12 h light-dark cycle. All experiments were conducted during the light phase. Subjects were fed standard rat chow following experimental sessions. Caloric intake was controlled to yield a mean body weight of about 250 grams. Animals used in these studies were maintained in accordance with the Guide for Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. The present study was approved by the Institutional Animal Care and Use Committee of the University at Buffalo.

Apparatus

Six small animal test chambers (Med-Associates Model ENV-008) housed in larger lightproof Malaguard sound attenuating cubicles (Med-Associates Model ENV-022M), were used for all experiments. Each box had a house light and exhaust fan. The chamber contained two levers mounted on opposite ends of one wall. Centered between the levers was a dipper that delivered 0.1 ml of sweetened condensed milk diluted 2:1 with tap water.

Procedure

LSD Discrimination. Twenty subjects were trained to discriminate LSD (0.1 mg/kg, 15 minute pretreatment time, intraperitoneal injection) from saline, as described previously (Fiorella et al., 1995). A non-resetting fixed ratio 10 (FR10) schedule of reinforcement was employed using the MED-PC version IV behavioral programming application. Drug-induced stimulus control was assumed to be present when, in five consecutive sessions, 83% or more of all responses prior to the delivery of the first reinforcer were on the appropriate lever. LSD-induced stimulus control was established after 25-30 training sessions. The LSD training dose produced approximately 99.5% drug-appropriate responding. After stimulus control was established with the training agents, tests were conducted once per week in each animal so long as performance did not fall below the criterion level of 83% correct responding in any one of the previous three training sessions. Half of the test sessions were conducted the day after saline training sessions with the remainder following LSD training sessions. During test sessions, no responses were reinforced and the session was terminated after the emission of ten responses on either lever. The distribution of responses between the two levers was expressed as a percentage of total responses

Discriminative Stimulus Effects in Rhesus Monkeys (flumazenil and midazolam discriminations, UTHSCSA)

Subjects

Six adult rhesus monkeys, weighing between 4.0 and 10.5 kg, were housed individually in stainless steel cages. Water was continuously available and monkeys received primate chow (Harlan Teklad, Madison, WI) daily as well as fresh fruit and peanuts several days per week.

Apparatus

Monkeys were seated in chairs that provided restraint at the neck. During experimental sessions, chairs were located in sound-attenuating, ventilated chambers that were equipped with several response levers, a food cup and an array of stimulus lights. Chairs were equipped with shoes containing brass electrodes, to which brief (250 ms) electric shock could be delivered from an a.c. shock generator located adjacent to the chambers.

Procedure

Flumazenil Discrimination. Monkeys consumed 5.6 mg/kg of diazepam in fruit punch 3 hours prior to daily sessions in which they discriminated between sc injections of 0.1 mg/kg (one monkey) or 0.32 mg/kg (two monkeys) of flumazenil and vehicle while responding under a fixed-ratio 5 schedule of food presentation. Daily training sessions consisted of several discrete, 15-minute cycles. Each cycle comprised a 10-minute pretreatment period, during which the chamber was dark and lever presses had no programmed consequence, followed by a 5-min response period, during which the chamber was illuminated green and monkeys could receive a 300 mg banana-flavored food pellet by responding five times on the appropriate lever as determined by the sc injection administered during the first minute of the 10-minute timeout (e.g., left lever after vehicle, right lever after flumazenil). Responses on the incorrect lever reset the response requirement on the correct lever. Test sessions were identical to training sessions except that various doses of flumazenil, CPDD 0063, CPDD 0064, or CPDD 0065 were administered during the first minute of the timeout and five consecutive responses on either lever resulted in the delivery of food. CPDD 0063, CPDD 0064, and CPDD 0065 were studied up to 2 hours after administration in tests comprising eight cycles.

Midazolam Discrimination. Monkeys discriminated between sc injections of 0.32 mg/kg of midazolam and saline while responding under a fixed-ratio 10 schedule of stimulus-shock termination. Daily sessions comprised multiple, 15-minute cycles. Each cycle comprised a 10-minute pretreatment period, during which the chamber was dark and lever presses had no programmed consequence, followed by a response period, during which the chamber was illuminated red and monkeys could postpone scheduled shock for 30 seconds by responding ten times on the appropriate lever as determined by the sc injection administered during the first minute of the 10-minute timeout (e.g., left lever after saline, right lever after midazolam). Failure to satisfy the response requirement within 15 seconds resulted in the delivery of a brief shock. The response period ended after 5 minutes or 4 shocks. Responses on the incorrect lever reset the response requirement on the correct lever. Test sessions were identical to training sessions except that various doses of flumazenil, CPDD 0063, CPDD 0064, or CPDD 0065 were administered during the first minute of the timeout and ten consecutive responses on either lever postponed the

sessions preceded by drug infusion. Discrimination training continued until at least 90% of the responses in the first trial were on the correct lever and at least 90% of the total trials (27/30) were avoidance trials for seven out of eight consecutive sessions. At this point testing began. During testing, sessions were conducted according to the following two-week schedule: SDTST, DSTDT, where T denotes test sessions. If the criteria for stimulus control were not met during the training sessions, test sessions were not conducted and the training sequence continued. Test sessions were identical to training sessions except that completion of the response requirement on either lever was reinforced. Dose was increased until either substitution for AMPH was seen (80% of the trials completed on the drug lever), response rate was decreased, or, in the absence of ether of these, obvious behavioral effects were observed.

Drugs

d-Amphetamine sulfate (Abbott Laboratories, N. Chicago, IL) was dissolved in sterile 0.9% saline to an infusion volume of 0.25 ml/kg. CPDD 0063 was prepared in sterile water. Low doses were given in the same standard infusion volume of 0.25 ml/kg, but for solubility reasons, the volume for the 10 mg/kg dose was increased to 0.75 ml/kg, to 1.7 ml/kg for the 17 mg/kg dose, and to 2.3 ml/kg for the 30 mg/kg dose. Doses of CPDD 0063 were usually tested once with either a saline or a drug training session in effect the day before, randomly determined. If in the first test session responding occurred predominately on the drug lever, that dose and at least one lower dose were tested again following a training session with the opposite pretreatment in effect.

CPDD 0064 was prepared in sterile water. Doses up to 10 mg/kg were given in the standard infusion volume of 0.25 ml/kg. For solubility reasons, the volume for 17 mg/kg was increased to 0.55 ml/kg. Doses of CPDD 0064 were usually tested once with either a saline or a drug training session in effect the day before, randomly determined. If in the first test session responding occurred predominately on the drug lever, the dose was tested again following a training session with the opposite pretreatment in effect.

CPDD 0065 was prepared in sterile water. Low doses were given in the same standard infusion volume of 0.25 ml/kg. For solubility reasons, the volume for 10 mg/kg was increased to 0.75 ml/kg and for 17 mg/kg to 0.85 ml/kg. Doses of CPDD 0064 were usually tested once with either a saline or a drug training session in effect the day before, randomly determined. If in the first test session responding occurred predominately on the drug lever, the dose was tested again following a training session with the opposite pretreatment in effect.

Drugs

Four doses (0.01, 0.03, 0.1, and 0.3 mg/kg/injection) of CPDD 0063 were studied in three monkeys. Five doses (0.01, 0.03, 0.1, 0.3, and 1.0 mg/kg/inj) of CPDD 0064 were studied in four monkeys. Five doses (0.003, 0.01, 0.03, 0.1, and 0.3 mg/kg/inj) of CPDD 0065 were studied in four monkeys. The dose ranges for each compound were increased until direct effects on behavior were observed. Cocaine and all test compounds were dissolved in physiological saline.

Discriminative Stimulus Effects in Rhesus Monkeys (amphetamine discrimination, UMMC)

Subjects

Three adult rhesus monkeys (*Macaca mulatta*) served as subjects. All monkeys had received other test drugs prior to the start of the present study. Monkeys were individually housed in stainless-steel cages with water available continuously. Feeding consisted of 110 to 200 g of Teklad Monkey Chow approximately three hours after each session and a chewable vitamin tablet 3 days/week.

Apparatus

During experimental sessions, each monkey was seated in a restraint chair and placed in a sound-attenuating cubicle that had two response levers (PRL-001, BRS/LVE, Laurel, MD) and a white houselight mounted on the ceiling. Above each lever was a set of white jewel lights. Shoes were attached to the foot rest of the chairs and were fitted with brass plates through which electric shocks could be delivered to the bottoms of the feet. Experimental events were programmed and recorded using an Apple Macintosh computer in an adjacent room.

Procedure

All monkeys had previously been trained in a discrete-trials shock avoidance paradigm to discriminate amphetamine (1.0 mg/kg) from saline. Each monkey was placed in the chair and moved to the test room. In the test room their feet were placed into shoes and held in place with a Velcro strap. Each monkey was given an infusion of either saline (0.25 ml/kg) or the training drug, followed by a 2.0 ml saline flush, intragastrically via a nasogastric tube. Monkeys then remained in the chair in the test room. Fifty-five minutes after the infusion, monkeys were placed in the experimental chambers. The session then began with a 5-min timeout, at the end of which the houselight and lever lights were illuminated (trial) and responding on the correct lever avoided electric shock (monkeys 8515 and Ou3) or delivered a 1-gram banana-flavored food pellet (monkey M163), and extinguished the lights. Responding on the incorrect lever reset the response requirement on the correct lever. The correct lever was determined by the pre-session infusion (drug or saline). If the response requirement (FR2, 8515; FR 5, M163, Ou3) was not met on the correct lever within 10 sec of the onset of the lights, shock (250 msec duration, 5 mA intensity) was delivered (8515 and Ou3). If the response requirement was not met within 4 sec of this shock, a second shock was delivered and the trial automatically ended. For M163, if the response requirement was not met within 10 seconds, the trial ended. Two consecutive trials in which 2 shocks were received or food was not obtained automatically ended the session. Trials were separated by a 30-sec timeout, and sessions lasted for 30 trials or 20 min, whichever came first.

Training sessions were conducted five days per week according to the following two-week schedule: SDDSS, DSSDD, where S denotes sessions preceded by saline infusion and D denotes

and continued, through a hollow restraining arm, to the outside rear of the cage. Animals were fed between 10 and 12 Purina monkey chows twice per day, and water was available *ad libitum*. Daily fresh fruit and other treats supplemented this diet. In accordance with IACUC requirements, environmental enrichment toys were also provided on a regular rotating basis.

Apparatus

Each animal wore a Teflon mesh jacket (Lomir, Québec, Canada) connected to a flexible stainless steel spring arm attached to the rear of the cage. The restraint and catheter protection devices are described in detail by Deneau *et al*. (1969) and allow relatively unrestricted movement within the cage. Catheters are thus protected as they pass through the restraint apparatus and join tubing passing through a roller infusion pump (Watson and Marlow Co., Model MHRK 55, Falmouth, UK). Operation of the infusion pump delivered 1 ml of drug solution over 5 seconds. Monkeys were individually housed in stainless steel cages, measuring 83.3 X 76.2 X 91.4 cm deep. A 15.4 cm square stimulus panel was located on the side of each cage, approximately 10 cm from the front and 19 cm from the bottom of the cage. Across the top of the stimulus panel, 1.5 cm apart, were three circular, 2.5 cm in diameter, translucent plastic stimulus lights that could be illuminated by 5 W colored bulbs. The two side lights could be illuminated red and the center light green. Below each of the two red stimulus lights was a response lever (Model 121-07; BRS-LVE, Beltsville, MD) capable of being operated by a force of 0.010 to 0.015 N. Experimental control was provided by an IBM computer programmed with Med-PC (Med-Associates, Fairfield, VT) software and located in an adjoining room.

Procedure

Reinforcing effects of CPDD 0063, CPDD 0064 and CPDD 0065 were evaluated in a substitution self-administration procedure in monkeys who were experienced with iv self administration of cocaine, MDMA and its stereoisomers, and several structural analogues. Test sessions and baseline sessions had the same general structure. At the start of each session, a red light was illuminated over one of two levers. When a monkey completed the fixed-ratio requirement of 10 presses on that lever (fixed-ratio [FR] 10), a 5-second, 1.0 ml injection of saline, cocaine (0.01 mg/kg), or a test compound was delivered. During drug infusion, the red stimulus light was extinguished and a center green light was illuminated for the duration of the infusion. Each injection was followed by a 1-minute timeout during which all stimulus lights were extinguished and responding had no programmed consequence. This schedule was previously used to engender contingent responding for MDMA and its enantiomers in these same subjects (see Fantegrossi et al 2002).

Twice daily experimental sessions (starting at 1000 and 1600 h) lasted 60 minutes each. On approximately half of the baseline sessions, the monkeys could respond for saline. All animals showed clear and consistent differential responses to saline and cocaine before test compounds were evaluated. In test sessions a dose of the test compound was made available for two to three discreet sessions. Other conditions were similar to those of the baseline sessions.

PROGRESS REPORT FROM THE TESTING PROGRAM FOR STIMULANT AND DEPRESSANT DRUGS (2002)

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INTRODUCTION

A research group within the Drug Evaluation Committee has been involved in the evaluation of stimulant and depressant compounds for approximately 19 years. The group currently includes laboratories at the University of Michigan (UM; Fantegrossi, Winger and Woods), The University of Texas Health Science Center at San Antonio (UTHSCSA; France, McMahon), The University of Mississippi Medical Center (UMMC; Woolverton), the State University of New York at Buffalo (SUNYB; Winter), and The University of Texas Medical Branch at Galveston (UTMB; Cunningham). As part of the Drug Evaluation Committee (J. Woods, Chair) of the College on Problems of Drug Dependence (CPDD), research is supported by both the CPDD and the National Institute on Drug Abuse (NIDA). One of the purposes of this group is to evaluate new compounds, generally classified as either stimulants or depressants, for their abuse liability and physical dependence potential; however, included this year were several drugs generally classified as hallucinogens. Compounds are received, coded and distributed by A. Coop at the University of Maryland School of Pharmacy (Baltimore) for blind testing in the various laboratories. Drugs are then evaluated for reinforcing effects in monkeys with previous histories of drug self-administration (UM), and for discriminative stimulus effects in amphetamine-trained monkeys (UMMC), midazolam-trained monkeys (UTHSCSA), and flumazenil-trained monkeys that receive diazepam daily (UTHSCSA). This year, compounds were also tested for effects on core temperature and spontaneous locomotor activity in mice (UM), for LSD-like discriminative stimulus properties in rats (SUNYB), and for effects on horizontal and vertical locomotor activity and for MDMA-like discriminative stimulus effects in rats (UTMB). This report includes the results of evaluation of CPDD 0063, CPDD 0064 and CPDD 0065. All studies were conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee, UM, UTHSCSA, SUNYB, UMMC, UTMB, and the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health.

METHODS

Reinforcing Effects in Rhesus Monkeys (UM)

Subjects

Subjects were rhesus monkeys (*Macaca mulatta*) experienced with self-administration of cocaine and 3,4-methylenedioxymethamphetamine (MDMA). Animals were surgically prepared with indwelling silicone rubber catheters using 10 mg/kg im ketamine and 2.0 mg/kg im xylazine as anesthetics. Catheters were implanted in jugular (internal or external), femoral or brachial veins as necessary. Catheters passed subcutaneously (sc) to the mid-scapular region, exited the body