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Original Article

Relationship between the dose of intravenous self-administration and the minimum effective dose for gross behavioral effects in rhesus monkeys: opiates vs CNS depressants

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ABSTRACT — In the development of drugs that affect the central nervous system (CNS), it is important to determine whether they have dependence potential and if so, to establish an effective dose within a range that does not manifest dependency. Methods for evaluating drug dependence include self-administration tests using experimental animals while assessment of the minimum effective dose (MED) involves gross behavioral observation. This study aims to examine the relationship between the most frequently self-administered dose (peak self-administered dose, PSAD) and the MED amongst different types of CNS depressants in order to optimize dose range selection for the evaluation of drug reinforcing effects. PSAD was investigated by intravenous self-administration in rhesus monkeys conducted as daily 2 hr-sessions under a fixed ratio 5 schedule with a 1-min time-out after each administration. MED was investigated by gross behavioral observation following cumulative dosing. For opiates, the PSAD was 0.016 mg/kg/infusion for morphine, 0.06 mg/kg/infusion for codeine, 0.25 mg/kg/infusion for butorphanol and 0.063 mg/kg/infusion for pentazocine. For anesthetics and sedatives, the PSAD was 0.5 mg/kg/infusion for pentobarbital, 0.25 mg/kg/infusion for thiopental, 0.06 mg/kg/infusion for ketamine and 0.063 mg/kg/infusion for midazolam. The PSAD/MED ratio was 1/63-1/32 for opiates and 1/8-1/2 for anesthetics and hypnosedatives. While previous research by Fujiwara *et al*. (2016) suggested that a dose range lower than the MED for gross behavioral effects should be used for intravenous self-administration in the evaluation of drug reinforcing effects, this study further indicates that the optimal dose range may vary depending on drug type.

Key words: Self-administration, Reinforcing effect, Minimum effective dose, Rhesus monkey

INTRODUCTION

The reinforcing effect of drug self-administration in animals is known to be useful in assessing the psychological dependence potential of drugs (Deneau *et al*., 1969; Balster and Bigelow, 2003; Ator and Griffiths, 2003). In drug self-administration, the relationship between the number of self-administrations and dose levels of a drug forms an inverted U-shape (no effect level to incapacitating level). Therefore, it is crucial to set optimal dose levels to ensure validity of the self-administration experiments. It was previously reported that the most frequent-

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ly self-administered dose levels of cocaine, pentobarbital, pentazocine, nicotine and caffeine were 1/250-1/8 of the MED for gross behavioral effects in rhesus monkeys (Fujiwara *et al*., 2016). This knowledge may be useful for the evaluation of reinforcing effects of new drugs under development. As for the dose range for assessing new drugs, while there is some variation, guidelines generally advocate for a wide dose range from threshold doses (showing no behavioral effects) up to doses corresponding to several-fold the human therapeutic dose (Ministry of Health and Welfare, 1975; European Medicines Agency, 2006; ICH, 2009; U.S. Department of Health and Human Services, 2017). This study examines the dose-response relationships observed in gross behavioral observation and intravenous self-administration experiments, comparing the results obtained between opiates and CNS depressants such as anesthetics and sedatives, in order to optimize the selection of dose levels for drug self-administration experiments.

MATERIALS AND METHODS

Subjects

In the gross behavioral observation, 4 male and 6 female rhesus monkeys (Macaca mulatta) between 5 and 21 years of age and weighing 4.1 to 7.8 kg were used. These monkeys were individually housed in stainless steel monkey cages with high-pressure melamine facing plate walls (68W × 86D × 86H cm, with stainless steel toy for environmental enrichment). In the self-administration, 4 male and 2 female rhesus monkeys between 5 and 16 years of age and weighing 5.0 to 8.6 kg were used. All monkeys had a history of intravenous self-administration of drugs including at least cocaine and pentobarbital but not other opiates or anesthetics. These animals were restrained in individual stainless steel cages (75W × 90D × 100H cm, with stainless steel toy for environmental enrichment) by metal harnesses and free-jointed metal arms in a monkey room (Deneau *et al*., 1969). Indwelling silicone catheters (OD: 2 mm, ID: 1 mm) were implanted into the jugular or femoral veins under pentobarbital anesthesia. Catheters exited from the midscapular region and were connected to silicone tubes passed through the restraining arm which was further connected to infusion apparatus located outside the cage (Fig. 1). All animals were fed 100-120 g of monkey chow (PS: Oriental Yeast Co., Ltd., Japan) and half a banana for environmental enrichment once daily. Tap water was continuously available from a fountain nozzle. The room temperature and humidity were set at 25 ± 2°C and 60 ± 20%, respectively. The room was illuminated from 7:00 AM to 7:00 PM.

All housing and experimental procedures were conducted according to the Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee (IACUC) of Ina Research Inc., which is fully accredited by AAALAC

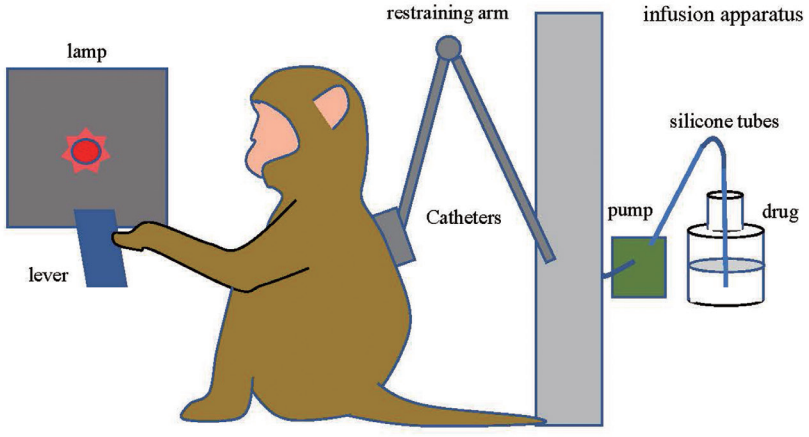

Fig. 1. Intravenous drug self-administration experiment in rhesus monkey. Animals were restrained by metal harnesses and free-jointed metal arms in individual cages in a monkey room. Indwelling silicone catheters were implanted into the jugular or femoral veins. Catheters exited from the midscapular region and were connected to silicone tubes passed through the restraining arm which was further connected to infusion apparatus located outside the cage. The drug is injected once through the catheter by pressing the lever five times. The beginning of the daily sessions was signaled by illumination of the red light above the lever.

International.

Apparatus

For the self-administration, the experiment was conducted in the home cages fitted with one response lever and one red light approximately 5 cm above the lever. A predetermined volume of dosing solution or saline was automatically infused through the catheter when the monkey pressed the lever (Deneau *et al*., 1969). Scheduling of infusions and collection of data were controlled by a personal computer system (MED-PC, Med Association, USA).

Procedures

Gross behavioral observation

The dosing solutions were administered at a rate of approximately 0.1 mL/second into the cephalic vein 3-4 times at intervals of 15 min for 2 chaired monkeys. Doses of the repeated administrations were cumulatively increased until gross behavioral changes were noted. Immediately after each administration, the animals were returned to their home cages and gross behavioral observation was conducted immediately and 10 min after administration, according to the methods and criteria of our laboratory. Observation items included salivation, retching, vomiting, reactivity to external stimuli, posture, pupil size and motor function such as locomotor activity, slowed motion and ataxia. Table 1 shows the test dose levels of each drug. For morphine, ketamine, codeine and midazolam, different monkeys were assigned. For thiopental and butorphanol, the same monkeys were assigned at intervals of 3 months.

Self-administration

Four to 5 monkeys were used for each drug. Intravenous self-administration of each drug or saline was observed for 2 hr (11:00 AM to 1:00 PM) a day. The drug was injected once through the catheter by pressing the lever five times (fixed ratio 5 schedule). The beginning of the daily sessions was signaled by illumination of the red light above the lever. Each self-administration was followed by a 1-min time-out period, during which time the red light was extinguished and responses had no consequence. Monkeys were first allowed to self-administer cocaine 0.03 mg/kg/infusion at a rate of approximately 0.1 mL/sec until the daily number of self-administrations attained 11 or more for 3 consecutive days and then saline 0.25 mL/kg/infusion until the daily number of self-administrations attained 10 or less for 3 consecutive days. The number of self-administrations of cocaine was limited to 20 times per day to avoid overdosing. After that, self-administration of the test drugs at 3-5 dose levels in descending order of dose level was observed for 4 days at each dose level. Since the number of drug self-administrations and gross behavioral changes can be affected by infusion speed of the dosing solutions (Wakasa *et al*., 1995), the same infusion speed was used for gross behavioral observation and drug self-administration in this study. Intervals of at least 2 weeks were set between each test drug.

Table 1. Dose levels of drugs in gross behavioral observation experiment

Drug	Cumulative dose (mg/kg)			
	1 st	2 nd	3 rd	4 th
Morphine	0.25	0.5	1	2
Codeine	0.5	1	2	4
Butorphanol	0.002	0.004	0.008	0.016
Thiopental	0.5	1	2	-
Ketamine	0.125	0.25	0.5	1
Midazolam	0.031	0.063	0.125	-

-: Not tested.

Drugs

Cocaine, morphine, codeine (Cocaine hydrochloride, Morphine hydrochloride hydrate and Codeine phosphate hydrate, Takeda Pharmaceutical Industry, Japan), butorphanol (Stadol injection, Bristol-Myers Squibb K.K., Japan), thiopental (Ravonal injection, Mitsubishi Tanabe Pharma Corporation, Japan), ketamine (Ketamine injection, Fujita Pharmaceutical Co., Ltd., Japan) and midazolam (Dormicum injection, Astellas Pharma Inc., Japan) were dissolved or diluted in isotonic physiological saline (Otsuka Normal Saline, Otsuka Pharmaceutical Factory Inc., Japan) and kept in light-protected bottles at room temperature.

Data analyses

For self-administration, the number of self-administrations over the last 3 days of cocaine, saline and the test drugs for each monkey was used to calculate the mean daily number of self-administrations of each drug for each monkey. For comparison of the minimum effective dose (MED) for gross behavioral effects and the dose with the highest mean daily number of self-administrations (peak self-administered dose, PSAD), the PSAD/MED ratios were analyzed for differences between opiates and CNS depressants using Student's t-test.

RESULTS

Gross behavioral observation

The gross behavioral changes observed were decreased grimacing at observer, slowed motion, ataxia and skin scratching with morphine; decreased grimacing at observer, muscle weakness, hypoactivity and skin scratching with codeine; hypoactivity with butorphanol; pale face with thiopental; slowed motion and ataxia with ketamine; and ataxia with midazolam. The MED for gross behavioral effects was 0.008 mg/kg for butorphanol, 0.063 mg/kg for midazolam, 0.5 mg/kg for ketamine, 1 mg/kg for morphine and thiopental and 2 mg/kg for codeine (Table 2).

Self-administration

Figures 2 and 3 and Tables 3 and 4 show the group and individual mean daily number of self-administrations for opiates and CNS depressants, respectively. The data of pentazocine and pentobarbital was derived from Fujiwara *et al*. (2016). The mean daily number of self-administrations of cocaine in the first self-administration period was higher than that of saline in all animals. For opiates (morphine, codeine, butorphanol and pentazocine), the inverted U-shaped dose-response curve was observed for all drugs and PSAD was notably higher than that of saline as a group mean (Fig. 2) and in all 4 animals (Table 3). The PSAD was 0.016 (range: 0.016-0.064) mg/kg/infusion for morphine, 0.06 (range: 0.015-0.06) mg/kg/infusion for codeine, 0.00025 (range: 0.00025-0.001) mg/kg/infusion for butorphanol and 0.063 (0.016-0.063) mg/kg/infusion for pentazocine. For CNS depressants (pentobarbital, thiopental, ketamine and midazolam), the inverted U-shaped dose-response curve was observed for all drugs except midazolam and PSAD was notably higher than that of saline as a group mean (Fig. 3) and in all 4 or 5 animals (Table 4). For midazolam, the inverted U-shaped curve was observed in 2 of 5 monkeys, but the dose-response curve for the group mean and remaining 3 monkeys showed a descending limb. The PSAD was 0.5 (range: 0.5-1) mg/kg/infusion for pentobarbital, 0.25 (range: 0.25-0.5) mg/kg/infusion for thiopental, 0.06 (range: 0.06-0.125) mg/kg/infusion for ketamine and 0.032 (0.016-0.032) mg/kg/infusion for midazolam.

A comparison of the MED for gross behavioral effects and the PSAD for self-administration is shown in Table 5. The PSAD/MED ratio was 1/63-1/32 (range: 1/133-1/8) for opiates and 1/8-1/2 (range: 1/8-1) for CNS depressants, and a statistically significant difference was noted between the two drug groups.

DISCUSSION

In this study, it was demonstrated that the peak self-administered doses (PSAD) for opiates and CNS depressants were 1/63-1/32 and 1/8-1/2 of the MED for gross behavioral effects, respectively.

Similar to other studies (Balster and Lukas, 1985; Woolverton *et al*., 2001; Do Carmo *et al*., 2008; Yanagita *et al*., 1977a, 1983a; Young and Woods, 1980, 1981; Broadbear *et al*., 2005; Winger *et al*. 1975) morphine, codeine, butorphanol, thiopental, ketamine and midazolam functioned as positive reinforcers. The inverted U-shaped curves were observed in all 4 monkeys with morphine, codeine, butorphanol, thiopental and ketamine, and in 2 of 5 monkeys with midazolam. PSADs in this study were comparable to other published substitution studies. For opiates, the PSAD was 0.016 mg/kg/infusion for morphine, 0.06 mg/kg/infusion for codeine and

Table 2. Minimal effective dose (MED) for gross behavioral observations in rhesus monkeys

Drug	No. of monkeys	MED (mg/kg)	Signs
Morphine	2	1	Decreased grimacing at observer Slowed motion Ataxia Skin scratching
Codeine	2	2	Decreased grimacing at observer Muscle weakness Hypoactivity Skin scratching
Butorphanol	2	0.008	Hypoactivity
Thiopental	2	1	Pale face
Ketamine	2	0.5	Slowed motion Ataxia
Midazolam	2	0.063	Ataxia

Drugs were administered intravenously by cumulative dosing at intervals of 15 min.

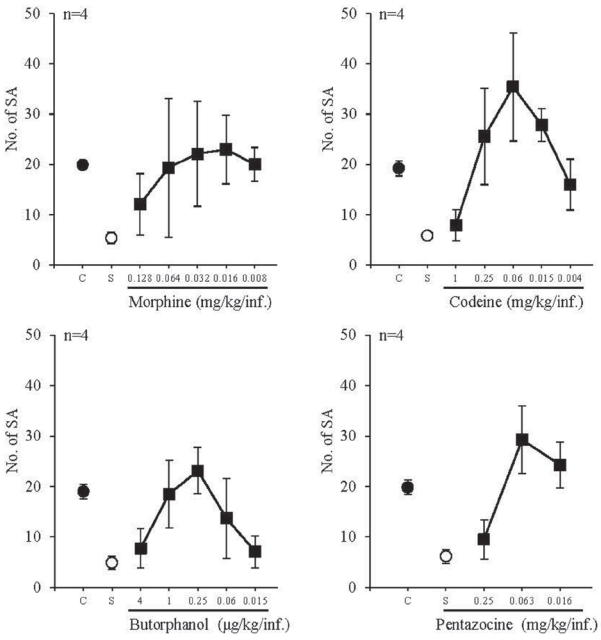

Fig. 2. Self-administration of opiates (morphine, codeine, butorphanol and pentazocine) in rhesus monkeys. Each monkey was allowed to self-administer cocaine at 0.03 mg/kg/inf. (C), saline (S) and the drugs in descending order of dose levels for 2 hr/day under a FR5 schedule with time-outs of 1 min. Each point represents the mean daily number of self-administrations (± S.D.) in the last 3 days.

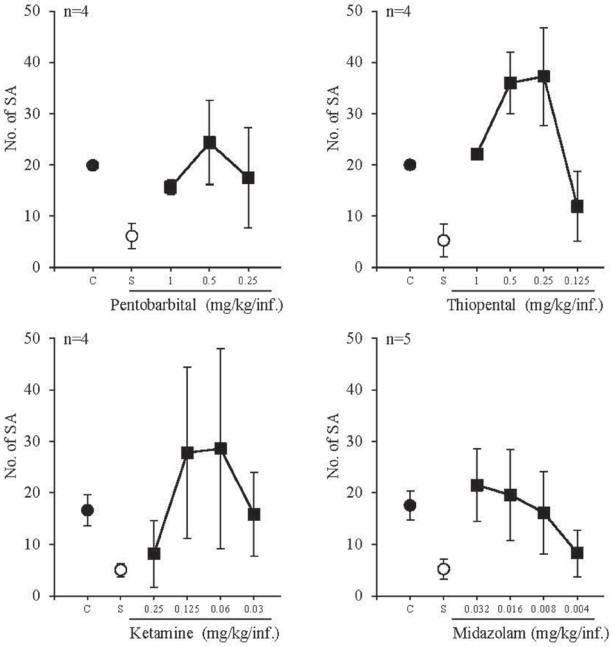

Fig. 3. Self-administration of CNS depressants (pentobarbital, thiopental, ketamine and midazolam) in rhesus monkeys. Each monkey was allowed to self-administer cocaine at 0.03 mg/kg/inf. (C), saline (S) and the drugs in descending order of dose levels for 2 hr/day under a FR5 schedule with time-outs of 1 min. Each point represents the mean daily number of self-administrations (± S.D.) in the last 3 days.

Table 3. Mean daily number of self-administrations of opiates (morphine, codeine, butorphanol and Pentazocine) in each monkey

Drug	Dose	Monkey				Monkey No.
	(mg/kg/inf.)	I	II	III	IV	
Morphine	Saline	6.0	5.7	3.7	6.0	I: No. 19 II: No. 26 III: No. 32 IV: No. 1407
	0.128	17.0	10.7	9.3	11.3	
	0.064	27.3	12.7	22.7*	14.7	
	0.032	23.3	18.7*	10.7	35.7	
	0.016	28.0*	16.3	8.0	39.7*	
	0.008	22.7	13.0	-	24.3	
Codeine	Saline	6.0	6.3	5.3	5.7	I: No. 19 II: No. 20 III: No. 26 IV: No. 1407
	1	8.3	4.7	6.7	12.0	
	0.25	34.7	12.3	25.7	29.7	
	0.06	49.7*	27.0	27.3	37.7*	
	0.015	31.7	27.7*	28.3*	23.7	
	0.004	15.0	22.3	10.0	16.7	
Butorphanol	Saline	6.3	4.3	5.7	3.3	I: No. 19 II: No. 26 III: No. 34 IV: No. 1407
	0.004	11.7	3.3	5.7	10.3	
	0.001	22.0	9.7	17.3	25.0*	
	0.00025	29.3*	18.3*	22.0*	23.0	
	0.00006	14.0	9.3	6.7	24.7	
	0.000015	7.7	4.0	5.3	11.3	
Pentazocine[a)]	Saline	3.3	7.7	7.0	6.7	I: No. 5 II: No. 1396 III: No. 1398 IV: No. 1405
	0.25	9.7	7.3	5.7	15.3	
	0.063	17.3*	24.3*	21.7	53.7*	
	0.016	6.0	19.7	37.3*	34.0	
	0.004	-	-	14.7	-	

-: Not tested.
*: The most frequent mean daily number of self-administrations.
The mean daily number of self-administrations obtained during the last 3 days at each dose.
[a)] Data derived from Fujiwara *et al*., 2016

Table 4. Mean daily number of self-administrations of CNS depressants (pentobarbital, thiopental, ketamine and midazolam) in each monkey

Drug	Dose	Monkey					Monkey No.
	(mg/kg/inf.)	I	II	III	IV	V	
Pentobarbital[a)]	Saline	8.7	2.7	6.0	6.3	-	I: No. 1 II: No. 5 III: No. 1396 V: No. 1414
	1	16.0*	15.0	17.3	14.0	-	
	0.5	15.7	22.3*	33.0*	15.7*	-	
	0.25	6.7	8.0	27.0	8.0	-	
Thiopental	Saline	4.3	9.3	1.7	5.7	-	I: No. 19 II: No. 20 III: No. 26 IV: No. 32
	1	23.3	22.3	21.7	21.3	-	
	0.5	42.0*	31.3*	40.3	30.3*	-	
	0.25	35.0	29.0	47.7*	-	-	
	0.125	7.3	19.7	8.7	-	-	
Ketamine	Saline	3.7	5.3	6.7	4.3	-	I: No. 19 II: No. 20 III: No. 26 IV: No. 1407
	0.25	1.7	16.0	4.3	10.7	-	
	0.125	8.7	30.7*	23.0*	48.7	-	
	0.06	19.0*	30.0	10.3	55.0*	-	
	0.03	13.7	17.3	6.3	26.0	-	
Midazolam	Saline	6.0	3.0	3.7	8.0	5.3	I: No. 19 II: No. 20 III: No. 26 IV: No. 32 V: No. 34
	0.032	30.7*	15.3*	25.3*	13.7	22.3	
	0.016	30.3	7.3	21.7	14.7*	24.0*	
	0.008	28.3	6.3	13.3	16.0	16.7	
	0.004	8.7	4.3	3.3	10.7	14.3	

-: Not tested.
*: The most frequent mean daily number of self-administrations.
The mean daily number of self-administrations obtained during the last 3 days at each dose.
[a)] Data derived from Fujiwara *et al*., 2016

Table 5. Relationship among the minimal effective doses (MED) in gross behavioral observations and the unit doses of the most frequent mean daily number of self-administrations (PSAD)

Group	Drugs	Doses (i.v.)			Ratio (range)		
		MED (mg/kg)	PSAD (range) (mg/kg/inf.)		PSAD/MED		Total
Opiates	Morphine	1	0.016	(0.016-0.064)	1/63	(1/63-1/16)	
	Codeine	2	0.06	(0.015-0.06)	1/33	(1/133-1/33)	1/63-1/32
	Butorphanol	0.008	0.00025	(0.00025-0.001)	1/32	(1/32-1/8)	(1/133-1/8)
	Pentazocine	2[a)]	0.063	(0.016-0.063)	1/32	(1/125-1/32)	
CNSdepressants	Pentobarbital	4[a)]	0.5	(0.5-1)	1/8	(1/8-1/4)	
	Thiopental	1	0.25	(0.25-0.5)	1/4	(1/4-1/2)	1/8-1/2*
	Ketamine	0.5	0.06	(0.06-0.125)	1/8	(1/8-1/4)	(1/8-1)
	Midazolam	0.063	0.032	(0.016-0.032)	1/2	(1/2-1)	

[a)] Data derived from Fujiwara *et al*., 2016

*: $p < 0.05$ vs the opiates group

0.00025 mg/kg/infusion for butorphanol; in comparison, previously reported values were 0.012 to 0.2 mg/kg/infusion for morphine (Balster and Lukas, 1985; Woolverton *et al*., 2001; Do Carmo *et al*., 2008), 0.06 to 1 mg/kg/infusion for codeine (Yanagita *et al*., 1977a; Young and Woods, 1980, 1981) and 0.00025 to 0.001 mg/kg/infusion for butorphanol (Balster and Lukas, 1985; Yanagita *et al*., 1983a). For CNS depressants, the PSAD was 0.25 mg/kg/infusion for thiopental, 0.06 mg/kg/infusion for ketamine and 0.032 mg/kg/infusion for midazolam in this study, while previously reported values were 0.125 to 0.5 mg/kg/infusion for thiopental (Winger *et al*., 1975), 0.03 to 1 mg/kg/infusion for ketamine (Woolverton *et al*., 2001; Young and Woods, 1981) and 0.01 mg/kg/infusion for midazolam (Broadbear *et al*., 2005). Furthermore, the study by Fujiwara *et al*. (2016) conducted with the same self-administration methods also reported PSADs of cocaine, pentazocine and pentobarbital that were comparable to other studies. Therefore, the findings indicate that our self-administration methods possess sufficient sensitivity for evaluation of drug reinforcing effects on par with other methods such as the substitution procedure.

In other published literature, there are some data on the PSAD/MED ratio of other opiates and CNS depressants such as loperamide, nufenoxole and dipotassium clorazepate. For opiates, the MED, PSAD and PSAD/MED were 0.25 mg/kg, 0.015 mg/kg/infusion and 1/17 for loperamide (Yanagita *et al*., 1979) and 1 mg/kg, 0.125 mg/kg/infusion and 1/8 for nufenoxole (Yanagita *et al*., 1983b), respectively. For dipotassium clorazepate, a benzodiazepine CNS depressant, the MED, PSAD and PSAD/MED were 2 mg/kg, 0.25-1 mg/kg/infusion and 1/8-1/2 (Yanagita *et al*., 1977b). Loperamide and nufenoxole are antidiarrheal drugs that do not cross the blood-brain barrier and have minimal effects on the CNS. It may be worth noting that some opiates, like these antidiarrheal drugs, may have low PSAD/MED ratios depending on the drug design. Further research will be needed on these cases. In summary, the most frequently self-administered dose levels (PSAD) in this study were lower than the MED for gross behavioral effects and the PSAD/MED ratios differed between representative opiates and CNS depressants. These results were useful in optimizing dose selection in drug self-administration.

Conflict of interest---- The authors declare that there is no conflict of interest.

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