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European Journal of Pharmacology 367 (1999) 175–181

ejp

Comparison between dopamine transporter affinity and self-administration potency of local anesthetics in rhesus monkeys

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Received 11 December 1998; accepted 22 December 1998

Abstract

Local anesthetics bind to dopamine transporters and inhibit dopamine uptake in rodent brain. Additionally, local anesthetics are self-administered in rhesus monkeys. The present study determined binding affinities of cocaine and five local anesthetics at dopamine transporters in rhesus monkey brain, and compared binding affinities to published self-administration potencies in rhesus monkeys. The affinity order at dopamine transporters was cocaine > dimethocaine > tetracaine > procaine ≥ chloroprocaine > lidocaine. The correlation between dopamine transporter affinities and self-administration potencies was significant. Binding affinities were also determined at sodium (Na^{2+}) channels in rhesus monkey brain. There was not a significant correlation between Na^{2+} channel affinities and self-administration potencies Local anesthetics with high dopamine transporter and low Na^{2+} channel affinities were self-administered, whereas those with either high or low affinity at both sites were not consistently self-administered. These data suggest that affinity at dopamine transporters is related to the reinforcing effects of local anesthetics in rhesus monkeys, and Na^{2+} channel effects may interfere with the reinforcing effect of these drugs.

Keywords: Local anesthetic; Dopamine transporter; Self-administration; Radioligand binding; (Rhesus monkey)

1. Introduction

The abuse of cocaine is thought to involve its action as a dopamine uptake blocker (Ritz et al., 1987; Kuhar et al., 1991; Kuhar, 1992; Woolverton and Johnson, 1992). Like cocaine, the local anesthetics dimethocaine, chloroprocaine, procaine and tetracaine have been shown to bind to dopamine transporters and inhibit the uptake of dopamine, whereas lidocaine does not exhibit these effects (Ritz et al., 1987; Izenwasser et al., 1994; Woodward et al., 1995). In addition, dimethocaine and procaine, but not lidocaine, have also been found to increase dialysate dopamine in in vivo microdialysis studies (Hernandez et al., 1991; Woodward et al., 1995). Considering this effect of some local anesthetics on dopamine function, it is perhaps not surprising that these compounds exhibit reinforcing effects. In drug self-administration studies in rhesus monkeys cocaine, dimethocaine, procaine, chloroprocaine and tetracaine maintained self-administration above saline levels, whereas lidocaine did not (Ford and Balster, 1977; Woolverton and Balster, 1979; Johanson, 1980; Woolverton and Balster, 1982). In addition, the potency order of local anesthetics in self-administration was similar to their affinity order for dopamine transporter binding, and their potency order to inhibit dopamine uptake in rat brain (Ritz et al., 1987; Woodward et al., 1995). Moreover, cocaine-dependent human subjects have reported that the subjective effects of a high dose of procaine were similar to cocaine (Adinoff et al., 1998). In another study, three out of four normal adult volunteers identified a high dose of intravenous procaine as cocaine, whereas intravenous lidocaine was identified as placebo (Fischman et al., 1983a; Fischman et al., 1983b). The dopaminergic effects of local anesthetics may contribute to these behavioral effects.

To date, all of the biochemical studies assessing the dopaminergic effects of local anesthetics have been con-

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0014-2999/99/$ - see front matter
PII: S0014-2999(98)00967-4

ducted in rat brain, whereas, with the exception of procaine (Collins et al., 1984), the self-administration data have been collected in rhesus monkeys. Considering that differences may exist between the biochemical effects of local anesthetics in rats and monkeys, one purpose of this study was to determine the binding affinities of several local anesthetics at dopamine transporters in rhesus monkey caudate and putamen. Since a primary site of action for local anesthetics is also the sodium (Na^{2+}) channel, binding affinities were also determined for these compounds at Na^{2+} channels in rhesus monkey frontal cortex. Additionally, ratios of binding affinities at dopamine transporters/binding affinities at Na^{2+} channels for cocaine and the local anesthetics were calculated. Finally, comparisons between binding affinities and self-administration potencies were made to assess the contribution of relative affinities at dopamine transporters and Na^{2+} channels to the reinforcing effects of local anesthetics in rhesus monkeys.

2. Materials and methods

2.1. Animals

A total of 9 rhesus monkeys, *Macaca mulatta*, were used for the in vitro studies. Monkeys had histories of cocaine or barbiturate self-administration, or exposure to oral methadone, and included 7 males (9032, 8713, 8217, 13296, 9125, 8805, 9001) and 2 females (11083, 9086). Monkeys 8217, 13296, 9125, 8805 and 9086 had self-administered cocaine within two months of sacrifice, and monkeys 9032, 8713 and 9001 had been drug-free for at least two months prior to sacrifice. Monkeys were euthanized via an overdose of pentobarbital. Brains were collected immediately after sacrifice and the caudate nucleus, putamen and frontal cortex were dissected according to the atlas of Snider and Lee (1961). Tissue was fast frozen on aluminum foil over solid CO_2 immediately after dissection with no additional preparation.

2.2. Tissue preparation

Frozen tissue was thawed and homogenized in buffer (Table 1) then centrifuged at $20{,}000 \times g$ for 20 min ([^{3}H]2β-carbomethoxy-3β-(4-fluorophenyl)tropane, CFT binding) or $1000 \times g$ for 10 min ([^{3}H]Batrachotoxinin A 20-α-benzoate, BTX binding) at 4°C. For [^{3}H]CFT binding, the resulting pellet was resuspended in fresh buffer and centrifuged an additional two times at $20{,}000 \times g$ for 20 min. For [^{3}H]BTX binding a P2 preparation was used. After the first centrifugation, the pellet was discarded and the supernatant was centrifuged at $20{,}000 \times g$ for 20 min.

Table 1
Displacement and saturation studies: parameters for in vitro assays

Site	[^{3}H]Ligand (nM)	[Ligand]	Nonspecific binding	Tissue (mg wet wt.)	No. washes (x/volumes)	Buffer[a] (pH 7.4)	Incubation (h/°C)	Filter soak time (min)	References
Dopamine transporter	CFT	1.0	100 μM (−)cocaine	4.0 C/P	1/10; 2/40	1) 50 mM Tris 2) 50 mM Tris 100 mM NaCl	2/4	40/ 0.1% BSA	Madras et al., 1989
Sodium channel	BTX	10	0.3 mM aconitine	2.5 FCTX	1/10 1/50	1a) 0.32 M sucrose 1b) HTS + 2) HTS +	1/37	15	Postma and Caterall, 1984

Abbreviations: CFT: 2β-carbomethoxy-3β-(4-fluorophenyl)tropane; BTX: Batrachotoxinin A 20-α-benzoate; C/P: caudate and putamen; FCTX: frontal cortex; BSA: bovine serum albumin; HTS +: 50 mM HEPES–Tris (pH 7.4), 5.4 mM KCl, 0.8 mM $MgSO_4$, 5.5 mM glucose, 130 mM choline.
[a] 1) = homogenization buffer, 2) = incubation buffer.
[b] Filters were presoaked in assay buffer unless otherwise indicated.

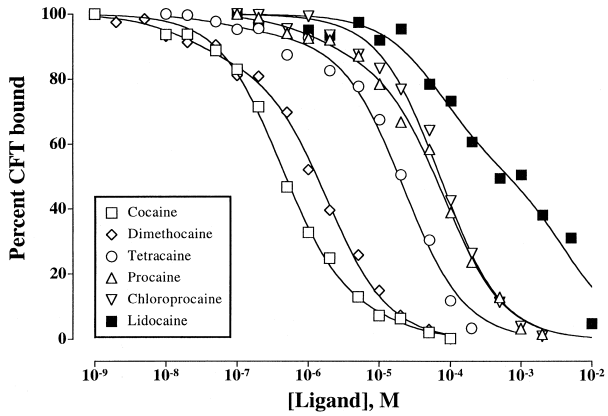

Fig. 1. Displacement of cocaine and the local anesthetics from dopamine transporters in rhesus monkey caudate and putamen. The *x*-axis represents the concentration of the displacer, and the *y*-axis the percent of [^{3}H]CFT bound. Each data point represents the mean (with less than 10% variation) for 4 monkeys.

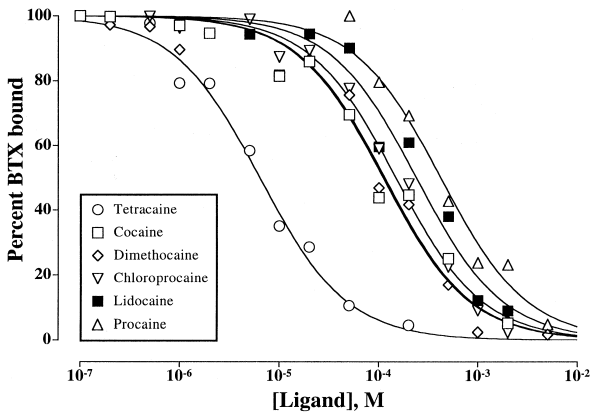

Fig. 2. Displacement of cocaine and the local anesthetics from sodium channels in rhesus monkey frontal cortex. The *x*-axis represents the concentration of the displacer, and the *y*-axis the percent of [^{3}H]BTX bound. Each data point represents the mean (with less than 10% variation) for 4 monkeys.

The resulting pellet was then resuspended in fresh buffer and centrifuged again at the same speed. After the final centrifugation the pellet was suspended at the appropriate tissue concentration for displacement or saturation assays with [^{3}H]CFT or [^{3}H]BTX.

2.3. Procedure

Specific conditions for displacement and saturation assays are presented in Table 1. For displacement studies the tissue was added to assays containing the radioligand (Dupont-NEN, Boston, MA; [^{3}H]CFT 84.5 Ci/mmol, [^{3}H]BTX 39.9 Ci/mmol) and various concentrations of cocaine or the other local anesthetics dissolved in assay buffer. To generate saturation isotherms samples were incubated with various concentrations of [^{3}H]CFT (0.50–64 nM) or [^{3}H]BTX (10–600 nM). For [^{3}H]BTX binding, 90 μg/ml of scorpion venom was added to each assay to enhance BTX binding. All assays were brought to their final volume with the addition of buffer, and were incubated under conditions described in Table 1. Reactions were terminated by rapid vacuum filtration through Whatman GF/C filters using a 24-well Brandel cell harvester (Brandel, Gaithersburg, MD). The filters were rinsed twice with 5 ml ice cold buffer, and deposited into Packard Top Count deep well plates. 500 μl of Microscint-20 cocktail (Packard Instruments, Downers Grove, IL) was added to each well. Bound radioactivity was determined using a Packard Top Count scintillation counter. Protein levels in tissue homogenate samples were determined using the bicinchoninic acid method (Smith et al., 1985; kits from Pierce, Rockford, IL). Absorbance (at 560 nm) was measured on a Beckman spectrophotometer (Beckman, Palo Alto, CA).

2.4. Data analysis

Each assay was performed in duplicate. Data were initially reduced and analyzed using iterative curve fitting (Prism, Graphpad, San Diego, CA). To compare the goodness-of-fit of one-site models to two-site models, all Hill coefficients were fixed to −1 to fit displacement data. The one-site model was assumed unless the mean square error was significantly reduced by using a two-site model ($P < 0.05$ using a univariate *F*-test). K_d and B_{max} values were derived from saturation studies. IC_{50} values were calculated for each compound from displacement studies. Comparisons of ligand selectivity for dopamine transporters

Table 2
IC_{50} values for local anesthetics at dopamine transporters in rhesus monkeys

Drug	Site 1, μM (95% CI)	% Site 1 (95% CI)[a]	Site 2, μM (95% CI)
Cocaine	0.31 (0.31–0.54)	65 (25–100)	31 (0.53–335)
Dimethocaine	0.02 (0.006–0.07)	14 (8–20)	1.8 (1.5 –2.2)
Tetracaine	0.14 (0.002–4.1)	7 (0–13)	29 (16–29)
Procaine	3.5 (0.19–8.2)	16 (3–21)	89 (61–101)
Chloroprocaine	0.46 (0.02–151)	7 (0–17)	93 (0.62–111)
Lidocaine	31 (11–328)	31 (30–65)	2895 (1647–11 260)

Data represent the mean and 95% confidence intervals (CI) ($n = 4$) and were derived as described in Section 2.
[a]The percentage of sites that were site 1.

Table 3
IC_{50} values for local anesthetics at sodium channels in rhesus monkeys

Drug	Site 1, μM (95% CI)
Cocaine	115 (79–154)
Dimethocaine	167 (166–337)
Tetracaine	6.6 (4.5 –9.0)
Procaine	528 (275–641)
Chloroprocaine	208 (117–209)
Lidocaine	315 (168–337)

Data represent the mean and 95% confidence intervals (CI) ($n = 3–4$) and were derived as described in Section 2.

and Na^{2+} channels were made using the IC_{50} value for displacement of the appropriate radioligand. In addition, a ratio of dopamine transporter IC_{50}/Na^{2+} channel IC_{50} was calculated for each compound. To calculate the ratio, the data for compounds that were described by a two-site fit were reduced to a one-site fit. Calculation of the ratio was based on IC_{50} values generated by fitting the data to a one-site model using a sigmoidal function with a variable Hill slope. Potencies in self-administration were determined two ways: 1) an average of the range of doses that maintained maximum levels of self-administration above saline; 2) an average of the range of doses that maintained responding similar to the baseline dose of cocaine (Ritz et al., 1987), across monkeys. A Pearson correlation was performed to determine if there was a significant relationship ($P < 0.05$) between potency in self-administration and dopamine transporter affinity or Na^{2+} channel affinity for a given drug.

2.5. Drugs

Cocaine HCl was obtained from the National Institute on Drug Abuse. Chloroprocaine was obtained from Astra Pharmaceutical Products (Westboro, MA). Procaine HCl, tetracaine HCl, lidocaine HCl, scorpion venom (*Leiurus quinquestriatus hebraeus*), and aconitine were purchased from Sigma (St. Louis, MO). Dimethocaine was a generous gift from Dr. Robert L. Balster (Medical College of Virginia, Richmond, VA). The non-radiolabled batrachotoxin used for the saturation assays was graciously supplied by Dr. John Daly (Laboratory of Bioorganic Chemistry, National Institutes of Health, Bethesda, MD).

3. Results

In saturation experiments using different concentrations of [^{3}H]CFT, the affinity of CFT at dopamine transporters in monkey striata was 18.9 nM (95% confidence intervals, 10–27). The B_{max} was 3061 (2334–3790) fmol/mg protein. In saturation experiments using different concentrations of [^{3}H]BTX the affinity of BTX at Na^{2+} channels was 85.5 nM (31–227). The B_{max} was 1383 (769–1997) fmol/mg protein.

Cocaine and the local anesthetics displaced [^{3}H]CFT in a concentration-dependent manner from dopamine transporters in monkey caudate and putamen (Fig. 1). Specific binding accounted for approximately 89% of total binding. Displacement of [^{3}H]CFT by cocaine and all of the local

Table 4
The ratio of dopamine transporter IC_{50} values to sodium channel IC_{50} values and potency in self-administration for local anesthetics in rhesus monkeys

Drug	Dopamine transporter	Sodium channel	Ratio	Potency in self-administration	
	One-site fit[a] μM (95% CI)	One-site fit μM (95% CI)		(mg/kg/inj)[b]	Relative potency[c]
Cocaine	0.61 (0.42--0.54)	111 (77–156)	0.005	0.005	1[d]
Dimethocaine	1.3 (1.1–1.4)	170 (88–152)	0.008	0.09	0.67[e]
Tetracaine	23.2 (18–30)	6.4 (4.6–9.0)	3.63	0.07	–[f]
Procaine	55.1 (46–66)	522 (283–597)	0.11	0.39	14.1[f,g]
Chloroprocaine	78.7 (67–93)	203 (118–189)	0.39	0.33	29[g,e]
Lidocaine	800 (513–1317)	210 (170–334)	3.81	not self-administered[f]	

Data represent the mean and 95% confidence intervals (CI) ($n = 3–4$) and were derived as described in Section 2.
[a]A one-site fit with a variable Hill slope was used to calculate the ratio since none of the drugs fit a two-site model for the displacement of [^{3}H]BTX. Hill slopes for dopamine transporter binding ranged from −0.61 (95% CI, −0.76 to −0.46) to −1.1 (−1.3 to −0.82). Hill slopes for sodium channel binding ranged from −0.96 (−1.3 to −0.60) to −1.3 (−1.7 to −0.86).
[b]All animals were trained under a fixed-ratio 10 self-administration procedure. Once responding for cocaine and saline was stable various doses of the local anesthetics were substituted for the cocaine baseline dose. For all drugs, 0.03 mg/kg/injection cocaine was the baseline dose. Doses reported in the table are an average of the doses that maintained maximum responding above saline levels, and functioned as positive reinforcers.
[c]Values reported in the table are taken from Ritz et al. (1987) and represent the average potency relative to the cocaine baseline dose for the doses that maintained responding similar to the cocaine baseline dose, and functioned as positive reinforcers.
[d]Weed and Woolverton, 1995.
[e]Woolverton and Balster, 1982.
[f]Woolverton and Balster, 1979.
[g]Johanson, 1980.

anesthetics was best described by a two-site model (Table 2). The rank order of IC_{50}s for these compounds at dopamine transporters was cocaine > dimethocaine > tetracaine > procaine > chloroprocaine > lidocaine (Table 4). Cocaine and the local anesthetics also displaced [^{3}H]BTX in a concentration-dependent manner from Na^{2+} channels in monkey frontal cortex (Fig. 2). Specific binding accounted for approximately 85% of total binding. In contrast to [^{3}H]CFT, displacement of [^{3}H]BTX was not described by a two-site model for any of the compounds tested (Table 3). The rank order of affinities at Na^{2+} channels was different from that for dopamine transporters with tetracaine > cocaine ≥ dimethocaine ≥ chloroprocaine ≥ lidocaine ≥ procaine (Table 4). All compounds except tetracaine and lidocaine had a higher affinity at dopamine transporters than Na^{2+} channels. Cocaine and dimethocaine had the lowest IC_{50} dopamine transporter/IC_{50} Na^{2+} channel ratios of 0.005 and 0.008, respectively (Table 4). Procaine and chloroprocaine had intermediate ratio values of 0.11 and 0.39, respectively (Table 4). The largest ratios were 3.81 for lidocaine and 3.63 for tetracaine (Table 4).

Table 5
Correlation between binding affinites and self-administration potencies in rhesus monkeys

	Pearson correlation coefficient	*P* value	*n*
Dopamine transporter			
Potency (mg/kg/inj)[a]	0.90	0.04	5
Relative potency[b]	0.98	0.02	4
Sodium channel			
Potency (mg/kg/inj)	0.81	0.10	5

[a]Self-administration potencies were based on the doses of the local anesthetics that maintained maximum responding above saline levels. Cocaine, dimethocaine, chloroprocaine, procaine and tetracaine were included in the correlation. Self-administration potencies were compared to IC_{50} values (Table 4).
[b]Self-administration potencies were based on the relative potencies for the local anesthetics reported in Ritz et al. (1987). Cocaine, dimethocaine, chloroprocaine and procaine were included in the correlation. Self-administration potencies were compared to relative IC_{50} values (relative to the IC_{50} for cocaine).

4. Discussion

The primary purpose of the present study was to determine the binding affinities of several local anesthetics at dopamine transporters in rhesus monkey brain. The rank order of affinities at dopamine transporters was cocaine > dimethocaine > tetracaine > procaine > chloroprocaine > lidocaine. Other studies have reported a similar in vitro affinity order for local anesthetics at dopamine transporters in rats (Ritz et al., 1987; Woodward et al., 1995). Additionally, the in vitro potency order for local anesthetics to inhibit dopamine uptake and to increase dialysate dopamine in in vivo microdialysis studies in rats (Hernandez et al., 1991; Woodward et al., 1995) was consistent with the affinity order of these drugs at dopamine transporters in the present study. Moreover, the potency order for the self-administration of cocaine and the local anesthetics in rhesus monkeys was also similar to the affinity order of these drugs in the present study with cocaine > tetracaine ≥ dimethocaine ≥ chloroprocaine ≥ procaine; lidocaine was not self-administered (Table 4). When the doses that produced maximum responding were used as measures of reinforcing potencies, the correlation for binding affinities and potencies in self-administration was significant for dopamine transporters, but not for Na^{2+} channels (Table 5), suggesting that the dopamine transporter actions of local anesthetics contribute to their reinforcing effects. Ritz et al. (1987) reported a similar finding when they compared dopamine transporter binding affinities of several compounds in rats to self-administration potencies of the same compounds in monkeys. Additionally, the correlation between dopamine transporter affinities in monkeys and self-administration potencies in the present study was also significant when the method of Ritz et al. (1987) was used to estimate reinforcing potencies (Tables 4 and 5). It should be noted that the animals used in the present study varied in drug histories and age, and that there are reports of drug- and age-related effects on dopamine transporter densities in rodents (Sharpe et al., 1991; Shimizu and Prasad, 1991; Koff et al., 1994; Boulay et al., 1996) monkeys (Farfel et al., 1992) and healthy human subjects (Volkow et al., 1994; Volkow et al., 1996; Wang et al., 1997; however, see Wilson et al., 1996). It seems unlikely that these effects are a concern in the present study since there were no differences in the affinities of local anesthetics for dopamine transporter binding across monkeys.

Since a primary mechanism of action of local anesthetics is the blockade of Na^{2+} channels, binding affinities were also determined at this site in rhesus monkey brain. The rank order of affinities at Na^{2+} channels was different from the rank order at dopamine transporters with tetracaine > cocaine ≥ dimethocaine ≥ chloroprocaine ≥ lidocaine ≥ procaine. Other studies have reported a similar in vitro affinity order for local anesthetics at Na^{2+} channels in rats (Creveling et al., 1983; Postma and Caterall, 1984; McNeal et al., 1985; Reith et al., 1986). There was no significant relationship between Na^{2+} channel affinities and self-administration potencies when self-administration potencies were determined using doses that maintained maximum responding.

It is possible that a mix of Na^{2+} channel and dopamine transporter effects may be meaningful in evaluating the reinforcing effects of local anesthetics. Moreover, the degree of separation between dopamine transporter and Na^{2+} channel affinities may be an important consideration. To determine the relative contribution of dopamine transporter affinity and Na^{2+} channel affinity to the reinforcing ef-

fects of local anesthetics a ratio of dopamine transporter IC_{50}/Na^{2+} channel IC_{50} was calculated. Cocaine, dimethocaine, procaine and chloroprocaine had higher affinities at dopamine transporters relative to Na^{2+} channels and were positive reinforcers (Woolverton and Balster, 1982). Cocaine and dimethocaine had the greatest separation between their affinities at dopamine transporters and Na^{2+} channels, indicated by their low dopamine transporter IC_{50}/Na^{2+} channel IC_{50} ratio values. The separation between dopamine transporter and Na^{2+} channel affinities for procaine and chloroprocaine was smaller compared to cocaine and dimethocaine, indicated by their intermediate ratio values. In contrast, tetracaine and lidocaine had higher affinities at Na^{2+} channels relative to dopamine transporters and had the highest dopamine transporter IC_{50}/Na^{2+} channel IC_{50} ratio values. The evidence for a mutual influence of dopaminergic and Na^{2+} channel effects to the reinforcing effects of local anesthetics is strongest for tetracaine. Tetracaine binds to dopamine transporters with high affinity but was not self-administered by all monkeys tested (Woolverton and Balster, 1979). One possibility is that the high affinity of tetracaine for Na^{2+} channels may have interfered with its reinforcing effects. In addition to tetracaine, the local anesthetic piperocaine consistently produces responding below saline levels in self-administration studies in rhesus monkeys despite its potency to inhibit dopamine uptake in rats ($IC_{50} = 13$ μM; Woolverton and Balster, 1982; Woodward et al., 1995). Although piperocaine was a potent inhibitor of dopamine uptake it also displaces [^{3}H]BTX from Na^{2+} channels with high affinity ($IC_{50} = 13.2$ μM; McNeal et al., 1985). Thus, a minimal separation between dopamine transporter affinities and Na^{2+} channel affinities may result in decreased reinforcing effects of local anesthetics.

Based on their biochemical and behavioral profiles the local anesthetics tested in the present study can be divided into three categories: 1) local anesthetics that have high affinity at dopamine transporters relative to Na^{2+} channels and are self-administered, 2) local anesthetics that have high affinity at both dopamine transporters and Na^{2+} channels and are not consistently self-administered, 3) local anesthetics that have low affinity at both dopamine transporters and Na^{2+} channels and are not self-administered. The mechanism by which a mixture of pharmacological actions determines self-administration should be pursued.

Acknowledgements

This research was supported by NIDA grants DA-10352 (W.L.W.) and DA-05807 (K.M.W.). All procedures were approved by the University of Mississippi Medical Center Institutional Animal Care and Use Committee. The authors gratefully acknowledge the excellent technical assistance of Rebeccah Nothdurft. We also thank Dr. Karen Anderson and Dr. Lisa Bellavance for their comments on an earlier version of this manuscript.

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