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Further evidence that the delayed temporal dopaminergic effects of LSD are mediated by a mechanism different than the first temporal phase of action

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Abstract

Activation of 5-HT_{2A} receptors is thought to mediate the hallucinogenic effects of LSD. Nevertheless, in a previous report we provided evidence that a delayed temporal phase of the behavioral pharmacology of LSD is mediated by D_2-like dopamine receptor stimulation. In this study rats were trained to discriminate LSD with either a 30 min preinjection time (LSD-30, $N=12$) or a 90 min preinjection time (LSD-90, $N=13$) from saline, using a two-lever, food-reinforced operant conditioning task. We then tested a large number of agonists and antagonists belonging to distinct pharmacological classes in these animals. As anticipated, classical hallucinogens such as psilocin and mescaline substituted only in LSD-30 rats, and not in LSD-90 rats. The dopamine receptor agonists ABT-724, aripiprazole, dihydrexidine, WAY 100635, and SKF 38393, fully or partially mimicked LSD-90, but not LSD-30. The results reported here support and extend our previous conclusion that the delayed temporal effects of LSD are mediated by activation of a dopaminergic system.

Keywords: Drug discrimination; Rats; LSD; Delayed temporal phase; 5-HT_{2A}; 5-HT_{1A}; Dopamine; Dopamine D_1; Dopamine D_2; Dopamine D_4; GABA; $GABA_B$

1. Introduction

Since 1943, when Albert Hofmann first observed the hallucinogenic effects of lysergic acid diethylamide (LSD) in humans (Hofmann, 1979), scientists have attempted to identify its mechanisms of action. The pharmacology of LSD is multifaceted and divergent, implicating a variety of different but probably interrelated neuronal mechanisms to account for the various components of the LSD experience. It is perhaps not surprising therefore, that the use of many physiological, pharmacological, and behavioral procedures has failed to elucidate completely the mechanisms underlying the complex spectrum of biobehavioral actions of LSD.

The effect of LSD in humans has been described by Freedman (1984) as occurring in two temporal phases. We recently reported (Marona-Lewicka et al., 2005) that the behavioral effect of LSD in rats also occurs in two distinct, time-dependent temporal phases that are mediated by two different neurochemical systems. In the initial phase, activation of serotonin2A (5-HT_{2A}) receptors plays an essential role, but that is followed by a later temporal phase where the effects of LSD are mediated by D_2 dopamine receptor stimulation. This response is reproducible, and when injected 90 min before training sessions, LSD generates a stable cue with essentially identical ED50s over several generations of rats.

The vast majority of compounds investigated in drug discrimination studies produce a unitary pharmacology. Thus, it has been widely accepted that the discriminative cue of LSD involves serotonin 5-HT_{2A} receptor activation. LSD, however, possesses more than one pharmacological action, and we were the first to report (Marona-Lewicka et al., 2005) that it produced a time-dependent dopaminergic component. In our initial report, however, we had not elucidated whether direct or indirect activation of the dopaminergic system was required for expression of this delayed dopaminergic phase. Thus, this study describes more precisely how different components of dopaminergic systems, as well as other drugs that earlier were

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evaluated in LSD-trained rats, affected the later temporal phase of the discriminative stimulus effect of LSD.

The drug discrimination (DD) procedure is the most frequently used *in vivo* model of hallucinogen activity. The paradigm has several advantages over other animal models of hallucinogenic activity, accounting for the frequency with which it is employed. Discrimination behavior is specific within a given pharmacological class, reliably sensitive to low doses of drug, and objectively quantified and related to the subjective effects of drugs as reported by humans (Appel et al., 1982; Colpaert, 1999). Any drug with activity at multiple receptors may differ, however, in its apparent stimulus properties, depending upon whether it is used for training or is tested in subjects trained with drugs that may share certain of its pharmacological elements.

LSD displays high affinity for serotonin 5-HT_{2A} and 5-HT_{2C} receptors (Titeler et al. 1988). Although LSD interacts with many other receptors (the 5-HT_1 family, 5-HT_5, 5-HT_6, 5-HT_7, α-adrenergic, and dopamine receptors; see on-line database: http://kidb.cwru.edu/pdsp.php), activation of 5-HT_{2A} receptors is thought to be the key feature that is essential for the hallucinogenic properties of LSD. In animals, this receptor is considered an important mediator of the effect of hallucinogens, a conclusion heavily based on drug discrimination studies.

During the past four decades, a large number of compounds that belong to different pharmacological classes of drugs have been tested in rats trained to discriminate LSD from saline or LSD from other drugs, in substitution and/or combination tests. Hallucinogens from different structural classes clearly mimicked LSD (Appel et al., 1982; Young et al., 1982; Colpaert et al., 1982; Oberlender et al., 1984; Cunningham et al., 1985; Freedman, 1986; Nichols, 1986; White, 1986; Arnt, 1989; Callahan and Appel, 1990; Huang et al., 1994; Monte et al., 1996, 1997; Chojnacka-Wojcik and Klodzinska, 1997; Blair et al., 2000; Rabin et al., 2002; Nichols et al., 2002; Smith et al., 2003; Benneyworth et al., 2005). Furthermore, antagonists with predominant affinity at the 5-HT_{2A} receptor were able to block the discriminative cue generated by LSD injected 10–30 min prior to tests (Colpaert et al., 1982, 1985; Colpaert and Janssen, 1983; Minnema et al., 1984; Callahan and Appel, 1990; Appel et al., 1999; Nichols et al., 2002). Importantly, recent human studies have shown that most of the hallucinogenic properties of psilocybin, an indoleamine hallucinogen, are mediated by 5-HT_{2A} receptors (Vollenweider et al., 1997, 1998).

In our previous study of the pharmacology of the later temporal phase of LSD (Marona-Lewicka et al., 2005), M100907 fully blocked drug appropriate responding in rats trained with a preinjection time of 30 min (LSD-30); however, it produced only partial (36%) inhibition of the LSD-90 cue (rats trained to discriminate LSD injected 90 min before tests). M100907 does have appreciable affinities for rat 5-HT_{2C} and other biogenic amine receptors, however, including α_1 and histamine H_1 receptors, and some of these receptors could modulate the LSD cue (e.g. Marona-Lewicka and Nichols, 1995). Thus, in recent experiments we have used the slightly less potent but more selective 5-HT_{2A} antagonist MDL 11,393 (Pehek et al., 2006) to confirm the role of the 5-HT_{2A} receptor in mediation of the discriminative stimulus effects in LSD-30- and LSD-90-trained rats.

The goals of the present study were to characterize the involvement of dopaminergic systems and other neurotransmitters in the discriminative stimulus effects in rats of the later temporal phase of LSD. Thus, experiments was designed to test the similarities of the discriminative stimulus properties produced by LSD-30 and LSD-90, as well as to elaborate further the differences between the two distinct temporal phases occurring after LSD treatment. We employed a large number of agonists and antagonists that belong to distinct pharmacological classes. Most of the tests were run at the same time, in parallel in both LSD-30 and LSD-90 groups.

2. Material and methods

2.1. Animals

Male Sprague Dawley rats (Harlan Laboratories, Indianapolis, IN) weighing 180–200 g at the beginning of the study were used as subjects. Rats were divided into two groups and trained to discriminate LSD (186 nmol/kg, 0.08 mg/kg, i.p.) with a 30 min preinjection time (LSD-30) and LSD (372 nmol/kg, 0.16 mg/kg, i.p.) with a 90 min preinjection time (LSD-90) from saline, using a two-lever, food-reinforced operant conditioning task. We try to maintain a stable number of animals for each training drug or training condition ($N=12$); in particular each group used for tests consisted of two subgroups, one with older rats (15–28 months old), and one with younger animals (5–15 months old). New animals are routinely added about every 6 months. Once they have been shaped, learned the discrimination task, and a dose–response curve obtained for the training drug, they are incorporated into the colony, replacing older animals. None of the rats had previously received drugs or behavioral training. Water was freely available in the individual home cages and a rationed amount of supplemental feed (LabDiet-5001, PMI, Nutrition International, LLC, Brentwood, MO) was made available after experimental sessions so as to maintain approximately 80% of free-feeding weight. Lights were on from 07:00 to 19:00. The laboratory and animal facility temperature was 22–24 °C and the relative humidity was 40–50%. Experiments were performed between 09:00 and 17:00 each day, Monday–Friday. Animals used in these studies were maintained in accordance with the U.S. Public Health Service Policy on Humane Care and Use of Laboratory Animals as amended August 2002 and the protocol was approved by the Purdue University Animal Care and Use Committee.

2.2. Apparatus

Six standard operant conditioning chambers (model E10-10RF, Coulbourn Instruments, Lehigh Valley, PA) consisted of modular test cages enclosed within sound-attenuated cubicles with background white noise and fans for ventilation. A white house light was centered near the top of the front panel of the cage, which also was equipped with two response levers, separated by a food hopper (combination dipper pellet trough, model E14-06, module size 1/2), all positioned 2.5 cm above the floor. Solid state logic in an adjacent room, interfaced

through a Med Associates (Lafayette, IN) interface to a personal computer, controlled reinforcement and data acquisition with a locally written program.

2.3. Discrimination training and testing

A fixed ratio (FR) 50 schedule of food reinforcement (45 mg dustless pellets, Research Diets, Inc., NJ) in a two-lever paradigm was used. The drug discrimination procedure details have been described elsewhere (Marona-Lewicka and Nichols, 1994). At least one drug and one saline session separated each test session. Rats were required to maintain the 85% correct responding criterion on training days in order to be tested. In addition, test data were discarded when the accuracy criterion of 85% was not achieved on one of the two training sessions following a test session. Training sessions lasted 15 min and test sessions were run under conditions of extinction, with rats removed from the operant chamber when 50 presses were emitted on either lever. If 50 presses on one lever were not completed within 5 min the session was ended and scored as a disruption. All test drugs were administered i.p. 30 min prior to test sessions. In addition, mescaline and cocaine were tested when administered both 30 and 90 min prior to tests. For combination tests antagonists were injected 30 min before training drug administration. For a dose–response effect of training drug all groups of rats were tested when animals first passed the required criteria, and later at least once during each eight month period of use.

2.4. Drugs

The training drug LSD [(+)-lysergic acid diethylamide tartrate, NIDA] was administered at a dose of 0.08 mg/kg (186 nmol/kg), or 0.16 mg/kg (372 nmol/kg.). Dihydrexidine hydrochloride, mescaline, DET (*NN*-diethyltryptamine), psilocin, DOI [1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride], DOM [1-(2,5 dimethoxy-4-methylphenyl)-2-aminopropame hydrochloride], MDMA (3,4-methylenedioxymethamphetamine hydrochloride), BECA2C [4-(2,5-dimethoxy-4-bromophenyl)-1,2,3,4-tetrahydroisoquinoline hydrochloride], iso-LSD tartrate salt, and WAY 100635 (*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl) cyclohexanecarboxamide) were synthesized in our laboratory. Other drugs used for this study include: clonidine hydrochloride, yohimbine hydrochloride, baclofen [(*R*,*S*)-4-amino-3-(4-chlorophenyl)butanoic acid], RU 24969 [5-methoxy-3-(1,2,5,6-tetrahydro-4-pyridinyl)1*H*-indole hemisuccinate], SKF 38393 [1-phenyl-2,3,4,5-tetrahydro-(*H*)-3-benzazepine-7-8-diol hydrobromide], cyproheptadine hydrochloride, mCPP (*m*-chlorophenylpiperazine hydrochloride) WB 4100 [2-(2,6-dimethoxyphenoxyethyl)aminomethyl-1,4-benzodioxane hydrochloride] were purchased from TOCRIS (Ellisville, MO). D-amphetamine sulfate, cocaine hydrochloride, and fenfluramine hydrochloride were from Sigma (St. Louis, MO). Other drugs and sources were: lisuride hydrogen maleate (Schering, AG), haloperidol (Mylan Pharmaceuticals, Inc., Morgantown, WV), and MDL 11,939 (alpha-phenyl-2-(2-phenylethyl)-4-piperidine-methanol; a generous gift from Acadia Pharmaceuticals). All drug solutions were prepared by dissolving the compounds in sterile saline (0.9% NaCl) at a concentration that allowed the appropriate dose to be given in a volume of 1 ml/kg, identical to the volume of the saline injection. A small amount of ascorbic acid was added to the DHX hydrochloride solution to prevent oxidative degradation. A stock solution of aripiprazole (5 mg/ml) was made by dissolving aripiprazole (OPC-14597) in a minimal volume of 50% L-lactic acid, and diluting with distilled water (final pH 6.2–6.7).

2.5. Data analysis

Data from the drug discrimination study were scored in a quantal fashion, with the lever on which the rat first emitted 50 presses in a test session scored as the "selected" lever. The percentage of rats selecting the drug lever (% SDL) for each dose of test compound was determined. Full, partial, and no substitution

Table 1
Compounds that produced full or partial substitution in LSD-90-trained rats

Test drug	Dose		*N*	% D	% SDL
	mg/kg	μmol/kg			
Baclofen	0.25	1.17	8	0	25
	0.50	2.34	8	12	43
	1.00	4.68	9	11	87.5
	2.00	9.36	8	25	83
WAY 100635	1.08	2.0	13	46	29
	2.69	5.0	13	54	33
	5.39	10.0	14	38	56
	10.78	20.0	7	29	80
ABT-724	0.040	0.1	9	22	29
	0.080	0.2	9	33	33
	0.205	0.5	7	14	14
	0.410	1.0	8	25	67
OPC 14597 Aripiprazole	2	4.46	7	14	17
	4	8.92	11	18	44
	8	17.80	10	10	78
	16	35.70	10	60	75
DHX	0.25	0.83	13	23	20
	0.50	1.65	12	25	33
	1.00	3.29	12	33	38
	2.00	6.58	11	45	67
SKF 38393	1.50	5.14	9	33	33
	1.87	6.42	8	25	50
	3.75	12.85	9	33	50
	7.50	25.70	9	45	60
MDMA	0.78	4.26	8	12.5	14
	1.51	8.25	8	25	33
	3.00	16.38	8	50	50
(+)-Amphetamine	0.25	1.35	6	0	0
	0.50	2.70	6	0	0
	1.00	5.40	6	0	33
	2.00	10.80	6	33	50
Cocaine (30 min)	2.5	7.36	10	30	25
	5.0	14.71	9	33	33
	7.5	22.07	8	50	50
	10.0	29.42	8	62.5	33
Cocaine (90 min)	2.5	7.36	6	17	40
	5.0	14.71	7	28.5	40
	10.0	29.42	9	33	67

N — number of rats tested.
% D — percent of rats that disrupted behavior and were not able to emit 50 presses during a 5 min test session.
% SDL — percent of rats selecting the drug appropriate lever.

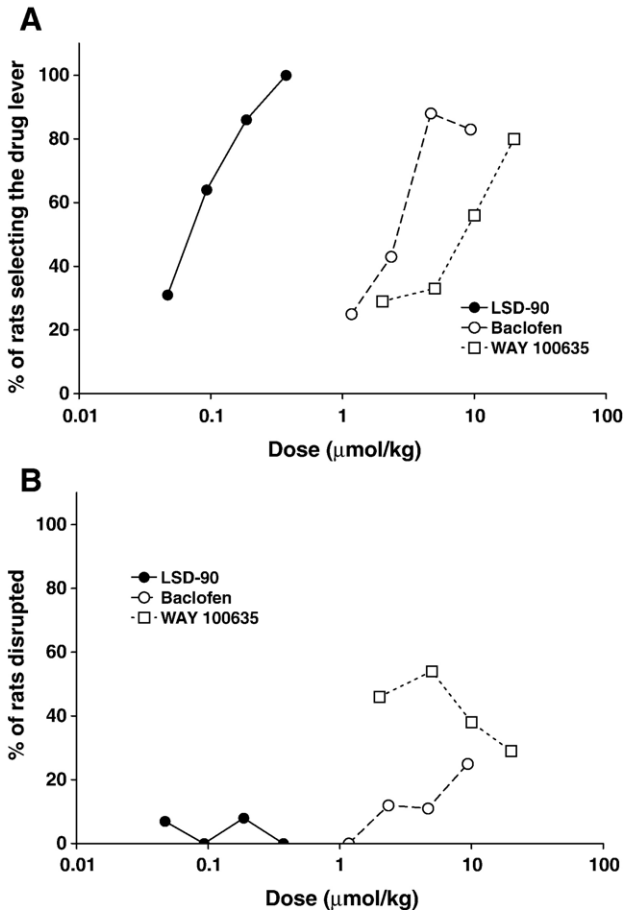

Fig 1. (A) Results from substitution tests in rats trained to discriminate LSD (filled circles) administered 90 min prior to training using the $GABA_B$ receptor antagonist, baclofen (open circles) and D_4 agonist/5-HT_{1A} antagonist, WAY 100635 (squares). (B) Percentage of rats disrupted during substitution tests in LSD-90 rats. Symbols are the same as on (A).

were statistically determined using a binomial test (Zar, 1999) as follows. When a one-sided 5%-level binomial test cannot reject the hypothesis of a 7% or lower LSD–lever response rate, the result is defined as "no substitution." When a one-sided binomial test cannot reject the hypothesis of a 95% or greater LSD–lever response rate, the result is defined as "full substitution." When both of these hypotheses are rejected, the result is defined as partial substitution. The values of 7% and 95% were determined from an assessment of the animal's accuracy during training conditions of saline and LSD, respectively, over one three-month period of time while the tests were being conducted. To illustrate the binomial test, when 12 animals are used, the partial substitution range is between 3 and 9 SDL (25–75%). When the number of animals tested is increased to 15, the partial substitution range widens slightly and the cutoffs for "no substitution" and "full substitution" are 27% and 80%, respectively. By contrast, if only eight rats are used, the partial substitution range narrows to 3–5 animals (37.5–62.5%). The use of a larger number of animals does not appreciably widen the partial substitution range because the training accuracies for saline and LSD are incorporated into the binomial test calculations. If training accuracy could be improved, then fewer animals would be needed, but these accuracies are typical for our colonies of rats.

If the drug was one that completely substituted for the training drug the method of Litchfield and Wilcoxon (1949) was used to determine the ED50 and 95% confidence interval (95% C.I.). If the percentage of rats disrupted (% D) was 80% or higher, the ED50 value for disruption was determined. The same method was used to determine the inhibition ED50 and 95% confidence interval (95% C.I.) if the maximum percentage of rats selecting

Table 2
Compounds that produced full or partial substitution in LSD-30-trained rats (for details and definitions see the Method section)

Test drug	Dose		*N*	% D	% SDL	ED50 (95% C.I.)	
	mg/kg	μmol/kg				mg/kg	μmol/kg
Mescaline	2	8.06	10	20	12.5	8.08	32.6
	3	12.11	11	0	9	(5.5–11.9)	(22–48.5)
	4	16.15	12	8	36		
	5	20.18	8	12.5	14		
	6	24.22	8	12.5	29		
	8	32.32	17	41	50		
	10	40.37	11	55	40		
	12	48.44	9	11	87.5		
Lisuride	0.0063	13.75	12	17	50	PS	PS
	0.0125	27.50	14	21	55		
	0.0250	55.00	17	12	60		
	0.0500	110.00	18	22	36		
	0.1000	220.00	14	64	40		
isoLSD	0.08	0.17	10	0	20	0.139	0.296
	0.16	0.34	11	0	55	(0.093–0.210)	(0.197–0.446)
	0.32	0.68	11	0	91		
DMT	1	3.14	8	0	37.5	3.24	10.16
	2	6.28	11	18	22	(1.5–6.98)	(4.71–21.93)
	4	12.56	13	23	40		
	8	25.12	19	37	83		
	16	50.25	15	73	75		
Fenfluramine	0.25	0.94	13	23	20	PS	PS
	0.50	1.87	9	11	37.5		
	1.00	3.74	11	36	29		
	1.25	4.68	11	36	71		
	1.375	5.14	11	36	57		
mCPP	0.2	0.86	8	0	0	PS	PS
	0.4	1.72	12	17	30		
	0.8	3.43	12	25	67		
	1.6	6.86	10	40	50		
Baclofen (BAC)	0.25	1.17	7	0	0	PS	PS
	0.50	2.34	7	14	33		
	1.00	4.68	7	28	60		
	2.00	9.36	7	54	75		
DET	0.25	1.16	8	0	25	2.53	0.55
	0.50	2.31	9	11	63.5	(1.12–5.71)	(0.24–1.23)
	1.00	4.63	8	0	63.5		
	2.00	9.26	10	10	67		
	3.00	13.89	12	8	91		
Psilocin	0.125	0.61	9	11	25	1.01	0.21
	0.250	1.23	9	11	37.5	(0.69–1.46)	(0.14–0.3)
	0.375	1.84	11	18	89		
	0.500	2.45	9	11	100		
ABT-724	0.040	0.1	13	31	44.0	PS	PS
	0.080	0.2	13	38	62.5		
	0.205	0.5	12	8	45.0		
	0.410	1.0	10	30	43.0		

If the drug was one that completely substituted for the training drug the method of Litchfield and Wilcoxon (1949) was used to determine the ED50 and 95% confidence interval (95% C.I.).

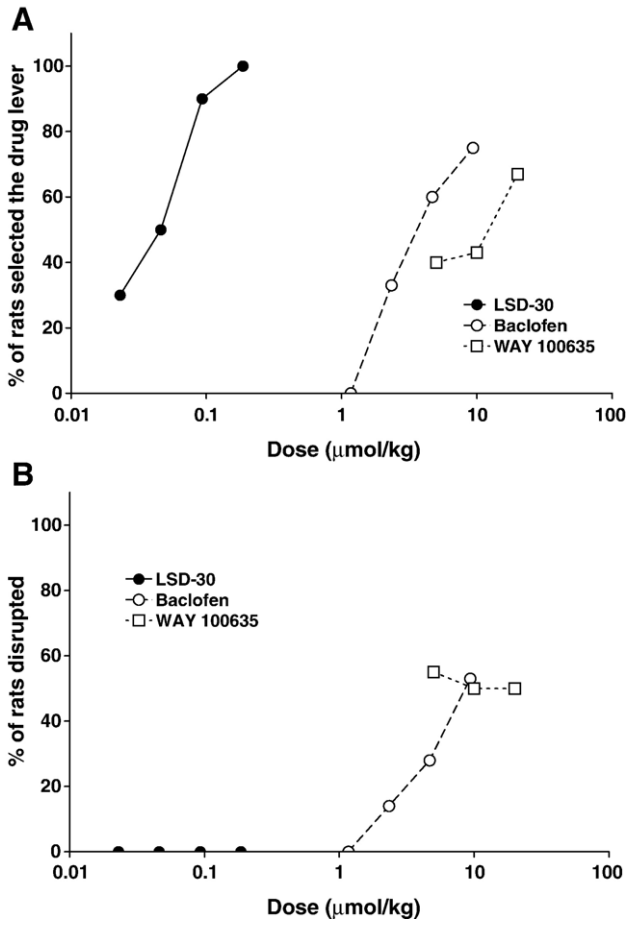

Fig. 2. (A) Results from substitution tests in rats trained to discriminate LSD (filled circles) administered 30 min prior to training using the $GABA_B$ receptor antagonist, baclofen (open circles) and D_4 agonist/5-HT_{1A} antagonist, WAY 100635 (squares). (B) Percentage of rats disrupted during substitution tests in LSD-30 rats. Symbols are the same as on (A).

the saline lever was not significantly different from the saline training condition, as determined by the binomial test, for at least one dose of antagonist used in a combination test.

3. Results

From a large number of compounds tested in rats trained to discriminate LSD-90 from saline, only the $GABA_B$ agonist baclofen, and WAY 100635 (a 5-HT_{1A} antagonist-D_4 agonist), were able to mimic fully the training drug (Table 1, Fig. 1A,B). Baclofen substituted for LSD-90 with an ED50 of 2.31 μmol/kg (0.49 mg/kg; 95% C.I. 1.15–4.63 μmol/kg; 0.24–0.99 mg/kg), calculated from the ascending part of the cue. The baclofen ED50 indicates that this drug is more than 40 times less potent than LSD. The ED50 for LSD in LSD-90-trained rats is 0.057 μmol/kg (95% C.I 0.037–0.087 μmol/kg), [0.025 mg/kg (95% C.I 0.016–0.038 mg/kg)]. WAY 100635 is about 120-fold less potent than LSD, with an ED50 of 6.69 μmol/kg (95% C.I. 2.39–18.73 μmol/kg); 3.6 mg/kg (95% C.I. 1.29–10.08 mg/kg).

In contrast, in rats trained to discriminate LSD injected 30 min before testing, baclofen and WAY 100635 (Table 2, Fig. 2A,B) produced only partial substitution (67% at 20 μmol/kg, and 60% at 5 μmol/kg, respectively), but both drugs also generated a relatively high degree of disruption: 53% for 9.36 μmol/kg of baclofen and 50–55% for all doses tested of WAY 100635 (Fig. 2B). The maximal percentage substitution of baclofen in LSD-30 rats was bordering on full substitution at 75%, at a dose of 9.36 μmol/kg, but this dose of baclofen produced more than 50% disruption, and an ED50 was therefore not calculated.

Table 1 lists all the compounds that produced partial substitution in LSD-90-trained rats. It is important to highlight the fact that all of these compounds have a "dopaminergic" profile: ABT-724 is a D_4 dopamine receptor agonist, aripiprazole (OPC 14597) is an antipsychotic drug with agonist and antagonist properties at D_2-like receptors, and dihydrexidine (DHX) and SKF 38393 are dopamine D_1 receptor agonists. Only ABT-724 produced partial substitution in LSD-30-trained rats (Table 2), although it generated a bell shaped dose–response cue with a maximum 62% substitution at the 0.2 μmol/kg dose (Table 2). Aripiprazole, DHX, and SKF 38393 produced predominantly saline appropriate responding in LSD-30-trained rats (Table 3).

In LSD-90 rats, racemic MDMA ("ecstasy"), an entactogen with 5-HT, dopamine, and norepinephrine releasing/reuptake blocking properties produced 50% drug lever selection with 50% disruption (Table 1); in LSD-30 rats it induced only 40% substitution with the same degree of disruption at the same dose tested (Table 3). (+)-Amphetamine, a dopamine, norepinephrine, and 5-HT releasing agent, and cocaine, which blocks all three neurotransmitter reuptake proteins, produced the same degree of substitution as MDMA, with a similar degree of disruption (Table 1). We recorded an increase in drug appropriate lever selection, however, when cocaine was injected 90 min before the test (Table 1). Neither of these compounds produced significant drug lever selection in LSD-30-trained rats.

Table 2 presents data from substitution tests in LSD-30 rats for all compounds that fully or partially mimicked LSD during

Table 3
Compounds that produced predominantly saline appropriate lever selection in LSD-30-trained rats

Test drug	Dose		*N*	% D	% SDL
	mg/kg	μmol/kg			
DHX	0.25	0.83	12	0	8
	0.50	1.65	10	20	25
	1.00	3.29	9	33	33
	2.00	6.58	10	50	40
SKF 38393	1.50	5.14	10	0	0
	1.87	6.42	11	0	0
	3.75	12.85	9	33	0
	7.50	25.70	8	50	25
WAY 100635	2.70	5.0	11	55	40
	5.39	10.0	14	50	43
	10.78	20.0	12	50	67
MDMA	0.75	4.10	8	12.5	14
	1.50	8.19	8	37.5	20
	3.00	16.38	10	50	40
OPC 14597 Aripiprazole	2	4.46	11	36	14
	4	8.92	11	36	0
	8	17.80	11	64	25

Abbreviations are the same as in Table 1.

Table 4
Compounds that produced predominantly saline lever selection in LSD-90-trained rats

Test drug	Dose		N	% D	% SDL
	mg/kg	μmol/kg			
Clonidine	0.1	0.38	11	27	0
	0.2	0.75	11	72	0
	0.4	1.50	11	91	0
	0.6	2.25	10	90	0
Yohimbine	2.0	5.01	10	20	0
	4.0	10.02	10	30	0
	8.0	20.02	10	70	0
Lisuride	0.0125	0.028	4	75	0
	0.02500	0.055	9	44	20
	0.0500	0.110	9	22	28
	0.1000	0.220	8	63	67
Mescaline (30 min)	2	8.06	10	20	12.5
	3	12.11	10	20	12.5
	4	16.15	11	27	25
	5	20.18	12	25	22
	6	24.27	10	33	28.5
	8	32.32	10	33	28.5
	10	40.37	10	40	33
	12	48.44	9	44	20
Mescaline (90 min)	4	16.15	9	44	20
	8	32.32	9	56	25
	12	48.44	9	56	0
DOM	0.25	1.01	6	0	17
	0.50	2.02	6	33	25
	1.00	4.04	6	50	33
Fenfluramine	0.25	0.94	7	29	20
	0.50	1.87	8	50	50
	1.00	3.74	10	60	50
mCPP	0.2	0.86	10	20	13
	0.4	1.72	12	12	30
	0.8	3.43	12	12	30
	1.6	6.86	11	27	25
RU 24969	0.5	1.74	10	20	0
	1.0	3.48	12	17	10
	2.0	6.96	16	37.5	0
	4.0	13.92	11	55	0
isoLSD	0.08	0.17	13	25	44
	0.16	0.34	8	0	38
	0.32	0.68	10	50	40
DET	0.25	1.16	6	33	25
	0.50	2.31	6	33	25
	1.00	4.63	6	67	50
	2.00	9.26	6	83	0
	3.00	13.89	7	100	0
Psilocin	0.125	0.61	6	50	33
	0.250	1.23	8	50	25
	0.375	1.84	8	75	0
	0.500	2.45	5	80	0

Abbreviations are the same as in Table 1.

the first temporal phase of the LSD effect. The hallucinogens mescaline, DMT, and psilocin fully mimicked LSD (Table 2), whereas all of these drugs induced predominantly saline appropriate responding in LSD-90 rats (Table 4). Even with a pre-injection time of 90 min, mescaline produced only saline appropriate lever pressing in LSD-90 rats, although at this time point mescaline generated much more disruption (44–56%) than when tested 30 min after injection (Table 4).

Surprisingly, lisuride (a semisynthetic ergot derivative), used in the past by several laboratories as a dopamine agonist and antiparkinsonian drug, did not produce significant substitution in LSD-90 rats (Table 4), but partial substitution occurred in LSD-30 rats (Table 2), with 60% of the maximum substitution and a bell shaped dose–response curve. The 8-epimer of LSD, isoLSD, fully mimicked LSD-30 (Table 2), although with a potency about seven times lower than LSD itself (LSD ED50 0.044 μmol/kg *vs* 0.296 μmol/kg for isoLSD). By contrast, isoLSD produced mostly saline appropriate responding in LSD-90-trained rats (Table 4).

Fenfluramine, an appetite suppressant and 5-HT releasing agent that partially mimicked LSD-30 (Table 2; see also Fiorella et al., 1995), with a bell shaped dose–response curve, also failed to mimic LSD-90 (Table 4). mCPP, a 5-HT_{2C} receptor agonist with 5-HT releasing properties, generated its highest degree of substitution (67%) at 3.43 μmol/kg in LSD-30 rats (Table 2), but produced a maximum of only 30% substitution at the same dose in LSD-90 rats (Table 4).

RU 24969, a 5-$HT_{1B/1D}$ agonist, produced only saline appropriate lever selection in LSD-90 rats (Table 4), whereas it elicited 50% drug lever responding in LSD-30 rats (Cunningham and Appel, 1987). We previously reported (Marona-Lewicka and Nichols, 1995) that the α_2-adrenoceptor antagonist, yohimbine, partially mimicked LSD in LSD-30 rats, although, earlier Colpaert (1984) reported full substitution for yohimbine in LSD-trained rats. In LSD-90 rats, only saline appropriate lever

Table 5
Results from combination tests in LSD-90-trained rats

Test drug	Dose of combined drug		N	% D	% SDL
	mg/kg	μmol/kg			
OPC 14597 Aripiprazole	0.5	1.12	11	18	100
	1.0	2.23	11	18	67
	2.0	4.46	11	9	70
	4.0	8.92	11	18	67
	8.0	17.80	10	50	60
Cyproheptadine	0.5	1.74	11	9	90
	1.0	3.48	13	23	90
	2.0	6.97	12	8	82
	4.0	13.94	13	15	73
WB 41001	0.5	1.31	6	33	75
	1.0	2.62	6	33	75
	2.0	5.24	6	67	100
Yohimbine	1.0	2.51	18	11	100
	2.0	5.01	16	31	91
	4.0	10.02	18	44	90
	8.0	20.04	18	50	100
WAY 100635	0.4	0.74	12	17	100
	0.8	1.48	11	9	90
	1.6	2.96	10	10	100
Propranolol	5	16.9	9	0	100
	10	33.8	9	0	89
	20	67.6	15	33	70
MDL 11,939	0.25	1.02	10	0	90
	0.50	2.04	10	20	88
	1.00	4.07	10	20	100
	2.00	8.15	10	10	67

All compounds were injected 30 min before administration of LSD (0.372 μmol/kg, with the exception of the combination with WAY 100635, where a dose of 0.186 μmol/kg was used), with testing 90 min later.
Abbreviations are the same as in Table 1.

selections occurred for yohimbine and the α_2-adrenoceptor agonist clonidine (Table 4). Both of these α_2 adrenergic agents strongly suppressed response rate in LSD-90 rats.

Combination tests (Tables 5 and 6) with the highly selective 5-HT_{2A} antagonist, MDL 11,393 confirmed that a 5-HT_{2A} antagonist effectively blocks only the LSD-30, and not the LSD-90 cue. Even the nonselective 5-HT antagonist, cyproheptadine, which significantly inhibited the LSD-30 effect (Table 6), had no effect against the LSD-90 cue (Table 5). Thus, we provide further evidence that activation of the 5-HT_{2A} receptor is not essential for the delayed effect of LSD treatment.

4. Discussion

The data reported in Tables 1–4 are generally self-explanatory, and require little elaboration. In essence, we have shown that a variety of standard ligands with diverse pharmacological properties fail to substitute in LSD-90 rats, even though some of them produced positive or "false" positive effects in LSD-30 rats. We confirmed that LSD generates time-dependent cues in the drug discrimination assay and that these cues are mediated by different mechanisms. Compounds producing no substitution or weak partial substitution require no further explanation in this context. In addition, drugs with significant activity in several serotonergic systems (5-HT_{1A} or 5-$HT_{1B/1D}$ agonists, or fenfluramine, a 5-HT releaser/reuptake blocker) that produced partial substitution in LSD-30 rats induced only saline appropriate responses in LSD-90 rats, accompanied with a decreased response rate.

We reported earlier that full substitution in LSD-90-trained animals by the D_2-like agonists apomorphine, quinelorane, and *N*-propyldihydrexidine was contrasted with their failure to substitute in animals trained to discriminate LSD-30. Further, we demonstrated that the LSD-30 cue could be blocked by standard 5-HT_{2A} receptor antagonists, whereas the LSD-90 cue was blocked by dopamine D_2-like antagonists (Marona-Lewicka et al., 2005). The results reported here, where we tested a variety of different types of pharmacological ligands, extend and strengthen those earlier findings.

An important result in the present study is that elevated extracellular levels of dopamine do not reproduce the mechanism responsible for the LSD-90 cue. Three compounds that raise extracellular dopamine levels, MDMA, amphetamine, and cocaine, produced only partial substitution in LSD-90-trained rats, with a fairly high degree of disruption. Dopamine D_1 receptor agonists produced partial substitution in LSD-90 rats, but predominantly saline lever responding in LSD-30 rats.

WAY 100635, a purported "selective 5-HT_{1A} antagonist," surprisingly produced full substitution in LSD-90 rats. Although LSD has high affinity for both the 5-HT_{1A} and 5-HT_{2A} receptors, drug discrimination studies have produced asymmetric substitution effects. That is, LSD partially substituted for the 5-HT_{1A} full agonist 8-OH-DPAT [8-hydroxy-2-(di-*n*-propylamino)-tetralin] in animals trained to discriminate 8-OH-DPAT from saline (Arnt, 1989). Nevertheless, 8-OH-DPAT did not substitute for LSD in rats trained to discriminate LSD from saline. Furthermore, Appel et al., (2004) and Reissig et al. (2005) have reported that WAY 100635 did not block the discriminative stimulus of LSD in rats trained to discriminate LSD from saline. These results suggest that the 5-HT_{1A} receptor does not play a significant role in mediating the cue properties of LSD in rats. Thus, the substitution of WAY 100635 is probably not due to its affinity for the 5-HT_{1A} receptor, but rather we believe can be explained by our recent finding that it is a potent dopamine D_4 agonist (Chemel et al., 2006a,b). Indeed, preliminary studies in our laboratory have shown that rats can be trained to discriminate WAY 100635, and that the discriminative cue is mediated by dopamine D_4 receptor activation (Chemel et al., 2006b). This conclusion is consonant with our prior studies showing that the LSD-90 cue is mediated by a dopamine D_2-like mechanism.

More perplexing, however, is our finding that the GABA-B agonist baclofen fully substituted in LSD-90 rats, although a dopamine mechanism may still be implicated. GABA-B receptors are localized on dopaminergic neurons and their stimulation by baclofen can attenuate dopaminergic activity (Westerink et al., 1996; Xi and Stein, 1999). This mechanism has been proposed as a likely explanation for the ability of baclofen to attenuate cocaine self-administration by rats under several different conditions (Roberts et al., 1996; Roberts and Andrews, 1997; Shoaib et al., 1998; Campbell et al., 1999; Brebner et al., 1999). Baclofen did not, however, attenuate the discriminative stimulus effects of cocaine or methamphetamine (Munzar et al., 2000).

Baclofen induced a significant leftward displacement of the dose–response curve for apomorphine-induced stereotypy in rats (Sandoval and Palermo-Neto, 1995), and by itself induced stereotyped behavior (Steiniger and Kretschmer, 2003).

Table 6
Results from combination tests in LSD-30-trained rats

Test drug	Dose of combined drug		*N*	% D	% SDL
	mg/kg	μmol⁄kg			
OPC 14597 (Aripiprazole)	0.5	1.12	9	33	83
	1.0	2.23	9	33	83
	2.0	4.46	14	50	71
	4.0	8.92	8	50	50
	8.0	17.80	10	60	50
Cyproheptadine	0.5	1.74	10	0	80
	1.0	3.48	12	17	60
	2.0	6.97	10	10	44
	4.0	13.94	10	20	37
WB 41001	0.5	1.31	8	0	100
	1.0	2.62	8	0	87.5
	2.0	5.24	8	0	80
WAY 100635	0.4	0.74	20	10	44
	0.8	1.48	10	0	50
	1.6	2.96	11	18	78
Propranolol	5.0	16.9	9	22	100
	10.0	33.8	8	50	100
	20.0	67.6	14	43	87.5
MDL 11,939	0.25	0.85	11	0	72
	0.50	1.69	11	9	60
	1.00	3.38	11	18	44
	2.00	6.77	11	36	14

All compounds were injected 30 min before administration of LSD (0.186 μmol/kg) and tested 30 min later.

GABAergic manipulation facilitates the progressive activation of the different dopaminergic pathways involved in stereotypic behaviors, thus increasing those components that appear at a high level of dopaminergic pathway activation. Moreover, although baclofen minimally affected glutamate levels in the medial prefrontal cortex, nucleus accumbens, or ventral tegmental area in normal animals, it dose-dependently increased glutamate levels in each of these regions in animals sensitized to cocaine or amphetamine (Jayaram and Steketee, 2004). Taken together, we suggest that the chronic treatment of rats with LSD appears to produce a persisting behavioral state that is characterized by sensitivity to dopamine agonists (unpublished results), and bears many of the behavioral features of amphethamine sensitivity. This speculation obviously requires further elucidation, but would be generally consistent with our results.

In conclusion, we have expanded our characterization of the LSD-90 cue, and provided additional evidence that the cue is mediated by a dopaminergic process. The full substitution of the mixed 5-HT_{1A} antagonist/D_4 agonist WAY 100635 suggests that the cue may be mediated, at least in part, by dopamine D_4 receptor activation, a finding that is currently the subject of further attention in our laboratory.

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