Psychopharmacology (2005) 180: 427–435
DOI 10.1007/s00213-005-2183-9

ORIGINAL INVESTIGATION

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Distinct temporal phases in the behavioral pharmacology of LSD: dopamine D_2 receptor-mediated effects in the rat and implications for psychosis

Received: 13 July 2004 / Accepted: 6 January 2005 / Published online: 19 February 2005

Abstract *Rationale:* The effect of LSD in humans has been described as occurring in two temporal phases. The behavioral effects in rats also occur in two temporal phases: an initial suppression of exploration followed by increased locomotor activity. *Objectives:* We decided to investigate this phenomenon from the perspective that the pharmacology might have relevance to the neurochemical mechanisms underlying psychosis. *Methods:* Twenty-five male Sprague–Dawley rats were trained to discriminate LSD (186 nmol/kg, 0.08 mg/kg, i.p.) with a 30-min preinjection time (LSD-30, *N*=12) and LSD (372 nmol/kg, 0.16 mg/kg, i.p.) with a 90-min preinjection time (LSD-90, *N*=13) from saline, using a two-lever, food-reinforced operant conditioning task. *Results:* LSD (186 or 372 nmol/kg, 0.08 or 0.16 mg/kg) given 30 min prior to training produced a cue that was completely antagonized by 5-HT_{2A} antagonists and lasted no longer than 1 h. LSD (372 nmol/kg, 0.16 mg/kg) injected 90 min before training produced a cue that was not fully blocked by 5-HT_{2A} antagonists, but instead was significantly inhibited by haloperidol. In these rats, substitution no longer occurred with the 5-HT_2 agonists DOI or LSD (30 min preinjection), but full substitution was obtained with the D_2 agonists apomorphine, *N*-propyldihydrexidine, and quinelorane. *Conclusion:* The discriminative stimulus effect of LSD in rats occurs in two phases, and these studies provide evidence that the later temporal phase is mediated by D_2 dopamine receptor stimulation. A second temporal phase that involves dopaminergic pathways would be consistent with the widespread belief that excessive dopaminergic activity may be an underlying cause of paranoid psychosis.

Keywords LSD · Drug discrimination · 5-HT_{2A} · Serotonin · Dopamine D_2 · Schizophrenia · Rat

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Introduction

LSD is the most potent known hallucinogenic substance, with high affinity for a number of brain receptors (see Nichols 2004 for a review). Although virtually all research on hallucinogens, both clinical and preclinical, has focused on a unitary pharmacology for these substances, Freedman (1984) has described the effects of LSD in humans as occurring in two temporal phases: a "psychedelic experience" in the early phase, with "meaningfulness and portentousness" as the prime characteristics, and a second phase that is "clearly a paranoid state." This latter phase develops about 4–6 h after LSD administration, and at times out to 10 h postdrug, subjects given LSD "...regularly report... they had been at the least self-centered, and usually suspicious, with ideas of reference or even paranoid convictions" (Freedman 1984). Freedman noted on several occasions that this effect continued to be unnoticed and unstudied in research with hallucinogens. Further, he drew parallels between this later paranoid phase and amphetamine psychosis in man and emphasized the possibility that clues to understanding psychosis might be found in this "paranoid" phase of LSD intoxication.

We decided to investigate this phenomenon from the perspective that the pharmacology might have relevance to the neurochemical mechanisms underlying psychosis. Furthermore, we reasoned that Freedman's observation might have relevance to the use of 5-HT_{2A} antagonists in the treatment of some forms of psychosis because LSD and other hallucinogens are thought to act principally as 5-HT_{2A} agonists (Nichols 2004). It also seemed possible that the incidence of "bad trips" with LSD could be related to

the nature of the underlying pharmacology in the later temporal phase of LSD intoxication.

In contrast to this observation about LSD, there is no indication in the literature of a parallel in the psychopharmacology of hallucinogenic phenethylamines or simple tryptamines. That is, reports of intoxication with these other agents appear to describe a unitary psychopharmacology with typical dose–response curves (Shulgin and Shulgin 1991, 1997). We therefore speculated that the two temporal intoxication "phases" of LSD were not related simply to 5-HT_2 agonist activity. These observations led us to hypothesize that a "two-phase" temporal action was unique to LSD and was related to some pharmacological property of LSD that distinguished it from other chemical classes of hallucinogens.

A significant pharmacological difference between LSD and other types of hallucinogens is the direct dopaminergic effect of LSD, which is not a component of the pharmacology of phenethylamine- and tryptamine-type hallucinogens. Dopaminergic effects of LSD have been recognized for many years (see Watts et al. 1995 and references therein), but the relevance of these actions to the psychopharmacology of LSD has never been fully appreciated. In view of the recognized role of dopamine in psychosis (Seeman 1987; Bennett 1998; Carlsson 2001), we felt that a closer focus in this area might be fruitful. Early drug discrimination studies did note that LSD substituted partially (70–80%) for the nonselective dopamine agonist apomorphine, and that apomorphine was able to generate 50% substitution in LSD-trained rats (Holohean et al. 1982). These authors concluded that although serotonin plays a prominent role in the discriminative stimulus effects of LSD, dopamine receptor activation is a secondary factor in the LSD cue. Holohean et al. (1982) further suggested that the dopaminergic effect of LSD might become evident in drug discrimination only when rats were trained to "attend" to dopamine receptor activation. There has never been any evidence from drug discrimination experiments, however, that the nature of the LSD discriminative cue might be time-dependent.

Evidence from animal studies of time-dependent behavioral pharmacology for LSD comes from the studies of Mittman and Geyer (1991), who showed that LSD-induced behavioral effects in rats occurred in two temporal phases: an initial suppression of behavioral responding, followed by a subsequent increase in locomotor activity that was not observed with other serotonergic agonists. Using pretreatment with ritanserin and/or propanolol, these workers concluded that the two temporal phases of LSD effects in rats were mediated by different serotonergic or β-adrenergic receptors. Due to the limited number of pretreatments that were employed in that study, and the possibility of dopamine–serotonin functional interactions, it is not possible to deduce clearly a mechanistic basis for the effects of LSD observed in that study. Nevertheless, the observed increase in the later phase of locomotor activity that was specific to LSD would seem to suggest a catecholamine-based effect.

We have previously shown that preadministration (but not coadministration) of the 5-HT_2 agonists DOI (3 h) or LSD (2 h) prior to amphetamine potentiated the amphetamine interoceptive cue in a drug discrimination paradigm in rats by shifting the amphetamine dose–response curve to the left (Marona-Lewicka and Nichols 1997). Those results suggested that the enhanced behavioral response to amphetamine might be due either to an increased sensitivity of dopaminergic neurons or to an enhanced release of dopamine by amphetamine (Marona-Lewicka and Nichols 1997).

Nevertheless, the acute (15–30 min prior to testing) discriminative cue of LSD is clearly mediated by activation of the 5-HT_{2A} receptor (Colpaert et al. 1982; Colpaert and Janssen 1983; Glennon et al. 1983, 1984; Glennon 1999; Ismaiel et al. 1993; Leysen et al. 1982; Schreiber et al. 1994; Winter 1994; Winter and Rabin 1988; Winter et al. 1999). Furthermore, time–response curves in rats given LSD 30 min prior to training showed a maximum of the 5-HT_{2A}-mediated discriminative cue at about 30 min, with a loss of the cue by about 1 h. We reasoned, therefore, that if rats discriminated an LSD cue at longer pretreatment times, it would likely be mediated by other pharmacological processes. In particular, if LSD sensitized the dopamine system, as we had shown in our amphetamine studies (Marona-Lewicka and Nichols 1997), rats might also be more sensitive to the dopaminergic effects of LSD at later time periods.

In the present study, a drug discrimination procedure in rats was used to evaluate the discriminative stimulus effect of LSD administered 90 min prior to testing, at a time when the 5-HT_{2A} receptor-mediated cue was no longer significant. These rats were compared with rats trained using our standard protocol where LSD is administered 30 min prior to training.

Material and methods

Animals

Twenty-five 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, N=12) and LSD (372 nmol/kg, 0.16 mg/kg, i.p.) with a 90-min preinjection time (LSD-90, N=13) from saline, using a two-lever, food-reinforced operant conditioning task. 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 ~80% of free-feeding weight. Lights were on from 0700 to 1900 h. The laboratory and animal facility temperature was 22–24°C, and the relative humidity was 40–50%. Experiments were performed between 0900 and 1700 h each day, Monday–Friday. Animals used in these studies were maintained in accordance with the US 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.

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 fans for ventilation and background white noise. A white house light was centered near the top of the front panel of the cage, which was also equipped with two response levers, separated by a food hopper (combination dipper pellet trough, model E14-06, module size one half), 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.

Discrimination training and testing

A fixed ratio (FR) 50 schedule of food reinforcement (45-mg dustless pellets, Research Diets, Inc., New Jersey) 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 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. For the time-dependency test, the training dose of either 186 or 372 nmol/kg (0.08 or 0.16 mg/kg) LSD was administered to LSD-30-trained rats 5, 10, 15, 20, 30, 45, 60, 90, and 120 min prior to the test session.

For the time-dependency test in LSD-90 trained rats, the dose of 372 nmol/kg (0.16 mg/kg) of LSD was injected 15, 30, 60, 90, 120, 180, and 240 min prior to the test session. Test drugs were administered i.p. 30 min prior to test sessions except for DOI, which was given 75 min prior to tests. For combination tests, antagonists were injected 30 min before training drug administration.

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). LY 163502 (quinelorane dihydrochloride dihydrate) was a generous gift from Eli Lilly & Co. (Indianapolis, IN). *N*-Propyldihydrexidine (Brewster et al. 1990) and 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride (DOI) were synthesized in our laboratory. Other drugs used for this study include apomorphine hydrochloride (Sigma, St. Louis, MO), haloperidol (Mylan Pharmaceuticals, Inc., Morgantown, WV), and MDL 100,907 (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 apomorphine hydrochloride solution to prevent oxidative degradation. A stock solution of haloperidol (0.5 mg/ml) was made by dissolving haloperidol in a minimal volume of 50% L-lactic acid and diluting with distilled water (final pH 6.2–6.7).

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 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 assesment of the animals' accuracy during training conditions of saline and LSD, respectively, over a 3-month period of time during which the tests were conducted. To illustrate the binomial test, when 12 animals are used, the partial substitution range is between three and nine 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 three to five 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 could be used, 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 ED_{50} and 95% confidence interval (95% CI). If the percentage of rats disrupted (%D) was 80% or higher, the ED_{50} value for disruption was determined. The same method was used to determine the inhibition ED_{50} and 95% confidence interval (95% CI) if the maximum percentage of rats selecting 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. In addition to both LSD-trained groups, data were taken from three additional groups of rats routinely used in our laboratory for comparison of response rates (number of presses per minute). These groups were trained to discriminate the following drugs: MMAI (8 μmol/kg, 1.71 mg/kg), (+)-amphetamine (5.4 μmol/kg, 1 mg/kg), and DOI (1.12 μmol/kg, 0.4 mg/kg). For these comparisons, a mean and SEM were calculated for each group from 22 consecutive drug or saline training sessions, for *N*=8–13 rats per group.

Results

At the beginning of our study, we attempted without success to train animals to discriminate saline from LSD injected at longer times (60 or 90 min) using the same dose as for the LSD-30 group. LSD at the dose of 186 nmol/kg (0.08 mg/kg) injected 90 min before testing did not generate a cue that was robust enough to serve as a training stimulus. Thus, we decided to increase the LSD training dose to 372 nmol/kg (0.16 mg/kg), commonly used in drug discrimination procedures in other laboratories (Arnt 1986; Colpaert 1984; Meert et al. 1989; Doat et al. 2003). All 13 rats successfully acquired the LSD (372 nmol/kg, 0.16 mg/kg) vs saline discrimination when LSD was injected 90 min before a training session. The mean number of sessions to criterion (85% correct responding for eight of ten consecutive sessions) was 46 (range 34–58). This number was somewhat higher than the mean number of sessions to criterion obtained for rats trained to discriminate LSD administered 30 min before training sessions from saline (30; range 15–45 sessions), although the difference was not significant. The mean response rate (±SEM) in LSD-90-trained rats during the 15-min training session was similar for both drug and saline treatment (199±20 vs 210±21 presses/min, respectively). Figure 1 shows the comparison of mean response rates during the 15-min training session for different groups of rats trained to discriminate LSD-30, LSD-90, DOI, MMAI, and (+)-amphetamine from saline. Rats trained to discriminate LSD administered 90 min before training emit more than twice as many presses per minute as any of the other drug groups ($P<0.01$, Student's *t*-test for LSD-90 vs each group comparison).

The time course for both the LSD-90 and the LSD-30 cue is illustrated in Fig. 2. In rats trained to discriminate LSD (186 nmol/kg, 0.08 mg/kg) from saline given 30 min prior to training, more than 80% of the rats selected the drug-appropriate lever in the period between 10 and 60 min. A

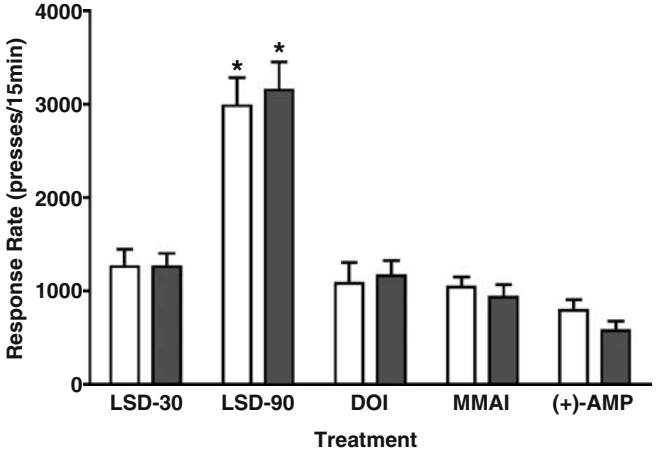

Fig. 1 Comparison of the mean response rates for groups (*N*=10–14 per group) trained to discriminate various drugs from saline, calculated from 22 consecutive sessions for each treatment. *Open bars* represent the number of presses after saline pretreatment during 15-min training sessions; *filled bars* show the number of presses after pretreatment with the training drug taken from 15-min training sessions. Training drugs were as follows: LSD-30 (186 nmol/kg, 0.08 mg/kg), LSD-90 (372 nmol/kg, 0.16 mg/kg), MMAI (8 μmol/kg, 1.71 mg/kg), DOI (1.12 μmol/kg, 0.4 mg/kg), and (+)-amphetamine (5.4 μmol/kg, 1 mg/kg) (see details in Materials and methods)

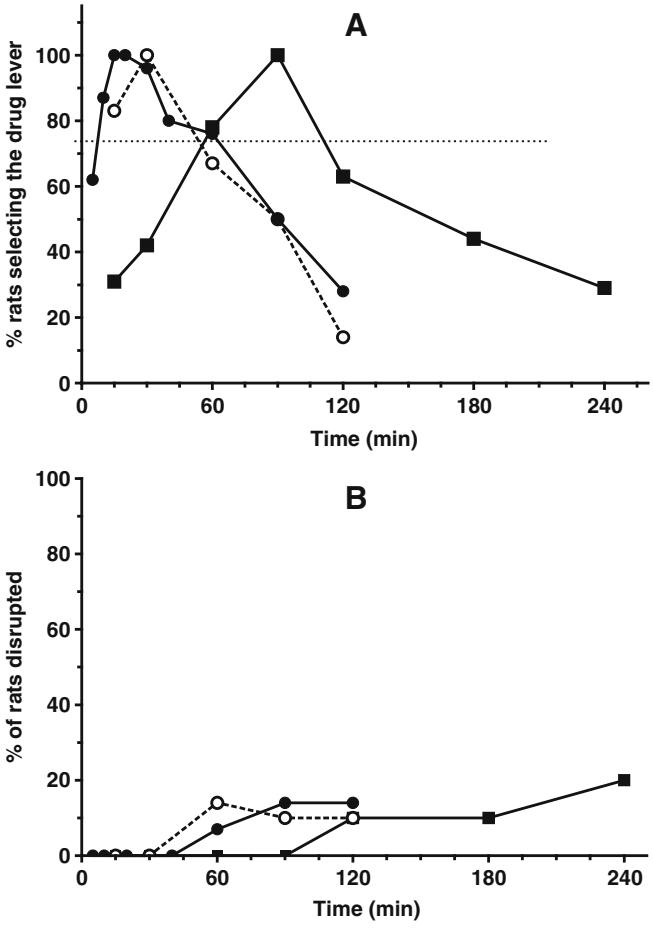

Fig. 2 **A** Time courses of the discriminative cues of LSD tested in rats trained to discriminate LSD administered either 30 or 90 min prior to training. *Filled circles* represent LSD at the dose of 186 nmol/kg (0.08 mg/kg), and *open circles* show the effect of the 372-nmol/kg (0.16 mg/kg) dose in LSD-30 rats. *Filled squares* represent LSD (372 nmol/kg, 0.16 mg/kg) tested at different preinjection times in LSD-90 rats. *N*=12–13 rats per group. **B** Percentage of rats disrupted during time-course tests. *Symbols* are the same as in **A**. *N*=12–13 rats per group

rapid onset of the cue in the LSD-30 group was evident, as 11 of 12 rats tested selected the drug lever only 10 min after injection. Doubling the dose of LSD to 372 nmol/kg (0.16 mg/kg) did not change the time course in LSD-30-trained rats.

LSD given 90 min before training sessions generated a cue that became fully recognized by rats (more than 75% of rats selected the drug-appropriate lever) no earlier than 60 min after LSD administration and lasting up to 100 min postinjection. Although there is a small period of overlap in the LSD-30 and LSD-90 cues, the nature of the cues is fundamentally different. Figures 3 and 4 show the results of inhibition tests of the LSD-30 (Fig. 3) and LSD-90 cues (Fig. 4) with the selective 5-HT_{2A} antagonist MDL 100,907 and the D_2/D_1 dopamine receptor antagonist haloperidol. MDL 100,907 was able to fully block drug-appropriate responding when administered 30 min before LSD in LSD-30 rats; however, it produced only partial inhibition of the LSD-90 cue (36% inhibition). In contrast, haloperidol was ineffective in LSD-30 rats up to a dose of 485 nmol/kg (0.2 mg/kg), but in LSD-90 rats, the inhibitory effect of haloperidol was quite evident, producing 78% inhibition of the LSD-90 cue. A 970-nmol/kg (0.4 mg/kg) dose of haloperidol induced sedative and cataleptic effects that produced disruption of behavior in all animals tested in both LSD-30- and LSD-90-trained rats (Figs. 3b and 4b). Thus, the highest effective dose of haloperidol for inhibition of the discriminative stimulus effects of LSD was 485 nmol/kg (0.2 mg/kg).

The results of substitution tests of 5-HT_2 agonists in LSD-90 rats are presented in Fig. 5. LSD produced full substitution (more than 75% of rats selected the drug-appropriate lever) only when administered 90 min before the test, with an ED_{50} of 82.5 nmol/kg (43–160 nmol/kg, 95% CI) [0.035 (0.018–0.069) mg/kg]. LSD administered 30 min before testing produced only partial substitution in LSD-90 rats. Although 57% of the rats selected the drug lever in this test at the 372-nmol/kg (0.16 mg/kg) dose, our statistical criterion classifies the response as partial substi-

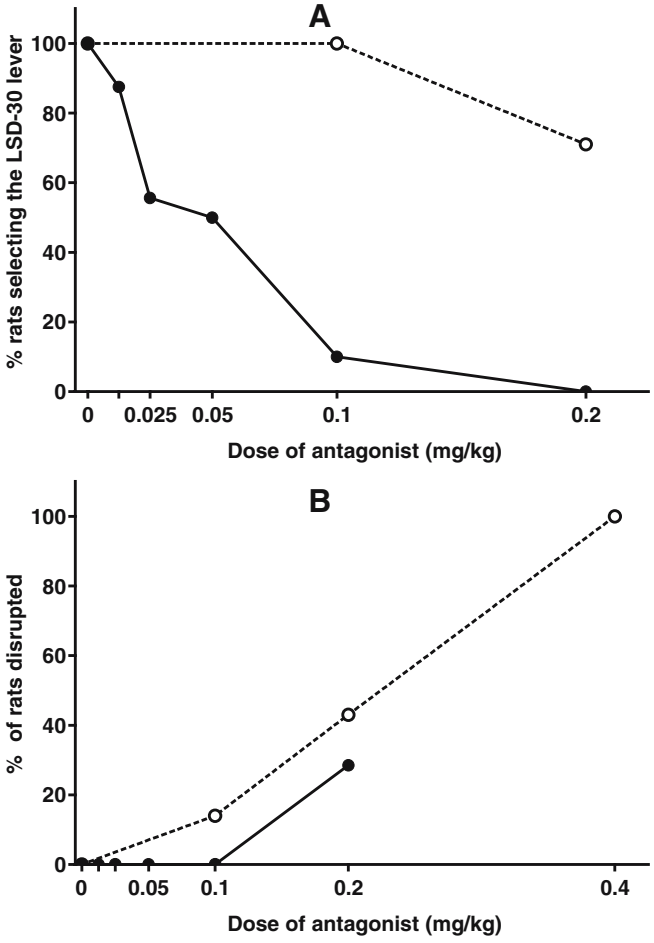

Fig. 3 **A** Results from combination tests in rats trained to discriminate LSD administered 30 min prior to training using the serotonin 5-HT_{2A} antagonist MDL 100,907 (*filled circles*) and the D_2 antagonist haloperidol (*open circles*). The doses of antagonists were administered 30 min before the training drug (60 min before the test). **B** Percentage of rats disrupted during combination test in LSD-30 rats. *Symbols* are the same as in **A**

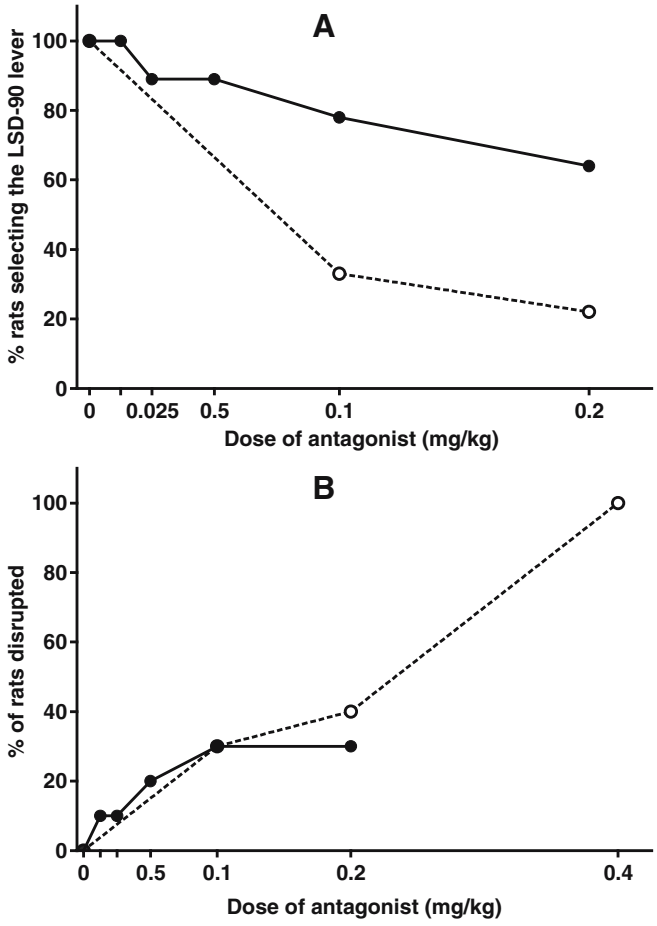

Fig. 4 **A** Results from combination tests in rats trained to discriminate LSD administered 90 min prior to training using the serotonin 5-HT_{2A} antagonist MDL 100,907 (*filled circles*) and the D_2 antagonist haloperidol (*open circles*). The doses of antagonists were administered 30 min before the training drug (120 min before the test). **B** Percentage of rats disrupted during combination tests in LSD-90 rats. *Symbols* are the same as in **A**

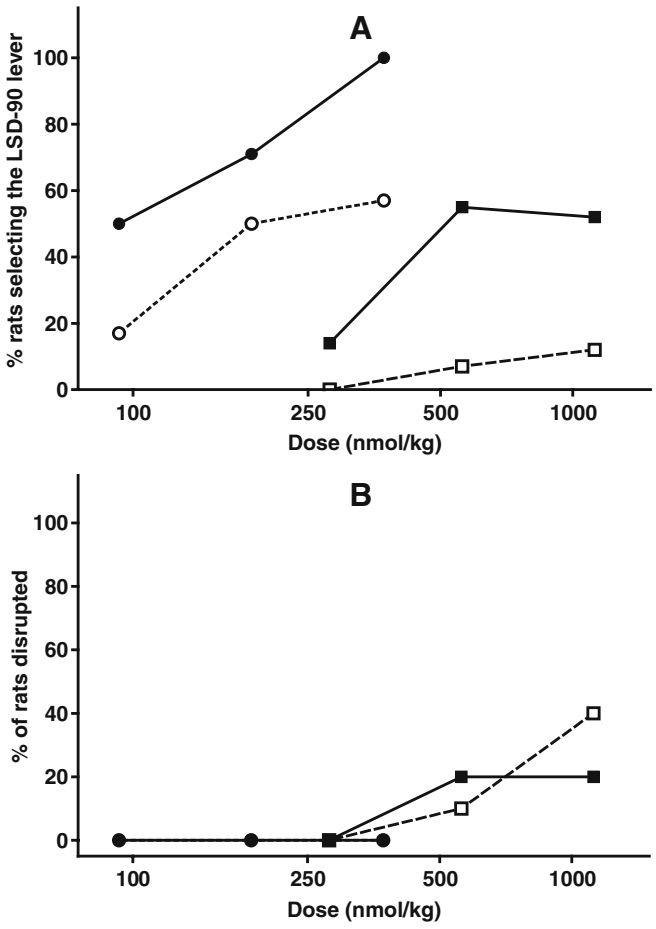

Fig. 5 **A** Dose–response curves for substitution tests of the 5-HT_2 agonists LSD and DOI in rats trained to discriminate LSD administered 90 min prior to training. LSD was injected 30 (*open circles*) or 90 min (*filled circles*), and DOI was injected 30 (*filled squares*) or 75 min (*open squares*) prior to testing. **B** Percentage of rats disrupted during substitution tests in LSD-90 rats. *Symbols* are the same as in **A**

tution (see Materials and methods for details). DOI, which is a more selective 5-HT_2 agonist than LSD, produced a maximum of 55% substitution in LSD-90 rats when injected 30 min before testing, but only 12% substitution when administered 75 min before testing (Fig. 5a). DOI injected at the longer time before testing had a tendency to produce more disruption of behavior in working animals than did DOI administered 30 min before testing (Fig. 5b).

The data obtained from substitution experiments with D_2 agonists in LSD-90 rats are shown in Fig. 6. All compounds tested generated greater than 75% drug-appropriate responding (full substitution, $P<0.05$), with potencies that paralleled their selectivity and activity at D_2 dopamine receptors. The most potent dopamine agonist tested was quinelorane, with an ED_{50} (95% CI) of 0.93 nmol/kg (0.2–3.6 nmol/kg) [0.00033 (0.000071–0.0013) mg/kg]. In addition, quinelorane produced a dose-dependent sedative effect that was evident as an increasing percentage of behavior disruption with increasing dose (Fig. 6b). *N*-Propyldihydrexidine, with selective agonist activity at postsynaptic D_2 receptors (Mottola et al. 2002), fully mimicked the training drug with an ED_{50} (95% CI) of 587 nmol/kg (420–820 nmol/kg) [0.22 (0.16–0.31) mg/kg], whereas the non-selective D_2/D_1 dopamine agonist apomorphine had an ED_{50} (95% CI) of 1.3 μmol/kg (0.8–2.2 μmol/kg) [0.49 (0.3–0.83) mg/kg].

Consistent with these results, only partial cross substitution of LSD occurs in LSD-30- and LSD-90-trained rats. Although LSD injected 30 min before tests engendered a modest degree of drug lever responding in LSD-90 rats (Fig. 5), LSD administered 90 min before tests produced an even lower degree of substitution in LSD-30-trained animals [maximum SDL of 46 and 48% for 186 nmol/kg (0.08 mg/kg) and 372 nmol/kg (0.16 mg/kg), respectively]. Full substitution did not occur in either testing situation, clearly showing that these two cues are mediated by different mechanisms.

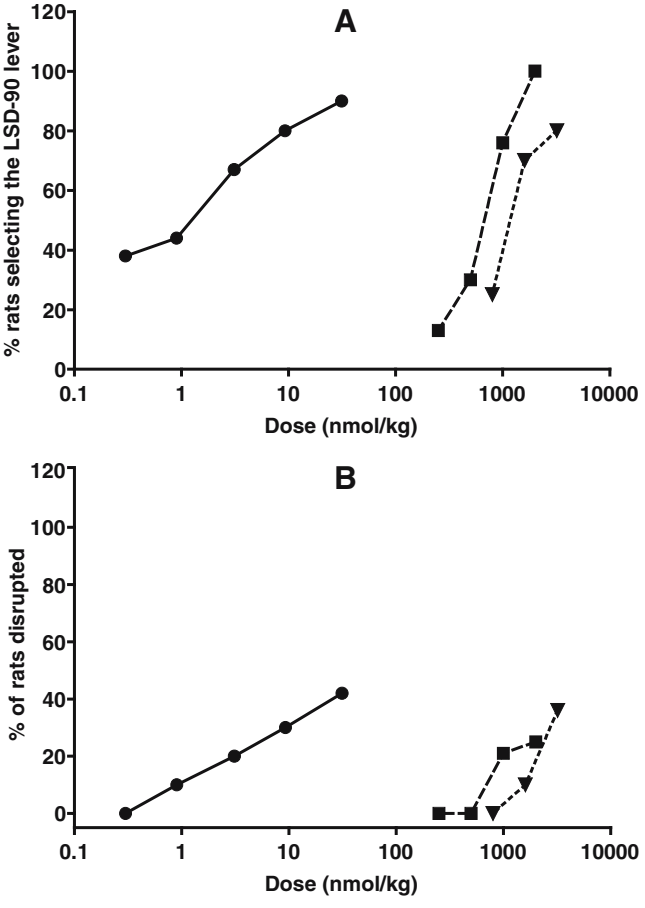

Fig. 6 **a** Dose–response curves for substitution tests of dopamine agonists with different selectivity and affinity at D_2 receptors in rats trained to discriminate LSD administered 90 min prior to training. Apomorphine(*filledtriangles*),*N*-propyldihydrexidine(*filledsquares*), and quinelorane (*filled circles*) were injected 30 min before testing. **b** Percentage of rats disrupted during substitution tests in LSD-90 rats. *Symbols* are the same as in **a**

Discussion

The results of these experiments indicate that the in vivo effects of LSD occur in two temporal phases, the first lasting about 1 h and mediated by 5-HT_{2A} receptors, and the second developing about 1 h after drug administration and lasting ~60–100 min. Thus, our results in animal behavior experiments confirm and extended the hypothesis based on clinical observations of the psychological effects of LSD in humans. Freedman (1984, 1986) described the clinical effects of LSD in humans as occurring in two temporal phases: a "psychedelic experience" in the early phase and a second phase that is "clearly a paranoid state." Behavior acutely induced by LSD in rats also occurs in two temporal phases: an initial suppression of exploratory behavior, followed by a subsequent increase in locomotor activity that is not observed with other serotonergic agonists (Adams and Geyer 1982, 1985; Mittman and Geyer 1989, 1991; Wing et al. 1990).

For the first time, we have shown that rats can be trained to discriminate LSD from saline when administered 90 min before training. There was no significant difference in the mean number of sessions to attain criterion by LSD-90-trained rats compared with LSD-30-trained rats, with our value being similar to those reported by others for a 15-min preinjection time for LSD (Appel et al. 1999; Callahan and Appel 1990; Cunningham et al. 1985; Holohean et al. 1982; Kuhn et al. 1978; Young et al. 1982). Our time course study showed that LSD (0.08 or 0.16 mg/kg; 186 or 372 nmol/kg), when given 30 min prior to training, produced a cue that had largely dissipated by 1 h and was completely gone by 2 h. This duration of effect is consistent with an earlier report by Jarbe (1980) of the time course of drug effect in pigeons trained to discriminate between saline and LSD given 15 min prior to training.

By contrast, LSD (0.16 mg/kg; 372 nmol/kg) injected 90 min prior to training generated a discriminative cue that became significant (more than 75% of rats selected the drug lever) no earlier than 1 h postinjection, with a maximum at 90 min and then slowly declining. Although there was a period of overlap in the two discriminative cues, LSD clearly generated two distinct cues with a nature that was dependent on the time of drug administration but not on the training dose of LSD.

Other hallucinogenic drugs used in drug discrimination studies generally produce a unitary pharmacology. In cases where a training drug cue has been found to possess more than one pharmacological component, different time dependency of the components has not been reported. The hallucinogenic tryptamine psilocybin produced a time-course curve with significant discriminative effects between 7.5 and 45 min that was completely gone by 2 h (Koerner and Appel 1982). The discriminative stimulus generated by hallucinogenic phenylalkylamines such as DOI or DOM is monophasic and lasts for several hours after drug administration, although these drugs have a slower onset of action than LSD. Fiorella et al. (1995) demonstrated that a 15-min pretreatment time with DOM did not generate a cue that was robust enough to serve as a training stimulus, but that drug-appropriate responding increased with increasing pretreatment times. They reported that the maximum effect for LSD occurred at 15 min postinjection and lasted up to 30 min. In our laboratory, DOI injected 30 min before training produced a maximal and stable discriminative stimulus effect that lasted up to 6 h postinjection (unpublished data). Thus, LSD administered 90 min prior to training generated a discriminative cue distinct from those obtained during training with either a 15- or 30-min preinjection time.

The LSD-30 cue was completely antagonized by selective 5-HT_{2A} antagonists, whereas the D_2 antagonist haloperidol was ineffective. By contrast, haloperidol antagonized the discriminative stimulus effect of LSD in LSD-90 rats by reducing drug-appropriate responding to 18%. Although MDL 100,907 did produce a maximum 36% inhibition in LSD-90 rats, the same dose completely abolished drug-appropriate responding in LSD-30 rats. Nevertheless, it is noteworthy that MDL 100,907 did produce some inhibition of the LSD-90 cue, suggesting that even though the cue may be primarily dopamine-mediated, the dopaminergic effects may be driven by, or synergistic with, 5-HT_{2A} receptor activation. This observation may have relevance to the use of mixed D_2/5-HT_{2A} antagonists to treat schizophrenia (Ichikawa et al. 2001; Meltzer et al. 1989; Meltzer 1999; Schotte et al. 1996).

It is well documented from substitution and antagonism studies that activation of serotonin 5-HT_{2A} receptors is responsible for the discriminative stimulus effects generated by LSD administered 15–30 min before training (Colpaert et al. 1982; Colpaert and Janssen 1983; Glennon et al. 1983, 1984; Glennon 1999; Ismaiel et al. 1993; Leysen et al. 1982; Schreiber et al. 1994; Winter 1994; Winter and Rabin 1988; Winter et al. 1999). By contrast, our combination tests in experiments using dopamine D_2 and 5-HT_{2A} antagonists provide compelling evidence that a dopaminergic pathway mediates the LSD-90 cue. Indeed, the strongest support for the hypothesis that the LSD-90 cue is mediated by direct activation of dopamine receptors comes from the substitution tests. In LSD-90 rats, full substitution occurred with three dopamine D_2 receptor agonists: apomorphine, *N*-propyldihydrexidine, and quinelorane, where quinelorane was the most potent compound with an ED_{50} of 0.93 nmol/kg (0.00033 mg/kg). None of these dopaminergic compounds produced significant generalization in LSD-30 rats. Surprisingly, the indirect dopamine releasing agent (+)-amphetamine did not produce significant drug lever selection in LSD-90 rats, but we speculate that the dopamine may not be released onto the relevant receptor population for this cue [2 mg/kg; 10.8 μmol/kg of (+)-amphetamine produced a maximum 50% SDL in LSD-90 rats, unpublished data].

Results from substitution tests with 5-HT_2 receptor agonists also provide evidence that the LSD-90 cue is not mediated through the 5-HT_{2A} receptor. In LSD-30 rats, extending the preinjection time of the 5-HT_2 agonist DOI from 30 to 75 min resulted in a leftward shift of the DOI cue, consistent with the suggestion by Fiorella et al. (1995) that increasing the preinjection time of hallucinogenic amphetamines potentiates their discriminative stimulus effects. By contrast, after a 30-min preinjection time, DOI produced

only 55% substitution in LSD-90 rats; extending the preinjection time to 75 min reduced the degree of substitution to only 12% (Fig. 4). Thus, only LSD, but not DOI, produced full substitution in LSD-90-trained rats when administered at preinjection times longer than 1 h.

In summary, we have found that the nature of the LSD cue is fundamentally different when animals are trained to discriminate the drug administered 90 min prior to training from when LSD is injected 15 or 30 min prior to training. With the 90-min preinjection time, the cue is no longer mediated by stimulation of 5-HT_{2A} serotonin receptors. Instead, the main mechanism is stimulation of dopamine D_2-like receptors, but possibly made more robust by activation of 5-HT_{2A} receptors, as evident by some inhibition of the cue by MDL 100,901. Animals trained with the larger dose of LSD (a dose commonly used in drug discrimination studies with LSD) also have a significantly higher response rate than after saline or injection with any other training drug that we have routinely used for DD studies in our laboratory.

Thus, the discriminative stimulus effect of LSD in rats occurs in two phases, and our studies here provide evidence that the later temporal phase is mediated by D_2 dopamine receptor stimulation. Our results in rats parallel Freedman's observations of two temporal intoxication phases in man, with the latter resembling a "paranoid" psychotic phase. Our observations that this second temporal phase involves dopaminergic pathways would be consistent with the widespread belief that excessive dopaminergic activity may be an underlying cause of paranoid psychosis. Although the early idea was ultimately abandoned that hallucinogens mimicked psychosis (i.e., were "psychotomimetics"), it may be, at least for LSD, that some aspects of that characterization may prove to be not so far off the mark.

Acknowledgements These studies were supported by grant DA-02189 from NIDA.

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