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ORIGINAL INVESTIGATION

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Complex discriminative stimulus properties of (+)lysergic acid diethylamide (LSD) in C57Bl/6J mice

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Abstract *Rationale:* The drug discrimination procedure is the most frequently used in vivo model of hallucinogen activity. Historically, most drug discrimination studies have been conducted in the rat. With the development of genetically modified mice, a powerful new tool has become available for investigating the mechanisms of drug-induced behavior. The current paper is part of an ongoing effort to determine the utility of the drug discrimination technique for evaluating hallucinogenic drugs in mice. *Objective:* To establish the training procedures and characterize the stimulus properties of (+)lysergic acid diethylamide (LSD) in mice. *Methods:* Using a two-lever drug discrimination procedure, C57Bl/6J mice were trained to discriminate 0.45 mg/kg LSD vs saline on a VI30 sec schedule of reinforcement, with vanilla-flavored Ensure serving as the reinforcer. *Results:* As in rats, acquisition was orderly, but the training dose was nearly five-fold higher for mice than rats. LSD lever selection was dose-dependent. Time-course studies revealed a rapid loss of the LSD stimulus effects. The 5-$HT_{2A/2C}$ receptor agonist, 2,5-dimethoxy-4-bromoamphetamine [(−)DOB] (1.0 mg/kg), substituted fully for LSD and the 5-HT_{1A} receptor agonist, 8-hydroxy-2-(di-*n*-propylamino)-tetralin (8-OH-DPAT) (1.6 mg/kg), substituted partially for LSD. Pretreatment with the 5-HT_{2A} receptor-selective antagonist, MDL 100907, or the 5-HT_{1A}-selective antagonist WAY 100635, showed that each antagonist only partially blocked LSD discrimination. Substitution of 1.0 mg/kg (−)DOB for LSD was fully blocked by pretreatment with MDL 100907 but unaltered by WAY 100635 pretreatment. *Conclusions:* These data suggest that in mice the stimulus effects of LSD have both a 5-HT_{2A} receptor and a 5-HT_{1A} receptor component.

Keywords LSD · (−)DOB · 5-HT_{2A} receptor · 5-HT_{1A} receptor · Drug discrimination · Serotonin · Mice

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Introduction

Beginning in 1943 when Hoffmann first observed the hallucinogenic effects of lysergic acid diethylamide (LSD) in humans, scientists have attempted to identify the mechanism of action of this and other hallucinogens. In the years that followed, evidence linking the actions of LSD to serotonergic systems began to appear. Early findings included recognition of the structural resemblance between LSD and serotonin (Gaddum and Hammeed 1954), the observation that LSD inhibits the action of 5-HT on smooth muscle (Gaddum 1953), reports of changes in brain levels of 5-HT (Freedman 1961) and its metabolite, 5-HIAA (Rosecrans et al. 1967), as well as electrophysiological studies showing that systemically administered LSD potently suppressed the firing rate of cells in the dorsal raphe nucleus (Aghajanian et al. 1968, 1970). In 1971, Hirschhorn and Winter reported that LSD and mescaline could serve as discriminative stimuli in rats. While the association between serotonin and LSD was established fairly early, the specific receptor(s) mediating the hallucinogenic effects of LSD remained unknown.

Although other animal models exist, the drug discrimination paradigm is the most frequently used in vivo model of hallucinogenic activity. In this paradigm, animals (generally rats) are trained to press one of two levers when given a drug (e.g., LSD) and the alternate lever when given saline. Drug-induced interoceptive cues serve as the stimuli that the animal learns to discriminate. Correct lever choices are

intermittently rewarded with food (or water). The drug discrimination 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, objectively quantified and related to the subjective effects of drugs as reported by humans (Appel et al. 1982; Colpaert 1999).

Using the drug discrimination paradigm, Glennon et al. (1983) presented evidence that the discriminative stimulus effects of LSD and other hallucinogens act via the 5-HT_2 receptor. In this study, rats were trained to discriminate 5-dimethoxy-4-methylamphetamine [(−)DOM] from saline. In substitution tests, LSD, mescaline and 5-methoxy-*N*,*N*-dimethyltryptamine were shown to substitute for the stimulus effects of (−)DOM. Pretreatment with the 5-HT_2 receptor antagonists, ketanserin and pirenperone, blocked both (−)DOM discrimination and substitution of the other hallucinogens for (−)DOM, suggesting a major role for the 5-HT_2 receptor in mediating the discriminative stimulus effects of hallucinogens. Subsequent studies looking at the correlation between the affinities of hallucinogens for various 5-HT receptor subtypes and their potencies as hallucinogens in humans or as discriminative cues in animals further supported the association between hallucinogen activity and the 5-HT_2 receptor (Glennon et al. 1984; Titeler et al. 1988).

While all classical hallucinogens bind with high affinity to the 5-HT_2 receptor, few bind with equally high affinity to the 5-HT_{1A} receptor; LSD is an exception. Because LSD has high affinity for both the 5-HT_2 and the 5-HT_{1A} receptor, many drug discrimination studies have investigated a possible role for the 5-HT_{1A} receptor in the stimulus effects of LSD. The results have been variable, but in general suggest that there is not a large 5-HT_{1A} component to the LSD stimulus in the rat. The 5-HT_{1A} receptor agonist 8-hydroxy-2-(di-*n*-propylamino)-tetralin (8-OH-DPAT) has been found to either partially substitute (Arnt 1989; Winter and Rabin 1988) or not substitute at all for LSD (Cunningham and Appel 1987) in rats. Arnt (1989) trained two separate groups of rats to discriminate either LSD from saline or 8-OH-DPAT from saline. LSD partially substituted for 8-OH-DPAT in animals trained to discriminate 8-OH-DPAT from saline. However, 8-OH-DPAT did not substitute for 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. Furthermore, Appel et al. (2004) reported that the selective 5-HT_{1A} receptor antagonist, WAY 100635, did not block the discriminative stimulus effects of LSD in rats trained to discriminate LSD from saline. Consistent with this observation is a report that the selective 5-HT_{2A} receptor antagonist MDL 100907 fully blocked LSD discrimination in rats (Winter et al. 2004).

In contrast, in monkeys trained to discriminate LSD from saline, substitution studies showed that 5-methoxy-*N*,*N*-dimethyltryptamine, but not mescaline, generalized to LSD (Nielsen 1985). Furthermore, pretreatment with ketanserin did not block the LSD stimulus and only the highest dose of pirenperone tested significantly attenuated LSD discrimination. The failure of mescaline, a 5-HT_2 agonist (Newton et al. 1996), to substitute for LSD suggests that in monkeys the 5-HT_2 receptor may not mediate the cue properties of LSD. 5-Methoxy-*N*,*N*-dimethyltryptamine substitution for LSD might be explained by the fact that, like LSD, it has a high affinity for the 5-HT_{1A} receptor (Blair et al. 2000), and this common action at the 5-HT_{1A} receptor may be responsible for the substitution to LSD. The lack of blockade of the LSD stimulus by 5-HT_2 antagonists ketanserin and pirenperone is consistent with an involvement by receptors other than the 5-HT_2 receptor. The discriminative stimulus effects of LSD have also been explored in pigeons and the results of this study indicate that both the 5-HT_2 and 5-HT_{1A} receptors are involved in the stimulus properties of LSD (Walker et al. 1991). The above studies suggest that the extent to which the 5-HT_2 and 5-HT_{1A} receptors mediate the discriminative stimulus effects of LSD may vary from species to species.

The goals of the present study were to characterize the discriminative stimulus effects of LSD in mice. Given that earlier work suggested between-species variability in the receptors mediating the discriminative stimulus effects of LSD, it was important to characterize the LSD cue in mice prior to utilizing the model as an in vivo assay of hallucinogen activity, for example, in genetically modified mice. To our knowledge, no other studies have been published describing the discriminative properties of LSD in mice. The present paper describes LSD vs saline discrimination in C57BL/6J mice, the most common strain used for genomic manipulation.

Materials and methods

Subjects

Subjects were 18 male C57Bl/6J mice, ~2 months old at the beginning of training. Food restriction, which began 2 weeks prior to the onset of training, consisted of free access to food for 4 h/day Monday to Thursday and free feeding from Friday, following training, through Sunday afternoon. Food was made available immediately after training each day. Mice had free access to water except during the training session. Mice were maintained on a 12:12 light/dark cycle with light onset at 0600 h. All experiments were done in compliance with the guide for the Principles of Laboratory Animal Care (NIH publication No. 85-23, revised 1996).

Apparatus

Training and experimentation were conducted in six commercially available mouse operant conditioning chambers (ENV 307A; Med Associates, Georgia, VT, USA). Each of these experimental chambers was housed in a sound-attenuated chamber. The experimental chambers were equipped with two ultra-sensitive response levers (ENV 310 M) and a liquid

dipper (ENV 302 M) centered between the two levers. The equipment and experimental parameters were programmed using MED Associates software and controlled by MED Associates interface and MS-DOS compatible computers.

Training and testing procedures

Mice were auto-shaped to lever-press for food reinforcement under a fixed ratio 1 (FR1) schedule of reinforcement during daily 2-hr training sessions. Vanilla-flavored Ensure (a milk-based nutritional supplement), diluted 1:1 in water, was used as the reinforcer. During these sessions, mice were reinforced on either lever. A few mice that were slow in acquiring lever-pressing behavior were hand-shaped. After lever-pressing behavior was acquired, preferences were eliminated by reinforcing the animal on the non-preferred lever until their responses were ~50% on each lever during the first 2.5 min of the 20-min training session. Gradually, over the next 12 training sessions, the FR schedule was incremented from an FR1 through FR5, FR10, FR15 and then to a VI30 sec schedule, where it remained for the duration of the study. Drug discrimination training began when the animals reached an FR5. The initial training dose of LSD was 0.25 mg/kg s.c. Over the course of the first 2 months of training, the training dose of LSD was increased incrementally to as high as 1.0 mg/kg, and then lowered to a training dose of 0.85 mg/kg. Throughout training, a time-out (TO) contingency was in effect, which consisted of a 5-sec period following an incorrect response during which reinforcement was not available. The purpose of the TO contingency was to punish the strategy of lever switching as a means of determining the correct lever. For half the mice, the right lever was LSD correct and the left lever, saline correct. For the remaining mice, the reverse was true. Mice were trained 5 days/week. LSD and saline training were given on alternate days. Acquisition of the discrimination was monitored twice weekly by calculating percent correct (number of correct responses/total responses) during 2.5-min extinction test sessions at the beginning of the training session. For the remainder of the training session, the VI30 sec schedule of reinforcement was in effect.

Initially, both the pre-injection interval and the training session were 20 min in length. An initial time course experiment revealed a surprisingly short duration of action for the discriminative stimulus effects of 0.85 mg/kg LSD. For subsequent training, the dose of LSD was lowered to 0.45 mg/kg, the pre-injection interval was reduced to 10 min and the training session was shortened to 15 min. Training continued until the animals averaged 85% or greater following both LSD and saline during the initial 2.5-min extinction periods.

Test sessions followed a minimum of 4 training days, with the fourth day being a saline training day. Unless indicated, all mice were tested in each experimental condition. In order to be included in the results, the mouse must have made at least five responses during the extinction test session. For this reason, where possible, experimental test sessions were lengthened to 5 min, as opposed to the 2.5-min sessions used to measure acquisition.

Time course of LSD and (−)DOB

To determine the onset and duration of the discriminative stimulus effects of LSD and 5-dimethoxy-4-bromoamphetamine [(−)DOB], mice were given a single s.c. injection of drug and tested at various time points subsequent to injection. For the first LSD time course, mice (n=18) were injected with 0.85 mg/kg LSD and tested at 5, 20, 35, 50, 65 and 80 min post-injection. Tests consisted of 5-min sessions during which the animals were reinforced for responding on either lever on a VI30 sec schedule of reinforcement. At the end of the 5-min test session, the animals were returned to the home cage until the next time interval. All mice were tested at each time interval.

The time intervals for the second LSD time-course experiment, after administration of 0.45 mg/kg LSD, were spaced more closely together and the test duration was shortened to 2.5 min. For this reason, the data were collected during two test sessions, separated by 1 week of training. On week 1, mice (n=18) were injected with 0.45 mg/kg LSD and tested at 5, 20, 35, 50 and 65 min subsequent to injection. On week 2, the same mice were injected with 0.45 mg/kg LSD and tested at 10 and 25 min post-injection. After retraining, the time course of the (−)DOB stimulus was examined. Mice (n=18) were injected with 1.0 mg/kg (−)DOB and tested at 10, 35, 60, 90, 120 and 180 min post-injection. All mice were tested at each time interval.

Dose–response curve and substitution studies

After acquisition, a dose–response curve was determined for four doses of LSD (0.45, 0.23, 0.11, and 0.06 mg/kg) and saline using a 10-min pre-injection interval. Test doses of LSD were administered in random order over a 5-week period. One 5-min extinction test was given per week. After testing, mice (n=18) were returned to their cages.

To examine the extent to which the 5-$HT_{2A/2C}$ and 5-HT_{1A} receptors contribute to the discriminative stimulus effects of LSD, substitution tests were conducted with the 5-$HT_{2A/2C}$ receptor-selective agonist (−)DOB and the 5-HT_{1A} receptor-selective agonist 8-OH-DPAT. A cumulative dosing procedure was used for these experiments (Bertalmio et al. 1982; Schechter 1997). For each test dose, mice (n=16) were injected with drug and returned to their home cages for 10 min. At the end of 10 min, the mice were placed in the experimental chambers and tested for choice behavior for 5 min. During the test session, responses on both levers were reinforced under a VI30 sec schedule of reinforcement. Upon completion of the test session, the mice were removed from the experimental chamber, injected with the next dose of drug, and returned to their home cages for 10 min until the next 5-min test. For 8-OH-DPAT, saline, 0.2, 0.2, 0.4, and 0.8 mg/kg were

administered sequentially, resulting in cumulative doses of 0.2, 0.4, 0.8 and 1.6 mg/kg. Doses of 0.125, 0.125, 0.25, and 0.5 mg/kg of (−)DOB were administered, resulting in cumulative dose of 0.125, 0.25, 0.5 and 1.0 mg/kg. Mice received a minimum of 1 week of retraining between substitution studies.

Antagonism of LSD cue

To further examine the role of specific 5-HT receptors in mediating the discriminative stimulus effects of LSD, antagonist studies were conducted using selective 5-HT antagonists. Mice (n=16) were pretreated for 20 min prior to LSD (0.15, 0.45, 0.9 mg/kg and saline) with the 5-HT_{2A} receptor-selective antagonist MDL 100907 (0.25 mg/kg), the 5-HT_{1A} receptor-selective antagonist WAY 100635 (1.0 mg/kg), or saline. Test sessions were initiated 10 min after LSD injection. Additionally, a single experiment was conducted with pretreatment of 0.125 mg/kg MDL 100907 prior to a dose of 0.45 mg/kg LSD. Ten minutes after LSD or saline injections, 5-min extinction tests were performed. Mice were tested once per week and received training the remainder of the week. Testing with one antagonist was completed prior to beginning testing with the other antagonist. All mice were tested under each condition.

Characterization of (−)DOB substitution

To examine the contribution of 5-HT_{2A} and 5-HT_{1A} receptors in the ability of (−)DOB to substitute for LSD, mice were divided into two groups and pretreated with either saline or MDL 100907 (0.25 mg/kg) and the following week with saline or WAY 100635 (1.0 mg/kg) prior to (−)DOB. For each experiment, half of the mice (n=8) were pretreated with saline and the other half (n=8) with antagonist. Twenty minutes after pretreatment, all mice were given 1.0 mg/kg (−)DOB. Ten minutes after (−)DOB injection, the mice were placed in the experimental chambers and tested in a 5-min extinction session.

Drugs

Drugs were administered s.c. at an injection volume of 10 ml/kg. All drugs were dissolved in distilled water, except MDL 100907, which was dissolved in three drops of 2% tartaric acid and then diluted in distilled water. LSD and (−)DOB were provided by the National Institute of Drug Abuse. MDL 100907 was a gift from Marion Merrell Dow. WAY 100635 nd CH-DPAT were purchased from Tocris Cookson (Baldwin, MO, USA).

Statistics

Results are presented as mean±SEM. Data were analyzed using either a one- or two-way repeated-measures analysis of variance. Post-hoc comparisons were done using the Student Newman–Keuls for multiple comparisons test. A p-value of less than 0.05 was considered to be statistically significant. An unpaired Student's t-test was used to compare the effect of antagonist pretreatment to vehicle pretreatment on (−)DOB substitution.

Results

Discrimination training

The initial training dose of LSD, 0.25 mg/kg, was gradually increased to 1.0 mg/kg over the course of the first 26 days of training. By day 39 of training, the mice averaged 85% or greater correct lever pressing for both training cues during the 2.5-min extinction test sessions (Fig. 1). Although the mice discriminated 1.0 mg/kg LSD well, this dose of LSD produced significant rate suppression so the training dose was incrementally lowered, reaching 0.85 mg/kg LSD at training day 52. At 0.85 mg/kg, the mice continued to discriminate at 85% correct or greater and their average rate of responding increased from 18 to

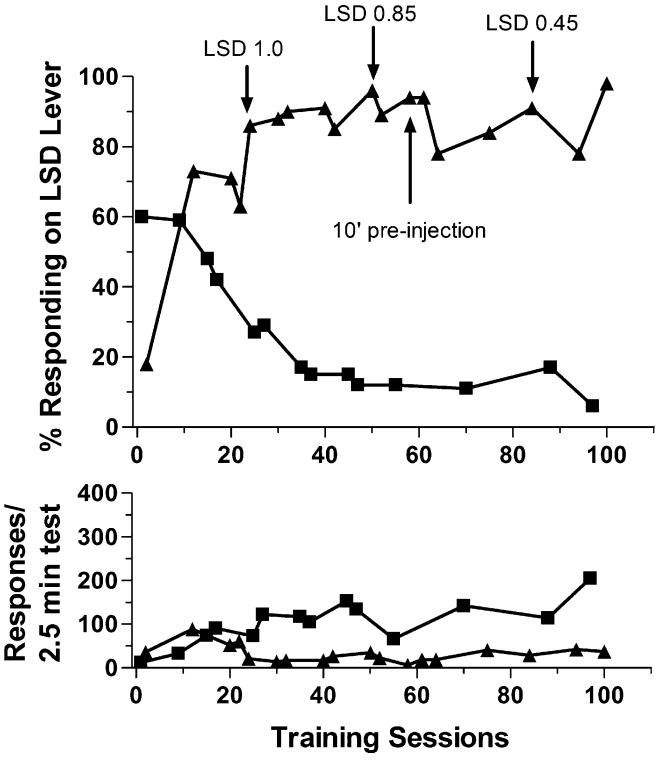

Fig. 1 LSD–saline acquisition curve. Eighteen mice were trained to discriminate 0.45 mg/kg LSD from saline on a VI30 sec schedule of reinforcement. *Upper panel* shows choice behavior measured during 2.5-min extinction tests given twice weekly at the beginning of training. The data are plotted as the mean percent responding on the LSD lever following administration of either LSD (*filled triangles*) or saline (*filled squares*). The *arrows* indicate the sessions at which the training dose was changed to the indicated dose (mg/kg) or when the pre-injection interval was dropped from 20 to 10 min. The *lower panel* depicts the mean number of total responses the mice made during the 2.5-min extinction period

31 responses per 2.5-min extinction test. These training conditions continued until completion of the first time course. The results of this experiment showed that LSD has a very short duration of action in the mouse such that the intensity of the cue changes dramatically over the course of a 20-min training session (see "Time Course for LSD and (−)DOB" below). For this reason, several changes were made in the training procedure, including shortening both the pre-injection interval (from 20 to 10 min) and the length of the training session (from 20 to 15 min) and reducing the dose of the training drug from 0.85 to 0.45 mg/kg LSD. The training dose of LSD was reduced because the 0.85 mg/kg dose disrupted lever pressing at 10 min post-injection. Comparing response rates during the 2.5-min extinction session on the last day of 0.85 mg/kg LSD with the first day of 0.45 mg/kg, the mean difference in number of responses was 20.9 (from 19.3 to 40.2 responses). A *t*-test indicated that this was a significant difference (t=3.109, p=0.0064).

Time course for LSD and (−)DOB

The time courses for the discriminative stimulus effects of 0.85 and 0.45 mg/kg LSD and 1.0 mg/kg (−)DOB are shown in Fig. 2. Choice behavior changed as a function of time for all drug treatments. The time course for a single injection of 0.85 mg/kg LSD shows that percent LSD lever selection fell from 84% at peak to 60% 35 min post-injection. This suggests that under the initial training procedures, where mice were trained from 20 to 40 min following LSD administration, the intensity of the LSD stimulus was changing and, for most of the training session, was weak. The 0.45-mg/kg LSD time course shows that, while stimulus intensity changed during the 10- to 25-min post-injection training period, percent LSD lever selection remained at or above 80%. The (−)DOB time course shows that percent LSD lever responding in mice treated with 1.0 mg/kg (−)DOB peaked at 95% on the LSD lever and remained above 85% for greater than 60 min post-injection. These data indicate that (−)DOB has a much longer duration of action in the mouse than does LSD, a result that is consistent with rat studies of LSD and (−)DOM (Fiorella et al. 1995a).

Dose–response function

A dose–response curve was determined by testing several doses of LSD (0.45, 0.23, 0.11 and 0.06 mg/kg) and saline 10 min post-injection. As shown in Fig. 3, percent LSD lever responding was dose-dependent [F(4,17)=108, p<0.05]. All doses of LSD tested yielded a significantly higher percent of LSD lever responding than saline discrimination (p<0.05, Newman–Keuls). Response rate was found to decrease significantly with increasing doses of LSD [F(4,17)=10.9, p<0.05].

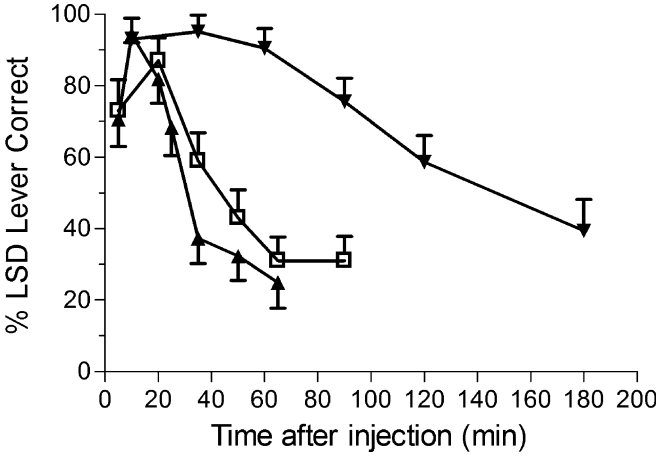

Fig. 2 Time course for the discriminative stimulus effects of LSD and (−)DOB. Mice were injected with either 0.45 mg/kg LSD (*filled triangles*), 0.85 mg/kg LSD (*open squares*), or 1.0 mg/kg (−)DOB (*filled inverted triangles*), and choice behavior was measured at various times following injection. Data represent the mean percent responding±SEM on the LSD lever for the mice that reached criterion (five responses) in each consecutive test session; 0.45 mg/kg LSD (n=16), 0.85 mg/kg LSD (n=15), (−)DOB (n=12)

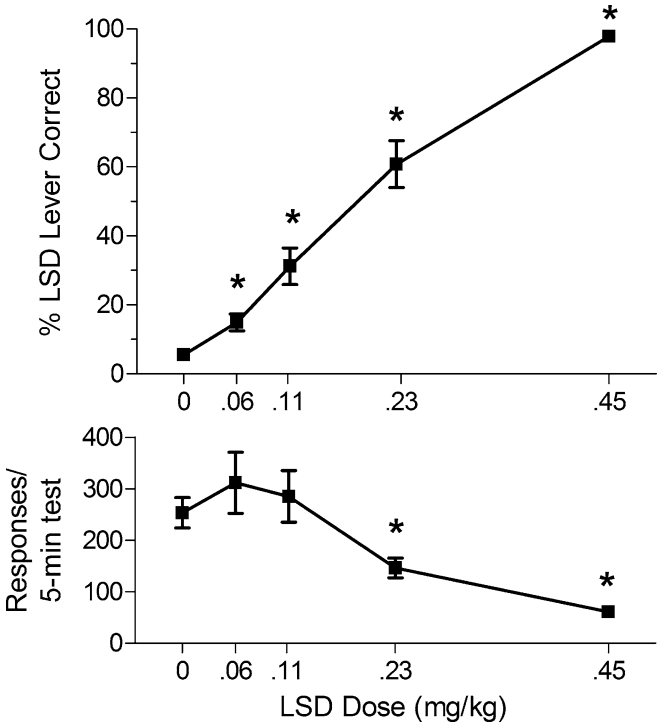

Fig. 3 LSD dose–response curve. Data represent mean responding± SEM on the LSD lever (*upper panel*) and the mean total number of responses (*lower panel*) made during a 5-min extinction tests as a function of varying doses of LSD. Tests were conducted 10 min following LSD injection. *Asterisks* indicate groups treated with a dose of LSD that yielded lever selection or rate of response significantly different than vehicle control (p<0.05, Newman–Keuls)

Substitution studies

Figure 4 shows the results of substitution tests with (−) DOB and 8-OH-DPAT using a cumulative dose–response design. Increasing doses of (−)DOB produced a dose-dependent increase in LSD lever selection with 1.0 mg/kg (−)DOB yielding 87% responding on the LSD lever [$F(4,9)=36.6$, $p<0.0001$]. Doses of 0.5 and 1.0 mg/kg (−)DOB differed significantly from saline pretreatment ($p<0.05$, Newman–Keuls). Response rates decreased significantly as a function of increasing doses of (−)DOB [$F(4,9)=6.6$, $p<0.0004$] with 0.5 and 1.0 mg/kg (−)DOB significantly reducing response rates relative to saline administration ($p<0.05$, Newman–Keuls).

Substitution tests with 8-OH-DPAT also yielded a dose-dependent increase in LSD lever selection with the highest dose, 1.6 mg/kg, producing 60% LSD lever responding [$F(4,15)=18.9$, $p<0.0001$]. Post-hoc analysis indicated that 0.4, 0.8 and 1.6 mg/kg 8-OH-DPAT produced a significant increase in LSD lever responding relative to saline ($p<0.05$, Newman–Keuls). Response rates decreased significantly as a function of increasing doses of 8-OH-DPAT relative to saline administration [$F(4,15)=22.2$, $p<0.0001$].

Antagonist studies

To examine the role of the 5-HT_{2A} receptor in regulating LSD discrimination in mice, the 5-HT_{2A} receptor-selective antagonist MDL 100907 (0.25 mg/kg) was administered prior to either saline or increasing doses of LSD. A 2 (Pretreatment Condition) × 3 (Test Dose) repeated-measures ANOVA of choice behavior indicated the following significant terms: Pretreatment Condition [$F(1,11)=32.4$, $p<0.0001$], Test Dose [$F(2,11)=81.8$, $p<0.0001$] and Pretreatment Condition × Test Dose interaction [$F(2,22)=10.2$, $p<0.0007$]. LSD lever selection following 0.25 mg/kg MDL 100907 pretreatment was significantly reduced relative to saline pretreatment at both 0.15 and 0.45 mg/kg LSD (Newman–Keuls, $p<0.05$) (Fig. 5). This reduction in choice behavior was surprisingly only partial, with 44% correct lever response at 0.45 mg/kg LSD. In this experiment, five of the 16 mice tested did not reach the criterion of five responses and, of the remaining mice, rate dropped to 19 responses per 5-min test. In an earlier experiment, mice were tested at a dose of 0.45 mg/kg LSD following 0.125 mg/kg MDL 100907. With this dose of MDL 100907, LSD lever selection was reduced to 51%± 8.8 ($n=17$) and the mean total response was 79.7±18.1.

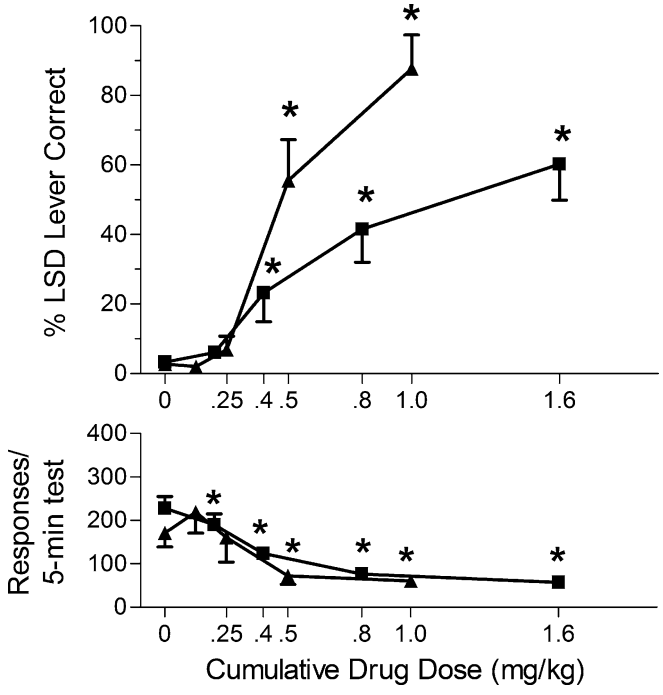

Fig. 4 Substitution tests. Mice were administered (−)DOB (*filled triangles*) or 8-OH-DPAT (*filled squares*) using a cumulative dosing regimen, with tests performed 10 min subsequent to drug injections. *Upper panel* shows mean percent LSD lever responding±SEM and the *lower panel* depicts the mean total responses±SEM made during the 5-min VI30 sec tests. Data reflect responses only from mice that met the response criteria following each subsequent injection of (−) DOB ($n=10$) or 8-OH-DPAT ($n=16$). *Asterisks* indicate data points where mean percent responding or rate of response was significantly different than vehicle control ($p<0.05$, Newman–Keuls)

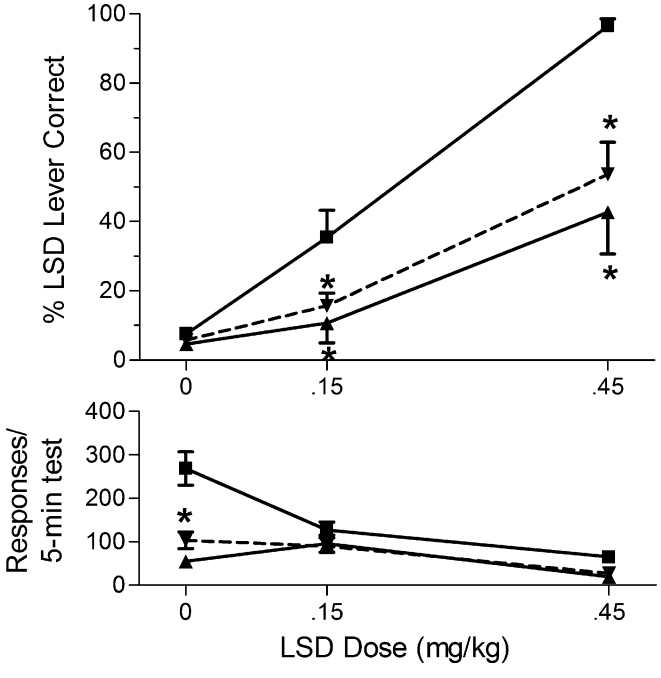

Fig. 5 Antagonism of LSD cue. Mice were pretreated for 20 min with either MDL 100907 (0.25 mg/kg, *filled triangles*), WAY 100635 (1.0 mg/kg, *inverted filled triangles*) or saline (*filled squares*), followed by an injection of saline or LSD (0.15 or 0.45 mg/kg). Ten minutes after saline or LSD injection, 5-min extinction tests were given. *Upper panel* shows mean percent LSD lever responding±SEM. *Asterisks* indicate doses of LSD at which antagonist pretreatment significantly reduced LSD lever selection relative to saline pretreatment ($p<0.05$, Newman–Keuls). Data points represent independent experiments. Only those mice ($n=11$) that responded under all conditions are included. The *lower panel* depicts the mean total responses±SEM made during the 5-min extinction test

Thus increasing the dose of MDL 100907 to 0.25 mg/kg did not significantly reduce LSD lever selection, but did significantly lower response rates. To determine if LSD antagonism could be surmounted by increasing the dose of LSD, mice were pretreated with 0.25 mg/kg MDL 100907 followed by 0.9 mg/kg LSD. This combination of drugs disrupted lever pressing such that only four mice reached the minimum requirement of five responses during the 5-min test session. For these four mice, the percent LSD lever selection averaged 97.5±2.5%.

The finding that MDL 100907 only partially blocked the training dose of LSD further suggests a role for another receptor in mediating the discriminative stimulus properties of LSD. Since LSD also acts as an agonist at the 5-HT_{1A} receptor and 8-OH-DPAT partially substituted for LSD, the effect of antagonizing the 5-HT_{1A} receptor with WAY 100635 was investigated. A 2 (Pretreatment Condition)×3 (Test Dose) repeated-measures ANOVA of choice behavior indicated the following significant terms: Pretreatment Condition [$F(1,12)=23.5$, $p<0.0004$], Test Dose [$F(2,12)=165.2$, $p<0.0001$] and Pretreatment Condition×Test Dose interaction [$F(2,24)=6.7$, $p<0.0048$]. As shown in Fig. 5, LSD lever selection following WAY 100635 pretreatment (1.0 mg/kg) was significantly reduced relative to saline pretreatment at both 0.15 and 0.45 mg/kg LSD (Newman–Keuls, $p<0.05$); however, WAY 100635 only partially blocked LSD discrimination (58 vs 95%) at the training dose of LSD. Increasing the test dose of LSD to 0.9 mg/kg, after WAY 100635 pretreatment, significantly disrupted lever pressing, such that only three mice met the criterion of five responses. The mean percent LSD lever selection for these three animals was 95±4.7%.

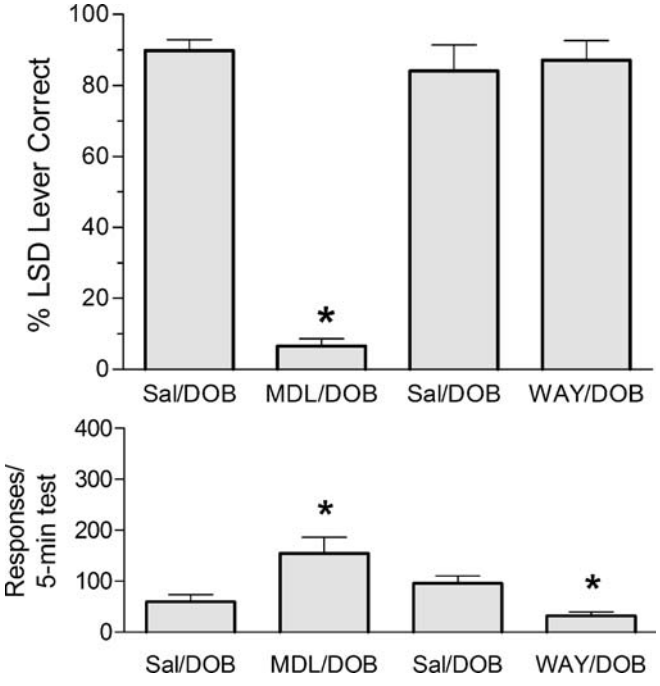

Fig. 6 Characterization of (−)DOB substitution. The ability of a single dose of MDL100907 (0.25 mg/kg), WAY100635 (1.0 mg/kg) or saline to block the mean percent LSD lever responding±SEM induced by 1.0 mg/kg (−)DOB is shown in the *upper panel*. Antagonist pretreatment was 20 min prior to (−)DOB injection. Mean total response is depicted in the *lower panel*. *Asterisks* indicate conditions significantly different ($p<0.05$, Student's *t*-test) from saline pretreatment. In these statistical analyses, antagonist pretreatment was compared to the saline pretreatment on the same test day

Characterization of (−)DOB substitution

To determine the role of the 5-HT_{2A} and 5-HT_{1A} receptors in the substitution of (−)DOB for LSD, mice were pretreated either with 0.25 mg/kg MDL 100907, 1.0 mg/kg WAY 100635 or saline followed by 1.0 mg/kg (−)DOB. As shown in Fig. 6, MDL 100907 significantly blocked substitution of (−)DOB for LSD ($t=23.4$, $p<0.0001$) relative to saline pretreatment (7 vs 90%, respectively) while WAY 100635 had no effect relative to saline pretreatment (87 vs 84%, respectively). Pretreatment with MDL 100907 also significantly increased rate of response relative to saline pretreatment ($t=2.6$, $p<0.02$). WAY 100635 pretreatment significantly reduced response rates relative to saline pretreatment ($t=3.8$, $p<0.002$).

Discussion

These experiments demonstrate that mice can be trained to discriminate LSD from saline. However, the training dose for mice (0.45 mg/kg) is considerably higher than the dose used previously in rats (0.08–0.1 mg/kg) in our laboratory (unpublished results) as well as others (Fiorella et al. 1995b; Marona-Lewicka et al. 2002). Comparable species differences were found with (±)DOI discrimination in rats and mice. Mice were trained on 2.5 mg/kg (±)DOI (Smith et al. 2003), while rats discriminated a dose of 0.075 mg/kg (Smith et al. 1999). These differences in dosage may be due to interspecies differences in pharmacokinetics; we are not aware of experiments that compare metabolism and excretion rates of LSD in rodents.

The stimulus time courses for both LSD and (−)DOB are consistent with these drugs having a more rapid pharmacokinetic profile in mice than rats. The temporal profile of 0.45 mg/kg LSD showed that the LSD lever responding peaks at 94% correct 10 min after s.c. injection and drops precipitously to only 40% correct by 35 min. (−)DOB produced a much more lasting effect, with full substitution observed as long as 60 min (88% LSD lever correct) post-injection. This time course is similar to that previously observed with (±)DOI in mice (Smith et al. 2003). Previous work in rats trained to discriminate LSD (0.1 mg/kg) from saline showed that (−)DOM (0.8 mg/kg) substituted fully for LSD as much as 180 min post-injection (Fiorella et al. 1995a). Our studies in rats trained to discriminate 0.085 mg/kg LSD showed that 60 min post-injection of this dose, rats had a mean LSD lever response of 81%, which dropped to 20% at 120 min post-injection (unpublished data). These results demonstrate that the hallucinogens LSD, (−)DOB, (±)DOI and (−)DOM seem to have similar time course profiles, relative

to each other, in rat and mouse, but the duration of action is consistently shorter in the mouse. We therefore suggest that the potency differences between rats and mice are the result of a more rapid metabolism and/or excretion of drugs, rather than species differences in receptor pharmacology.

Past experiments in rats have led to the hypothesis that the LSD-induced discriminative stimulus is predominantly mediated by activation of the 5-HT_{2A} receptor. The highly selective 5-HT_{2A} receptor antagonist, MDL 100907, has been shown in rats to completely block the stimulus properties of hallucinogens, including LSD and (±)DOI (Schreiber et al. 1994; Winter et al. 2004). However, in the current experiments in mice, 0.25 mg/kg MDL 100907 produced only a partial blockade of the LSD discriminative stimulus. One possible interpretation of this data is that the dose of MDL 100907 was insufficient. However, this seems unlikely since doubling the dose of MDL 100907 from 0.125 to 0.25 mg/kg caused only a marginal drop in LSD lever choice, but led to substantial behavioral disruption. Therefore we conclude that the stimulus effects of 0.45 mg/kg LSD are only partially mediated by activation of 5-HT_{2A} receptors.

Given that MDL 100907 only partially blocked LSD discrimination and the 5-HT_{1A} receptor agonist, 8-OH-DPAT, partially substituted for LSD, we hypothesized that the LSD discriminative stimulus contained a significant 5-HT_{1A} receptor component. This was confirmed by partially blocking the LSD stimulus with the 5-HT_{1A} receptor-selective antagonist WAY 100635, suggesting that the interoceptive state caused by LSD is more complex in mice than in rats. This is the first time that the discriminative stimulus of LSD has been clearly shown to include a 5-HT_{1A} receptor component in rodents. Although LSD has a high affinity for rat 5-HT_{1A} receptors, these receptors do not appear to play a significant role in LSD discrimination in rats (see "Introduction"). There has been little pharmacological characterization of mouse receptors, so we can only speculate that the observed effects may be due to species differences in receptor activation. Another possibility is that the observed 5-HT_{1A} receptor component is the result of the training regime, since the training dose is so much higher in mice than in rats. It is conceivable that if mice could be trained to discriminate a lower dose of LSD, a different pharmacological profile would emerge, e.g., a less prominent 5-HT_{1A} receptor component.

Despite our new finding of a more complex LSD discriminative stimulus in mice, substitution experiments demonstrated clearly that (−)DOB fully substituted for LSD. This finding is consistent with past experiments in rats, but is surprising in light of the partial substitution by a 5-HT_{1A} receptor agonist in these mice. (−)DOB is a highly selective 5-$HT_{2A/2C}$ receptor agonist, with little potency at the 5-HT_{1A} receptor. Additionally, the substitution of (−)DOB for LSD was entirely mediated by activation of the 5-HT_{2A} receptor, as shown by full blockade of (−)DOB with MDL 100907 and insensitivity to WAY 100635.

In conclusion, these data illustrate that the stimulus properties of 0.45 mg/kg LSD in mice contain components that are common to both hallucinogenic drugs and non-hallucinogenic drugs. This is consistent with the broad spectrum of receptors with which LSD is known to interact. It is likely that the high training dose and rapid stimulus kinetics generated a mixed spectrum of stimuli that the mice learned to discriminate. Fiorella et al. (1995b) proposed that using substitution of (−)DOM for the LSD stimulus would have a dual effect of enhancing the specificity of the drug discrimination paradigm by focusing on mechanisms common to both hallucinogens and by de-emphasizing properties unique to the LSD stimulus. Our results reinforce this conclusion. The use of (−)DOB as a test drug, substituting for LSD, emphasized that the relevant mechanism mediating the common stimulus is activation of 5-HT_{2A} receptors, and that the stimulus related to 5-HT_{1A} receptor activation is likely external to the hallucinogenic activity of LSD. This study strengthens the concept of using generalization in drug discrimination to isolate mechanisms specific to hallucinogens, without undue concern about non-specific effects. Given the complex nature and temporal profile of the LSD stimulus demonstrated in the current study, this design might be especially relevant to research with mice.

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References

Aghajanian GK, Foote W, Sheard M (1968) Lysergic acid diethylamide: sensitive neuronal units in the midbrain raphe. Science 161:706–708

Aghajanian GK, Foote WE, Sheard MH (1970) Action of psychotogenic drugs on single midbrain raphe neurons. J Pharmacol Exp Ther 171:178–187

Appel J, White F, Holohean A (1982) Analyzing mechanism(s) of hallucinogenic drug action with drug discrimination procedures. Neurosci Behav Rev 6:529–536

Appel JB, West WB, Buggy J (2004) LSD, 5-HT (serotonin), and the evolution of a behavioral assay. Neurosci Biobehav Rev 27:693–701

Arnt J (1989) Characterization of the discriminative stimulus properties induced by 5-HT1 and 5-HT2 agonists in rats. Pharmacol Toxicol 64:165–172

Bertalmio AJ, Herling S, Hampton RY, Winger G, Woods JH (1982) A procedure for rapid evaluation of the discriminative stimulus effects of drugs. J Pharmacol Methods 7:289–299

Blair JB, Kurrasch-Orbaugh D, Marona-Lewicka D, Cumbay MG, Watts VJ, Barker EL, Nichols DE (2000) Effect of ring fluorination on the pharmacology of hallucinogenic tryptamines. J Med Chem 43:4701–4710

Colpaert FC (1999) Drug discrimination in neurobiology. Pharmacol Biochem Behav 64:337–345

Cunningham KA, Appel JB (1987) Neuropharmacological reassessment of the discriminative stimulus properties of D-lysergic acid diethylamide (LSD). Psychopharmacology (Berl) 91:67–73

Fiorella D, Palumbo PA, Rabin RA, Winter JC (1995a) The time-dependent stimulus effects of *R*(−)-2,5-dimethoxy-4-methamphetamine (DOM): implications for drug-induced stimulus control as a method for the study of hallucinogenic agents. Psychopharmacology (Berl) 119:239–245

Fiorella D, Rabin RA, Winter JC (1995b) Role of 5-HT_{2A} and 5-HT_{2C} receptors in the stimulus effects of hallucinogenic drugs II: reassessment of LSD false positives. Psychopharmacology 121:357–363

Freedman DX (1961) Effects of LSD-25 on brain serotonin. J Pharmacol Exp Ther 134:160–166

Gaddum JH (1953) Antagonism between lysergic acid diethylamide and 5-hydroxytryptamine. J Physiol 121:15P

Gaddum J, Hammeed K (1954) Drugs which antagonize 5-hydroxytryptamine. Br J Pharmacol 9:240–248

Glennon RA, Young R, Rosecrans JA (1983) Antagonism of the effects of the hallucinogen DOM and the purported 5-HT agonist quipazine by 5-HT2 antagonists. Eur J Pharmacol 91:189–196

Glennon R, Titeler M, McKenney J (1984) Evidence for 5-HT_2 involvement in the mechanism of action of hallucinogenic agents. Life Sci 35:2502–2511

Hirschhorn ID, Winter JC (1971) Mescaline and lysergic acid diethylamide (LSD) as discriminative stimuli. Psychopharmacologia 22:64–71

Marona-Lewicka D, Kurrasch-Orbaugh DM, Selken JR, Cumbay MG, Lisnicchia JG, Nichols DE (2002) Re-evaluation of lisuride pharmacology: 5-hydroxytryptamine1A receptor-mediated behavioral effects overlap its other properties in rats. Psychopharmacology (Berl) 164:93–107

Newton RA, Phipps SL, Flanigan TP, Newberry NR, Carey JE, Kumar C, McDonald B, Chen C, Elliott JM (1996) Characterisation of human 5-hydroxytryptamine2A and 5-hydroxytryptamine2C receptors expressed in the human neuroblastoma cell line SH-SY5Y: comparative stimulation by hallucinogenic drugs. J Neurochem 67:2521–2531

Nielsen EB (1985) Discriminative stimulus properties of lysergic acid diethylamide in the monkey. J Pharmacol Exp Ther 234:244–249

Rosecrans JA, Lovell RA, Freedman DX (1967) Effects of lysergic acid diethylamide on the metabolism of brain 5-hydroxytryptamine. Biochem Pharmacol 16:2011–2021

Schechter MD (1997) Discrete versus cumulative dosing in dose-response discrimination studies. Eur J Pharmacol 326:113–118.

Schreiber R, Brocco M, Millan MJ (1994) Blockade of the discriminative stimulus effects of DOI by MDL 100907 and the "atypical" antipsychotics, clozapine, risperidone. Eur J Pharmacol 264:99–102

Smith RL, Barrett RJ, Sanders-Bush E (1999) Mechanism of tolerance development to 2,5-dimethoxy-4-iodoamphetamine in rats: down-regulation of the 5-HT2A, but not 5-HT2C, receptor. Psychopharmacology (Berl) 144:248–254

Smith RL, Barrett RJ, Sanders-Bush E (2003) Discriminative stimulus properties of 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane [(±)DOI] in C57BL/6J mice. Psychopharmacology (Berl) 166:61–68

Titeler M, Lyon R, Glennon R (1988) Radioligand binding evidence implicates the brain 5-HT2 receptor as a site of action for LSD and phenylisopropylamine hallucinogens. Psychopharmacology 94:213–216

Walker EA, Yamamoto T, Hollingsworth PJ, Smith CB, Woods JH (1991) Discriminative-stimulus effects of quipazine and l-5-hydroxytryptophan in relation to serotonin binding sites in the pigeon. J Pharmacol Exp Ther 259:772–782

Winter JC, Rabin RA (1988) Interactions between serotonergic agonists and antagonists in rats trained with LSD as a discriminative stimulus. Pharmacol Biochem Behav 30:617–624

Winter JC, Eckler JR, Rabin RA (2004) Serotonergic/glutamatergic interactions: the effects of mGluR2/3 receptor ligands in rats trained with LSD and PCP as discriminative stimuli. Psychopharmacology 172:233–240