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A Comparison of the Stimulus Effects of Morphine and Lysergic Acid Diethylamide (LSD)[1]

IRA D. HIRSCHHORN[2] AND JOHN A. ROSECRANS

Department of Pharmacology, Medical College of Virginia, Richmond, Va., 23298

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HIRSCHHORN, I. D. AND J. A. ROSECRANS. *A comparison of the stimulus effects of morphine and lysergic acid diethylamide (LSD).* PHARMAC. BIOCHEM. BEHAV. 2(3) 361–366, 1974. – Morphine and lysergic acid diethylamide (LSD) each was used as a discriminative stimulus for rats. After the injection of drug (morphine or LSD), depression of one lever of an operant test chamber resulted in positive reinforcement according to a variable interval schedule of 15 sec (VI-15 sec). When saline was given, responses on the opposite lever were reinforced. Discriminated responding occurred when either morphine or LSD served as the discriminative stimulus. When animals which were trained to discriminate morphine from saline were given LSD, they pressed predominantly the saline-correct lever. Similarly, LSD discrimination did not generalize to morphine. Two 5-hydroxytryptamine (5-HT) antagonists, cyproheptadine and methysergide, and one acetylcholine (Ach) antagonist, atropine, did not effect morphine or LSD discrimination. The narcotic antagonist, naloxone, blocked the stimulus effect of morphine, but did not alter LSD discrimination. These results indicate that the morphine and LSD stimuli are dissimilar and that the integrity of 5-HT or Ach nervous systems is not essential for morphine or LSD to serve as a discriminative stimulus.

Morphine Lysergic acid diethylamide (LSD) Discriminative stimulus Cyproheptadine Methysergide Atropine Naloxone

MANY DRUGS can serve as discriminative stimuli in laboratory animals. Among these are morphine [4,6] and lysergic acid diethylamide (LSD-25) [5]. These two drugs produce very different effects in man and in animals and belong to different pharmacological classes; morphine is classified as a narcotic analgesic and LSD as a hallucinogen. The mechanisms by which either drug produces its various effects are not known, but several investigators have suggested that at least some of the effects of both drugs are mediated through putative 5-hydroxytryptamine (5-HT) nervous systems in the brain [1, 7 8]. Previously, we reported that p-chlorophenylalanine (PCPA), a drug which markedly reduces the quantity of 5-HT in the brain, antagonized the discriminative stimulus effect of morphine [6]. In the present experiments, we sought to further test the hypothesis that the neuronal pathways which contain 5-HT must be functioning normally for morphine to serve as a discriminative stimulus, and to test whether the same condition is necessary for LSD discrimination. This hypothesis was tested by the administration of 5-HT receptor blockers to rats trained to discriminate between drug (LSD or morphine) and nondrug (saline) states. In addition, we compared the morphine and LSD stimuli in stimulus generalization tests and investigated the effects of atropine (cholinergic antagonist) and naloxone (narcotic antagonist) on the morphine and LSD cues.

METHOD

Animals

Male Sprague-Dawley rats (Flow Research Animals, Dublin, Va.) were approximately 9 weeks old at the beginning of the experiments. They were housed in individual cages in air-conditioned quarters with an automatically timed cycle of 12 hr of light and 12 hr of darkness. The animals were maintained at 70–80% of their expected free feeding weights by adjusted feedings following each experimental session. Water was freely available in the home cages.

Procedure

Discrimination training procedures were similar to those previously described [5]. First, animals were trained to press both bars of a standard operant test chamber (Lehigh Valley Electronics). The reinforcer was sweetened condensed milk diluted 2:1 with tap water. After bar pressing was established on both levers, drug administration began. Each daily session was preceded by the injection of either a drug or normal saline. Depression of one of the 2 levers resulted in reinforcement after the administration of drug (morphine or LSD) and responses on the opposite lever were reinforced following saline. For one-half of the animals in

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any group, the right lever was reinforced when drug was given and the left lever was correct following saline; these conditions were reversed for the remaining animals. Discrimination training began with 4 preliminary training sessions of 15 min duration in which drug and saline administration were alternated daily and each correct bar press resulted in reinforcement. Subsequent sessions, also of 15 min duration, were composed of an initial 2.5 min period during which no responses were reinforced and a latter 12.5 min period in which responses on the correct lever were reinforced according to a variable interval schedule of 15 seconds (VI-15 sec). During these sessions, 2 days of drug treatment were followed by 2 days of saline treatment (double alternation). Two groups of 6 rats each were used. One group received LSD tartrate (0.15 μ mol/kg) and normal saline as the 2 treatments and the other received morphine sulphate (10 mg/kg) and saline. LSD was administered 5 min before the session and morphine was given 45 min before the session. Saline was administered at a time before the session which corresponded to the time of administration of either drug, respectively.

Drug interaction and stimulus generalization experiments were accomplished in sessions designated as test sessions. After 40 discrimination training sessions, discriminated responding was relatively stable. The same animals continued to receive morphine and saline or LSD and saline according to a double alternation sequence. However, test sessions were now interposed among discrimination training sessions. Test sessions were sessions of 2.5 min duration in which no responses were reinforced. An odd number of training sessions, generally 3, separated any 2 test sessions.

Drugs

LSD tartrate was obtained from the National Institutes of Mental Health. All other drugs were obtained from commercial sources. Cyproheptadine HCL (50 mg) was dissolved in 0.5 ml of absolute ethanol. This was diluted with 0.7 ml of a polyethoxylated vegetable oil-absolute alcohol vehicle [2] and then with 3.3 ml of 0.9% sodium chloride to make a solution of 10 mg/ml, which was further diluted with normal saline to make solutions of lesser concentrations. All other drugs were dissolved in 0.9% sodium chloride. With the exceptions of atropine sulfate and LSD tartrate, which were calculated as free bases (0.15 μ moles of LSD = 72 μg), all drug doses refer to the salts. Drugs were injected intraperitoneally in a volume of 1 ml/kg with the exception of atropine sulfate which was administered subcutaneously in the same volume.

RESULTS

The development of discriminated responding when either LSD or morphine was used as a discriminative stimulus is shown in Fig. 1. When the data are represented as they are in this figure, discriminated responding is manifested by a greater percentage of responses on the drug-correct lever following the administration of drug than after saline. Thus, discriminated responding was evident from the first session block when LSD and saline were the discriminative stimuli (70% LSD-correct responses after LSD; 47% LSD-correct responses after saline) and from the third session block when morphine and saline were the stimuli (64% morphine-correct responses after morphine; 23% morphine-correct responses after saline). Wilcoxon's signed ranks test for paired observations [3], when applied to all 10 session blocks, indicates that the discriminated responding is significant in both cases ($p<0.01$, 2-tail).

Figure 2 represents the data obtained when animals which had been trained to discriminate morphine and saline were given various doses of LSD and those which had been trained with LSD and saline were given morphine. Each dose of morphine tested in the LSD-trained animals (Fig.

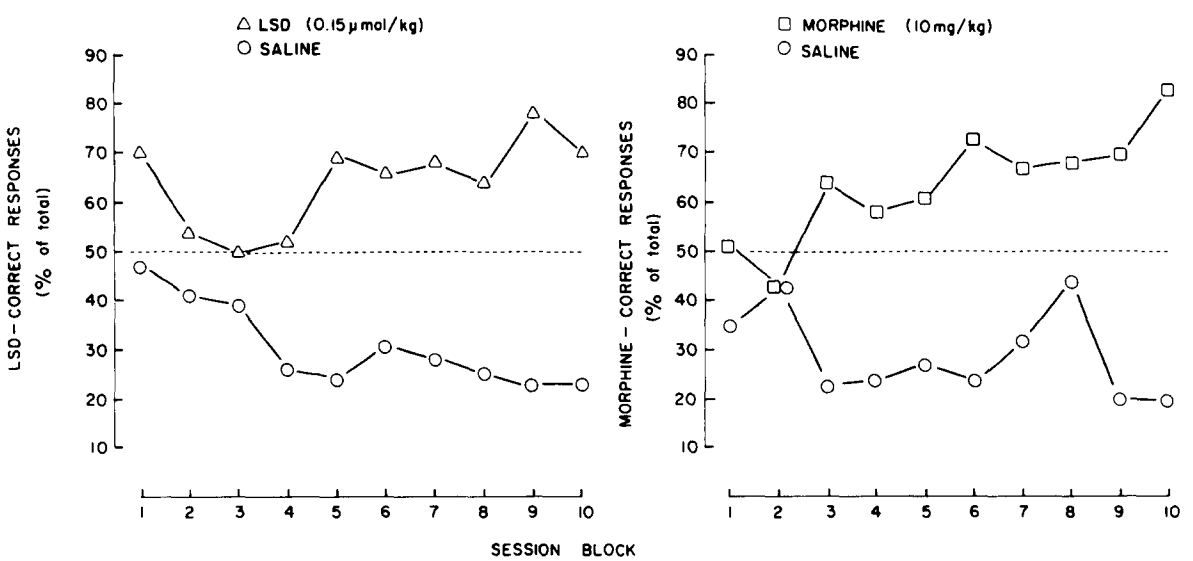

FIG. 1. Discriminated responding following the administration of LSD (a) or morphine (b). One group of 6 rats received 0.15 μ mol/kg of LSD or saline 5 min before the session. A second group of 6 rats were given 10 mg/kg of morphine or saline 45 min before the session. On any given day, one-half of the animals of either group were given drug and the remainder given saline. Ordinate: number of responses in the first 2.5 min of the session on the LSD or morphine-correct lever expressed as a percentage of total response. Abcissa: successive blocks of 4 sessions.

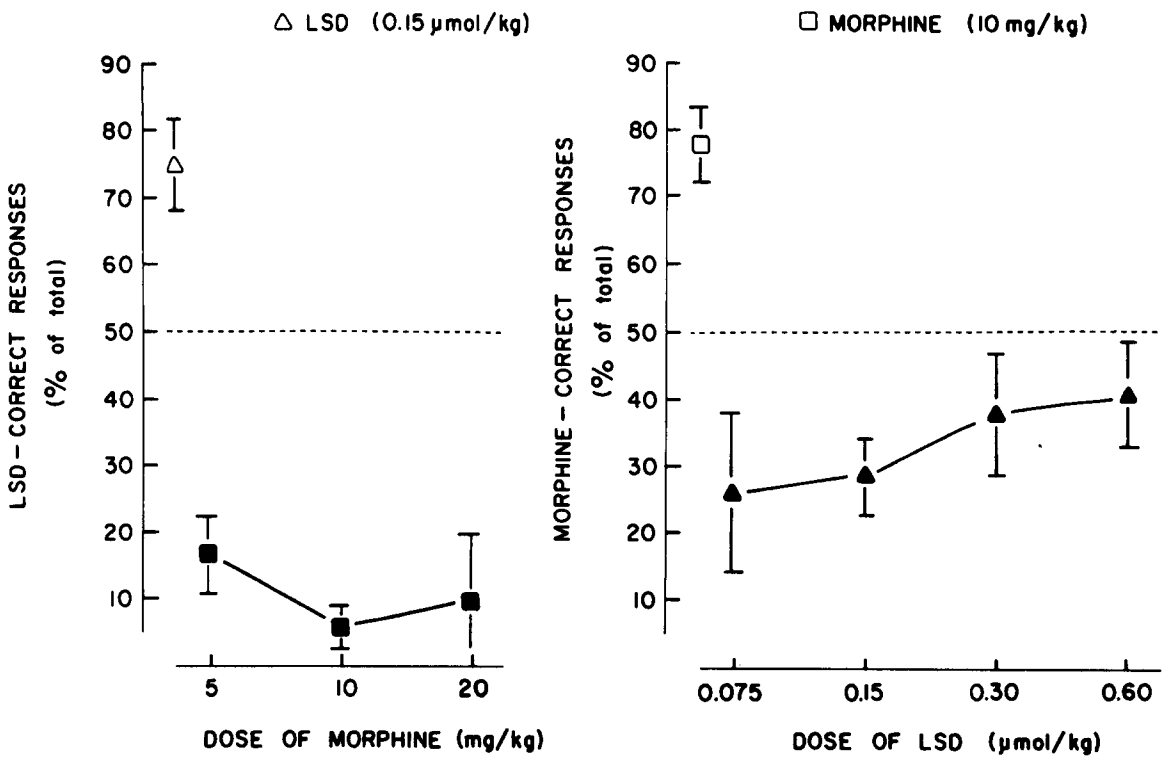

FIG. 2. Generalization of discriminated responding following the administration of LSD (a) or morphine (b). Two and one-half min test sessions during which no responses were reinforced were interposed among discrimination training sessions subsequent to those represented by Fig. 1. Animals which learned to discriminate LSD and saline received various doses of morphine 45 min before the test sessions. Those animals which discrimined morphine and saline were given LSD 5 min before the session. Each point is the mean of two determinations in each of 6 animals. Vertical lines indicate + S.E.M. The open triangle and open square represent responding following LSD in LSD-trained animals and after morphine in morphine-trained animals, respectively. Ordinate: number of responses on LSD- or morphine-correct lever expressed as a percentage of total responses. Abcissa: Dose of morphine sulfate or LSD tartrate plotted on a log scale.

2a) produced responding appropriate to saline treatment, i.e. a very low percentage of LSD-correct responses. Increasing doses of LSD appear to have resulted in a concomitant increase in the percentage of morphine-correct responses made by the morphine-trained animals (Fig. 2b). These data suggest that a higher dose of LSD might produce a majority of responses on the morphine-correct lever. However, depression of response rate prohibited the testing of a higher dose of LSD.

Figure 3 indicates that atropine did not decrease discriminated responding following LSD (Fig. 3a) or morphine (Fig. 3b). In fact, a slight increase in discrimination after each drug is suggested by the data. Atropine caused an increased variability in responding as indicated by larger standard errors, following saline administration, but no consistent change in lever choice pattern was noted.

The results obtained with cyproheptadine and methylsergide are shown in Table 1 and Fig. 4, respectively. Neither 5-HT antagonist markedly altered LSD or morphine discrimination. Data from only one dose of cyproheptadine, 3 mg/kg, are shown because the next higher dose tested, 6 mg/kg, severely depressed the response rate. No effect of methysergide on lever choice after saline injection was observed, but as in the case of atropine an increase in variability was apparent.

Naloxone (Fig. 5) caused a decrease in the percentage of morphine-correct responses following morphine and the magnitude of the decrease was proportional to the dose of naloxone. LSD discrimination was not greatly changed by naloxone nor was responding following saline administration in either group of animals.

The absolute rates of responding after the various drug pretreatments are presented in Table 2. During discrimination training (no pretreatment), the response rates under the drug and saline conditions were similar in each group. Both decreases and increases in response rate were observed after drug pretreatments. These changes of response rate had no apparent effect upon lever choice.

DISCUSSION

Morphine or LSD can serve as a discriminative stimulus in the rat when either drug state is paired with the injection of saline. The development of discriminated responding followed a similar time course for both drugs (Fig. 1). Thus, the doses of LSD and morphine used in the present study appear to be approximately equal in terms of their ability to produce discriminated responding. However, the results of stimulus generalization and drug interaction experiments

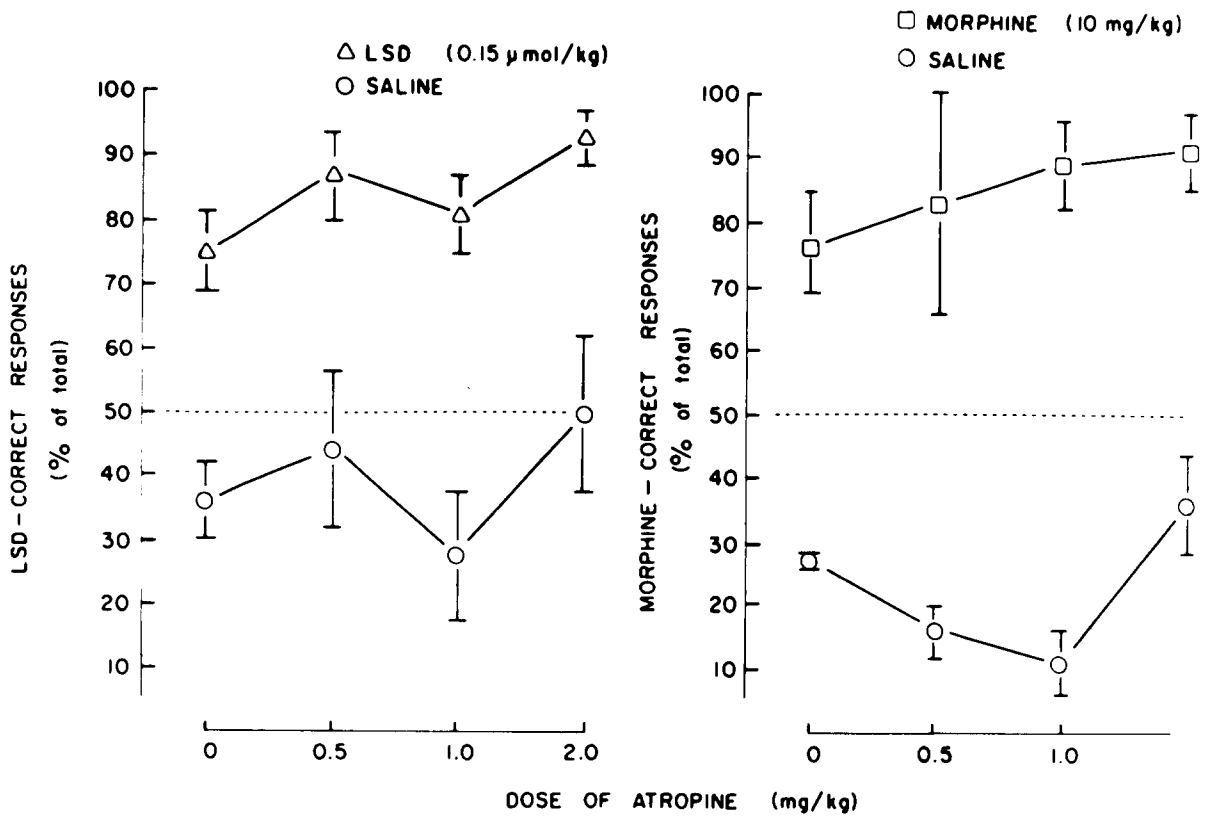

FIG. 3. Effect of atropine on LSD and morphine discrimination. Atropine sulfate was injected 15 min prior to test session. Each point is the mean of one determination in each of 6 animals. Abcissa: Dose of atropine plotted on a log scale. Test sessions and other details are as described in Fig. 2.

TABLE 1

EFFECT OF CYPROHEPTADINE HCL* ON LSD OR MORPHINE DISCRIMINATION

	Dose of Cyproheptadine (mg/kg) 0	3
	LSD-correct responses (% of total)	
LSD‡	87.2 ± 4.2†	90.6 ± 9.4
Saline	30.9 ± 3.0	17.4 ± 10.1
	Morphine-correct responses (% of total)	
Morphine§	98.4 ± 1.3	97.5 ± 2.5
Saline	20.4 ± 4.5	7.2 ± 4.6

*Cyproheptadine was administered, i.p., 1 hr before session. Time of administration of LSD, morphine and saline were same as for discrimination training.

†Data are presented as mean ± S.E.M.

‡n = 5

§n = 6

indicate that the stimulus effects of morphine and LSD are, in other ways, very different.

When animals which were trained to discriminate LSD from saline were given various doses of morphine, they pressed predominantly the saline-correct bar (Fig. 2a). Similarly, LSD produced saline-appropriate responding in animals which had learned to discriminate morphine and saline (Fig. 2b). These data provide no evidence for stimulus generalization between morphine and LSD. These results are not surprising in view of the fact that, in man, the perceived effects of these two drugs are very dissimilar.

Neither cyproheptadine or methysergide, both of which are 5-HT antagonists, nor atropine, an anticholinergic agent, blocked morphine or LSD discrimination. The only drug which did alter discriminated responding in the present study was naloxone. This narcotic antagonist clearly blocked the stimulus effect of morphine, but did not effect LSD discrimination. The present finding that 5-HT antagonists do not effect the morphine or LSD stimulus does not support the hypothesis that the neuronal pathways which contain 5-HT must be functioning normally for morphine or LSD to serve as a discriminative stimulus. The results with morphine are in disagreement with the results of a previous study [6] in which parachlorophenylalanine (PCPA), a depletor of 5-HT, blocked morphine discrimination. However, although it is known that these drugs antagonize the effects of 5-HT in the periphery and that they can penetrate the blood brain barrier, the extent to which cyproheptadine

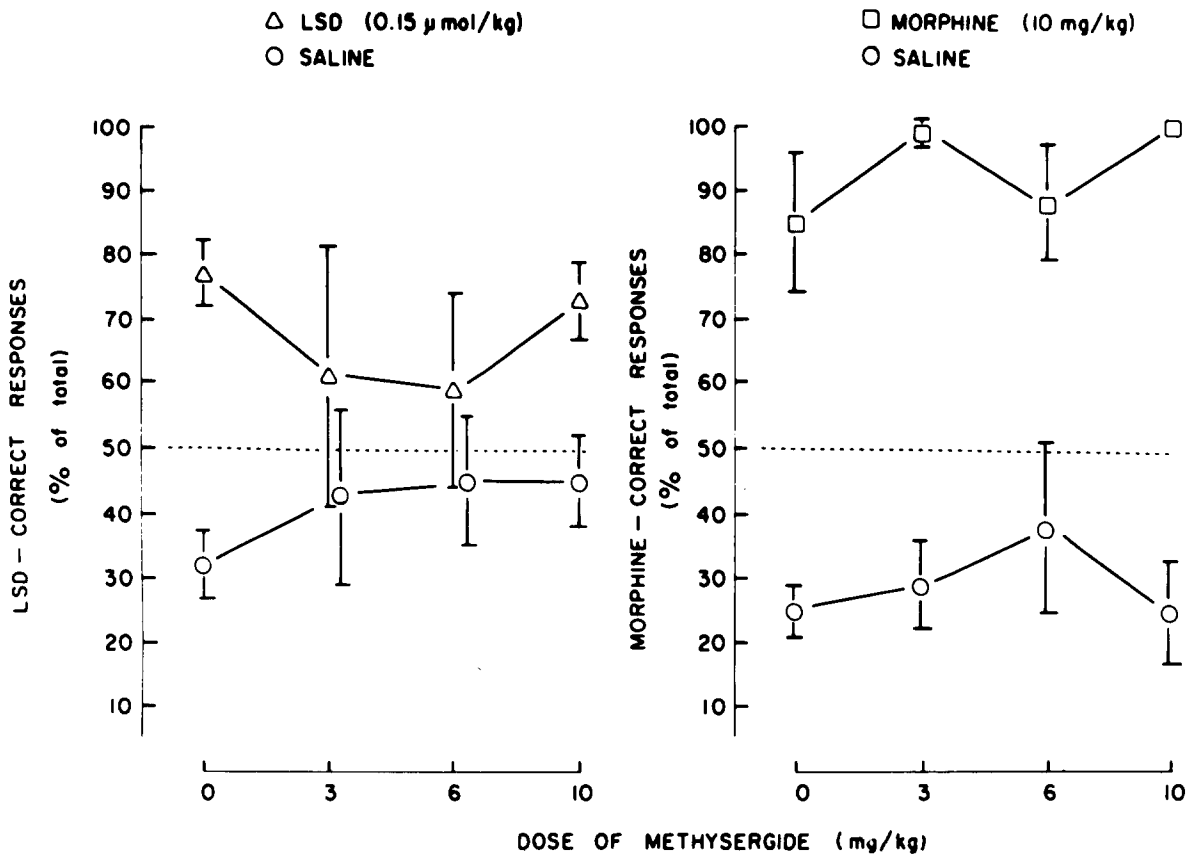

FIG. 4. Effect of methysergide on LSD and morphine discrimination. Methysergide was injected 35 min before the test session. Each point is the mean of one determination in each of 6 animals. Abcissa: Dose of methysergide plotted on a log scale. Test sessions and other details are as in Fig. 2.

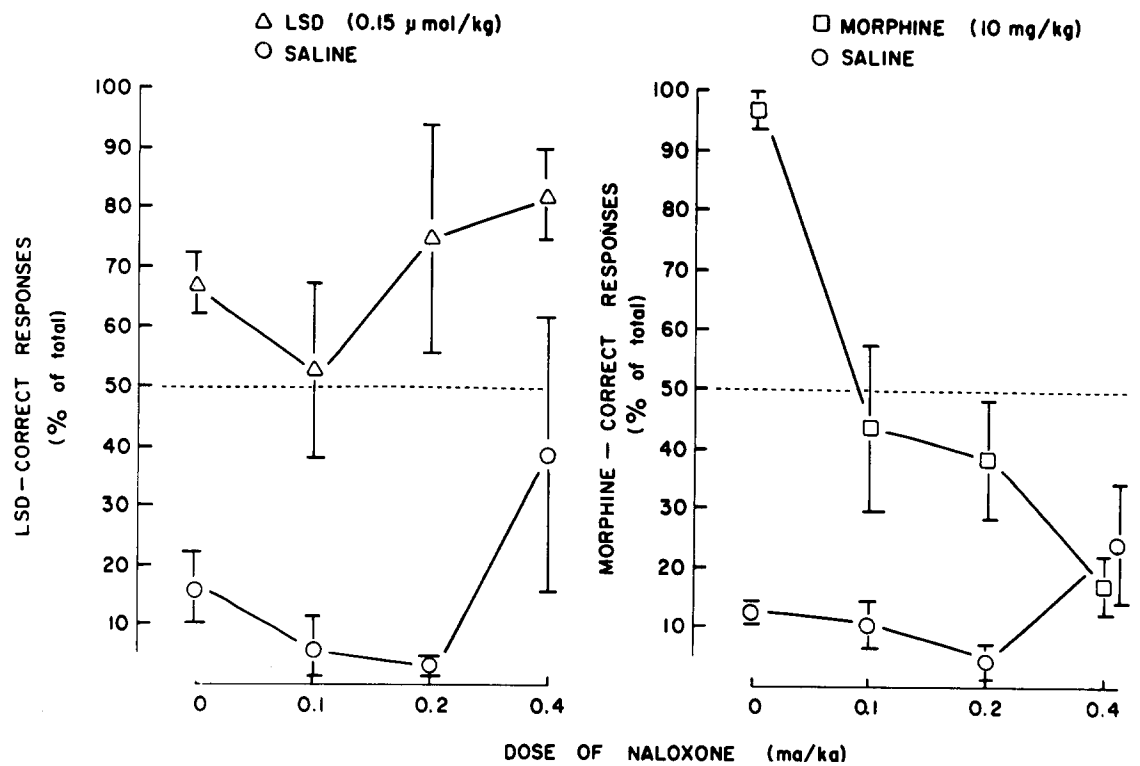

FIG. 5. Effect of naloxone on discriminated responding following the administration of LSD or morphine. Naloxone HCL was injected 30 min before the session. Each point is the mean of one determination in each of 5 animals in the LSD group and 6 animals in the morphine group. Abcissa: Dose of naloxone HCL plotted on a log scale. Test sessions and other details are as in Fig. 2.

TABLE 2

EFFECT OF DRUG PRETREATMENTS ON RATE OF RESPONDING IN FIRST 2.5 MIN OF THE SESSION (UNREINFORCED)

Pretreatment (mg/kg)		Replications	Rate (responses/min) Saline	Morphine (10 mg/kg)	Saline	LSD (0.15 μmol/kg)
none		20	8.2	8.2	5.3	4.2
atropine	(0.5)	1	7.3	3.2	3.0	4.1
	(1.0)	1	7.1	5.0	3.7	5.6
	(2.0)	1	7.5	2.3	5.3	7.5
methysergide	(3)	1	10.3	10.2	4.3	2.1
	(6)	1	5.9	3.3	7.4	4.5
	(10)	1	6.0	4.8	6.5	2.8
cyproheptadine	(3)	1	10.6	5.3	2.3	1.6
naloxone	(0.1)	1	19.6	13.7	5.2	7.5
	(0.2)	1	20.3	8.6	4.6	6.0
	(0.4)	1	11.2	11.1	3.5	2.1

and methysergide actually block 5-HT receptors in the brain is not known. In addition, it cannot be determined whether the blockade of morphine discrimination in the earlier study was caused by 5-HT depletion or by some other effect of PCPA. Problems such as these probably contribute to the contradictory results which are common in experiments investigating the relationship between putative neurotransmitter systems and drug effects. Such conflicting results are well illustrated by the literature on morphine [9]. Thus, although the present study does not support the hypothesis that the stimulus effects of morphine and LSD are mediated by 5-HT containing neurons, neither does it entirely refute this possibility. Perhaps the development of more specific and clearly defined methods of altering the function of serotonergic neurons will contribute to answering this question.

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