Lysergic Acid Diethylamide (LSD) and Lisuride: Differentiation of Their Neuropharmacological Actions

Abstract. *The nonhallucinogenic ergot derivative lisuride exerts many pharmacological effects that are similar to those of its hallucinogenic congener, lysergic acid diethylamide (LSD). Animals trained to discriminate between the presence of one drug and the other can be used to differentiate the actions of these compounds on a neuronal level. The discriminative stimulus effect of LSD (the LSD cue) is similar to that of the serotonin agonist quipazine, whereas the lisuride cue is similar to that of the dopamine agonist apomorphine. These data support the hypothesis that serotonin is intricately involved in the hallucinogenic effects of LSD.*

The potent hallucinogenic effects of lysergic acid diethylamide (LSD) have long been attributed to alterations in the functioning of central monoamine neurotransmitters, particularly serotonin [5-hydroxytryptamine (5HT)] (*1*). Recently, however, some investigators have questioned this hypothesis (*2*). Strikingly similar decreases in rat brain 5HT turnover produced by LSD and lisuride hydrogen maleate (LHM), a structurally related but nonhallucinogenic ergot derivative (*3*), led Pieri *et al.* (*2*) to conclude that ". . . the biochemical changes induced by both lisuride and LSD may solely represent an epiphenomenon unrelated to the hallucinosis." Other experiments have also demonstrated similar (serotonergic) actions of LSD and LHM including ability to (i) depress the discharge rate of 5HT-containing neurons in the rat dorsal raphe nucleus (*4*); (ii) inhibit the specific binding of tritiated LSD and 5HT to homogenates of rat or rabbit brain frontal cortex (*5*); and (iii) elicit the "serotonin syndrome" in rats (*6*). Indeed, the differences in the effects of LSD and LHM on any of the monoaminergic systems that have been described are primarily quantitative; yet LSD is a potent hallucinogen and LHM is not. If these systems are involved in hallucinogenic drug action, qualitative differences in their response to LSD and LHM must not only exist but should indicate actions essential for hallucinosis.

We used a drug discrimination procedure to distinguish between the "states" produced by LSD and LHM. Animals are trained to discriminate between the presence and absence of a drug (drug versus saline) or the presence of one drug or another drug (drug versus drug). The drug discrimination procedure has several advantages over other behavioral procedures used to study drug actions. (i) It is exquisitely sensitive to low doses of drugs (*7*). (ii) It is specific within pharmacological classes and to particular neuronal actions (*8, 9*). (iii) It is reliable and stable over time (*9*). (iv) It is the only animal behavior that parallels verbal reports of drug effects in humans (*9*). Using the drug discrimination procedure and, in particular, a drug versus drug discrimination (*10*), we have compared the discriminative stimulus effects of LSD and LHM. We report that, although LSD and LHM produce similar effects in rats, the neuronal substrates of these effects can be differentiated in that the LSD discriminative stimulus is mediated primarily by central 5HT neurons, whereas the LHM discriminative stimulus is mediated primarily by central dopamine neurons.

In experiment 1, two groups of water-deprived male rats were trained to discriminate either LSD at 0.08 mg/kg (N = 24) or LHM at 0.08 mg/kg (N = 39) from saline (*11*). On any session, they were given an intraperitoneal injection of either a training drug or saline 15 minutes before being placed in experimental chambers containing two levers. Initially, each rat was trained to respond following an injection of saline by being given water each time it pressed one lever [fixed ratio 1 (FR 1)]; similar training was conducted on the other lever after LSD or LHM. The schedule on each lever was increased gradually to FR 32 (that is, 32 lever presses were required for each water reinforcer). Discrimination training was then begun on two levers; for half of the animals, responding on the right lever following drug and on the left lever following saline was correct; for the other half, the conditions were reversed. Before the first reinforcer, the only cue the animal can use to determine the correct lever is the drug-induced state. For this reason, the ability to discriminate was always assessed before the first reinforcer was given or, in test sessions, during extinction periods in which no water was delivered, and the session ended after 32 responses had occurred on one of the levers. After the LSD and LHM discriminations were acquired, each group of rats was divided into three equal subgroups (8 in each subgroup for LSD discrimination and 13 in each subgroup for LHM discrimination). Animals in each subgroup were trained to discriminate 0.02, 0.08, or 0.32 mg/kg of drug by progressively altering the dose (*11, 12*). Thus, at the end of training (55 sessions), the rats were accurately discriminating a wide range of doses of LSD or LHM from saline.

Animals trained to discriminate a particular dose of drug (LSD or LHM) from saline were given various doses of LHM and LSD to assess the similarity of the two ergots (Fig. 1). In each group, the percentage of responding on the lever

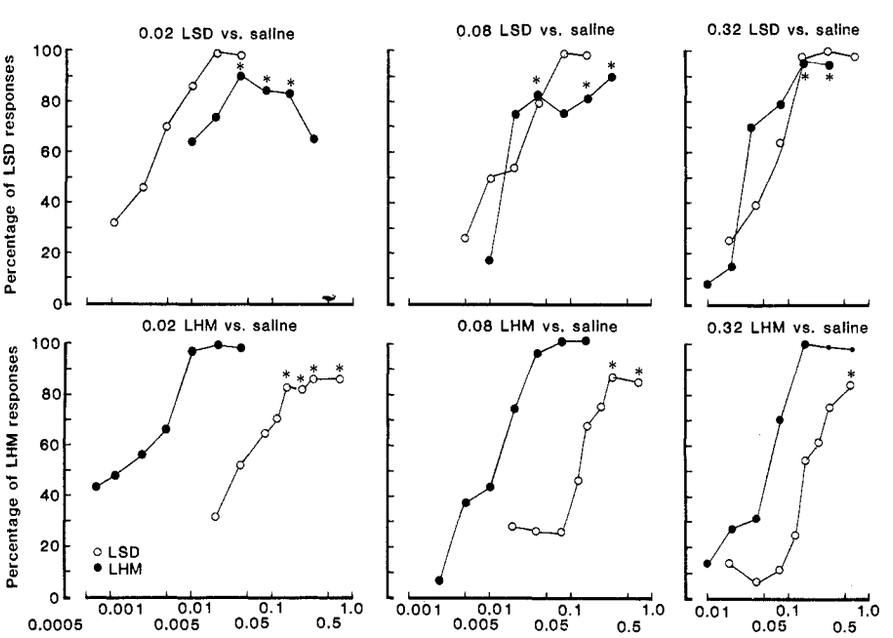

Fig. 1. Results of substitution tests with LSD and LHM in groups of rats trained to discriminate one of three doses (0.02, 0.08, or 0.32 mg/kg) of either LSD (top) or LHM (bottom) from saline. Asterisks represent significant substitutions (*13*).

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appropriate for drug elicited by at least one dose of the novel drug was significant (*13*). Nevertheless, the extent of responding following the novel drug on the lever appropriate for drug (substitution) was usually less than that for the training drug. These data led us to examine the possibility that the stimulus effects of LSD and LHM might be sufficiently different to allow rats to discriminate between them. Because rats can learn to discriminate quantitative differences in drug effects (*10, 14*), three groups of rats (eight rats in each group) were trained to discriminate between LSD at 0.08 mg/kg and LHM at 0.02, 0.04, or 0.08 mg/kg, respectively. These doses were chosen on the basis of experiment 1 and previous results (*4–6*) indicating that LHM is one to five times as potent as LSD. The methods were the same as those described previously, except that an FR 16 schedule was used.

Each of the three groups learned to discriminate LSD from LHM; the mean number of sessions to attain a criterion of 85 percent correct for five consecutive sessions depended on dosage ratio: 21, 16, and 14 sessions in the groups receiving LHM at 0.02, 0.04, and 0.08 mg/kg, respectively. Substitution tests were then conducted with saline, LSD, LHM, the dopamine agonist apomorphine, and the 5HT agonist quipazine (Fig. 2). In the group receiving LHM at 0.08 mg/kg (Fig. 2, bottom), the discrimination seemed to be based on the quantitative difference of amount of drug effect; that is, at equivalent doses, LHM is much more potent than LSD. Thus, during tests with a relatively high dose of LHM, responding occurred primarily on the lever appropriate for LHM, whereas during tests with low doses of LHM, most responding occurred on the lever appropriate for LSD. In contrast, in the other groups, the discriminations were based on qualitative differences between the effects of LSD and LHM; that is, during saline tests and tests with low doses of LSD, choices were not based on the drug cue (chi-square test). As the dose of LSD increased, the percentage of responding on the LSD lever increased to levels above those expected by chance. Discrimination of all doses of LHM was nearly perfect. As the dose of quipazine increased, rats responded as though they had received LSD; as the dose of apomorphine increased, they responded as though they had received LHM.

Thus, the effects of LSD and LHM are sufficiently different to enable rats to discriminate between them. Observations that the 5HT agonist quipazine elicits responding appropriate for LSD, whereas the dopamine agonist apomorphine elicits responding appropriate for LHM, suggest that the discriminative stimulus effects of LSD are mediated primarily by 5HT receptors, whereas those of LHM are mediated primarily by dopamine receptors. This interpretation is supported by data demonstrating that (i) 5HT agonists substitute completely for LSD, but only partially for LHM; (ii) 5HT antagonists block the LSD discriminative stimulus, but not the LHM discriminative stimulus; (iii) dopamine agonists substitute completely for LHM, but not at all for LSD; and (iv) dopamine antagonists block the LHM discriminative stimulus but not the LSD discriminative stimulus (*11*). This does not mean that LSD and LHM act only at 5HT and dopamine receptors, respectively, but does imply that the discriminative stimulus effects of these drugs are mediated primarily by actions on one or the other of these neuronal systems.

Thus, quantitative differences between the actions of LSD and LHM (*1–6*) can result in qualitative differences in the states produced by these ergots. Our results parallel those seen in humans in that LHM exerts much greater potency as a dopamine agonist (in Parkinsonism) and does not generally produce LSD-like hallucinogenic reactions (*15*). These comparisons of LSD and LHM have important implications for current theories of the action of hallucinogenic drugs. First, the fact that the two ergots exert similar effects on 5HT turnover suggests that drug-induced decreases in 5HT turnover are not sufficient for inferring hallucinogenic efficacy. Second, since LHM is more potent than LSD at depressing raphe neuronal firing, actions at postsynaptic 5HT receptors may be at least as important as the raphe in determining hallucinogenic potency. This is also suggested by recent findings that LSD and other hallucinogens sensitize some postsynaptic 5HT receptors to the actions of 5HT, whereas LHM and other nonhallucinogenic ergots do not (*16*). Third, since our findings support those indicating that LHM is as potent as LSD at 5HT receptors (*2, 4–6*) and much more potent than LSD at dopamine receptors (*2, 5, 6, 11, 17*), the recent hypothesis that potency of hallucinogenic effects is determined by synergistic actions at 5HT and dopamine receptors (*18*) cannot be correct. Finally, the data confirm the long-standing theory that the actions unique to hallucinogenic drugs such as LSD are mediated by central 5HT neuronal systems.

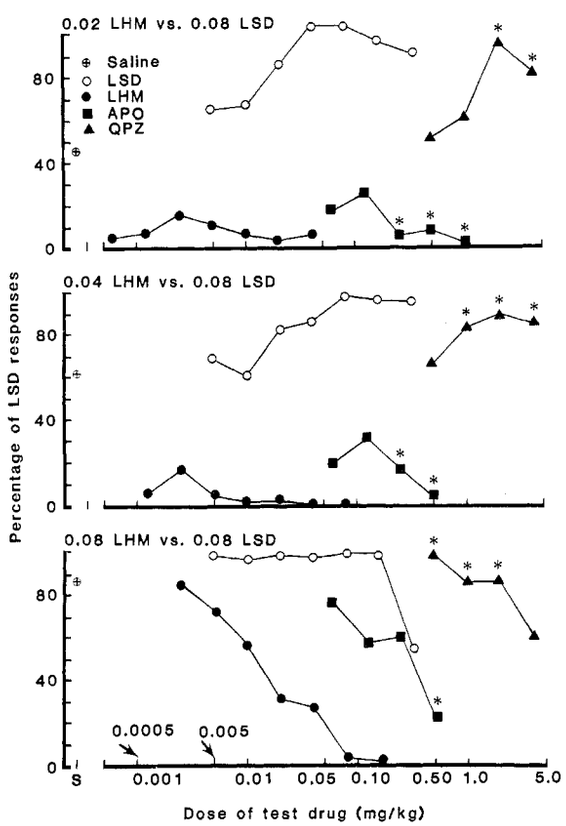

Fig. 2. Results of substitution tests with LSD, LHM, quipazine (*QPZ*), and apomorphine (*APO*) in rats trained to discriminate LSD (0.08 mg/kg) from either LHM at 0.02 mg/kg (top), 0.04 mg/kg (middle), or 0.08 mg/kg (bottom). Note that zero percent LSD responses is equivalent to 100 percent LHM responses. Asterisks represent significant substitution of quipazine for the LSD cue, or significant substitution of apomorphine for the LHM cue ($P < .05$, *t*-test for correlated measures).

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References and Notes

1. For review, see D. X. Freedman and A. E. Halaris, in *Psychopharmacology: A Generation of Progress*, M. A. Lipton, A. DiMascio, K. F. Killam, Eds. (Raven, New York, 1978), p. 347.
2. L. Pieri, M. Pieri, W. Haefely, *Nature (London)* **252**, 586 (1978).
3. W. M. Herrmann, R. Horowski, K. Dannehl, U. Kramer, K. Lurati, *Headache* **17**, 54 (1977); R. Horowski, *Eur. J. Pharmacol.* **51**, 157 (1978).
4. M. A. Rogawski and G. K. Aghajanian, *Life Sci.* **24**, 1289 (1979); J. R. Walters, M. D. Baring, J. M. Lakoski, in *Dopaminergic Ergot Derivatives and Motor Function*, K. Fuxe and D. B. Calne, Eds. (Pergamon, New York, 1979), p. 207.
5. K. Fuxe, B. B. Fredholm, S. Ogren, L. F. Agnati, T. Hokfelt, J. Gustafsson, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **37**, 2181 (1978); M. R. Rosenfeld and M. K. Makman, *J. Pharmacol. Exp. Ther.* **216**, 526 (1981); E. K. Silbergeld, R. E. Hruska, R. Weir, S. Kennedy, in *Dopaminergic Ergot Derivatives and Motor Function*, K. Fuxe and D. B. Calne, Eds. (Pergamon, New York, 1979), p. 223.
6. E. K. Silbergeld and R. E. Hruska, *Psychopharmacology* **65**, 233 (1979).
7. J. B. Appel, F. J. White, D. M. Kuhn, in *Stimulus Properties of Drugs: Ten Years of Progress*, F. C. Colpaert and J. A. Rosecrans, Eds. (Elsevier/North-Holland, Amsterdam, 1978), p. 7.
8. H. Barry III, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **33**, 1814 (1974); J. A. Rosecrans and R. A. Glennon, *Neuropharmacology* **18**, 981 (1979).
9. R. A. Glennon and J. A. Rosecrans, *Neurosci. Biobehav. Rev.* **5**, 197 (1981).
10. H. Barry III and E. C. Krimmer, *Neuropharmacology* **18**, 991 (1979); J. A. Goas and J. E. Boston, *Pharmacol. Biochem. Behav.* **8**, 235 (1978); D. A. Overton, *Psychopharmacology* **53**, 195 (1977).
11. F. J. White and J. B. Appel, *Psychopharmacology*, in press; *J. Pharmacol. Exp. Ther.*, in press.
12. F. C. Colpaert, C. J. E. Niemegeers, P. A. J. Janssen, *J. Pharmacol. Exp. Ther.* **212**, 474 (1980); D. A. Overton, *Science* **205**, 720 (1979).
13. These data were analyzed with Dunnett's procedure for comparing all means to a single control mean with $P < .05$ as the error rate between experiments [C. W. Dunnett, *Biometrics* **20**, 482 (1964)]. A significant substitution means that the percentage of responding on the drug lever produced by the novel drug was not significantly different from that obtained with the training drug.
14. D. A. Overton, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **33**, 1800 (1974); J. B. Trost and D. P. Ferraro, in *Neurobiology and Influences on Behavior*, J. M. Singh and H. Lal, Eds. (Stratton, New York, 1974), p. 223.
15. G. Gopinathan *et al.*, *Neurology* **31**, 371 (1981).
16. R. B. McCall and G. K. Aghajanian, *Life Sci.* **26**, 1149 (1980).
17. W. Kehr, *Eur. J. Pharmacol.* **41**, 261 (1977); ——— and W. Speckenbach, *Naunyn-Schmeideberg's Arch. Pharmacol.* **301**, 163 (1978).
18. B. L. Jacobs, M. A. Trulson, A. D. Stark, G. R. Christoph, *Commun. Psychopharmacol.* **1**, 243 (1977); M. E. Trulson and B. L. Jacobs, *Science* **205**, 515 (1979).

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Electric Pulse–Induced Fusion of 3T3 Cells in Monolayer Culture

Abstract. *Swiss mouse 3T3-C2 fibroblasts, grown to confluence in monolayer culture, are shown to fuse when exposed to electric fields. Exposure to five repetitive electric pulses of about 1 kilovolt per centimeter with a duration of 50 microseconds caused approximately 20 percent of the cells to become fused (multinucleate) when 1 millimolar magnesium was present in the medium. The effects of minimum thresholds of field strength, pulse duration, and number of pulses were determined. Cell disruption was observed when the electric field exceeded 2.0 kilovolts per centimeter or the pulse was of longer duration than 120 microseconds.*

The major nonthermal effect of an electric field on a closed bilayer vesicle is the induction of a membrane potential (*1*). When cells are clustered, as during the development of the mold *Dictyostelium* or in Pronase-treated red cell ghosts that have been stacked by dielectrophoresis, strong electric pulses induce fusion of cell membranes (*2*). In this report we present evidence that this phenomenon is a general property of cells in close contact. No chemical or physical treatments are required. The technique may be extended to different types of cells, and fusions have also been observed with human myeloma and *Dictyostelium* cells in our laboratory (*3*). The efficiency of cell fusion induced by voltage pulses is generally high (10 to 20 percent), and experiments with 3T3 cells suggest that the fused cells are viable. Thus the technique may find application in the study of genetic transfer between cells.

The electric pulses were generated by a Cober 605 pulse generator connected to two electrodes. The signal was monitored as previously described (*4*). The electrodes were either two platinum wires or two stainless steel razor blades, parallel to each other at a distance of 3 mm. The electrodes were immersed in the cell-bathing buffer and seated firmly against the bottom of the plastic cell-culture dish; the polarity of the electrodes could be reversed by a switch. The temperature increase due to Joule heating was estimated to be less than 2°C. The initial temperature was 22° ± 2°C. Square wave pulses of controlled voltage and duration could be applied to the cell monolayers and the impulses repeated at intervals ranging from 1 to 15 seconds.

Swiss mouse 3T3-C2 fibroblasts were grown on plastic dishes as described (*5*) and were used in experiments 2 to 8 days after they reached confluence. For the electric pulse experiments, the culture medium was replaced with 3.5 ml of 272 m*M* sucrose in 7 m*M* phosphate buffer (*p*H 7.2), with or without 1 m*M* $MgSO_4$. This change of medium reduced ionic strength and, thus, the Joule heating of the cultured cells. After the cells had been treated with the electric pulses the sucrose solution was removed and replaced with the culture medium. The pulsed cells were then returned to the incubator (37°C; 95 percent air and 5 percent CO_2) for at least 2 hours. The monolayers were subsequently washed with phosphate-buffered saline (PBS), fixed with methanol, and stained with Giemsa blue. Cells were scored as fused if a continuous membrane surrounded at least two Giemsa blue–stained nuclei as viewed by phase-contrast microscopy. The viability of the pulsed and control cells was routinely monitored by trypan blue exclusion and by the incorporation of tritiated leucine into pulsed cells. The monolayers were incubated with 200 μCi of L-[4,5-^{3}H(N)]leucine (New England Nuclear) for 2 hours at 37°C. The cells were then extensively washed with PBS and autoradiographed with Ilford L4 emulsion for 1 week at −70°C.

When an electric pulse of 1.6 kV/cm

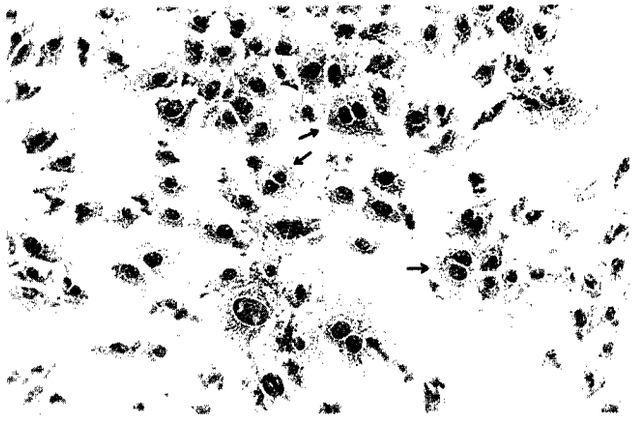

Fig. 1. Electric field–induced fusion of 3T3-C2 fibroblasts in monolayer culture. The cells were treated with five electric pulses of 1.6 kV/cm field strength and 100-μsec duration. A significant fraction of cells fused. Several binucleate cells are identified (see arrows for examples of fused cells). Fused cells were judged viable by the criteria discussed in the text.

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