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The Effect of Serotonin Depletion on the Discriminability of LSD[1]

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WHITE, F. J., M. A. SIMMONS, K. B. WEST, A. M. HOLOHEAN AND J. B. APPEL. *The effect of serotonin depletion on the discriminability of LSD.* PHARMAC. BIOCHEM. BEHAV. 13(4) 569–574, 1980.—Nine groups of rats were trained to discriminate LSD (0.12 mg/kg) from saline in a two-lever, water-reinforced, drug discrimination procedure. After stable discriminative performance was obtained (>95% correct), groups were administered one of several treatments which lower the concentration of serotonin (5-HT) in brain: (1) 12.5, 25, 50, 100 or 200 μg of 5,7-dihydroxytryptamine (5,7-DHT) intraventricularly (IVT); (2) 3×100 mg/kg of p-chlorophenylalanine (PCPA) intraperitoneally (IP); or (3) 20 mg/kg of p-chloroamphetamine (PCA) IP. Control rats received either IVT injections of 5,7-DHT vehicle or IP injections of PCA or PCPA vehicles. Beginning 12 days after treatment, lever preference following various doses of LSD was determined. The results indicated that only the 200 μg dose of 5,7-DHT and PCPA caused a significant potentiation of LSD-lever responding at the 0.03 mg/kg dose of LSD while all treatments except 12.5 and 25 μg of 5,7-DHT resulted in significant depletion of 5-HT. Moreover, amount of 5-HT and percent LSD responding following 0.03 mg/kg LSD were not significantly correlated. It was concluded that 5-HT depletion, *per se*, cannot account for supersensitivity to the behavioral effects of LSD.

Behavior Depletion Drug discrimination 5,7-Dihydroxytryptamine LSD p-Chloroamphetamine p-Chlorophenylalanine Serotonin Supersensitivity

OVER the past quarter of a century, substantial evidence has accumulated in support of the hypothesis that the biochemical [15], behavioral [2], and hallucinogenic [36,37] effects of d-lysergic acid diethylamide (LSD) depend, at least in part, on the functioning of the serotonergic (5-HT) neuronal system (for review see [16]). For example, depleting central 5-HT concentration either by electrolytic lesions of 5-HT-containing neurons in the midbrain raphe nuclei or administration of p-chlorophenylalanine (PCPA), an inhibitor of tryptophan hydroxylase [26], increases sensitivity to the disruptive effects of LSD on the bar-pressing behavior of rats maintained under fixed-ratio (FR) schedules of reinforcement [4,5].

Recently, the serotonin neurotoxin 5,7-dihydroxytryptamine (5,7-DHT) has been a useful tool in the investigation of the functional activity of 5-HT. This compound, when injected intraventricularly (IVT), in conjunction with the catecholaminergic uptake inhibitor desmethylimipramine (DMI), results in a relatively selective destruction of serotonergic pathways [9]; at a dose of 200 μg 5,7-DHT causes substantial depletion of whole brain 5-HT (60%) and concomitant potentiation of LSD-induced disruption of bar-pressing [13, 24, 25]. In contrast, intraperitoneal (IP) administration of the halogenated amphetamine p-chloroamphetamine (PCA 20 mg/kg), which also selectively depletes 5-HT (76%) and may be neurotoxic [22], does not potentiate the disruptive effects of LSD [25]. Since PCA and 5,7-DHT alter 5-HT levels through different mechanisms [17,18] and affect different cell groups within the raphe [8, 21, 22, 32], heightened sensitivity to LSD (as measured by disruption of schedule-controlled bar-pressing) may not depend on 5-HT depletion *per se* but, rather, on the specific pattern of depletion [25].

Another behavioral procedure which has been useful in delineating the neuronal mechanisms that may mediate the effects of LSD is the drug discrimination procedure [6, 28, 48]. In this situation, animals are trained to make differential responses contingent solely on the presence or absence of a drug. It is highly sensitive in that rats can be trained to discriminate doses of LSD as low as 0.005 mg/kg from saline [20]. Moreover, the ability to make such a discrimination depends specifically on the interaction of LSD with central serotonergic systems [28, 38, 48]. However, there has been only one study involving the effects of 5-HT *depletion* on the discriminability of LSD; PCPA (300 mg/kg) was shown to increase sensitivity to low (subthreshold) doses of LSD [12] but, because only two rats were tested, these results must be viewed as suggestive. It should be noted that Browne [11] has shown that depleting serotonin with either PCPA or electrolytic raphe lesions results in increased sensitivity to the

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TABLE 1

SUMMARY OF EXPERIMENTAL PROCEDURES AND BIOCHEMICAL RESULTS

Treatment	Dose	Volume (μl)	Phase	N*	n†	5-HT %	5-HT ng/g‡	NE %	NE ng/g‡	DA %	DA ng/g‡
5,7-DHT	12.5 μg	10	II	13	8	126	411 ± 39	99	441 ± 4	99	974 ± 7
	25 μg	20	II	13	6	122	399 ± 94	103	455 ± 12	102	999 ± 20
	50 μg	10	III	13	10	41	133 ± 8#	101	449 ± 6	99	966 ± 4
	100 μg	20	III	13	8	32	106 ± 9#	102	450 ± 11	99	966 ± 2
	200 μg	20	I	14	9	21	69 ± 19#	103	455 ± 10	97	950 ± 8
IVT Cont.	—	20	I	5	5	—	327 ± 29§	—	443 ± 10§	—	979 ± 13§
		10	II	5	3	—		—		—	
		10	III	5	3	—		—		—	
PCA	20 mg/kg	—	I	12	10	53	192 ± 48#	105	467 ± 13	98	944 ± 7
PCPA	900 mg/kg¶	—	II	12	10	59	211 ± 13#	104	459 ± 6	101	974 ± 7
IP Cont.	—	—	I	5	5	—	360 ± 33§	—	443 ± 15§	—	966 ± 4§
			II	5	5	—		—		—	

*Number of rats assigned to each condition.
†Number of rats from which data were obtained.
‡Data represent nanograms per gram wet tissue (mean ± standard error).
§Data from control groups from the different phases were pooled.
¶3 × 100 mg/kg administered 3 times during the course of testing (see Text).
#Significantly different from control values ($p<0.01$).

discriminable effect of mescaline, a phenethylamine hallucinogen with stimulus properties similar to those of LSD [12, 23, 41, 48]. The present study was designed to investigate the effects of 5-HT depletion induced by a variety of techniques on the discriminability of LSD.

METHOD

Subjects

Male albino rats (Sprague-Dawley) weighing between 280–350 g at the inception of the experiment were maintained at 80–85% of expected free-feeding weight by restricting water intake. They were housed individually with free access to laboratory chow in a room with controlled temperature (21–23°C), humidity (40–50%) and lighting (12 hour light-dark cycle).

Apparatus

Eight sound- and light-attenuated chambers (LVE 143-24) were used. Each was equipped with two response levers on one wall separated by a dipper which delivered 0.1 ml of water. Illumination was provided by a 28 V white house light; an exhaust fan supplied both ventilation and masking noise. Electromechanical and solid-state programming and recording circuits were located in an adjoining room.

Behavioral Training

All rats were first trained to discriminate LSD from saline using a procedure described in detail elsewhere [28, 45, 46]. Fifteen minutes prior to each experimental session animals were injected IP with either LSD (0.12 mg/kg as the tartrate) dissolved in isotonic saline or an equivalent volume of isotonic saline alone (1.0 ml/kg). During initial training only one lever was present and responses on that lever were reinforced with presentation of the water-filled dipper under a fixed ratio 1 (CRF) schedule. The lever present depended on the solution that was injected: for half of the rats the left lever was designated LSD-appropriate and was present following LSD injections and the right lever was present following saline injections. The right lever was designated LSD-appropriate for the remaining animals. Saline and LSD injections alternated irregularly with the constraint that no more than three consecutive sessions occur with the same substance. Animals received equal numbers of LSD and saline sessions.

The schedule of reinforcement then was increased gradually to FR 32, i.e., every thirty-second response was followed by dipper presentation. After stable response rates were obtained, the two response levers were presented simultaneously. Discrimination training consisted of reinforcing only correct (stimulus-appropriate) responses under the FR 32 schedule; responses on the incorrect lever had no programmed consequences. Experimental sessions lasted 20 min and were conducted five days per week. Rats received 18 hr free access to water following the final session of the week. Training continued under this procedure for 40 sessions, at which time the percent correct responding for each group of animals was about 95%. This measure is expressed as the number of responses on the correct lever prior to the delivery of the first reinforcer divided by the total number of responses on both levers during this period (% correct=no. correct responses÷total responses×100%).

Treatment Protocol

After reliable discriminations were obtained (>85% correct), different groups were subjected to one of the following treatments: (1) 12.5, 25, 50, 100 or 200 μg of 5,7-DHT (IVT); (2) 300 mg/kg of PCPA (IP); (3) 20 mg/kg of PCA (IP); (4) IVT vehicle (control) or (5) IP vehicle (control). Space and time limitations precluded the many groups from being trained and tested simultaneously; rather, the experiment was conducted in three successive phases as shown in Table 1.

Surgical Procedures

Surgical and pharmacological manipulations were per-

formed as follows: 5,7-dihydroxytryptamine creatinine sulfate was dissolved in a vehicle solution of 0.05% saline plus 0.5 mg/ml ascorbic acid. The solution was injected into the right lateral ventricle via a microliter syringe (1 μl/min) according to the following coordinates: 3 mm posterior to bregma, 1 mm lateral, 4.2 mm ventral from the dura [35]. In order to prevent damage to noradrenergic (NE) neurons, injections of 5,7-DHT were preceded one hour by IP injection of 25 mg/kg desmethylimipramine (DMI HCl, as the salt) dissolved in isotonic saline. Rats also received 0.05 mg/kg methyl atropine prior to being anesthetized with 300–350 mg/kg chloral hydrate. The animals were treated with 5,7-DHT or vehicle as shown in Table 1. Doses are expressed as the salt.

Pharmacological Procedures

Rats received IP injections of 20 mg/kg PCA HCl (as the salt), in 0.05% saline (1 ml/kg) or vehicle as indicated in Table 1 preceded by 12.5 mg/kg of chlorpromazine HCl (as the salt) to prevent convulsions.

In the PCPA groups, rats were given IP injections of 100 mg/kg PCPA methyl ester, as the free base, dissolved in 0.05% saline for three consecutive days (3×100 mg/kg) or vehicle controls (Table 1). To maintain depletion of 5-HT throughout the course of the experiment these animals also received "booster" treatments of PCPA (or vehicle) consisting of three consecutive days of 100 mg/kg every two weeks [3]; thus, the rats received a total of 900 mg/kg PCPA over the course of testing.

Behavioral Testing

Those animals that underwent surgery were allowed 12 days of post-operative recovery in home cages before behavioral testing was conducted. PCA-treated animals were tested on the twelfth day after treatment. PCPA-treated rats were tested on the fourth day after the last PCPA injection. On test days, animals were given one of the following doses of LSD: 0.015, 0.030, 0.060, 0.090 or 0.120 mg/kg (doses calculated as the salt). Dosage for each animal was randomly varied within groups to control for possible time course effects. Each animal received each dose twice with no animal receiving any dose twice before receiving all other doses at least once. No reinforcers were delivered during test sessions, which terminated either after 32 responses were made on one or the other of the two levers or after 20 min, whichever occurred first. Data for each rat were used only when at least 10 responses were made. Following test sessions animals were given 10–15 min free access to water in home cages.

Biochemical Determinations

Following behavioral testing, the animals were sacrificed, brains were removed and whole brain concentrations of 5-HT, norepinephrine (NE) and dopamine (DA) were determined using a procedure developed by Shellenberger and Gordon [42] with slight modifications. The animals were decapitated, the brains removed and homogenized in 3 volumes of 0.4 N perchloric acid containing 1.0 g sodium metabisulfate and 0.5 g (ethylene dinitrite) tetraacetic acid, disodium salt (EDTA) per liter. The homogenates were left to stand on ice for 10 min and then centrifuged at 28,000 g for 15 min. The supernatants were saved and buffered to pH 7.5–8.0 by adding a Tricine-EDTA-NaOH solution. Approximately 275–300 mg activated alumina was added. Samples were shaken for 20 min and centrifuged at 500 g for 5 min. The resulting supernatant was transferred for extraction and assay of 5-HT using the butanolheptane method of Bogdanski [10] as modified by Lovenberg and Engelman [29]. 5-HT fluorescence was read in an Aminco-Bowman spectrophotofluorometer, activation peak 295 nm, fluorescence at 540 nm. After washing the alumina with deionized distilled water, the catecholamines were eluted using 0.5 N perchloric acid and oxidized in a single sample step. NE fluorescence was read at activation peak 380 nm, fluorescence at 495 nm. DA was read at activation peak 325 nm, fluorescence at 380 nm. Assays were conducted 55–60 days following treatment with 5,7-DHT or PCA; 14 days after the last PCPA injection.

Statistical Methods

Behavioral test data are expressed as the percentage of LSD-appropriate responses; the number of responses on the LSD lever divided by the total number of responses on both levers (×100%). Whole brain 5-HT, DA and NE for pretreated and control rats were calculated and are expressed as ng/g of wet tissue. Since control animals were run at separate times and received different amounts of IVT vehicle, a one-way analysis of variance (ANOVA) was conducted to test for possible differences between the control groups. The statistical significance between control and experimental groups was evaluated using two-way ANOVA with repeated measures on one variable; one ANOVA between all 5,7-DHT groups and their control (IVT vehicle) and one ANOVA between PCPA, PCA and IP controls along with Dunnett's procedure (*a priori* planned) for comparing individual means with control means [47]. Amine levels were compared with a one-way ANOVA followed by Dunnett's procedure. A Pearson product-moment correlation between percent LSD responding at the 0.03 mg/kg (30 μg/kg) dose of LSD and amount of whole brain 5-HT was also obtained.

RESULTS

The data that are presented are from those animals that survived surgery and successfully completed *all* behavioral testing (Table 1). Since there were no significant differences between IVT control groups or between IP groups, control data were pooled to form one IVT group and one IP group.

Figure 1 shows the results of dose-response testing for all 5,7-DHT groups and the IVT control group. It is clear that a significant potentiation, i.e., increased LSD-appropriate responding, occurred only after 200 μg of 5,7-DHT at both the 0.03 mg/kg ($p<0.01$) and the 0.06 mg/kg doses of LSD ($p<0.05$). Figure 2 shows the dose-response curves for the PCA, PCPA and IP control groups. PCPA caused a significant increase in LSD-lever responding at the 0.03 mg/kg dose of LSD ($p<0.01$) but PCA had no effect on ability to discriminate LSD. There were no effects of any treatment on saline accuracy. Whole brain concentrations of 5-HT, DA and NE are shown in Table 1. Analysis of these assay data revealed that 50 μg, 100 μg and 200 μg of 5,7-DHT as well as both PCA and PCPA significantly depleted 5-HT ($p<0.01$). There were no differences between any of the groups with respect to whole brain levels of the catecholamines.

The Pearson product-moment correlation between percent LSD lever responding at the 0.03 mg/kg (30 μg/kg) dose of LSD (the dose at which behavioral differences were most evident) and amount of whole brain 5-HT revealed a nonsignificant correlation coefficient (−0.226).

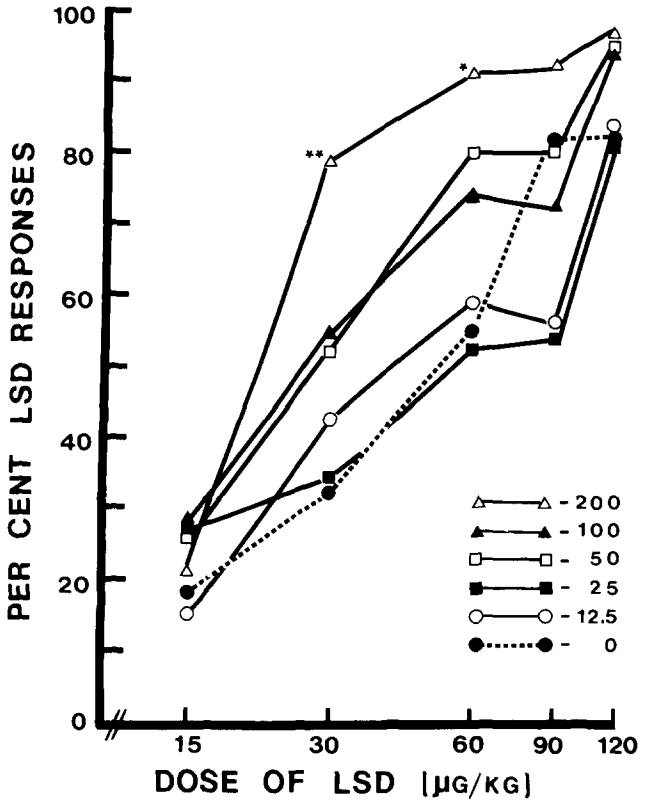

FIG. 1. Dose-response curves for all 5,7-DHT groups and the IVT control group. On the ordinate is the percent of responses made on the LSD lever during extinction testing. On the abscissa is dose of LSD plotted on a log scale. Each point represents the mean of all animals in the group that completed behavioral testing (see Table 1). Each rat received each dose twice. Significant effects are represented by asterisks (**$p<0.01$; *$p<0.05$).

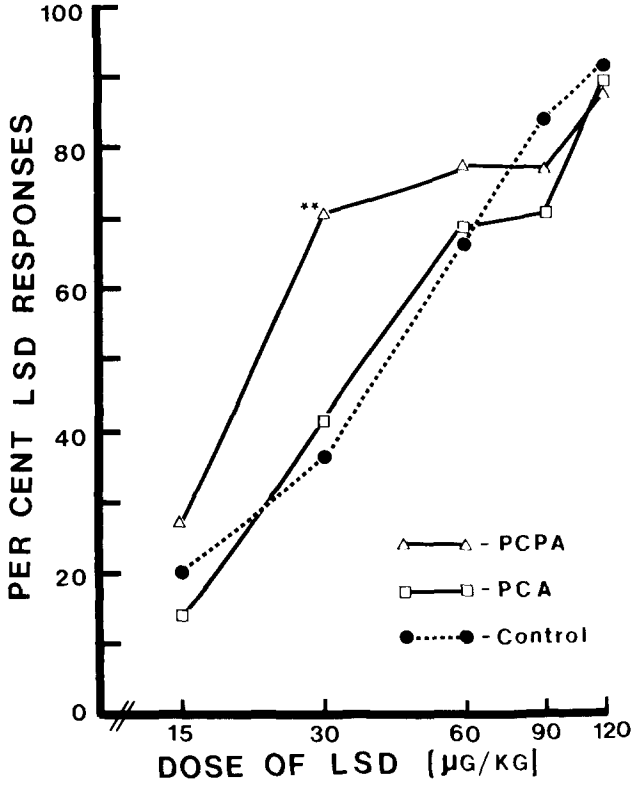

FIG. 2. Dose-response curves for PCPA, PCA and IP control groups. On the ordinate is the percent of responses made on the LSD lever during extinction testing. On the abscissa is dose of LSD plotted on a log scale. Each point represents the mean of all animals in the group that completed behavioral testing (See Table 1). Each rat received each dose twice. Significant effects are represented by asterisks (**$p<0.01$).

DISCUSSION

The results of this study indicate that: (1) IVT injection of 5,7-DHT at doses of at least 200 µg (96 µg free base) results in a highly significant increase in sensitivity to the discriminative stimulus effect of LSD, particularly the relatively low dose of 0.03 mg/kg, (2) PCPA, but not PCA, also significantly heightens sensitivity to 0.03 mg/kg of LSD, and (3) concentration of 5-HT (in whole brain) following these various agents is not significantly correlated with behavioral sensitivity; that is, although PCA as well as 50 µg and 100 µg 5,7-DHT substantially deplete 5-HT (to 53, 41 and 32% of control, respectively), they do not increase sensitivity to LSD. In fact, concentration of 5-HT in these groups is lower than after PCPA (58% of control), a drug that does induce supersensitivity. Thus, amount of 5-HT depletion does not appear to be the determining factor in producing heightened sensitivity to LSD following pretreatment with 5-HT depleting agents.

The same conclusion is supported by studies utilizing other behavioral techniques. For example, 5,7-DHT and PCPA increase the amount of disruption of bar-pressing maintained under an FR 32 schedule following low doses of LSD [3, 13, 25], mescaline [3], psilocybin [3], quipazine [3] and DOM [13], all of which act, at least in part, as 5-HT agonists. Yet PCA, which induced even greater depletion than 5,7-DHT or PCPA, failed to enhance behavioral disruption following LSD [3,25], mescaline [3], psilocybin [3] or quipazine [3]. In another study [43] PCPA and 5,7-DHT induced similar amounts of depletion, but only 5,7-DHT treatment resulted in supersensitivity to a complex group of LSD-induced behaviors (tremor, rigidity, Straub tail, hindlimb abduction, reciprocal forepaw treading and lateral headweaving) known collectively as the "serotonin syndrome" [44]. However, it should be noted that, in our laboratory, PCPA was found to increase the ability of LSD to induce these behaviors [27].

The results of all of these studies raise the question of why PCA does not increase sensitivity to the behavioral effects of LSD. They also suggest that pattern (rather than amount) of depletion may be responsible for increases in sensitivity or that the disparate mechanisms by which PCA, PCPA and 5,7-DHT deplete 5-HT are important determinants of this phenomenon.

While either PCPA, PCA or 5,7-DHT cause neurochemical changes in the parameters associated with brain 5-HT, i.e., decreases in tryptophan hydroxylase, 5-HT and 5-hydroxyindole acetic acid (5-HIAA), each agent produces these changes differently: PCPA depletes 5-HT primarily by inhibiting tryptophan hydroxylase [26]; 5,7-DHT is a potent neurotoxin which destroys 5-HT neurons throughout the neuraxis including the B-7 (dorsal), B-8 (median) and B-9 (bilateral pontine) raphe nuclei, as designated by Dahlstrom

and Fuxe [14], as well as their terminal projections [18, 19, 21]. Apparently, PCA has two separate actions of 5-HT neurons [17,39]: an immediate effect which includes release of 5-HT, inhibition of 5-HT uptake and inhibition of tryptophan hydroxylase [39] and a more prolonged neurotoxic effect which is relatively selective to B-9 cells [8, 21, 22, 32]. Thus, differences between the effects of 5,7-DHT and PCA on raphe nuclei may account for the finding that 5,7-DHT, but not PCA, heightens sensitivity to LSD.

PCA, PCPA and 5,7-DHT also have different effects on sub-cellular components of the 5-HT neuron. Whereas 5,7-DHT destroys terminal, axonal and somatic portions of the neuron [18,21], PCA has a more selective cytotoxic action; that is, this agent affects B-9 cell bodies [8, 21, 22, 32] and, possibly, certain terminals [39]. Furthermore, while PCA decreases terminal 5-HT, it simultaneously increases axonal 5-HT levels [30]. Although PCPA is also more effective in depleting 5-HT in terminal regions than in cell bodies [1,31], it does deplete both dorsal (30%) and medial (60%) raphe nuclei [31]. Thus, PCA, which does not increase sensitivity to LSD, is the only depleting agent we have tested that does not deplete 5-HT throughout the neuron which suggests that depletion throughout the neuron may be an important determinant of behavioral supersensitivity.

The fact that only the 200 μg dose of 5,7-DHT induced heightened sensitivity to LSD even though both 100 μg and 50 μg 5,7-DHT (48 and 24 μg, free base) significantly depleted 5-HT again suggests that site of depletion may determine supersensitivity. This is because, at smaller doses, IVT 5,7-DHT (50 μg, free base) produces damage in 5-HT cells which are proximal to the ventricle, but does not affect more distal terminal regions [34].

Other data that might be particularly relevant to the present results indicate that both 5,7-DHT and PCPA increase the specific binding of d-(^{3}H)LSD and (^{3}H)5-HT to rat brain membranes [7,18]. In fact, 5,7-DHT actually increases the total number of LSD and 5-HT binding sites [18,33]. Furthermore, there are also regional differences with respect to 5-HT receptor supersensitivity [33]. This drug-induced increase in LSD and 5-HT binding could certainly potentiate the actions of LSD and other 5-HT agonists on certain postsynaptic 5-HT receptors [18,33] and could, therefore, explain our results. While the effect of PCA on d-(^{3}H)LSD and (^{3}H)5-HT binding is not known, it has been demonstrated that fenfluramine, which is structurally and functionally similar to PCA [21], can actually *decrease* the number of (^{3}H)5-HT binding sites [40].

In conclusion, converging lines of evidence suggest that the relationship between 5-HT depletion and the behavioral effects of LSD (and of several related compounds) is not simple: depletion of 5-HT does not, *per se* induce behavioral supersensitivity. In fact, drug discrimination, FR disruption [25] and the "serotonin syndrome" [43] have shown that sensitivity depends on the method of depletion. Careful scrutiny of related biochemical and histological evidence involving 5,7-DHT, PCA and PCPA leads to the conclusion that differences in regional effects of these compounds and resultant changes in the affinity or density of 5-HT and LSD receptors may be important in determining behavioral sensitivity to LSD. Future studies, involving more discrete lesioning procedures (e.g., topical administration of minute quantities of 5,7-DHT or PCA) and regional assays might prove useful in testing this hypothesis.

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