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Alterations in the Behavioral Effects of LSD by Pretreatment with *p*-Chlorophenylalanine and α-Methyl-*p*-Tyrosine*

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Abstract. The bar-pressing behavior of hungry rats was maintained by a fixed-ratio schedule of food reinforcement. At 5 and 12 days after pretreatment with *p*-chlorophenylalanine (PCPA), a subtreshold dose (20 μg/kg) of lysergic acid diethylamide (LSD) was found to disrupt this behavior. No such disruption occurred when PCPA pretreatment was followed by either a distracting external stimulus (tone) or a low dose of D-amphetamine (0.3 mg/kg). Sensitivity to LSD was apparently unaffected by pretreatment with α-methyl-*p*-tyrosine (AMPT).

Key-Words: Lysergic Acid Diethylamide — *Para*-Chlorophenylalanine — *Alpha*-Methyl-*Para*-Tyrosine — Operant Conditioning.

The action of d-lysergic acid diethylamide (LSD) has been linked to the endogenous monoamine serotonin (5HT) for at least 17 years (Gaddum, 1953; Woolley and Shaw, 1954). Direct interactions were observed at certain neuromuscular effector sites, such as the gut or uterus, and in the clam heart where 5HT is the "natural" neurotransmitter (Freedman and Aghajanian, 1966). More recently, doses as low as 130 μg/kg of LSD have been shown to increase 5HT in the rat brain (Freedman, 1961) and, unlike nonhallucinogenic congeners of LSD (UML, BOL, etc.), to decrease 5-hydroxyindoleacetic acid (5HIAA), the principe metabolite of 5HT in the brain (Freedman *et al.*, 1970).

Although its presence in relatively high concentrations in various brain regions suggests that 5HT may have some neuroregulatory or neurotransmitter function, direct evidence for the existence of "seroton-

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ergic" neurons in the central nervous system has been presented only recently (Dahlstrom and Fuxe, 1965). Lesions in the medial forebrain bundle (MFB) at the level of the lateral hypothalamus (Harvey *et al.*, 1963) or in the midbrain raphé nuclei (Sheard and Freedman, 1966) not only destroy these neurons, but deplete 5HT in the telencephalon; stimulation of the raphe increase 5HIAA and decreases 5HT (Aghajanian *et al.*, 1967).

While the behavioral effects of LSD have been analyzed extensively in recent years (Jarvik, 1967; Appel, 1968), there have been few systematic attemps to relate these effects to the concentration, distribution or metabolism of brain monoamines. We therefore undertook a series of experiments in which one quantitative behavioral effect of relatively low doses of LSD, the disruption of bar pressing maintained by a fixed-ratio (FR) schedule of reinforcement, was altered by pretreatment with agents known to affect brain monoamine concentration (Appel and Freedman, 1964). Pretreatment with reserpine (1.4 mg/kg) and tetrabenazine (4 mg/kg), "tranquilizing" drugs that significantly deplete both 5HT and norepinephrine (NE) in the brain, was found to enhance the effects of a threshold dose of 40 μg/kg of LSD in rats. Benzquinamide (15 mg/kg), a "tranquilizer" pharmacologically similar to tetrabenazine, affected neither sensitivity to LSD nor concentration of either amine.

Although these data suggested that brain amine concentration might be correlated with the extent of LSD effect, they did not clarify the specific relationship between LSD and 5HT, most obviously because drugs like reserpine and tetrabenazine deplete NE as well as 5HT in brain (Freedman, 1966). Moreover, reserpine has a variety of peripheral autonomic in addition to central effects which, even when they can no longer be directly observed in the rat, might affect sensitivity to compounds like LSD. Our most recent experiments have involved pretreatment with agents that are markedly different from most tranquilizers behaviorally, pharmacologically and biochemically, and that have more specific effects on brain monoamines. In this paper, we describe the effects of pretreatment with *p*-chlorophenylalanine (PCPA) and α-methyl-*p*-tyrosine (AMPT), compounds which deplete brain 5HT or NE, respectively, by inhibiting the biosynthesis of the amine (Koe and Weissman, 1966; Spector *et al.*, 1965).

Methods

Subjects. Naive, male, albino Sprague-Dawley rats obtained from Charles River Laboratories, Wilmington, Massachusetts, were used in a series of four behavioral and biochemical experiments. They were housed in individual cages in a room of constant temperature (75° F) and humidity (40—50%) and maintained on a 12 h (7:00 A.M. — 7:00 P.M.)

day-night cycle. Each animal was approximately 90 days old and weighed 250—300 g at the beginning of the experiment.

Apparatus. The behavioral studies were run in three boxes which were slightly modified versions of apparatus which is commercially available (R. Gerbrands Co.). Each box contained a single lever or bar located in the center of one of its sides (about 5 cm above the floor); a force of 10—15 g activated the lever and defined a bar-pressing response. A dim 28-v D.C. house light was mounted behind a white, translucent panel directly above the lever. "White" noise of moderate intensity was provided through a 10.16 cm speaker mounted outside of the box. To the right and slightly below the lever, a dipper delivered 0.05 ml of a liquid diet of sweetened evaporated milk and vitamins into a feeding cup. Each box was housed in an outer chest that provided sound and light attenuation. All experimental events were programmed by switching and timing circuits in an adjoining room. Responses were recorded on electromagnetic counters and cumulative recorders.

Preliminary Training. Upon arrival in the laboratory, each rat was weighed, coded, and placed into its home cage. For a period of at least one week it was allowed free access to both food and water. During the second week, each rat was weighed, handled, and given about 20 g of dry Purina Laboratory Chow each day to stabilize its weight. All solid food was removed for a period of two days at the beginning of the third week: on the second of these deprivation days, each animal was given a small amount (less than 1 ml) of the diet used as a reinforcer in the behavioral experiments.

For an animal scheduled to be used in either a behavioral or behavioral-biochemical experiment, testing began on the third day of the third week. After the animal was placed in the apparatus, the house light and the "white" noise were turned on. The feeding dipper was then presented for 10 sec every 20 sec until the rat ate. This normally required less than 10 min. When the animal ate as soon as the dipper was presented, the duration of the 10 sec reinforcement "cycle" was gradually shortened to 3 sec. The automatic recycling circuit was disconnected and a shaping procedure (Ferster and Skinner, 1957) was instituted to condition the bar-pressing response. Normally, conditioning required less than 20 min. The rat was then allowed to work for milk on a continuous reinforcement (CRF) schedule for the remainder of its 45-min experimental session. After each session, each animal was weighed and given enough additional solid food (5—10 g) to maintain its post-deprivation weight. If, however, an animal did not learn to press the bar on the first day, no additional food was given and shaping was continued for a second day. The ratio of responses required for each reinforcement was raised very slowly from one (CRF or FR 1) to 40 (FR 40). When an animal's bar-pressing rate

stabilized on FR 40, daily intraperitoneal (i.p.) injections of 0.1 ml isotonic saline solution (NaCl) were begun. When scheduled, the supplementary diet was changed from solid, dry food to mash. Each animal was run 7 days per week for 45 min on FR 40, given enough food each day to maintain its pretesting, post-deprivation weight, and given NaCl for several days before the behavioral experiments were begun.

Biochemical Procedures. Trained animals were sacrificed at appropriate times to obtain biochemical information. Behaviorally naive animals were also sacrificed to provide necessary supplemental information concerning brain 5HT and NE concentrations. The animals were sacrified by decapitation and the brains quickly removed; either the whole brain was homogenized directly or the brain was separated into forebrain (telencephalon and diencephalon) and hindbrain sections (midbrain, pons, cerebellum and medulla) by a single transection (dorsally, just anterior to the corpora quadrigemina and ventrally, just anterior to the mammilary bodies). Brain tissue was homogenized in 4 vol cold

Table 1. *Summary of the experimental design*

Experiment	Groups		Subgroups		
	Test day	Sacrifice day	Pretreatment drug	Test condition	*n*
1	5	6	PCPA	LSD	5
			PCPA	NaCl	5
			NaCl	LSD	4
	12	13	PCPA	LSD	5
			PCPA	NaCl	5
			NaCl	LSD	4
	20	21	PCPA	LSD	6
			PCPA	NaCl	6
			NaCl	LSD	7
2	5	—	PCPA	Tone	5
			PCPA	—	5
			NaCl	Tone	4
3	5	—	PCPA	*d*-Amphet.	8
			PCPA	NaCl	8
			NaCl	*d*-Amphet.	7
4	8 h	—	AMPT	LSD	5
			AMPT	NaCl	4
			NaCl	LSD	4
	22 h	—	AMPT	LSD	4
			AMPT	NaCl	4
			NaCl	LSD	4

0.1 N HCl. Fore- and hindbrain concentrations of 5HT were determined by the method of Bogdanski *et al.* (1956). For determination of 5HT and 5HIAA in whole brain, a modification (Rosecrans *et al.*, 1967) of the method of Bogdanski *et al.* (1956) for 5HT and of Udenfriend *et al.* (1958) for 5HIAA was used. For simultaneous determination of 5HT and NE in whole brain, the method of Mead and Finger (1961) was used.

Behavioral and Pharmacological Procedures. The experimental design is summarized in Table 1. In three experiments, we investigated the interaction of PCPA pretreatment with (1) LSD, (2) an external stimulus (tone) and (3) d-amphetamine. In a fourth experiment, we investigated the interaction of AMPT pretreatment with LSD.

1. PCPA-LSD Interaction

In the three experiments involving PCPA pretreatment, each animal was given 5g of mash which was prepared from ground Purina Laboratory Chow and water in its home cage immediately after its daily testing period; its consistency was such that it neither spilled nor stuck to the bottom or sides of the feeding jar in which it was administered (Fisher Scientific Co., No. 1-338). Response rates were allowed to stabilize once more on FR 40 and the subjects were sub-divided into groups equated with respect to average bar-pressing rate during the last three days of training prior to pretreatment, this figure being the base line or control rate for each animal. In the first two subgroups of Table 1 (PCPA-LSD, PCPA-NaCl), each animal was given 100 mg/kg of DL-*para*-chlorophenylalanine (PCPA[1]), mixed with its mash for three successive days (Day 0, 1, and 2)—a total of 300 mg/kg of PCPA. All animals consumed all of the drug. The remaining subjects (NaCl-LSD) continued to receive mash. For the several days which intervened between the last pretreatment (or mash) day and the test day (Table 1), all animals were given 0.1 ml NaCl before being run on FR 40 but no other drugs. An i.p. injection of 20 μg/kg of LSD[2] was given to each animal in the appropriate subgroups on the test day (Table 1). The animals were sacrificed by decapitation on the day after the test day. Their brains were extracted, dissected, homogenized and assayed for 5HT.

2. PCPA-External Stimulus Interaction

Two behavioral "control" experiments were run in order to analyze further the effects of PCPA pretreatment. In the first of these, the possibility that hypersensitive, PCPA pretreated animals would be more

1 Obtained from Mann Research Laboratories, New York, N.Y.

2 0.1 mg/ml ampules prepared by Sandoz Pharmaceuticals, Hanover, N.Y., and obtained from the National Institutes of Mental Health, Center for Studies of Narcotics and Drug Abuse.

distracted by any stimulus than nontreated controls was tested by the introduction of a tone from time to time on the fifth day after pretreatment. Fourteen rats that had been trained to respond on FR 40 at a stable and rapid rate were treated in exactly the same manner as the animals in the first subgroups of Experiment 1 on Days 0—4, i.e., they were given PCPA or mash on Days 0—2 and where run on FR 40 on Days 0—4. On Day 5, all animals were run as usual except that a 1000 cps tone of approximately 60 db was presented through the speaker that normally delivered "white" noise to the appropriate subgroups (Table 1). This tone was of 3 sec duration and occurred at an average rate of once every $^1/_2$ min (VI $^1/_2$) throughout the 45-min session.

3. PCPA-Amphetamine Interaction

In this experiment, we explored the possibility that PCPA-pretreated animals might be hypersensitive to small doses of other "excitatory" drugs by giving a subthreshold dose of D-amphetamine, a compound which, while normally not hallucinogenic at low doses, acts somewhat like LSD on FR (Appel and Freedman, 1965). Twenty-three rats were treated in exactly the same manner as those of the first part of Experiment 1 (and those of Experiment 2), except that 0.3 mg/kg of D-amphetamine[3] was substituted for LSD or tone on Day 5 after the first of three days of 100 mg/kg of PCPA pretreatment (PCPA-D-amphetamine).

4. AMPT-LSD Interaction

Finally, the hypothesis that catechol-as well as indoleamine depletion might enhance the behavioral effects of LSD was tested by pretreating animals with AMPT[4]. Thirteen rats trained to respond on FR 40 were accustomed to receiving NaCl injections immediately before and eating mash immediately after each session and were divided into three groups on the basis of their base line rates of bar pressing. Five animals were given a single dose of 200 mg/kg of AMPT in their mash eight hours prior to being injected with 20 μg/kg of LSD (AMPT-LSD), four more rats (AMPT-NaCl) received AMPT eight hours before testing but were given no LSD and four animals (NaCl-LSD) were given LSD but no AMPT (Table 1). Another 12 rats, divided into three similar subgroups of four animals each, were given AMPT (or mash) at 22 rather than eight hours prior to LSD (or NaCl) on their last day of testing. Biochemical data were obtained on a separate group of 16 animals, eight of which received mash and eight received 200 mg/kg of AMPT. Four

3 Prepared from D-amphetamine sulfate and saline in our laboratory.

4 L-*Alpha*-methyl-*para*-tyrosine was obtained from Merck, Sharpe and Dohme, Inc., Ranhway, N.J.

rats in each group were sacrificed at eight hours and the remaining animals at 22 h after AMPT (or mash).

Results

Behavioral Effects of PCPA. In contrast to non-specific monoamine depletors such as reserpine and tetrabenazine, both of which have been widely used as tranquilizers, PCPA induces a marked increase in excitability, irritability and sensitivity in the rat (Tenen, 1967). In our experiments, pretreated animals became vicious, difficult to handle, and hypersensitive to daily injections of saline. During bar-pressing sessions PCPA causes a maximum decrease of 25% in overall rate of bar pressing on FR 40 (Fig. 1), i.e., to 75% of control. The onset and amount of disruption of bar pressing followed the course of treatment. In Fig. 1, the first dose of 100 mg/kg of PCPA was given immediately after the performance shown on Day 0. On Day 1, there was very little, if any drug effect. On Day 2, 24 h after the second PCPA treatment (a total of 200 mg/kg), the rate of bar pressing falls to about 80% of control and on Day 3, following a total of 300 mg/kg of PCPA it reaches its lowest level (75%). Recovery begins and continues thereafter. While these effects are small, they occur consistently in more than 100 animals we have observed in these and other experiments.

Fig. 2 shows cumulative records of the behavior of a single animal in the PCPA-NaCl group (Day 5). The control performance (Day 0) at the upper left does not change on Day 1 (after 100 mg/kg of PCPA). On Days 2 and 3 (center panels) the cumulative effects of 200 and 300 mg/kg of PCPA, respectively, can be seen. These curves are characteristic of PCPA but of few, if any, other compounds, and certainly not of LSD (Freedman *et al.*, 1964). Both post-reinforcement "pausing" and fixed-ratio "straining" (Ferster and Skinner, 1957) seem to increase as the session progresses, i.e., they are most pronounced towards the middle and end of each session. On Days 4 and 5 (lower panels) a gradual return to normal patterns of bar pressing occurs.

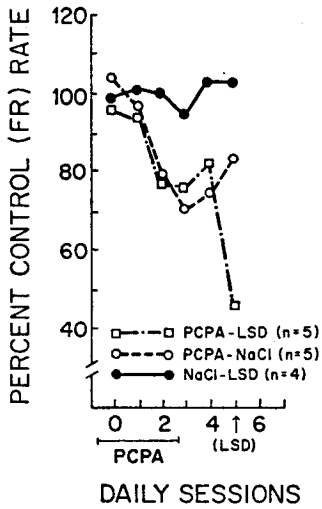

Fig. 1. Average daily percentage of baseline rate of bar pressing on an FR 40 schedule of milk reinforcement in rats given 20 μg/kg of LSD or NaCl on the 5th day following the first of three days of pretreatment with 100 mg/kg of PCPA or mash

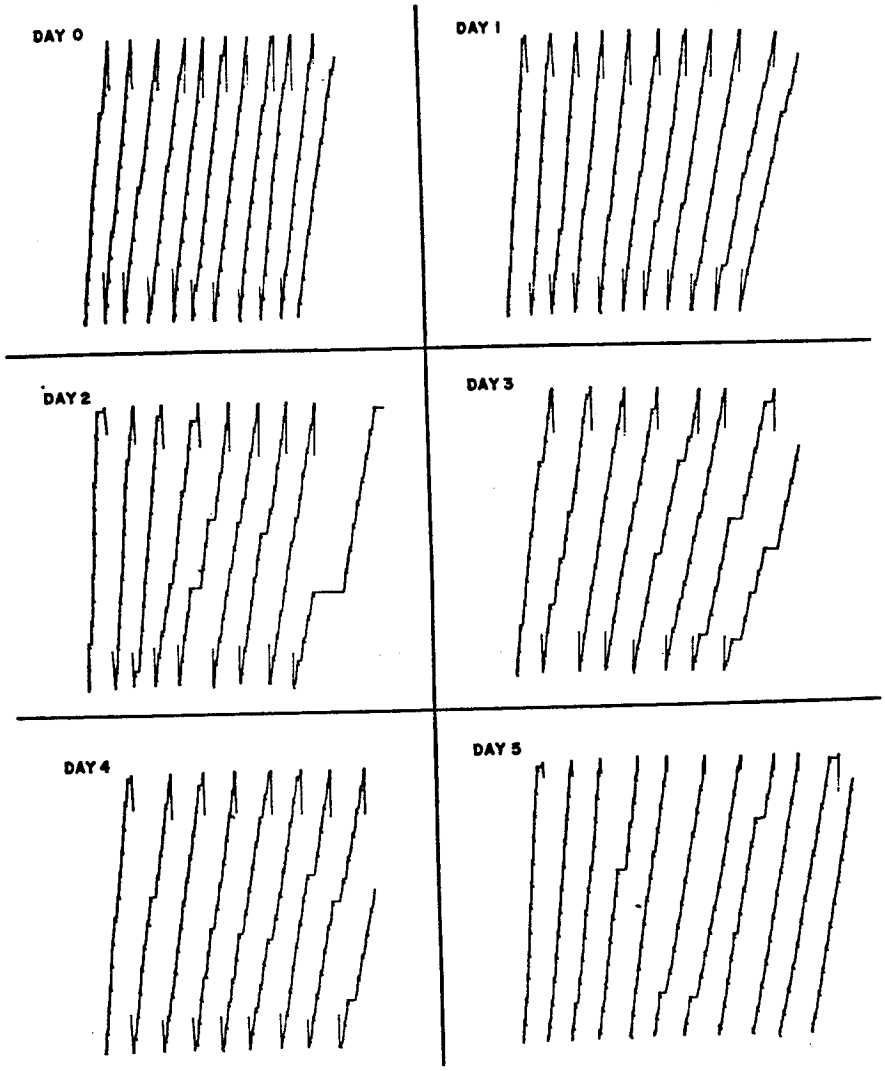

Fig. 2. Cumulative records of the FR 40 performance of a single rat in the PCPA-NaCl subgroup during five days of experimentation. The slopes of these curves, which are reset and compressed after 500 responses, are proportional to the rate of bar pressing; reinforcements are indicated by vertical "pips"

Effect of PCPA Pretreatment on Sensitivity to LSD. Three days of pretreatment with 100 mg/kg of PCPA enhance sensitivity to LSD. Fig. 1 shows that the rate of bar pressing of animals pretreated with PCPA and given 20 μg/kg of LSD on Day 5 (PCPA-LSD) is lower than

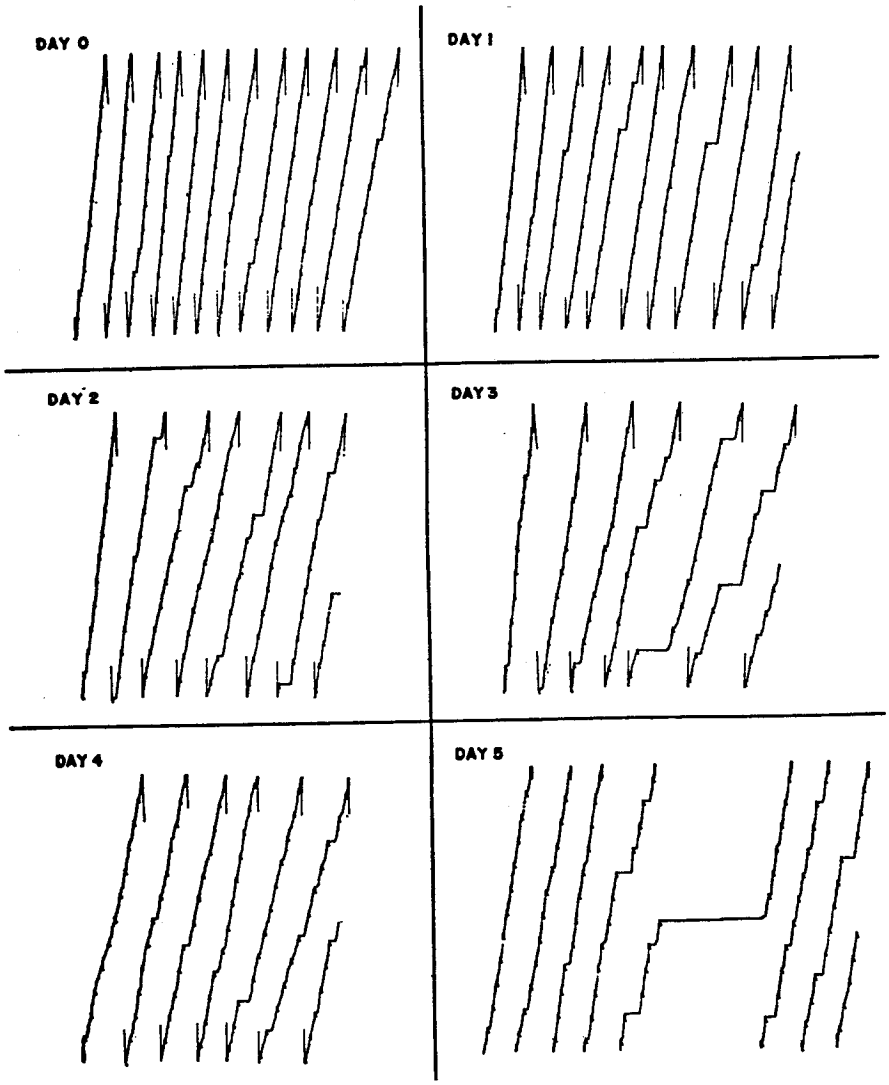

Fig. 3. Cumulative records of the behavior of a single rat given PCPA in its mash after the performances shown on Days 0, 1, and 2, and then given 20 μg/kg of LSD immediately before being run on Day 5

either that of the animals pretreated with PCPA but given no LSD (PCPA-NaCl) or animals given LSD alone. A t-test between the scores of the PCPA-LSD and PCPA-NaCl subgroups was significant ($t = 3.29$, $df = 14$, $p < 0.01$).

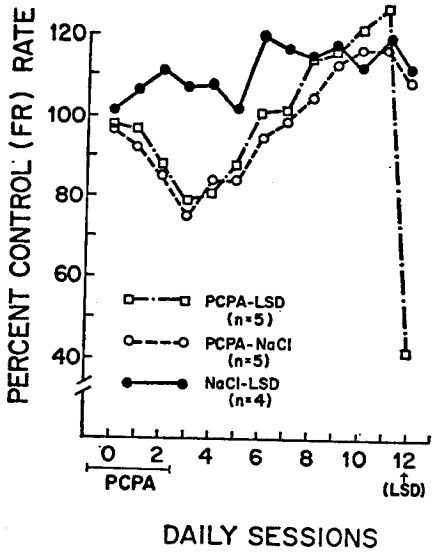

Fig. 4. Average daily percentage of baseline rate of bar pressing on FR 40 in rats given 20 μg/kg of LSD or NaCl on the 12th day, after three days of pretreatment with 100 mg/kg of PCPA each

A dose of 20 μg/kg of LSD has no effect on bar pressing for food under the conditions of the present series of studies (Fig. 1, NaCl-LSD subgroup). Fig. 3 shows the behavior of a single animal given LSD (on Day 5) after pretreatment with PCPA (Days 0—2). The behavior of this subject on Days 0—4 is much like that of the animal shown in Fig. 2, i.e., there is an increased frequency of pausing on Day 2 and, more prominently, on Day 3, and some recovery on Day 4. Following LSD (Day 5) there is, in addition to an increase in PCPA-like effects (1), an increase in disturbed patterns of responding early in the session characterized particularly by changes in local rates, i.e., different slopes between reinforcements, as well as post-reinforcement pausing; (2) a prominent long pause or period of nonresponding which begins about 30 min after administration of LSD. While such periods are characteristic of higher doses of LSD and other psychotomimetics (Freedman *et al.*, 1964; Appel and Freedman, 1965, 1968), the time of onset of the long pause during Day 5 (Fig. 3) is somewhat later than usual for doses of LSD that are, by themselves, sufficient to disrupt FR behavior (Freedman *et al.*, 1964).

Fig. 4 shows the effect of 20 μg/kg of LSD 12 days after the first initiation of PCPA pretreatment. There is a decline in the average bar-pressing rate of the PCPA-LSD group to 42% of normal on Day 12

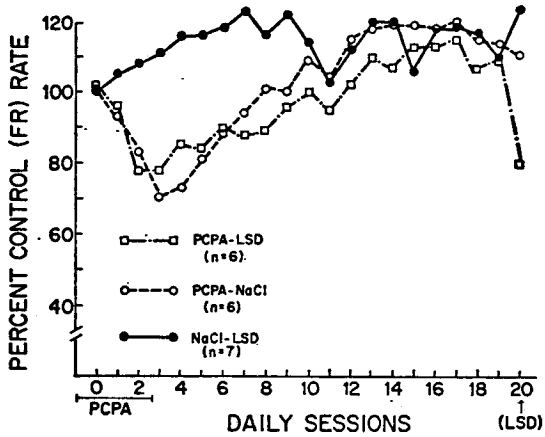

Fig. 5. Average percentage of baseline rate of bar pressing for 20 days following pretreatment with PCPA

even though performance completely recovers to normal by Day 11. A *t*-test between the rates of the PCPA-LSD and PCPA-NaCl groups on Day 12 was highly significant ($t = 7.79$, $df = 8$, $p < 0.01$).

Twenty one days after administration, PCPA fails to increase sensitivity to LSD significantly. Fig. 5 shows that the rates of animals in the PCPA-LSD group are reduced to only 80% of controls, which is not significantly different from either of the two control groups (e.g., the results of the *t*-test between PCPA-LSD and PCPA-NaCl scores are: $t = 2.30$, $df = 10$, $0.05 < p < 0.10$).

Effects of PCPA on Brain Serotonin. Figs. 6 and 7 show levels of 5HT at 6, 13, and 21 days after the initiation of PCPA pretreatment and, for comparison, the data of Koe and Weissman (1966) at a comparable dosage.

Under the conditions of the present experiment, 5HT is depleted to about 8—15% of normal on Day 6 and to at least that extent on the last day of behavioral testing (Day 5). On Day 13, when the behavioral effects of PCPA have dissipated but a significant enhancement of the response to LSD still obtains, brain 5HT concentrations are essentially normal. These biochemical effects are somewhat greater and longer lasting than those observed by Koe and Weissman (1966), perhaps because of the difference in route of administration of PCPA (i.p. vs p.o.).

The data from both control and PCPA-treated animals are more orderly, i.e., have lower standard deviations, in the fore- than in the hindbrain. Rank order correlations were run on the rate of FR responding

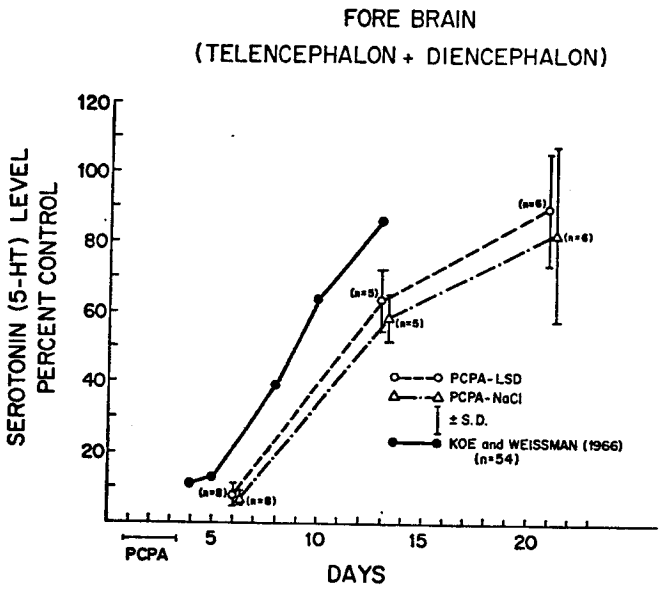

Fig. 6. Average concentration of 5HT in the forebrain of rats of the PCPA-LSD and PCPA-NaCl groups in the behavioral experiments expressed as a percentage of the mean concentration of 5HT in the NaCl-LSD (control) group. The data of Koe and Weissman (1966) on whole brain are also shown for purposes of comparison

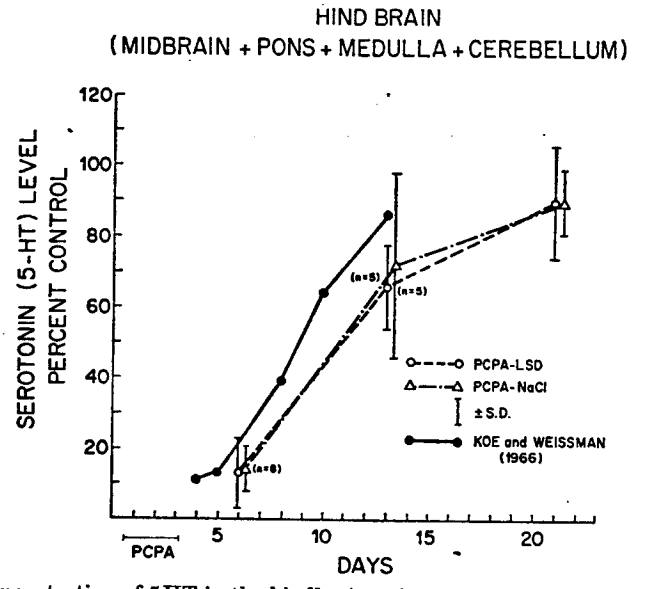

Fig. 7. Concentration of 5HT in the hindbrains of the same animals shown in Fig. 6

Fig. 8. at var

Fig. 9.

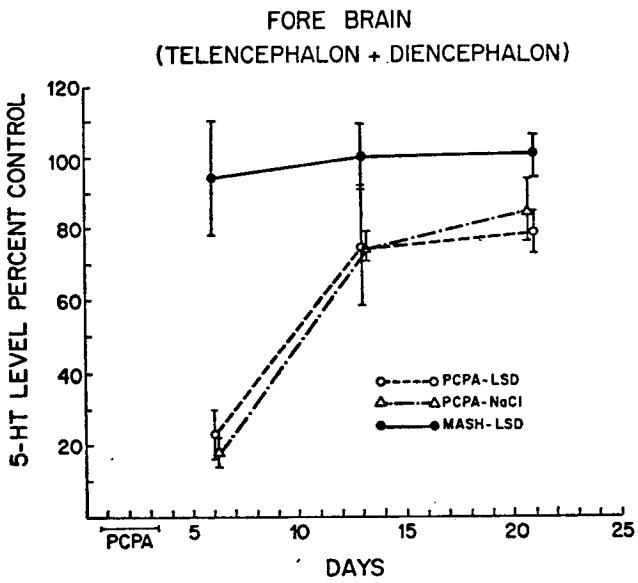

Fig. 8. Average concentration of 5HT in the forebrain of behaviorally naive rats at various times after pretreatment with PCPA. The data are expressed as a percentage of the mean concentration of the mash-NaCl control group

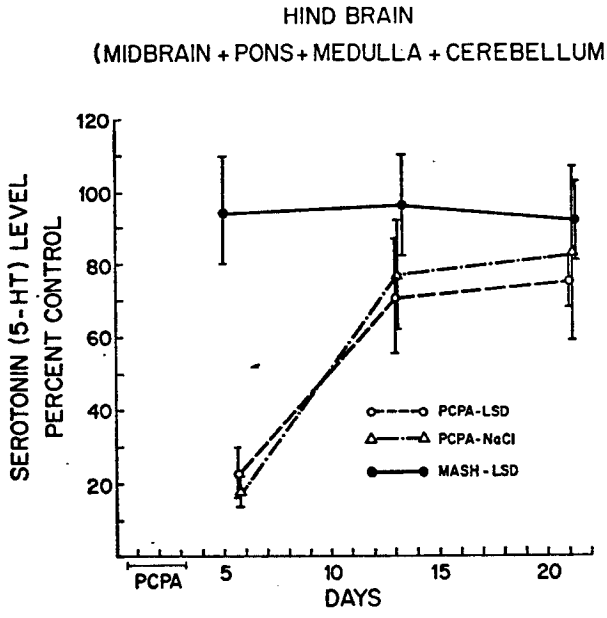

Fig. 9. Average concentration of 5HT in the hindbrain of the animals shown in Fig. 8

and 5HT concentration in both sections of the brain of all animals. The concentration of 5HT in the forebrain was directly and significantly correlated with behavioral performance: the lower the 5HT level, the lower the rate ($r_s = 0.50$, $p < 0.05$). In the hindbrain, the correlation between amine level and FR rate was in the same direction but was not significant ($r_s = 0.33$).

A second determination of brain 5HT after PCPA and PCPA plus LSD was made in the fore- and hindbrains in 48 naive (untrained) rats in order to (1) provide a more typical control group (mash-NaCl), (2) evaluate the effect, if any, of 20 μg/kg of LSD on 5HT in fore- and hindbrain, and (3) determine the extent to which behavioral training and transportation to the biochemical laboratory might affect 5HT concentration. The animals in this experiment were housed in individual cages in a climate-controlled room maintained at a 12-h (7:00 A.M.—7:00 P.M.) day-night cycle. They were deprived and fed mash or mash containing PCPA in exactly the same way as the animals used for behavioral testing except that no animal received any milk or injection until the appropriate time before sacrifice and assay. Each group of 16 rats was subdivided, so that four animals received mash and 0.1 ml NaCl (mash-NaCl) and four animals in the second subgroup (PCPA-LSD) received 100 mg/kg of PCPA each day for three days and 20 μg/kg of LSD (i.p.). The third subgroup (PCPA-NaCl) was treated like the second but was given NaCl (rather than LSD), and finally, four rats (mash-LSD) received mash and 20 μg/kg of LSD. Figs. 8 and 9 show the amounts of 5HT in fore- and hindbrains in three groups of treated animals at 6, 13, and 20 days after PCPA or mash pretreatment expressed as per cents of the 5HT levels of the mash-NaCl groups. Two facts are apparent when these curves are compared with those of Figs. 6 and 7: (1) serotonin, while obviously depleted, is at higher concentrations than in either those of the preceding experiment or that of Koe and Weissman (1966). None of these differences, however, is statistically significant ($p > 0.05$ in every case). (2) A dose of 20 μg/kg of LSD does not affect 5HT levels (solid lines); the mash-LSD group is therefore a valid control in the first behavioral experiment. Thus, it is probable that behavioral testing, handling, and associated events have little, if any, effect on brain 5HT concentrations.

PCPA-External Stimulus Interaction. Fig. 10 shows that, while PCPA had a slightly disruptive effect on the average rate of bar pressing, particularly in the PCPA-tone group, the tone had little, if any additional effect. A comparison was made between the data of PCPA-tone and PCPA-no tone groups on Day 5 and was not significant ($t = 1.19$, $df = 8$, $0.3 < p < 0.4$).

PCPA-d-Amphetamine Interaction. d-Amphetamine appears to have had a slight disruptive effect on some of the animals in both PCPA-

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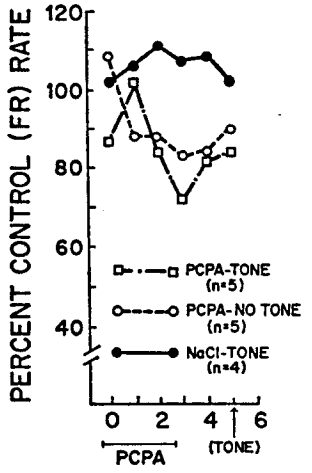

Fig. 10

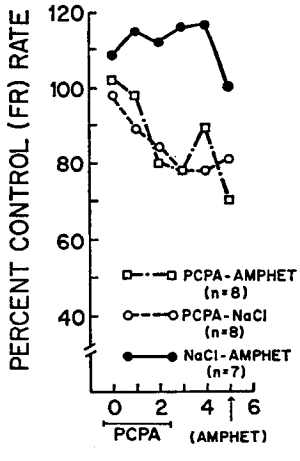

Fig. 11

Fig. 10. Average percentage of baseline rate of bar pressing on FR 40 in rats presented with a tone on the fifth day after PCPA pretreatment as compared with control animals given PCPA and no tone or tone and no PCPA

Fig. 11 Average percentage of baseline rate of bar pressing on FR 40 in animals given 0.3 mg/kg of *d*-amphetamine on the fifth day after PCPA as compared with controls

Table 2. *Percentage of base line rate of bar pressing and concentration of NE following pretreatment with alpha-methyl-para-tyrosine (AMPT)*

	8 h				22 h			
	Mean		s.d.		Mean		s.d.	
	% FR	% Control NE	% FR	% Control NE	% FR	% Control NE	% FR	% Control NE
AMPT-LSD	15	—	6	—	90	—	17	—
AMPT-NaCl	14	24	2	7.5	97	48	16	2.4
NaCl-LSD	104	—	15	—	102	—	5	—

D-amphetamine and NaCl-D-amphetamine groups (Fig. 11). There was, however, no significant difference in the behavior of animals pretreated with PCPA and later given 0.3 mg/kg of D-amphetamine, and animals which received only PCPA ($t = 1.10$, $df = 14$, $0.2 < p < 0.3$).

AMPT-LSD interaction. The behavioral data are summarized in Table 2. Rate of bar pressing for food on FR 40 is severely disrupted (14—15% of normal) eight hours after 200 mg/kg of AMPT when NE level is 24% of control (*s.d.* = 7.5%). There is, however, no difference in the behavior of animals given AMPT plus a small dose of LSD and those given AMPT alone (AMPT-NaCl). At 22 h, the rate of responding on FR 40 is no longer disrupted by AMPT (Table 2) but the average NE level is still only 48% of normal (*s.d.* = 2.4%). There is again no difference between the performance of animals given AMPT plus 20 μg/kg of LSD and those given AMPT alone ($t = 0.46$, $p > 0.7$).

Discussion

Certain *para*-chloro-arylalkylamines deplete 5HT in the brain while having relatively little effect on other monoamines. Examples are 4-chloro-N-methyl-amphetamine (Pletscher *et al.*, 1964) and PCPA. The latter has been much studied as an inhibitor of tryptophan hydroxylase (Jequier *et al.*, 1967) and thus of 5HT biosynthesis (Pletscher *et al.*, 1965; Koe and Weissman, 1966), the result of which is a decrease in the concentration of 5HT.

While the behavioral effects of 4-chloro-N-methyl-amphetamine have not, to our knowledge, been much studied, the results with PCPA (Tenen, 1967) tend to support the observation of Lints and Harvey (1969), derived from lesion studies (Harvey and Lints, 1965; Lints and Harvey, 1969), that 5HT plays some sort of behaviorally inhibiting role; its depletion leads to hyperexcitation, hyperirritability or hypersensitivity. In the cat, for example, chronic administration of PCPA causes insomnia (Delormé *et al.*, 1966) and, according to Ferguson *et al.* (1970), increases in sexual behavior, aggression, appetite, emotionality, drive, frequency of sleep-like EEG patterns in the awake state, and what appear to be hallucinations. In the rat, PCPA may also cause insomnia (Mouret *et al.*, 1967; Rechtschaffen *et al.*,1969); its most profound effect is hypersensitivity to pain (Tenen, 1967) and probably to other external stimuli. This hypersensitivity could explain why the drug facilitates active avoidance behavior at low shock intensities (Tenen, 1967) and certain kinds of nonspatial discrimination learning (Stevens *et al.*, 1967). In the rat hypersexuality has also been reported after PCPA administration by Tagliamonte *et al.* (1969), and by Sheard (1969), who also observed increased aggressiveness and abnormal patterns of resting behavior; these changes were correlated with altered 5HT metabolism in the forebrain (Sheard, 1969).

Of more direct relevance to our results is the report of Rosen and Buga (1969) that PCPA disrupts bar pressing maintained by a multiple fixed-interval fixed-ratio schedule of food reinforcement. While we con-

firm that PCPA can disrupt bar pressing during FR, particularly toward the end of the 45-min experimental session, we believe that this disruption is not a direct effect of the drug on motivation or disposition to respond for food, but is rather a reflection of drug-induced hyperirritability. Our animals behaved as if bar pressing were more difficult than normal after the drug.

It is important to note that neither behavioral hypersensitivity nor *maximal* 5HT depletion can fully explain the fact that PCPA enhances the effects of LSD, since this enhancement is more clearly seen 12 days after pretreatment when behavioral hypersensitivity and disruptions in bar-pressing rate can no longer be observed and when levels of 5HT have recovered to about 60% of normal. The intriguing question is whether enhanced sensitivity to LSD is related to depletion of 5HT or to some other effect of PCPA (Lipton *et al.*, 1967). Support for the hypothesis that 5HT is involved is derived from the fact that the behavioral effects of LSD are enhanced only when 5HT is depleted, since the interaction of PCPA and LSD does not occur 21 days after pretreatment when 5HT is almost normal. In addition to serving as a "control" for the peripheral effects of PCPA, a study of the effects of lesions in the dorsal raphé nuclei on bar-pressing behavior maintained by FR reinforcement (Appel and Sheard, unpublished data) has provided further support for this hypothesis. In properly lesioned animals, there is both a 40—50% depletion of 5HT and an enhanced sensitivity to 20 μg/kg of LSD—the same situation as in our 12-day animals.

The relationship between 5HT and LSD would be more firmly established if we could reverse the effect of PCPA pretreatment on sensitivity to LSD by administering the 5 HT precursor, 5-hydroxytryptophan (5HTP). Unfortunately, our attempts to do so have thus far met with little succes, probably because the doses of 5HTP we have used (25—75 mg/kg) interfere with performance on FR. But even if it were beyond dispute that 5HT is involved in the behavioral effects of LSD and other psychotogens, the nature of this involvement is far from clear. Largely on the basis of studies in peripheral tissues, LSD was originally regarded as a 5HT antagonist, but Aghajanian *et al.* (1970) and others (Aghajanian and Freedman, 1968; Anden *et al.*, 1968) have recently argued that LSD may have a 5HT-like effect at postsynaptic receptors and may specifically inhibit serotonergic fibers (Aghajanian *et al.*, 1968) through some sort of "neural feedback mechanism" (Aghajanian *et al.*, 1970). In this regard, it is interesting that electrical stimulation of raphé nuclei produces behavioral effects similar to those of LSD in unanesthetized rats (Aghajanian and Sheard, 1968; Sheard and Aghajanian, 1968).

It is known that PCPA also depletes brain NE to some extent, particularly in the mouse (Welch and Welch, 1967). However, NE

depletion after PCPA occurs *before* there is any sign of 5HT diminution in the brain. In our experiments, while there is an enhanced sensitivity to LSD when brain 5HT is depleted by 40% with PCPA, there is no such interaction when brain NE is depleted by 48% with AMPT. It therefore seems unlikely that the effects of PCPA on NE metabolism can be invoked to explain our results. Nevertheless, NE and other monoamines may be involved in the action of LSD, particularly because of neurophysiological interactions between serotonergic and adrenergic pathways in the brain. While AMPT had no influence on sensitivity to LSD in this study, the drug apparently *diminished* LSD-induced excitation in rabbits (Horita and Hamilton, 1969).

In conclusion, these experiments do not prove that brain 5HT concentration controls sensitivity to LSD or that the physiological role of LSD is closely related to that of 5HT. The fact that PCPA, as well as nonspecific 5HT-depleting tranquilizers (Appel and Freedman, 1964), but apparently not AMPT at the doses we studied, enhance sensitivity to LSD may be of relevance when the mode of action of psychotogenic drugs is finally elucidated.

References

Aghajanian, G. K., Foote, W. E., Sheard, M. H.: Action of psychotogenic drugs on single midbrain raphé neurons. J. Pharmacol. exp. Ther. **171**, 178–187 (1970).

— — — Lysergic acid diethylamide: sensitive neuronal units in the midbrain raphé. Science **161**, 706–708 (1968).

— Rosecrans, J. A., Sheard, M. H.: Serotonin: release in the forebrain by stimulation of midbrain raphé. Science **156**, 402–403 (1967).

— Sheard, M. H.: Behavioral effects of midbrain raphé stimulation: dependence on serotonin. Comm. Behav. Biol., Part A, **1**, 37–41 (1968).

Anden, N.-E., Corrodi, H., Fuxe, K., Hokfelt, T.: Evidence for a central 5-hydroxytryptamine receptor stimulation by lysergic acid diethylamide. Brit. J. Pharmacol. **34**, 1–7 (1968).

Appel, J. B.: The effects of "psychotomimetic" drugs on animal behavior. In: Psychopharmacology: a review of progress 1957–1967, pp. 1211–1222. D. Efron, ed. Washington, D.C.: U.S. Government Printing Office 1968.

— Freedman, D. X.: Chemically-induced alterations in the behavioral effects of LSD-25. Biochem. Pharmacol. **13**, 861–869 (1964).

— — The relative potencies of psychotomimetic drugs. Life Sci. **4**, 2181–2186 (1965).

— — Tolerance and cross-tolerance among psychotomimetic drugs. Psychopharmacologia (Berl.) **13**, 267–274 (1968).

Bogdanski, D. F., Pletscher, A., Brodie, B. B., Udenfriend, S.: Identification and assay of serotonin in brain. J. Pharmacol. exp. Ther. **117**, 82–88 (1956).

Dahlstrom, A., Fuxe, K.: Evidence for the existence of monoamine containing neurons in the central nervous system. Acta physiol. scand. **62**, 1–55 (1965).

Delormé, F., Froment, J. L., Jouvet, M.: Suppression du sommeil par la P. chloromethamphétamine et la P. chlorophenylalanine. C.R. Soc. Biol. (Paris) **180**, 2347–2351 (1966).

Ferguson, J., Hendriksen, S., Cohn, H., Mitchell, G., Barchas, J., Dement, W.: "Hypersexuality" and behavioral changes in cats caused by administration of p-chlorophenylalanine. Science **168**, 499—501 (1970).

Ferster, C. B., Skinner, B. F.: Schedules of reinforcement. New York: Appleton-Century-Crofts 1957.

Freedman, D. X.: Effects of LSD-25 on brain serotonin. J. Pharmacol. exp. Ther. **134**, 160—166 (1961).

— Aspects of the biochemical pharmacology of psychotropic drugs. In: Psychiatric Drugs, pp. 32—57. P. Solomon, *ed.* New York: Grune and Stratton 1966.

— Aghajanian, G. K.: Biochemical pharmacology of LSD-25. Lloydia **29**, 309—314 (1966).

— Appel, J. B., Hartman, F. R., Molliver, M. E.: Tolerance to behavioral effects of LSD-25 in rat. J. Pharmacol. exp. Ther. **143**, 309—313 (1964).

— Gottlieb, R., Lovell, R. A.: Psychotomimetic drugs and brain 5-hydroxytryptamine metabolism. Biochem. Pharmacol. **19**, 1181—1188 (1970).

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

Harvey, J. A., Heller, A., Moore, R. Y.: The effect of unilateral and bilateral medial forebrain bundle lesions on brain serotonin. J. Pharmacol. exp. Ther. **140**, 103—110 (1963).

— Lints, C. E.: Lesions in the medial forebrain bundle: delayed effects on sensitivity to shock. Science **148**, 250—252 (1965).

Horita, A., Hamilton, A. E.: Lysergic acid diethylamide: dissociation of its behavioral and hyperthermic actions by DL-α-methyl-*p*-tyrosine. Science **164**, 78—79 (1969).

Jarvik, M. E.: The behavioral effects of psychotogens. In: LSD, Man, and Society, pp. 186—206. R. C. Debold and R. C. Leaf, *eds.* Middletown, Connecticut: Wesleyan University Press 1967.

Jequier, E., Lovenberg, W., Sjoerdsma, A.: Tryptophan hydroxylase inhibition: the mechanism by which *p*-chlorophenylalanine depletes rat brain serotonin. Molec. Pharmacol. **3**, 274—278 (1967).

Koe, B. K., Weissman, A.: *p*-Chlorophenylalanine: a specific depletor of brain serotonin. J. Pharmacol. exp. Ther. **154**, 499—516 (1966).

Lints, C. E., Harvey, J. A.: Altered sensitivity to foot-shock and decreased brain content of serotonin following brain lesions in the rat. J. comp. physiol. Psychol. **67**, 23—31 (1969).

Lipton, M. A., Guroff, R. G., Udenfriend, S.: *p*-Chlorophenylalanine-induced chemical manifestations of phenylketonuria in rats. Science **156**, 248—250 (1967).

Mead, J. A. R., Finger, K. F.: A simple extraction method for the determination of both norepinephrine and serotonin in brain. Biochem. Pharmacol. **6**, 52—53 (1961).

Mouret, J., Bobillier, P., Jouvet, M.: Effects de la parachlorophenylalanine sur le sommeil du rat. C.R. Soc. Biol. (Paris) **161**, 1600—1603 (1967).

Pletscher, A., Bartholini, G., Bruderer, H., Burkhard, W. P., Gey, K. F.: Chlorinated arylalkylamines affecting the cerebral metabolism of 5-hydroxytryptamine. J. Pharmacol. exp. Ther. **145**, 344—350 (1964).

— DaPrada, M., Bartholini, G., Burkard, W. P., Bruderer, H.: Two types of monoamine liberation by chlorinated arylalkylamines. Life Sci. **4**, 2301—2308 (1965).

Rechtschaffen, A., Lovell, R. A., Freedman, D. X., Whitehead, P. K., Aldrich, M.: Effects of parachlorophenylalanine (PCPA) on sleep in rats. Psychophysiology **6**, 233 (1969).

Rosecrans, J. A., Lovell, R. A., Freedman, D. X.: Effects of lysergic acid diethylamide on the metabolism of brain 5-hydroxytryptamine. Biochem. Pharmacol. **16**, 2011—2021 (1967).

Rosen, A. J., Buga, J.: Effects of brain serotonin manipulation on multiple schedule performance in the rat. Arch. int. Pharmacodyn. **180**, 299—308 (1969).

Sheard, M. H.: The effect of *p*-chlorophenylalanine on behavior in rats: relation to brain serotonin and 5-hydroxyindoleacetic acid. Brain Res. **15**, 524—528 (1969).

— Aghajanian, G. K.: Stimulation of midbrain raphé neurons: behavioral effects of serotonin release. Life Sci. **7**, 19—25 (1968).

— Freedman, D. X.: The effects of CNS lesions on norepinephrine serotonin, and acetylcholine in brain. Brain Res. **3**, 292—294 (1966/1967).

Spector, S., Sjoerdsma, A., Udenfriend, S.: Blockade of endogenous norepinephrine synthesis by α-methyl-tyrosine, an inhibitor of tyrosine hydroxylase. J. Pharmacol. exp. Ther. **147**, 86—95 (1965).

Stevens, D. A., Resnick, O., Krus, D. M.: The effects of *p*-chlorophenylalanine, a depletor of brain serotonin, on behavior. I. Facilitation of discrimination learning. Life Sci. **6**, 2215—2220 (1967).

Tagliamonte, A., Tagliamonte, P., Gessa, G. L., Brodie, B. B.: Compulsive sexual activity induced by *p*-chlorophenylalanine in normal and pinealectomized rats. Science **166**, 1433—1435 (1969).

Tenen, S. S.: The effects of *p*-chlorophenylalanine, a serotonin depletor on avoidance acquisition, pain sensitivity and related behavior in the rat. Psychopharmacologia (Berl.) **10**, 204—219 (1967).

Udenfriend, S., Weissbach, H., Brodie, B. B.: Assay of serotonin and related metabolites, enzymes, and drugs. In: Methods of Biochemical Analysis, vol. VI, pp. 95—130. D. Glick, *ed.* New York: Interscience 1958.

Welch, A. S., Welch, B. L.: Effect of *p*-chlorophenylalanine on brain noradrenaline in mice. J. Pharm. Pharmacol. **19**, 632—633 (1967).

Woolley, D. W., Shaw, E. A.: A biochemical and pharmacological suggestion about certain mental disorders. Proc. nat. Acad. Sci. (Wash.) **40**, 228—231 (1954).

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