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EARLY EXPERIENCE AND LSD-25*

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A. INTRODUCTION

The purpose of this study was to determine the influence of variations in infantile experience on the behavioral effects of LSD-25 in adult rats. Bovard (4) has reported that handling rat pups during infancy modifies the balance of hypothalamic activity in favor of the anterior (parasympathetic) regions. Such modification reduces adult pituitary-adrenal and sympathetic-adrenal medulla responses to stress. Hence, handling is judged to reduce emotional reactivity to stress. Conversely, leaving rat pups to develop unmolested in the maternal nest appears to maximize sensitivity to external stimuli. According to Purpura (13), LSD-25 decreases reactivity of the nonspecific afferent system in cats. Hence, presumably, LSD-25 reduces an organism's emotional responsiveness to stress.

The possibility that variations in infantile experience may influence effects of LSD-25 was suggested by some research on schizophrenia. Clinical observers claim that schizophrenes commonly are reared in an overprotective and restrictive manner (2, 3, 5, 11). Others have alluded to the presence in schizophrenes of a psychotogenic agent, such as LSD-25 (1, pp. 91-119; 8, pp. 120-145). LSD-25 is related structurally to some of the breakdown products of epinephrine found in humans (7). Winter and Flataker (16) have added credibility to the notion that a psychotogenic agent exists. They have demonstrated that serum from the blood of psychotic patients (largely schizophrenic) and LSD-25 have similar effects on the behavior of rats. If *both* early experience and psychotogenic agent are involved in the development of schizophrenia, the nature of the interaction effect between these variables would be of heuristic interest.

B. METHOD

1. *Subjects and Procedure*

Litters of rats were randomly split to form sociological litters. The pups in one set of sociological litters were handled in infancy; pups in the other

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set were left in the nest unmolested or ignored. Testing began when the rats were 53 days old, on which day the groups handled and ignored were further subdivided. Half of the rats in each group were given injections of LSD-25; the other half were given "dummy" injections. Rats were then tested for general activity, water consumption, and avoidance learning.

Forty Holtzmann albino rats were used as subjects. They were raised from infancy by the experimenter and were housed in a research room in which an electric motor produced a constant background sound. The racks of cages were completely surrounded by a homogeneous field of white cloth curtains. A single ceiling lamp (three feet to the side of the cages) was allowed to burn eight hours per day. Light distribution was equalized (somewhat) by placing sheet metal over the top row of cages.

Two days after the birth of the rats, the natural litters were distributed among all the mothers in order to equalize genetic factors in the experimental and control groups. The litters were then randomly designated as "gentled" (i.e., handled) or "ignored."

Treatment was begun at once; i.e., on the second day. When pups were to be handled, the mother was removed from the home cage and was placed in a carrying cage. At first, three pups at a time were stroked from head to tail (at the rate of about 50 strokes per minute) for three minutes per day. When the rats were larger, each rat was handled individually. After the rats had been handled, they were replaced in the cage and, after a complete litter had been handled, the mother was returned to the cage. Handling or "gentling" was continued past weaning until the rats were 40 days old. At that point, experimental handling was discontinued until the 53rd day, when testing was begun.

Control rats (i.e., the rats ignored) were handled only for cage rearrangement and weaning. Treatment and housing were identical for all rats.

On the 21st day after birth, the rats were weaned, marked, and weighed. Within treatment groups, they were then assigned to smaller, separate cages, two rats to a cage. Cage mates were of the same sex. Solid, sheet-metal cage dividers separated the animal pairs.

2. *Apparatus*

Three sets of apparatus were used. The first set consisted of a tabletop, 60×30 inches, covered by a sheet of light gray wallboard. Fifty squares, 6×6 inches, were marked on the wallboard with a china marking pencil. The table was surrounded by black curtains situated one foot from its edges

and was placed directly beneath the overhead light in the room previously described.

On a similarly enclosed table nearby was a cage to which an eudiometer tube (100 cc capacity) filled with tap water was attached.

The third set of apparatus was located in a room directly adjacent to the research room. It consisted of a black runway 39 inches long, 5-1/2 inches wide, and 4 inches deep. A black starting box 8-1/2 inches long and a white goal box 11 inches long were closed off from the main runway by guillotine doors. Both starting box and runway floors consisted of electrified grids. The apparatus was capable of providing an electric charge based on 115 volts passed through a resistor of 150,000 ohms.

3. *Testing*

Testing began on Day 53 and continued for 10 days. On each day, two rats from the group of handled or "gentled" rats and two rats from the "ignored" group were randomly selected from one sex. Thus, on any given test day, all rats tested were males or all were females. One of the two "gentled" rats was assigned to an LSD group and one was assigned to a "distilled water" group. The "ignored" rats were similarly assigned. Prior to testing, each rat was deprived of food and water for 48 hours.

a. Group G-LSD. This group consisted of "gentled" rats. After weighing on the test day, each rat in this group was given an intraperitoneal injection of LSD-25. The dosage was 0.5 milligram of LSD per kilogram of body weight (i.e., 2 ml of an aqueous solution of LSD-25 per kilogram of body weight).

b. Group G-H_2O. This group consisted of "gentled" rats which were treated with an injection of 2 ml of distilled water per kilogram of body weight.

c. Group I-LSD. This group consisted of "ignored" rats treated with LSD-25.

d. Group I-H_2O. This group consisted of "ignored" rats treated with distilled water.

The four rats employed each day were tested in systematic order. (*a*) Thirty seconds after injection, the rat was placed on the tabletop and the number of squares the animal traversed was recorded for each of 20 consecutive minutes. A square was counted if the rat's left hind foot was placed in it. (*b*) The animal was placed in a carrying cage for two minutes. (*c*) He was placed for 20 minutes in the cage containing the eudiometer tube, and a record of the amount of water consumed was obtained. (*d*) The rat was allowed to rest one minute. (*e*) Then the rat was given 20 trials in the

runway. Fifteen seconds after the rat was placed in the starting box, the guillotine door was raised. Such action simultaneously activated a buzzer, the shock stimulus, and a chronograph. When the animal crossed the threshold of the goal box, the guillotine door was lowered (stopping buzzer, shock, and chronograph). The rat was permitted to remain in the goal box for 30 seconds, after which the next trial was begun. For trials 4 through 20, the shock grid was not electrified; only the buzzer and the chronograph were operative. The time recorded on the chronograph for the 17 nonshock trials constituted the third measure employed in this study.

C. Results

The general findings show (*a*) no significant weight differences between handled and ignored groups; (*b*) a significant interaction effect between LSD-25 and early experience during the initial stage of tabletop activity; and (*c*) a trend, on the part of "ignored" animals given LSD-25, toward greater running speed in the avoidance task.

Table 1 summarizes the weight data. A series of Kruskal-Wallis H tests (14) was performed to determine whether there were significant differences among the groups in weight *after deprivation* (immediately prior to testing). Separate tests were performed on the weights for the four males in each group, on those for the six females in each group, and on the weights for each of the four groups. A final Mann-Whitney U test (14) was done on

TABLE 1
MEAN WEIGHTS OF GROUPS AFTER DEPRIVATION

Group	Males ($N = 16$)	Females ($N = 24$)	Males and Females ($N = 40$)
G-LSD	194.55	151.68	168.83
G-H_2O	201.25	146.83	168.60
Total			168.72
I-LSD	194.40	156.07	171.30
I-H_2O	201.32	159.70	176.35
Total			173.83

these same weight data considering all 20 gentled animals as one group (regardless of type of injection) and all 20 ignored animals as one group (again disregarding injection). A U test performed on weight measures four days before the start of testing (with the animals satiated) revealed no significant differences between the gentled and the ignored animals; at that point the means were 183.1 and 183.3 respectively.

1. *Activity Measure*

The highly significant interaction effect shown in Table 2 confirms the hypothesis that the effects of LSD-25 on the activity of animals are significantly altered by variations in early experience.

Barlett's test (12) showed that the hypothesis of homogeneity of variance was tenable for the tabletop activity data. An analysis of variance, using the factorial design model discussed by Lindquist, was therefore applied to the untransformed data. The F for between-groups variance proved to be significant beyond the .01 level of significance. The mean number of boxes traversed by each animal in each of the groups during the 20 minutes of tabletop activity was as follows: G-LSD, 115.2; I-LSD, 209.9; G-H_2O, 272.4; I-H_2O, 218.7. Table 2 shows the partitioning of the between-groups sum of squares.

TABLE 2
PARTITIONING OF BETWEEN-GROUPS SUM OF SQUARES FOR TABLETOP ACTIVITY (20 MINUTES)

Source of variation	Sum of squares	*df*	Means squares	*F*
Early experience (G *vs.* I)	2059.2	1	2059.2	.4
Chemical (LSD *vs.* H_2O)	82355.7	1	82355.7	16.8**
Interaction	53655.6	1	53655.6	10.9**
Within groups	176776.3	36	4910.5	

** Significant at .01 level.

Lindquist's method of testing the differences between particular pairs of means, after an analysis of variance procedure, reveals that four of the six possible pairings are significant; the two comparisons yielding *insignificant* t's are those performed between groups I-LSD and I-H_2O and between groups G-H_2O and I-H_2O. Figure 1 shows the tabletop activity curves of the four groups over the 20-minute period. It is of special interest that the gentled and the ignored groups given dummy injections (groups G-H_2O and I-H_2O) present similar curves of activity which are not significantly different from one another, while comparable groups (G-LSD and I-LSD) given LSD-25 yield curves significantly different from one another. LSD-25 did not appear simply to have exaggerated subtle differences between gentled and ignored animals, because the slopes of the pertinent curves are not similar and, in addition, there is the reversal in activity measures of gentled and ignored groups depending on whether they have been given LSD or not. Although the total number of boxes traversed by the two ignored groups during the

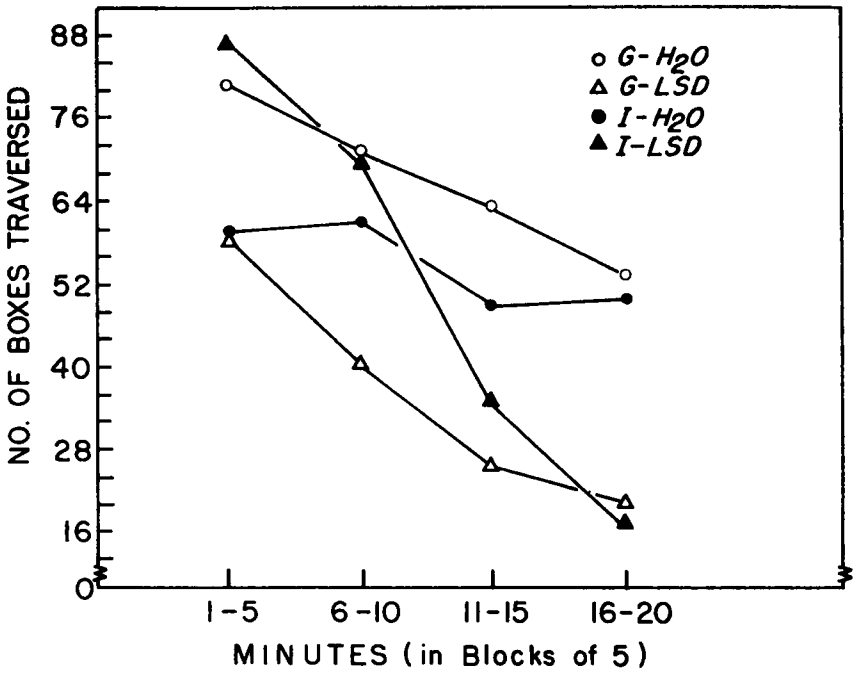

FIGURE 1
NUMBER OF BOXES TRAVERSED AS A FUNCTION OF TIME AFTER INJECTION AND SUBSTANCE INJECTED

20-minute period is not significantly different, the slopes of the curves produced by these groups are highly divergent, group I-LSD's range being the largest of the groups and group I-H_2O's being the smallest.

Two additional analyses of variance were performed on the tabletop activity data. The first analysis included only the data for the first 10-minute period, while the second included only the data for the second 10-minute period (see Table 3). As the data show, the interaction effect is maintained only during the first stages of LSD-25 intoxication and dissipates as the drug effect becomes maximal. The mean numbers of boxes traversed for the first and the second 10 minutes of tabletop activity (respectively) are as follows: G-LSD, 86.1, 29.1; I-LSD, 158.7, 51.1; G-H_2O, 148.0, 124.4; I-H_2O, 120.7, 98.0.

2. *Water-Consumption Measure*

The analysis of variance on the transformed drinking measures showed significance (at the .01 level) only for the main effect due to type of chemical

TABLE 3
PARTITIONING OF BETWEEN-GROUPS SUMS OF SQUARES FOR FIRST AND SECOND PERIODS OF TABLETOP ACTIVITY

Source of variation	Minutes 1-10 Sums of squares	*df*	Means squares	*F*	Minutes 11-20 Sums of squares	*df*	Means squares	*F*
Early experience (G *vs.* I)	5130	1	5130	2.1	60	1	60	0
Chemical (LSD *vs.* H_2O)	1428	1	1428	.5	50197	1	50197	9.8
Interaction	24950	1	24950	10.1**	5736	1	5736	1.1
Within groups	89141	36	2476	—	185331	36	5148	—

** Significant at .01 level.

(water or LSD-25) used (see Table 4). Because Bartlett's test indicated that homogeneity of variance was not tenable for the data on water consumption, a log transformation that corrected for heterogeneity of variance was employed. Correcting the water-intake measures for body-weight differences did not notably affect the analysis. Neither the early experience nor the interaction effects were significant. The mean number of cubic centimeters of water drunk by each animal during the 20-minute period in the water cage for the four groups was as follows: G-LSD, .54; I-LSD, .37; I-H_2O, 2.72; G-H_2O, 2.36. As is patent, LSD-25 inhibited water consumption in

TABLE 4
PARTITIONING OF BETWEEN-GROUPS SUMS OF SQUARES FOR WATER DRINKING (MINUTES 20 THROUGH 40 AFTER INJECTION)

Source of variation	Sums of squares	*df*	Means squares	*F*
Early experience	.00	1	.00	0
Chemical (LSD *vs.* H_2O)	1.43	1	1.43	51.52**
Interaction	.02	1	.02	.70
Within groups	1.00	36	.03	—

** Significant at .01 level.

the rats regardless of type of early experience. The water-drinking period (from the 20th to the 40th minute after LSD injection) occurred at the height of LSD intoxication when the animals were virtually immobile. Differential results as between gentled and ignored animals may have been found with a smaller dose of LSD-25. The hypotonic distilled-water injection did not appear to mask differences among groups.

3. *Runway Measures*

The analysis of variance did not yield significance at the .05 level. The obtained *F* was 2.75, while the *F* required for significance was 2.86. How-

ever, the data emphasize the trend for ignored animals which are given LSD to be fastest. The other three groups yield results that are highly similar to one another. The trend toward greater speed among the I-LSD subjects parallels their heightened motility during the initial phase of tabletop activity. Heterogeneity of variance is significant for speed of running scores. A log transformation to correct for heterogeneity was applied and an analysis of variance was performed, the score for each animal being the mean of the 17 nonshock trials in the runway. The mean running times (in hundredths of a second) for the groups were as follows: G-LSD, 31.04; I-LSD, 23.84; I-H_2O, 29.23; G-H_2O, 31.87. These measures were obtained during the 20-minute period starting at 40 minutes after injection. During this time, LSD intoxication had passed its peak.

D. Discussion

The discrepancy in behavior between gentled and ignored animals at the initial and the terminal stages of LSD-25 intoxication is an interesting one. Those rats which were gentled and given LSD-25 might be expected to be inured to novel or to noxious stimulation, and, presumably, therefore, to be relatively immobile and imperturbable. In the case of ignored rats given LSD-25, two conflicting effects would presumably be operative—ignoring serving to render these animals excitable and LSD-25 serving to render them insensitive.

In the case of the gentled animals given LSD-25, both agents, following from the views of Bovard (4) and Purpura (13), would serve to lessen excitability. Combining both the effects of early experience and LSD-25, we would expect ignored LSD animals to be more active than gentled LSD animals. The hyperactivity of ignored, LSD-25 injected rats (as compared with the gentled LSD-25 controls) appears to corroborate Bovard's view of the effects of early experience. The fact that the control groups (G-H_2O and I-H_2O) did not differ may highlight the "latent" nature of the effects of varying early experience, differences becoming evident only when a markedly novel stimulus (LSD-25) is introduced. The effects of differences in handling are nullified when the relatively large dose of LSD-25 reaches full potency. At that point (approximately 20 minutes following injection), all the animals given the drug are relatively immobilized. It is instructive to note that the behavioral effects of handling were evident under the initial and the terminal stages of LSD-25 intoxication despite the fact no weight differences were observed among groups prior to testing. Relating this to Bovard's ideas,

it would appear that the sequelae of handling relating to "emotionality" do not covary with the anabolic or growth sequelae.

In regard to schizophrenia, our results highlight the notion that the toxic element hypothesized to be active in that disorder does not act *in vacuo*. Rather, the interaction between toxic agent and the experiential background of the individual must be studied. Speculatively, this interaction may determine which variety of schizophrenic symptoms the individual displays, or one might question, on the basis of the present results, whether the initial and the terminal stages of the disorder differ, behaviorally, in those schizophrenics with overprotective or restrictive parents as opposed to those where overprotection is not involved.

E. Summary

The question of an interaction effect between early experience and LSD-25 was studied. It was found that a significant interaction effect occurs during the first 10 minutes after LSD-25 injection. Ignored rats injected with LSD-25 show significantly greater activity on a tabletop than do gentled rats injected with LSD-25, despite the fact that control groups not given LSD-25 are not significantly different from one another. The implications of these findings for Bovard's hypotheses concerning the later effects of "gentling" and "ignoring" organisms in infancy were considered. Speculations concerning the possible ramifications of these findings for the study of schizophrenia were also discussed.

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