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Effects of LSD-25 and Dimethysergide Bimaleate on a Conditioned Reflex in the Rat

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INTRODUCTION

The experimental objects of this study were conceived with two purposes.

The first purpose was to study the effects of different amounts of LSD-25 on spontaneous and motivated behaviour (conditioned reflex) in the rat.

The second purpose was to study the effect of another drug having an antiserotonin action and a molecular structure similar to LSD-25 upon the same performances in the rat in order to verify the hypothesis which ascribes the effects of LSD-25 to its property of antagonizing serotonin (COOK AND WEIDLEY 1956). For this purpose we used dimethysergide bimaleate (Deseril).

The literature concerning this subject reports changes in both spontaneous behaviour and conditioned reflexes produced by the action of LSD-25. Changes of spontaneous behaviour were reported by WINTER AND FLATAKER (1956). The authors described a syndrome characterized by increase of motor activity followed in few minutes by immobility although the movements were still possible. The animal was able to give an adequate response if tested with a climbing-pole apparatus. An increase of both spontaneous activity and defensive hostility was reported by NORTON (1957) in the cat.

BRUNEAUD AND LION (1959) observed in the rat an increase of induced aggressivity after a dose of 0.1 mg/kg. The same dose changes the affectivity of kangaroo rat by reducing its characteristic sociability and digging behaviour (HALEY AND MAVIS 1964).

In the rat BRIMBLECOMBE (1963) observed a significant increase of emotionality evaluated by open-field test, with a dose of 0.5 mg/kg of LSD-25.

The authors who have studied the action of LSD-25 on conditioned reflexes do not report consistent findings. An inpairment of performance was observed by BRIDGER (1958) and MAFFII (1959); RAY AND MARRAZZI (1961) and WINTER AND FLATAKER (1956) observed a decrease in conditioned response in some experiments. BLOUGH (1957) observed an increase of time-response for a conditioned reflex in the pigeon with LSD-25 but obtained an improvement of discrimination tests in the same animal.

Monkeys were found to show an impairment in performing delayed-alternation problems following an injection of LSD-25 (JARVIK AND CHOROVER 1960). The same authors demonstrate that initial doses as low as 0.005 mg/kg decrease accuracy of response and increasingly larger doses also tend to depress the rate of performance. JARVIK AND CHOROVER (1960) observed an increase of distractability due to environmental stimuli induced by injection of LSD-25. KEY (1964) also showed in cats an impairment of performance rate in an avoidance conditioned reflex following doses of 5–10–20 mg/kg of LSD-25 (i.m.). KEY (1964) explained this result by an increase of the "level of significance or meaning attached to the sensory stimuli" that is the gradient of conditioned stimulus generalisation. A change in gradient of generalisation would induce in the cat a reduction of arousal threshold not only for unconditioned auditory stimuli but also for different sounds.

Contrary results were reported by JARRARD (1963), who observed an impairment of rat performance in both food-reward and shock-avoidance situations following the injection of larger doses of LSD-25, while smaller doses induced an increase in performance rate.

The changes of spontaneous behaviour and conditioned reflexes induced by LSD-25 have been variously explained. Some authors postulate that the changes are explained by *d*-lysergic acid diethylamide (LSD-25) and serotonin interaction. Indeed, GADDUM (1954), COSTA (1956), and SHORE *et al.* (1955) showed *in vivo* and *in vitro* an antagonism between these drugs. COOK AND WEIDLEY (1956) showed an antagonism between LSD-25 and serotonin in their effect on conditioned reflexes in the rat.

The literature concerning experiments on conditioned behavior does not report any studies comparing the effects of LSD-25 with other drugs antagonizing serotonin.

MATERIAL AND METHODS

The experiments described in this paper were performed with the method of conditioned avoidance response (barrier-crossing-response). In comparison with other avoidance situations (lever-pressing-response, pole-climbing-response) this method is preferable because it does not require either hard muscular effort or training in unusual movements.

The subjects were 10 male albino rats of the Wistar strain which averaged 230–250 g in body weight at the start of the experiment. The apparatus consisted of a shuttle-box in plexiglas with a transparent band on the ceiling, so that the animal could be observed during the experiments. The grid floor consisted of 40 bars of rustless steel. The box was divided by means of a wall having a small door measuring 6 × 5 cm. The unconditioned stimulus (U.S.) consisted of an electric shock (0.8–1 mA) delivered through a grid scrambler to the floor of the box. The conditioned stimulus consisted of a sound produced by a bell fixed centrally on the partition wall.

The animal was placed in the experimental cage for 5 min in order to familiarize it with the environment. Then it was trained with the shock avoidance procedure. The sound stimulus (conditioned stimulus, C.S.) lasted 6 sec. After 3 sec from the beginning of the sound the animal received the electric shock (unconditioned stimulus, U.S.). It was possible for the animal to stop the electric shock by passing through the

door into the opposite compartment of the box. In this way it produced a capacity variation in an electronic circuit, setting in action a relay, which discontinued the stimulus.

The training lasted 10 min and 40 shocks were given, administered at 6 sec intervals. The responses were automatically registered by a writing apparatus which discriminated conditioned from unconditioned responses. An electro-mechanical meter marked the response-time giving a rate proportional to its amount.

The experiment started when the rats had obtained a constant performance rate (conditioned responses 70%) in 3 training-sessions.

d-Lysergic acid diethylamide was injected intraperitoneally into 10 rats. Each subject served as its own control and was on another occasion injected with a physiological saline solution. The doses were 0.05, 0.1, 0.5 mg/kg. Dimethysergide bimaleate was also injected intraperitoneally into 10 rats at the same doses.

All the rats were injected 5 min before the beginning of the experiment according to data in the literature (WINTER AND FLATAKER 1956; JARRARD 1963) which indicate that the effect appears after 5 min and is maximal after 10 min.

RESULTS

We observed the effect of drugs on spontaneous behaviour in a familiar environment and on responsiveness to conditioned and unconditioned stimuli.

Spontaneous behaviour

Before starting the experiment the untrained rat explores the cage, passes through the door, stands upright on his hind legs in order to explore the walls, shows piloerection, exophthalmos and a typical tail posture. These phenomena are usually interpreted as emotional symptoms and are not observed when the animal is accustomed to the environment.

The rats injected by physiological saline solution showed the same behaviour as untrained rats. After i.p. injection of LSD-25, the behaviour of the rats changed. With 0.1 mg kg the exploring activity increased and about 1 min after the injection piloerection and exophthalmos appeared. With 0.5 mg/kg motor activity increased for a short time. After a few minutes the rats remained in a corner of the box with flexed legs so that the abdomen touched the floor and they showed marked piloerection. Changes of performance were not observed with a dose of 0.05 mg/kg.

The rats injected with dimethysergide bimaleate did not show any difference in their behaviour in comparison with controls, whatever the dose which was used.

Conditioned reflex

During the experiment the performance of rats injected with 0.05 and 0.1 mg/kg of LSD-25 was similar. During the interval-time they stayed expectantly in front of the partition wall, passing quickly through the door when they heard the bell sound (C.S.). Therefore the shock avoidance rate was higher than in controls.

Table 1 reports the results. The rats injected with 0.05 mg/kg averaged 34.8 shock avoidances on 40 sound-shock cycles, *i.e.*, 87.0%, while the rats injected with physiological saline solution (controls) averaged 28.3 shock-avoidances, that is 78.75%.

TABLE 1

COMPARATIVE RESULTS OF EXPERIMENTS ON THE EFFECTS OF INCREASING DOSES OF LSD-25 ON CONDITIONED RESPONSES*

Rats	Control		LSD-25 0.05 mg/kg		LSD-25 0.1 mg/kg		LSD-25 0.5 mg kg	
	no. of responses	%	no. of responses	%	no. of responses	%	no. of responses	%
1	37	92.50	36	90.00	40	100.00	28	70.00
2	20	50.00	36	90.00	36	90.00	10	25.00
3	40	100.00	40	100.00	40	100.00	25	62.50
4	32	80.00	37	92.50	39	97.50	7	17.50
5	17	42.50	27	67.50	26	65.00	23	57.50
6	22	55.00	30	75.00	32	80.00	10	25.00
7	14	35.00	24	60.00	27	67.50	6	15.00
8	33	82.50	38	95.00	40	100.00	27	67.50
9	34	85.00	40	100.00	39	97.50	26	65.00
10	34	85.00	40	100.00	40	100.00	24	60.00
Mean	28.3	78.75	34.8	87.00	35.9	89.75	18.6	46.50
				χ^2 29.09 ($P<0.1$)		χ^2 42.70 ($P<0.1$)		χ^2 49.58 ($P<0.1$)

* For each dose in the left column we report the number of conditioned responses for 40 trials. In the right column we report the percentage of the same data. In each column we also give the results of statistical analysis (χ^2) comparing the performance rates of injected and control rats.

We evaluated the ratio between these results with the χ^2 test and obtained a figure of 29.09 ($P<1\%$) for a dose of 0.05 mg/kg .With the dose of 0.1 mg/kg the average of shock-avoidances was 35.9, that is 89.75% ($\chi^2=42.70$; $P<1\%$). Therefore both the doses induced an improvement of performance when compared with the controls. Statistical analysis shows that the improvement is highly significant. The dose of 0.1 mg/kg induced the greater improvement. With 0.5 mg/kg we obtained opposite results: the average of shock-avoidances was then 18.6, that is 46.5% ($\chi^2=49.58$; $P<1\%$). Therefore the impairment of performance was significant with this dose according to statistical analysis.

In Table 2 we report the performance rate of rats injected with dimethysergide bimaleate. With 0.05 mg/kg the shock-avoidance average was 32.1 (80.25%). With 0.1 mg/kg the average was 31.8 (79.50%) and with 0.5 mg/kg it was 30.9 (77.25%). There were no significant changes in comparison with the controls which averaged 31.5 shock-avoidances in 40 sound-shock cycles (78.75%). This is proved by the analysis of χ^2; the results were respectively: 0.19, 0.03, and 0.18 with the 3 doses of injected drug. With any dose of both drugs no changes were observed in comparison with the controls as far as the responses to the unconditioned stimulus (electric shock) were concerned.

We also considered the response-time estimated from the beginning of the sound to movement into the opposite box compartment. The average time taken by controls was 2.28 sec, while with 0.05 mg/kg and 0.1 mg/kg of LSD-25 we observed shorter response-times, that is to say respectively 2.03 sec with the dose of 0.05 mg/kg and 1.74 sec with the dose of 0.1 mg/kg (Table 3). We observed opposite results in rats injected with 0.5 mg/kg, in which the response-time was higher than in controls (2.95 sec).

The rats injected with dimethisergide bimaleate did not show any significant changes

TABLE 2

COMPARATIVE RESULTS OF EXPERIMENTS ON THE EFFECTS OF INCREASING DOSES OF DIMETHYSERGIDE BIMALEATE ON CONDITIONED RESPONSES*

Rats	Control		Dimethysergide bimaleate 0.05 mg/kg		Dimethysergide bimaleate 0.1 mg/kg		Dimethysergide bimaleate 0.5 mg/kg	
	no. of responses	%	no. of responses	%	no. of responses	%	no. of responses	%
1	38	95.00	39	97.50	31	77.50	34	85.00
2	40	100.00	38	95.00	38	95.00	39	97.50
3	40	100.00	36	90.00	35	87.50	39	97.50
4	39	97.50	39	97.50	39	97.50	36	90.00
5	21	52.50	34	85.00	33	82.50	26	65.00
6	22	55.00	19	47.50	25	62.50	20	50.00
7	14	35.00	16	40.00	18	45.50	18	45.50
8	33	82.50	35	87.50	33	82.50	33	82.50
9	34	85.00	33	82.50	35	87.50	30	75.00
10	34	85.00	32	80.00	37	77.50	34	85.00
Mean	31.5	78.75	32.1	80.25	31.8	79.50	30.9	77.25
				$\chi^2$0.19		$\chi^2$0.03		$\chi^2$0.18

* For each dose in the left column we report the number of conditioned responses for 40 trials. In the right column we report the percentage of the same data. In each column we also give the results of a statistical analysis (χ^2) comparing the performance rates of injected and control rats.

TABLE 3

RESPONSE-TIMES AVERAGED BY CONTROL RATS AND BY THE RATS INJECTED WITH LSD-25

Rats	Response-time			
	Control	LSD-25 0.05 mg/kg	LSD-25 0.1 mg/kg	LSD-25 0.5 mg/kg
1	2.09	2.07	1.81	2.56
2	2.46	1.89	1.66	3.35
3	1.67	1.69	1.44	2.82
4	2.19	1.97	1.38	3.32
5	2.63	2.89	2.34	2.76
6	2.65	2.07	1.88	3.51
7	2.77	2.48	2.62	3.41
8	2.06	1.83	1.46	2.79
9	2.09	1.78	1.21	2.50
10	2.14	1.66	1.61	2.43
Mean time	2.28	2.03	1.74	2.95
		$F = 16.37$		

of response-time in comparison with the controls. Indeed, the controls averaged a response-time of 2.18 sec and the rats injected with dimethysergide bimaleate averaged 2.05 sec with a dose of 0.05 mg/kg, 2.17 sec with 0.1 mg/kg and 2.10 with the dose of 0.5 mg/kg (Table 4).

Statistical analysis was also performed by the F method (Fisher). We employed this method, which analyses at the same time the data obtained by all experimental groups, because it takes into account the individual changes within an experimental group, and

TABLE 4

THE RESPONSE-TIMES AVERAGED BY CONTROL RATS AND BY THE RATS INJECTED WITH DIMETHYSERGIDE BIMALEATE

Rats	*Response-time*			
	control	*dimethysergide bimaleate 0.05 mg/kg*	*dimethysergide bimaleate 0.1 mg/kg*	*dimethysergide bimaleate 0.5 mg/kg*
1	2.40	2.19	2.44	2.43
2	1.70	1.82	2.01	1.62
3	1.84	1.89	1.95	1.51
4	1.66	1.71	1.90	2.18
5	2.55	1.84	2.04	2.40
6	2.63	2.68	2.26	2.56
7	2.77	2.54	2.84	2.65
8	2.06	2.04	2.14	2.05
9	2.09	1.76	2.04	1.73
10	2.14	2.04	2.17	1.86
Mean time	2.18	2.05	2.17	2.10
		$F = 0.30$		

therefore removes a possible source of error. For the data obtained in rats injected with LSD-25, F was 16.37; this rate is very significant ($P < 0.01$) and shows a remarkable change of response-time in animals treated by injection of different doses of LSD-25.

The same statistical analysis of data obtained with dimethysergide bimaleate gave $F = 0.30$ (not significant). There is no change of response-time in rats injected with this drug.

DISCUSSION

We will consider separately the results obtained.

1. Effects of LSD-25 in increasing doses, from 0.05 mg/kg to 0.5 mg/kg on the spontaneous behaviour of rats

The reported data show that all doses of LSD-25 induce changes in spontaneous behaviour, that is piloerection and exophthalmos. These signs are more obvious with higher doses. Smaller doses also increase spontaneous activity. Higher doses, by contrast, reduce spontaneous activity. These experimental data confirm reports in the literature (WINTER AND FLATAKER 1956; NORTON 1957; BRUNEAUD AND LION 1959; HALEY AND MAVIS 1964; BRIMBLECOMBE 1963).

2. Effects of dimethysergide bimaleate in similar doses on spontaneous behaviour of the rat

A drug with a molecular structure like that of LSD-25 and having an anti-serotonin action (dimethysergide bimaleate) did not induce significant changes in the spontaneous behaviour of the rat.

3. Effects of LSD-25 in increasing doses, from 0.05 mg/kg to 0.5 mg/kg on a conditioned reflex of avoidance

The data obtained in this experiment show the different effects of various doses of LSD-25 on conditioned avoidance-response. Our findings are in contrast with most

results given in the literature (BRIDGER 1958; MAFFII 1959; RAY AND MARRAZZI 1961; WINTER AND FLATAKER 1956; KEY 1964) but agree with those of JARRARD (1963). JARRARD explained the different results of other authors by suggesting that these authors experimented only with higher doses. This hypothesis cannot, however, explain the results of RAY AND MARRAZZI (1961), who observed in rats an impairment of performance in a conditioned reflex of avoidance with a dose of 0.1 mg/kg (that is with the dose inducing the highest improvement of performance in our experiment).

Possibly other authors did not observe an improvement in performance with smaller doses of the drug because of differences in experimental conditions or because performance of the task chosen could not be improved further.

4. Effect of dimethysergide bimaleate in the same doses on a conditioned reflex of avoidance

We did not observe any significant changes in conditioned responses on animals injected with dimethysergide bimaleate. This result does not support the hypothesis suggested by some authors (GADDUM 1954; COSTA 1956; SHORE 1955; COOK AND WEIDLEY 1956) *viz.*, that the action of LSD-25 is produced by antagonising serotonin. However, dimethysergide bimaleate is a known serotonin antagonist and as this drug produced no significant effect in our experiments it would seem unlikely that LSD-25 also acts in this way.

JARRARD (1963) observed marked changes in the response-time induced by different doses of LSD-25. He also reported some clinical researches on man proving that "one effect of the drug was to distort the sense of time" (JARRARD 1963).

We suggest a different hypothesis. One effect of LSD-25 observed in our experiment was to increase emotional expressions at somatic level and these symptoms are likely to be proportional to the dose (BRIMBLECOMBE 1963; BRUNEAUD AND LION 1959). On the other hand we observed that small doses of LSD-25 have a facilitating action on a conditioned response while higher doses have an inhibitory effect. We can suppose that a dose inducing emotional changes would also facilitate an avoidance-response to a painful stimulus. In the animals in which we established an association between a painful stimulus and the conditioning signal the increased emotivity could also increase the meaning of the latter and induce a greater number of conditioned responses. This happened with smaller doses. The inhibition induced by higher doses could also be explained by the same mechanism as an excessive state of emotional alarm together with the impairment of motor activity and reaction-time can interfere with an adequate avoidance response (WINTER AND FLATAKER 1956; BARUK *et al.* 1958). This hypothesis is based on theories of emotion accepted by some modern psychologists (ABBAGNANO 1962; TORRE 1965). These theories distinguish the emotions according to the meaning they assume in relation to the determining situation.

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SUMMARY

The authors studied the effects of LSD-25 at doses of 0.05 mg/kg, 0.1 mg/kg and 0.5

mg/kg, and of dimethysergide bimaleate (Deseril) at the same doses, on spontaneous behaviour and on a conditioned reflex of avoidance in rats.

They obtained the following results:

(1) LSD-25 induced changes in spontaneous behaviour including piloerection and exophthalmos. This effect was proportionally increased with the dose. Smaller doses increased motor activity, while higher doses inhibited it.

(2) Dimethysergide bimaleate in the same doses did not induce any change in spontaneous behaviour.

(3) LSD-25 in doses of 0.05 mg/kg and 0.1 mg/kg improved performance in a conditioned reflex of avoidance, reducing the shock rate and the response-time. With a dose of 0.5 mg kg, on the contrary, we obtained an increase of the shock rate and a lengthening of the response-time.

(4) Dimethysergide bimaleate did not have any effect on the same conditioned reflex.

The authors suggest that the action of LSD-25 does not result from the anti-serotonin action of this drug because another drug with known anti-serotonin action (dimethysergide bimaleate) did not induce significant changes under the same experimental conditions.

In discussing the biphasic dose-dependent activity of LSD-25 on the animal's behaviour as proved in this study the authors suggest that the drug, in the doses used, acts mainly on emotivity.

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