

Evidence of Catecholamine Mediation in the 'Aberrant' Behaviour Induced by Lysergic Acid Diethylamide (LSD) in the Rat

Since the first report on the properties of LSD¹ several hypotheses have been advanced to explain the potent psychotomimetic action of this compound. Based on its profound serotonin blocking activity on peripheral tissues² it was postulated that LSD could antagonize serotonin³ in the brain. Later theories proposed a serotonin-like central action^{4,5}. However the advent of D-2-bromo-lysergic acid diethylamide (BOL-148) showed this substance to be a more potent serotonin antagonist than LSD^{6,7} and the discovery that it was without psychotomimetic activity cast doubt on the serotonin deficiency theory. From a study of 18 derivatives of lysergic acid CERLETTI⁸ found a striking parallelism between the central activity in animals and the psychotic effects in man. BRODIE et al.⁹ pointed out that besides being an indole, LSD possessed a phenylethylamine skeleton and suggested that its main action was through stimulation of central adrenergic receptors. This contention was reiterated by COSTA et al. in 1962¹⁰.

It is clear that despite much discussion the mode of action of LSD on central nervous function is still unresolved. One reason is the lack of a suitable experimental model in animals by means of which the typical effects of LSD as seen in man can be reproduced. Attempts towards this problem have made use of the pyretogenic effect of LSD in rabbits^{11,12} which is produced with doses similar in order to the human psychotic dose. However the influence on temperature regulation in the rabbit is difficult to compare with the behavioural action in man.

In the rat behavioural effects of LSD can be demonstrated only by the administration of relatively high doses. The pattern of arousal exhibited however is both reproducible and typical. The behaviour consists of a strong excitement with clearly recognizable symptoms including a stereotyped component with accompanying sympathetic stimulation. Using such 'aberrant' behaviour it has been possible to develop a method for the study of drug influences on the action of LSD. Although it may be questionable whether such behavioural disturbances induced in animals are representative of the psychotomimetic action in man, there seems to be no reason why, for lack of a better model, these effects cannot be subjected to closer examination in the hope of gaining some insight into the mode of action of LSD.

Method. Experiments were carried out in male Sprague-Dawley rats (170–220 g). The rats were kept in the laboratory for at least 24 h before use, at a constant room temperature of 22°C. Food and water were available ad libitum up to 3 h before the test. Half an hour before injection of LSD the animals were removed from their home cages (housed 3 to a cage) and transferred to the experimental cage. The latter consisted of a Plexiglass container 21 × 36 × 15 cm fitted with a steel wire mesh floor raised 2 cm above the bottom of the cage. Experimental trials consisted of one control group injected with 5 or 3 mg/kg s.c. of LSD tartrate, and test groups injected with the same dose of LSD as well as the test substance. All groups consisted of 6 rats. Observation of the behaviour commenced immediately after the administration of LSD and lasted for 3 h. For the purpose of facilitating observation 3 rats were allocated to each experimental cage, 4 sets of 3 rats being observed simultaneously. For each drug tested a fresh LSD control was set up. The behaviour was assessed using a scoring system under blind conditions.

Two aspects of activity were rated. (a) Aberrant behaviour, and (b) locomotor excitation. The scoring system for (a) and (b) respectively was on a 0–8 continuum.

For LSD induced aberrant behaviour (LSD-AB): 0, normal activity as exhibited by 0.9% saline treated rats; 2, slight sniffing and side to side head movements; 4, strong sniffing and head movements, licking and jaw movements; 6, incessant sniffing, licking, biting of wire mesh floor; 8, as for 6 with the addition that the animals lie with their jaws firmly clamped on the wire mesh floor, practically immobile except for sporadic twitches of the body; occasionally interrupted by aggressive-like reactions. Locomotor excitation was scored as: 2, slight; 4, moderate; 6, strong; 8, very strong.

The score for each separate rat was assessed at 10 min intervals for the first hour and then subsequently every 20 min up to the termination of the trial. The scores obtained were totalled thus yielding 6 values for each group. These values were then compared for significance against the respective control using the Wilcoxon's Two-Sample Rank Order Test (non-parametric).

All drugs were injected by the s.c. route unless otherwise stated. Details of pretreatment times are given in the legend to the Figures. LSD tartrate was dissolved in 0.9% saline. Test drugs were dissolved in the same medium except where otherwise stated. Injection volume was 10 ml/kg body weight.

Drugs tested. Dibenamine hydrochloride, phenoxybenzamine hydrochloride, pronethalol hydrochloride, propranolol hydrochloride. Reserpine (base), chlorpromazine hydrochloride, perphenazine tartrate, BOL-148 (D-2-bromo-lysergic acid hydrogen tartrate), Deseril (1-methyl-D-lysergic acid hydrogen maleinate), desmethylinipramine hydrochloride, disulfiram (tetraethylthiuram disulphide), parachloro-phenyl-alanine, α -methyl-para-tyrosine (base). The amino acids, and disulfiram were administered as an aqueous suspension. Reserpine was mixed with an equal amount of tartaric acid, 5 drops of absolute ethyl alcohol, and made up to volume with distilled water.

Results. Following the injection of 5 mg/kg LSD into rats a typical and profound excitation is produced. Within

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⁸ A. CERLETTI, in *Neuro-Psychopharmacology*, First Int. Meet. Neuro-Psychopharmac., Rome 1958 (Eds. P. B. BRADLEY, P. DENIKER and C. RADOUCO THOMAS; Elsevier, Amsterdam 1959), vol. 1, p. 117.

⁹ B. B. BRODIE, S. SPECTOR and P. A. SHORE, *Pharmac. Rev.* 11, 548 (1959).

¹⁰ E. COSTA, G. L. GESSA, C. HIRSCH, R. KUNTZMAN and B. B. BRODIE, *Ann. N.Y. Acad. Sci.* 96, 118 (1962).

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¹² A. HORITA and J. H. GOGERTY, *J. Pharmac. exp. Ther.* 122, 195 (1958).

5 min the rats exhibit piloerection, mydriasis and commence a light but persistent aimless sniffing. This is accompanied by an increase in locomotor activity in the initial phase of drug action, but which is gradually super-
 vined by an intensification of sniffing. Incessant head movements from side to side, together with compulsive licking and chewing movements appear within 10–15 min after administration. This activity culminates in what appears to be an abnormal or 'aberrant' pattern of behavioural characterized as a strong tendency to bite or gnaw the wire mesh floor of the cage (see Figure 1). The maximum intensity of this syndrome is reached 45–60 min after receiving LSD. At this point locomotor activity may be impaired and the animals exhibit a reduced pelvic elevation with the hind limbs placed flat on the floor in a splayed position. Occasionally when rats are in contact with each other, some exhibit an aggressive-like reaction denoted as a rearing on the hind legs and facing the other animal with both fore-paws extended. This is accompanied by frequent teeth chattering and vocalization. Actual biting however is rarely seen. After about 80–90 min this activity subsides until 100–120 min few symptoms are observed. The animals then appear completely exhausted.

The effects of α - and β -adrenergic-blocking agents, namely dibenamine, phenoxybenzamine, propranolol and pronethalol on LSD-induced aberrant behaviour are given in Figure 2.

A clear dose related inhibitory action was obtained for all compounds with the exception of dibenamine. This was the weakest antagonist of this group and produced clear inhibition of LSD-AB only in the highest dose. At this level locomotor excitation was also reduced. Phenoxybenzamine was particularly effective, producing a profound inhibition of LSD-AB in all doses tested, and qualitatively it produced a concomitant depressant action on locomotor activity similar to dibenamine. The 2 β -adrenergic-blocking agents however, unlike the α -blocking drugs only produced an inhibition of LSD-AB whilst in the same doses left locomotor activity unimpaired.

Although these 4 drugs are established adrenolytics, 2 of them, dibenamine and phenoxybenzamine are known to antagonize serotonin in peripheral tissues. Thus it seemed interesting to investigate the action of known serotonin antagonists on this phenomenon. The results of 2 such compounds, deseril and BOL-148, are given in Figure 3.

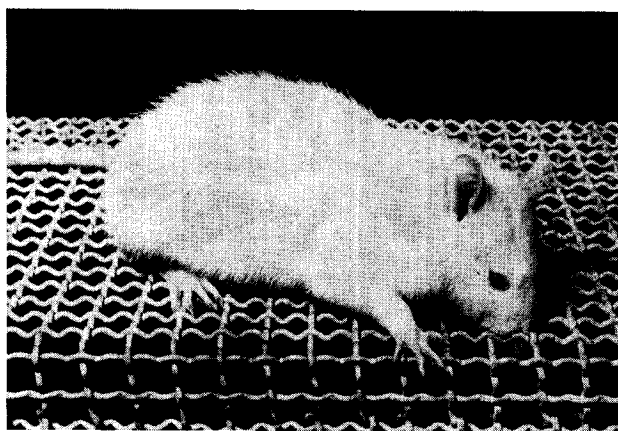


Fig. 1. LSD-induced aberrant behaviour in the rat. See text for description.

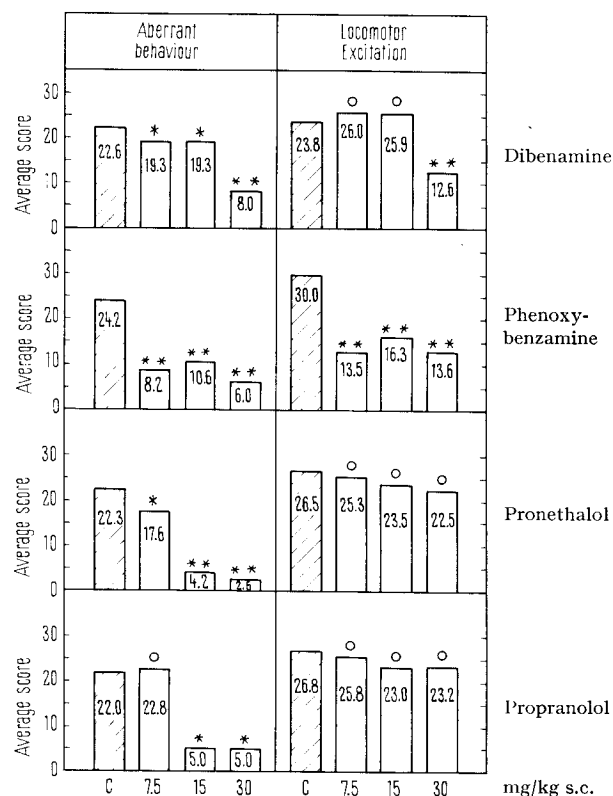


Fig. 2. Influence of adrenergic blocking drugs on LSD-induced behaviour. C, LSD 5 mg/kg s.c. Doses of investigated compounds administered 2 h before LSD. Statistical significance (p): 0, > 0.05, *, < 0.05, **, < 0.01. Results expressed as the average total score obtained for each group in 3 h.

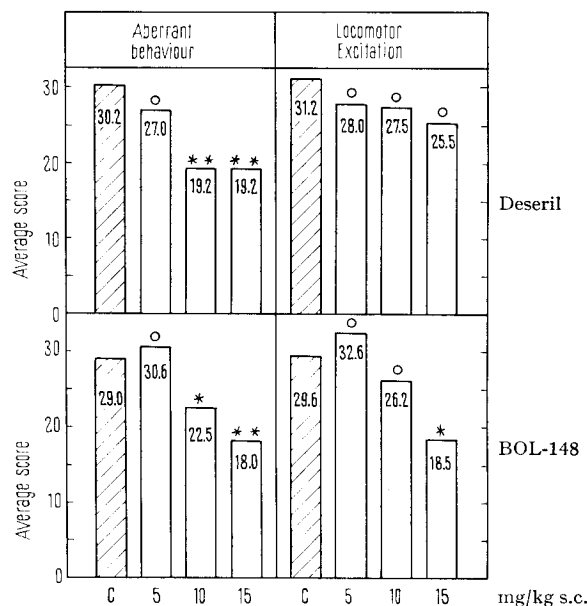


Fig. 3. Influence of serotonin antagonists on LSD-induced behaviour: C, LSD 5 mg/kg s.c. Doses of investigated compound administered 1 h before LSD. Statistical significance (p): 0, > 0.05, *, < 0.05, **, < 0.01. Results expressed as the average total score obtained for each group in 3 h.

Both substances exerted only weak inhibitory actions on LSD-AB in the doses tested and did not antagonize the locomotor excitation, with the exception of BOL-148 which produced weak impairment in the highest dose.

The action of 4 psychotropic drugs on LSD-AB are given in Figure 4.

The 2 phenothiazines, perphenazine and chlorpromazine, exerted a dose related inhibition of the aberrant behaviour as well as reducing the locomotor excitation. The action of perphenazine was particularly strong. Animals pretreated with this substance were practically inactive.

Following a 20 h pretreatment with reserpine a striking potentiation of the behavioural effects of LSD was observed. The effect of reserpine showed a clear dose response. The rats exhibited marked sniffing, salivation, and compulsive gnawing of the wire mesh floor. In many cases the animals were aggressive showing a rage reaction, spitting, and vocalization. A slight but significant reduction in locomotor activity was also obtained.

A similar but less intense effect was produced by desmethylinipramine. In contrast to reserpine, desmethylinipramine also increased locomotor activity by a significant extent. It should be noted that in this experiment only 3 mg/kg of LSD was injected so that the potentiating effect could be better revealed.

The effect of 3 inhibitors of biogenic amine synthesis in vivo are given in Figure 5. Para-chloro-phenylalanine in doses known to almost completely inhibit the synthesis

of serotonin in the rat brain, without influencing brain levels of catecholamines was found to exert no influence on either LSD-AB or locomotor excitation. In contrast a single dose of α -methyl-para-tyrosine, a potent inhibitor of catecholamine synthesis in the brain but without effect on cerebral serotonin concentrations, produced a strong inhibition of LSD-AB and to a lesser extent LSD-induced locomotor activity. Within 20–30 min after LSD the α -methyl-para-tyrosine treated animals showed practically no aberrant behaviour.

In disulfiram treated rats only a weak reduction of LSD-AB was observed, this slight effect being evident only in the first 30 min following LSD. Disulfiram has been shown to inhibit conversion of dopamine to nor-adrenaline.

Discussion. The results show that α - and β -adrenergic-blocking drugs, α -methyl-para-tyrosine, and the major tranquilizers perphenazine and chlorpromazine inhibit LSD induced aberrant behaviour.

The β -adrenergic-blocking drugs, pronethalol and propranolol, exert a selective antagonism of LSD-AB since in contrast to dibenamine and phenoxybenzamine, no influence on the locomotor activity induced by LSD was demonstrated. This observation suggests that the mechanism subserving LSD-AB is particularly sensitive to compounds interacting with the β -adrenergic system.

It was previously pointed out that phenoxybenzamine and dibenamine in addition to their adrenergic proper-

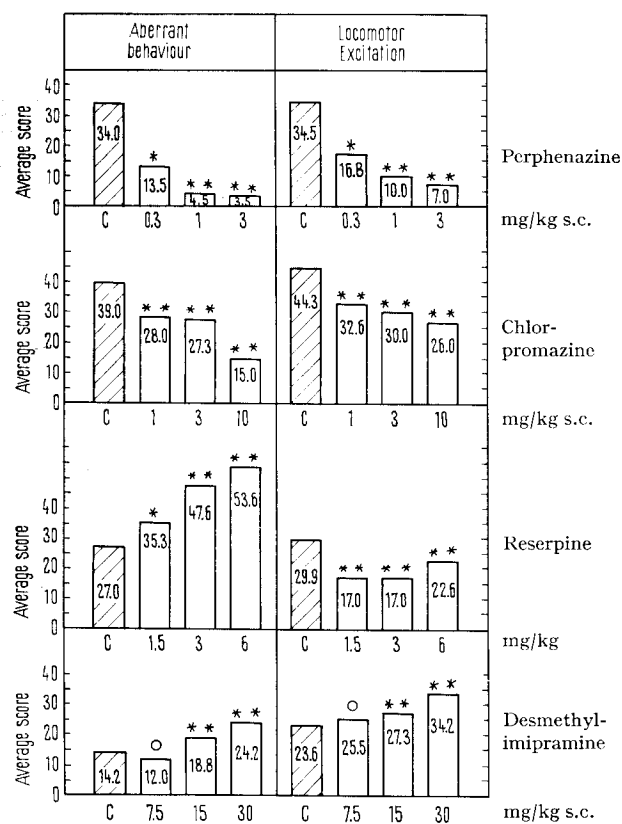


Fig. 4. Influence of psychotropic drugs on LSD-induced behaviour. C, LSD 5 mg/kg s.c. Reserpine given i.p. 20 h before LSD. All other compounds administered 2 h before LSD. LSD 3 mg/kg s.c. was used in the experiment with desmethylinipramine. Statistical significance (p): 0, > 0.05, *, < 0.05, **, < 0.01. Results expressed as the average total score obtained for each group in 3 h.

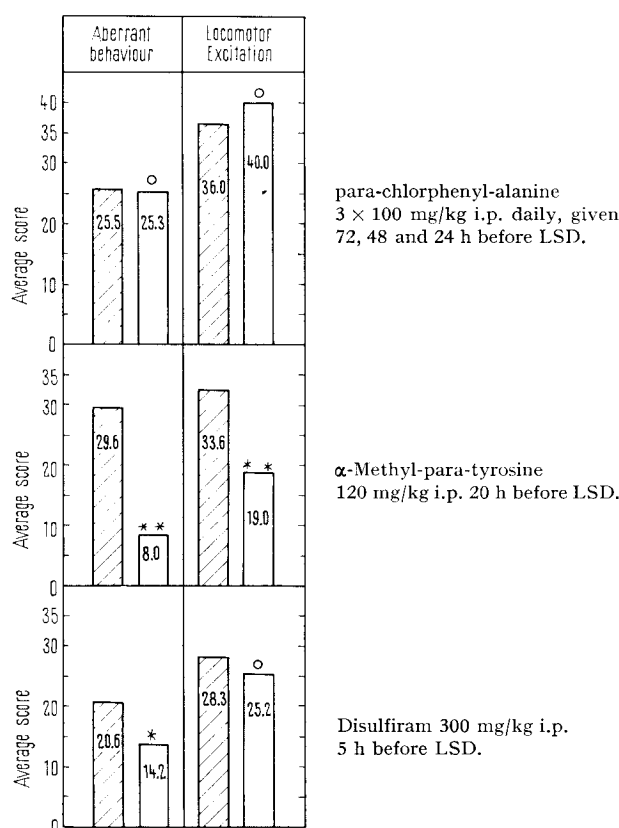


Fig. 5. Influence of biogenic amine synthesis inhibitors on LSD-induced behaviour. C, LSD 5 mg/kg s.c. Statistical significance (p): 0, > 0.05, *, < 0.05, **, < 0.01. Results expressed as average total score obtained for each group in 3 h.

ties, possess serotonin antagonistic characteristics¹³⁻¹⁵. That this latter property was not responsible for their profound inhibitory action on LSD-AB is indicated by the weak antagonism obtained with BOL-148 and deseril. DOEPFNER¹⁶ has pointed out that deseril does not enter the brain as readily as LSD. However in the present experiments relatively high doses were adopted. Furthermore BOL-148 has been shown to exert clear antagonism of the potentiating action of serotonin on barbiturate anaesthesia¹⁷ which is of central origin. The fact that BOL-148 has also been detected in the brain in the same way as has LSD indicates that it is unlikely that these drugs were not present in the brain in sufficient amounts to exert an antagonism to serotonin.

Confirmatory evidence that serotonin does not play a major role in the central action of LSD was obtained with para-chloro-phenylalanine. This compound has been shown to be a potent and selective inhibitor of serotonin biosynthesis¹⁸. The dose regimen adopted in the present investigations is known to cause almost complete reduction in brain concentrations of serotonin without affecting catecholamine levels. Despite this fact para-chloro-phenylalanine failed to exert any influence on LSD-AB or the accompanying locomotor excitation.

It would appear therefore that the antagonism against LSD-AB produced by the adrenergic blocking agents reflects a true central adrenergic action, and furthermore suggests that the action of LSD is subserved by an adrenergic component.

Similarly the clear and significant inhibition of LSD-AB by α -methyl-para-tyrosine, a selective inhibitor of catecholamine biosynthesis¹⁹, gives strong support to the contention that catecholamine mediation is responsible for the excitement observed with LSD in the present experimental situation. It is of interest that besides causing an increase in brain levels of serotonin, LSD produces a significant concomitant lowering of noradrenalin and dopamine concentrations in the rat brain²⁰⁻²². Thus it could be that LSD release catecholamines centrally.

The question arises, which of the 2 amines, noradrenaline or dopamine contributes to the effects of LSD? Disulfiram, a compound which inhibits the conversion of dopamine to noradrenaline^{23,24}, produced only a small although significant attenuation of LSD-AB. Since after disulfiram brain levels of noradrenaline but not those of dopamine are reduced, it could be argued that a persistence of aberrant behaviour in this experiment suggests a dopamine mediated effect. However GOLDSTEIN²⁵ has pointed out that disulfiram does not completely inhibit brain noradrenaline synthesis, and thus the possibility exists that in the present experiment a sufficient reduction of this catecholamine was not achieved. The definite proof concerning the responsible catecholamines implicated in the observed action of LSD must await further data from studies on the biochemical mechanisms involved.

The action of perphenazine and chlorpromazine in antagonizing LSD-AB may reflect an action on central adrenergic mechanisms. Chlorpromazine possesses adrenergic properties but is also a potent dopamine antagonist^{26,27}. Recent studies with thioridazine carried out on the present experimental situation show that it exerts similar inhibitory effects as does chlorpromazine and perphenazine. Chlorpromazine and thioridazine are known to produce an elevation of homovanillic acid and dopamine in the corpus striatum²⁸⁻³¹. It should be of interest therefore to examine catecholamine concentrations in the basal ganglia in animals treated with LSD, before and after pretreatment with neuroleptics in order to ascertain if this could be the site of action.

The profound stimulation induced by LSD in reserpinized rats confirms earlier findings^{17,32}. Why reserpine should potentiate the behavioural action of LSD is not clear. In reserpinized animals there is a constant overflow of newly synthesized catecholamine in adrenergic neurones³³⁻³⁵ as a result of impaired intraneuronal storage. Uptake of catecholamine into the axoplasm however still occurs. Accordingly, it may be possible that LSD either sensitizes the central adrenergic receptors to the action of the available catecholamine, inhibits the re-uptake mechanism of catecholamine into the neurone, and/or possesses monoamine-oxidase inhibitory properties. This could possibly explain the stronger effect of LSD in reserpinized rats as compared to those animals receiving LSD alone. However until more detailed investigations have been carried out on this phenomenon, the reserpine potentiation of LSD can only be speculated upon.

Desmethylinipramine is a tricyclic antidepressant which is believed to act by inhibition of the re-uptake of noradrenaline into the neurone after release by nervous impulse³⁶⁻³⁹. Since LSD appears to act through catecholamine mediation in producing aberrant behaviour, the potentiation of this effect by desmethylinipramine should be expected and supports the assumption of the LSD mechanism.

As stated in the introduction it is possible that the aberrant behaviour produced by LSD in the rat does not reflect the psychotomimetic action in man. However the

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behavioural syndrome produced parallels the strong central effects previously reported by CERLETTI.

The work presented here supports the concept that LSD acts, at least partly, through catecholamine mediation.

Zusammenfassung. Der Einfluss verschiedener Pharmaka auf das durch LSD hervorgerufene bizarre Verhalten der Ratte wurde untersucht. Die Resultate zeigen, dass α - und β -Blocker sowie α -Methyltyrosine eine starke Hemmung dieses Verhaltens erzeugen, im Gegensatz zu den Serotonin-Antagonisten Deseril, BOL-148 und Para-

chlorphenylalaninen, die nur schwache oder keine Hemmung hervorrufen. Es wird gefolgert, dass das bizarre Verhalten durch Wirkung von LSD an Katecholaminstrukturen hervorgerufen wird. Die Wirkung von 4 psychotropen Pharmaka am bizarren Verhalten wird im Rahmen dieser Hypothese diskutiert.

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