Br. J. Pharmac. (1974), 52, 419-426

REVERSAL LEARNING ENHANCED BY LYSERGIC ACID DIETHYLAMIDE (LSD): CONCOMITANT RISE IN BRAIN 5-HYDROXYTRYPTAMINE LEVELS

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1 Small doses of lysergic acid diethylamide (LSD) (12.5-50 μg/kg) consistently facilitated learning of a brightness discrimination reversal.

2 2-Bromo-lysergic acid diethylamide (BOL-148), a structural analogue of LSD, with similar peripheral anti-5-hydroxytrypamine activity but no psychotomimetic properties, had no effect in this learning situation at a similar dose (25 μg/kg).

3 LSD, but not BOL-148, caused a small but significant increase in brain 5-hydroxytryptamine levels, but had no effect on the levels of catecholamines in the brain at 25 μg/kg.

Introduction

The behavioural effects of lysergic acid diethylamide (LSD) in laboratory animals have been studied in various experimental situations (Appel, 1968; Freedman, 1969; Himwich & Alpers, 1970). While it would be premature to conclude that the behavioural properties of LSD are well understood, some tentative conclusions may be reached. First, the behavioural effects of LSD are dose related; doses in the range 10-40 μg/kg increase responsiveness to sensory stimuli (Key, 1964b; Appel, 1971), while higher doses produce a variety of effects characterized as response perseveration, regression to earlier patterns of behaviour and 'pausing' (Appel, 1971; Castellano, 1971). Behaviour involving relatively difficult discriminations between several kinds of sensory stimuli or stimuli scaled along a common dimension is particularly sensitive to small doses of LSD. Both enhancement and deterioration of performance have been reported (Key, 1961; Roberts & Bradley, 1967; Appel, 1971), probably influenced by the amount of novel extraneous stimulation in the experimental situation; animals treated with LSD are easily distracted, and this may lead to a deterioration in performance (Key, 1964a).

Several attempts have been made to relate the electrophysiological effects of LSD to specific neurotransmitter substances present in the brain (Aghajanian, Foote & Sheard, 1970; Boakes, Bradley, Briggs & Dray, 1970). The excitant effects of iontophoretically applied 5-hydroxytryptamine (5-HT) on neurones are antagonized by LSD. After large doses of LSD levels of 5-HT were increased and noradrenaline levels decreased (Rosecrans, Lovell & Freedman, 1967; Leonard & Tonge, 1969). Chronic administration of LSD; in doses as low as 20 μg/kg has been found to cause an increase in brain 5-HT and a fall in the level of its principal metabolite, 5-hydroxyindoleacetic acid (Diaz & Huttunen, 1971). The administration of *p*-chlorophenylalanine, which inhibits synthesis of 5-HT, intensified the behavioural effects of LSD (Appel, Lovell & Freedman, 1970). Finally, a lesion in the raphé nucleus, which resulted in a widespread depletion of 5-HT in the brain, intensified effects of systemically administered LSD (Appel, Sheard & Freedman, 1970; Kuhar, Roth & Aghajanian, 1971).

In contrast to the considerable amount of behavioural, biochemical and neuropharmacological data which exists in isolation, few attempts have been made to study biochemical changes in animals whose behaviour has been observed before and after the administration of LSD.

In the present experiments, the behavioural measure chosen was performance in a brightness discrimination reversal learning task under conditions of minimal interference from extraneous stimuli. This precaution was taken in case any effect on discriminative ability, for example, were to be obscured by randomly interpolated distracting stimuli. Finally, an attempt was made to assess both the behavioural and biochemical specificity of those effects which were found by comparing them with the effects of 2-bromo-

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lysergic acid diethylamide (BOL-148). The estimation of brain amine levels was carried out by gas liquid chromatography (Martin & Ansell, 1973).

Methods

Behaviour

Male Wistar SPF rats, approximately 90 days old on arrival in the laboratory, were used. They were supplied by the Biochemistry Department, University of Birmingham. The apparatus (Fig. 1) consists of two parallel runways connected at each end by a goalbox, constructed of black perspex, except for transparent goalbox lids through which the animals may be observed. The liquid reward (0.2 ml water) is delivered to a small well in each goalbox via a self-filling syringe. Above each runway there is a lighting unit, separated from the alley by a semi-opaque plastic sheet. The whole apparatus is used in a sound-attenuated darkened room, lit only by a small red light.

On arrival at the laboratory, the animals were individually caged and given food and water *ad lib.* for a few days. During this time and throughout the experiment they were handled for approximately 2 min each day. After this short period of adaptation, measurements were taken of daily water consumption. After 10 days the mean water consumption was computed for each rat; thereafter, 60% of the *ad lib.* drinking level was given daily. Food *ad lib.* was available throughout the experiment and a regular check on body weight was maintained; overall weight loss was less than 5% of the starting level. Water deprivation was continued for 12 days before any training was given in the apparatus.

Starting on the 13th day after water deprivation, each subject was placed in the apparatus for 5 min each day; 0.2 ml water was made available every time the animal walked through an alleyway and entered the goalbox. One alley was illuminated and the other left dark; the position of these was altered from day to day. After 6 days most subjects drank readily in the goalbox; any that did not took no further part in the experiment.

Training in the acquisition of a light-dark discrimination was then begun. The light alley was the positive, rewarded stimulus (S^+) for half the animals, with the dark alley unrewarded (S^-) for these and rewarded (S^+) for the others. They were run for 10 min each day for 8 successive days. The position of the S^+ alley was varied between sessions to prevent the animal from learning a position discrimination. About 10% of the subjects failed to learn the discrimination or refused to run; these were removed from the experiment. All other animals reached 75-95% accuracy; this seems to be asymptotic for the conditions used in this experiment since further training does not lead to improvement.

Reversal training took place in a single session after completion of acquisition training. The animals were rested for 3 days between acquisition and reversal. Drugs were administered intraperitoneally as a total of 1 ml in saline (0.9% w/v NaCl solution) on the fourth day and animals were run after the appropriate post-injection time interval. One hundred and twenty reversal trials were given; all correct responses to the new positive stimulus (i.e. the former S^-) were rewarded. A trial by trial record of choices was kept for each animal. A measurable degree of reversal can be obtained in about 25 minutes. Three experiments are described here, as follows.

Dose-response experiment Six subjects were assigned to each of 5 drug conditions; these groups were matched on the basis of discrimination performance during the last few days of initial training. LSD (Delysid-Sandoz) was administered 15 min prior to reversal at 6.25, 12.5, 25 and 50 μg/kg (i.p.); a fifth group of subjects was given physiological saline. This part of the experiment was carried out blind; the injections were prepared by one experimenter and the subjects injected and run by another who had no knowledge of what had been injected. A few animals did not complete 120 reversal trials; these were rejected from the experiment. There was no relationship between the drug dose and the tendency not to run to completion.

Time study In the second experiment, the effects of LSD on reversal learning at various times after injection were investigated. The training and testing were as before, but a single dose of 12.5 μg/kg LSD was employed. As before, 5 groups of 6 animals each were matched on the basis of performance during the initial discrimination training. Time intervals of 5, 15, 45 and 90 min after drug administration were allowed before reversal for the 4 LSD groups; a physiological saline treated group was also employed, run 5 min after injection.

Comparison with BOL-148 The last experiment was designed to control for the peripheral anti-5-HT effects of LSD by comparison with a similar dose of BOL-148. Twenty animals were run with the methods described above, 15 min after injection. Three drug conditions were used. One group was treated with 25 μg/kg BOL-148

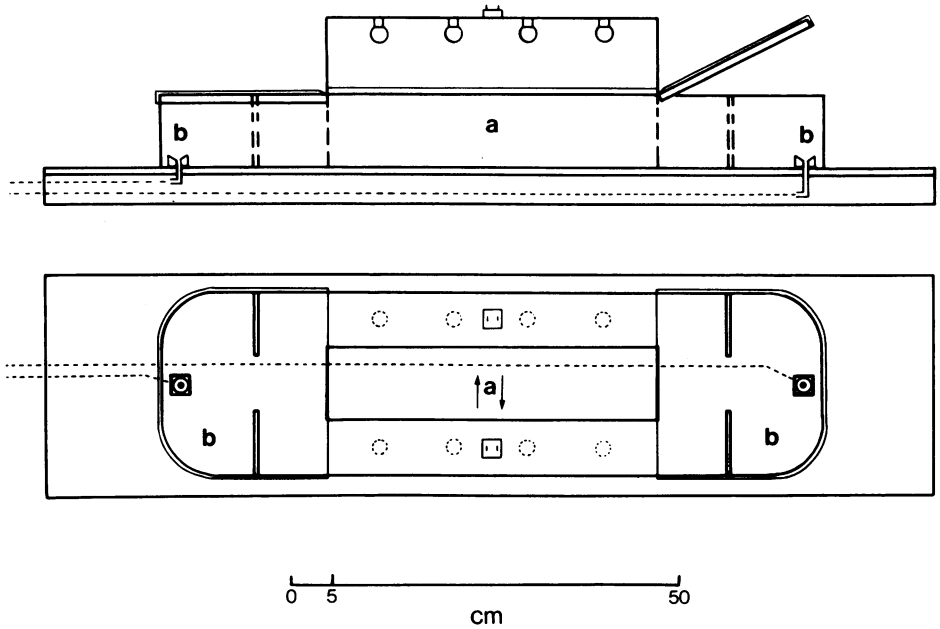

Fig. 1 Diagram of discrimination apparatus. (a) Illuminated alleyways; (b) liquid reward devices.

(Sandoz) ($n = 7$), one group with 25 μg/kg LSD ($n = 6$) and the third with saline ($n = 7$).

Biochemistry

Two biochemical experiments were carried out. In the first, the animals used had taken part in a complete behavioural experiment. The second experiment was conducted with animals which had been handled and water-deprived exactly as above, but had received no discrimination training.

The animals were killed 45 min after injection of the drug; the brains were removed at room temperature and stored at −17°C until the assays were performed. All analyses were carried out by a new gas chromatographic method in which the brains were homogenized in a butanol medium; the catecholamines were adsorbed on to alumina and after elution subjected to ion-pair extraction; 5-HT was purified by solvent extraction. The amines were then converted to their trifluoroacetyl derivatives and subjected to gas chromatography with electron capture detection (Martin & Ansell, 1973).

Results

Behaviour

Dose-response experiment The results of the first experiment are shown in Figure 2. For all groups, the learning curves are of similar shape; however, at doses above 6.25 μg/kg, the LSD treated groups showed accelerated learning. This effect is particularly clear in the last 60 trials. Analysis of variance performed on data normalized with an arc-sin transformation confirmed this. The drug treatment main effect was significant between 0.05 and 0.10 probability levels but the drug x

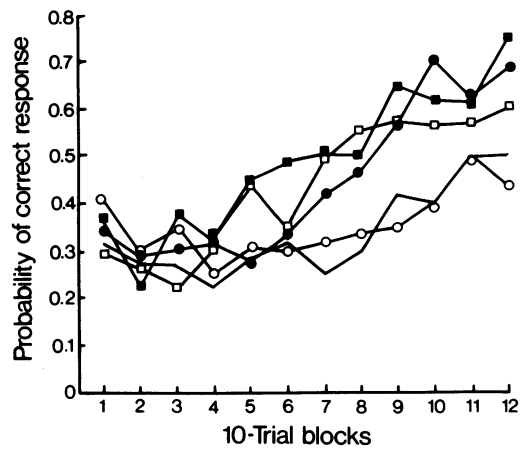

Fig. 2 Effects of various doses of LSD-25 on reversal learning. Continuous line, saline; LSD-25 (μg/kg): (○) 6.25; (●) 12.5; (□) 25.0; (■) 50.0. $n = 6$ per group.

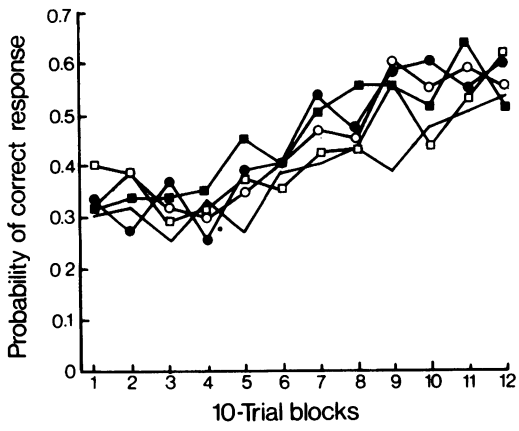

Fig. 3 Effect of time of administration on the action of LSD-25 (12.5 μg/kg) on reversal learning. Continuous line, saline, 5 min; (○) LSD-25, 5 min; (●) LSD-25, 15 min; (□) LSD-25, 45 min, (■) LSD-25, 90 min.

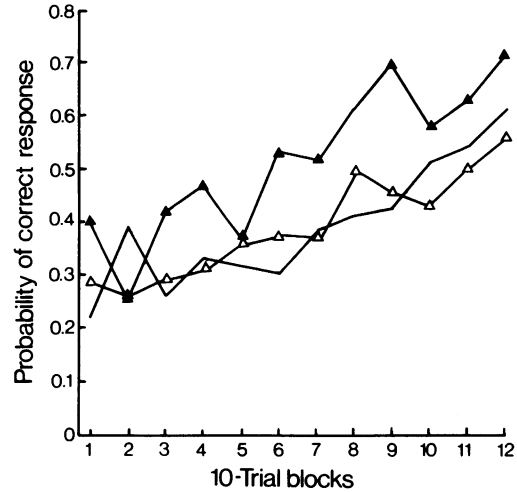

Fig. 4 Comparison between effects of LSD-25 and BOL-148 on reversal learning. Continuous line, saline; (▲) LSD-25 (25 μg/kg); (△) BOL-148 (25 μg/kg).

trials interaction effect was more significant ($P < 0.01$). The trial blocks effect was highly significant ($P < 0.001$), as was the between subjects effect, indicating considerable individual variation in reversal performance. Individual dose-group comparisons were carried out using *t* tests for correlated data on mean scores. These showed that the group treated with 6.25 μg/kg LSD did not differ significantly from the control group. The other drug treatment groups were each different from saline ($P < 0.005$ (2-tail) for each comparison) but not from each other, indicating that there was no dose level effect. Light condition during reversal (light or dark S^+) was found not to influence performance, nor to interact with the drug response in any way.

Time study The results of the time study also showed facilitation of performance with LSD. Each drug group was superior to the saline control (Fig. 3); *t* tests carried out on group mean data showed that these differences were significant for 5, 15, 45 and 90 min groups, $P < 0.0025$, 0.005, 0.0125 and 0.0025 respectively (1-tail tests).

Comparison with BOL-148 The results of the BOL-148 control experiment showed that LSD again facilitated performance but that BOL-148

Table 1 Effects of LSD and BOL-148 on amine levels in rat brain

Amine	*Ratios*		
	LSD / Saline	*LSD / BOL*	*BOL / Saline*
5-Hydroxytryptamine	106.3 ±6.6	111.6* ±6.0	96.6 ±4.4
Noradrenaline	93.4 ±9.9	94.4 ±3.3	98.5 ±9.3
Dopamine	96.0 ±12.8	91.7 ±8.9	105.9 ±11.7

The drugs were given i.p. at 25 μg/kg and the animals killed 45 min later.
The ratios are expressed as a % and are given with s.e. mean where $n = 5$, except in the 5-hydroxytryptamine determinations on the BOL-148 group where $n = 4$.
* Significant at the 0.05 level; all other results are not significant.

Table 2 Effects of LSD and BOL-148 on amine levels in rat brain: a study with littermates

Amine	*Ratios*		
	LSD Saline	*LSD BOL*	*BOL Saline*
5-Hydroxytryptamine	113.9* ±5.9	123.1* ±9.2	94.7 ±9.2
Noradrenaline	92.9 ±6.1	92.2 ±8.4	103.9 ±10.1
Dopamine	98.5 ±6.3	96.7 ±6.0	102.1 ±4.3

The drugs were given i.p. at 25 μg/kg and the animals killed 45 min later.
The ratios are expressed as a % and are given with s.e. mean where $n = 6$.
* Significant at the 2.5% level, all other results are not significant.

treatment did not differ from saline (Figure 4). Analysis of variance was carried out on arc-sin transformed data; the drug effect was significant ($P < 0.025$), and the drug x trials interaction effect also approached significance ($P < 0.10$). The effect of trials was again highly significant ($P < 0.001$). Individual group comparisons showed that the effect of drug treatment was due to the superiority of the LSD group; there was no difference between the BOL-148 and saline control groups.

Biochemistry

The results of the first biochemical experiment are shown in Table 1. The animals used here were killed immediately after collection of the behavioural data in the BOL-148 comparison experiment described above. There was an increase in the levels of brain 5-HT in the LSD treated group, although this was only significant ($P < 0.05$) when compared to the BOL group. The levels of the catecholamines in the brain were not significantly different from the control group in either case.

In the second biochemical experiment, the animals were handled and deprived of water but not trained. The LSD treated group (Table 2) was found to have a significantly higher level of brain 5-HT when compared with both the BOL and the saline groups ($P < 0.025$). There were no significant differences in the catecholamine levels in the brain between the groups.

Discussion

Behaviour

LSD consistently facilitated the learning of a brightness discrimination reversal problem at several dose levels. These results and the dose levels employed were similar to those reported recently (Schechter & Winter, 1971; Bignami, 1972). The behavioural parameter mediating these effects cannot yet be identified with certainty, but a preliminary trial-by-trial analysis of the reversal learning data suggests that the facilitation is due to faster extinction, i.e. the rats cease to make the previously correct response. Facilitation could occur because of a motivational change such as increased or decreased thirst; the first possibility should result in slower extinction, which is not consistent with the data reported here. On the other hand, there is evidence that a decreased motivational level does facilitate reversal learning (Bruner, Mandler, O'Dowd & Wallach, 1958; Kendler & Lachman, 1958). If LSD acts in this way, it should reduce learning in the acquisition phase of the experiment, prior to reversal. Unpublished data from this laboratory indicate that LSD also facilitates learning during acquisition of the initial brightness discrimination. For these reasons neither an increase nor a decrease in thirst provides a suitable explanation.

A more likely explanation stems from the well documented observation that LSD has an arousing effect on the electrocorticogram, correlated with increased responsiveness to sensory stimuli (Bradley & Elkes, 1957; Bradley & Key, 1958). However, since LSD is known to have an arousing effect which is shared by amphetamine, this substance should also be tested in a reversal learning situation.

In the time study, facilitatory effects of LSD were still present 90 min after injection although the plasma half life of the drug has been estimated to be no greater than 20 min in the rat (Freedman, 1969). This time was considered by Freedman to be correlated with the termination of the behavioural effects of the drug. Brawley &

Duffield (1972) have recently reviewed results from other species, including man, which are also consistent with this view. At the same time, it seems unlikely that our own results obtained at 90 min are invalid since similar effects were observed by Jarrard (1963). A further complication is that the environment in which the drug is administered clearly influences the duration of the behavioural effects. Sparber & Tilson (1971) reported that 10 mg/kg mescaline hydrochloride or LSD disrupted operant behaviour between 10 and 25 min after injection, if the animal was returned immediately after injection to the operant chamber. On the other hand, if the animal was returned to the home cage for a few minutes after the onset of a behavioural effect, replacement in the operant chamber led to the immediate reinstatement of normal behaviour, i.e. termination of drug effects in a much shorter period of time.

BOL-148, which is 2-bromo LSD, has powerful anti-5-HT effects on isolated organ preparations, but it does not antagonize the effects of 5-HT in the brain (Boakes *et al.*, 1970). In the present study, BOL-148 was without effect on reversal learning when compared with a similar dose of LSD. A similar result has been obtained in other behavioural situations (Roberts & Bradley, 1967; Schechter & Winter, 1972). At this dose level, BOL-148 is not psychotomimetic.

Biochemistry

The first biochemical experiment was carried out on animals which had been used in the behavioural experiments; they were not from the same litter. Because of the limitations imposed by the collection of the behavioural data there were, in some cases, differences in the time that the animals were killed after the drug treatment. In an attempt to control for this factor and the inter-animal variations in the brain amine levels, the second experiment was carried out using littermates but without full behavioural training. In these experiments LSD produced an increase in the whole brain 5-HT levels 45 min after a single dose of 25 μg/kg; no effects were observed on the levels of noradrenaline and dopamine. BOL-148 at the same dose level had no effect on the levels of noradrenaline, dopamine or 5-HT.

The increases in brain 5-HT levels after LSD treatment are consistent with the findings cited in the introductory section.

The turnover of brain noradrenaline appears to be slightly increased after LSD (Andén, Corrodi, Fuxe and Hökfelt, 1968), although the small depletion of noradrenaline which has been reported (Leonard & Tonge, 1969) is not supported by this work at much lower dose levels.

A single dose of BOL-148 of 25 μg/kg showed no significant effects on brain amine levels in this study which is consistent with previous reports (Freedman, 1961).

The results of these experiments together with other data in the literature suggest that the behavioural effects of LSD in the reversal learning situation, were mediated centrally by an action of 5-HT sensitive neurones, possibly in a way suggested by the results of iontophoretic studies, where antagonism by LSD, but not by BOL-148 to the excitatory effects of 5-HT has been demonstrated (Boakes *et al.*, 1970). It should be stressed that the generality of these conclusions is limited by the fact that only one kind of behaviour has been investigated. Furthermore, no direct correlation between absolute levels of brain 5-HT and the behaviour of individual animals has been established. The answer to this problem must await the outcome of further experiments.

We should like to thank the Honorary Director of the M.R.C. Neuropharmacology Unit, Professor P.B. Bradley, and Dr G.B. Ansell for their interest and encouragement in this work.

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(Received May 15, 1974)