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Lysergic Acid Diethylamide, Amphetamine and Chlorpromazine on Water Maze Discrimination in Mice

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Abstract. The effects of a psychodysleptic (lysergic acid diethylamide), a psychoanaleptic (amphetamine) and a psycholeptic (chlorpromazine) have been evaluated on the performance of mice previously trained in a Y water maze, following two procedures: Light Procedure, corresponding to a type of innate behaviour, and to a "learning without errors", and Dark Procedure, corresponding to a type of acquired behaviour.

The disrupting effect of lysergic acid diethylamide and chlorpromazine was much more marked in the Dark than in the Light Procedure, so that the results can be interpreted as a return to an innate behaviour pattern.

Lysergic acid diethylamide causes the reappearance of a "coming and going" pattern of behaviour normally observed only during the pre-training sessions.

With chlorpromazine a deconditioning effect limited to the Dark Procedure is evident at low doses, while at the highest dose immobilization at the starting point in both procedures was observed.

Key-Words: Lysergic Acid Diethylamide — Amphetamine — Chlorpromazine — Discrimination — Mice.

Introduction

Since its introduction the water maze has proved to be a useful tool for studying learning and retention problems and analysing swimming performance, because it yields relatively consistent results and the motivation to performance is relatively innocuous to the animals.

Earlier experiments with mice have included those on discrimination learning problems by Waller *et al.* (1960) by Meier and Foshee (1963) studying the performances of different strains in a three choice point water maze, and by Latz *et al.* (1967) utilizing a T maze in order to analyse the effects of some antidepressant drugs on learning.

More recently, Krivanek and McGaugh (1968) have used a Y water maze for brightness discrimination learning, in studying the effects of Pentylenetetrazol on memory storage, and Sansone *et al.* (1969) have studied, in a similar maze, discrimination learning in different strains trained to swim towards light or dark.

In preliminary experiments (Sansone *et al.*, 1969) with the Y water maze using light as the discriminatory signal, it was possible to utilize the animal preferences to achieve both "learning without errors" and the acquisition of a behaviour pattern opposite to the innate behaviour.

Using the same technique we have examined the effect of some psychotropic drugs on the phases of the acquired behaviour, and the way the initial innate preferences may reappear under the effects of these drugs, even in apparently well trained animals.

Three drugs, belonging to three different groups of psychotropic drugs have been tested: psychodysleptics or psychotomimetics (lysergic acid diethylamide), psychoanaleptics or psychostimulants (amphetamine), and psycholeptics (chlorpromazine).

Methods

The apparatus consisted of two automated Y water mazes. The sequence of the trials was automatically programmed and the responses were recorded by means of photocells located in the middle of each alley, and a four channel recorder.

The walls were of black plexiglas. At the end of every alley there was a swimming platform that could be raised above the water level. Above the platform there was a 3 W lamp, behind an opalescent screen.

The temperature of the water ranged between 18 and 20° C.

At fixed intervals the platform on which the animal stood was automatically submerged, while another platform in randomized succession was raised in one of the alleys.

The programmer controlling the movement of the platforms also controlled the lighting and the switching off of the lights in the alleys.

During the experiments the maze was covered with a black cloth to isolate it from the surrounding environment.

All animals, before beginning the learning trials, underwent five pre-training sessions, consisting of 5 trials each in a straight alley. This pre-training was performed switching off the signals and placing a light of medium intensity on the top of the pool.

During each trial the times taken to cover the distance (70 cm) between two platforms were recorded. The few animals who did not succeed in covering this distance in a mean time of 3—5 sec were excluded from the following experiments.

At the end of the pre-training sessions the animals were randomly distributed in two groups and trained with the Light (L) Procedure or the Dark (D) Procedure.

In the L Procedure the apparatus was programmed in such a way that a platform was submerged and light was switched off every minute,

Table 1a. *Training in D Procedure. Mean percent performances ± S.E., in the training test, of 16 animals trained for 7 sessions of 5 trials*

	I	II	III	IV	V	VI	VII
Correct choices	26 ± 1.64	33 ± 1.38	52 ± 1.32	64 ± 0.94	81 ± 0.72	95 ± 0.41	100
Multiple errors	56 ± 1.60	45 ± 1.33	24 ± 0.94	13 ± 0.94	5 ± 0.64	0	0
Simple errors	18 ± 0.94	21 ± 0.84	23 ± 0.66	22 ± 0.72	13 ± 0.70	5 ± 0.44	0
Coming and going errors	—	—	—	—	—	—	—
Remain at the starting point	—	—	—	—	—	—	—

Table 1b. *Training in L procedure. Mean percent performances ± S.E., in the training test, of 16 animals trained for 7 daily sessions of 5 trials*

	I	II	III	IV	V	VI	VII
Correct choices	74 ± 0.94	74 ± 0.94	88 ± 0.72	90 ± 0.40	95 ± 0.41	100	100
Multiple errors	2 ± 1.94	1 ± 1.98	0	0	0	0	0
Simple errors	24 ± 0.84	25 ± 0.70	12 ± 0.64	10 ± 0.64	5 ± 0.64	0	0
Coming and going errors	—	—	—	—	—	—	—
Remain at the starting point	—	—	—	—	—	—	—

Difference between D and L Procedure for the first three training sessions: $p < 0.01$, and for the seven training sessions: $p < 0.05$.

while, at the same time, another platform rose and the corresponding light switched on.

In the D Procedure the two lights corresponding to the two submerged platforms were on, while the one corresponding to the raised platform was switched off.

Table 2a. *Pharmacological effect in D Procedure. Mean percent performances (± S.E.) of animals during sessions of 10 trials for chlorpromazine, of 5 trials for lysergic acid diethylamide*

	C	LSD 5	C	CPZ 4	C	CPZ 6	C	CPZ 8	C
Correct choices	100	12 ± 0.64	100	67 ± 0.80	100	44 ± 0.20	100	5 ± 0.88	100
Multiple errors	0	0	0	0	0	0	0	0	0
Simple errors	0	0	0	26 ± 0.80	0	45 ± 0.26	0	15 ± 0.90	0
Coming and going errors	—	83 ± 0.75	—	0	—	0	—	—	—
Remain at the starting point	—	5 ± 1.88	—	7 ± 1.88	—	11 ± 0.86	—	80 ± 0.26	—

Table 2b. *Pharmacological effect in L Procedure. Mean percent performances (± S.E.) of 8 animals during sessions of 10 trials for chlorpromazine, of 5 trials for lysergic acid diethylamide*

	C	LSD 5	C	CPZ 4	C	CPZ 6	C	CPZ 7	C
Correct choices	100	100	100	100	100	100	100	18 ± 1.78	100
Multiple errors	0	0	0	0	0	0	0	0	0
Simple errors	0	0	0	0	0	0	0	0	0
Coming and going errors	—	—	—	—	—	—	—	—	—
Remain at the starting point	—	—	—	—	—	—	—	82 ± 0.26	—

Difference between the performances after 8 mg/kg of Chlorpromazine: n.s.

For each type of training (L and D Procedure), the animals were trained for seven learning sessions, at 24 h intervals. Each session consisted of 5 or 10 trials, at 1 min intervals.

The pharmacological tests were carried out in well trained animals, after a minimum of 8 consecutive sessions.

The result (Tab. 1—2) refer to the mean (± S.E.) of a group of 16 animals for the training and 8 animals for the pharmacological

tests, and are presented according to the type and number of errors made by the animals.

The pharmacological data in each test was compared with that of the same group of animals on the day just preceding and following the tests.

After preliminary tests, and from previously published data (Uyeno, 1970), the interval between the intraperitoneal administration and the beginning of the session was fixed at 15 min for lysergic acid diethylamide, 45 min for chlorpromazine and 45 min for amphetamine.

Results

Training. According to previous results (Sansone *et al.*, 1969) a marked difference was observed in the learning curves using the two training procedures.

The animals trained with the L Procedure (Fig.1) (correct choice entering the lighted alley) achieve high levels of performance (from the first session). At this stage the percent correct choices exceeds the 50% level (which represents the normal chance level), while a very low percentage of multiple errors (when mice return into the start alley at least once during each trial) occurs only in the first two sessions.

Conversely, the animals trained with the D Procedure (Figs. 2 and 3) (correct choice entering the dark alley) start 50% below the level, and multiple errors are high, although they then decrease until the fifth session.

All the animals achieve high levels of correct choices (100%) during the last two sessions in both types of training.

Lysergic Acid Diethylamide. The effect of lysergic acid diethylamide on discriminative behaviour has been investigated in pretrained mice, alternating a session in which the drug was previously injected with two control sessions.

In the tests performed with increasing doses a disruptive effect on behaviour was seen with doses of 5 mg/kg in the D Procedure (Fig.2) while the L Procedure was unaffected.

In contrast with the results of the control sessions, the animals trained with the D Procedure preferred to remain in the start alley for a prolonged time, when the platform was submerged and the corresponding alley was lighted; the animals tested under these conditions have always shown a particular pattern of "coming and going errors", covering the length of the start alley many times.

In some other cases a reduction of crossing errors was observed, because the animals remained at the starting point during the whole trial. In such cases the animals have been taken off and returned to the platform in order to avoid drowning.

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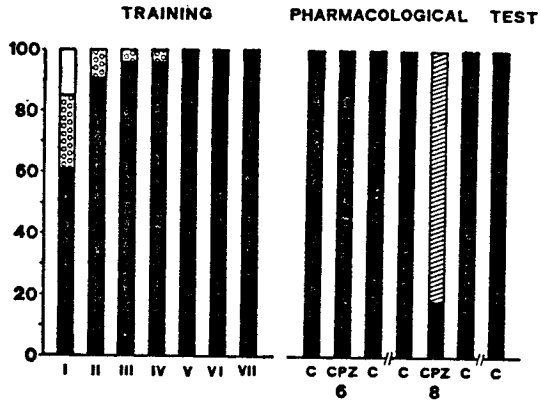

Fig. 1. Training in the L Procedure and behavioural effect of chlorpromazine. Mean percent of the performances of a group of 8 animals, during daily sessions of 10 trials. Effect of 2 doses of chlorpromazine: 6 mg and 8 mg/kg. In contrast with the D Procedure (Figs. 1 and 3), in the L Procedure a high percent of correct choices from the first training session can be observed

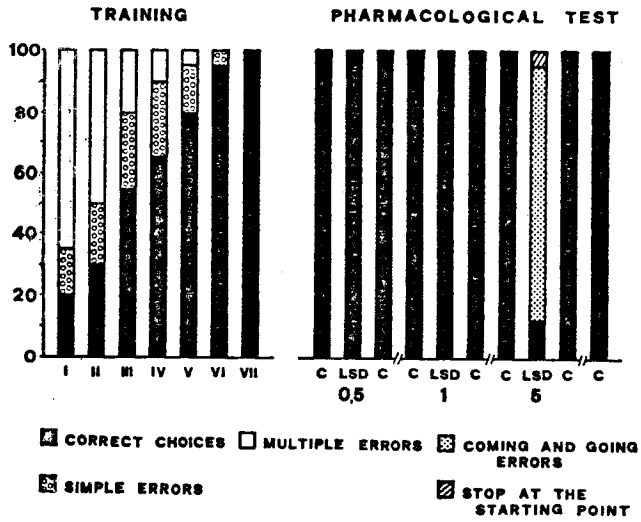

Fig. 2. Training in the D Procedure and behavioural effect of lysergic acid diethylamide in the Y Water maze. Mean percent of the performances of a group of 8 animals during daily sessions of 5 trials. Performances in 7 training sessions (I—VII). Effect of three doses of LSD: 0,5 mg, 1 mg and 5 mg/kg e.p. Each pharmacological test is preceded and followed by a control session at 24 h of interval

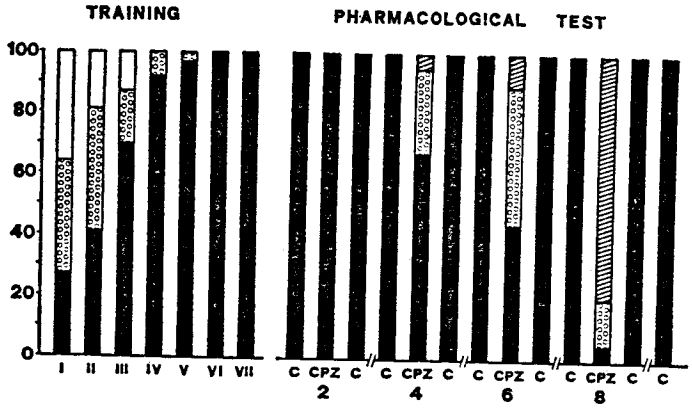

Fig. 3. Training in the D Procedure and behavioural effect of chlorpromazine. Mean percent of the performances of a group of 8 animals during daily sessions of 10 trials. Effect of 4 doses of CPZ: 2 mg, 4 mg, 6 mg, 8 mg/kg. Compare the strong disturbing effect on the D Procedure with the limited effect on the L Procedure, which appears only at the highest sedative dose of 8 mg/kg

When the crossings took place and the alley was left, the animals made, in the great majority of the cases, the correct choice. This behaviour pattern ("coming and going errors") typically occurs during the first pretraining sessions.

Amphetamine. In order to compare the effect of lysergic acid diethylamide with that of amphetamine, the same animals were injected with increasing doses of this drug.

No difference between L and D Procedure was observed.

In the animals injected with 5 mg/kg increased motor activity and tremor were evident. In the discrimination tests the animals injected with amphetamine behave normally, with no errors and normal swimming time.

At higher doses (10 mg/kg) the loss of motor coordination became so important that the animals were not able to remain on the starting platform or to return there after falling into the water. This effect and the lack of motivation, made continuation of the experiment impossible since it was necessary to take the animals off the maze to prevent drowning.

Chlorpromazine. The effect of chlorpromazine on discriminative behaviour was studied by comparing the results of two different learning procedures, in which sessions consisted of 5 and 10 trials.

In mice trained with the D procedure (Fig.3), a loss of discriminative ability was recorded with doses of 4 and 6 mg/kg with the reappearance of simple errors.

The initial level of CR fell to 87% with the dose of 4 mg/kg, in the 5 trials session, and to 74%, in the 10 trials session.

A higher dose (6 mg/kg) provoked a fall of the initial level of CR to 65% in the first, and to 55% in the second case.

With these doses immobility at the starting point was observed, prolonging the whole trial. When the same doses (4 and 6 mg/kg) were used in the L procedure, the high percentage of correct responses was unaffected (Fig.1). Only an increase in the time employed to reach the platform of the goal alley was observed. This increase, already clear in the animals trained for 5 trials (from 3 to 10 sec) became more marked for a series of 10 trials (from 3 to 20 sec).

Discussion

The parmacological tests, performed with two drugs, a neuroleptic, and an hallucinogen permit some conclusions to be drawn:

1. There are different drug sensitivities of the two types of behaviour induced by the two Procedures: one is the L Procedure and corresponds to "learning without errors", or to the consolidation of an innate behaviour; the other, the D Procedure, corresponds to the learning of a new behaviour and to the acquisition of a newly acquired behaviour.

2. The data obtained from experiments using lysergic acid diethylamide and chlorpromazine show the resistence of an innate consolidated behaviour, and the sensitivity of a newly acquired behaviour to the specific disrupting effect of these drugs.

Chlorpromazine. Following the initial experiments of Courvoisier (1956) many observations have shown that previously conditioned rats behave "like an untrained animal" (Courvoisier *et al.*, 1957).

If the data suggest that the drug provokes a "loss of conditioning" or a "deconditioning effect" (Delay and Deniker, 1957; Bovet, 1957), this may be subject to discussion.

Some authors, desiring to give a more unitary picture of the effects of the drug e.g. on spontaneous behaviour (aggressivity, global motor activity), and acquired behaviour (pole climbing, maze), proposed diminution in motivation (Archer, 1954), or lack of reward effects (Guha, *et al.*, 1953).

Our results are more consistent with the deconditioning hypothesis, than with that of a loss of motivation, which appeared mainly in the higher dosage.

The results may be interpreted in terms of a double effect of chlorpromazine.

a) A "deconditioning" effect, already clear with low doses (4 mg/kg), observed in the D Procedure.

b) A "sedative" effect, only with the highest dose, when animals remain at the starting point.

Amphetamine. The results obtained with Amphetamine represent an extreme case of deconditioning for loss of motivation or of motor control.

Lysergic Acid Diethylamide. Lysergic acid diethylamide specifically modifies acquired behaviour but other effects suggest a different kind of action.

It is possible to relate the effects of lysergic acid diethylamide to the particular type of hallucinogenic psychoses induced by anticholinergic drugs (parasympatholytic with central action) and of mescaline.

Our observations may be compared with previous data, showing the difference between the disrupting effects of chlorpromazine [deconditioning by indifference to the surroundings (Bovet, 1959)] and anticholinergic agents (deconditioning by dissociation from the surroundings) (Overton, 1966; Oliverio, 1967).

While the effect of chlorpromazine can be described as an inhibition of the acquired behaviour, the effect of lysergic acid diethylamide represents a reappearance of primitive innate behaviour.

The observations on lysergic acid diethylamide may be interpreted on the basis of the data given for mescaline by Sivadijan (1934), who has described the "hallucination" of the rat placed in the cage of Warner, or by Courvoisier (1956) who, in a more systematic way, has described the "disoriented responses" of the animal in the pole climbing situation.

The data of Cook (1957) and Uyeno (1970) which demonstrated a disrupting action of lysergic acid diethylamide agree with our observations that an innovated acquired behaviour is much more strongly modified than the innate behaviour.

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