Europ.J.Pharmacol. 13, 341-346 (1971). LSD 2118

EUROPEAN JOURNAL OF PHARMACOLOGY 13 (1971) 341–346. NORTH-HOLLAND PUBLISHING COMPANY

AN EXAMINATION OF THE EFFECT OF CENTRAL NERVOUS SYSTEM STIMULANT AND ANTI-DEPRESSANT DRUGS ON OPEN FIELD PERFORMANCE IN RATS

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Received 9 March 1970 Accepted 19 November 1970

B.D. GUPTA, P.C. DANDIYA, M.L. GUPTA and A.K. GABBA, *An examination of the effects of central nervous system stimulant and anti-depressant drugs on open field performance in rats*, European J. Pharmacol. 13 (1971) 341–346.

The Open Field performance of rats treated with five different doses of amphetamine, iproniazid or imipramine was compared with those treated with the same doses of lysergide (LSD) or placebo. The analysis of variance performed on the ambulation, rearing and preening scores produced significant differences between the groups. It was found that selective stimulation of ambulation with suppression of other interrupting responses, such as rearing and preening, was a typical stereotyped Open Field behaviour which was a function of increasing doses of, not only LSD, but also of iproniazid. Similarly, selective stimulation of rearing at the cost of preening was a behavioural function of increasing doses of amphetamine and of lower doses of imipramine.

Open Field Test Lysergide Amphetamine Iproniazid Imipramine

1. INTRODUCTION

When a rat is subjected to an Open Field Test, which provides a sudden and marked emergency situation, massive autonomic responses, such as freezing and defecation result (Broadhurst, 1957; Holland and Gupta, 1966); autonomic depressant drugs which block such responses produce a higher rate of exploratory locomotor activity (Dicker et al., 1957; Steinberg et al., 1961; Rushton and Steinberg, 1963). A high intensity of noise is an important stimulus in the Open Field Test. King (1964) has reported that noise is an arousal-provoking stimulus, which augments psychomotor activity (Lindsley, 1960) by increasing arousal of the cerebral cortex (Bradley, 1958). CNS-stimulant drugs have been shown to increase ambulation in the Open Field Test (Broadhurst et al., 1959). Besides defecation and ambulation, a rat exhibits several other responses in the Open Field Test, such as rearing and preening. Rearing has been measured as a function of CNS-excitability (Lat, 1965; Holland and Gupta, 1966); increasing doses of CNS-stimulant drugs augment the rearing response (Gupta and Gregory, 1967). Investigations on the study of preening behaviour of rats are rather scanty. Nevertheless, Dandiya et al. (1969) have assumed that preening is a complex stereotyped response.

Dandiya et al. (1969) found that lysergic acid diethylamide (LSD), when given to rats, elicited abnormal sterotyped behaviour consisting of selective stimulation of ambulation; larger doses suppressed rearing and preening. In the present study, attempts were made to extend this approach to other stimulant drugs, i.e. iproniazid, amphetamine and imipramine; the data obtained with these drugs was compared to data obtained with LSD (Dandiya et al., 1969) in order to make (i) a more critical, psychological analysis of the Open Field performance in (ii) a better appreciation of the differential or common features of these drugs under this specific stressful situation.

2. METHODS

2.1. *Subjects*

The subjects were naive male albino rats of Haffkine's strain weighing 100 to 150 g. No attempt was made to control their early environment; temperature, lighting, handling, etc. were allowed to vary in an attempt to obtain a heterogeneous population. The experimental design was composed of twenty five groups (five treatments, five doses of each treatment); each group being composed of five rats. Thus, in all 125 animals were subjected to the experiment.

2.2. *Apparatus*

The apparatus used for the Open Field Test was similar to that used by Dandiya et al. (1969). Briefly, it consisted of a circular open arena made up of wood measuring 32¾ inches (84 cm) in diameter, enclosed by a sunmica wall 12½ inches (27.5 cm) high. The floor, which had sunmica surface, was marked out in three concentric circles divided into segments by lines radiating from the floor centre. The wall was fixed on the outermost of these circles. The markings provided 25 floor units of approximately equal size and were used to score the ambulation of the animal moving around in the arena during the test. The placement by the rat of all four of its limbs in a segment was taken as one unit of ambulation. In the "Open Field Test" two types of stimuli were presented to the animal, white noise with an intensity of 78 dB was presented by an oscillator through 5 loudspeakers and light of 165 foot candles intensity was produced by five photographic lamps. A translucent glass screen enclosed the arena from all the sides; the front side having a glass door through which the subject was placed in the arena and the experimenter observed the rat under test. By the side of the arena, there was a small table on which three hand operated counters were fixed to record the number of ambulation, rearing and preening responses. There was a clock on the table, for recording the testing period. The ambulation response was defined as a "walking around" score derived from the number of radial segments of the arena which were crossed by the subject. Rearing response was defined by the number of times the rat stood on its hind legs during the test.

2.3. *Procedure*

Double distilled water was used as a carrier for all the drugs administered. According to the body weight of the animal the required volume of the drug solution was injected intraperitoneally. The doses and the absorption period of the treatments are listed in table 1. The doses selected were such that the difference between the logarithms of the doses were uniform.

As soon as the absorption period elapsed the treated subject was carried to the apparatus. Each animal was tested in the Open Field Test for only two minutes and the scores on ambulation, rearing, preening and defecation were recorded.

Table 1
Drugs, doses and the latency period.

No.	Drug	I	II	III	IV	V	Latency between treatment and test (hr)
1	Amphetamine	0.65	1.10	1.80	3.0	5.0	1.00
2	LSD	0.002	0.008	0.032	0.136	0.50	0.40
3	Iproniazid	6.250	12.50	25.0	50.0	100.0	+
4	Imipramine	3.80	7.500	15.0	30.0	60.0	4.00

Each drug group was composed of 25 rats divided into five dose groups; each of these dose groups was injected intraperitoneally with one of the five doses of the drug before subjecting to the Open Field Test. Under columns I to V are log doses in mg/kg of the body weight of the animal.

* Iproniazid was divided into three equal parts, the first part was given 28 hr, second part 24 hr, and the last part 20 hr before the test.

Table 2
Ambulation scores after treatment in the Open Field Test.

Groups	Placebo	Amphetamine	LSD	Iproniazid	Imipramine
$\bar{X}$	47.40	84.04	124.48	113.40	55.56
Placebo		4.70 *	9.88 *	8.46 *	1.04
Amphetamine			5.18 *	3.76 *	3.65 *
LSD				1.42	8.83 *
Iproniazid					7.41 *

Treatment group mean differences as measured by t test. Each drug group was composed of 25 rats divided into five dose groups. The mean responses (in 25 rats) following treatment were compared with each other. The relevant mean response is given below the treatment and the relevant t value between any pair of the treatments is given against that pair. For example, 3.76 is the t value for the comparison of the amphetamine with the iproniazid group.

* Significant at the 1% level.

3. RESULTS

An analysis of variance was performed on each of the measures. Analysis of ambulation scores showed that "treatment" (F-ratio = 13.23; p = 0.01), "dose" (F-ratio = 12.18; p = 0.01) and "treatment X dose interaction" (F-ratio = 6.04; p = 0.01) produced significant differences between the groups. Further analysis of the "treatment" effect by Student's t-test against the residual variance of the analysis of var-

Table 3
Mean scores due to drug X dose interaction on ambulation.

Drugs	Doses				
	I	II	III	IV	V
Amphetamine	77.0	95.2	94.8	78.8	74.4
LSD	92.6	106.6	119.6	104.6	179.0
Iproniazid	65.6	69.6	123.6	85.8	222.4
Imipramine	59.0	56.2	59.0	51.8	51.0

Dose group means of the treatments. Each dose group was composed of 5 rats.

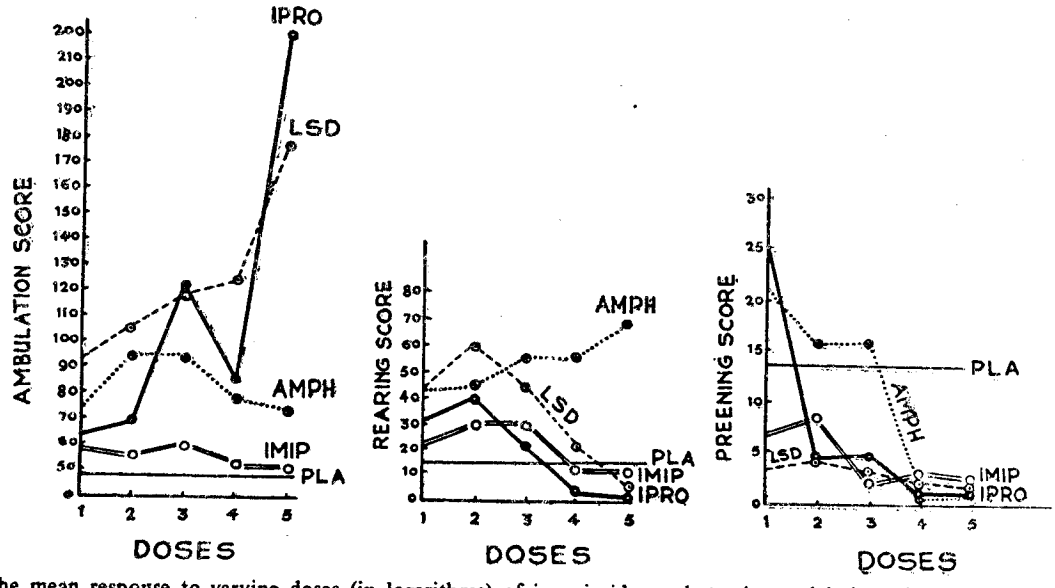

Fig.1. The mean response to varying doses (in logarithms) of iproniazid, amphetamine and imipramine with reference to the placebo and LSD on scores of ambulation, rearing and preening (from left to right) obtained from 125 rats in the Open Field Test. The Abscissa represents five doses (in logarithms) of each drug as given in table 1. IPRO, iproniazid; LSD, lysergide; AMPH, amphetamine sulphate; IMIP, imipramine; PLA, placebo.

Table 4
Rearing scores after treatment in the Open Field Test.

Groups	Placebo	Amphetamine	LSD	Iproniazid	Imipramine
$\bar{X}$	13.64	53.92	34.92	19.60	21.98
Placebo		10.09 *	5.34 *	1.49	2.09 **
Amphetamine			4.76 *	8.60 *	8.04 *
LSD				3.84 *	3.24 *
Iproniazid					0.59

Treatment group mean differences as measured by t test. Each drug group was composed of 25 rats divided into five dose groups. The mean responses (in 25 rats) following treatment were compared with each other. The relevant mean response is given below the treatment and the relevant t value between any pair of the treatments is given against that pair. For example, 8.60 is the t value for the comparison of the amphetamine with the iproniazid group.

* Significant at the 1% level. ** Significant at the 5% level.

iance is outlined in table 2. The relevant group means due to "treatment" X dose" interaction are shown in table 3. Analysis of rearing scores showed that "treatment" (F-ratio = 32.21; p = 0.01), "dose" (F-ratio = 4.92; p = 0.01) and "treatment X dose interaction" (F-ratio = 4.73; p = 0.01) produced significant differences between groups. Table 4 shows the t-test comparisons of the treatment groups and table 5 outlines the relevant groups means due to the "treatment X dose" interaction. Analysis of preening scores also showed that treatment (F-ratio = 7.47; p = 0.01), "dose" (F-ratio = 6.58; p = 0.01) and treatment X dose interaction (F-ratio = 2.90; p = 0.01) produced significant differences between groups. Treatment group differences, as measured by Student's test, are outlined in table 6. The relevant group means due to the "treatment X dose" interaction are shown in table 7. Since analysis of defecation scores failed to produce any significant effect, the data were not analysed further.

Table 5
Mean scores due to drug X dose interaction on rearing.

Drugs	Doses				
	I	II	III	IV	V
Amphetamine	42.0	45.2	55.6	56.6	70.2
LSD	43.2	59.8	44.2	21.4	6.0
Iproniazid	31.6	40.4	20.4	4.2	1.4
Imipramine	22.4	30.0	29.6	20.0	15.8

Dose group means of the treatments. Each dose group was composed of 5 rats.

Table 6
Preening scores after treatment in the Open Field.

Groups	Placebo	Amphetamine	LSD	Iproniazid	Imipramine
$\bar{X}$	12.95	10.88	2.72	7.24	4.48
Placebo		0.94	4.65 *	2.60 **	3.85 *
Amphetamine			3.71 *	1.65	2.91 *
LSD				2.05 **	0.80
Iproniazid					1.25

Treatment group mean differences as measured by t test. Each drug group was composed of 25 rats divided into five dose groups. The mean responses (in 25 rats) following treatment were compared with each other. The relevant mean response is given below the treatment and the relevant t value between any pair of the treatments is given against that pair. For example, 1.65 is the t value for the comparison of the amphetamine with the iproniazid group.

* Significant at the 1% level. ** Significant at the 5% level.

Table 7
Mean scores due to drug X dose interaction on preening.

Drugs	Doses				
	I	II	III	IV	V
Amphetamine	21.0	15.6	15.8	1.0	1.0
LSD	3.2	3.8	3.0	2.4	1.2
Iproniazid	25.2	4.2	4.6	1.2	1.0
Imipramine	7.0	8.6	2.0	2.6	2.2

Dose group means of the treatments. Each dose group was composed of 5 rats.

4. DISCUSSION

The results obtained clearly indicate that Open Field performance, as defined by the frequency of ambulation, rearing, and preening can be modified by amphetamine, iproniazid, imipramine and LSD. The finding, that the drugs failed to affect the defecation response of rats, is consistent with the similar results reported by Broadhurst et al. (1959). The results of the present experiments have raised the following salient points:

i) On ambulation responses, iproniazid produced an LSD-like augmenting effect which was higher than that of amphetamine; imipramine failed to affect this response. The results with amphetamine agree with earlier reports by Lat (1965) and Gupta and Holland (1969). In the present study, it was observed that iproniazid-treated subjects behaved similarly to those under LSD (Dandiya et al., 1969) but differently to those treated with amphetamine; the former drug group exhibited increased ambulation in the outermost circle of the Open Field in a fixed pattern whereas the amphetamine group explored the whole arena of the Test apparatus and displayed additional intermittant responses, such as rearing on the walls during exploration. Lat (1965) and Randrup and Mukvad (1969) have also reported that stereotyped locomotion of rats was restricted to part of the cage where the subject ran forth and back many times in contrast to the normal rats which covered the whole arena of the cage. It is known that stereotyped, repetitive thoughts and acts are observed in psychotic patients (Bleuler, 1961) and that LSD addicts exhibit obsessive–compulsive acts with general excitation. The stereotyped behaviour may, therefore, be defined as drug-induced hyperactivity associated with repetitive, irrelevant acts performed in a fixed pattern; LSD and iproniazid induce such a pattern of behaviour in rats, whereas amphetamine produces exploratory ambulation in the Open Field situation.

ii) The difference between the behavioural effects of iproniazid and amphetamine is further highlighted by results observed on rearing responses – a behaviour recognised by Holland et al. (1966) to be an exploratory response in rats. Amphetamine facilitated this response to the greatest extent, whereas iproniazid failed to affect it. Other investigators have also reported the facilitatory effect of amphetamine on rearing (Lat, 1965; Holland and Gupta, 1967; Gupta and Gregory, 1967; Gregory et al., 1967). LSD and imipramine also increased rearing responses. However, as shown in the figure, facilitation of rearing is a function of only the lower doses of these drugs. Thus, if rearing performance is a function of CNS-excitability (Lat, 1965; Holland and Gupta, 1966), and drug like amphetamine facilitates rearing by modifying CNS-excitability (Lat, 1965), the present results suggest that LSD and imipramine, in smaller doses, act as CNS-stimulants.

iii) The drugs, no matter what type of effect they produced on ambulation and rearing, inhibited the preening performance of rats.

iv) The dose-response functions of the drugs are illustrated in the figure. Approximately linear facilitation of ambulation with a simultaneous inhibition of rearing and preening is a function of increasing doses of iproniazid, an effect similar to that of LSD. Thus, if LSD is a psychotic drug (Dandiya et al., 1969), iproniazid may also be classified, of course tentatively and cautiously, as a psychotic drug as far as its Open Field behavioural effect is concerned. The stereotyped ambulation due to psychotic drugs can very well be differentiated from the exploratory ambulation produced by CNS-stimulants (Broadhurst et al., 1959) or tranquillizers (Rushton and Steinberg, 1963). This qualitative behavioural difference is evident from the dose-response relationship in the amphetamine group; increasing doses augmented rearing and decreased preening fairly linearly; effect on ambulation was non-linear (middle doses producing a higher score than those of the lower and higher doses). Lat (1965) has also reported similar results on

ambulation describing it as a typical CNS-stimulant effect.

Since reduction of preening in association with a parallel augmentation of rearing is a positive function of the drug induced CNS-excitation, it may be argued that preening response may be a behavioural correlate of CNS-inhibition. Sinha et al. (1958) successfully measured alternation behaviour of rats in T-maze as a behavioural correlate of CNS-inhibition which could be facilitated by CNS-depressant and inhibited by CNS-stimulant drugs. In the present study, similar inhibition of preening was produced by each CNS stimulant drug. These results may not only be taken to support the prediction of Eysenck (1964) that there is a CNS excitation-inhibition balance which determines the intensity of a particular response to a situation, but may also suggest that since rearing may be a behavioural measure of CNS-excitation, the preening response may be determined by the level of CNS-inhibition.

ACKNOWLEDGEMENTS

The authors are grateful to the Indian Council of Medical Research for financial support and to the Pharmaceutical firms – Sandoz Ltd., Basel, Switzerland; Merck Sharp and Dohme Research Lab. West Point, Pa., U.S.A.; Hoffmann-LaRoche, Montreal, Canada and Geigy, Basel, Switzerland – for the supply of LSD, Amphetamine sulphate, Iproniazid Phosphate (Marsalid) and imipramine respectively.

REFERENCES

Bleuler, E., 1961, Dementia praecox of the group of schizophrenias (International University Press, New York); Behavioural stereotypies induced by pharmacological agents, Pharmackopsychiat. Neuro-Psycho Pharmakol. 1, 18–26.

Bradley, P.B., 1958, The central action of certain drugs in relation to the reticular formation of the brain, in: Reticular Formation of the Brain, eds. H.H. Jasper and X. Ors (Little and Brown, Boston) 123–149.

Broadhurst, P.L., 1957a, Determinants of emotionality in the rat. 1. Situational factors, Brit. J. Psychol. 48, 1–12.

Broadhurst, P.L., S.D. Sinha and S.D. Singh, 1959, The effect of stimulant and depressant drugs on a measure of emotional reactivity in the rat, J. Genet. Psychol. 95, 217–226.

Dandiya, P.C., B.D. Gupta, M.L. Gupta and S.K. Patni, 1969, Effects of LSD on Open Field Performance in rats, Psychopharmacologia 15, 333–340.

Dicker, S.E., H. Steinberg and R.H.J. Watson, 1957, Effect of methylpentynol on the activity of rats, J. Physiol. 137, 88–89.

Eysenck, H.J., 1964, Psychological theory and measurement of drug effects, Neuropsychopharmacol. 3, 9–16.

Gregory, K., B.D. Gupta and H.C. Holland, 1967, The effects of drugs on activity in two strains of rats selectively bred for high and low emotionality, Life Sci. 6, 981–988.

Gupta, B.D. and K. Gregory, 1967, The effect of drugs and their combinations on the rearing response in two strains of rats. Psychopharmacologia 11, 365–371.

Gupta, B.D. and H.C. Holland, 1969, An examination of the effects of stimulant and depressant drugs on escape/avoidance conditioning in strains of rats selectively bred for emotionality/non-emotionality; Intertrial activity, Intern. J. Neuropharmacol. 8, 227–234.

Holland, H.C. and B.D. Gupta, 1966, Some correlated measures of activity and reactivity in two strains of rats selectively bred for differences in the acquisition of a conditioned avoidance response, Animal Behavior 14, 574–580.

Holland, H.C. and B.D. Gupta, 1967, An examination of the effects of some central and autonomic nervous system stimulant and depressant drugs on one form of exploratory activity in rats, Life Sci. 6, 63–70.

Holland, H.C., B.D. Gupta and E. Weldon, 1966, A note on rearing and an environmental constraint, Activitas nervosa superior 8–2, 140–144.

King, A.R., 1964, Some effects of drugs on rats in varying conditions of sensory stimulation, Psychopharmacologia 6, 338–346.

Lat, J., 1965, The spontaneous exploratory reactions as a tool for psychopharmacological studies, Proc. 2nd Intern. Pharmacol. Meeting, 22–23 August 1963 (Czechoslovak Medical Press, Praha) pp. 47–66.

Lindsley, D.B., 1960, Attention, consciousness, sleep and wakefulness, in: Handbook of Physiology, ed., J. Field (American Physiological Society, Washington) chapter LXIV.

Randrup, A. and I. Mukvad, 1969, Biochemical, anatomical and psychological investigations of stereotyped behaviour induced by amphetamines, Symposium on Amphetamines and related compounds, March 1969, Milan (Raven Press, New York).

Rushton, R. and H. Steinberg, 1963, Mutual potentiation of amphetamine and amylobarbitone measured by activity in rats, Brit. J. Pharmacol. 21, 295–305.

Sinha, S.N., C.M. Franks and P.L. Broadhurst, 1958, The effect of a stimulant and a depressant drug on a measure of reactive inhibition, J. Exptl. Psychol. 56, 349–354.

Steinberg, H., R. Rushton and C. Tinson, 1961, Modification of the effects of an amphetamine-barbiturate mixture by the post experience of rats, Nature 192, 533–535.