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Evaluation of Psychotropic Drugs with a Modified Open Field Test[1]

Jomar M. Cunha[2] *and Jandira Masur*[3]

Departamento di Psicobiologia, Escola Paulista de Medicina, São Paulo

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Abstract. The open field test used to study the behavioral alterations induced by psychotropic drugs is based mainly in the defecation, ambulation, rearing and grooming scores presented by animals subjected to the test. Because of the criticisms raised against the defecation score as a measure of the central effects of drugs, in the present experiment a modification of the test is proposed for the rat. The main points that characterize the new procedure are: (1) defecation scores are not considered; (2) besides ambulation, rearing and grooming, immobility was also recorded; (3) the total time of observation was increased, and (4) the stimuli, usually presented simultaneously in the open field (light and noise), were presented separately. The results obtained suggest that it is possible to differentiate classes of psychotropic drugs, without taking the defecation and grooming scores into consideration. Besides stimulants that evoked a characteristic pattern of behavior, neuroleptics could be differentiated from anxiolytics. Similarities, according to the dose, between barbiturates and anxiolytics were detected. Under LSD a peculiar pattern of behavior characterized by a large reactivity to light was observed.

One of the procedures used to evaluate the effects of psychotropic drugs in rats is the open field test (4–6, 8, 10). Its use was also proposed as a screening test for hallucinogenic drugs (1) and consists in exposing the animal for a period of 2–4 min to a well-illuminated arena provided with a sound generator. The behavioral response of the animal to such an

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Table 1. Open field behavior of control 1 (C1), control 2 (C2) and control 3 (C3)

		Ambulation frequency	Rearing frequency	Immobility time, sec	Grooming time, sec
C1	dark	89 ± 7	30 ± 3	17 ± 9	17 ± 4
C2	dark	91 ± 5	32 ± 2	15 ± 4	9 ± 4
C3	dark	98 ± 8	25 ± 9	7 ± 2	6 ± 2
C1	odor	38 ± 7	15 ± 2	74 ± 22	36 ± 8
C2	–	45 ± 7	16 ± 2	39 ± 15	32 ± 11
C3	odor	47 ± 8	10 ± 3	50 ± 15	31 ± 10
C1	sound	17 ± 4	7 ± 1	114 ± 18	50 ± 12
C2	sound	28 ± 7	11 ± 1	90 ± 18	47 ± 18
C3	–	42 ± 9	10 ± 2	58 ± 20	49 ± 12
C1	light	47 ± 4	8 ± 1	116 ± 14	12 ± 4
C2	light	60 ± 4	13 ± 2	64 ± 17	9 ± 4
C3	light	54 ± 9	8 ± 2	110 ± 16	12 ± 4

The figures indicate mean ± SE.

environment is usually measured using scores for defecation, ambulation, rearing and grooming. The interpretation of the results is commonly based on the assumption that these measures, mainly defecation and ambulation, reflect the emotional state of the rat.

Recently, serious criticisms were raised to the defecation score as a measure of the central effect of psychotropic drugs (11). It was found that the time-action parameter was crucial in determining open field defecation. By changing the time elapsing between drug administration and exposition to the open field, a reversal of the drug effects on defecation was obtained. This finding, and data showing that several psychotropic drugs increase homecage defecation, could indicate that decreased defecation in the open field may be the counterpart of a previous increment of fecal elimination generated by central or peripheral actions of the drug (11). The same authors (11) also argued that the results obtained in the open field lacks specificity, which speaks against its usefulness as a screening test.

These results indicate that the traditional way of using and interpreting the open field test may be inadequate. However, in our opinion, submitting an animal pretreated with a psychotropic drug to different stimuli (e.g. novel environment, light, sound) is worthwhile, and could provide useful data.

The present work suggests a modified open field test. The main points characterizing this different approach are: (1) because of its difficult interpretation, defecation is not evaluated; (2) besides ambulation, rearing and grooming, immobility was also recorded; (3) the total time of observation is markedly increased, and (4) the stimuli are presented separately.

Material and Methods

Animals

250 naive male Wistar rats from our colony were used. They were raised in litters reduced to 6 pups. After weaning at 25 days of age they were maintained in groups of 3 in wire cages and at a room temperature of 23 ± 1 °C. When tested, they were 90–110 days old and weighed 270–350 g.

Apparatus

The open field used was a modification of that described elsewhere (7). It consisted in an arena 80 cm in diameter and 40 cm in height, having 3 loudspeakers emitting a noise of 78 dB. It was provided with 3 lamps of 60 W each and 2 red lamps. The open field was also provided with a system that released vanillin odor, by passing air through constant pressure, by a solution of vanillin. The releasing tube descended from the ceiling of the apparatus and ended 42 cm above the floor of the arena. The apparatus was isolated with transparent plastic and completed with a renovation air system.

Drugs

The drugs studied were: (-) Δ^9-*trans*-tetrahydrocannabinol (Δ^9-THC); lysergic acid diethylamide (LSD-25); psylocibin (PSY); *d*-amphetamine sulphate (AMPH); caffeine (CAF); chlorpromazine hydrochloride (CPZ); haloperidol (HALO); diazepam (DZP), and pentobarbital sodium (PTB). Δ^9-THC was suspended in 0.7% Tween 80-saline. The other drugs were dissolved in distilled water. The doses used and the interval between drug administration and open field exposure can be seen in table VI.

Procedure

3 days before being tested the rats were maintained in individual cages. At different times after drug intraperitoneal injection, each animal was introduced in the middle of the arena, and observed for a period of 20 min. For each dose 10 rats were used. During the first 5 min the observation was made in the dark (the 2 red lamps allowed observation). At the end of the first 5 min, vanillin odor (0.5% solution) was released for 15 sec. After 5 min more the loudspeakers were turned on. At the end of another 5 min, the arena was illuminated by the 3 lamps, the sound being maintained.

With the help of an events record, measures were taken for ambulation frequency (number of floor units entered), rearing frequency (number of times the animal stood on hindlegs), grooming time (seconds that the animal touches the forepaws to the head, snout or genitals) and immobility time (seconds of lack of movement, including the head).

Three control-solvent groups were performed, each one made up by 10 rats. In the first (control 1), the procedure was the same as described. The animals of the second group (control 2) were not exposed to the vanillin olfactory stimulus, while for the third group (control 3), sound was not provided. For the 3 groups the total time of exposition to the open field was 20 min, the only difference among them being the absence of the olfactory (control 2) or auditory (control 3) stimulus.

In each experimental session (performed in the afternoon), different drugs and doses were tested, a control rat being run in each session. After the exposure of each rat an interval of 20 min was made, when the arena was cleaned and the renovation air system was turned on.

Statistical Analysis

The scores obtained in the first 5 min (dark) under drug action were compared with the first 5 min of control 1 using Duncan's new multiple-range test (12). The same procedure was used for odor, noise and light periods, always compared with the correspondent data of control 1. Controls 1, 2 and 3 were also compared with Duncan's new multiple-range test.

Results

Table I shows that there was no significant difference among controls 1, 2 and 3. That is, the rats which were not exposed to the vanillin odor or to the sound behaved similarly to the rats exposed to the olfactory and acustic stimuli. The observation of the control rats (table I) shows that ambulation and rearing decreased throughout the first 15 min. The introduction of light induced an increase of ambulation. Grooming and immobility increased throughout the exposition to the open

field, being grooming decreased by the introduction of light.

The data comparing control 1 (C_1) with those after psychotropic drugs are shown in tables II–V and summarized in table VI. Grooming seems not to be an adequate measure to differentiate classes of psychotropic drugs, as all of them reduced it. Amphetamine and caffeine increased ambulation and rearing while decreasing immobility. Chlorpromazine and haloperidol acted by decreasing ambulation and rearing and by increasing immobility. After diazepam a reduction in rearing and increase in immobility but without concomitant reduction in ambulation was observed. Pentobarbital in the smallest dose (10 mg/kg) similarly to the higher dose of diazepam, also decreased rearing and increased immobility without a reduction in ambulation. In the higher dose pentobarbital reduced ambulation and rearing concomitantly

Table II. Influence of drugs on ambulation frequency in the open field

Drugs	Dose, mg/kg	Dark	Odor	Sound	Light
AMPH	1.0	156 ± 5*	105 ± 4*	86 ± 9*	114 ± 8*
	2.0	163 ± 11*	135 ± 14*	133 ± 17*	143 ± 14*
CAF	10.0	103 ± 5	61 ± 7*	46 ± 4*	69 ± 4*
	20.0	119 ± 7*	82 ± 6*	62 ± 7*	87 ± 6*
HALO	0.125	48 ± 6*	19 ± 4*	8 ± 3	21 ± 5*
	0.25	22 ± 3*	1 ± 1*	1 ± 1*	5 ± 2*
CPZ	1.5	71 ± 7	34 ± 7	12 ± 3	41 ± 6
	3.0	34 ± 6*	14 ± 4*	4 ± 2	22 ± 4*
DZP	1.25	88 ± 12	35 ± 9	22 ± 5	44 ± 8
	2.5	83 ± 6	22 ± 6	16 ± 3	43 ± 5
PENTO	10.0	95 ± 13	48 ± 7	19 ± 3	54 ± 7
	20.0	25 ± 7*	8 ± 4*	7 ± 3	17 ± 10*
LSD	0.1	70 ± 10	38 ± 6	28 ± 4	66 ± 6*
	0.2	63 ± 9	28 ± 9	17 ± 5	62 ± 7*
THC	1.25	97 ± 8	34 ± 6	17 ± 5	42 ± 6
	2.5	62 ± 10*	15 ± 6*	12 ± 4	25 ± 6*
PSY	0.5	78 ± 7	27 ± 8	28 ± 5	46 ± 6
	1.0	41 ± 7*	17 ± 6*	8 ± 2	26 ± 5*
C1	–	89 ± 7	38 ± 7	17 ± 11	47 ± 4

The figures indicate mean ± SE.

* Differed from control at a level of 0.01 (Duncan's new multiple-range test).

with an increase of immobility, as the neuroleptics did. A peculiar effect was obtained with both doses of LSD. They induced a decrease in rearing and a drastic increase in immobility, but did not change the other behavioral parameters. However, when light was turned on, ambulation increased to levels above control. This peculiar pattern was not seen after the other hallucinogens. The smallest dose of THC did not alter behavior, while 2.5 mg/kg decreased ambulation and rearing while increasing immobility, as neuroleptics did. The smallest dose of psylocibin did not alter ambulation but reduced rearing and increased immobility similarly to the higher dose of diazepam and the smallest dose of pentobarbital. The higher dose of psylocibin acted by reducing ambulation and rearing while increasing immobility, similarly to both neuroleptics and to the higher dose of pentobarbital.

Table III. Influence of drugs on rearing frequency in the open field

Drugs	Dose, mg/kg	Dark	Odor	Sound	Light
AMPH	1.0	47 ± 5*	26 ± 4*	11 ± 1*	15 ± 2*
	2.0	53 ± 5*	28 ± 7*	21 ± 4*	20 ± 4*
CAF	10.0	34 ± 2	19 ± 3	15 ± 2*	10 ± 1
	20.0	34 ± 3	22 ± 2*	17 ± 2*	12 ± 2*
HALO	0.125	19 ± 2*	8 ± 3*	5 ± 3	6 ± 2
	0.25	10 ± 2*	1 ± 1*	0*	1 ± 1*
CPZ	1.5	25 ± 3	11 ± 2	7 ± 2	10 ± 1
	3.0	9 ± 5*	3 ± 1*	1 ± 1*	3 ± 1*
DZP	1.25	23 ± 4*	11 ± 3	10 ± 2	8 ± 2
	2.5	13 ± 2*	3 ± 1*	4 ± 1	9 ± 2
PENTO	10.0	14 ± 2*	6 ± 1*	4 ± 1	7 ± 1
	20.0	2 ± 1*	1 ± 1*	1 ± 1*	1 ± 1*
LSD	0.1	5 ± 2*	3 ± 1*	4 ± 1	6 ± 1
	0.2	2 ± 1*	1 ± 1*	1 ± 1*	4 ± 1*
THC	1.25	24 ± 2	10 ± 9	8 ± 2	8 ± 2
	2.5	7 ± 2*	2 ± 1*	2 ± 1*	2 ± 1*
PSY	0.5	14 ± 3*	4 ± 1*	0*	7 ± 2
	1.0	4 ± 1*	0*	0*	2 ± 1*
C1		30 ± 3	15 ± 2	7 ± 1	8 ± 1

The figures indicate mean ± SE.

* Differed from control at a level of 0.01 (Duncan's new multiple-range test).

Discussion

To evaluate the results obtained in the present experiment, it is interesting to compare them with the data of other authors showing the effects of psychotropic drugs through the classical open field procedure (11). In that experiment, the rats were exposed to the open field for a 3-min period under light and noise, simultaneously, and ambulation, rearing, grooming and defecation were recorded. Under 0.1 mg/kg of LSD it was reported that the rats showed a decrease of rearing without altering ambulation. This pattern was shared by diazepam (1.25 and 2.5 mg/kg) and pentobarbital (5.0 and 10 mg/kg). In the present experiment, through the dissociation of the stimuli and the measurement of immobility, a peculiar pattern for LSD could be detected. 0.1 and 0.2 mg/kg of LSD decreased rearing, increased

Table IV. Influence of drugs on immobility time (sec) in the open field

Drugs	Dose, mg/kg	Dark	Odor	Sound	Light
AMPH	1.0	1 ± 1	14 ± 8*	37 ± 14*	19 ± 6*
	2.0	1 ± 1	4 ± 3*	6 ± 5*	1 ± 1*
CAF	10.0	4 ± 1	18 ± 8*	23 ± 10*	25 ± 12*
	20.0	0	8 ± 5*	7 ± 3*	7 ± 2*
HALO	0.125	75 ± 18*	150 ± 25*	189 ± 25*	168 ± 26*
	2.5	185 ± 16*	274 ± 7*	285 ± 7*	267 ± 9*
CPZ	1.5	60 ± 18*	112 ± 27	192 ± 19*	113 ± 16
	3.0	157 ± 28*	220 ± 22*	255 ± 16*	204 ± 17*
DZP	1.25	46 ± 18	106 ± 34	144 ± 33	149 ± 25
	2.5	91 ± 18*	204 ± 29*	209 ± 23*	146 ± 22
PENTO	10.0	92 ± 27*	145 ± 22*	206 ± 11*	164 ± 19*
	20.0	202 ± 28*	248 ± 29*	260 ± 18*	261 ± 11*
LSD	0.1	123 ± 21*	171 ± 16*	175 ± 15*	112 ± 14
	0.2	161 ± 17*	182 ± 20*	243 ± 9*	136 ± 13
THC	1.25	25 ± 11	107 ± 20	191 ± 25*	132 ± 20
	2.5	151 ± 22*	230 ± 14*	243 ± 13*	214 ± 12*
PSY	0.5	78 ± 21	194 ± 29*	187 ± 24*	157 ± 28*
	1.0	187 ± 20*	250 ± 12*	262 ± 12*	213 ± 10*
Cl	-	17 ± 9*	74 ± 22	114 ± 18	116 ± 14

The figures indicate mean ± standard error.

* Differed from control at a level of 0.01 (Duncan's new multiple-range test).

immobility and did not alter ambulation in the dark, odor and sound periods, but induced an increase of ambulation, above control levels under light. This reaction to light differentiated LSD from diazepam and pentobarbital. Through the traditional use of the open field this reaction to light cannot be detected, as all stimuli are presented together.

Another point that deserves attention concerns the measure of immobility. The data obtained with LSD, diazepam and pentobarbital show that immobility is not necessarily the counterpart of ambulation. This apparent paradox can be explained if one considers that the rats can run fast and then stop, giving high scores on both ambulation and immobility measures. These drugs increased immobility without a concomitant alteration in ambulation.

In conclusion, without considering defecation, the data of the present experiment

Table V. Influence of drugs on grooming time (sec) in the open field

Drugs	Dose, mg/kg	Dark	Odor	Sound	Light
AMPH	1.0	1 ± 1*	8 ± 3*	11 ± 4*	9 ± 2
	2.0	0*	1 ± 1*	4 ± 3*	2 ± 2
CAF	10.0	5 ± 3*	26 ± 6*	47 ± 7	19 ± 8
	20.0	2 ± 1*	13 ± 3*	23 ± 5*	19 ± 7
HALO	0.125	17 ± 8	14 ± 3*	29 ± 10*	5 ± 3
	2.5	8 ± 3*	1 ± 1*	3 ± 3*	1 ± 1*
CPZ	1.5	3 ± 1*	20 ± 10*	26 ± 11*	18 ± 7
	3.0	1 ± 1*	2 ± 3*	4 ± 3*	4 ± 3
DZP	1.25	4 ± 2*	15 ± 5*	21 ± 7*	10 ± 6
	2.5	7 ± 6*	15 ± 9*	17 ± 9*	8 ± 4
PENTO	10.0	2 ± 1*	5 ± 3*	3 ± 2*	3 ± 2
	20.0	0*	0*	3 ± 3*	0*
LSD	0.1	0*	1 ± 1*	8 ± 5*	7 ± 4
	0.2	0*	1 ± 1*	1 ± 1*	0*
THC	1.25	1 ±1*	12 ± 5*	8 ± 4*	7 ± 3
	2.5	1 ± 1*	0*	0*	0*
PSY	0.5	2 ± 1*	3 ± 2*	2 ± 1*	1 ± 1*
	1.0	1 ± 1*	0*	0*	0*
C1	–	17 ± 4	36 ± 8	50 ± 12	12 ± 4

The figures indicate mean ± SE.

* Differed from controls at a level of 0.01 (Duncan's new multiple-range test).

Table VI. Effects of drugs on ambulation, rearing, immobility and grooming in the open field: arrows indicate significant differences compared to control during dark (D), odor (O), sound (S) and light (L)

Drug	Dose mg/kg	Interval between drug administration and test, min	Ambulation				Rearing				Immobility				Grooming			
			D	O	S	L	D	O	S	L	D	O	S	L	D	O	S	L
AMPH	1.0	30	↑	↑	↑	↑	↑	↑	↑	↑	–	↓	↓	↓	↓	↓	↓	–
	2.0		↑	↑	↑	↑	↑	↑	↑	↑	–	↓	↓	↓	↓	↓	↓	–
CAF	10.0	30	–	↑	↑	↑	–	–	↑	–	–	↓	↓	↓	↓	↓	–	–
	20.0		↑	↑	↑	↑	–	↑	↑	↑	–	↓	↓	↓	↓	↓	↓	–
HALO	0.125	60	↓	↓	–	↓	↓	↓	–	–	↑	↑	↑	↑	–	↓	↓	–
	0.250		↓	↓	↓	↓	↓	↓	↓	↓	↑	↑	↑	↑	↓	↓	↓	↓
CPZ	1.5	60	–	–	–	–	–	–	–	–	↑	–	↑	–	↓	↓	↓	–
	3.0		↓	↓	–	↓	↓	↓	↓	↓	↑	↑	↑	↑	↓	↓	↓	–
DZP	1.25	30	–	–	–	–	↓	–	–	–	–	–	–	–	↓	↓	↓	–
	2.5		–	–	–	–	↓	↓	–	–	↑	↑	↑	–	↓	↓	↓	–
PENTO	10.0	10	–	–	–	–	↓	↓	–	–	↑	↑	↑	↑	↓	↓	↓	–
	20.0		↓	↓	–	↓	↓	↓	↓	↓	↑	↑	↑	↑	↓	↓	↓	↓
LSD	0.1	10	–	–	–	↑	↓	↓	–	–	↑	↑	↑	–	↓	↓	↓	–
	0.2		–	–	–	↑	↓	↓	↓	↓	↑	↑	↑	–	↓	↓	↓	↓
THC	1.25	45	–	–	–	–	–	–	–	–	–	–	↑	–	↓	↓	↓	–
	2.5		↓	↓	–	↓	↓	↓	↓	↓	↑	↑	↑	↑	↓	↓	↓	↓
PSY	0.5	10	–	–	–	–	↓	↓	↓	–	↑	↑	↑	↑	↓	↓	↓	↓
	1.0		↓	↓	–	↓	↓	↓	↓	↓	↑	↑	↑	↑	↓	↓	↓	↓

allowed a reasonable differentiation among most of the classes of drugs studied considering only the measures of ambulation, immobility and rearing. Thus, stimulants could be differentiated from all other classes of drugs through their general excitatory effects. The distinction between neuroleptics and the anxiolytic drug used can be made considering that while haloperidol and chlorpromazine induced a general depressant effect, diazepam although affecting rearing and immobility in the same direction, did not alter ambulation. On the other hand, the higher dose of the anxiolytic induced the same effect as the smallest dose of the barbiturate. This result is in accordance with the statement that barbiturates and anxiolytics may share the same effects (9). Concerning the hallucinogens it was possible to differentiate LSD from all other classes of drugs by its peculiar influence on the reaction to light. This effect of LSD was not shared by the other psychotomimetic drugs used (THC and psylocibin), which confirms the difficulty of screening hallucinogenic drugs (11). On the other hand, the resemblance of the effects of THC with those of chlorpromazine and

haloperidol, confirms the observations of similarities of the effects of THC and neuroleptics (2, 3).

References

1 Brimblecombe, R.W.: Effects of psychotropic drugs on open field behaviour in rats. Psychopharmacologia *4:* 139–147 (1963).

2 Carlini, E.A.: Acute and chronic behavioral effects of *Cannabis sativa;* in Pharmacology and the future of the man. Proc. 5th Int. Congr. Pharmacology, San Francisco 1972.

3 Carder, B. and Deikel, S.M.: Similarities between Δ^9-tetrahydrocannabinol (Δ^9-THC) and reserpine-like drugs. Behavl Biol. *17:* 313–332 (1976).

4 Dandiya, P.G.; Gupta, B.D.; Gupta, M.P., and Patni, S.K.: Effects of LSD on open field performance in rats. Psychopharmacologia *15:* 333–340 (1969).

5 Janssen, P.A.J.; Jageneau, A.H.M., and Schellekens, K.H.L.: Influence of haloperidol (r 1625) and of chlorpromazine on the behaviour of rats in an unfamiliar 'open field' situation. Psychopharmacologia *1:* 389–392 (1960).

6 Masur, J.; Martz, R.M.W., and Carlini, E.A.: Effects of acute and chronic administration of *Cannabis sativa* and (-) Δ^9-*trans*-tetrahydrocannabinol on the behavior of rats in an 'open field' arena. Psychopharmacologia *19:* 388–397 (1971).

7 Masur, J.: Sex differences in 'emotionality' and behavior of rats in the open field. Behavl Biol. *7:* 749–754 (1972).

8 Masur, J. and Martz, R.M.W.: Failure of chlorpromazine to protect rats against the effects of shock administration during the second month of life. Psychopharmacologia *28:* 301–307 (1973).

9 Meyers, F.H.; Jawetz, E., and Goldfren, A.: A review of medical pharmacology (Lange, Los Altos 1968).

10 Ryall, R.W.: Effects of drugs on emotional behaviour in rats. Nature, Lond. *182:* 1606–1607 (1958).

11 Silva, M.T.A. and Calil, H.M.: Screening hallucinogenic drugs: systematic study of three behavioral tests. Psychopharmacologia *42:* 163–171 (1975).

12 Steel, R.G.D. and Torrie, J.H.: Principles and procedures of statistics (McGraw-Hill, New York 1960).

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Jomar M. Cunha, Departamento de Psicobiologia, Escola Paulista de Medicina, Rua Botucatu, 862, 04023 São Paulo (Brasil)