EFFECTS OF PSYCHOTROPIC DRUGS ON EMOTIONAL BEHAVIOR: EXPLORATORY BEHAVIOR OF NAIVE RATS IN HOLED OPEN FIELD

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Testing drugs on intact animals is considered to be the best method for investigation (1) and since there have been a few reports concerning the action of psychotropic drugs on unlearned behavior, the authors were interested in their effects on emotional behavior of animals. Hall's open-field test, the optimum conditions of which were established by Broadhurst, has been used for many years to study behavior (2-4).

This test is generally accepted as a valid measure of emotion in rats. The principle of the test is that the novel situation of the open field evokes in the animal a pattern of behavior characterized by exploration (ambulation and rearing), emotional defecation and urination (5). It has been considered that the exploration evoked under an unfamiliar environment is modified with psychological factors such as curiosity, fear and anxiety as well as with psychotropic drugs (6-11). For example, chlorpromazine and reserpine produced a graded parallel reduction in all types of behavior in rats in open fields, although haloperidol and perphenazine were capable of inhibitory ambulation and rearing without ataxia (7, 12). Boissier and Simmon have investigated the effects of psychotropic drugs on head-entering behavior of mice into the holes in unlearned field (13). Thereafter, Krnjevic and Videk have presented the method of investigation of the behavior in rats confronted by a hole on the animal cage (14). In this paper a new method for investigation is presented on reference to the exploratory behavior of inexperienced rats placed in a square holed open field. The suitability of the method is examined and effects of various kind of psychotropic drugs are discussed.

As a result, this method has proved to be useful for investigation of the effects of psychotropic drugs.

MATERIALS AND METHODS

Subjects: Four hundred eight weeks old male Wistar rats weighing 120-130 g were purchased. The animals were kept for 8-10 days, housed five per in home cages 45 cm in length, 25 cm in width and 18 cm in height. In order to reduce extraneous noise, the home cages were kept in a separate room illuminated artificially from 8:30 am. to 8:00 pm. and maintained at 22.0–23.0 °C. Apart from a once daily provision of food and water,
the rats were left undisturbed before the test. Observation of behavior was performed in the laboratory under the same atmospheric conditions as in the animal room.

**Drugs:** All drugs were injected i.p. except for morphine injected s.c. in a volume of 0.2 ml/100 g body wt. either dissolved in 0.9\% W/V physiological saline or suspended in 0.5\% tragacanth solution.

The following drugs were used: haloperidol (Serenace), perphenazine (P.Z.C.), chlorpromazine (Wintermine), imipramine (Tofranyl), desmethyl-imipramine (Pertofran), diazepam (powder), chlordiazepoxide (powder), meprobamate (Atraxin), pentobarbital (Mintal), methamphetamine (Philopon), mescarine and morphine.

Doses were calculated on the basis of the salt.

**Apparatus:** The apparatus consisted of a horizontal vinylchloriden plain field (55 x 55 cm), where 9 holes were made and spaced 20 cm apart. The horizontal plain was placed 90 cm above the floor. The apparatus was situated in the dark room and illuminated by a 20 W fluorescent light placed 70 cm above the central part of the field.

The apparatus for the rotating cylinder test consisted of a wooden rod 90 cm in length, revolving 7.5 times per min. The rod was divided into 6 compartments 15 cm apart by wooden disks 30 cm in diameter.

**Procedure:** Eight rats were used for each dose of drugs and vehicles. To minimize risks of error in the experiment time, drugs or vehicles were injected alternatively. Thirty min after administration, unless otherwise stated, each rat was transferred carefully to the center of the field. After 10 sec, behavior was televised continuously for 6 min. The set had been placed in an adjacent room. When an animal walked the distance between two holes, ambulation of the animal was counted as one score.

The behavior of a rat plunging the head into the hole more deeply than at the level of the rostral edge of the eye lid was referred to as head-dipping behavior. The number of fecal boluses excreted during observation time was recorded as defecation score. In this study, effects of the drugs were mainly evaluated according to ambulation and head-dipping behavior.

In the rotating cylinder test, rats were trained on the rotating cylinder three times for 3 min at intervals of 30 min. Drugs were injected only to those rats which did not fall from the cylinder on the third training. Thirty min after the drug administration these same rats were placed on the rotating cylinder. The rats falling from the rotating cylinder more than twice in 3 min were labelled expected response.

Through all experiments, an uniformed experimenter observed the behavior.

**RESULTS**

**Exploratory behavior of naive rats in the holed open field situation:** Fig. 1 shows the holed open field situation used in this experiment. As shown in Fig. 2, head-dipping behavior of rats was promoted on the field with the holes 6.0 cm in diameter as compared to those 2.8 cm in diameter which was almost same size to head of rat, however, the diameter of the hole did not appear to influence ambulation, rearing and defecation. Scores of
ambulation and defecation obtained in the holed open fields showed approximately the same values as those obtained in the plain open field, although rearing was significantly reduced in the holed open field. The holes 6.0 cm in diameter were spaced 20 cm apart, because intervals of 10 cm might make it difficult to distinguish this exploratory behavior from other behavior such as displacing or preening, which ought to be considered as an expression of general behavior. In the smaller box (35 × 35 cm) ambulation and head-dipping behavior lessened, while in the larger box (75 × 75 cm), it increased.

Effects of neuroleptics and thymoleptics: Figs. 3 and 4 show the effects of several neuroleptics and thymoleptics on ambulation and the head-dipping behavior. During the first 3 min of observation, haloperidol (0.1–2.5 mg/kg), perphenazine (0.1–2.5 mg/kg) and chlorpromazine (1.0–5.0 mg/kg) reduced both ambulation and head-dipping with a very low dosage. Rats treated with these drugs displayed motor incoordination and signs of sedation such as lowered body tone, splaying of limbs and passivity. Thymoleptics slightly reduced ambulation and head-dipping without producing any motor incoordination in a dose of 10 mg/kg. During the next 3 min of observation, all neuroleptics and thymoleptics reduced all behavior more strikingly than those during the first 3 min.

Effects of tranquilizers: Fig. 5 shows the effects of several tranquilizers on ambulation and head-dipping behavior. During the first 3 min of observation, diazepam (0.5–10 mg/kg), meprobamate (10–40 mg/kg) and particularly chlordiazepoxide (0.5–10 mg/kg) caused a marked increase in ambulation at doses below 10 mg/kg, but none of these drugs produced motor incoordination. On the other hand, head-dipping behavior was little affected with these drugs during the first 3 min. During the next 3 min, however, chlor-
diazepam and diazepoxide particularly lessened both types of behavior. Sixty, 120 and 180 min after administration of 10 mg/kg of chlordiazepoxide, ambulation and head-dipping were observed with different groups of rats respectively and the same pattern as observed 30 min after drug administration was evidenced. Rats fell down from the rotating cylinder and all exploratory behavior was reduced significantly, under chlordiazepoxide (40 mg/kg), diazepam (40 mg/kg) or meprobamate (160 mg/kg) treatments. Ambulation and head-dipping behavior were accordingly promoted with a below ataxic dose of pentobarbital.

Since injection of chlordiazepoxide and diazepam solutions is said to cause the transient local irritation in rats, effect of irritability on the subsequent exploration was observed by scoring exploratory behavior 30 min after intraperitoneal treatment with 0.2 ml of 0.05 N-hydrochloric acid. This treatment only slightly reduced both ambulation and head-dipping behavior during the first 3 min.
Effects of neuroleptics and thymoleptics on exploratory behavior in rats. Drugs were administered i.p. to rats 30 min prior to placing them into the holed open field. Per cent values are in respect to the basal value (saline) of ambulation and head-dipping, respectively. (N = 8)

Fig. 3. Following 3–6 min observation of the effects of neuroleptics and thymoleptics on exploratory behavior in rats. (N = 8)
Fig. 5. Effects of tranquilizers and hypnotics on exploratory behavior in rats. Drugs were administered i.p. to rats 30 min prior to placing them into the holed open field. (N=8)

Fig. 6. Effects of psychostimulants and psychotomimetics on exploratory behavior in rats. Methamphetamine and mescaline were administered i.p., morphine was administered s.c. 30 min prior to placing them into the holed open field. (N=8)
Effects of miscellaneous centrally acting drugs: Results are given in Fig. 6. During the first 3 min of observation, morphine (1.0–4.0 mg/kg) and mescaline (50–100 mg/kg) showed a tendency to promote ambulation, however, methamphetamine (1.0–4.0 mg/kg) had little effect. In the following 3 min these three drugs caused a remarkable increase in ambulation.

Methamphetamine enhanced head-dipping behavior, however morphine and mescaline slightly reduced the behavior. Rats injected with methamphetamine revealed an increase in motor activity, however, at a higher dosage than introduced in this figure, methamphetamine reduced locomotor activity and promoted gnawing movement. Rats treated with morphine showed a slight reduction in general motor activity but those treated with mescaline failed to show any changes in motor activity except slight catatonic behavior.

DISCUSSION

Holed open field methods resulted in new findings in the effects of psychotropic drugs on exploratory behavior in rats. Those rats placed in the field expressed two kinds of characteristic exploratory behavior, one was ambulatory walking around from hole to hole, another was head-dipping behavior into the hole. The head-dipping behavior was so clearly observed that it was quite distinguishable from other general behavior. Observation utilizing television was essential to this kind of experiment as any noise made by the observer resulted in a freezing reaction in the rats. Even if a faint noise was made the ambulatory and head-dipping behavior ceased temporarily. There are several reports of investigations concerning exploratory behavior in rats (10, 15) and familiarity of a rat with an environment.

Table 1. Summary of effects of psychotropic drugs on exploratory behavior in rats in holed open field.

| Drugs         | Dose  | Ambulation Duration | Head-dipping Duration (min) | Defecation |
|---------------|-------|----------------------|----------------------------|------------|
|               | mg/kg i.p. | 0–3 | 3–6 | 0–3 | 3–6 | 0–6 |
| Haloperidol   | 0.1–2.5 |     |     |     |     |     |
| Perphenazine  | 0.1–2.5 |     |     |     |     |     |
| Chlorpromazine| 0.5–5.0 |     |     |     |     |     |
| Diazepam      | 0.5–10.0| ↑   |     |     |     |     |
| Chlordiazepoxide | 0.5–10.0 | ↑   |     |     |     |     |
| Meprobamate   | 10.0–40.0| →  |     |     |     |     |
| Imipramine    | 2.5–10.0 | ↓   |     |     |     |     |
| Desmethyl-imip. | 2.5–10.0 | ↓   |     |     |     |     |
| Methamphetamine | 1.0–4.0 | →  |     |     |     |     |
| Morphine      | 1.0–4.0 | ↑   |     |     |     |     |
| Mescaline     | 50.0–100.0 | ↑ |     |     |     |     |
| Pentobarb.-Na | 0.5–10.0 |     |     |     |     |     |

→ = Control values ± 25%  ↓ = Control values – (26~50)%
↑ = Control values + (26~50)%  ↓ = Control values – (51~100)%
↑ = Control values + (51~100)%
is apt to decrease fear, anxiety and curiosity when in an open field (16). There is, however, no report observing exploratory behavior of rats in the holed open field.

Parallel decrease of ambulation and head-dipping behavior accompanied by motor incoordination or sedation was observed with neuroleptics. In contrast, a transient and distinct increase of ambulation, and little change of head-dipping were observed with administration of tranquilizers. This finding is of particular interest, for these drugs have the general clinical property of allaying fear and anxiety, though differing from each other in many ways (16, 17).

Methamphetamine caused an increase in ambulation and head-dipping, and these changes in behavior became conspicuous with lapse of observation time. Morphine and mescaline, which produce euphoria in humans, promoted ambulation and demoted head-dipping behavior in rats (Table 1).

To interpret the meaning of ambulation and head-dipping behavior, (a separate unpublished experiment), rats which had been placed in the situation once a day for 6 min during 24 consecutive days were investigated in order to be made accustomed to the situation. Exploration and head-dipping of the rats were, respectively, increased and decreased in proportion to repetition of the experience in the holed open field. These results suggest that ambulation has some relation to fear and anxiety, while head-dipping may be related to the curiosity.

From the behavioral observations, it may be stated that neuroleptics depress psychotic function as well as motor function, tranquilizers diminish fear, anxiety and curiosity, and psychostimulants elevate the 'psychotic function.'

It can be concluded from over-all results that ambulation and head-dipping behavior in rats are a significant expression of the central effect of psychotropic drugs.

**SUMMARY**

The effects of psychotropic drugs have been studied on exploratory behavior in rats in the holed open field.

1. Effects of psychotropic drugs and other centrally acting drugs on ambulation and head-dipping behavior in naive rats were investigated using a newly designed open field.
2. Neuroleptics (haloperidol, perphenazine and chlorpromazine) and thymoleptics (imipramine and desmethylimipramine) reduced ambulation and head-dipping behavior.
3. Tranquilizers (diazepam, chlordiazepoxide and meprobamate) caused an increase in ambulation, but little affected the head-dipping behavior.
4. Psychotomimetics (morphine and mescaline) enhanced ambulation, however reduced head-dipping behavior.
5. Psychostimulant (methamphetamine) promoted both types of behavior.

**REFERENCES**

1) KRNJEVIC, H. AND VideC, M.: Psychopharmacologia 10, 308 (1967)
2) HALL, C.S.: J. comp. physiol. Psychol. 18, 385 (1934)
3) HALL, C.S.: J. comp. physiol. Psychol. 22, 345 (1936)
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4) Broadhurst, P.L.: Br. J. Physiol. 48, 1 (1957)
5) Bindra, D. AND Thompson, W.R.: J. comp. physiol. Psychol. 46, 315 (1953)
6) Berlyne, D.E.: Br. J. Psychol. 41, 68 (1950)
7) Ryall, R.W.: Nature 182, 1606 (1958)
8) Blimblecombe, R.W.: Psychopharmacologia 4, 193 (1963)
9) Markot, A.S. AND Spencer, P.S.J.: Br. J. Pharmac. Chemother. 25, 432 (1965)
10) Rushton, R. AND Steinberg, H.: Br. J. Pharmac. Chemother. 21, 295 (1963)
11) Rushton, R. AND Steinberg, H.: Nature 221, 1312 (1966)
12) Janssen, P.A.J., Jageneau, A.H.M. AND Shellekens, K.H.L.: Psychopharmacologia 1, 389 (1960)
13) Boissier, J.R. AND Simon, P.: Arch. int. Pharmacodyn. Thér. 147, 372 (1962)
14) Krnjevic, H. AND Videk, M.: Psychopharmacologia 10, 389 (1967)
15) Berlyne, D.E.: Conflict, Arousal and Curiosity, McGraw-Hill, New York (1960)
16) Steinberg, H., Rushton, R. AND Tinson, C.: Nature 192, 533 (1961)
17) Berger, F.M.: Clin. Pharmac. Ther. 4, 209 (1963)