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# An Improved Method for Detecting Drug Effects in the Open Field

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**Abstract.** A double test cross-over design was applied to the testing of rats in the open field. When used to examine the effects of atropine, chlorpromazine and lysergic acid diethylamide (LSD) on open field behaviour, this design proved 4–40 times more sensitive than the previously popular single test design. In no case was the double test design less sensitive. Results are discussed in relation to screening of medically useful compounds.

**Key words:** Open field test – Rats – Atropine – Chlorpromazine – LSD – Double test cross-over – Test sensitivity

The well-known open field test has been widely used in recent years, presumably due to its simplicity and ease of quantification (Archer, 1973; Walsh and Cummins, 1976). Because a range of behaviour may be measured, it is ideally suited for use in screening of untested drugs. When used in this way the test has typically been administered as a single short exposure with subjects divided into two groups to receive either drug or control treatment (Ader, 1965; Aldous et al., 1974; Brimblecombe, 1963; Singer, 1963).

When screening new drugs, test sensitivity is obviously very important. A potentially more sensitive design would involve testing each subject twice with some subjects receiving drugs on the first, and some on the second exposure. This cross-over design allows subjects to act as their own control, thus reducing the error variance by statistical partitioning (Winer, 1962). An additional advantage derives from the observation that drug effects may depend on the baseline level of performance from which they are measured (Dews, 1958). If baselines vary between two exposures to the open field, then better results may be obtained by exposing animals to treatment on both baselines.

The experiments reported here were designed to compare this double test cross-over design with the single test method. Experiment 1 consisted of retesting subjects without any differential between-test manipulation in order to observe test-retest fluctuations in levels of behaviour. This experiment was carried out four times to determine whether such fluctuations may be consistently obtained. This type of experiment has previously been performed (Broadhurst, 1957; Denenberg, 1969; Hall, 1941).

In Experiment 2 various doses of atropine, chlorpromazine and lysergic acid diethylamide (LSD) were administered using the cross-over design. Previous experiments using a single test method in these laboratories have shown that atropine will increase ambulation, decrease defaecation and decrease preening with a lowest effective dose (LED) of about 2.0 mg/kg; chlorpromazine will reduce ambulation and rearing (LED, 1.0 mg/kg) and LSD will increase ambulation and decrease defaecation (LED, 0.05 mg/kg; Aldous et al., 1974).

Walsh and Cummins (1976) pointed out the diversity of apparatus used in the open field test and have stressed the importance of test-retest reliability in the behavioural measures used. The most systematic test of reliability of open field behaviour was carried out by Ivinskis (1968), in apparatus very similar to that employed here. He concluded in tests with albino rats that only defaecation, ambulation, rearing and a measure of washing may be considered reliable measures. Of those measures, ambulation and rearing showed a high positive inter-subject correlation while others were uncorrelated (Ivinskis, 1966).

## Materials and Methods

*Subjects.* Male albino rats of the Porton strain were used, weighing 190 ± 20 g on the first test day and aged about 40 days. They were housed in communal cages 56 × 32 × 19 cm, ten rats per cage, in a

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room illuminated at all times, and were allowed constant access to food and water.

*Apparatus.* The open field consisted of a circular arena with a diameter of 82-cm and 45-cm walls. The floor was divided into 19 squares of approximately equal area. The walls and floor were white and the apparatus was illuminated by four 150-W lamps situated 165 cm above the floor. White noise at 88 dB was used and the operator was screened from the rat by a muslin curtain.

*Injections.* Drugs were administered to each subject by subcutaneous injection into the groin 30 min before the start of each test except in the case where LSD was given in Experiment 2, when an interval of 15 min was used, corresponding to that used by Aldous et al. (1974). All drugs were dissolved in 0.85% saline injected at 1.0 ml/kg.

In each replication of Experiment 1, 16 rats were used, each being injected with saline 30 min before each test. All these animals were tested by the same experimenter. In each replication of Experiment 2, 16 rats were used, divided into two equal groups. Subjects of Group DC were injected with drug before Test 1 and saline before Test 2. Subjects of Group CD were injected with saline before Test 1 and drug before Test 2. A double-blind procedure was employed such that neither the experimenter administering the injection nor the experimenter observing the open field behaviour knew which rats had received drug and which saline.

Drugs were administered at the following doses (all in mg/kg). Atropine: 4.0, 2.0 (three replications), 1.0, 0.5 (two replications), 0.2 (two replications), 0.1, 0.05 (two replications); chlorpromazine: 2.0, 0.5, 0.2; LSD: 0.5, 0.1, 0.05, 0.02, 0.01 (with 48-h inter-test interval) and 0.01 (with inter-test interval of 168 h).

*Observation.* Each subject was exposed to the open field for 3 min on two occasions. The second test followed the first by 48 h, except in the case of a single replication of Experiment 2 when an interval of 168 h was used. The subjects were placed into one of the peripheral squares of the field and during the next 3 min behaviour was recorded manually by the experimenter. Only measures found to be reliable by Ivinskis (1968) were taken, as defined below:

Defaecation: Number of boli passed.

Ambulation: Number of squares traversed with a criterion of all four paws in a new square.

Rearing: Number of times lifting front legs off floor with back legs extended.

Preening: Number of occurrences of a behavioural sequence consisting of washing face, ears and side of body, in that order.

*Analysis.* For each replication of Experiment 1, Student's *t*-test for paired observations was used to compare performance on each measure between day 1 and day 2.

In Experiment 2 the performance on Test 1, which is equivalent to the single test design often employed previously, was compared by means of Student's *t*-test, between the drug group and the control.

The correct method for analysis of the combined results of the two tests of Experiment 2 is to use a Latin square principle with repeated measures. This design is clearly explained by Winer (2nd ed., p. 712, plan 5) for a 3 × 3 Latin square. However, since in a 2 × 2 design the interaction (Winer's AB) is eliminated from the analysis, the computationally similar analysis for a 'two-factor experiment with repeated measures in one factor' may be substituted and produces identical results (Winer, 1971, p. 518, Table 7.2 – 1). Most researchers will be familiar with this common design, but it is worth pointing out that in the 2 × 2 case the values obtained for the test session effect and the drug effect (both within-subject effects) are identical whether the former is considered as a main effect (Winer's B) and the latter as an interaction (Winer's AB) or vice versa. This is because the analysis really comprises a Latin square design with three main factors and no interaction. However, in the 3 × 3 design and bigger, this is no longer the case and the full Latin square design must be used.

In the present case, the two-factor repeated measures design was used and three *F*-ratios were computed for group (DC *vs* CD), drug (drug *vs* control) and test session (Test 1 *vs* Test 2). The *F*-test on the drug factor is equivalent for the double test procedure to the *t*-test between drug and control groups carried out on the Test 1 results. This drug factor *F*-ratio is the only one reported.

## Results

### *Experiment 1*

Mean scores for each behaviour are given in Table 1 for each test on each replication. Ambulation and rearing scores were lower on Test 2 of each replication ($t > 2.92$, $P < 0.01$, in each case). Defaecation and preening scores did not vary systematically between the two tests, although on replication a there was significantly less preening on Test 2 ($t = 2.52$, $P < 0.05$).

The four behavioural measures were intercorrelated in each test. Rearing was positively correlated with ambulation on both tests ($r > 0.52$, $P < 0.01$, in each case), but none of the other correlations were significant.

### *Experiment 2*

Mean scores for each group at each dose level are shown for the various drugs in Tables 2, 4 and 6. Those measures not significantly affected by any dose of a given drug were not included in the table of results for that drug.

A comparison of the single and double test methods is presented in Tables 3, 5 and 7, which show those measures found to be significant ($P \leq 0.05$) by each method of analysis. The criterion of significance where multiple replications were run at the same dose level is two replications significant out of two or three run.

Comparing performance on the two tests, results were similar to those found in Experiment 1. Defaecation and preening scores did not differ systematically between Tests 1 and 2. Ambulation and rearing scores were always higher on Test 1, except in the case of LSD where control ambulation scores were about equal on the two tests when a 48-h inter-test interval (ITI) was used.

With all drugs the effects tended to be greater at higher doses. Atropine tended to increase ambulation and to decrease defaecation and preening (Table 2). With each of these measures significant results were obtained at a lower dose with the double test method (respective LEDs 0.2, 0.5 and 0.1 mg/kg) than with the single test method (LEDs 4.0, 2.0 and 4.0 mg/kg). The former method was 20 times more sensitive in the case

**Table 1.** Mean scores on open field behaviours in a double test with no drug

| Replication | Test | Ambulation | Defaecation | Rearing | Preening |
|---|---|---|---|---|---|
| a | 1 | 52.4 | 2.1 | 14.3 | 0.9 |
| | 2 | 27.2 | 2.6 | 4.2 | 0.3 |
| b | 1 | 55.2 | 3.4 | 11.6 | 0.7 |
| | 2 | 29.4 | 1.9 | 5.1 | 0.8 |
| c | 1 | 39.2 | 2.9 | 8.0 | 0.9 |
| | 2 | 21.1 | 1.8 | 3.6 | 0.8 |
| d | 1 | 34.3 | 3.6 | 7.8 | 0.6 |
| | 2 | 19.5 | 2.6 | 3.2 | 0.5 |

**Table 2.** Effects of atropine on open field behaviour

| Dose (mg/kg) | Test | Ambulation | | Defaecation | | Preening | |
|---|---|---|---|---|---|---|---|
| | | C | D | C | D | C | D |
| 1.0 | 1 | 40.6 | 75.4 | 3.63 | 1.00 | 1.13 | 0.25 |
| | 2 | 31.8 | 61.1 | 1.63 | 1.00 | 0.50 | 0.20 |
| 2.0 | 1 | 45.9 | 68.1 | 2.38 | 0.96 | 1.00 | 0.46 |
| | 2 | 25.3 | 43.8 | 1.50 | 0.59 | 0.59 | 0.29 |
| 1.0 | 1 | 45.6 | 56.4 | 2.38 | 0.88 | 0.75 | 0.25 |
| | 2 | 27.3 | 52.9 | 3.63 | 0.75 | 1.13 | 0.13 |
| 0.5 | 1 | 34.8 | 53.3 | 2.76 | 1.01 | 0.94 | 0.94 |
| | 2 | 22.3 | 28.4 | 1.75 | 1.13 | 0.50 | 0.82 |
| 0.2 | 1 | 51.7 | 53.7 | 2.13 | 0.75 | 0.94 | 0.38 |
| | 2 | 25.9 | 28.1 | 1.94 | 1.50 | 0.44 | 0.13 |
| 0.1 | 1 | 38.7 | 47.5 | 2.88 | 1.38 | 1.25 | 0.75 |
| | 2 | 29.3 | 22.1 | 1.25 | 1.38 | 0.63 | 0.25 |
| 0.05 | 1 | 24.6 | 32.5 | 3.01 | 3.32 | 0.63 | 0.38 |
| | 2 | 17.3 | 9.8 | 1.69 | 2.07 | 0.44 | 0.63 |

Each replication consisted of two groups of eight rats, injected 30 min before each 3-min test. The interval between Test 1 and Test 2 was 48 h. Subjects treated with drug on Test 1 were treated with saline on Test 2 and vice versa

**Table 3.** Comparison of the single and double test methods in detecting the effects of atropine on open field behaviour

| Dose mg/kg | Ambulation | | Defaecation | | Preening | |
|---|---|---|---|---|---|---|
| | Single | Double | Single | Double | Single | Double |
| 4.0 | ↑ | ↑ | ↓ | ↓ | ↓ | ↓ |
| 2.0 | ↑ | ↑ | – | ↓ | – | ↓ |
| 1.0 | – | ↑ | – | ↓ | – | ↓ |
| 0.5 | – | ↑ | – | ↓ | – | – |
| 0.2 | – | – | – | ↓ | – | ↓ |
| 0.1 | – | – | – | – | – | ↓ |
| 0.05 | – | – | – | – | – | – |

Drug effects significant at 0.05 are indicated by ↑ (increase) or ↓ (decrease); – indicates no significant change

**Table 4.** Effects of chlorpromazine on open field behaviour

| Dose (mg/kg) | Test | Ambulation | | Rearing | | Preening | |
|---|---|---|---|---|---|---|---|
| | | C | D | C | D | C | D |
| 2.0 | 1 | 48.0 | 17.5 | 8.5 | 4.1 | 1.25 | 0.50 |
| | 2 | 29.1 | 13.0 | 8.4 | 1.5 | 0.50 | 0.25 |
| 0.5 | 1 | 45.6 | 25.6 | 9.8 | 6.8 | 0.75 | 0.63 |
| | 2 | 23.1 | 23.0 | 4.6 | 3.0 | 2.00 | 0.38 |
| 0.2 | 1 | 39.8 | 43.0 | 10.1 | 13.9 | 1.75 | 1.00 |
| | 2 | 27.8 | 15.1 | 6.0 | 1.5 | 1.00 | 1.00 |

Each replication consisted of two groups of eight rats, injected 30 min before each 3-min test. The interval between Test 1 and Test 2 was 48 h. Subjects treated with drug on Test 1 were treated with saline on Test 2 and vice versa

**Table 5.** Comparison of the single and double test methods in detecting the effects of chlorpromazine on open field behaviour

| Dose mg/kg | Ambulation | | Rearing | | Preening | |
|---|---|---|---|---|---|---|
| | Single | Double | Single | Double | Single | Double |
| 2.0 | ↓ | ↓ | ↓ | ↓ | – | ↓ |
| 0.5 | ↓ | ↓ | – | – | – | ↓ |
| 0.2 | – | – | – | – | – | – |

Drug effects significant at 0.05 are indicated by ↑ (increase) or ↓ (decrease); – indicates no significant change

f defaecation, four times with ambulation and 40 times with preening (Table 3).

Chlorpromazine reduced ambulation, rearing and preening at higher doses (Table 4). Reduction of ambulation and rearing were significant by both methods at 0.5 mg/kg and 2.0 mg/kg, respectively. Significant reduction of preening was found by the double test method at 0.5 mg/kg and above, but no significant effect on preening was found at any dose by the single test method (Table 5).

With LSD and a 48-h ITI, ambulation was increased and defaecation and preening decreased (Table 6). Often the effect on ambulation was only present on Test 1. These effects were detected at LEDs of 0.02, 0.05 and 0.02 mg/kg, respectively, by the double test method. With the single test method, the LED's were 0.02, 0.5 and 0.1 mg/kg, respectively. Thus while the double test method was ten times more sensitive in the case of preening and five times in the case of defaecation, there was no difference in the sensitivity of detection of the effects on ambulation. With an ITI of 168 h and a dose of 0.01 mg/kg, however, the double test method detected a significant

**Table 6.** Effects of LSD on open field behaviour

| Dose (mg/kg) | Test | Ambulation | | Defaecation | | Preening | |
|---|---|---|---|---|---|---|---|
| | | C | D | C | D | C | D |
| a) Injected 15 min pretest ITI = 48 h | | | | | | | |
| 0.5 | 1 | 40.0 | 81.4 | 4.38 | 0.00 | 1.13 | 0.00 |
| | 2 | 43.0 | 59.8 | 1.88 | 0.38 | 0.63 | 0.00 |
| 0.1 | 1 | 34.5 | 62.8 | 2.25 | 1.00 | 1.38 | 0.00 |
| | 2 | 29.6 | 22.6 | 1.38 | 0.50 | 0.75 | 0.00 |
| 0.05 | 1 | 31.9 | 52.6 | 4.00 | 1.75 | 0.50 | 0.13 |
| | 2 | 35.8 | 42.1 | 2.88 | 1.13 | 0.38 | 0.00 |
| 0.02 | 1 | 29.8 | 47.1 | 1.63 | 2.25 | 0.88 | 0.38 |
| | 2 | 26.8 | 26.3 | 2.25 | 0.25 | 0.38 | 0.13 |
| 0.01 | 1 | 36.0 | 43.4 | 2.00 | 2.38 | 0.25 | 0.88 |
| | 2 | 27.3 | 34.4 | 2.38 | 2.25 | 0.38 | 0.38 |
| b) Injected 15 min pretest ITI = 168 h | | | | | | | |
| 0.01 | 1 | 43.5 | 50.0 | 3.38 | 2.75 | 1.00 | 1.25 |
| | 2 | 26.6 | 40.9 | 4.00 | 3.88 | 0.38 | 0.13 |

Each replication consisted of two groups of eight rats. Subjects treated with drug on Test 1 were treated with saline on Test 2 and vice versa

**Table 7.** Comparison of the single and double test methods in detecting the effects of LSD on open field behaviour

| Dose (mg/kg) | Ambulation | | Defaecation | | Preening | |
|---|---|---|---|---|---|---|
| | Single | Double | Single | Double | Single | Double |
| a) Injected 15 min pretest ITI 48 h | | | | | | |
| 0.5 | ↑ | ↑ | ↓ | ↓ | ↓ | ↓ |
| 0.1 | ↑ | ↑ | – | ↓ | ↓ | ↓ |
| 0.05 | – | – | – | ↓ | – | ↓ |
| 0.02 | ↑ | ↑ | – | – | – | ↓ |
| 0.01 | – | – | – | – | – | – |
| b) Injected 15 min pretest ITI 168 h | | | | | | |
| 0.01 | – | ↑ | – | – | – | – |

Drug effects significant at 0.05 are indicated by ↑ (increase) or ↓ (decrease); – indicates no significant change

increase in ambulation while the single test did not (Table 7).

## Discussion

The results of Experiment 1 are in general agreement with those quoted in the literature, indicating that the present experiments are typical of those conducted using this type of apparatus. A reduction in ambulation and rearing is nearly always found in tests (Ivinskis, 1970; Ader, 1969). Defaecation has sometimes been found to decrease over a series of tests (Hall, 1934; Evans and Hunt, 1942), but over a short series it is usual to find no difference (Ivinskis, 1970; Ader, 1968). It is also usual to demonstrate no consistent variation in preening over tests (Ivinskis, 1970).

It should be noted that one proposed advantage of the double test method, that of measuring effects from two different baselines, can only operate in the case of ambulation and rearing since the other measures do not vary from Test 1 to Test 2.

Intercorrelations of measures in Experiment 1 agree with past results. Although a negative correlation has occasionally been shown between ambulation and defaecation (Yoshioka, 1932), it is more usual to find these measures uncorrelated (Satinder, 1968; Ivinskis, 1966, 1968).

The series of replications that constituted Experiment 1 allows us to estimate the reliability of changes between Test 1 and Test 2. The effects on ambulation, defaecation and rearing were very consistent. With preening, however, a significant decrease over tests was obtained on only one of the four replications. This may have arisen as a result of a statistical error of type 1.

In Experiment 2 the effects of the drugs on open field behaviour were as previously observed. The most important point about the present findings was that in most cases the double test method was more sensitive in detecting these effects than the widely used single test method, in that drug effects could be demonstrated at doses 4–40 times lower.

There were two exceptions to this general superiority of the double test method. When LSD was used with a 48-h ITI the double test method was no better than the single in detecting an increase of ambulation, although it was considerably better in the case of preening and defaecation. It was observed that control ambulation scores were about the same on Test 1 and Test 2 in experiments with LSD, whereas with other drugs and in Experiment 1, ambulation scores were much lower on Test 2. Since LSD tends to increase ambulation, this suggests that the effects of the drug had not completely worn off when Group DC were run on Test 2. A single replication of the experiment with LSD at 0.01 mg/kg and an ITI of 168 h showed that under these circumstances the superiority of the double test method over the single test method was restored. This shows that it is important to avoid a carry-over of drug effect if the double test method is to be efficient. In addition it provides evidence for behavioural effects of LSD in the rat after 48 h.

The second exception to the superiority of the double test method was in the effects of chlorpromazine on rearing and ambulation. These baselines decrease from Test 1 to Test 2 and chlorpromazine decreases both measures. Since decreases in performance are

often easier to detect from a high baseline, it is not surprising that the effect on Test 1 was greater than that on Test 2. Despite these adverse baseline effects, however, the double test method was still as good as the single.

Previous LEDs for these drugs in the open field are far higher than the LEDs for effects in man. Atropine produces hallucinations in humans at doses of about 1 mg/kg and other undesirable side effects at doses as low as 0.1 mg/kg (Longo, 1966). Chlorpromazine at 1.0 mg/kg produces marked sedation (Delay and Deniker, 1952). LSD at doses as low as 0.001 mg/kg produces a range of impairments described by Klee et al. (1961). The double test method allows us to detect behavioural changes in the open field at doses comparable to these LEDs for effects in man, except in the case of LSD where there remains a considerable difference. Nonetheless, the authors are aware of no more sensitive behavioural test to detect effects of LSD in rodents.

At the present time there is great awareness of the need for early detection of behavioural side effects of new therapeutic drugs (e.g., Secretary's Commission on Pesticides, p. 406). Therefore it is concluded that the double test cross-over design for the open field test should be used whenever sensitivity of detection is important. Thus, in addition to being one of the simplest and most economical of behavioural tests, the open field becomes one of the most sensitive.

## References


Ader, R.: Effects of early experience and differential housing on behaviour and susceptibility to gastric erosions in the rat. J. Comp. Physiol. Psychol. **60**, 233–238 (1965)

Ader, R.: Adrenocortical function and the measurement of "emotionality". Ann. N. Y. Acad. Sci. **159**, 791–805 (1969)

Aldous, F. A. B., Barrass, B. C., Brewster, K., Buxton, D. A., Green, D. M., Pinder, R. M., Rich, P., Skeels, M., Tutt, K. J.: Structure activity relationships in psychotomimetic phenylalkylamines. J. Med. Chem. **17**, 1100–1111 (1974)

Archer, J.: Tests for emotionality in rats and mice: A review. Anim. Behav. **21**, 205–235 (1973)

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

Broadhurst, P. L.: Determinants of emotionality in the rat: 1. Situational factors. Br. J. Psychol. **48**, 1–12 (1957)

Delay, J., Deniker, R.: Le traitement de psychoses par une methode neurolytique dérivé déhybernothérapie. Ann. méd-psychol. **110**, 564 (1952)

Denenberg, V. H.: Open field behaviour in the rat: What does it mean? Ann. N. Y. Acad. Sci. **159**, 852–859 (1969)

Dews, P. B.: Analysis of effects of pharmacological agents in behavioural terms. Fed. Proc. **17**, 1024–1030 (1958)

Evans, J. T., Hunt, J. McV.: The 'emotionality' of rats. Am. J. Psychol. **55**, 528–545 (1942)

Hall, C. S.: Emotional behaviour in the rat. 1: Defaecation and urination as measures of individual differences in emotionality. J. Comp. Psychol. **18**, 385–403 (1934)

Hall, C. S.: Temperament: a survey of animal studies. Psychol. Bull. **38**, 909–943 (1941)

Ivinskis, A.: A note on the open field test of emotionality. Aust. J. Psychol. **18**, 276–280 (1966)

Ivinskis, A.: Reliability of behavioural measures obtained in the open field. Aust. J. Psychol. **20**, 173–177 (1968)

Ivinskis, A.: A study of validity of open field measures. Aust. J. Psychol. **22**, 175–183 (1970)

Klee, G. D., Bertino, J., Weintraub, W., Callaway, E.: The influence of varying dosage on the effects of lysergic acid diethylamide (LSD-25) in humans. J. Nerv. Ment. Dis. **132**, 404–409 (1961)

Longo, V. G.: Behavioural and electroencephalographic effects of atropine and related compounds. Pharmacol. Rev. **18**, 965–996 (1966)

Satinder, K. P.: A note on the correlation between open field and escape avoidance behaviour in the rat. J. Psychol. **69**, 3–6 (1968)

Secretary's Commission on Pesticides and their relationship to environmental health. Report: Washington DC: US GPO (1969)

Singer, J. E.: Sympathetic activation, drugs and fear. J. Comp. Physiol. Psychol. **56**, 612–615 (1963)

Walsh, R. N., Cummins, R. A.: The open field test: A critical review. Psychol. Bull. **83**, 482–502 (1976)

Winer, B. J.: Statistical principles in experimental design. New York: McGraw-Hill 1962, 2nd ed. 1971

Yoshioka, J. G.: Learning versus skill in rats. J. Genet. Psychol. **41**, 406–416 (1932)


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