Repeated Test of Mice in the Photocell Activity Cage After Different Time Intervals

By NATHAN WATZMAN, HERBERT BARRY, III, WILLIAM J. KINNARD, JR., and JOSEPH P. BUCKLEY

A total of 384 mice, given 2-hr. tests twice in the photocell activity cage under the same conditions, showed lower activity scores (with square root transformation) in the second test. This behavioral carry-over effect was greater for subgroups given the second test at an interval of 1 or 3, instead of 7 or 14 days, after the first. Chlorpromazine produced a greater decrement in activity in the first 0.5 hr. of the second test than of the first. In both tests, aggregations of five, rather than single animals, showed a greater drug decrement in the first hour and some recovery from the drug effect in the last 0.5 hr. The most stable data were obtained in the first 0.5 hr. A high correlation between tests, especially with a 1-day interval, indicated that a repeated-test design can provide a sensitive measure of drug effects.

IT IS HIGHLY desirable to give repeated tests to the same animals when evaluating the effects of psychotropic agents on behavior. Not only is it an economic advantage, but a more sensitive test is obtained, because the consistency of individual performances usually found in repeated tests means a small variation in scores against which the effects of drugs can be measured.

The disadvantage of repeated tests on the same animals is that both drug and behavioral carry-over effects may occur from one test to the next, especially when there is a short time interval between sessions. In order to assess this disadvantage, it is necessary to measure the degree of this carry-over effect at different intersession intervals, with the experimental conditions held constant in both sessions. This was the purpose of the study reported in the present paper.

The photocell activity cage, which has been used extensively to study the depressant properties of chlorpromazine (1–3), was used as the test apparatus.

METHOD

Subjects.—The subjects were 384 male, Swiss-Webster mice (Taconic Farms, N. Y.) weighing approximately 18–20 Gm. Housing and testing were in separate rooms, both with the temperature controlled between 73 and 75°F. Food and water were available *ad libitum*.

Apparatus.—The experimental work was performed in four 6-beam photocell activity cages, circular in shape (Actophotometers, Metro Industries, Inc.). The electronic circuits of each cage are designed so that a single digital counter (General Controls, Des Plaines, Ill.) is activated whenever an animal blocks, or moves through any beam, regardless of whether any of the other beams are interrupted. Therefore, an animal continuously blocking a single beam could not preclude the registration of counts emanating from the other beams. The experimental room was sound attenuated, and was effectively shielded from the noise of the counters which were placed in an adjoining room.

Experimental Design.—Each animal, or group of animals, was tested in 2 sessions, both 2 hr. in duration. Several experimental conditions were varied in a complete factorial design, so that all levels of each variable were equally represented as follows.

Intersession Interval.—1, 3, 7, and 14 days.

Drug Condition.—Chlorpromazine (4 mg./Kg.) or placebo (saline, 0.1 ml./10 Gm. body weight), given orally 30 min. before the start of the session. Each animal was assigned to the same condition (drug or placebo) in both tests.

Test Aggregation.—Singly, or in groups of five mice. They were housed during the intersession interval under the same aggregation conditions as during testing.

Different Units.—Four activity cages operated simultaneously.

Replication.—The complete design was repeated once.

This experimental design resulted in 128 different combinations of conditions, divided equally between single animals and groups of five.

Statistical Treatment of Data.—Normalization of skewed frequency distributions by means of data transformation is an acceptable statistical technique in research. Extremely high raw scores are likely to lead to a misinterpretation of relative magnitudes, if they are farther from the median values than the lowest scores. Logarithmic, or square root, transformation of data may provide a nonskewed, normal distribution of scores by reducing the magnitude of the high scores more than the low ones. The parametric tests of statistical significance, including the analysis of variance, depend on the assumption that the frequency distribution of scores is nonskewed.

A comparison of the raw, square root, and logarithmic forms of these experimental data indicated that the raw scores were skewed in a positive direction, with the extreme high scores being much farther from the median than were the extreme low scores, whereas the logarithmic scores were skewed in the opposite, negative direction. The square root scores were skewed to the least degree (positive direction), especially in the first hour of the 2-hr. session. Also, activity appeared to be the most consistent and correlations between the two sessions highest for the scores in square root form. Irwin (4) has used square root transformation of data on locomotor activity in the treadwheel.

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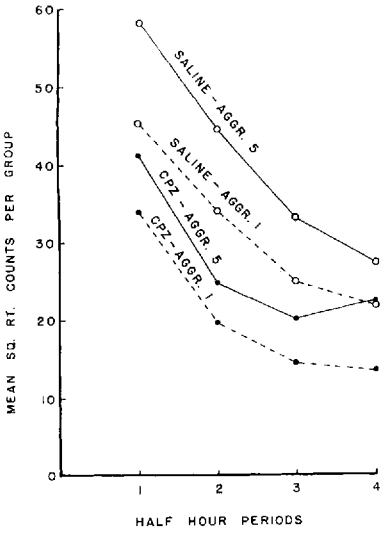

Fig. 1.—The effects of chlorpromazine and aggregation on the spontaneous activity of mice.

In the present study, the number of counts for each 0.5 hr., hour, and the 2-hr. total in both sessions were punched on IBM cards, converted to square root scores by the IBM 7090 computer, and tested for statistical significance by the BMD 02V analysis of variance program on the same computer. Each fixed factor (drug, aggregation, intersession interval) was tested for statistical significance in relation to the pooled interactions, which included the interaction of the randomly selected variables (test units with replication) and either or both variables with one or more of the fixed variables (5). Since each animal was tested in both sessions, the pooled interaction term for testing the difference between the sessions, and interactions of any other variables with sessions, included the session variable. Correlations between the first and second session for all the animals, and for each level of the selected variables, were computed by the BMD 03D program. Stability of performance within the same session was computed according to the split-half method (6), for 0.5-hr. intervals, utilizing a specialized program written for the IBM 7090 computer. This method correlates the performance of an animal, or aggregation of animals, in the even-time segments (second and fourth 0.5-hr. periods) with the scores recorded in the odd-time segments (first and third 0.5-hr. periods). The test of statistical significance for the difference between correlation coefficients was that described by Edwards (7).

RESULTS

Figure 1 shows the effects of two variables (drug and aggregation) on activity in each 0.5-hr. period, with scores for the other experimental variables averaged together. In the first 0.5 hr., there was a highly significant difference between the chlorpromazine and saline conditions ($F = 174$, $df = 1,117$, $p < 0.01$) and between animals tested singly and in groups of five ($F = 90$, $df = 1,117$, $p < 0.01$). There was also a significant interaction between drug and aggregation, indicating that chlorpromazine had a greater effect on the activity of aggregated than single mice for the first 0.5 hr. ($F = 5.76$, $df = 1,117$, $p < 0.05$). This interaction was also significant for the second 0.5 hr. ($F = 5.31$, $df = 1,117$, $p < 0.05$), but not for the last two 0.5-hr. periods. These findings confirm results reported previously by Watzman *et al.* (2), utilizing raw scores.

In the last 0.5 hr. of the session, the animals tested in groups of five showed some recovery from the depressant effects of the drug. Out of 32 groups of aggregated animals under chlorpromazine, 25 showed higher activity in the fourth than in the third 0.5 hr., and only seven groups were lower. This recovery from the depressant effects of the drug was statistically significant ($\chi^2 = 9.0$, $df = 1$, $p < 0.01$) and is in contrast with the lower activity in the fourth than third 0.5-hr. period found with each of the other three conditions shown in Fig. 1. In general, all of these experimental effects decreased in statistical significance in the later 0.5-hr. periods, due to a decrease in stability of the scores. However, the difference between animals tested singly and in groups of five continued to be highly significant, even in the last 0.5-hr. period ($F = 25$, $df = 1,117$, $p < 0.01$).

The replication showed no consistently significant difference from the original experiment. There was a significant difference among the four test units ($F = 9$, $df = 3,117$, $p < 0.01$) for the entire 2 hr., but no consistently significant interaction between this variable and any of the others.

The main subject of interest in the present experiment is the comparison between the first and second test session, shown in Fig. 2. The activity counts were preponderantly lower in the second session ($F = 48$, $df = 1,112$, $p < 0.01$ for the total 2 hr.). This decrement in second-session performance was greater for the groups tested at shorter time intervals (1 and 3 days). This difference, generally, persisted throughout the second session, but was statistically significant for the first 0.5 hr. only, measured by the interaction of sessions with the four intervals ($F = 3.06$, $df = 3,112$, $p < 0.05$). When the test for linearity of the scores is applied, with the assumption that the 1, 3, 7, and 14-day intervals represent a progressive function, the interaction between tests and intervals is statistically significant

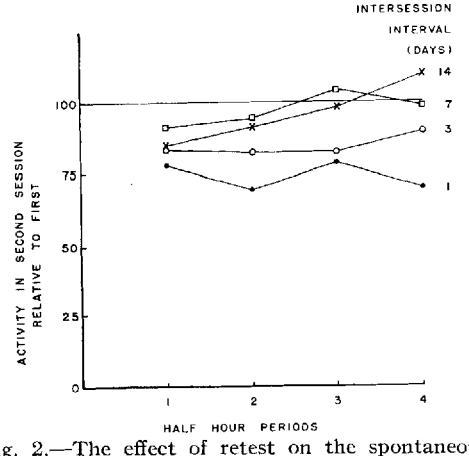

Fig. 2.—The effect of retest on the spontaneous activity of mice at 1, 3, 7, and 14-day intervals.

($p < 0.05$) in each of the 0.5-hr. periods, except the third ($F = 4.6$, 6.0, 3.7, and 6.5, respectively, $df = 1,112$).

In the first 0.5 hr., chlorpromazine produced a 23% decrement in activity in the first session and a 32% decrement in the second session. This difference was statistically significant, as indicated by the interaction between sessions and drug conditions ($F = 4.68$, $df = 1,112$, $p < 0.05$). This difference was approximately equal for the groups given the two tests at different intervals ($F < 1$ for the three-way interaction among drug conditions, sessions, and intervals). In the last three 0.5-hr. periods, the drug-induced decrement in activity was approximately equal in the two sessions, as indicated by the absence of any significant interaction between sessions and drug conditions. The other variables did not interact significantly with the two sessions, and therefore showed similar patterns in both tests.

There was a high degree of stability of performance within each session, with the split-half correlation being 0.75 for session *1* and 0.74 for session *2*. The over-all correlation between the two sessions, pooling all of the other parameters together, was 0.74 for the total 2 hr. As would be expected, the correlation between sessions was greater for the shorter intersession intervals, with the stability scores for the total 2 hr. being 0.84, 0.78, 0.80, and 0.61, respectively, for the groups given the second session 1, 3, 7, and 14 days after the first. The correlation between sessions was highest in the first 0.5 hr. of the session (0.73) and lowest in the fourth 0.5 hr. (0.35), and this difference was statistically significant at the 1% level. For the total 2 hr., this stability score showed little difference between saline (0.62) and drug treatment (0.57), but in the first 0.5 hr., the saline scores were significantly more stable, with correlations between the sessions of 0.68 for saline and 0.46 for chlorpromazine ($p < 0.05$). In the first 0.5 hr., the correlation between sessions was higher for the animals tested in groups of five (0.74) than those tested singly (0.60), but this difference was not statistically significant.

DISCUSSION

The present experiment clearly shows a behavioral carry-over effect from one session to the next for both saline and chlorpromazine animals. After an interval of 1 or 3 days between tests, activity in the second session was substantially lower than in the first, in all four 0.5-hr. periods. Even at the 7 and 14-day intervals, the recovery of the original activity level was not complete, as shown by significantly lower activity in the first 0.5 hr. for these two groups pooled together ($F = 20$, $df = 156$, $p < 0.01$.)

The dosage of chlorpromazine used (4 mg./Kg., orally) greatly decreased the activity of mice under all the experimental conditions tested. However, the magnitude of this drug effect was somewhat altered by certain experimental conditions. The greater decrement produced by chlorpromazine in the first two 0.5-hr. periods, for animals tested in groups of five rather than singly, confirms findings previously reported (2). Since the present experiment used square root transformations which produced normally distributed scores, this interaction between drug condition and aggregation appears to be a general phenomenon, not depending on the skewed distribution of the raw scores used in the prior study. The present experiment also showed that the aggregated condition, which produced a greater decrement under the drug in the first hour, produced an earlier recovery from the drug effect, indicated by the statistically significant increase in activity from the third to the fourth 0.5 hr. The stimulating aggregation situation, in which activity was decreased to a greater degree by chlorpromazine, apparently caused a more rapid recovery from the depressant effects of the drug.

The present experiment also demonstrated a greater depressant effect of chlorpromazine on activity in the first 0.5 hr. of the second session than of the first session. Adler (8), also utilizing the photocell activity cage, found a greater depressant effect of tetrabenazine on the motor activity of rats in the second of two tests with a 1-week intertrial period. Rushton *et al.* (9) found different magnitudes of drug effect on exploratory activity of rats, depending on whether the drug test was on their second rather than first exposure to the test situation, and also depending on whether the drug test was preceded by a drug or placebo test in the same situation. The stimulating effect of an amphetamine–barbiturate mixture was apparently greater in the second test, when the same drug was given in both tests. In the present experiment, and in Adler's study (8), the animals were given the same drug, or placebo, condition in both tests, so that it is not possible to determine whether the greater depressant drug effect in the second test was due solely to a greater familiarity with the test situation, or to the prior administration of the drug associated with this test situation. However, this effect was clearly not due to accumulation of the drug from the first session, because the greater drug effect was found in the second session after the 14-day interval, as well as after the 1-day interval.

The behavioral and drug carry-over effects, from the first to the second session, indicate that the experimenter should be careful when using the same animals more than once in tests of drug effects. It is obviously necessary to counterbalance the sequence, giving placebo first to half the animals and the drug first to the other half. On the other hand, the high over-all correlation in activity between the first and second sessions, especially in the early part of the sessions, shows that the use of repeated tests on the same animal can increase the sensitivity of the test of drugs, or other conditions which are experimentally varied in the different test sessions. With the factorial design as used in the present experiment, the advantages of repeated testing can be fully obtained, even when there is a decrement in performance in the second session and a different magnitude of drug effect in the two sessions. It should be emphasized that, in the present experiment, these differential effects in the two sessions were fairly small, relative to the magnitude of the over-all drug effects and to the effects of other conditions, such as aggregation and 0.5-hr. intervals. The same general pattern of depressant drug effects was found in both sessions. Therefore, the effects of the drug could be tested adequately with a greatly reduced number of animals by the use of the repeated test design, giving chlorpromazine in one session and placebo in the other, at least for this dosage of this compound and under these conditions.

The most stable data were obtained in the first

0.5 hr. of the test, and with the shortest interval between sessions. These conclusions are based on the higher correlations between the two tests for the first 0.5 hr. and for the 1-day interval. The disadvantage of the greater decrement in activity after the shortest intersession interval is offset by the generally higher correlations between the first and second sessions found for the 1-day group, as well as by the practical advantage of using a shorter interval. These findings may be of use to experimenters in selecting the optimum conditions for testing the effect of chlorpromazine on the activity of mice in the photocell cage.

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Preliminary Investigations of *Heracleum mantegazzianum*

By EUGENE C. LEE, PHILIP CATALFOMO, and LEO A. SCIUCHETTI

Preliminary investigations of the air-dried roots of *Heracleum mantegazzianum* grown under greenhouse conditions were conducted. Seed germination requires moist cold treatment. The germination rate appears to be directly related to the length of the moist cold period. Gibberellin seed treatment did not substitute for the cold requirement. Thin-layer chromatography revealed the presence of 6 coumarins which were tentatively identified as bergapten, isobergapten, pimpinellen, isopimpinellen, sphondin, and umbelliferone. Results also indicate that the plant can cause photosensitization.

EXCELLENT reviews concerning the distribution, chemistry, or pharmacological properties of the naturally occurring coumarins are available (1–5); included are phytochemical studies revealing the presence of coumarins in a number of *Heracleum* species. A notable aspect is the involvement of furocoumarins in certain cases of phytophotodermatitis; several of them occur in *Heracleum* species. The distribution of photodynamically active furocoumarins were recently reviewed by Pathak *et al.* (6). Although phytochemical investigations of the coumarins of *Heracleum* species has been extensive, the species *Heracleum mantegazzianum* Somm. et Lev. is a noteworthy exception. However, during the course of this investigation, a report by Beyrich (7) revealed the presence of phellopterin and other coumarins in this species, but the results are not entirely consistent with those reported in this investigation. Since *H. mantegazzianum* has been reported to evoke phytodermatitis, a preliminary investigation was undertaken to determine the presence of photosensitizing coumarins and related compounds.

To obtain sufficient plant material for this and subsequent studies the plant was propagated under greenhouse conditions. Seeds of the *Umbelliferae* have been noted for germination difficulties, and germination standards for cultivated members of this order have been set much lower than those of other plants (8). The seeds of some *Heracleum* species have a requirement for after ripening in moist cold (9). Attempts have also been made to obviate the cold requirement in the dormancy of certain seeds by chemical means, especially with the gibberellins (10). Since no report could be found in the literature concerning the cold requirement of the effects of gibberellic acid on seed germination of *H. mantegazzianum*, preliminary germination studies were also conducted.

EXPERIMENTAL AND RESULTS

Germination Studies.—Preliminary studies were designed to compare the effect of cold treatment *versus* treatment with gibberellic acid on the germination rate of the seeds. Three groups of seeds (39 per group) were planted in flats containing a mixture of 1 part sand and 2 parts sandy loam plus 50 Gm. of complete fertilizer.[1] Group *A* was pretreated by storage at 2–5° for 74 days; group *B* was soaked for 20 hr. in a solution of gibberellic acid (100 p.p.m.); and group *C*, soaked in distilled water, was considered the control group. The flats were maintained under normal greenhouse conditions and germination was allowed to occur at a temperature range of 18–27° for 38 days. Ger-

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[1] Organic Morcrop, Chas. Lilly Co., Seattle, Wash. (Analysis: 5% total nitrogen, 3% available phosphate, 2% available potash.)