Effects of Centrally Acting Drugs on Confinement Motor Activity

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A test procedure is described for use in quantitating the effects of drugs which stimulate or depress central nervous system function. Motor activity of rats is measured by photoelectric cells while the rats are confined in a small plastic chamber. The chamber is designed to permit the rats to exhibit "up and down" exploratory movements but not movement from place to place, *i.e.*, locomotor activity. Drugs tested include caffeine, *d*-amphetamine, phenmetrazine, methylphenidate, pipradrol, tranylcypromine, chlorpromazine, prochlorperazine, trifluoperazine, and chlordiazepoxide. Advantages of the test include a high degree of reproducibility, selectivity, and sensitivity. Two of the drugs mentioned, namely caffeine and tranylcypromine, were especially potent by this test, in spite of the fact that they are essentially inactive by the conventional photoelectric cell locomotor activity test.

ONE OF THE more useful test procedures available for quantitating the effects of drugs on animal behavior involves measurement of alterations in motor activity. In general, the value of a particular motor activity measuring device resides in its sensitivity, selectivity, and reproducibility. Devices which utilize the photoelectric cell counter, first introduced for this purpose by Winter and Flataker (1), have proven to be both selective and reproducible for measuring changes in *locomotor* activity, *i.e.*, movement of animals from place to place. Such devices have, however, proven to be of little value in quantitating the effects of known central nervous system stimulants such as caffeine or tranylcypromine, which cause only minor overt changes in locomotor activity. To quantitate the stimulant effects of these compounds it was necessary to exaggerate their influence on motor activity. This was accomplished by restricting the locomotor activity of rats through confinement in a photoelectric cell activity chamber small enough to permit "up and down" movements of the animals, but not movements from place to place. This report is concerned with the effects of a variety of drugs on such "confinement motor activity" and describes the advantages of this approach for the evaluation of drugs influencing animal behavior.

METHODS

All experiments were performed on adult male albino rats (150–250 Gm.) of the Sprague-Dawley strain. All of the drugs tested were administered by the oral route and were tested at their time of peak activity as determined by the confinement motor activity (CMA) test. Drugs tested included chlorpromazine (Thorazine[1]), prochlorperazine (Compazine[1]), trifluoperazine (Stelazine[1]), chlordiazepoxide (Librium[2]), caffeine, *d*-amphetamine (Dexedrine[1]), phenmetrazine (Preludin[3]), methylphenidate (Ritalin[4]), pipradrol (Meratran[5]), and tranylcypromine (Parnate[1]). Motor activity was measured as follows.

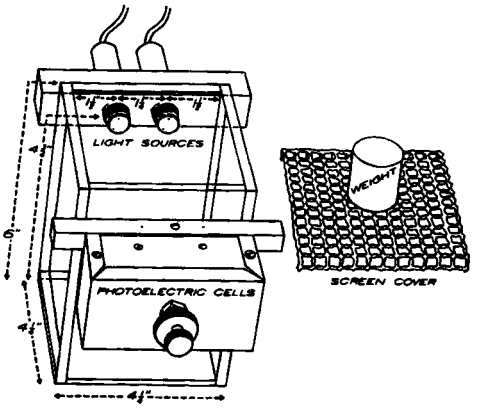

Fig. 1.—Schematic diagram of an individual photoelectric cell counting chamber.

APPARATUS

The device employed for the measurement of changes in motor activity is illustrated in Figs. 1–3. It consists of banks of five plastic chambers. The dimensions of each chamber are 4.25 × 4.25 × 6 in.[6] They are constructed of 0.25-in. thick clear plastic. Each plastic chamber is enclosed in a separate rack. Attached to this rack are two photoelectric cells and two light sources for each chamber. The center of each light source is 4.75 in. from the base of the chamber and 1.5 in. in from each side of the chamber. The centers of the light sources are thus approximately 1.25 in. apart in the same plane. Each pair of photoelectric cells

Received January 6, 1964, from the Research and Development Division, Smith Kline & French Laboratories, Philadelphia, Pa.
Accepted for publication January 22, 1964.
[1] Trademark of Smith Kline and French Laboratories.
[2] Trademark of Roche Laboratories Division, Hoffmann-LaRoche, Inc.
[3] Trademark of Geigy Company, Inc.
[4] Trademark of Ciba Pharmaceutical Products, Inc.
[5] Trademark of Wm. S. Merrell Company.
[6] In a preliminary series of experiments the height of the light beams and size of the plastic chambers were varied. On the basis of the results from these experiments the parameters described above were selected as being optimal in terms of maximum sensitivity and reproducibility.

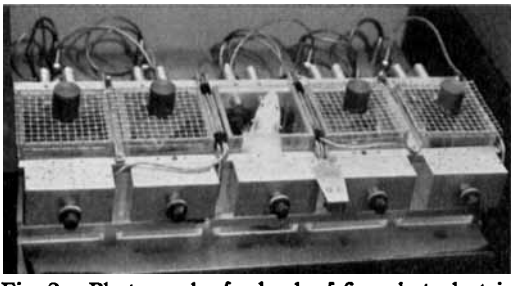

Fig. 2.—Photograph of a bank of five photoelectric cell counting chambers.

is connected to a single counter so that interruption of one or both light beams results in a single count. The top of each chamber is closed with a wire screen. A weight is attached to the screen to prevent the rat from raising it. A tray covered with paper is fitted under each bank of five chambers. The paper is changed after each occupancy to maintain an environment as constant as possible. Two hinged lids, one covering the front and one the back of each bank of five chambers, are closed to provide a minimum of light input from the room and to cut down distraction for the animals placed in the individual chambers.

Procedure for Testing Compounds Effecting a Reduction in Motor Activity

A group of 70 rats is randomly divided into seven groups. Five groups containing 10 rats each are treated with various doses of the drug under investigation. The sixth and seventh groups containing 10 rats each are pooled to make a group of 20. These are treated with equivalent volumes of the vehicle employed, water or tragacanth depending on solubility of the drug, and serve as controls. At the time of peak drug effect, determined by this same procedure, the rats are placed in the activity counting chambers for a period of 15 minutes. This test interval was selected on the basis of a series of experiments on chlorpromazine which is described under the *Results* section. The depressant dose-50 (DD_{50}) is defined as the dose of drug effective in reducing the average 15-minute counts of the treated rats to 50% of the average 15-minute counts of the control rats tested concomitantly.

Procedure for Compounds Producing an Increase in Motor Activity

The procedure is similar to that described for motor activity depressants, except that the rats are placed in the chambers 15 minutes before the time of peak drug effect and are kept in the chambers for a period of 25 minutes rather than 15 minutes. This test interval is based on a series of experiments performed on *d*-amphetamine which is described under *Results*. Only counts taken during the last 10 minutes of this 25-minute period are evaluated. The stimulant dose-200 (SD_{200}) is defined as the dose of drug which causes a 200% increase in the average 10-minute counts of treated rats, over and above the average 10-minute count of control rats tested concomitantly.

Procedure for Statistical Analysis of Data

Graphs were made for each of several drugs by plotting the mean count of activity at each dose against log dose. The resulting log dose-response curves were consistently concave upward. Furthermore, it was noticed that there was a definite tendency for the variability of the activity counts to increase as the mean activity count increased. These two observations suggested that a transformation of the activity counts would be necessary for proper analysis of the data. A logarithmic transformation provided dose-response curves of satisfactory linearity and uniformity of variability among different groups.

For each drug a straight line was fitted to the mean log activity counts *versus* log dose by the usual least-squares procedure. The parameters of this straight line and the mean log activity count of the control group were used to estimate the dose of the drug which would produce the desired change in activity from control counts by means of the following formulas:

$$x_0 = \log SD_{200} = \frac{\bar{y}_c + 0.47712 - a}{b}$$

or

$$x_0 = \log DD_{50} = \frac{\bar{y}_c - 0.30103 - a}{b}$$

In these formulas $\bar{y}_c$ is the mean log activity count of the control group, a is the intercept, and b is the slope of the fitted straight line; 0.47712 is log 3 and 0.30103 is log 2. The form of this estimate made possible the use of Fieller's theorem (2) to calculate fiducial limits for the log SD_{200} or log DD_{50} and thus for the SD_{200} or DD_{50} itself. Application of this theorem resulted in the following formula for the fiducial limits:

$$x_0 + \frac{(x_0 - \bar{x})g}{1 - g} \pm \frac{ts}{b(1 - g)} \left[(1 - g)\left(\frac{1}{n_c} + \frac{1}{\sum_{i=1}^{k} n_i} \right) + \frac{(x_0 - \bar{x})^2}{SS_x} \right]^{1/2}$$

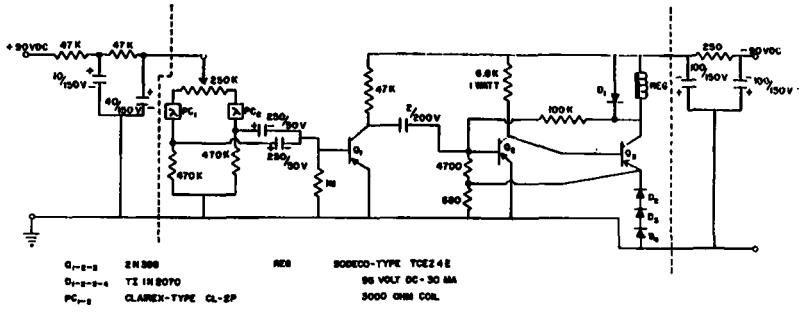

Fig. 3.—Wiring diagram for photoelectric cell and counter circuitry.

In this formula x refers to log dose; it is assumed that k doses were tested with n_i animals receiving the ith dose and n_c animals in the control group; $\bar{x}$ is thus the mean log dose tested. The symbol s stands for the pooled within-group standard deviation of the log activity counts. The symbol t stands for the value of Student's t with degrees of freedom appropriate to s and at the chosen significance level (for our purposes this was the 5% level). SS_x is customarily known as the corrected sum of squares for log dose and is computed as

$$SS_x = \sum_{i=1}^{k} n_i x_i^2 - \left[\left(\sum_{i=1}^{k} n_i x_i\right)^2 \Big/ \sum_{i=1}^{k} n_i\right]$$

where x_i is the logarithm of the ith dose. The term g is computed as

$$g = t^2 s^2 / b^2 SS_x$$

It provides an indication of the significance of the slope of the dose-response curve, and all of its terms have been defined above. In accordance with common practice, g was ignored if its value was 0.05 or less, and this resulted in the following simpler formula for the fiducial limits:

$$x_0 \pm \frac{ts}{b}\left[\frac{1}{n_c} + \frac{1}{\sum_{i=1}^{k} n_i} + \frac{(x_0 - \bar{x})^2}{SS_x}\right]^{1/2}$$

Although either of the fiducial limit formulas can be simplified somewhat in particular situations, the form in which they are presented here applies to any spacing of the doses and any inequality in number of animals per dose.

RESULTS

Determination of Optimum Periods of Both Acclimation in the Chamber and Actual Testing in the Chamber

Two series of trial experiments, each consisting of seven different tests, were performed on *d*-amphet-

TABLE I.—INFLUENCE OF VARYING ACCLIMATION PERIOD AND TEST PERIOD ON SD_{200} OF *d*-AMPHETAMINE

Acclimation Period, Min.	Test Period, Min.	SD_{200} (95% Fiducial Limits) mg./Kg. Oral	Ratio: Upper Confidence Limit / Lower Confidence Limit
None	0–15	12.6 (2.5 to 2600)	1,032.0
		2.7 (0.9 to 17.5)	19.2
		4.1 (1.1 to 87.3)	80.8
		7.1 (1.5 to 517.8)	340.6
		5.2 (0.9 to 4899)	5,512.0
		1.7 (0.6 to 10.3)	17.4
		7.7 (0.2 to 302.5)	1,681.0
None	0–25	2.2 (0.8 to 11.0)	13.7
		1.0 (0.4 to 2.9)	6.2
		1.6 (0.6 to 5.6)	8.7
		2.7 (0.9 to 21.7)	25.3
		2.0 (0.5 to 32.5)	62.5
		0.8 (0.3 to 2.8)	8.0
		2.4 (0.9 to 11.9)	13.4
5	6–15	0.7 (0.2 to 5.2)	31.7
		0.7 (0.2 to 2.5)	11.2
		1.1 (0.4 to 4.5)	11.3
		1.5 (0.6 to 6.5)	11.1
		0.9 (0.3 to 5.2)	19.3
		0.8 (0.3 to 3.3)	12.2
		1.4 (0.5 to 9.3)	21.7
15	16–25	0.17 (0.04 to 0.46)	10.7
		0.24 (0.11 to 0.47)	4.1
		0.46 (0.23 to 0.93)	4.0
		0.44 (0.22 to 0.93)	4.3
		0.39 (0.05 to 0.88)	16.3
		0.19 (0.06 to 0.47)	4.4
		0.36 (0.15 to 0.80)	8.4
None	Ratio of 16–25 min. count / 0–15 min. count	0.063 (0.00003 to 0.3932)	12,528.8
		0.128 (0.0268 to 0.3519)	13.1
		0.640 (0.274 to 1.662)	6.1
		0.360 (0.152 to 0.810)	5.3
		0.249 (0.0005 to 4.3798)	9,418.1
		0.1687 (0.0010 to 1.2768)	1,244.3
		0.2476 (0.0528 to 0.8083)	15.3
None	Ratio of 16–25 min. count / 0–5 min. count	0.0571 (0.0003 to 0.2950)	1,011.0
		0.1238 (0.0308 to 0.3142)	10.2
		0.6044 (0.2988 to 1.2956)	4.3
		0.3211 (0.1377 to 0.6949)	5.0
		0.2061 (0.0030 to 1.6679)	552.6
		0.1387 (0.0034 to 0.7203)	209.0
		0.2291 (0.0635 to 0.6228)	9.8

TABLE II.—INFLUENCE OF VARYING ACCLIMATION PERIOD AND TEST PERIOD ON DD_{50} FOR CHLORPROMAZINE

Acclimation Period, Min.	Test Period, Min.	DD_{50} (95% Fiducial Limits) mg./Kg. Oral	Ratio: Upper Confidence Limit / Lower Confidence Limit
None	0–15	3.8 (1.9 to 7.2)	3.8
		5.0 (2.2 to 11.4)	5.1
		4.8 (2.5 to 9.0)	3.6
		6.4 (3.0 to 14.4)	4.8
		3.8 (1.7 to 7.8)	4.5
		6.2 (3.0 to 13.1)	4.3
		3.0 (1.4 to 6.0)	4.2
5	6–15	4.5 (1.5 to 13.5)	9.0
		5.0 (1.1 to 21.8)	19.0
		3.8 (1.2 to 6.9)	3.5
		6.1 (2.7 to 14.4)	5.3
		2.6 (0.8 to 7.0)	9.3
		6.4 (2.4 to 18.3)	7.7
		2.0 (0.8 to 4.6)	5.9

amine and chlorpromazine, respectively. Rats treated orally with various doses of *d*-amphetamine were placed in the chambers 15 minutes after drug administration; counts were then taken at 5-minute intervals for a total of 25 minutes. Rats treated orally with various doses of chlorpromazine were placed in the chambers 270 minutes after drug administration. Counts were also recorded for these animals at 5-minute intervals for a total of 25 minutes. In both instances groups of 20 control rats were tested concomitantly.

Mean counts, averaged for the seven tests, were determined for each dose of drug tested for each interval analyzed. In addition, the ratios of mean counts for two different intervals, again averaged for the seven tests, were also determined. The mean counts for a specific interval or the ratios of mean counts for two different intervals were then plotted against log dose and evaluated graphically. On the basis of this preliminary analysis several intervals or ratios of intervals which demonstrated a reasonable dose-response relationship were selected for a detailed statistical analysis. The results of this analysis are presented in Tables I and II. It is apparent from the data presented that maximum potency with minimum variability occurred in the tests on *d*-amphetamine when the rats were acclimated for a period of 15 minutes and tested 10 minutes thereafter. In the case of chlorpromazine, the optimum test period was 15 minutes with no period of acclimation.

A number of stimulants and depressants were evaluated by these procedures; the results of these experiments are presented in Tables III and IV, respectively.

DISCUSSION

The principle involved in measuring the motor activity of rats in a restricted area of movement has several inherent advantages. Rats placed in such an environment demonstrate exaggerated attitudes of behavior involving increased exploration during the beginning of their confinement and markedly reduced exploration or complete inactivity toward the end of their confinement. Thus, drugs which reduce motor activity may be tested against a background of increased activity and, conversely, drugs which increase motor activity may be tested against a background of markedly reduced activity. These alterations in baseline activity are more or less "physiological" in nature in contrast to increases or decreases in baseline activity which are brought about by drug treatment, nociceptive stimulation, etc. Another, perhaps more practical, advantage of this test method is the compactness and relatively small cost of the equipment involved. Ten CMA testing chambers cost about the same as two conventional photoelectric cell counting chambers; the latter are 13 in. in diameter and contain six light sources and six photoelectric cells. Similarly, 10 CMA chambers occupy the same amount of space as two conventional counting chambers.

The stimulant effects reported for caffeine and tranylcypromine testify to the enhanced sensitivity of the CMA test procedure. Caffeine was tested previously in doses of 5 to 350 mg./Kg. by the conventional photoelectric cell method (Tedeschi, unpublished observation) and was ineffective in producing an increase in locomotor activity. Indeed, a graded decrease in activity was observed in doses of 100 to 400 mg./Kg. This is in sharp contrast to the 200% increase caused by caffeine in the CMA test at a dose of 5 mg./Kg. Similarly Green and co-workers (3) reported that daily administration of tranylcypromine to rats in a dose of 5 mg./Kg. twice a day failed to produce a consistent or significant effect on motor activity. These workers employed the conventional photoelectric cell counting chamber described above. As shown in Table III, an oral dose of 5 mg./Kg. of tranylcypromine in the CMA test was effective in producing a 200% increase in activity.

d-Amphetamine was especially potent in the CMA test. Its SD_{200} of 0.24 mg./Kg. makes it at least 10 times as potent as any of the other stimulants tested. It is of interest that in the conventional motor activity test, an oral dose of 5 mg./Kg. of *d*-amphetamine induces only a 40% increase in activity (Tedeschi, unpublished observations).

TABLE III.—STIMULANT DOSE-200 FOR VARIOUS CENTRAL NERVOUS SYSTEM STIMULANTS

Compd.	Pretreatment Time,[a] Min.	SD_{200} (95% Fiducial Limits) mg./Kg. Oral
Caffeine	75	5.1 (1.5 to 15.8)
d-Amphetamine	15	0.24 (0.11 to 0.47)
Phenmetrazine	15	4.2 (1.7 to 9.7)
Methylphenidate	15	5.1 (2.5 to 9.7)
Pipradrol	15	3.0 (1.0 to 8.8)
Tranylcypromine	525	5.0 (3.1 to 8.2)

[a] Refers to the time after drug administration that the rats were placed in the counting chambers. Counts were recorded during a 10-minute interval following a 15-minute acclimation period in the chamber.

TABLE IV.—QUANTITATION OF THE EFFECTS ON CONFINEMENT MOTOR ACTIVITY OF VARIOUS CENTRAL NERVOUS SYSTEM DEPRESSANTS IN RATS

Compd.	Pretreatment Time, Min.	DD_{50} (95% Fiducial Limits) mg./Kg. Oral
Chlorpromazine	270	6.8 (4.5 to 10.4)
Prochlorperazine	270	4.3 (2.3 to 8.7)
Trifluoperazine	300	1.2 (0.6 to 3.5)
Chlordiazepoxide	60	13.2 (6.6 to 25.4)

The CMA test is also effective for quantitating the effects of drugs which reduce motor activity. The sensitivity of this test for the three phenothiazines evaluated is not, however, significantly greater than that of the conventional photoelectric cell method. For example, it was previously reported (4) that trifluoperazine, prochlorperazine, and chlorpromazine had oral DD_{50}'s of 1.5 (0.9–5.7), 5.3 (3.5–8.8), and 8.5 (5.2–16.5) mg./Kg. These doses were expressed in terms of the bases of these compounds and should, of course, be compared with their DD_{50}'s in terms of the base by the CMA test. Conversion of DD_{50}'s in Table IV to bases resulted in the following for the above-mentioned compounds, respectively, 1.0 (0.5–3.0), 3.6 (1.9–7.3), and 6.1 (4.0–9.3) mg./Kg. Although the DD_{50}'s by the CMA test are consistently less than the DD_{50}'s by the conventional procedure, there is no significant difference in potency for any one drug between the two tests.

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Extrapolation of Appearance of Tablets and Powders from Accelerated Storage Tests

By J. THURO CARSTENSEN, J. B. JOHNSON, W. VALENTINE*, and J. J. VANCE

A descriptive system for tablet and powder appearance utilizing tristimulus reflectances allows formal treatment of appearances. Cases are cited where responses to accelerating conditions can be treated by Arrhenius treatment.

WE HAVE, for a period of years, attempted to find a standard means of describing the appearance of a tablet in a numerical fashion, and to find "average" storage times at 55, 45, and 37° which would correspond to 2 years at 25°. Fully realizing that this will vary from compound to compound, it is nevertheless important to have a standard storage period if a large number of compounds have to be screened for compatibility with common tablet ingredients.

Several investigators (1–4) have found dyed liquid or tablet decompositions to be first order in part or *in toto*, in response to light and heat stress.

The question of whether a white tablet or powder mixture would adhere to such a scheme has been treated below by (*a*) visual observation and use of a suitable scoring system and by (*b*) reflectance measurements.

EXPERIMENTAL

As part of a routine compatibility program, the compatibility of new investigational compounds with various excipients or lubricants is tested in the following manner. The drug is mixed and ground with the excipient in question, and half of the powder mixture is transferred to glass vials which are plugged and sealed. To the other half is added 5% water, careful mixing is performed in a mortar, and the moist mix is transferred to vials as above. The plugged aluminum sealed vials are wax sealed to insure an adequate moisture barrier. As shown in Table II, 11 such two-component systems are prepared. In addition, the drug is set up *per se*. The ratio of drug to excipient is 1:5 by weight, or, in the case of lubricants, 20:1. The samples are stored at 55, 37, 25, and 5° and observed at various time intervals. The accelerated samples are stored at 5° after 10 days at 55° and 2 months at 37° to enable retrieval of the sample at later times for further comparison.

The following scoring system is used:

Degree of Color.—*1*, Unchanged; *2*, hardly noticeable darker; *3*, very slightly darker; *4*, slightly darker; *5*, darker; *6*, much darker (color change). When change toward lighter color occurs, a similar scoring system is used.

Degree of Fineness.—*1*, Unaltered; *2*, very slight particle size increase, does not adhere to glass, flow characteristics slightly altered from control; *3*, slight increase in particle size, movement not quite free flowing, and/or tendency for particles to adhere to glass; *4*, definitely increased in particle size, but still free flowing (like a granulation); *5*, caked—does not move or moves as an entity and discrete particles detectable; *6*, fluidized and solidified—no discrete particles detectable.

In the case of the photometric reflectance measurements a Color Coder[1] was used. Flat-faced bevelled tablets of two different drugs (*a*) one containing 60 mg. of active drug per 170-mg. tablet weight ($^9/_{16}$ in.) and (*b*) one containing 1 mg. of active drug per 100 mg. tablet weight ($^1/_4$ in.) were ex-

Received November 11, 1963, from Hoffmann-LaRoche, Inc., Nutley, N. J.
Accepted for publication January 2, 1964.
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[1] Automatic Control Devices, Bethel, Conn.