Comparison of Different Photobeam Arrangements for Measuring Spontaneous Activity of Mice

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Effects of chlorpromazine (1, 2, 4, 8 mg./Kg., p.o.) and aggregation (one or five animals) were tested on activity of mice in a circular photocell activity cage. The use of two right-angle (crisscross) beams yielded a better delineation of low drug doses than the use of three parallel beams. Fewer counts were recorded from the middle of three parallel beams than from the same beam in the crisscross arrangement, suggesting that the tendency of rodents to stay near the periphery was enhanced when the audible clicks of the counter were generated by interruptions of the peripheral beams. Both beam arrangements showed higher counts for aggregated than single animals and also showed a significant dose-aggregation interaction, with chlorpromazine decreasing activity of aggregated more than single animals. The findings indicate certain optimal conditions for testing the effects of chlorpromazine on activity.

THE PHOTOCELL activity cage summates, on digital counters, light beam interruptions due to animals in motion. This instrument has been used extensively in the preliminary evaluation of potential psychotropic agents, for measuring their ability to alter the normal spontaneous locomotor activity of small animals. Variations of this apparatus, used for testing drug effects, have included a rectangular single-beam cage (1), and a circular arena with two beams (2) or six beams (3, 4) activating a single counter. Investigation of the conditions which maximize the sensitivity and reliability of this measuring instrument may contribute scientific knowledge about the interactions of experimental variables with drug effects and also may be of practical use in evaluating compounds.

The present paper reports on the use of a photocell activity cage with a separate counter recording from each of several beams. Thus, it is possible to compare different arrangements of beams recording the activity of the same animals simultaneously. The principal objective of this study was to determine whether photocell beams in different locations show significant differences in the magnitude of drug effects that are recorded. Studies with the six-beam photocell activity cage (3, 4) gave evidence that chlorpromazine (CPZ) may have a greater effect on activity of animals tested in aggregations of five rather than singly; the present study provided a test of this drug–aggregation interaction in the independent-beam photocell activity cage.

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EXPERIMENTAL

Subjects.—The subjects were 360 male, Swiss-Webster albino mice (Taconic Farms, New York, N. Y.).

Apparatus.—Spontaneous locomotor activity was measured by the photocell cage available from Aidiation Electronics, Alexander, Va. This unit is a circular arena 13 in. in diameter and physically similar to the six-beam Actophotometer (Metro Industries, New York, N. Y.). The counters, which produce an audible clicking sound, are mounted on the front of the apparatus, near the base of beam D, shown in Fig. 1. Spontaneous activity can be measured by a single beam, or a combination of any two or three beams operating simultaneously. Also, the positions of any of the light sources may be changed so that a parallel or right-angle (crisscross) arrangement of the beams may be utilized. The experimental room was sound attenuated.

Experimental Design.—The animals were divided into two groups, one tested with two beams in a crisscross arrangement (Fig. 1, left), the other with three beams in a parallel arrangement (Fig. 1, right). Each group was subdivided into five subgroups which were given one of four dosages of CPZ (1, 2, 4, 8 mg./Kg. orally) or saline placebo (0.9%, 0.1 ml./10 Gm. body weight). These groups were further subdivided into two test aggregation conditions: singly or in groups of five. Thus, the experiment comprised a complete factorial design with every combination of two beam arrangements, five dosage levels (including placebo), and two aggregation conditions; the design was repeated 6 times. The animals were given a 60-min. test session in the photocell cage in a dark environment, beginning 60 min. after drug administration.

Statistical Treatment of Data.—The number of activity counts for the first 30-min. period were punched on IBM cards, then converted into square roots, and evaluated by the BMDO2V analysis of variance program on the IBM 7090 computer. In accordance with prior findings (3, 4), the use of the square root transformation, for the first 0.5 hr.

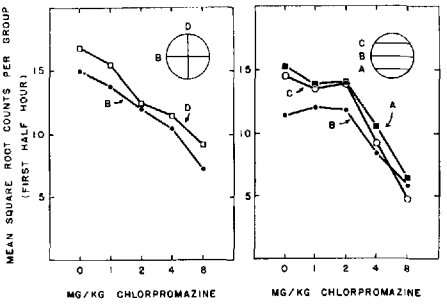

Fig. 1.—Effects of CPZ and beam arrangement on spontaneous activity recorded from each separate beam in the crisscross and parallel arrangements.

of the session, yielded a minimally skewed distribution of scores, with stable performance and large drug effects. For testing the dosage conditions (0, 1, 2, 4, 8 mg./Kg.), and interactions between dosage conditions and any other variables, the statistical significance test was based on the linear orthogonal polynomials, with 1 degree of freedom (5, 6), as used in the prior papers by Watzman *et al.* (3, 4). This test for linear trend assumes a progressive dose-response relationship.

RESULTS

Figure 1 shows the effects of the different dose conditions on activity recorded by each beam, separately for the crisscross and parallel beam arrangements. Different activity counts were recorded on beam B, depending on whether it was one of two crisscross beams or the middle one of three parallel beams. The analysis of variance, comparing this one beam in the different arrangements, shows a statistically significant over-all difference, with higher counts in the crisscross arrangement ($F = 10.1$, d.f. $= 1, 95$, $p < .01$).

A comparison between different beams simultaneously recording the activity of the mice also reveals some highly significant differences. The left graph of Fig. 1 shows a consistent difference between the two crisscross beams, with higher activity recorded on beam D ($F = 25.5$, d.f. $= 1, 50$, $p < .001$). This over-all difference was due to a highly significant interaction between beams and aggregation ($F = 17.5$, d.f. $= 1, 50$, $p < .001$), with beam D recording higher activity levels than beam B for the aggregated but not for the singly tested mice. No significant interaction was found between these two beams and the dosage conditions.

With the parallel arrangement, as shown in the right graph of Fig. 1, the two peripheral beams did not differ significantly from each other, but the middle beam produced lower activity counts, in spite of the fact that it covers the longest span. The quadratic term in the analysis of variance, which provides a comparison between the middle and peripheral beams, shows a significant over-all difference ($F = 20.8$, d.f. $= 1, 100$, $p < .001$). The greater drug decrement for the peripheral beams, which registered substantially higher activity counts than the middle beam for the groups given placebo and the lower doses but not for the groups given the higher doses, resulted in a significant interaction of beams with dosage conditions ($F = 8.35$, d.f. $= 1, 100$, $p < .01$). No significant interaction was found between these three beams and aggregations.

For testing the effects of drug doses and aggregation on activity, the two crisscross or three parallel beams were averaged together. For both arrangements (Fig. 2), the difference among dosage conditions was highly significant, with a similar order of magnitude ($F = 58.1$, d.f. $= 1, 45$, $p < .001$ for the crisscross arrangement; $F = 62.1$, d.f. $= 1, 45$, $p < .001$ for the parallel arrangement). However, an important difference may be seen in the pattern of drug effect. The crisscross arrangement shows a progressive decrease in activity with increasing doses, including a clear delineation between placebo and the lowest doses, but the parallel arrangement shows no consistent decrease in activity with the two lower doses. An analysis of variance limited to the placebo and lower two doses showed a significant dose–response relationship for the crisscross arrangement ($F = 10.9$, d.f. $= 1, 25$, $p < .01$) but not for the parallel arrangement ($F < 1$, d.f. $= 1, 25$).

Figure 2 also shows that higher activity counts were recorded with aggregated than single animals; this aggregation effect was greater with the crisscross arrangement ($F = 86.2$, d.f. $= 1, 45$, $p < .001$) than with the parallel arrangement ($F = 20.6$, d.f. $= 1, 45$, $p < .001$). With both beam arrangements, the drug produced a greater activity decrement in aggregated than single animals, as shown by a significant linear trend for the interaction between dosage and aggregation ($F = 8.76$, d.f. $= 1, 45$, $p < .01$ for the crisscross arrangement; $F = 6.64$, d.f. $= 1, 45$, $p < .05$ for the parallel arrangement).

DISCUSSION AND CONCLUSIONS

The natural tendency of rodents to stay near the periphery of the arena is indicated by the lower number of counts on the middle beam than on the peripheral beams in the parallel arrangement. Figure 1 shows that a significantly larger number of counts, with a superior delineation of small CPZ doses, was recorded from the same location (beam B) when it was one of two crisscross rather than one of three parallel beams. The different locations in which the animals activated the clicking noise of the counters thus produced a significant difference in locomotor behavior, with the crisscross and parallel arrangements. Mice have been shown to behave

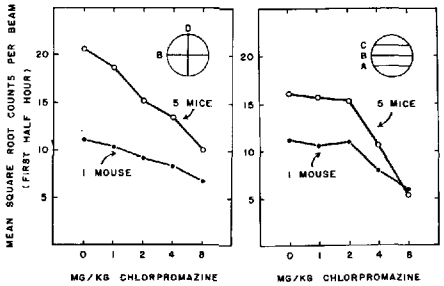

Fig. 2.—Effects of CPZ and aggregation on spontaneous activity; the separate beam recordings in the crisscross and parallel arrangements are pooled together.

so as to elicit noises of low intensity and pitch, comparable to the clicking sound of the counters (7). The present results indicate that the greater coverage of the peripheral portion of the arena, with the parallel beams, enhanced the natural tendency to stay near the periphery, and thus reduced the number of counts on the middle beam. With the crisscross arrangement, the more uniform coverage of all quadrants of the arena by the noise-producing beams apparently elicited a more consistent pattern of activity and dose–response relationship.

The higher counts produced by beam D than beam B, for the aggregated animals tested in the crisscross arrangement, indicates that the aggregated animals tended to clump together near the periphery, either near the counters (at the base of beam D) or away from them, thus activating beam D more often than beam B. The lack of any difference between the two peripheral beams (A and C), in the parallel arrangement, indicates that there was no consistent tendency either to approach or avoid the counters. A further comparison of the parallel beams also shows that the peripheral beams recorded higher counts than the middle beam for the animals under placebo and the low CPZ doses, but not for those under the highest dose. Apparently, the high doses of CPZ reduced the tendency to stay at the periphery of the arena.

With both beam arrangements there was a large dose–aggregation interaction, with CPZ producing a greater depression in activity of grouped than single mice. This indicates a greater tranquilizing effect of this compound in the stimulating social situation, in agreement with prior findings (3, 4). In the photocell activity cage with six crosscross beams recording on a single counter, the less consistent dose–aggregation interaction (4) may be due to failure of the single counter to record fully the high rate of beam interruptions during the intense activity of grouped animals in the placebo condition.

The six-beam Actophotometer is closely similar in dimensions and appearance to the independent beam instrument used in the present study. The counter which recorded activity in the six-beam unit was placed in a separate room and was inaudible to the animals. Apparently this condition of silence does not necessarily improve the delineation of drug effects; the present crisscross arrangement compares favorably with the six-beam unit in detecting the effects of low doses of CPZ. However, a separate experiment, with all other conditions equalized, would be necessary to test the effect of the audible counter clicks on spontaneous activity.

The superiority of the crisscross arrangement in detecting effects of small doses of CPZ, plus the greater drug effect with the peripheral than middle beam in the parallel arrangement, suggest that the most sensitive measure of effect of CPZ would be with an arrangement of two pairs of peripheral beams at right angles to each other, forming a tic-tac-toe pattern. The optimal conditions should probably include the use of animals in aggregations of five rather than singly as well as a separate counter for each beam.

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Distribution of Quaternary Ammonium Salts Between Chloroform and Water

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The apparent partition coefficients, $K_{app.}$, of some alkylsulfate salts of six quaternary ammonium compounds and one tertiary amine are reported. The $K_{app.}$ of the corresponding bisulfate salts were determined by extrapolation. Some comparisons of molecular structures to the $K_{app.}$ are discussed. A method of analysis of the quaternary cations in the presence of long chain anions is reported. The relationship of longer crystal spacings of the sodium salts of alkylsulfates to the molecular weight is shown.

IN PREVIOUS communications (1, 2) it was reported that the partition of organic salts or complexes was determined by the molecular weight of the organic ions, the branching effect of the aliphatic amine cations, and the nature of the organic solvent system used. The authors showed that partitioning into the organic layer from the aqueous layer could be increased by the addition of proton donor molecules.

In several communications Levine and co-

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