Keyphrases

Ion-exchange resin—binding
Bilirubin binding—ion-exchange resins
Kinetics—bilirubin-resin binding
Colorimetric analysis—spectrophotometer

Modified Confinement Motor Activity Test For Use in Mice

By GERALD MILNER

The modification of a confinement motor activity (CMA) unit, for use with mice, is described. Its use in quantitating the effect of drugs on the motor activity in mice is illustrated by experiments with amitriptyline and alcohol. Amitriptyline (10 mg./kg.), given orally, was found to significantly increase CMA when compared with control groups. Alcohol (25 ml./kg., 10% solution) depressed CMA in mice, but not to a statistically significant extent. When the amitriptyline and alcohol were given together, a significant depression of CMA was recorded. A positive joint action is suggested—amitriptyline, when taken by man, may add to the effects of alcohol.

MEASUREMENTS OF alterations in locomotor activity have proved valuable in testing for drug effects on animal behavior. In 1964 Tedeschi *et al.* described a photoelectric cell counting chamber (1) for measuring confinement motor activity (CMA) in rats. This consisted of a chamber so small as to prevent the rat moving from place to place, but high enough to permit the typical "up-and-down" exploratory behavior of the confined rodent. The rat's movements were counted by means of two photoelectric cells. The advantages of the CMA chamber over conventional locomotor activity units are that it is more compact, cheaper, and more sensitive—particularly when testing stimulant drugs such as caffeine, tranylcypromine, and amphetamine. Photoelectric cell activity chambers have been shown to be generally more satisfactory than mechanical tests, such as those involving rocking floors (2).

Mice are probably the most widely used and useful laboratory animals; they are small, easily maintained and bred, cheap, and can be used in large numbers for individual experiments. With many drugs, particularly alcohol, prediction of behavioral effects in man from their effects in mice have proved valid (3). The present paper describes the development of a confinement motor activity chamber for mice. Its use is illustrated by experiments involving amitriptyline and alcohol.

Amitriptyline is an iminodibenzyl drug (with a chemical formula closely related to chlorpromazine) and in doses of 30 and 50 mg./kg. body weight has been shown to significantly increase the length of loss of righting reflexes in mice given alcohol (4, 5). With alcohol levels of 25 ml./kg. of a 25% solution, the average length of loss of righting reflexes in an adult mouse is 1.2 hr., whereas a dose of 25 ml./kg. of 20% alcohol just fails to cause coma. The significant increase in the length of alcoholic coma brought about by amitriptyline might be due only to the sedation caused by each drug. A positive joint action of alcohol and amitriptyline is indicated in the experiments described in this paper, where low doses of the drugs were used.

EXPERIMENTAL

Development of a CMA Unit for Mice—Preliminary experiments enabled a rough estimate of the best size of chamber to be made. Tests were then

Received May 23, 1968, from Claremont Hospital, Mental Health Services, Claremont, Western Australia.
Accepted for publication July 24, 1968.
The author acknowledges Dr. A. S. Ellis, Mental Health Services, Western Australia; Dr. I. Oswald, University of Western Australia, Department of Psychiatry; Dr. W. Laurie, Public Health Laboratory of Western Australia, who provided facilities for this research. Smith, Kline and French Laboratories (Philadelphia, Pa.) kindly supplied a 10-chamber unit for use with rats. Dr. M. F. Lockett (Department of Pharmacology, University of Western Australia) gave advice on the experimental methodology. Precision Electronics of Subiaco, Western Australia, built the CMA unit for use with mice. Geigy, Roche, and Sandoz pharmaceutical companies assisted in a number of ways.
This work forms part of a thesis submitted for the Doctor of Medicine degree, University of Western Australia.

performed using albino mice (Prince Henry strain, obtained from the Institute of Medical and Veterinary Science, Adelaide, South Australia). The laboratory temperature was held at 21.5 ± 1° and constant illumination was maintained. The experiments were performed at the same time each day. The mice used were matched for weight and sex; none of them had ever previously been dosed with a drug. The various factors were (*a*) weight: 20 ± 2 g., 25 ± 2 g., 30 ± 2 g.; (*b*) floor size, *i.e.*, the dimensions of the CMA chamber floor: 3.8 × 5 cm. (1.5 × 2 in.), 3.8 × 4.4 cm. (1.5 × 1.75 in.), 3.8 × 3.8 cm. (1.5 × 1.5 in.); (*c*) height, *i.e.*, the distance from the CMA chamber floor to the horizontal light beams [the two beams are parallel, 1.9 cm. (0.75 in.) apart, and each is focused on a separate photoelectric cell]: 5.6 cm. (2.25 in.), 5 cm. (2 in.) 4.4 cm. (1.75 in.); (*d*) replication, *i.e.*, with each combination of other factors a given group of mice was tested in the CMA chambers for 15 min. in each of 3 consecutive hr.: first replication, second replication, third replication; (*e*) 5-min. scores, during each replication, the score for each mouse was recorded as follows: 0–5 min., 6–10 min., 11–15 min.

In this series of preliminary experiments 2,430 different scores were collected (10 mice per group × 3 different weights × 3 different floor sizes × 3 heights × 3 replications at hourly intervals × three, 5-min. scores). A computer analysis of variance of these data was performed in order to discover which set of conditions combined sensitivity with minimum variability of scores. The optimal conditions proved to be: (*a*) weight = 25–30 g.; (*b*) floor size = 3.8 × 4.4 cm.; (*c*) height = 5 cm.; (*d*) second replication; (*e*) score at the end of 10 min.

Description of Apparatus—A 10-chamber CMA unit for mice was made—it measures only 61 × 20 × 12.7 cm. (24 × 8 × 5 in.); there are 10 mechanical counters (each connected to two photoelectric cells so that interruption of one or both light beams results in a single count); each chamber has opaque sides so that the individual mouse is isolated from, and not disturbed by, its fellows (an improvement over the original rat CMA chambers which had transparent sides); a single removable tray makes up the floor of all 10 chambers. The tray can be covered with blotting paper that is changed after each experiment to ensure uniform conditions. This compact equipment is illustrated in Figs. 1 and 2.

Subsequent trials have shown that variability can be further reduced by allowing the mice to become accustomed to the chamber by being put in it for 5 min. twice a day for 3 or more days. The mice can then be allocated to different treatment groups on the basis of their basal activity scores, so that each group contains an equal proportion of high and low responders.

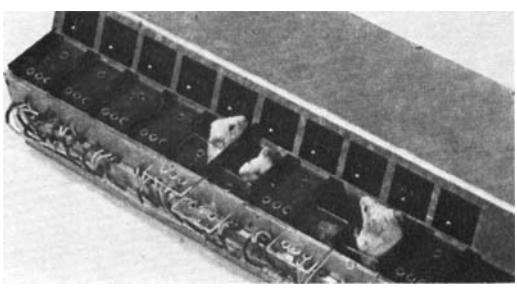

Fig. 1—Ten-chamber confinement motor activity unit for use with mice.

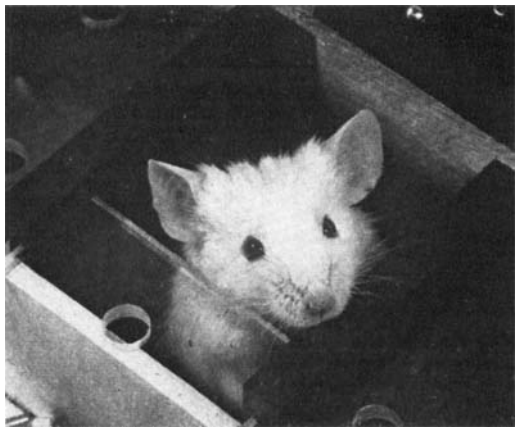

Fig. 2—Close-up of a mouse in one chamber of the confinement motor activity unit.

AMITRIPTYLINE AND ALCOHOL

In a series of three consecutive experiments a total of 120 male albino mice (26 ± 2 g.) were tested in the CMA chambers. The mice were fasted overnight, then dosed by stomach tube in four treatment groups: Group I received amitriptyline (10 mg./kg.) solution and ethyl alcohol (25 ml./kg., 10% solution); Group II received amitriptyline (10 mg./kg.) solution and placebo (5% glucose solution equal in volume to the alcohol); Group III received placebo (water equal in volume to the amitriptyline solution) and alcohol (25 ml./kg., 10%); Group IV received water and glucose as in Groups II and III (double placebo conditions).

The average total 5-min. score for the double placebo-treated groups was 2,858 when put in the CMA chambers 0.5 hr. after dosing. The alcohol and placebo group total scores averaged 2,514; this slight depression of activity was not statistically significant. The average total score for mice given amitriptyline plus placebo in place of alcohol was 4,968. This elevation of CMA was statistically significant at the 1% level of probability. The three groups of 10 mice given alcohol plus amitriptyline showed a significant depression of activity; the total score of each group averaged 719 ($p < 0.01$). Table I shows the scores obtained in the three experiments, and Table II the analysis of variance of the results. Figure 3 represents the results graphically.

The analysis of variance also revealed that only

TABLE I—TOTAL 5-MIN. CMA SCORES OF GROUPS OF 10 MICE GIVEN AMITRIPTYLINE (10 mg./kg.), ALCOHOL (25 ml./kg., 10%), AND PLACEBO SOLUTIONS

Expt.	Placebo	Alcohol Plus Placebo	Placebo and Drug	Alcohol Plus Amitriptyline
A	2,511	3,409	4,900	513
B	2,900	2,323	5,498	1,222
C	3,164	1,810	4,506	423
Average	2,858	2,514	4,968	719

TABLE II—ANALYSIS OF VARIANCE OF THE SCORES OF THREE EXPERIMENTS COMPARING THE EFFECT ON CMA PERFORMANCE OF FOUR CONDITIONS OF DRUG, ALCOHOL, AND PLACEBO

Source	*df*	*MS*	*F* Ratio
Conditions	3	911,595.32	11.30[a]
Replications	2	29,465.83	0.36[b]
Interaction	6	30,909.05	0.37[b]
Residual	108	83,440.39	
Total	119		

[a] $p < 0.01$. [b] Not significant.

the conditions effect was significant, *i.e.*, there was no "between replications" difference. The significance of the individual comparisons was ascertained by Duncan's multiple-range test. Amitriptyline plus alcohol resulted in lower CMA scores than any of the other three combinations. The difference between amitriptyline plus alcohol (Group I) and alcohol plus placebo (Group III) was significant at the 0.05 level of confidence.

DISCUSSION

The confinement motor activity unit has been shown by Tedeschi *et al.* to reveal the stimulant effects in rats of caffeine and tranylcypromine, although these drugs seem essentially inactive when tested by conventional photoelectric cell locomotor activity chambers. The present study has shown that the CMA unit, when modified for use with mice, enables the measurement of the excitation caused by low doses of the antidepressant drug, amitriptyline. Similar results were reported by Boissier *et al.* (6) who measured the rate of exploration of peg-board holes by mice. Higher doses of amitriptyline were shown to produce sedation.

When the stimulant dose of amitriptyline is combined with a low dose of alcohol (which by itself produced very little sedation), a reversal of drug effect is seen, the mice getting significantly low CMA scores, apparently a joint drug action.

Prediction of human response on the basis of animal experiments is possible when the laboratory tests are efficient, have high reproducibility, and adequacy of experimental design. Amitriptyline is of proven value in the relief of psychotic depression (7), but frequently causes sedation and drowsiness as side effects, especially in the first few days of administration. When prescribing amitriptyline, the possibility that it may add to the effects of alcohol should be considered.

SUMMARY

1. The confinement motor activity test, originally described for use in rats, has been appropriately modified for use in quantitating the motor activity of mice.

2. Amitriptyline in a dose of 10 mg./kg. body weight (orally) was shown to significantly increase the activity of mice.

3. Alcohol in a dose of 25 mg./kg. body weight of a 10% solution did not significantly depress the activity of mice in this test.

4. Amitriptyline (10 mg./kg.), plus alcohol (25 ml./kg., 10% solution) caused a significant depression of the activity of mice.

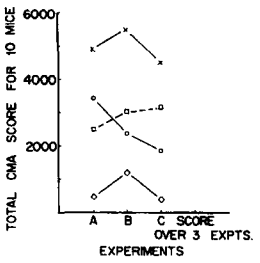

Fig. 3—Confinement motor activity scores in mice dosed with combinations of alcohol, placebo, and amitriptyline. Key: ×, amitriptyline, 10 mg./kg. + placebo (5% glucose); □, water + 5% glucose (double placebo); ○, alcohol 25 ml./kg., 10% + placebo (water); ◇, amitriptyline 10 mg./kg. + alcohol 25 ml./kg., 10%.

REFERENCES

(1) Tedeschi, D. H., Fowler, P. J., Cromley, W. H., Pauls, J. F., Eby, R. Z., and Fellows, E. J., *J. Pharm. Sci.*, **53**, 1046 (1964).
(2) Lal, H., Shefner, A., and Wenzel, D. G., *Arch. Intern. Pharmacodyn.*, **150**, 192(1964).
(3) Lockett, M. F., and Milner, G., *Brit. Med. J.*, **1**, 921 (1965).
(4) Halliwell, G., Quinton, R. M., and Williams, F. E., *Brit. J. Pharmacol.*, **23**, 330(1964).
(5) Milner, G., *Lancet*, **1**, 222(1967).
(6) Boissier, J. R., Simon, P., Fichelle-Pagny, J., and Laroff, J. M., *Therapie*, **20**, 759(1965).
(7) Ayd, F. J., Jr., *Dis. Nerv. Syst.*, **26**, 719(1965).

Keyphrases

Motor activity test—mice
Confinement motor activity unit—modifications
Amitriptyline, alcohol, effects—motor activity
Alcohol effect—amitriptyline activity