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A METHOD FOR RECORDING THE EFFECT OF DRUGS ON THE ACTIVITY OF SMALL MAMMALS OVER LONG PERIODS OF TIME

BY

ELIZABETH E. SHILLITO

From the Department of Pharmacology, Wyeth Institute of Medical Research, Taplow, Maidenhead, Berks.

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The activity of animals is a useful parameter on which to determine the effect of drugs since it can indicate the sedative or stimulant properties of the compounds or give a lead as to their method of action. Most methods of measuring the activity of animals take the form of Dew's cages with a light beam, or jiggle cages, and these generally involve the animal entering a new cage or some unfamiliar surroundings. Thus, with the disturbance of handling and the impact of an unknown area, the activity of the animal is very far from normal. Under these conditions the experiments measure the effect of drugs on the locomotion of animals in new surroundings, and possibly this is part of exploratory behaviour.

This paper describes a method of recording the activity of a group of small mammals as they live permanently in an area. The cage is a vertical wall cage made from galvanized steel sheet. It consists of eight units one on top of each other. Each unit consists of a nest box connected to a feeding chamber by two runs. The runs of each unit are connected by holes so that the animals can climb up and down the whole enclosed area. The nest boxes can be closed by shutters and the front of the cage is made of two pieces of Perspex (Fig. 1).

The movement of the animals is recorded by a capacitor system. Six probes are placed in the control area of the cage, under some of the holes in the runs through which the animals climb (Fig. 1). Each probe extends the width of the cage and consists of a brass rod within a glass tube. Outside the cage each probe is connected to a series-tuned circuit the details of which are given by Haines (1961).

When an animal goes near or past a probe, it alters the capacitance of the probe which unbalances an electric circuit and a dot is made on a moving graphite-backed recording paper. There is a time recorder incorporated in the writing mechanism so that the activity of the animals within the cage is sampled in relation to time. The records are then analysed by counting the dots and these results can be used in various ways. Counting can be done visually but print-out counters can also be used.

The cage is kept in a reversed daylight room in which the lights go off at 1000 and come on at 2200 hr. Any observations are made in red light which does not alter the activity rhythm of the animals. Food is put on each shelf of the food chambers and two water troughs, one at the top and one at the bottom of the cage, provide a drinking source.

Present address: Agricultural Research Council Institute for Animal Physiology, Babraham, Cambridge.

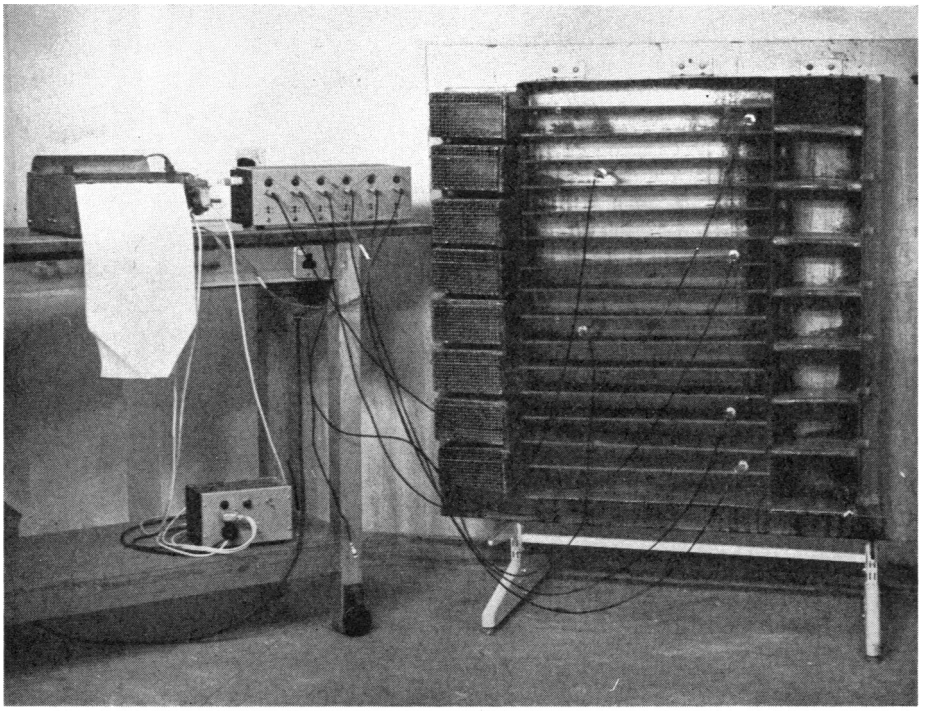

Fig. 1. Photograph of the activity cage.

Most of the experiments have used white mice of Schofield strain. One group of ten animals of the same sex is used at a time. The mice are left in ordinary cages in the reversed daylight room for 1 week in order to adjust to the lighting conditions. Then they are put in the cage and left for a week to settle down. During this time at least two trial records of their activity are taken.

Once a group of mice is established in the wall cage and the trial records indicate that their activity is reasonably stable and distributed throughout the cage, experiments to test the effects of compounds can proceed. One record is run on the first day as an undisturbed control. On the second day the mice are given the vehicle in use by the route to be used, either intraperitoneal or subcutaneous injection or orally. This record is a disturbed control and gives an indication of the disturbance caused by handling and injection. On the third day the test compound is administered and on the fourth day an undisturbed after-drug control is run. Alternatively the effect of daily dosing can be determined or any drug interaction can be studied.

The mice are strongly nocturnal and become active as soon as the lights go out. Until this time they are ideally in the nest boxes, so just before 1000 hr the shutters to the nest boxes are closed. This time is marked on the recording paper. Any stray mice are then persuaded to go to the nest boxes if they are running around elsewhere in the cage. The animals are removed from the nest box, weighed, injected and returned to the cage. The

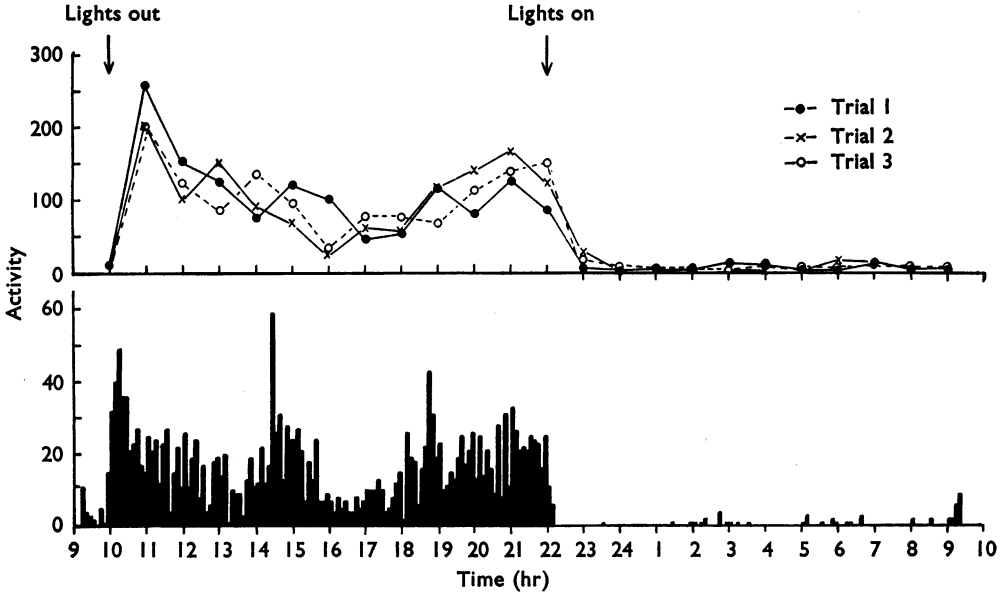

Fig. 2. Above, graph to show the activity of a group of mice over 24 hr, on three successive days. ●——●, trial 1; ×——×, trial 2; ○——○, trial 3. Below, histogram to show the activity of ten mice over 24 hr.

shutters are then opened and the mice are free to run around again. This time is also marked on the recording paper. Any other form of disturbance such as feeding and renewing the water is also recorded on the paper.

The analysis of the records can be made in several ways. The number of dots made on the paper are counted during every 6 min, so there are ten estimates to 1 hr. Hourly totals and a total 24 hr count are also made, and a simple histogram of the activity is plotted and also a graph of hourly activity (Fig. 2).

It can be seen that this method provides a way of determining the effect of a drug for some time after administration. Also it shows if the animals make any compensatory activity at a much later hour, or if any drug effect carries on for more than 1 day. The following results demonstrate these points.

TABLE 1

THE ACTIVITY COUNT OF TEN MICE GIVEN 5 MG/KG OF METHYLAMPHETAMINE ON THREE SUCCESSIVE DAYS

	Activity Count				
	1 hr	2 hr	3 hr	3 hr total	24 hr total
Test day 1	536	223	245	1,004	2,864
Test day 2	268	136	155	559	2,021
Test day 3	178	117	203	498	2,079
Control	215	120	106	441	2,065

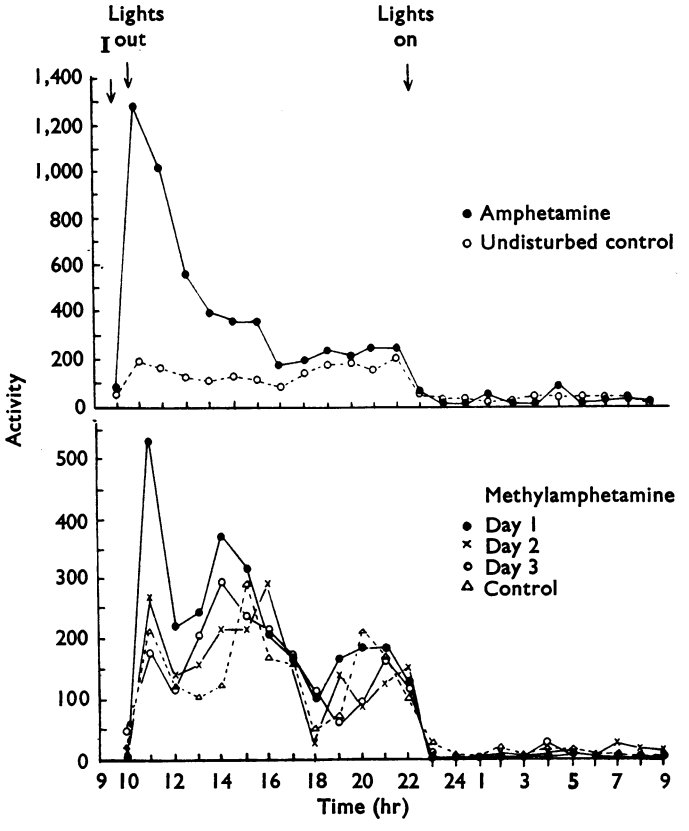

Fig. 3. Graphs to show the activity of groups of ten mice over 24 hr when given stimulants. Upper graph, comparison of the effects of amphetamine (10 mg/kg) with undisturbed controls. Lower graph, activities on three days of treatment with methylamphetamine (5 mg/kg, daily) compared with an undisturbed control day. Injections were at the first arrow (I).

The effect of stimulants

Experiments were carried out with amphetamine and methylamphetamine. At high doses it was found necessary to use only five mice because the increased activity made it difficult to count the number of dots accurately.

Amphetamine. This drug, 10 mg/kg intraperitoneally, increased the first peak of activity and activity was higher than control values for 6 hr. There was no compensation for the increase at a later time, so that the total count for 24 hr was higher than the control. The activity at the end of the period was normal. The numerical results are given in Table 2 and shown graphically in Fig. 3.

Methylamphetamine. This had a similar effect to amphetamine. In one experiment it was given intraperitoneally at 5 mg/kg on 3 successive days and the stimulant effect became less with the repeated doses.

The main reduction in response occurred in the first 2 hr after injection. These results are shown graphically in Fig. 3 and numerically in Table 1.

TABLE 2
THE ACTIVITY COUNTS OF GROUPS OF MICE GIVEN VARIOUS DRUGS
The vehicle control was 1% Tween 80

Treatment	Activity Count					
	0.5 hr	1 hr	2 hr	3 hr	3 hr total	24 hr total
Amphetamine, 10 mg/kg	436	1,021	812	460	2,293	4,504
Undisturbed control	112	166	125	99	390	1,859
Chlorpromazine, 2 mg/kg	55	77	93	82	252	1,244
Chlorpromazine, 5 mg/kg	26	38	31	20	89	1,354
Saline control	113	152	168	191	511	1,828
Undisturbed control	107	156	82	120	358	1,974
Chlordiazepoxide, 12.5 mg/kg	90	161	97	126	384	2,050
Undisturbed control	124	216	141	96	453	2,161
Hexobarbitone sodium, 100 mg/kg	0	34	87	123	344	1,699
Saline control	65	124	162	70	356	1,614
Undisturbed control	105	186	101	116	403	1,644
Meprobamate, 50 mg/kg	94	143	41	202	386	2,725
Meprobamate, 100 mg/kg	56	71	129	198	398	2,149
Undisturbed control	45	169	211	136	516	2,248
Imipramine, 10 mg/kg	104	157	65	76	298	1,419
Imipramine, 20 mg/kg	33	68	53	39	160	1,413
Undisturbed control	139	254	200	137	591	1,950
Vehicle control	143	214	107	85	406	1,915
Iproniazid, 60 mg/kg	51	92	111	121	324	1,829
Undisturbed control	99	214	95	122	431	2,059
Vehicle control	87	199	139	152	490	2,159

The effect of sedatives

Chlorpromazine. This was injected intraperitoneally at doses of 2 and 5 mg/kg. The mice were sedated, in relation to the dose, and this effect lasted up to 7 hr. There was no compensation for the loss of activity and so the total activity count was lower than that on the saline and undisturbed control records. The results are shown graphically in Fig. 4 and numerically in Table 2.

Chlordiazepoxide. Various intraperitoneal doses were used up to 12.5 mg/kg and no marked sedation was observed. The graph in Fig. 4 shows that the normal pattern of activity for the group of nine mice was followed. The numerical counts are given in Table 2.

Hexobarbitone sodium. 100 mg/kg were given intraperitoneally. This is the same dose as is used in drug interaction sleeping-time experiments. The mice lost their righting reflex and were inactive for about 45 min. This sedation was followed by a recovery excitement which explains the score seen on the graph in Fig. 4. There was some compensation for the early inactivity and so the total 24 hr count was very similar to, but slightly higher than, that for the saline controls. The numerical results are shown in Table 2.

Meprobamate. This was used at 50 and 100 mg/kg and it gave a slight sedation over the first 3 hr. Otherwise the activity was normal. The numerical results are given in Table 2.

Reserpine. Reserpine (2 mg/kg) was given intraperitoneally and the results are shown graphically in Fig. 5. The sedation started 3 hr after administration of the compound and the count from 1300–1400 hr was low. The activity stayed low and the normal pattern was disrupted as the night activity was higher than normal. On the following day, the activity was lower than usual until about 1900 hr which was 32 hr after the injection. The numerical results are given in Table 3.

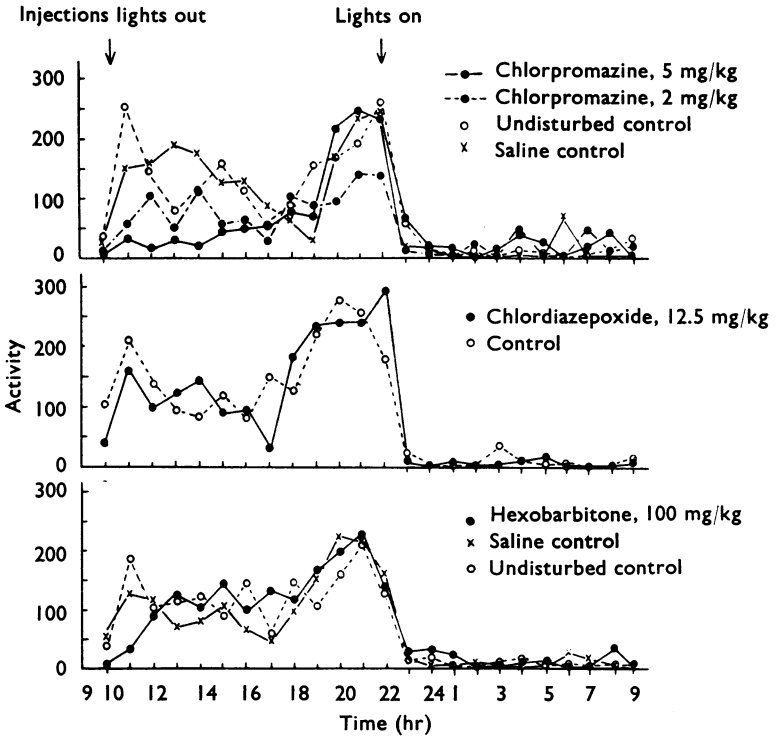

Fig. 4. Graphs to show the activity of groups of mice over 24 hr when given sedatives, compared with undisturbed controls and controls given saline.

TABLE 3
THE ACTIVITY COUNT OF TEN MICE GIVEN 2 MG/KG OF RESERPINE

Treatment	Activity count					
	0.5 hr	1 hr	2 hr	3 hr	3 hr total	24 hr total
Reserpine, 2 mg/kg	76	117	98	142	357	1,233
Saline	103	148	138	145	431	2,076
1st day after drug	18	96	60	91	247	1,155

The effect of antidepressant drugs

Imipramine and desipramine. These were both used at 10 and 20 mg/kg intraperitoneally. Both produced a sedation in the first 3 hr after the injection and which was related to dose. The results for imipramine are shown graphically in Fig. 5 and numerically in Table 2.

Iproniazid. This was tried at 60 mg/kg intraperitoneally, and it produced a slight sedation in the first 2 hr after administration. The results are shown in Fig. 5 and in Table 2.

DISCUSSION

This method can provide answers to various questions concerning the immediate or long-term effect of drugs on the activity of a settled population of animals, at any time of the day or night and under conditions which can be varied if necessary. The results have

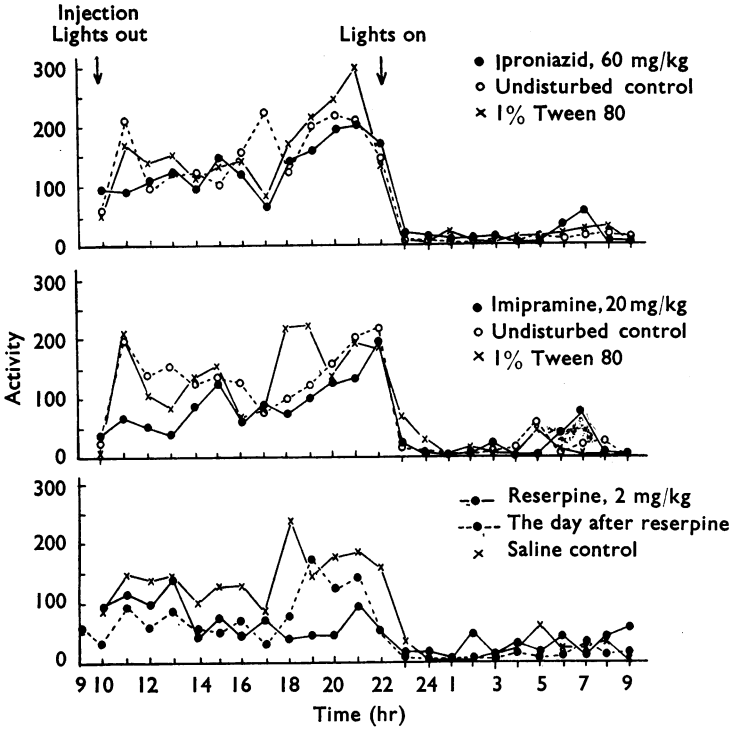

Fig. 5. Graphs to show the effects of various drugs on the activity of groups of mice recorded over 24 hr, compared with undisturbed controls and controls given saline or the vehicle (Tween 80).

shown the importance of the long-term recording which supplies more information on the test compound than is given by conventional methods. An example of this is the delayed sedation which lasted into the second day of the experiment when reserpine was given to mice. In addition to this, the opportunity to use the same mice repeatedly under the same conditions can give information such as that which was obtained, for example, with methylamphetamine. This gave a decreased response with repeated dosing.

The major advantage of this method is that the mice are returned to the area in which they were living before the drug was administered. In this way all effects of a new cage are removed, and the mice have no need to move in order to explore. The effects of disturbance and handling can be determined by control records, so that the immediate effects of the test compounds can be obtained by analysis of the records down to 6 min intervals. This was shown when hexobarbitone sodium was given to the mice, and the immediate complete sedation followed by a recovery excitement was clearly seen and recorded.

The activity of the mice can be only sampled by the probes. These have been placed in the most suitable positions in the cage, but even so it is necessary to make sure that the mice are moving through the cage evenly. It is important too that the group of mice should be large enough to make an accurate sample possible and not too large to make the counting of the dots inaccurate. It has been found that less than four mice is not suitable and that ten mice is the optimum number in a group. It is also important that they should all be of

one sex, because otherwise the activity pattern does not become constant. Aggression and reproduction also interfere with the results. However, the effect of drugs on group structure could be studied in this way.

The length of time which is needed to test a drug is a drawback to the method, but the advantages of having a group of animals living in permanent surroundings and under a normal activity cycle of lighting outweigh this disadvantage; for this is the type of situation in which drugs will be acting if they are of any therapeutic use.

Although only the use of white mice has been described, other small mammals can be put in the cage. Some previous experience with the short-tailed vole, *Microtus agrestis*, has shown that the activity patterns of diurnal animals can be obtained by this method, and consequently experiments to determine the factors which affect these patterns can be carried out.

SUMMARY

1. A method of recording the activity of a group of small mammals over a long period of time is described.

2. The effect of some standard compounds on the activity of mice living in this way is recorded, and the significance of these results is discussed.

I would like to acknowledge the many people who have worked with me while this method has been developed. In particular I am grateful to Mr John Haines who designed the electronic recording method. Margaret Bryant, Pat Mortimer, Melvyn Noller and Ann Carter have spent many hours counting dots and I am grateful for their patience and help. I would also like to thank Mr K. E. V. Spencer who has helped throughout in many ways.

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