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A method for investigating the effects of drugs on the exploratory behaviour of mice

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Summary

1. Exploratory behaviour in mice was observed when they were put on a wooden board to which twelve tunnels were fixed. The number of different tunnels entered (indicating exploration) and the total entries into tunnels were recorded over 5 min on 2 successive days.

2. Untreated mice entered more different tunnels in the first minute on the second day on the tunnel board, and this difference in behaviour was taken as an indication that exploration had occurred on the first day. When the behaviour of the treated mice on the second day was similar to that of inexperienced mice on the first day it was inferred that drug treatment had adversely affected exploration.

3. Haloperidol 4 mg/kg, chlorpromazine 8 mg/kg and thioridazine 16 mg/kg adversely affected exploration at doses which almost immobilized the mice.

4. Amphetamine at 8 mg/kg disrupted exploratory behaviour in the mice, although the mice were observed to move round the board very quickly.

5. With tranylcypromine 2 mg and nialamide 100 mg, increased exploratory behaviour by comparison with controls was recorded in the mice when they were tested 24 h after drug treatment.

6. Imipramine at 20 mg/kg reduced the total number of tunnels entered by the mice on the first day, but on the second day the mice behaved in a similar way to mice treated with monoamine oxidase inhibitors.

Introduction

When a rodent is put into a new area, it tends to move around investigating the features of the surroundings. This movement has been used as a basis for measuring the effects of drugs on " exploratory " behaviour, a category of behaviour which gives the animal information about its environment. The effects of drugs on the movement of rats in a Y-maze were first studied by Rushton, Steinberg & Tinson (1961) and have also been used by Marriott & Spencer (1965), Shillito (1967) and others. Changes in the behaviour of mice as a result of drug treatment on a square board with regularly spaced holes in it have been studied by Boissier & Simon (1964) and Joyce, Porsolt, Steinberg & Summerfield (1968). This last method involves counting the number of times each mouse dips its nose into the holes, but because mice tend to dip their noses into any depression of the surface and over the edge of boards both the above methods involve, as they must, locomotion by the animals. It seemed desirable to have a method which could distinguish exploration more

clearly from locomotion, particularly for use with drugs which cause an increase in activity but not necessarily an increase in exploration. To do this it is essential to observe some action of the mouse, which may be part of its general activity but which does not occur as a result of random locomotion. It is also necessary to be able to observe some behavioural change which will indicate that learning has taken place as a result of exploration. When mice are put on a board to which some tunnels are fixed, they enter the tunnels after some delay and they also enter only a few different tunnels on the first exposure to the board, although they may enter the same tunnel several times. On a second exposure the mice behave differently and quickly enter several different tunnels. In this situation a special behaviour pattern is elicited which can be distinguished from general locomotion and which also changes as a result of experience ; accordingly the following method was devised.

Methods

Male albino mice, supplied by Tucks, weighing between 15 and 20 g were used ; they were kept in treatment groups of ten animals to a cage. One control group was always used simultaneously with groups to which various doses of drugs had been administered. All tests were carried out on mice kept in reversed daylight conditions with red light from 10.00 to 22.00 h and white light from 22.00 to 10.00 h, for at least a week before the experiment, and some drugs were also tested on mice kept in daylight during the day and dark at night.

Mice were placed one at a time on the left hand corner of a wooden board measuring 61 cm × 61 cm, on to which twelve tunnels 7·5 cm long and 4 cm in diameter were fixed arranged in a symmetrical pattern. The tunnels were made from strips of plastic bent into semi-circles and screwed on to the board. Each tunnel was numbered (Fig. 1). Each mouse was watched for 5 min under red light on 2 consecutive days, at the same time each afternoon. Drugs were given by intraperitoneal injection 30 min before observation on the first day ; there was no drug treatment on the second day. When monoamine oxidase inhibitors were used, they were given 24 h before observation to allow for oxidase inhibition to occur. The following drugs were used, given in solutions adjusted to 10 ml/kg: chlorpromazine hydrochloride 2, 4 and 8 mg/kg ; thioridazine hydrochloride 2, 4, 8 and 16 mg/kg ; haloperidol 2, 4 and 8 mg/kg ; amphetamine sulphate 2, 4 and 8 mg/kg ; tranylcypromine sulphate 2 and 4 mg/kg ; nialamide 75, 100 and 200 mg/kg, pargyline hydrochloride 110 and 220 mg/kg ; imipramine hydrochloride 10 and 20 mg/kg. All the drugs were given in saline solution which was given to the controls, except for haloperidol, which was dissolved in ascorbic acid.

During the 5 min observation period, the number of each tunnel entered by the mouse was recorded in each minute ; from this the total number of tunnel entries was calculated, as well as the number of entries into different tunnels. The number of different tunnels entered in the first minute on day 1 and day 2 was considered to be the most stable indication of exploration and these values were compared by using Student's *t* test. Progress of exploration, defined as the number of different tunnels entered in successive minutes of the 5 min observation period, also gave an indication of exploration and this was shown by plotting the cumulative entries (Figs. 2–7). The total number of tunnels entered during 5 min varied between

groups because in some groups the mice seemed to enter a tunnel several times, particularly when they appeared to be confused and excited after drug treatment. Thus the number of different tunnels entered was considered to be a more valuable measure of exploration, particularly the results for the first minute of the observation period on the second day. General locomotion was not measured.

Results

When an untreated mouse was put on the tunnel board for the first time, it moved around the board walking slowly between the tunnels. The mouse sniffed the entrance and sides of each tunnel and touched them with the nose and front feet using the vibrissae, until it gradually entered the tunnel. In the first minute of observation only one or two tunnels were entered and in the following minutes the mice moved over the board, sometimes entering as many as six or eight different tunnels in 5 min. Generally the centre tunnels were entered before the peripheral ones, and there was much locomotion round and between the tunnels ; only occasionally did a mouse stay inside them. The total number of entries into tunnels varied between groups, some made twelve to fifteen entries while the more active groups made on average twenty to twenty-five.

The behaviour of untreated mice on the second day differed from that on the first day in that more different tunnels were entered quickly. Thus in the first

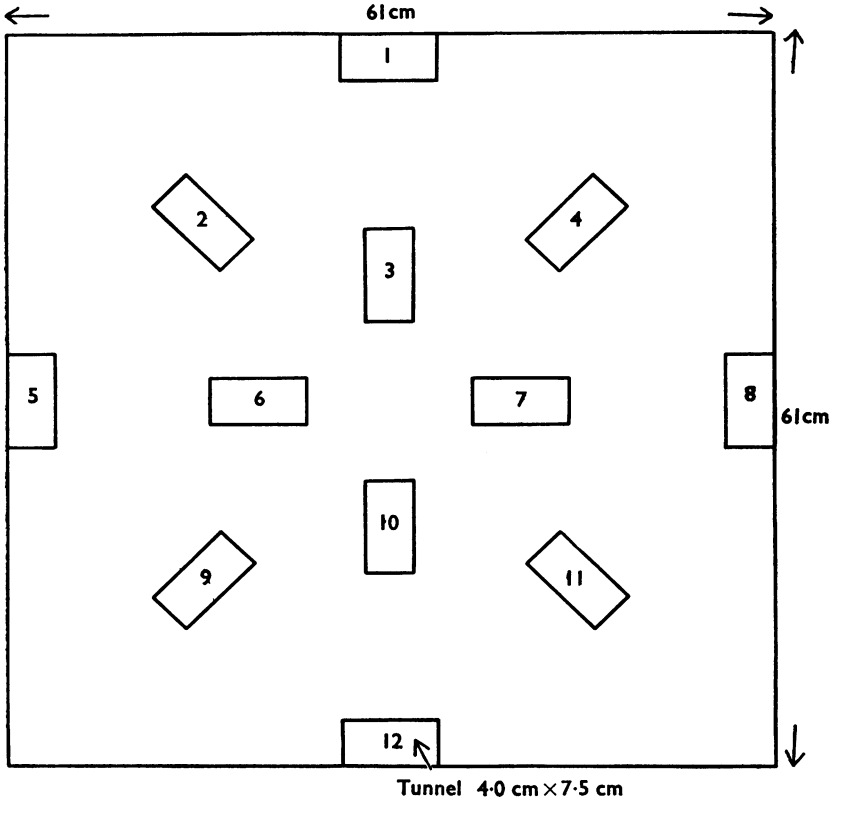

FIG. 1. Plan of the tunnel board.

minute three to five different tunnels were entered and this difference was statistically significant. The gait of the mice was faster and less hesitant than that of inexperienced mice, so that they were running rather than walking. When the results were drawn graphically, the slope of the cumulative curve for entries in successive minutes obtained on the second day was steeper than that for the first day (Figs. 2–7). This difference was taken as an indication that exploration had occurred on the first day. The number of entries by the drug-treated mice were compared with those of the controls, particularly the difference during the first minute. It was concluded that the drug treatment had affected exploration adversely when the slope for the second day entries of a group treated with a drug before the first test was the same as that of the first day control group. In these circumstances the mice were behaving as if they were inexperienced.

Single doses of " tranquillizers "

The effect of single doses of " tranquillizers " such as chlorpromazine, thioridazine and haloperidol was to reduce the number of different tunnels entered and the total number of entries was lower than in the control groups. Locomotion was decreased with the higher doses and the mice, generally, moved into the centre of the board. Mice treated with haloperidol 2 mg/kg, chlorpromazine 2 mg/kg and 4 mg/kg and thioridazine 4 mg/kg and 8 mg/kg entered two or three different tunnels on the first day on which they were given a drug, but they behaved like experienced control mice on the second day. At the highest doses (haloperidol 8 mg/kg, chlorpromazine 8 mg/kg and thioridazine 16 mg/kg) the mice were very sedated and almost immobilized, entering only one tunnel or none at all and then, on the second day, they behaved as if they were untreated inexperienced mice on the board for the first time. The results are shown in Table 1 and Figs. 2, 3 and 4.

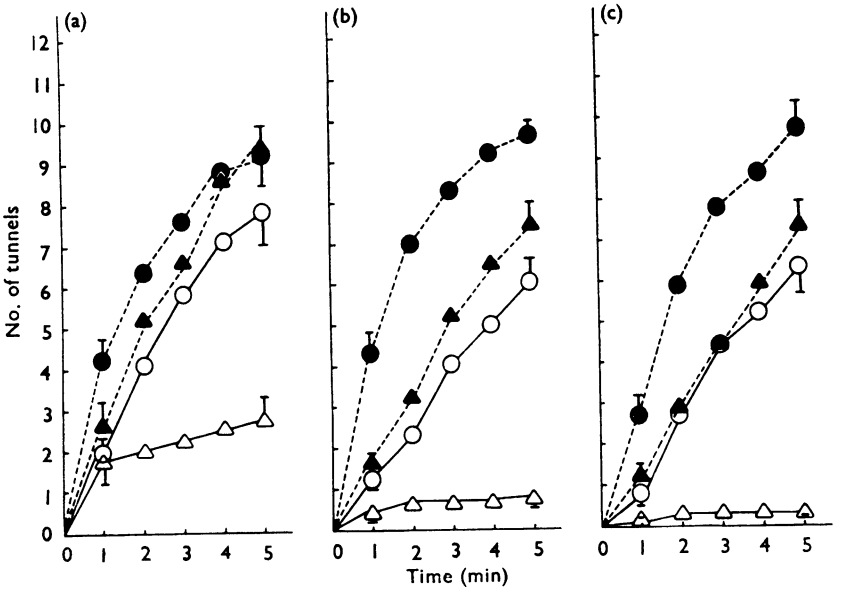

FIG. 2. Average number of different tunnels entered cumulatively over the 5 min observation period by mice treated with haloperidol (a) 2 mg, (b) 4 mg, (c) 8 mg on day 1 (△) and untreated on day 2 (▲), with a control group day 1 (○) and day 2 (●).

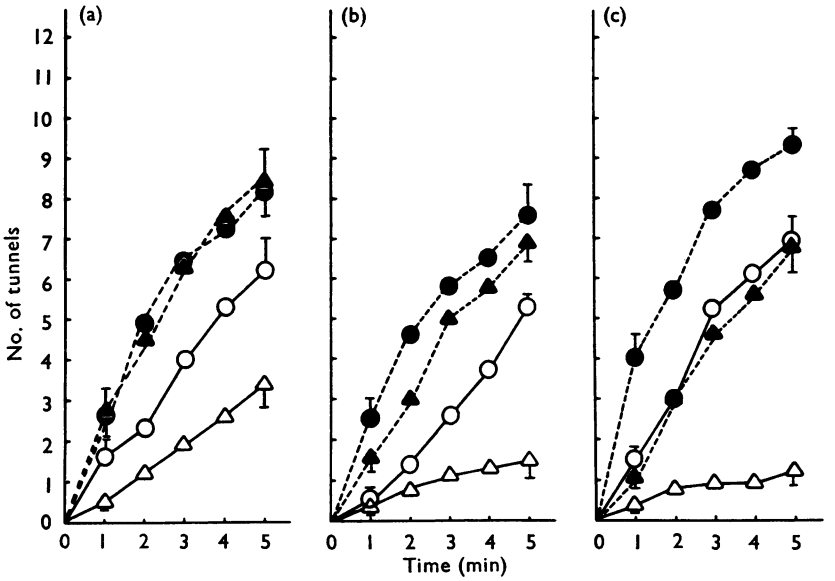

FIG. 3. Average number of different tunnels entered cumulatively over the 5 min observation period by mice treated with chlorpromazine (a) 2 mg, (b) 4 mg, (c) 8 mg on day 1 (△) and untreated on day 2 (▲), with a control group day 1 (○) and day 2 (●).

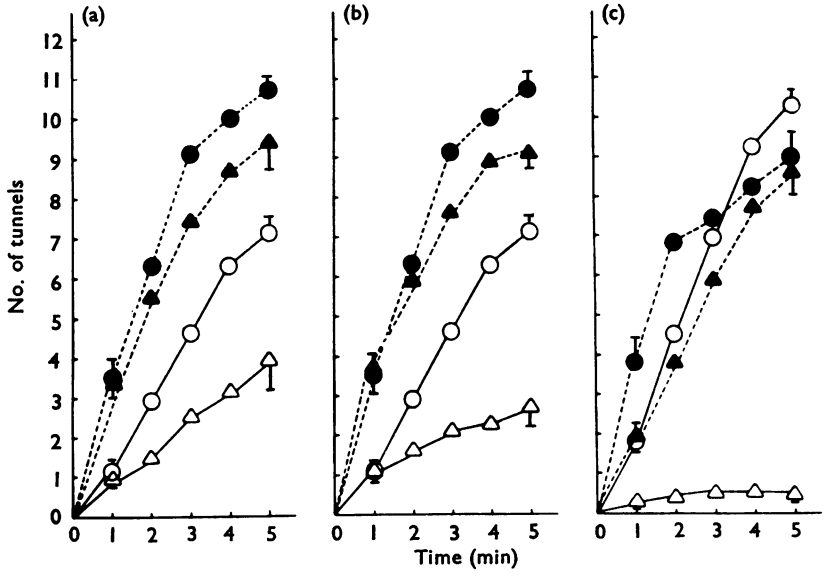

FIG. 4. Average number of different tunnels entered cumulatively over the 5 min observation period by mice treated with thioridazine (a) 4 mg, (b) 8 mg, (c) 16 mg on day 1 (△) and untreated on day 2 (▲), with a control group day 1 (○) and day 2 (●).

TABLE 1. *Average number* (±S.E.) *of different tunnels entered in the first minute and after 5 min and the total number of entries* (±S.E.) *into tunnels made by mice on the tunnel board over 5 minutes*

Drug and dose	day 1 and day 2	Progress of exploration: 1st min	Progress of exploration: 5 min	Average total entries
Haloperidol				
2 mg	1	1·7±0·52	2·7±0·59*	4·0±0·95*
	2	2·6±0·62	9·3±0·58†	23·2±1·81†
Control	1	1·9±0·31	7·8±0·84	18·1±2·04
	2	4·2±0·55‡	9·2±0·76	18·6±2·56
4 mg	1	0·44±0·24	0·77±0·28**	1·0±0·35**
	2	1·77±0·22**	8·22±0·52*‡	15·5±2·17**‡
Control	1	1·20±0·20	6·0±0·58	11·2±1·08
	2	4·3±0·56‡	9·6±0·43‡	24·0±1·91†
8 mg	1	0·1±0·1	0·3±0·15**	0·4±0·22**
	2	1·2±0·25*	7·3±0·68*‡	12·9±1·48**‡
Control	1	0·8±0·43	6·3±0·67	13·8±2·77
	2	2·7±0·49‡	9·7±0·68†	19·0±2·67
Chlorpromazine				
2 mg	1	0·5±0·16	3·4±0·61*	5·9±1·62*
	2	2·7±0·54	8·5±0·88‡	15·5±1·70‡
Control	1	1·6±0·45	6·2±0·81	11·3±1·66
	2	2·5±0·47	8·2±0·61	18·9±2·01‡
4 mg	1	0·4±0·16	1·5±0·54**	1·7±0·65**
	2	1·6±0·34	6·9±0·43‡	14·7±0·57‡
Control	1	0·5±0·16	5·3±0·26	9·0±1·14
	2	2·5±0·45‡	7·6±0·82†	15·5±2·04†
8 mg	1	0·4±0·16	1·2±0·39**	1·4±0·52**
	2	1·1±0·31**	6·8±0·69**‡	14·9±2·68*‡
Control	1	1·5±0·27	6·9±0·64	16·1±1·7
	2	4·0±0·56‡	9·3±0·42‡	22·6±1·23‡
Thioridazine				
4 mg	1	0·9±0·28	3·9±0·72**	8·4±1·74*
	2	3·4±0·43	9·4±0·62‡	20·2±1·73‡
8 mg	1	1·1±0·35	2·7±0·50**	3·8±0·91**
	2	3·6±0·40	9·1±0·43**‡	21·0±2·73‡
Control	1	1·1±0·23	7·1±0·48	13·8±0·87
	2	3·5±0·40‡	10·7±0·37‡	21·8±2·11‡
16 mg	1	0·27±0·14	0·46±0·21**	0·45±0·21
	2	1·91±0·34*	8·63±0·61	17·40±1·34
Control	1	1·8±0·20	10·2±0·39	23·1±1·31
	2	3·8±0·55‡	8·9±0·61	19·9±1·62
Amphetamine				
2 mg	1	1·0±0·39	5·0±0·92	13·7±2·7
	2	2·7±0·63	9·2±0·74	18·3±1·68
Control	1	1·3±0·39	8·2±0·65	16·8±1·36
	2	3·9±0·43‡	9·3±0·56	20·0±2·65
4 mg	1	0·62±0·41	4·12±1·15	15·7±4·62
	2	2·42±0·61	8·0±1·11†	18·7±3·57
Control	1	1·75±0·36	6·25±0·90	12·3±1·81
	2	3·12±0·39†	8·37±0·49†	16·1±2·2
8 mg	1	0·9±0·31	4·6±0·80**	12·9±2·14
	2	1·7±0·39	7·6±0·75†	15·0±1·72
Control	1	0·9±0·23	7·5±0·73	14·6±1·86
	2	2·7±0·47‡	8·0±0·68	16·2±1·14
Tranylcypromine				
2 mg	1	1·45±0·28	9·2±0·65	21·7±1·79**
	2	4·91±0·46	11·0±0·36**	28·7±1·64*†

[*Table is continued on facing page*

TABLE 1—*continued*

Drug and dose	day 1 and day 2	Progress of exploration: 1st min	Progress of exploration: 5 min	Average total entries
Tranylcypromine				
4 mg	1	1·72±0·24	9·0±0·36	19·09±1·74
	2	4·09±0·28	9·90±0·46	21·54±1·55
Control	1	1·1±0·18	7·5±0·69	13·9±1·64
	2	3·8±0·51‡	9·4±0·37†	21·2±2·61†
Pargyline				
110 mg	1	2·6±0·52	8·9±0·48	24·3±3·09
	2	4·1±0·50	10·4±0·50	26·2±2·17
220 mg	1	1·9±0·46	8·1±0·43	18·3±1·76
	2	3·1±0·28	9·9±0·72	21·6±1·41
Control	1	1·9±0·35	7·8±0·63	19·3±1·89
	2	3·6±0·43‡	9·2±0·66	22·1±2·34
Nialamide				
100 mg	1	2·19±0·24	9·76±0·28	22·09±1·30*
	2	4·38±0·20	10·33±0·23*	21·3±0·90
Control	1	1·8±0·47	7·8±0·36	17·4±1·93
	2	3·7±0·30†	9·2±0·53†	21·1±1·95
Imipramine				
20 mg	1	0·8±0·33	5·7±0·90	9·1±1·67*
	2	3·5±0·45†	9·5±0·40†	19·6±1·38
40 mg	1	0·4±0·22	2·6±0·67**	4·3±1·35*
	2	2·4±0·37†	8·7±0·45†	18·6±1·34
Control	1	1·4±0·22	7·2±0·65	14·0±1·60
	2	2·9±0·57†	9·3±0·52†	17·6±1·39

† Significant difference between day 1 and day 2 ($P<0·05$)
‡ Significant difference between day 1 and day 2 ($P<0·01$–$0·001$)
* Significant difference between treated and control of the same day ($P<0·05$)
** Significant difference between treated and control of the same day ($P<0·01$–$0·001$)

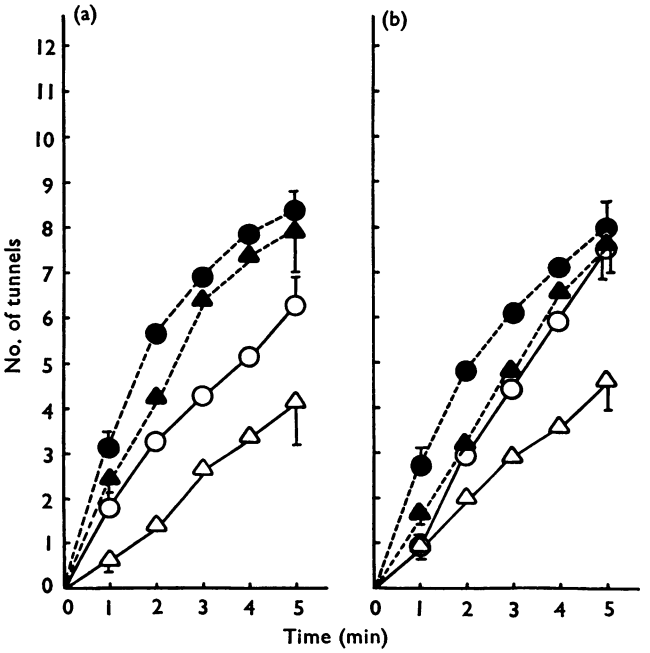

FIG. 5. Average number of different tunnels entered cumulatively over the 5 min observation period by mice treated with amphetamine (a) 4 mg, (b) 8 mg on day 1 (△) and untreated on day 2 (▲), with a control group day 1 (○) and day 2 (●).

Amphetamine

The effect of amphetamine 4 mg/kg and 8 mg/kg on the mice was to reduce the number of different tunnels entered in spite of an increase in locomotion which was apparent to an observer. On the second day the mice which had been given amphetamine 4 mg/kg on the preceding day behaved like second day controls, but those given 8 mg/kg behaved like inexperienced mice (Fig. 5). 2 mg/kg amphetamine did not change the behaviour of the mice on the tunnel board.

Monoamine oxidase inhibitors

The monoamine oxidase inhibitors increased the number of different tunnels entered on the first day, and this difference was maintained on the second day. These mice were observed for the first time 24 h after receiving the drug because of the delay in action of these compounds on monoamine oxidase. Tranylcypromine at 2 mg and 4 mg/kg was the most potent monoamine oxidase inhibitor used. Nialamide at 100 mg produced a similar increase but 75 mg/kg had no effect and 200 mg/kg made the mice very ill. Pargyline at 110 mg/kg also produced an increase but it was not as evident as with the other compounds and was not statistically significant. These results are shown in Fig. 6, and it is clear that the curves for the drug-treated groups always fell to the left of the control curves.

Imipramine

Imipramine at 10 mg had no obvious effect on the mice, but at 20 mg/kg the mice were slightly sedated on the first day so that the number of different tunnels entered was lower than that of the control group. On the second day, however, the

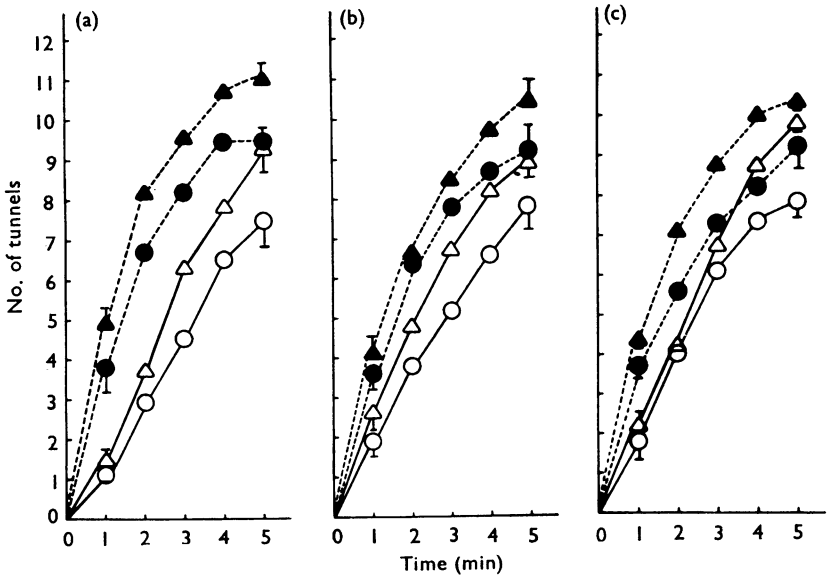

FIG. 6. Average number of different tunnels entered cumulatively over the 5 min observation period by mice treated with monoamine oxidase inhibitors (a) tranylcypromine 2 mg, (b) pargyline 110 mg, (c) nialamide 100 mg on day 1 (△) and untreated on day 2 (▲), with a control group day 1 (○) and day 2 (●). These mice were treated 24 h before observation.

treated mice moved through more different tunnels more quickly and appeared to behave in a similar way to the mice treated with monoamine oxidase inhibitors (Fig. 7).

Discussion

Exploratory behaviour is the way in which animals learn the character of the surroundings in which they live, for it is during exploration that the pathways, food supplies and objects of the area become familiar (Thorpe, 1956 ; Shillito, 1963). In rodents locomotion is an essential part of exploration as the animal needs to cover the area to learn its nature in any detail, but it is not necessary for a mouse or rat to move very much for it to be exploring.

If exploratory behaviour has occurred, it will be demonstrated by a change in the behaviour of the animal, thus showing that learning has taken place. This can be seen when a mouse or rat is put in the same situation on two successive days, or even with some longer time interval between trials. Normal rats behave differently on two successive trials in a Y-maze as they move much less on the second

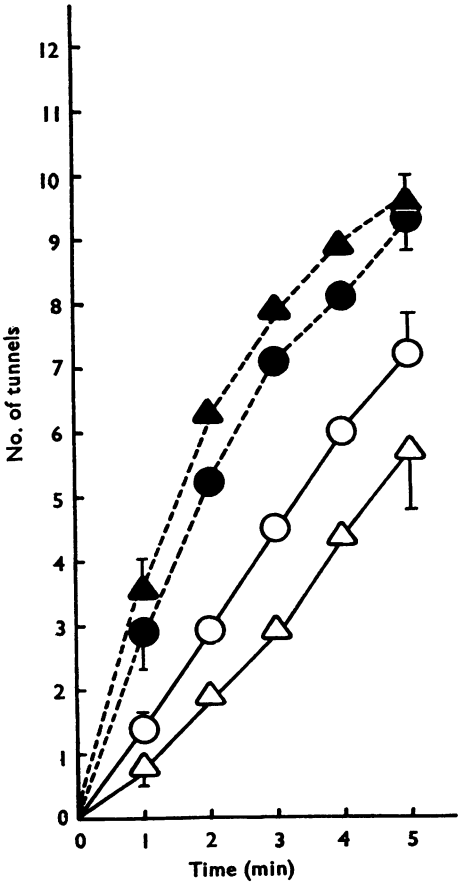

FIG. 7. Average number of different tunnels entered cumulatively over the 5 min observation period by mice treated with imipramine 20 mg on day 1 (△) and untreated on day 2 (▲), with a control group day 1 (○) and day 2 (●).

trial (Shillito, 1967). Rushton, Steinberg & Tinson (1963) and Rushton, Steinberg & Tomkiewicz (1968) have found more complicated changes in behaviour which they attribute to an interaction between maze experience and drugs. The experiments reported in this paper show that mice demonstrate learning on the tunnel board by moving more, and entering more different tunnels more quickly on the second day. This is probably because the mice no longer avoid the tunnels after one experience on the board on the previous day from which they received no adverse stimulation. So this change has been taken as an indication that exploration had occurred on the first day of the experiment, and that the mice recognize the environment on the second day. If the mice behaved as if they were in a strange situation on the second day, that is moving less round the board and entering a few different tunnels slowly, this was taken as an indication that exploration or retention of the experience had been affected on the first day.

When the tunnel board was designed, it was found that the diameter of the tunnels was important. Wide tunnels were not explored but the mice just ran through them, and narrow tunnels delayed entry a long time. Similarly the shorter tunnels were not entered but only investigated at both ends, and in longer tunnels the mice tended to stay inside. At first the arrangement of the tunnels was random, but after some experience a fixed arrangement was used which has considerable advantages when comparisons are made.

The results of the experiments using sedatives showed that a mouse had only to move a little to be able to explore and receive enough information for behaviour to be similar to control mice on the second day. In fact the doses of chlorpromazine, thioridazine and haloperidol which impaired exploratory behaviour almost immobilized the mice. At the lower doses, these drugs reduced locomotion on the board without affecting exploration, and the mice all showed that they had been able to learn on the previous day. So these drugs only impair exploratory behaviour when the mouse is too sedated to move.

Although it is known that amphetamine increases activity in mice (Dews, 1953 ; Shillito, 1966), the treated animals appeared to be confused. They entered the same tunnels repeatedly, and on the second encounter with the tunnel board they appeared to be inexperienced mice as if on the first encounter they had moved without exploring and learning the situation. Thus exploration was affected although locomotion was increased. Chance & Silverman (1964) describe the behaviour of amphetamine-treated rats by saying that " they seem to indulge in apparently aimless activities ", and perhaps this could be applied to the increased locomotion seen in these mice.

The effect of the monoamine oxidase inhibitors contrasts with that of amphetamine. They are often described as having " amphetamine-like " effects, but these experiments showed that the increase in locomotion was different from the increase caused by amphetamine 8 mg, and at 2 mg and 4 mg amphetamine did not change the behaviour of the mice on the board so it is not a matter of relative dose levels. The monoamine oxidase inhibitors were the only compounds used that were found to increase the number of different tunnels entered by the mice in the first minute on the first day, which was 24 h after drug treatment, so that the inhibition of the oxidase was complete. On the second day this difference was maintained and the treated mice still entered more different tunnels more quickly than the controls.

This second day effect was also shown by imipramine when the drug had been acting for the same length of time as the monoamine oxidase inhibitors on the first day. On day one, imipramine seemed to be slightly sedative and the progress of exploration was slower than in the controls. As both classes of drugs are effective in the treatment of depression, it is interesting that they influence this aspect of mouse behaviour in a similar way.

The value of these measurements lies in their independence from the effect of drugs on general locomotion in an area. In fact activity as such is not measured by this method although the mice move a lot between and round the tunnels. The constant change in behaviour that indicated whether the mouse was in a "known" situation or an "unknown" situation was the number of different tunnels which were entered quickly and this was taken as the measure of exploration. A mouse moves around an area for a number of reasons, one of which is exploration, but this activity does not appear to be directly correlated with the amount of exploratory behaviour which has occurred in a given situation and time.

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