Br. J. Pharmac. Chemother. (1967), **30**, 258–264.

THE EFFECT OF CHLORPROMAZINE AND THIORIDAZINE ON THE EXPLORATION OF A Y-MAZE BY RATS

BY

ELIZABETH E. SHILLITO

From the Agricultural Research Council Institute of Animal Physiology, Babraham, Cambridge

(*Received November* 9, 1966)

When a rodent is put into a situation with which it is unfamiliar, exploratory behaviour is elicited and the animal investigates the surroundings. In exploration, all the animal's sense organs are used to receive stimuli from the unfamiliar area and generally it moves through the area to increase the range of available stimuli. Many workers have used this movement as a measure of exploratory behaviour in the animal, and in particular Steinberg, Rushton & Tinson (1961), Rushton & Steinberg (1963), Rushton, Steinberg & Tinson (1963) and Marriott & Spencer (1965) have studied movement in a Y-maze by rats and the way in which it is affected by various drugs.

As a result of some differences found in the effects of chlorpromazine and thioridazine on the behaviour of mice and cats, which remained more active under thioridazine than under chlorpromazine a comparison was made between the effects of these two drugs on the exploration of a Y-maze by rats. Marriott & Spencer (1965) had found a decrease in the number of entries made into each arm of the maze when doses of chlorpromazine of 1 mg/kg, 4 mg/kg and 8 mg/kg were used. A similar procedure was followed as described by these workers.

METHODS

Male albino rats, supplied from Tucks, were used. They weighed between 80 g and 112 g and were housed in groups of 4 animals to a cage for 3 or 4 days before the experiments started. One day was allowed for conditioning to handling and injection. On this day they were taken to the laboratory and weighed and marked, and 3 hr later they were injected with saline and returned to their cages. After another 2 hr they were returned to the animal house. On the following day, the animals were taken to the laboratory and weighed 2–3 hr before they were injected with the drug. Groups of 8 animals were used at each dose level. In each experiment, one group was injected with saline and two groups were given one of the phenothiazines. The chlorpromazine and thioridazine were used as hydrochlorides dissolved in saline. The thioridazine turned blue on exposure to light and was kept out of the light when possible.

The rats were injected subcutaneously into the neck 35 min before they were put into the Y-maze. The maze was made of three-ply wood varnished inside. It consisted of three arms 15 in. long and 4 in. wide which fitted on to a central triangle. It stood on the floor and was lit by a 200 watt lamp in the ceiling 2 m above it. The animals were put into one arm of the maze and watched for 5 min. During this time the number of entries into each arm, the number of rears and the number of times washing started were recorded. Defaecations were also counted. The rats were returned to their own cages after each trial and the cages were returned to the animal room each

day. This procedure but without weighing and injection was repeated on the second day and in some experiments on a third day.

RESULTS

When the control rats were put into one arm of the Y-maze, they either paused at first and then moved, or moved at once entering each arm and standing up with the front feet against the walls of the apparatus. Their activity was higher on the first day than on the second day. Both the number of entries made by the rats and the frequency of rearing were recorded and the effects of drugs on these parameters were very similar. As the frequency of rearing varied more between individuals than the number of entries made, only the latter was used as a measure of drug effect. These results are shown in Table 1.

TABLE 1

AVERAGE NUMBER OF ENTRIES MADE IN THE INVESTIGATION OF A Y-MAZE BY RATS GIVEN TWO PHENOTHIAZINES

Drug and dose (mg/kg)	Average number of entries 1st day	2nd day	3rd day
Chlorpromazine			
1	9·0 ±1·66*	5·6 ±1·87	5·7 ±1·46
2	5·8 ±1·37**	6·8 ±1·12	6·7 ±1·50
Saline	17·1 ±1·27	6·3 ±2·03‡	5·0 ±1·54
1	4·0 ±1·27	4·25±1·12	
2	2·5 ±0·92*	5·12±1·8	
Saline	7·8 ±1·8	2·25±0·64†	
4	1·0 ±0·42**	12·8 ±2·0‡	
8	0·12±0·12**	11·2 ±1·9‡	
Saline	11·7 ±1·58	4·5 ±1·58†	
4	1·37±0·51*	2·0 ±0·96	
8	1·5 ±0·36*	6·6 ±1·87†	
Saline	7·0 ±1·77	1·6 ±0·58†	
4	2·12±0·61**	10·6 ±1·97†	
Saline	18·0 ±2·37	3·25±0·91‡	
Thioridazine			
2	11·75±2·52	4·12±1·52†	
4	11·3 ±1·5	3·87±0·91†	
Saline	13·6 ±2·45	5·75±1·12†	
2	10·8 ±1·87	6·8 ±2·24	
8	7·0 ±2·75	4·6 ±1·41	
Saline	12·3 ±2·5	5·2 ±1·38†	
4	6·5 ±2·0*	3·3 ±1·84	
16	6·6 ±1·8*	5·8 ±1·84	
Saline	14·0 ±1·46	4·8 ±1·34‡	
4	15·25±1·24	6·3 ±0·98‡	
Saline	18·0 ±2·37	3·25±0·91	
16	7·0 ±2·18*	3·2 ±2·0	
32	8·3 ±2·19*	5·7 ±1·9	
Saline	14·3 ±1·73	3·1 ±0·95‡	
8	17·0 ±1·12	6·25±1·8‡	4·1 ±0·56
32	9·62±2·12*	6·5 ±1·5	3·37±0·94
Saline	17·75±1·87	6·75±2·42†	5·25±0·93
64	7·2 ±2·2*	3·0 ±1·43	
Saline	17·0 ±1·62	10·2 ±1·23†	

Values are means and standard errors.

* On figures for the 1st day indicates significant difference from controls; *$P=<0{\cdot}05+P-<0{\cdot}01$; **$P=<0{\cdot}001$.

† On figures for the 2nd day indicates significant difference between days: †$P=<0{\cdot}05+P=<0{\cdot}01$; ‡$P=<0{\cdot}001$.

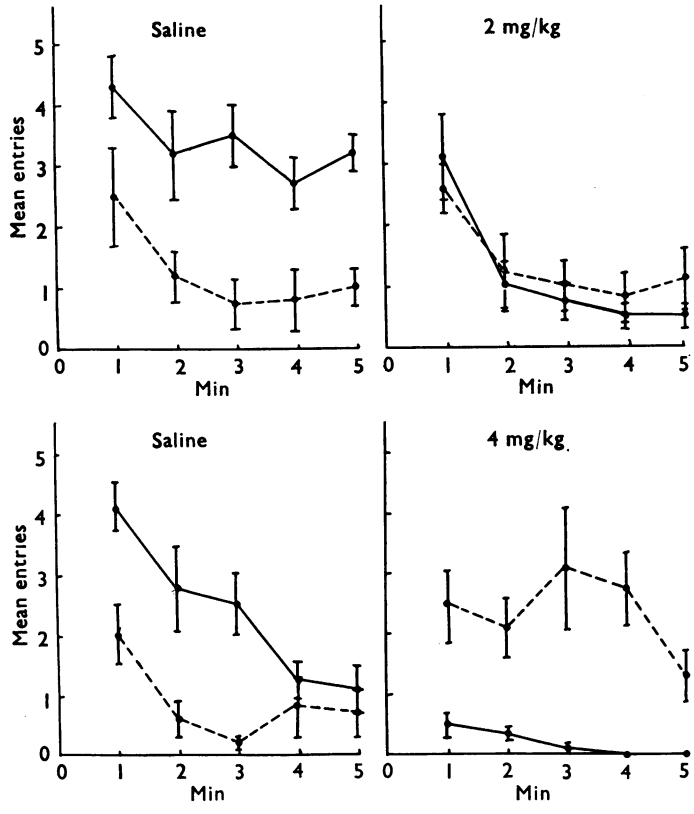

Fig. 1a. The effect of chlorpromazine on the exploration of a Y-maze by rats. ●——● on the day of injection. ●----● on the following day. Each group injected with drug is shown with the control group of its series. Vertical lines indicate standard errors.

Figures 1a and b show how movement decreased over the 5 min observation period. For statistical purposes the total number of entries made in each group has been used.

After moving around and in between visits to each arm, the rats sat and washed. Grooming behaviour out of its normal context has been interpreted as an indication of uncertainty and conflict within the animal. Individual variation was large, but as a rule it was lower on the second day and the only drug effect was an inhibition by doses of chlorpromazine high enough to depress activity in general. There was no correlation between the incidence of defaecation and any dose of phenothiazine.

The rats treated with chlorpromazine 4 mg/kg and 8 mg/kg appeared ataxic and showed loss of muscle tone, but at 1 mg/kg and 2 mg/kg these effects were absent.

Ataxia or loss of muscle tone were not evident with the thioridazine-treated animals and even when the rats moved about less they appeared alert and co-ordinated.

It can be seen from Table 1 that with the exception of thioridazine 1 mg/kg and 2 mg/kg, which had no effect, both phenothiazines at various doses caused a reduction in the number of entries into the arms of the Y-maze. With thioridazine 4 mg/kg and

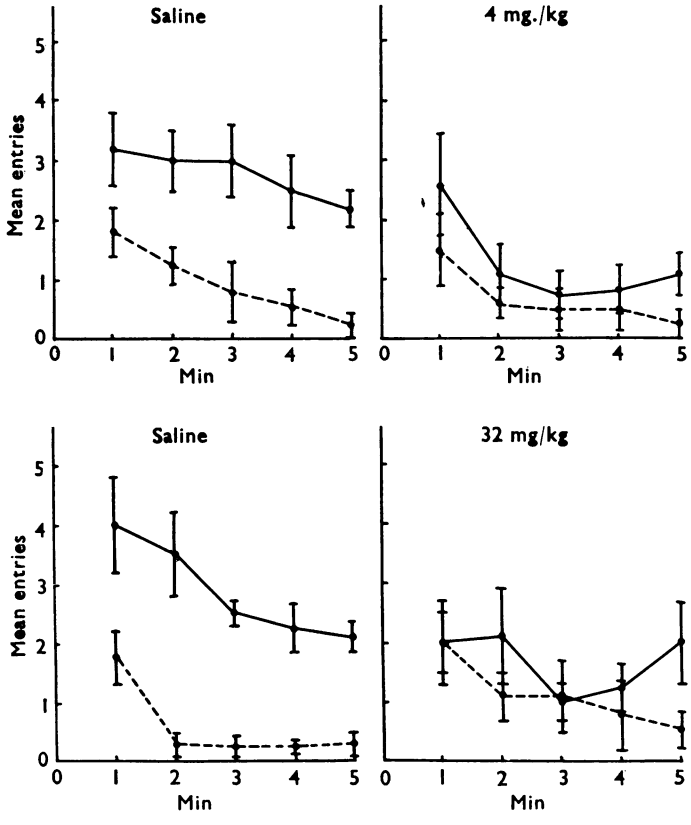

Fig. 1b. The effect of thioridazine on the exploration of a Y-maze by rats. ●——● = on the day of injection. ●----● = on the following day. Each group injected with drug is shown with the control group of its series. Vertical lines indicate standard errors.

8 mg/kg this reduction was not always significant but it was so with 16 mg/kg, 32 mg/kg and 64 mg/kg. After any dose of thioridazine the number of entries on the second day was normal. Chlorpromazine 1 mg/kg sometimes, and higher doses always, significantly depressed the number of entries on the day of injection. The following day, entries were normal after 1 mg/kg or 2 mg/kg, but with doses of 4 mg/kg and 8 mg/kg they were greatly increased in number, and they were often as frequent as in a rat which had not been in the maze before. These results are illustrated in the histograms in Fig. 2.

DISCUSSION

In a study of exploratory behaviour in the short-tailed vole (Shillito, 1963) exploration is described as a behaviour pattern which the animal possesses " for the purpose of learning." It is an instinctive behaviour pattern with a drive to know the topography of the surroundings. The consummatory situation for the drive is the reception of environmental stimuli, the novelty of which will determine whether the animal will start

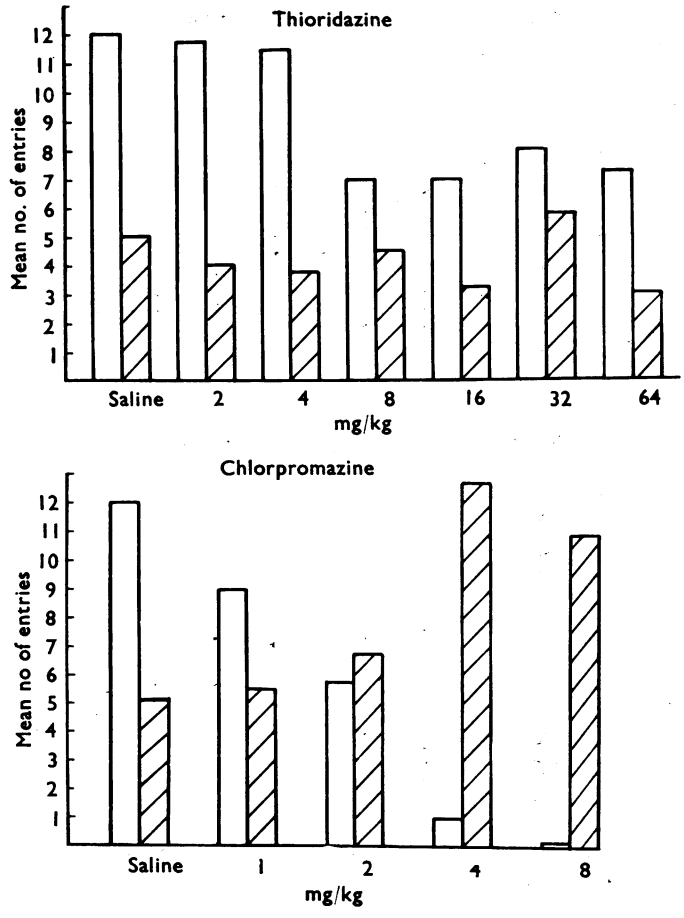

Fig. 2. The effect of chlorpromazine and thioridazine on the total number of entries made into the arms of a Y-maze by rats. On the day of injection (open block) and on the following day (shaded block). One example of a saline group is shown.

orientated investigation which follows a set pattern of behaviour sometimes described as a "new object reaction," or reconnaissance behaviour which is the way in which the known surroundings are checked.

This description of exploratory behaviour can be applied to rats in the Y-maze. In the first experiment they are in a "new situation" and investigation is elicited. As the maze is learnt movement decreases and the rats' behaviour shows less tension. On a second encounter, the movement of the rats is markedly decreased. There is a statistically significant difference in the number of entries made into the arms of the maze by the rats on the first or second encounter in a control group. The movement that occurs on the second day represents the random reconnaissance behaviour in which the surroundings are checked although they are known. The decrease in movement can be taken to demonstrate that learning has taken place. Behaviour on the third day is similar to that on the second day.

After adequate doses of thioridazine and the lower doses of chlorpromazine, activity was depressed on the first day without any change in the number of entries made on the second day. It seems that the animals treated in this way showed a reduced " new object reaction," but their investigation was sufficient for learning to have taken place.

With the higher doses of chlorpromazine (4 mg/kg and 8 mg/kg) the number of entries made by the rats were lower than any made by normal rats on either day of the experiment. There must have been very little perception of stimuli and on the second day movement was increased to the frequency of a normal rat on the first day. This increase in movement was not just a rebound phenomenon following heavy sedation as rats treated with chlorpromazine 8 mg/kg and tested on the day of injection and 48 hr, instead of 24 hr later, showed a similar reaction—namely, increased movement which was equal to that of a saline control group tested for the first time. So apparently no learning had taken place while the rats were under the influence of chlorpromazine.

It might be argued that the rats treated with thioridazine in doses exceeding 8 mg/kg, and which showed a low number of entries on the first day, had low entry figures on the second day because the effect of the drug had not worn off. The observation that on a third successive day the number of entries remained the same rules out this possibility.

These results indicate that the normally high level of activity, which is seen in rats when they are put into " new situations," is excessive to their requirements for perception of stimuli and learning to take place. In fact the movement observed in the groups of saline-treated animals showed a wide variation on the first day. It is obvious that all the activity was not necessarily exploratory behaviour, but was an expression of the nature of the animals. Thus control rats which moved a lot on the first day also tended to move more than less inactive controls on the second day, although the difference between the 2 days was considerable. It is the lack of movement which is correlated with the lack of exploratory behaviour. The stimulus of novelty elicits investigation but also may elicit fear which reduces movement. It is contrary to the evidence for neuroleptic action of the phenothiazines to suggest that the reduced movement in the drug-treated animals could have been the result of increased fear. It might, however, explain the variation in the control groups, particularly as this variation was reduced by the first day conditioning to handling and injection.

These experiments have shown that there is a qualitative difference in the response of rats to chlorpromazine, which in the right doses inhibited learning of the surroundings, and thioridazine, which, even at 16 times the effective dose of chlorpromazine, did not have this effect. The reduction in the number of entries made by the rats into the arms of the maze was comparable at chlorpromazine 2 mg/kg and thioridazine 16 mg/kg, 32 mg/kg and 64 mg/kg ; thus in the latter drug beyond 16 mg/kg there was no graded response to an increase in dose as was seen with chlorpromazine (Fig. 2). This shows that the difference between the two phenothiazines is not purely quantitative.

Whether the inhibition of learning is brought about by a blocking of sensory pathways, or by a degree of motor inhibition so severe that learning is brought to a standstill, is not certain. It is interesting that the comparable dose for these phenothiazines that is effective in inhibiting a learned sound-conditioned habit in rats is chlorpromazine 0.93 mg/kg and thioridazine 20 mg/kg (Haase & Janssen, 1965). This might indicate that the blocking of sensory pathways is a more probable cause than motor inhibition, in spite of

the fact that the two behavioural tests are not comparable as the exploratory behaviour is concerned with reception of stimuli and acquisition of learning rather than with a learnt response.

It is interesting that a difference in the action of these two drugs does not appear to be found in its usefulness in schizophrenic patients, and in the treatment of other psychotic states in which the dose is similar for both compounds (chlorpromazine 75–500 mg; thioridazine 30–600 mg; *British Pharmacopoeia*, 1963). A qualitative difference is, however, shown by the low incidence of Pakinsonian side-effects seen with thioridazine Furthermore, biochemical differences of a qualitative nature were shown by Laverty & Sharman (1965), thioridazine lacking the effect on dopamine turnover in the brain which is found with chlorpromazine.

SUMMARY

1. The effects of chlorpromazine and thioridazine have been compared on the exploration of a Y-maze by rats. The experiments were repeated on the following day without any injections.

2. Chlorpromazine 1 mg/kg and 2 mg/kg reduced the number of entries made by the rats in the Y-maze, but their behaviour on the following day was normal.

3. Chlorpromazine 4 mg/kg and 8 mg/kg greatly reduced the number of entries made by the rats, and the animals were ataxic and showed loss of muscle tone. On the second day the rats made a similar number of entries to that made by normal rats on the first day.

4. Thioridazine 4 mg/kg, 8 mg/kg, 16 mg/kg, 32 mg/kg and 64 mg/kg reduced the number of entries made by the rats, and at all doses their behaviour on the following day was normal.

5. It is concluded that rats under the effect of chlorpromazine 4 mg/kg and over are unable to learn the topography of the surroundings, but at lower doses and at all the doses tried of thioridazine the rats were able to learn the nature of the surroundings although their movement was decreased.

I would like to thank Dr. M. Vogt, F.R.S., for her help in many ways.

REFERENCES

HAASE, H. J. & JANSSEN, P. A. J. (1965). *The Action of Neuroleptic Drugs*. North Holland Publishing Company, Amsterdam.

LAVERTY, R. & SHARMAN, D. F. (1965). Modification by drugs of the metabolism of 3,4-dihydroxyphenylethylamine, noradrenaline and 5-hydroxytryptamine in the brain. *Br. J. Pharmac. Chemother.*, **24**, 759–772.

MARRIOTT, A. S. & SPENCER, P. S. J. (1965). Effects of centrally acting drugs on exploratory behaviour in rats. *Br. J. Pharmac. Chemother.*, **25**, 432–441.

RUSHTON, R. & STEINBERG, H. (1963). Mutual potentiation of amphetamine and amylobarbitone measured by activity in rats. *Br. J. Pharmac. Chemother.*, **21**, 295–305.

RUSHTON, R., STEINBERG, H. & TINSON, C. (1963). Effects of a single experience on subsequent reactions to drugs. *Br. J. Pharmac. Chemother.*, **20**, 99–105.

SHILLITO, E. (1963). Exploratory behaviour in the short-tailed vole *Microtus agrestis*. *Behaviour*, **21**, 145–154.

STEINBERG, H., RUSHTON, R. & TINSON, C. (1961). Modification of the effects of an amphetamine-barbiturate mixture by the past experience of rats. *Nature, Lond.*, **192**, 533–535.