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The effect of benzodiazepines and atropine on exploratory behaviour and motor activity of mice

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Summary

1. Male albino mice were watched in red light on a tunnel board to test exploration and their motor activity was assessed in an open cage, 30 min after intraperitoneal injection of drugs.

2. Atropine and methylatropine 5 or 10 mg/kg did not alter the motor activity of the mice, while chlordiazepoxide 25 or 50 mg/kg and diazepam 10 or 20 mg/kg increased the activity, especially at the lower of the two doses used.

3. All the compounds used except methylatropine adversely affected the exploratory behaviour.

4. When atropine 10 mg/kg was given with the benzodiazepines, the activity of the mice was reduced and exploratory behaviour was further impaired. Methylatropine did not have this effect.

Introduction

Although the benzodiazepines have been widely used as tranquillizing agents for 10 years, their psychopharmacological effects are still poorly understood. There are a few studies examining the role of the cholinergic nervous system in the action of these drugs. Frommel, Fleury, Schmidt-Ginzkey & Béguin (1960) reported that chlordiazepoxide has a very weak and questionable atropine-like central action in blocking tremorine excitation and salivation and in blocking the morphine Straub tail effect. On the other hand, the benzodiazepines do not potentiate and may even antagonize the peripheral effects of atropine-like drugs (Droppleman & McNair, 1968 ; Ahtee & Pohto, 1968).

Recently the combination of benzodiazepines and atropine-like drugs has been introduced in the treatment of gastric ulcer and related disorders (Head & Hammond, 1968). This paper deals with the effect of such combinations on the behaviour of mice. The potency of tranquillizing drugs is frequently evaluated by changes in motor activity, but such tests may overlook any effects on perception which the drugs might have. Accordingly the effects on exploratory behaviour in mice were determined, as well as recording effects on motor activity. Shillito (1963) defined exploratory behaviour as " a behaviour pattern which the animal possesses

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for learning its surroundings ". Since it requires a certain amount of perception, drug effects on perception can be detected by changes in exploratory behaviour.

Methods

Male albino mice weighing 15–20 g were used for the experiments. 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 drugs were tested on mice kept in reversed daylight conditions for 10 days before the experiment, so that their activity cycle was reversed. They were kept in red light from 10.00 h to 22.00 h and in white light from 22.00 h to 10.00 h. In these conditions the mice were in their normal activity period during the time of the experiments, which started about 14.00 h. All observations were made in red light.

Exploration was measured as described by Shillito (1968, 1970). Mice were placed one at a time on the left hand corner of a wooden board measuring 61 × 61 cm, on to which twelve plastic tunnels 7·5 cm long and 4 cm in diameter had been fixed and arranged in a symmetrical pattern. Each tunnel was numbered. Each mouse was watched for 5 min on two consecutive days. The number of each tunnel entered by the mouse was recorded for each minute of the observation period; from this the number of different tunnels entered was calculated and this gave the measure of exploration. The progress of exploration was defined as the number of different tunnels entered cumulatively over the 5 min observation period and was expressed graphically. The difference between the number of different tunnels entered in the first minute on 2 days showed whether exploratory behaviour had been affected.

After assessing the exploratory behaviour each mouse was put individually into an open metal box measuring 35 cm × 52 cm, height 12 cm. Here the motor activity was studied for 3 min using two kinds of criteria: the time which the mouse spent moving round the cage was recorded by dividing the 3 min observation period into 10 s periods and recording the dominant kinds of behaviour during each period. These were moving, resting or grooming. Furthermore, differences in the running speed of the mice were observed. The control mice moved rather slowly, stopping to sniff the surroundings, while the treated mice often ran round the cage very fast without any obvious purpose. Therefore the number of times the mouse crossed the observation box from end to end and ran back was also counted. The distance covered by such a run was at least 104 cm. Sometimes these observations were repeated on the second day.

The drugs used were: chlordiazepoxide hydrochloride and diazepam (Roche Products Ltd.), atropine sulphate and atropine methyl bromide (The British Drug Houses Ltd.). Diazepam was dissolved in a minimum volume of 1 N NaOH, neutralized with 1 N HCl and diluted further with saline. All the other drugs were dissolved in saline. Drugs were given by intraperitoneal injection 30 min before the observation on the first day. On the second day no injections were given.

The results of treated and control groups were compared statistically using the Student's *t* test. The number of different tunnels entered during the first minute of the exploration time on the 2 days was compared to yield a measure of the effect on exploratory behaviour. The number of 10 s periods during which the mice

were active and the number of crossings were compared to obtain measurements of activity.

Results

Motor activity

The control mice moved actively around the cage for about 2·5 min of the 3 min observation period. They crossed the cage about six times during these 3 min. The rest of the time they spent grooming or resting quietly. On the second day they tended to be less active although the differences were not statistically significant.

In doses of 25 mg/kg of chlordiazepoxide and of 5 to 10 mg/kg of diazepam, the benzodiazepines increased both the time the mice spent moving around and particularly the number of crossings (Fig. 1). When injected with higher doses, 50 mg/kg of chlordiazepoxide and 20 mg/kg of diazepam, the time the mice spent moving was less or equal to that seen in the controls, although the injected mice crossed the cage more often. When the doses were increased another 2–3 times, the mice became inactive, and they required only slightly higher doses to lose their righting reflex. With all doses of the benzodiazepines the movements of the mice appeared to be less co-ordinated than those of the controls. They staggered, sometimes ran very quickly and then remained still, and they spent less time than the controls sniffing the surroundings or grooming. When they were observed again 24 h later their motor activity was similar to or slightly higher than that of the controls.

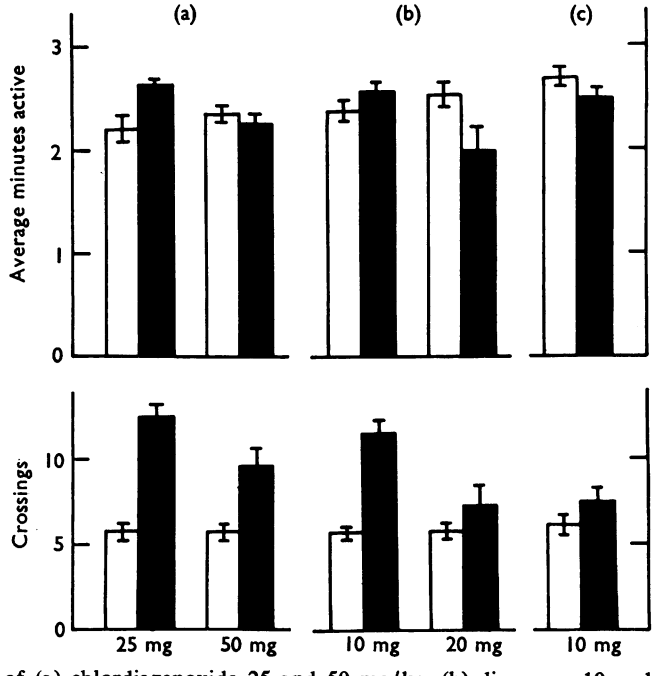

FIG. 1. Effect of (a) chlordiazepoxide 25 and 50 mg/kg, (b) diazepam 10 and 20 mg/kg and (c) atropine 10 mg/kg on motor activity of mice 30 min after intraperitoneal injection. Upper columns, average number of minutes active ; lower columns, average number of crossings. White columns, control mice ; black columns, treated.

The effect of atropine on the activity of mice was tested with doses of 2·5, 5·0, 7·5, 10 (Fig. 1) and 20 mg/kg. There was no effect on activity and exploration. Methylatropine did not cause any change in the activity of the mice.

When atropine 5 mg/kg and chlordiazepoxide 25 mg/kg were given together (Fig. 2), the mice behaved as if they had had only chlordiazepoxide. After 50 mg/kg of chlordiazepoxide and 10 mg/kg of atropine, however, the mice were very sedated, the time spent moving and the number of crossings were halved and some of the mice even lost their righting reflex. Mice treated with 10 mg/kg of atropine and 25 mg/kg of chlordiazepoxide tended to be quieter than the mice treated with chlordiazepoxide alone, although the changes were not significant. Methylatropine 10 mg/kg did not modify the effect of 50 mg/kg of chlordiazepoxide. A similar pattern of interaction was also seen with diazepam and atropine or methylatropine (Fig. 3), the doses of 10 mg and 20 mg/kg being equivalent to the two doses of chlordiazepoxide.

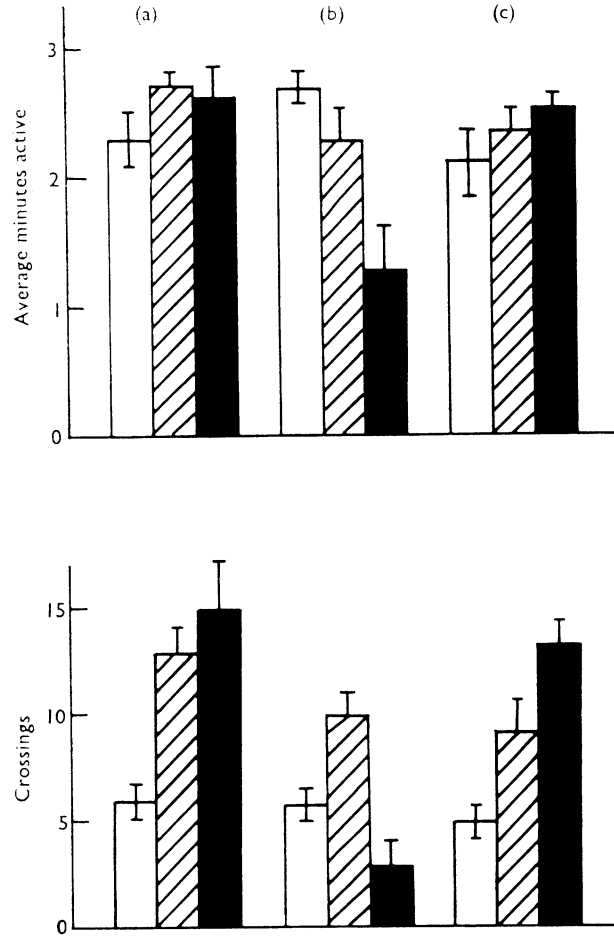

FIG. 2. Effect of (a) chlordiazepoxide 25 mg/kg and atropine 5 mg/kg, (b) chlordiazepoxide 50 mg/kg and atropine 10 mg/kg and (c) chlordiazepoxide 50 mg/kg and methylatropine 10 mg/kg on motor activity of mice 35 min after intraperitoneal injection. Upper columns, average number of minutes active; lower columns, average number of crossings. White columns, control mice; shaded, chlordiazepoxide alone; black, both drugs.

Toxicity

When the benzodiazepines were combined with methylatropine 10 mg/kg, one or two mice died from the group of ten also given chlordiazepoxide 50 mg/kg or diazepam 20 mg/kg. No deaths occurred when these doses of benzodiazepines were combined with 10 mg/kg of atropine. Therefore the effect of 10 mg/kg of atropine and methylatropine on the toxicity of benzodiazepines was studied. Again it was found that in groups of ten mice treated with 10 mg/kg of methylatropine and 50 mg/kg of chlordiazepoxide or 20 mg/kg of diazepam one or two mice died, whereas there were no deaths when atropine was used. However, atropine or methylatropine did not cause any significant change in the toxicity of higher doses of benzodiazepines up to 400 mg/kg. If anything the mice tolerated higher doses of benzodiazepines better when they were combined with atropine or methylatropine.

Exploratory behaviour

When a control mouse was put on the tunnel board for the first time, it moved around, walking between the tunnels. The mouse sniffed the entrance and sides of

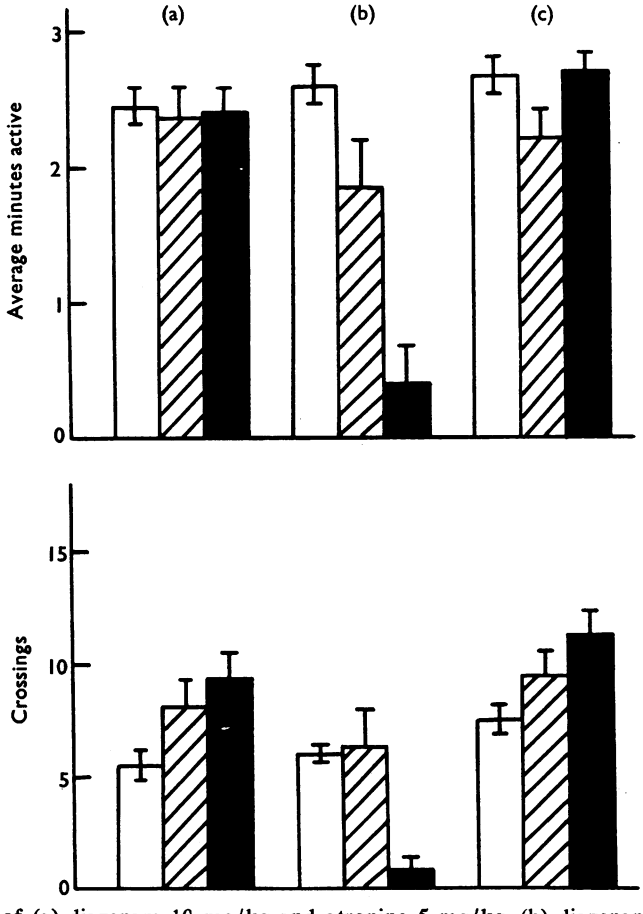

FIG. 3. Effect of (a) diazepam 10 mg/kg and atropine 5 mg/kg, (b) diazepam 20 mg/kg and atropine 10 mg/kg and (c) diazepam 20 mg/kg and methylatropine 10 mg/kg on motor activity of mice 35 min after intraperitoneal injection. Upper columns, average number of minutes active; lower columns, average number of crossings. White columns, control mice; shaded, diazepam alone; black, both drugs.

each tunnel and touched it with the nose, vibrissae and front feet and gradually the tunnel was entered. In the first minute of observation only one or two different tunnels were entered and about six or eight different tunnels were entered during 5 min. The total number of entries into the tunnels varied between groups, some made twelve to fifteen entries while more active groups made an average of twenty-five entries. Generally the control mice entered the centre tunnels before the peripheral ones and there was much movement around and among the tunnels.

On the second day, the untreated control mice behaved differently from the first day in that more different tunnels were entered quickly. This difference showed most clearly in the first minute of observation when three to five different tunnels were entered and this difference was statistically significant (P at least$<0{\cdot}05$). Experienced mice moved more quickly than inexperienced mice, so that they ran rather than walked round the tunnel board. When the results were drawn on a graph so that the number of different tunnels entered cumulatively was plotted for each minute, the slope of the line obtained for the second day was steeper than that for the first day. When this difference was also seen in the mice treated with drugs, it was assumed that exploration had not been altered by the drug treatment on the first day. But when the treated mice behaved overtly on the second day in a similar way to first day control mice, it was assumed that the drug treatment on day 1 had impaired exploration and the mice were behaving as inexperienced mice.

The effect of chlordiazepoxide and diazepam on the mice was similar for both drugs given, according to their relative potencies. Chlordiazepoxide 25 mg/kg and diazepam 10 mg/kg reduced the number of different tunnels entered. However, exploration did not seem to be affected, as on the second day of observation the mice behaved as if they had been on the board before, although one or two mice started off slowly. With chlordiazepoxide 50 mg/kg and diazepam 20 mg/kg the mice moved a lot around the tunnels on the first day, but fewer different tunnels were entered than with controls ($P<0{\cdot}001$), and on the second day the mice behaved as inexperienced animals (Fig. 4). The posture of the treated mice was very characteristic for both benzodiazepines. The mice moved low to the ground with their backs flattened and tails curved upwards in a convex semi-circle. The mice moved quickly with small, rapid steps. Both benzodiazepines showed a variation in potency in the different groups of mice, leading to a wider variation in effect. This is well known for the benzodiazepines (Dr. Parkes, personal communication).

When atropine was given at 2·5 mg/kg, and also at 5 mg and 10 mg/kg, the exploratory behaviour of the mice was blocked so that on the second day of observation the treated mice behaved as if they had not been on the board before (Fig. 5). Behaviourally the mice appeared to be normal after treatment, moving between the tunnels, although fewer different tunnels were entered than by the controls in the first minute ($P<0{\cdot}01$). Methylatropine at the same doses did not adversely affect exploratory behaviour.

Since atropine affected exploratory behaviour, the combination of the benzodiazepines and atropine was also expected to affect exploration. In fact the motor activity of the mice was reduced very severely by 10 mg/kg atropine given with the benzodiazepines, and the mice did not move on the board on the first day of observation. On the second day they behaved overtly like control mice on the first day (Fig. 6).

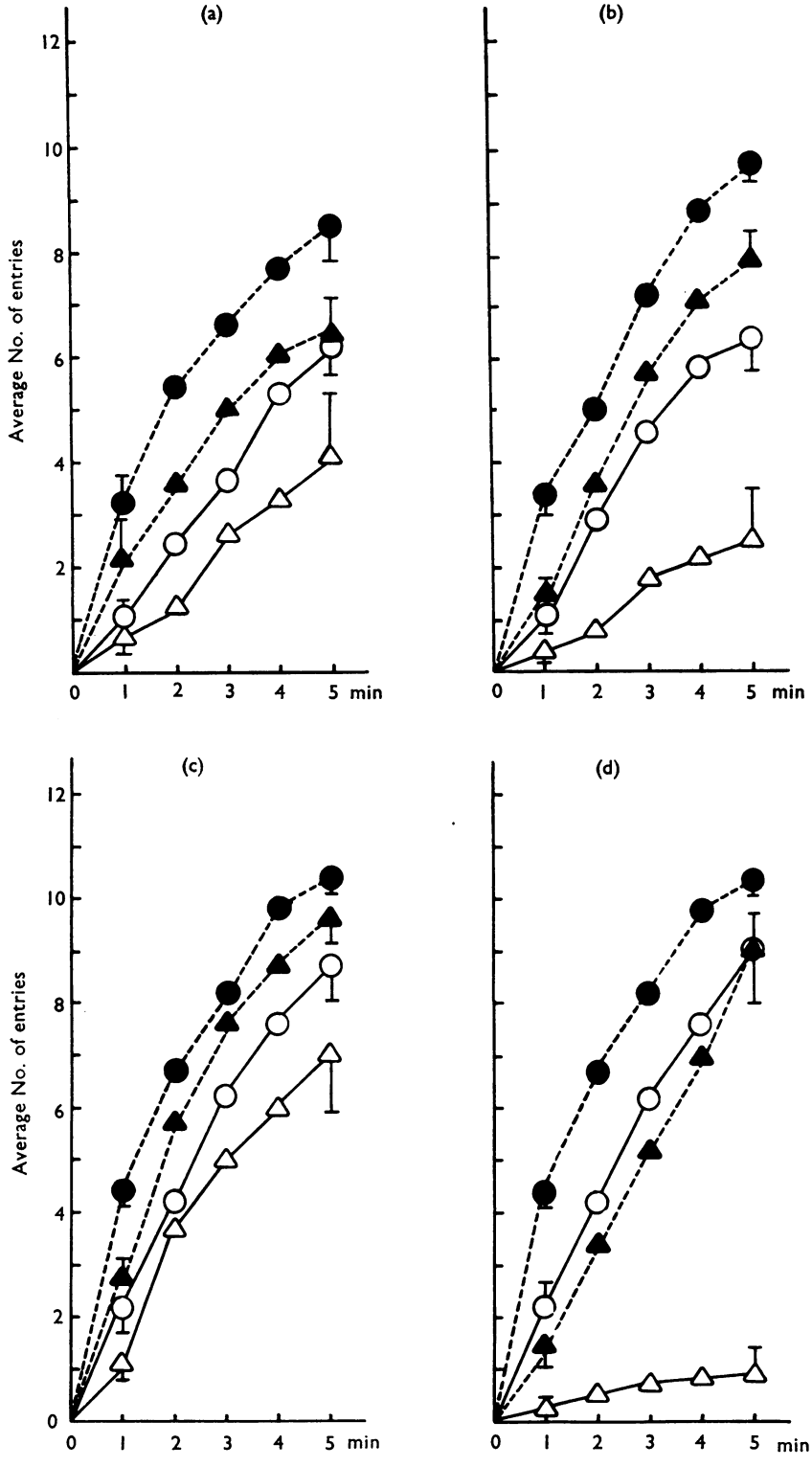

FIG. 4. Average number of different tunnels entered cumulatively over the 5 min observation period by mice treated on day 1 with (a) chlordiazepoxide 25 mg/kg and (b) 50 mg/kg or (c) diazepam 10 mg/kg and (d) 20 mg/kg. Treated mice: △, day 1 ; ▲, day 2. Control mice ; ○, day 1 ; ●, day 2.

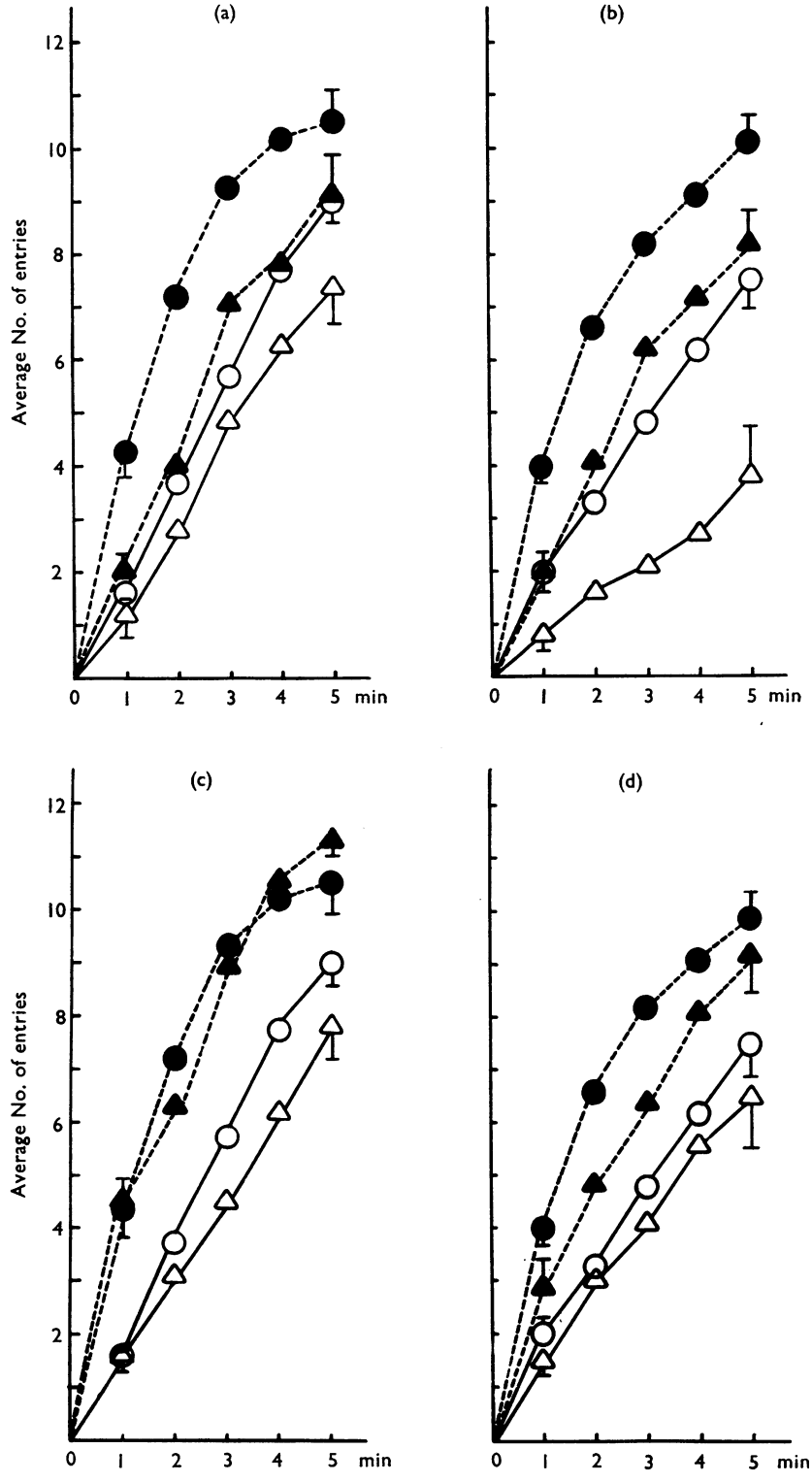

FIG. 5. Average number of different tunnels entered cumulatively over the 5 min observation period by mice treated on day 1 with (a) atropine 2·5 mg/kg and (b) 10 mg/kg or with (c) methylatropine 2·5 mg/kg and (d) 10 mg/kg. Treated mice: △, day 1 ; ▲, day 2. Control mice: ○, day 1 ; ●, day 2.

Discussion

The effects of drugs on the motor activity of mice has been used by many workers to assess the sedative or stimulant properties of compounds. However, the finding of normal motor activity after the administration of a drug may not mean that other activities such as exploratory behaviour have not been affected. When rodents explore a new area they move around to learn the nature of the new surroundings. Although there is a certain amount of correlation between locomotion and exploration, it is not necessarily true that because an animal has moved more it has explored more. Neither is it true that if an animal has moved less than usual it has not explored.

In this paper the effect of atropine and the benzodiazepines has been measured on activity and on exploratory behaviour. At the doses at which exploratory behaviour was blocked, the drugs used did not significantly alter the time the mice spent actively moving. Yet activity itself was not normal, as shown by the increase in the number of times the cage was crossed. Atropine did not alter the amount of time the mice spent in moving nor the number of crossings they made in the cage. If exploratory behaviour were correlated with this locomotion it would be expected that atropine would have no effect on exploration, while the benzodiazepines would increase exploration. In fact some doses of all these compounds affected exploratory behaviour adversely, so that with all doses of atropine and with chlordiazepoxide 50 mg/kg and diazepam 20 mg/kg, the treated mice behaved like inexperienced controls on the second day. This means that although the mice were able to move around the board, either their perception or memory was blocked so that learning was not evident.

When atropine and the benzodiazepines were combined, activity was profoundly affected. The mice were very sedated and hardly moved at all either on the tunnel

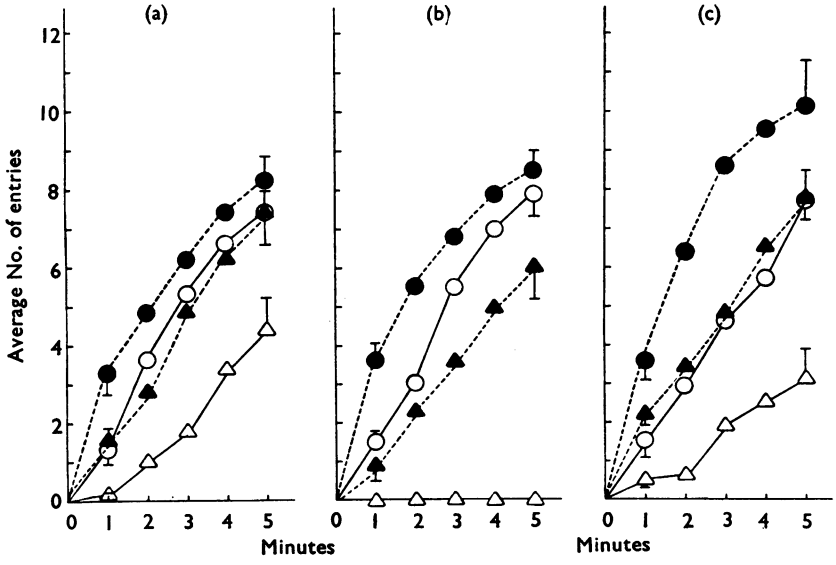

FIG. 6. Average number of different tunnels entered cumulatively over the 5 min observation period by mice treated on day 1 with (a) chlordiazepoxide 50 mg/kg alone, (b) chlordiazepoxide 50 mg/kg and atropine 10 mg/kg and (c) chlordiazepoxide 50 mg/kg and methylatropine 10 mg/kg. Treated mice: △, day 1 ; ▲, day 2. Control mice: ○, day 1 ; ●, day 2.

board or in the activity cage. These results are interpreted as a potentiation of the effects of the benzodiazepines by atropine, because at much higher doses of chlordiazepoxide or diazepam the mice also do not move. Atropine also prolonged the sleeping time of mice treated with large doses of benzodiazepines.

The effects of methylatropine were studied because the drug does not enter the central nervous system. The results showed that methylatropine did not affect activity or exploratory behaviour in mice, so it seemed that it was the central effects of atropine which were related to its effect on exploratory behaviour. The effect of methylatropine when given to mice with the benzodiazepines was more complicated. There was no potentiation of the sedative effect of the benzodiazepines, as with atropine, but some kind of summation of effects occurred because it was usual for one or two mice in a group of ten to die when the benzodiazepines were combined with methylatropine. However, the toxicity of higher doses of the benzodiazepines was not potentiated by methylatropine, neither did deaths occur when atropine was combined with them.

Maickel (1968) used rats to investigate the effects of a mixture of amphetamine and cholinergic drugs on the action of chlorpromazine. Measuring overt behaviour in an open field situation and motor activity on a wheel, he found that atropine alone did not affect either parameter and also did not alter the sedation produced with low doses of chlorpromazine. However, a combination of amphetamine and atropine was effective in reversing the sedation produced by high doses of chlorpromazine. In mice, atropine given with high doses of the benzodiazepines potentiated the sedation caused by the drug at those doses. Studies on the effects of drug mixtures on locomotion in mice have been made by Joyce, Porsolt, Steinberg & Summerfield (1968) using amphetamine-barbiturate mixtures. They found that an inverted V dose-response relation was obtained for the mixture used at a constant ratio of 1 : 20. Similar results were obtained by Rushton & Steinberg (1963) with the same mixture given to rats. These workers have also used mixtures of chlordiazepoxide and dexamphetamine in rats (1966). With this mixture the activity of the rats in a Y-maze was increased significantly compared with either drug alone. The rats given chlordiazepoxide alone were highly active at first and then became immobile in a " trance-like " state. Rushton & Steinberg (1966) suggested that the effect of the dexamphetamine was to prevent the " trance-like " state of the rats so that they continued to be highly active. The results reported in this paper showed that atropine appeared to reduce the increase in activity produced by lower doses of chlordiazepoxide. Steinberg, Rushton & Tinson (1961) have also shown that the effects of drug mixtures can be altered by the past experience of the rats used. Thus amphetamine and amylobarbitone act together to increase activity only in rats which are unfamiliar with the environment. Saline-treated rats explored more after experience of the Y-maze. Although these workers have not used trials on 2 successive days, their results show a similar trend to the results reported in this paper with mice.

Marriott (1968) has compared the effect of some stimulants on locomotor activity of rats in a Y-maze and in activity wheels. He found that neither amphetamine nor caffeine increased Y-maze activity at doses which increased wheel activity, while methylphenidate increased both measures. These results compare with our results in mice in which it was possible to distinguish between effects on locomotor activity and exploration. Thus it was demonstrated that atropine did not affect activity

although it blocked exploration, while chlordiazepoxide and diazepam increased activity and also blocked exploration.

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