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EFFECTS OF A SINGLE EXPERIENCE ON SUBSEQUENT REACTIONS TO DRUGS

BY

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The activity of rats in an unfamiliar environment was studied in order to determine how far their reactions to an amphetamine-barbiturate mixture depended on whether or not they had been under the influence of this mixture while exposed to the same environment once before. The environment consisted of a Y-shaped runway, and the activity studied was the number of entries into the arms of the Y during a three-minute trial; the two trials took place three days apart. At the first trial the drug mixture practically doubled activity. At the second trial rats which had been under the influence of the drug mixture at the first trial were again made more active by the drug mixture, but the drug mixture did not increase the activity of rats which had received only saline at the first trial. These results showed that a single brief exposure to an unfamiliar environment can markedly affect subsequent reactions to drugs, and interactions of this kind may have to be taken into account when it is desired to use animals repeatedly in tests of the action of drugs on behaviour. The drug mixture also produced ataxia which was assessed quantitatively by measuring the variability of the "splay" of the rats' footprints; ataxia was unaffected by previous experience.

In this investigation rats' reactions to a mixture of amphetamine and amylobarbitone in a controlled environment were studied after the animals had or had not been under the influence of the same drugs while exposed to the same environment once before.

Steinberg, Rushton & Tinson (1961) found that rats which were given twice-weekly trials in a simple Y-shaped runway for several weeks soon developed a relatively stable level of activity, as measured by the number of entries made into the arms of the Y in a 3-minute trial. However, when these "experienced" animals were tested after receiving drugs, they were insensitive to the stimulant effects of an amphetamine-barbiturate mixture, while "inexperienced" rats which had been similarly handled but had not been in the runway before made about three times as many entries under the influence of the drug mixture as inexperienced controls which had received saline. This showed that reactions to the drug mixture in this experimental situation were altered by the previous experience of the subjects. It had also been noted that in animals which were being tested repeatedly without drugs, activity at the second trial was usually significantly less than activity at the first trial, and this suggested that reactions to drugs also might be affected even at this early stage of familiarity with the runway. The present investigation was therefore undertaken in order to analyse the effects of first trial on second trial activity in the presence and in the absence of drugs.

Previous experiments showed that, although the drug mixture greatly increased exploratory activity, it also made the gait of most rats appear clumsy and incoordinated. For the present investigation a quantitative method of studying gait was developed so that effects of the experimental conditions on this could also be determined.

METHODS

Subjects

Sixty adult Lister hooded female rats were used, aged 120 to 130 days. They had been reared under standard conditions since weaning, had been little handled and had not been used in any previous experiments. Throughout the investigation they lived in their home cages with food and water *ad libitum*.

Plan of the experiment

The rats were allocated at random to two groups of thirty each. At the first trial in the runway one of these groups was given the drug mixture, and the other group was given saline. For the second trial, 3 days later, the two groups were further subdivided at random, so that fifteen rats from each group were tested under the influence of the drug mixture and the other fifteen under saline. Altogether there were, therefore, four groups of fifteen rats each, which groups differed according to whether the animals were under the influence of the drug mixture or of saline at the first and/or the second trial (Table 1).

TABLE 1

PLAN OF EXPERIMENT

Sixty rats were divided into four groups of fifteen rats. Each group was given two trials, three days apart, in a Y-shaped runway while under the influence of either saline or a drug mixture, as follows: 1, saline-saline; 2, saline-drug mixture; 3, drug mixture-saline; 4, drug mixture-drug mixture

Trial I	Saline (n=30)		Drug mixture (n=30)	
Trial II	Saline (n=15)	Drug mixture (n=15)	Saline (n=15)	Drug mixture (n=15)

Apparatus

For testing exploratory activity. The apparatus was similar to that previously described (Steinberg *et al.*, 1961). It consisted of a symmetrical, wooden, Y-shaped runway, 13 in. high and with arms 15 in. long and 5 in. wide. It was illuminated from above so that the light intensity on the floor of the runway was approximately 5.5 ft. candles. A trial consisted of placing a rat in the centre of the Y and leaving it in the apparatus for 3 min; the number of times it entered the arms with all four feet was the measure of exploration. There were no rewards. Two runways, identical in appearance, were used, and rats in the different groups were allocated to one or the other runway at random, but each rat was tested in the same apparatus and by the same experimenter on successive trials; the inter-observer reliability for the measure of exploration used was, in preliminary experiments, practically unity.

For studying gait. Another Y-shaped runway was devised which had the same dimensions as that already described, except that the arms were 30 in. long, to allow for a longer run. The floor of the runway was covered with white paper. Each rat's hind paws were smeared with Vaseline, the animal was placed in the centre of the Y and was removed as soon as it had reached the end of an arm and before it could retrace its steps. If a run was not continuous or complete, further trials were made, though this was rarely necessary because most rats readily ran from the centre of the Y to the end of an arm. After the trial the paper over which the rat had run was dusted with powdered charcoal which adhered to the grease

marks and so showed up footprints. The spacing of these prints was measured as described later. This method is a modification of those described by Shirley (1931) for infants and by Khairy (1961) for rats.

Procedure

Before their first trial in the runway rats were injected alternately either with saline or with a mixture of amphetamine sulphate (0.75 mg/kg) and amylobarbitone sodium (15 mg/kg) dissolved in saline, so that the volume injected, subcutaneously, was 2 ml./kg. This drug mixture was chosen because exploratory behaviour in a similar runway was known to be much more sensitive to the mixture than to the separate constituents (Steinberg *et al.*, 1961 ; Rushton & Steinberg, 1963). Rats receiving saline were given the same volume by the same route. Each rat was given a single 3 min trial in the smaller runway 35 min after the injection, and immediately afterwards it was placed in the larger runway where records of footprints were obtained. The rats were given their second injection 3 days later and the procedure was repeated. All experiments were carried out between 2.30 and 5.30 p.m.

RESULTS

Exploratory activity. Fig. 1 illustrates the results. At trial I exploratory activity was greatly increased by the drug mixture compared with saline ($t=5.62$; $d.f.=58$; $P<0.001$). A two-way classification analysis of variance on trial II scores showed

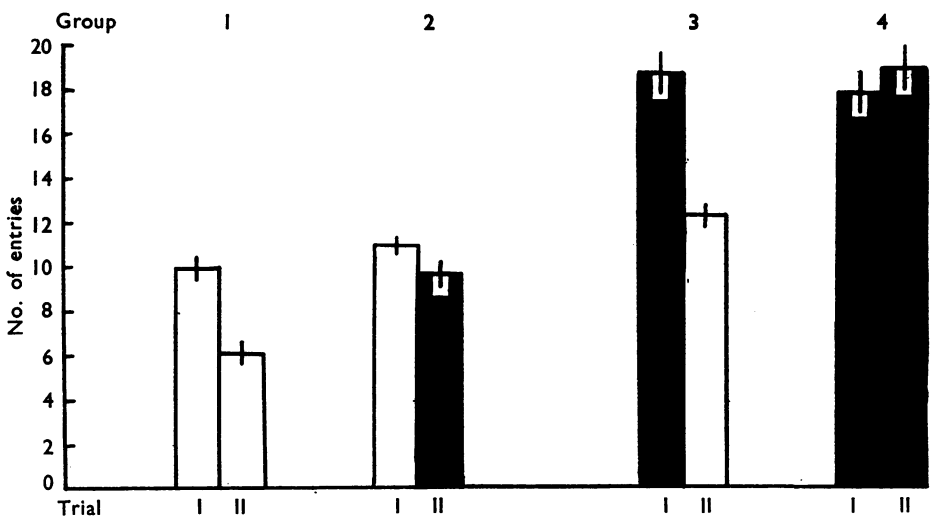

Fig. 1. Effect of a single experience on subsequent reactions to drugs. Rats were given two trials, 3 days apart, in a Y-shaped runway. The reactions tested were exploratory activities, scored as the number of entries into the arms of the Y in 3 min. Four groups of fifteen rats were tested with the rats of a group under the influence of either an amphetamine/barbiturate mixture (filled columns) or of saline (open columns) at the first and/or the second trial. The vertical lines indicate standard errors. The number of entries are the means per group of fifteen rats.

that activity depended on whether trial II took place under the influence of the drug mixture or of saline, but it also depended on whether the preceding trial (I) had occurred under the influence of the drug mixture or of saline (Table 2).

TABLE 2

TWO-WAY CLASSIFICATION ANALYSIS OF VARIANCE OF EXPLORATORY ACTIVITY AT TRIAL II

The analysis shows significant differences between conditions on trial I, and between conditions on trial II, but no significant interaction

Source	Sums of squares	d.f.	Mean square	F	P
Trial I					
Saline v. drug mixture	897·06	1	897·06	31·21	<0·001
Trial II					
Saline v. drug mixture	385·06	1	385·06	13·40	<0·001
Trial I × Trial II					
Interaction	38·41	1	38·41	1·34	>0·25
Error	1,609·20	56	28·74		
Total	2,929·73	59			

Among animals given saline at trial I there was, as expected, a drop in activity at trial II if the latter also took place under the influence of saline (group 1 ; $t=5.94$; $d.f.=14$; $P<0.001$). The rank correlation (Kendall's Tau) between trial I and trial II in this group was 0.585. If, however, trial II occurred under the influence of the drug mixture, activity was maintained at a level similar to that at the first saline trial (group 2), though it was still only about half the activity in trial I with the drug mixture ($t=4.34$; $d.f.=43$; $P<0.001$); it was also only about half the activity in trial II with the drug mixture if the first trial was also with the drug mixture ($t=3.55$; $d.f.=28$; $P<0.001$). For rats which did the first trial under the influence of the drug mixture, the results at trial II were different. The drug mixture prevented the drop in activity which normally followed a first trial with saline, and at the second trial with saline (group 3) the rats were as active as though it were their first saline trial. Furthermore, if the drug mixture was given for trial II as well as trial I there was no drop in activity at all (group 4); this is particularly striking since trial II with the drug mixture produced only about half as much activity when preceded by a trial using saline (group 2).

Gait. The footprints were measured in the following way. The second and third interdigital pads on the sole of the rat's hind foot show clearly in a normal print, and the small space between these was the point from which measurements were made (Fig. 2). Various preliminary measurements were made on sample prints in order to determine the measure which discriminated best between rats given saline and rats given the drug mixture and which were ataxic. The most reliable and sensitive measure was not of absolute size but of variability: the " splay," or distance between consecutive hind footprints measured at right angles to the direction of the path, was very regular in animals given saline, but it was much more variable in animals which were ataxic (Fig. 2). These distances could be measured fairly quickly by means of tracing graph paper. The variance was calculated for each rat's record, and the mean log variances were calculated and compared for the different groups of rats ; log variances were used because it would otherwise have been difficult to compare two values of s^2 if they were of different absolute size.

Table 3 summarizes the results. Rats whose gait was tested when they were under the influence of the drug mixture gave consistently larger log variances than rats given saline, regardless of whether it was the first or the second trial or whether a previous

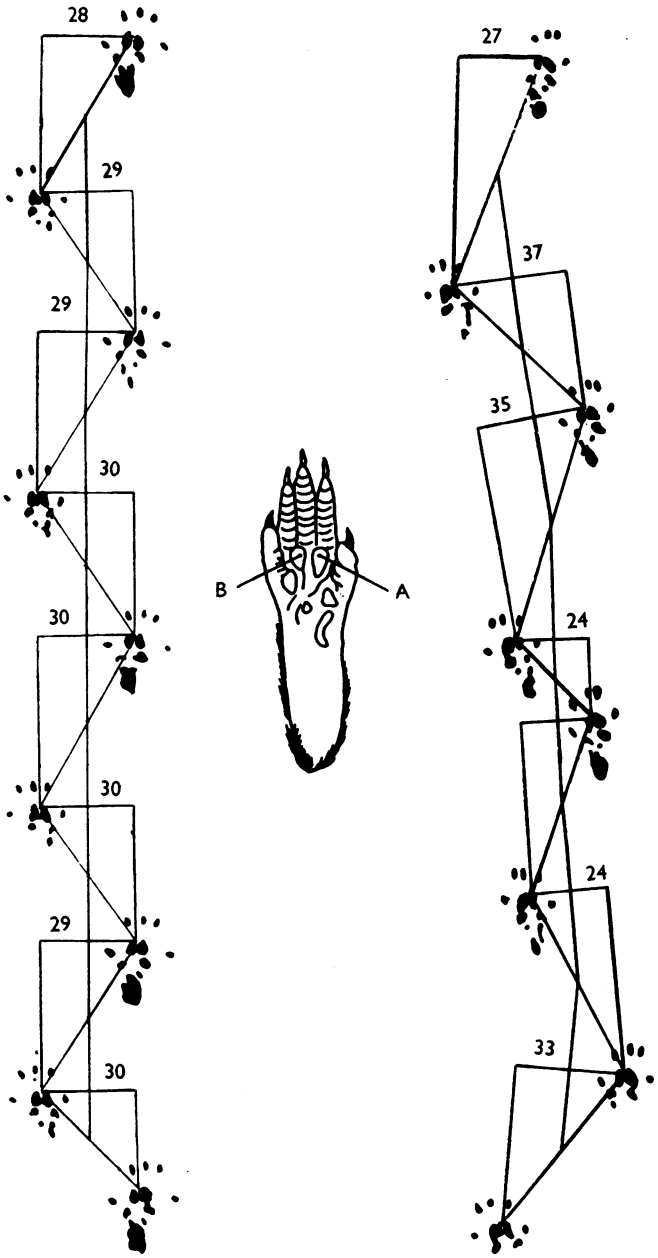

Fig. 2. Measurements used for studying gait, with prints of hind feet of rats given saline (left) and an amphetamine/barbiturate mixture (right). The numbers indicate the " splay " in mm. Irregularity of splay was used as a measure of ataxia. The diagram of the sole of the right hind foot (Greene, 1935) shows the second (A) and third (B) interdigital pads which determined the point from which measurements were made.

TABLE 3

RESULTS OF MEASURING FOOTPRINTS IN FOUR GROUPS OF RATS, OBTAINED AS DESCRIBED FOR FIG. 2

	Trial I			Trial II		
Groups	Mean splay (mm)	s^2 (mm)	log s^2	Mean splay (mm)	s^2 (mm)	log s^2
1, Saline-saline	28·2	6·23	0·702	27·8	5·03	0·549
2, Saline-drug mixture	29·0	0·04	0·741	24·5	30·18	1·384
3, Drug mixture-saline	26·9	25·39	1·145	30·2	5·89	0·728
4, Drug mixture-drug mixture	25·7	18·74	1·071	27·6	22·94	1·165

first trial had been under the influence of saline or of the drug mixture. A two-way classification analysis of variance of these data showed that the overall increase in the log variances due to the drug mixture on trial II, as compared with saline, was statistically significant ($F=34.33$; $d.f.=1.40$; $P<0.001$). However, there was no significant overall difference between trial I results compared with trial II results, nor was the interaction between " drug mixture " and " trial " conditions significant. These results therefore show that under *all* the conditions of the present investigation the drug mixture produced ataxia, as indicated by an increase in the variability of the width of gait.

DISCUSSION

The explanation of the measurements of exploratory activity is probably complex. For example, one of the effects of the drug mixture may have been to make the animals react as if they had not been in the situation previously ; in other words, the drug mixture might affect perception or memory in such a way that stimuli to which an animal is exposed while under its influence have no effect on subsequent activity. This interpretation is tenable on statistical grounds ; in a two-way classification analysis of variance, comparison of trial II scores of rats which had the drug mixture at trial I with trial I scores showed no significant differences. Bloch & Silva (1959) and Miller (1961) have reported that responses which animals learnt while under the influence of barbiturates were not retained when the effects of the drugs had worn off. An effect of this kind might therefore account for the trial II results of groups 3 and 4, as compared with groups 1 and 2. Furthermore, activity at trial II might be a function of the sheer amount of activity at trial I, in the sense that the more active the first trial the more active, relatively, the second trial, and *vice versa.* This would account for the fact that the activity in trial II is proportionately about the same, two-thirds, of trial I activity, both in group 1 and in group 3.

A fuller explanation must probably await a better understanding of the significance of exploratory behaviour in animals (Barnett, 1958 ; Bindra, 1959 ; Berlyne, 1960) and, in particular, of why exploratory activity is apt to decrease at the second trial (Glanzer, 1961), and of why the amphetamine-barbiturate mixture increases exploratory activity at all (Steinberg *et al.*, 1961) ; it may be because amphetamine alone increases activity (Rushton & Steinberg, 1963) while barbiturates act indirectly by reducing fear (Barry, Wagner & Miller, 1962), including perhaps fear of new environments.

Other experiments (for example, Steinberg *et al.*, 1961 ; Bindra & Mendelson, 1962 ; Schnitzer & Ross, 1963) have mostly been concerned with the effects of relatively repetitive or prolonged kinds of experience on various reactions to drugs ; the present investigation shows that even a single brief experience, lasting only 3 min, can markedly modify subsequent reactions to drugs, in particular by almost abolishing the otherwise considerable stimulation of exploratory activity by a drug mixture. When it is desired to use animals repeatedly in tests of the effects of drugs on behaviour, interactions of this kind may therefore have to be taken into account. The present findings are also congruent with the growing literature on the ways in which past experience, environment and similar factors can modify reactions to centrally acting drugs in man.

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