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THE EFFECTS OF PSYCHOTROPIC SUBSTANCES ON THE ORGANIZATION OF SPONTANEOUS EXPLORATORY LOCOMOTION OF RATS

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ABSTRACT

A system of enclosed maze alleys was presented 4 times without any barriers and thereafter 4 times with barriers dividing the system into two sections accessible from one to the other only by passing through an open field. The behavior of the drug-treated animals differed from that of the controls in a differential way, dependent not only upon the drug, but also upon the genetic lines (RHA/Verh vs RLA/Verh) of the rats and upon the two maze configurations. Chlorpromazine (1.5 mg/kg) reduced the maze center activity and exploration in both lines, the effect being more pronounced for the second maze configuration, indicating a loss of exploratory behavior which became especially evident with the more complex maze configuration. With pentobarbital (7.5 mg/kg) the maze center activity and exploratory efficiency (repetition index) were reduced in both rat lines for the first maze and both measures increased considerably for the second maze. The predominant effect of imipramine (7.5 mg/kg) was to depress activity. Chlordiazepoxide (7.5 mg/kg) which initially depressed activity subsequently had a pronounced stimulating effect only on the RLA rats, suggesting a specific anxiolytic action according to the behavioral characteristics of this rat line.

Precedent studies indicate that the exploratory activity of rats represents a very complex behavior, involving the acquisition and use of complex spatial cognitive maps, fear of novelty and non-specific activity drive[4,12]. The food rewarded testing arrangements used in these studies showed that the rats could also make use of extra-maze stimuli. Olton and Samuelson[12] showed that a rat easily remembers 16 different places in which food has already been collected or not. The organization of such behavior is of course of great importance for the orientation of an animal within its territory. Our approach was different from all other similar studies; we investigated spontaneous locomotion of rats in a variable system of completely enclosed maze alleys, without any extra-maze cues, offering the animals no other incentive than that inherent to ambulation through the maze. Under this condition the rats continued to run through the maze day after day, even after prolonged testing periods[16] and after a few repeated tests the animals acquired the ability to visit all maze corners with decreasing repetition of places already visited[2], which required the formation of internal cognitive spatial maps. In other studies it was shown that this behavior is very sensitive to the differential effects of nicotine and amphetamine[15].

The present study attempts to investigate whether other widely used psychopharmacological substances might also exert differential effects. This could demonstrate the usefulness of such a test both for studies in psychopharmacology and behavioral toxicology.

Single doses of chlorpromazine, pentobarbital, imipramine and chlordiazepoxide were chosen for this initial study. Earlier results[15] with single doses of nicotine and amphetamine showed differential effects of these drugs on different variables of the test. Like those studies the present investigation also included the two lines of rats which have been shown in this laboratory to differ over a series of behavioral aspects[6], one line (RLA/Verh) being more anxious, less active and showing poorer learning in aversive situations and better learning in non-aversive situations than the other line (RHA/Verh). Unlike most of our earlier experiments, in this test a small illuminated open field was included in the center of the maze during the second testing phase, as open fields are known to produce more initial fear of novelty than enclosed alleys.

METHOD

Animals

The 48 RHA/Verh and 48 RLA/Verh female rats used in the experiment were 5 months old at the beginning of the tests. They were taken from the 38th generation of the breeding colony of the Institute. The average weight of the RHA/Verh rats was 240 g, that of the RLA/Verh rats 210 g. They received water and food (NAFAG pellets) *ad libitum* throughout the entire testing period and a handling program of two weeks before the test which involved strictly all manipulations of the subsequent experiment except injections and maze exposures. They were housed groupwise in Macrolon cages under reversed light conditions, with illumination on between 6 p.m. and 8 a.m. Before the handling program the animals were assigned to the later experimental groups according to the split litter technique.

Apparatus

The maze system used differs from other mazes in that the walls and ceiling, including all electronic photocell circuitry, form a compact unit, which can be lifted from the floor. At the beginning of the experiment the animal is placed through the ceiling door into the maze, and at the end of the test, the maze is lifted up from the floor. The animal is then removed from the floor, and the floor is wiped off with a wet sponge. The barriers which can be placed between any sections of the maze allow changes of the alley configurations. This was done in this experiment so as to obtain the two maze configurations shown in Figure 1. The first maze, used in the first four testing days of the later experiment contained no barriers at all. The second configuration, used in the second four testing days of the experiments included a small open field in the center, which

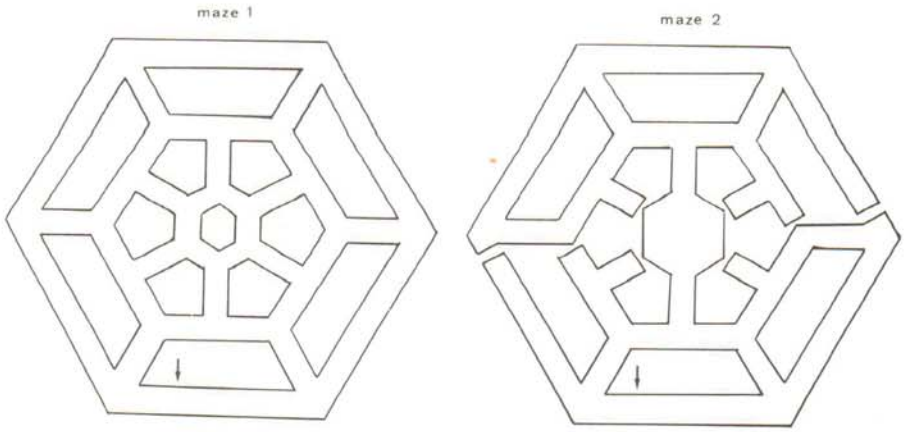

FIG. 1 – Two maze configurations. The hexagonal area in the middle of maze 2 is an illuminated open field with the walls about 50 cm high. All other alleys are completely enclosed.

was illuminated (75W bulb) and had the side walls extended to a height of about 50 cm. Furthermore, the remaining alleys were divided by barriers into two sections accessible from one to the other only by passing through the open field in the center.

Continuous recording of the position of the animal is achieved by 42 photocells placed at equal distances throughout the maze. On-line data acquisition and analysis with a PDP-11 laboratory computer allows immediate control of all locomotion and multiple derived indexes of special aspects of this behavior. The sequences for testing the individual animals according to a predetermined cross-over design and the prefixed daytimes of individual testing are also controlled by the processing system. The other details of this maze system have been described elsewhere[1].

Drugs and injections

Chlorpromazine-HCl (Siegfried AG, Zofingen), pentobarbital-Na (Siegfried AG, Zofingen), imipramine-HCl (Ciba-Geigy AG, Basel) and chlordiazepoxide (Hoffmann-La Roche AG, Basel) were all dissolved so as to obtain a uniform injection volume of 1 ml/kg body weight. The dosages were 1.5 mg/kg for chlorpromazine and 7.5 mg/kg each for pentobarbital, imipramine and chlordiazepoxide. Chlordiazepoxide was dissolved in "Special Librium Solvens" (Hoffmann-La Roche AG), all other substances in physiological saline, which was also used for injecting the control animals. All solutions were prepared freshly every day and injected intraperitoneally one hour before each test throughout the entire experimental program. After the injection each animal was placed in an individual cage and brought into the dimly illuminated testing room for the one hour "waiting" period until testing.

Experimental design

The testing program was split up into two subsequent phases. For each phase the 48 animals were divided into separate groups of 8 animals each according to a 2 × 3 design, with the two lines RHA/Verh and RLA/Verh and with the three treatments with saline and two different drugs. The two drugs used in the first phase were chlorpromazine and pentobarbital, the two drugs used in the second phase were imipramine and chlordiazepoxide. The animals were tested only on alternate days, in order to avoid possible cumulative drug effects, all RHA/Verh rats being tested on one day, and the RLA/Verh rats on the alternate day. All groups were tested in maze 1 during the first four days and in maze 2 during the second four days of the experiment which, therefore, included a total of 32 testing days within 8 weeks.

Data processing and statistics

Eleven different indexes of locomotion were obtained on-line from the PDP-11 computer for each run of an animal. These were then tabulated and separately subjected for each variable to an analysis of variance (ANOVA) in order to make a comparison between the two mazes and among the four testing days used for each maze. In order to evaluate effects of drug and rat line, between-group comparisons were subsequently made with the t-test[14].

RESULTS

No significant differences were found between the results of the control groups in the two experimental phases. The following 3 figures compare the day-to-day averages of the most important variables for the two lines of rats in the first phase of the experiment.

Figure 2 shows the daily amount of locomotion which is termed as activity. This was considerably higher in the RHA/Verh than in the RLA/Verh rats. It also increased in the RHA/Verh rats during the first testing days, declined considerably upon presentation of the second maze configuration with the open field and recovered again thereafter. In the RLA/Verh rats locomotion remained relatively modest throughout the experiment without any visible effects of the change of the maze configuration. Intrasession activity decrease appeared to be a more erratic measure than activity as evidenced by a generally high level of variance. However, the pronounced low average level for the RLA/Verh on the first testing day is remarkable and points to the low level of situational habituation seen in these animals upon first confrontation with the maze.

Figure 3 shows the activity spent in the center of the maze and the degree of preference for the peripheral parts of the maze. The center of the maze consisted of the small hexagonal alley shown in Figure 1 for the first maze configuration and of the open field for the second maze configuration. This activity increased in both rat lines for the small hexagonal alley, became immediately depressed upon introduction of the open field and recovered thereafter only partially. The outward preference coefficient (activity per photocell in the outer hexagonal and

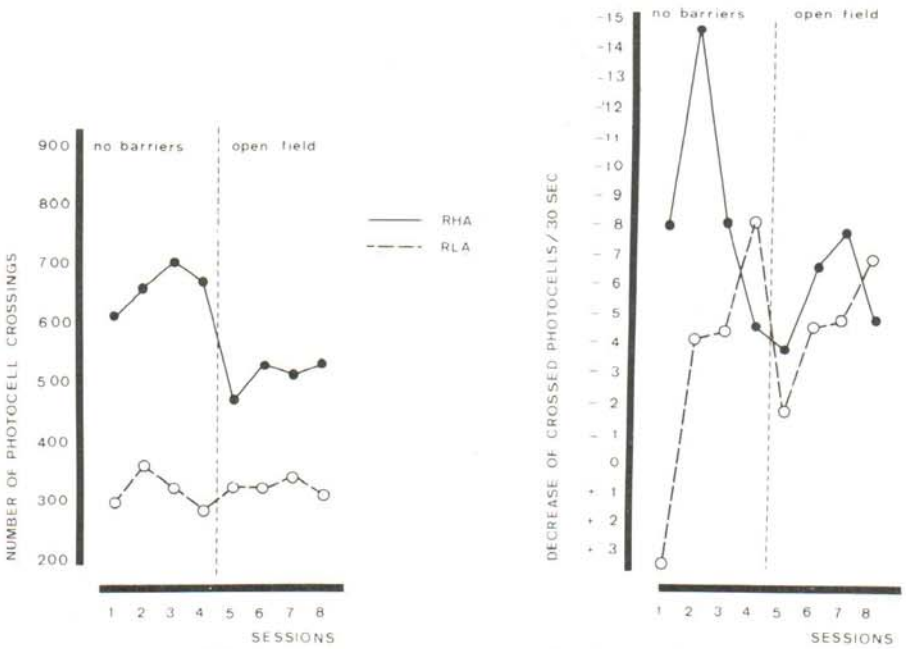

FIG. 2 – The activity measures in the two rat lines.

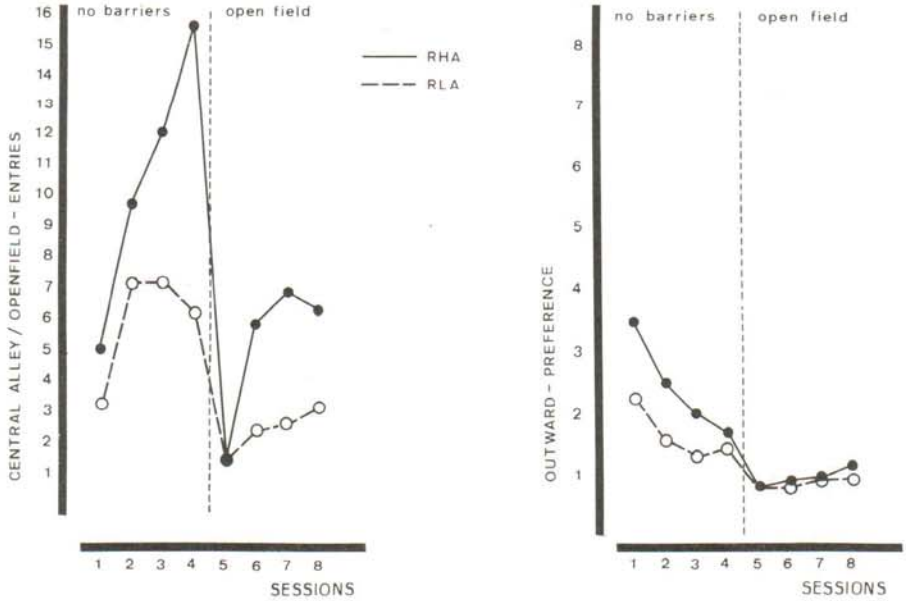

FIG. 3 – Maze center activity (enclosed alleys for the maze "no barriers" and open field for the second maze) and outward preference in the two rat lines.

radial alleys divided by activity per photocell in the middle hexagonal and inner radial alleys) decreased in both rat lines to values near 1.0 already during the presentation of the first maze configuration and it was not affected by the introduction of the open field.

Figure 4 summarizes the most important variables which characterize exploratory efficiency. The repetition index shown in the left part of the graph is a rather complex measure. It represents the regression coefficient over the number of crossed photocells accumulated in steps of 6 new photocells crossed for the first time. The minimum value of 1.0 for this index would be reached by an animal crossing each of the 42 photocells only once without any repetition. The repetition index declined for both rat lines during the first four maze presentations without barriers. It increased considerably for both lines upon the presentation of the more complex second maze configuration and then decreased again. Generally, this repetition index appeared to be lower in the RLA/Verh than in the RHA/Verh rats. The other variable characterizing exploratory efficiency measures the extent of the explored area by counting the photocells which were crossed during a single session. The introduction of the open field produced a transitory decrease of the explored area. Furthermore, the explored area was generally smaller for the RLA/Verh than for the RHA/Verh rats. Since the repetition index increased progressively with the extent of the explored area as would be expected, it must be concluded that the lower repetition indexes seen

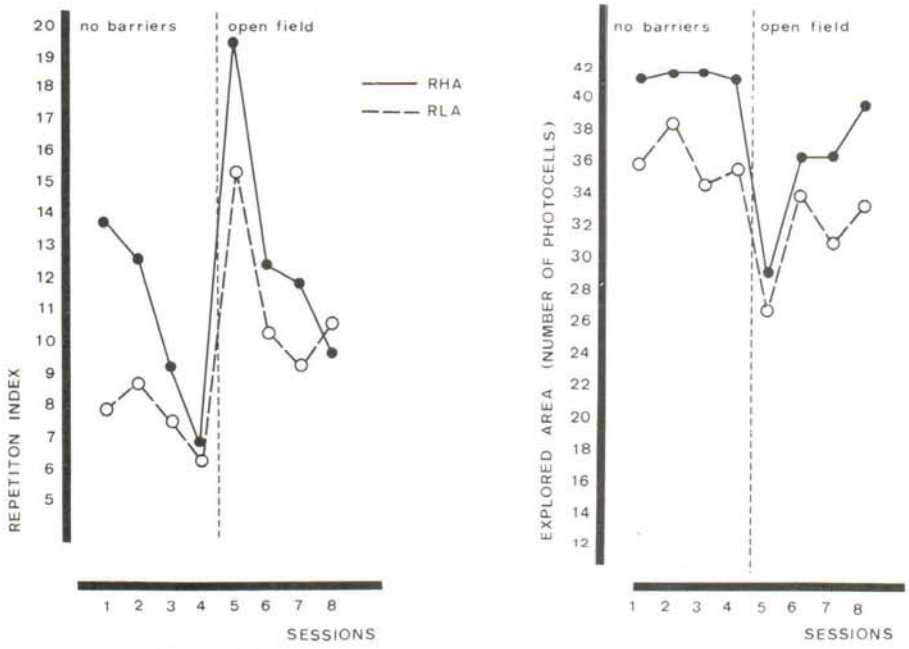

FIG. 4 – The exploratory efficiency indexes for the two rat lines.

previously in the RLA/Verh rats should not necessarily be taken as an indication of more efficient exploration by this rat line.

The effects of the four drugs in the two subsequent maze configurations are summarized in Table 1. Chlorpromazine had only modest effects on behavior in the first maze. In this phase the maze center was less frequently entered by the RHA/Verh rats and the explored area decreased in the RLA/Verh rats as compared to the controls. Mainly depression was seen in both strains for the second maze, with less open field entries and decrease of the explored area in both strains, general activity depression in the RLA/Verh rats and an increased repetition index in the RHA/Verh rats. The decreased repetition index in the RLA/Verh rats should not necessarily be taken as an improvement in view of the simultaneous reduction of the explored area.

TABLE 1

Statistical significance of changes induced by the drug treatments as compared to the relevant control groups (+ $p < 0.05$, ++ $p < 0.01$, 0 $p > 0.05$). The figures for the "maze center activity" represent entries into the central hexagonal alley for the first maze configuration and open field entries for the second maze configuration.

	Variables/Treatment	RHA/Verh		RLA/Verh	
		No barriers	Open field	No barriers	Open field
Chlorpromazine	Overall activity	0	0	0	− −
	Maze center activity	− −	− −	0	−
	Repetition index	0	+ +	0	−
	Explored area	0	−	−	−
Pentobarbital	Overall activity	0	+ +	0	+ +
	Maze center activity	− −	+ +	− −	+ +
	Repetition index	+ +	0	+	0
	Explored area	− −	+ +	0	+ +
Imipramine	Overall activity	− −	− −	−	− −
	Maze center activity	0	− −	0	0
	Repetition index	0	0	−	− −
	Explored area	0	0	0	0
Chlordiazepoxide	Overall activity	− −	0	− −	+
	Maze center activity	− −	0	− −	+ +
	Repetition index	0	0	−	0
	Explored area	− −	0	−	+ +

With pentobarbital the effects were almost identical for both rat lines, but differed considerably between the two maze configurations. With the first configuration the activity in the maze center was depressed and the repetition index was increased in both strains while the explored area was reduced in the RHA/Verh rats only. With the second maze the overall activity, open field entries and explored area increased above control in both strains and the repetition index recovered in both strains to the control level.

With imipramine the difference both between maze configurations and rat lines was modest. Depression of overall activity was significant in all cases. A reduction of the repetition index indicating improved exploratory efficiency was seen only in the RLA/Verh rats, but for both maze configurations. In the RHA/Verh rats in addition to the reduction of overall activity the drug produced also a reduction of open field entries.

With chlordiazepoxide a pronounced difference between the two rat lines was obtained for the second maze configuration. General reduction of activity and explored area was obtained in both rat lines for the first maze configuration. With the second maze, however, the RHA/Verh rats behaved completely like the controls, whereas the RLA/Verh rats increased in overall activity, open field entries and the explored area.

DISCUSSION

The three main different aspects of the tested behavior, the formation and use of cognitive spatial maps, fear of open field and basic activity level are well demonstrated with the two configurations of the maze used for the first time in this study. The results of the control groups are in line with earlier conclusions about the behavioral differences between the two rat lines[6]. The RLA/Verh rats are considerably less active and the depressive effect of the introduction of the open field is stronger than in the RHA/Verh rats. Repetition index decreases within both mazes from day to day are an indicator of spatial learning. The introduction of the open field drastically reduces activity in this portion of the maze and a subsequent disinhibition is similar to that observed in most open field experiments. The psychopharmacological interpretations of the findings of the study, however, should be made with caution, as only one dose of each drug was used and as chronic vs acute effects might confound the differentiation seen between the two mazes.

Chlorpromazine appears to reduce exploration especially in the second, more complex maze configuration. This is in line with the reduced exploration reported in several studies using hole board exploration such as the ones by File and Pope[9] and by File[10]. Ortiz and co-workers[13] have shown further, that the behavioral deficit in adaptive learning increases with increasing task complexity. No studies have so far compared the effects of this substance in the RHA/RLA--lines of other laboratories[5], but the present results suggest that the genetic factor might be of minor importance at least for this type of behavior. Low doses of barbiturates have been shown in many other studies to increase locomotor activity, as they did in the present experiment[17]. Disinhibition of open area avoidance was also seen by Morrison and Stephenson[11] after barbiturate administration. With imipramine the major effect in both rat lines was a depression of activity, which is well known to appear with tricyclic antidepressants in rats and mice.

The differential effect of chlordiazepoxide on the two rat lines in the second maze with the open field is of particular interest in view of the fact that the

RLA/Verh rats are generally more anxious than the RHA/Verh rats[6]. File[8] suggested that an increased rate of habituation might be responsible for the specific anxiolytic effects of this substance. This is particularly interesting as it also has been shown that the RLA/Verh rats habituate more slowly to an open field than the RHA/Verh rats[7].

Further pharmaco-genetic studies with these rat lines and with this test therefore appear justified. The differences in the brain chemistry between the two rat lines[3] make such pharmaco-genetic comparisons particularly interesting.

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