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The use of a plus-maze to measure anxiety in the mouse

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Abstract. To investigate whether an elevated plus-maze consisting of two open and two closed arms could be used as a model of anxiety in the mouse, NIH Swiss mice were tested in the apparatus immediately after a holeboard test. Factor analysis of data from undrugged animals tested in the holeboard and plus-maze yielded three orthogonal factors interpreted as assessing anxiety, directed exploration and locomotion. Anxiolytic drugs (chlordiazepoxide, sodium pentobarbital and ethanol) increased the proportion of time spent on the open arms, and anxiogenic drugs (FG 7142, caffeine and picrotoxin) reduced this measure. Amphetamine and imipramine failed to alter the indices of anxiety. The anxiolytic effect of chlordiazepoxide was reduced in mice that had previously experienced the plus-maze in an undrugged state. Testing animals in the holeboard immediately before the plus-maze test significantly elevated both the percentage of time spent on the open arms and the total number of arm entries, but did not affect the behavioral response to chlordiazepoxide. The plus-maze appears to be a useful test with which to investigate both anxiolytic and anxiogenic agents.

Key words: Anxiety – Plus-maze – Mouse – Benzodiazepine receptor – Stimulants – Exploration

Pellow et al. (1985) recently validated in rats a new test of anxiety developed from the work of Montgomery (1958) and of Handley and Mithani (1984). The test is based on the natural aversion of rodents for open spaces and uses an elevated plus-maze with two open and two closed arms. Two indices of anxiety are obtained: the number of entries into open arms expressed as a percentage of the total number of arm entries, and the amount of time spent on the open arms expressed as a percentage of the total time on both open and closed arms. The test is rapid and was found to be sensitive to the effects of both anxiolytic and anxiogenic agents, anxiolytic agents increasing and anxiogenic agents decreasing the two measures. The aim of the present study was to examine whether the plus-maze could also be used as a model of anxiety in the mouse. The effects of pharmacological agents from a number of different classes are examined. Chlordiazepoxide, sodium pentobarbital and ethanol are examined as agents that have anxiolytic effects (Sternbach 1979; Pohorecky 1981), and picrotoxin, caffeine, FG 7142 and *d*-amphetamine were used as agents with anxiogenic actions (File and Hyde 1979; Dorow et al. 1983; Charney et al. 1984; File and Lister 1984; Pellow and File 1984; Uhde et al. 1984).

As in the experiments of Pellow et al. (1985), animals were tested for 5 min in a holeboard apparatus immediately before testing them in the plus-maze. By using both the holeboard and the plus-maze we anticipated obtaining independent measures of locomotion, exploration and anxiety. With these indices it would be possible to discuss the specificity of a drug's effects by considering whether the drug is selectively a stimulant, sedative, anxiolytic or anxiogenic agent.

The relationship between the behavioral measures from the holeboard test and those from the plus-maze is examined in the first experiment. Experiments 1 and 3 examine whether the behavior of animals changes with repeated testing and whether the response to an anxiolytic is altered in animals familiar with the test apparatus. The effects of various pharmacological agents with known anxiolytic or anxiogenic actions on behavior in the holeboard and plus-maze are investigated in experiment 2. The final experiment investigates whether testing animals in the holeboard apparatus alters their subsequent behavior in the plus-maze.

Materials and methods

Animals

Male NIH Swiss mice weighing approximately 25 g were used in all experiments. They were housed in groups of five to seven and allowed free access to food and water. Animals were naive to both the holeboard apparatus and the plus-maze except in experiments 1 and 3, when varying degrees of familiarity with the apparatus was an experimentally controlled variable.

Drugs

Chlordiazepoxide hydrochloride (CDP), *d*-amphetamine hydrochloride, caffeine, picrotoxin, sodium pentobarbital and imipramine hydrochloride were obtained from Sigma and were dissolved in distilled water. FG 7142 (Ferrosan) was suspended in distilled water to which a drop of Tween 20 had been added. All drugs were administered intraperitoneally (IP) in an injection volume of 10 ml/kg. Ethanol was mixed with distilled water to give an injection volume of 20 ml/kg.

Apparatus

The plus maze was made of plexiglass and consisted of two open arms 30×5 cm and two enclosed arms $30 \times 5 \times 15$ cm. The arms extended from a central platform 5×5 cm. The open arms, the central platform, and the floor

of the closed arms were made of black plexiglass. The apparatus was mounted on a plexiglass base, raising it 38.5 cm above the floor. The principle difference between the apparatus used by Pellow et al. for their work in rats and the scaled down version used in the current experiments with mice is that the side walls of our apparatus were made of clear plexiglass rather than wood. This change was made to ensure that the light levels on the open and closed arms were approximately the same. The side walls were 14.5 cm high.

The holeboard apparatus was a plexiglass box $40 \times 40 \times 30$ cm, with four holes 3 cm in diameter equally spaced in the floor. Infra-red photocells directly beneath each hole provided automated measures of the number of head-dips and time spent head-dipping. Four pairs of infra-red photocells mounted in the walls of the box provided an automated measure of locomotor activity. The apparatus was interfaced with a PDP-11 microcomputer running SKED-11 software.

Experiment 1

In the first experiment 91 NIH mice received an IP injection of distilled water (10 ml/kg). Twenty-five minutes later each mouse was tested individually for 5 min in the holeboard apparatus. Immediately after the end of the holeboard test each mouse was placed in the centre of the plus-maze facing an open arm. During the 5 min test the number of entries into each type of arm and the time spent in each arm were scored using a keyboard interfaced with a PDP-11 microcomputer. A mouse was taken to have entered an arm when all four legs were on the arm. At the end of the test, the number of entries into the open arms was expressed as a percentage of the total number of arm entries. The time spent on the open arms was also expressed as a percentage of the time spent on both the open and the closed arms. It should be noted that since all animals spent some time on the central platform the denominator of the latter measure was always less than 5 min.

A BMDP statistical package was used to factor analyse the data. A principal component solution and an orthogonal rotation of the factor matrix were employed.

The final part of experiment 1 investigated the effects of familiarity with the apparatus on the behaviour of animals in the plus-maze. It also allowed an evaluation of the test-retest reliability. Twenty-six mice were tested individually for 5 min in the holeboard 25 min after receiving an IP injection of distilled water (10 ml/kg). The mice were tested in the plus-maze immediately after the holeboard test and the behaviour scored as described above. The procedure was repeated every 48 h until each animal had been tested on four occasions.

Experiment 2

The second experiment investigated the effects of various psychotropic drugs on the behaviour of mice in the plus-maze. In each case animals were injected with the drug or its vehicle 25 min before being tested individually in the holeboard. Immediately after the holeboard test each animal was placed in the plus-maze facing an open arm for a 5 min test. The behavior was scored as described in experiment 1.

The following drugs were used: chlordiazepoxide hydrochloride (5 and 10 mg/kg), ethanol (0.8 and 1.6 g/kg), sodium pentobarbital (7.5, 15 and 30 mg/kg), caffeine (15, 30 and 60 mg/kg), FG 7142 (5 and 10 mg/kg), picrotoxin (1 and 2 mg/kg), imipramine (7.5 and 15 mg/kg), *d*-amphetamine (1, 2 and 4 mg/kg).

Table 1. Correlations between the six measures obtained from the plus-maze and holeboard tests ($N=91$)

	1	2	3	4	5	6
1 % Entries made into open arms	*					
2 % Time spent in open arms	0.86	*				
3 Total number of entries	0.39	0.39	*			
4 Number of head-dips	0.11	0.13	0.31	*		
5 Time spent head-dipping	0.13	0.15	0.12	0.50	*	
6 Locomotor activity	0 04	0.04	0.39	0.16	-0.22	*

Values greater than 0.27 are significant, $P<0$ 01

Table 2. Rotated factor loadings for the variables from the holeboard and plus-maze tests

	Factor 1	Factor 2	Factor 3
% Entries made into open arms	0.953	0.000	0.000
% Time spent in open arms	0.951	0.000	0.000
Total number of entries	0.421	0.000	0.676
Number of head-dips	0.000	0.845	0.000
Time spent head-dipping	0.000	0.872	0.000
Locomotor activity	0.000	0.000	0.909

Factor loadings less than 0.25 have been replaced by 0.000

Table 3. The behavior of mice in a holeboard and a plus-maze following repeated testing every 48 h. Scores are means ± SEM ($N=26$). Test-retest correlation coefficients for each test relative to the first test are given below each mean

	Test 1	Test 2	Test 3	Test 4
% Entries into open arms	42.1 ± 2.6	40.9 ± 2.9	41.9 ± 2.9	43.3 ± 2.6
	—	0.57**	0.46*	0.41
% Time on open arms	33.9 ± 2.4	30.0 ± 2.7	28.8 ± 2.6	31.7 ± 3.1
	—	0.64**	0.55**	0.29
Total entries	18.9 ± 0.8	21.3 ± 1.1	22.8 ± 1.3	24.9 ± 1.1[a]
	—	0.44*	0.45*	0.34
Number of head-dips	65.4 ± 2.8	52.1 ± 4.9	46.9 ± 4.8	37.2 ± 3.2[a]
	—	0.51**	0.65**	0.47**
Time spent head-dipping	26.7 ± 2.2	25.2 ± 3.0	27.4 ± 3.1	23.6 ± 2.5
	—	0.61**	0.47**	0.63**
Locomotor activity	159 ± 4	169 ± 6	158 ± 5	151 ± 7
	—	−0.02	0.34	0.30

[a] Significant effect of repeated testing (see text).

* $P<0.05$, ** $P<0.01$

Experiment 3

The third experiment examined whether the effects of an anxiolytic (chlordiazepoxide) were altered in animals that

Table 4. The effects of FG 7142 (5–10 mg/kg), caffeine (15–60 mg/kg), picrotoxin (1–2 mg/kg), amphetamine (1–4 mg/kg), imipramine (7.5–15 mg/kg), sodium pentobarbital (7.5–30 mg/kg), chlordiazepoxide (5–10 mg/kg), and ethanol (0.8–1.6 g/kg) on the behavior of mice in a holeboard apparatus and a plus-maze. Scores are means ± SEM, $N = 7$–11 per group

Drug	Plus-maze			Holeboard		
	Open/total number	Open/total time	Total entries	Number of head-dips	Time head-dipping	Motor activity
Vehicle	41.9 ± 6.0	39.5 ± 5.0	17.9 ± 2.0	71.2 ± 6.2	45.5 ± 6.3	136 ± 10
FG 5	27.2 ± 3.7	19.7 ± 2.8	14.9 ± 1.6	63.9 ± 7.7	31.0 ± 6.0	154 ± 8
FG 10	38.0 ± 5.1	29.5 ± 5.8[a]	15.6 ± 1.0	58.7 ± 5.8	29.8 ± 2.4	136 ± 7
Vehicle	43.1 ± 2 8	36.8 ± 2.6	19.2 ± 1.3	71.6 ± 5.1	47.4 ± 3.8	137 ± 6
CAF 15	34.9 ± 5.6	33.7 ± 4.8	20.4 ± 2.1	56.3 ± 5.4	20.6 ± 4.0	160 ± 14
CAF 30	37.8 ± 3.1	32.9 ± 3.0	20.0 ± 2.2	48.0 ± 5.5	22.5 ± 4.5	155 ± 8
CAF 60	26.5 ± 5.6[a]	20.2 ± 6.0[a]	20.1 ± 2.6	47.9 ± 9.2[a]	19.7 ± 4.2[a]	143 ± 8
Vehicle	42.9 ± 3.2	36.5 ± 2.7	19.2 ± 1.3	69.9 ± 4.8	45.0 ± 3.9	136 ± 6
PIC 1	36.8 ± 4.1	35.1 ± 4.4	17.7 ± 1.3	54.8 ± 7.0	26.1 ± 5.0	131 ± 14
PIC 2	18.5 ± 8.1[a]	15.5 ± 6.6[a]	12.2 ± 2.6[a]	30.8 ± 7.6[a]	13.6 ± 4.4[a]	79 ± 19[a]
Vehicle	41.0 ± 2.7	33.7 ± 2.6	19.5 ± 1.3	58.8 ± 3.7	32.7 ± 2.1	150 ± 7
AMP 1	44.5 ± 6.1	39.2 ± 5.7	23.7 ± 2.2	56.6 ± 7.8	28.7 ± 5.1	162 ± 9
AMP 2	37.2 ± 7.1	38.4 ± 7.1	29.4 ± 3.0	72.1 ± 6.6	29.7 ± 5.3	175 ± 11
AMP 4	36.6 ± 9.9	38.6 ± 9.4	43.1 ± 2.9[a]	27.0 ± 3.7[a]	7.3 ± 1.1[a]	269 ± 11[a]
IMI 7.5	40.2 ± 6.0	34.5 ± 6.4	17.0 ± 2.1	66.9 ± 5.3	32.1 ± 2.8	140 ± 7
IMI 15	31.7 ± 5.6	28.0 ± 5.8	16.3 ± 1.6	61.8 ± 7.5	27.3 ± 4.3	152 ± 6
PB 7.5	42.9 ± 4.7	35.8 ± 4.7	19.9 ± 1.8	53.1 ± 5.1	27.3 ± 3.7	144 ± 12
PB 15	50.4 ± 5.4	41.9 ± 6.9	19.6 ± 2.3	52.9 ± 9.3	31.5 ± 6.7	137 ± 13
PB 30	61.1 ± 8.5[a]	54.8 ± 7.5[a]	30.7 ± 3.1[a]	40.3 ± 9.6	15.6 ± 5.8	244 ± 14[a]
Vehicle	39.0 ± 8.1	31.4 ± 6.7	18.3 ± 1.9	74.1 ± 6.5	42.8 ± 6.0	134 ± 12
CDP 5	53.5 ± 3.2	46.8 ± 3.9	35.3 ± 3.8	65.0 ± 9.6	46.3 ± 6.9	151 ± 9
CDP 10	63.2 ± 4.2[a]	53.5 ± 4.8[a]	36.4 ± 3.6[a]	68.0 ± 10 0	36.6 ± 6.1	160 ± 31
ET 0.8	39.0 ± 4.6	29.0 ± 4.0	18.7 ± 2.2	63.9 ± 3.2	37.9 ± 3.7	122 ± 17
ET 1.6	55.7 ± 4.9[a]	49.4 ± 5.5[a]	31.3 ± 2.6[a]	56.8 ± 6.4	30.3 ± 2.3	145 ± 24

[a] Significant overall drug effect, see text

had previously been tested in the plus-maze. A group of 17 animals was tested in the holeboard for 5 min, 25 min after receiving an IP injection of distilled water. Immediately after the holeboard test they were tested for 5 min in the plus-maze. A second group of 16 animals received the same handling but were not tested in either the holeboard or the plus-maze. Forty-eight hours later eight or nine animals from each group received an IP injection of the vehicle and the remaining animals received 7.5 mg/kg chlordiazepoxide. After 25 min each animal was tested for 5 min in the holeboard and immediately afterwards was tested in the plus-maze.

The data from the final test day were analysed using analysis of variance with drug condition and familiarity with the apparatus as independent measures.

Experiment 4

The fourth experiment examined whether the behavior of animals in the plus-maze was affected by testing them in the holeboard first. It also examined whether the effects of CDP on behavior in the plus-maze were altered by exposing mice to the holeboard. Thirty-eight animals were divided into four groups each containing nine or ten animals. Chlordiazepoxide (7.5 mg/kg) was administered IP to two of the groups and the other two groups received the vehicle. One group from each treatment was tested for 5 min in the holeboard, 30 min after injection. Immediately after the holeboard test the animals were tested for 5 min in the plus-maze. The other two groups were tested in the plus-maze for 5 min, 35 min after their injection but were not tested in the holeboard. Behavior was scored as described in experiment 1.

Results

Experiment 1

The correlations between the different behavioral measures are shown in Table 1. Three factors emerged from the analysis corresponding to those with eigenvalues greater than 1 and these accounted for 83.4% of the variance. Each behavioral index loaded on to one or other of the three factors (see Table 2). We consider factor 1 to be an index of anxiety, since the two measures of anxiety used by Pellow et al. load on this factor. Factor 2 appears to be a measure of directed exploration, since it comprises the two measures of head-dipping. Factor 3 appears to be a measure of locomotion with contributions from locomotor activity in the holeboard test and from the total number of arm entries in the plus-maze.

The mean scores and standard deviations for the behavioral measures from the plus-maze are: percentage of entries into the open arms = 40.7 ± 13.7, percentage time in the open arms = 33.5 ± 12.6, and the total number of entries = 19.2 ± 5.1. Undrugged animals spent less time in the open

than in the closed arms and made fewer entries into open than into closed arms ($P<0.001$).

Neither of the two indices of anxiety were altered by repeatedly testing the animals. There was a progressive increase in the total number of arm entries with repeated testing [$F(3,75)=9.3$, $P<0.0001$] but animals' motor activity in the holeboard apparatus did not vary over the 4 days. There was a progressive decrease in the number of head-dips in the holeboard from the first to the fourth test [$F(3,75)=21.3$, $P<0.0001$], although the reduction in the time spent head-dipping was not significant (see Table 3).

The test-retest correlations between the scores on the first and the subsequent tests are also shown in Table 3.

Experiment 2

The effect of the various drug treatments on the behavior of animals in the holeboard and plus-maze is shown in Table 4.

FG 7142 significantly reduced the proportion of time spent in the open arms [$F(1,23)=4.3$, $P<0.05$] and did not significantly affect the total number of arm entries or any of the behavioral measures from the holeboard test. The 5 mg/kg dose of FG 7142 appeared to be more effective than the 10 mg/kg dose, although this difference was not significant ($P>0.05$, Dunnett).

Caffeine significantly reduced the proportion of entries into [$F(1,35)=3.1$, $P<0.05$] and the proportion of time on [$F(1,35)=3.4$, $P<0.05$] the open arms. The drug did not affect the total number of entries. In the holeboard test caffeine significantly reduced the number of head-dips [$F(1,35)=4.0$, $P<0.02$] and the time spent head-dipping [$F(1,35)=12.0$, $P<0.0001$], but did not significantly alter locomotor activity.

Picrotoxin significantly reduced the percentage of entries made into open arms [$F(1,33)=6.56$, $P<0.005$], the percentage of time spent on the open arms [$F(1,33)=7.03$, $P<0.005$], and the total number of arm entries [$F(1,33)=4.3$, $P<0.05$]. In the holeboard test the number of head-dips [$F(1,33)=10.4$, $P<0.001$], the time spent head-dipping [$F(1,33)=13.6$, $P<0.001$] and locomotor activity [$F(1,33)=6.63$, $P<0.005$] were all reduced.

Amphetamine did not significantly alter either index of anxiety but significantly increased the total number of arm entries [$F(1,41)=23.9$, $P<0.001$]. The drug caused significant reductions in the number of head-dips [$F(1,41)=10.6$, $P<0.001$] and the time spent head-dipping [$F(1,41)=11.2$, $P<0.001$], due almost entirely to the effects of the highest dose. Amphetamine-treated animals showed a dose-related increase in locomotor activity [$F(1,41)=33.4$, $P<0.001$].

Imipramine did not significantly alter any of the behavioral measures.

Sodium pentobarbital caused a dose-related increase in the proportion of entries into open arms [$F(1,38)=3.3$, $P<0.05$], the proportion of time spent on the open arms [$F(1,38)=3.2$, $P<0.05$] and the total number of arm entries [$F(1,38)=5.6$, $P<0.005$]. In the holeboard test the number of head-dips was not altered by the drug treatment. A reduction in the time spent head-dipping just failed to reach significance [$F(1,38)=2.7$, $P=0.06$]. Locomotor activity was increased [$F(1,38)=15.4$, $P<0.001$] due entirely to the effects of the highest dose.

Chlordiazepoxide caused dose-related increases in the proportion of entries made into open arms [$F(1,18)=4.7$, $P<0.025$], the proportion of time spent in the open arms [$F(1,18)=4.7$, $P<0.025$] and in the total number of arm entries [$F(1,18)=10.2$, $P<0.005$]. Behavior in the holeboard was not significantly altered.

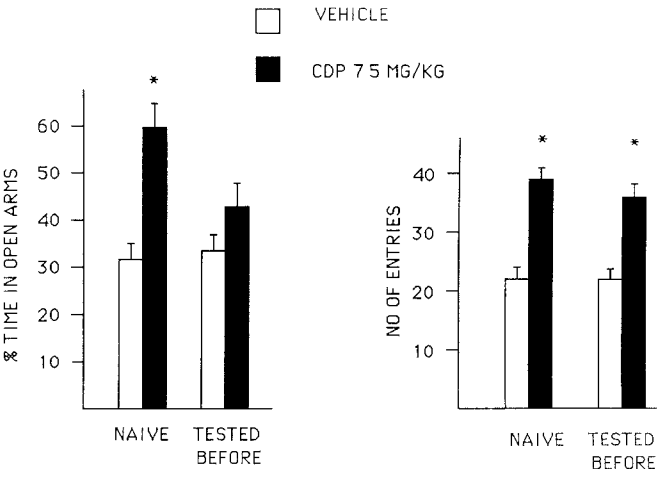

Fig. 1. The effect of chlordiazepoxide (*CDP*) on the percentage of time spent on the open arms (*left*) and on the total number of arm entries (*right*) during a 5-min test on a plus-maze. Mice were either naive to the apparatus, or had been tested once before, 24 h earlier, in an undrugged state. Scores are means ±SEM. * Significantly different from animals treated with vehicle ($P<0.05$, Dunnett)

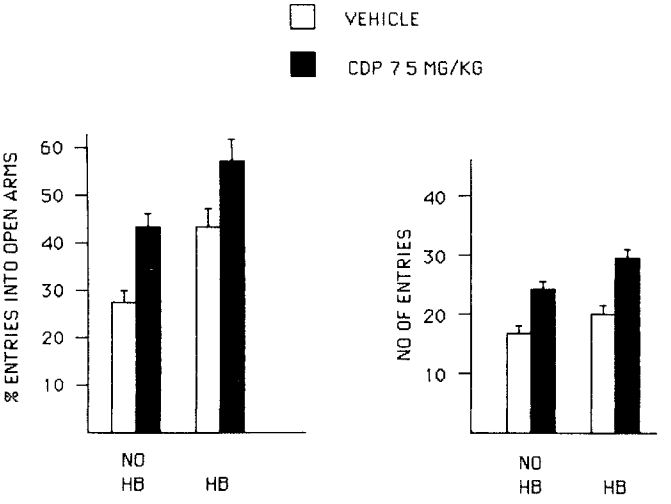

Fig. 2. The effect of chlordiazepoxide (*CDP*) on the percentage of entries made into the open arms of a plus-maze (*left*) and on the total number of arm entries (*right*) during a 5-min test. Mice either received a 5-min test in a holeboard apparatus (*HB*) immediately before being tested in the plus-maze, or remained in their home cage prior to testing (*NO HB*). Scores are means ±SEM

Ethanol significantly increased the proportion of time spent in the open arms [$F(1,17)=3.7$, $P<0.05$] and the total number of arm entries [$F(1,17)=10.7$, $P<0.005$]. Changes in the other behavioral measures did not reach significance.

Experiment 3

There were significant drug x familiarity interactions for both the proportion of entries made into the open arms [$F(1,29)=4.8$, $P<0.05$] and the proportion of time spent

in the open arms [$F(1,29)=4.0$, $P<0.05$, see Fig. 1]. Although chlordiazepoxide increased both these measures (as we had observed in experiment 3), the increase was significantly greater in animals that had not been tested before (see Fig. 1).

Chlordiazepoxide significantly increased the total number of arm entries [$F(1,29)=41.3$, $P<0.0001$] regardless of whether or not the animals had been tested before (i.e., there was no drug x familiarity interaction, see Fig. 1).

In the holeboard test, there was a significant familiarity effect for both the number of head-dips [$F(1,29)=23.9$, $P<0.0001$], and the time spent head-dipping [$F(1,29)=6.0$, $P<0.025$], familiar animals having reduced levels of exploration. A drug x familiarity interaction on the number of head-dips just failed to reach significance [$F(1,29)=3.96$, $P<0.06$]. Chlordiazepoxide significantly increased locomotor activity [$F(1,29)=8.5$, $P<0.01$].

Experiment 4

Experience in the holeboard [$F(1,34)=18.6$, $P<0.001$] and chlordiazepoxide [$F(1,34)=17.8$, $P<0.001$] both increased the proportion of entries made into the open arms of the plus-maze. Increases in the proportion of time spent on the open arms were also observed as a result of testing animals in the holeboard [$F(1,34)=15.7$, $P<0.001$] or of treating them with chlordiazepoxide [$F(1,34)=6.3$, $P<0.02$]. The holeboard test [$F(1,34)=8.7$, $P<0.01$] and chlordiazepoxide [$F(1,34)=36.6$, $P<0.0001$] each increased the total number of arm entries. In no measure was there any indication of a drug x holeboard experience interaction (see Fig. 2).

Discussion

The first experiment clearly showed that undrugged mice have a consistent preference for the closed arms. The factor analysis indicated that the six parameters from the holeboard and plus-maze tests contributed to three different measures. The two indices of anxiety used by previous investigators, (i.e., the percentage of arm entries made into the open arms, and the time spent on the open arms expressed as a percentage of the total time spent on either type of arm) correlated highly with each other ($r=0.86$) as documented by Pellow et al. (1985) in rats, and both loaded on factor 1. A previous study (File 1983) using rats in the holeboard test showed a high correlation between the number of head-dips and the duration of head-dipping, and in the present study with mice these two measures of directed exploration loaded on factor 2. Motor activity in the holeboard and the total number of arm entries in the plus-maze, which both appear to be measures of locomotion, were significantly correlated with one another and both loaded on factor 3.

Results of several of the subsequent experiments support the idea that the various measures are distinct by showing that they can vary independently. For example, in experiment 1, although the total number of arm entries increased with repeated testing, there was no indication of any change in either index of anxiety, and the number of head-dips made in the holeboard test decreased steadily over the four test sessions. That the indices of anxiety did not change with increased familiarity with the apparatus is, perhaps, surprising, but is consistent with the observations of Pellow et al. (1985) who tested rats on three occasions. The test-retest correlations for the 26 mice used in the present study were lower than was found by Pellow et al. for the six rats that they tested.

Of the drugs tested in experiment 2, FG 7142 and caffeine both reduced the percentage of time spent on the open arms, consistent with previous reports of their anxiogenic actions (Dorow et al. 1983; Charney et al. 1984; Uhde et al. 1984). Neither drug significantly altered the total number of arm entries. Picrotoxin, which has been shown to possess anxiogenic actions in other tests (Prado de Carvalho et al. 1983; File and Lister 1984), decreased both indices of anxiety, but also decreased the total number of arm entries and all of the behavioral measures from the holeboard test. An anxiogenic interpretation of picrotoxin's action should be made with caution, since all the behavioral measures were depressed.

Amphetamine, which has been reported to have an anxiogenic action in other tests including the plus-maze with rats (Pellow et al. 1985), failed to alter either index of anxiety in the present experiment although the total number of arm entries was increased. The dissociation between this drug's effect on directed exploration and on locomotor activity that has been reported previously (Robbins and Iversen 1973; File and Wardill 1975) was confirmed in the holeboard test in the present study, in which an increase in locomotor activity was observed with a decrease in exploratory head-dipping.

Imipramine failed to alter the behavior of mice in the plus-maze, consistent with previous reports in rats (Pellow et al. 1985; Briley et al. 1986). It is possible that effects may have been seen with higher doses.

Chlordiazepoxide, ethanol and sodium pentobarbital each increased the percentage of time spent on the open arms and the total number of arm entries, consistent with their reported anxiolytic actions. All three drugs also increased the total number of arm entries, but only sodium pentobarbital significantly increased locomotor activity in the holeboard test.

In experiment 3, as in experiment 1, undrugged animals that had been tested in the plus-maze 2 days earlier did not differ in either index of anxiety from those that were unfamiliar with the apparatus. Pellow et al. (1985) obtained similar results and suggested that it may be possible to use the same animals twice. However, in contrast to the behavior of undrugged animals the effect of the anxiolytic chlordiazepoxide was reduced in mice that had been tested in the plus-maze once before (in an undrugged state). Although it is possible that this effect of familiarity with the apparatus may diminish as the interval between tests increases, the results of this experiment suggest that caution should be exercised in repeatedly testing animals.

In the holeboard 7.5 mg/kg chlordiazepoxide significantly increased locomotor activity, while in experiment 2 no significant effect was observed on this measure with doses of 5 and 10 mg/kg. The apparent discrepancy between the two experiments may have resulted from the different doses used. Chlordiazepoxide has a biphasic dose-response curve on measures of locomotion and exploration (Ahtee and Shillito 1970; Noland and Parkes 1973).

In experiment 4 testing animals in the holeboard immediately before the plus-maze test significantly increased both indices of anxiety and also the total number of arm entries. Interestingly, the size of this behavioral manipulation was

as great as that of a 7.5 mg/kg dose of the anxiolytic chlordiazepoxide. The effect of chlordiazepoxide, however, was no different in animals that had been tested in the holeboard than in those that had not. The holeboard manipulation appeared to increase the percentage of entries into open arms to a greater extent than the total number of arm entries.

In conclusion, the results of the present study using mice are in broad agreement with those obtained by Pellow et al. (1985) with rats. Undrugged animals show a preference for the closed arms of the plus-maze, and this preference is increased in animals given anxiogenic agents such as caffeine and FG 7142, and decreased in animals that have received anxiolytic agents such as chlordiazepoxide and ethanol. The test is rapid and offers a number of advantages over other paradigms used to assess anxiety that involve food or water deprivation or shock administration. In particular, drug effects on appetite or sensitivity to pain are unlikely to interfere with experimental results. The test relies on spontaneous behaviour and, therefore, animals do not have to be trained. Finally in the strain of mice used, the test can detect both anxiolytic and anxiogenic drug effects. Since the behavior of mice and their response to anxiolytics varies with genotype (e.g., Crawley and Davis 1982; Crabbe 1983), experiments are currently in progress using different strains of mice on the plus-maze, investigating their behavior when undrugged and also following treatment with various anxiolytics.

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