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Influence of Prior Experience on Mice Behavior Using the Four-Plate Test

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HASCOET, M., M. BOURIN AND A. COUETOUX DU TERTRE. *Influence of prior experience on mice behavior using the four-plate test.* PHARMACOL BIOCHEM BEHAV **58**(4) 1131–1138, 1997.—A single prior undrugged exposure to the four-plate test reduces punished responding on retest at intervals ranging from 24 h to 42 days. Furthermore, prior experience attenuates the anxiolytic response to the benzodiazepines diazepam (0.25 to 2 mg/kg) and lorazepam (0.5 to 4 mg/kg). The result was first discussed in term of "one trial tolerance." The anxiety baseline was increased during the retest, which counteracted the anxiolytic action of benzodiazepines. To ascertain if memory processes are also implicated, the cholinergic drugs scopolamine and oxotremorine were used. Additional experiments with the GABAergic inverse agonist FG7142 and with the 5-HT_1A receptor agonist 8-OH-DPAT were also performed. Administration of scopolamine and 8-OH-DPAT–induced weak impairment of memory, when administered before the second trial, but no effect was seen with cognition enhancing agents. © 1997 Elsevier Science Inc.

Four-plate test Prior experience—Anxiety Memory Retest—Mice

THE four-plate test is used for the measurement of anxiety in mice (1,6). This test is based on passive avoidance where mice must stop moving to avoid electric foot shocks. The floor of the experimental cage consists of four metallic plates, and each time mice cross from one plate to another they receive a mild electric shock. The consequence is a marked decrease in exploratory activity. With anxiolytic-treated mice, punished responding still persists. The procedure is simple and is widely used as an animal model of anxiety, and there is no need for training animals.

However, preliminary experiments (unpublished results) have shown that a previous experience of the test can significantly abolish the usual expected response of control animals during reexposure, i.e., the number of punished crossings decrease dramatically. In addition, mice with previous experience of the four-plate test have no anxiolytic response to diazepam administration. Influence of prior exposure to the elevated plus-maze is well documented (14–18,27). A marked attenuation, or even abolition of the response to anxiolytic compounds, was induced by a previous single undrugged experience to the test. This phenomenon was called "the one-trial tolerance" (16). The term of one-trial tolerance was used to describe the loss of anxiolytic efficacy of benzodiazepines following a prior experience of the elevated plus-maze in contrast to the normal 3-week treatment of benzodiazepine that is necessary to obtain tolerance to anxiolytic effects in the test (15). It has been reported that experience to the open arms was the crucial factor (17), and there was evidence for a role of learning as administration of high amnesic doses of chlordiazepoxide before the first exposure prevented the phenomenon (17). To assess the generalization of this phenomenon to other tests of anxiety, the punished drinking test was used (18). However, benzodiazepines still demonstrated anxiolytic activity during reexposure. Rodgers and Shepherd (28) demonstrated the abolition of anxiolytic response to diazepam in a light/dark test of exploration following prior experience of the elevated plus-maze.

The following experiments were designed, using the four-plate test, to understand and to characterize the lack of activity of mice during a second test trial. In initial experiments, the duration of this effect was considered by variation of the time interval between two exposures to the four-plate test. In a second set of experiments, we tried to understand if the effect observed in the four-plate test is related or not to the "one-trial tolerance" phenomenon. Several experiments were carried out with the benzodiazepines diazepam and lorazepam and with drugs known to produce experimental amnesia, or reported to have antiamnesic actions. The influence of different neurotransmitters systems, including acetylcholinergic, GABAergic, and serotoninergic systems, will be considered.

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METHODS

Animals

Male mice (Swiss strain) purchased from R. Janvier (Le Genest) were used. Their average body weight on the first day of study was 22 ± 2 g. These animals were housed in groups of 20 at constant room temperature (20°C) and had free access to food and water. All experiments were conducted within the guidelines of the French Ministry of Agriculture for experiments with laboratory animals by law No. 87.848.

Drugs

Scopolamine hydrochloride, 0.003 to 4 mg/kg (Sigma Chemical Co.); methylscopolamine bromide, 0.125 to 32 mg/kg (Sigma Chemical Co.), adrafinil, 2 to 64 mg/kg (Lafon, France); oxotremorine sesquifumarate, 0.003 to 0.125 mg/kg (Research Biochemicals Incorporated; R.B.I.), lorazepam, 0.03 to 4 mg/kg (Sanofi Pharma, France); diazepam, 0.25 to 8 mg/kg (Roche, France); FG7142 (N-methyl-β-carboline-carboxamide), 1 to 32 mg/kg (R.B.I.); (±) 8-OH-DPAT [8-hydroxy-2-(di-n-propylamino) tetralin] 0.5 to 8 mg/kg, (R.B.I.). The solutions were ultrasonically dispersed in distilled water except for adrafinil, lorazepam, diazepam, FG7142, and 8-OH-DPAT, which were dissolved in Tween 80 at 5% concentration. All drugs or vehicle were administered IP in a volume of 0.5 ml/20 g of body weight. Controls animals received vehicles only.

Psychopharmacological Tests

Actimeter test. The spontaneous activity of naive animals was recorded using a photoelectric actimeter (5). This apparatus consists of transparent cages in which the animal's activity is measured by light beams connected to a photoelectric cell. The activity is recorded during a 10-min test period. The actimeter test was performed independently of the four-plate test to examine the effect of drugs on the spontaneous locomotor activity of mice. The use of the actimeter test allows us to eliminate the influence of stimulation or sedation in the interpretation of the four-plate test results.

The four-plate test. The test was performed in naive mice. This apparatus consists of a cage with a floor composed of four metal plates connected to a device that can generate electric shocks (0.6 mA, 0.5 s). Following a 15-s latency period, the animal is subjected to an electric shock after crossing from one plate to another. The number of crossings is recorded during a 1-min test period (11). The top of the cage is covered by a transparent perspex lid that prevents escape behavior.

Experimental procedure. Testing mice in the four-plate test consisted of two separate trials over 2 consecutive days. The two trials were called "test" and "retest."

Preliminary experiment: to assess the optimum time, different time intervals were used between the two separate trials (test and retest phases) after administration of distilled water to mice. A 24-h interval was then chosen (see Results section). (a) Trial 1: in the first experiment drugs were administered IP 30 min before the first trial (test phase). This allowed us to determine the effect of the drug per se in the four-plate test and the effect on the acquisition of information. (b) Trial 2: in the second experiment, drugs were administered IP 30 min before the second trial (retest phase). This allowed us to estimate the influence of the first exposition to the test.

Analysis of data. The mean number of responses for each group and for each test was calculated and the final results were expressed as percentage of the value observed in control animals or as mean ± SEM (standard error of the mean) (see the text). Data were evaluated by nonparametric statistical methods due to a nonnormal distribution. Statistical analysis of the data was performed by application of the Kruskal–Wallis test for independent groups, followed by an "a posteriori" Newman–Keuls test, except for the study of determination of time interval between trials (for this analysis the Wilcoxon test for paired data was used).

All analyses were conducted using the PCSM program (Deltasoft) for IBM-compatible computers.

RESULTS

Preliminary Experiment: Determination of the Time Interval Between Trials

Seven groups of naive mice ($n = 10$) received distilled water 30 min before the first test phase. The time interval between two trials was: 24 h, 3 days, 7 days, 21 days, 28 days, or finally, 42 days (Fig. 1). Results showed no statistical differences between groups for the first trial in the four-plate test. Then, mice were tested again for the second trial (retest phase); they demonstrated a dramatic decrease in the number of punished crossings (5.2 ± 0.55 shocks during the test phase vs. 1 ± 0.2 shocks during retest phase for the "24-h interval" group) ($p \leqslant 0.01$ using the Wilcoxon test). An interval of 24 h was then chosen between the two trials for facilitation of the experiment, and to have similar body weights between groups of mice.

Effects of Cholinergic Drugs

Scopolamine induced a dose-dependent increase in spontaneous motility from the dose of 0.03 mg/kg (145% $p \leqslant 0.05$*) with a maximum effect at the dose of 1 mg/kg (188% $p \leqslant 0.01$ *) (Table 1). Experiment a: when scopolamine was administered 30 min before the test phase, it induced a dose-dependent increase in punished crossings during the test phase, but did not change the second trial in comparison with appropriate controls (Table 2). Experiment b: scopolamine significantly increased the punished crossings from the dose of 0.125 mg/kg to 4 mg/kg when administered 30 min before the retest phase (3.9 ± 0.8 punished crossings for 4 mg/kg during retest phase vs. 0.7 ± 0.3 for controls, $p \leqslant 0.01$) (Table 3).

Methylscopolamine significantly increased locomotion in mice at 16 and 32 mg/kg (Table 1). Methylscopolamine did not modify punished behavior in the four-plate test, either ad-

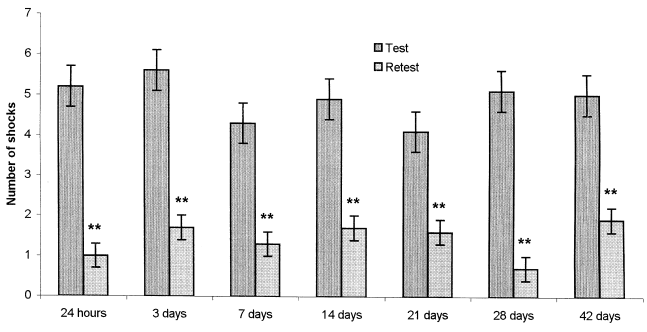

FIG. 1. Variation of time interval between test and retest phase. Mice received saline solution, IP, 30 min before the test. Results are expressed as the mean of 10 mice per group. Statistical differences between the day of test and retest phase were analyzed using the nonparametric Wilcoxon test for paired data. $p \leqslant 0.01$*.

TABLE 1

SPONTANEOUS LOCOMOTOR ACTIVITY OF MICE AFTER DRUG INJECTIONS

Doses (mg/kg)	*H*-test	Controls	0.003	0.007	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32
Scopo-		100%	105%	118%	126%	145%*	146%*	163%*	—	169%*	188%†	202%†	207%†	—	—	—
lamine	30.6627	127 ± 6	133 ± 11	150 ± 14	160 ± 11	184 ± 13*	185 ± 12*	207 ± 19*	—	214 ± 17*	—	—	—	—	—	—
	22.8493	147 ± 7	—	—	—	—	—	—	—	—	277 ± 17†	298 ± 19†	304 ± 12†	—	—	—
Methylsco-		100%	—	—	—	—	—	96%	—	105%	—	111%	122%	119%	132%*	154%†
polamine	29.1835	125 ± 11	—	—	—	—	—	120 ± 8	—	132 ± 7	—	139 ± 10	152 ± 11	150 ± 11	166 ± 11*	193 ± 10†
Oxotrem-		100%	85%*	80%*	71%†	32%†	20%†	26%*	—	—	—	—	—	—	—	—
orine	53.9898	155 ± 11	132 ± 13*	124 ± 13*	109 ± 9†	50 ± 7†	30 ± 5†	40 ± 24*	—	—	—	—	—	—	—	—
Adrafinil		100%	—	—	—	—	—	—	—	—	—	115%	128%*	124%	136%*	163%†
	31.2720	166 ± 12	—	—	—	—	—	—	—	—	—	191 ± 22	213 ± 15*	206 ± 12*	222 ± 17*	271 ± 18†
Lorazepam		100%	—	—	—	78%	67%*	34%†	19%†	24%†	12%†	15%†	15%†	—	—	—
	62.2596	201 ± 16	—	—	—	157 ± 15	135 ± 17*	68 ± 15†	39 ± 6†	49 ± 10†	23 ± 5†	31 ± 5†	30 ± 6†	—	—	—
Diazepam		100%	—	—	—	—	106%	102%	117%	106%	80%*	46%†	26%†	20%†	—	—
	57.3445	186 ± 9	—	—	—	—	198 ± 24	189 ± 15	217 ± 30	197 ± 30	149 ± 39*	86 ± 14†	49 ± 8†	37 ± 6†	—	—
FG 7142		100%	—	—	—	—	—	—	—	—	84%	87%	97%	95%	72%	69%
	9.8036	158 ± 8	—	—	—	—	—	—	—	—	132 ± 6	137 ± 14	154 ± 19	151 ± 25	114 ± 24	109 ± 16
8-OH-DPAT		100%	—	—	—	—	—	—	—	108%	143%	122%	65%*	47%†	—	—
	29.2756	145 ± 15	—	—	—	—	—	—	—	157 ± 14	207 ± 24	177 ± 24	94 ± 15*	68 ± 12†	—	—

Drugs were injected, IP, 30 min before the actimeter test. Results are expressed as the percentage of activity in comparison with control values ($n = 10$). Statistical analyses were performed using the nonparametric Kruskal–Wallis, *H*-test, followed by the a posteriori, Newman–Keuls test. *$p \leq 0.05$ and †$p \leq 0.01$.

TABLE 2

EFFECT OF MICE RESPONDING DURING THE FIRST AND SECOND TRIAL WHERE DRUGS WERE ADMINISTERED 30 MIN BEFORE THE FIRST EXPOSURE TO THE TEST (TRIAL 1)

Doses (mg/kg)	*H*-test	Controls	0.003	0.007	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32
Scopolamine	T 5.8522	4.6 ± 0.2	4.5 ± 0.3	3.9 ± 0.4	4.7 ± 0.4	4.1 ± 0.2	—	—	—	—	—	—	—	—	—	—
	R 6.3061	1.4 ± 0.3	0.7 ± 0.3	0.6 ± 0.2	0.8 ± 0.2	0.6 ± 0.2	—	—	—	—	—	—	—	—	—	—
	T 29.5145	3.4 ± 0.3	—	—	—	—	—	4.9 ± 0.5	5.7 ± 0.6†	7.1 ± 0.7†	7.2 ± 0.4†	—	—	—	—	—
	R 9.8680	0.6 ± 0.2	—	—	—	—	—	0.6 ± 0.2	1 ± 0.3	0.5 ± 0.2	1.5 ± 0.3	—	—	—	—	—
	T 24.26	4.7 ± 0.3	—	—	—	—	—	—	—		8.9 ± 0.5†	6.3 ± 0.5†	7.8 ± 0.5†	—	—	—
	R 5.6242	0.7 ± 0.3	—	—	—	—	—	—	—		0.4 ± 0.2†	1.1 ± 0.2	1.0 ± 0.3			
Methylscopolamine	T 3.0730	4.5 ± 0.3	—	—	—	—	—	—	4.1 ± 0.3	3.8 ± 0.3	—	3.9 ± 0.3	4.5 ± 0.4	—	—	—
	R 3.2329	0.5 ± 0.2	—	—	—	—	—	—	0.9 ± 0.5	0.7 ± 0.3	—	0.6 ± 0.2	0.2 ± 0.1	—	—	—
Oxotremorine	T 13.1334	6.2 ± 0.6	5.7 ± 0.4	3.8 ±0.5*	3.9 ± 0.5*	4.6 ± 0.7*	—	—	—	—	—	—	—	—	—	—
	R 7.4468	2.4 ± 0.3	2.4 ± 0.4	2.0 ± 0.2	1.5 ± 0.4	1.7 ± 0.4	—	—	—	—	—	—	—	—	—	—
Adrafinil	T 19.9206	4.9 ± 0.5	—	—	—	—	—	—	—	—	—	4.8 ± 0.5	5.3 ± 0.3	—	5.9 ± 0.4	7.0 ± 0.5*
	R 5.5680	0.5 ± 0.2	—	—	—	—	—	—	—	—	—	0.4 ± 0.2	0.2 ± 0.1	—	0.5 ± 0.2	0.7 ± 0.4
Lorazepam	T 12.1057	4.7 ± 0.3	—	—	—	4.6 ± 0.5	5.5 ± 0.4	6.2 ± 0.4	7.7 ± 1.1	—	—	—	—	—	—	—
	R 4.5003	2.5 ± 0.3	—	—	—	2.0 ± 0.3	1.9 ± 0.3	2.2 ± 0.4	2.7 ± 0.3	—	—	—	—	—	—	—
	T 11.3626	3.9 ± 0.4	—	—	—	—	—	—	—	8.0 ± 0.6*	6.7 ± 1.2*	7.3 ± 0.9*	6.0 ± 1.3*	—	—	—
	R 22.8253	0.7 ± 0.2	—	—	—	—	—	—	—	1.8 ± 0.5†	2.4 ± 0.6†	3.6 ± 0.6†	4.1 ± 0.4†	—	—	—
Diazepam	T 6.8161	4.9 ± 0.4	—	—	—	—	—	—	5.2 ± 0.2	5.7 ± 0.3	60 ± 0.5	6.0 ± 0.6	6.4 ± 0.8	—	—	—
	R 28.6248	0.5 ± 0.3	—	—	—	—	—	—	0.2 ± 0.1	1.2 ± 0.1†	1.5 ± 0.3†	1.9 ± 0.4†	2.2 ± 0.2†	—	—	—
FG 7142	T 3.5355	4.8 ± 0.2	—	—	—	—	—	—	—	—	—	4.4 ± 0.3	4.1 ± 0.3	4.6 ± 0.2	—	—
	R 3.0958	1.5 ± 0.3	—	—	—	—	—	—	—	—	—	0.9 ± 0.2	0.9 ± 0.2	0.8 ± 0.2	—	—
8-OH-DPAT	T 1.9803	4.3 ± 0.4	—	—	—	—	—	—	—	—	4.8 ± 0.3	5.0 ± 0.4	4.4 ± 0.3	—	—	—
	R 6.1706	0.3 ± 0.2	—	—	—	—	—	—	—	—	0.5 ± 0.2	0.9 ± 0.2	0.8 ± 0.1	—	—	—

Drugs or vehicles were injected, IP, 30 min before the first trial of the four plates test. Results are expressed as the percentage of activity in comparison with control value ($n = 10$). Statistical analyses were performed using the nonparametric Kruskal–Wallis *H*-test, followed by a posterior Newman–Keuls test. *$p \leqslant 0.5$* and †$p \leqslant 0.01$.

TABLE 3

EFFECT OF MICE RESPONDING DURING THE FIRST AND SECOND TRIAL WHERE DRUGS WERE ADMINISTERED 30 MIN BEFORE THE SECOND EXPOSURE TO THE TEST (TRIAL 2)

Doses (mg/kg)	*H*-test	Controls	0.003	0.007	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32
Scopolamine	T 4.3051	5.0 ± 0.3	4.7 ± 0.4	4.6 ± 0.3	5.0 ± 0.3	5.9 ± 0.6	—	—	—	—	—	—	—	—	—	—
	R 1.9307	1.3 ± 0.4	1.0 ± 0.2	0.8 ± 0.2	0.7 ± 0.2	0.9 ± 0.2	—	—	—	—	—	—	—	—	—	—
	T 0.5636	4.2 ± 0.2	—	—	—	—	—	3.9 ± 0.3	4.1 ± 0.3	4.1 ± 0.2	4.0 ± 0.4	—	—	—	—	—
	R 17.85	0.6 ± 0.2	—	—	—	—	—	1.5 ± 0.3†	2.1 ± 0.4†	2.4 ± 0.3†	3.2 ± 0.7†	—	—	—	—	—
	T 3.6832	4.5 ± 0.3	—	—	—	—	—	—	—	—	5.0 ± 0.3†	4.8 ± 0.4	4.2 ± 0.3	—	—	—
	R 9.7183	0.7 ± 0.3	—	—	—	—	—	—	—	—	2.2 ± 0.6*	3.0 ± 0.9*	3.9 ± 0.8*	—	—	—
Methylscopolamine	T 1.1861	4.2 ± 0.2	—	—	—	—	—	—	4.0 ± 0.3	4.0 ± 0.4	—	4.4 ± 0.5	3.9 ± 0.2	—	—	—
	R 4.9020	0.2 ± 0.1	—	—	—	—	—	—	0.7 ± 0.2	0.6 ± 0.2	—	1.0 ± 0.3	0.6 ± 0.3	—	—	—
Oxotremorine	T 2.8896	5.3 ± 0.5	4.6 ± 0.3	4.3 ± 0.4	4.8 ± 0.2	4.5 ± 0.5	—	—	—	—	—	—	—	—	—	—
	R 7.1961	1.3 ± 0.3	0.9 ± 0.2	1.1 ± 0.2	0.6 ± 0.3	0.4 ± 0.2	—	—	—	—	—	—	—	—	—	—
Adrafinil	T 2.2752	4.9 ± 0.4	—	—	—	—	—	—	—	—	—	5.1 ± 0.4	5.2 ± 0.4	—	4.7 ± 0.4	5.5 ± 0.3
	R 9.8155	0.5 ± 0.3	—	—	—	—	—	—	—	—	—	0.7 ± 0.4	0.7 ± 0.3	—	0.4 ± 0.2	1.6 ± 0.5
Lorazepam	T 3.3428	4.8 ± 0.4	—	—	—	—	—	—	—	4.4 ± 0.2	4.6 ± 0.3	4.2 ± 0.3	3.9 ± 0.3	—	—	—
	R 3.0632	0.7 ± 0.3	—	—	—	—	—	—	—	1.0 ± 0.3	0.7 ± 0.3	0.6 ± 0.3	1.1 ± 0.3	—	—	—
Diazepam	T 7.1913	4.9 ± 0.3	—	—	—	—	—	—	5.1 ± 0.2	5.4 ± 0.2	4.5 ± 0.2	4.9 ± 0.4	—	—	—	—
	R 5.5191	0.6 ± 0.2	—	—	—	—	—	—	0.6 ± 0.2	0.1 ± 0.1	0.5 ± 0.2	0.4 ± 0.2	—	—	—	—
FG 7142	T 5.8004	5.0 ± 0.2	—	—	—	—	—	—	—	—	—	4.0 ± 0.4	4.3 ± 0.3	4.1 ± 0.4	—	—
	R 14.1523	0.7 ± 0.2	—	—	—	—	—	—	—	—	—	0.4 ± 0.2	0.0 ± 0	0.0 ± 0	—	—
8-OH-DPAT	T 3.4955	5.1 ± 0.3	—	—	—	—	—	—	—	—	5.1 ± 0.3	5.0 ± 0.2	4.5 ± 0.2	—	—	—
	R 13.3015	0.6 ± 0.2	—	—	—	—	—	—	—	—	1.5 ± 0.3†	2.3 ± 0.4†	2.1 ± 0.2†	—	—	—

Drugs or vehicles were injected, IP, 30 min before the first trial of the four plates test. Results are expressed as the percentage of activity in comparison with control value ($n = 10$). Statistical analyses were performed using the nonparametric Kruskal–Wallis *H*-test, followed by a posterior Newman–Keuls test. *$p \leqslant 0.5$* and †$p \leqslant 0.01$.

ministered before the test phase or before the retest phase (Tables 2 and 3).

Oxotremorine significantly decreased spontaneous motility from 0.003 mg/kg to 0.125 mg/kg (26% of activity in comparison with control for 0.125 mg/kg, $p \leq 0.05$* (Table 1). Experiment a: when administered 30 min before the first trial, oxotremorine weakly decreased the number of punished crossings for the doses of 0.007 to 0.03 mg/kg ($p \leq 0.5$*) (Table 2). Experiment b: oxotremorine had no effects in comparison with appropriate controls when administered 30 min before the retest phase (Table 3).

Effects of Stimulant Drugs, Acting on the Noradrenergic System

Adrafinil significantly increased spontaneous motility from 4 to 32 mg/kg (Table 1). Experiment a: adrafinil increased punished crossings for the doses of 32 mg/kg ($p \leq 0.05$*) when administered before trial 1 (Table 2). Experiment b: adrafinil did not modify responding when administered before trial 2 (Table 3).

Effects of Drugs Acting on the GABAergic System

Lorazepam dramatically decreased spontaneous motility in mice from the doses of 0.06 mg/kg to 4 mg/kg (Table 1). Experiment a: lorazepam (0.03 mg/kg to 4 mg/kg), administered 30 min before the first trial, significantly increased the number of punished crossings, with a maximum effect at the dose of 0.5 mg/kg (8 ± 0.6 punished crossings vs. 3.9 ± 0.4 for control mice, $p \leq 0.05$*). The same effect was observed during trial 2, with a maximum effect at the dose of 4 mg/kg (4.1 ± 0.4 punished crossings vs. 0.7 ± 0.2 for controls, $p \leq 0.01$*) (Table 2). Experiment b: no effect was seen in comparison with controls when lorazepam was administered 30 min before trial 2 (Table 3).

Diazepam decreased spontaneous motility from 1 mg/kg to 8 mg/kg (20% of activity in comparison with controls, $p \leq 0.01$*). Experiment a: diazepam weakly increased punished crossings in mice, but this effect did not reach statistical significance (6.4 ± 0.8 for 4 mg/kg vs. 4.9 ± 0.4 for controls). On the other hand, it significantly increased punished crossings during the second trial (trial 2) 24 h later (2.2 ± 0.2 for 4 mg/kg vs. 0.5 ± 0.3 for controls) (Table 2). Experiment b: diazepam, at the dose range chosen, did not modify behavior in comparison with controls when administered before trial 2 (Table 3).

FG 7142 had no effect on spontaneous activity (Table 1). FG 7142 did not modify punished behavior in the four-plate test either in experiments a or b (Tables 2 and 3).

Effects of a Serotoninergic Drug

8-OH-DPAT decreased spontaneous motility in mice at the doses of 4 and 8 mg/kg (Table 1). Experiment a: 8-OH-DPAT (1 to 4 mg/kg) had no effect on both phases when administered 30 min before the first trial (Table 2). Experiment b: 8-OH-DPAT increased punished responding during trial 2 when administered before the second trial, with a maximum effect for 2 mg/kg (2.3 ± 0.4 punished crossings vs. 0.6 ± 0.2 for controls, $p \leq 0.01$*)

DISCUSSION

During a second exposure to the four-plate test, all mice demonstrated a dramatic decrease in punished exploratory activity, with an inhibition of the punished crossings. Furthermore, mice immediately tried to escape from the box. This effect appeared as soon as mice were placed in the box test, even before receiving any electric shocks. Then, mice adopted a freezing attitude. This decrease in activity was persistent even at the "42-day interval" between the two sessions.

Slotnick and Jarvik (30) have already found similar results in the four-plate test, but the testing procedure was different. During the second trial, no shock was applied to the mice. The authors noticed a decrease of about 83% in activity during the second exposition to the test, 24 h later, for saline-treated animals, when compared with the first trial.

The inhibition of activity during the second session seems to be an interesting indication of shock-induced fear and of the implication of memory processes. It could also help to understand underlying mechanisms between anxiety and memory, and their interplay.

The preliminary experiment demonstrated that prior exposure to the four-plate test could markedly influence the future responses of mice. It would seem that on initial exposure, the animals have acquired some information about the test, and that this effect persists even at 42 days. Some experiments on reexposure to test have been already performed using the elevated plus-maze, but with this test the score of saline control animals did not change from the first trial to the reexposure. That is one point of discordance with our results. But it is possible that the two tests generate different kinds of anxiety, and that punishment induces strong behavioral inhibition and reinforces learning. The elevated plus-maze test is based upon the natural aversion of rodents to height and open spaces (23,25). With this test, Rodgers and Shepherd (27,28) found that prior experience of the plus-maze eliminated the anxiolytic response to diazepam and reduced or even abolished the anxiolytic effect of chlordiazepoxide in mice (23). This effect did not seem to be specific to the plus-maze. We have observed the same results where lorazepam and diazepam, at nonsedative doses, did not demonstrate any effect in comparison with controls when administered 30 min before the second trial of the four-plate test. The knowledge of the test, where there is no possibility to escape from the electric shocks, reduced the tendency to explore the box during reexposure. This resulted in a negative response to diazepam and lorazepam on the second trial. It might be difficult to distinguish between decrease in punished activity and enhanced anxiety. But during the first seconds of the reexposure, the fact that mice tried to escape from the box seems a factor of fear and of apprehension of the test. From our own conception of the four-plate test, anxiety always appears as a decrease in activity where mice stay immobile in a corner of the box.

Da Cunha et al. (9) have reported that the time spent in the open arms of an elevated plus-maze decreased with the number of sessions. Using another test developed for the studies of anxiolytic compounds, we have here demonstrated, as in previous studies (9,27,28), that reexposure to the test induced an anxiogenic-like behavioral profile in control animals. The anxiety baseline was increased during the retest, which counteracted the anxiolytic action of benzodiazepines. Mice developed some kind of anticipatory anxiety reaction. Previous exposures to the test have developed a kind of phobic state against which the benzodiazepines are ineffective (18). The term "one-trial tolerance" has been used to characterize this phenomenon as the effect seen was equivalent to 21 days pretreatment with chlordiazepoxide (14).

The behavioral background of mice is important for the functioning of the GABA–benzodiazepine system, and this system can also be influence by environmental stress (4). In naive rats, the stress of handling was found to decrease

$GABA_A$ receptor binding (3) and benzodiazepine receptor binding in the cortex (24). According to File et al. (16), the beneficial effect of benzodiazepines markedly depends on the baseline condition of the animals and is modified by the rodent's past experiences. In a recent study (19), Gonzalez and File made the hypothesis that previous experience of the plus-maze changed the state of the benzodiazepine receptor by releasing an endogenous inverse agonist.

On the other hand, no "one-trial tolerance" was found in the punished drinking test. Diazepam still demonstrated anxiolytic effects whatever the number of exposures to the test (18). Several tests, like the Geller-Seifter conflict test, require previous training procedure. In our model, based on operant conditioning (20), rats were submitted to 3 months of daily training before treatment where diazepam and alprazolam demonstrated strong anxiolytic effects. Those procedures do not induce lack of efficacy of benzodiazepine treatment. If the elevated plus-maze test is only based upon mice exploration, the four-plate test also implicates electric shocks as for the above conflict procedures. Punishment cannot be the only explanation for the lack of "one-trial tolerance" in conflict operant paradigms with or without training sessions. This will need further experimentation.

The results from the preliminary experiment have shown that even when the trials in the four-plate test were separated by even more than 1 month (42 days), the phenomenon was also observed. This very long effect confirmed the implication of learning. Furthermore, when 75 mg/kg of chlordiazepoxide was administered to rats, no tolerance to the test was then observed during reexposure. This was due not only to sedative effects, but also to the amnesic effects of benzodiazepines (17). One other hypothesis of discussion is that mice have habituated to the four-plate test, and the decrease in activity during the second exposure may only be the consequence of the knowledge of no possible escape. Reexposure to the four-plate test might be a tool for the evaluation of memory and for the screening of nootropic drugs in mice. Deterioration of central cholinergic neurotransmitter systems are considered to contribute to the memory impairment that occurs in Alzheimer's disease (8). The cholinergic blocker scopolamine and other anticholinergics have been found to disrupt learning in rats (21). From the results, it appeared that scopolamine produced an increase activity in the four-plate test when administered before or after the first trial. The exact nature of this effect is not clear. It can implicate either amnesia or anxiolytic-like effect. Injected immediately after the first trial of an elevated plus-maze (22), scopolamine increased during reexposure the time to move from the open arm to the closed one when mice were placed initially in the open arm (transfer latency). The same effect was seen in experimental amnesic mice. Posttraining reduction in muscarinic cholinergic activity could result in an impairment in memory storage. On the contrary, methylscopolamine, either administered before the test or retest phase, did not modify the number of punished crossings, indicating a central effect of scopolamine, as methylscopolamine does not cross the blood–brain barrier. Methylscopolamine was found to have no effect in a mnesic processes (2).

Scopolamine also induced an increase in spontaneous activity (see Table 1), which could interfere with the interpretation of the four-plate test results. For this purpose, adrafinil, a psychostimulant that has no effect on memory (12), was also used. When administered 30 min before reexposure to the four-plate test, it did not increase punished crossing, in contrast to scopolamine. A promesic effect would be expected with oxotremorine, but the detection of such effects was not possible using the modified four-plate test. Indeed, the baseline of animal crossings was to low on the second trial. In the same way, no effect was seen with the $GABA_A$ receptor inverse agonist FG 7142.

The role of the serotoninergic system has been considered in amnesic processes and in learning (10,13), but contradictory effects of 5-HT depletion have been observed, depending on the particular behavioral test employed (29). Behavioral studies have revealed that stimulation of 5-HT_1A receptors impairs performance in different tests used to assess learning and memory (26). In our study, 8-OH-DPAT, the 5-HT_1A receptor agonist, administered before the second trial, seemed to act like scopolamine with an impairment of memory in mice. In a passive avoidance task (7) 8-OH-DPAT was found to interfere with the mechanism related to the acquisition of memory.

In conclusion, the modified four-plate test would not be useful for studying memory and for the screening of nootropic drugs, as it was impossible to detect cognition enhancing agents. Furthermore, it was difficult to distinguish nonspecific effects.

The present study shows that prior four-plate test experience altered the behavioral and pharmacological reactions of mice during the second trial (reexposure). The underlying mechanism is not understood and will require further experimentation.

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