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A New Approach to the Light/Dark Test Procedure in Mice

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HASCÖET, M. AND M. BOURIN. *A new approach to the light/dark test procedure in mice.* PHARMACOL BIOCHEM BEHAV **60**(3) 645–653, 1998.—The effect of the known anxiolytic agents diazepam and alprazolam and a putative anxiogenic agent, FG 7142, were assessed in a fully automated and computer-integrated two-compartment light/dark apparatus. In addition, psychostimulant drugs (amphetamine, adrafinil, amineptine, and caffeine) were tested to determinate the influence of increasing locomotor activity on the indices of anxiety. Some modifications, such as using a soiled apparatus, have been made from the initial model to reduce any neophobic response to the test situation. These results have been compared to results obtained after cleaning between trials. In addition, strain differences have been assessed by comparing the effect of Swiss mice with the C57Bl/6J strain. The role of each parameter as an index of anxiety is discussed. The time spent in the lit area and exploratory behaviors seemed to be the most reliable parameter for assessing anxiolytic-like activity. Diazepam and alprazolam were found to have an anxiolytic profile. FG 7142 did not demonstrate any intrinsic effect. Amphetamine was reported to be anxiogenic, and amineptine, adrafinil, and caffeine only had a psychostimulant profile. We conclude that the light/dark test may be useful for identifying putative anxiolytic and anxiogenic agents, but an additional test such as an open field or an actimeter test must be performed as a control with regard to the problem of sedation and change in exploration. The Swiss strain of mice has been found a suitable strain to be used in the test. © 1998 Elsevier Science Inc.

Light/dark two-compartment test Anxiety Mice Anxiolytics Anxiogenics Psychostimulants Procedure

ANXIETY and/or fear can be induced by the novelty of a situation. They can be evaluated by the intensity of behavior of mice in an unknown area, by the quantity of unfamiliar food consumption (10) or by social interaction with another unknown animal (25). Generally, anxiolytic drugs such as benzodiazepines release punished behavior induced by mild electric shocks in tests like the operant conflict test (27,30). Other tests include the elevated plus maze, based on the natural aversion of rodents for height and open spaces (35,37) and the light/dark test (17,20), which uses the aversion of rodents for brightly lit large spaces.

Although the light/dark test was based on the initial model described by Crawley and colleagues (5,17,19,20), many authors have used it with several modifications. First, the nature of these modifications were structural, with changes in the size of the box (31), with the addition of a tunnel between the two compartments (4,23), with the light compartment included in the dark one (38), and computer-assisted data collection. Secondly, additional parameters such as indices of anxiety have been introduced. Five parameters are now available to assess the anxiolytic profile of drug treatment: the latency time for the first passage from the light compartment to the dark one, the number of transitions between the two compartments, the movement in each compartment, the time spent in the dark (or light), and sometimes the number of rears is reported. The use of various versions of the test and the variation in the parameters studied result in discrepancies between results reported in the literature, and it is now obvious that several parameters require careful analysis.

In the present study, a fully automated and computer-integrated two-compartment light and dark apparatus was used (9). As some modifications from the initial procedure were performed, the parameters are discussed in comparison to data reported in the literature. In addition, because natural strain differences exist for behavioral trait, C57Bl/6J and Swiss mice strains were compared for their activity in the light/dark paradigm. Two well-known benzodiazepines, diazepam and alprazolam, were used as references for anxiolytic-like activity. The effect of a GABAergic inverse agonist, FG 7142, was also studied. Psychostimulant drugs were tested in

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the light/dark test to determine the influence of increasing locomotor activity on the indices of anxiety. Indeed, anxiolytic effects measured in behavioral tests of anxiety are sometimes confounded by changes in locomotor activity (22). In view of this fact, the effect of amphetamine, caffeine, adrafinil, and amineptine were investigated in the light/dark test. In addition, an actimeter test was performed for all drugs cited, because this paradigm provides an independent measure of locomotor activity.

METHOD

The ethical rules of the French Ministry of Agriculture for experiments with laboratory animals (No. 87.848) were followed at all times.

Materials

Animals. Male mice were obtained from the following sources: Swiss mice (4 weeks old) purchased from R. Janvier (Le Genest, France), C57Bl/6J (4 weeks old) from R. Janvier and IFFA CREDO (69592 L'Arbresle, France). Their average body weight on the day of the study was 22 ± 2 g for the Swiss mice and 16 ± 2 g for the C57Bl/6J mice. These animals were housed in groups of 20, at constant temperature (20°C), with standard light cycle (lights on between 0700 and 1900 h), and had free access to food and water.

Drugs. Adrafinil, 2 to 32 mg/kg (Lafon, France), alprazolam 0.03 to 4 mg/kg, (Pharmacia Upjohn, France), amineptine, 0.5 to 16 mg/kg (Servier, France), caffeine 2 to 32 mg/kg, (Research Biochemicals Incorporated), dextroamphetamine sulfate, 1 to 32 mg/kg (Research Biochemicals Incorporated), diazepam, 0.06 to 4 mg/kg (Roche, France), and FG7142 (N-methyl-β-carboline-carboxamide), 0.5 to 32 mg/kg (Research Biochemicals Incorporated) were used.

All drugs were ultrasonically dispersed in distilled water except for adrafinil, alprazolam, diazepam, and FG7142, which were dissolved in 5% concentration of Tween 80. All drugs or vehicle were administered IP in a volume of 0.5 ml/20 g of body weight. Control animals received vehicle only.

Psychopharmacological Tests

Part 1. Preliminary experiments were performed to investigate any effect that the drugs might have on spontaneous locomotor activity.

Actimeter test. The spontaneous activity of naive animals was recorded using a photoelectric actimeter (6). This apparatus consisted of transparent cages in which the animals activity was measured by light beams connected to a photoelectric cell. The activity was recorded during a 10-min test period. The actimeter test was performed independently of the light–dark test to examine the effect of drugs on the spontaneous locomotor activity of mice.

Part 2. Experiments were performed in the light–dark apparatus under three different conditions: (a) clean vs. soiled apparatus (Swiss strain of mice treated with diazepam). For clean apparatus, at the end of each session, any faecal pellets were removed and the floor of the boxes were wiped with detergent and dried. For soiled apparatus, mice other than those used for testing, were placed in the boxes 30 min before experiments were conducted; (b) two different strains of mice treated with diazepam (soiled apparatus); and (c) comparison of the effects of anxiolytic/anxiogenic drugs with psychostimulant agents (Swiss strain, soiled apparatus).

Light/Dark Exploration Test in Mice

Apparatus. The apparatus consisted of a fully automated box monitored by computer. It was constructed by OSYS, Orga system (Changé, France). The light–dark apparatus consisted of four Perspex test boxes, an RS 232C/RS 422 interface together with a software management of the experiments. An open-topped rectangular box (46 × 27 × 30 cm high), was divided into a small (18 × 27 cm) area and a large (27 × 27) area with an opening door (7.5 × 7.5 cm) located in the center of the partition at floor level. The small compartment was painted black and illuminated under a dim red light (60 W; 4 lx), whereas the large compartment was painted white and brightly illuminated with a 60 W (400 lx) light source. The compartments were equipped with infrared beam sensors (four in the white area, three in the black one), enabling the detection of locomotion in each zone, time spent in each zone, latency of the first crossing from one compartment to the other and shuttle crossings between both compartments. The data from these four parameters were directly collected by computer.

Procedure

The test was performed in a quiet, darkened room. The mice were kept in this room at least 1 h before the test. After injection (saline or treatment), mice were placed in their home cage. To reduce any neophobic response to the test situation, the light–dark compartments are previously dirty with mice other than those used during the test. Mice are always tested in a soiled apparatus, and there is no cleaning between trials [Experiments (b) and (c)]. Naive mice are placed individually in the middle of the light area facing away from the opening. A 5-min test is given, during which the four parameters are recorded.

Analysis of Data

The mean number of responses for each group and for each test was calculated, and the final results were expressed as a percentage of the value observed in control animals or as mean ± SEM (standard error of the mean). For the analysis of movements in both compartments, data collected were expressed as movement by unity of time (movements/time spent in the area) to avoid false interpretation of results (see Discussion section). All 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" Steel test (1) to detect any significant differences between groups.

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

RESULTS

Doses of drugs were selected on the basis of preliminary studies using the actimeter test (see Table 1) to assess the influence of spontaneous locomotor activity in the light–dark test. Results of the light–dark test are shown in Figs. 1 and 2 for the benzodiazepines diazepam and alprazolam, in Table 2 for the comparison between strain and between soiled and cleaned apparatus, and in Table 3 for the other compounds studied.

Under control conditions, vehicle-treated mice displayed a consistent pattern of spending about 55% of the 5-min test in the dark area.

TABLE 1
SPONTANEOUS LOCOMOTOR ACTIVITY OF MICE AFTER DRUG INJECTIONS

Drugs	Controls	Doses (mg/kg)											
		0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64
Amphetamine	100%	—	—	90%	95%	63%	90%	196%†	195%†	—	—	—	—
	114 ± 8	—	—	102 ± 10	97 ± 11	72 ± 13	103 ± 14	224 ± 28	223 ± 32	—	—	—	—
Caffeine	100%	—	—	—	—	—	—	—	148%*	137%*	170%†	148%*	150%*
	105 ± 8	—	—	—	—	—	—	—	155 ± 14	144 ± 12	179 ± 14	156 ± 13	161 ± 13
Amineptine	100%	—	—	—	—	103%*	133%	117%	125%	163%†	191%†	—	—
	155 ± 6	—	—	—	—	159 ± 17	206 ± 14	182 ± 15	193 ± 22	253 ± 19	296 ± 25	—	—
Adrafinil	100%	—	—	—	—	—	—	115%	128%*	124%*	136%*	163%†	—
	166 ± 12	—	—	—	—	—	—	191 ± 22	213 ± 15	206 ± 12	222 ± 17	271 ± 18	—
Alprazolam	100%	134%	151%*	123%†	78%†	44%†	20%†	13%†	24%†	—	—	—	—
	153 ± 15	203 ± 13	230 ± 14	187 ± 17	118 ± 10	67 ± 7	31 ± 7	19 ± 3	37 ± 6	—	—	—	—
Diazepam	100%	—	106%	102%	117%	106%	80%*	46%†	26%†	20%†	—	—	—
	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%	—
	158 ± 8	—	—	—	—	—	132 ± 6	137 ± 14	154 ± 19	151 ± 25	114 ± 24	109 ± 16	—

Drugs were injected, IP, 30 min before the actimeter test. Results are expressed as the percentage of activity in comparison with control values ($n = 12$). Statistical analyses were performed using the nonparametric Kruskal–Wallis, H-test, followed by the "a posteriori," Steel test.
$p \leq 0.05$ and †$p \leq 0.01$.

Part 1: Spontaneous Locomotor Activity

The benzodiazepine alprazolam induced a significant increase in locomotor activity at the doses of 0.06 and 0.125 mg/kg ($p < 0.01$). However, higher doses (0.25 to 4 mg/kg) significantly reduced activity in this test. Diazepam administration slightly reduced activity at 1 mg/kg. This effect was more marked at 2, 4, and 8 mg/kg ($p < 0.01$). The benzodiazepine receptor inverse agonist, FG7142, did not induce any significant effects.

All four psychostimulant compounds (amphetamine, amineptine, adrafinil, and caffeine) produced a significant increase in spontaneous locomotor activity in the actimeter test from the dose of 4 mg/kg to 32 mg/kg for adrafinil, 2 to 4 mg/kg for amphetamine, 4 to 64 mg/kg for caffeine, and 8 to 16 mg/kg for amineptine.

Part 2: Light/Dark Experiments

Soiled/Clean Apparatus. In a soiled apparatus, treatment with diazepam increased the time spent in the light compartment for the doses of 1 mg/kg to 4 mg/kg ($p < 0.01$, $H = 32.91$). Sedative effects, consisting of a significant reduction in transitions ($p < 0.01$, $H = 67.77$) and locomotion, occurred at doses of 2 and 4 mg/kg ($H = 49.4$ for movements in dark area and $H = 39.01$ for movements in light area). This effect was less marked than the one observed in the actimeter test. The dose of 4 mg/kg produced an increased latency for the mice to enter into the dark ($p < 0.05$, $H = 30.56$). In a clean apparatus, diazepam treatment did not demonstrate any anxiolytic effect for nonsedative doses, with an increase of time spent in the light only for 2 and 4 mg/kg ($p < 0.05$, $H = 17.37$). For the other parameters, data did not reach statistical significance.

Strain difference investigations. Movement values of C57Bl/6J mice (either coming from IFFA CREDO or Janvier farms) were slightly elevated in comparison to Swiss mice tested under the same conditions (soiled apparatus). Under control conditions, IFFA CREDO C57Bl/6J mice (vehicle-treated mice) demonstrated a higher base line of time spent in the dark compartment (67%), but demonstrated little anxiolytic effect for 1 mg/kg ($p < 0.05$, $H = 14.9$). Janvier C57Bl/6J mice did not show significant anxiolytic effects at any dose.

Standard conditions (soiled apparatus). Effect of GABAergic receptor ligands (benzodiazepines and β-carbolines): Diazepam treatment: see the Soiled/clean apparatus section.

Alprazolam treatment displayed the same profile of action as diazepam, with an increase in the time spent in the light area from 0.5 mg/kg to 2 mg/kg ($p < 0.05$ for 0.5 mg/kg, and $p < 0.01$ for other doses, $H = 34.50$). Sedative effects appeared to 0.5 mg/kg, with a greater magnitude than with diazepam ($p < 0.01$, $H = 61.83$ and $H = 84.21$ for movements in dark and light, respectively). Latency time increased as early as the dose of 0.5 mg/kg ($p < 0.01$, $H = 67.77$). Furthermore, as was seen in the actimeter test, an increase in activity was seen for the dose of 0.06 mg/kg, with increased transitions ($p < 0.05$, $H = 94.06$) and movements in the light compartment ($p < 0.05$, $H = 84.21$) without any modifications in the time spent in each compartment.

Treatment with FG 7142, the GABAergic inverse agonist, failed to modify any of the parameters studied in the light–dark test. A small nonsignificant increase in the time spent in the dark area was observed.

Effects of psychostimulants—amphetamine, amineptine, adrafinil, and caffeine: In the light–dark test, while adrafinil values for locomotion in light and dark areas and for transi-

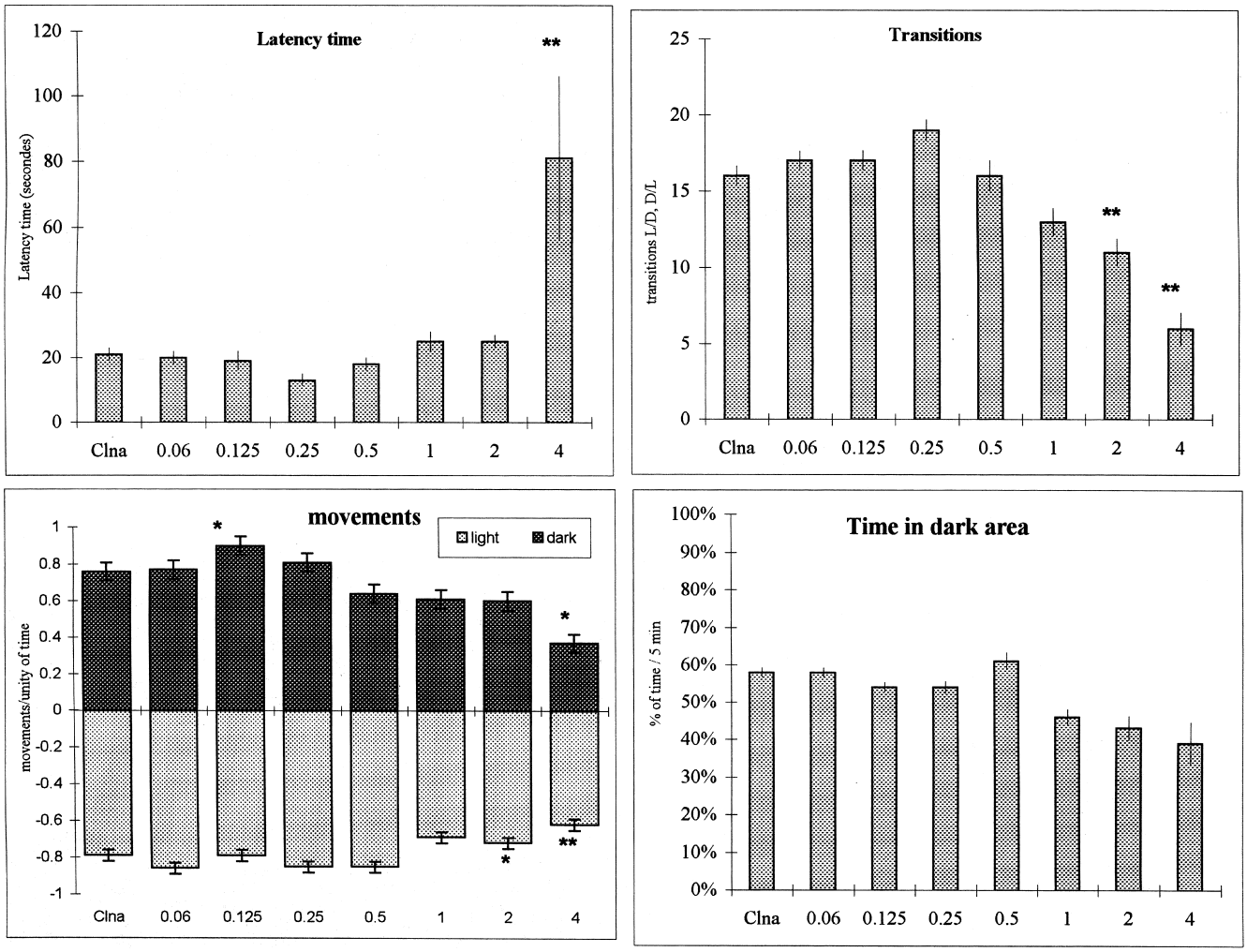

FIG. 1. Effects of diazepam on behavioral parameters in the light/dark test in mice. Drugs were injected IP, 30 min before the test ($n = 18$). Statistical analyses were performed using the nonparametric Kruskal–Wallis H-test, followed by the "a posteriori," steel test for comparison with the control group. $^{*}p < 0.05$ and $^{\dagger}p < 0.01$.

tions between the two compartments were slightly elevated for doses of 16 and 32 mg/kg, no dose reached significance. Amineptine increased the number of transitions and the movements, with a higher activity in the brightly lit area. We noted a small nonsignificant tendency of adrafinil- and amineptine-treated mice to spend more time in the black compartment of the light–dark test. High doses of caffeine (16 and 32 mg/kg, $p < 0.01$, $H = 17.49$) decreased the latency to enter the dark compartment. Exploration activity (i.e., movement and transitions) was increased in the dose range of 8 to 32 mg/kg ($p < 0.01$ $H = 34.16$ for the dark area and $p < 0.01$, except for 32 mg/kg $p < 0.05$, $H = 22.53$ for the light area). A nonsignificant increase of time spent in the dark area was observed from 2 to 32 mg/kg. Amphetamine-treated mice demonstrated increased activity in both compartments for the dose of 8 to 32 mg/kg in the dark area ($p < 0.05$ for 8 mg/kg and $p < 0.01$ for the other doses, $H = 9.36$) and 4 to 32 mg/kg for the light area ($p < 0.01$ except for 32 mg/kg $p < 0.05$, $H = 33.86$). Latency time to enter the dark compartment was increased for 8 and 16 mg/kg ($p < 0.01$, $H = 8.55$). Administration of 4, 8, or 16 mg/kg induced a dramatic increase ($p < 0.01$, $H = 33.86$) in the time spent in the dark area in comparison with control values.

DISCUSSION

Crawley and Goodwin (20), developed an animal model of anxiety based on the natural aversion of rodents for large and brightly lit areas. This test is now widely used, but numerous variations of the procedure have been reported in the literature, with a lack of standardization among research groups. The variability of results may be due to a number of factors, including routes of administration, species differences, sex of animal, or the environment in which the test is conducted.

First, we have noted some differences in the size of the two compartments, even if generally the dark compartment was about 1/3 of the total box size. Crawley and collaborators (5,17,19,20) used a box with total dimensions being 44 × 21 × 21 cm, and Costall et al. (12,15) used a 45 × 27 × 27 cm box. In the present experiment, the total size of the box is 46 × 27 × 30 cm, with 1/3 dark and 2/3 light. The results obtained using these three sizes of box were nearly the same. What was

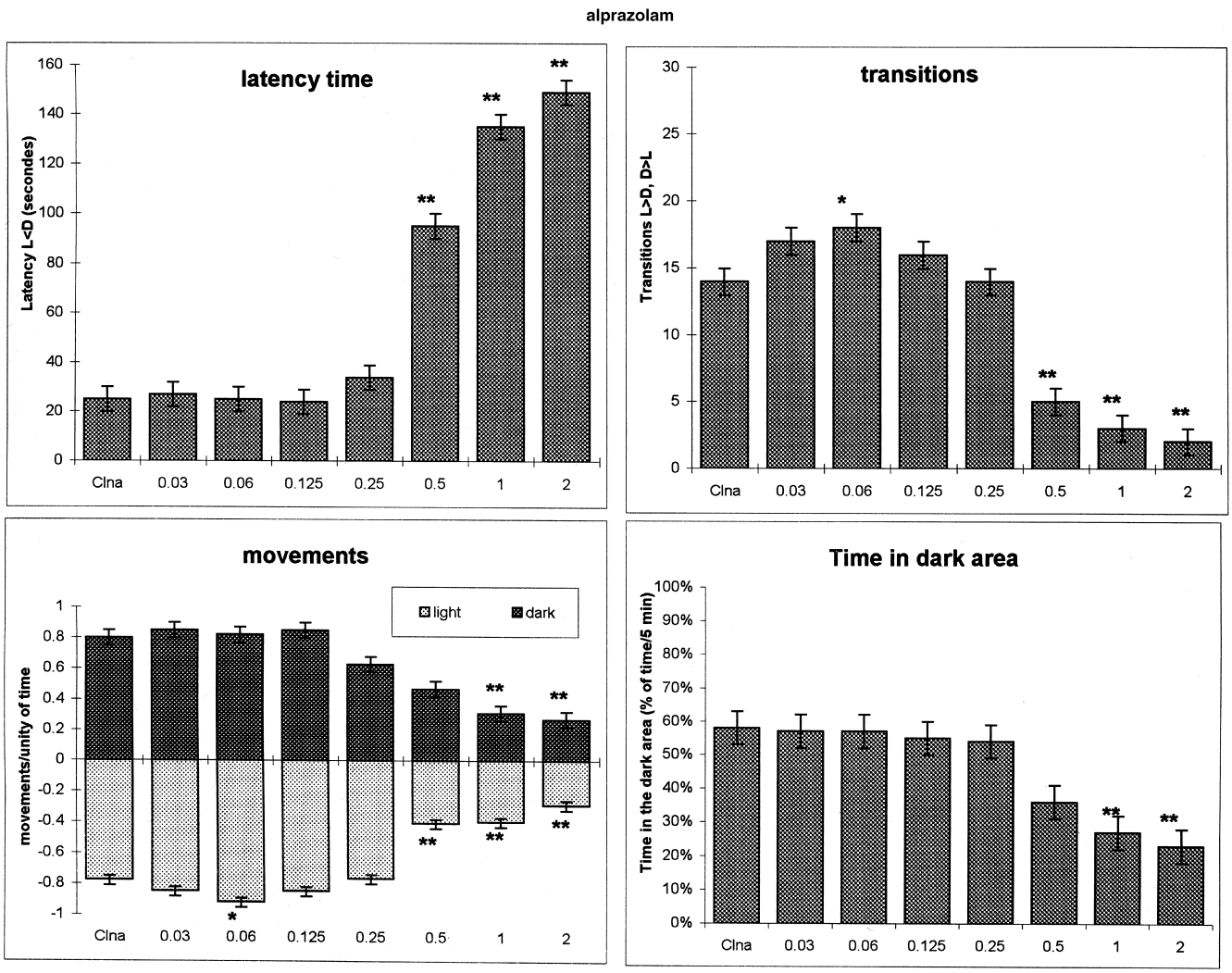

FIG. 2. Effect of alprazolam on behavioral parameters in the light/dark test in mice. Drugs were injected IP, 30 min before the test ($n = 18$). Statistical analyses were performed using the nonparametric Kruskal–Wallis H-test, followed by the "a posteriori," steel test for comparison with the control group. $^{*}p < 0.05$ and $^{\dagger}p < 0.01$.

surprising and required some thought in the analysis of the results when compared to other studies were the different results obtained when the two compartments were of equal size (4) or when the light area was included in the dark one (38). Some authors used an inverse light:dark cycle (12,14–16,26). As first performed in Crawley's procedure, a standard light: dark cycle was chosen for the present experiments. Results obtained with the anxiolytic reference drug, diazepam, were in agreement with expected results with an increase of exploration and an increase in the time spent in the light area. These effects were counteracted by the flumazenil (data not shown), which did not demonstrate an intrinsic effect in the light–dark test when administrated alone.

It has been reported that the light–dark paradigm shows an anxiolytic effect of diazepam and other anxiolytic drugs only in certain inbred mouse strains (19). One strain that has been reported to show a robust effect is the C57Bl/6J which demonstrated a maximum diazepam response of 129% in mean exploratory behavior (19). Strains with a low number of baseline transitions generally show weak responses to anxiolytics (18). However, the results of the present study call this theory into question. C57Bl/6J mice were obtained from two different sources and tested in comparison with the Swiss mouse strain. All strains showed the same baseline transition activity. Swiss strain showed a decrease in the time spent in the dark area at 1 mg/kg (46%), as did the C57Bl/6J from IFFA CREDO; however, this effect was less significant. On the other hand, C57Bl/6J (from Janvier) did not show any significant anxiolytic behavior at any dose. All strains showed a similar baseline activity in movements in each compartment. These results demonstrate that the Swiss strain does display anxiolytic behavior, and that the activity is stronger than that observed with the C57Bl/6J strain in the present study.

From data collected in the literature, it was difficult to compare the effects of movements in each compartment. Indeed, movements were expressed whatever the time spent in the compartment under consideration. However, it seems obvious that mice that spend less time in one compartment demonstrate few movements, and vice versa. It was, therefore, surprising to see false sedative effects or false psychostimulant

TABLE 2

EFFECTS OF DIAZEPAM ON BEHAVIORAL PARAMETERS IN THE LIGHT/ DARK TEST IN MICE: INFLUENCE OF SOILED APPARATUS AND OF MICE STRAINS

		Light/Dark Test Parameters				
	Doses	Latency	Transitions	Movements/Unity of Time		Time in Dark Area/5 min
Drugs	mg/kg IP	L----D	L----D	D	L	%
Soiled apparatus, Swiss strains, Janvier, (France)	0	21 ± 2	16 ± 1	0.76 ± 0.02	0.79 ± 0.03	58% ± 4
	0.06	20 ± 2	17 ± 1	0.77 ± 0.05	0.86 ± 0.04	58% ± 4
	0.125	19 ± 2	17 ± 1	0.90 ± 0.02*	0.79 ± 0.03	54% ± 4
	0.25	13 ± 2	19 ± 1	0.1 ± 0.64	0.85 ± 0.06	54% ± 5
	0.5	18 ± 2	16 ± 1	0.64 ± 0.05	0.85 ± 0.06	61% ± 5
	1	25 ± 3	13 ± 1	0.61 ± 0.03	0.69 ± 0.06	46% ± 6†
	2	25 ± 2	11 ± 1†	0.60 ± 0.04	0.72 ± 0.04*	43% ± 10†
	4	81 ± 25*	6 ± 1†	0.37 ± 0.08*	0.62 ± 0.05†	39% ± 17†
Kruskal–Wallis *H*-value		$H = 30.56$	$H = 67.77$	$H = 49.40$	$H = 39.01$	$H = 32.91$
Cleaned apparatus, Swiss strain	0	21 ± 2	19 ± 2	0.72 ± 0.05	0.96 ± 0.06	63% ± 2
	0.06	24 ± 4	16 ± 1	0.72 ± 0.04	0.88 ± 0.04	57% ± 2
	0.125	22 ± 2	16 ± 1	0.68 ± 0.04	0.85 ± 0.04	57% ± 2
	0.25	27 ± 3	20 ± 2	0.84 ± 0.04	0.90 ± 0.04	54% ± 3
	0.5	23 ± 3	17 ± 1	0.85 ± 0.04	0.98 ± 0.05	56% ± 3
	1	27 ± 4	18 ± 2	0.83 ± 0.05	0.98 ± 0.07	52% ± 3
	2	40 ± 10	15 ± 2	0.72 ± 0.06	0.74 ± 0.07	44% ± 5*
	4	38 ± 7	9 ± 3*	0.67 ± 0.07*	0.57 ± 0.08*	39% ± 8*
Kruskal–Wallis *H*-value		$H = 5$	$H = 5.34$	$H = 33.79$	$H = 41.25$	$H = 17.37$
C57Bl/6J, Janvier (France)	0	12 ± 4	17 ± 1	0.83 ± 0.03	0.91 ± 0.13	57% ± 7
	0.125	17 ± 3	17 ± 1	0.94 ± 0.06	0.93 ± 0.01	58% ± 3
	0.25	19 ± 3	16 ± 1	0.95 ± 0.06	0.88 ± 0.03	56% ± 3
	0.5	14 ± 3	17 ± 1	0.87 ± 0.03	0.85 ± 0.06	52% ± 3
	1	20 ± 4	18 ± 2	1.01 ± 0.06	0.90 ± 0.06	49% ± 1
	2	19 ± 38	9 ± 2*	0.56 ± 0.09	0.59 ± 0.09	47% ± 6
Kruskal-Wallis *H*-value		$H = 5.34$	$H = 14.79$	$H = 17.36$	$H = 11.60$	$H = 9.09$
C57Bl/6J, IFFA CREDO (France)	0	18 ± 5	17 ± 1	0.87 ± 0.06	1.11 ± 0.09	67% ± 3
	0.125	13 ± 3	18 ± 1	0.90 ± 0.06	1.04 ± 0.03	64% ± 3
	0.25	9 ± 2	17 ± 1	0.93 ± 0.03	0.98 ± 0.16	65% ± 3
	0.5	25 ± 8	15 ± 1	0.73 ± 0.06	0.91 ± 0.06	58% ± 3
	1	33 ± 8	15 ± 1	0.79 ± 0.06	0.89 ± 0.06	53% ± 3*
	2	16 ± 4	9 ± 1†	0.55 ± 0.09	0.69 ± 0.12*	58% ± 6
Kruskal-Wallis *H*-value		$H = 11.36$	$H = 25.82$	$H = 14.52$	$H = 12.51$	$H = 14.09$

Drugs were injected, IP, 30 min before the light/dark test ($n = 12$). Statistical analyses were performed using the nonparametric Kruskal–Wallis, *H*-test, followed by the "a posteriori," Steel test. *$p \leq 0.05$ and †$p \leq 0.01$. (D = dark compartment; L = light compartment).

effects. To avoid this problem, in the present study, results of movements/exploratory behavior in each area were expressed as a function of time spent in the compartment under consideration. This approach resulted in a more reliable idea of the indices of exploration and made the comparison between treatments easier. One other cause of difficulty is the role of neophobia in this test situation. In the present study, mice (Swiss strain), were tested in clean or soiled apparatus to investigate any effects these conditions might have on the response to diazepam. It is clear from the results that cleaning between trials masked the anxiolytic behavior observed in soiled apparatus at 1 mg/kg. Anxiolytic-like effects were only obtained at sedative doses when cages were cleaned between trials. In our procedure, mice were always tested in a dirty apparatus, smelling of mouse urine and faeces, without cleaning between trials. This kind of procedure had already been used with the actimeter test to measure the spontaneous locomotor activity of mice. Measures would reflect more purely the influence of dark or bright areas on exploration activity rather than the influence of a clean new area smelling of detergent. A soiled apparatus removed or at least reduced, the neophobia factor. Rather, the aversive stimulus is the novel environment. The novelty and dubiousness generated emotional factors, that, at short term, limited the exploratory behavior. Models based on spontaneous responses are linked with uncontrollable stress where animals cannot escape from a novel aversive environment (28). The test was based on an ethological view, and does not explicitly involve animals' pain or discomfort with the exception of Imaizumi's studies (31,32), where mice were first placed in the light area. This change in procedure did not allow the author to take the latency to leave the white compartment as a index of anxiety as did Costall et al. (11–13,15). Mice placed in a uniform noncompartmentalized apparatus did not demonstrate more activity than

TABLE 3

EFFECTS OF DRUGS ON BEHAVIORAL PARAMETERS IN THE LIGHT/DARK TEST IN MICE

Drugs	Doses mg/kg I.P.	Light/Dark Test Parameters					
		Latency	Transitions	Movements/Unity of Time		Time in Dark Area/5 Min	
		L ----- D	L ----- D	D	L	D	%
Amphetamine	0	21 ± 3	17 ± 0.7	0.75 ± 0.02	0.82 ± 0.04	167 ± 4	56%
	1	18 ± 2	17 ± 0.7	0.75 ± 0.04	0.87 ± 0.05	183 ± 6	61%
	2	24 ± 2	18 ± 1.5	0.76 ± 0.05	0.98 ± 0.04	193 ± 8	64%
	4	20 ± 3	16 ± 2.7	0.75 ± 0.05	1.30 ± 0.06†	231 ± 8	77%†
	8	52 ± 23†	4 ± 1.1†	0.99 ± 0.14*	1.32 ± 0.15†	229 ± 25	76%†
	16	36 ± 6†	3 ± 0.5†	0.93 ± 0.09*	1.03 ± 0.10†	242 ± 11	81%†
Kruskal–Wallis *H*-value		H = 8.55	H = 46.2	H = 9.36	H = 23.57	H = 33.86	
amineptine	0	21 ± 3	14 ± 0.7	0.68 ± 0.04	0.73 ± 0.02	171 ± 7	57%
	0.5	28 ± 4	16 ± 0.9	0.79 ± 0.05	0.84 ± 0.05	169 ± 4	56%
	1	22 ± 4	16 ± 0.9	0.86 ± 0.06	0.82 ± 0.04	166 ± 6	55%
	2	29 ± 9	16 ± 0.7	0.80 ± 0.05	0.84 ± 0.07	170 ± 8	57%
	4	23 ± 2	18 ± 1.0†	0.96 ± 0.04†	0.96 ± 0.02†	179 ± 5	60%
	8	18 ± 3	17 ± 0.7	0.84 ± 0.03	0.98 ± 0.04†	192 ± 6	64%
	16	17 ± 3	22 ± 2.0†	1.08 ± 0.07†	1.16 ± 0.04†	186 ± 11	62%
Kruskal–Wallis *H*-value		H = 6.89	H = 18.25	H = 25.84	H = 37.78	H = 9.9	
adrafinil	0	21 ± 3	16 ± 0.7	0.75 ± 0.02	0.82 ± 0.04	167 ± 4	56%
	1	24 ± 5	17 ± 1.0	0.85 ± 0.06	0.86 ± 0.06	169 ± 6	56%
	2	23 ± 5	13 ± 0.6	0.84 ± 0.04	0.80 ± 0.03	168 ± 6	56%
	4	21 ± 3	15 ± 0.7	0.78 ± 0.04	0.86 ± 0.04	174 ± 5	58%
	8	28 ± 5	16 ± 0.9	0.87 ± 0.05	0.88 ± 0.04	174 ± 4	58%
	16	21 ± 2	15 ± 1.0	0.81 ± 0.05	0.82 ± 0.03	176 ± 7	59%
	32	17 ± 2	19 ± 0.6	0.91 ± 0.05	0.97 ± 0.03	180 ± 5	60%
Kruskal–Wallis *H*-value		H = 3.98	H = 26.25	H = 8.5	H = 12.28	H = 4.16	
caffeine	0	25 ± 3	13 ± 0.9	0.58 ± 0.04	0.68 ± 0.05	166 ± 7	55%
	2	21 ± 3	15 ± 0.8	0.65 ± 0.03	0.81 ± 0.03	186 ± 3	62%
	4	19 ± 3	16 ± 1.0	0.68 ± 0.04	0.83 ± 0.03	185 ± 5	62%
	8	15 ± 2	20 ± 0.8†	0.87 ± 0.04†	0.95 ± 0.03†	188 ± 7	63%
	16	12 ± 2†	17 ± 0.8†	0.86 ± 0.03†	0.94 ± 0.03†	195 ± 6	65%
	32	14 ± 3†	18 ± 1.0†	0.85 ± 0.02†	0.94 ± 0.06*	176 ± 9	60%
Kruskal–Wallis *H*-value		H = 17.49	H = 23.25	H = 34.16	H = 22.53	H = 8.36	
FG 7142	0	20 ± 4	16 ± 0.9	0.72 ± 0.03	0.79 ± 0.02	162 ± 6	54%
	0.5	24 ± 4	15 ± 0.9	0.66 ± 0.03	0.86 ± 0.05	180 ± 5	60%
	1	29 ± 5	14 ± 0.8	0.75 ± 0.03	0.69 ± 0.03	165 ± 6	55%
	2	24 ± 2	14 ± 0.9	0.79 ± 0.05	0.83 ± 0.04	173 ± 6	58%
	4	23 ± 2	16 ± 1.0	0.80 ± 0.02	0.82 ± 0.05	173 ± 5	58%
	8	27 ± 5	15 ± 0.9	0.80 ± 0.05	0.90 ± 0.03	179 ± 4	60%
	16	21 ± 3	15 ± 0.9	0.73 ± 0.04	0.84 ± 0.04	180 ± 7	60%
	32	27 ± 4	13 ± 0.4	0.70 ± 0.03	0.76 ± 0.02	175 ± 4	58%
		H = 3.19	H = 9.79	H = 13.65	H = 19.45	H = 5.92	

Drugs were injected, IP, 30 min before the light/dark test ($n = 12$). Statistical analyses were performed using the nonparametric Kruskal–Wallis, *H*-test, followed by the "a posteriori," Steel test. *$p \leq 0.05$ and †$p \leq 0.01$. (D = dark compartment; L = light compartment).

saline control animals (20). Furthermore, benzodiazepines did not modify the number of transitions from one side to the other if they were identically illuminated (17), or if they were placed in a single chamber (12). Anxiolytic agents selectively increase exploration, rather than general activity (39). As with many experimental protocols, drugs that affect general motor function will affect light–dark performance, such that parallel experiments for general locomotion in an automated locomotor activity apparatus serve as necessary controls, as was conducted in the present study. Here, diazepam- and alprazolam-treated mice demonstrated more activity in the light–dark box than in the actimeter chamber (see Table 1 and Figs. 1 and 2). Following the administration of 4 mg/kg of diazepam, mice demonstrated 26% of spontaneous activity in the actimeter test in comparison with saline controls, but had still 78% of activity–exploratory behavior in the brightly lit side of the light–dark two compartments test. In parallel, mice demonstrated 48% of activity in the dark area. The same effect was seen with alprazolam, with 51% of activity in the light compartment compared with vehicle-treated animals, but only 20% of spontaneous activity in the actimeter test for 1 mg/kg-treated mice. That brings us to the question of the real significance of all parameters collected with the light–dark test. To this end, a full exploration of the literature was conducted to-

gether with studies with anxiolytic reference drugs, anxiogenics, and psychostimulant drugs. The number of transitions between the two compartments was the more controversial parameter. Crawley had first reported that shuttle activity between the two compartments was the index of anxiety. This parameter was used as such by some authors (2–4), while others (34,36,41) reported no significant changes after treatment with anxiolytics. In the present investigation, transitions were more dependent on sedative or psychostimulant effects of drug treatment. Decreases in transition were seen with diazepam and alprazolam, but at sedative doses. Increased transitions were seen with caffeine and amineptine that corroborated actimeter results.

Thus, in summary, from all data, the time spent in the lit area and exploratory behaviors seemed to be the more reliable parameters to assess anxiolytic-like activity (12,34,36,41). Young and Johnson found that this parameter provided the most consistent dose–effect results. Data concerning time spent in the light compartment demonstrated a stable baseline in vehicle-treated animals from which drug effects could be assessed (41). Mice were reported to pass 60% of the time in the dark (36). In the present study a stable percentage of time spent in dark area of about 56% was observed in saline control animals (see Table 2). Only the benzodiazepines, diazepam and alprazolam, increased time spent in the lit area. Previous studies with tests for anxiolytics with mice and rats (8,30) have shown that the anxiolytic-like effects of diazepam appear at 1 mg/kg (mice) and at lower doses in rats. As was seen in the present work, diazepam-induced anxiolytic effects also appeared at 1 mg/kg, coinciding with the onset of sedation in the locomotor activity apparatus. However, movements in the light and in the dark compartment were not statistically changed, indicating an anxiolytic effect at this dose.

Few data were available concerning the latency time parameter. This index was not used by Crawley (5,17,19,20), in contrast to some other authors (11–13,15,16). The real sense of this parameter is difficult to appreciate and is rarely discussed in literature. Two hypotheses of definition could be advanced. Increase in latency time could be the result of disinhibitory behavior and decreased anxiolysis, where animals spend more time in exploring the white area. The other explanation is the influence of sedation, where animals are unable to move quickly to the dark compartment (41). It is not always easy to appreciate one or the other hypothesis, as benzodiazepines sometimes demonstrated a narrow margin between anxiolysis and sedation. In a recent study (40), the authors found 7-nitroindazole (7-Ni), a nitric oxide synthase inhibitor, to have anxiolytic-like properties as it enhanced time spent in the light compartment. On the other hand, it reduced transitions that might also be due to sedative effects, but the authors found the latency time unchanged after medication with 7-Ni, and concluded that sedation did not interfere with the results. In the present study we found the latency for the initial movement from the white to the dark compartment increased for diazepam and alprazolam, as has been reported elsewhere (2,41). Generally benzodiazepines demonstrate a delayed latency, but Costall et al. (13) found a reduction in latency time. The chronic administration of diazepam in the study under consideration could be the reason for this discrepancy. The only case in our study where we found a reduction in latency time was with caffeine. With regard to the other parameters, caffeine seems only to be a psychostimulant with enhanced transitions and total movements, but not in time spent in the dark area. This is in contrast with the results of Imaizumi et al. (31), who found an anxiogenic profile for the adenosine receptor antagonist at a dose of 20 mg/kg. In the present study, amphetamine was found to be stimulant and anxiogenic producing a significant dramatic increase in the time spent in the dark area, in accordance with the results of Pellows et al. (37), who found an anxiogenic action in rats using the elevated plus maze. On the other hand, Young and Johnson (41) demonstrated that in the light–dark test amphetamine did not significantly change the time spent in the dark area. FG 7142, an inverse agonist of the benzodiazepine receptor, was effective in reversing the anxiolytic action of diazepam (21) but did not demonstrate intrinsic anxiogenic activity. These findings agree with the present study, where no effect was seen with the FG 7142. Nevertheless, an increase in the dark time was reported by Kilfoil et al. (34) using FG 7142.

Interesting results were obtained with the pure psychostimulant adrafinil (24,29) and the stimulant antidepressant amineptine (7). No change in latency time was noticed. Enhanced transitions and movements in both compartments were noted. The psychostimulant effect did not induce any increase in the time spent in the dark, showing that this parameter is specific for anxiolytic activity. The stimulant profile of action of adrafinil and amineptine in the black and white test is different from that of amphetamine, which was an anxiogenic psychostimulant.

In conclusion, the black and white test may be a useful test to predict anxiolytic-like or anxiogenic-like activity in mice. Transitions have been reported to be an index of activity–exploration because of habituation over time, and the time spent in each compartment to be a reflection of aversion (4). The effects of drugs should be assessed carefully (33) with regard to the problem of sedation, stimulation, and change in exploration induced by anxiolytic effects. An additional test such as an open field or an actimeter test must be performed in addition as a control. Many discrepancies seen in literature may be due to difference in procedure, mice strain, and so on, but the best measures seem to be the percentage of time spent in each compartment and the movements/exploratory behavior in each compartment.

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