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A Fully Automated Light/Dark Apparatus Useful for Comparing Anxiolytic Agents

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YOUNG, R. AND D. N. JOHNSON. *A fully automated light/dark apparatus useful for comparing anxiolytic agents*. PHARMACOL BIOCHEM BEHAV **40**(4) 739–743, 1991.—The effects of known anxiolytic agents and putative anxiolytic agents were assessed in mice in a fully automated 2-compartment light/dark test. Significant increases in lit area activities (e.g., time spent in the lit area, locomotor activity, rearing behavior) were used as possible indicators of anxiolytic-like action. The measurement found most consistent and useful for assessing antianxiety-like activity was the time mice spent in the lit area. The benzodiazepine, diazepam; the 5-HT1A agent, ipsapirone; and the 5-HT3 receptor antagonist, ondansetron, produced significant anxiolytic-like activity between doses of 1.0 to 10.0 mg/kg, 17.8 to 31.6 mg/kg, and 0.0001 to 1.0 mg/kg respectively. The 5-HT1A receptor agonist, 8-OH DPAT, also exhibited anxiolytic-like action between doses of 0.0005 to 3.16 mg/kg. In contrast, the peripheral 5-HT3 receptor agonist, N-phenylbiguanide; the antidepressant, imipramine; the neuroleptic, chlorpromazine; and the CNS stimulant, *S*(+)-amphetamine, did not display antianxiety-like activity. The positive results obtained for the three types of compounds (benzodiazepine, 5-HT1A, and 5-HT3) indicate that this fully automated light/dark apparatus may be useful for identifying known and putative anxiolytic agents.

Anxiolytic Light/dark test Serotonin Diazepam Ipsapirone Ondansetron (GR38032F) 8-OH DPAT N-Phenylbiguanide

A number of animal test procedures have been used to identify compounds that may have anxiolytic potential [for review, see (5)]. In one such test, mice are placed on the brightly lit side of a 2-compartment chamber, where either two-thirds or one-half of the area is lit and the remaining area is dark [e.g., (1, 2, 9)]. When placed in the lit area, mice will move quickly through a passageway to the dark area and spend most of their time and exhibit most of their behavioral activities (i.e., locomotor and rearing) in this area. In contrast, the administration of anxiolytics—such as diazepam, chlordiazepoxide, and buspirone—produce an increase in latency for mice to enter the dark area and increases in their time spent and behavioral activities in the lit area. Movements between the two areas (i.e., transitions) have also been reported to increase [e.g., (1, 3, 4, 8)].

In the first descriptions of the light/dark test that used anxiolytics in mice, photocells across the partition were used to detect transitions between the two areas (4–6). Later studies used human observers to record transitions of the mice and the amount of time mice spent in each area [e.g., (1)]. Video recordings of mice behavior in the chamber have also been used to detect increases in locomotor and rearing activities in the lit area that were accompanied by decreases of these behaviors in the dark area (2,3). More recently, a test system has been designed that integrates photoelectric cell activation and a microcomputer to automatically record and then summarize locomotor activity in each area. Again, however, video recordings were used to determine time mice spent and rearings in each area and transitions (9).

In the present study, a fully automated and computer-integrated 2-compartment light/dark apparatus for mice is described and characterized for its pharmacological selectivity to anxiolytic agents. In this test system, latency to enter the dark area, transitions between the two areas, time spent in each area, locomotor activities in each area, and rearings in each area are detected by infrared photocells and are transmitted to computers that automatically store and then summarize each of the five behavioral parameters (eight total measurements). In order to evaluate the system, the effects of the known and putative anxiolytics—diazepam, ipsapirone, and ondansetron (GR38032F)—were studied. For comparative purposes, the effects of the 5-HT1A receptor agonist, 8-OH DPAT; the peripheral 5-HT3 receptor agonist, N-phenylbiguanide; the antidepressant, imipramine; the neuroleptic, chlorpromazine; and the central nervous system stimulant, *S*(+)-amphetamine, were also evaluated.

METHOD

Animals

Female ICR-DUB albino mice, 17–35 g, obtained from Dominion Labs (Dublin, VA), were used. Thirty mice were normally housed in each cage and given free access to food and

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TABLE 1

EFFECTS OF KNOWN AND PURPORTED ANXIOLYTIC DRUGS ON EXPLORATORY BEHAVIOR IN MICE

Compound[a]	Dose mg/kg (IP)	Mean Transitions (± S.D.)	Mean Lit Side Locomotor Counts (± S.D.)	Mean Total Locomotor Counts (± S.D.)	Mean Lit Side Rears (± S.D.)	Mean Total Rears (± S.D.)	Mean Latency (S.D.)
Vehicle	10 ml/kg	15.3 (8.6)	438.6 (282.6)	1503.6 (278.6)	21.2 (14.7)	65.2 (19.6)	21.6 (12.9)
Diazepam	0.316	17.0 (8.3)	552.3 (387.6)	1455.7 (265.7)	17.0 (13.9)	65.6 (25.3)	36.3 (18.6)
	1.0	18.9 (9.1)	724.4 (421.8)	1439.8 (265.6)	29.6 (16.3)	52.4 (22.7)	31.1 (16.7)
	5.0	16.6 (7.5)	887.0 (377.9)*	1529.6 (249.3)	36.3 (18.7)	55.7 (19.6)	78.0 (27.3)*
	10.0	16.3 (9.1)	819.9 (455.3)*	1282.8 (289.3)	46.3 (21.2)*	65.8 (28.7)	83.1 (28.7)*
Vehicle	10 ml/kg	16.9 (8.2)	522.8 (275.6)	1556.7 (321.4)	11.4 (9.1)	82.6 (24.6)	20.9 (10.2)
Ipsapirone	0.1	12.0 (8.0)	280.7 (206.7)	1431.0 (263.7)	6.1 (7.8)	70.8 (23.4)	20.7 (12.7)
	1.0	17.0 (10.1)	428.5 (286.5)	1769.3 (325.6)	15.3 (12.5)	80.1 (25.9)	12.2 (10.5)
	3.16	21.9 (8.7)	544.4 (323.7)	1617.7 (303.6)	23.7 (17.3)	95.6 (25.3)	19.3 (12.6)
	5.62	19.1 (9.3)	598.0 (326.8)	1628.4 (287.5)	20.7 (15.6)	86.0 (18.7)	23.6 (10.9)
	10.0	18.6 (9.1)	526.7 (299.4)	1352.3 (291.7)	22.0 (12.7)	69.7 (22.3)	48.7 (28.7)
	17.8	20.2 (7.9)	838.6 (365.7)*	1722.0 (332.4)	40.2 (20.1)*	84.8 (27.6)	50.2 (28.5)
	31.6	19.3 (8.2)	949.6 (310.5)*	1536.3 (275.7)	43.0 (17.3)*	89.7 (29.3)	81.1 (21.1)*
Vehicle	10 ml/kg	21.6 (11.2)	508.9 (255.6)	1395.8 (209.3)	22.0 (9.6)	86.2 (26.8)	17.4 (7.9)
8-OH DPAT	0.00005	19.3 (7.5)	573.4 (232.1)	1421.9 (225.7)	21.6 (8.7)	80.7 (23.5)	15.9 (6.9)
	0.0001	18.7 (9.7)	623.8 (285.6)	1523.8 (285.6)	30.3 (10.3)	100.8 (30.3)	20.0 (10.3)
	0.0005	20.3 (8.7)	750.9 (195.3)*	1440.6 (210.7)	28.4 (8.7)	85.4 (21.7)	35.4 (15.1)
	0.001	23.8 (10.1)	799.5 (310.7)	1514.0 (311.7)	50.3 (12.3)*	90.0 (27.3)	85.8 (25.3)*
	0.01	17.8 (10.3)	930.3 (379.7)*	1539.8 (278.7)	62.5 (14.1)*	79.5 (25.4)	85.0 (18.4)*
	0.10	22.0 (9.6)	908.4 (365.4)*	1492.6 (228.7)	69.6 (12.6)*	91.4 (21.7)	92.2 (18.7)*
	1.0	21.6 (7.5)	877.0 (372.5)	1525.9 (275.4)	50.4 (12.9)	82.6 (23.5)	114.4 (33.4)*
	1.78	22.1 (9.2)	949.4 (390.5)*	1611.6 (301.7)	53.7 (15.3)	73.3 (22.1)	65.6 (20.1)
	3.16	19.6 (7.3)	1059.4 (400.3)*	1538.7 (279.7)	64.5 (18.6)*	88.9 (24.8)	112.0 (35.3)*
Vehicle	10 ml/kg	18.8 (9.5)	428.0 (235.4)	1269.3 (288.6)	10.1 (8.7)	65.2 (22.3)	38.4 (15.6)
Ondansetron	0.000005	13.1 (8.3)	422.6 (207.9)	1231.3 (212.7)	12.7 (7.9)	59.3 (21.1)	32.3 (10.7)
	0.00001	10.6 (8.6)	477.6 (295.4)	1260.0 (277.4)	11.1 (9.1)	66.8 (25.7)	72.4 (21.7)
	0.00005	12.1 (6.7)	440.9 (221.5)	1211.7 (209.3)	15.4 (8.3)	55.5 (20.7)	80.7 (15.3)
	0.0001	8.6 (6.5)	578.9 (321.6)	1123.9 (301.4)	21.0 (12.3)	50.9 (18.3)	139.7 (32.3)*
	0.001	7.0 (9.3)	402.6 (286.9)	1074.3 (249.6)	10.1 (8.3)	35.5 (19.3)	117.1 (33.3)*
	0.01	18.4 (10.3)	686.9 (355.1)*	1415.5 (287.9)	22.6 (12.5)*	58.5 (22.1)	79.0 (39.7)
	0.10	16.7 (7.6)	675.9 (325.7)*	1326.3 (315.4)	20.7 (13.3)	47.1 (20.9)	88.6 (21.4)*
	1.0	15.6 (8.2)	575.6 (289.7)	1320.9 (299.9)	19.7 (10.7)	58.3 (19.9)	75.9 (41.7)
	10.0[b]	0.7 (1.4)*	246.3 (156.2)*	251.3 (104.8)*	0.0 (0.0)*	0.0 (0.0)*	222.3 (20.1)*

*Indicates values that are significantly different from vehicle.
[a]For each vehicle treatment and each dose of each drug n = 7.
[b]Four mice died, n = 3.

water. The mice were kept in the Robins vivarium on a 12-h light and 12-h dark cycle with lights off at 1800 h and on at 0600 h. On test days, 60 to 63 mice were used. The mice were naive to the test apparatus.

Apparatus

Experiments were performed in a sound-attenuated room illuminated with a 25-watt red bulb. Behavioral testing was conducted with three, 2-compartment automated test chambers (Digiscan, Model RXYZCM16, Omnitech Electronics Inc., Columbus, OH). The outer open-topped chamber measured 42 × 42 × 30 cm high. The inner compartment (i.e., dark area) measured 40 × 23.5 × 20.5 cm high. Access between the lit and dark area was provided by a 7.5 × 7.5-cm passageway. A 90-watt light bulb located 30 cm above the box was used to provide light to one compartment (hereafter called the lit area). The intensity of light was constant at 260 lux. Interruptions of the infrared beams in the chamber were automatically recorded by the Digiscan analyzer and then transmitted to a "VAX cluster" which consisted of a VAX 11/785 computer and a VAX 85-30 computer (Digital Equipment Corp., DEC, Maynard, MA). Computer software programs were written for summarizing (mean, standard deviation) data and performing statistical analyses (see below).

Procedure

At approximately 0900 h on the day of an experiment, the mice were taken from a vivarium holding room to the sound-attenuated, darkened room. The mice were then randomized into dose groups (n = 7/dose) according to a table of random numbers (10) and placed into individual holding cages. The mice stayed in the dark room for a subsequent 4-h period.

Starting at approximately 1230 h, each mouse received an IP dose of either saline, diazepam, ipsapirone, 8-OH DPAT, ondansetron, N-phenylbiguanide, imipramine, chlorpromazine, or *S*(+)-amphetamine. Thirty min later, the animal was placed at the center of the lit area, and behavioral activity was tallied over

TABLE 2

EFFECTS OF REFERENCE DRUGS ON EXPLORATORY BEHAVIOR IN MICE

Compound[a]	Dose mg/kg (IP)	% Time in Lit Area (± S.D.)	Mean Transitions (± S.D.)	Mean Lit Side Locomotor Counts (± S.D.)	Mean Total Locomotor Counts (± S.D.)	Mean Lit Side Rears (± S.D.)	Mean Total Rears (± S.D.)	Mean Latency (S.D.)
Vehicle	10 ml/kg	29 (7.5)	12.7 (11.3)	446.4 (344.9)	1512.0 (273.2)	21.3 (31.1)	83.2 (29.0)	35.0 (41.2)
Phenylbiguanide	1.0	30 (12.6)	19.0 (9.9)	532.1 (314.2)	1732.0 (358.5)	13.9 (9.1)	56.6 (16.9)	24.3 (22.1)
	3.16	29 (11.9)	15.3 (6.7)	530.0 (274.8)	1578.4 (238.6)	17.1 (19.9)	66.1 (19.4)	22.4 (18.0)
	10.0	38 (10.6)	14.6 (9.9)	592.7 (244.9)	1623.0 (330.1)	20.4 (14.2)	53.4 (18.5)	38.0 (41.8)
	31.6	46 (19.4)	18.5 (9.2)	599.7 (183.2)	965.2 (244.3)	19.7 (16.9)	63.9 (21.1)	39.2 (31.5)
Vehicle	10 ml/kg	27 (8.2)	22.0 (8.7)	561.6 (280.7)	1493.3 (265.7)	33.7 (18.9)	86.7 (25.3)	27.9 (9.7)
Imipramine	0.01	38 (11.9)	19.7 (9.3)	625.6 (320.6)	1490.9 (289.6)	26.9 (15.6)	78.8 (18.9)	30.3 (18.9)
	0.10	44 (14.3)	17.1 (10.2)	721.6 (389.7)	1618.9 (309.2)	34.3 (25.4)	83.0 (22.9)	40.9 (31.5)
	1.0	33 (13.8)	11.2 (8.7)	344.8 (295.6)	1122.5 (289.9)	5.5 (3.6)*	40.7 (20.7)*	29.3 (15.6)
	3.16	59 (18.7)*	7.7 (9.2)*	401.5 (325.7)	828.5 (325.6)*	5.5 (3.4)*	21.2 (15.6)*	143.0 (60.7)*
Vehicle	10 ml/kg	28 (9.1)	22.5 (8.8)	542.8 (310.7)	1793.3 (379.7)	27.4 (22.4)	87.0 (28.6)	22.4 (18.1)
Chlorpromazine	0.01	32 (9.3)	17.7 (10.1)	502.7 (325.9)	1587.7 (295.8)	19.9 (20.6)	65.8 (19.7)	33.6 (20.4)
	0.10	44 (18.7)	11.1 (9.3)	482.7 (300.8)	1071.7 (298.6)	11.7 (15.3)	46.7 (22.4)	39.3 (19.7)
	1.0	60 (15.3)*	8.9 (9.6)*	384.7 (256.3)	794.7 (325.6)*	7.1 (10.2)*	19.5 (18.6)*	134.9 (75.4)*
	3.16	83 (8.2)*	0.5 (1.3)*	189.0 (201.7)*	233.3 (108.7)*	0.0 (0.0)*	0.7 (1.3)*	250.2 (20.4)*
Vehicle	10 ml/kg	29 (7.8)	20.1 (6.1)	519.3 (244.1)	1535.2 (257.8)	27.1 (19.3)	103.8 (32.2)	21.9 (10.0)
S(+)-	0.10	28 (9.7)	19.4 (7.2)	466.6 (330.7)	1518.1 (279.5)	22.3 (21.9)	94.7 (22.1)	28.7 (17.1)
Amphetamine	1.0	32 (9.2)	19.4 (7.0)	567.7 (283.5)	1749.9 (260.6)	23.7 (18.4)	100.6 (26.8)	25.0 (16.0)
	3.16	34 (8.6)	13.6 (9.4)	544.6 (278.2)	1889.3 (490.7)*	10.4 (11.7)*	47.0 (24.0)*	70.1 (106.5)
	5.62	31 (9.1)	8.4 (9.6)*	540.9 (483.2)	2144.6 (668.2)*	14.1 (16.5)*	63.7 (25.4)*	63.6 (105.4)

[a]For each vehicle treatment and each dose of each drug n = 7.
*Indicates values that are significantly different from vehicle.

a 5-min period by the Digiscan analyzer. Eight behavioral measures were recorded and analyzed: the time spent in the lit and dark areas, locomotor activity counts in each area, number of rearings in each area, number of transitions between the two areas, and latency to make the first transition from the lit area to the dark area. In addition, total locomotor activity counts and total rears were calculated and analyzed. Statistical analyses were performed by using Dunnett's *t*-test. All significant differences were determined by using a p value of ≤ 0.05.

Drugs

Diazepam (Hoffmann-La Roche, Nutley, NJ), ipsapirone HCl (Troponwerke GmbH and Co., Federal Republic of Germany), 8-hydroxy-dipropylaminotetralin HBr (8-OH DPAT, Research Biochemicals Inc., Natick, MA), ondansetron (synthesized by Chemical Research Group, A.H. Robins, Richmond, VA), N-phenylbiguanide HCl (K and K Labs, Plainview, NY), imipramine HCl (Ciba-Geigy, Summit, NJ), chlorpromazine HCl and S(+)-amphetamine sulfate (Smith Kline and French, Philadelphia, PA) were dissolved in 0.9% saline. Doses were expressed as mg of base per kg of body weight. Injections were given intraperitoneally (IP) in a constant volume of 10 ml/kg.

RESULTS

Results of light/dark tests are shown in Figs. 1 through 4 and Tables 1 and 2. Under control conditions, vehicle-treated mice displayed a consistent pattern of spending 26% to 30% of the 5-min test in the lit area (Figs. 1–4 and Table 2). The administration of diazepam, ipsapirone, 8-OH DPAT, and ondansetron produced significant increases in time mice spent in the lit area between doses of 1.0 to 10.0 mg/kg, 17.8 to 31.6 mg/kg, 0.0005 to 3.16 mg/kg, and 0.0001 to 1.0 mg/kg, respectively (Figs. 1–4). The dose-dependent increases produced by at least three of these drugs (ipsapirone, 8-OH DPAT, ondansetron) reached an asymptotic level of approximately 50–70% of time spent in the lit area. All four compounds also produced increased latencies for the mice to enter the dark area and significant increases in lit area locomotor and rearing activities (Table 1). The significant increases in lit compartment activities produced by those doses of the four compounds were accompanied by significant decreases of these activities in the dark area (data not shown). It should be noted however, that the total amount of these activities was not significantly altered. Finally, transitions between the two compartments were unaffected by the four drugs.

Statistical analyses of the results obtained with the four remaining compounds are shown in Table 2. N-Phenylbiguanide did not significantly affect any of the behavioral measurements. The administration of imipramine at 3.16 mg/kg and chlorpromazine at 1.0 or 3.16 mg/kg increased the time mice spent in the lit area and increased their latencies to enter the dark area; however, they did so only with concomitant decreases in total locomotor activity and rearing behavior. Visual observation of the mice revealed that the drugs produced an ataxic or sedative effect. Lastly, the injection of *S*(+)-amphetamine did not significantly change the percent of time mice spent in the lit area. It did however, at the two highest doses (3.16 and 5.62 mg/kg) tested, significantly increase total locomotor activity counts and significantly decrease total rearing behavior.

DISCUSSION

In the present study, the use of a fully automated and computer-integrated light/dark apparatus for mice is described and

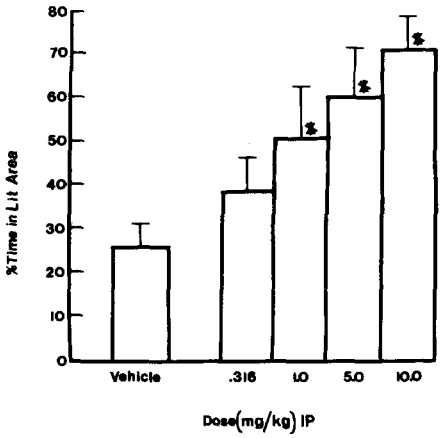

FIG. 1. The effect of diazepam on the time mice spent in the lit area (± standard deviation). The * indicates values that are significantly different from vehicle; $p<0.05$, Dunnett's t-test.

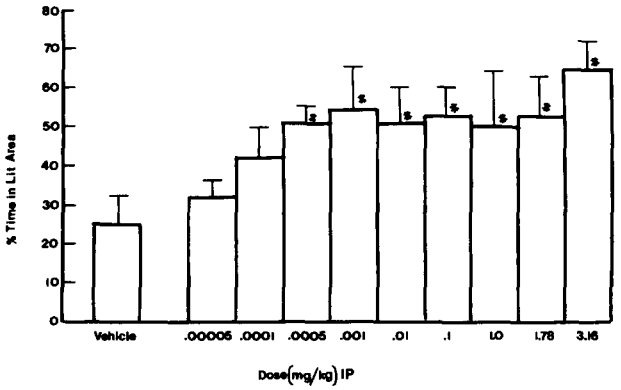

FIG. 3. The effect of 8-OH DPAT on the time mice spent in the lit area (± standard deviation). The * indicates values that are significantly different from the vehicle; $p<0.05$, Dunnett's t-test.

characterized for its pharmacological selectivity and sensitivity to anxiolytic drugs. Over the course of the study, the data obtained from vehicle-treated animals provided a stable baseline from which drug effects could be assessed. Thus the present system may offer distinct advantages over testing schemes previously reported, since it is faster and not susceptible to potential intra- or interobserver variability.

The two-compartment light/dark test is designed to exploit the tendency of rodents to explore a novel environment when confronted with the aversive properties of a brightly lit area. The aversive nature of the lit compartment is inferred from the inhibition of rodent exploratory behaviors. However, whether this situation is relevant to 1) "anxiety" in rodents and, more importantly, 2) the action of anxiolytics to treat the human condition is not known with certainty. Nonetheless, the administration of the known anxiolytics (diazepam and ipsapirone), the putative anxiolytic (ondansetron), and the 5-HT1A receptor agonist (8-OH DPAT) produced increases in time mice spent in the lit area and increases in locomotor activity and rearing behavior in this area with concomitant decreases of these activities in the dark area. The increases in behavioral activity in the lit area were not due to a generalized increase in motor behavior since total activity remained unchanged. A comparison of minimum effective doses (M.E.D.) based on the lit area measurements that were increased by the drugs reveals an order of potency of ondansetron ≥ 8-OH DPAT > diazepam > ipsapirone. The data obtained with 8-OH DPAT suggests that this compound may possess anxiolytic-like activity.

The results with diazepam, ipsapirone, and ondansetron are in agreement with those of previous investigations of the light/dark effects of these drugs [e.g., (1, 2, 4, 7, 9)]. Some of these latter studies [e.g., (1,4)] have also reported increases in transitions between the two compartments by rodents after anxiolytic administration, while other studies [e.g., (2, 7, 9)] have reported no change. In the present investigation, transitions were unaffected by the drugs, and thus these data suggest that the time mice spent in the lit area and behavioral activities, such as locomotor and rearing behaviors, may be more useful measures of the "anxiolytic" potential of a compound than transitions between the 2 compartments. In fact, the measurement found most consistent and useful for assessing anxiolytic-like action was the time mice spent in the lit area. This conclusion is based on the

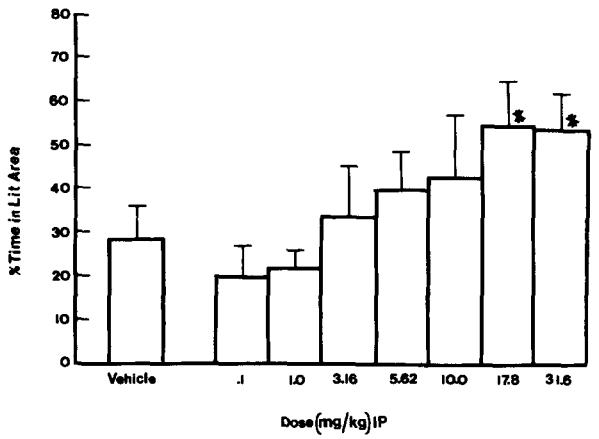

FIG. 2. The effect of ipsapirone on the time mice spent in the lit area (± standard deviation). The * indicates values that are significantly different from vehicle; $p<0.05$, Dunnett's t-test.

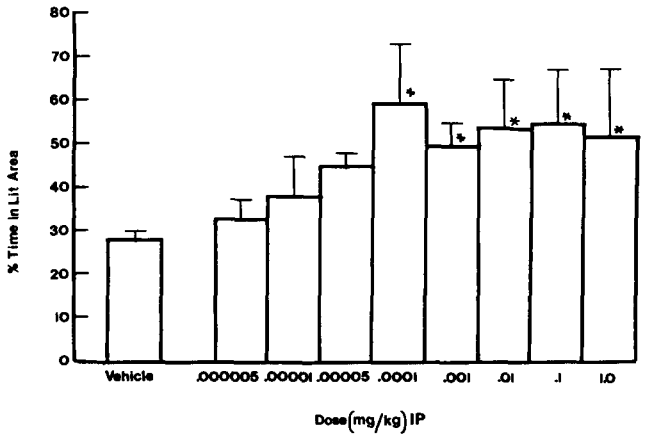

FIG. 4. The effect of ondansetron on the time mice spent in the lit area (± standard deviation). The * indicates values that are significantly different from vehicle; $p<0.05$, Dunnett's t-test.

fact that this parameter provided the most consistent dose-effect results with the drugs (Figs. 1–4 and Table 1).

Clear differences in the exploratory activities of the anxiolytics and the remaining compounds were demonstrated in this study. The administration of the peripheral 5-HT3 receptor agonist, N-phenylbiguanide, between doses of 1 to 31.6 mg/kg, did not significantly alter behavioral measurements. The lack of activity by N-phenylbiguanide was not unexpected, however, since it does not readily penetrate the blood-brain barrier. The effects of other types of CNS active agents were also examined. Specifically, the antidepressant, imipramine, and the antipsychotic, chlorpromazine, produced increases in time mice spent in the lit area only at doses that impaired total locomotor or rearing behavior. In addition, the CNS stimulant, *S*(+)-amphetamine, did not significantly alter the time mice spent in the lit area, but it did significantly increase total locomotor activity counts and concomitantly decrease total rearing behavior. The lack of anxiolytic-like action by imipramine, chlorpromazine, and *S*(+)-amphetamine is consistent with earlier reports of the behavioral effects produced by these drugs in the light/dark exploratory procedure [e.g., (2,9)]. When taken together, these data suggest that anxiolytic agents produce a selective profile of effects on animal behavior in the 2-compartment apparatus.

In summary, the fully automated 2-compartment light/dark chamber may offer technical improvements over previously reported light/dark chambers. Using this system, anxiolytics that are thought to act by a benzodiazepine-receptor related mechanism (diazepam) and agents that are thought to act by 5-HT1A (ipsapirone and 8-OH DPAT) and 5-HT3 (ondansetron) mechanisms produced significant increases in lit area activities in mice that may be indicative of anxiolytic-like action. The measurement found most useful for assessing antianxiety-like activity was the time mice spent in the lit area. Compounds from other therapeutic classes (imipramine, chlorpromazine, and *S*(+)-amphetamine) did not exhibit a similar profile of behavioral activity. In agreement with previous studies, the 2-compartment light/dark test may be useful for identifying known and putative anxiolytic agents.

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