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Pharmacological Evaluation of a Modified Open-Field Test Sensitive to Anxiolytic Drugs

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REX, A., J. P VOIGT, M. VOITS AND H. FINK. *Pharmacological evaluation of a modified open-field test sensitive to anxiolytic drugs*. PHARMACOL BIOCHEM BEHAV **59**(3) 677–683, 1998.—In a recent study it has been shown that benzodiazepine receptor agonists attenuate novelty-induced suppression of feeding and increase the percentage of animals feeding in the open field. Food-deprived rats were placed in one corner of the open field containing food in the center. The number of rats beginning to eat in the first 5 min was recorded. In the present study this test was validated pharmacologically using known "anxiolytic" or "nonanxiolytic" drugs. The following substances (effective doses, given IP) increased the number of rats feeding within 5 min in the center of the open field: meprobamate (30.0–300 mg/kg), 8-OH-DPAT (10 and 30 μg/kg), ipsapirone (1.0 and 2.0 mg/kg), ritanserin (0.125–0.5 mg/kg), tropisetron (0.1–10.0 μg/kg), ondansetron (0.3–3.0 μg/kg), lisuride (0.28–0.55 mg/kg), morphine (0.3 and 1.0 mg/kg), propranolol (0.3 and 1.0 mg/kg), clozapine (1.0 mg/kg). Drugs without "anxiolytic" effects in other animal models or in humans, including amphetamine, apomorphine, haloperidol, sulpiride, and mCPP did not increase the incidence of food intake in this test. Ethanol and hexobarbital, in nonsedative doses, had no effect in this paradigm. Drugs and doses effective in the modified open-field test caused no increase in food intake in an independent food consumption test using food-deprived rats staying in the familiar cages. The results suggest that the modified open-field test can detect "anxiolytic" drug properties and is valid for the assessment of "anxiolytic" effects from different classes of drugs.

Anxiety Open field Animal model of anxiety Rat Food deprivation Food intake

THERE is considerable interest in the development of anxiolytics. New drugs acting at benzodiazepine receptors, serotonin receptors, or neuropeptide receptors may have a possible therapeutic relevance in the treatment of anxiety (3,4). Thus, animal models of anxiety are widely used, but most of them need well-trained animals and/or induce "anxiety"/"fear"/"panic" under nonethological conditions. Additionally, the test procedures are often time consuming, and common animal models of anxiety are often insensitive to substances other than benzodiazepines (6). Especially serotonergic drugs with supposed "anxiolytic" effects caused inconsistent results in several animal models [e.g., (20)]. However, the efficacy of 5-HT_{1A} agonists in general anxiety disorder is well established (9,35) and clinical trials suggest antianxiety actions of ritanserin (3).

In the search for a simple model with a more ethological approach to "anxiety" or "fear" we adapted two models of anxiety assessing feeding in an open field (2,5). These models purport to elicit anxiety developed against food intake by hungry rats in a novel aversive environment. Recently, we demonstrated that diazepam and abecarnil produced similar "anxiolytic" effects in this modified open-field test and the elevated plus-maze test (30), a well-established model of anxiety.

Nevertheless, possible confounding factors in tests of anxiolytic activity involving feeding are the ability of drugs to induce a hyperphagia or hypophagia in rodents [e.g., (25)] or to have effects on locomotor activity.

For this reason, possible drug-induced changes in feeding behavior and locomotor activity were determined by performing an independent food consumption test and by measuring locomotor activity during the modified open-field test.

The aim of the present study was the pharmacological validation of the modified open-field test using known "anxi-

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olytic" and "nonanxiolytic/anxiogenic" agents acting at serotonin receptors, dopaminergic receptors, and miscellaneous drugs with known "anxiolytic" side effects.

METHODS

Animals

Male Wistar rats (Meichsner, Berlin, Germany), 200 ± 25 g were used for the experiments. The animals were group housed, 10 per cage (45 × 60 × 25 cm), at room temperature (22°C) and under a 12 L:12 D cycle (light on at 0600 h). Standard pellet food (Altromin 1326) and water were freely available.

Separate groups of rats were used for each dose (n = 9–11). All animals were used once.

Drugs

The following drugs were used: amphetamine (Berlin-Chemie, Germany), apomorphine (WoelmPharma, Germany), clozapine (Sandoz, Switzerland), ethanol (Berlin-Chemie, Germany), hexobarbital (AWD, Germany), haloperidol (Gideon-Richter, Hungary), ipsapirone (Tropon, Germany), lisuride (Schering, Germany), mCPP (Research Biochemical Inc., USA), meprobamate (Philopharm, Germany), morphine (AWD, Germany), 8-OH-DPAT (Research Biochemical Inc., USA), ondansetron (Glaxo Group, UK), *d*, l-proprandolol (AWD, Germany), ritanserin (Jannssen, Belgium), sulpiride (Schuerholz, Germany), and tropisetron (Ciba Geigy, Switzerland). The drugs were dissolved in saline or suspended in vehicle [saline containing 1% Cremophor EL (BASF, Germany)] immediately prior to use (Table 1). Drugs were injected IP in a volume of 5 ml/kg body weight.

Modified Open-Field Test

The experiments were performed in a sound proof, brightly illuminated (≈1500 lx) observation chamber between 0900 and 1000 h using a white wooden open field (100 × 100 × 40 cm). Locomotor activity was assessed simultaneously using 1-min intervals by interruptions of 10 equally spaced infrared light beams in the open field. The rats were food-deprived 20 h prior to testing. One hour before testing the animals were transferred into the observation chambers. A Petri dish (diameter 8 cm) filled with standard food pellets (Altromin 1326) was placed in the center of the open field. Following the injection and a substance-specific pretreatment period, the animals were placed individually in one corner of the unfamiliar open field, facing the center. Each rat was observed from outside by remote monitoring for 5 min and the time of the initial feeding (latency to eat) was recorded. The number of rats taking food (% of rats in a group feeding) during the 5 min was registered.

The open field was cleaned after each animal using a disinfectant, and the Petri dish was filled with fresh pellets for each animal. To exclude batch and seasonal variation, control groups (n = 9–11), treated with saline or vehicle, were tested at irregular intervals between drug-treated groups.

Food Consumption Test

Rats were singly housed for 6 days and on the seventh day food deprived for 20 h (same duration as for the modified open-field test), with water freely available. Only drugs showing an effect in the modified open field were tested in the food consumption test. The animals received an injection with the dose that induced the strongest effect in the modified open-field test. Following the drug-dependent waiting period (same duration as for the modified open-field test) the animals were allowed to feed. The amount of food eaten during the following 30 min was determined and compared to the controls.

Statistics

The data from the modified open-field test (%) were analyzed using a two-tailed Fisher's exact test. Locomotor activity data, the latency-to-eat data and data from the food consumption test were analyzed with the Welch's test [shown as means ± SEM, t-value; degrees of freedom (df), p-value]. Differences of the means with $p < 0.05$ were considered as statistically significant.

RESULTS

Modified Open-Field Test

The incidence of food intake observed in 11 control groups injected either with saline or vehicle was 7.8%. Eight rats of 102 control rats fed during the 5 min of the test session. The averaged latency to start eating was 295 ± 5 s. The incidence of food intake and the latency to start eating showed no difference between saline (3 from 41) or vehicle (5 from 61)-treated controls. Additionally, there was no batch variation or seasonal difference regarding the behavior in the open field. Therefore, the data of the controls were pooled. The incidence of food intake could be maximally increased to 70% (diazepam) or 80% (meprobamate), while the latency to start eating could be decreased most by diazepam [193 ± 27 s, t (9) = 3.715, p = 0.0024] and meprobamate [118 ± 16 s, t (10) = 10.559, $p < 0.0001$]. Diazepam as a reference drug for anxiolytic action, increased the incidence of food intake following the administration of 2.5 and 5.0 mg/kg. The same doses reduced the latency to start eating in the open field [193 ± 27 s, t (9) = 3.715, p = 0.0024, and 265 ± 14 s, t (11) = 2.018, p = 0.0034].

The 5-HT_{1A} agonists 8-OH-DPAT and ipsapirone increased the percentage of rats taking food. The inverted U-shape dose–response curves with maximal effects 0.03 mg/kg and 2.0 mg/kg, respectively (Fig. 1). The latency to start eating was reduced following the administration of 0.03 mg/kg 8-OH-DPAT [239 ± 26 s, t (9) = 2.115, p = 0.0318] and 2.0 mg/kg ipsapirone [247 ± 31 s, t (10) = 1.883, p = 0.0446] (Table 1). The 5-$HT_{1b/2C}$ agonist mCPP (0.1 −3.0 mg/kg) had no effect compared to the controls (Table 1).

The 5-HT_2 antagonist ritanserin had a maximal effect on the incidence of food intake and the latency to eat at a dose of 0.25 mg/kg [230 ± 33 s, t (9) = 1.947, p = 0.0417] (Fig. 1, Table 1). The "anxiolytic" effects of the 5-HT_3 antagonists tropisetron and ondansetron occurred at low doses of 0.1–10.0 μg/kg and 0.3–3.0 μg/kg, respectively (Fig. 1, Table 1). The latency to eat was reduced by both drugs, peaking at doses of 10.0 μg/kg [226 ± 31 s, t (9) = 2.197, p = 0.0278] tropisetron and 0.3 μg/kg [214 ± 17 s, t (10) = 4.571, p = 0.0005] ondansetron.

The direct dopamine agonist apomorphine (1.0 and 3.0 mg/kg), the indirect dopamine agonist amphetamine (0.5 and 1.0 mg/kg), the dopamine receptor blocker haloperidol (0.05–1.25 mg/kg), the D_2 antagonist sulpiride (4.0 and 8.0 mg/kg) did not increase the feeding in the open field (Table 1). However, other dopaminergic drugs as the ergot derivative lisuride (0.28–0.55 mg/kg) and the atypical neuroleptic clozapine (1.0 mg/kg) increased the percentage of rats feeding in the open field and decreased the latency to begin eating, maximally effective at doses of 0.55 mg/kg [233 ± 27 s, t = 2.257, p = 0.0004] and 1.0 mg/kg [240 ± 21 s, t = 2.548, p = 0.0145], respectively (Table 1, Fig. 2).

TABLE 1

COMPARISON OF THE LATENCY TO START EATING AND THE LOCOMOTOR ACTIVITY IN THE MODIFIED OPEN-FIELD TEST FOLLOWING ACUTE TREATMENT WITH KNOWN ANXIOLYTIC AND NONANXIOLYTIC DRUGS COMPARED TO SALINE/VEHICLE-TREATED CONTROLS WELCH'S TEST

Drug (Dissolved or Suspended)	Dose (mg/kg)	Latency To Eat (s)	Motor Activity (Squares/ 5 min)	Drug (Dissolved or Suspended)	Dose (mg/kg)	Latency To Eat (s)	Motor Activity (Squares/ 5 min)	Drug (Dissolved or Suspended)	Dose (mg/kg)	Latency to Eat (s)	Motor Activity (Squares/ 5 Min)
Saline/ vehicle	0.9%	295 ± 5	64 ± 11	Ondansetron (in vehicle)	0.0001	291 ± 9	61 ± 10	Sulpiride (in vehicle)	4.0	288 ± 12	33 ± 9*
					0.0003	214 ± 17*	78 ± 8		8.0	300 ± 0	43 ± 15
					0.001	263 ± 18	69 ± 12				
Diazepam	0.625	282 ± 18	46 ± 21		0.003	269 ± 18	59 ± 16				
(in vehicle)	1.25	282 ± 14	65 ± 15					Ethanol	0.3	300 ± 0	89 ± 11
	2.5	193 ± 27*	72 ± 14					(in saline)	1.0	300 ± 0	61 ± 6
	5.0	265 ± 14*	58 ± 15	mCPP	0.1	300 ± 0	50 ± 12		3.0	300 ± 0	45 ± 10
	10.0	300 ± 0	89 ± 10	(in vehicle)	1.0	300 ± 0	54 ± 10				
					3.0	300 ± 0	48 ± 12				
								Hexobarbital	3.0	300 ± 0	68 ± 12
8-OH-DPAT	0.003	292 ± 8	51 ± 18					(in saline)	10.0	300 ± 0	56 ± 15
(in vehicle)	0.01	267 ± 22	78 ± 7	Amphetamine	0.5	300 ± 0	144 ± 16*		30.0	300 ± 0	21 ± 9*
	0.03	239 ± 26*	85 ± 14	(in saline)	1.0	300 ± 0	180 ± 21*				
	0.1	291 ± 9	61 ± 11								
	0.3	300 ± 0	103 ± 16*					Meprobamate	10.0	289 ± 9	56 ± 12
	1.0	300 ± 0	72 ± 9	Clozapine	0.3	300 ± 0	33 ± 21	(in vehicle)	30.0	265 ± 23	82 ± 17
				(in vehicle)	1.0	240 ± 21*	82 ± 11		100.0	118 ± 16*	129 ± 21*
					3.0	252 ± 22*	9 ± 4*		300.0	198 ± 17*	30 ± 8*
Ipsapirone	0.5	276 ± 18	31 ± 8		10.0	300 ± 0	11 ± 3*				
(in vehicle)	1.0	269 ± 19	79 ± 7								
	2.0	247 ± 31*	81 ± 11					Morphine	0.1	298 ± 2	58 ± 17
	4.0	273 ± 26	63 ± 14	Haloperidol	0.05	300 ± 0	53 ± 11	(in vehicle)	0.3	261 ± 21	52 ± 15
				(in saline)	0.125	300 ± 0	18 ± 9*		1.0	245 ± 29*	100 ± 19
					0.5	300 ± 0	26 ± 5*		3.0	294 ± 6	71 ± 11
Ritanserin	0.0625	283 ± 18	52 ± 8		1.25	300 ± 0	3 ± 1*				
(in vehicle)	0.125	231 ± 34*	61 ± 12								
	0.25	230 ± 33*	68 ± 10					Propranolol	0.1	292 ± 8	72 ± 12
	0.5	255 ± 22	68 ± 15	Apomorphine	1.0	300 ± 0	63 ± 11	(in vehicle)	0.3	249 ± 36	61 ± 13
	1.0	242 ± 38	51 ± 5	(in vehicle)	3.0	300 ± 0	48 ± 12		1.0	270 ± 14	73 ± 9
									3.0	275 ± 20	66 ± 14
									10.0	300 ± 0	99 ± 13*
Tropisetron	0.00003	294 ± 6	69 ± 13	Lisuride	0.1	285 ± 15	49 ± 14				
(in vehicle)	0.0001	287 ± 11	72 ± 11	(in vehicle)	0.2	260 ± 27	43 ± 25				
	0.0003	261 ± 24	46 ± 16		0.28	264 ± 19	95 ± 11*				
	0.001	236 ± 22*	71 ± 9		0.4	255 ± 17*	34 ± 15				
	0.003	228 ± 29*	63 ± 11		0.55	233 ± 27*	36 ± 16				
	0.01	226 ± 31*	60 ± 10		0.8	300 ± 0	36 ± 17				

*$p < 0.05$. Data expressed as mean ± SEM.

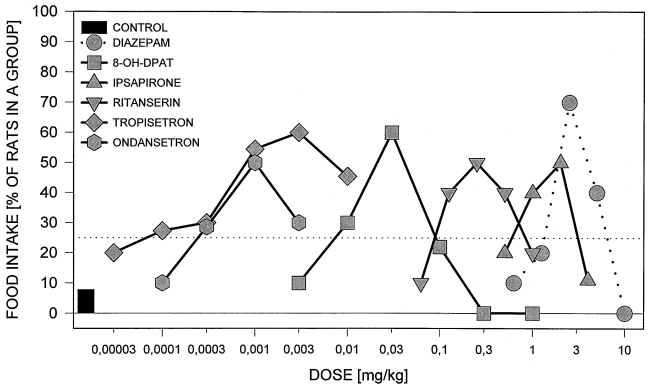

FIG. 1. Changes in the incidence of food intake in the modified open field following a food deprivation of 20 h and an acute treatment with 8-OH-DPAT [□] (0.003, 0.01, 0.03, 0.1, 0.3, 1.0 mg/kg), ipsapirone [△] (0.5, 1.0, 2.0, 4.0 mg/kg), ritanserin [▽] (0.0625, 0.125, 0.25, 0.5, 1.0 mg/kg), tropisetron [◇] (0.00003, 0.0001, 0.0003, 0.001, 0.003, 0.01 mg/kg), or ondansetron [x] (0.0001, 0.0003, 0.001, 0.003 mg/kg) and diazepam [○] (0.625, 1.25, 2.5, 5.0, 10.0 mg/kg) compared to the controls [■] (Fisher exact test, $n = 9$–11). The data are expressed as percent (% of rats feeding in a group). An increase in the incidence of food intake exceeding 27% of the rats feeding (·····) was considered as statistically significant ($p < 0.05$).

The following drugs with "anxiolytic" properties induced an increase in the percentage of rats feeding in the open field and decreased the latency to start eating, similar to the effects of diazepam (Fig. 3, Table 1): the carbaminacidderivative meprobamate induced the strongest effects on the feeding in the open field following treatment with 30.0–300.0 mg/kg, peaking at 100.0 mg/kg. Meprobamate reduced the latency to begin eating, also peaking at 100 mg/kg [118 ± 16 s, t (10) = 10.559, $p < 0.0001$] (Fig. 3, Table 1).

Lower effects on the food intake in the open field were produced by the β-blocker propranolol (0.3 and 1.0 mg/kg) and the opiate morphine (0.3 and 1.0 mg/kg) (Fig. 3). The latency to begin eating was reduced by morphine [1.0 mg/kg, 245 ± 29 s, t (11) = 1.961, $p = 0.0378$] (Table 1). The barbiturate hexobarbital (3.0–30.0 mg/kg) and ethanol (0.3–3.0 mg/kg) had no effects on percentage of rats feeding and subsequently on the latency to start feeding when given in the used nonsedative doses (Table 1).

Locomotor Activity

Control rats crossed 64 ± 11.5 squares during the 5-min test period. Diazepam caused a slight but not significant increase in locomotor activity, compared to the controls (Table 1).

8-OH-DPAT [0.3 mg/kg, t (17) = 2.009, $p = 0.0295$] induced a hyperlocomotion (Table 1) in the open field. None of the other serotonergic drugs altered the locomotor activity.

Amphetamine [0.5 mg/kg, t (19) = 4.12, $p = 0.0003$ and 1.0 mg/kg, t (14) = 4.893, $p < 0.0001$] induced the highest rises in locomotor activity to more than 125 squares crossed per 5 min. Haloperidol [0.125 mg/kg, t (47) = 3.237, $p = 0.0011$, 0.5 mg/kg, t (105) = 3.145, $p < 0.0001$ and 1.25 mg/kg, t (111) = 5.323, $p < 0.0001$] produced sedative effects. Lisuride induced a hyperlocomotion at a dose of 0.28 mg/kg, t (39) = 1.993, $p = 0.0027$. Sulpiride [4.0 mg/kg, t (47) = 2.181, $p = 0.0171$] and higher doses of clozapine (3.0 mg/kg, t (116) = 4.699, $p < 0.0001$ and 10.0 mg/kg, t (118) = 4.648, $p < 0.0001$] had sedative effects on the behavior in the open field (Table 1).

Propranolol [10.0 mg/kg, t (28) = 2.055, $p = 0.026$] markedly stimulated locomotor activity with approximately 100 crossed squares. Meprobamate [100 mg/kg, t (14) = 2.742, $p = 0.0079$] also increased locomotor activity in the open field, whereas a dose of 300 mg/kg, t (58) = 2.5, $p = 0.0077$, caused sedation. The highest dose of hexobarbital [30 mg/kg, t (47) = 3.025, $p = 0.002$] had sedative effects in the open field (Table 1).

Food Consumption Test

Control rats in their home cages ate approximately 3 g of the standard pellets within 30 min following 20 h food deprivation (Fig. 4).

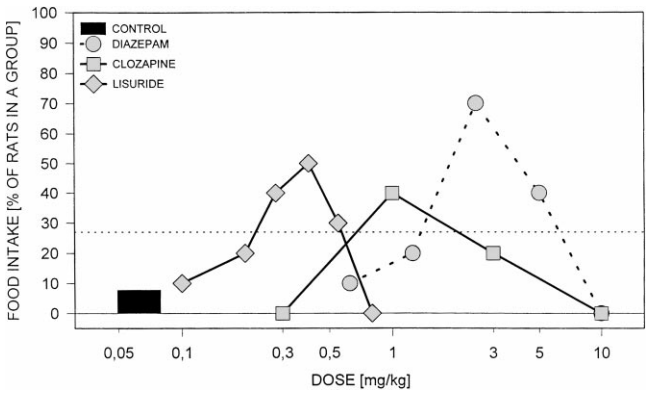

FIG. 2. Changes in the incidence of food intake in the modified open field following a food deprivation of 20 h and an acute treatment with Clozapine [□] (0.3, 1.0, 3.0, 10.0 mg/kg) or lisuride [◇] (0.1, 0.2, 0.28, 0.4, 0.55, 0.8 mg/kg) compared to the controls [■] and diazepam [○] (0.625, 1.25, 2.5, 5.0, 10.0 mg/kg) (Fisher exact test, $^*p < 0.05$, $n = 9$–11). The data are expressed as percent (% of rats feeding in a group). An increase in the incidence of food intake exceeding 27% of the rats feeding (·····) was considered as statistically significant ($p < 0.05$).

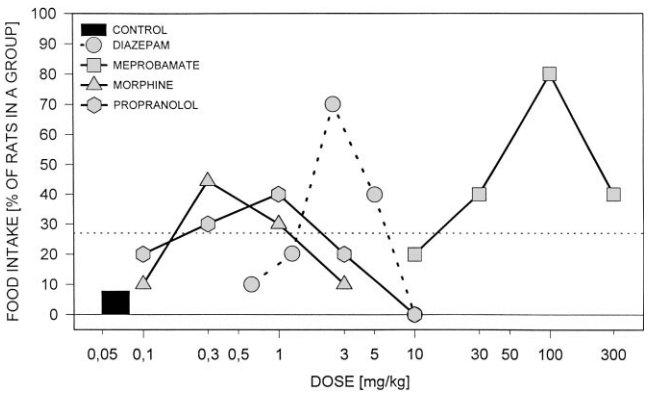

FIG. 3. Changes in the incidence of food intake in the modified open field following a food deprivation of 20 h and an acute treatment with meprobamate [□] (10.0, 30.0, 100.0, 300.0 mg/kg), morphine [△] (0.1, 0.3, 1.0, 3.0 mg/kg) or propranolol [x] (0.1, 0.3, 1.0, 3.0, 10.0 mg/kg) compared to the controls [■] and diazepam [○] (0.625, 1.25, 2.5, 5.0, 10.0 mg/kg) (Fisher exact test, $^*p < 0.05$, $n = 9$–11). The data are expressed as percent (% of rats feeding in a group). An increase in the incidence of food intake exceeding 27% of the rats feeding (·····) was considered as statistically significant ($p < 0.05$).

Diazepam (2.5 mg/kg) as well as all tested serotonergic drugs with an anxiolytic action—8-OH-DPAT (0.03 mg/kg), ipsapirone (2.0 mg/kg), ritanserin (0.25 mg/kg), ondansetron (0.001 mg/kg), and tropisetron (0.003 mg/kg)—failed to modify the food consumption in the familiar cage following the food deprivation (Fig. 4).

The food intake of food-deprived rats in the familiar home cage following treatment with clozapine (1.0 mg/kg), lisuride (0.4 mg/kg), meprobamate (100.0 mg/kg), or propranolol (1.0 mg/kg) was not increased compared to the vehicle-treated rats (Fig. 4).

Morphine [0.3 mg/kg, t (16) = 4.6, p = 0.0064] markedly decreased the amount of food eaten in the familiar cage compared to the controls.

DISCUSSION

The anxiolytic profile of a compound is best characterized by using several animal models of anxiety basing on different "anxious" stimuli. Animal models of anxiety using the natural neophobia of rodents appear to be more sensitive for antianxiety effects of nonbenzodiazepine than paradigms based on punished behavior (20).

To determine the reliability of the modified open-field test based upon suppression of feeding in a novel and aversive environment, the test was pharmacologically validated. The parameters measured were the percentage of rats of one group starting to feed and the latency to start eating. An increasing number of rats taking food and a decrease of the latency to start eating, a parameter used to determine the "anxiolytic" drug effects (2), were considered as an "anxiolytic" action. The results show that both parameters proved to be reliable for the determination of "anxiolytic" effects in this test with the incidence of food intake to be more sensitive. The reduction of measurement down to a "yes–no decision" simplifies the analysis of the anxiety-related behavior. Preliminary experiments including the time and the amount of eating and the frequency of feeding did not deliver more information on the anxiety-related behavior, due to the short duration of the test (5). The amount of feeding between the groups with a suspected anxiolytic effect was not different. Less than 10% of the vehicle-treated rats fed in the open field. In the preliminary experiments even a longer test period of 10 or 15 min did not increase the incidence of food intake. Diazepam, with robust "anxiolytic" effects in a wide range of animal models, proved to be effective in our modified open-field test and was used as a reference for "anxiolytic" action (30). The results demonstrate that this paradigm covers a wide range of agents that are anxiolytic active in humans or animal models, for example, the carbamate acid derivative meprobamate (7), as well as serotonergic drugs like ondansetron (20).

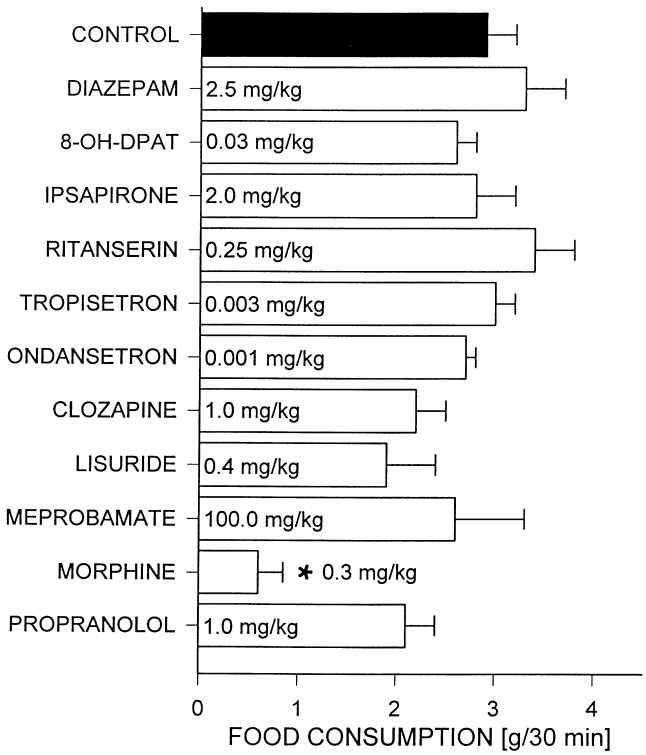

FIG. 4. Food consumption of single-housed rats under a fixed food regimen (20 h food deprivation) in 30 min following an acute treatment with doses that induced the highest "anxiolytic" effects in the modified open field compared to saline/vehicle treated controls (Welch's test, $*p < 0.05$). Data expressed as mean ± SEM.

8-OH-DPAT induced an "anxiolytic" effect in our test. correspondingly, "anxiolytic" effects of 8-OH-DPAT in the social interaction, on exposure to the elevated plus-maze and in an ultrasonic vocalization test were described in the same dose range (9,20). However, no anxiolytic or anxiogenic effects following treatment with 8-OH-DPAT (24) have been demonstrated. The effects induced by 8-OH-DPAT may be dependent on the animal species, the doses, the test, and the procedural variables in the test used (24,31). Ipsapirone showed in our model an eloquent effect in a narrow dose range of 1.0–2.0 mg/kg, despite the ineffectiveness of this compound in the elevated plus-maze test (9,20). However, our results bear comparison with the effects of ipsapirone in a saltwater-drinking test in a dose range from 0.5–5.0 mg/kg, in the ultrasonic vocalization test, social interaction test, and in the attenuation of defensive burying behavior by ipsapirone (7,9,20). Additionally, ipsapirone is effective in the treatment of anxiety in humans (8). The 5-HT_2 antagonist ritanserin induced "anxiolytic" effects in our model (0.125–0.5 mg/kg). Similar results were obtained in a wide range of models of anxiety at doses from 0.25 to 10.0 mg/kg (1,18,26). Anxiolytic effects of ritanserin have been described in humans (3) also. Tropisetron and ondansetron, 5-HT_3 antagonists, induced stable "anxiolytic" effects in low doses at 0.1–10.0 μg/kg and 0.3–3.0 μg/kg, respectively, in our test. The effective dose range was similar compared to the social interaction test, in the dark–white box test (6,13,20) and a elevated "zero-maze" study (33). However, it should be noted that ondansetron was ineffective in tests, using punished responding (20) and in higher doses (0.1–1.0 mg/kg) in the social interaction test (14). In our test, ondansetron showed a bell-shaped dose–response curve with no "anxiolytic" activity at doses higher than 0.01 mg/kg. It is known, that the 5-$HT_{2C/1B}$ agonist mCPP often induces "anxiogenic"/proaversive effects, for example, in the social interaction test (37) and the elevated "zero-maze" (33). Additionally, in clinical trials mCPP induces anxiety (38). In our modified open-field test mCPP had no effect, and these results support the view that drugs with no "anxiolytic" effects can be screened out in our test.

The dopamine agonists amphetamine and apomorphine and the dopamine antagonists haloperidol and sulpiride were ineffective in our paradigm. These drugs do not have "anxiolytic" effects [e.g., (19,22,27,29)]. Thus, this may provide sup-

port for our idea that this test is suitable to discard drugs without "anxiolytic" effects. The ergot derivative lisuride showed "anxiolytic" effects in doses from 0.28 to 0.55 mg/kg in our modified open-field test. Lisuride is known to be a potent dopamine agonist. Besides that, lisuride possesses a high affinity to the 5-HT_{1A} receptor acting as an agonist (15). Therefore, an "anxiolytic" side effect of this compound could be expected. The atypical neuroleptic clozapine was effective in our test (1.0 mg/kg), and has been shown to be effective in the treatment of anxiety in humans (34) and in classical conflict tests in doses of 0.3–1.0 mg/kg (1,20) likely by a nonselective antagonism at the 5-HT_{2C} receptors.

Meprobamate, an anxiolytic drug, widely used in the prebenzodiazepine era, induced robust "anxiolytic" effects in doses from 30.0–300 mg/kg, corresponding with the effective doses in other "anxiety" tests, for example, the conditioned defensive burying model (7) and the Geller-Seifter test (19).

The $\beta_{1/2}$-blocker propranolol induced an anxiolytic-like effect (0.3 and 1.0 mg/kg) in our paradigm, similar to animal models of punished responding (11) and social interaction (17). It is known that propranolol has fear-reducing effects in anxiety combined illnesses (12,36). This anxiolytic-like action may be caused by the nonselective 5-$HT_{1/2C}$ antagonist affinities of propranolol (20). We observed anxiolytic-like effects of morphine in the modified open-field test (0.3 and 1.0 mg/kg). Arguments for anxiolytic effects of morphine were provided by clinical observations in the treatment of anxiety-inducing pains (21,32) and from a study showing "anxiolytic" properties of morphine in a conditioned emotional response paradigm (23).

Hexobarbital and ethanol used in nonsedative doses, 0.03–0.3 mg/kg and 0.3-3.0 mg/kg respectively, failed in our test. However, barbiturates have been shown to be effective in paradigms using punished behavior, for example, in classical conflict tests (19) and in an open-field test (5). Ethanol, in the doses used, has shown "anxiolytic" effects on the elevated plus-maze (10), whereas other authors failed to find "anxiolytic" effects in a water-lick test and in a punished responding model in monkeys [e.g., (28)].

Possible confounding factors in this model of anxiety could be drug-induced effects on feeding and/or changes in locomotor activity. Both factors have been assessed.

The independent food consumption test was designed to determine possible drug effects on the feeding behavior. The test was performed using the maximal effective "anxiolytic" doses resulting from the modified open-field test and the same period of food deprivation as in the modified open-field test was used.

A drug-induced increase in food intake should be detected in the familiar cage as well as in the aversive open field. All drugs showing an "anxiolytic" effect in the modified open field were tested in the food consumption test. Diazepam and 5-HT_{1A} agonists are known to produce hyperphagia in nonstarving rats (25) in a familiar surrounding, but these effects do not occur in food-deprived rats (5,25). It appears that fasting produced an increase in feeding with a ceiling effect and could not be further stimulated by drug action (16). In contrast, morphine, given in the most effective "anxiolytic" dose, reduced the food intake in the familiar home cage, but increased the percentage of rats taking food in the aversive open field. The hyperphagic or anorectic drug effects occurring in nonfood-deprived rats staying in the home cage did not have an important role in food-deprived animals. Most control animals do not feed in the open field, and it is obvious that the novel environment suppresses the food intake, an effect diminished by anxiolytics. Our food consumption test revealed that the increase of feeding in the open field is presumable not caused by drug-induced effects on feeding mechanisms, because a treatment with the most effective "anxiolytic" doses of all drugs did not change food consumption in the familiar cages compared to the similar food-deprived controls.

Changes in locomotor activity can alter or conceal anxiety-related behavior, and determination of motor activity is important for all animal models of behavior based on exploratory behavior. Locomotor activity has been measured simultaneously using 1-min intervals during the modified open-field test. Interestingly, in all groups, except for amphetamine- and apomorphine-treated animals, we found a similar pattern of movement with a high motor activity during the first third of the test and a hypoactivity during the last part, in which the feeding occurred. Therefore, the time the animals eat does not interfere with the locomotion index, for example, an increase in food intake was not causing a decrease in overall locomotor activity. In advance, it was assumed that hyperactivity could increase the probability of rats to approach the food and to eat, or eating might decrease locomotor activity generally. We could not find a general decrease in locomotor activity in groups showing a higher incidence of food intake. However, the results indicate that there is no simple relationship between locomotor activity and the incidence of food intake. It is demonstrated that an increase of locomotion is not necessarily linked with an increase in feeding in our test (apomorphine, amphetamine). The spatial pattern of locomotion did not change despite the increase in activity induced by these drugs. On the contrary, drugs with potential "anxiolytic" properties but also inducing strong sedative effects may mask an "anxiolytic" behavior. In cases when sedative effects and an "anxiolytic" effect occur at the same doses, an independent measurement of locomotion should be performed. In our test moderate sedative side effects did not prevent obligatory "anxiolytic" effects in the modified open field, for example, clozapine. The modified open-field test is based to some extent on exploratory behavior. In this case, the moderate locomotor effects seen in our experiments reduce the problem of the extent to which the behavioral response may be considered as an anxiolytic effect, rather than a change in motor activity.

CONCLUSIONS

The modified open-field test in the open field is a simple and inexpensive paradigm, employing an ethological induction of anxiety. The pharmacological validation of the paradigm, employing an ethological induction of anxiety. The pharmacological validation of the paradigm, using various drugs with known "anxiolytic" or "nonanxiolytic" properties proved the reliability of the model. It allows the rapid assessment of potential anxiolytics. As a drug with suspected anxiolytic properties has to be tested in a battery of several models of anxiety, we believe that this test may be helpful for the detection of new anxiolytic drugs in association with other well-established models of anxiety (e.g., X-maze or black–white box).

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REFERENCES

1. Benvenga, M. J.; Leander, J. D.: Olanzapine, an atypical antipsychotic, increases rates of punished responding in pigeons. Psychopharmacology (Berlin) 119:133–138; 1995.
2. Bodnoff, S. R.; Suranyi-Cadotte, B.; Quirion, R.; Meaney, M. J.: A comparison of the effects of diazepam versus several typical and atypical anti-depressant drugs in an animal model of anxiety. Psychopharmacology (Berlin) 97:277–279; 1989.
3. Bressa, G. M.; Marini, S.; Gregori, S.: Serotonin S2 receptors blockage and generalized anxiety disorders. A double-blind study on ritanserin and lorazepam. Int. J. Clin. Pharmacol. Res. 7:111–119; 1987.
4. Briley, M.; Chopin, P.; Moret, C.: Effect of serotonergic lesion on "anxious" behavior measured in the elevated plus-maze test in the rat. Psychopharmacology (Berlin) 101:187–189; 1990.
5. Britton, D. R.; Thatcher Britton, K.: A sensitive openfield measure of anxiolytic drug activity. Pharmacol. Biochem. Behav. 15:577–582; 1981.
6. Broekkamp, C. L. E.; Berendsen, H. H. G.; Jenck, F.; Van Delft, A. M. L.: Animal models for anxiety and response to serotonergic drugs. Psychopathology 22(Suppl. 1):2–12; 1989.
7. Craft, R. M.; Howard, J. L.; Pollard, G. T.: Conditioned defensive burying as a model for identifying anxiolytics. Pharmacol. Biochem. Behav. 30:77580780; 1988.
8. Cutler, N. R.; Sramek, J. J.; Keppel Hesselink, J. M.; Krol, A.; Roeschen, J.; Riskels, K.; Schweizer, E.: A double-blind, placebo-controlled study comparing the efficacy and safety of ipsapirone versus lorazepam in patients with generalized anxiety disorder: A prospective multicenter trial. J. Clin. Psychopharmacol. 13:429–437; 1993.
9. De Vry, J.: 5-HT_{1A} receptor agonists: Recent developments and controversial issues. Psychopharmacology (Berlin) 121:1–26; 1995.
10. Durcan, M. J.; Lister, R. G.; Eckardt, M. J.; Linnoila, M.: Behavioral interactions of fluoxetine and other 5-hydroxytryptamine uptake inhibitors with ethanol in tests of anxiety, locomotion and exploration. Psychopharmacology (Berlin) 96:528–533; 1988.
11. Durell, L. A.; Krantz, D. S.; Barrett, J. E.: The antianxiety effect of beta-blockers on punished responding. Pharmacol. Biochem. Behav. 25:371–374; 1986.
12. Dyck, J. B.; Chung, F.: A comparison of propranolol and diazepam for preoperative anxiolysis. Can. J. Anaesth. 38:704–709; 1991.
13. Eglen, R. M.; Lee, C.-H; Khabbaz, M.; Fontana, D. J.; Daniels, S.; Kilfoil, T.; Wong, E. H. F.: Comparison of potencies of 5-HT_3 receptor antagonists at inhibiting aversive behavior to illumination and the von Bezold-Jarische reflex in the mouse. Neuropharmacology 33:227–234; 1994.
14. File, S. E.; Johnston, A. L.: Lack of effects of 5-HT_3 receptor antagonists in the social interaction and elevated plus-maze tests of anxiety in the rat. Psychopharmacology (Berlin) 99:248–251; 1989.
15. Fink, H.; Rex, A.; Yamaguchi, M.; Turner, J.; Stephens, D. N.: Serotonin 1_A receptor activity of lisuride. Behav. Pharmacol. 3(Suppl. 1): 95; 1992.
16. Fletcher, P. J.; Davies, M.: Effects of 8-OH-DPAT, buspirone and ICS 205-930 on feeding in a novel environment: Comparisons with chlordiazepoxide and FG 7142. Psychopharmacology (Berlin) 102:301–308; 1990.
17. Gao, B.; Cutler, M. G.: Effects of acute and subchronic administration of propranolol on the social behaviour of mice: An ethopharmacological study. Neuropharmacology 31:749–756; 1992.
18. Gao, B.; Cutler, M. G.: Effects of acute and subchronic administration of ritanserin on the social behaviour of mice. Neuropharmacology 32:265–272; 1993.
19. Geller, I.; Seifter, J.: The effects of meprobamate, barbiturates, *d*-amphetamine and promazine on experimentally induced conflict in the rat. Psychopharmacologia 1:482–492; 1960.
20. Griebel, G.: 5-Hydroxytryptamine-interacting drugs in animal models of anxiety disorders: More than 30 years of research. Pharmacol. Ther. 65:319–395; 1995.
21. Hashimoto, Y.; Takarada, M.; Tanioka, H.; Rigor, B. M.: Treatment of cancer pain of the head and neck by continuous intravenous infusion of high-dose morphine: Report of two cases. J. Oral. Maxillofac. Surg. 48:398–400; 1990.
22. Hijzen, T. H.; Houtzager, S. E.; Joordens, R. J.; Oliver, B.; Slangen, J. L.: Predictive validity of the potentiated startle response as a behavioral model for anxiolytic drugs. Psychopharmacology (Berlin) 118:150–154; 1995.
23. Hill, H. E.; Belleville, R. E.; Pescor, F. T.; Wikler, A.: Comparative effects of methadone, meperedine and morphine on conditioned suppression. Arch. Int. Pharmacodyn. 163:341–352; 1966.
24. Hogg, S.; Andrews, N.; File, S. E.: Contrasting behavioural effects of 8-OH-DPAT in the dorsal raphé nucleus and ventral hippocampus. Neuropharmacology 33:343–348; 1994.
25. Hutson, P. H.; Dourish, C. T.; Curzon, G.: Evidence that the hyperphagic response to 8-OH-DPAT is mediated by 5-HT1A receptors. Eur. J. Pharmacol. 150:361–366; 1988.
26. Meert, T. F., Jannssen, P. A. J.: Psychopharmacology of ritanserin: Comparison with chlordiazepoxide. Drug Dev. Res. 18:119–144; 1989.
27. Molewijk, H. E.; van der Poel, A. M.; Olivier, B.: The ambivalent behaviour "stretched approach posture" in the rat as a paradigm to characterize anxiolytic drugs. Psychopharmacology (Berlin) 121:81–90; 1995.
28. Patel, J. B.; Migler, B.: A sensitive and selective monkey conflict test. Pharmacol. Biochem. Behav. 17:645–649; 1982.
29. Rex, A.; Marsden, C. A.; Fink, H.: Validation of the elevated plus maze using dopaminergic drugs. Naunyn Schmiedebergs Arch. Pharmacol. Suppl. 347:R130; 1993.
30. Rex, A.; Stephens, D. N.; Fink, H.: 'Anxiolytic' action of diazepam and abecarnil in a modified open-field test. Pharmacol. Biochem. Behav. 53:1005–1011; 1996.
31. Rodgers, R. J.; Cole, J. C.: The elevated plus-maze: Pharmacology, methodology and ethology. In: Cooper, S. J.; Hendrie, C. A., eds. Ethology and psychopharmacology, New York: John Wiley & Sons; 1994:9–44.
32. Semsroth, M.: Indirekte Kalorimetrie bei beatmeten Kindern. 3. Teil. Klinische Anwendung eines neuen Messverfahrens, Infusionsther. Klin. Ernahr. 13:44–62; 1986.
33. Shepard, J. K.; Grewal, S. S.; Fletcher, A.; Bill, D. J.; Dourish, C. T.: Behavioral and pharmacological characterization of the elevated "zero-maze" as an animal model of anxiety. Psychopharmacology (Berlin) 116:56–64; 1994.
34. Shopsin, B.; Klein, H.; Aaronson, M.; Collora, M.: Clozapine, clorpromazine and placebo in newly hospitalized, acutely schizophrenic patients. Arch. Gen. Psychiatry 36:657–664; 1979.
35. Straughan, J. L.; Conradie, E. A.: Buspirone—Frontrunner of a new genre of anxiolytics. S. Afr. Med. J. 74:441–444; 1988.
36. Turner, P.: Clinical psychopharmacology of beta-adrenoceptor antagonism in treatment of anxiety. Ann. Acad. Med. Singapore. 20:43–45; 1991.
37. Whitton, P.; Curzon, G.: Anxiogenic-like effect of infusing 1-(3-chlorophenyl) piperazine (mCPP) into the hippocampus. Psychopharmacology (Berlin) 100:138–140; 1990.
38. Zuardi, A. W.: 5-HT-related drugs and human experimental anxiety. Neurosci. Biobehav. Rev. 14:507–510; 1990.