Pharmacology Biochemistry & Behavior, Vol. 34, pp. 293–296. © Pergamon Press plc, 1989. Printed in the U.S.A. 0091-3057/89 $3.00 + .00

Isolation-Induced Social Behavioral Deficit Test: Effect of Tranquillizing Drugs

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Received 17 November 1988

FRANCES, H. AND C. LIENARD. *Isolation-induced social behavioral deficit test: Effect of tranquillizing drugs.* PHARMACOL BIOCHEM BEHAV **34**(2) 293–296, 1989.—Mice were reared in isolation for one week. Then, one isolated and one group-reared mouse were observed together under an inverted beaker for two minutes. The number of escape attempts of the isolated mouse were half of those of the grouped mouse. This is considered as a social behavioral deficit. The present study was undertaken to assess the effect of neuroleptics and various anxiolytic agents on this behavioral deficit. Neither acute administration of chlorpromazine, levomepromazine, sulpiride, flupentixol, pipotiazine, pimozide and haloperidol nor the subchronic (5 days) administration of flupentixol, pipotiazine and pimozide impaired the behavioral deficit. Diazepam and triazolam increased, chlordiazepoxide, hydroxyzine and buspirone did not modify the behavioral deficit. It is concluded that neuroleptics and anxiolytic agents did not impair the isolation-induced social behavioral deficit either because of inadequate doses or duration of administration or because this behavioral state is unresponsive to neuroleptics and anxiolytic agents.

Neuroleptics Anxiolytic agents Mice Social behavioral deficit

THE isolation-induced social behavioral deficit test has been previously described (7). Mice were isolated for one week, then an isolated mouse was observed together with a group-reared mouse under an inverted beaker. The two mice attempted to escape, however the escape attempts of the isolated mice were only half of those of the grouped mice. This reduction in the escape attempts of the isolated mice was named the isolation-induced social behavioral deficit. Tricyclic antidepressant drugs did not impair this social behavioral deficit (8). The agonists of the serotonergic (5-HT_{1B}) receptors: 5-methoxy-3(1,2,3,6-tetrahydro-4-pyridinyl)-1H-indole (RU 24969), 1-(3-trifluoromethylphenyl)piperazine (TFMPP) and 1-(3-chlorophenyl)piperazine (m-CPP) made the social behavioral deficit disappear (7). The present experiments were undertaken to assess the effect of neuroleptics and various anxiolytic agents on this behavioral deficit.

METHOD

Animals

Male Swiss NMRI mice (20–24 g) from CERJ, Genest St. Isle, 53940, France, were used in all experiments. Mice were either housed in groups of 10 in home cages of $30 \times 20 \times 10$ cm or isolated in home cages of $24 \times 10 \times 8$ cm for 7–9 days. Mice were four weeks old at the beginning of the isolation period. The room was thermostatically maintained at $21 \pm 1°C$ with a 12 hours light/dark schedule. Food and water were freely available.

Drugs

The drugs used were chlorpromazine hydrochloride, levomepromazine and pipotiazine (Specia: Paris, France), flupentixol dihydrochloride (Labaz: Paris, France), pimozide (Cassenne: Paris, France), sulpiride (Delagrange: Chilly-Mazarin, Paris), buspirone (Bristol Myers: Paris, France), diazepam and chlordiazepoxide (Roche: Neuilly-sur-Seine, France), hydroxyzine (U.C.B.: Nanterre, France), triazolam (Upjohn: Paris-la-Défense, France). Pimozide and haloperidol were dissolved in hot tartaric acid and then diluted with demineralized water. The other drugs were either dissolved in demineralized water or suspended in arabic gum; they were administered by intraperitoneal route (IP) in a volume of 0.25 ml/20 g body weight.

Experimental Procedure: Social Behavioral Deficit

Mice were tested in pairs (one isolated mouse + one grouped mouse) under a transparent beaker (height 14 cm; diameter: 10 cm) inverted on a rough surface glass plate. The number of escape attempts was counted for 2 minutes. An escape attempt was defined in any of the following ways: 1) The two forepaws were leaned against the beaker wall or 2) The mouse was sniffing, its nose into the spout of the beaker or 3) The mouse was scratching the glass floor. There was no minimal duration for one attempt. For a long lasting attempt, a new attempt was counted for each period of 3 seconds. All mice were used only once. Behavioral observations were taped by an observer blind to the treatments received by the mice. Drugs were administered only to the isolated mice. Control isolated mice received water. Drugs were administered 30 minutes before the test. In subchronic experiments drugs

were given once a day during 4 days and, in addition, 30 minutes before testing.

Spontaneous Motor Activity

Immediately after administration of drugs or water, the animals were placed in individual cages and, 30 minutes later, in photocell actimeter cages. Performances were measured 10, 20 and 30 minutes later (2).

Statistical Analysis of the Results

For the experiments described in Tables 1, 2 and 3, the scores of the isolated mice were compared to those of the grouped mice of the same experiment using the Student's *t*-test.

RESULTS

None of the neuroleptics studied increased the score of isolated mice up to the level of grouped mice (Table 1). On the contrary, the score of isolated mice was further reduced by the highest doses of neuroleptics; this reduction was probably linked to a sedative effect since chlorpromazine (4 mg/kg) and haloperidol (0.5 mg/kg) decreased motor activity to, respectively 50% and 40% of that of controls. After subchronic treatment, neither of the following: flupentixol, pipotiazine, pimozide made the difference between isolated and grouped mice disappear (Table 2).

In the dose-ranges studied, none of the following anxiolytic agents modified the isolation-induced social behavioral deficit: chlordiazepoxide, hydroxyzine, buspirone (Table 2). Diazepam and triazolam increased the behavioral deficit but the doses of 4 and 8 mg/kg of diazepam and 0.03 mg/kg triazolam reduced significantly the spontaneous motor activity suggesting that the increased deficit may result from sedation.

TABLE 1

EFFECT OF ACUTE ADMINISTRATION OF NEUROLEPTICS ON THE SOCIAL BEHAVIORAL DEFICIT

Drugs	mg/kg	n	Attempted Escapes Mean ± S.E.M. Grouped Mice	Isolated Mice	%
Chlorprom-azine	0	10	19.3 ± 2.4	8.5 ± 0.9‡	44
	0.25	10	22.8 ± 3.9	9.8 ± 1.6†	43
	1	10	22.4 ± 3.0	11.8 ± 2.7*	53
	4	9	33.8 ± 2.6	1.6 ± 0.4‡	5
Levomeprom-azine	0	10	19.1 ± 2.5	8.3 ± 0.9‡	43
	0.03	10	17.5 ± 3.6	6.4 ± 1.1†	37
	0.125	10	18.3 ± 3.1	7.5 ± 1.3†	41
	0.5	9	18.4 ± 3.3	2.7 ± 1.2‡	15
Sulpiride	0	10	21.5 ± 2.5	13.7 ± 1.8*	64
	0.25	10	23.9 ± 3.5	11.8 ± 1.8†	49
	1	10	23.9 ± 3.1	14.4 ± 2.3*	60
	4	10	29.2 ± 2.4	12.0 ± 1.0‡	41
	16	10	23.6 ± 2.8	7.6 ± 1.0‡	32
	64	9	22.8 ± 3.3	7.8 ± 2.7†	34
Flupentixol	0	10	21.2 ± 2.5	13.4 ± 2.8*	63
	0.003	10	20.3 ± 2.7	7.9 ± 0.9‡	39
	0.015	11	19.3 ± 1.3	12.1 ± 1.9‡	63
	0.06	20	17.5 ± 1.5	10.6 ± 1.1‡	61
	0.125	10	23.0 ± 2.2	12.4 ± 2.4†	54
	0.25	10	22.6 ± 3.0	10.5 ± 2.4†	46
	0.5	9	24.2 ± 2.7	12.9 ± 3.0*	53
Pipotiazine	0	20	20.4 ± 1.7	11.0 ± 1.6‡	54
	0.003	10	23.7 ± 3.3	10.6 ± 1.7†	45
	0.015	10	23.7 ± 3.2	9.9 ± 1.8†	42
	0.06	10	22.3 ± 2.8	8.2 ± 1.5‡	37
	0.25	10	18.0 ± 2.4	7.7 ± 1.3†	43
	1	10	19.3 ± 2.1	5.8 ± 1.6‡	30
	4	10	17.3 ± 2.5	2.2 ± 0.9‡	13
Pimozide	0	10	21.3 ± 2.4	8.3 ± 0.9‡	39
	0.007	10	19.5 ± 2.7	13.1 ± 2.5*	67
	0.03	10	16.0 ± 2.1	9.3 ± 1.7*	58
	0.125	9	21.8 ± 2.9	11.9 ± 2.9*	55
	0.5	10	20.9 ± 3.0	13.1 ± 2.2*	63
Haloperidol	0	10	29.6 ± 3.4	16.6 ± 2.5†	56
	0.25	10	22.2 ± 2.5	7.6 ± 1.9‡	34
	0.5	10	20.7 ± 3.5	2.7 ± 1.0‡	13
	1	10	25.4 ± 3.1	5.8 ± 1.3‡	23

*$p<0.05$; †$p<0.01$; ‡$p<0.001$.

Means ± S.E.M. of attempted escapes. Isolation duration was 7–9 days. Levels of significance were determined by *t*-tests compared to the grouped mice in the same experiment. % is percentage of attempted escapes of isolated mice in regard to grouped mice in the same experiments. n = number of pairs of mice.

DISCUSSION

The chosen neuroleptics belong to several classes; derivatives of phenotiazine: chlorpromazine, levomepromazine, pipotiazine, of benzamide: sulpiride; of butyrophenones: haloperidol and pimozide and of thioxanthene: flupentixol. The doses were chosen merely under the sedative doses. However, the very small number of escape attempts observed with the highest doses may be the reflect of a sedative effect.

The chosen anxiolytic agents were various: three benzodiazepines: diazepam, triazolam, chlordiazepoxide, a pyrimidinylpiperazine derivative: buspirone with an anxiolytic action in the clinic (12) and hydroxyzine of which antianxiety action is only one of the properties. None of these anxiolytic agents reduced the social behavioral deficit. The increased deficit observed with the higher doses of diazepam and triazolam may result from a sedative effect since spontaneous motor activity was reduced to 61 and 60% of that of controls with, respectively, 4 mg/kg diazepam and 0.03 mg/kg triazolam.

The significance of the social behavioral deficit test is not firmly established. The reduced number of escape attempts of the isolated mice when paired with grouped mice is a social phenomenon since the number of escape attempts of isolated mice was not smaller than that of grouped mice when the animals were individually tested (7). This behavioral deficit or inhibition seems to be the consequence of an hyperreactivity to the stimulus "other mouse" (9). This explanation is in accordance with observations by several groups of an hyperreactivity induced by isolation (5, 10, 11).

It is surprising that the neuroleptics and anxiolytic agents did not act. In fact, if the deficit was the result of an anxiety founded upon fear or mistrust towards the unknown grouped mouse, then the anxiolytic agents and the light anxiolytic activity of neuroleptics (15) would have probably removed the deficit.

The reduced escape attempts of isolated mice may be seen as a behavioral inhibition. The paradoxical stimulating effect of low doses of pimozide, pipotiazine and sulpiride in rats (4), corresponding to the energizing properties of the same compounds used at low dosage in man (3, 13, 16) was expected to alleviate the behavioral deficit. However, these treatments given either acutely

TABLE 2

EFFECT OF SUBCHRONIC ADMINISTRATION OF NEUROLEPTICS ON THE SOCIAL BEHAVIORAL DEFICIT

Drugs	mg/kg	n	Attempted Escapes Mean ± S.E.M. Grouped Mice	Isolated Mice	%
Flupentixol	0	9	22.7 ± 3.3	9.4 ± 1.4†	41
	0.015	10	24.4 ± 2.0	11.6 ± 2.2‡	48
	0.03	10	26.0 ± 3.2	10.5 ± 1.0‡	40
	0.06	10	23.0 ± 2.8	6.3 ± 1.4‡	27
Pipotiazine	0	19	23.1 ± 3.0	12.6 ± 2.8*	55
	0.015	10	23.0 ± 2.7	10.1 ± 1.9†	44
	0.06	10	22.5 ± 1.6	12.0 ± 2.4†	53
	0.25	19	25.1 ± 2.8	11.4 ± 1.8‡	45
	0.5	9	21.2 ± 2.6	8.3 ± 2.1‡	39
	1	10	23.9 ± 3.1	13.6 ± 2.6*	57
Pimozide	0	9	23.0 ± 2.1	15.4 ± 2.6*	67
	0.03	10	21.5 ± 3.0	11.3 ± 1.5†	53
	0.06	10	24.5 ± 2.0	12.8 ± 2.3†	52

*$p<0.05$; †$p<0.01$; ‡$p<0.001$.

Results were expressed as indicated in Table 1. Drugs were given at the doses reported in the table once a day during 4 days and, in addition, 30 minutes before testing.

TABLE 3

EFFECT OF ACUTE ADMINISTRATION OF VARIOUS TRANQUILLIZING DRUGS ON THE SOCIAL BEHAVIORAL DEFICIT

Drugs	mg/kg	n	Attempted Escapes Mean ± S.E.M. Grouped Mice	Isolated Mice	%
Diazepam	0	10	21.5 ± 2.0	10.2 ± 1.8†	47
	2	9	25.0 ± 2.4	14.2 ± 1.9‡	57
	4	10	30.3 ± 2.7	12.4 ± 2.7‡	41
	8	9	26.0 ± 2.0	6.2 ± 1.1‡	24
Triazolam	0	10	18.2 ± 2.5	10.7 ± 1.8*	59
	0.002	10	20.4 ± 3.5	9.2 ± 1.0*	45
	0.008	10	18.0 ± 2.4	10.4 ± 1.9*	58
	0.03	10	18.4 ± 2.4	2.9 ± 0.9‡	16
Chlordiaze-poxide	0	20	20.0 ± 2.7	9.7 ± 1.9†	49
	1	20	20.3 ± 2.8	11.5 ± 2.1†	57
	4	20	19.6 ± 1.6	13.1 ± 2.8†	67
	16	20	20.9 ± 3.1	12.0 ± 3.1*	57
Hydroxyzine	0	10	24.9 ± 2.5	13.7 ± 2.8*	55
	0.5	10	25.0 ± 2.5	11.1 ± 1.6‡	44
	1	10	24.1 ± 2.7	8.1 ± 2.4‡	34
	2	10	22.9 ± 2.3	12.1 ± 2.8†	53
	4	9	23.1 ± 3.4	8.7 ± 1.5†	38
Buspirone	0	9	24.6 ± 2.4	13.3 ± 2.4†	54
	0.25	10	21.6 ± 2.7	8.8 ± 2.7†	41
	1	19	22.4 ± 1.9	12.2 ± 1.9‡	54
	2	20	24.7 ± 2.2	10.9 ± 1.5‡	44
	4	20	24.1 ± 2.6	12.8 ± 1.4‡	53
	8	10	24.5 ± 3.0	8.2 ± 2.5‡	33

*$p<0.05$; †$p<0.01$; ‡$p<0.001$.

Results were expressed as indicated in Table 1.

or subchronically did not reduce the deficit.

In spite of these negative findings, it is impossible to assert that the isolation-induced social behavioral deficit was not the consequence from an anxious state because the lack of effect of neuroleptics and tranquillizing drugs in this test may also result from inadequate doses or duration of treatments. However, at least for some drugs (diazepam, chlordiazepoxide), the doses were in the dose-range active in mice in other tests. Diazepam (1 mg/kg) and chlordiazepoxide (8 mg/kg) were active on a test of conditioned inhibition: the four-plates test (1). Chlordiazepoxide was active at the dose of 10 mg/kg in mice in the elevated plus-maze (14); diazepam (1 mg/kg) and chlordiazepoxide (20 mg/kg) were active in the social interaction test (6).

Alternatively, the possibility remains that this social deficit represents a behavioral state different from those which are sensitive to neuroleptics and tranquillizing agents.

These negative results may lead to a positive finding in that they state the limit of the specificity of this test. The social behavioral deficit is unmodified by either tricyclic antidepressants (8), or major and minor tranquillizers (this present study). Until now, the only drugs which made the difference between isolated and grouped mice disappear were clomipramine (one dose), indalpine and the agonists of 5-HT_{1B} receptors: m-CPP, TFMPP and RU 24969: drugs which are all related to serotonin (7). So, the specificity of this test appears high. The next step will be to study if this model may correspond to a known human psychiatric trouble.

ACKNOWLEDGEMENT

This research was supported by the Institut National de la Santé et de la Recherche Médicale.

REFERENCES

1. Aron-Samuel, C. D'un test pharmacologique utilisable pour la sélection de tranquillisants. Thèse pour le Doctorat en Médecine, Paris, Copedith, 1970.
2. Boissier, J. R.; Simon, P. Action de la cafeine sur la motilité spontanée de la souris. Arch. Int. Pharmacodyn. 158:212–221; 1965.
3. Breier, A.; Wolkowitz, O. M.; Doran, A. R.; Roy, A.; Boronow, J.; Hommer, D. W.; Pickar, D. Neuroleptic responsivity of negative and positive symptoms in schizophrenia. Am. J. Psychiatry 144:1549–1555; 1987.
4. Carnoy, P. Déficits motivationnels et cognitifs induits par de faibles doses d'apomorphine chez le rat: sensibilité aux neuroleptiques "désinhibiteurs". Thèse de Doctorat de l'Université René Descartes de Paris, 1987.
5. Einon, D.; Morgan, M. J. Habituation under different levels of stimulation in socially reared and isolated rats: a test of the arousal hypothesis. Behav. Biol. 22:553–558; 1978.
6. File, S. E. How good is social interaction as a test of anxiety? In: Simon, P.; Soubrié, P.; Widlocher, D., eds. Animal models of psychiatric disorders. vol. 1. Selected models of anxiety, depression and psychosis. Paris: Karger; 1988:151–166.
7. Francès, H. New animal model of social behavioral deficit: Reversal by drugs. Pharmacol. Biochem. Behav. 29:467–470; 1988.
8. Francès, H.; Danti, S.; Fermanian, J. Effect of tricyclic antidepressant drugs in the isolation-induced social behavioural deficit test. Prog. Neuropsychopharmacol. Biol. Psychiatry, in press; 1989.
9. Francès, H.; Lièнard, C.; Fermanian, J.; Lecrubier, Y. Isolation-

induced social behavioral deficit: A proposed model of hyperreactivity with a behavioral inhibition. Pharmacol. Biochem. Behav. 32:637–642; 1989.
10. Gentsch, C.; Lichtsteiner, M.; Feer, H. Behavioural comparisons between individually- and group-housed male rats: effects of novel environments and diurnal rhythm. Behav. Brain Res. 6:93–100; 1982.
11. Gentsch, C.; Lichtsteiner, M.; Feer, H. Individually housed rats exceed group-housed animals in rotational movements when exposed to a novel environment. Experientia 39:1412–1414; 1983.
12. Goldberg, H. L.; Finnerty, R. J. The comparative efficacy of buspirone and diazepam in the treatment of anxiety. Am. J. Psychiatry 136:1184–1187; 1979.
13. Lecrubier, Y. Multiple pharmacological mechanisms and clinical targets for neuroleptics: should a more operational classification be considered? Psychiatry Psychobiol. 4:232–238; 1987.
14. Lister, R. G. The use of a plus-maze to measure anxiety in the mouse. Psychopharmacology (Berlin) 92:180–185; 1987.
15. Rickels, K.; Schweizer, E. E. Current pharmaco-therapy of anxiety and panic. In: Meltzer, H. Y., ed. Psychopharmacology: The third generation of progress. New York: Raven Press; 1987:1193–1203.
16. Van Kammen, D. P.; Hommer, D. W.; Malas, K. L. Effect of pimozide on positive and negative symptoms in schizophrenic patients: Are negative symptoms state dependent? Biol. Psychiatry 18:113–117; 1987.