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New Animal Model of Social Behavioral Deficit: Reversal by Drugs

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FRANCÈS, H *New animal model of social behavioral deficit Reversal by drugs* PHARMACOL BIOCHEM BEHAV **29**(3) 467–470, 1988 —A new model of social behavioral deficit and its reversal by drugs is described Mice isolated for one week or more (isolated mice) behave differently from controls (grouped mice) When observed together under an inverted beaker, the isolated mice make significantly less escape attempts than the grouped mice This behavioral deficit is a social one because it exists only when the isolated and grouped mice are tested together but not when they are tested singly Several drugs impair this social behavioral deficit, particularly the 5-HT1B receptors agonists

Isolation Social behavior 5-HT1B agonists

IN man, isolation may be reached through diverse situations (geography, bereavements, divorce, prison) Results from epidemiological studies suggest that a relationship may exist between isolation and suicidal attempts Thus, the behavioral consequences of isolation appear very important

In rodents, prolonged isolation has been extensively studied A review by Valzelli [16] indicates that the most reproducible consequence of isolation is aggressiveness However, prolonged isolation produces not only aggressiveness but a complex syndrome of behavioral changes including an increase in general reactivity to environmental stimuli, deviation and/or decrease of sexual activity [2,7], impairment of exploratory activity [14,15] and also impairment of learning capacity [4,16]

Although behavioral studies of socioenvironmental deprivation are numerous, they are concerned with animals observed alone or with other "isolated" animals But studies of the social behavior of mice previously deprived of a social environment are lacking That is why in these experiments, the behaviour of previously isolated mice is studied and compared in two situations isolated mice are tested either alone or in the presence of a "normal" mouse

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 30×20×10 cm or isolated in home cages of 24×10×8 cm Mice were 4 weeks old at the beginning of isolation The room was thermostatically maintained at 21±1°C with a 12 hour light/dark schedule Food and water were freely available

Experimental Procedure

Mice were tested either individually or in pairs 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 the following way (1) the two forepaws were leaned against the beaker wall, (2) the mouse was sniffing the beaker, (3) the mouse was scratching the glass floor There was no minimal duration for one attempt When an attempt lasted a long time, a new attempt was counted for each period of 3 seconds However, the escape attempts were very rapid movements and the longest duration observed lasted between 3 and 6 seconds (counted as 2 attempts) All mice were used only once except when studying the effect of repetition (Results paragraph 3, Fig 3) Behavioural observations were taped by an observer blind to the treatments received by the mice. However, it was impossible to be blind about which were the grouped and which were the isolated mice since they looked different The hairs of the isolated mice were agglomerated in small separated clusters as if neglected On the contrary, the hairs of the grouped mice were smooth and glossy

Drugs

Drugs used were clomipramine chlorhydrate, imipramine chlorhydrate (Ciba-Geigy Rueil-Malmaison, France), indalpine (Pharmuka Gennevilliers, France), fenfluramine chlorhydrate (Biopharma Neuilly/Seine, France), clenbuterol (Boehringer-Ingelheim, Reims, France), 5,methoxy-N-N-dimethyltryptamine (5 MeODMT—Sigma La Verpillière, France), 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT—Research Biochemical Inc , Wayland, USA), 5-methoxy-3 (1,2,3,6-tetrahydropyridin-4-yl) 1-H indole (Ru

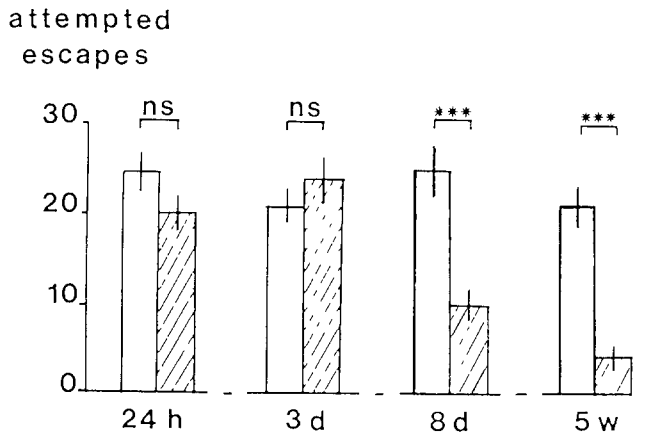

FIG 1 Attempted escapes (mean±S E M) of the grouped (clear bars) and isolated (striped bars) mice tested in pairs in relation to the duration of isolation 24h (24 hours), 3d, 8d (3 or 8 days) and 5w (5 weeks) Groups (n=10) differed significantly, $F(7,72)=15\ 8$, $p<0\ 001$ Levels of significance between pairs were determined by t-tests $^{***}p<0\ 001$ There were no significant differences among the scores of the grouped mice For the isolated mice, the mean score was significantly different from the 24h group at 8d ($p<0\ 001$) and at 5w ($p<0\ 001$) but not at 3d

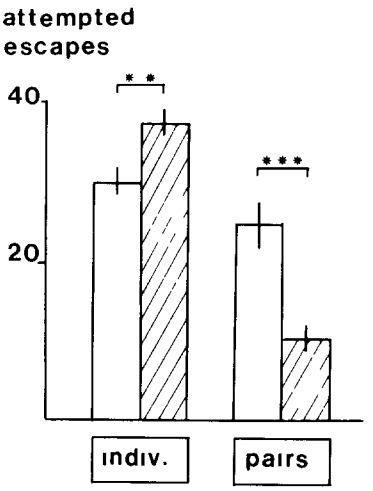

FIG 2 Attempted escapes (mean±S E M) of the grouped (clear bars) and isolated (striped bars) mice tested either individually (indiv) or in pairs (pairs) Duration of isolation was 8 days Groups (n=20) differed significantly, $F(3,76)=37\ 37$, $p<0\ 001$ Levels of significance were determined by t-tests $^{**}p<0\ 01$, $^{***}p<0\ 001$ The scores of the grouped mice tested in pairs were significantly ($p<0\ 05$) lower than those of the grouped mice tested individually The scores of the isolated mice tested in pairs were highly significantly ($p<0\ 001$) lower than those of the isolated mice tested individually

24 969)—Roussel-Uclaf, Paris-La Défense, France), m-chlorophenylpiperazine (m-cpp—Aldrich Chemical Co Strasbourg, France), 1-(3-trifluoromethylphenyl) piperazine (TFMPP—Aldrich Chemical Co Strasbourg, France), diazepam (Roche Neuilly/Seine, France) Drugs were dissolved in water or suspended in arabic gum and administered by intraperitoneal route except 8-OH-DPAT (subcutaneous route) in a volume of 0 25 ml/20 g body weight

Statistical Analysis of the Results

For the experiments described in Table 1, the scores of the isolated mice were compared to those of the grouped mice of the same experiment using the Student's t-test For the experiments described in Figs 1, 2 and 3 a one way analysis of variance followed by a Student's t-test was used

RESULTS

After one week or more of isolation, isolated and grouped mice behaved differently when observed in pairs under the beaker The grouped mice made numerous escape attempts The isolated mice made fewer escape attempts or some attempts which were not counted as they were very weak with only one forepaw on the beaker wall, the back of the mouse staying horizontal The isolated mouse was very often inactive in the middle of the beaker or sniffed at the grouped mouse

(1) Effect of the duration of isolation (Fig 1) After one or three days, there was no difference in the behavior of isolated and grouped mice observed together The difference was significant after an 8 days isolation and also after five weeks However, after five weeks, isolated mice were strongly aggressive

(2) Effect of the "social component" (Fig 2) Mice isolated for 8 days were tested alone under the beakers and compared to grouped mice tested alone In these conditions, the isolated mice made more escape attempts than the grouped mice

(3) Effect of repetition (Fig. 3) The 10 same pairs of mice (one isolated + one grouped mouse) were tested 5 times after 8 days of isolation The first 4 tests were performed with one hour intervals between them and the fifth one on the following day The mean score of all mice decreased. The decrease was weaker (and not significant) for isolated than for grouped mice The level of escape attempts remained steady from the 2nd test for isolated mice, and from the 4th test for grouped mice There was an habituation, however, this habituation did not eliminate the difference in behavior between the two groups of mice

(4) Effect of drugs on the isolation-induced social behavioral deficit (Table 1) In this set of experiments, the duration of isolation was 7–9 days All mice were used only once Drugs were administered 30 minutes before the test to the isolated mice only The grouped mice received demineralized water Imipramine, a classical tricyclic antidepressant, was inactive, failing to reverse the social behavioral deficit. On the contrary, at the dose of 32 mg/kg it decreased the attempted escapes of the isolated mice Diazepam, inactive at the doses of 2 and 4 mg/kg, increased the behavioral deficit at the dose of 8 mg/kg. Clenbuterol (agonist at the beta 2-adrenergic receptors and potential antidepressant), fenfluramine, and 5-MeODMT (stimulants of the serotonergic system) were inactive at the doses studied Indalpine 2 mg/kg (but not 0 5 mg/kg) and clomipramine (2 mg/kg) reduced the differences between the grouped and the isolated mice 8-OH-DPAT, agonist at the 5-HT1A receptors, accentuated at the doses of 0 5 and 2 mg/kg (but not at 0.1 mg/kg) the behavioral deficit MCPP (2 mg/kg) and Ru 24 969 (3 mg/kg) reversed the behavioral deficit of the isolated mice TFMPP reduced the deficit in escape attempts of isolated mice at the dose of 2 mg/kg but not at 0 125 or 0 5 mg/kg

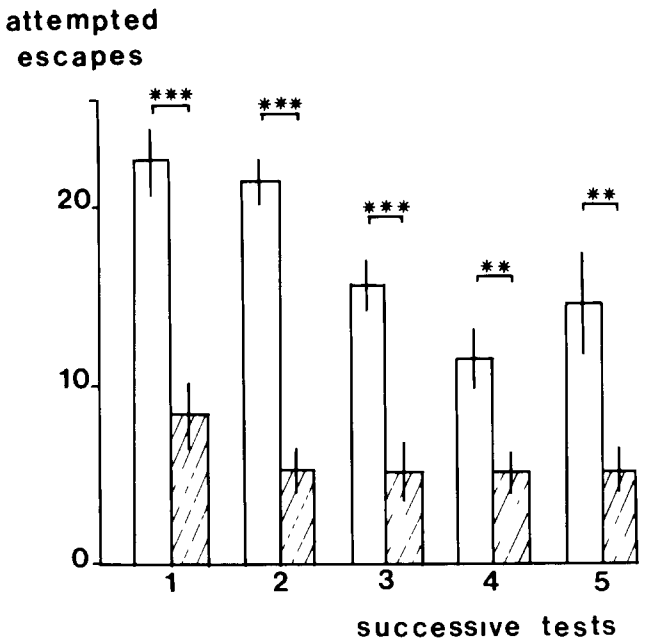

FIG 3 Attempted escapes (mean±S E M) of the same pairs of grouped (clear bars) and isolated (striped bars) mice successively tested Tests 1, 2, 3 and 4 were performed with a one hour interval between them and the 5th on the following day Duration of isolation was 8–9 days Groups (n=10) differed significantly, $F(9,90)=17\ 98$, $p<0\ 001$ Levels of significance were determined by t-tests $^{**}p<0\ 01$, $^{***}p<0\ 001$ For the isolated mice there was no significant difference between any of the successive tests For the grouped mice, the mean score was significantly different from the first test on the 3rd ($p<0\ 01$), the 4th ($p<0\ 001$) and the 5th ($p<0\ 05$) tests but not on the 2nd test

TABLE 1

MODIFICATION BY DRUGS OF THE SOCIAL BEHAVIORAL DEFICIT INDUCED, IN MICE, BY ISOLATION

		Attempted Escapes Mean ± S E M		
Drugs	mg/kg	Grouped Mice	Isolated Mice	%
Water	—	28 0 ± 3 7	13 4 ± 2 1†	48
Imipramine	16	26 4 ± 3 1	10 2 ± 2 1‡	39
	32	29 8 ± 2 7	3 0 ± 1 6‡	10
Clomipramine	2	26 5 ± 3 0	19 0 ± 2 9 NS	71
	8	27 3 ± 3 0	16 8 ± 4 1*	61
	32	32 9 ± 2 1	0 88 ± 0 4‡	2 6
Clenbuterol	0 25	19 9 ± 1 9	7 7 ± 1 5‡	38
Diazepam	2	26 7 ± 2 4	14 2 ± 1 9‡	53
	4	30 3 ± 2 7	12 4 ± 2 7‡	41
	8	29 5 ± 2 0	5 2 ± 1 1‡	17
Fenfluramine	8	27 3 ± 3 9	12 7 ± 3 0†	46
5-MeODMT	4	19 8 ± 1 8	4 8 ± 0 8‡	24
Indalpine	0 5	25 0 ± 2 8	9 3 ± 2 3‡	37
	2	30 3 ± 2 5	22 4 ± 3 6 NS	74
MCPP	0 1	23 0 ± 2 3	13 0 ± 2 2†	56
	1	25 8 ± 3 0	14 5 ± 2 9†	56
	2	28 7 ± 2 4	24 9 ± 3 1 NS	86
8-OH-DPAT	0 1	22 9 ± 2 3	9 9 ± 2 6‡	43
	0 5	31 3 ± 3 2	1 8 ± 0 6†	5 7
	2	24 0 ± 2 8	1 2 ± 0 9‡	5
Ru 24 969	3	24 3 ± 3 3	21 5 ± 4 0 NS	88
TFMPP	0 125	26 0 ± 3 1	11 4 ± 2 6†	44
	0 5	24 9 ± 1 5	14 7 ± 2 8†	59
	2	30 6 ± 2 7	25 4 ± 2 3 NS	83

Means ± S E M of attempted escapes Isolation duration was 7–9 days Levels of significance were determined by t-tests $^{*}p<0\ 05$, $^{\dagger}p<0\ 01$, $^{\ddagger}p<0\ 001$ compared to the grouped mice in the same experiment % Percentage of attempted escapes of isolated mice in regard to grouped mice in the same experiment Ten or 20 mice in each group

DISCUSSION

These experiments described a behaviour which has not previously been reported Adult mice which had been isolated for at least one week behaved differently than mice housed together under standard conditions The behavioral differences were opposite depending on the experimental protocol Isolated mice, individually placed under an inverted beaker, attempted to escape from the beaker more often than grouped mice On the contrary, when an isolated mouse and a grouped mouse were placed together under an inverted beaker, the isolated mouse attempted to escape less often than the grouped mouse

The higher mean score of the isolated mice than that of the grouped mice when tested individually may have been a consequence of increased reactivity to the environment, already described [17] The reduced number of escape attempts of the isolated mice when tested together with grouped mice was a highly reliable effect, persisting even after repeated testing of the same pair The reduction in scores of either group of mice with repeated testing may have been due to habituation or learned helplessness, a kind of behavioral despair closely related to that described by Porsolt [11] Of course, these proposals are only speculative

A duration of isolation of 7–9 days was chosen to study the effect of drugs. A shorter duration (1 or 3 days) was not enough to induce this behaviour and a longer duration (5 weeks) produced, in addition, an aggressiveness undesirable in regard to the parameters under study

The reduced number of escape attempts of isolated mice when tested together with a grouped mouse was probably not the result of either motor or cognitive impairment since, tested individually, the isolated mice were not less active than the grouped mice The effect may have been the consequence of more responding to the conspecific than to the new inescapable situation

Whatever the significance of this behavior, its reproductibility permits the study of its reversal by drugs

Among the drugs studied, the classical antidepressant, imipramine and the potential one, clenbuterol, were inactive after a single administration Chronic treatments have not been tested, therefore a relationship between this deficit and a "depressive state" cannot be excluded. The minor tranquilizer, diazepam, did not impair the behavioral deficit, which was probably not the consequence of an anxious state The higher doses of diazepam increased the deficit and this

may have reflected the sedative properties of this drug Fenfluramine releases serotonin from nerve endings, inhibits its reuptake and also affects dopamine metabolism [3,9] but was inactive in this test 5-MeODMT, a centrally acting 5-HT receptor agonist known to bind to 5-HT1A receptors [13] merely accentuated the social behavioral deficit In the same way, 8-OH-DPAT, an agonist specific for 5-HT1A receptors [5], accentuated the social behavioral deficit Clomipramine and indalpine, potent serotonin uptake inhibitors [8] with antidepressant properties, reduced the deficit at 2 mg/kg but not at the other doses tested In addition to these antidepressants, the three drugs which clearly antagonize this behavior are agonists at the 5-HT1B receptors Ru 24 969 [6], TFMPP [1, 10, 12] and MCPP [12]

In conclusion, the new test presented in this study implies a social behavioral component which is modified by isolation This test needs further experimentation and analysis for its significance to be adequately or fully explained Drugs acting on the serotonergic system, and more precisely the 5-HT1B receptors, reverse this behavioral deficit Additional analysis of this behavior and of its pharmacology are in progress in our laboratory

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