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Isolation-Induced Social Behavioral Deficit: A Proposed Model of Hyperreactivity With a Behavioral Inhibition

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FRANCES, H., C. LIENARD, J. FERMANIAN AND Y. LECRUBIER. *Isolation-induced social behavioral deficit: A proposed model of hyperreactivity with a behavioral inhibition.* PHARMACOL BIOCHEM BEHAV **32**(3) 637–642, 1989.—The behavior of mice isolated for 7–9 days (isolated mice) was compared to that of mice reared in groups (grouped mice). The method consisted of counting the number of escape attempts of the mice placed under an inverted beaker. When individually observed the isolated mice attempted to escape slightly but significantly more often than the grouped mice. When a pair of mice (one isolated + one grouped) were tested together, the number of escape attempts of the isolated mice was half of that of the grouped mice: this phenomenon was named the isolation-induced social behavioral deficit. These opposed behaviors may mean the same thing: an hyperreactivity to the novelty. In a variety of new situations under the beaker (presence of a lifeless object, of a grouped mouse or of an isolated mouse), the isolated mice were more reactive than the grouped mice. In conclusion, the social behavioral deficit test may be seen as a model of hyperreactivity with a behavioral inhibition.

Isolation Social behavior Inhibition Hyperreactivity

ISOLATION induces in rodents a complex syndrome of behavioral changes including an increase in general reactivity to environmental stimuli and aggressiveness (15,17).

The social behavioral deficit of mice previously deprived of a social environment has been recently described (8). When a mouse isolated for a short period of time (7–9 days) was introduced together with a group-housed mouse under an inverted beaker, a quantitative difference in their behavior was observed. The escape attempts of the isolated mice were only half those of the grouped mice. This difference did not result from motor or cognitive impairments since in a previous work we demonstrated that the escape attempts of the isolated mice tested alone under the beaker were slightly but significantly higher (41.1 ± 1.6) than those of the grouped mice tested alone (35.0 ± 2.0; $p<0.05$).

The present experiments were carried out to further understand the significance of this behavior.

HABITUATION

Previously we showed that after repetitive testing (each hour) of the same pairs of mice (one isolated mouse + one grouped mouse), a significant habituation occured only for grouped mice. This raised the following question: either the isolated mice were unable to habituate or the score of the isolated mice tested in pairs was too small to be further reduced. To answer this question, we tested isolated and grouped mice individually and repeatedly.

DURATION OF THE TEST

The duration of the test was two minutes: this choice was justified in the course of a lengthened experiment demonstrating that the difference in the behavior of grouped and isolated mice was observed during the first two minutes of testing and not thereafter. The importance of the first minutes of test raised the question of the novelty of the situation. This novelty consisted of two events: a new place and an unknown partner. These two events will be studied separately. The influence of the novelty of the experimental place on the social behavioral deficit has been studied by comparing pairs of mice placed for the first time in the experimental place with pairs of mice having a previous exposure to the test environment either of the isolated mice only or of both isolated and grouped mice. The influence of stimuli under the beaker has been compared in grouped and isolated mice. The stimuli were an inanimate object, a similar mouse (grouped for the grouped, isolated for the isolated), a dissimilar mouse (isolated for the grouped, grouped for the isolated) or there was no stimulus at all. Then the influence of a previous social contact has been studied by comparing the social behavioral deficit in pairs of mice

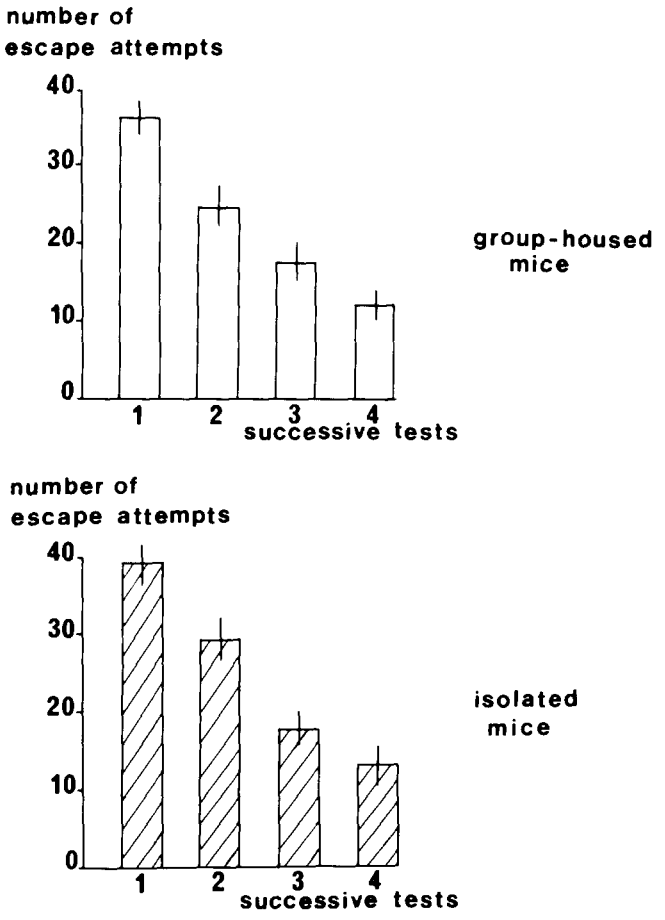

FIG. 1. Effect of repetition on the mean (±S.E.M.) number of escape attempts of 10 grouped (open bars) and 10 isolated (hatched bars) mice in the course of four successive tests (duration = 2 minutes) separated by one hour intervals. Mice were individually tested.

without previous social contact for the isolated mice with pairs of mice habituated to each other and with pairs of mice habituated with randomly chosen mice.

It was previously shown (17) that isolation of male mice induced an aggressiveness which increased with the duration of isolation rearing. In our situation test, the percentage of pairs of mice in which struggles occurred was about 20–40%.

Obviously, when the mice were fighting, they could not attempt to escape and this phenomenon constitutes a potential error for our measurement. So the influence of struggles on the measurement of the number of escape attempts has been investigated by comparing the intensity of the social deficit in pairs of mice with and without struggles. Then, a correlation has been searched between escape attempts after one week of isolation and aggressiveness after three weeks of isolation in the same isolated mice.

METHOD

Animals

Male Swiss NMRI mice (20–24 g at the beginning of the experiment), from CERJ, Genest St. Isle 53940 (France), were either housed in groups of 10 in home cages of 30 × 20 × 10 cm or

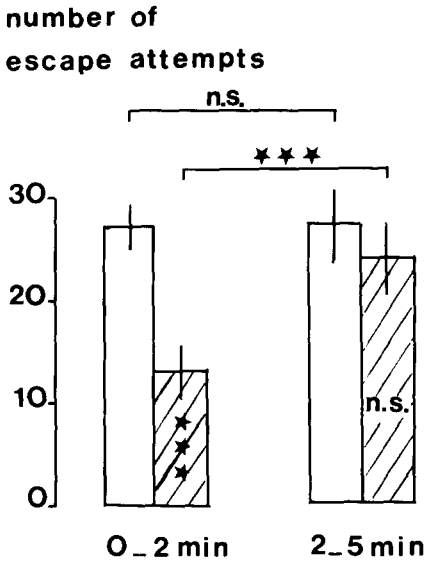

FIG. 2. Effect of the duration of the test on the number of escape attempts of the grouped (open bars) and the isolated (hatched bars) mice tested in pairs (n = 9 pairs of mice). $F(3,32) = 5.10$, $p < 0.01$ (Student's *t*-test: $^{***}p < 0.001$).

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 hours light/dark schedule. Food and water were freely available.

Experimental Procedure

General conditions. Mice were tested either individually or in pairs (details are given in the Particular Protocols section) under a transparent beaker (1 liter, height: 14 cm; diameter: 10 cm) inverted on a rough surface glass plate. The number of escape attempts was counted for 2 minutes (except if otherwise stated). An escape attempt was defined as any one of the following: 1) the two forepaws were leaned against the beaker wall, 2) the mouse was sniffing, its nose into the spout of 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).

Particular Protocols

Experiment 1—Habituation. Ten group-housed and 10 isolated mice were observed alone during the first two minutes following introduction under the beaker in 4 successive tests separated from each other by one-hour intervals.

Experiment 2—Duration of the test. Nine pairs of mice (one grouped + one isolated) were observed during five minutes. The scores obtained during minutes 1 and 2 were summed and scores obtained during minutes 3, 4 and 5 were summed.

Experiment 3—Novelty of the experimental place. Two protocols were used. In the first, 20 isolated mice were individually observed during 2 minutes under the beaker, then 1 or 48 hours later the test was performed with pairs of mice: an isolated informed mouse and a group-housed mouse for which the exper-

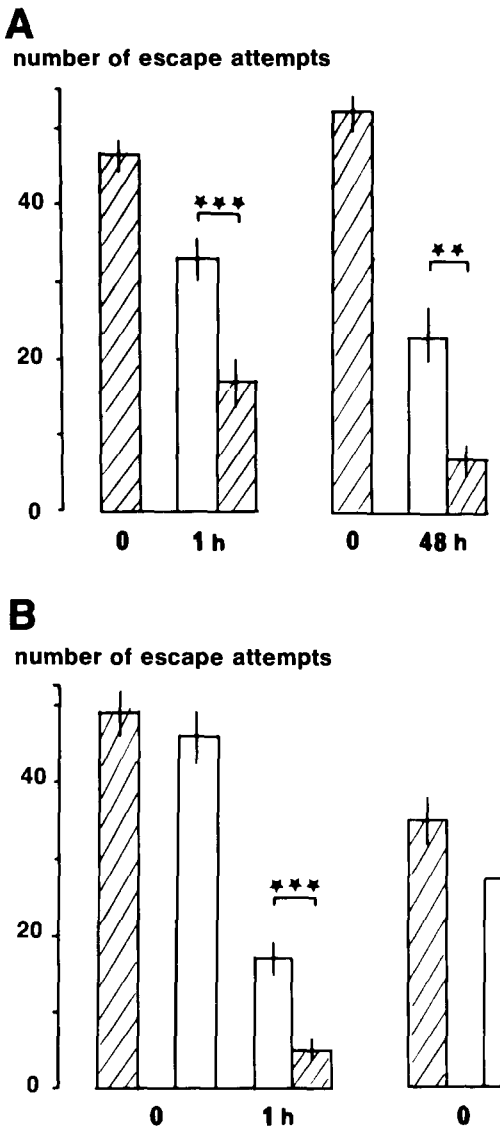

FIG. 3. Effect of a previous exposure to the experimental place on the number of escape attempts of the (hatched bars) isolated and (open bars) grouped mice. Upper panel (A): only isolated mice have been individually tested one hour (left) or 48 hours (right) before being tested in pairs. Lower panel (B): both isolated and grouped mice have been individually tested one hour (left) or 48 hours (right) before being tested in pairs (n = 10 pairs of mice in each group). Student's *t*-test: $^{**}p<0.01$; $^{***}p<0.001$.

imental place was novel. In the second protocol, 20 isolated and 20 grouped mice were individually observed during 2 minutes under the beaker, then 1 or 48 hours later the test was performed with pairs of informed isolated and grouped mice.

Experiment 4—Influence of stimuli under the beaker. In this set of experiments, several different situations were compared. 1) The mouse was alone under the beaker; 2) The mouse was alone under the beaker which contained a pill-box (4 cm high; 3 cm diameter); 3) The mouse was paired with a similar mouse (grouped for the grouped and isolated for the isolated; 4) The mouse was paired with a dissimilar mouse (grouped for the isolated and isolated for the grouped). The same groups were constituted for grouped mice. The performances of isolated mice and of grouped mice were compared in each of these situations.

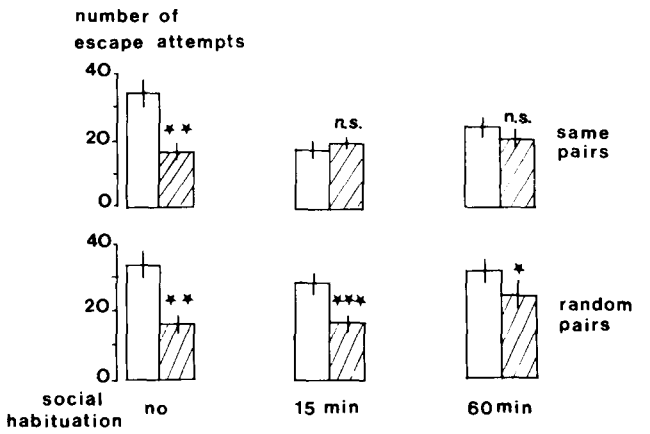

FIG. 4. Effect of previous social experience on the isolation-induced social behavioral deficit. The pairs of mice [grouped (open bars), isolated (hatched bars)] were tested without social habituation (left), with an habituation of 15 minutes (middle) or 60 minutes (right). The same pairs were used for social habituation and test (upper panel). Randomly chosen pairs were tested in the lower panel. Tests were performed 5 hours after the end of social habituation (n = 10 pairs of mice in each group). Student's *t*-test: $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$.

Experiment 5—Previous social experience. These experiments were performed to test the influence of a preliminary social contact. Two protocols were used. In the first, 10 pairs of mice (isolated + grouped) were placed together for 15 or 60 minutes in a home-cage with litter, drink and food and then pairs consisting

TABLE 1

ESCAPE ATTEMPTS OF ISOLATED MICE IN VARIOUS SITUATIONS

	Isolated Alone	Isolated + Pill-box	Isolated + Isolated	Isolated + Grouped
n	65	19	62	24
mean ± S.E.M.	41.1 ± 1.6 A	24.7 ± 1.5 B	14.9 ± 1.5 C	11.9 ± 1.3 D

ANOVA $F(3,166) = 73.23$, $p<0.001$.

A/B $p<0.001$; A/C $p<0.001$; A/D $p<0.001$; C/D: NS; B/C $p<0.001$, B/D $p<0.001$.

ESCAPE ATTEMPTS OF GROUPED MICE IN VARIOUS SITUATIONS

	Grouped Alone	Grouped + Pill-box	Grouped + Grouped	Grouped + Isolated
n	40	20	30	24
mean ± S.E.M.	35.0 ± 2.0 A′	27.9 ± 1.6 B′	31.4 ± 1.0 C′	22.1 ± 1.5 D′

ANOVA $F(3,110) = 9.61$, $p<0.001$.

A′/B′ $p<0.001$; A′/C′ $p<0.05$; A′/D′ $p<0.001$; C′/D′ $p<0.001$; B′/C′: NS; B′/D′ $p<0.05$.

n = number of mice.

Mean ± S.E.M. = mean number of escape attempts ± standard error to the mean A/A′ $p<0.05$.

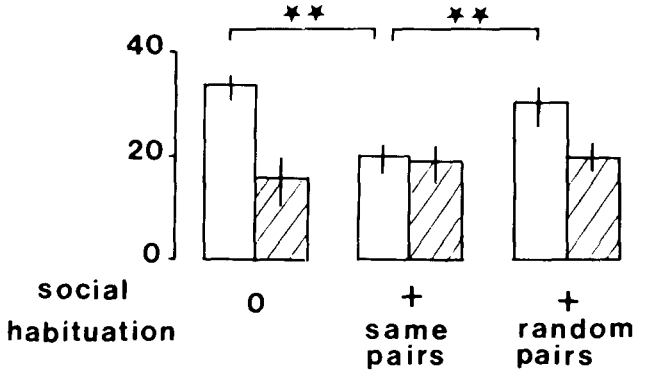

FIG. 5. Influence of the previous habituation to the partners on the scores of the isolated and grouped mice. The pairs of mice [grouped (open bars), isolated (hatched bars)] were tested without social habituation (left), with habituation with the same partner (middle) or with a randomly chosen partner (right). Tests were performed 5 hours after the end of social habituation (n = 20 pairs of mice in each group). Student's *t*-test: $^{**}p<0.01$. The results presented on this figure are a combination of the results from Fig. 4: the scores of mice habituated for 15 or 60 minutes were mixed for habituation with the same partner on one hand and with a randomly chosen partner on the other hand.

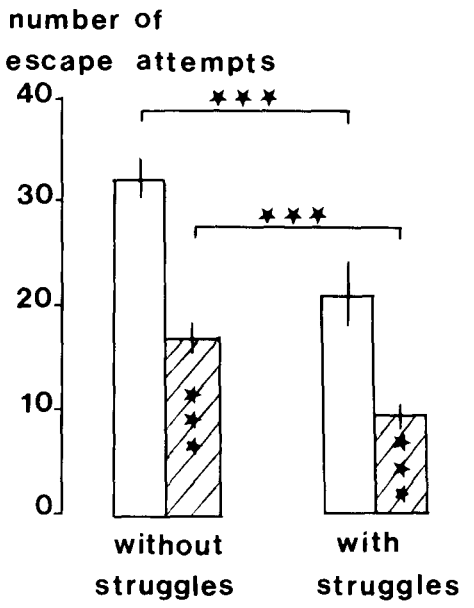

FIG. 6. Effect of aggressiveness on the number of escape attempts of isolated (hatched bars) and grouped (open bars) mice. n = 34 pairs without struggles; 36 pairs with struggles; $F(3,136)=39.95$, $p<0.001$. Student's *t*-test: $^{***}p<0.001$.

of the same partners were observed under the beaker 5 hours later. In the second protocol, 10 pairs of mice (isolated + grouped) were placed together for 15 or 60 minutes but the partners in the pairs observed under the beaker 5 hours later were randomly chosen. Ten pairs (isolated + grouped) with no previous social experience were also tested.

Experiment 6—Influence of struggles on the measure of social behavioral deficit. After many studies of this phenomenon, in pairs consisting of a group and isolation-reared mouse, reported here and elsewhere, it was possible to extract the data for pairs with or without struggles. Struggles were defined as attacks from the isolated mouse which resulted either in bites when the grouped mouse did not retaliate or in fights when it did. We compared the number of escape attempts in 36 pairs with struggles and 34 pairs without struggles randomly selected.

Experiment 7—Relation between aggressiveness and social behavioral deficit. Mice (one isolated + one grouped) were tested a first time and the number of escape attempts of each mouse in each pair noted as usual. Forty-one pairs consisting of the same partners were retested in the same conditions after two additional weeks of isolation for the mice in the isolated group. During the second test, escape attempts were not noted but the total duration of periods of aggressiveness by the isolated mice was recorded. Aggressiveness was defined as one of the following: 1) the mice fought; 2) the isolated mouse bit the grouped mouse; 3) the isolated mouse was in a dominant position, i.e., its forepaws were placed on the back of the grouped mouse or 4) the isolated mouse showed tail-rattling.

Statistical Analysis of the Results

Results were analyzed using the one-way analysis of variance followed by the Student's *t*-test for 2 by 2 comparisons in Figs. 2 and 6 and Table 1. The Student's *t*-test was used alone in Figs. 3, 4, 5 and Table 1. A correlation coefficient has been calculated for Fig. 7.

RESULTS

Experiment 1: Habituation

Isolated mice tested individually and repeatedly are as able of habituation as grouped mice (Fig. 1).

Experiment 2: Duration of the Test

The difference in the scores of grouped and isolated mice was significant during the first 2 minutes and not thereafter (Fig. 2). The disappearance of the difference was accounted for by the higher score of isolated mice. The discrepancy between the behavior of the two sets of mice was therefore probably linked to the novelty of the situation. This experiment justified the choice of 2 minutes as a suitable duration for this test.

Experiment 3: Novelty of the Experimental Place

The social behavioral deficit was still present and highly significant in each of the two protocols used: when only isolated mice were previously informed (Fig. 3A), when both isolated and grouped mice were previously exposed to the test environment (Fig. 3B). Thus, the novelty of the place did not appear to be a decisive factor for the social behavioral deficit of the isolated mice.

Experiment 4: Influence of Stimuli Under the Beaker

The results are presented in Table 1. The presence of a pill-box under the beaker reduced the number of escape attempts of the isolated mice (40%) and also, to a lesser extent that of the grouped mice (20%). The pill-box was explored by the mice and this may be an explanation for the reduced number of escape attempts since the time spent in exploration of the pill-box was not spent in attempting to escape. The presence of an isolated or a grouped mouse under the beaker reduced dramatically the escape attempts of the isolated mouse (71% and 64% respectively). This reduction was significantly higher than that induced by the presence of a pill-box (40%).

For grouped mice, the presence of another unknown grouped

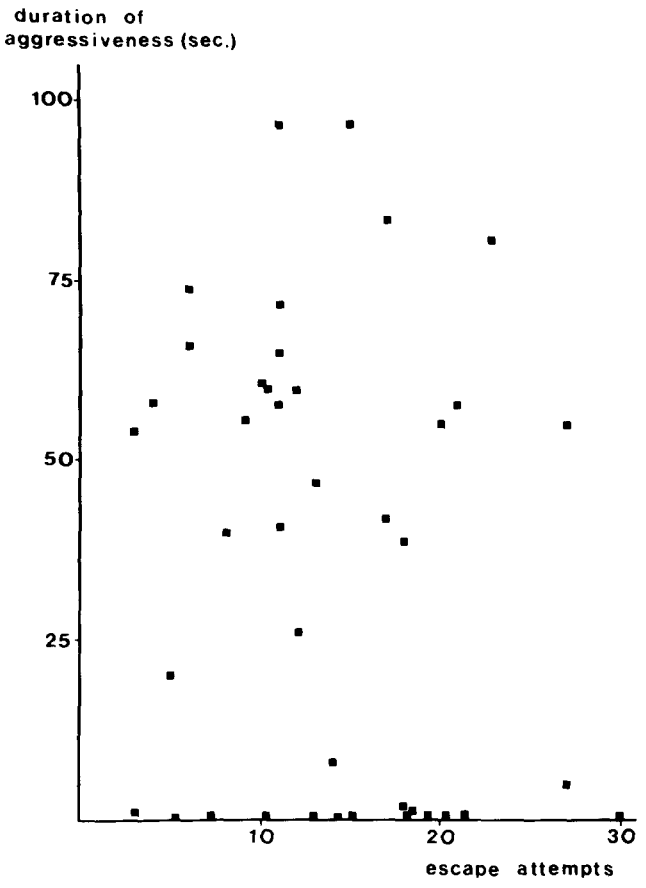

FIG. 7. Relation between the number of escape attempts and the duration of aggressiveness in isolated mice. The abscissa shows the number of escape attempts of isolated mice tested during 2 minutes in pairs with grouped mice after 7 days of isolation. The ordinate shows the duration of aggressiveness (seconds) of the same isolated mice tested during 2 minutes in pairs with the same grouped mice (same pairs) after 2 additional weeks of isolation, for the isolated mice. Correlation coefficient n = 0.20.

mouse under the beaker reduced the number of escape attempts (10%) but no more than the presence of a pill-box (20%).

The score of a grouped mouse tested when paired with an isolated mouse was significantly lower (37%) than when the partner was another grouped mouse (10%): the grouped mice differentiated a grouped from an isolated mouse.

These results show that the decisive factor to dramatically reduce the number of escape attempts of the isolated mice beyond that seen with the pill-box stimulus appeared to be the presence of another mouse either grouped or isolated. In the following set of experiments, we tested the importance of the fact that the partner was a known or an unknown mouse.

Experiment 5: Previous Social Experience

The difference between grouped and isolated mice disappeared when pairs were habituated to each other either 15 or 60 minutes (Fig. 4), but persisted when the pairs were randomly chosen.

Since no difference appeared between a social habituation of 15 or 60 minutes, the results were averaged in Fig. 5. This figure shows that the social habituation did not modify the number of escape attempts of isolated mice. However, the mean score of grouped mice was significantly reduced in the group "same pairs," probably on account of the prior aggressiveness of the isolated mice.

Experiment 6: Influence of Struggles on the Measure of the Social Behavioral Deficit (Fig. 6)

Struggles reduced the number of escape attempts of the grouped and also of the isolated mice: however, the difference (grouped-isolated) persisted with or without struggles. Aggressiveness reduced the number of escape attempts and increased the variability of the results but did not impair the appearance of the phenomenon.

Experiment 7: Relation Between Aggressiveness and the Social Behavioral Deficit

The results (Fig. 7) showed no significant correlation between the isolation-induced social behavioral deficit after one week of isolation and the duration of aggressiveness after three weeks of isolation.

DISCUSSION

Taken together these results show that a short duration isolation (7–9 days) is sufficient to induce in mice an hyperreactivity. Numerous previous studies show that isolated mice are more reactive than grouped ones to environmental stimuli [noise (1), restraint (19)] and particularly to the novelty: the spontaneous motor activity is increased during the first minutes and reduced afterwards (3–6, 10, 11, 13, 16, 18). The present results are similar although expressed in two opposite but noncontradictory ways.

Mice individually tested under the beaker made a number of escape attempts slightly but significantly higher than those of the grouped mice. In the same way, isolated mice tested with a pill-box (inanimate object) or a homologous or heterologous conspecific (animated object) showed an hyperreactivity: the reduction in escape attempts induced by the objects is more important than in grouped mice. The duration of this hyperreactivity was brief since it disappeared after two minutes suggesting the importance of the novelty in this hyperreactivity. The behavioral alterations in isolated animals appears more clearly in a social situation (12,14). The social behavioral deficit is significant with a small number of mice (10 pairs) whereas the increase in escape attempts of isolated mice tested alone needs greater samples (N.S. with 10 values—Fig. 1; $p<0.05$ with 65 grouped and 40 isolated mice—Table 1) underlining the interest of this test. This social behavioral deficit is a stable phenomenon since preliminary information of the mouse about the experimental place (Experiment 3) or previous short social experience (Experiment 5) did not impair its appearance in randomly chosen pairs. The disappearance of the social behavioral deficit in pairs of known partners likely results from the reduction in the score of grouped mice previously attacked by their isolated partner during the social habituation period. The social behavioral deficit may be partly explained by an increase in the search for social contact in the isolated mouse (7, 12, 14) or by a feeling of fear or mistrust which would be in competition with the escape attempts. Aggressiveness is a well known consequence of isolation in male mice, particularly in Swiss albino mice (15). Aggressiveness was a difficulty in measuring the social behavioral deficit since it reduces the scores of isolated as well as that of grouped mice and increased the variability of the results; however, aggressiveness did not impair the appearance of the deficit. No relation appeared between the

social behavioral deficit after one week of isolation and aggressiveness after 3 weeks in the same pairs.

Preliminary pharmacological studies of this behavioral model show that the deficit was unchanged with tricyclic antidepressants (9) and disappeared with the agonists of the 5-HT1B receptors (8). Taken together these results suggest that an isolation of 7–9 days is sufficient to modify the behavior of mice in a direction that may be interpreted as an hyperreactivity. It is suggested that this isolation-induced social behavioral deficit may be seen as a model of hyperreactivity with behavioral inhibition possibly resulting from attention to the partner including interest, mistrust and/or fear.

The actual behavioral and previous pharmacological results prompt us to thoroughly investigate the role of 5-HT1B receptors stimulation in hyperreactivity.

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