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Effect of Isolation on Brain Monoamines and the Behavior of Mice in Tests of Exploration, Locomotion, Anxiety and Behavioral 'Despair'

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HILAKIVI, L. A., M. OTA AND R. G. LISTER. *Effect of isolation on brain monoamines and the behavior of mice in tests of exploration, locomotion, anxiety and behavioral 'despair.'* PHARMACOL BIOCHEM BEHAV **33**(2) 371–374, 1989.—We have recently found that, besides an increase in aggression, isolation increases social interaction in NIH Swiss mice. In the present study the effect of isolation in other behavioral paradigms and their relation to brain monoamine concentrations were investigated. Mice, isolated for 0–20 days, were tested in the holeboard test of exploration and locomotor activity, the plus-maze test of anxiety, and Porsolt's swim test of behavioral 'despair.' Isolation reduced exploratory head-dipping, increased locomotor activity and increased the preference of mice for the open arms of the plus-maze. The immobility time in the swim test was shortened in mice isolated for 2 or 5 days, suggesting an improved ability to cope with stressful situations. Monoamine assays failed to show significant changes in the noradrenaline, dopamine, HVA, 5-HT or 5-HIAA contents in the amygdala, hypothalamus, hippocampus or brain stem. The results indicate that isolation of NIH Swiss mice for less than three weeks induces several behavioral changes, but is not particularly stressful.

Isolation Exploration Locomotion Anxiety Behavioral 'despair' 5-HT Noradrenaline Dopamine

ISOLATION is the most common means to induce aggressive behavior in otherwise nonaggressive laboratory animals (12). Besides the increase in aggressiveness, surprisingly little is known about the other behavioral effects of isolation. The traditional view is that isolation in rodents, as in primates, constitutes a stress in terms of social deprivation and that it has its action on behavior and endocrinology by increasing irritability of these animals (8). Isolation in adult male mice, however, may not be markedly stressful because of their territoriality. In fact, housing male mice in small cages in groups probably is a highly artificial situation (1). Our recent observation in adult male mice suggested that isolation increases social interaction (11). Other studies have reported increased behavioral reactivity to environmental stimuli, disturbances in sexual and exploratory behavior, and elevated locomotor activity [e.g., (4, 16, 18–20)] due to isolation.

The role of social isolation in generating anxiety and depression is relatively unknown. The present study examined in mice the effects of isolation on behavioral 'despair,' measured by the Porsolt's swim test (15,23), anxiety, measured by the plus-maze test (7, 10, 14), and exploration and locomotor activity, measured by the holeboard test (5). In addition, the concentrations of monoamines in the brain of mice isolated for various time periods were determined. The most consistent finding concerning putative neurotransmitters and isolation is a decrease in cerebral serotonin turnover [see (1,21)].

METHOD

Animals

Male NIH Swiss mice, weighing approximately 22–24 g, were maintained on a 12-hr light:12-hr dark cycle and allowed ad lib access to food and water. Prior to the experiments they were either housed individually for 20, 10, 5 or 2 days or in groups of ten.

Behavioral Testing

The experiments were carried out in two parts: animals isolated for 0, 2 or 5 days were studied in the first part and animals isolated for 0, 10 or 20 days were studied in the second part.

Swim Test

Sixty-eight naive mice, isolated for 0, 2 or 5 days, and 62 naive animals, isolated for 0, 10 or 20 days were used. Each mouse was placed in a plastic cylinder (height 17 cm, inside diameter 21 cm) containing 8 cm of water maintained at about 25°C. Each mouse

was in the water for a total of 10 min which included a 2-min acclimation period at the beginning of the test during which very little immobility was observed (15), immediately followed by an 8-min test. The room in which the experiment was carried out was maintained at about 25°C and was dimly lit. A mouse was judged to be immobile when it was floating motionless in the water making only those movements necessary to keep its head above the water. The time spent immobile was scored using a keyboard linked to PDP-microcomputer running SKED-11 software (State Systems, Kalamazoo, MI).

Holeboard

The holeboard apparatus was made of Plexiglas (40 × 40 × 30 cm) and had four holes 3 cm in diameter equally spaced in the floor. Infra-red photocells in the walls of the box and directly beneath each hole provided automated measures of locomotor activity (number of beam interruptions), of the number of exploratory head-dips made and the duration of head-dipping.

The holeboard testing, which took place in a dimly lit room, involved placing a mouse in the center of the floor and allowing it to explore for 5 min. Forty-three naive mice, isolated for 0, 2 or 5 days, and 30 naive mice, isolated for 0, 10 or 20 days, served as subjects.

Plus-Maze

The plus-maze was made of Plexiglas and consisted of two open arms 30 × 5 cm and two enclosed arms 30 × 5 × 15 cm. The arms extended from a central platform 5 × 5 cm. The open arms, the central platform, and the floor of the closed arms were made of black Plexiglas. The apparatus was mounted on a Plexiglas base, raising it 38.5 cm above the floor. The clear Plexiglas side walls of the closed arms were 14.5 cm high.

Immediately after the end of the holeboard test each mouse was placed in the center of the plus-maze facing an open arm. During the 5-min test the number of entries into each type of arm and the time spent in each arm were scored using a keyboard interfaced with a PDP-11 microcomputer. A mouse was taken to have entered an arm when all four legs were on the arm. In addition to the total number of arm entries, the number of entries into the open arms expressed as a percentage of total arm entries and the time spent on the open arms expressed as a percentage of the time spent on both the open and closed arms were measured.

Monoamine Assays

Separate groups of 6–7 mice per group were isolated at the same time as the above experiments were performed but were not behaviorally tested. Instead they were killed on the test day by decapitation, and the brain stem, amygdala, hippocampus and hypothalamus were dissected out. These brain areas were then frozen and stored at −70°C until they were assayed for 5-hydroxytryptamine (5-HT), noradrenaline, dopamine, 5-hydroxyindoleacetic acid (5-HIAA) and homovanillic acid (HVA).

The concentrations of the monoamines and their metabolites were determined using HPLC with electrochemical detection (using a potential of 0.85 V). The tissue was weighed and homogenized in 0.1 M perchloric acid to which the internal standard 5-hydroxy-N-methyltryptamine was added. Following centrifugation at 12000 × g for 2 min the supernatant was directly injected into the chromatograph.

Statistical Analysis

A BMDP statistical package was used to analyze the data.

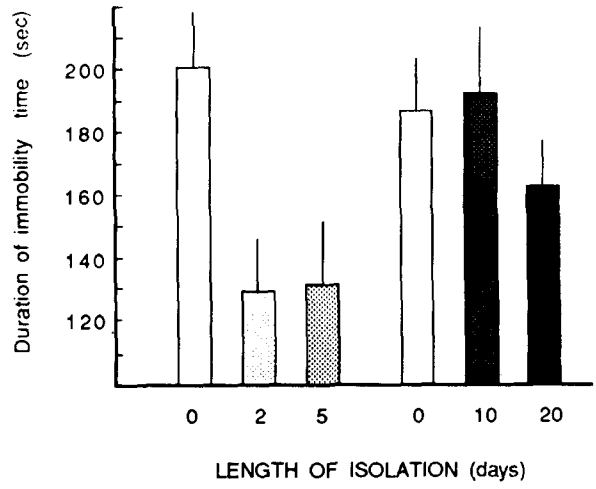

FIG. 1. The effect of isolation for 0, 2 and 5 days or 0, 10 and 20 days on immobility during the swim test. The means+SEM of 10–23 mice per group are shown.

Results for the swim test, the holeboard test,the plus-maze test and the neurochemical assays were analyzed using one-way analysis of variance. Prior to the analysis the swim test data were log-transformed. Between-group comparisons were made using Newman-Keuls' test.

RESULTS

Swim Test

Isolation for 2–5 days. There was a significant difference in immobility between the groups, $F(2,65)=4.79$, $p<0.02$: mice isolated for 2 ($p<0.05$) and 5 days ($p<0.05$) floated for a shorter time than the group-housed controls (Fig. 1).

Isolation for 10–20 days. The immobility time did not differ between the group-housed animals and animals isolated for 10–20 days (Fig. 1).

Holeboard

Isolation for 2–5 days. Isolation-dependent decreases in the number of exploratory head-dips, $F(2,40)=19.05$, $p<0.0001$, and the time spent head-dipping, $F(2,40)=6.33$, $p<0.004$, were found in mice isolated for 2–5 days (Fig. 2). Locomotor activity was significantly higher in the mice isolated for 5 days ($p<0.01$) than in the controls, $F(2,40)=6.13$, $p<0.005$ (Fig. 2).

Isolation for 10–20 days. Mice isolated for 10–20 days spent less time head-dipping than their controls, $F(2,27)=10.33$, $p<0.0005$. Although the number of exploratory head-dips was lower in the mice isolated for 10 and 20 days, these reductions failed to reach significance (Fig. 2). Locomotor activity scores were increased with isolation, $F(2,27)=3.92$, $p<0.04$.

Plus-Maze

Isolation for 2–5 days. Isolation for 2–5 days did not affect the proportion of entries on open arms or the proportion of time spent on the open arms. The total number of arm entries was elevated with isolation, $F(2,40)=6.09$, $p<0.005$ (Fig. 3).

Isolation for 10–20 days. The proportion of entries made onto the open arms, $F(2,27)=4.45$, $p<0.02$, was higher in animals isolated for 10–20 days than in the controls (Fig. 3). Similarly, the

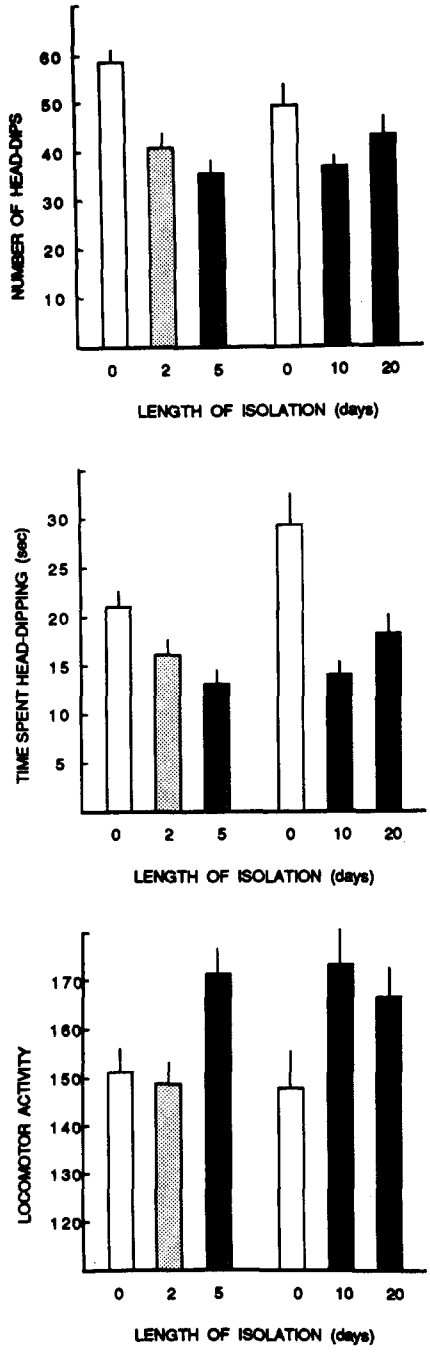

FIG. 2. The effect of isolation for 0, 2 and 5 days or 0, 10 and 20 days on exploratory head-dips (number and duration) and locomotor activity scores during a 5-min test in a holeboard. The means+SEM of 10–15 mice per group are shown.

time spent on open arms, $F(2,27) = 5.83$, $p<0.008$, expressed as a percentage of the time spent on both open and closed arms, was higher in the isolated animals (data not shown). Animals isolated for 10–20 days also made a greater number of arm entries than the controls, $F(2,27) = 13.95$, $p<0.0001$ (Fig. 3).

Monoamine Assays

Isolation did not induce any significant changes in the mono-

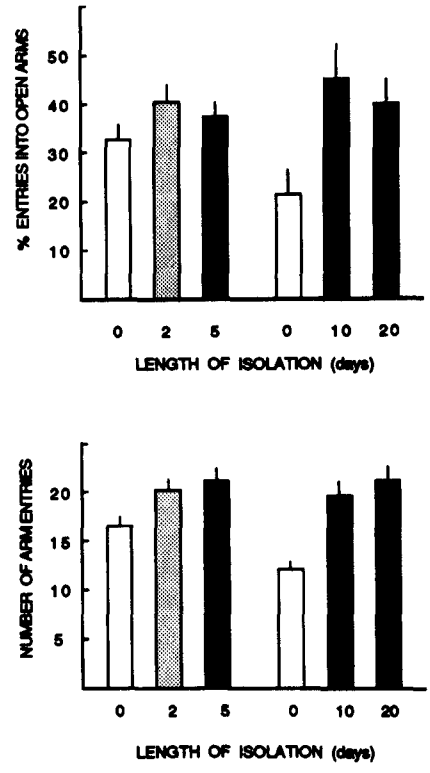

FIG. 3. The effect of isolation for 0, 2 and 5 days or 0, 10 and 20 days on the percentage of entries made into the open arms and on the total number of arm entries during a 5-min plus-maze test. The means+SEM of 10–15 mice per group are shown.

amine concentrations in any of the brain areas studied (data not shown).

DISCUSSION

The increased locomotor activity found in the isolated animals in the present study has been reported by several other investigators [e.g., (4, 18, 20)]. We also found an isolation-induced decrease in the time spent making exploratory head-dips. This decrease was evident following only 2 days of isolation. The data extend the findings of Valzelli (19) who showed an impairment in the exploratory activity of mice following four weeks of isolation. Sahakian *et al.* (16), however, found that rats isolated for more than 6 weeks from weaning had higher levels of exploration in another paradigm in which exploration and locomotor activity were assessed independently. The apparent discrepancy seems likely to be a result of the different species used as well as the different isolation regimen. It might be argued that the reduction in exploration caused by isolation was secondary to the increase in locomotor activity. However, a number of studies suggest that these two measures are capable of varying independently (3, 5, 10). The locomotor activity increase observed in the present study was less than that which we have observed following low doses of ethanol, which increase both locomotor activity and exploration. We think it is unlikely, therefore, that the reduced exploration was a direct result of the increased locomotion.

The mice isolated for 10 or 20 days increased the percentage of time they spent in the open arms of the plus-maze. This finding is

somewhat difficult to interpret since the mice also showed increased activity both on the plus-maze and in the holeboard. It may not, therefore, reflect a specific anxiolytic effect of isolation. Certainly there was no indication that the isolation increased anxiety.

In the swim test, the behavioral 'despair' measure, the time spent immobile in the water [see (23)], was found to be shorter in mice which were isolated for 2–5 days, but not for 10–20 days as compared to group-housed controls. Hence, isolation did not induce behavioral 'despair.' The reduction in the floating time with short-term isolation probably was not related to changes in behavior observed in the holeboard and plus-maze tests, because the holeboard and plus-maze performances were both altered, but even more profoundly, after 10–20 days of isolation than after an isolation lasting for a shorter time, whereas immobility in the water was shortened only in mice isolated for 2–5 days. In other studies we have failed to demonstrate any correlation between behavior in the swim test and the holeboard or plus-maze tests (Hilakivi and Lister, submitted). Since the swim test is suggested to measure an animal's ability to adapt to a stressful situation (9), short-lasting isolation may improve coping mechanisms for a stress in these animals. A balance between a stress associated with group housing and stress associated with long-term social deprivation could conceivably account for this pattern of results.

Recently, isolation of Swiss mice for 8 days was suggested to induce behavioral changes, described as learned helplessness by Frances (6). The behavioral measure in the experiment was 'an escape attempt' which was defined as rearing against a transparent beaker, sniffing or scratching the glass floor. When the animals were tested alone they showed more of these activities than the controls, but when tested in pairs control animals made more 'escape attempts' than isolated mice. Instead of interpreting the behavior of isolated mice as reflecting increased depression, it may have been a consequence of increased social investigation. Our earlier studies indicated that in NIH Swiss mice, isolation increases social interaction but does not affect locomotor activity of a mouse pair (11). Hence the isolated mice of Frances (6) may have preferred sniffing their partners to investigating the beaker. The learned helplessness interpretation of her data would appear highly questionable, and our data using the test of behavioral 'despair' provides no evidence to support her conjecture.

In the present study isolating the mice for a period shorter than three weeks did not produce any significant alterations in the central monoamine concentrations; only tendencies in the directions reported in previous experiments (4, 17, 21). Because the isolated mice showed changes in behavior, these changes do not seem to be clearly related to the monoamine concentrations in the brain. However, other functional changes (e.g., alterations in receptor number) may have occurred (12). It should also be noted that alterations in monoamine concentrations and other behavioral changes may result from longer periods of isolation than those used in the present study.

In conclusion, several observations from the present study support the suggestion of Brain (1) that isolation is not markedly stressful to NIH Swiss mice. Firstly, isolation showed no indication of being anxiogenic in the plus-maze test (on the contrary there was some evidence that it was anxiolytic). Secondly, immobility in the swim test was not lengthened following isolation, and this might be expected following stress (9,22). Finally, none of the alterations in brain monoaminergic function associated with stress [e.g., increased 5-HT turnover (2)] were observed in isolated mice.

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