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Altered Behavioral Responses to Intense Foot Shock in Socially-Isolated Rats

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NISHIKAWA, T. AND M. TANAKA. *Altered behavioral responses to intense foot shock in socially-isolated rats.* PHARMAC. BIOCHEM. BEHAV. 8(1) 61–67, 1978. – Male Wistar rats were isolated immediately after weaning for 12 weeks and exposed to electric foot shock of various intensities. The shock-elicited jumping behavior was measured every ten minutes for one hr. The frequency of jumping in isolated rats was lower than that in grouped and the difference between two groups was the greatest with the most intense shock. In these experimental situations, there was no significant difference in monoamine turnover rate between the two groups while noradrenaline turnover markedly increased in both groups. Chlorpromazine suppressed the jumping in a dose-dependent manner in both groups with stronger suppression in isolated rats. Methamphetamine facilitated the jumping in grouped rats dose-dependently while the drug rather depressed it in the isolated. From these results and the behavioral similarity between the isolated and 6-hydroxydopamine treated rats under foot shock situation, it was suggested that the receptor supersensitivity of central catecholaminergic neurons was involved in the behavioral change in isolated rats.

Social isolation Foot shock Jumping Catecholamine Receptor supersensitivity Model schizophrenia

SOCIAL isolation in animals especially in rodents induces aggressive behavior and the biochemical mechanisms underlying the altered behavior have been investigated [1, 5, 6, 7, 10, 13, 15, 21]. In these studies, aggressive behavior was reported to be closely related to the turnover rate of monoamines especially serotonin [5, 6, 10, 15, 21]. Valzelli [22] proposed the term isolation syndrome much more adequate for such altered behavior, and also suggested that psychoneurosis in man is similar to this syndrome.

We supposed that prolonged isolation from the early postnatal period may result in changes of brain composition such as receptor sensitivity or enzyme activity as a result an altered monoamine turnover rate because of decreased neuronal activity following sensory deprivation. This change of brain composition may result in a behavioral change in isolated animals when the animals are exposed to stressful situations. If this hypothesis was demonstrated experimentally, social isolation might become an interesting laboratory model for understanding the pathophysiology of psychosis like schizophrenia from the standpoint of view that psychosis consists of the interactions of both environmental influences and abnormal biological processes.

The present study was undertaken to test this hypothesis using rats isolated immediately after weaning.

METHOD

General

Social isolation. Animals were male Wistar rats, weaned at three weeks after birth, and immediately divided randomly into two groups, the isolated and the grouped. The isolated rats were kept individually in the 210 × 320 × 135 mm plastic cages containing wood shavings covered with thick paper to prevent visual and tactile contact, but they retained access to olfactory and auditory stimuli. The grouped control rats were housed three or four per standard 265 × 425 × 150 mm plastic cage containing wood shavings. Behavioral observations were done for the rats isolated for 3, 9, 12 and 15 weeks, but biochemical determination and the drug administration study were done for the rats isolated for 12 weeks.

6-hydroxydopamine treatment. Male Wistar rats weaned at three weeks after birth and housed in groups, were injected 6-hydroxydopamine (6-OHDA) intraventricularly. The behavioral and biochemical determinations of 6-OHDA treated rats were performed at 13–15 weeks after birth.

All animals were kept at constant room temperature (24 ± 1°C) and humidity (50 ± 10%). Lights were on from 7:00 a.m. to 7:00 p.m. and food and water were given ad libitum. Cages were changed once each week.

Behavioral Observations

All rats were subjected only once to the behavioral observations during 10:00 a.m.–3:00 p.m. but muricide was observed on the rats subjected to open-field test at the next day of the test.

Open-field test. The modified Hall's open-field apparatus [8,16] was used. The rat was placed in a corner of the open-field, and its behavior was recorded manually on ambulation, rearing, grooming, defecation and urination once every minute for six min at 3, 9 and 12 weeks of isolation.

Muricide. Muricide was observed by putting a mouse in a rat's cage for 30 min.

Electric foot shock. The electric foot shock apparatus was a box with a 90 × 90 cm floor of stainless-steel rods, 0.5 cm in diameter and spaced 1.9 cm apart, center to center, and 80 cm high plastic walls. The box was divided

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into 25 independent small compartments by transparent plastic walls. Shock was delivered by a constant voltage source (pulse wave, 60 Hz) at various intensities (0 V, 40 V, 60 V and 70 V) for a duration of 10 sec (each 10 sec train consisted of 80 msec on time and 420 msec interval) every 30 sec for one hour.

Each rat was put into one compartment. Usually 14 rats were subjected to the experiment at a time. When the shock was delivered, the rats struggled, vocalized, defecated and jumped. The frequency of shock-elicited jumping behavior was measured manually with blind method every ten min for one hr. Immediately after foot shock, rats were decapitated and biochemical assays were performed.

Biochemical Assays

The levels of monoamines in perchloric acid extracts of one cerebral hemisphere minus cerebellum were assayed fluorimetrically by the method of Shellenberger and Gordon [19] for noradrenaline (NA) and dopamine (DA), and by the method of Curzon and Green [4] modified by Kōno [12] for serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA). On the other hemisphere 3-methoxy-4-hydroxyphenylethyleneglycol sulphate (MHPG-SO_4) was assayed according to the method of Meek and Neff [14] with a slight modification.

Drug Administration

Chlorpromazine hydrochloride (Wintermin, Shionogi and Co., LTD., commercial injection preparation) and methamphetamine hydrochloride (Dainippon Pharmaceutical Co., LTD.) were dissolved in physiological saline. Chlorpromazine and methamphetamine were injected intraperitoneally 60 and 10 minutes respectively before placing the animals in the foot shock apparatus. 6-OHDA hydrobromide (Sigma Chemical Company) was dissolved in saline with ascorbic acid (1 mg/ml) and injected stereotaxically in right lateral ventricle (100 μg/10 μl; applied as the free base). Control rats received only the solvent in the same manner.

Statistical Analyses

Statistical analyses were performed by Mann-Whitney U test (two-tailed) in the behavioral study and Student's t-test (two-tailed) in the biochemical study.

RESULTS

Isolation-Induced Behavioral Changes

Open-field test. As shown in Fig. 1, the behavioral differences between the two groups were statistically significant for ambulation at the 9th week and the 12th week of isolation, and for rearing at the 3rd week and the 12th week of isolation, though the differences between the two groups were not large. There was no significant difference on other parameters in the open-field test as grooming, defecation and urination between the two groups except for the defecation at the 12th week (isolated rats showed lower defecation; $p<0.05$).

Muricide. Muricide occurred with only one in isolated rats for 12 weeks from 90 rats tested in these experimental situations.

Shock-elicited jumping behavior. On the other hand, isolation-induced behavioral changes became very characteristic in the intense foot shock situation. When the

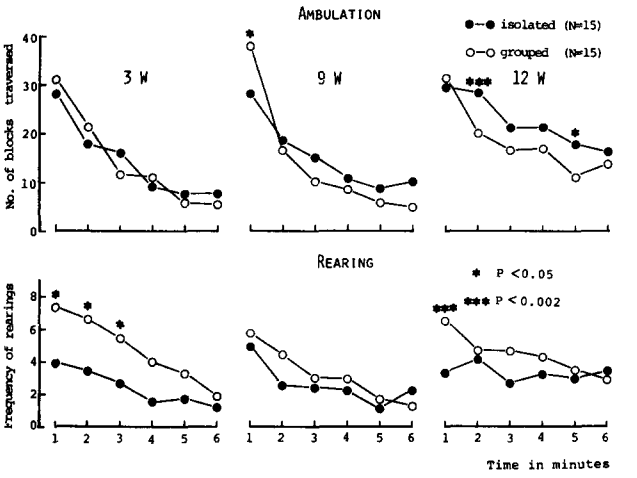

FIG. 1. Effect of isolation on ambulation and rearing in an open-field test. The rats were tested after 3, 9 and 12 weeks of isolated or grouped housing.

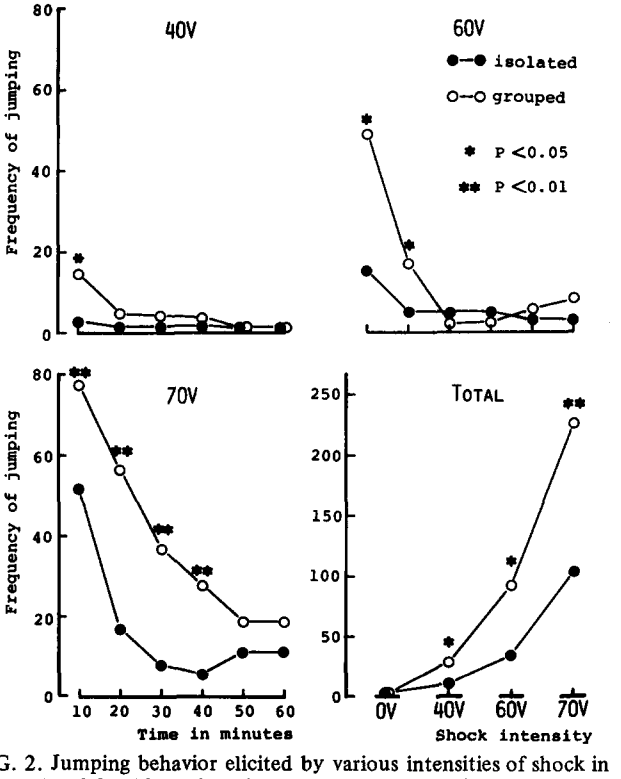

FIG. 2. Jumping behavior elicited by various intensities of shock in rats isolated for 12 weeks. The rats were tested by 40, 60 and 70 V of shock intensity. Total is the sum of the shock-elicited jumping behavior for one hr. Each point represents the mean value on seven rats except grouped rats at 70 V (N = 5).

electric foot shock was delivered, the rats struggled, vocalized, defecated and jumped. In terms of the shock-elicited jumping behavior, as shown in Fig. 2, isolated rats showed a lower frequency of jumping than did grouped rats at each shock intensity and these differences became greater at greater shock intensity. This behavioral difference also became significant depending on the periods of isolation as shown in Fig. 3, wherein the difference was very small at the 3rd week of isolation, occurred at the 9th

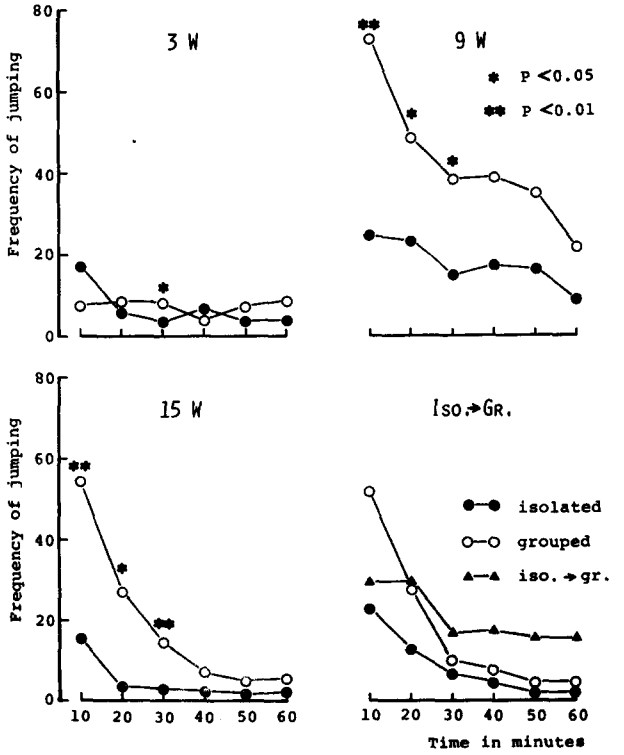

FIG. 3. Jumping behavior elicited by 70 V foot shock in rats at various periods of isolation. The rats were tested after 3, 9 and 15 weeks of isolated or grouped housing. In the lower right, all the rats were tested at 12 weeks after weaning and the iso. → gr. rats were isolated for 11 weeks and subsequently kept in grouped housing for one week. The isolated rats were housed individually for 12 weeks. There was no significant difference between iso. → gr. and each of the other two groups at initial 10 and 20 min although significant difference between isolated and grouped rats was observed ($p<0.05$), but significant difference appeared between iso. → gr. and isolated rats at 60 min ($p<0.05$). Number of rats in each group is seven except iso. → gr. (N = 5) because two rats died during the initial ten min and were excluded from the data.

week, continued up to the 15th week and diminished after placement of the rats into grouped housing.

The next biochemical and drug administration studies were performed to investigate the mechanism of the altered behavior in rats isolated for 12 weeks because of two reasons. First, behavioral difference between the two groups was the largest at 12 weeks. Second, there was no significant change on brain monoamine levels by isolation itself at 12 weeks although transitory elevation of NA concentration was observed at 6 and 9 weeks of isolation [17].

Changes of Brain Monoamines and the Metabolites Elicited by Electric Foot Shock

Control values ± SEM of brain NA, DA, 5-HT, MHPG-SO_4 and 5-HIAA levels (expressed as 100% in Fig. 4) obtained from grouped unshocked rats were 0.347 ± 0.020 μg/g (N = 6), 0.922 ± 0.036 μg/g (N = 6), 0.495 ± 0.017 μg/g (N = 6), 0.123 ± 0.008 μg/g (N = 7) and 0.441 ± 0.032 μg/g (N = 7) respectively. As shown in Fig. 4-A, 5-HT and 5-HIAA levels were almost unchanged under these foot shock conditions. Although, as shown in Fig. 4-B, the NA level decreased and MHPG-SO_4 level increased markedly with increasing shock intensity, there was no significant difference between the two groups. Brain DA level also increased as a function of shock intensity slightly but there was no significant difference between the two groups.

Influences of Methamphetamine and Chlorpromazine on Shock-Elicited Jumping Behavior in Isolated Rats

As shown in Fig. 5-A, methamphetamine facilitated jumping behavior in grouped rats in a dose-dependent manner. When the drug was administered to the isolated rats, jumping behavior was facilitated slightly at a dose of 0.5 mg/kg but rather depressed at doses of 1.0 mg/kg and 5.0 mg/kg; in addition, the rats tended to die with administration of high doses of methamphetamine and at a dose of 5.0 mg/kg all the rats died within 40 min. As shown in Fig. 5-B, chlorpromazine did not depress jumping behavior in grouped rats at all at a dose of 0.2 mg/kg but markedly depressed jumping in the isolated rats at the same dose. A higher dose, 5.0 mg/kg, depressed jumping behavior both isolated and grouped rats to the same extent.

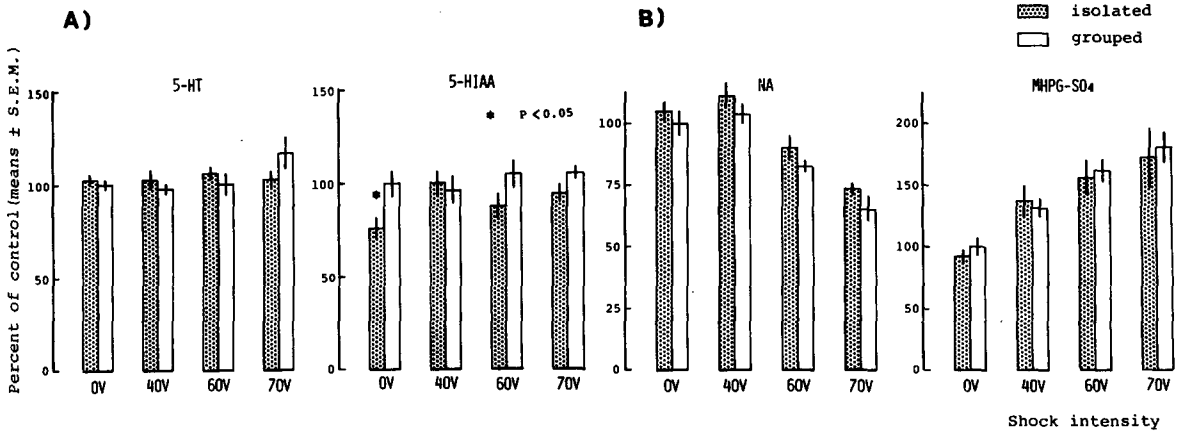

FIG. 4. Changes in brain monoamines and the metabolites elicited by electric foot shock. Isolation period is 12 weeks. Monoamines and the metabolites were determined on 5–7 rats in each group.

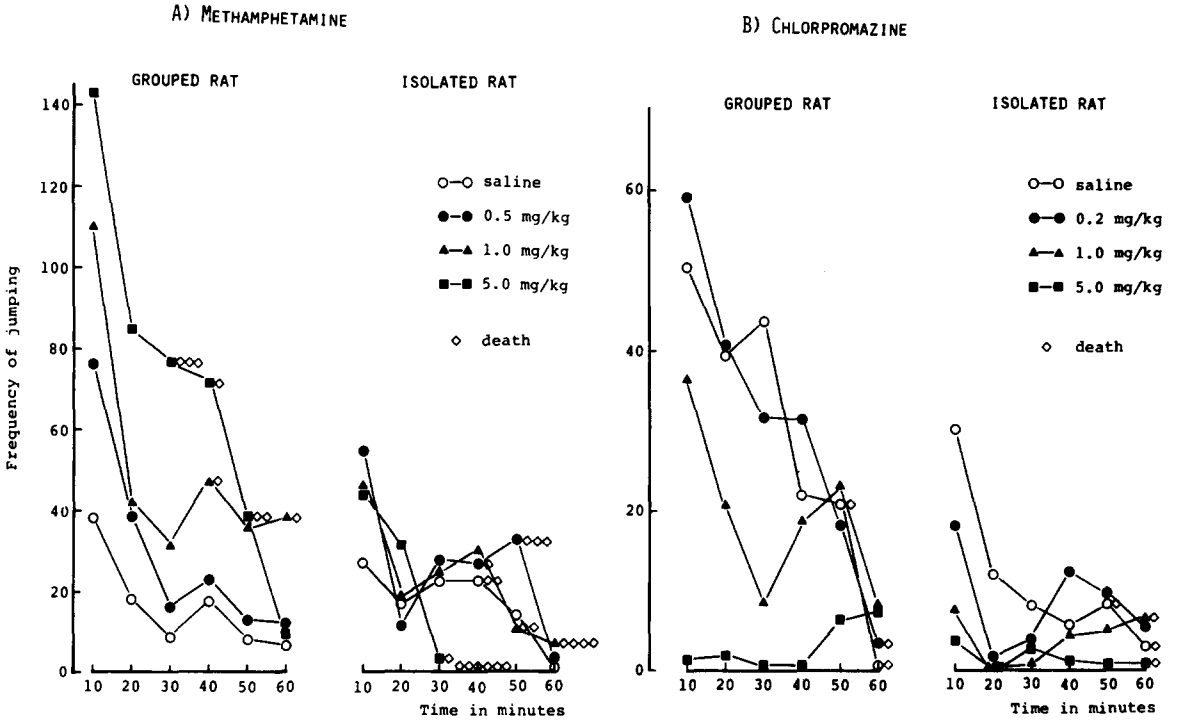

FIG. 5. Effects of various doses of methamphetamine or chlorpromazine on jumping behavior in response to 70 V of foot shock. Isolation period is 12 weeks. Number of rats in each group at the beginning of the test is seven and the mean value is shown. If a rat died, the mean value was calculated by excluding the dead rat from the data at the time when the rat died.

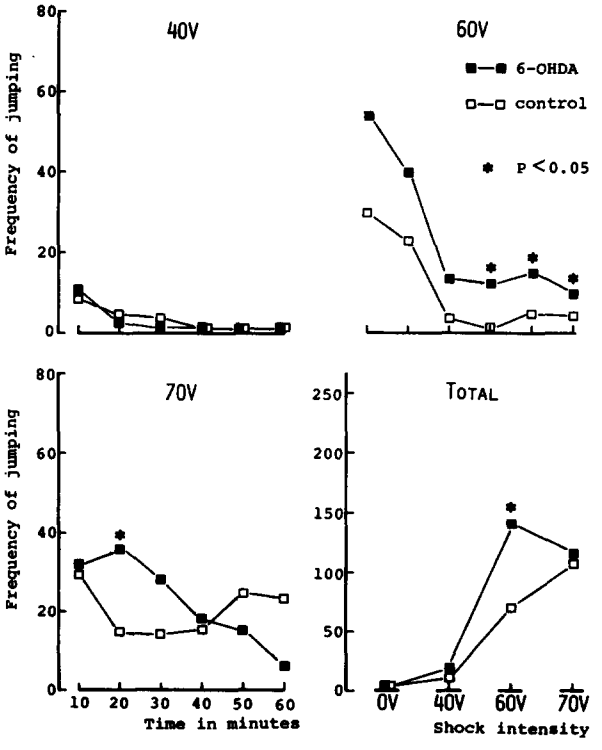

FIG. 6. Shock-elicited jumping behavior by various intensities in rats 21 days after 6-OHDA treatment. Number of rats in each group is 6–7.

Changes in Brain Monoamine Levels After 6-OHDA Administration

Brain NA level decreased to 46% ($p<0.01$), 33% ($p<0.01$) and 36% ($p<0.01$) of control value and brain DA level decreased to 82% ($p<0.01$), 70% ($p<0.05$) and 85% (NS) of control value at 2, 7 and 21 days after intraventricular injection of 6-OHDA respectively, but brain 5-HT level was not significantly affected.

Shock-Elicited Jumping Behavior in 6-OHDA Treated Rats

Shock-elicited jumping behavior was examined under various intensities of shock at 21 days after 6-OHDA treatment. Whereas isolation decreased jumping behavior, 6-OHDA treatment increased shock-elicited jumping, as shown in Fig. 6. The frequency of jumping by 6-OHDA treatment was the greatest at 60 V shock.

Influence of Methamphetamine and Chlorpromazine on Shock-Elicited Jumping Behavior in 6-OHDA Treated Rats

As shown in Fig. 7-A, methamphetamine at a high dose (5.0 mg/kg), administered to 6-OHDA pretreated rats, depressed jumping behavior rather than facilitated it as in isolated rats and occurrence of these phenomena depended on the period after 6-OHDA administration. On the other hand as shown in Fig. 7-B, chlorpromazine did not depress jumping behavior in 6-OHDA pretreated rats (21 days) at all, but facilitated jumping behavior in the initial ten min dose-dependently.

DISCUSSION

Various reports were concerned with aggressive behavior induced by social isolation as mentioned in introduction.

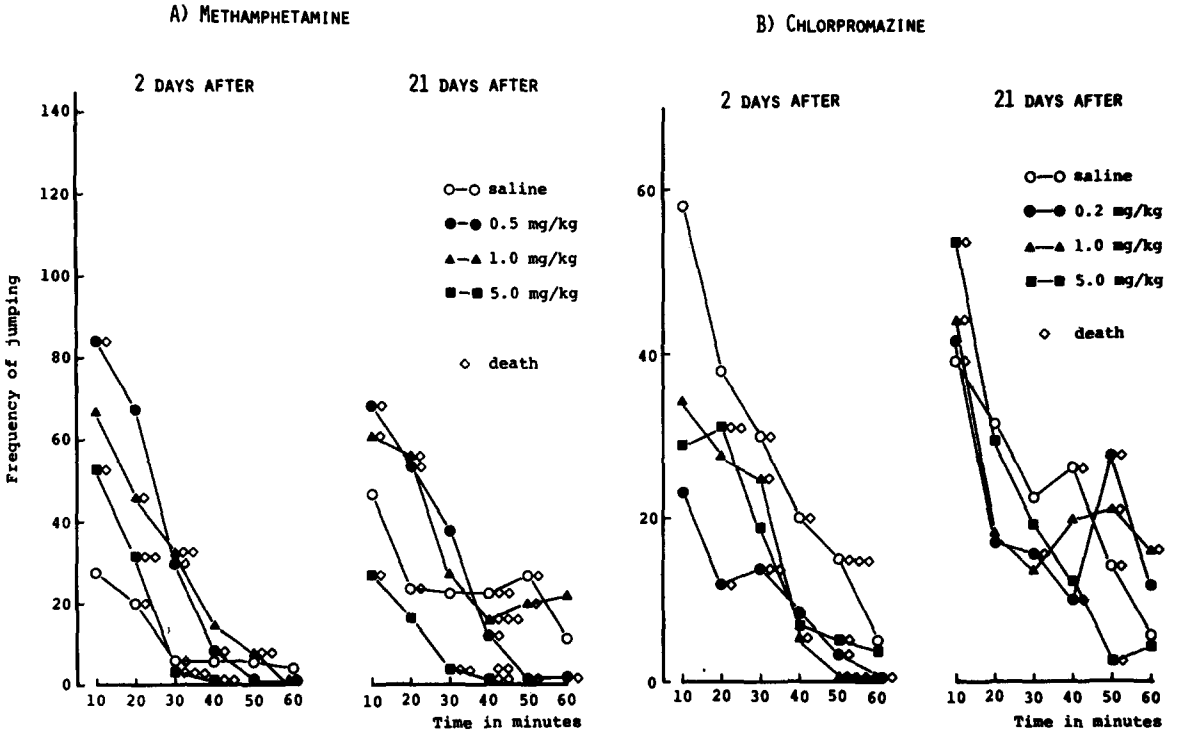

FIG. 7. Effect of various doses of methamphetamine or chlorpromazine on jumping behavior in response to 70 V of foot shock in 6-OHDA pretreated rats. Number of rats in each group is seven at the beginning of the test. Same calculation was done as described in the legend of Fig. 5.

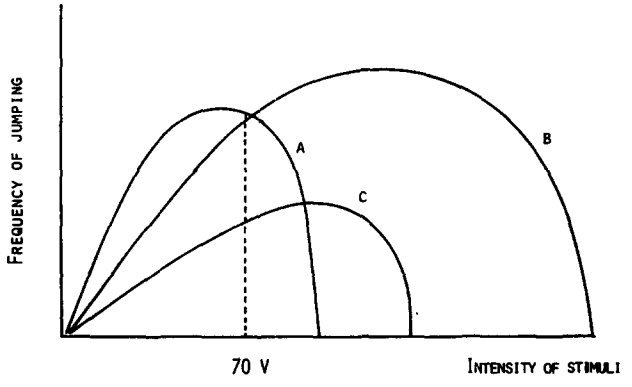

FIG. 8. Schema of stimulus-response curve in jumping behavior. Frequency of jumping in total period shown in Figs. 2 and 6 is plotted as for the response to the stimuli under 70 V of shock. As for the response to the stimuli over 70 V of shock, it is plotted considering the response with administration of methamphetamine which would stimulate inner environment of the animals chemically, namely the jumping after death with administration of methamphetamine is estimated as zero.

Valzelli and Garattini [21] reported that rats isolated for a period of six weeks developed three different patterns of behavior toward mice: friendly, muricidal and indifferent. As shown in their findings and our observation, isolation in rats did not always induce aggressive behavior toward mice. We have been interested in more common behavioral and biochemical changes induced by prolonged isolation itself [16,17].

In an open-field test which might be the behavioral responses to mild stimuli, the differences between isolated and grouped rats were observed but not large and the occurrence of the isolation effect on behavior were not always related to the duration of isolation. On the other hand, we found that the marked behavioral difference between the two groups was obtained by counting the jumping behavior in rats on being exposed to the intense foot shock stimuli.

The jumping behavior elicited by foot shock would participate possibly in the activity of noradrenergic neurons since the marked increase of NA turnover was observed in the foot shock situation. However, no difference in contents of NA and MHPG-SO_4 was seen between the two groups. Considering this finding, it is unable to explain the behavioral change in isolated rats by the difference in NA turnover rate. Methamphetamine, a stimulant of catecholaminergic neurons [20] facilitated jumping in isolated rats at low doses but high doses rather depressed it and finally the rats died. These phenomena might occur at a portion where the shock-elicited jumping behavior almost reached the top of the response to increasing shock intensity. The result of methamphetamine administration and the biochemical findings suggested that catecholaminergic hyperactivity especially receptor supersensitivity might play a role in the isolation-induced behavioral changes under foot shock situations. While the result of chlorpromazine administration, blocker of catecholaminergic transmission [20], opposed the idea of the participation of the hyperactivity because jumping behavior in isolated rats was depressed by administration of even a small dose of the drug.

Further study was done to clarify the possible role of receptor supersensitivity of catecholaminergic neurons on the altered behavioral responses in socially isolated rats by

comparing with 6-OHDA treated rats. Ungerstedt [23] reported that the receptor supersensitivity after denervation of dopaminergic pathways in 6-OHDA treated rats developed depending on the period after 6-OHDA using rotometer.

The reactivity of 6-OHDA pretreated rats was similar to that of isolated rats in such findings that low doses of methamphetamine facilitated jumping behavior but high doses of this drug depressed jumping and then animals died. As this phenomenon occurred depending on the similar time effect after 6-OHDA treatment reported by Ungerstedt [23], the catecholaminergic receptor supersensitivity might play an important role in the response to methamphetamine administration. However, the behavioral responses in 6-OHDA treated rats to stimuli under 70 V of foot shock was different from that in isolated rats, and the responses to chlorpromazine also differed clearly. Considering these findings, it was suggested that receptor supersensitivity of central catecholaminergic neurons might play an important role, however, not only supersensitivity but also other factors were involved in the behavioral change in isolated rats under foot shock situations. It might be reasonable that supersensitivity occurred due to lowered nerve impulse flow because of the lack of social contacts for so long a period.

It might be suitable to adopt the stimulus-response theory of psychology [9] to explain the meaning of the altered behavioral responses under foot shock situations. When the rats were exposed to the stimulus of foot shock, the rats struggled, vocalized, defecated, and jumped: of these behaviors, only the jumping constituted the effective adaptive behavior for escaping from this stimulus.

The results in the present experiment would be schematized as shown in Fig. 8. The stimulus-response curve (S-R curve) in grouped control rats is indicated as curve B, where the jumping frequency continues to increase depending on the increase of the shock intensity. The frequency still increase up to the highest dose of methamphetamine administration at 70 V, which would be stimulate inner environment of the animals chemically. Accordingly the frequency of jumping in grouped rats does not reach a top of the curve even at a 70 V shock. In isolated rats as shown in curve C, the reactivity against stimuli under 70 V of shock was lower than that of grouped animals. As the jumping frequency in isolated rats would be almost reach a top of the curve at 70 V shock, disruption of adaptive behavior occurred by higher doses of methamphetamine administration at 70 V shock and then the jumping frequency decreases. Namely, in isolated rats the top of inverted-U shaped S-R curve is shifted to the left and the height of the whole curve is lower than that in grouped rats. As shown in curve A, the reactivity of 6-OHDA pretreated rats was similar to that of isolated rats in that the top of inverted-U S-R curve shifted to the left, however the ascending portion of the curve in 6-OHDA treated rats was higher than that in isolated rats, and the response to chlorpromazine also differed clearly.

We found that these different S-R curve could be drawn by counting the shock-elicited jumping behavior among the grouped, isolated and 6-OHDA treated rats adopting the stimulus-response theory of psychology. Furthermore the shift of the top of S-R curve to the left reflects sharply differences of condition of catecholaminergic neurons especially receptor sensitivity as discussed above. These findings are new and important characteristics of isolated animals which have not been described yet in isolation syndrome integrated by Valzelli [22].

Hopefully social isolation in rats might become an animal model of schizophrenia because a considerable amount of the literature on schizophrenia is concerned with the role of environment and experience [2,18] in the pathogenesis and dopaminergic receptor supersensitivity was noted recently about the pathophysiology of this disease [3,11].

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