〈Regular Article〉

Effects of Partial Isolation on Mice Behavior

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ABSTRACT **Background**: The effects of an isolated human environment with little societal contact are a topic of interest in clinical psychiatry. Studies using animal models are important for investigating the effects of such an environment. Considering that the modern human environment that allows partial and limited communication even for those who are isolated from the society, raising mice in social isolation, a method that has been conventionally adopted with mice, is not an accurate simulation.

Method: Therefore, in our experiment, to better simulate the partial isolation that is often observed in humans, we devised a method of dividing the breeding cage into two compartments using a transparent sheet, raising four mice in one section and a single mouse in the other (we defined it as physical isolation). We then compared the behavioral patterns of group-reared, conventionally socially isolated, and physically isolated mice to determine the effects of limited communication restrictions on individual mice.

Result: When the new rearing method of physical isolation was adopted, there was no significant difference in the time spent around cages with and without familiar mice, or around cages with strange mice and cages with familiar mice, as observed in group rearing, confirming that social behavior is suppressed in the same way as in social isolation. However, there was no significant increase in immobility time on the forced swim test or tail suspension test, as observed in social isolation, suggesting no increase in anxiety or depression. In the cotton bud biting test, the number of attacks was significantly lower than the other two rearing methods, confirming a decrease in aggression.

Conclusion: Our findings, which show that mice placed in such an environment may experience less stress than when being raised in groups, despite suffering problems with their development of sociability, are of considerable interest.

doi：10.11482/KMJ-E202248105　*(Accepted on August 17, 2022)*

Key words：Social withdrawal, Sociability, Anxiety and depressive like behaviour, Social isolation, Partial isolation, Stress, Group housing, Animal model

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INTRODUCTION

The effects of an isolated human environment with little societal contact are a topic of interest in clinical psychiatry. This can be observed in what is commonly called social withdrawal, and in lifestyle patterns of patients with schizophrenia and autism spectrum disorder. In clinical situations, it is known that a patient's tendency to not leave the house further exacerbates any anxiety or obsession about a variety of domestic and social situations and reduces the opportunities to become more involved with society. Some individuals in this situation may gradually gain the ability to engage in social activities, while others may continue to lead isolated lives for extended periods with minimal interaction with other people. This diversity is believed to stem from the balance between a person's innate traits, upbringing, and their disorder, as well as the effects of isolation from society on his or her mind.

While studies of factors that contribute to social withdrawal exist[1], few have investigated the effects of a lifestyle with few societal contacts. The reason for this scarcity of studies is assumed to be the wide variety of conditions that influence isolation, and the difficulty in making observations because of subjects' reclusiveness. Studies using animal models are therefore important for investigating the effects of such an environment.

When mice are used to study the effects of isolation from society, researchers generally raise an individual mouse after weaning, during the murine equivalent of adolescence, in complete isolation from other mice. Compared to isolation from society, temporary isolation prior to weaning is often used to investigate the effects of high stress experienced during infancy. In humans, it is believed that the effects of social isolation must be investigated separately from the effects of dysfunctional mother-child relationships. Therefore, in this study, we isolated the mice after weaning.

According to a questionnaire survey conducted in 2019 by Japan's Ministry of Health, Welfare and Labour, the mean age of the initial onset of social withdrawal in people was 19.5 years, with 14 years (after puberty) being the mode age. Therefore, mice isolated after weaning can be used as a model for social isolation in humans. Previous studies have shown that a mouse that has been raised in isolation after weaning demonstrates abnormal social behaviors, such as increased anxiety and depression-like behaviors[2, 3], excessive aggressive behaviors[4, 5], reluctance to show interest in other individuals, and difficulty in achieving social proximity[6]. Studies have also shown lower pain sensitivity related to heat stimulation[7, 8] and hyperactivity in new environments[2, 9].

Raising mice in sequestration in this way, however, differs sharply from the isolated environment seen in people's actual lives. Today, it is extremely rare for an individual to be completely isolated from others. Even in regions that are generally regarded as being withdrawn from population centers, individuals are usually still able to visit neighborhood stores and interact with family members. Even for those who do not engage in these daily social activities, observing other people's activities via TV and the Internet, or engaging in limited communication with an unspecified but large number of other people via social media are still available. Considering that the modern human environment that allows partial and limited communication even for those who are isolated from society, we feel that raising mice in complete social isolation, a method that has been conventionally adopted with mice, is not an accurate simulation.

Therefore, in our experiment, to better simulate the partial isolation that is often observed in humans, we devised a method of dividing the breeding cage into two compartments using a transparent sheet, raising four mice in one section and a single mouse in the other. Thus, we created an environment in which contact-based physical communication was

prevented for a single mouse, but visual, auditory, and olfactory communication, as well as awareness of other mice were permitted. We then compared the changes in behavioral patterns of mice that had been subjected to conventional social isolation and those raised alongside others in groups and investigated the effects of limited communication restrictions on individual mice, comparing the differences between these effects and those due to complete isolation.

The purpose of this study was to bring the rearing environment of mice close to the partial isolation that is often observed in humans, and to clarify how this environment affects the behavior of mice.

MATERIALS AND METHODS

Animals

Three-week-old male mice (C57BL/6) were used in this study. The animals were purchased from Charles River Laboratories (Kanagawa, Japan) and housed in cages with food and water provided ad libitum under a 12 h light/dark cycle at 23-26℃. We divided the breeding cage (235 × 325 × 170 mm) into two compartments (117.5 × 325 × 170 mm) by placing a transparent plastic sheet in the middle to prevent the mice from moving between the compartments. We then randomly divided the mice into three groups for 6 weeks. In the first group, three mice were raised in one compartment, and the adjoining compartment was left empty (the group housing group or the GH group: n = 13 animals). In the second group, one mouse was raised in one compartment, and three mice were raised in the adjoining compartments (physical isolation group, or PI group: n = 10 animals). In the third group, one mouse was raised in one compartment and the adjoining compartment was left empty (social isolation group, or SI group: n = 10 animals) (Fig. 1A). Behavioral experiments were conducted over a 3-week period (Fig. 1B).

Every effort was made to minimize the number of animals used and thus minimize suffering. These experiments complied with the U.S. National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (NIH Publication No. 80-23, revised in 1996) and were approved by the. Animal Research Committee of Kawasaki Medical School (approval number: 20-056)

Behavioral tests

All behavioral tests were performed during the light phase (9:00-16:00). Each mice was tested in a random order. After testing, the apparatus was cleaned with 70% ethanol and 80 ppm hypochlorous acid solution (made with CL-4104; CLEA, Tokyo, Japan) to prevent any bias due to olfactory cues. Each animal was subjected to the hot plate test, rotatod test, neurological screening, cotton bud biting test, Y-maze test, passive avoidance test, sociability assessment test, tail suspension test, and Porsolt forced-swim test only once.

General health screening

The body weight of the mice were recorded weekly from week 3 to week 9, and the rectal temperature were recorded at week 9.

Hot plate test

The hot plate test was used to evaluate nociception (sensitivity to a painful stimulus). Each mouse was placed on a plate (**ND-3LA; AS ONE, Tokyo, Japan**) heated to 55.0℃ ± 0.3℃, and the latency to the first paw response was recorded. The paw responses included foot shakes or paw licks. A latency period of 30 s was defined as complete analgesia and was used as the cutoff time to prevent tissue injury[10].

Rotarod test

Motor function was quantitated using the rotarod test in mice according to Dunham and Miya (1957). Each mouse was placed on a rod (**RTR-M5; Melquest, Toyama, Japan**) that rotated at a set

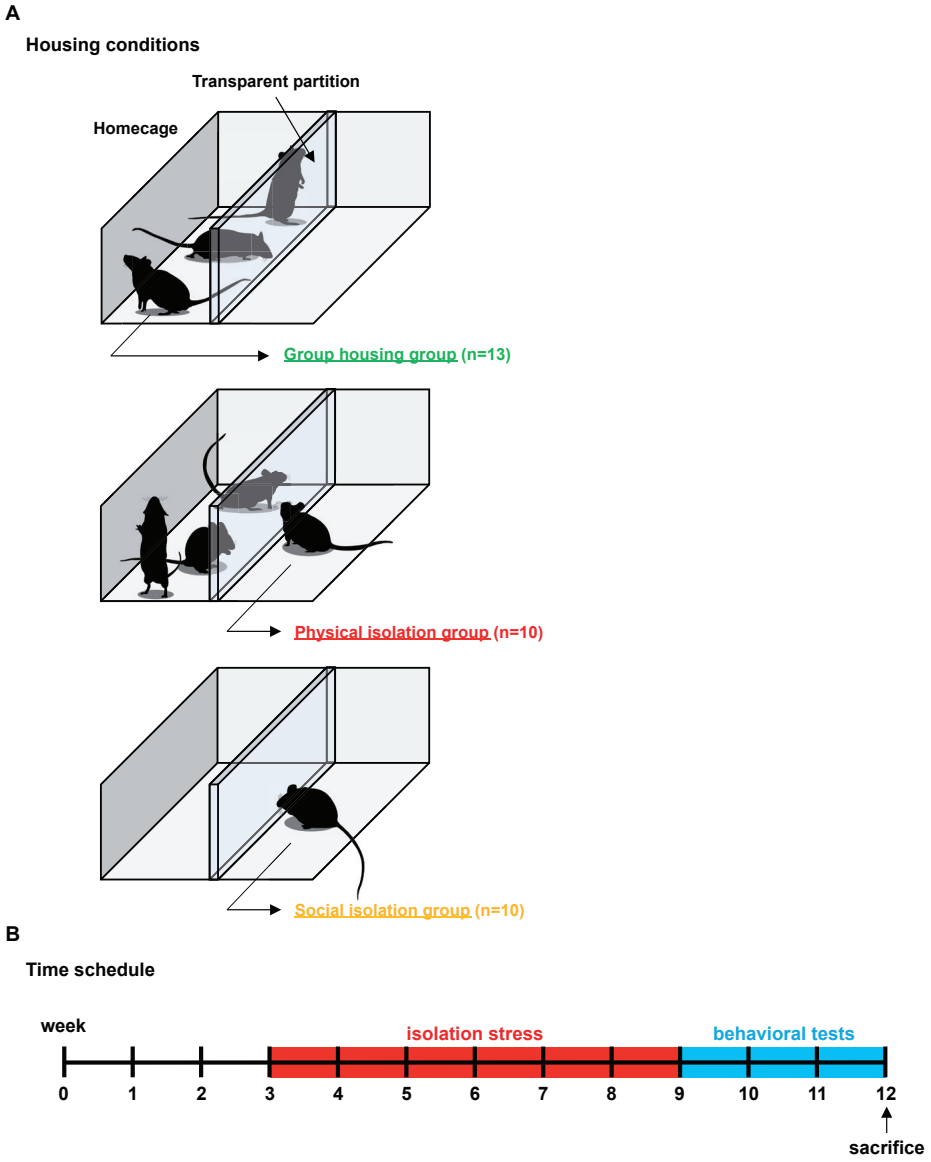

Fig. 1. The housing conditions and protocol of this study. (A) Three-week-old male mice were housed in groups, physically isolated, or socially isolated throughout the experimental period. (B) Behavioral tests were conducted over a 3-week period.

speed, and the time until the mouse fell off, or after 5 min had elapsed, was designated as a single trial. The rotational speed of the rod began at 4 rpm and was raised by 20 rpm every minute. The number of trials was 5 and the intertrial interval was 20 minutes. All mice were subjected to the test without any pre-trial training.

Neurological screening

Neuromuscular strength was examined using grip strength and wire hang tests. In the grip strength test, a grip strength meter (of our own making) was used to assess forelimb grip strength. Each mouse was lifted and held by the tail such that their forepaws could grasp a wire grid; they were

then gently pulled backward until they released the grid. The peak force applied by the forelimbs was recorded in Newtons (N/g). In the wire hang test, the mouse was placed on a wire grid such that they would hang to it. The grid was then turned over, and the time until the mouse fell from the grid was measured (maximum: 60 s). Five trials were made for both test, and the maximum value was used as the result.

Cotton bud biting test

Aggressive behavior was examined using a cotton bud biting test. Each mouse was held in the experimenter's hand, and a sterilized cotton bud was held close to their faces. Biting of the cotton bud was considered an aggressive behavior. The mice were tested 10 times. An analysis was conducted on the total number of biting attacks[11].

Y-maze test

Spatial working memory was measured using a Y-maze apparatus (of our own making) (arm length: 40 cm, arm bottom width: 3 cm, arm upper width: 10 cm, wall height: 12 cm). Each mouse was placed at the center of the Y-maze field. Visual cues were placed around the maze in the testing room and remained constant throughout the testing sessions. The mice were examined without prior learning. The number of entries and alterations was recorded and analyzed automatically using ANY-MAZE software (Stoelting Company, Wood Dale, Illinois, USA). Data were collected for a total of 10 min.

Passive avoidance test

To evaluate learning and memory, the passive avoidance test was used. For this test, the chamber (**MPB-M020; Melquest**) was divided into one lit compartment and one dark compartment. On the first day, each mouse was placed in the lit compartment. The time until the mouse entered the dark compartment was measured. Electrical stimulation was applied immediately after a mouse entered the dark compartment. On the second day, the mouse were placed in the lit compartment once again, and the time until they entered the dark compartment was measured.

Sociability assessment test

Before the test, the mouse were placed inside a plastic box (60 cm × 30 cm × 40 cm) and allowed to freely explore the entire space for 5 min. Then, a sociability test was conducted. A mouse (stranger) that had so far not been in contact with the test mouse, was placed inside a transparent cage that had 10 cm × 10 cm openings. The test mouse was placed inside another cage containing no mice and allowed to explore inside for 6 min. Subsequently, a social preference test was conducted. The mouse that had been used for the sociality test (familiar) was kept inside the cage, after which a mouse (stranger) that had thus far experienced no contact with the test mouse was placed inside another cage for the first time. The test mouse was allowed to explore for 6 min under these conditions (Fig. 2A). In these two tests, the total distance traveled and the time spent around each of the installed cages were measured.

Tail suspension test

Each mouse was suspended by the tail 60 cm above the floor in a white plastic chamber, using adhesive tape placed < 1 cm from the tip of the tail. Mouse behavior was recorded for 6 min. Images were captured by video camera, and immobility time was measured. In this test, the immobile period was defined as the period when the animals stopped struggling for ≥ 1 s. Data acquisition and analysis were performed automatically using ANY-MAZE software[10].

Porsolt forced-swim test

The apparatus for the Porsolt forced-swim test

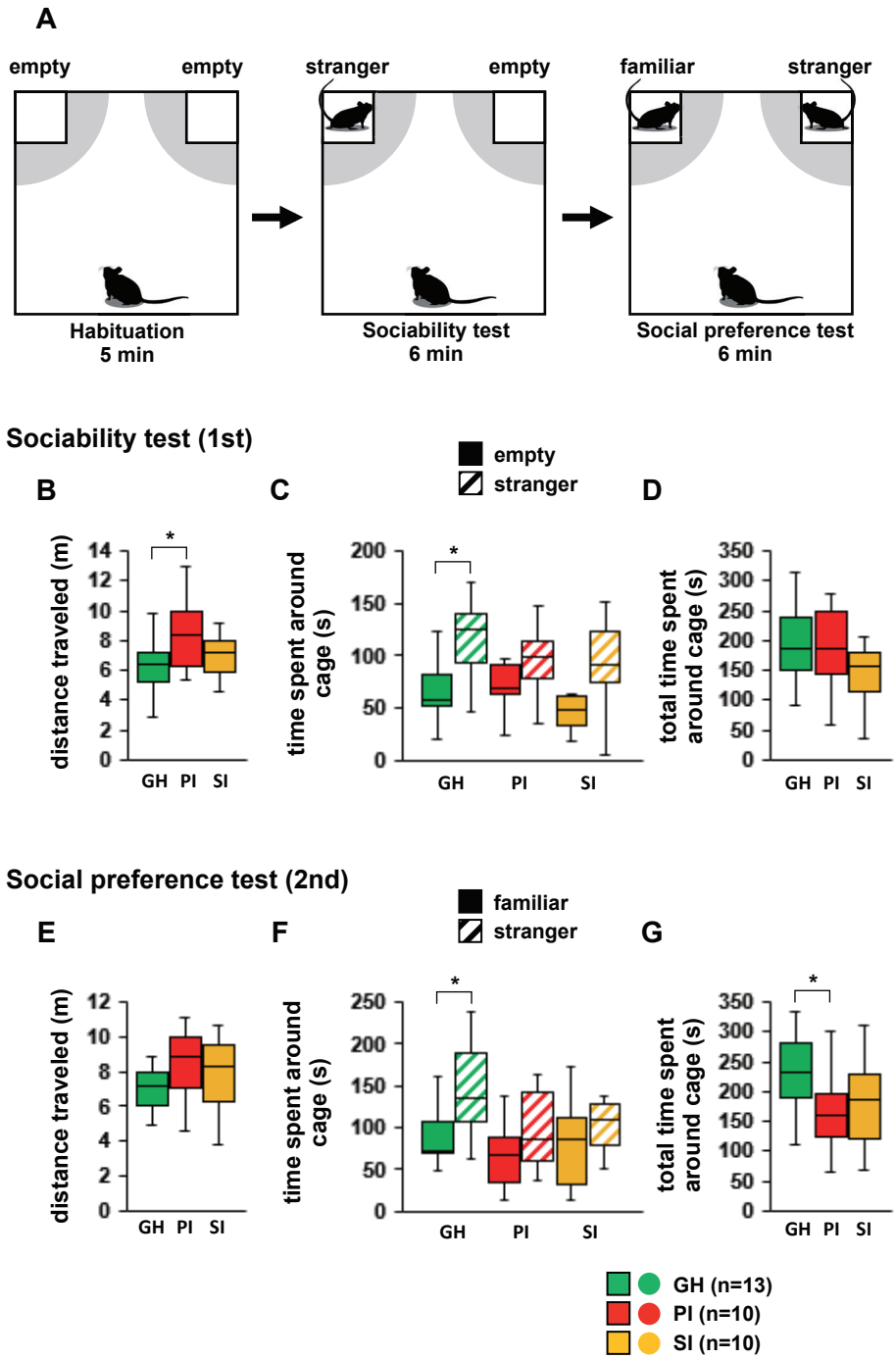

Fig. 2. Procedures of Sociability Assessment test(A), effect of isolation in the test(B-G). (A) The procedure of sociability and social preference tests. (B-D) Sociability test (1st). (B)The total distance traveled, (C) the time spent around cage containing stranger and empty cage, (D) the total time spent around either cage. (E-G) Social preference test. (E) The total distance traveled, (F) the time spent around cage containing familiar and time spent around cage containing stranger, (G) the total time spent around either cage.* , significant difference among groups ($p < 0.05$).

consisted of four Plexiglas cylinders (20-cm height × 10-cm diameter). The cylinders were filled with water (23℃) up to a height of 7.5 cm in accordance with the methods of previous studies[12, 13] Each mouse was placed into the cylinders, and their behavior was recorded over a 6-min test period. As in the tail-suspension test, the immobility time was evaluated using ANY-MAZE software[10].

Statistical Analyses

Data were analyzed using one-way analysis of variance (ANOVA), followed by Ryan's test. Statistical significance was set at $p < 0.05$. The data are presented as box plots. All statistical analyses were carried out with SPSS software (IBM, Armonk, NY, USA).

RESULTS

General health screening

We compared the general health of three groups of mice: GH, PI, and SI. There was no significant difference in body weight among the three groups (week9; GH: 26.5 ± 0.5g, PI: 28 ± 0.7g, SI: 27.5 ± 0.5g, $p = 0.096$). There was no significant difference in body temperature among the three groups ($F_{2, 30} = 0.884$, $p = 0.424$; GH vs. PI, $p = 0.195$; GH vs. SI, $p = 0.630$; PI vs. SI, $p = 0.436$).

Hot plate test

Effect of isolation on the sensitivity to a painful stimulus in the hot plate test was evaluated. The SI group showed a significantly longer reaction latency than the GH and PI groups, but no significant difference was observed between the other two groups (Fig. 3A, $F_{2, 30} = 7.884$, $p = 0.002$; GH vs. SI, $p = 0.013$; PI vs. SI, $p < 0.001$; GH vs. PI, $p = 0.141$).

Rotarod test

We found no significant difference in fall latency among the three groups in the rotarod test used to evaluate motor function (try1; GH: 10.6 ± 0.9s, PI: 12 ± 1.2s, SI: 10.5 ± 1.3s, try2; GH: 13.3 ± 1.2s, PI: 13.3 ± 1.5s, SI: 13.1 ± 1.4s, try3; GH: 12.7 ± 1.3s, PI: 14.0 ± 1.9s, SI: 12.5 ± 0.8s, try4; GH: 16.2 ± 1.4s, PI: 15.4 ± 1.6s, SI: 16.7 ± 0.9s, try5; GH: 15.4 ± 1.4s, PI: 15.7 ± 2.0s, SI: 15.7 ± 1.6s, $p = 0.948$).

Neurological screening

We compared the neuromuscular strength of GH, PI, and SI groups. Among the three groups, no difference was observed in grip strength test (GH: 79.2 ± 26.2N/g, PI: 70.0 ± 20.9N/g, SI: 67.5 ± 11.2N/g, $F_{2, 30} = 0.924$, $p = 0.408$) and wire hang

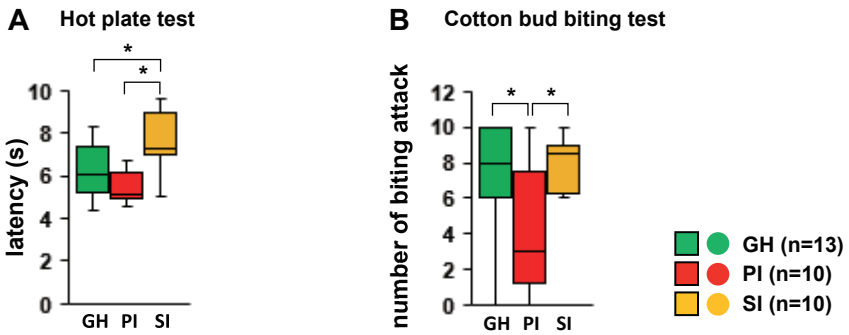

Fig. 3. Effect of isolation in the tests of evaluate pain sensitivity(A) and aggression(B). (A) The latency to the first paw response in the hot plate test. (B) The number of biting attacks on the cotton bud in the cotton bud biting test.* , significant difference among groups ($p < 0.05$).

test (GH: 30.3 ± 24.4s, PI: 27.6 ± 25.8s, SI: 37.8 ± 25s, $F_{2, 30} = 0.412$, p = 0.666).

Cotton bud biting test

Aggressive behaviors were evaluated in GH, PI, and SI groups. The PI group showed significantly fewer incidents of aggressive behavior than the other two groups (Fig. 3B, $F_{2, 30} = 3.534$, p = 0.042; GH vs. PI, p = 0.019; SI vs. PI, p = 0.040; GH vs. SI, p = 0.849).

Y-maze test

We examined the effect of isolation on the short-term spatial working memory of GH, PI, and SI groups by monitoring spontaneous alteration behavior in the Y-maze test. There was no significant difference among the three groups regarding the total distance traveled (Fig. 4A, $F_{2, 30} = 2.033$, p = 0.149) or the alteration (Fig. 4C, $F_{2, 30} = 2.907$, p = 0.070). The PI group markedly increased the number of arm entries (Fig. 4B, $F_{2, 30} = 4.316$, p = 0.022; GH vs. PI, p < 0.001; SI vs. PI, p = 0.037; GH vs. SI, p = 0.614).

Passive avoidance test

Learning function and the ability to register memory were evaluated in the GH, PI, and SI groups. The entry latency to the dark compartment on the first day was significantly longer in the SI group than in the other two groups (Fig. 4D, $F_{2, 30} =$

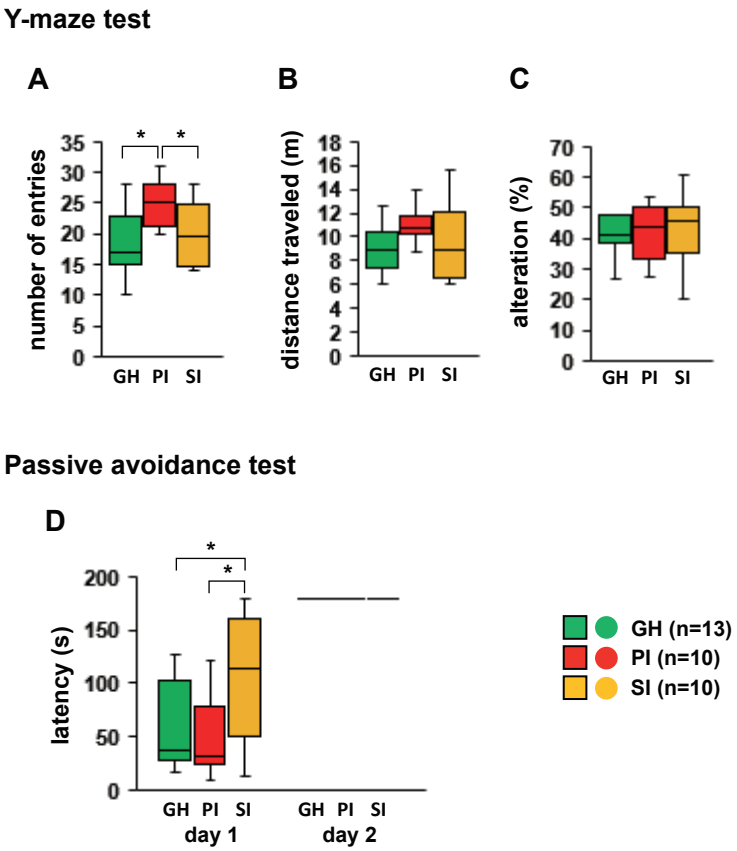

Fig. 4. Effect of isolation in the learning and memory test. (A-C) Y-maze test. (A) The number of arm entries, (B) the total distance traveled, and (C) the percentage of alterations. (D) The latency to enter the dark compartment in the passive avoidance test.*, significant difference among groups (p < 0.05).

3.982, p = 0.024; GH vs. SI, p = 0.027; PI vs. SI, p = 0.014; GH vs. PI, p = 0.679). On the second day, however, no entry into the dark compartment was observed in any of the three groups.

Sociability assessment test

In the sociability test (Fig. 2A), mice from the PI group traveled significantly longer distances than those from the GH group (Fig. 2B, $F_{2, 30}$ = 0.018, p = 0.053; GH vs. PI, p = 0.018; GH vs. SI, p = 0.526; PI vs. SI, p = 0.092). Moreover, those from the GH group spent significantly more time around the cage that had a stranger inside it than around an empty cage (Fig. 2C, GH, p = 0.076; PI, p = 0.534; SI, p = 0.151). A similar tendency was seen in the two other groups, although no significant difference was observed. No significant difference was observed among any of the groups in terms of the time spent around either of the two cages (Fig. 2D, $F_{2, 30}$ = 1.051, p = 0.362; GH vs. PI, p = 0.794; GH vs. SI, p = 0.172; PI vs. SI, p = 0.172).

In the social preference test, no significant difference in the total distance traveled was observed among the three groups (Fig. 2E, $F_{2, 30}$ = 0.631, p = 0.539; GH vs. PI, p = 0.316; GH vs. SI, p = 0.392; PI vs. SI, p = 0.888). Mice from the GH group spent a significantly longer time around the cage with the stranger mouse inside than around the cage with the familiar mouse inside (Fig. 2F, GH, p = 0.034; PI, p = 0.090; SI, p = 0.182). In the two other groups, although such tendencies were evident, no statistically significant difference was observed. Mice from the GH group spent a significantly longer time around either cage than those from the PI group (Fig. 2G, $F_{2, 30}$ = 2.994, p = 0.065; GH vs. PI, p = 0.028; GH vs. SI, p = 0.096; PI vs. SI, p = 0.577).

Tail suspension test and Porsolt forced-swim test

Effect of isolation on depressive-like behavior were evaluated. In the tail suspension test (Fig. 5A, Fig. 5B), the percentage of time spent immobile was significantly higher in the SI group than in the other two groups (Fig. 5A, $F_{2, 30}$ = 6.466, p = 0.005; GH vs. SI, p = 0.001; PI vs. SI, p = 0.016; GH vs. PI, p = 0.440). In the Porsolt forced swim test (Fig. 5C, Fig5D), the percentage of time spent immobile was significantly higher in the SI than in the GH group (Fig. 5C, $F_{2, 30}$ = 3.078, p = 0.061; GH vs. SI, p = 0.020; GH vs. PI, p = 0.452; PI vs. SI, p = 0.121).

DISCUSSION

The significance of this study is that it devised a mice model demonstrating the effects of a solitary living environment on behavior that is more similar to a human environment than the social isolation traditionally practiced in mice.

In this study, we proposed a new rearing method called physical isolation (PI), in which a mouse is aware of the presence of other mice visually by smell and sound but cannot communicate with them through contact. Then changes in rodent behavior were evaluated. The results showed no increase in anxiety or depression-like behaviors that have been suggested in the past to be higher due to stress. Aggression, which has also been reported to increase, was found to decrease. We also noted certain abnormalities in the sociability of the rodents.

No significant difference was observed among the PI, SI, or GH groups in terms of body weight, body temperature, or physical and learning capabilities. To measure physical capabilities, we performed the rotarod, wire hang, and grip strength tests, and to evaluate learning capabilities, we performed the Y-maze and passive avoidance tests. We found no significant differences in any of these abilities. In the passive avoidance test, the SI group showed longer latency. However, this is assessed as anxiety/depressive behavior, which is described below. Since no significant differences were observed among the three groups in this study, we assumed that the behavioral changes observed were psychologically

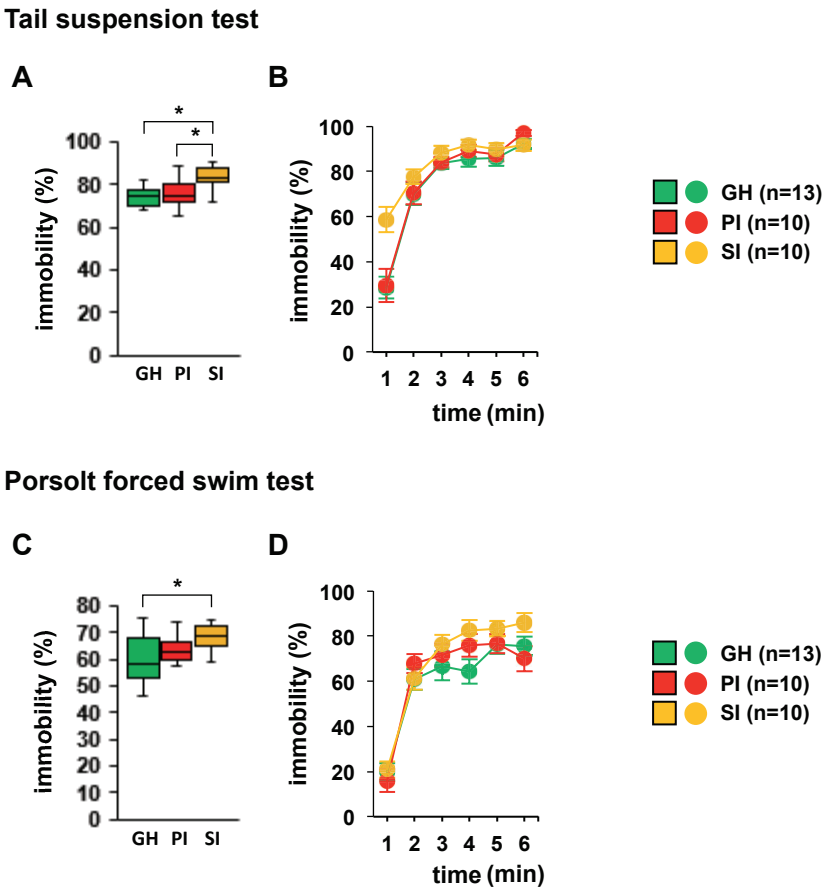

Fig. 5. Effect of isolation in anxiety and depressive behavior Test. (A, B) Tail suspension test. (A) The proportion of total time spent immobile and (B) the proportion of time spent immobile in each 1-min period. (C, D) Porsolt forced swim test. (C) The proportion of total time spent immobile and (D) the proportion of time spent immobile in each 1-min period. (B, D) Date are presented as means ± standard errors. *, significant difference among groups ($p < 0.05$).

based.

Lower pain sensitivity was seen in the SI group than in the GH and PI groups, although no significant differences were observed between the GH and PI groups. A hot plate test was performed for evaluation. Previous studies have reported that social isolation after weaning has sedative effects[7, 14], and the SI group results in this study are consistent with this, but the PI group results differ. Our findings suggest the possibility that, due to visual stimulation, the mice judged themselves to belong to a group, as well as the possibility that communication based on non-tactile senses affected the mice's reaction to pain stimulation.

Significantly lower aggression was observed in the PI mice compared to the GH mice, as shown by the cotton bud biting test. Social isolation after weaning increases aggression[4]. The central serotonin system is said to be involved[15], and this phenomenon is interpreted as an exacerbation of aggression due to stress. Some autism spectrum disorder model mice, in addition to a reduction in sociability, showed an increase in aggression, while others showed a reduction or no change[16, 17]. Therefore,

lower aggression in PI may represent some type of abnormality in social behavior. Another possibility is that PI may offer a lower stress environment than GH. The possibility that aggression is higher when raising multiple mice has been pointed out for some time, with struggles between individual animals being regarded as one reason[18, 19].

A higher activity volume was observed in the PI group than in the GH group. The Y-maze test showed no significant change in alternation, and that there were more entries. The sociability test also revealed that the total activity volume was high, which is in line with previous studies that have reported high voluntary activity in SI mice. Moreover, with the Y-maze test, some reports have shown low alternation or increased entries[20, 21]. However, our experiments did not confirm these changes. As described previously, low aggression was seen in PI, as were few anxious and depressive reactions (described later). This suggests that PI is an environment that exerts less stress than a group-rearing environment. In other words, it is possible that the increased volume of activity observed with PI on this occasion, which is also generally seen with SI, may not be caused by stress, but is instead indicative of a sociability disorder.

Sociability was shown to be impaired in the PI and SI groups. In the GH group, the rodents frequently chose to spend more time on the periphery of the cage with a stranger mouse inside than with an empty cage, and on the periphery of a cage with a new mouse inside than a cage with a familiar mouse inside. However, neither SI nor PI mice showed significant selectivity towards a cage with an unknown mouse or a cage with a new, stranger mouse. Previous studies have revealed that social isolation after weaning causes a loss of social behavior[22]. The impairment of social behavior seen in PI mice suggests the importance of contact-based communication for the development of sociability.

Our experiments confirmed that immobility time in the tail suspension test and the Porsolt forced-swim test was significantly longer in SI mice than in GH mice. However, the PI mice showed no significant difference from the GH group in any of the tests. Previous studies have pointed out that social isolation likely influences subjects with chronic stress and increases anxiety and depression-like behavior[23–26]. An increase in anxiety and depression-like reactions was also observed in SI mice but not in PI mice. This suggests that the PI environment may not constitute a large stress factor for a mouse.

Rearing mice in the partially isolated environment of PI that we adopted provides valuable information to help us understand and treat the behavior of people who have become less connected to society.

Social behaviors were confirmed to have been inhibited when adopting a new rearing method called PI. However, no higher levels of anxiety or depressive behavior, which are usually seen along with social isolation, were observed, and aggression was lower rather than higher.

PI is an environment in which visual and sound communication is possible. Our findings suggest that contact-based communication plays an important role in the development of sociability. The fact that anxiety and depressive behaviors were not higher suggests that physical isolation, unlike social isolation, may not be a particularly stressful environment for mice, even with limited communication taking place. In other words, a mouse may not experience a great deal of stress, provided communication is possible or it is fully aware of the presence of other mice, even if communication is limited.

Aggression was lower in the PI mice than in the GH mice. One reason for this might be related to impaired sociability. Another possibility, however, is that less stress is encountered in PI than in raising multiple mice together. In other words, physical isolation may be an environment that imposes little

or no stress, where no stress is experienced from group living, and no stress is experienced from isolation.

Physical isolation has aspects that resemble the low level of communication seen in socially withdrawn individuals and in groups of people on the autistic spectrum, as well as remote and partial communication sometimes seen in the general population. We believe that our findings, which show that mice placed in such an environment may be less stressed than when being raised in groups, despite suffering problems with their development of sociability, are of considerable interest.

In considering the future rearing of mice, and especially in evaluating sociability, we posit that physical isolation may be a useful raising method that can inhibit sociability with decreased stress levels. We feel this type of partial isolation to be of benefit for identifying which elements of solitary living serve as a source of stress. For example, by raising mice in isolation while showing them video material of mice being raised in groups, it might become possible to raise mice in an environment in which all forms of communication have been shut off, but in which they can still recognize a group.

The limitation of this study is that the factors responsible for the effects on behavior could not be clearly established due to the rather complex rearing method of PI, and further research is needed.

The authors have no conflicts of interest directly relevant to the content of this article

CONCLUSION

A new rearing method, called PI, inhibited social behaviors of the mice compared to group rearing, but suggested that the stress it inflicted may be the same or even less.

The effects of this partially isolated environment on mice provide valuable information for encouraging understanding and treatment of behaviors of people whose connection with society has become limited.

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