Video Article

A Mouse Model of Subchronic and Mild Social Defeat Stress for Understanding Stress-induced Behavioral and Physiological Deficits

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Abstract

Stressful life events often increase the incidence of depression in humans. To study the mechanisms of depression, the development of animal models of depression is essential. Because there are several types of depression, various animal models are needed for a deeper understanding of the disorder. Previously, a mouse model of subchronic and mild social defeat stress (sCSDS) using a modified chronic social defeat stress (CSDS) paradigm was established. In the paradigm, to reduce physical injuries from aggressors, the duration of physical contact between the aggressor and a subordinate was reduced compared to in the original CSDS paradigm. sCSDS mice showed increased body weight gain, food intake, and water intake during the stress period, and their social behaviors were suppressed after the stress period. In terms of the face validity of the stress-induced overeating and overdrinking following the increased body weight gain, the sCSDS mice may show some features related to atypical depression in humans. Thus, a mouse model of sCSDS may be useful for studying the pathogenic mechanisms underlying depression. This protocol will help establish the sCSDS mouse model, especially for studying the mechanisms underlying stress-induced weight gain and polydipsia- and hyperphagia-like symptoms.

Video Link

The video component of this article can be found at <http://www.jove.com/video/52973/>

Introduction

Many kinds of stressful events occur throughout the lives of humans. Excessive stress often leads to harmful physiological consequences in humans and animals. In humans, stressful events are major risk factors for precipitating psychiatric disorders such as depression¹. A Global Burden of Disease study indicated that depression is one of the most disabling disorders in terms of disability-adjusted life years (DALYs) and years lived with disability². In addition, depression accounts for the largest proportion of suicide DALYs³. People suffering from depression find it difficult to manage their lives, and as a result, their quality of life often worsens. Therefore, there is a strong need to develop effective therapeutics to improve the quality of life in these patients.

Many studies have been performed on major depressive disorders, and have revealed that the genetic contribution to disease susceptibility is approximately 30–40%, which is explained by the effects of multiple loci of small effects⁴. Because of the complex pathogenic mechanisms underlying depression, the detailed etiology of the disease remains elusive. Clinical reports indicate that there are subtypes of depression such as melancholic and atypical depression⁵, which show reduced and increased body weight, respectively⁶. Although 25–30% and 15–30% of patients with depression represent pure melancholic and atypical features, respectively, most of them have mixed features of both subtypes⁷. Therefore, major depression has a wide range of symptoms. In order to find biomarkers and develop objective therapeutics for the various types of depression in humans, it is important to establish several different animal models of depression⁸.

Animal models of depression have been established using several approaches including learned helplessness, chronic unpredictable mild stress, and chronic social defeat stress (CSDS)^{9–12}. Toyoda and colleagues established the CSDS models of rats and mice^{13–17} in order to elucidate the metabolism and behaviors that are associated with depression. Given that animal models of depression are evaluated by face validity¹⁸, the context within which the model is established is important. Moreover, Golden *et al.*¹⁹ reported the methods for creating CSDS mice in detail. It is known that the deficits in social behavior of CSDS mice can be recovered by chronic treatment, but not by acute treatment, with antidepressants, and that they share symptoms similar to those in patients with depression in terms of the regulation of brain-derived neurotrophic factor⁶.

Goto *et al.*¹³ previously developed the subchronic and mild social defeat stress (sCSDS) mouse model by modifying the methods of Golden *et al.*¹⁹. The sCSDS mice show polydipsia- and hyperphagia-like symptoms following gains in body weight and increased body water content¹³. In this report, the protocol for establishing the sCSDS mouse model is provided and we discuss the utility of this model.

Protocol

The animal studies were approved by and met the guidelines of both the Animal Care and Use Committee of Ibaraki University and the Ministry of Education Culture, Sports, Science, and Technology (MEXT), Japan (Notification No.71). A complete overview of the protocol is shown in **Figure 1**.

1. Apparatus

1. Prepare two kinds of cages: a single cage (width [W] × depth [D] × height [H] = 143 mm × 293 mm × 148 mm), and a “social defeat (SD)” cage (W × D × H = 220 mm × 320 mm × 135 mm).
2. As shown in **Figure 2**, divide the SD cage into two compartments with an acrylic transparent board divider (5-mm thick) with 15 circular holes (3 × 5 matrix: 8 mm in diameter).
3. Obtain wood-shaving chips made from spruce trees, purified-diet food pellets, and a drinking water bottle. In addition, obtain paper towels, a mask, and latex gloves.
4. For the social interaction test, prepare an open-field arena (W × D × H = 40 cm × 40 cm × 40 cm) made of gray polyvinylchloride, a weight made of steel (190 g), and a plastic interaction box (W × D × H = 10 cm × 10 cm × 13 cm; 100 g) with three wire-mesh windows (W × D = 5 cm × 5 cm) (**Figure 3**).
5. Place a CCD camera (2.8–12 mm; F = 1:1.4; 1/3 inch CCD) and an automated tracking system in the behavioral testing room in the animal facility. Place an appropriate shelf for the mouse cages in the behavioral testing room to habituate the mice to the environment of the testing room for at least 30 min.

2. Habituation to the Environment

1. Use male C57BL/6JmsSlc (B6) mice that are 7 weeks old and male Slc:ICR (ICR) mice that are over 5 months old and deliver to the facility from an animal breeding company.
2. Independently move two groups of B6 mice ($n = 12$ in each group) to the facility; use one group (screener B6 mice) for screening aggressive ICR mice and another group (subject B6 mice) for developing the sCSDS model.
3. Introduce ICR mice ($n = 12$) to the facility in order to screen their aggressive behaviors.
4. House the mice individually in single cages for 1 week under a 12-hr light-dark cycle (around 100 lux fluorescent light, lights on at 08:00) with constant temperature (around 23 °C) and humidity levels (around 40%) in order to habituate them to the environment. Partition each cage by placing white-colored plastic boards between the cages so that the mice are not affected by the behaviors of neighboring mice.
5. Make purified-diet food pellets and reverse osmosis water available ad libitum. Use AIN-93G chow because the ingredients of other non-purified diet pellets may vary.
6. Measure body weight, food intake (FI), and water intake (WI) of the mice every day. Calculate the body weight gain (BWG) from the initial day.

3. Screening of Aggressive ICR Mice

1. After habituation for 1 week as mentioned above, screen the ICR mice (resident) using resident-intruder tests for a 3 min trial with screener B6 mice (intruder; 8 weeks of age) in the afternoon (14:00-17:00) under lighting of around 300 lux in the housing room.
 1. Specifically, test each ICR mouse for three trials per day for 5 consecutive days (15 trials in total) toward many different B6 mice as possible to find which ICR mice show high aggression toward the intruders. During the tests, record the attack latency and duration of the aggressive behavior (rapid motions with attack biting).
2. Identify hyper-aggressive ICR mice by checking the damage wounds on B6 intruder mice after each trial.
3. Calculate the ratio of trials in which the attack latency is less than 30 sec as a first index of the aggression score.
4. Calculate the ratio of trials in which the attack latency is less than 3 min as a second index of the aggression score.
5. Evaluate the aggression scores from the first index. Use the second index when the first index is equal.
6. Select the ICR mice that had high aggression scores without hyper-aggressive behaviors as aggressive ICR mice. Use the aggressive ICR mice repeatedly throughout the next set of experiments until approximately 12 months of age; however, perform the screening process for aggressive ICR mice as described above before every experiment to confirm the aggressiveness of ICR mice.
7. After each session of the screening, record the degree of injury of the screener B6 mice. If a mouse is wounded, isolate the mouse in a single cage and observe the progress by checking its body weight gain, food intake, and water intake. In the case of severe wounds which affect physiology and behavior in mice, euthanize them according to local IACUC guidelines.

4. sCSDS

1. Introduce subject B6 mice 7 days before (day -6) the day of the initial stress exposure (day 1) and house the mice individually in single cages to habituate them to the environment.
2. Three days before day 1 (on day -2), move the aggressive ICR mice into a compartment of each SD cage, which is divided by an acrylic divider to let the mice establish their territories in the SD cages (the same number of subject B6 mice).
3. Divide the SD cages by using white-colored partitioning boards, as in the single-cage condition described above.
4. For non-stressed control B6 mice, prepare SD cages (half the number of mice) to keep the mice in pairs; place two mice into each compartment divided by the divider in an SD cage for 10 days.

5. After the daily BWG, FI, and WI measurements on day 1, place subject B6 mice into one of the compartments of the SD cages (resident's home-cage) in the afternoon (14:00–16:00) under lighting of around 300 lux in the housing room and measure the physical contact time from the first attack bite to complete the resident-intruder test.
6. On day 1, set the physical contact time to 5 min from the first attack bite and have observers count the number of attack bites by ICR mice that are directed preferentially at the back or flanks of the opponent as described by Miczek *et al.*²⁰ to determine the degree of physical stress to B6 mice.
7. After the physical contact time, rescue subject B6 mice, and check and record their fur status and wounds. Then, place the B6 mice into another compartment next to the ICR mice in the SD cages until exposure to physical stress the next day.
8. Because the acrylic divider in the SD cage is transparent and contains holes, expose subject B6 mice to various emotional stresses, including visual, auditory, and olfactory stimuli, from the ICR mice in the neighboring compartment of the SD cage for 24 hr every day.
9. Measure the BWG, FI, and WI of control B6 mice daily and then place them into each compartment in the SD cages for 1 day.
10. On day 2, after the daily measurements of BWG, FI, and WI, introduce subject B6 mice into the territories of other ICR mice to expose them to physical stress.
11. Set the duration of physical contact at 4.5 min from the first attack bite on day 2, and count the number of attack bites.
12. Move control B6 mice into different compartments and change the pair combinations in order to shuffle the placement and partner of the pairs every day.
13. Decrease the duration of physical contact by 0.5 min per day, so that the duration on day 10 is set to 0.5 min from the first attack bite.
14. On day 7, replace approximately half of the wood shavings in all of the compartments in the SD cages.
15. After exposure to the last physical stress on day 10, move subject B6 mice into single cages. Similar to the subject B6 mice, move control B6 mice into single cages on day 10.
16. If an ICR mouse does not show any attack bites until 5 min in each trial on days 1 to 10, terminate the trial. Replace the ICR mouse with spare aggressive ICR mice and conduct an alternative trial for the subject B6 mouse.
17. If a mouse is wounded, isolate the mouse in a single cage and observe the progress by checking its body weight gain, food intake, and water intake. Follow your IACUC guidelines for analgesia. In the case of severe wounds which affect physiology and behavior in mice, euthanize them according to local IACUC guidelines.

5. Social Avoidance Test (Social Interaction Test)

1. Perform behavioral tests for social avoidance in the morning (09:00–12:00) on day 11.
2. Thirty minutes before the test, transfer the cages of control and subject B6 mice onto a shelf (dim light of less than 1 lux) in the behavioral testing room under lighting of less than 20 lux to allow them to habituate to the environment.
3. To reduce order-effects, alternately test control and subject B6 mice.
4. Clean an open-field arena (illuminated with a light of 20 lux at the center of the field) and a plastic interaction box using paper towels soaked with 70% ethanol before the behavioral test for each mouse to remove feces, urine, and any odors.
5. Place an unfamiliar ICR mouse (not used as an aggressor) from a single cage near the open-field apparatus.
6. Place a B6 mouse into the open field of the vacant interaction box as shown in **Figure 4**. Monitor and analyze its behavior for 2.5 min using the automatic analysis system (described in step 5.10).
7. After the first trial, remove the B6 mouse from the field and place it into its home cage.
8. Following this, place the unfamiliar male ICR mouse (social target) into the interaction box and then introduce the B6 mouse into the open field and monitor its behavior for 2.5 min.
9. After the second trial, return both the B6 and ICR mice to their home cages, and clean the field and the interaction box as mentioned above.
10. Repeat these steps for each B6 mouse to test its social behavior. During each trial, record top-view movies using a CCD camera.
 1. After the behavioral tests, calculate the time spent in the interaction zone (in seconds) and in the corner zone (in seconds) for each trial as shown in Goto *et al.*¹³. Judge the position of the mouse based on the center of the mouse.
 2. Calculate the social interaction scores as $100 \times (\text{interaction time, with target})/(\text{interaction time, without target})$, following the methods published in Krishnan *et al.*²¹.

Representative Results

To monitor the degree of physical stress for over the 10-day periods, the number of attack bites by ICR mice was manually counted by a researcher. **Figure 5A** indicates the individual values for the number of attack bites received. There was considerable variability in the early stage (approximately 10–120 bites on day 1), but this variability was reduced in the later stage (approximately 5–20 bites on day 10). **Figure 5B** indicates that the average number of attack bites received gradually decreased over time, because the duration of the physical contact decreased (from 5 min to 0.5 min).

The subject mice showed weight gain changes, as well as changes in their daily consumption of food and water after exposure to sCSDS mice for 10 days. There was a significant difference in the total body weight gain between the control and subject mice ($p < 0.0001$, unpaired two-tailed Student's *t*-test). The total BWG of the control mice was approximately 0.5 g, whereas that of the subject mice was approximately 2.0 g (**Figure 6**). There was a suggestive difference in the total FI between the two groups of mice ($p = 0.0904$, unpaired two-tailed Student's *t*-test). The control and subject mice consumed approximately 30 g and 33 g of food, respectively (**Figure 7**). There was a significant difference in the total WI between the two groups ($p < 0.0001$, unpaired two-tailed Student's *t*-test). The total WI of the subject mice (approximately 80 g) was two times greater than that of the control mice (approximately 40 g), as shown in **Figure 8**.

For the social interaction score, there was a significant difference between the two groups ($p = 0.0033$, unpaired two-tailed Student's *t*-test). The control mice had a score of >100 , whereas the subject mice had a score of <100 (**Figure 9**).

Figure 1. Experimental schedule of the subchronic and mild social defeat stress (sCSDS) paradigm. [Please click here to view a larger version of this figure.](#)

Figure 2. Picture of the social defeat (SD) cage used in the subchronic and mild social defeat stress (sCSDS) experiments. [Please click here to view a larger version of this figure.](#)

Figure 3. Picture of the plastic interaction box with three wire-mesh windows that was used in the social interaction test. In order to prevent the mice from moving the box, a weight made of steel was placed on the box. [Please click here to view a larger version of this figure.](#)

Figure 4. Schematic representation of the social interaction test. [Please click here to view a larger version of this figure.](#)

Figure 5. Number of attack bites received from day 1 to day 10. The physical stress levels were evaluated by a researcher who counted the number of attack bites. **(A)** Individual data are represented ($n = 23$). Although there is a high amount of variability (approximately 10–120 bites) for the 5-min period in the early stage, this variability gradually decreased toward the late stage because the duration of the physical contact decreased (from 5 min to 0.5 min). **(B)** Data ($n = 23$) are shown as the mean $\pm$ the standard error of the mean. Although the number of attack bites was approximately 35–40 in the early stage, this number decreased to approximately 15–20 in the late stage. [Please click here to view a larger version of this figure.](#)

Figure 6. Body weight gain from day 1 to day 10. There is a significant difference between the control ($n = 24$) and subchronic and mild social defeat stress (sCSDS) ($n = 23$) mice in terms of the total body weight gain ($***p < 0.0001$, unpaired two-tailed Student's t -test). [Please click here to view a larger version of this figure.](#)

Figure 7. Food intake from day 1 to day 10. Although it was not significant, there is a suggestive difference between control ($n = 24$) and subchronic and mild social defeat stress (sCSDS) ($n = 23$) mice in terms of the total food intake ($†p = 0.0904$, unpaired two-tailed Student's t -test). [Please click here to view a larger version of this figure.](#)

Figure 8. Water intake from day 1 to day 10. There is a significant difference between control ($n = 24$) and subchronic and mild social defeat stress (sCSDS) ($n = 23$) mice in terms of the total water intake (** $p < 0.0001$, unpaired two-tailed Student's t -test). [Please click here to view a larger version of this figure.](#)

Figure 9. Social interaction score on day 11. There is a significant difference between control ($n = 24$) and subchronic and mild social defeat stress (sCSDS) ($n = 23$) mice for the social interaction score (** $p = 0.0033$, unpaired two-tailed Student's t -test). [Please click here to view a larger version of this figure.](#)

Discussion

There were definitive differences in body weight between sCSDS mice and CSDS mice subjected to the standard CSDS protocol (5 to 10 min of physical contact with aggressors per day)¹⁹. The sCSDS mice showed increased BWG during the stress period, whereas the standard CSDS mice showed a decrease in body weight during the stress period^{21,22,23}. There is a substantial difference between the two protocols in terms of the total duration of physical contact with aggressors during the 10-day stress period. While the original CSDS paradigm includes 50 or 100 min of physical interaction between the aggressor and the subordinate mice in total (a full-scale CSDS), the sCSDS method included 27.5 min of this interaction in total (a half-scale CSDS). Although the intensity of the physical stress on the standard CSDS mice was unknown¹⁹, it was confirmed that the average number of attack bites received was approximately 30 per day in sCSDS mice. As mentioned in Goto *et al.*¹³, sCSDS mice are a milder model of depression compared to the standard CSDS mice (the social aversion is sustained for more than 1 month after the last defeat stress)²¹ because the sCSDS mice recover normally within 1 month after the last defeat stress. Additionally, Savignac *et al.*²⁴ reported that the relatively short physical contact with aggressors could accelerate the BWG of subject mice during the stress period. Interestingly, Warren *et al.*²³ have reported that emotionally stressed mice that only witnessed the physical contact of other mice during the CSDS paradigm showed decreased BWG without physical stress. In this manner, the original and modified CSDS paradigms produce various models showing a broad range of BWG phenotypes¹³. These manifestations are similar to those observed in patients suffering from melancholic and atypical depression, including losses and gains in body weight, respectively⁶. In addition, several lines of evidence suggest that severe stressful life events including

physical abuse are strongly associated with depression in humans^{25,26,27}. In terms of the face validity¹⁸ for stress-induced weight gain and overeating, the sCSDS mice may have some features related to atypical depression in humans.

However, there are some limitations of the sCSDS mice as a model of depression. In animal models, depression-like alterations (e.g., anhedonia in the sucrose preference test, despair-like behaviors in the forced swim and/or tail suspension tests) are used to evaluate the models. Although there were no differences between sCSDS and non-stressed mice in terms of their despair-like behaviors¹³, anhedonia should be analyzed in the future. Moreover, molecular changes in the hypothalamus-pituitary-adrenal (HPA) system, immune system, and central nervous system should be investigated. In addition, drug experiments with antidepressants should be conducted. As mentioned above, the face, construct, and predictive validities for a model of depression should be accumulated for the sCSDS mice.

The sCSDS mice showed excessive WI behaviors (stress-induced polydipsia-like symptoms). In patients suffering from psychiatric disorders, psychogenic polydipsia and/or water intoxication with polyuria and polydipsia are commonly observed²⁸. Although there are some treatments including fluid restriction and behavioral and pharmacologic therapies²⁹, the underlying pathophysiology of these symptoms remains unclear. Therefore, animal models of polydipsia are essential for understanding the mechanisms of stress-induced polydipsia. Polydipsic STR/N mice³⁰ and schedule-induced polydipsia models³¹ are well-known models of polydipsia. In addition to these models, the sCSDS mice are a useful model of polydipsia that can be used to understand the features of stress-induced polydipsia.

Furthermore, the authors believe that feeding the mice a purified diet (AIN-93G), rather than an unpurified diet, is exceedingly important for establishing the sCSDS mice. Because gut microbiota can influence animal behaviors³², the intestinal bacterial flora of each animal and indigenous bacterial flora of each animal facility should be investigated in the future. Until now, evidence regarding the use of sCSDS mice as a model of depression was lacking, and while the previous findings of Goto *et al.*^{13,14} support their use, additional research on this model should be conducted to increase the validity of sCSDS mice as a model of depression. However, the results of the current study suggest that the use of sCSDS mice is advantageous because the sCSDS mice can help us further understand the mechanisms underlying stress-induced weight gain and polydipsia- and hyperphagia-like symptoms.

Disclosures

The authors declare that they have no competing financial interests.

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