Behavioural Processes 122 (2016) 21–25

Contents lists available at ScienceDirect

Behavioural Processes

journal homepage: www.elsevier.com/locate/behavproc

# Subchronic and mild social defeat stress alter mouse nest building behavior

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ARTICLE INFO

*Article history:*
Received 22 July 2015
Received in revised form 8 October 2015
Accepted 25 October 2015
Available online 31 October 2015

*Keywords:*
Attention
Depression
Mouse
Nest building
Social defeat stress

ABSTRACT

Behavioral and physiological evaluations of animal models of depression are essential to thoroughly understand the mechanisms of depression in humans. Various models have been developed and characterized, and the socially defeated mouse has been widely used for studying depression. Here, we developed and characterized a mouse model of social aversion using a subchronic and mild social defeat stress (sCSDS) paradigm. Compared to control mice, sCSDS mice showed significantly increased body weight gain, water intake, and social aversion to dominant mice on the social interaction test. We observed nest building behavior in sCSDS mice using the pressed cotton as a nest material. Although sCSDS mice eventually successfully built nests, the onset of nest building was severely delayed compared to control mice. The underlying mechanism of this significant delay in nest building by sCSDS mice is unclear. However, our results demonstrate that nest building evaluation is a simple and useful assay for understanding behavior in socially defeated mice and screening drugs such as antidepressants.



## 1. Introduction

Many developed nations have emphasized improving mental health due to increased health problems related to various social stresses. According to the World Health Organization, more than 350 million people suffer from depressive disorders worldwide (WHO, 2012). In particular, chronic social and psychological stresses increase the incidence of depressive disorders in humans; however, our understanding of the precise mechanisms of depression is limited. Uncovering them will require the establishment and characterization of an appropriate animal model. Recently, chronic social defeat models of mice and rats have been widely used to clarify the basic mechanisms of depression (Tsankova et al., 2006; Krishnan et al., 2007; Iio et al., 2011). There are several assessments to evaluate model appropriateness including the open field test, elevated plus maze test, forced swim test, and tail suspension test (Porsolt et al., 1977; Steru et al., 1985; Dere et al., 2002; Rygula et al., 2005). For the chronic social defeat stress (CSDS) mouse model, the social interaction test has been frequently used to observe their typical social aversion behaviors toward dominant mice (Krishnan et al., 2007). This test identifies animals who are susceptible and resilient to social stress, and some molecular and physiological differences have been identified in these two groups (Berton and Nestler, 2006; Krishnan et al., 2007). For example, Hodes et al. (2014) reported that vulnerability to social stress in CSDS mice is related to peripheral interleukin-6 expression.

We have recently developed and characterized a subchronic and mild social defeat stress (sCSDS) mouse model (Goto et al., 2014, 2015a). We found that the feed quality affects the social aversion behavior of sCSDS mice in social interaction testing, but there is no information about feed nutrients improving resilience to social stress (Goto et al., in press). Other environmental factors also affect aversion behavior in socially defeated mice, and another reproducible behavioral test would be useful for measuring stress vulnerability in the social defeat mouse model using relatively small numbers of animals.

In this study, we tried to develop a more simple and reproducible method to assess the behavioral deficits of sCSDS mice and focused on nest building behaviors. Mouse nest building is a complex and innate goal-directed behavior to protect them from predators and maintain their body temperature (Kinder, 1927; Van de Weerd et al., 1997; Heller et al., 2014). Furthermore, some authors have

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http://dx.doi.org/10.1016/j.beproc.2015.10.018
0376-6357/

found that nest building requires attention processes mediated by the frontal cortex and hippocampus (Kolb, 1984; Carter et al., 2000; Deacon et al., 2002, 2003; Filali and Lalonde, 2009). Notably, severe nest building deficits were observed in mouse models of psychiatric disorders such as schizophrenia (Takao et al., 2013). In the present study we observed the effects of sCSDS on mouse nest building behavior and physiological parameters including body weight gain (BWG) and food and water intake (FI and WI, respectively).

## 2. Materials and methods

### 2.1. Animals

Animal experiments were performed as previously reported (Goto et al., 2014). Male C57BL/6JJmsSlc (B6) mice (7 weeks old) and male Slc:ICR (ICR) mice (older than 5 months) were acquired from SLC Japan (Shizuoka, Japan), and individually housed in single cages with corn-cob bedding. The AIN-93G pellet diet (Oriental Yeast, Tokyo, Japan) and standard laboratory pellet diet (MF diet, Oriental Yeast) were fed to B6 and ICR mice, respectively. This study met the guidelines of the Animal Care and Use Committee of Ibaraki University, Japan.

### 2.2. Experimental design

The sCSDS paradigm and behavioral tests were performed as previously reported (Goto et al., 2014). Nest building was assessed after social defeat stress on day 10, and social interaction was tested on day 11.

### 2.3. sCSDS model

A modified sCSDS paradigm (Goto et al., 2014) was performed from 10:30 am to 13:30 pm in accordance with a standardized protocol (Golden et al., 2011). Detailed methods were described previously (Goto et al., 2014, 2015a, in press). Briefly, the duration of physical contact was set at 5 min after the first attack bite on day 1, and then was reduced 0.5 min per day from days 2 to 10. From days 1 to 10, subject mice (B6) were exposed to a different ICR aggressor mouse each day beginning at 11:00 am. After physical contact, test mice were moved into the neighboring compartment with a another aggressor for 24 h in a two-compartment social defeat (SD) cage (220 mm × 320 mm × 135 mm; Natsume Seisakusho, Tokyo, Japan).

### 2.4. Nest building assessment

On the final day of sCSDS (day 10), nest building behavior was evaluated beginning at 11:10 am. Nest building behavior was assessed using a standardized scoring scale as previously described (Deacon, 2006, 2012). Compressed cotton (Nestlet, Ancare; UK agent, Lillico) was used as a nest material. Nestlets (~3 g) were put in the compartments of the SD cages. Finished nest structure scoring was done as according to standard criteria (Deacon, 2006, 2012). In this experiment, we assessed the nests using a Deacon score from 1 to 5 (Deacon, 2006) every hour in the light period (11:10 am–19:00 pm). Finally, we also assessed the nest scores after a dark period (10:00 am on day 11, about 23 h after starting the test).

### 2.5. Social interaction test

The social interaction test was performed on day 11 as described previously (Goto et al., 2014). Social interaction scores (target presence/target absent) were calculated as 100 × (interaction time, target present)/(interaction time, target absent) (Krishnan et al., 2007).

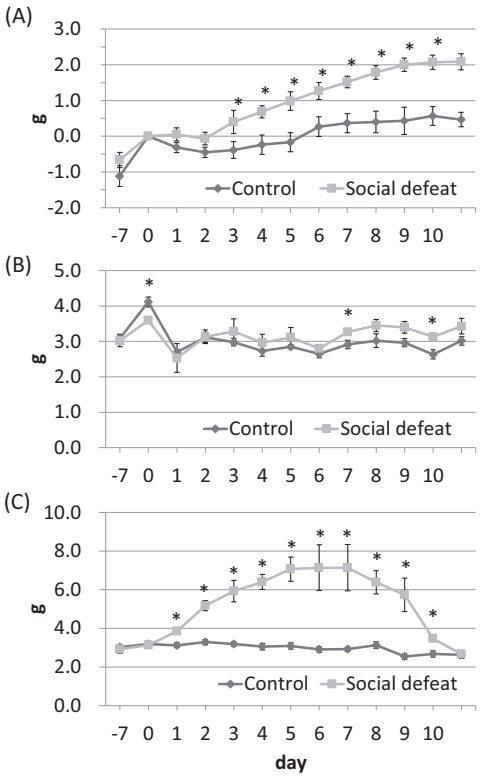


**Fig. 1.** Effects of subchronic and mild social defeat stress (sCSDS) on general physiological features. (A) Temporal changes in body weight gain ($n = 6$ per group). Two-way repeated measures ANOVA revealed a significant effect during exposure to stress ($F_{1,119} = 12.734$, $p = 0.0051$). (B) Temporal changes of food intake ($n = 6$ per group). Two-way repeated measures ANOVA revealed no significant effect during exposure to stress ($F_{1,119} = 3.159$, $p = 0.1059$). (C) Temporal changes of water intake ($n = 6$ in each group). Two-way repeated measures ANOVA showed a significant effect during exposure to stress ($F_{1,119} = 35.635$, $p = 0.0001$). Data are expressed as the mean ± SEM (*$p < 0.05$ versus control).

### 2.6. Statistical analysis

BWG, FI, and WI were analyzed using two-way repeated measures analysis of variance (ANOVA) followed by Bonferroni post-hoc tests to compare factor in terms of "stress." The other data were tested with unpaired two-tailed Student's *t*-tests. Non-parametric tests were used to assess nest building, and the Mann–Whitney *U* test was employed for independent data sets (Deacon, 2006, 2012; Heller et al., 2014). Data were analyzed by Excel Toukei 2006 for Windows (Social Survey Research Information Co., Ltd., Tokyo, Japan) and are shown as means ± standard errors of the mean (SEMs). The significance threshold was set at $p < 0.05$.

## 3. Results

### 3.1. BWG, FI, and WI

We assessed BWG, FI, and WI to evaluate the influence of the sCSDS model on general health. Two-way repeated measures ANOVA revealed a significant effect of stress on BWG for the 10 consecutive days in the stress period ($F_{1,119} = 12.734$, $p = 0.0051$). As shown in Fig. 1A, the mean body weight of defeated mice was higher than that of control mice. Fig. 1B shows that there was no significant difference in FI between the two groups for these 10 days