JARQ 55 (1), 77-83 (2021) https://www.jircas.go.jp

The Effects of Chronic Social Defeat Stress on Solid Gastric Emptying and Defecation in C57BL/6J Mice

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Abstract

Stress contributes to mental disorders as well as functional gastrointestinal disorders. In functional gastrointestinal disorders, gastrointestinal motility is thought to play a role. Therefore, we evaluated the effects of chronic social defeat stress on gastrointestinal motility in C57BL/6J mice, by measuring gastric emptying, fecal pellet output, and gastrointestinal transit. In mice subjected to 10 days of social defeat stress, serum corticosterone concentrations were significantly higher, relative tissue weights of spleen and adrenal gland tissues were significantly heavier, and thymus sizes were smaller than in the control mice. Stressed mice exhibited social avoidance behavior in a social interaction test and anxiety-like behavior and lower locomotor activity in an elevated plus maze test. In gastric emptying test, stressed mice displayed increased gastric emptying rate with significant suppression of short-time (30 min.) test diet intake. Fecal pellet output and gastrointestinal transit were not different in control and stressed mice. These results suggest that chronic social defeat stress influences gastric motility. Thus, the social defeat stress model may be useful for studying psychiatric disease and functional gastrointestinal disorders simultaneously.

Discipline: Animal Science
Additional key words: anxiety-like behavior, social interaction

Introduction

Social stress is a part of modern society, contributing to the increase in stress-related diseases (Huhman 2006). The response to stress can result in functional gastrointestinal disorders, such as functional dyspepsia and irritable bowel syndrome. Chronic stress also contributes to psychiatric disorders, such as depression and anxiety (Gros et al. 2009), which are known to be highly comorbid with functional gastrointestinal disorders (Pinto-Sanchez et al. 2015, Van Oudenhove et al. 2010). In some cases, antipsychotic drugs are used to treat functional gastrointestinal disorders (Dekel et al. 2013, Jackson et al. 2000). Nevertheless, it is unclear why these diseases, psychiatric disorders and functional gastrointestinal symptoms, show close relationships under conditions of stress.

In the animal industry, animals during weaning periods are subject to physiological and psychosocial stresses, such as maternal separation, handling by humans, and social mixing with unfamiliar partners at the relocation site. Therefore, it is important to understand the physiological and behavioral problems induced by stress (Toyoda 2017).

Gastrointestinal dysmotility is important in the etiology of functional gastrointestinal disorders. Up until now, the relationship between stress and the development of gastrointestinal disorders has been examined in experimental animal models of acute restraint stress. Acute restraint stress delays gastric emptying (GE) and stimulates defecation in mice (Martinez et al. 2004) and rats (Nakade et al. 2005, Nakade et al. 2007). However, the effects of chronic stress on GE and fecal output have not been fully clarified. An increasingly used model is

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Received 17 December 2019; accepted 23 March 2020.

chronic social defeat stress (CSDS) (Hammack et al. 2012, Iio et al. 2012, Rygula et al. 2005, Warren et al. 2013), which occurs when an animal experiences daily social encounters with an aggressive partner and is housed in a building where it exposed to continuous sensory contact with the aggressors. CSDS has been utilized as a more natural stress, because it mimics the social conflicts that repeatedly occur in our lives (Tamashiro et al. 2005), and thus it constitutes an appropriate model for studying psychiatric disorders and gastrointestinal dysfunction caused by stress.

In our previous study, we found that chronic social defeat stress downregulates the expression of tight junction genes in the colon (Yamagishi et al. 2019), and modifies the diversity of microbiota in cecal contents and feces (Aoki-Yoshida et al. 2016). In order to investigate how CSDS influences gastrointestinal motor function, we examined the effects of CSDS on solid GE, fecal pellet output, and the gastrointestinal transit of administered feed in C57BL/6J mice.

Materials and methods

1. CSDS exposure and experimental design

Male retired-breeder ICR mice >4 months of age (Japan SLC, Inc.) were purchased and screened for use as the stressors as previously described (Golden et al. 2011). The selected aggressive ICR mice were then habituated for 3 days in social defeat cages (22 × 32 × 13.5 cm; Natsume Seisakusho Co., Ltd.) that had been separated into two compartments with clear perforated plastic dividers. The mice were fed a conventional diet (MF; Oriental Yeast Co., Ltd.) and given *ad libitum* access to tap water. Male C57BL/6JJmsSlc (B6) mice 6 weeks of age (Japan SLC, Inc.) were singly housed and acclimated for 1 week to standard housing conditions on a 12 h light/dark cycle (lights on at 07:00). The B6 mice had free access to a diet of AIN-93G (Oriental Yeast Co., Ltd.) and distilled water. Body weights and food/water intakes were measured every day at 10:00. The B6 mice were then assigned to either a control or stress group so that the average body weights of the groups were equal.

All experiments were approved by the experimental animal ethics committee of the National Agriculture and Food Research Organization (Tsukuba, Japan; Permit Nos. 14013101 and 14113017) and conducted in accordance with their guidelines. The 10-day CSDS paradigm was performed according to an established protocol (Golden et al. 2011). The 10-min. daily interaction with the aggressor (i.e., defeat session) was shortened if the target B6 mouse was wounded and/or bleeding occurred during the first 5 min. after an attack. All defeat sessions were conducted between 10:00 and 13:00. Three experiments were used to study the animals (Fig. 1). In experiments 1and 2, mice (n = 6/group) were subjected to behavioral tests and blood and tissue sampling. In experiment 2, GE was also measured. In experiment 3, mice (control group, n = 5; stress group, n = 6) were subjected to behavioral tests and measurement of gastrointestinal transit.

2. Behavioral tests

In all experiments, social interaction (SI) test was performed on day 11 (d11) as previously described (Yamagishi et al. 2019). On d12, elevated plus maze (EPM) test was performed as previously described (Goto et al. 2014) with a minor change. The times spent in open and closed arms, the total distance traveled, and the frequency of entries into each arm during a 5 min. test session were measured with Time EP1 software (O'Hara & Co. Ltd.). Behavioral tests were conducted between 10:00 and 14:00. For these tests, B6 mice were placed in the testing room 30 min. before the test and acclimated to the room illuminated with 20 lux.

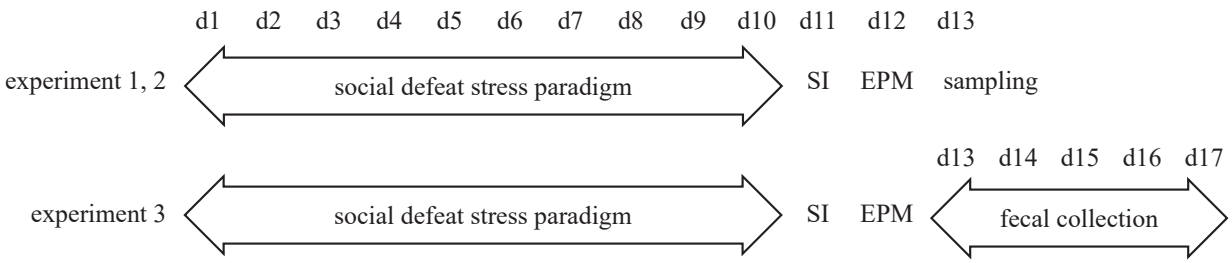

Fig. 1. Experimental design of the CSDS paradigm, behavioral testing, and sampling
SI: social interaction test
EPM: elevated plus maze test
In experiments 1 and 2, mice (n = 6/group) were used for behavioral testing and blood/tissue sampling; GE was measured in mice in experiment 2.
In experiment 3, mice (control group, n = 5; stress group, n = 6) were used for behavioral testing and measurement of gastrointestinal transit.

3. Measurement of GE

In the morning on d13 of experiment 2, B6 mice were given AIN-93G containing 1% chromium oxide (Cr_2O_3; Wako Pure Chemical Industries, Ltd.) as a meal marker for the solid phase. To encourage mice to eat the test diet as soon as it is provided, on d12, the mice were given 75% of the amount of food consumed on d11. The mice were euthanized by decapitation 30 min. after the start of eating, and their stomachs and small intestines were collected. The remainder of the test diet was collected, and the intake amount was calculated. The Cr content in tissue samples was measured by the colorimetric method (Takemasa 1992). The percentage of GE was calculated as the Cr concentration in the small intestine/total Cr concentration in the stomach and small intestine × 100.

4. Measurement of defecation

(1) fecal pellet output

In all experiments, the number of fecal pellets produced during the SI test was counted (Barone et al. 2008, Julio-Pieper et al. 2010).

(2) gastrointestinal transit

B6 mice were given AIN-93G containing 1% Cr_2O_3 after EPM test on d12 of experiment 3. Feces were collected from the floors of the mouse cages at 12, 24, 36, 48, 72, 96, and 120 h after the test diet was provided. The remainder of the test diet was collected after 24 h, and the intake amount was calculated. The Cr content in feces samples was measured (Takemasa 1992) and the recovery rate (%) of Cr from the fecal samples was calculated as the total amount of Cr from collected feces/Cr intake from the test diet × 100.

5. Tissue sampling and measurement of plasma hormone concentration

For experiments 1 and 2, B6 mice were euthanized by decapitation and trunk blood was collected in tubes containing EDTA-2K (Terumo) and kept on ice. The blood samples were centrifuged at 1,500 × g at 4°C for 20 min., and the plasma was stored at −80°C for subsequent analyses of hormone concentrations. The spleen, thymus, and bilateral adrenal glands were removed from each mouse and weighed for analyses of stress-sensitive tissues (Ochi et al. 2008, Reber et al. 2006, Tramullas et al. 2012). Plasma corticosterone concentrations and adrenocorticotropic hormone (ACTH) levels were measured using a corticosterone enzyme-linked immunosorbent assay kit (Enzo Life Science, Inc.) and an ACTH (rat, mouse) enzyme immunoassay kit (Phoenix Pharmaceuticals, Inc.), respectively.

6. Statistical analysis

All statistical analyses were conducted according to a randomized block design using the GLM procedure of SAS (version 9.4; SAS Institute, Inc.). Each experiment was treated as a block in the statistical analysis. CSDS was a fixed effect for analyses of body weight gain, food and water intake, plasma hormone concentrations, tissue weights, EPM results, GE, and fecal pellet output. The results from the SI tests were analyzed with CSDS, exploration, and the CSDS by exploration interaction as the fixed effects. For the response criteria with a significant CSDS by exploration interaction, the means were separated by the PDIFF option with a Tukey–Kramer adjustment. For the analysis of gastrointestinal transit data, each mouse was considered as a block, and CSDS, time, and CSDS by time interaction were the fixed effects. The results were considered significant at $P < 0.05$ and marginally significant at $0.05 \leq P \leq 0.10$. The data are expressed as means ± the standard errors of the means (SEMs).

Results

1. Effects of CSDS on dietary consumption and body and tissue weights

Figure 2 shows the mean changes in body weight (a), food intake (b), and water intake (c) for mice in the control and stress groups during the experiment. During the social defeat stress paradigm, stressed mice had significantly greater daily water consumption (control, 3.79 ± 0.18; stress, 5.51 ± 0.30, $P < 0.01$) and daily body weight gain (control, 0.09 ± 0.02; stress, 0.17 ± 0.02, $P < 0.01$), although there was no significant difference between the groups in food intake (control, 2.77 ± 0.07; stress, 2.75 ± 0.06) as previously reported (Goto et al., 2014). After the stress paradigm, there was no significant difference between the groups in body weight gain (control, 0.18 ± 0.02; stress, 0.19 ± 0.02) and food (control, 2.53 ± 0.09; stress, 2.69 ± 0.07) and water intake (control, 3.99 ± 0.16; stress, 4.39 ± 0.21).

The spleen and adrenal gland tissues from mice in the stress group were significantly heavier than those from the control mice, although the thymus tissues from mice subjected to CSDS were significantly lighter than those from the control mice (Table 1).

2. Stress-related behaviors and plasma hormone concentrations

Table 2 lists the results from the behavioral tests. Although CSDS did not significantly affect SI behaviors, it induced social avoidance. Mice in the stress group stayed longer in the avoidance zone when a target mouse

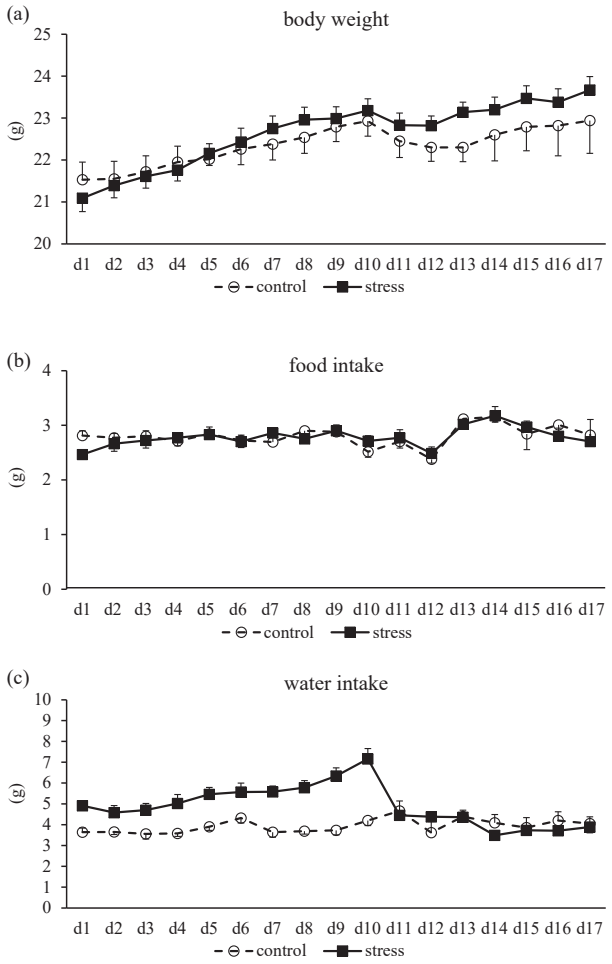

Fig. 2. Changes in body weight, food intake, and water intake

Body weight (a), food intake (b), and water intake (c) were measured every day.

Averages during the CSDS paradigm were calculated using the data of three experiments (n = 17 controls, 18 stress group).

For average after the CSDS paradigm, only the data of experiment 3 were used (n = 5 controls, 6 stress group).

Data are the means ± SEMs.

Table 1. Effect of CSDS on stress-sensitive tissue weights

	control	stress
(mg/g BW)		
spleen	2.40 ± 0.15	4.00 ± 0.36 **
adrenal gland	0.18 ± 0.02	0.23 ± 0.02 **
thymus	1.28 ± 0.14	0.92 ± 0.05 **

Data are the means ± SEMs (n = 12/group).
BW: body weight
**$P < 0.01$

was present; there was no significant difference in the time spent in the avoidance zone in the no target condition. In the EPM test, mice that were subjected to CSDS exhibited anxiety-like behaviors, such as spending less time in the open arms and more time in the closed arms than the control mice. Furthermore, the mice in the stress group had lower locomotor activities, such as travelling significantly shorter total distances and making fewer entries into each of the arms than the control mice.

The increase in stress-related behaviors in mice subjected to CSDS was accompanied by significantly higher plasma levels of corticosterone than in the control mice (Fig. 3 (a)). However, the concentrations of ACTH did not differ between the groups (Fig. 3 (b)).

3. Effect of CSDS on short time intake and GE

Gastric motor function was estimated by measuring GE. Although all mice began feeding immediately after the Cr-containing test diet was provided, the short-time (30 min.) intake was significantly lower in mice subjected to CSDS than in the control mice (Fig. 4 (a)). However, the GE rate was significantly increased in mice from the stress group compared with that in the controls (Fig. 4 (b)).

4. Effect of CSDS on defecation

The fecal pellet output during the 5 min of the SI test was measured as an estimate of colonic motor function. The number of fecal pellets from mice in the stress group did not differ from that of the control mice (0.12 ± 0.08 versus 0.06 ± 0.06, respectively). To further examine whether CSDS produces a constipation-like effect, gastrointestinal transit was estimated by the recovery of Cr in fecal samples in experiment 3. The average consumption of the test diet did not differ between the stress and control groups (control, 2.38 ± 0.16; stress, 2.49 ± 0.08). The recovery rates of orally consumed Cr in feces over 5 days did not differ between the groups (Fig. 5). Thus, CSDS did not affect gastrointestinal transit.

Discussion

This study evaluated the effects of CSDS on gastrointestinal motor function. GE and gastrointestinal transit rates were estimated by marking the solid phase with Cr_2O_3, which is not absorbed by the gastrointestinal tract and is widely used as an external indicator for determining the nutritive value of diets (Garcia-Rico et al. 1999, Milton Bell & Keith 1992, Nakayama et al. 1960). Ten days of social defeat stress resulted in no alteration of fecal pellet output or gastrointestinal transit, but changed short-time feeding and the GE rate.

Table 2. Effect of CSDS on behavior

	control	stress
social interaction test		
time spent in interaction zone (s)		
no target	63.7 ± 4.2	58.6 ± 4.9
target	102.1 ± 4.7	83.0 ± 8.8
time spent in avoidance zone (s)		
no target	22.5 ± 4.8	19.0 ± 3.7
target	10.2 ± 1.5	23.3 ± 7.9 #
elevated plus maze test		
anxiety behavior		
time spent in open arms (%)	17 ± 2	11 ± 1 **
time spent in closed arms (%)	38 ± 3	51 ± 3 **
locomotor activity		
total distance traveled (cm)	1,113 ± 48	865 ± 48 **
total number of entries into arms	20 ± 1	15 ± 1 **

Social interaction tests were performed on d11 and elevated plus maze tests on d12.
Data are the means ± SEMs (control group, n = 17; stress group, n = 18).
$^{**}P < 0.01$; $\#P = 0.07$

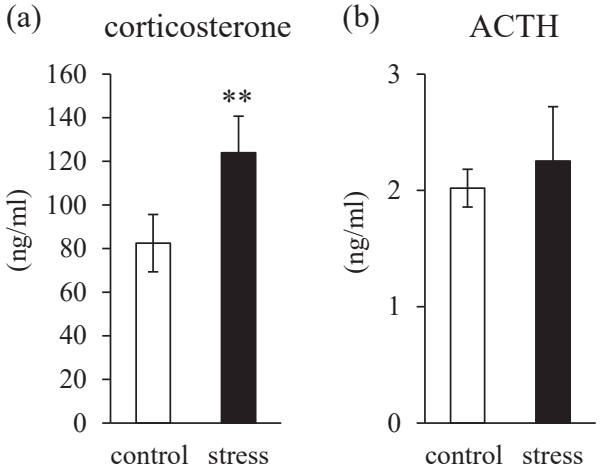

Fig. 3. Effect of CSDS on stress-related hormone concentrations in plasma
Trunk blood was collected on d13, and plasma was prepared with EDTA-2K to measure corticosterone (a) and ACTH (b) concentrations.
Data are the means ± SEMs (n = 12/group).
$^{**}P < 0.01$

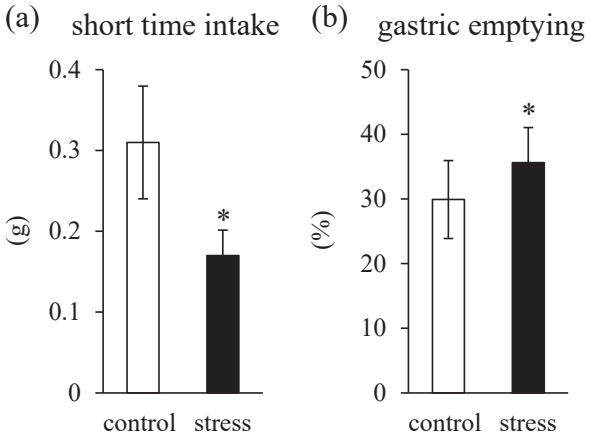

Fig. 4. Effect of CSDS on short time intake and GE rate
In experiment 2 (n = 6/group), Cr containing test diet intake for short time (a) and solid GE rate (b) were measured on d13.
Data are the means ± SEMs.
$^{*}P < 0.05$

The findings reveal that CSDS may affect upper gastrointestinal motor function in mice. Additionally, CSDS increased plasma corticosterone concentrations and adrenal gland/spleen tissue weights, along with thymic atrophy, well-known chronic stress-induced physiological markers (Ochi et al. 2008, Reber et al. 2006, Tramullas et al. 2012). Although plasma ACTH concentrations were not significantly increased, the hypothalamic-pituitary-adrenal axis was likely activated by the CSDS exposure, which led to social avoidance and anxiety-like behaviors in these mice.

Functional dyspepsia is defined as abnormal sensations and movement in the upper gastrointestinal tract, experienced as discomfort and pain, a restriction of meal size, and delayed GE, in the absence of an observable structural abnormality. In the present study, mice subjected to CSDS did not consume as much during the

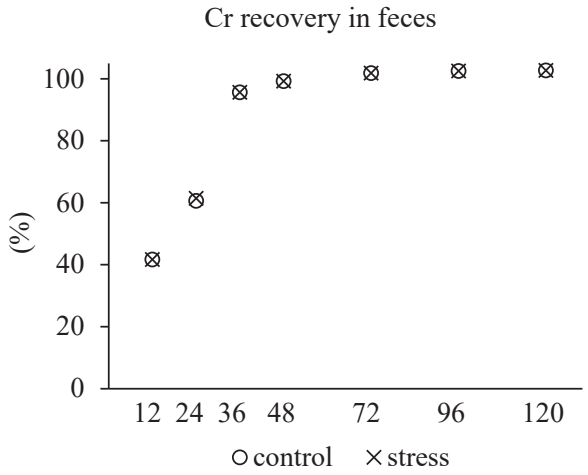

Fig. 5. Effect of CSDS on gastrointestinal transit
In experiment 3 (n = 5 controls, 6 stress group), after the Cr containing test diet was provided on d12, gastrointestinal transit was measured from 12 h to 120 h.
Data are the means ± SEMs.

short-time (30 min.) meal, suggesting that gastric accommodation was disturbed and meal volume restricted. However, the relationship between gastric motor function and the smaller meal size in these mice is not clear. Alternatively, CSDS had no effect on cumulative daily food intake during and after the CSDS paradigm. We previously reported that subchronic and mild social defeat stress accelerated food intake after the stress period (Goto et al. 2014). These inconsistent results may be related with the difference in the length and/or intensities of social defeat stress.

Acute stress has been shown to delay GE in experimental animal models (Nahata et al. 2014, Nakade et al. 2005), whereas the effects of chronic stress on gastric function differ. For example, 5 days of chronic restraint stress do not alter GE or gastric motility (Babygirija et al. 2010, Zheng et al. 2009). In contrast, Ochi et al. (2008) found that continuous stress induced by a water-soaked cage delayed GE for 24 h, but accelerated it after 3 to 5 days. These differences may reflect the different types (physical or psychiatric), strengths, and durations of the stresses. The results shown here indicate that exposure to chronic social stress increases the solid GE rate, supporting the findings of Ochi et al. (2008). However, the short-time test diet intake was significantly diminished in stressed mice and the GE rate might appear to increase.

Acute and chronic stresses in mice and rats also affect defecation differently. Whereas acute restraint stress increases defecation (Martinez et al. 2004, Tache et al. 1993), chronic (19 days) stress promotes a constipation-like phenotype observed as a decrease in defecation during the SI test (Tramullas et al. 2012). However, the results of the present study revealed that fecal pellet output was not altered by CSDS, which is consistent with a previous observation (Savignac et al. 2011). Moreover, CSDS did not significantly affect the transit of contents through the gastrointestinal tract. Thus, CSDS does not affect colonic motor function or defecation.

In summary, this study showed that CSDS which induces anxiety-like and social avoidance behaviors changes the GE rate, accompanied by suppression of short time food intake in C57BL/6J mice. Thus, the CSDS model may be useful for further studies examining the relationship between psychiatric diseases and gastric motility disorders associated with stress. Additional studies are needed to clarify the underlying mechanisms regulating these changes.

Acknowledgments

This work was partially supported by the Research Project on Development of Agricultural Products and Foods with Health-promoting Benefits (NARO, MAFF of Japan), and by the Council for Science, Technology and Innovation (CSTI), Japan, under the Cross-ministerial Strategic Innovation Promotion Program (SIP) “Technologies for creating next-generation agriculture, forestry and fisheries” (Grant No. 14532924).

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