Effects of dietary intake of heat-inactivated *Lactobacillus gasseri* CP2305 on stress-induced behavioral and molecular changes in a subchronic and mild social defeat stress mouse model

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ABSTRACT

The intestinal ecosystem is involved in the pathogenesis of mood disorders such as depression. Inestinal microbes can affect the central nervous system through the gut–brain axis, which raises the possibility of using probiotics for preventing depression. In this study, we examined the effect of heat-inactivated *Lactobacillus gasseri* CP2305 (CP2305) in a subchronic and mild social defeat stress (sCSDS) mouse model. sCSDS suppressed food intake. However, dietary CP2305 intake rescued it, suggesting that CP2305 improved the decreased appetite in sCSDS mice. sCSDS did not alter the gene expression of brain-derived neurotrophic factor, nerve growth factor, and neurotrophin-3 in the hippocampus. However, dietary CP2305 provided following sCSDS increased the gene expression of these neurotrophins in the hippocampus. These findings suggest that CP2305 supplementation would aid in preventing psychosocial stress–induced disorders.

Depression is one of the most common diseases, and several antidepressants have been widely used as therapeutics (40). However, these drugs fail to improve the quality of life in some patients (14), leading to the need for developing alternative approaches. Several studies have described significant relationships between diets and depressive symptoms in humans and animal models of depression (9). We previously found that dietary conditions influenced stress resilience in a mouse depression model exposed to subchronic and mild social defeat stress (sCSDS) (17). Additionally, the gut environment and microbiome are altered by sCSDS in mice (3). Collectively, diets and intestinal microbiota may provide targets for alternative treatments for depression.

Previous studies have suggested that probiotic microorganisms such as certain lactic acid bacteria (LABs) significantly modulate communication in the gut–brain axis through neural, endocrine, and immune signaling pathways (9), thereby allowing them to alter stress sensitivity (10). Studies employing experimental animals have shown that administering specific probiotics eases stress-induced glucocorticoid and inflammatory cytokine responses in conjunction with decrease in depression- and anxiety-related behaviors (4, 7, 15). Dietary supplements of *Lactobacillus* have been found to increase psychosocial stress resilience in sCSDS mice (23) and gene expression of neurotrophic factors such as brain-derived neurotrophic factor (BDNF) in the hippocampus (38). Moreover, patients with major depression were found to have lower *Bifidobacterium* and *Lactobacillus* counts in their gut than healthy controls did (1). Therefore, probiotic approaches would contribute to preventing and/or curing depression.

*Lactobacillus gasseri* CP2305 (CP2305) is a LAB that was isolated from the stool of a healthy adult; it has been studied for its beneficial effects on the...
Experimental design. All experiments were conducted between November 30, 2017, and April 19, 2018. The time schedule for test diet feeding, the sCSDS paradigm, behavioral tests, and dissections is shown in Fig. 1. On the basis of body weight, the 30 intruder mice were equally divided into three groups: no-sCSDS healthy control (negative control; C) group, sCSDS control (positive control; S) group, and sCSDS with CP2305 treatment (T) group. From the intruder mice, the C and S mice were fed powder-formed AIN-93G and T mice were fed powder-formed AIN-93G supplemented with CP2305 cell preparation from the first day (Day 0) until the end of the experiment (Day 47). Twenty of the 30 ICR mice were selected as aggressors. The selection criteria for aggressors were adopted from previous studies (16, 18).

sCSDS. The sCSDS paradigm was performed from Day 28 in accordance with our previously published protocol (16, 18), with some modifications. The aggressor mice were transferred to a social defeat cage (cage size, 220 mm × 320 mm × 135 mm; Natsume Seisakusho, Tokyo, Japan) with an acrylic compartment. Aggressors were segregated into compartments of the social defeat cages 3 days before the start of sCSDS to establish their individual territories. From Day 28, intruders of the S and T groups were exposed to a different ICR aggressor mouse each day at 10:00 am. After physical contact, the intruders were carried into the neighboring compartment with another aggressor for 24 h. These intruder and aggressor mice were separated by the acrylic partition, allowing no physical contact with each other. Hence, intruder mice were exposed continuously to emotional stressors from the aggressors, including visual, auditory, and olfactory stimuli. B6 mice in group C were kept in pairs in each compartment in social defeat cages with the dividers during the sCSDS period. The duration of physical contact was set at 5 min after the first attack bite on Day 28 and was then gradually reduced 0.5 min per day from Day 29 to Day 37. Thus, only 0.5 min of physical contact was set for Day 37. The sCSDS challenge of 0.5 min was continued from Day 37 until the forced swim test (Day 44). Intruder mice were drifted into the other compartment of the social defeat cage every day to change the combination of aggressor mice and environments.

Social interaction test. The social interaction test was performed on Day 38 (Fig. 1). The method followed was similar to that published previously (16,
was performed from Day 38 to Day 40 (Fig. 1). This test was performed as a modification of the two-bottle preference test for mice (39). On Day 38, intruder mice in the social defeat cage were offered two bottles containing solutions of 1% sucrose (Sigma-Aldrich Japan, Tokyo, Japan) or distilled water for 24 h. Fluid consumption was determined on the basis of weight and expressed as a percent of the total fluid intake from the sucrose solution. The positions of the water and sucrose bottles were alternated on Day 39 to avoid side preferences; this reverse test was also continued for 24 h and was then completed on Day 40.

**Nest building test.** The nest building test was started on Day 40 at 10:00 am (Fig. 1). This method was similar to those described previously (28, 29), with some modifications. Compressed cotton (Nestlet, Ancare; Bellmore, NY, USA) was used as a nesting material; 3 g Nestlets were inserted into the intruder compartments of the social defeat cages. Nest structure was scored using a Deacon score from one to five (11) every hour during the light period (0–7 h). During the next day (Day 41), the final structure of their nest was scored at 9:00 am (23 h after starting the test).

**Tail suspension test.** The tail suspension test was performed on Day 41 (Fig. 1) according to a previously described procedure (16). The total freezing time was measured with the TimeFZ4 software (O’Hara & Co.).

**Forced swim test.** This test was performed on Day 18, with a few modifications. This test consisted of two trials: a no-target test without an ICR mouse in the interaction box and a target test with an ICR mouse in the interaction box. An open field arena (400 mm × 400 mm × 300 mm) made of grayish polyvinylchloride (O’Hara & Co., Tokyo, Japan) was used and illuminated with 20 lux at the center of the field. A wire-mesh interaction box (100 mm × 100 mm × 150 mm) was placed at one side of the arena. An interaction zone was defined as the 6- to 7-cm-wide area surrounding the interaction box. A corner zone was defined as two corner regions (8 cm × 8 cm) of the side across from the interaction box.

First, B6 mice were transported to the behavior testing room 30 min before the tests for habituation to the conditions of the room. Then, the B6 mouse was gently placed in the corner of the field without a social target. Its behavior was monitored using a CCD camera (O’Hara & Co.). After a 150-s trial, the mouse was returned to its home cage. Next, an unfamiliar male ICR mouse was set into the interaction box and the second trial was performed for 150 s (with a social target). After the trial, the mouse was returned to its home cage, and the open field arena and interaction box were cleaned with alcohol and wiped to dry the field. The time spent in the interaction zone (in seconds) was measured in each trial with the TimeOFCR1 software (O’Hara & Co.). Social interaction scores (% target absent) were calculated as 100 × (interaction time, target present) / (interaction time, target absent).

**Sucrose preference test.** The sucrose preference test was performed from Day 38 to Day 40 (Fig. 1). This test was performed as a modification of the two-bottle preference test for mice (39). On Day 38, intruder mice in the social defeat cage were offered two bottles containing solutions of 1% sucrose (Sigma-Aldrich Japan, Tokyo, Japan) or distilled water for 24 h. Fluid consumption was determined on the basis of weight and expressed as a percent of the total fluid intake from the sucrose solution. The positions of the water and sucrose bottles were alternated on Day 39 to avoid side preferences; this reverse test was also continued for 24 h and was then completed on Day 40.
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Measurement of gene expression in the hippocampus. The methods used for total RNA extraction and cDNA synthesis have been described in earlier studies (35). Real-time polymerase chain reaction (PCR) was performed using a Rotor-Gene 6200 system (Qiagen, Tokyo, Japan). The primers and TaqMan probes used in the current study are listed in Table 1. Optimal primers and probes were designed using a freely available online tool (https://www.roche-applied-science.com/) or designed at Bioresearch Technologies Japan (Tokyo, Japan). The methods employed for PCR analyses have been adapted from previous studies (37).

Neurotransmitter concentration in the brain. The concentrations of neurotransmitters and their associated substrates such as acetylcholine, adrenaline, dopamine, gamma aminobutyric acid, kynurenic acid, 3-hydroxykynurenine, serotonin, tryptophan, and tyrosine were measured using an ultra-pressure liquid chromatography apparatus equipped with a binary solvent manager, an autosampler, and a column heater and tandem mass spectrometry (Acquity TQD UPLC-MS/MS; Nihon Waters, Tokyo, Japan). Frozen brain samples were weighted and inserted into 2 mL screw-cap tubes (Watson, Tokyo, Japan), to which 5 volumes of 0.2 mol/L perchloric acid was immediately added. The suspensions were homogenized at 3,000 r.p.m. for 30 s by using the Micro Smash MS-100 (Tomy, Tokyo, Japan) with a 5.5 φ

| Table 1 Primers and probes used in this study |
|-----------------------------------------------|
| Genes name                                     | Primers (5′–3′) | Probe number | GenBank accession number |
| Brain-derived neurotrophic factor (BDNF)       | F: cacttttgagcaegteatc  | 42           | *                        |
| Nerve growth factor transcript variant A (NGF) | R: tcttaagtgtttctctg   |              |                          |
| Neurotrophin 3, transcript variant 1 (NT-3)    | F: egacgctctggaaatagctc | 29           | NM_001164034.1           |
| Neurotrophic tyrosine kinase, receptor, type 1 (Ntrk1) | R: tggacatctctgatc   | 20           | NM_001033124.1           |
| Neurotrophic tyrosine kinase, receptor, type 2, transcript variant 1 (Ntrk2) | F: tgcccagagcaggataaatg   | 76           | NM_001025074.2           |
| Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) | R: aaagtctctgcctgctatg | –            | NM_000804.2              |

Listed probe numbers indicate the product number of the Universal ProbeLibrary Set, Human and Extension Set sold by Roche Applied Science. Primers and probe for GAPDH were designed and synthesized by Bioresearch Technologies Japan (Tokyo, Japan).

*The primers for BDNF were synthesized from consensus sequences of all variants of BDNF, that is, variant 1 (NM_007540.4), variant 2 (NM_001048139.1), variant 3 (NM_001048141.1), variant 4 (NM_001048142.1), variant 5 (NM_001285416.1), variant 6 (NM_001285417.1), variant 7 (NM_001285418.1), variant 8 (NM_001285419.1), variant 9 (NM_001285420.1), variant 10 (NM_001285421.1), variant 11 (NM_001285422.1), and variant 12 (NM_001316310.1).

Dissection and sampling. Intruder mice were euthanized by exsanguination under deep anesthesia with an intraperitoneal injection of sodium pentobarbital (3.24 mg/head; Somnopentyl, Kyoritsu, Tokyo, Japan). Before exsanguination, blood was collected from the abdominal vein. The brain was removed, and the hippocampus and prefrontal cortex were immediately separated. The left side of the hippocampus was soaked in RNA-later solution (Sigma-Aldrich Japan) overnight at 4°C. The samples were then stored at −80°C until subsequent RNA extraction. The right side of the hippocampus and prefrontal cortex was stored at −80°C until neurotransmitter analyses.

Dissections were initiated at 10:00 am. Ten replicates of the three groups (C, S, and T) were set, and dissection was performed for every replicate. All dissections were completed by 2:00 pm at least.

Measurement of gene expression in the hippocampus. The methods used for total RNA extraction and cDNA synthesis have been described in earlier studies (35). Real-time polymerase chain reaction (PCR) was performed using a Rotor-Gene 6200 system (Qiagen, Tokyo, Japan). The primers and TaqMan probes used in the current study are listed in Table 1. Optimal primers and probes were designed using a freely available online tool (https://www.roche-applied-science.com/) or designed at Bioresearch Technologies Japan (Tokyo, Japan). The methods employed for PCR analyses have been adapted from previous studies (37).

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Frozen brain samples were weighted and inserted into 2 mL screw-cap tubes (Watson, Tokyo, Japan), to which 5 volumes of 0.2 mol/L perchloric acid was immediately added. The suspensions were homogenized at 3,000 r.p.m. for 30 s by using the Micro Smash MS-100 (Tomy, Tokyo, Japan) with a 5.5 φ...
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Japan). Each sample was analyzed in duplicate.

**RESULTS**

**Body weight, water intake, and food intake**

Time course analysis of body weight and total water intake and food intake during the experiment is shown in Fig. 2. Body weight gain did not significantly differ among the three groups during the pre-feeding period of the test diet. During the sCSDS period (Days 28–36), body weight gain significantly differed among the groups ($F_{2,27} = 4.0723$, $P = 0.0285$; Control, $0.71 \pm 0.47$ g vs. sCSDS, $1.43 \pm 1.30$ g, $P = 0.2243$; Control vs. sCSDS + CP2305, $1.80 \pm 0.60$ g, $P = 0.0276$; sCSDS vs. sCSDS + CP2305, $P = 1.000$). The total water intake during the prefeeding period of the test diet (Days 0–27) did not significantly differ among the groups (data not shown).

| Reference materials | Retention time (min) | Quadrupole (m/z) | Dwell (s) | Cone voltage (V) |
|--------------------|----------------------|------------------|-----------|-----------------|
| Kynurenic acid     | 0.85                 | 190.2            | 0.036     | 28              |
| Tyrosine           | 2.96                 | 182.3            | 0.036     | 28              |
| Tyrosine-d10 ($^{13}$C$_9$, $^{15}$N$_1$) | 2.96 | 192.3 | 0.036 | 28 |
| 3-Hydroxykynurenine| 3.16                 | 225.3            | 0.036     | 28              |
| Tryptophan         | 3.48                 | 205.3            | 0.036     | 28              |
| Tryptophan-d13 ($^{13}$C$_9$, $^{15}$N$_1$) | 3.48 | 218.3 | 0.036 | 28 |
| GABA               | 6.67                 | 104.4            | 0.036     | 18              |
| Adrenaline         | 7.67                 | 184.3            | 0.057     | 28              |
| Acetylcholine      | 8.03                 | 146.3            | 0.057     | 30              |
| Dopamine           | 8.76                 | 154.3            | 0.077     | 28              |
| Serotonin          | 10.54                | 177.3            | 0.077     | 30              |

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| Serotonin          | 10.54                | 177.3            | 0.077     | 30              |

GABA, gamma aminobutyric acid
with CP2305 on social avoidance behavior in sCSDS mice, social interaction (SI) test was performed on Day 38 and their scores and their distributions were plotted (Fig. 3A). The SI score did not significantly differ among the groups. The control group consisted of three stress-susceptible (SI score < 100%) and six stress-resilient (SI score > 100%) mice (susceptible proportion = 33.3%), the sCSDS group consisted of four stress-susceptible and six resilient mice (susceptible proportion = 40.0%), and the sCSDS + CP2305 group consisted of three stress-susceptible and seven stress-resilient mice (susceptible proportion = 30.0%; Fig. 3A). The proportion of resilient mice did not significantly differ among the three groups ($P = 1.0000$).

We evaluated sucrose preference (Days 39 and 40) to analyze the anhedonia-like symptoms in the mice (Fig. 3B). There was no significant difference but tendency ($P < 0.1$) between the three groups in the sucrose preference (Control group vs sCSDS group, $P = 0.08$). The control group consisted of three stress-susceptible (sucrose preference ratio $< 100\%$) and seven stress-resilient (sucrose preference ratio $> 100\%$) mice (susceptible proportion $= 30.0\%$), the sCSDS group consisted of seven stress-susceptible and three resilient mice (susceptible proportion $= 70.0\%$), and the sCSDS + CP2305 group consisted of five stress-susceptible and five stress-resilient mice (sucrose preference ratio $= 50.0\%$). The proportion of resilient mice did not significantly differ between the three groups ($P = 1.0000$).

The nest building test was conducted on Day 40 to evaluate goal-directed behavior in the mice (Fig. 4). The nest scores significantly differed among the three groups at 1 h after commencement of the test ($P = 0.0468$), and the sCSDS + CP2305 group showed slightly suppressed nest building compared to that by the control group ($P = 0.0670$).

The immobility in the tail suspension test was analyzed on Day 41 in order to evaluate depression-like behavior in the mice (Fig. 3C). Immobility time did not significantly differ among the three groups.

Immobility was analyzed in the forced swim test on Day 44 to evaluate depression-like behavior in the mice (Fig. 3D). Immobility time did not significantly differ between the three groups.

### Gene expression of neurotrophins and their receptors in the hippocampus

Gene expression of neurotrophins (BDNF, NGF and NT-3) and their receptors (NtrkA and NtrkB) was analyzed (Table 3), because the reduction of these...
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sessions in the hippocampus. However, CP2305 supplementation following sCSDS significantly increased hippocampal gene expressions of BDNF, NGF, and NT-3 compared with that noted for the control and sCSDS groups. Dietary CP2305 did not alter NtrkA and NtrkB gene expression in the hippocampus. According to the results, orally CP2305 administration stimulated the hippocampal neurotrophin expressions, and these stimulation might be improved the behavioral performances.

Biochemical analysis
We analyzed serum corticosterone levels as a measure of stress (Fig. 5). The levels did not significantly vary among the three groups. Furthermore, we analyzed several critical neurotransmitters and their related molecules involved in mental disorders in the hippocampus and the prefrontal cortex (Table 4). Hippocampal tyrosine and tryptophan levels were significantly reduced by sCSDS compared to the levels in the control, but the other levels analyzed were not influenced by sCSDS and sCSDS + CP2305.

Fig. 3 Effects of the treatments on social interaction (A), sucrose preference (B), tail suspension (C), and forced swimming (D). C, control; S, sCSDS; T, sCSDS + CP2305. Dagger (+) indicates a tendency toward significance (P < 0.1) of data between mouse groups.

Fig. 4 Effects of the treatments on nest building behavior. C, control; S, sCSDS; T, sCSDS + CP2305. Asterisk (*) indicates significant differences between groups (P < 0.05).
sCSDS mice in the social interaction test was affected by several environmental factors, including feed (17). Furthermore, control B6 mice used in the current study showed relatively lower social interaction scores compared with those in our previous studies (16, 17). Similarly, sCSDS did not severely suppress nesting behavior (Fig. 4), although our previous studies revealed that sCSDS and acute social defeat model of mice were severely suppressed to build their own nests (28, 29). These differences might be due to the several alterations in the animal facilities and the experimenters (Ohsawa et al. unpublished data). sCSDS mice slightly showed anhedonia-like features (Fig. 3B); thus, the current sCSDS paradigm induced some restricted deficits in B6 mice. CP2305 intake did not change a number of depression-like behavioral features in B6 mice (Fig. 3).

For biochemical characterization, the gene expression of neurotrophins and their receptors in the hippocampus (Table 3) and serum corticosterone levels (Fig. 5) were investigated. Previous studies reported that hippocampal BDNF gene expression was reduced by chronic social defeat stress (CSDS) (36), whereas in the current study, sCSDS did not influence its hippocampal expression (Fig. 5A). This difference might be due to the varying stress paradigms; one reason could be that the physical stress of sCSDS is milder than that of CSDS (16). CP2305 treatment following sCSDS increased hippocampal bdnf, ngf, and nt-3 expression (Fig. 5A–C). Because we did not use a control group whose mice were fed a CP2305-containing diet without sCSDS in this study, it is unclear whether dietary CP2305 intervention alone would increase hippocampal neurotrophin gene expression. In particular, hippocampal BDNF plays a critical role in learning and memory (13), and decrease in its expression has also been observed in depressive patients (12). Therefore, dietary

**Table 3** Effects of the treatments on the gene expression of neurotrophins and their receptors

| Genes | Control | Stress | Stress+CP2305 |
|-------|---------|--------|--------------|
| bdnf  | 1.04 ± 0.10<sup>a</sup> | 1.03 ± 0.12<sup>a</sup> | 1.62 ± 0.15<sup>b</sup> |
| ngf   | 1.08 ± 0.15<sup>a</sup> | 0.69 ± 0.05<sup>a</sup> | 1.64 ± 0.12<sup>b</sup> |
| nt-3  | 1.12 ± 0.17<sup>a</sup> | 1.37 ± 0.22<sup>a</sup> | 2.57 ± 0.46<sup>b</sup> |
| ntrka | 1.48 ± 0.39 | 2.97 ± 1.28 | 2.67 ± 0.83 |
| ntrkb | 1.05 ± 0.11 | 0.98 ± 0.18 | 0.89 ± 0.16 |

BDNF, brain-derived neurotrophic factor; NGF, nerve growth factor; NT-3, neurotrophin-3; NtrkA, tropomyosin receptor kinase A; NtrkB, tropomyosin receptor kinase B; GAPDH, glyceraldehyde-3-phosphate dehydrogenase. Different superscripts indicate significant differences between groups (P < 0.05).

**Fig. 5** Effects of the treatments on serum corticosterone levels. C, control; S, sCSDS; T, sCSDS + CP2305.

**DISCUSSION**

In this study, we examined the effect of dietary intake of CP2305 on psychosocial stress in an sCSDS mouse model. This dietary intake was found to influence some aspects of stress-induced symptoms in the sCSDS mice. The sCSDS mice showed marked reduction in total food intake during Days 28–36, while dietary CP2305 supplementation rescued the sCSDS-induced lowered food intake (Fig. 2C). Depressive patients show several deficits, including those in food intake behaviors (e.g., decreased or increased appetite) (2). Reduced appetite, namely, anorexia, lowers the general QOL and nutritional conditions in depressive patients. Hence, dietary CP2305 supplementation may increase motivation toward food intake and should be studied in the clinical setting in the future.

In the current study, we failed to obtain depression-like behavior such as social avoidance in the social interaction test and prolonged immobility in the tail suspension test and forced swim test (Fig. 3A, C, D) in sCSDS mice. Social avoidance of
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Tryptophan depletion results in the lowering of plasma and brain tryptophan levels (24) and a concomitant decrease in brain serotonin synthesis (33), with changes in cognitive functions, along with depression- and anxiety-like behaviors in rats (6) and transient mood effects in humans (41). There is a probability that the sCSDS-induced tryptophan decrease in the hippocampus showed some significant features related to the effects of chronic but mild psychosocial stress.

In conclusion, we found that oral heat-inactivated CP2305 supplementation rescued the decline in food intake due to sCSDS and increased gene expression of neurotrophic factors such as BDNF in the hippocampus. Therefore, CP2305 supplementation could provide a potent treatment option for psychosocial stress–induced anorexia and decreased hippocampal BDNF levels.

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| Table 4 Concentrations of the amino acids, neurotransmitters, and kynurenic acid in the prefrontal cortex and hippocampus of the mice |
| Region | Control | Stress | Stress + CP2305 |
| Hippocampus (μg/g) | | | |
| Tyr | 43.8 ± 2.3 | 37.5 ± 2.0* | 41.9 ± 1.7 |
| Trp | 2.26 ± 0.31 | 1.46 ± 0.25* | 1.52 ± 0.22 |
| 5-HT | 0.0750 ± 0.0095 | 0.0789 ± 0.0123 | 0.0580 ± 0.0091 |
| DA | ND | ND | ND |
| GABA | 32.1 ± 4.9 | 32.6 ± 3.5 | 37.1 ± 4.8 |
| ACh | 0.174 ± 0.046 | 0.239 ± 0.052 | 0.145 ± 0.028 |
| KA | ND | ND | ND |
| 5-HT/Trp | 3.64 ± 0.40 | 7.89 ± 2.80 | 6.06 ± 1.81 |
| Prefrontal cortex (μg/g) | | | |
| Tyr | 20.7 ± 2.0 | 22.6 ± 2.3 | 21.1 ± 0.9 |
| Trp | 2.25 ± 0.20 | 3.26 ± 0.61 | 2.23 ± 0.24 |
| 5-HT | 0.0502 ± 0.0061 | 0.0603 ± 0.0071 | 0.0555 ± 0.0057 |
| DA | 0.570 ± 0.164 | 0.648 ± 0.175 | 0.756 ± 0.197 |
| GABA | 33.1 ± 1.8 | 33.2 ± 2.3 | 32.0 ± 2.5 |
| ACh | 0.311 ± 0.056 | 0.334 ± 0.063 | 0.338 ± 0.076 |
| KA | 0.0152 ± 0.0037 | 0.0144 ± 0.0030 | 0.0121 ± 0.0032 |
| 5-HT/Trp | 2.42 ± 0.35 | 2.75 ± 0.55 | 3.28 ± 0.64 |

All values have been provided in terms of mean ± SE.
*: Significantly different from control, $P < 0.05$ (one-way ANOVA followed by the Dunnett test).
ND, not detected; Tyr, tyrosine; Trp, tryptophan; 5-HT, serotonin; DA, dopamine; GABA, gamma aminobutyric acid; ACh, acetylcholine; KA, kynurenic acid.

CP2305 intervention could provide an alternative therapeutic option to psychotic drugs. Previous studies have reported that supplementation with probiotic LABs stimulated $bdnf$ expression in the mouse hippocampus (5, 22). In addition, Riezzo et al. (30) reported that long-term supplementation with live $L. reuteri$ DSM-17938 stimulated an increase in BDNF concentration in the serum of humans.

Brain serotonin and dopamine levels are thought to be critical in the occurrence of depressive disorders (8, 27). Biomarkers of serotonin deficiency have been identified in subpopulations of depressive patients (19). Furthermore, brain serotonin deficiency increases vulnerability to social defeat stress in mice (31). Dopaminergic neurons in the ventral tegmental area projecting to the nucleus accumbens play a critical role in susceptibility to psychosocial stress (21). In the current study, the serotonin and dopamine levels in the hippocampus and prefrontal cortex of the sCSDS and sCSDS + CP2305 groups did not significantly differ from those of the control group (Table 3). Compared to control mice, sCSDS mice exhibited lower concentrations of the precursors tryptophan and tyrosine in the hippocampus but not in the prefrontal cortex (Table 3). In particular, tryptophan, a precursor of serotonin, is a limiting factor in serotonin synthesis in the brain (20). Acute tryptophan depletion results in the lowering of plasma and brain tryptophan levels (24) and a concomitant decrease in brain serotonin synthesis (33), with changes in cognitive functions, along with depression- and anxiety-like behaviors in rats (6) and transient mood effects in humans (41). There is a probability that the sCSDS-induced tryptophan decrease in the hippocampus showed some significant features related to the effects of chronic but mild psychosocial stress.

In conclusion, we found that oral heat-inactivated CP2305 supplementation rescued the decline in food intake due to sCSDS and increased gene expression of neurotrophic factors such as BDNF in the hippocampus. Therefore, CP2305 supplementation could provide a potent treatment option for psychosocial stress–induced anorexia and decreased hippocampal BDNF levels.

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