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RESEARCH ARTICLE

Fermented mixed grain ameliorates chronic stress-induced depression-like behavior and memory deficit

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Abstract

Fermented mixed grain (FG) has beneficial anti-cancer, antioxidant, and anti-inflammatory effects. In this study, we investigated the effects of FG on gut inflammation, brain dysfunction, and anxiety/depression-like behavior induced by unpredictable chronic mild stress (UCMS) in mice. Mice were administered mixed grain or FG for 3 weeks and were then exposed to UCMS for 4 weeks. FG administration ameliorated stress-induced anxiety/despair-like behavior. FG administration also prevented UCMS-induced memory impairment. Additionally, the mRNA levels of 5-HTR1A and IL-6 were restored to normal levels in the brains of FG-administered mice. FG administration also inhibited intestinal damage in stressed mice compared with that in the UCMS (without FG) group. These results suggest that FG can alleviate stress-induced intestinal damage, brain dysfunction, and cognitive impairment.

Keywords Fermented grain · Depression-like behaviour · Memory impairment · Chronic mild stress

Introduction

Individuals are frequently exposed to stress in their daily lives, and such stress can significantly impact both their mental and physical health (Shields and Slavich, 2017). Stress represents a significant risk factor for depression and anxiety, as well as for diseases such as irritable bowel syndrome and diarrhea, while also affecting memory formation (Enck et al., 2016; Tafet and Nemeroff, 2016). In rodent models, unexplained chronic mild stress (UCMS) can trigger intestinal barrier dysfunction, inflammatory responses, anxiety-like behavior and cognitive decline (Nollet, 2021; Willner, 2017).

Psychological and physical stressors increase intestinal motility and permeability, leading to intestinal barrier dysfunction (Foster et al., 2017). The regulation of stress-related responses is critical to the gut-brain axis (GBA) and such stress responses can be reproduced in animal models (Foster et al., 2017). In the gut, stress modifies the gut microbiome and alters the levels of metabolites, such as short-chain fatty acids, choline and bile acids. These changes can lead to behavioral impairments and negatively impact brain development and function (Bharwani et al., 2016, van de Wouw et al., 2018).

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The gut microbiome composition can be altered by various factors, including diet (Foster et al., 2017). Grains are a source of bioactive components such as phenolic compounds and dietary fibers, which can regulate the gut microbiota (Slavin, 2003). Consumption of grains can have positive effects on health.

Ethanol extracts of rice bran and whole-grain adlay seeds decreased pro-inflammation cytokines and chemokine in ulcerative colitis-induced mice (Lo et al., 2022). In addition, rice bran extract increased tight junction protein levels, and whole grain adlay seeds extract improved diarrhea. Bread made with whole wheat flour alleviated hepatic oxidative stress and damage and improved lipid metabolism in high-fat diet-fed mice (Sun et al., 2023). It also increased microbiota diversity. Wheat bran extract also showed neuroprotective effects and alleviated memory impairment in 3 × Tg AD mice (Lee et al., 2015).

Fermented soybean foods contain various biological functional components that affect the gut microbiota, linked to the pathogenesis of various neurological disorders, including depression, anxiety, autism, AD, and PD (Jang et al., 2021). Mice fed fermented milk rich in γ-aminobutyric acid (GABA) improved memory and sleep quality (Yu et al., 2020), which may be related to alteration in the gut microbiota and increased short-chain fatty acid (SCFA) levels.

Fermentation of grains is carried out using microorganisms that are generally recognized as safe (GRAS), such as lactic acid bacteria, molds, and yeasts (Bourdichon et al., 2012). The resulting fermented grains contain essential GABA, vitamins, and phenols (Melini, Melini et al., 2019). GABA is a major neurotransmitter inhibiting neuronal excitability by inhibiting nerve transmission (Heaney and Kinney, 2016). Exposure to chronic stress decreases the GABAergic interneuronal network, potentially leading to the development of cognitive disorders (Jie et al., 2018). Vitamins can reduce oxidative stress and improve cognitive functions, and polyphenols are known to have neuroprotective effects by regulating the blood–brain barrier (Balland et al., 2022; Suh et al., 2020).

The present study investigated whether FG could alleviate gut inflammation, brain dysfunction, and anxiety/depression-like behavior induced by UCMS in mice. The study employed intestinal tissue analysis and behavioral analysis to evaluate the effectiveness of FG in treating stress-related health issues.

Materials and methods

Non-fermented mixed grains and fermented mixed grains

Non-fermented mixed grains (NFG) and fermented mixed grains (FG) were provided by CJ CheilJedang Co. (Seoul, Republic of Korea). The NFG and FG were comprised of wheat germ (40%), wheat bran (30%), oats (10%), barley (5%), brown rice (5%), lentils (5%), and quinoa (5%). FG were subject to solid-state fermentation at 37 °C for 48 h using *Bacillus amyloliquefaciens* 245 (BA 245), which is found in traditional yeast (Heo et al., 2020). The compositions of NFG and FG are presented in Table 1.

Table 1 Composition of non-fermented and fermented mixed grains

Ingredients (%)	Non-fermented mixed grains	Fermented mixed grains
Carbohydrate	63.03	57.87
Fiber	22.63	24.9
Crude protein	17.74	23.45
Crude fat	6.05	7.87
Moisture	9.85	6.77
Ash	3.32	4.04
Sugars (fructose, glucose, sucrose, maltose, lactose, mg/g)	32.87	81.52
Free amino acids (mg/100 g)		
Threonine	16.73	47.98
Cystine	0.04	–
Tyrosine	5.52	56.71
Arginine	44.91	41.35
Alanine	32.57	221.85
Proline	8.29	36.14
Lysine	9.07	80.09
Histidine	14.75	35.32
Isoleucine	3.39	57.65
Leucine	4.11	114.72
Methionine	1.35	16.2
Phenylalanine	4.02	103.49
Tryptophan	29.57	30.31
Valine	6.68	119.06
Glutamic acid	54.91	224.9
Aspartic acid	24.87	54.87
Serine	6.07	33.22
Glycine	14.97	33.6
Low molecular peptide		
Below 30 kDa	7.44 (41.95)	21.02 (89.66)
Below 10 kDa	4.37 (24.64)	18.67 (79.60)

Animals

Male C57BL/6N mice were purchased from Samtako BIO (Osan, Republic of Korea). The mice were maintained under standardized conditions: 23 ± 2 °C, 50 ± 10% humidity, and a 12 h/12 h photoperiod. Mice were divided into four groups of 10 mice each: control (CTL), unpredictable chronic mild stress (UCMS), non-fermented mixed grains-UCMS (NFGU), and fermented mixed grains-UCMS (FGU). The NFGU and FGU groups were treated with normal feed containing NFG (150 mg/kg/day) or FG (100 mg/kg/day) for 3 weeks before an unpredictable chronic mild stress (UCMS) experiment. The CTL and UCMS groups were fed a normal diet (Rat and mouse diet, Samtako BIO, South Korea). Food and water were provided ad libitum to all the groups. All experiments were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee of Sejong University.

Unpredictable chronic mild stress model

The unpredictable chronic mild stress (UCMS) protocol used was adapted from previous studies (Jung et al., 2014; Mineur et al., 2006). The stress groups were exposed to two or three stressors per day for four weeks. The stressors included restraint (30 min), cage tilting (45°, 2 h), wet bedding overnight, empty cage overnight, water deprivation (3 h), and water bath (13 °C, depth 2 cm, 5 min) on a randomized schedule. Stressors were continued during the behavior test phase.

Behavioral tests

Mice were subjected to behavioral tests after two weeks of UCMS exposure. Behavioral tests were performed using the ANY-maze version 6.0 (Stoelting Co., Wood Dale, IL, USA), except for rotarod and forced swimming tests.

Rotarod test

The rotarod test (RRT) was performed to measure basal locomotor activity, as previously described, with slight modifications (Kim et al., 2015). Mice were placed on a 9 cm diameter stationary cylinder of the rotarod apparatus. In the habituation stage, mice were subjected to four trials, with the rod rotating at 4 rpm for 60 s. The next day, the rod rotations were increased from 4 to 29 rpm for 300 s in the test stage. The test was repeated eight times, with at least a 5 min rest between repetitions. The latency to fall from the rotarod was measured manually.

Open field test

The open-field test (OFT) was performed to measure locomotor activity as previously described with slight modifications (Bouwknecht et al., 2007; Jung et al., 2017). Mice were placed in an unclear acrylic arena (40 × 40 × 40 cm). Mice were first placed in the corner and allowed to explore freely for 20 min. Afterward, mice were returned to their cages. The total distance traveled was measured to determine locomotor activity.

Light and dark box test

The light and dark box test (LDB) was performed to measure anxiety, as described previously, with slight modifications [47]. Mice were placed in a light and dark box apparatus (45 × 26 × 26 cm) at approximately 500 lx. The apparatus comprised one-third dark and two-thirds light compartments. Mice were first placed in the light zone and allowed to explore freely for 5 min. Afterward, the mice were returned to their cages. The time spent in the light zone and the number of transitions between zones were analyzed to determine anxiety.

Forced swimming test

The forced swimming test (FST) was performed to measure depression-like behavior, as previously described with slight modifications (Porsolt et al., 1977). Mice were placed in a water-filled glass cylinder (height 26 cm, diameter 18 cm, and water depth of 20 cm at 24 ± 1 °C). The test lasted for 6 min and was recorded using a Logitech QuickCam Pro 9000 (Logitech, Lausanne, Switzerland). The immobility time was recorded during the last 4 min of the test. Afterward, the mice were dried using paper towels, placed in a drying cage, and returned to their cages once they were completely dry. The duration of immobility was measured to determine depression-like behavior and was analyzed manually.

Novel object recognition 1 h test

The novel object recognition 1 h test (NOR) was performed to determine recognition memory, as previously described, with slight modifications (Jung et al., 2017). The test was performed in an open-field arena with two objects. In the training phase, the same two objects were placed at the one-third location of the diagonal of the arena. The mice were placed in a corner far away from the objects and allowed to freely explore the arena for 5 min. After 1 h, one different type of object replaced the one of the previous objects. The position of the novel object was randomized for each mouse. The mice were placed in a corner far away from the objects

and allowed to explore freely for 5 min. Afterward, the mice were returned to their cages. The exploration time for each object was measured to determine recognition memory.

Intestine histology

The animals were sacrificed by cervical dislocation once the behavioral studies were concluded. The ileum and brains were immediately removed and placed in ice-cold isotonic saline. Formalin-fixed, paraffin-embedded tissue sections parallel to the longitudinal axis of the bowel were processed for staining with hematoxylin and eosin (H&E) and then examined using light microscopy. Villi height and crypt depth were measured using the H&E-stained sections.

Intestinal permeability

The intestinal permeability test was conducted as previously described (Zhong et al., 2010). Ileum has a role in the digestion and absorption of nutrients mainly, and the colon has a role in the absorption of water and electrolytes mainly (Steegenga et al., 2012). Morphology and functional alteration of the ileum can be changed by diet (Navarrete et al., 2015). Therefore, we focused on the ileum to elucidate FG and NFG effects on digestion and absorption. Freshly isolated ileum was placed in modified Krebs–Henseleit buffer (KHB, 8.4 mM HEPES, 119 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$, 2.5 mM $CaCl_2$, and 11 mM glucose, pH 7.4). First, one end of the ileal segment was ligated with a suture. The other end of the ileal segment was ligated after injecting Fluorescein isothiocyanate (FITC)-dextran (FD-4, 100 µL, 40 mg/mL, molecular weight 4000 g/mol) into the lumen using a gavage needle. The ileum was placed in 2 mL of KHB and incubated at 37 °C for 20 min. FD-4 penetrating from the lumen into the incubation buffer was measured using a microplate fluorescence reader at excitation and emission wavelengths of 485 and 530 nm, respectively. The FD-4 permeability was comparatively analyzed.

Intestinal myeloperoxidase (MPO) assay

Intestinal myeloperoxidase (MPO) activity was measured in ileal tissues using an MPO activity colorimetric assay kit (BioVision, Milpitas, CA, USA). The samples were processed according to the manufacturer's instructions, and the final values were expressed as MPO mU/mL.

RNA isolation and quantitative real-time PCR (qRT-PCR) analysis

Ileum and brain tissues were dissected and immediately stored at − 80 °C until RNA extraction was performed. Total RNA was isolated using TRIzol reagent (Invitrogen, Waltham, MA, USA). RNA concentrations and ratios were measured using a NanoDrop™ ND-1000 spectrophotometer (Thermo Fisher Scientific, USA). Then, cDNA was synthesized using a high-capacity cDNA reverse transcription kit (Applied Biosystems, Waltham, MA, USA). After cDNA synthesis, quantitative real-time PCR (qRT-PCR) analyses were performed using SYBR Green MG 2X qPCR MasterMix (MGmed, Republic of Korea) and a CFX Connect Real-Time PCR system (Bio-Rad, Hercules, CA, USA). GAPDH was used for normalization. The primers used in these analyses are listed below: 5-HTR1A (Forward-CATCGCGCTAGACAGGTACTG, Reverse-CAATGAGCCAAGTGAGCGAGA), GABAB1b (Forward-ACGTCACCTCGGAAGGTTG, Reverse-CCCGGCACACATATTCAATCT), Occludin (Forward-TGAAAGTCCACCTCCTTACAGA, Reverse-CCGGATAAAAAGAGTACGCTGG), Claudin (Forward-GCAAGGTGTATGAATCTGTGCT, Reverse-GTCAAGGTAACAAAGAGTGCCA), ZO-1 (Forward-CGCCAAATGCGGTTGATC, Reverse-TTTACACCTTGCTTAGAGTCAGGGTT), IL-6 (Forward-TCCAGAAACCGCTATGAAGTTC, Reverse-CACCAGCATCAGTCCCAAGA), GAPDH (Forward-GACGGCCGCATCTTCTTGT, Reverse-CAGTGCCAGCCTCGTCCCGTACAA).

Statistical analysis

Statistical package for the social sciences (SPSS) statistics version 23 software (SPSS Inc, Chicago, IL, USA) was used for statistical analyses. Data were analyzed using one-way analysis of variance (ANOVA) followed by Duncan's post hoc multiple comparison tests. The exploration time (%) between two objects of NOR was compared using Student's t-test. All behavioral data are presented as mean ± standard error of the mean (SEM). Statistical significance was set at $p < 0.05$.

Results and discussion

Effects of FG administration on basal motor activity

Mice were treated with either NFG or FG for three weeks prior to the 5 weeks UCMS procedure to investigate their preventive effects on chronic stress. Behavioral tests were conducted for 3 weeks, 2 weeks after the start of the UCMS procedure (Fig. 1A). RRTs and OFT were performed to evaluate basal motor activity. The final RRT results were obtained by averaging the latency to fall of mice in the last four trials out of eight RRT trials performed on the test day. No differences in the latency to fall were observed across all mouse groups (Fig. 1B). The OFT was performed for 20 min, and the distance traveled was expressed in units of

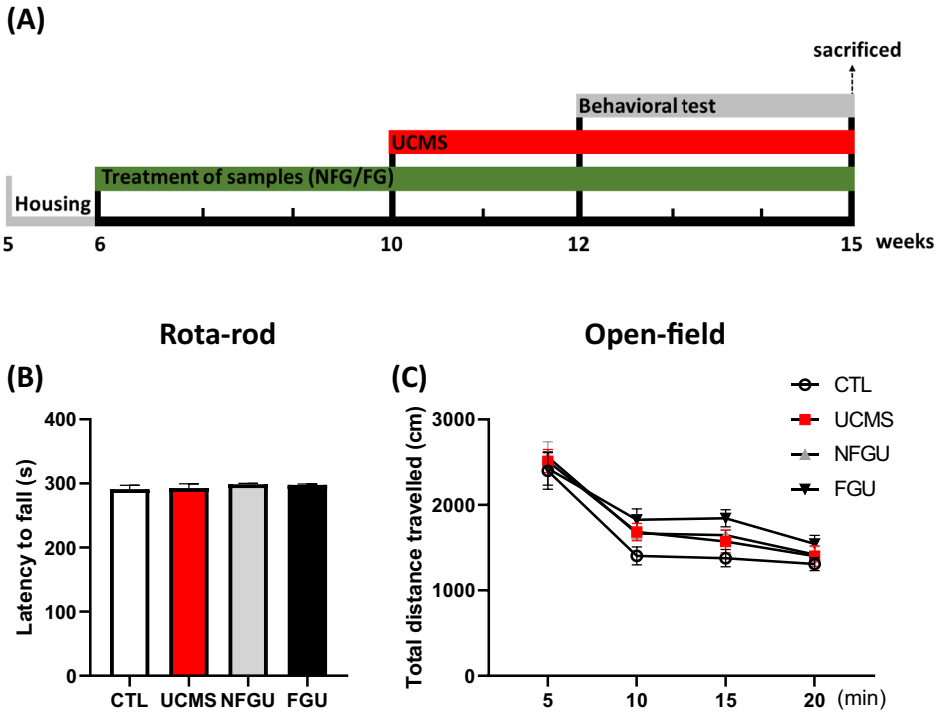

Fig. 1 Locomotor activity compared among treatment groups. **A** Schematic overview of the in vivo behavioral tests. **B** Latency to fall in RRT. **C** Total distance travelled in OFT. CTL control, UCMS unpredictable chronic mild stress, NFGU non-fermented mixed grain-UCMS, FGU fermented mixed grain-UCMS. Data are mean ± SEM

5 min. No differences were observed in the total distance traveled between the groups (Fig. 1C). These results suggest that NFG or FG administration had no impact on basal motor activity in mice.

Effects of FG administration on anxiety, depression-related behavior, and memory impairment

LDB and FST were performed to determine the effect of NFGU and FGU on anxiety and depression-like behavior induced by UCMS. In the LDB, the UCMS group spent slightly less time in the light zone than the CTL group. In contrast, the FGU group spent significantly more time in the light zone than the UCMS group (Fig. 2A). It indicated that the administration of FG led to a significant reduction in anxiety-like behaviors. No significant differences were observed between the groups in the transition numbers between the light and dark areas (Fig. 2B). In the FST, the UCMS group showed a statistically significant increase in immobility time compared with the CTL group. The NFGU group showed a slight decrease in immobility, and the FGU group showed a statistically significant decrease in immobility time compared with the UCMS group (Fig. 2C). These results suggest that FG administration improves anxiety and depression-like behavior induced by chronic stress in mice.

NOR was performed to determine whether NFGU and FGU suppress memory impairment induced by chronic stress. No differences were observed in the ratio of exploration of the two identical objects between all mice groups during the training session (Fig. 2D). In the test session, the ratio of exploring novel objects in the CTL group was significantly higher than that of exploring familiar objects. In contrast, the UCMS group did not show any difference between the ratios of exploration time of the novel and familiar objects. While the NFGU group recognized the novel object more than the familiar one, this difference was not statistically significant, according to our analysis. The FGU group's ratio of exploration time of a novel object was significantly higher than that of a familiar object (Fig. 2E), confirming that FG administration can recover the memory deficit induced by chronic stress.

Effects of FG administration on the mRNA levels of genes related to brain function

The mRNA levels of neuronal markers in the prefrontal cortex (PFC) and amygdala (AMG) were measured to correlate the behavioral effects of FG administration with biochemical changes. PFC and AMG are interconnected brain regions that form the PFC-AMG pathway, which plays an important role in regulating emotions and behavior (Andolina et al., 2013; Liu et al., 2020). Serotonin, a neurotransmitter that modulates mood and attention, affects the PFC and AMG (Passetti et al., 2003; Wellman et al., 2007). Cognitive dysfunction has been reported in cases of reduced expression of 5-HT1A receptors (Glikmann-Johnston et al., 2015; Leiser et al., 2015). The monoamine neurotransmitter 5-Hydroxytryptamine receptor 1A (5-HTR1A) in the UCMS group significantly decreased

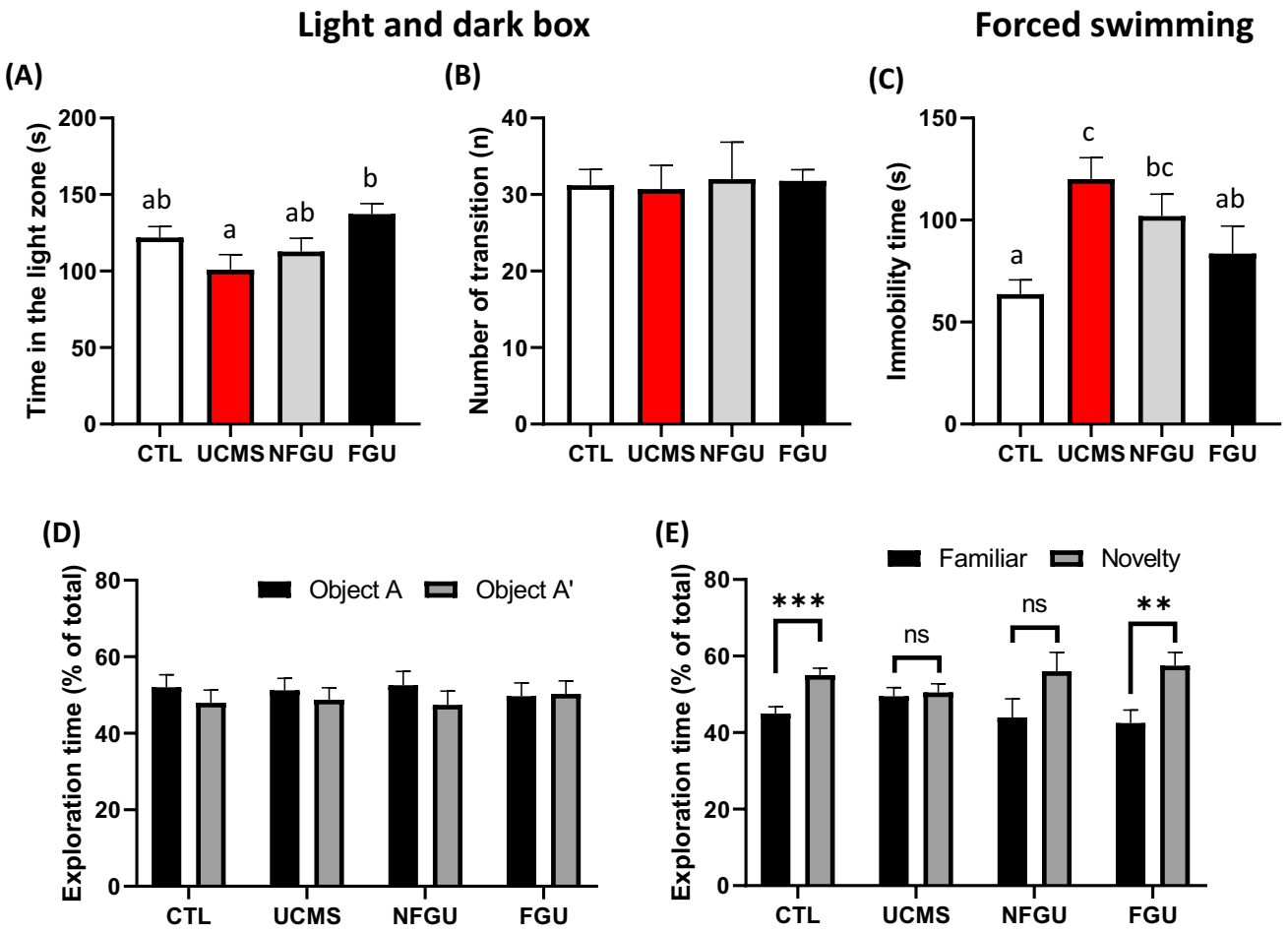

Fig. 2 FG attenuates UCMS-induced anxiety, depression-like behavior, and memory impairment. **A** Time in the light zone in LDB. **B** Number of transitions in LDB. **C** Immobility time in FST. **D–E** Exploration time (% of total) in training (**D**) and test (**E**) on NOR. CTL control, UCMS unpredictable chronic mild stress, NFGU non-fermented mixed grain-UCMS, FGU fermented mixed grain-UCMS. Data are mean ± SEM

compared with the CTL group. In contrast, 5-HTR1A significantly increased in the FGU group compared to the UCMS groups (Fig. 3A) in the PFC and AMG.

GABA, the primary inhibitory neurotransmitter, plays a critical role in the modulation of memory (Heaney and Kinney, 2016). GABA acts mainly via ionotropic $GABA_A$ receptors and G-protein-coupled $GABA_B$ receptors, which are located at both pre/postsynaptic sites (Bowery, 1993). The aberrant function of $GABA_B$ receptors has been implicated in various clinical disorders such as anxiety, depression, and cognitive deficits (Davies et al., 1991; Meeren et al., 2002; Olpe et al., 1993). In the PFC, the decreased mRNA levels of $GABA_{B1b}$, a GABA receptor isoform, in the UCMS-treated groups were not recovered in the FG group compared to those in the CTL group. However, in the AMG, $GABA_{B1b}$ levels in the FGU group were significantly higher than that of the UCMS group (Fig. 3B).

Stress leads to the release of pro-inflammatory cytokines. IL-6 is produced under stress conditions in the hypothalamus. The inflammatory cytokine Interleukin-6 (IL-6) was measured to evaluate the effects of FG on UCMS-induced inflammation in the brain. A significant increase in IL-6 mRNA levels was observed in the UCMS groups compared with the CTL group. However, IL-6 levels in the NFGU and FGU groups did not increase (Fig. 3C).

Effects of FG administration on UCMS-induced intestinal damage

H&E staining and measurement of the villus-to-crypt length ratio were performed to evaluate the degree of intestinal damage. The UCMS group showed severe damage in H&E-stained sections, and the length ratio was decreased compared with that of the CTL group. The

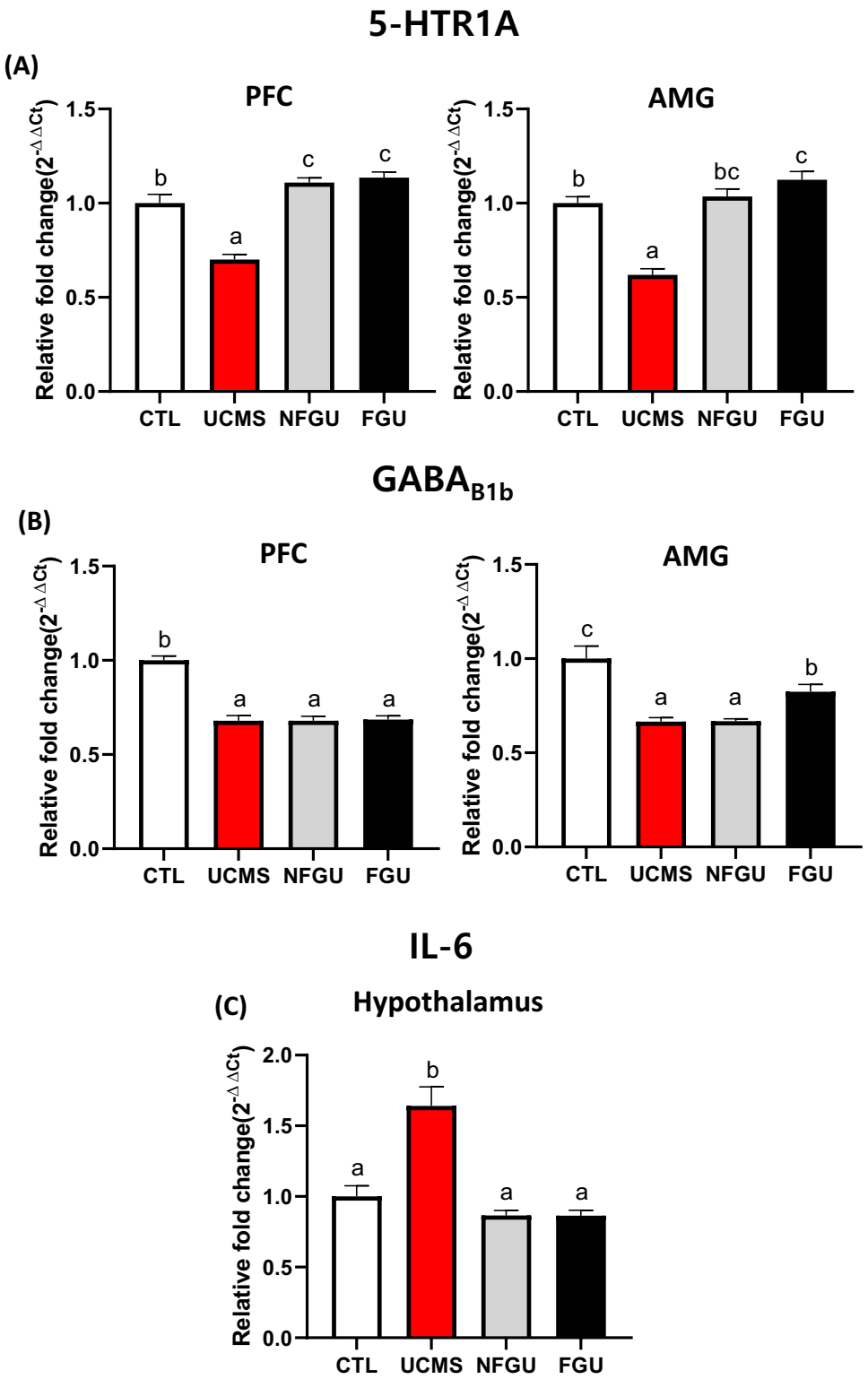

Fig. 3 FG treatment recovers the reduction of 5-HTR1A and $GABA_{B1b}$ induced by UCMS. **A** Expression of 5-HTR1A mRNA in PFC and AMG. **B** Expression of $GABA_{B1b}$ mRNA in PFC and AMG. **C** Expression of IL-6 mRNA in hypothalamus. CTL control, UCMS unpredictable chronic mild stress, NFGU non-fermented mixed grain-UCMS, FGU fermented mixed grain-UCMS. Data are mean ± SEM

histological analysis presented in Fig. 4A and B demonstrates the protective effects of FG on UCMS-induced intestinal epithelial disruption. The FGU group showed recovery from damage compared to the UCMS group, and the length ratio of the villi to crypts was significantly longer in the FGU group compared to that of the UCMS group (Fig. 4A and B). FD-4, commonly used to measure intestinal permeability, was denoted by the relative fluorescence unit (RFU). The RFU of UCMS was significantly higher than that of the CTL group. The RFU

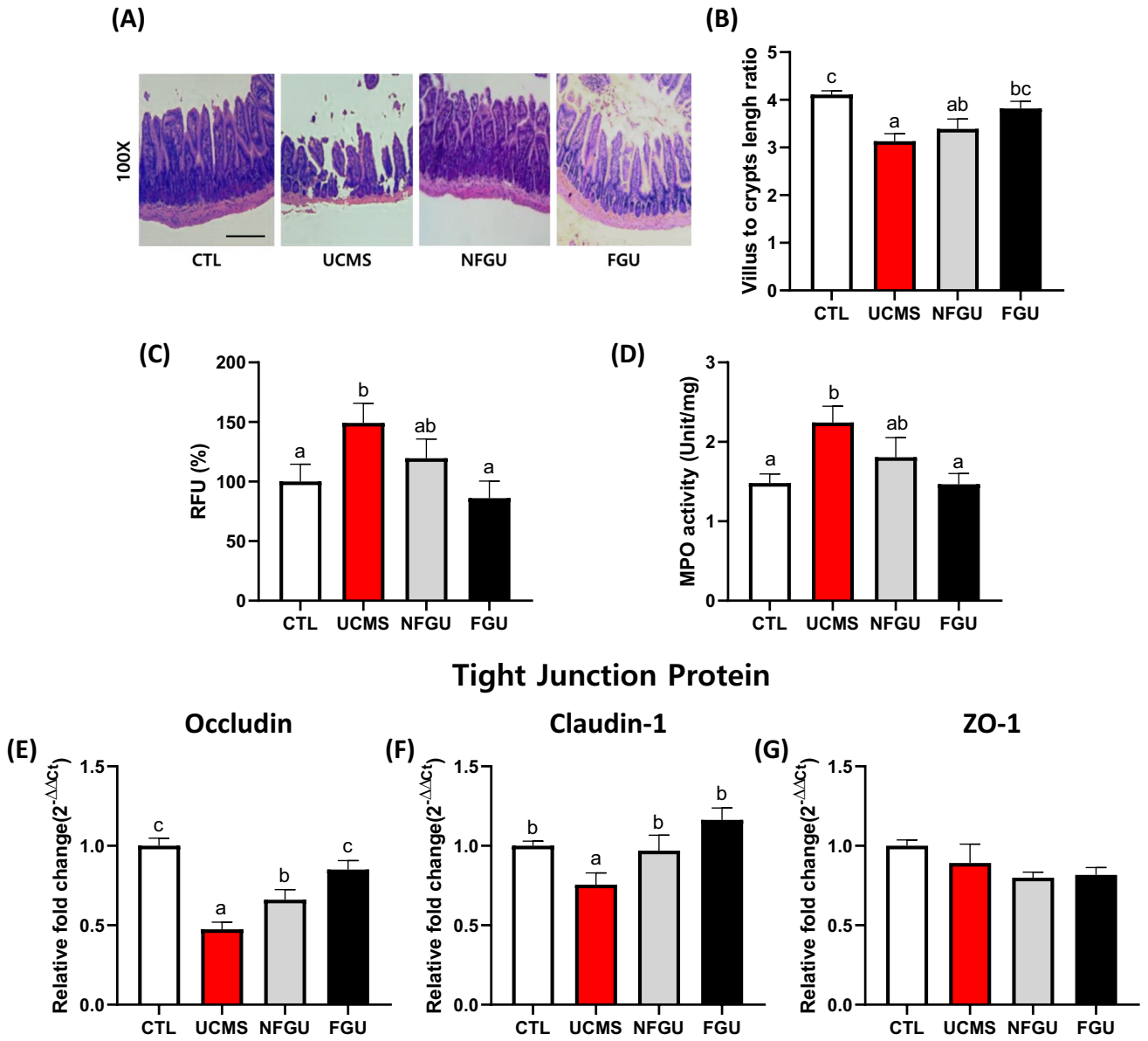

Fig. 4 FG prevents UCMS-induced memory impairment. **A** Representative H&E-stained colon sections. Scale Bars: 50 μm. **B** The length ratio of villi to crypts. **C** Intestinal epithelial permeability to FD-4. **D** The ileal MPO activity levels compared to the CTL group. **E–G** Expression of genes related to the tight junction in the gut. CTL control, UCMS unpredictable chronic mild stress, NFGU non-fermented mixed grain-UCMS, FGU fermented mixed grain-UCMS. Data are mean ± SEM

of the FGU group was significantly lower than that of the UCMS group (Fig. 4C). The intestinal MPO level is an oxidative stress biomarker. Elevated intestinal MPO levels were observed in both animal models of IBD and patients with IBD (Hegazy and El-Bedewy, 2010; Kruidenier et al., 2003; Zhang et al., 2021). The UCMS group showed significantly increased MPO activity compared to the CTL group. The MPO activity levels in the FGU group were significantly lower than those in the UCMS group (Fig. 4D). To evaluate whether FG treatment affects tight junctions in stressed mice, the mRNA levels of tight junction proteins (Claudin-1, occludin, and ZO-1) were examined. Occludin and Claudin-1 mRNA levels were significantly lower in the UCMS group than in the CTL group. Both NFGU and FGU showed higher mRNA levels of occludin and claudin than the UCMS group. The mRNA levels of ZO-1 were not statistically significant in any group (Fig. 4E–G). Our results suggest that FG treatment could reinforce the integrity of tight junctions, reduce intestinal permeability, and alleviates oxidative stress, ultimately leading to improved gut-blood barrier function.

The amino acid content of FG

The amino acid content of FG was quantitatively analyzed compared to that of NFG (Table 1). Histidine (14.75 mg/100 g to 35.32 mg/100 g), Isoleucine (3.39 mg/100 g to 57.65 mg/100 g), Leucine (4.11 mg/100 g to 114.72 mg/100 g), Lysine (9.07 mg/100 g to 80.09 mg/100 g), Methionine (1.35 mg/100 g to 16.2 mg/100 g), Phenylalanine (4.02 mg/100 g to 103.49 mg/100 g), Threonine (16.73 mg/100 g to 47.98 mg/100 g), Tryptophan (29.57 mg/100 g to 30.31 mg/100 g), and Valine (6.68 mg/100 g to 119.06 mg/100 g), which are essential amino acids, were increased by at least 2 to 27 times in FG, except for tryptophan. The other of amino acids, Aspartic acid (24.87 mg/100 g to 54.87 mg/100 g), Glycine (14.97 mg/100 g to 33.6 mg/100 g), Glutamic acid (54.91 mg/100 g to 224.9 mg/100 g), Proline (8.29 mg/100 g to 36.14 mg/100 g), Serine (6.07 mg/100 g to 33.22 mg/100 g), Alanine (32.57 mg/100 g to 221.85 mg/100 g), and Tyrosine (5.52 mg/100 g to 56.71 mg/100 g), except for Arginine (44.91 mg/100 g to 41.35 mg/100 g), increased by at least twofold to tenfold. Cystine (0.04 mg/100 g to 0 mg/100 g) was not detected in FG. Fermented grains have a higher amino acid content than non-fermented grains (Erbas et al., 2005; Hamad and Fields, 1979; Kang et al., 2011), and amino acids are required for neurotransmitter production (Maher, 2000). Phenylalanine is used as a precursor to tyrosine, and Leucine and valine called branched-chain amino acids, positively affect fatigue, depression, anxiety, and psychological distress (Chen et al., 2016; Koochakpoor et al., 2021). It is undetermined which substances in fermented grains have anti-stress activity, but amino acids with large changes in fermented grains could be associated with anti-stress activity.

The potential of FG for stress relief

Acknowledging that our study only examined the stress-relieving phenotype by FG administration, not how FG relieved stress, represents a limitation of this study that could benefit from further exploration. Future studies should focus on analyzing how fermented grains affect stress relief. However, we anticipate that fermented grains may exert these effects through several mechanisms.

First, chronic stress or dietary intake can alter gut microbiota (Foster et al., 2017; Madison and Kiecolt-Glaser, 2019). Our findings suggest that fermented grain has a stress-relief effect through cognition-related behavioral analyses and several gut and brain factors. Considering the microbiome-gut-brain axis, the beneficial gut microbiota could also be expected to increase (Horn et al., 2022). Future research on the intestinal microbiome and its metabolite levels should be performed to elucidate the underlying mechanisms of the beneficial effects of FG consumption.

Second, fermented grains are rich in dietary fiber, polyphenols, and nutrients such as vitamins, minerals, and antioxidants, essential for brain health (Berding et al., 2021; Kim et al., 2016; Vauzour, 2012). These nutrients have been shown to protect neurons from oxidative stress and inflammation, which are implicated in depression and memory impairment (Kumar et al., 2022; Mota de Carvalho et al., 2018). The polyphenol content of FG used in this study was analyzed in the previous study (Heo et al., 2020). The FG has eight times higher polyphenol content than NFG and increased radical scavenging antioxidant activity. In other research results, Polyphenols and flavonoids have stress-relieving effects and radical scavenging activity (Bouayed, 2010; Liu et al., 2013; Zhu et al., 2012). It is possible that the polyphenols contained in FG affected stress relaxation.

In conclusion, while the stress recovery mechanism is not fully understood, several potential explanations exist for the therapeutic effects of fermented grain on stress-induced depression and memory impairment. Fermented grain has the potential to be developed as an ingredient of functional food that helps relieve stress and improve mental health.

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Declarations

Conflict of interest None of the authors of this study have any financial interest or conflict with industries or parties.

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