Lactobacillus rhamnosus GG ameliorates noise-induced cognitive deficits and systemic inflammation in rats by modulating the gut-brain axis

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Research Article

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Abstract

Background

Environmental noise exposure is linked to neuroinflammation and imbalance of the gut microbiota. Promoting gut microbiota homeostasis may be a key factor in relieving the deleterious non-auditory effects of noise. This study aimed to investigate the effect of *Lactobacillus rhamnosus* GG (LGG) intervention on noise-induced cognitive deficits and systemic inflammation in rats.

Methods

Learning and memory were measured using the Morris water maze, while 16S rRNA sequencing and gas chromatography-mass spectrometry were used to analyze the gut microbiota and short-chain fatty acid (SCFA) content. Endothelial tight junction proteins and serum inflammatory mediators were assessed to explore the underlying pathological mechanisms.

Results

The results indicated that LGG intervention ameliorated noise-induced memory deterioration, promoted the proliferation of beneficial bacteria, inhibited harmful bacteria, improved dysregulation of SCFA-producing bacteria, and regulated SCFAs levels. Mechanistically, noise exposure led to a decrease in tight junction proteins in the gut and hippocampus and an increase in serum inflammatory mediators, which were significantly alleviated by LGG intervention.

Conclusions

Taken together, LGG intervention reduced gut bacterial translocation, restored gut and blood-brain barrier functions, and improved gut bacterial balance in rats exposed to chronic noise, thereby protecting against cognitive deficits and systemic inflammation by modulating the gut-brain axis.

Introduction

The intestine has the most abundant and diverse bacterial community in the body [1]. The gut microbiota, known as the "second brain", affects normal physiology, synaptic, immune, and barrier functions, and host behavior, including cognition, through the microbiome-gut-brain axis [2]. Dysbiosis of the gut microbiota frequently leads to brain[3–5] and gut diseases, such as inflammatory gut disease[6]. Our previous study demonstrated that noise intervention changed the gut microbiota, increased gut and brain endothelial barrier dysfunction, and accelerated neurochemical and inflammatory dysregulation in an Alzheimer's disease (AD) mouse model[7]. Moreover, noise exposure leads to changes in the relative abundance of species belonging to the family *Lactobacillaceae* (including the genus *Lactobacillus*) [8],

although other factors may also cause *Lactobacillus* disorders. For instance, the abundance of *Lactobacillus* is reduced in mice with high-fat diet induced steatohepatitis[9].

Lactobacillus Rhamnosus GG (LGG) is a probiotic originally isolated from the human gut. In recent years, studies have shown that LGG can tolerate the environment of the digestive tract and colonize the gut, participating in the regulation of intestinal microbiota homeostasis [10, 11]. Sanborn et al.[12] reported that cognitive performance improved in older adults after supplementation with probiotic LGG, suggesting that LGG intervention may delay aging-related cognitive decline. Additionally, supplementation with *Lactobacillus* and *Bifidobacterium* reportedly improved spatial learning, memory deficits, and oxidative stress in AD rats[13]. Moreover, LGG plays a key role in protecting from of inflammatory injury and intestinal barrier dysfunction [14]. For example, LGG reportedly regulates the expression of tight junction proteins occludin and zonula occludens-1 to alleviate impaired barrier function[15]. Notably, one study demonstrated that LGG colonization in early life reduced inflammation, increased the abundance of SCFA-producing bacteria, and promoted the production of SCFAs in young mice, with the beneficial effects persisting up to eight months[16].

The above studies suggest potential beneficial effects of LGG on cognitive function, systemic inflammation, intestinal barrier function, and gut metabolism. Thus, the purpose of this research was to investigate whether intervention with LGG may improve noise-induced cognitive deficits and systemic inflammation by modulating the gut-brain axis in rats exposed to chronic noise.

Materials And Methods

LGG culture

LGG was cultured anaerobically at 37 °C for 24 h in MRS medium (pH 6.2 ± 0.2, autoclaved at 121 °C for 15 min). Subsequently, the bacteria were centrifuged at 4000 × *g* for 15 min at 4 °C and the precipitate was collected. The bacteria were resuspended at a concentration of 1×10^8 CFU/mL after the precipitate was washed with saline 3 times. LGG suspension was refrigerated at 4 °C.

Animals and experimental groups

A total of 48 healthy, male, six-week-old Wistar rats were provided by Beijing Viton Lihua Laboratory Animal Technology Co., Ltd. (Beijing, China) (animal license number: 2016-0006). The rats were maintained under standard housing conditions with an ambient temperature of 23 ± 2 °C and 50 - 60% humidity. The rats were acclimated for five days prior to the experiment. Rats were fed a standard laboratory rodent diet and had free access to water. The rats were randomly assigned to the following groups (n = 12): Control, LGG, Noise, and Noise + LGG. The Control and Noise groups received 1 mL normal saline by gavage daily. The LGG and Noise + LGG groups received 1 mL LGG suspension by gavage daily. For 56 days, the Noise and Noise + LGG groups were exposed to 88 dB sound pressure level (SPL) white noise 4 h/day, whereas the Control and LGG groups were exposed to background noise (< 40 dB SPL).

Noise exposure set-up

Noise was produced by a noise generator (BK 3560 C, Brüel & Kjær Instruments, Nærum, Denmark), which was then amplified by a power amplifier and broadcast via a loudspeaker. The frequency range of the generator's noise signal was 20-20,000 Hz. In a reverberation chamber, the rats were housed in wire mesh cages in the center of the sound field and exposed to the noise via the loudspeaker hung above the cage.

Morris water maze testing

The Morris water maze (MWM) test was conducted in accordance with a previous study [8], using hidden platform training (spatial learning) and a probe trial (spatial memory). During the platform training phase, the rats sought a hidden platform 2 cm below the water surface. The rats were set in one of the four quadrants of the pool, facing the wall (alternating clockwise in each trial), and were allowed to stay on the platform for 10 s after finding it. If the rats did not find the platform within 60 s, they were manually placed on the platform for 10 s. For four days in a row, the rats finished four trials per day. On day 5 during the probe trial session, the platform was removed and each rat was allowed to swim freely for 60 s. Video cameras were used to record the movements of the rat and to obtain the target quadrant distance/total distance, number of crossings over the target quadrant, time spent in the target quadrant during training sessions, and escape latency.

Transmission electron microscopy and hematoxylin-eosin (HE) staining of colon tissue

A sample of colon tissue (1-2 mm^3) was excised from each mouse, to which 2.5% glutaraldehyde was added, and the sample was stored at 4 °C. Tissues were fixed with osmium acid, dehydrated using an alcohol gradient, permeabilized with encapsulant epoxy, polymerized at 60 °C for 48 h, and then stored at room temperature for approximately 20 days. Ultrathin sections (50 nm) were sliced, double stained with uranyl acetate and lead citrate at room temperature for 15 min, dried at room temperature overnight, and observed using transmission electron microscopy.

Additionally, a sample of colon tissue was fixed with 4% paraformaldehyde, dehydrated using an ethanol gradient, rendered transparent with xylene, embedded in paraffin, cooled to -20 °C, and sliced into sections (5 μm). Subsequently, sections were heated at 45 °C in a water bath, gently flattened, and dried at 60 °C. After staining with HE, the sections were rendered transparent with xylene for 10 min, sealed with neutral resin, and observed under an optical microscope.

Western blot analysis

Frozen colon and hippocampal tissues were homogenized and centrifuged. Western blotting analysis was conducted using standard procedures, employing rabbit anti-occludin (1:1000, Bioworld, USA), rabbit anti-CLDN1 (1:2000, Bioworld, USA), and rat anti-GAPDH (1:10,000, Bioworld, USA) antibodies, and GADPH as an internal reference standard.

Enzyme-linked immunosorbent assay (ELISA)

Serum was obtained by centrifuging intracardiac blood for 10 min at 3000 × *g*. The serum was stored at -80 °C until further analysis. Serum levels of β-amyloid peptides (Aβ)1-40, Aβ1-42, nuclear factor-kappa B (NF-κB), interleukin (IL)-10, IL-17, D-lactic acid (D-LA), and endotoxin (lipopolysaccharide; LPS) were measured using corresponding ELISA kits (Biotop, Beijing, China), in accordance with the manufacturer's instructions.

Sequencing of 16S ribosomal RNA genes in microbiota

Amplicon sequencing was employed to sequence the 16S rRNA genes of the microbiota, as previously described [8]. Briefly, microbial DNA was isolated from the colon contents using the cetyltrimethylammonium bromide (CTAB) and sodium dodecyl sulfate (SDS) technique. Barcodes were used to amplify distinct portions of the 16S rRNA genes using specific primers (16S V4: 515F-806R, 18S V4: 528F-706R, and 18S V9: 1380F-1510R). PCR reactions were performed using Phusion® High-Fidelity PCR Master Mix (New England Biolabs, Ipswich, MA, USA) and bacterial diversity was assessed using the Illumina HiSeq platform (Illumina Inc., San Diego, CA, USA). Analysis was performed using Novogene Bioinformatics Technology (Beijing, China). QIIME software (http://qiime. org/) was used to examine the sequences. For each operational taxonomic unit (OTU), each representative sequence was annotated with classification information from the Ribosomal Database Project Classifier.

GC-MS analysis

GC-MS was performed using a TRACE™ 1310 ISQ LT GC-MS (Thermo Fisher Scientific, Waltham, MA, USA) with an HP-INNOWAX GC column (30 m × 0.25 mm ID × 0.25 μm; Agilent Technologies, Santa Clara, CA, USA) under the following conditions: sample volume, 1 μL; split ratio, 10:1; inlet temperature, 250 °C; ion source temperature, 230 °C; transmission line temperature, 250 °C; quadrupole temperature, 150 °C. The initial oven temperature was 90 °C, which was increased to 120 °C with a ramp rate of 10 °C/min, then to 150 °C with a ramp rate of 5 °C/min, and finally increased to 250 °C with a ramp rate of 25 °C/min, and was held at 250 °C for 2 min. Helium was used as the carrier gas at a flow rate of 1.0 mL/min. The SCFA concentration was calculated from the respective peak areas of the sample and the internal standard, isohexanoic acid.

Statistical analysis

The data are presented the mean ± standard deviation. Statistical analysis was performed using IBM SPSS Statistics v.22.0 and GraphPad Prism v.7 software. Data were analyzed using one-way analysis of variance (ANOVA), and differences were considered statistically significant when $P < 0.05$. The Kruskal-Wallis test was used when data were not normally distributed. For two-way comparisons between groups, the least significant difference test was used if the variances were equal, and the Games-Howell test was used if they were not. Correlations were identified using Spearman's rank correlation analysis.

Results

Effect of LGG intervention on cognitive impairment after noise exposure

To assess changes in cognitive ability, all rats underwent MWM testing after noise exposure for 56 days. The escape latency of rats in the Noise group was prolonged on the second day of training compared to that of rats in the Control group ($P < 0.05$). Rats in the Noise + LGG group showed a statistically significant ($P < 0.05$) decrease in escape latency on the first day of training compared with rats in the Noise group (Figure 1A). Additionally, the time spent in the target quadrant, number of platform crossings, and target quadrant distance/total distance were reduced in the Noise group (Figure 1B-D), and the number of traverses in the target quadrant and time spent in the target quadrant were increased in the Noise + LGG group (Figure 1C, D). These results showed that LGG intervention had a protective effect against noise-induced cognitive impairment in rats.

Effect of LGG intervention on gut microbiota after noise exposure

The composition of the gut microbiota in each treatment group was detected via 16S rRNA high-throughput sequencing, and gut microbiota richness and diversity (α-diversity) were reflected by Chao1 and ACE indices. The Chao1 and ACE indices were elevated in the Noise group (Supplemental Figure S1A, B), indicating that gut microbiota abundance in rats was increased after noise exposure, suggesting an overgrowth of gut bacteria in noise-exposed rats. Comparatively, the Chao1 and ACE indices were reduced in the Noise + LGG group, indicating that LGG intervention could regulate the gut microbiota in rats towards homeostasis. β diversity was obviously decreased after noise exposure compared with the control group (Tukey's test, $P < 0.05$; Supplemental Figure S1C). However, β diversity in the Noise + LGG group was significantly increased ($P < 0.05$) compared with the Noise group, indicating that LGG intervention could regulate both the richness and variety of the gut microbiota in rats.

Principal coordinate analysis (PCoA) revealed that the formation of the gut microbiota differed among the treatment groups (Supplemental Figure S1D). The distribution of the microbiota of the LGG group was relatively concentrated, while that of the Noise group was relatively dispersed, indicating that noise exposure and LGG intervention affected the abundance and structure of the gut microbiota. Based on the species annotation results, the top 10 most abundant bacteria of each treatment group were selected for clustering analysis based on the weighted UniFrac distance matrix (unweighted pair group method with arithmetic mean; UPGMA). Subsequently, the UPGMA results were combined with the relative abundance of the gut microbiota in each treatment group (Supplemental Figure S1E).Firmicutes, Bacteroidetes, and Proteobacteria were the most abundant phyla in all treatment groups. Compared to the Control group, the relative abundance of Bacteroidetes was increased while that of Firmicutes was decreased in the Noise group. Meanwhile, the abundance of Firmicutes was increased while that of Bacteroidetes and Proteobacteria was decreased after LGG intervention compared with the Noise group.

In order to further identify differences in the gut microbiota between the four treatment groups, changes in the composition of the gut microbiota were further assessed using the linear discriminant analysis effect size (LEfSe) test, and the dominant flora in each group were represented by cladograms (Figure 2A, B). The taxa with the greatest differences from phylum to genus were identified using linear discriminant

analysis (LDA) scoring (Figure 2C, D). The dominant species in the Control group belonged to the *Bacteroidaceae* and *Gammaproteobacteria*, while thosein the Noise group belonged to the *Gammaproteobacteria*. The dominant species in the LGG group belonged to the *Muribaculaceae*, *Erysipelotrichaceae*, and *Burkholderiacea,* and the genera *Allobaculum* and *Parasutterella*, while those in the Noise + LGG group included *Lactobacillus gasseri* and species belonging to the *Ruminococcaceae* and *Alloprevotella*. Furthermore, LEfSe was used to specifically analyze the abundance of differential genera in the Noise and Noise + LGG groups (Figure 2E, H), revealing that the abundance of species belonging to the *Gammaproteobacteria* was decreased while that of *Lactobacillus-gasseri* and species belonging to the *Ruminococcaceae* and *Alloprevotella* were increased in the Noise + LGG group compared with the Noise group.

Changes of SCFA levels and metabolites in gut microbiota after LGG intervention

SCFA levels in the feces of rats in each treatment group are shown in (Figure 3A-D). Levels of propionic acid, butyric acid, isobutyric acid, and isovaleric acid were reduced in the Noise group compared to those in the Control group, whereas levels of these SCFAs were increased after LGG intervention. These results indicated that LGG intervention inhibited the noise-induced decrease in SCFAs levels in rats.

Microbial metabolites are one of the main communication channels for crosstalk between bacteria and hosts. As the main product of bacterial fermentation, changes in SCFAs content alter the acidic environment in the gut, affect the normal growth of gut microbes, and influence the composition and structure of the gut microbiota. Therefore, correlations were analyzed between the SCFAs content in each treatment group and dominant bacteria at the phylum (Figure 3E), family (Figure 3F), and genus (Figure 3G) levels. The results revealed that the *Cyanobacteria* was negatively correlated with isobutyric acid and isovaleric acid. At the family level, *Lachnospiraceae* were significantly positively correlated with butyric acid, *Ruminococcaceae* were significantly positively correlated with isobutyric acid and isovaleric acid, and *Enterobacteriaceae* and *Eggerthellaceae*were negatively correlated with all SCFAs. At the genus level, *unidentified-Enterobacteriaceae* wasnegatively correlated with butyric acid, *Oscillibacter* species were positively correlated with isovaleric acid, and *Alloprevotella* species were positively correlated with hexanoic acid.

Changes in β-amyloid peptides and serum inflammatory cytokines after LGG intervention

Serum levels of Aβ1-40 and Aβ1-42 were higher after noise exposure than those in the Control group (Figure 4A, B). However, serum levels of Aβ1-40 and Aβ1-42 in the Noise + LGG group were notably lower ($P < 0.05$) than those in the Noise group. These results indicated that LGG intervention can regulate abnormal increases in Aβ serum levels. Serum inflammatory cytokine and mediator levels in each treatment group are shown in (Figure 4C-E). IL-17 and IL-10 are important inflammatory cytokines, and NF-κB is a main regulator of natural immunity and inflammation that can be activated by inflammatory cytokines. Serum levels of IL-17, NF-κB, and IL-10 were higher in the Noise group than in the Control group, but lower in the Noise + LGG group than in the Noise group. Serum levels of inflammation markers D-LA and LPS, which are related to gut mucosal damage, were remarkably higher in the Noise group than

those in the Control group (Figure 4F, G), suggesting injury to the gut mucosa. However, serum levels of D-LA and LPS were significantly reduced after noise and LGG intervention. These results showed that LGG intervention may inhibit the release of inflammatory cytokines into the blood and reverse the inflammation caused by noise exposure in rats, thus playing a protective role.

Effect of LGG intervention on epithelial barrier function after noise exposure

Histopathological analysis (Figure 5A) of colon tissues from each treatment group revealed complete mucosal structure, orderly arrangement of epithelial cells, and tightly arranged lamina propria glands in the Control and LGG groups. In the Noise group, the mucosal structure of the colon tissue was incomplete, the epithelium was exfoliated, and the lamina propria glands were short and arranged loosely and irregularly. The mucosal structure of the colon tissue in the Noise + LGG group was incomplete and the lamina propria glands were arranged irregularly, but the pathological changes were reduced compared to those in rats exposed to noise alone.

Comparison of colon tissues in each treatment group (Figure 5B) revealed normal colonic epithelial cells in the Control and LGG groups with more mitochondria and clear structures. Comparatively, the chromatin of colonic epithelial cells was marginalized in the Noise group, the connective gaps of epithelial cells were significantly widened, fewer mitochondria were present, and some mitochondria were vacuolated. However, the colonic epithelial cells in the Noise + LGG group were relatively normal and exhibited clear structures but fewer mitochondria than those in the Control group.

Analysis of tight junction proteins in the colon and hippocampus of each treatment group demonstrated that expression levels of occludin and CLDN1 in the Noise group were decreased compared to those in the Control group (Figure 5C, D). Further, expression levels of occludin and CLDN1 in the Noise + LGG group were increased compared to those in the Noise group. These results suggested that noise exposure led to impaired gut and blood-brain barrier functions in rats, which was ameliorated with LGG intervention.

Discussion

Our previous studies showed that noise exposure alters the gut microbiota [17], inducing oxidative inflammation and AD-like neuropathy [8]. The results of the current study confirmed that chronic low-intensity noise exposure could induce numerous microbiome-gut-brain axis events. Moreover, the results indicated that LGG intervention could ameliorate noise-induced gut microbiota disturbance, gut and blood-brain barrier dysfunction, cognitive impairment, and systemic inflammation, which may provide new insights into treating the neurological effects of environmental noise exposure (Figure 6).

LGG improves cognitive decline caused by noise exposure

In our previous studies [7,18], long-term high-intensity noise exposure negatively impacted spatial learning and memory and caused cognitive impairment in rats. Gut microbiota dysbiosis, increased

systemic inflammation, and reduced integrity of gut and blood-brain barriers may also be factors in noise-induced impairment of cognitive function via the microbiome-gut-brain axis. The results of the current research support that low-intensity noise exposure leads to cognitive decline and concur with two recent models of low-intensity noise exposure [19,20], while the cognitive deficits were improved by LGG intervention. Therefore, we hypothesize that the microbiome-gut-brain axis may play a key role in the amelioration of cognitive impairment caused by noise exposure.

LGG ameliorates dysfunction of gut microbiota and metabolites caused by noise exposure

The results of the current study indicate that noise exposure leads to abnormal changes in the gut microbiota and disrupts normal metabolism of SCFAs, which in turn damages the intestinal barrier, creating a vicious cycle [21]. LGG intervention can ameliorate gut microbiota imbalance and SCFAs metabolism abnormalities caused by noise. *Gammaproteobacteria* are related to increased intestinal permeability, inflammatory cell proliferation, and secretion of inflammatory mediators, leading to activation of the immune inflammatory response [22,23]. In addition to providing energy to the host [24], *Ruminococcaceae*are known to have beneficial effects on intestinal barrier function [25].
Further, *Lactobacillus-gasseri*, a symbiotic lactic acid bacterium, can inhibit the NF-κB signaling pathway and increase intestinal barrier integrity [26]. *Lactobacillus-gasseri* can also regulate gut bacterial dysbiosis, alleviate colonic inflammation, and improve cognitive dysfunction in mice [27]. Moreover, the relative abundance of *Alloprevotella* has been negatively associated with inflammation [28] and may also enhance antioxidant capacity [29]. In this study, LGG intervention increased the abundance of *Alloprevotella* and probiotic bacteria belonging to the *Firmicutes*, such as *Ruminococcaceae* and *Lactobacillus-gasseri*, which are known to produce SCFAs [30,31]. In addition, the abundance of species belonging to the *Enterobacteriaceae* and *Eggerthellaceae* in the *Gammaproteobacteria* was negatively correlated with isobutyric acid, isovaleric acid, and hexanoic acid levels, whereas LGG decreased the abundance of harmful *Gammaproteobacteria*. These results show that LGG can mitigate the negative effects of noise by increasing the abundance of *Firmicutes* and reversing the *Firmicutes/ Bacteroidetes* ratio, which is considered an important indicator of gut microbiota health [32]. These variations were followed by beneficial changes in gut microbiota diversity and improved SCFAs levels.The study findings demonstrate that LGG intervention can reshape the gut microbiota structure, increase the SCFAs content, and maintain the normal intestinal microenvironment by enriching beneficial bacteria and inhibiting pathogenic bacteria.

LGG alleviates gut-blood-brain barrier damage and systemic inflammation by modulating the gut-brain axis

The results of the present research indicate that noise exposure increases gut and blood-brain barrier permeability and systemic inflammatory responses in rats, which is consistent with our previous research [8]. D-LA is used as a serum marker of intestinal permeability [33] and LPS can be used as an indicator of gut microbiota translocation [34]. Gut mucosal inflammation is positively correlated with D-LA and LPS levels, which are also associated with increased gut mucosal permeability [35]. Consistently,

noise exposure significantly increased serum levels of LPS and D-LA, pro-inflammatory cytokine IL-17, and inflammatory mediators NF-κB, Aβ1-40, and Aβ1-42. Interestingly, noise exposure led to elevated levels of anti-inflammatory cytokine IL-10. NF-κB can drive the differentiation of monocytes into macrophages, with the M1 type producing pro-inflammatory cytokines and the M2 type producing anti-inflammatory cytokines, such as IL-10 [36]. We hypothesize that gut microbiota dysbiosis after noise exposure and excessive release of LPS activates NF-κB, which in turn drives the differentiation of monocytes into macrophages, with the M1 phenotype leading to a massive release of IL-17, thus causing an inflammatory response. Subsequently, the M2 phenotype produces IL-10 to resist the body's exposure to inflammation.

LGG can act on the gut epithelium to form a barrier and block the invasion of harmful bacteria in the intestine [37], regulate the NF-κB signaling pathway, and exert certain anti-inflammatory effects [38]. Therefore, serum levels of D-LA, LPS, and NF-κB decreased after LGG intervention. The reduced levels of IL-17 and IL-10 may be due to less LPS being released after improvement of intestinal damage, thus downregulating NF-κB expression, inhibiting macrophage differentiation, and balancing inflammatory and anti-inflammatory cytokines. In addition, LGG intervention reduced serum levels of Aβ, preventing abnormal entry of Aβ into the brain through the damaged blood-brain barrier, which can damage neurons, exacerbate neuroinflammation, and lead to cognitive dysfunction [39,40]. These results further suggest that LGG regulates gut microbiota homeostasis and SCFAs levels, inhibits the release of inflammatory cytokines, and protects the intestinal barrier. Therefore, LGG intervention may prevent local inflammation in the gut, avoiding systemic inflammation induced via the gut-brain axis pathway that could impair neurological function.

Conclusions

Taken together, the study findings indicate that noise exposure disturbs intestinal microbiota homeostasis, SCFAs metabolism, and upregulates systemic low-grade inflammation, which may be the cause of intestinal and brain epithelial barrier deficiencies. LGG intervention can ameliorate cognitive deficits and systemic inflammation in rats exposed to noise, possibly through linked changes in the microbiome-gut-brain axis. This research adds to our knowledge of the etiological signaling pathways participating in negative non-auditory effects of environmental noise.

Abbreviations

Aβ: β-amyloid peptides; AD: Alzheimer’s disease; D-LA: D-lactic acid; IL: interleukin; LGG: *Lactobacillus Rhamnosus* GG; LDA: linear discriminant analysis; LEfSe: linear discriminant analysis effect size; LPS: lipopolysaccharide; MWM: Morris water maze; NF-κB: nuclear factor-kappa B; OTU: operational taxonomic unit; PCoA: principal coordinate analysis; SCFA: short-chain fatty acid; SPL: sound pressure level; UPGMA: unweighted pair group method with arithmetic mean.

Declarations

Authors' Contributions: Conceptualization, B.C., S.J., and F.W.; X.L., P.Z., and W. C. shared first authors; methodology, X.L., P.Z., W.C., and X.S., software, W.C.; validation, S.J. and F.W.; formal analysis, H.Y.; investigation, B.C.; resources, K.M. and F.W.; data curation, J.Y. and Y.F.; writing original draft preparation, W.C.; writing review and editing, X.L. and P.Z.; visualization, P.Z. and X.L.; supervision, X.G.; project administration, X.S.; funding acquisition, B.C.

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Ethics approval and consent to participate

All animal experiments were conducted according to the KU Leuven ethical guidelines and approved by the KU Leuven Committee on Animal Care.

Consent for publication

No conflict of interest exits in the submission of this manuscript, and manuscript is approved by all authors for publication.

Competing Interests:

The authors have declared that no competing interest exists.

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Figures

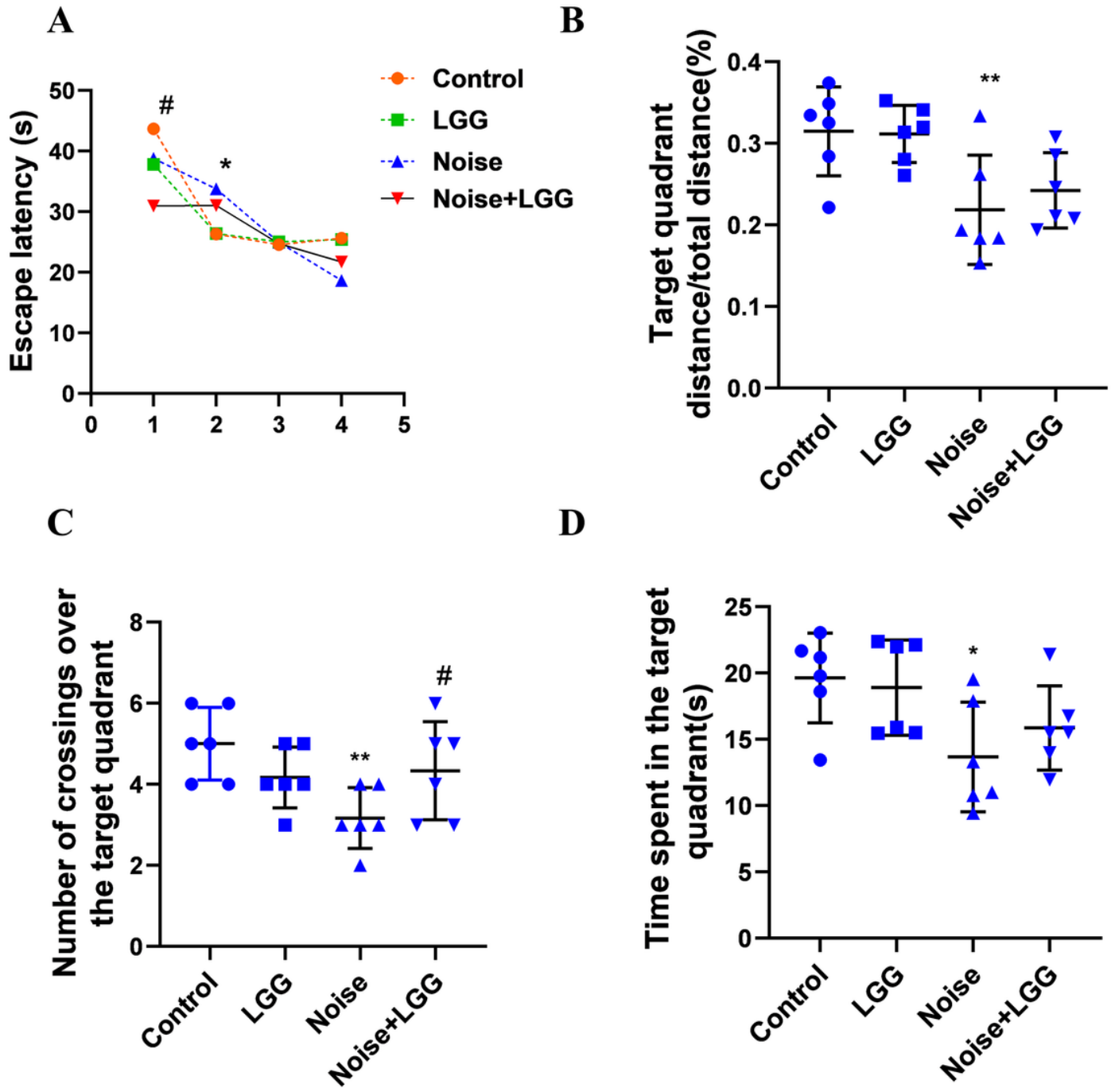

Figure 1

Effects of LGG intervention improved learning and memory abilities in rats. (A) Effects of noise exposure on escape latency in the training phase; (B) Effects of noise exposure on target quadrant distance / total distance in the probe trial; (C) Effects of noise exposure on number of crossings over the target quadrant in the probe trial; (D) Effects of noise exposure on time spent in the target quadrant in the probe trial. n = 6. *P < 0.05, **P < 0.01 vs. Control, Noise group; #P < 0.05 vs. Noise, Noise + LGG group.

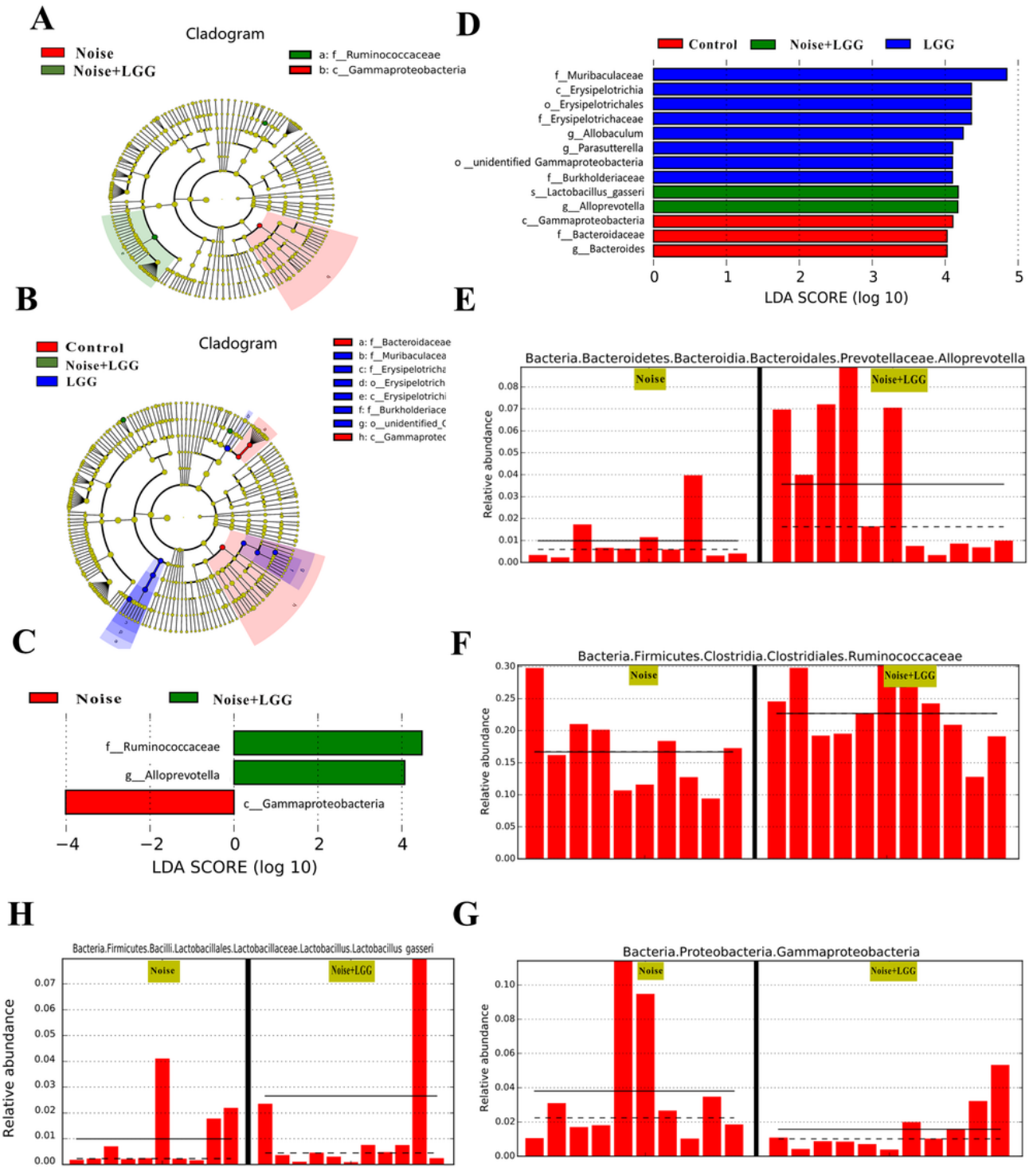

Figure 2

LGG regulates the composition of gut microbiota. (A, B) Cladogram based on linear discriminant analysis effect size (LEfSe) analysis. The central point represents the root of the tree (bacteria), and each ring represents the next lower taxonomic level (phylum to genus). The diameter of each circle represents the relative abundance of the taxon (n = 10). (C, D) The most differentially abundant taxa in each group

identified by linear discriminant analysis (LDA) scores generated from the LEfSe analysis (n = 10). (E, H). Changes in the abundance of different microbiota in the Noise and Noise + LGG groups (n = 10).

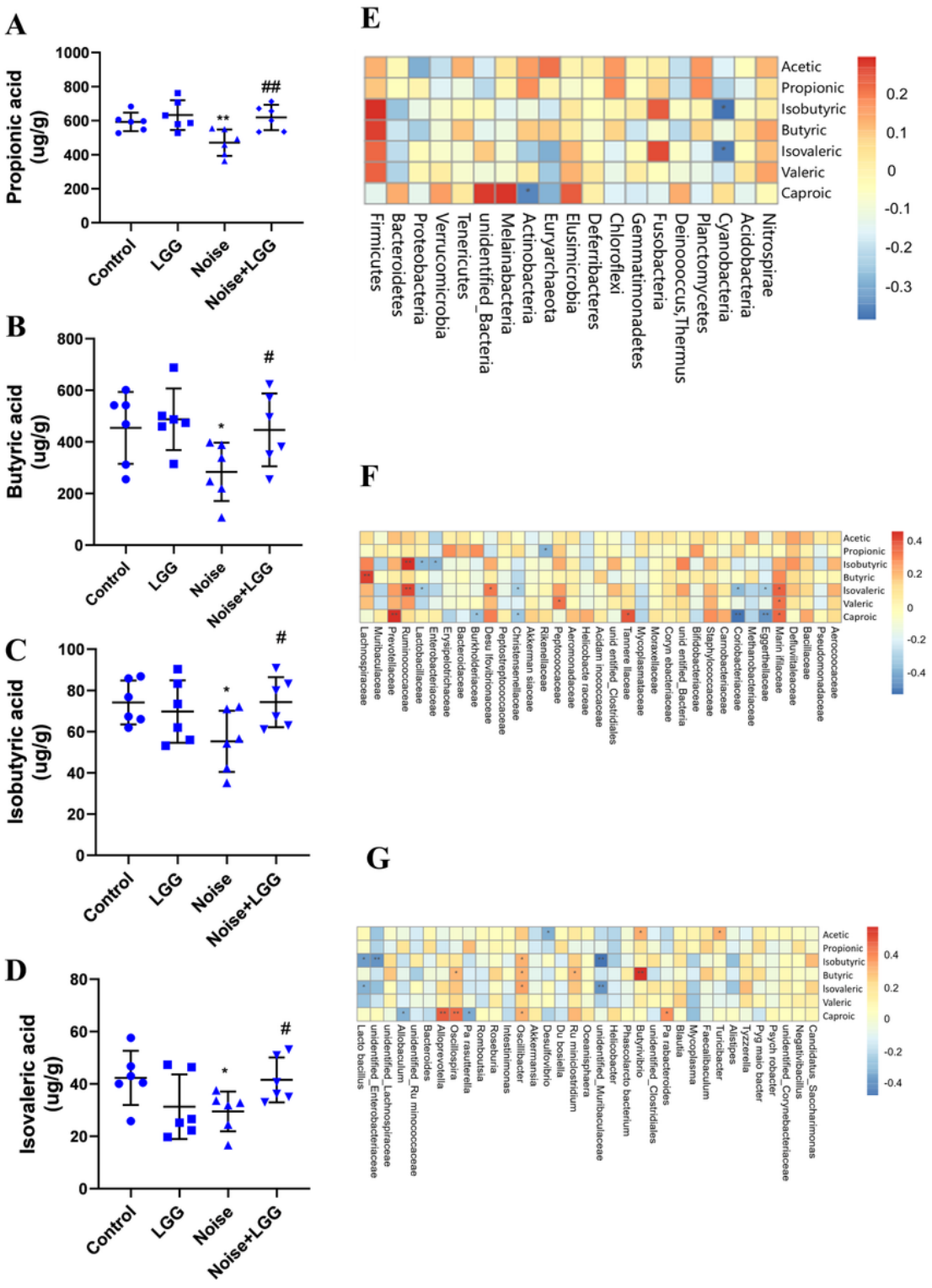

Figure 3

Analysis of the level of SCFAs and its correlation with gut microbiota. LGG intervention reversed the reduction in SCFAs levels caused by noise exposure (A-D). (A) Propionic, (B) Butyric, (C) Isobutyric, and

(D) Isovaleric acids. n = 6. *P < 0.05, **P < 0.01 vs. Control, Noise group; #P < 0.05, ##P < 0.01 vs. Noise, Noise + LGG group. (E-G) Correlation analysis of SCFAs and gut microbiota at phylum, family, and genus levels. n = 10. *P < 0.05, **P < 0.01.

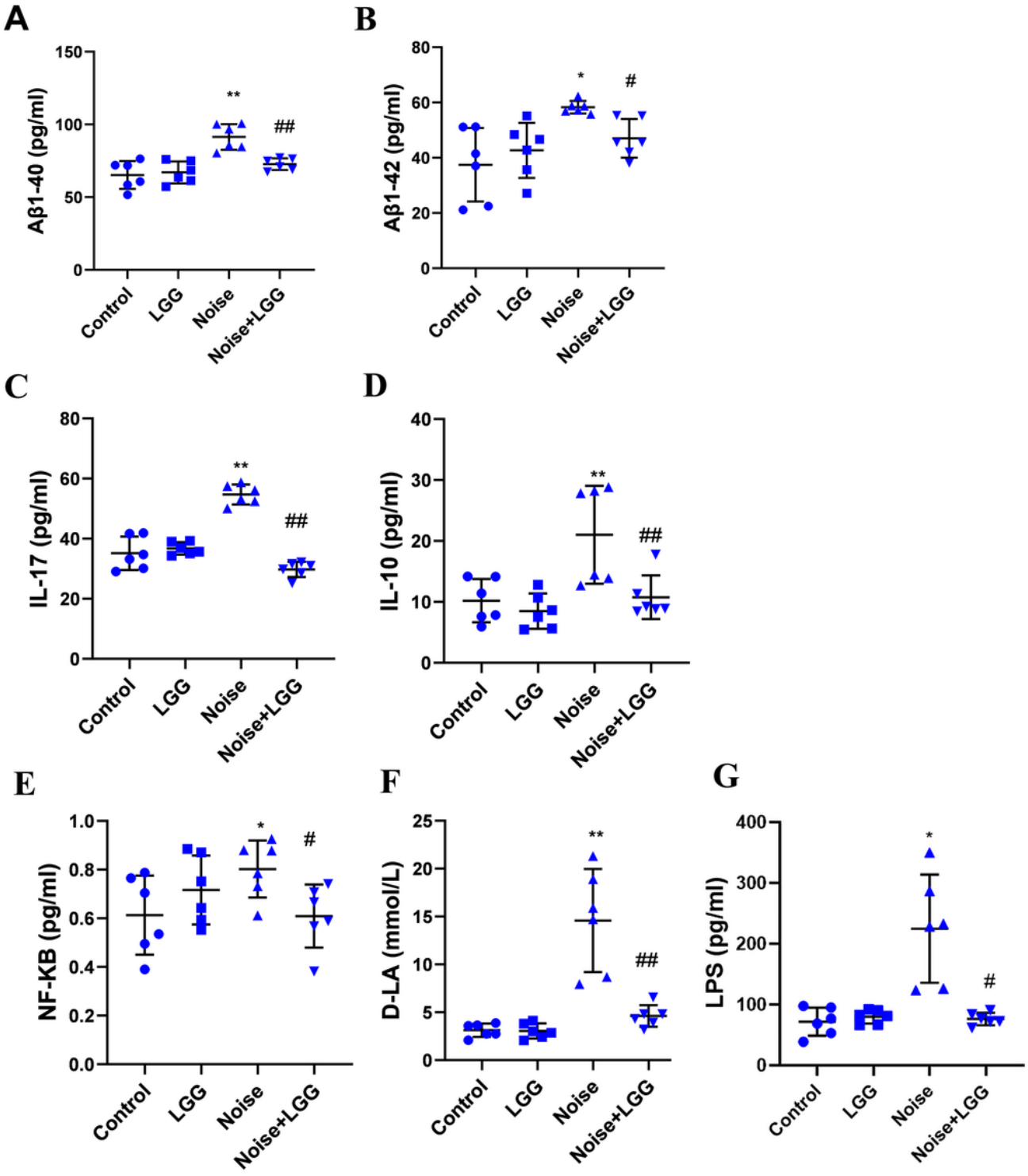

Figure 4

LGG intervention reduces serum inflammatory cytokines and inflammatory markers. (A, B) Changes in serum Aβ1-40 and Aβ1-42 in each group. (C-E) Changes in serum IL-17, IL-10, and NF-κB in each group. (F) Changes in serum D-LA in each group. (G) Changes in serum LPS in each group. n = 6. *P < 0.05, **P < 0.01 vs. Control, Noise group; #P < 0.05, ##P < 0.01 vs. Noise, Noise + LGG group.

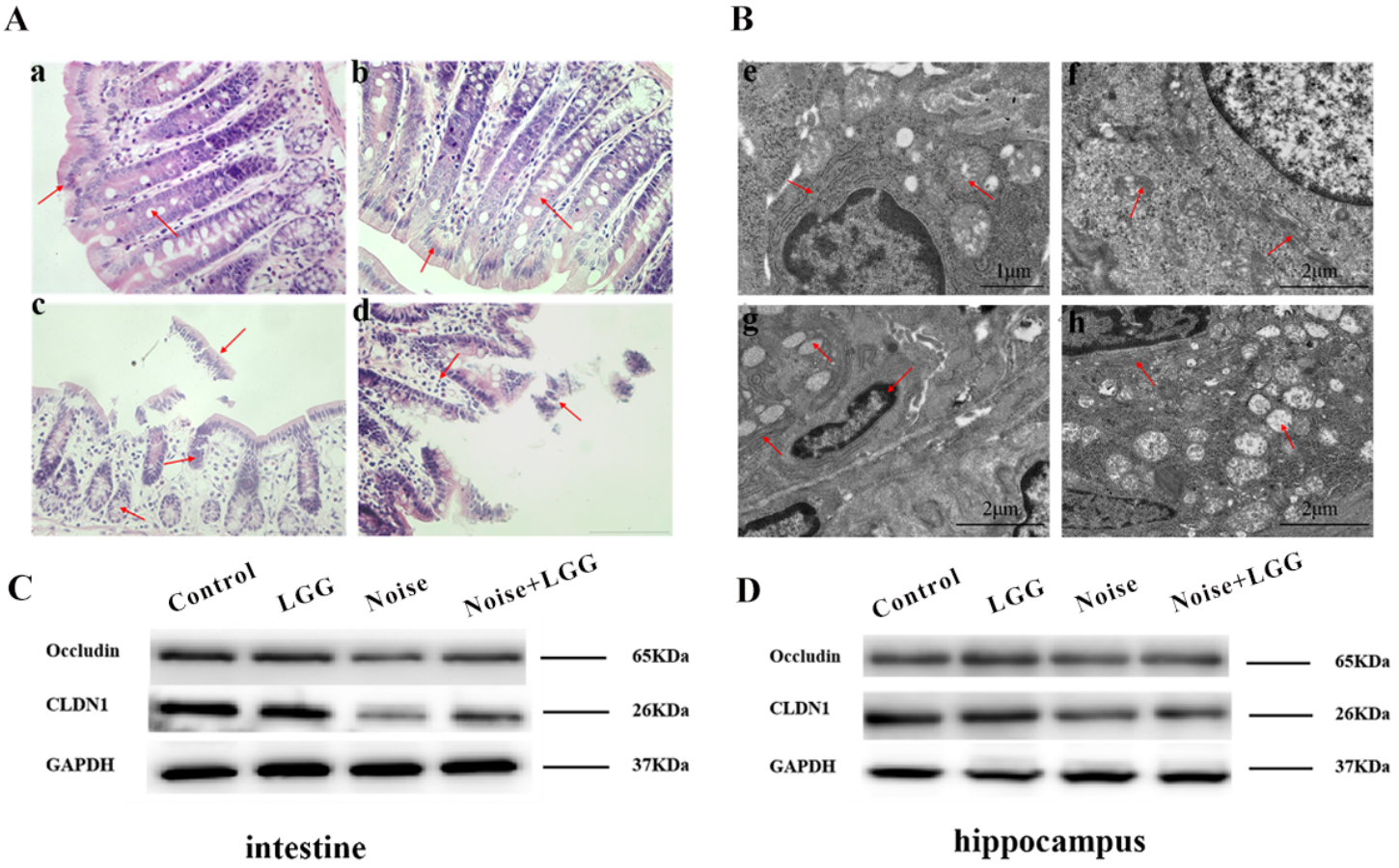

Figure 5

Protective effect of LGG intervention on gut and blood-brain barrier in rats. (A) Histopathological changes in HE-stained rat colon sections. (a) Control, (b) LGG, (c) Noise, and (d) Noise + LGG groups. (B) The ultrastructural colon changes were observed with transmission electron microscopy (magnification × 5000). (e) Control, (f) LGG, (g) Noise, and (h) Noise + LGG groups. (C) The protein levels of occludin and CLDN1 in the colon tissues of rats. (D) The protein levels of occludin and CLDN1 in the hippocampal tissues of rats.

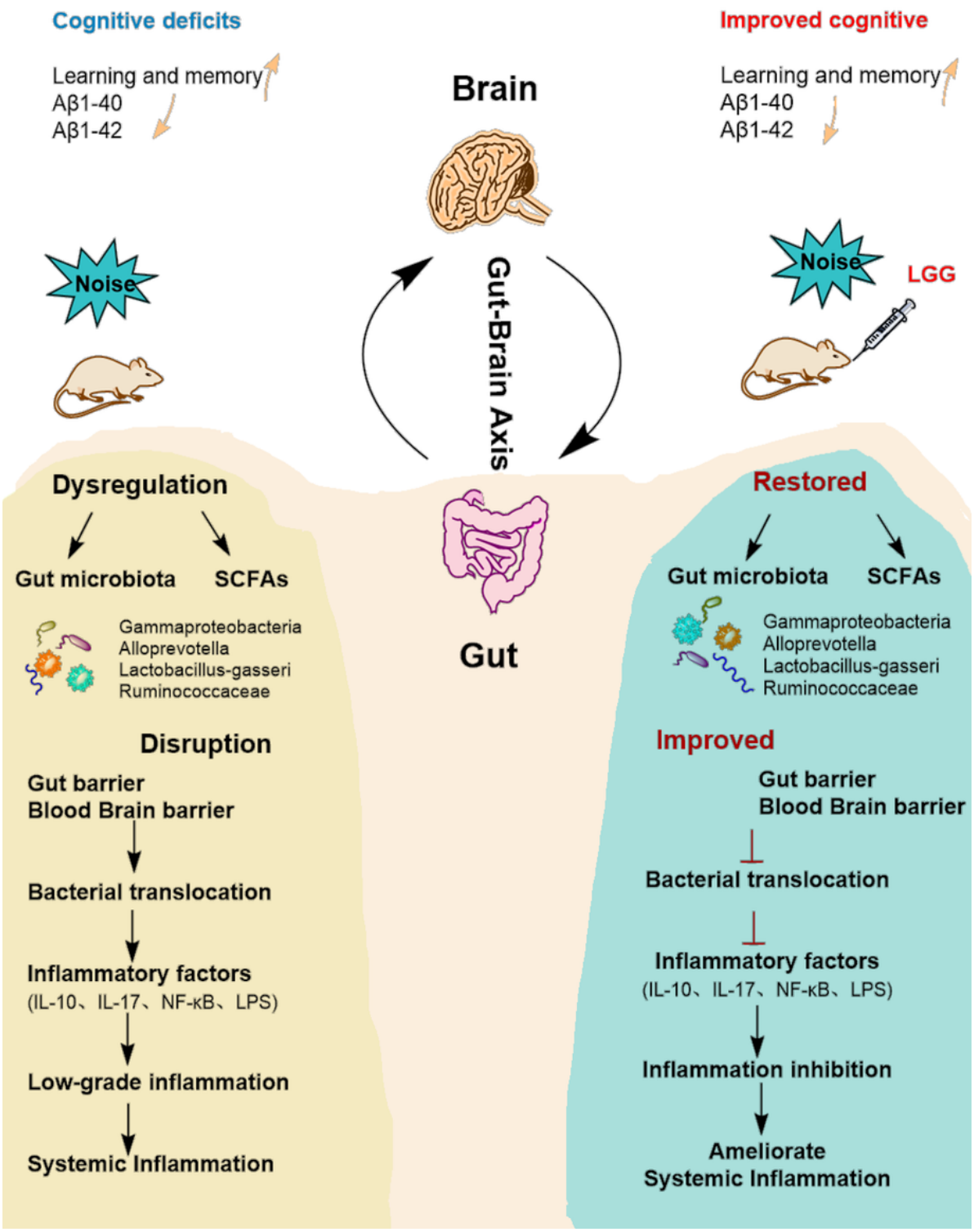

Figure 6

LGG intervention ameliorates systemic inflammation and cognitive impairment. Analysis of fecal microbiota showed significant differences in composition and diversity between the two groups. LGG intervention significantly increased the abundance of beneficial bacteria (*Ruminococcaceae*, *Lactobacillus-gasseri*, and *Alloprevotella*) and decreased harmful strains (*Gammaproteobacteria*). LGG colonization increased the abundance of SCFA-producing bacteria (*Ruminococcaceae*, *Alloprevotella*)

and the content of SCFAs. In addition, LGG intervention also improved cognitive function and reduced systemic inflammation through the microbial-gut-brain axis by repairing gut-blood-brain barrier damage, thus avoiding bacterial translocation, reducing inflammatory cytokine levels, and improving learning and memory abilities in rats. LGG: *Lactobacillus rhamnosus* GG; SCFAs: short-chain fatty acids; IL-10: interleukin 10; IL-17: interleukin 17; LPS: lipopolysaccharide.

Supplementary Files

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