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Streptomyces rimosus-rich soil exposure alleviates depression-like behaviors by modulating neuroinflammation and synaptic plasticity in mice with stress

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Soil contains a wide range of microbial communities. Recently, direct exposure to soil and soil microbes has been reported to have a positive effect on emotional integrity. Among the soil microbes, *Streptomyces rimosus* is known to produce geosmin, which have a unique odor and positive effects on mental status. In this context, this study aimed to investigate the effects of direct exposure to soil containing *S. rimosus* on depression-like behavior and depression-related factors in the mouse. To induce depression, the mice were exposed to chronic restraint stress (CRS) for 14 days, and direct soil exposure continued for 17 days from the first day of CRS. The results showed that direct exposure to soil containing *S. rimosus* alleviated the CRS-induced depression-like behavior. Additionally, *S. rimosus*-rich soil exposure reduced the activation of microglia and astrocyte in the depression-related brain area, and reduced the mRNA expression levels of cytokines including interleukin (IL)-6, interferon-γ, and IL-17 A. Moreover, *S. rimosus*-rich soil contact increased synaptic plasticity, which was reduced by CRS. The same effects were not observed in the group exposed to sterilized soil. Collectively, the current study suggests that *S. rimosus* soil contact can be a beneficial psychological therapeutic strategy for patients with mental illnesses.

Keywords *Streptomyces rimosus*, Soil microbiome, Depression, Neuroinflammation, Synaptic plasticity

Humans and the soil interact directly and indirectly with each other[1]. For example, soil helps humans sustain their lives by directly providing necessities such as food, building materials, and fossil fuels[2]. Additionally, soil-supported ecosystems play a role in the carbon cycle[3]. There has been emerging evidence showing that the horticultural activity of touching soil has beneficial effects on both physical and mental health[4–6]. In a recent study, mice exposed to unsterilized soil had higher serum levels of interferon-γ, which is an important biomarker of immune response, compared to intact mice[7]. However, this phenomenon was not observed in mice exposed to sterilized soil. These studies imply that soil microbes may be a crucial factor in the health benefits of soil exposure.

Streptomyces rimosus, a gram-positive filamentous bacterium ubiquitous in soil environments, represents a particularly interesting candidate for understanding soil-mediated neurobiological benefits. Beyond its well-established role in antibiotic production, *S. rimosus* produces a complex array of secondary metabolites that may have significant neuroactive properties[8]. The growing body of evidence suggests that certain soil bacteria, including *S. rimosus*, may influence neural function through direct or indirect pathways that affect neurotransmitter systems, immune-brain interactions, and stress response mechanisms[9–11]. Given that neuroinflammation and synaptic dysfunction are central to depression pathophysiology, soil microbes with

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anti-inflammatory and neuromodulatory properties present a novel therapeutic avenue that warrants systematic investigation.

Similarly, our group has previously reported changes in psychophysiological responses after exposure to soil inoculated with *S. rimosus*, a representative soil microbe[5]. *S. rimosus* produces geosmin and 2-methylisoborneol (2-MIB), which play a role in stabilizing brain waves in response to an earthy odor[12]. Our previous study revealed that *S. rimosus*-rich soil exposure increased alpha wave activity in the frontal lobe of the brain, which may be closely related to relaxation. Moreover, the group exposed to soil inoculated with *S. rimosus* had lower serum C-reactive protein concentrations than the group exposed to sterile soil. Although our previous study found a beneficial effect of a single bout *S. rimosus* exposure on mental health under normal conditions, it is still unclear whether repeated contact with soil inoculated with *S. rimosus* can alleviate depression and its related pathophysiology under stressed conditions[5].

Depression is a mental disorder characterized by persistent low mood, sadness, and malaise[13]. According to the World Health Organization, depression is a common disorder that affects approximately 280 million individuals[14]. Although the pathological mechanism of depression remains unclear, hypothalamic-pituitary-adrenal axis dysfunction, neurotransmitter dysregulation, and neuroinflammation are considered primary factors[15,16]. In particular, neuroinflammation has emerged as a significant pathology in depression because many studies have reported depression in patients suffering from immune diseases, infectious diseases, or those exposed to cytokines[17–20]. In addition, synapses are damaged by increased cytokine levels caused by neuroinflammation and synaptic plasticity is reduced, which is the main target mechanism of drug development[21,22].

Based on previous studies, we hypothesized that exposure to soil inoculated with *S. rimosus* could alleviate depression by suppressing neuroinflammation and promoting synaptic plasticity. This study investigated the effects of direct exposure to soil rich in *S. rimosus* on depressive behavior, inflammatory responses, and synaptotoxicity in the brains of mice with chronic restraint stress (CRS).

Materials and methods

Preparation of the soil and *S. rimosus*

The preparation process of soil and *S. rimosus* is the same as[5]. Soil was sterilized by autoclaving at 121 °C for 15 min, and sterility was confirmed by the absence of microbial growth on media following inoculation. Briefly, sterilized peatmoss (2000 ml) and perlite (800 ml) were mixed with 200 ml of water and 50 ml of *S. rimosus* culture medium suspension, with a dry cell weight of 6.68 ± 0.02 g/L. To ensure consistency throughout the exposure period, all soil mixtures were freshly prepared by combining sterile soil with bacterial suspension cultured under identical and strictly controlled conditions. In the sterilized soil group, an equal amount of bacteria-free medium was added instead of the *S. rimosus* culture medium.

Animals

Six-week-old female C57BL/6 J mice were purchased from Daehan Biolink (Eumseong, Republic of Korea). Mice were accommodated at a maintained condition (temperature: 23 ± 1 °C, humidity: 60 ± 10% a 12 h light/dark cycle, and water and food ad libitum). All animal studies were performed in accordance with the "Guide for the Care and Use of Laboratory Animals, 8th edition" (National Institutes of Health, 2011) and approved by the "Animal Care and Use Guidelines" of Kyung Hee University, Seoul, Republic of Korea (Approval number: KHSASP-22-585). The study was conducted in compliance with the ARRIVE guidelines.

Experimental design

The mice were randomly divided into four groups: (1) normal (NOR) group (non-CRS and non-soil exposure), (2) CRS group (CRS induced and non-soil exposure), (3) CRS + sterilized soil group (CRS induced and sterilized soil exposure), (4) CRS + soil with *S. rimosus* group (CRS induced and exposed to sterilized soil inoculated with *S. rimosus*). Mice were divided into 12 mice per group. Restraint stress was induced for 6 h every day for a total of 14 days using mouse restrainer[23]. Immediately after restraint stress exposure, mice were directly exposed to sterilized soil or soil with *S. rimosus* for 1 h. Direct soil exposure was conducted for 14 days during restraint stress and immediately before behavioral experiments and mouse sacrifice, for a total of 17 days. 1220 ml of soil samples were placed in each 38 cm × 24 cm × 18 cm cage, and then 12 mice were exposed together for 1 h.

Assessment of depression-like behaviors

Tail suspension test (TST)

Mice were suspended in a visually isolated area with the tip of their tail firmly clamped to a metal bar. After 6 min of video recording, immobility time was measured. Measurements were taken for 4 min, excluding the first 2 min, by a highly trained observer who was unaware of the group[24].

Forced swim test (FST)

Each mouse was placed in a glass cylinder (25 cm × 14 cm) containing 20 cm of water at a temperature of 22 ± 1 °C. The water was changed between each swim session and the mouse was forced to swim for 6 min. The immobility time was measured by video surveillance during the last 4 min of the 6 min test[23].

Tissue preparation

One day after the behavior test, the mice were anesthetized. Six mice per group were perfused transcardially with 0.05 M phosphate-buffered saline (PBS) and subsequently fixed with pre-chilled 4% PFA in 0.1 M phosphate buffer. Whole brain tissues were post-fixed with 4% PFA overnight, immersed in a solution containing 30% sucrose in 0.05 M PBS, and stored at 4 °C until sectioning. The frozen brains were coronally sectioned on a cryostat at 25 μm and then stored in a storage solution at 4 °C. The following Bregma coordinates were used

for each brain region based on Paxinos and Franklin's mouse brain atlas: prefrontal cortex (Bregma + 1.70 to + 2.00 mm), hippocampus (Bregma − 1.20 to − 2.10 mm), and hypothalamus (Bregma − 0.50 to − 1.50 mm)[25]. For the analysis of the prefrontal cortex, cortical layers II–V were included. The remaining six mice per group were decapitated, and the hippocampus, prefrontal cortex and hypothalamus in their brains was isolated and stored at − 80 °C until used for extracting mRNA.

Materials and reagents

Rabbit anti-ionized calcium-binding adapter molecule-1 (Iba-1) was purchased from Fujifilm Wako (Chuo-Ku, Japan; Cat. No. 019-19741). Rabbit anti-glial fibrillary acidic protein (GFAP; Cat. No. PA3-16727) and Alexa 488 (Cat. No. A11008) were purchased from Invitrogen (Waltham, USA). Biotinylated goat anti-rabbit antibody, avidin–biotin complex (ABC) solution, normal goat serum and streptavidin-Daylight 594 (Cat. No. DI-1594) were purchased from Vector Labs (California, United States). TRIzol reagent was purchased from Life technologies (California, USA). Rabbit anti-postsynaptic density protein 95 (PSD-95) was purchased from Abcam (Cambridge, UK; Cat. No. AB18258). Mouse anti-synaptophysin (SYP; Cat. No. S5768), paraformaldehyde (PFA), 3,3-diaminobenzidine (DAB) and all other reagents were purchased from Sigma-Aldrich (St. Louis, USA) unless noted.

Immunofluorescence

Three to four brain sections per animal were washed with 0.05 M PBS and incubated with a rabbit anti-Iba1 antibody (1:1000) overnight at 4 °C in the presence of 0.3% triton X-100. After rinsing in 0.05 M PBS, the sections were incubated with anti-rabbit Alexa 488 (1:500) for 1 h. To examine the activation of astrocyte, GFAP immunofluorescence staining was performed. GFAP staining was the same as the Iba-1 staining method, using rabbit anti-GFAP as the primary antibody and anti-rabbit Daylight 594 as the secondary antibody. The images were photographed using an K1-Fluo confocal microscope (Nanoscope Systems, Republic of Korea). The areas of Iba-1-, and GFAP-positive cells in the hippocampus, prefrontal cortex and hypothalamus were analyzed using the ImageJ software (National Institutes of Health, USA). Quantification was performed based on the area fraction of positive staining in each region.

Immunohistochemistry

Three to four brain sections per animal were rinsed in 0.05 M PBS and incubated with 1% hydrogen peroxide (H_2O_2) in 0.05 M PBS for 15 min. Subsequently, sections were replaced with anti-Iba-1 antibody (1:1000) in 0.3% Triton X-100 and 1% normal goat serum in 0.05 M PBS overnight at 4 °C. They were subsequently incubated in an ABC solution with biotinylated anti-rabbit immunoglobulin G antibodies (1:500). DAB was used to develop the color of each section, and images were photographed using an Olympus BX51 Fluorescence Microscope (Olympus, Tokyo, Japan). The area of Iba-1-positive cells were analyzed using the ImageJ software (National Institutes of Health, USA). Quantification was performed based on the area fraction of positive staining in each region.

mRNA extraction and real-time PCR (RT-PCR) analysis

Total RNAs of hippocampus, prefrontal cortex and hypothalamus were extracted using the TRIzol reagent according to the manufacturer's instructions. Total RNA was reverse-transcribed to cDNA using TOPscript™ RT DryMIX (Enzynomics, Republic of Korea), and RT-PCR was performed using TOPreal™ qPCR 2X PreMIX (SYBR Green; Enzynomics, Republic of Korea), and the CFX Connect Real-Time PCR System (Bio-Rad Laboratories, USA). Primers synthesized by COSMO Genetech (Seoul, Republic of Korea) were interleukin-6 (IL-6), interferon-γ (IFN-γ) and interleukin-17 A (IL-17 A) are listed in Table 1.

Statistical analysis

Differences among the groups were analyzed statistically by one-way analysis of variance (ANOVA) followed by Dunnett's post-hoc test using GraphPad Prism 8.0 software (GraphPad Software Inc., USA). All values are presented as mean ± standard error of the mean (S.E.M.). The differences were considered statistically significant at $p < 0.05$ and are expressed in each figure.

Results

Exposure to soil with *S. rimosus* improves depression-like behaviors in CRS-induced mice

Depression-like behaviors in mice were evaluated using the TST and FST. In the TST, the immobility time increased owing to CRS. Additionally, compared to the CRS group, the immobility time was significantly reduced in the CRS + soil with *S. rimosus* group. However, the CRS + sterilized soil group showed no significant changes compared to the CRS group [one-way ANOVA, $F(3, 44) = 4.373$; $p = 0.0064$, NOR vs. CRS; $p = 0.0493$, CRS vs. soil with *S. rimosus* (Fig. 1A)]. Similar to the TST results, the FST results showed that the immobility

Gene symbol	Primer sequence (forward)	Primer sequence (reverse)	References
IL-6	5′-CCG GAG AGG AGA CTT CAC AG-3′	5′-TTG CCA TTG CAC AAC TCT TT-3′	NM_001314054.1
IFN-γ	5′-GCG TCA TTG AAT CAC ACC TG-3′	5′-GAG CTC ATT GAA TGC TTG GC-3′	NM_008337.4
IL-17 A	5′-GCC CTC AGA CTA CCT CAA CC-3′	5′-ACA CCC ACC AGC ATC TTC TC-3′	NM_010552.3

Table 1. Oligonucleotide sequences used in RT-PCR experiments.

time was increased by CRS and was significantly reduced in the CRS + soil with *S. rimosus* group, but there was no significant difference in the CRS + sterilized soil group [one-way ANOVA, F (3, 44) = 3.513; $p = 0.0113$, NOR vs. CRS; $p = 0.0493$, CRS vs. soil with *S. rimosus*, (Fig. 1B)].

Exposure to soil with *S. rimosus* inhibits microglial activation in CRS-induced mouse brain

To evaluate the inhibitory effects on microglial cell-mediated neuroinflammation, we assessed microglial activation by staining for Iba-1, a microglial marker. CRS significantly increased the area of Iba-1-posivitve cells in the dentate gyrus (DG), prefrontal cortex, and paraventricular nucleus (PVN). In the hippocampal DG, there was no significant difference between the CRS + sterilized soil group and the CRS group, but the area of Iba-1-posivitve cells was significantly decreased in the CRS + soil with *S. rimosus*. These significant results in the CRS + soil with *S. rimosus* group were also observed in the prefrontal cortex and PVN [one-way ANOVA, DG: F (3, 20) = 37.69; $p < 0.0001$, NOR vs. CRS; $p < 0.0001$, CRS vs. soil with *S. rimosus*; prefrontal cortex: F (3, 20) = 7.941; $p = 0.0046$, NOR vs. CRS; $p = 0.0078$, CRS vs. soil with *S. rimosus*; PVN: F (3, 20) = 12.93; $p = 0.0099$, NOR vs. CRS; $p < 0.0001$, CRS vs. soil with *S. rimosus*, (Fig. 2)].

Exposure to soil with *S. rimosus* inhibits astrocyte activation in CRS-induced mouse brain

Astrocytes, which are glial cells, play a major role in neuroinflammation along with microglia[26]. Therefore, we evaluated the level of activation of GFAP, an astrocyte marker, in the DG and PVN. In the DG, the number of GFAP-positive cells was significantly higher in the CRS group than in the NOR group. Additionally, there was no significant difference between the CRS + sterilized soil group and the CRS group; however, the areas of GFAP-positive cells were significantly reduced in the CRS + soil with *S. rimosus* group [one-way ANOVA, F (3, 20) = 8.812; $p = 0.0022$, NOR vs. CRS; $p = 0.0146$, CRS vs. soil with *S. rimosus*, (Fig. 3A, C)]. In the PVN, the areas of GFAP-positive cells, which were increased by CRS, were significantly decreased only in the CRS + soil with *S. rimosus* group [one-way ANOVA, F (3, 20) = 8.790; $p = 0.0141$, NOR vs. CRS; $p = 0.0175$, CRS vs. soil with *S. rimosus*, (Fig. 3B, D)].

Exposure to soil with *S. rimosus* reduces mRNA expression levels of cytokines in CRS-induced mouse brain

To determine the effects of soil exposure on cytokines due to activation of microglia and astrocytes in depressed situations, the mRNA expression levels of IL-6, IFN-γ, and IL-17 A in each region were evaluated. The IL-6, IFN-γ, and IL-17 A mRNA expression levels were significantly increased in the CRS group compared to the NOR group in the hippocampus, prefrontal cortex, and hypothalamus. As results in the hippocampus, the mRNA expression levels of IL-6, IFN-γ, and IL-17 A were significantly decreased in the CRS + soil with *S. rimosus* group compared to the CRS group. Additionally, there was no significant difference between the CRS + sterilized soil and the CRS groups [one-way ANOVA, IL-6: F (3, 20) = 3.702; $p = 0.0206$, NOR vs. CRS; $p = 0.0286$, CRS vs. soil with *S. rimosus*; IFN-γ: F (3, 20) = 11.68; $p < 0.001$, NOR vs. CRS and CRS vs. soil with *S. rimosus*; IL-17a: F (3, 20) = 4.426; $p = 0.0231$, NOR vs. CRS; $p = 0.0145$, CRS vs. soil with *S. rimosus*, (Fig. 4A, B, C)]. In the prefrontal cortex, the mRNA expression levels of IL-6, IFN-γ, and IL-17 A were significantly decreased in the CRS + soil with *S. rimosus* group compared to the CRS group. Additionally, in the CRS + sterilized soil group, the mRNA expression levels of IFN-γ in the prefrontal cortex were significantly decreased compared to the CRS group. There were no significant differences in the levels of other cytokines in sterilized soil [one-way ANOVA, IL-6: F (3, 20) = 6.257; $p = 0.0094$, NOR vs. CRS; $p = 0.0017$, CRS vs. soil with *S. rimosus*; IFN-γ: F (3, 20) = 13.07; $p < 0.001$, NOR vs. CRS, CRS vs. sterilized soil and CRS vs. soil with *S. rimosus*; IL-17a: F (3,

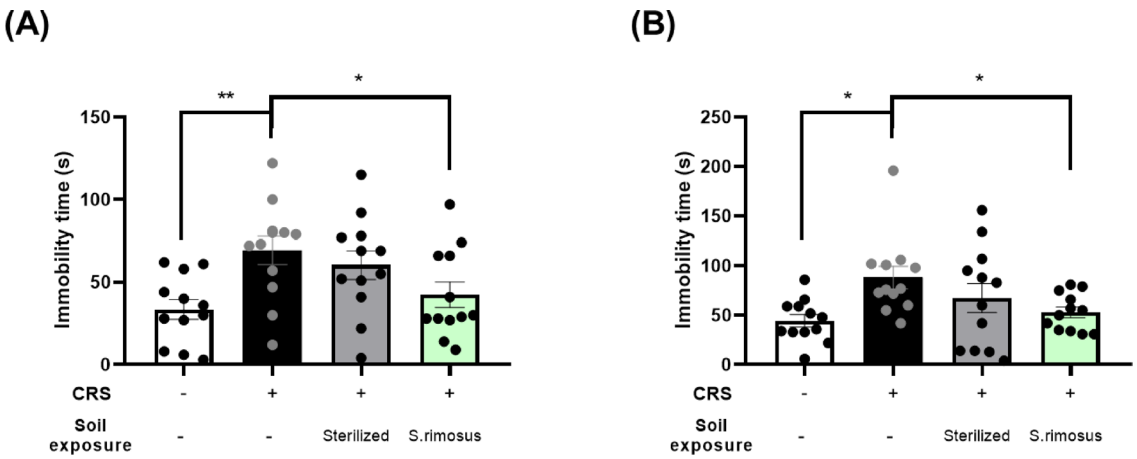

Fig. 1. Effects of exposure of soil with *S. rimosus* on improving depression-like behavior. CRS-induced mice were exposed to sterilized soil or soil with *S. rimosus* for 17 days. Depression-like behavior was assessed using TST (**A**) or FST (**B**). Data were analyzed by one-way ANOVA followed by Dunnett's post hoc test. $^{*}p < 0.05$ and $^{**}p < 0.01$ compared to CRS group. Values are expressed as mean ± SEM. *TST* tail suspension test, *FST* forced swim test.

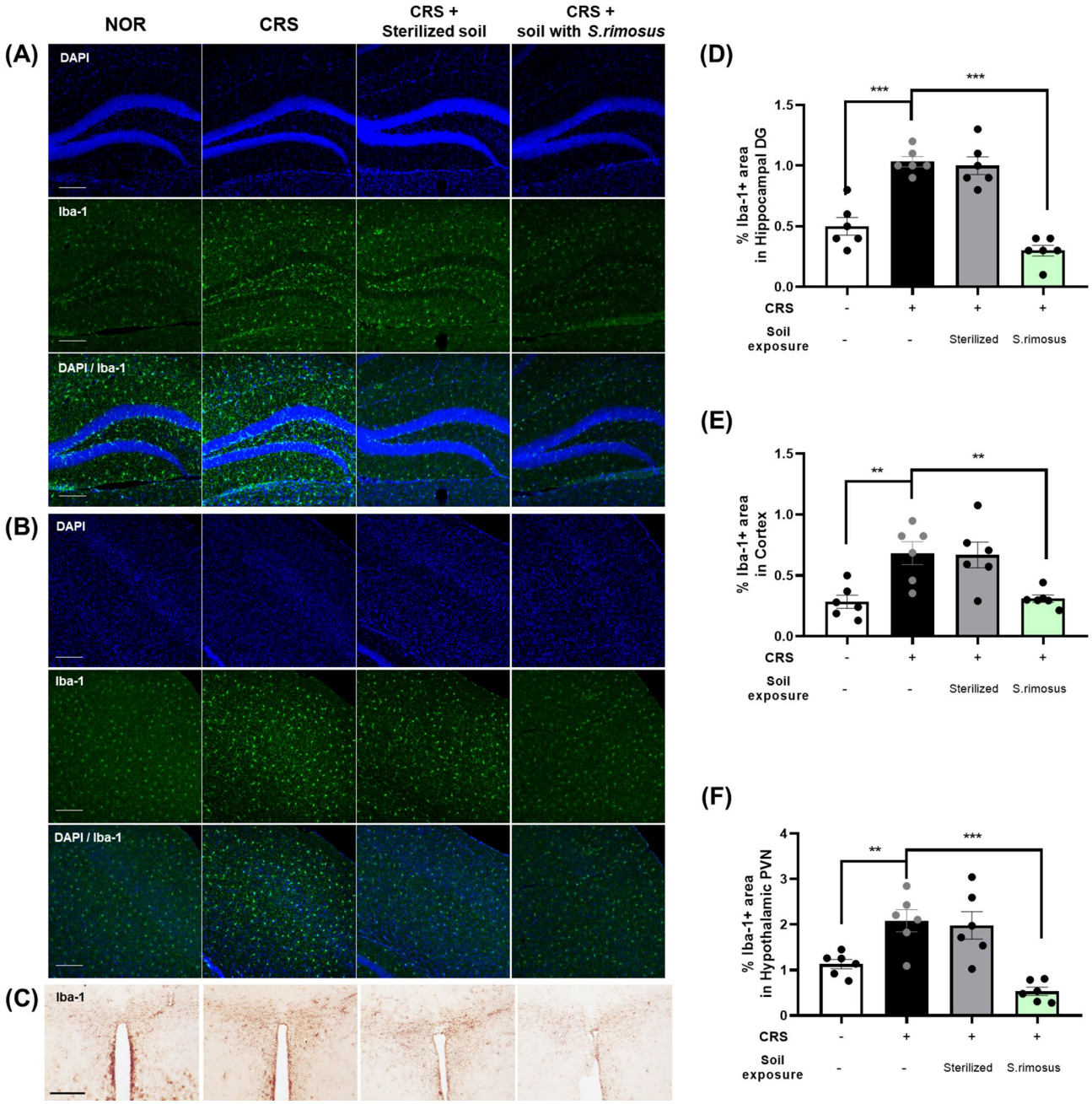

Fig. 2. Inhibitory effects of exposure to soil with *S. rimosus* on microglia activation in mouse brains. Representative images are shown for Iba-1 staining in hippocampal DG (**A**; scale bar = 100 μm), prefrontal cortex (**B**; scale bar = 100 μm), and hypothalamic PVN (**C**; scale bar = 50 μm). Quantitative graphs of Iba-1 are shown in DG (**D**), prefrontal cortex (**E**), and PVN (**F**). Data were analyzed by one-way ANOVA followed by Dunnett's post hoc test. $^{**}p < 0.01$ and $^{***}p < 0.001$ compared to CRS group. Values are expressed as mean ± SEM. *DG* dentate gyrus, *PVN* paraventricular nucleus.

20) = 6.211; $p = 0.0335$, NOR vs. CRS; $p = 0.0124$, CRS vs. soil with *S. rimosus*, (Fig. 4D, E, F)]. Lastly, in the results of hypothalamus, the expression levels of IL-6, IFN-γ, and IL-17 A were significantly decreased in the CRS + soil with *S. rimosus* group compared to the CRS group. No significant changes were observed in the hypothalamus due to soil sterilization [one-way ANOVA, IL-6: F (3, 20) = 4.240; $p = 0.0331$, NOR vs. CRS; $p = 0.0143$, CRS vs. soil with *S. rimosus*; IFN-γ: F (3, 20) = 6.811; $p = 0.0169$, NOR vs. CRS; $p = 0.0037$, CRS vs. soil with *S. rimosus*; IL-17a: F (3, 20) = 6.988; $p = 0.0023$, NOR vs. CRS; $p = 0.0027$, CRS vs. soil with *S. rimosus*, (Fig. 4G, H, I)].

Exposure to soil with *S. rimosus* regulates synaptic plasticity in CRS-induced mouse brain

To evaluate the effects on postsynapses, the optical density (OD) of PSD95 in hippocampal CA3 was measured. The CA3 subregion was selected due to its well-established role in synaptic plasticity and memory formation,

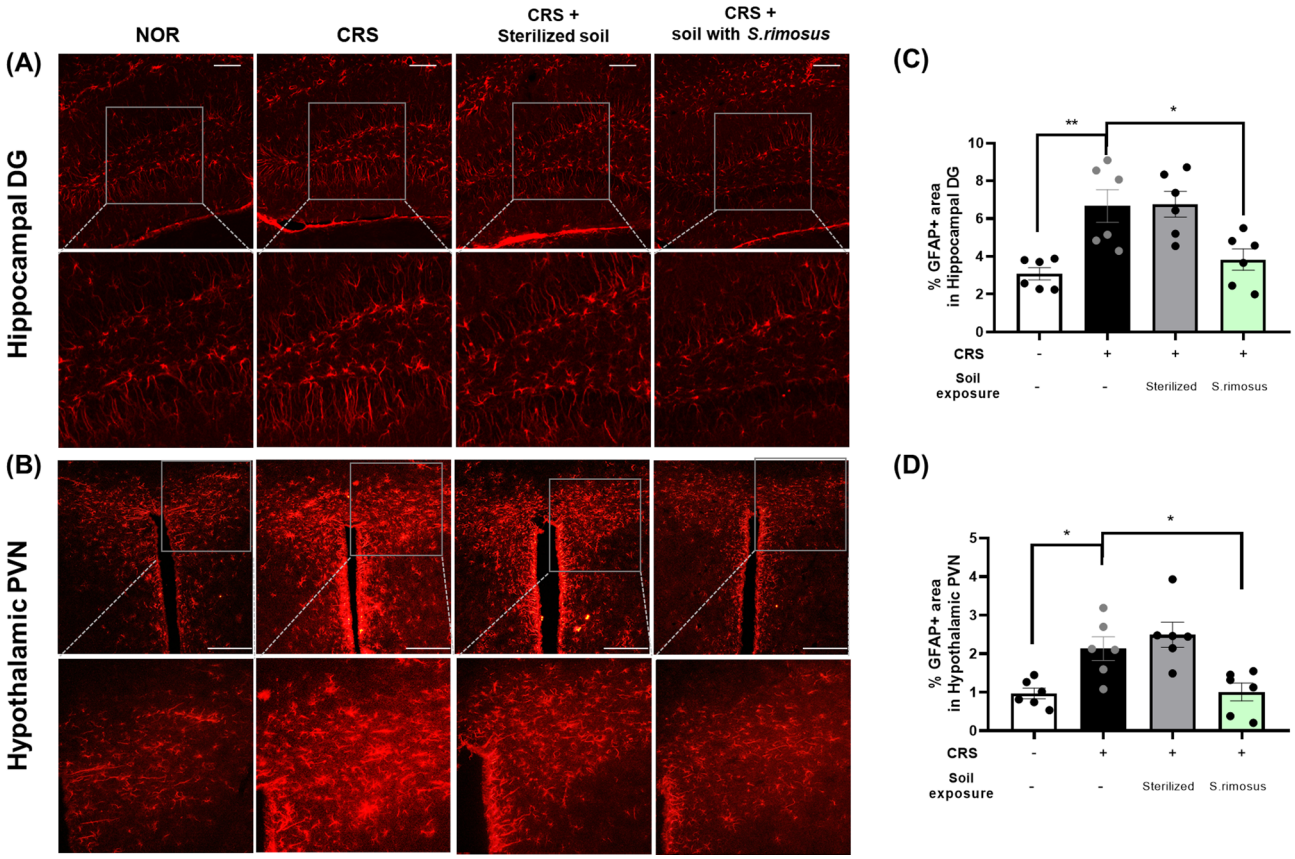

Fig. 3. Inhibitory effects of exposure to soil with *S. rimosus* on astrocyte activation in mouse brains. Representative images are shown for GFAP staining in DG (**A**; scale bar = 100 μm) and PVN (**B**; scale bar = 50 μm). Quantitative graphs of GFAP are shown in hippocampal DG (**C**) and hypothalamic PVN (**D**). Data were analyzed by one-way ANOVA followed by Dunnett's post hoc test. $^{*}p < 0.05$ and $^{**}p < 0.01$ compared to CRS group. Values are expressed as mean ± SEM. *DG* dentate gyrus, *PVN* paraventricular nucleus.

which are closely related to depressive-like behaviors. The OD of PSD95 was significantly lower in the CRS group than in the NOR group. Additionally, there was no difference between the CRS + sterilized soil group and the CRS group; however, the OD of PSD95 significantly increased in the CRS + soil with *S. rimosus* group [one-way ANOVA, F (3, 20) = 6.491; $p = 0.0028$, NOR vs. CRS; $p = 0.0180$, CRS vs. soil with *S. rimosus*, (Fig. 5A, C)]. The effects on the presynapses was evaluated using SYP staining. In the hippocampal CA3 region, SYP expression was significantly decreased by CRS. There was no significant difference in the CRS + sterilized soil group, but the optical density of SYP significantly increased in the CRS + soil with *S. rimosus* group compared to that in the CRS group [one-way ANOVA, F (3, 20) = 7.140; $p = 0.0029$, NOR vs. CRS; $p = 0.0415$, CRS vs. soil with *S. rimosus*, (Fig. 5B, D)].

Discussion

The current study demonstrates that exposure to soil inoculated with *S. rimosus* can have antidepressant effects by inhibiting neuroinflammation and enhancing synaptic plasticity. We found that exposure to soil containing *S. rimosus* significantly alleviated depression-like behavior, and reduced activation of microglia and astrocyte as well as mRNA expression levels of inflammatory cytokines such as IL-6, IFN-γ, and IL-17 A in the brain. Moreover, exposure to soil containing *S. rimosus* enhanced synaptic plasticity, which was attenuated by the CRS treatment.

Many studies have evaluated the impact of soil on health, but most have used intake-based methods such as oral administration or mixing into feed[27,28]. They revealed positive physiological effects, including changes in the gut microbiota and immune system of mice when they ingested sterilized or live soil[6]. However, these administration methods differ significantly from the way people typically encounter soil in their daily lives[7]. Therefore, we exposed mice to soil instead of bedding to mimic natural soil exposure as closely as possible. Thus, the results of our research focused on the benefits of natural exposure to soil. The main difference between our study and previous studies on ingestion-based administration is that exposure to sterile soil did not induce behavioral or histological changes in our study. These results are consistent with those of a previous study using a similar soil exposure method and suggest that microbiomes play a significant role when humans are naturally exposed to soil[7].

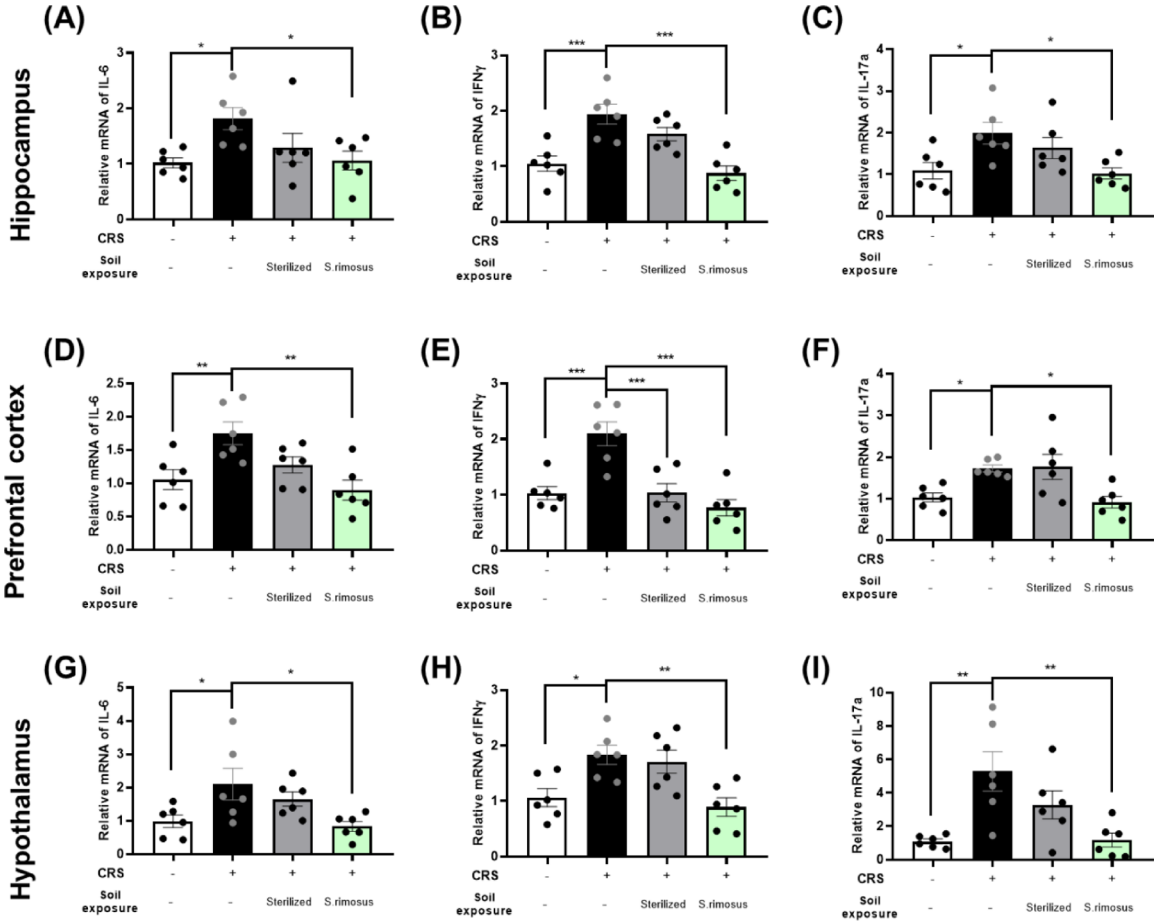

Fig. 4. Effect of exposure to soil with *S. rimosus* on reducing mRNA expression levels of cytokines in mouse brains. mRNA expression levels of IL-6, IFN-γ, and IL-17 A in hippocampus (**A**–**C**), prefrontal cortex (**D**, **E**), and hypothalamus (**G**, **H**) were measured by RT-PCR. Data were analyzed by one-way ANOVA followed by Dunnett's post hoc test. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ compared to CRS group. Values are expressed as mean ± SEM.

S. rimosus produces geosmin and 2-MIB, which are responsible for its earthy odor[12]. One clinical study found that inhalation of geosmin or 2-MIB positively changed brain waves[29]. Moreover, *Mycobacterium vaccae*, another strain that produces geosmin and 2-MIB, has been reported to alleviate neuroinflammation and anxiety-like behaviors, similar to the results of the current study[30–32]. Therefore, we expected that the positive effects of *S. rimosus* on depression observed in the current study would be due to olfactory stimulation by geosmin and 2-MIB. Furthermore, the region-specific effects observed in glial activation and cytokine expression may be partially explained by the anatomical connections of the olfactory system. The olfactory bulb projects directly to brain regions such as the prefrontal cortex, hippocampus, and amygdala, which are known to be involved in mood regulation and emotional processing. Thus, olfactory stimulation by volatile compounds produced by *S. rimosus*, such as geosmin and 2-MIB, may differentially influence neuroinflammatory responses depending on the neural circuitry and sensitivity of each brain region.

Neuroinflammation is one of the main mechanisms underlying depression development[33]. The inflammatory hypothesis of depression was proposed more than 20 years ago and is still accepted today[34]. Glial cells are representative cells that regulate inflammation in the central nervous system and are activated under inflammatory conditions[35,36]. Activated glial cells secrete various inflammatory cytokines that negatively affect neuronal and synaptic plasticity, leading to depression-like behavior[37]. Therefore, alleviating neuroinflammation and strengthening synaptic plasticity are essential for improving depression.

Therefore, in the present study, we focused on the prefrontal cortex, hippocampus, and hypothalamus, which are major brain regions implicated in the pathophysiology of depression, stress response, and neuroinflammation. The prefrontal cortex regulates cognitive and emotional processes, and alterations in this region are strongly implicated in depression[38]. In particular, layers II to V of the prefrontal cortex contain principal excitatory neurons and are highly responsive to chronic stress and inflammatory signaling[39]. Therefore, we analyzed glial and cytokine changes across these layers to capture the neuroinflammatory landscape relevant to depressive symptoms. The hippocampus is composed of distinct subregions, each with specialized functions. The DG is primarily involved in adult neurogenesis and is highly susceptible to stress-induced neuroinflammation, making

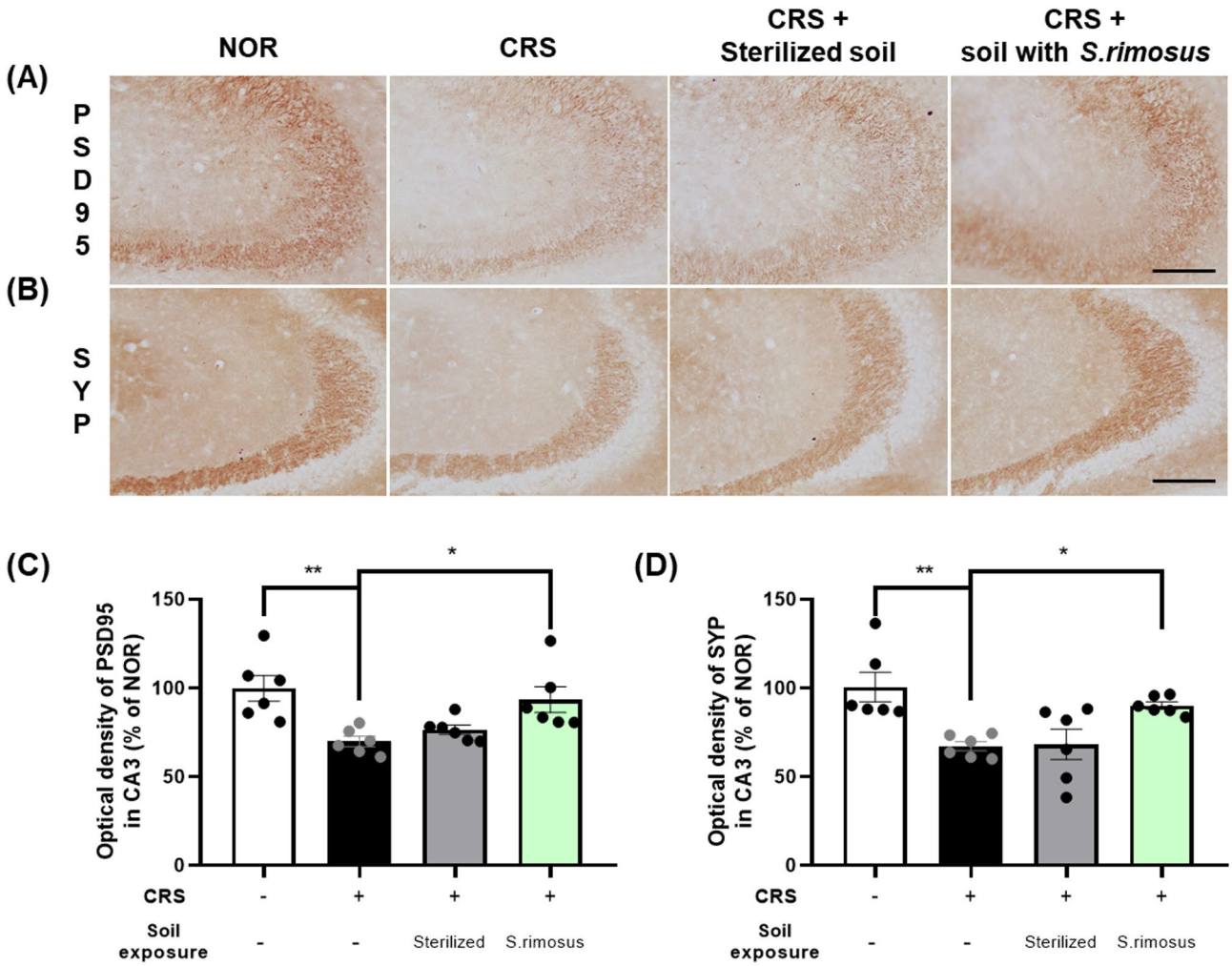

Fig. 5. Synaptic protective effects of exposure of soil with *S. rimosus* in hippocampal CA3. Representative images are shown for PSD95 (**A**) and SYP (**B**) optical density in hippocampal CA3 (scale bar = 20 μm) staining. Quantitative graph of PSD95 expression is indicated in (**C**), and quantitative graph of SYP expression is indicated in (**D**). Data were analyzed by one-way ANOVA followed by Dunnett's post hoc test. $^{*}p < 0.05$ and $^{**}p < 0.01$ compared to CRS group. Values are expressed as mean ± SEM.

it a relevant target for evaluating glial activation and inflammatory cytokine expression[40]. In contrast, the CA3 subregion plays a key role in synaptic integration and transmission, and has been widely used to study synaptic plasticity[41,42]. Based on these functional distinctions, we assessed neuroinflammation in the DG and synaptic markers in the CA3 region to capture region-specific pathological changes associated with depressive behaviors.

The region-specific effects observed in glial activation and cytokine expression may be partially explained by the anatomical connections of the olfactory system. The olfactory bulb projects directly to brain regions such as the prefrontal cortex, hippocampus, and amygdala, which are involved in mood regulation and emotional processing[43,44]. Thus, olfactory stimulation by volatile compounds produced by *S. rimosus*, such as geosmin and 2-MIB, may differentially influence neuroinflammatory responses depending on the neural circuitry and sensitivity of each brain region.

Our findings provide evidence for an integrated mechanism by which *S. rimosus* exposure alleviates depression-like behaviors. We observed that S. rimosus exposure led to significant reductions in inflammatory cytokines (IL-6, IFN-γ, and IL-17 A) and glial activation across key brain regions involved in mood regulation. This neuroinflammation suppression was accompanied by enhanced synaptic plasticity, as evidenced by improved synaptic markers in the hippocampal CA3 regions. The regional specificity of these effects—particularly in the prefrontal cortex, hippocampus, and hypothalamus—corresponds to the functional domains affected in depression: cognitive-emotional processing, memory-mood regulation, and stress response, respectively. This integrated neurobiological response ultimately manifested as improved behavioral outcomes in depression-related tasks, suggesting that the anti-inflammatory and synaptic plasticity-enhancing effects of S. rimosus work synergistically to restore normal brain function and alleviate depressive symptoms.

The current study had several limitations. First, only female mice were used. Previous studies have reported that geosmin causes brainwave changes only in women, though the exact reason for this sex-specific response

is not yet known[29]. Since our experiment was conducted exclusively with female mice based on this finding, additional experiments using male mice are needed to determine whether similar antidepressant effects occur in males or if the response is indeed female-specific. Additionally, this study showed that *S. rimosus* had a significant effect on soil but did not reveal a clear mechanism. Based on previous studies, olfactory stimulation is expected to be effective; however, additional research is needed to verify this hypothesis.

Our study demonstrated that when directly exposed to soil with *S. rimosus*, depression-like behaviors were suppressed. Our study showed that exposure to soil containing *S. rimosus* inhibits neuroinflammation and prevents synaptic damage to the brain. Therefore, this study suggests that the depression-alleviating effect of natural soil exposure is caused by *S. rimosus*.

Data availability

All the raw data in this research can be obtained from the corresponding authors upon reasonable request.

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References

1. Banerjee, S. & van der Heijden, M. G. A. Soil microbiomes and one health. *Nat. Rev. Microbiol.* **21**, 6–20. https://doi.org/10.1038/s41579-022-00779-w (2023).
2. Amundson, R. et al. Soil science. Soil and human security in the 21st century. *Science* **348**, 1261071. https://doi.org/10.1126/science.1261071 (2015).
3. Bai, Y. & Cotrufo, M. F. Grassland soil carbon sequestration: current understanding, challenges, and solutions. *Science* **377**, 603–608. https://doi.org/10.1126/science.abo2380 (2022).
4. Ottman, N. et al. Soil exposure modifies the gut microbiota and supports immune tolerance in a mouse model. *J Allergy Clin Immunol* 143, 1198–1206 e1112, (2019). https://doi.org/10.1016/j.jaci.2018.06.024
5. Kim, S. O. et al. Psychophysiological and metabolomics responses of adults during horticultural activities using soil inoculated with streptomyces rimosus: A pilot study. *Int. J. Environ. Res. Public. Health.* **19** https://doi.org/10.3390/ijerph191912901 (2022).
6. Li, N. et al. Sterile soil mitigates the intergenerational loss of gut microbial diversity and anxiety-like behavior induced by antibiotics in mice. *Brain Behav. Immun.* **115**, 179–190. https://doi.org/10.1016/j.bbi.2023.10.014 (2024).
7. Kummola, L. et al. Comparison of the effect of autoclaved and non-autoclaved live soil exposure on the mouse immune system: effect of soil exposure on immune system. *BMC Immunol.* **24**, 29. https://doi.org/10.1186/s12865-023-00565-0 (2023).
8. Kim, R., Yang, S., Lee, C. H. & Park, S. A. Horticultural activity in soil inoculated with streptomyces rimosus improved depressive mood with altered electroencephalogram and serum metabolism in adults. *Sci. Rep.* **15**, 2197. https://doi.org/10.1038/s41598-024-79159-8 (2025).
9. Fonken, L. K. et al. Mycobacterium vaccae immunization protects aged rats from surgery-elicited neuroinflammation and cognitive dysfunction. *Neurobiol. Aging.* **71**, 105–114. https://doi.org/10.1016/j.neurobiolaging.2018.07.012 (2018).
10. Sanchez, K. et al. Mycobacterium vaccae immunization in rats ameliorates features of age-associated microglia activation in the amygdala and hippocampus. *Sci. Rep.* **12**, 2165. https://doi.org/10.1038/s41598-022-05275-y (2022).
11. Loupy, K. M. et al. Comparing the effects of two different strains of mycobacteria, Mycobacterium vaccae NCTC 11659 and M. vaccae ATCC 15483, on stress-resilient behaviors and lipid-immune signaling in rats. *Brain Behav. Immun.* **91**, 212–229. https://doi.org/10.1016/j.bbi.2020.09.030 (2021).
12. Jachymova, J., Votruba, J., Viden, I. & Rezanka, T. Identification of streptomyces odor spectrum. *Folia Microbiol. (Praha).* **47**, 37–41. https://doi.org/10.1007/BF02818563 (2002).
13. Malhi, G. S., Mann, J. J. & Depression *Lancet* **392**, 2299–2312, doi:https://doi.org/10.1016/S0140-6736(18)31948-2 (2018).
14. *Depressive disorder (depression)*, (2023). https://www.who.int/news-room/fact-sheets/detail/depression
15. Cui, L. et al. Major depressive disorder: hypothesis, mechanism, prevention and treatment. *Signal. Transduct. Target. Ther.* **9** https://doi.org/10.1038/s41392-024-01738-y (2024).
16. Duan, Z. et al. Antitumor activity of Mianserin (a tetracyclic antidepressant) primarily driven by the Inhibition of SLC1A5-mediated glutamine transport. *Invest. New. Drugs.* **40**, 977–989. https://doi.org/10.1007/s10637-022-01284-w (2022).
17. Wang, H. et al. Microglia in depression: an overview of microglia in the pathogenesis and treatment of depression. *J. Neuroinflammation.* **19**, 132. https://doi.org/10.1186/s12974-022-02492-0 (2022).
18. Felger, J. C. & Lotrich, F. E. Inflammatory cytokines in depression: Neurobiological mechanisms and therapeutic implications. *Neuroscience* **246**, 199–229. https://doi.org/10.1016/j.neuroscience.2013.04.060 (2013).
19. Pryce, C. R. & Fontana, A. Depression in autoimmune diseases. *Curr. Top. Behav. Neurosci.* **31**, 139–154. https://doi.org/10.1007/7854_2016_7 (2017).
20. Elpers, H. et al. Major depressive disorders increase the susceptibility to self-reported infections in two German cohort studies. *Soc. Psychiatry Psychiatr Epidemiol.* **58**, 277–286. https://doi.org/10.1007/s00127-022-02328-5 (2023).
21. Rao, J. S., Kellom, M., Kim, H. W., Rapoport, S. I. & Reese, E. A. Neuroinflammation and synaptic loss. *Neurochem Res.* **37**, 903–910. https://doi.org/10.1007/s11064-012-0708-2 (2012).
22. Mottahedin, A. et al. Effect of neuroinflammation on synaptic organization and function in the developing brain: implications for neurodevelopmental and neurodegenerative disorders. *Front. Cell. Neurosci.* **11**, 190. https://doi.org/10.3389/fncel.2017.00190 (2017).
23. Kim, J. S. et al. Inulae Flos has Anti-Depressive effects by suppressing neuroinflammation and recovering dysfunction of HPA-axis. *Mol. Neurobiol.* **61**, 8038–8050. https://doi.org/10.1007/s12035-024-04094-8 (2024).
24. Kim, J. H. et al. Coadministration of 6-Shogaol and Levodopa alleviates parkinson's Disease-Related pathology in mice. *Biomol. Ther. (Seoul).* **32**, 523–530. https://doi.org/10.4062/biomolther.2024.075 (2024).
25. Franklin, G. P. K. B. J. *Paxinos and Franklin's the Mouse Brain in Stereotaxic Coordinates* (Academic, 2012).
26. Patani, R., Hardingham, G. E. & Liddelow, S. A. Functional roles of reactive astrocytes in neuroinflammation and neurodegeneration. *Nat. Rev. Neurol.* **19**, 395–409. https://doi.org/10.1038/s41582-023-00822-1 (2023).
27. Li, M. et al. Soil intake modifies the gut microbiota and alleviates Th2-type immune response in an ovalbumin-induced asthma mouse model. *World Allergy Organ. J.* **17**, 100897. https://doi.org/10.1016/j.waojou.2024.100897 (2024).
28. Liddicoat, C. et al. Naturally-diverse airborne environmental microbial exposures modulate the gut Microbiome and May provide anxiolytic benefits in mice. *Sci. Total Environ.* **701**, 134684. https://doi.org/10.1016/j.scitotenv.2019.134684 (2020).
29. Kim, M. et al. Gender differences in electroencephalographic activity in response to the earthy odorants Geosmin and 2-Methylisoborneol. *Appl. Sci.* **7**, 876 (2017).
30. Hassell, J. E. et al. Immunization with a heat-killed Preparation of Mycobacterium vaccae NCTC 11659 enhances auditory-cued fear extinction in a stress-dependent manner. *Brain Behav. Immun.* **107**, 1–15. https://doi.org/10.1016/j.bbi.2022.09.003 (2023).

31. Hassell, J. E. Jr. et al. Treatment with a heat-killed Preparation of Mycobacterium vaccae after fear conditioning enhances fear extinction in the fear-potentiated startle paradigm. *Brain Behav. Immun.* **81**, 151–160. https://doi.org/10.1016/j.bbi.2019.06.008 (2019).
32. Frank, M. G. et al. Immunization with Mycobacterium vaccae induces an anti-inflammatory milieu in the CNS: Attenuation of stress-induced microglial priming, alarmins and anxiety-like behavior. *Brain Behav. Immun.* **73**, 352–363. https://doi.org/10.1016/j.bbi.2018.05.020 (2018).
33. Troubat, R. et al. Neuroinflammation and depression: A review. *Eur. J. Neurosci.* **53**, 151–171. https://doi.org/10.1111/ejn.14720 (2021).
34. Roohi, E., Jaafari, N. & Hashemian, F. On inflammatory hypothesis of depression: what is the role of IL-6 in the middle of the chaos? *J. Neuroinflammation*. **18**, 45. https://doi.org/10.1186/s12974-021-02100-7 (2021).
35. Haroon, E., Miller, A. H. & Sanacora, G. Inflammation, glutamate, and glia: A trio of trouble in mood disorders. *Neuropsychopharmacology* **42**, 193–215. https://doi.org/10.1038/npp.2016.199 (2017).
36. Greenhalgh, A. D., David, S. & Bennett, F. C. Immune cell regulation of glia during CNS injury and disease. *Nat. Rev. Neurosci.* **21**, 139–152. https://doi.org/10.1038/s41583-020-0263-9 (2020).
37. Quan, L., Uyeda, A. & Muramatsu, R. Central nervous system regeneration: the roles of glial cells in the potential molecular mechanism underlying remyelination. *Inflamm. Regen*. **42**, 7. https://doi.org/10.1186/s41232-022-00193-y (2022).
38. Pizzagalli, D. A. & Roberts, A. C. Prefrontal cortex and depression. *Neuropsychopharmacology* **47**, 225–246. https://doi.org/10.1038/s41386-021-01101-7 (2022).
39. Lee, J., Kim, S. W. & Kim, K. T. Region-Specific characteristics of astrocytes and microglia: A possible involvement in aging and diseases. *Cells* **11** https://doi.org/10.3390/cells11121902 (2022).
40. Wu, A. & Zhang, J. Neuroinflammation, memory, and depression: new approaches to hippocampal neurogenesis. *J. Neuroinflammation*. **20**, 283. https://doi.org/10.1186/s12974-023-02964-x (2023).
41. Magarinos, A. M. & McEwen, B. S. Stress-induced atrophy of apical dendrites of hippocampal CA3c neurons: comparison of stressors. *Neuroscience* **69**, 83–88. https://doi.org/10.1016/0306-4522(95)00256-i (1995).
42. Qiao, H., An, S. C., Ren, W. & Ma, X. M. Progressive alterations of hippocampal CA3-CA1 synapses in an animal model of depression. *Behav. Brain Res.* **275**, 191–200. https://doi.org/10.1016/j.bbr.2014.08.040 (2014).
43. Zhou, G. et al. Human hippocampal connectivity is stronger in olfaction than other sensory systems. *Prog Neurobiol.* **201**, 102027. https://doi.org/10.1016/j.pneurobio.2021.102027 (2021).
44. Gascuel, J. et al. Hypothalamus-olfactory system crosstalk: orexin a immunostaining in mice. *Front. Neuroanat.* **6**, 44. https://doi.org/10.3389/fnana.2012.00044 (2012).

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Author contributions

Jin Hee Kim: Conceptualization, Methodology, Validation, Investigation, Writing - Original Draft; Jin Se Kim: Methodology, Validation, Investigation; Hyeyoon Eo: Writing - Review & Editing; Sowon Yang: Resources; Choong Hwan Lee: Conceptualization, Sin-Ae Park: Conceptualization, Funding acquisition, Myung Sook Oh: Project administration, Supervision, Writing - Review & Editing.

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Declarations

Competing interests

The authors declare no competing interests.

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