Gut microbiota regulate stress resistance by influencing microglia-neuron interactions in the hippocampus

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A R T I C L E   I N F O

A B S T R A C T

Communication among the brain, gut and microbiota in the gut is known to affect the susceptibility to stress, but the mechanisms involved are unclear. Here we demonstrated that stress resistance in mice was associated with more abundant *Lactobacillus* and *Akkermansia* in the gut, but less abundant *Bacteroides, Alloprevotella, Helicobacter, Lachnoclostridium, Blautia, Roseburia, Colidextibacter* and *Lachnospiraceae NK4A136*. Stress-sensitive animals showed higher permeability and stronger immune responses in their colon, as well as higher levels of pro-inflammatory cytokines in serum. Their hippocampus also showed more extensive microglial activation, abnormal interactions between microglia and neurons, and lower synaptic plasticity. Transplanting fecal microbiota from stress-sensitive mice into naïve ones perturbed microglia-neuron interactions and impaired synaptic plasticity in the hippocampus, translating to more depression-like behavior after stress exposure. Conversely, transplanting fecal microbiota from stress-resistant mice into naïve ones protected microglia from activation and preserved synaptic plasticity in the hippocampus, leading to less depression-like behavior after stress exposure. These results suggested that gut microbiota may influence resilience to chronic psychological stress by regulating microglia-neuron interactions in the hippocampus.

1. Introduction

Chronic stress can precipitate many psychiatric disorders, such as depression and anxiety (Liu et al., 2020; Seo et al., 2017). Individuals differ substantially in their resilience to stress (Cathomas et al., 2019; Dion-Albert et al., 2022; Fleshner et al., 2011), and the same is true of laboratory animals (Dudek et al., 2020; Menard et al., 2017; Zhang et al., 2021). Greater resilience to stress has been linked to lower risk of depression (Monti and Rudolph, 2017). Understanding the molecules and processes underlying such resilience may lead to new therapies against depression, anxiety and other stress-related disorders.

Studies in rodents have established that stress-induced behavior reflects alterations in the diversity and abundance of microbiota (Ma et al., 2021; Nikolova et al., 2021; Taylor and Holscher, 2020) and in inflammatory responses (Dantzer et al., 2018; Gao et al., 2018) in the gut. Individuals with irritable bowel syndrome, who are at greater risk of depression and anxiety (Hu et al., 2021; Koller et al., 2023), show alterations in gut microbiome which appear to be related to hyperactive immune responses in the intestine and greater intestinal permeability (Franzosa et al., 2019). Previous studies have shown that changes in gut microbiota can affect stress sensitivity or resistance (Han et al., 2023; Pearson-Leary and Zhao, 2020). Perhaps one of the strongest indications

that the gut microbiome can influence responses to stress is the fact that transplanting fecal microbiota from stress-sensitive rats, but not from stress-resistant ones, increases depression-like behaviors in naïve animals (Li et al., 2019; Pearson-Leary et al., 2020). In addition, mice lacking any gut microbiota are hypersensitive to stress (Sudo et al., 2004).

Several lines of evidence suggest that gut microbiota affect stress responses through microglia, the immune cells of the brain (Schramm and Waisman, 2022). By permeabilizing the intestine, stress facilitates the entry of metabolites from gut microbes into the circulation, from where they can enter the brain and activate microglia to adopt a pro-inflammatory state and disrupt microglia-neuron interactions, reducing synaptic transmission in the hippocampus and giving rise to behaviors characteristic of depression (Gareau et al., 2008; Mossad et al., 2022; Teitelbaum et al., 2008). Blocking microglial activation with minocycline can prevent stress from triggering depression-like symptoms (Han et al., 2019; Leschik et al., 2021). Together, these studies strongly suggest that the microbiota-gut-brain axis activates microglia and injures neurons in the brain, particularly in the hippocampus.

How exactly the gut microbiome induces these changes in the brain is unclear. The present study explores this question by comparing the fecal microbiomes as well as microglial and neuronal function in the hippocampus and prefrontal cortex between mice resistant or sensitive to chronic unpredictable mild stress (CUMS). We demonstrate that transplanting fecal microbiota from stress-resistant animals into naïve ones can protect the recipients from stress-induced neuropathology and depression-like behavior, whereas fecal microbiota from stress-sensitive animals exert the opposite effects. Our findings identify particular microbial genera that may be associated with stress resilience and they highlight the importance of microglia-neuron interactions in the hippocampus for such resilience. These insights may lead to dietary and molecular treatments against depression, anxiety and other stress-induced disorders.

2. Materials and methods

2.1. Animals

Male C57BL/6 mice (eight weeks old, specific pathogen-free) were purchased from Changsha Tianqin Biotechnology (Changsha, China), assigned unique numbers, and caged individually in temperature- and humidity-controlled rooms on a standard 12-h light-dark cycle. The mice were allowed to acclimate for one week prior to experiments, then they were habituated to 1 % sucrose solution for 48 h before experiments. Before each experiment, weight-matched animals were randomly allocated to experimental or control groups.

All experiments were approved by the Institutional Animal Care and Use Committee at the Guizhou University of Traditional Chinese Medicine (Guiyang, China). All animal experiments were performed in accordance with ARRIVE guidelines (Percie du Sert et al., 2020) and with the "Guidelines for the Care and Use of Laboratory Animals" from the US National Institutes of Health.

2.2. CUMS

CUMS may mimic the stressful experiences that trigger depression in humans more faithfully than the monostressors often used in animal models (Muscat et al., 1988). Mice were subjected to CUMS for three weeks, consisting of daily exposure to 2–3 stressors in random order, as previously described (Zhang et al., 2021). These stressors included empty water bottles (12 h), food deprivation (12 h), tail clipping (10 min), restraint (2 h), lights-off for 3 h during the daylight phase, cage shaking (1 h), cage tilting (45°, 24 h), reversal of the light–dark cycle (24 h), strobe lighting (12 h), damp bedding (24 h), and a soiled cage (24 h). Mice not subjected to CUMS served as naïve controls.

2.3. Body weight and coat score

Mice were weighed weekly and physical appearance was evaluated in terms of the coat score (Cao et al., 2013). The total coat score was calculated as the sum of individual scores for the head, neck, forepaws, dorsal coat, ventral coat, hind paws, and tail. Animals were also assigned a "coat score" of 0 if they were unkempt or 1 if they were well-groomed.

2.4. Behavioral testing and classification as stress-resistant or stress-sensitive

All behavioral tests were conducted as previously described (Zhang et al., 2021). After CUMS as described in section 2.2, animals were first subjected to a sucrose preference test (SPT), which assesses anhedonia (Zurita et al., 1996), followed by a forced swimming test (FST) and tail suspension test (TST), which assess behavioral despair (Nomura et al., 1982; Steru et al., 1985). CUMS mice were preliminarily classified into anhedonia-high resilience and anhedonia-low resilience groups according to their sucrose preference in the SPT. If their sucrose preference in the SPT were less than one standard deviation of the mean for naïve controls, these mice were considered as anhedonia-low resilience group. Otherwise, animals were as anhedonia-high resilience group. These CUMS animals were further divided into behavioral despair low and high resilience groups based on immobility time in FST and TST. If their immobility time in FST and TST were higher than one standard deviation of the mean for naïve controls, these mice were considered as behavioral despair-low resilience group. Otherwise, animals were as behavioral despair-high group. If the both indicators (anhedonia and behavioral despair) were clustered to the high resilience group, mice were considered to stress resilience (SR). If the both indicators (anhedonia and behavioral despair) were clustered as the low resilience group, mice were considered as stress sensibility (SS) (Nasca et al., 2015).

After their assignment into resistant or sensitive groups, animals underwent the open field test (OFT) or elevated plus maze test (EPMT), depending on the experiment.

In experiments involving transplantation of fecal microbiota (see section 2.6), animals underwent the SPT and TST, followed by CUMS for three weeks. Finally, they underwent the SPT, TST, OFT, EPMT and FST.

2.5. Fecal microbiome sequencing

All fecal samples were collected at the same time (4:00 p.m.) to avoid circadian influences on the microbiome (Pearson-Leary et al., 2020). Fecal samples were frozen on dry ice and stored at −80 °C until analysis. The composition of the fecal microbiome was analyzed using 16S rRNA sequencing, which was performed by Majorbio Bio-Pharm Technology (Shanghai, China). Sequencing experiments involved the following steps: DNA extraction, PCR amplification and product purification, real-time quantitative PCR, MiSeq library construction, and MiSeq sequencing. PCR amplification was carried out using TransStart FastPfu DNA Polymerase (TransGen Biotech, Beijing, China) and an ABI GeneAmp® 9700 system (Thermo Fisher Scientific, Wilmington, DE, USA). Bacterial DNA fragments were amplified using forward primer-338 (5′-GTACTCCTACGGGAGGCAGCA-3′) and reverse primer-806 (5′-GTGGACTACHVGGGTWTCTAAT-3′). The DNA was quantified using a Nanodrop spectrophotometer (Thermo Fisher Scientific), and paired-end sequencing of the V3–V4 region of 16S rRNA was performed using an Illumina MiSeq system. Purified amplicons were pooled in equimolar amounts and subjected to paired-end sequencing on an Illumina MiSeq PE300 platform platform (Illumina, San Diego, CA, USA) according to the standard protocols by Majorbio Bio-Pharm Technology. The raw sequencing reads were deposited into the NCBI Sequence Read Archive (submission ID SUB12872846, BioProject ID PRJNA936460).

The taxonomy of representative sequences for operational taxonomic

units (OTUs) was determined using RDP Classifier 2.2 (SourceForge, San Diego, CA, USA) against the 16S rRNA gene database using a confidence threshold of 0.7. The statistical significance of differences in OTU abundance was assessed in SPSS 22.0 (IBM, Chicago, IL, USA) using the Kruskal–Wallis test, followed by non-parametric Bonferroni-Dunn *post hoc* testing. OTUs of interest were identified using SINA Aligner 45. Rarefaction curves and indices of alpha diversity (observed OTUs, Chao1 richness, Shannon index, Good's coverage) were calculated based on OTUs using Mothur 1.30.1, and analyzed by non-parametric kruskal-Wallis test. Bray–Curtis similarity matrices were calculated at the OTU level, and Bray-Curtis distances were entered into principal component analysis in Vegan 2.5–3 in order to assess similarity among microbial communities.

2.6. Fecal microbiota transplantation

Fecal pellets were pooled from 10 stress-resistant mice, 9 stress-sensitive mice or 15 naïve mice. The three sets of pooled pellets were weighed, resuspended in 1 mL phosphate-buffered saline (PBS) per 300 mg of feces, vortexed for 1 min, and centrifuged at 500 *g* for 5 min. The supernatant was collected and aliquots (150 μl) were given to naïve recipient mice by oral gavage once daily for five weeks as described (Chevalier et al., 2020). Recipient mice had fasted for 2 h prior to fecal transplantation. After the mice had received fecal slurry for two weeks, some were subjected to CUMS for three weeks, while others were not.

2.7. Transcriptome profiling of hippocampus, prefrontal cortex and colon

Mice were perfused with 0.9 % NaCl, the whole brain was removed, and the hippocampus and cortex were isolated. The colon was also removed, repeatedly rinsed with 75 % ethanol and frozen. Total RNA was extracted from all three tissues using TRIzol® according to the manufacturer's instructions (Invitrogen, Carlsbad, CA, USA). RNA samples were used to construct the sequencing library if they satisfied the following quality conditions: ratios of optical density (OD) at 260 nm to OD at 280 nm and of OD at 260 nm to OD at 230 nm, $\geq$ 2.0; RNA integrity number, $\geq$ 6.5; and ratio of 28S to 18S, $\geq$ 1.0. RNA sequencing was performed by Majorbio Bio-Pharm Technology. All sequences were uploaded to the NCBI Sequence Read Archive (submission ID SUB12536246, BioProject ID PRJNA928573).

The remaining data were analyzed to determine the level of each transcript according to the "fragments per kilobase of exon per million mapped reads" method (Maekawa et al., 2019). Genes in hippocampus, prefrontal cortex and colon that were differentially expressed in naïve, stress-sensitive and stress-resistant mice were identified using EdgeR software. Enrichment of these differentially expressed genes in certain Gene Ontology (GO) terms or certain Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways was analyzed using, respectively, Goatools or Kobas.

Levels of mRNAs encoding genes of interest were measured by reverse-transcribing the Trizol-extracted RNA using a cDNA conversion kit (catalog no. 630, Takara, Tokyo, Japan), then amplifying the cDNA using a Bio-Rad CFX 96 system (Bio-Rad Laboratories, Hercules, CA, USA) and the primers in Supplementary Table 1. The threshold amplification cycle number (Ct) was determined from the linear phase of the amplification plot. Differences in gene expression were determined using the $-\Delta\Delta$Ct method (Schmittgen and Livak, 2008) and normalized to β-actin as the internal standard. Each sample was analyzed in triplicate.

2.8. Microglia and synapses in hippocampus and prefrontal cortex

Mice were anesthetized with 1 % pentobarbital, transcardially perfused with PBS containing heparin, then brains were removed, fixed in 4 % paraformaldehyde for 48 h, washed with PBS, and dehydrated in 30 % sucrose as described (Zhang et al., 2017). Sagittal sections 20 μm

thick containing the hippocampus or prefrontal cortex were obtained using a freezing microtome (CM1900; Leica Microsystems, Wetzlar, Germany). Six sequential slices were collected into different wells of 12-well plates containing PBS with 0.02 % sodium azide, and stored at 4 °C until analysis.

Sections were washed three times in PBS, blocked with 0.2 % Triton X-100 for 1 h, then incubated overnight at 4 °C with primary antibodies diluted in PBS containing 0.2 % Triton X-100 and 5 % bovine serum albumin. Sections were incubated with antibodies against ionized calcium binding adaptor molecule-1 (Iba1; 1:400 dilution; Wako, Japan) and CD68 (1:300; Abcam, UK) to examine microglial morphology and activation, or against postsynaptic density 95 (PSD95; 1:300; Cell Signaling Technology, Danvers, MA, USA) and synapsin (1:200, Cell Signaling Technology) to examine synaptic clusters. Next, sections were incubated for 2 h at room temperature with Alexa-conjugated secondary antibodies (1:300; Invitrogen, USA), followed by incubation for 5 min with 4′,6-diamidino-2-phenylindole (DAPI; 1:10,000; Roche, Switzerland).

Stained sections were imaged using a fluorescence microscope (Olympus IX73, Japan), and images were analyzed using Image J 1.45J (US National Institutes of Health, Bethesda, MD, USA). A threshold for positive staining was defined to exclude background staining, and both the percentage of total area that was positively stained and the numbers of cells were averaged across five fields of view per mouse at 40 × magnification.

2.9. Inflammatory cytokines in serum, hippocampus and prefrontal cortex

Levels of inflammatory cytokines in mouse serum were detected using the Proteome Profiler Mouse Cytokine Array Kit (catalog no. ARY006, R&D Systems, USA) on a Luminex 200 liquid chip (Millipore, USA) according to the manufacturers' protocols.

Hippocampal and prefrontal cortex tissues were quickly dissected, lysed in RIPA lysis buffer (catalog no. R0010, Solarbio, Beijing, USA) containing phenylmethanesulfonyl fluoride (catalog no. IP0280, Solarbio, Beijing, USA). Lysates were centrifuged for 15 min at 1000 *g* at 4 °C, total protein concentration in the supernatant was estimated using the BCA Protein Assay (Boster), and the supernatants were assayed for IL-1β using an enzyme-linked immunosorbent assay (catalog no. EK0394, Boster, Wuhan, China) according to the manufacturer's instructions.

2.10. Neuronal and synaptic proteins in hippocampus and prefrontal cortex

Protein from hippocampus or prefrontal cortex tissue, obtained as described in section 2.10, was analyzed using western blotting. Equal amounts of protein were fractionated by sodium dodecylsulfate-polyacrylamide gel electrophoresis and electrophoretically transferred to a polyvinylidene fluoride membrane for western blotting as described (Jiang et al., 2022). Membranes were incubated overnight at 4 °C with primary antibodies against CX3CL1, CX3CR1, CD200, CD200R, SIRPα, CD47, GluA2, CamKII β, PSD95 and actin (Supplemental Table 2), followed by incubation for 30 min at room temperature with the corresponding secondary antibody. Finally, membranes were scanned with a V370 scanner (Epson, Shenzhen, China), and bands were quantitated using AlphaEaseFC 4.0 software (Alpha Innotech, Shanghai, China).

2.11. Statistical analysis

All statistical analyses were performed using GraphPad Prism 6.0 (GraphPad, USA), and results that were associated with P < 0.05 were considered statistically significant. Data were analyzed for normal distribution using the Shapiro-Wilk test and reported as mean ± standard error of the mean (SEM). Differences among stress-sensitive, stress-resistant or naïve animals were assessed for significance using one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test for

multiple comparisons. Differences among mice that received fecal transplants from stress-sensitive, stress-resistant or naïve animals were assessed for significance using repeated-measures two-way ANOVA followed by Tukey's *post hoc* test for multiple comparisons. The results of statistical analyses are listed in Supplementary Tables 3–9.

To identify associations between gut microbial composition and depression-like symptoms, linear regression was performed using the abundance of each bacterial genus in the gut as the independent variable and the following dependent variables: sucrose preference, immobility time in the TST and FST, time in open-arms of the EPMT, time in the center of the OFT, and coat score. Correlations were assessed using Spearman rank correlation.

3. Results

3.1. Differences in gut microbiomes between stress-resistant and stress-sensitive mice

After three weeks of CUMS, animals that we classified as stress-sensitive showed obvious depression-like symptoms, such as anhedonia, behavioral despair and anxiety, while those animals that we classified as stress-resistant did not (Fig. 1A–B, Supplementary Fig. S1). The fecal microbiota of stress-resistant animals showed higher Chao, Ace, and Simpson indices of alpha diversity than microbiota from naïve mice, and higher Chao and Shannon indices than microbiota from stress-sensitive animals (Fig. 1C). Beta diversity based on Bray–Curtis distances differed significantly not only between either stressed group and naïve controls, but also between the two stressed groups (Fig. 1D), suggesting that stress-resistant and stress-sensitive mice possess unique gut microbiome signatures.

Among the five bacterial phyla and 12 bacterial families that we identified across all three mouse groups, the gut of stress-resistant mice showed significantly higher abundance of *Muribaculaceae* and *Lactobacillaceae,* but significantly lower abundance of *Lachnospiraceae, Bacteroidaceae, Helicobacteraceae* and *Oscillospiraceae* than the gut of stress-sensitive mice (Fig. 1E).

The gut of stress-resistant mice contained significantly higher abundance of *Lactobacillus, Prevotellaceae UCG-001* and *Akkermansia* than the gut of the other two mouse groups (Fig. 1F), and the abundance of these genera correlated negatively with depression-like symptoms (Table 1). Conversely, the gut of stress-resistant mice contained significantly lower abundance of *Bacteroides, Alloprevotella, Helicobacter, Lachnoclostridium, Blautia, Roseburia, Colidextibacter* and *Lachnospiraceae NK4A136* than the gut of stress-sensitive mice, and the abundance of these genera correlated positively with depression-like symptoms.

3.2. Association of stress resistance with lower expression of immune response proteins and higher expression of tight junction proteins in the colon

Three-way comparison of colon RNA sequences from stress-resistant, stress-sensitive and naïve mice (Supplementary Fig. 2A) linked stress sensitivity to upregulation of 422 genes, most of them involved in hypersensitivity, immune defense and inflammatory responses; and to downregulation of 435 genes, most of them involved in haptoglobin binding, the extracellular space and functioning of the cell membrane (Supplementary Figs. 2B–D). Conversely, stress resistance was linked to upregulation of 158 genes, mostly involved in synaptic function, phagocytosis and cell junctions; and downregulation of 215 genes, mostly involved in stress responses, immune responses, inflammatory responses and pyrotosis (Supplementary Figs. 2E–F).

Pairwise comparison of colon RNA sequences from stress-resistant and stress-sensitive mice associated sensitivity with upregulation of genes involved in immune responses and immunoglobulin production (Supplementary Figs. 2G–H). Using multichannel gene set enrichment analysis (GSEA), we determined that transcripts in the colon of stress-

sensitive mice were enriched in genes involved in the following processes compared to transcripts from colon of stress-resistant animals (Fig. 2A): inflammatory bowel disease, defense response to bacteria, bacterial invasion of epithelial cells, Toll-like receptor signaling, NF-KB signaling, NOD-like receptor signaling and tight junctions.

We validated these results by showing that levels of mRNAs encoding the intestinal immunity-related molecules interleukin (IL)-1β, tumour necrosis factor (TNF)-α, Toll-like receptor (TLR)-4 and 5, nuclear factor (NF)-κB, NOD-like receptor family, pyrin domain containing 3 (NLRP3) and indoleamine-2, 3-dioxygenase (IDO)-1 were higher in the gut of stress-sensitive mice than in the gut of the other two mouse groups (Fig. 2B). In fact, the level of mRNA encoding TLR4 was even lower in stress-resistant mice than in naïve animals.

In opposition to the trend seen with immune molecules, levels of mRNAs encoding four marker proteins related to tight junctions (TJP1, OCLN, CLND2 and CLND4) were less abundant in stress-sensitive mice than in the other two mouse groups (Fig. 2C). At the same time, the level of mRNA encoding the tight junction protein CLND15 was higher in stress-resistant mice than in naïve ones.

These results associate stress resistance with weaker immune responses and stronger tight junctions in the colon, and they imply that stress sensitivity involves altered intestinal permeability that triggers peripheral immune responses (Fig. 2D). To verify this idea, we assayed mouse serum for immune-related molecules. In support of our idea, the serum of stress-sensitive mice showed higher levels of interleukin (IL)-6, IL-1β, interferon (IFN)-γ, monocyte chemoattractant protein-1 and matrix metalloprotease-9 than the serum of stress-resistant or naïve animals. Conversely, the level of IL-4 was significantly higher in stress-resistant mice than in stress-sensitive animals (Fig. 2E).

3.3. Association of stress sensitivity with higher microglial activation and altered microglia-neuron interactions in the hippocampus and prefrontal cortex

The differential expression of genes involved in immune responses and permeability in the colon led us to ask whether the three mouse groups might also differ in levels of microglial activation in the brain. Pairwise comparison of hippocampus and prefrontal cortex RNA sequences from stress-resistant and stress-sensitive mice associated sensitivity with genes involved in tissue-specific immune responses, responses to lipopolysaccharide, and innate immune responses (Supplementary Figs. 3A–F). Multichannel GSEA plots showed that the transcriptomes in both hippocampus and prefrontal cortex were enriched for genes involved in long-term depression, NF-κB signaling, and neuroactive ligand-receptor interactions in stress-sensitive mice compared to stress-resistant animals (Fig. 3A and B).

We validated these results by showing that hippocampal levels of mRNAs encoding several markers of microglial activation (TLR4, CSFR1, CD74, CD11b and CD11c) (Lan et al., 2017) were higher in stress-sensitive mice than in stress-resistant or naïve animals (Fig. 3C and D). In contrast, CD74 was the only one of these markers upregulated in the prefrontal cortex of stress-sensitive animals (Fig. 3E).

Microglia maintain neuronal survival and synaptic function through interactions involving several pairs of ligands and receptors on their surfaces (Manich et al., 2019; Ohnishi et al., 2010; Pawelec et al., 2020; Vainchtein et al., 2018), which include Cx3CL1/Cx3CR1, CD200/CD200R, SIRPα/CD47 and IL-33/IL33R. In hippocampus, level of mRNA encoding CX3CL1 were lower in stress-resistant than naïve animals, while the level of mRNA encoding CD200 was higher than in naïve animals. Levels of mRNAs encoding CX3CL1, CX3CR1, CD47 or SIRPα were higher in stress-sensitive animals than in stress-resistant or naïve animals, while the levels of mRNAs encoding CD200 or CD200R1 was lower than in stress-resistant or naïve animals (Fig. 3F). In the prefrontal cortex, the level of mRNA encoding IL-33 was lower in the two stressed groups than in naïve animals (Fig. 3G).

The hippocampus and prefrontal cortex of stress-sensitive mice
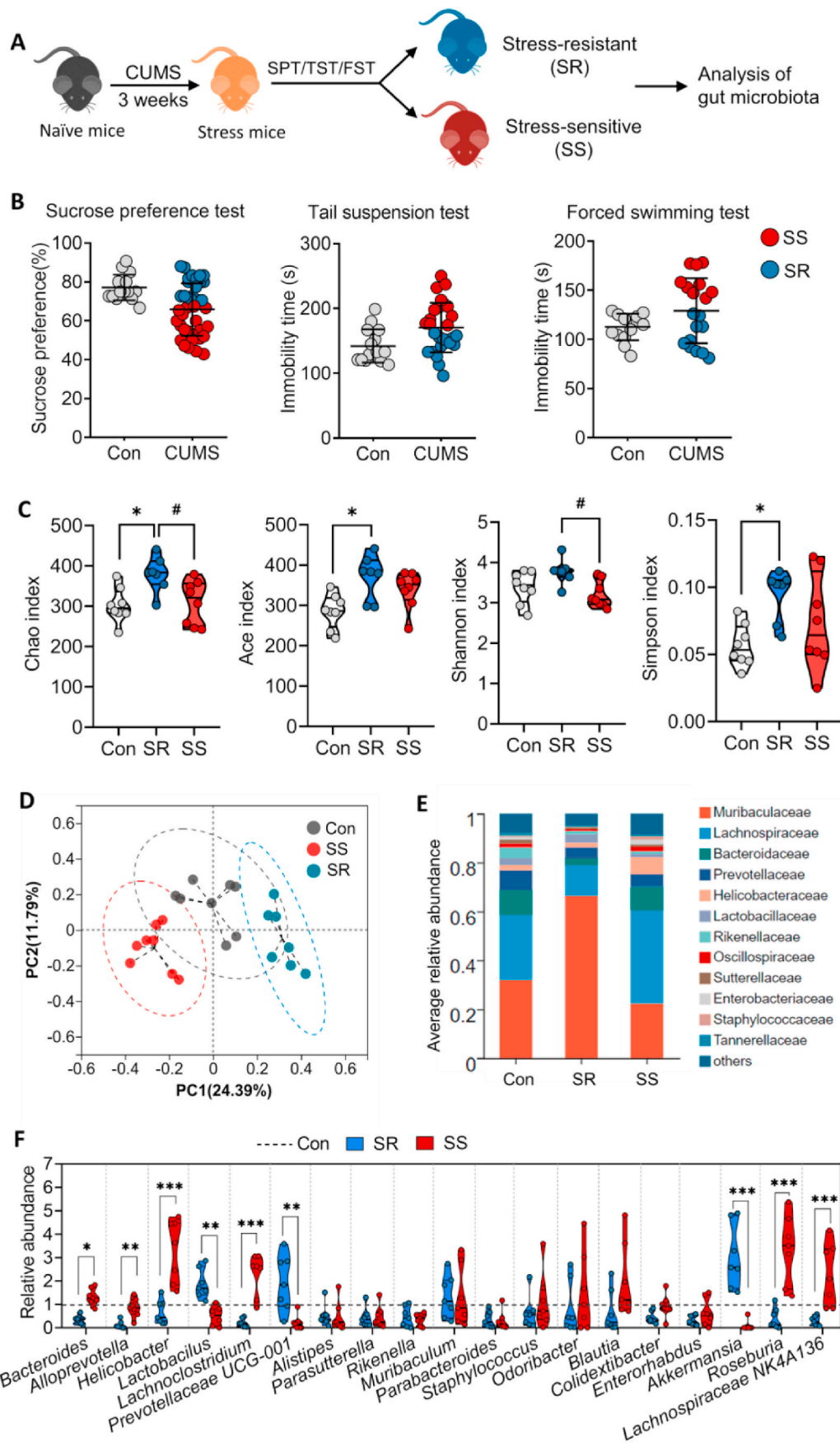
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Fig. 1. Microbiome profiles from the gut of naïve, stress-resistant or stress-sensitive mice. **(A)** Schematic for classification of mice as stress-resistant (SR) or stress-sensitive (SS) following exposure to chronic unpredictable mild stress (CUMS). FST, forced swimming test; SPT, sucrose preference test; TST, tail suspension test. **(B)** Results on the three behavioral tests from panel (A) are shown for the mice ultimately classified as SR and the animals ultimately classified as SS. Con, naïve mice. **(C)** Alpha diversity of operational taxonomic units in fecal microbiota, quantified in terms of the Chao, Ace, Shannon and Simpson indices. Results are shown for 7–8 animals per group. **(D)** Beta diversity of operational taxonomic units in fecal microbiota as assessed using Bray–Curtis distances. The dashed ovals represent confidence intervals for each group. Results are shown for 7–8 animals per group. PC, principal component. **(E)** Relative abundance of microbial families in fecal microbiota. Results are shown for 7–8 animals per group. **(F)** Relative abundance of bacterial genera in the gut. Results are shown for 7–8 animals per group. Abundance in the two stressed groups was normalized to that in the naïve group. Panels (C) and (F): Data are shown as violin plots. The horizontal line within the violin plots represents the median, upper, and lower quartiles. The width of the plot depicts the density and distribution shape of the data points. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ vs. Con group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. SR group, based on one-way ANOVA with Tukey's multiple-comparisons test.

contained larger numbers of microglia (identified by anti-Iba1 staining) and activated microglia (identified by anti-CD68 staining), a larger proportion of microglia that were compact and ameboid, and a higher concentration of IL-1β and TNF-α than the same brain regions in stress-resistant or naïve animals (Fig. 4A–K). Proliferation, CD68 expression, and compact ameboid morphology are characteristics of activated microglia (Streit and Xue, 2016). The hippocampus, but not prefrontal cortex, of stress-sensitive mice showed shorter microglial branches and a smaller proportion of microglia that were radially ramified (Fig. 4H and I), which are additional signs of microglial activation (Streit and Xue, 2016).

These results link stress sensitivity in mice to hyperactivation of microglia and perturbation of microglia-neuron interactions in hippocampus.

3.4. Association of stress sensitivity with reduced synaptic plasticity in the hippocampus

The differences in interactions between microglia and neurons among the three mouse groups led us to ask whether the groups might also differ in synaptic plasticity in the brain, given that microglia are responsible for pruning synapses, guiding the formation of new synapses, and regulating the function of neurons (Bar and Barak, 2019; Cserep et al., 2021; Eyo et al., 2017). Indeed, the transcriptomes in both hippocampus and prefrontal cortex were enriched for genes involved in regulation of synaptic plasticity in stress-sensitive mice compared to stress-resistant animals (Fig. 5A and B). Several genes associated with synaptic plasticity were differentially expressed in hippocampus and prefrontal cortex between stress-resistant and stress-sensitive mice (Fig. 5C), including GluA2, CamKIIβ and PSD95, which were less abundant in stress-sensitive animals than in the other two groups (Fig. 5D). In addition, the hippocampus of stress-sensitive mice contained fewer postsynaptic clusters of PSD95 and synapsin than the other two groups (Fig. 5E and F). These results link stress sensitivity to lower synaptic plasticity in the hippocampus (Fig. 5G).

3.5. Fecal microbiota from stress-resistant mice promote hippocampal synaptic plasticity and confer resistance to stress

The results above link stress sensitivity not only to a particular composition of the gut microbiome but also to alterations in microglial alteration and microglia-neuron interactions. This led us to ask whether transplanting gut microbiome from stress-resistant mice into naïve animals could prevent such alterations and thereby protect the recipients from stress-induced depression-like behaviors. If so, it would provide evidence that the gut microbiota directly influence the observed cellular and phenotype effects of stress in our mouse model.

We transplanted fecal microbiota from stress-resistant, stress-sensitive or naïve mice into another group of naïve mice, which were concurrently challenged or not with three weeks of stress (Fig. 6A). Before stress exposure, mice that received each type of fecal transplant did not differ significantly from one another in sucrose preference (Fig. 6B) or immobility time in the TST (Fig. 6C); but mice that received fecal microbiota from stress-sensitive animals spent less time in open-arms in the EPMT, showed shorter latency to immobility in the FST

and spent less time in the central area of the OFT than mice that received fecal microbiota from naïve animals. After CUMS, mice that received fecal microbiota from stress-resistant animals showed higher sucrose preference in the SPT, longer latency to immobility time and shorter immobility time in the TST and FST, as well as shorter time in open-arms in the EPMT than mice that received fecal microbiota from stress-sensitive or naïve animals (Fig. 6D–H). On all these tests, mice that received fecal microbiota from stress-sensitive animals showed stronger depression-like symptoms than mice that received fecal microbiota from naïve animals, including lower coat score (Fig. 6I). However, none of the three types of fecal microbiota was able to prevent CUMS-induced loss of body weight (Fig. 6J).

These results suggest that fecal microbiota from stress-resistant mice can protect recipient animals from the behavioral effects of stress, leading to milder depression- and anxiety-like behaviors. Fecal microbiota from stress-sensitive animals exerts the opposite effects.

We found that these phenotypic effects of fecal microbiota transplantation from stress-sensitive or stress-resistant animals involved similar alterations in microglia-neuron interactions in the hippocampus as when we subjected mice to CUMS without fecal transplantation. Regardless of whether the recipient mice were stressed or not, transplantation of fecal microbiota from stress-sensitive animals upregulated hippocampal levels of CX3CL1, CX3CR1, SIRPα and CD47, while downregulating hippocampal levels of CD200, CD200R, GluA2, CamKIIβ, PSD95 (Fig. 7A–G). It also reduced the density of PSD95^{+}-synapsin^{+} clusters in stressed recipient mice (Fig. 7H).

Conversely, transplantation of fecal microbiota from stress-resistant mice downregulated CX3CL1 in hippocampus of recipient mice not concurrently exposed to CUMS (Fig. 7B), and it downregulated CX3CL1 and upregulated CD200 in hippocampus of recipient mice that were concurrently exposed to CUMS (Fig. 7B and C). It did not, however, significantly affect hippocampal levels of CX3CR1, CD200R, SIRPα or CD47, regardless of whether recipient animals were concurrently stressed or not (Fig. 7B–D). It upregulated GluA2 in hippocampus of recipient mice not exposed to CUMS, but it did not alter hippocampal expression of CamKIIβ or PSD95 in recipient mice, regardless of whether they were stressed or not (Fig. 7E–G). It also increased the density of PSD95^{+}-synapsin^{+} clusters in hippocampus, regardless of whether the recipient mice were stressed or not (Fig. 7H).

These results provide strong evidence that the gut microbiota, by influencing microglia-neuron cross-talk and synaptic function in the hippocampus, can exacerbate or mitigate stress-induced depression-like behaviors.

4. Discussion

Here we provide evidence in a mouse model that resilience to stress depends on the composition of gut microbiota. Stress alters the distribution of bacterial genera in the gut, and these changes in microflora are associated with increased permeability and immune responses in the intestine, as well as with hyperactivation of microglia, perturbations in microglia-neuron interactions and less synaptic plasticity in the hippocampus. These neurophysiological changes, in turn, are associated with behaviors reminiscent of depression and anxiety. To what extent stress induces these changes in the gut microbiota and in the brain varies from

Table 1
Analysis of correlations between the relative abundance of bacterial genera in the gut and depression-like symptoms.

Taxon	Anhedonia		Behavioral despair				Anxiety				Others			
	Sucrose preference		Immobility time in TST		Immobility time in FST		Time in open-arms in EPM		Time spent in the center in OFT		Coat score		Traveled distance in OFT	
	R-value	P-value	R-value	P-value	R-value	P-value	R-value	P-value	R-value	P-value	R-value	P-value	R-value	P-value
Bacteroides	−0.8068	0.0019	0.7841	0.0009	0.8295	0.0002	−0.4908	0.0747	−0.5770	0.0316	−0.6998	0.0053	−0.4472	0.1021
Lactobacillus	0.6096	0.0206	−0.6562	0.0108	−0.7070	0.0047	0.3884	0.1699	0.3629	0.2023	0.3664	0.1975	0.5224	0.0553
Alistipes	0.0395	0.9121	−0.0324	0.9751	−0.1036	0.7243	0.2937	0.3062	0.3349	0.2418	0.1012	0.7305	0.1720	0.5565
Helicobacter	−0.8744	<0.0001	0.6489	0.0120	0.7180	0.0038	−0.3524	0.2166	−0.4663	0.0927	−0.5928	0.0254	−0.5103	0.0622
Prevotellaceae UCG-001	0.8817	0.0011	−0.7674	0.0267	−0.7666	0.0014	0.6830	0.0071	0.4494	0.1067	0.5423	0.0451	0.2357	0.4116
Parasutterella	−0.0412	0.8876	0.2660	0.3579	0.0569	0.8468	0.1862	0.5237	−0.3448	0.2273	−0.0614	0.8346	0.2379	0.4126
Lachnoclostridium	−0.7949	0.0007	0.8366	0.0002	0.8176	0.0004	−0.5290	0.0517	−0.5771	0.0307	−0.7204	0.0037	−0.3856	0.1733
Akkermansia	0.9122	0.0002	−0.8301	0.0002	−0.8545	<0.0001	0.0607	0.3958	0.6650	0.0095	0.5121	0.0612	0.2794	0.3335
Rikenella	0.3210	0.2644	−0.2887	0.3166	−0.1850	0.5265	0.2416	0.4051	0.2417	0.4050	0.3652	0.1991	−0.0760	0.7963
Blautia	−0.5006	0.0682	0.6393	0.0138	0.5003	0.0684	−0.2964	0.3031	−0.4604	0.0975	−0.4882	0.0765	−0.2856	0.3221
Parabacteroides	0.1087	0.7114	0.0387	0.8955	−0.0027	0.9987	−0.1128	0.7008	0.2281	0.4328	0.3318	0.2465	0.2760	0.3393
Roseburia	−0.7867	0.0008	0.7887	0.0008	0.8941	<0.0001	−0.2540	0.8281	−0.6252	0.0115	−0.7435	0.0023	−0.6346	0.0148
Odoribacter	−0.1122	0.7025	0.3587	0.2078	0.2364	0.4155	−0.1304	0.6568	−0.0195	0.9481	−0.2172	0.4556	−0.1303	0.6568
Lachnospiraceae NK4A136	−0.5345	0.0490	0.7724	0.0012	0.7070	0.0047	−0.3406	0.0811	−0.5053	0.0653	−0.6519	0.0115	−0.3508	0.2187
Enterorhabdus	−0.2402	0.4079	0.3505	0.2191	0.2114	0.4669	−0.1531	0.6012	−0.1984	0.4959	−0.1959	0.5020	−0.2430	0.4025
Alloprevotella	−0.7696	0.0013	0.6962	0.0057	0.7800	0.0010	−0.2426	0.3945	−0.5959	0.0245	−0.5905	0.0262	−0.6342	0.0148
Muribaculum	0.0258	0.9778	0.1923	0.5090	−0.0269	0.9328	0.2603	0.3687	−0.0940	0.7547	0.02375	0.4134	0.2189	0.4520
Colidextibacter	−0.6594	0.0103	0.4785	0.0834	0.6390	0.0139	−0.1378	0.6384	−0.3261	0.2549	−0.4505	0.1059	−0.4401	0.1152
Staphylococcus	−0.2346	0.4195	0.2181	0.4538	0.3429	0.2300	0.3900	0.1681	0.0337	0.9736	−0.0943	0.7483	−0.4035	0.0148

Notes: Data are based on 8 stress-naïve mice, 8 stress-resistant mice and 7 stress-sensitive animals. Sucrose preference served as an indicator of anhedonia; immobility time in the tail suspension test and forced swimming test, as indicators of behavioral despair; and time in open-arms of the elevated plus maze and time in the center of the open field test, as indicators of anxiety. Correlations were considered significant if |R| > 0.3 and P < 0.05, in which case they were colored blue if positive or pink if negative.

one individual to the next (Li et al., 2019; Pearson-Leary et al., 2020): consistently, we identified stress-resistant mice whose gut microbiome differed from stress-sensitive animals, and the differences were associated with lower microglial activation and greater synaptic plasticity in the hippocampus. Transplanting fecal microbiota from stress-resistant or stress-sensitive mice into naïve animals made the recipients resistant or more sensitive to concurrent stress, leading to milder or stronger depression-like behaviors.

Our work suggests that at least some of the variations in stress resistance among individuals are due to differences in gut microbiota, and it implies that increasing the abundance of "beneficial" bacteria in the gut may increase resilience to stress by limiting microglial activation, preserving healthy microglia-neuron interactions and promoting synaptic plasticity in the hippocampus. Our findings may provide strategies for treating or even preventing depression and anxiety.

Our results verify and extend several previous studies linking alterations in gut microbiota to altered function of neurons and microglia in the brain and to the tendency to engage in depression-like behavior (Borkent et al., 2022; Ge et al., 2022; Ortega et al., 2023; Pearson-Leary et al., 2020; Wang et al., 2020; Westfall et al., 2021). The gut of stress-sensitive mice in our study contained more abundant *Bacteroides, Alloprevotella, Helicobacter, Lachnoclostridium, Blautia, Roseburia, Colidextibacter* and *Lachnospiraceae NK4A136* than the gut of naïve controls. *Bacteroides, Lachnoclostridium, Helicobacter, Blautia* and *Roseburia* have been linked to stress response, anxiety and depression (Chung et al., 2019; Kabeer et al., 2017; Radjabzadeh et al., 2022; Xu et al., 2021, 2022; Zhang et al., 2022): increases in their abundance can induce intestinal inflammation and destroy the intestinal barrier, leading to infiltration by immune cells and changes in behavior (Cheng et al., 2023; Korenblik et al., 2023; Ortega et al., 2023). The gut of stress-resistant mice in our study contained lower levels of these bacteria. Further study should verify and extend our observation of an association between greater abundance of *Alloprevotella, Colidextibacter* and *Lachnospiraceae NK4A136* in the gut and stronger depression-like symptoms.

The gut of stress-resistant mice in our study showed higher abundance of *Lactobacillus, Prevotellaceae UCG-001* and *Akkermansia. Lactobacillus* and *Akkermansia* can suppress intestinal inflammation by downregulating inflammatory cytokines and chemokines and upregulating tight junction proteins (Hashikawa-Hobara et al., 2022; Liu et al., 2022). The two genera are less abundant in individuals with generalized anxiety disorder and depression than in healthy individuals (Bravo et al., 2011; Deng et al., 2022; Li et al., 2019). Further work should verify and extend our observation of an association between greater abundance of *Prevotellaceae UCG-001* and milder anxiety- and depression-like symptoms.

Our results in fecal transplantation studies are consistent with a study in which transferring gut microbiota from individuals with depression into germ-free rats induced behaviors reminiscent of anxiety and depression (Kelly et al., 2016). Our results are also consistent with a study in which mice were stressed through repeated exposure to social defeat (Pearson-Leary et al., 2020). Transplanting fecal microbiota from stress-sensitive mice into naïve ones in that study induced anxiety- and depression-like behaviors. Like previous studies in animals (Bravo et al., 2011; Chevalier et al., 2020; Pearson-Leary et al., 2020), our work suggests that increasing the abundance of beneficial bacteria in the gut can help relieve depression and anxiety.

Stress-sensitive animals in our study showed upregulation of intestinal immunity mediators such as TLR4, NF-κB and IDO-1; as well as higher levels of IL-6, IL-1β, IFN-γ, monocyte chemoattractant protein-1 and matrix metalloprotease-9 in serum. These results are consistent with the idea that gut microbiota act *via* not only neural but also humoral pathways to influence brain physiology and behavior (Li et al., 2019; Pearson-Leary et al., 2020; Wong et al., 2016).

Stress in our mice was associated with more extensive activation of microglia in hippocampus and, to a lesser extent, in prefrontal cortex. Both brain regions help regulate emotion and perception of stress (Costa
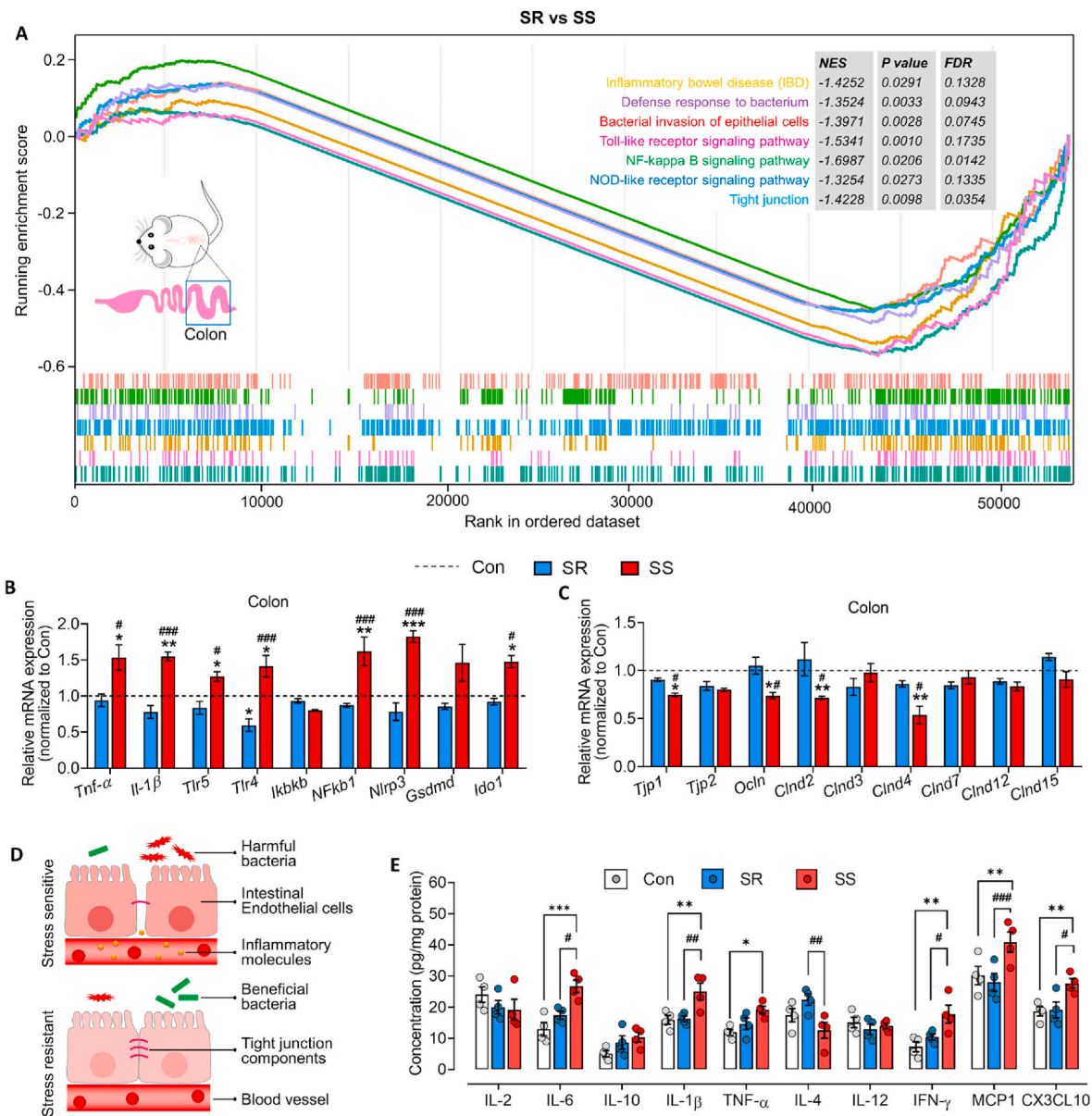
Fig. 2. Differences between stress-sensitive and stress-resistant mice in immune responses and the epithelial barrier in the gut.(A) Multichannel gene set enrichment analysis, indicating enrichment of pathways involving immune responses and tight junctions in colon transcriptomes of stress-sensitive (SS) mice compared to stress-resistant (SR) mice. (B and C) Levels of mRNAs encoding (B) intestinal immunity molecules and (C) tight junction components in the colon of naïve mice (Con), SR mice and SS mice. Fold-expression was normalized to that in Con animals. Results are shown for triplicate samples from three animals per condition.(D) Schematic illustrating potential differences between SS and SR mice in the gut microbiome and intestinal epithelium.(E) Levels of inflammatory cytokines in serum. Results are shown for triplicate samples from four animals per condition.Data are mean ± standard error of the mean (SEM). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs. Con group, #$P < 0.05$, ##$P < 0.01$, ###$P < 0.001$ vs. SR group, based on one-way ANOVA with Tukey's multiple-comparisons test.

et al., 2015). Microglial hyperactivation leads to the release of pro-inflammatory cytokines positively associated with symptoms of anxiety and depression (Yirmiya et al., 2015). For example, stress increased levels of IL-1β in the hippocampus of our mice, and reducing these levels may strengthen resilience to stress (Wong et al., 2016). Our results single out microglia as potential key mediators of differences in stress resistance from one individual to the next.

Stress in our animals was also associated with perturbations of the microglia-neuron interactions that maintain neuronal health and function (Chamera et al., 2020; Cserep et al., 2021; Eyo et al., 2017; Jiang et al., 2022). We found that stress sensitivity was linked to hippocampal upregulation of two mediators of these interactions, CX3CR1 and CD47, while stress resistance was linked to their downregulation. A previous study also found CX3CR1-deficient mice to exhibit less depression-like

behavior after stress (Hellwig et al., 2016; Milior et al., 2016). In the present study, transplanting fecal microbiota from stress-sensitive mice into naïve mice upregulated CX3CL1, CX3CR1, SIRPα and CD47 and downregulated CD200 and CD200R in hippocampus and sensitized the recipients to concurrent CUMS. Conversely, transplanting fecal microbiota from stress-resistant mice into naïve animals downregulated CX3CL1 and upregulated CD200 in hippocampus and promoted the resistance of the recipients to concurrent CUMS.

Finally, our experiments link stress to inhibition of synaptic plasticity in the hippocampus. Whether this causes the observed perturbations in microglia-neuron interactions or simply occurs in parallel with it requires further investigation. In any case, our findings are consistent with numerous animal studies linking stress resilience to synaptic plasticity (Heshmati et al., 2020; Lee et al., 2021; Leschik et al., 2021), and they
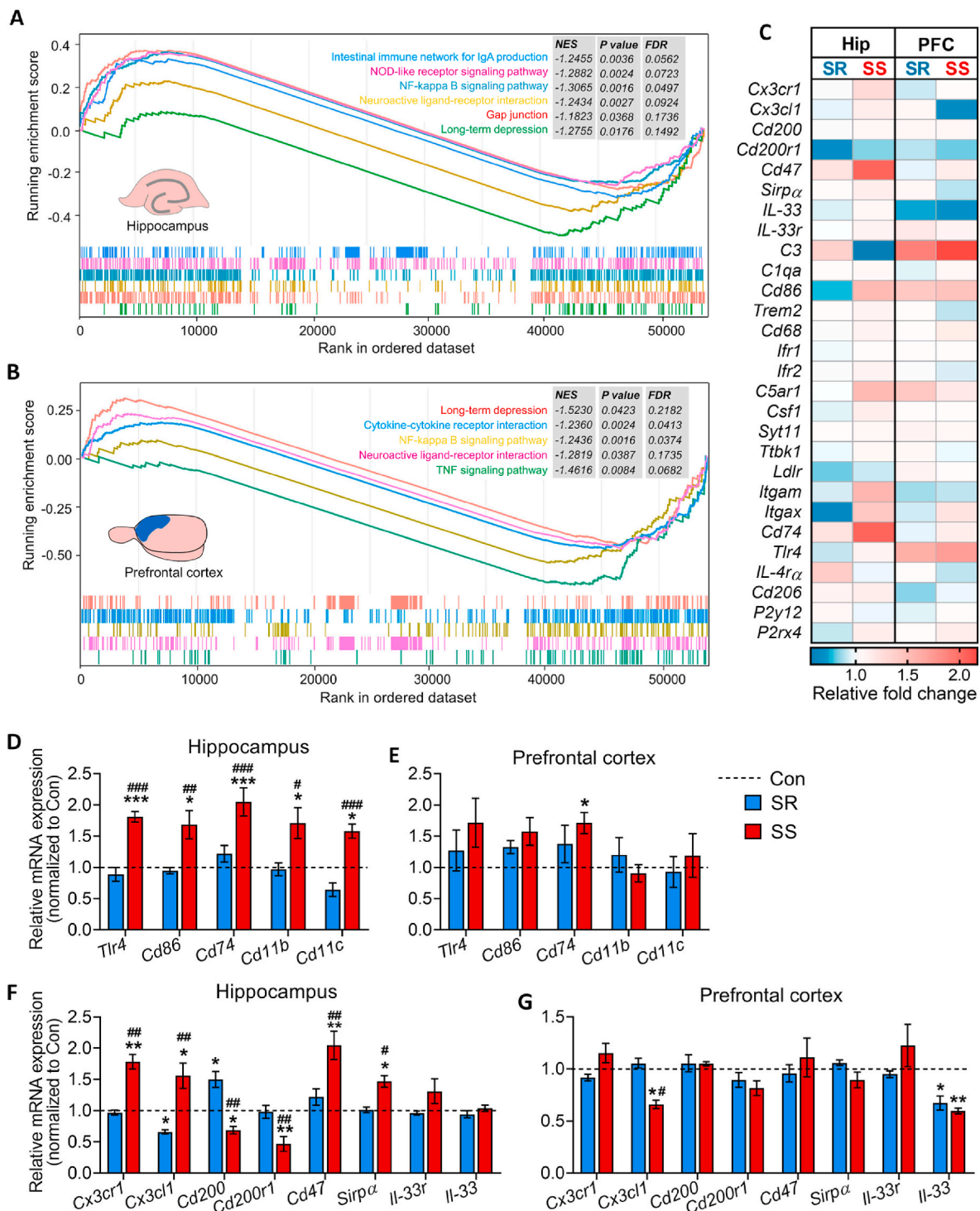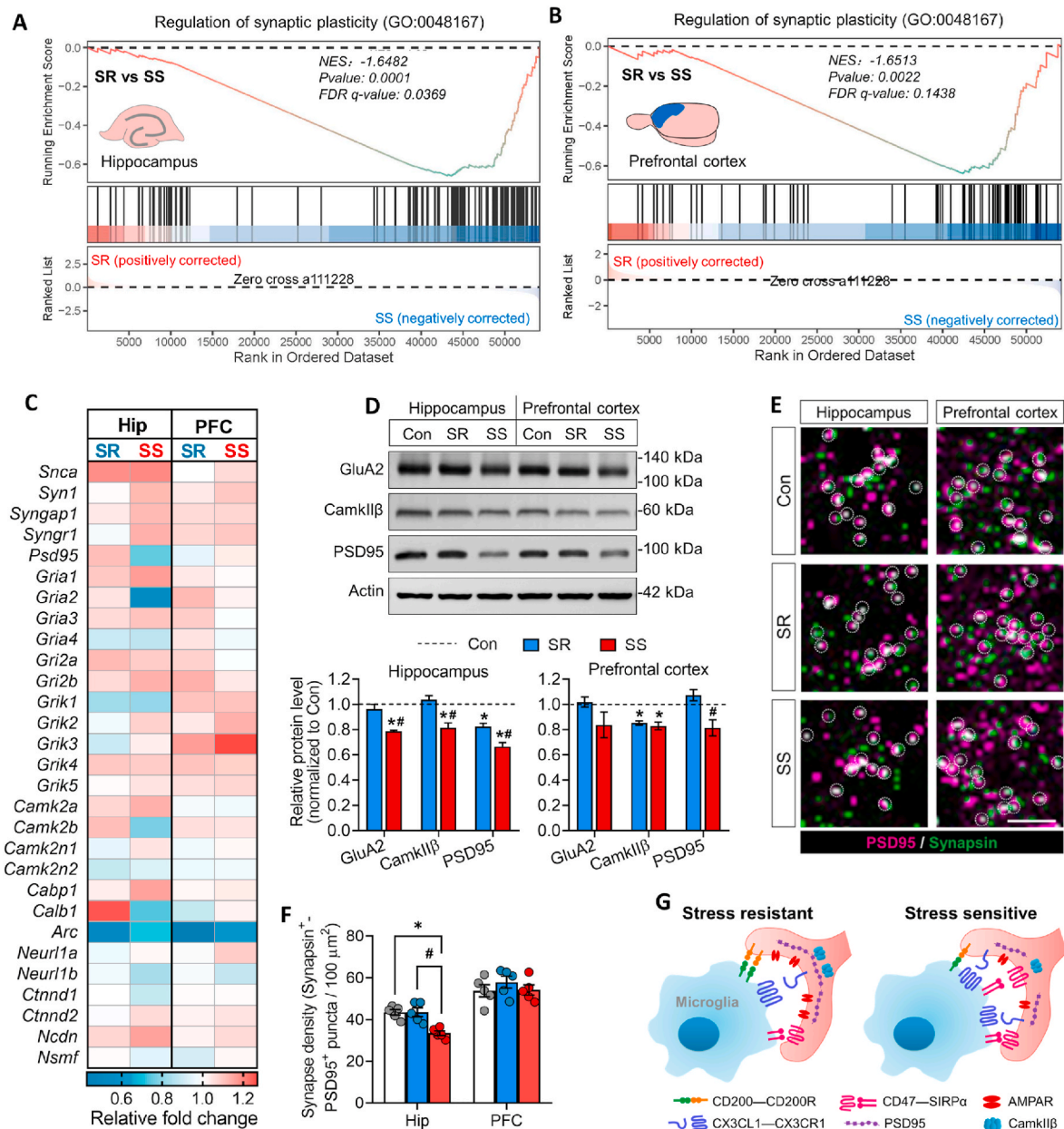
Fig. 3. Differences between stress-sensitive and stress-resistant mice in neuro-immune homeostasis in hippocampus and prefrontal cortex.
(A and B) Multichannel gene set enrichment analysis, indicating enrichment of pathways related to neuro-inflammation in transcriptomes of (A) hippocampus and (B) prefrontal cortex of stress-sensitive (SS) mice compared to stress-resistant (SR) animals.(C) Fold-differences in expression of genes related to neuro-immune homeostasis in the hippocampus (Hip) and prefrontal cortex (PFC) of SR and SS mice, relative to naïve controls (Con).**(D** and **E)** Quantitative PCR validation of differential expression of genes involved in microglial activation in hippocampus and prefrontal cortex. Fold-expression was normalized to that in the Con group.**(F** and **G)** Levels of mRNAs encoding ligand-receptor pairs that mediate cross-talk between neurons and microglia in hippocampus and prefrontal cortex were confirmed using q-PCR. The fold-expression of each gene was normalized to the Con group.Data are shown as mean (panel C) or mean ± standard error of the mean (SEM) (panels D–G). Transcriptome and gene-level analysis data were derived from 3 to 4 animals per group. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs. Con group; #$P < 0.05$, ##$P < 0.01$, ###$P < 0.001$ vs. SR group, based on one-way ANOVA with Tukey's multiple-comparisons test.
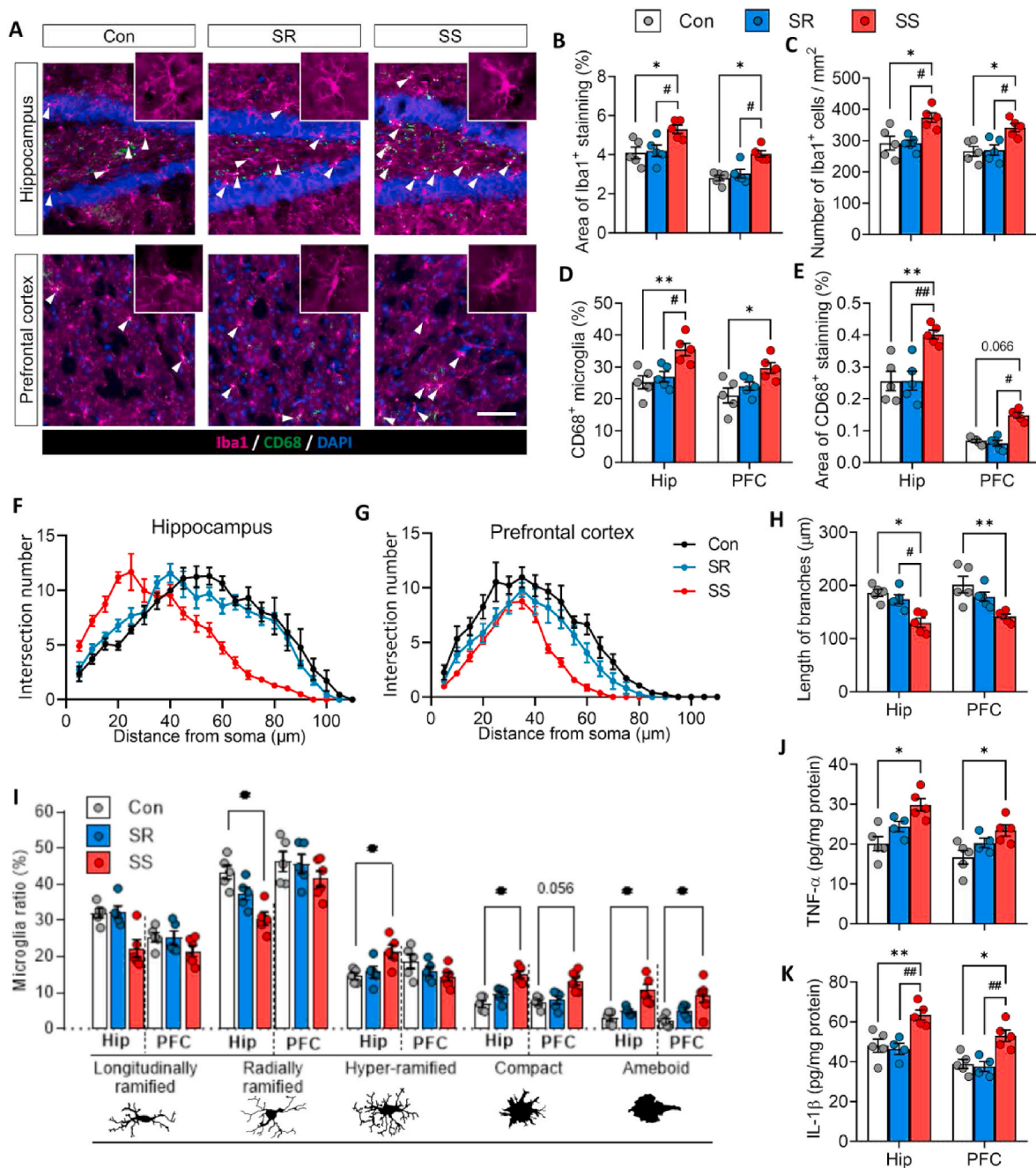
Fig. 4. Differences between stress-sensitive and stress-resistant mice in microglial activation in hippocampus and prefrontal cortex. **(A)** Representative fluorescence micrographs showing the morphology and density of microglia and CD68 expression in the hippocampus and prefrontal cortex of naïve mice (Con), stress-resistant (SR) mice and stress-sensitive (SS) mice. Sections were immunostained against Iba1 as a marker of microglia (purple) and CD68 as a marker of activated microglia (green). Nuclei were counterstained with DAPI (blue). The white arrowheads indicate Iba1$^+$-CD68$^+$ cells. Scale bar, 50 μm. **(B)** Quantification of the percentage of total area occupied by Iba1$^+$ cells (all microglia) in hippocampus (Hip) and prefrontal cortex (PFC). **(C)** Quantification of the density of Iba1$^+$ cells in hippocampus (Hip) and prefrontal cortex (PFC). **(D)** Quantification of the percentage of microglia occupied by CD68$^+$ in hippocampus (Hip) and prefrontal cortex (PFC). **(E)** Quantification of the percentage of total area occupied by CD68$^+$ in hippocampus (Hip) and prefrontal cortex (PFC). **(F and G)** The number of intersections of microglial branches in the hippocampus and prefrontal cortex as a function of distance from the soma, as determined by Sholl analysis. **(H)** Quantification of the length of microglial branches in hippocampus (Hip) and prefrontal cortex (PFC). **(I)** Quantification of the proportions of microglia in hippocampus (Hip) or prefrontal cortex (PFC) that had the indicated morphologies. **(J and K)** Levels of inflammatory cytokines (TNF-α and IL-1β) in hippocampus (Hip) and prefrontal cortex (PFC). Results are shown for triplicate samples from 4 to 5 animals per condition. Data are shown as mean (panel G) or mean ± standard error of the mean (SEM). Microglial morphological analysis data were obtained from five animals per group. *$P < 0.05$, **$P < 0.01$ vs. Con group; $^#P < 0.05$, $^{##}P < 0.01$ vs. SR group, based on one-way ANOVA with Tukey's multiple-comparisons test. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

support hippocampal microglia and neurons as key mediators of such resilience and, therefore, as determinants of its variability from one person to the next. Further work is needed to examine the effects of

subdiaphragmatic vagotomy on the outcomes in mice after FMT from stress-resistant/sensitive mice because several reports showed that subdiaphragmatic vagotomy blocked onset of depression-like behaviors

Fig. 5. Differences between stress-sensitive and stress-resistant mice in synaptic plasticity in hippocampus and prefrontal cortex.(A and B) Gene set enrichment analysis, indicating enrichment of processes related to regulation of synaptic plasticity (GO: 0048167) in transcriptomes of (A) hippocampus and (B) prefrontal cortex of stress-sensitive (SS) mice compared to stress-resistant (SR) animals.(C) Fold-differences in expression of genes related to synaptic plasticity in the hippocampus (Hip) and prefrontal cortex (PFC) of SR and SS mice, relative to naïve controls (Con). Results are from three animals per condition.(D) Western blotting of total lysates from hippocampus and prefrontal cortex to detect the synaptic plasticity proteins GluA2, CamKIIβ and PSD95. Levels were normalized to those of β-actin in Con animals. Results are shown for triplicate samples from three animals per condition.(E) Representative fluorescence micrographs showing the density of clusters containing synapsin and PSD95 in the hippocampus and prefrontal cortex of Con, SR and SS mice. Synapsin served as a marker of synapses (green), while PSD95 served as a marker of excitatory postsynaptic membrane (purple). Dotted circles indicate colocalization of synapsin and PSD95 in clusters. Scale bar, 5 μm.(F) Quantification of clusters containing synapsin and PSD95 in hippocampus (Hip) and prefrontal cortex (PFC). Results for each group were obtained from five mice, for each of which five hippocampal slices were examined at 40× magnification. Each dot in the bar graph represents the average of all micrographs for one mouse.(G) Schematic illustrating how neuron-microglial interactions and synaptic plasticity may differ between SS and SR mice.Data are shown as mean (panel A) or mean ± standard error of the mean (SEM). $*P < 0.05$, vs. Con group, $\#P < 0.05$ vs. SR group, based on one-way ANOVA with Tukey's multiple-comparisons test. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

in mice after FMT from mice with depression-like behaviors (Pu et al., 2021; Wang et al., 2023).

This study provides the first evidence that gut microbiota influence microglia-neuron interactions in the hippocampus and thereby affect risk of anxiety- or depression-like symptoms in response to chronic psychological stress. Our findings identify numerous bacterial genera, signaling factors and pathways as well as other molecules that should be

analyzed in more depth to elucidate how anxiety and depression arise and how they can be treated.

Availability of data and materials

Data can be obtained from the corresponding authors upon reasonable request.
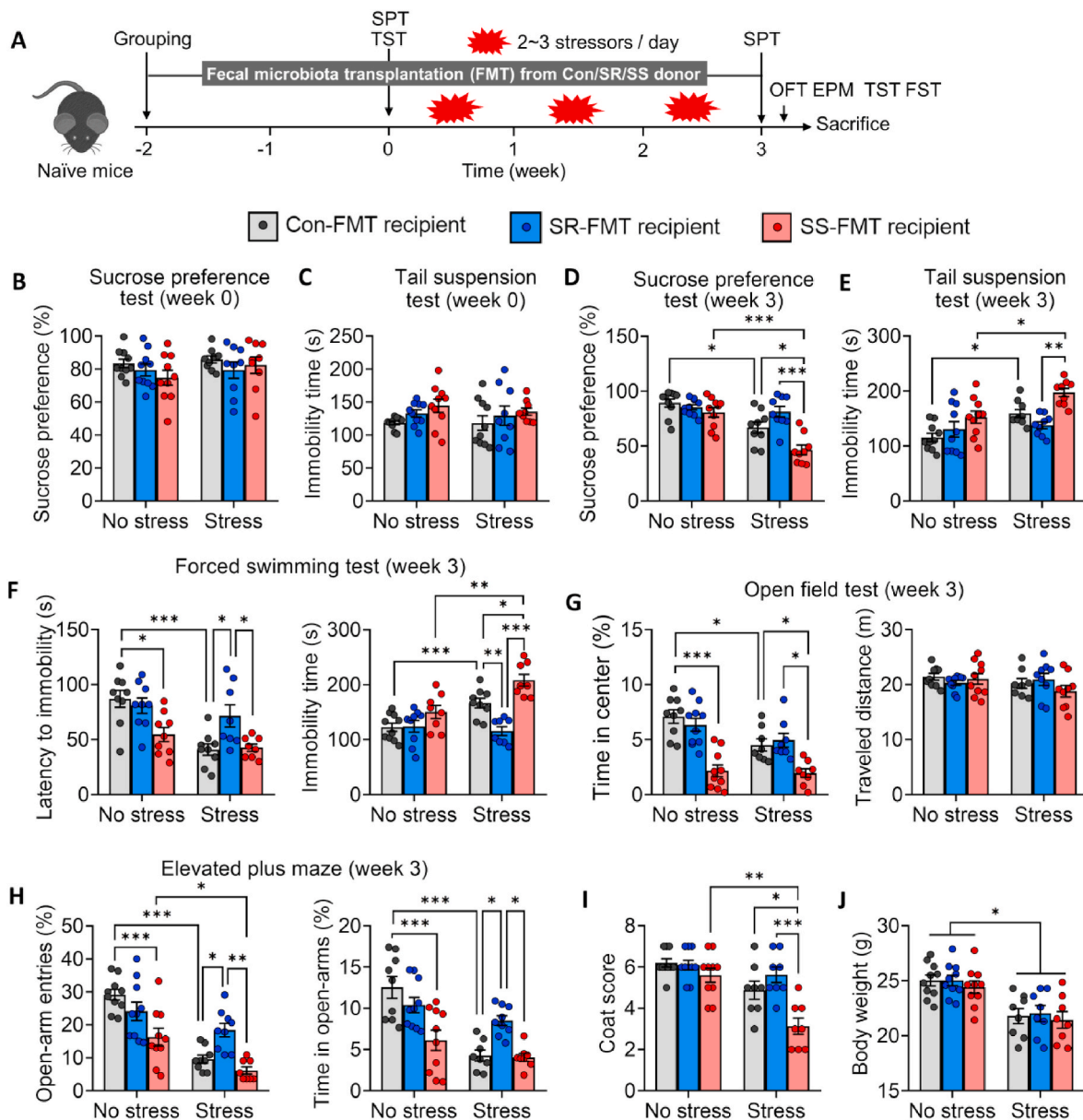
Fig. 6. Fecal microbiota transplantation (FMT) from stress-resistant or stress-sensitive mice can mitigate or exacerbate the effects of stress on recipient animals. **(A)** Schematic of the experimental process. The fecal microbiota from naïve control (Con), stress-resistant (SR) or stress-sensitive (SS) mice were transplanted into naïve mice, which were concurrently subjected to stress ("Stress") or not ("No stress"). Depression-like and anxiety-like behaviors were analyzed in the tail suspension test (TST), forced swimming test (FST), sucrose preference test (SPT), open field test (OFT) and elevated plus maze test (EMPT). **(B–C)** Comparison of recipient mice before stress exposure (week 0) in terms of (B) sucrose preference and (C) immobility time in the TST. **(D–E)** Comparison of recipient mice after stress exposure (week 3) in terms of (D) sucrose preference and (E) immobility time in the TST. **(F–J)** Comparison of recipient mice that were subjected to stress or not in terms of (F) latency and time spent immobile in the FST, (G) time in the central area and total distance traveled in the OFT, (H) percentage of total entries that were into open-arms and time spent in open-arms in the EPMT, (I) coat score, and (J) body weight. Data are mean ± standard error of the mean (SEM) for 8–10 animals per group. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, based on two-way ANOVA with Tukey's multiple-comparisons test.

Ethics approval

All experiments were approved by the Institutional Animal Care and Use Committee at the Guizhou University of Traditional Chinese Medicine.

Consent for publication

Not applicable.

CRediT authorship contribution statement

Haili He: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Hui He:** Conceptualization, Data curation, Investigation, Methodology, Software, Writing – review & editing. **Li Mo:** Data curation, Formal analysis, Investigation, Methodology, Software, Writing – review & editing. **Qingsong Yuan:** Conceptualization, Methodology, Resources, Writing – review & editing. **Chenghong Xiao:** Formal analysis, Methodology, Writing – review & editing. **Qiman Ma:** Visualization, Writing – review & editing. **Saini Yi:** Methodology. **Tao Zhou:** Funding acquisition, Resources, Writing – review & editing. **Zili**
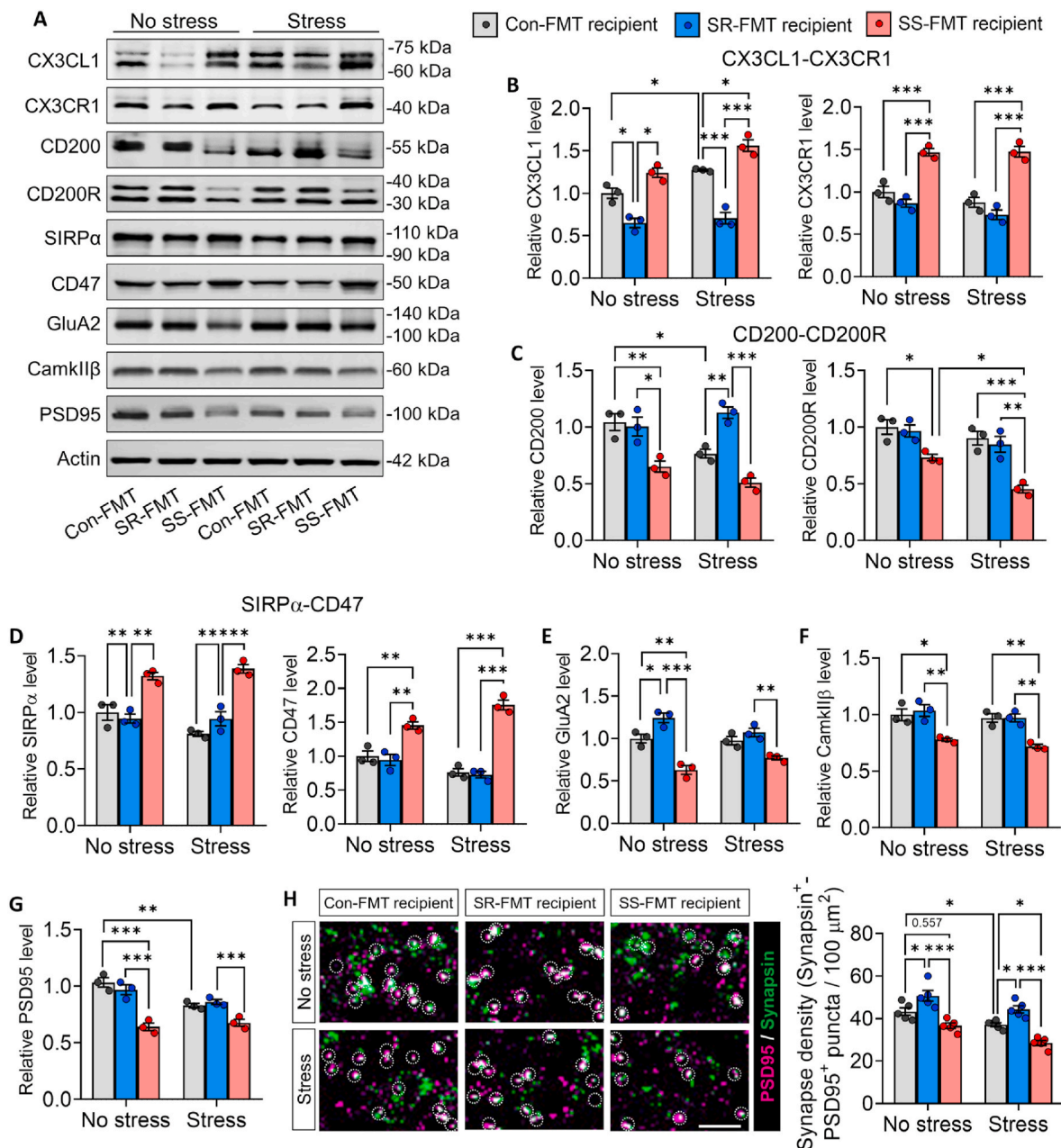
Fig. 7. Fecal microbiota transplantation (FMT) from stress-resistant or stress-sensitive mice can negatively or positively regulate microglia-neuron interactions in the hippocampus of recipient animals. **(A)** Western blotting of total hippocampal lysates against three ligand-receptor pairs that mediate cross-talk between neurons and microglia (Cx3CL1/Cx3CR1, CD200/CD200R and SIRPα/CD47) and against three synaptic plasticity markers (GluA2, CamkIIβ and PSD95) in recipient animals that were subjected to stress concurrently with fecal microbiota transplantation ("Stress") or not ("No stress"). Abbreviations are the same as those in the legend of Fig. 6. **(B–G)** Quantitation of the individual proteins shown in panel (A). Levels were normalized to those of non-stressed mice that received fecal microbiota from naïve animals. Results are shown for triplicate samples from three animals per condition. **(H)** Representative fluorescence micrographs (*left*) and quantification (*right*) of clusters containing synapsin and PSD95 (dotted circles) in the hippocampus of recipient mice that were subjected or not to stress concurrently with fecal microbiota transplantation. Quantification was performed as described in the legend of Fig. 6D. Scale bar, 5 μm. Data are mean ± standard error of the mean (SEM). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, based on two-way ANOVA with Tukey's multiple-comparisons test.

You: Funding acquisition, Project administration, Writing – review & editing. **Jinqiang Zhang:** Formal analysis, Funding acquisition, Project administration, Visualization, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could appear to have influenced the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbih.2024.100729.

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