Brain, Behavior, and Immunity 102 (2022) 266–278

Contents lists available at ScienceDirect

Brain Behavior and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

Full-length Article

Colitis-associated microbiota drives changes in behaviour in male mice in the absence of inflammation

Fernando A. Vicentini [a,b,d,e,2], Jake C. Szamosi [f], Laura Rossi [f], Lateece Griffin [a,e], Kristoff Nieves [b,d,e], Dominique Bihan [g], Ian A. Lewis [g], Quentin J. Pittman [a,b,c,e], Mark G. Swain [b,h], Michael G. Surette [f], Simon A. Hirota [b,c,d,e,1], Keith A. Sharkey [a,b,e,*,1]

[a] Hotchkiss Brain Institute, Cumming School of Medicine, University of Calgary, Calgary, AB, Canada
[b] Snyder Institute for Chronic Diseases, Cumming School of Medicine, University of Calgary, Calgary, AB, Canada
[c] Alberta Children's Hospital Research Institute, Cumming School of Medicine, University of Calgary, Calgary, AB, Canada
[d] Inflammation Research Network, Cumming School of Medicine, University of Calgary, Calgary, AB, Canada
[e] Department of Physiology and Pharmacology, Cumming School of Medicine, University of Calgary, Calgary, AB, Canada
[f] Farncombe Family Digestive Health Research Institute, McMaster University, Hamilton, ON, Canada
[g] Department of Biological Sciences, University of Calgary, Calgary, AB, Canada
[h] Division of Gastroenterology and Hepatology, Department of Medicine, Cumming School of Medicine, University of Calgary, Calgary, AB, Canada

ARTICLE INFO

Keywords:
Anxiety
Colitis
Depression
Fecal microbiota transplant
Gut-brain axis
Inflammatory bowel disease
Neuroinflammation

ABSTRACT

Inflammatory bowel diseases (IBD) are chronic inflammatory conditions of the gastrointestinal tract. IBD are associated with a high prevalence of cognitive, behavioural and emotional comorbidities, including anxiety and depression. The link between IBD and the development of behavioural comorbidities is poorly understood. As the intestinal microbiota profoundly influences host behaviour, we sought to determine whether the altered gut microbiota associated with intestinal inflammation contributes to the development of behavioural abnormalities. Using the dextran sulphate sodium (DSS) model of colitis, we characterized intestinal inflammation, behaviour (elevated plus maze and tail suspension test) and the composition of the microbiota in male mice. Cecal contents from colitic mice were transferred into germ-free (GF) or antibiotic (Abx)-treated mice, and behaviour was characterized in recipient mice. Gene expression was measured using qPCR. DSS colitis was characterized by a significant reduction in body weight and an increase in colonic inflammatory markers. These changes were accompanied by increased anxiety-like behaviour, an altered gut microbiota composition, and increased central *Tnf* expression. Transfer of the cecal matter from colitic mice induced similar behavioural changes in both GF and Abx-treated recipient mice, with no signs of colonic or neuroinflammation. Upon characterization of the microbiota in donor and recipient mice, specific taxa were found to be associated with behavioural changes, notably members of the Lachnospiraceae family. Behavioural abnormalities associated with intestinal inflammation are transmissible via transfer of cecal matter, suggesting that alterations in the composition of the gut microbiota play a key role in driving behavioural changes in colitis.

1. Introduction

Patients with inflammatory bowel diseases (IBD) exhibit a high prevalence of cognitive, behavioural and emotional disorders when compared to the general population (Bernstein et al., 2019; Marrie et al., 2017; Mikocka-Walus et al., 2016a). Characterized by chronic, unpredictable, relapsing inflammation within specific regions of the gastrointestinal (GI) tract, IBD has been increasing worldwide (Ng et al., 2018)

Abbreviations: Abx, Antibiotic; CRH, Corticotropin-releasing hormone; DSS, Dextran sulphate sodium; EPM, Elevated plus maze; GF, Germ free; GI, Gastrointestinal; IBD, Inflammatory bowel disease; Lcn-2, Lipocalin-2; MT, Microbiota transpant; OTU, Operational taxonomic unit; PBS, Phosphate-buffered saline; PCoA, Principal coordinate analysis; PCR, Polymerase chain reaction; SCFA, Short-chain fatty acid; SPF, Specific-pathogen free; TST, Tail suspension test.

* Corresponding author at: Department of Physiology and Pharmacology, University of Calgary, 3330 Hospital Drive NW, Calgary, AB T2N 4N1, Canada.
E-mail address: ksharkey@ucalgary.ca (K.A. Sharkey).

[1] Co-senior authors.

[2] Present address: Farncombe Family Digestive Health Research Institute, McMaster University, Hamilton, ON, Canada.

https://doi.org/10.1016/j.bbi.2022.03.001
Received 21 October 2021; Received in revised form 10 February 2022; Accepted 3 March 2022
Available online 5 March 2022

and so has the number of people suffering from the behavioural comorbidities that accompany this condition. IBD profoundly impacts quality of life and the occurrence of these comorbidities worsens the burden of the disease (Mikocka-Walus et al., 2016b; Szigethy et al., 2021). Among the comorbidities associated with IBD, anxiety and depression are among the most common (Bernstein et al., 2019; Mikocka-Walus et al., 2016a). In order to explain how cognitive, behavioural and emotional changes may be linked to inflammation in the GI tract, it has been proposed that neural, humoral and/or immune signals activate peripheral and central signalling pathways of the gut-brain axis (Carabotti et al., 2015; Collins, 2020). However, the mechanisms underlying the altered signaling remain poorly understood.

The intestine harbors an immense population of commensal bacteria, termed the gut microbiota, with an abundance reaching 10^{11} cfu/g in the distal colon (Donaldson et al., 2016). Over the past decade, a plethora of studies has suggested that the gut microbiota plays a fundamental role in host physiology, including modulating brain functions and behaviour (Clarke et al., 2013; Cryan et al., 2019; Heijtz et al., 2011). In both IBD and psychiatric conditions, such as major depressive disorder, alterations in the composition of the gut microbiota have been described (Halfvarson et al., 2017; Jiang et al., 2015; Ryan et al., 2020; Yang et al., 2020). Interestingly, transferring the microbiota from major depressive disorder patients into germ-free (GF)-recipient rodents has been shown to be sufficient to transfer some phenotypic aspects of the disease (Kelly et al., 2016; Zheng et al., 2016). Thus, it is apparent that the gut-brain axis is critically regulated by the gut microbiota, hence the concept of the microbiota-gut-brain axis (Cryan et al., 2019).

While it is apparent that alterations in the microbiota-gut-brain axis contribute to changes in behaviour and potentially contribute to the pathogenesis of psychiatric disorders (Jang et al., 2021; Thomann et al., 2020), it has yet to be determined whether inflammation-associated alterations in the intestinal microbiota directly contribute to the behavioural abnormalities. We hypothesized that intestinal inflammation induces changes in the microbiota that influences the onset of behavioural abnormalities. To test this hypothesis, we used a well-established preclinical model of colitis and assessed changes in behaviour and microbiota composition. Upon observing colitis-associated changes in behaviour, as we have reported previously (Nyuyki et al., 2018), we determined if this effect was driven by changes in the intestinal microbiota composition by: i) performing compositional analysis on the cecal contents from colitic and naïve mice, ii) transferring cecal contents from colitic mice into GF mice, iii) transferring cecal contents from colitic mice into antibiotic (Abx)-treated recipient mice, and iv) measuring the expression of proinflammatory and stress-related genes in the brain to identify pathways responsible for the behavioural abnormalities.

2. Materials and methods

2.1. Animals

Specific-pathogen free (SPF) male C57Bl/6 mice (8–12 weeks of age) from Jackson Laboratories (Bar Harbor, ME, USA) were used in the colitis and Abx exposure experiments. Mice were randomized upon arrival and housed (n = 5/cage) in the animal facility at the University of Calgary at 22 ± 2°C on a 12 h light–dark cycle. SPF mice were allowed to acclimatize for one week in the animal facility before any experiments were performed. Mice had free access to sterilized food and water. Germ-free male C57Bl/6 mice (6–9 weeks of age) were provided by the University of Calgary International Microbiome Centre (Mager et al., 2020). Germ-free mice were housed under the same conditions as SPF mice after microbiota transplant (MT). All animal procedures were approved by the University of Calgary Health Sciences Animal Care Committee (#AC19-0124) and are in accordance with the guidelines established by the Canadian Council on Animal Care.

2.2. Induction of gut inflammation

Dextran sulphate sodium (DSS) was used as a colitogenic agent. DSS-induced colitis is a well-established method to study gut inflammation in mice (Chassaing et al., 2014). DSS (2.5% w/v; AAJ1448922, ThermoFisher Scientific) was dissolved in sterilized water at the beginning of each experiment. Mice had free access to the bottles containing DSS solution for 5 days. After this period, mice were switched to regular autoclaved water for 2 days. On day 7, mice were used for behavioural tests and euthanized for further analyses.

2.3. Assessment of colonic inflammation

Assessment of colonic macroscopic damage score was performed according to a previous publication (Cluny et al., 2010) with minor modifications. Mice were euthanized with isoflurane overdose followed by cervical dislocation. The colon was removed and placed in dish for assessment of macroscopic damage score. Final score was built based on (i) body weight loss compared to day 0 (1 = 0–5%, 2 = 5.1–10%, 3 = 10.1–15%, 4 = >15%), (ii) colon length compared to control average (1 = 75–85%, 2 = 65–74.9%, 3 = <65%), (iii) presence of erythema, fecal blood, diarrhea (1 = each positive), (iv) length of inflamed colon (inflamed percentage of total length), and (v) bowel thickness (in mm).

Fecal lipocalin-2 (Lcn-2) concentration was measured as a non-invasive technique for assessing colonic inflammation. Elevated fecal Lcn-2 levels have been described in colitis, and thus considered a good marker of gut inflammation (Chassaing et al., 2012). Feces were collected on the last experimental day directly into a pre-weighed tube. Fecal weight was determined and samples were frozen in dry ice and stored at −80°C until further processing. Next, feces were dissolved in phosphate-buffered saline (PBS) containing 0.1% Tween 20 to a final concentration of 100 mg/mL. For optimal homogenization, samples were minced and vortexed at high speed for 20 min. After, tubes were centrifuged at 12,000 rpm for 10 min at 4°C. Supernatant was collected and diluted in 1:10 in 1% BSA in PBS. Next, the Mouse Lcn-2 DuoSet ELISA kit (DY1857, R&D Systems) was used and the assay was carried out according to manufacturer's instructions. Final concentration of Lcn-2 was determined using a standard curve.

Gene expression of proinflammatory cytokines was assessed in the colon via qPCR. Colonic samples were collected after the macroscopic damage score were performed. A small piece of colon was removed, placed in a tube and fixed in dry ice immediately. Samples were stored at −80°C until further processing. All tools used were cleaned with RNase AWAY (ThermoFisher Scientific). RNA extraction was performed using QIAzol (Qiagen) and purified with the RNeasy Mini Kit (Qiagen) according to the manufacturer's instructions. Briefly, samples were initially digested in QIAzol and homogenized with stainless steel beads (Qiagen) for 5 min in 50 Hz (TissueLyser LT; Qiagen). Next, samples were mixed with chloroform and centrifuged (12,000 rpm for 15 min at 4°C). The upper, transparent phase was pipetted into new tubes, and submitted to the kit columns tubes for final RNA isolation. RNA concentration was determined using NanoDrop (ThermoFisher Scientific), and solutions containing 1 μg/ml of RNA were prepared for reverse transcriptase reactions. cDNA was generated using High-Capacity cDNA Reverse Transcription Kit (ThermoFisher Scientific) with RNaseOUT (ThermoFisher Scientific) according to manufacturer's instructions. Due to the administration of normal drinking water for 2 days following DSS treatment, no additional steps were necessary to reduce the DSS interference with the amplification step. qPCR was performed using the TaqMan Fast Advanced Master Mix (ThermoFisher Scientific) with the following primers: *Tnf* (Mm00443258_m1; ThermoFisher Scientific), *Il1b* (Mm00434228_m1; ThermoFisher Scientific), and *Il6* (Mm00446190_m1; ThermoFisher Scientific). *Gapdh* (Mm99999915_g1; ThermoFisher Scientific) was used as reference gene. QuantStudio 3 System (ThermoFisher Scientific) was used to run the qPCR plates. Comparative analyses were done using the delta-delta Ct method, with

results expressed in fold change over control.

2.4. Microbiota transplant

To perform MT in both GF and Abx-treated mice, we first generated a stock of microbiota from donor animals. A cohort of mice (n = 10/group) was either treated with DSS (as described above) or left as controls. On the experimental day, mice were euthanized with isoflurane overdose followed by cervical dislocation, and the ceca were removed. Cecal matter were pooled by group into a tube containing cold 20% glycerol in sterile PBS to a final concentration of 1 g/15 mL. Pooled samples were then vortexed and aqueous phase was aliquoted into 2 mL tubes, frozen in dry ice, and later stored at −80°C. At each MT gavage day, one aliquot per group was thawed and used once.

To achieve microbiota transfer in GF mice, animals received one gavage (100 μL/mouse) at the beginning of the experiment under sterile conditions. After MT, mice were housed in sterilized IVC cages with ventilation of HEPA filtered air. Until the behavioural experiments, handling of the mice was done only by the experimenter, inside the biosafety cabinet, with the use of sterilized equipment.

For MT in Abx-treated mice, first, mice were treated with Abx as described previously (Miller et al., 2019; Rakoff-Nahoum et al., 2004). Abx mix consisted of ampicillin (1 g/L; A9518, Sigma-Aldrich), neomycin (1 g/L; N1876, Sigma-Aldrich), vancomycin (0.5 g/L; 94747, Sigma-Aldrich), and metronidazole (1 g/L; M3761, Sigma-Aldrich) diluted in autoclaved water. Mice received Abx treatment for 14 days. Metronidazole was gradually added into the Abx mix solution to avoid massive body weight loss. Briefly, 0.25 g/L (25% of final concentration) of metronidazole was added starting on day 2; 0.50 g/L (50% of final concentration) was added at day 6; and 1.0 g/L (full concentration) was added at day 9. Abx solution was refilled every week. Control mice had regular, autoclaved water. After 14 days of treatment, Abx solution was exchanged for autoclaved water. After a 24 h Abx washout period, mice were gavaged (200 μL/mouse) every other day with microbiota from donors. MT gavages were performed 4 times before any experimental test.

2.5. Behavioural assessment

The elevated plus maze (EPM) and the tail suspension test (TST) were used to assess animal behaviour. All behavioural tests were performed in the morning, from 7 am to 12 pm. On the experimental day, mice were brought to the behavioural room and allowed to rest and habituate for at least 2 h prior the test. All analyses were done by an experimenter blinded to the treatment groups.

For the EPM test, mice were randomly selected and placed on the EPM apparatus. The apparatus consisted of a cross-shaped platform with an acrylic surface elevated 50 cm above the floor. The 4 arms were composed of two opposite open arms with no walls and two enclosed arms by a 20 cm wall, all connected to a central area of 7 × 7 cm. Each arm was 21.5 cm long and 7 cm wide. Light intensity was set to 70–75 lux on the open arms and 15–20 lux on the closed arms. The recording camera was set above the apparatus. In each session, the animal was placed on the centre zone facing the open arm opposite to the experimenter and recorded for 5 min. At the end of the session, the subject mouse was returned to a new home cage. The apparatus was cleaned with 70% ethanol in between subject mouse. The TopScan software (CleverSys) was used for analyses of the recorded videos, assessing the animals' time spent in the open and closed arms, and total locomotion through the maze.

For the TST, mice were first prepared in a transport cage and had a plastic tube (2.5 cm long × 0.5 cm diameter) placed on their tail to prevent tail-climbing behaviour. An adhesive tape was then used to attach the animals to the horizontal bar of the apparatus. The adhesive tape used was 15 cm long and wrapped 2 cm of the tip of the mouse tail. The behavioural apparatus consisted of a box (30 × 26 × 60 cm) with an open front where the recording camera was positioned. The horizontal bar in which the animals were suspended was 50 cm high. Mouse behaviour was recorded for 6 min, and analysis of immobility time was assessed during the final 4 min of observation. Absence of escape-oriented behaviour (i.e., hanging passively, motionless) was considered immobility. Tail climbing was considered an exclusion criterion.

2.6. Microbiota analyses

Cecal matter were collected and frozen in dry ice for bacterial composition analyses via 16S rRNA sequencing. First, genomic DNA was extracted from the raw sample as described previously (Stearns et al., 2015) with some modifications. Samples were transferred to screw-cap tubes containing 2.8 mm ceramic beads, 0.1 mm glass beads, guanidinium thiocyanate EDTA N-lauroylsarkosine buffer and PBS, then bead beat and centrifuged. The supernatant was further cleaned up using the MagMAX Express 96-Deep Well Magnetic Particle Processor with the Multi-Sample kit (4413022; ThermoFisher Scientific). PCR was then used to amplify the v3 region of the 16S rRNA gene. The template was 50 ng of purified DNA and the reaction was made up with 1 U of Taq, 1x buffer, 1.5 mM MgCl2, 0.4 mg/mL BSA, 0.2 mM dNTPs, and 5 pmoles each of 341F (CCTACGGGNGGCWGCAG) and 518R (ATTACCGCGGCTGCTGG) primers. The primers were adapted for Illumina as described by Bartram et al. (2011). The thermocycler was set at 94 °C for 5 min, 25 cycles of 94 °C for 30 s, 50 °C for 30 s and 72 °C for 30 s, with a final extension of 72 °C for 10 min. Amplicons were visualized on a 1.5% agarose gel and positive ones were normalized using the SequalPrep normalization kit (A1051001; ThermoFisher Scientific). Due to the administration of normal drinking water for 2 days following DSS treatment, no additional steps were necessary to reduce the DSS interference with the amplification step. Subsequently, amplicons were pooled and sequenced on the Illumina MiSeq platform at the McMaster Genomics Facility.

Reads were processed using Cutadapt on the raw reads to filter and trim the adapter and PCR primer sequences with a minimum quality score of 30 and a minimum read length of 100 bp (Martin, 2011). DADA2 was used to determine sequence variants (Callahan et al., 2016). The trimmed reads were processed through DADA2 in batches per Illumina run. The resulting sequence variant tables were then merged, bimeras were removed and taxonomy was assigned using the SILVA database version 1.3.2. All sequences not assigned to Kingdom Bacteria or Archaea (potential host sequences), or lacking phylum assignment were removed. The remaining sequences were clustered at 99% identity using the DECIPHER package (Wright, 2016), applying the UPGMA method. Representative sequences were generated for each cluster using ambiguity codes where necessary, and taxonomy was re-assigned to the representative sequences using the assignTaxonomy function in DADA2. A count table based on these 99% identity clusters was used for all downstream analyses. Before filtering, 438 operational taxonomic units (OTUs) were found in 66 samples analyzed. A total of 6,370,332 reads were registered, with sample read depths ranging from 16,908 to 180,911. Here again, any suspected host OTUs were removed as described above, plus all OTUs assigned to Family Mitochondria. Relative abundance values were calculated and all OTUs whose mean raw abundance across all samples was 10 or less were removed. After filtering, 6,339,510 reads were observed, with sample depths ranging from 16,757 to 180,321.

For alpha diversity, we rarefied the samples to the minimum sample depth (16,757) before calculating Simpson's diversity index. Principal coordinate analysis (PCoA) plots were based on Bray-Curtis distances calculated from relative abundances. The effect of group and treatment on community variability was tested with a PERMANOVA implemented in the adonis function in the vegan (Oksanen et al., 2019) package. Differential abundance between DSS and Control groups was tested using both the ANCOM package (Mandal et al., 2015) and DESeq2 (Love et al., 2014).

All microbiome data were analyzed in R (Development Core Team, 2019). The phyloseq (McMurdie and Holmes, 2013), ggplot2 (Wickham, 2016), and tidyverse (Wickham et al., 2019) packages were used for data organization and plotting.

2.7. Gene expression measurements in brain samples

After euthanasia, mice were perfused with ice-cold, sterile saline and the brain was extracted from the skull and placed in a cold petri dish. Hypothalamus, amygdala, and hippocampus were microdissected by a trained researcher and immediately fixed in dry ice. All tools used were cleaned with RNase AWAY (ThermoFisher Scientific) and samples were stored at −80°C until further processing. Samples were processed for qPCR similarly to colonic tissue, described above. Besides the above-mentioned primers for *Tnf*, *Il1b*, and *Il6*, additional primers used were: *Nos2* (Mm00440502_m1; ThermoFisher Scientific), *Crh* (Mm04206019_m1; ThermoFisher Scientific), *Crhr1* (Mm00432670_m1; ThermoFisher Scientific), *Nr3c1* (Mm00433832_m1; ThermoFisher Scientific), and *Bdnf* (Mm01334042_m1; ThermoFisher Scientific). *Gapdh* was used as the reference gene. Comparative analyses were done using the delta-delta Ct method, with results expressed in fold change over control.

2.8. Fecal short-chain fatty acids (SCFA) measurement

Fecal samples were collected directly into sterile tubes and stored at −80°C until sample processing. SCFA were measured as described previously (Bihan et al., 2019). In brief, SCFA were extracted (1:2 ratio wet sample weight [mg] to extraction solvent [μL]) from fecal samples with ice-cold extraction solvent (50% water/acetonitrile, v/v) spiked with stable isotope-labeled SCFA internal standards (IS) (acetic acid-1,2-13C2 (2 mM, final concentration), propionic acid-13C3 (0.5 mM, final concentration), butyric acid-1,2-13C2 (1 mM, final concentration), isobutyric-d7 acid (25 mM, final concentration), valeric-d9 acid (50 mM, final concentration) and isovaleric-d9 acid (25 mM, final concentration)), derivatized with N-(3-dimethylaminopropyl)-N'-ethyl-carbodiimide hydrochloride and aniline, and then submitted to ultrahigh-performance liquid chromatography-mass spectrometry analysis (UHPLC-MS). The UHPLC-MS platform consisted of a Vanquish ultrahigh-performance liquid chromatography system coupled to a TSQ Quantum Access MAX triple quadrupole Mass Spectrometer (ThermoFisher Scientific) equipped with an electrospray ionization probe. SCFAs were separated on a Hypersil GOLD TM C18 column (200 × 2.1 mm, 1.9 μm; ThermoFisher Scientific) by using a binary solvent system composed of liquid chromatography-mass spectrometry grade water and methanol, both containing 0.1% (%v/v) formic acid, and monitored with the mass spectrometer operating in positive ionization mode and selected reaction monitoring mode. Data analyses, on the converted mzXML files, were conducted in El-MAVEN (Agrawal et al., 2019), and the absolute quantification of native SCFA concentration was based on the ^{12}C:IS signal intensity ratio and the respective IS concentration.

2.9. Statistical analyses

Shapiro Wilk and Kolmogorov-Smirnov tests were used to assess data normality. Student's *t* test was used for analysis between two groups. Analyses of two variables were performed applying two-way ANOVA followed by Sidak's multiple comparisons test. The specific statistical test and *n* number are described in every figure legend and the accompanying Table of Statistical Values (Supplementary Table 1). Complete statistical analysis done with DESeq2 is shown at Table of DESeq2 output (Supplementary Table 2). $p < 0.05$ was considered statistically significant. Bar graphs and PCoA plots display biological replicates as individual dots. Statistical analyses were performed with GraphPad Prism 9 (GraphPad Software).

3. Results

3.1. Dextran sulphate sodium (DSS)-induced colitis is associated with changes in the composition of the intestinal microbiota, increased anxiety-like behaviour and increased Tnf in distinct brain regions

To assess the effects of intestinal inflammation on the microbiota and behaviour, colitis was induced in adult mice by the administration of DSS (Fig. 1a). Body weight loss and higher colonic macroscopic damage scores were observed in DSS-treated mice compared to control mice (Fig. 1b, 1c). An increase in both fecal lipocalin-2 (Lcn-2) (Chassaing et al., 2012) and colonic proinflammatory gene expression (*Tnf*, *Il1b*, and *Il6*) confirmed the presence of intestinal inflammation in DSS-treated mice (Fig. 1d, 1e). There were no changes ($p > 0.05$) in the expression of the reference gene *Gapdh* between the experimental and control animals (data not shown). Evaluating the cecal microbiota via 16S rRNA gene sequencing, we observed a shift in the composition associated with DSS treatment through analysis of β-diversity displayed using PCoA matrices (Fig. 1f). The PCoA matrices were generated based on all the microbiota analyses performed in the study to provide a holistic representation of the data. Next, we assessed behaviour in colitic mice. As we have shown previously (Nyuyki et al., 2018), DSS-treated mice exhibited an increase in anxiety-like behaviour, as demonstrated by the reduced time spent and number of entries in the open arm in the EPM test (Fig. 1g). Moreover, a reduction in general locomotion was also observed in the DSS group in the EPM test (Fig. 1g). Although peripheral inflammation has been associated with changes in despair behaviour (Lopes et al., 2020; Takahashi et al., 2019), no alterations in immobility time were recorded on the TST (Fig. 1h). Together our data suggest colitis-induced changes in microbiota composition are associated with increased anxiety-like behaviour in mice.

To determine the mechanism(s) by which colitis-associated changes in the microbial composition drive behavioural alterations, we assessed gene expression in distinct brain regions (e.g., hippocampus, hypothalamus, and amygdala) that are involved in the control of both anxiety- and depression-like behaviour (Cryan et al., 2019; Krishnan and Nestler, 2008). As neuroinflammation is a known driver of behavioural alterations (Walker et al., 2014), we first measured *Tnf*, *Il1b*, *Il6*, and *Nos2* as markers of a proinflammatory milieu in the different brain regions (Fig. 1i). We observed a consistent increase in *Tnf* expression in the DSS-treated mice throughout the different brain regions analyzed (Fig. 1i). Although *Tnf* expression was elevated, no other alteration of proinflammatory markers were measured in the DSS-treated group, except an unexpected reduction in *Nos2* expression ($p = 0.06$) in the hippocampus (Fig. 1i).

3.2. Colitis-associated behaviours are transmissible via microbiota transfer (MT) into germ-free (GF) recipient mice

To address whether the changes in the microbiota composition directly contribute to the behavioural abnormalities observed in colitis (Fig. 1), we collected cecal contents from DSS-treated and control mice (Fig. S1), which were defined as microbiota donors. The donor cecal contents were then administered to GF-recipient mice by oral gavage (Fig. 2a). Following a 3-weeks period of engraftment, we assessed behaviour by comparing the two groups (GF MT Control and GF MT DSS). Prior to assessing behaviour, we confirmed that the microbiota transfer did not alter any parameters of intestinal inflammation. Indeed, no clinical signs of colitis or measures of colonic inflammation were observed in either group, as evidenced by the absence of alterations in body weight or increased fecal Lcn-2 levels over the course of the experiment. While fecal Lcn-2 levels were reduced in the GF MT DSS group (Fig. 2d), neither group exhibited levels of Lcn-2 suggestive of an overt inflammatory state (Pulakazhi Venu et al., 2021). Furthermore, upon completion of behavioural studies, analysis of isolated colonic tissues revealed no changes in colonic macroscopic damage scores or the

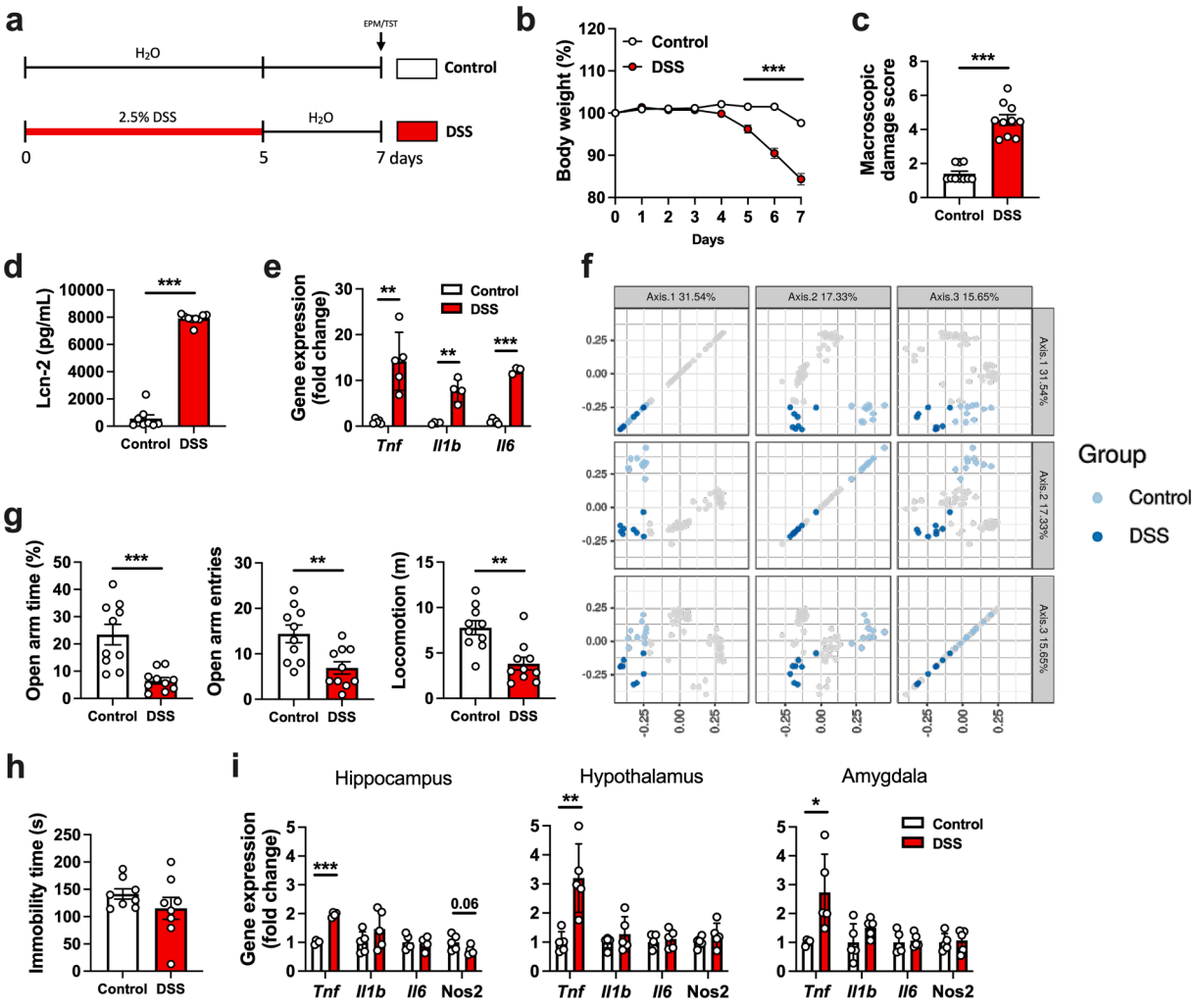

Fig. 1. Dextran sulfate sodium (DSS) administration induces colonic inflammation accompanied by a shift in the microbiota composition, increased anxiety-like behaviour, and elevated *Tnf* expression in the brain. (**a**) Experimental design. Mice were administered DSS (2.5% w/v) in their drinking water for 5 days followed by 2 days of regular water (DSS group). The control group received regular water throughout the experiment. All experiments and sample harvesting were done on day 7. (**b**) Daily body weight variation (two-way ANOVA followed by Sidak's multiple comparisons test). (**c**) Colonic macroscopic damage score. (**d**) Levels of lipocalin-2 (Lcn-2) in the feces. (**e**) Gene expression of pro-inflammatory cytokines in the colon. (**f**) Principal coordinate analysis (PCoA) displaying gut bacteria composition (16S rRNA gene sequencing) based on Bray-Curtis dissimilarity metric. Each dot represents the bacterial composition from the cecal matter of one biological replicate. The three main axes that explain the major variations are shown with the percentage of variation for each axis. Individual gray dots represent data from other experiments that were also considered when generating the PCoA matrix (see Materials and methods for details). PERMANOVA: $p < 0.001$ considering Control vs DSS. (**g**) Anxiety-like behaviour as measured by the elevated plus maze test (EPM). (**h**) Immobility time measured during the tail suspension test (TST). (**i**) Gene expression of pro-inflammatory mediators in the hippocampus, hypothalamus, and amygdala. Data are expressed as mean ± SEM. All statistical values are shown in the Supplementary Table of Statistical Values. *Gapdh* was used as reference gene in all gene expression analyses and was verified to be unchanged between experimental and control datasets. (b-d, f-h) n = 8–10; (e, i) n = 3–5. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; Student's *t* test unless otherwise stated. Tnf: tumor necrosis factor; Il1b: interleukin 1β; Il6: interleukin 6; Nos2: inducible nitric oxide synthase (iNOS).

expression of proinflammatory cytokines (Fig. 2b-e). The efficiency of microbiota transfer was confirmed by β-diversity analysis (Fig. 2f). Individual recipient samples were grouped into two distinct clusters which were closely associated with their respective microbiota donor signatures (Fig. 2f). Following confirmation of successful microbiota transfer and the absence of intestinal inflammation, we assessed behaviour. In the EPM test, GF mice receiving microbiota from mice with colitis, but not those administered cecal matter from control mice, presented with anxiety-like behaviour, as indicated by a reduction in the time spent in the open arm of the EPM (Fig. 2g). No differences in the number of open arm entries or locomotion were observed between groups (Fig. 2g). Interestingly, GF mice receiving microbiota from mice with colitis also exhibited significantly greater immobility times in the TST, suggesting the manifestation of despair behaviour (Fig. 2h). Unlike the naïve and DSS-treated donors, *Tnf* expression was not increased in any brain region assessed in the GF MT DSS group presenting with behavioural alterations (Fig. 2i). However, GF MT DSS mice exhibited lower expression of *Nos2* in the hippocampus and amygdala, and increased *Il6* in the hippocampus (Fig. 2i). Together our data suggest that colitis-associated behavioural changes are transmissible via microbiota transfer, and that inflammation-induced changes in the composition of the intestinal microbiota likely contribute to behavioural abnormalities.

3.3. *Colitis-associated behaviours are transmissible via MT into antibiotic (Abx)-treated recipient mice*

Because GF mice have an immature immune system (Honda and Littman, 2016), we next evaluated the effects of the transfer of the colitis-associated microbiota into Abx-treated recipient mice in order to further support the transmissibility of the behavioural abnormalities.

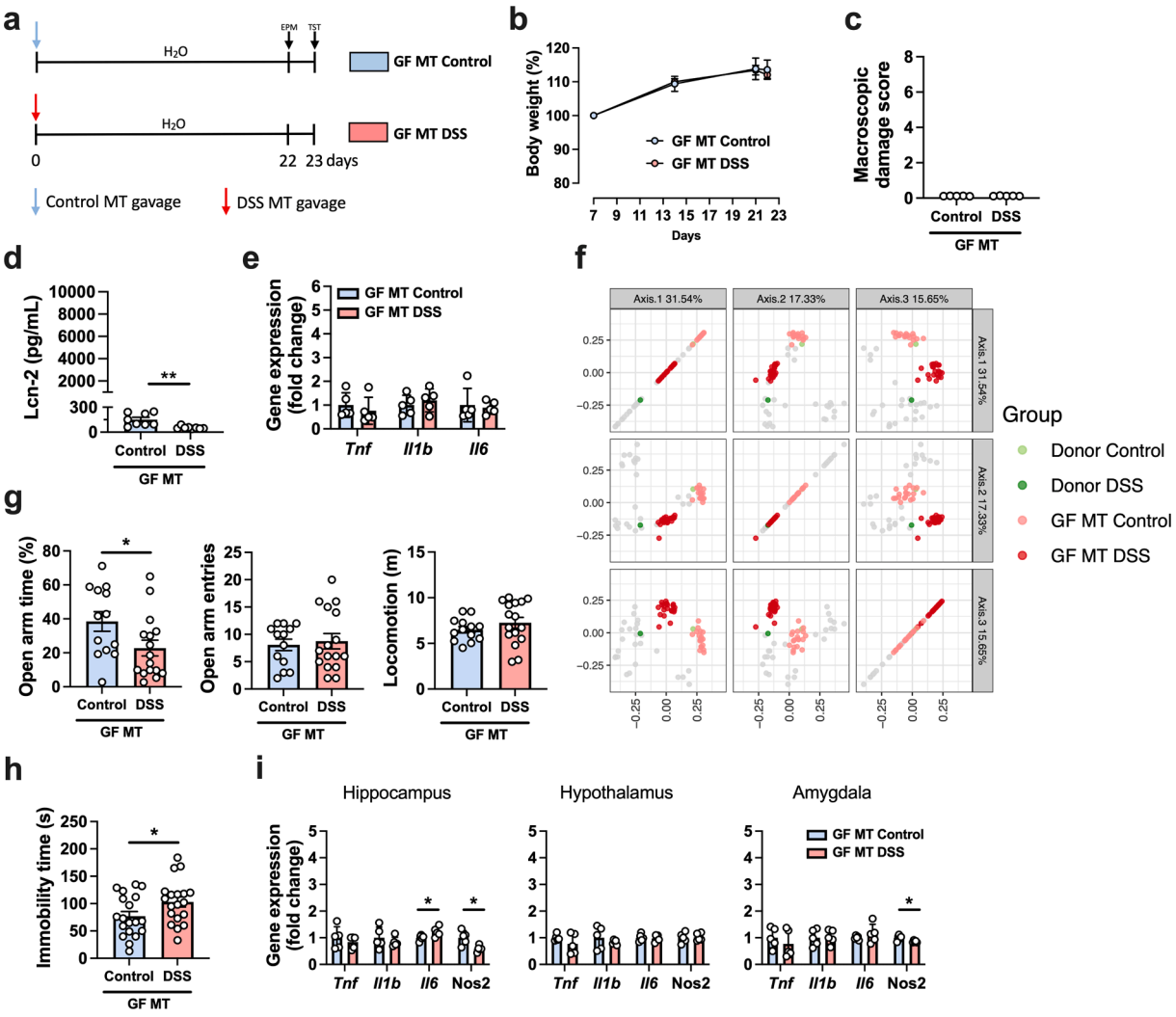

Fig. 2. Transfer of colitis-associated microbiota into germ-free (GF) recipient mice induces anxiety-like and despair behaviour in the absence of colonic or brain inflammation. (**a**) Experimental design. Microbiota transplant (MT) was performed in GF mice with either microbiota derived from control (GF MT Control group) or DSS-treated mice (GF MT DSS group). Donor microbiota was collected from cecal matter and transferred into recipient mice via gavage. Gavage was performed on day 0 and behaviour experiments and tissue harvesting were done on days 22 and 23. (**b**) Weekly body weight variation (two-way ANOVA test). (**c**) Colonic macroscopic damage score. (**d**) Levels of lipocalin-2 (Lcn-2) in the feces. (**e**) Gene expression of pro-inflammatory cytokines in the colon. (**f**) Principal coordinate analysis (PCoA) displaying gut bacteria composition (16S rRNA gene sequencing) based on Bray-Curtis dissimilarity metric. Each dot represents the bacterial composition from the cecal matter of one biological replicate. The three main axes that explain the major variations are shown with the percentage of variation for each axis. Individual gray dots represent data from other experiments that were also considered when generating the PCoA matrix (see Materials and methods for details). PERMANOVA: $p < 0.001$ considering GF MT Control vs GF MT DSS. (**g**) Anxiety-like behaviour assessment as measured by the elevated plus maze test (EPM). (**h**) Immobility time measured during the tail suspension test (TST). (**i**) Gene expression of pro-inflammatory mediators in the hippocampus, hypothalamus, and amygdala. Data are expressed as mean ± SEM. All statistical values are shown in the Supplementary Table of Statistical Values. *Gapdh* was used as reference gene in all gene expression analyses and was verified to be unchanged between experimental and control datasets. (b, d) n = 8–10; (c, e, i) n = 5; (f-h) n = 15–20. *$p < 0.05$, **$p < 0.01$; Student's *t* test unless otherwise stated. Tnf: tumor necrosis factor; Il1b: interleukin 1β; Il6: interleukin 6; Nos2: inducible nitric oxide synthase (iNOS).

Mice were treated with Abx and subsequently administered cecal microbiota from DSS-treated or control mice (Fig. 3a). As in our previous experiments, microbiota transfer did not elicit any changes in body weight (Fig. 3b), nor did it trigger any detectable colonic inflammation (Fig. 3c-e). Microbial transfer efficiency was confirmed by β-diversity analysis, wherein each recipient group clustered separately from each other, and with close proximity to its respective donor (Fig. 3f). Behavioural testing revealed that Abx-treated mice receiving cecal matter from colitic mice presented with anxiety-like behaviour, as indicated by less time spent and reduced entries in the open arm of the EPM, and also lower mobility throughout the test (Fig. 3g). Additionally, mice receiving colitic microbiota presented with increased despair behaviour, as indicated by greater immobility time in the TST (Fig. 3h). Interestingly, Abx MT DSS mice did not exhibit any changes in inflammatory gene expression in any brain region assessed (Fig. 3i), despite presenting with robust behavioural abnormalities. These data also strongly suggest that colitis-induced alterations in the composition of the microbiota contribute to generation of behavioural abnormalities.

3.4. The transmissibility of colitis-associated behavioural abnormalities are not associated with changes in stress- and neuroplasticity-associated gene expression

Since we did not observe a strong inflammatory signature in the recipient animals, which suggests that neuroinflammation is not the driver of the transmissible behavioural abnormalities, we next tested

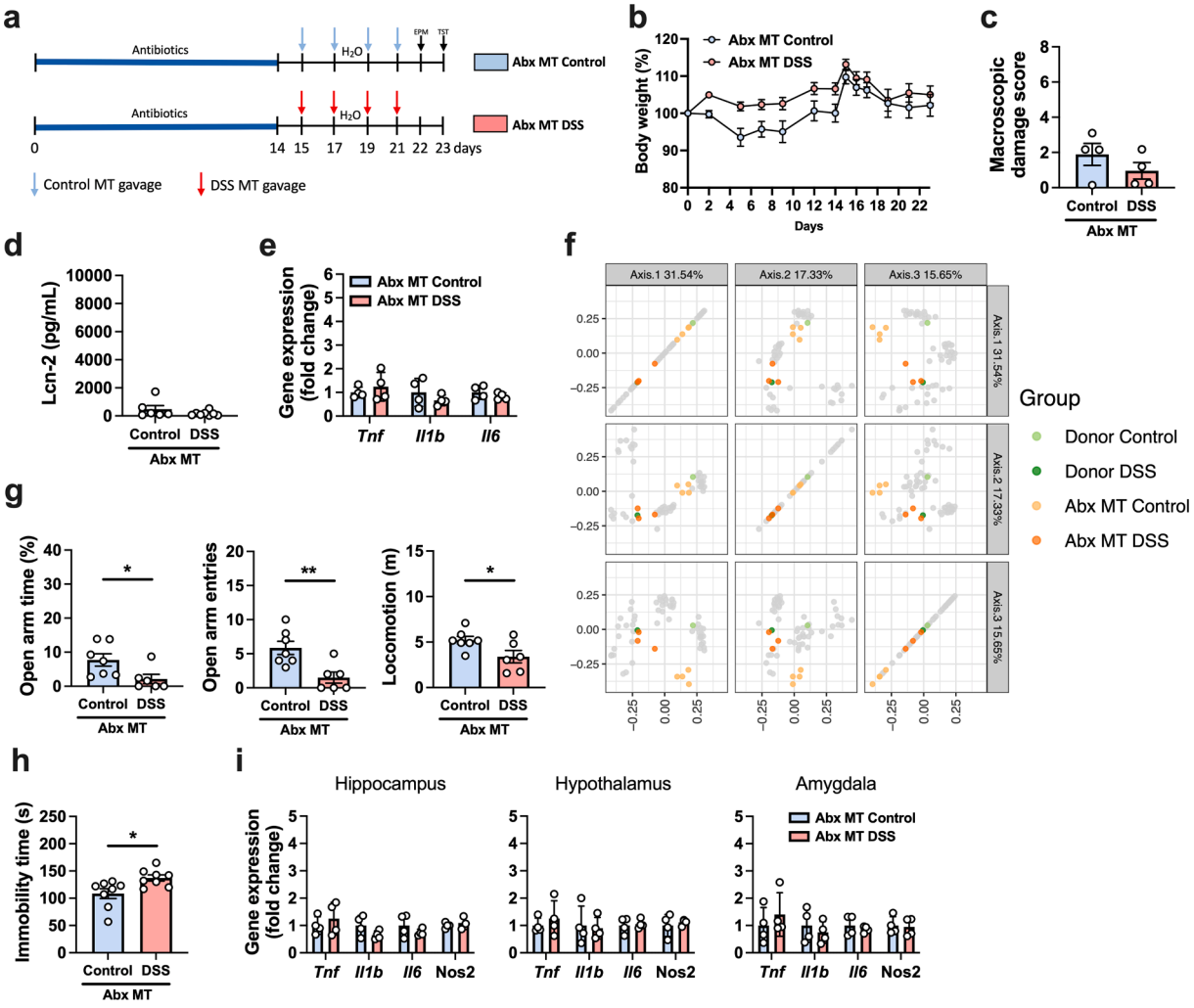

Fig. 3. Colitis-associated microbiota induces anxiety-like and despair behaviour in antibiotic (Abx)-treated recipient mice with no associated colonicorbrain inflammation. (**a**) Experimental design. Microbiota transplant (MT) was performed in Abx-treated mice with either microbiota derived from control (Abx MT Control group) or DSS-treated mice (Abx MT DSS group). Donor microbiota was collected from cecal matter and transferred into recipient mice via gavage. First, recipient mice were treated with a cocktail of Abx as described in Materials and methods. Following 14 days of Abx treatment, bottles were swapped to regular water, and mice had 24 h of Abx washout period before the first MT gavage. Following, mice were gavaged every other day to a total of 4 MT gavage. Behaviour experiments and tissue harvesting were performed on days 22 and 23. (**b**) Body weight variation (two-way ANOVA test). (**c**) Colonic macroscopic damage score. (**d**) Levels of lipocalin-2 (Lcn-2) in the feces. (**e**) Gene expression of pro-inflammatory cytokines in the colon. (**f**) Principal coordinate analysis (PCoA) displaying gut bacteria composition (16S rRNA gene sequencing) based on Bray-Curtis dissimilarity metric. Each dot represents the bacterial composition from the cecal matter of one biological replicate. The three main axes that explain the major variations are shown with the percentage of variation for each axis. Individual gray dots represent data from other experiments that were also considered when generating the PCoA matrix (see Materials and methods for details). PERMANOVA: $p < 0.05$ considering Abx MT Control vs Abx MT DSS. (**g**) Anxiety-like behaviour assessment as measured by the elevated plus maze test (EPM). (**h**) Immobility time measured during the tail suspension test (TST). (**i**) Gene expression of pro-inflammatory mediators in the hippocampus, hypothalamus, and amygdala. Data are expressed as mean ± SEM. All statistical values are shown in the Supplementary Table of Statistical Values. *Gapdh* was used as reference gene in all gene expression analyses and was verified to be unchanged between experimental and control datasets. (b, d, g, h) n = 6–10; (c, e, f, i) n = 3–4. *$p < 0.05$, **$p < 0.01$; Student's *t* test unless otherwise stated. Tnf: tumor necrosis factor; Il1b: interleukin 1β; Il6: interleukin 6; Nos2: inducible nitric oxide synthase (iNOS).

whether genes related to stress responses (e.g., corticotropin-releasing hormone [*Crh*], corticotropin-releasing hormone receptor 1 [*Crhr1*], and glucocorticoid receptor [*Nr3c1*]) were altered in colitic animals or recipients of microbiota transfer (Fig. 4). The stress response has a major influence on anxiety and depression incidence (Krishnan and Nestler, 2008). DSS-treated mice had minor changes in stress response-related genes, with lower levels of *Crh* expression in the hippocampus (Fig. 4a). Interestingly, GF MT DSS mice exhibited increased expression of *Crh* in the hypothalamus (Fig. 4b), which was not observed in the Abx MT DSS (Fig. 4c). Abx MT DSS mice displayed alterations in the expression of the *Crhr1* and *Nr3c1* in the amygdala (Fig. 4c). In addition, no signal of neuroplasticity, as measured by *Bdnf* expression (Lu et al., 2013), was observed in any group in the brain regions analyzed (Fig. 4).

3.5. DSS-induced colitis and MT leads to distinct intestinal microbiota signatures

We next identified microbial community members that are associated with the transmissible behaviours. First, when comparing DSS-treated to control mice, α-diversity analysis showed that intestinal inflammation was associated with a greater data dispersion and a reduction in the Simpson's diversity index (Fig. S2a). However, this reduction in Simpson's diversity was not maintained in GF and Abx-treated recipient mice, suggesting that the alterations in α-diversity were driven by active intestinal inflammation, but likely do not contribute to the behavioural abnormalities. Next, we evaluated the distribution of the microbiota composition in a PCoA matrix based on

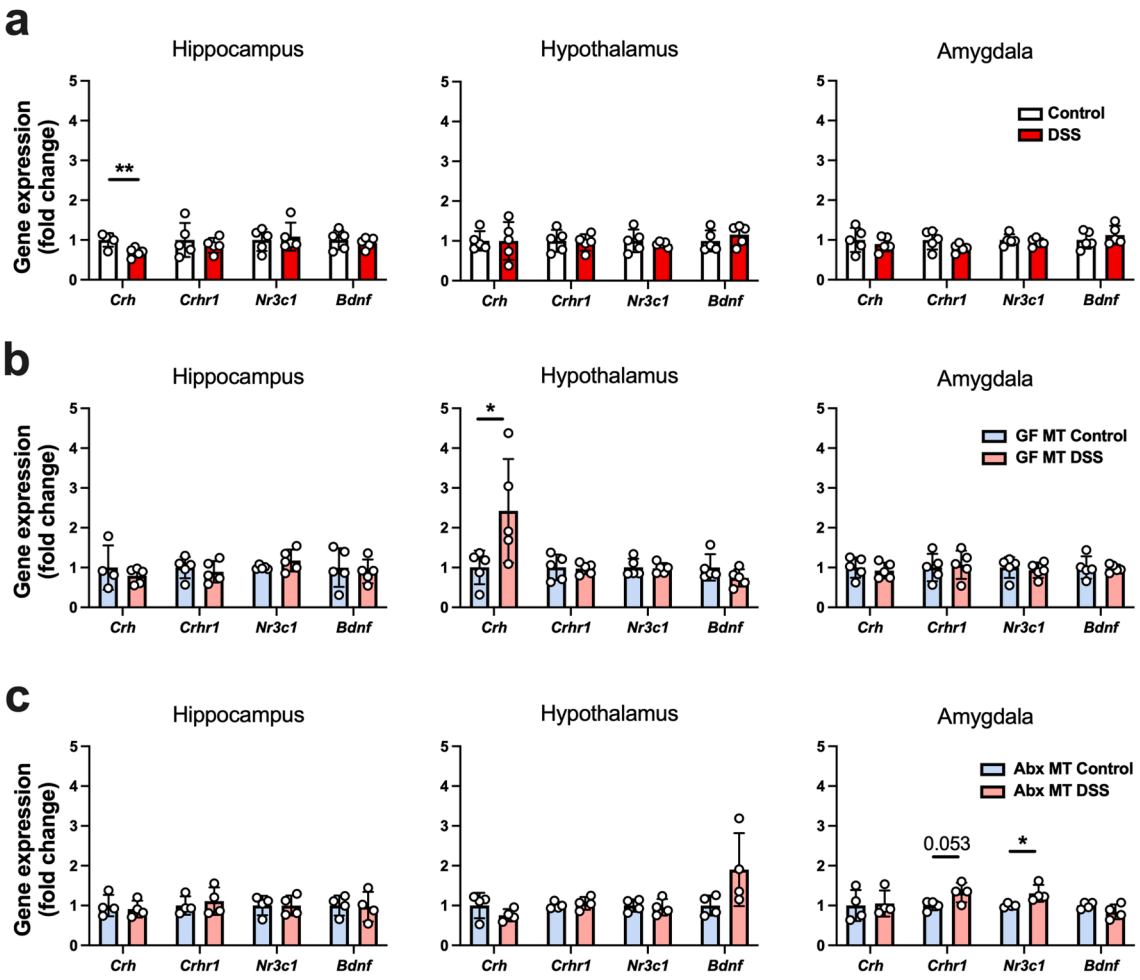

Fig. 4. Minor changes were observed in the expression of genes related to stress responses in dextran sulfate sodium (DSS)-treated mice and germ-free (GF) and antibiotic (Abx) recipient mice after microbiota transfer (MT). Expression of target genes linked to stress responses and neuroplasticity were measured by qPCR in the hippocampus, hypothalamus, and amygdala of (**a**) DSS-treated mice, and (**b**) GF and (**c**) Abx recipient mice after MT, and their respective controls. Data are expressed as mean ± SEM. All statistical values are shown in the Supplementary Table of Statistical Values. n = 4–5. *p < 0.05, **p < 0.01; Student's *t* test. Crh: corticotropin-releasing hormone; Crhr1: corticotropin-releasing hormone receptor 1; Nr3c1: nuclear receptor subfamily 3, group C, member1 – glucocorticoid receptor (GR); Bdnf: brain-derived neurotrophic factor.

our analysis of β-diversity (Fig. 5a). We identified a main separation of two groups on the PCoA axis 1 (accounting for 31.54% of the variation), in which all recipient mice clustered separately from control and DSS-treated mice. Moreover, we observed that axis 2 and 3, accounting for 17.33% and 15.65% of the variation respectively, clearly separate the data into control vs. DSS-treated, independent of the experiment (i.e., initial donor analysis, GF recipients, Abx-treated recipients). These data suggest that treatment (i.e., control vs. DSS) heavily influenced microbiota composition, and that microbiota engraftment after MT was efficient and robust. While assessing specific taxa responsible for the observed differences in composition, we identified the most abundant alterations in Lachnospiraceae, Ruminococcaceae and Muribaculaceae (Fig. S2b). Further analysis of these taxa revealed changes in specific OTUs that were common to either all DSS (Fig. 5b) or recipient groups (Fig. 5c). Since alterations in the composition of the microbiota can change the metabolic landscape in the gut, we assessed the fecal concentration of short-chain fatty acids (SCFA) in the different groups (Fig. S3). We observed an increase in fecal levels of propionate and isovalerate in the DSS group when compared to Control mice, whilst levels of propionate were reduced, and levels of isovalerate were increased in the GF MT DSS as compared to its respective control. No changes were observed in the Abx MT groups. Thus, both intestinal inflammation and the subsequent microbiota transfer favour a microbiota signature that is likely responsible for the behavioural phenotypes observed, which may include species from the Lachnospiraceae, Ruminococcaceae and Muribaculaceae taxa.

4. Discussion

The present findings demonstrate the importance of the gut microbiota as a driver of behavioural alterations in the context of intestinal inflammation. Here, we show that alterations in the composition of the intestinal microbiota induced by intestinal inflammation are sufficient to transmit behavioural abnormalities after MT into GF and Abx-treated recipient mice. While DSS-treated mice had markers of neuroinflammation, a known driver of behavioural changes, the mice that received colitic microbiota and subsequently presented with anxiety-like and despair behaviours had no evidence of inflammation within the three brain regions assessed. The behavioural changes were found to be associated with major shifts in bacteria taxa, namely the Lachnospiraceae, Ruminococcaceae and Muribaculaceae families. Combined, these data suggest that mechanisms other than neuroinflammation are driving the microbiota-associated behavioural changes in recipient mice.

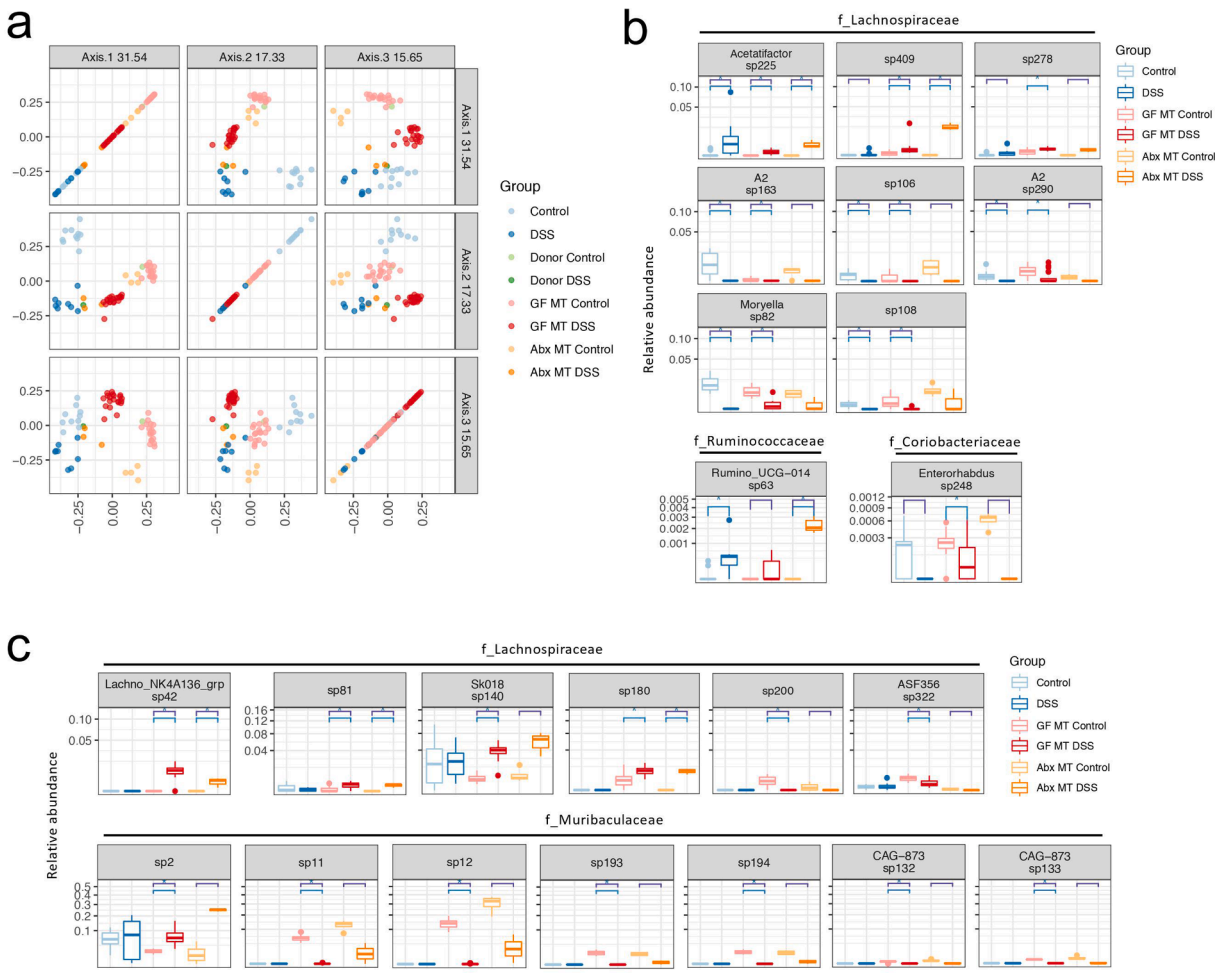

Fig. 5. Colitis-associated microbiota clusters differently from control microbiota exhibiting major changes in the Lachnospiraceae family. (**a**) Principal coordinate analysis (PCoA) displaying gut bacteria composition (16S rRNA gene sequencing) based on Bray-Curtis dissimilarity metric. Each dot represents the bacterial composition from the cecal matter of one biological replicate. The three main axes that explain the major variations are shown with the percentage of variation for each axis. PERMANOVA: $p < 0.001$ considering treatment variation in all groups (except donors). (**b**) Relative abundance changes of specific OTU that trend similarly on the 3 different groups associated with colitic microbiota or (**c**) in recipient mice only. Two statistical methods were used to assess statistical significance, with DESeq analysis presented in purple and ANCOM analysis shown in blue. Data are expressed as box plots. Control and DSS groups: n = 8–10; GF MT Control and GF MT DSS groups: n = 15–20; Abx MT Control and Abx MT DSS groups: n = 3–4. All statistical values are shown in the Supplementary Table of Statistical Values and Table of DESeq2 output.

IBD have long been associated with increased incidence and prevalence of comorbidities, such as anxiety and depression (Marrie et al., 2017; Mikocka-Walus et al., 2016a), but remarkably, these often go undiagnosed and are poorly treated (Bernstein, 2018). Due to the importance of these behavioural conditions in IBD patients (Bernstein et al., 2019), it is imperative to identify putative biological mechanisms driving the associated changes in behaviour. In the current study, we found that DSS-induced colitis drives changes in anxiety-like behaviour, results which are in agreement with previous reports (Bercik et al., 2011b; Chen et al., 2015; Emge et al., 2016; Hassan et al., 2014; Nyuyki et al., 2018; Painsipp et al., 2011; Salvo et al., 2020). In addition to DSS-induced colitis, similar results have been described with different experimental models of intestinal inflammation, including models of infectious colitis (Bercik et al., 2010; Haj-Mirzaian et al., 2017). Therefore, rodent models of colitis are robust tools to identify mechanisms by which intestinal inflammation modulates anxiety-related behaviours. Although the incidence of anxiety has been highly associated with depression (Lamers et al., 2011), we did not see an increase of despair behaviour in colitic mice, as measured by the immobility response in the TST. While these data appear contrary to some published reports (Haj-Mirzaian et al., 2017; Heydarpour et al., 2016; Reichmann et al., 2015; Takahashi et al., 2019), the field is rife with inconsistent findings. For example, reports using the TST and forced-swim test to quantify despair behaviour in mice present mixed results, with animals exhibiting no alteration in behaviour, or altered responses for either higher passive or active coping strategies (Haj-Mirzaian et al., 2017; Hassan et al., 2014; Heydarpour et al., 2016; Matisz et al., 2020; Painsipp et al., 2011; Reichmann et al., 2015; Takahashi et al., 2019). In contrast, in rat models of colitis, authors have consistently reported increased despair behaviour and anhedonia through the use of both forced-swim test and the sucrose preference test (Chen et al., 2015; Dempsey et al., 2019). Analyzing the combined body of literature, including our work, we suggest that a more comprehensive analysis of depression-like behaviour in mice is necessary for better conclusions, with future studies incorporating a combination of tests that each interrogate different behavioural traits (e.g., anhedonia, apathy, social aversion) (Planchez et al., 2019). As it stands, the data currently published in the field are consistent and robust regarding measurements of anxiety-like behaviour, but still provide inconclusive results when considering the manifestation of depression-like behaviours in rodent models of intestinal inflammation.

Previous reports have associated changes in brain physiology with peripheral inflammation arising from animal models of liver inflammation, arthritis, colitis, lipopolysaccharide-induced endotoxemia, and

viral infections (Blank et al., 2016; D'Mello et al., 2013; D'Mello et al., 2015; Lopes et al., 2020; O'Connor et al., 2009; Riazi et al., 2015; Süß et al., 2020). Several mechanisms have been proposed to explain how peripheral inflammation affects the central nervous system (Bercik et al., 2011b; Blank et al., 2016; Chen et al., 2015; D'Mello et al., 2013; Süß et al., 2020), with many associated with induction of neuroinflammation, and its consequent neuroplastic effects. As peripheral inflammation is known to affect brain regions differently (Süß et al., 2020), we measured the expression of proinflammatory markers in different brain regions associated with the neurobiology of anxiety and depression (Calhoon and Tye, 2015; Krishnan and Nestler, 2008). In models of peripheral inflammation induced by lipopolysaccharide, levels of *Tnf*, *Il1b*, and *Il6* are elevated, indicating a robust, classic neuroinflammatory response (Henry et al., 2008; O'Connor et al., 2009). We found elevated levels of *Tnf* expression in the hippocampus, hypothalamus, and amygdala in our DSS-treated mice. Others have also associated colitis with increased levels of TNF in specific brain regions, including the hippocampus and cortex (Haj-Mirzaian et al., 2017; Han et al., 2018; Heydarpour et al., 2016; Riazi et al., 2008; Sroor et al., 2019; Takahashi et al., 2019). Moreover, elevated brain TNF has also been strongly correlated with other models of peripheral inflammation (e.g., arthritis models) (Lopes et al., 2020; Süß et al., 2020). Interestingly, DSS-induced increases in TNF expression in the brain have also been correlated with iNOS function (Heydarpour et al., 2016). In our experiments, we did not observe any changes in *Nos2* expression in colitic mice, thus we did not replicate the previously reported positive correlation between *Tnf* and *Nos2* expression. Moreover, although our data clearly demonstrate increased *Tnf* expression, we saw no changes in *Il6* or *Il1b* transcripts. While IL-6 has been reported to be elevated in the hippocampus and cortex in the context of colitis in other reports (Dempsey et al., 2019; Haj-Mirzaian et al., 2017; Han et al., 2018; Takahashi et al., 2019), the lack of *Il1b* gene induction in our study is in agreement with previous reports (Dempsey et al., 2019; Haj-Mirzaian et al., 2017; Han et al., 2018; Riazi et al., 2008; Sroor et al., 2019; Takahashi et al., 2019).

While it is clear that the behavioural alterations induced by colonic inflammation are accompanied by an increase in central expression of *Tnf*, the transmissibility of the behavioural alterations was not associated with any increases in colonic or central inflammation. This suggests that different mechanisms are driving the behavioural abnormalities observed in the recipients of the colitis-associated microbiota. As stress responses are tightly regulated by microbiota and deemed fundamental in the onset of psychiatric conditions (Dean and Keshavan, 2017; Krishnan and Nestler, 2008; Sudo et al., 2004), we measured the expression of stress-related genes in different brain regions in our mice. GF rodents are known to have an unbalanced stress response (Clarke et al., 2013; Crumeyrolle-Arias et al., 2014; Sudo et al., 2004). We observed that GF mice, but not Abx-treated recipients of the colitis-associated microbiota exhibited increased *Crh* expression in the hypothalamus. Elevated corticotropin-releasing hormone (CRH) expression by hypothalamic neurons has been associated with behavioural abnormalities, including in psychiatric conditions such as major depressive disorder (Füzesi et al., 2016; Krishnan and Nestler, 2008; Zhu et al., 2014). Moreover, other models of microbiota manipulation have been shown to modulate hypothalamic CRH expression (Ait-Belgnaoui et al., 2012; Crumeyrolle-Arias et al., 2014). Thus, a colitis-associated microbiota regulates *Crh* expression in GF recipients in the absence of intestinal inflammation. However, as alluded to above, *Crh* expression was not altered in Abx-treated recipients that also presented with behavioural changes, suggesting additional mechanism(s) are responsible for the transmissible phenotype.

There is considerable growing interest in developing therapeutic approaches to target the intestinal microbiota for a variety of health-related conditions, including psychiatric disorders (Messaoudi et al., 2011; Pinto-Sanchez et al., 2017; Su et al., 2020; Tillisch et al., 2013). The search for the potential psychobiotics (i.e., a live organism that, when ingested in adequate amounts, produces a health benefit in patients suffering from psychiatric illness (Dinan et al., 2013)) is fundamental for the development of microbiota-based treatment strategies. Therefore, providing causative links between inflammation-associated changes in the composition of the intestinal microbiota and changes in brain function support the rationale for future clinical studies investigating the efficacy of psychobiotics for alleviating psychiatric comorbidities in IBD patients. It is well established that IBD is associated with changes in the intestinal microbiota composition (Halfvarson et al., 2017; Ryan et al., 2020). Furthermore, MT has been shown to transfer the behavioural signatures of the donor to recipients (Bercik et al., 2011a). Our findings clearly demonstrated that the alterations in the composition of the intestinal microbiota induced during acute DSS-induced colitis are sufficient to drive behavioural changes when transferred to GF mice. Since GF mice exhibit abnormalities in brain development and alterations in immune system function, it was imperative that we validated the transmissibility of the behavioural abnormalities in another system. As such, we performed experiments where we transferred the colitis-associated microbiota into Abx-treated mice. In these studies, we observed the same induction of anxiety-like and despair behaviours as observed in GF recipients. Interestingly, a difference in baseline behaviour was observed when comparing the Control groups among the different methodological approaches. For instance, Control mice exhibited an average of 23.4% of open arm time in the EPM test (Fig. 1g), whereas GF MT Control spent more time (38.5%; Fig. 2g) and the Abx MT Control spent less time (7.7%; Fig. 3g). Other studies have demonstrated the reduced anxiety-like behaviour in GF mice, which is linked to their impaired neurodevelopment (Heijtz et al., 2011; Vuong et al., 2020). In contrast, we posit that the Abx-treated mice had a lower baseline behaviour as a consequence of the stress induced by receiving multiple oral gavage administrations, as stress has been shown to potentiate the anxiety-like phenotype (Gacias et al., 2016; Li et al., 2019). Despite these differences in the baseline behaviours of the control groups, we clearly observed the impaired anxiety-like behaviour phenotype derived from the colitis-associated microbiota. Remarkably, we also observed a reduction in locomotion is exhibited by DSS and Abx MT DSS mice, but not by the GF MT DSS mice. These data further support the presence of the increased anxiety-like behaviour, as reduction in locomotion can be associated with an overall sickness behaviour phenotype, whereas the reduction only in the specific parameter, such as open arm time, is suggestive of an increase in the approach-avoidance conflict, which is the main anxiety-like trait measured on the EPM test (Walf and Frye, 2007). Thus, the use of additional, redundant methodological strategies are important to demonstrate the reproducibility of the behavioural phenotype.

Furthermore, we observed that both DSS-treated and colitis-associated microbiota recipients (both GF and Abx-treated mice) exhibited similar changes in major bacterial taxa including the Lachnospiraceae, Ruminococcaceae and Coriobacteriaceae families. Of note, changes in specific taxa from Lachnospiraceae and Muribaculaceae were only observed in GF or Abx-treated recipient mice administered colitis-associated cecal material. Interestingly, alterations in Lachnospiraceae and Ruminococcaceae have been associated with psychiatric conditions (Nikolova et al., 2021; Simpson et al., 2021) and GI disorders associated with psychiatric comorbidities (De Palma et al., 2017; Peter et al., 2018). In preclinical studies, Gacias et al. suggested that behavioural abnormalities (anxiety- and depression-like behaviour) induced by microbial disturbances in mice are linked to the bacterial Lachnospiraceae and Ruminococcaceae families (Gacias et al., 2016). In clinical settings, De Palma and colleagues demonstrated the correlation between a genus belonging to the Lachnospiraceae family (*Blautia*) with gut and brain dysfunction in humans (De Palma et al., 2017). Additionally, in a recent meta-analysis evaluating the correlation of microbiota composition and psychiatric conditions, Nikolova et al. reported that changes in members of the Lachnospiraceae, Ruminococcaceae, and Coriobacteriaceae families are one of the strongest correlations with major depressive disorder

(Nikolova et al., 2021). The identification of a unique bacterium, or most likely, a consortium of bacteria within these taxa are particularly relevant targets for future studies related to the gut-brain axis.

Previous reports have shown that the microbiota impacts host metabolomics (Gacias et al., 2016; Vojinovic et al., 2019; Wikoff et al., 2009; Yang et al., 2020) and that specific microbe-derived metabolites likely regulate behaviour (Chu et al., 2019). Moreover, it has been shown that IBD patients have a different fecal metabolomic profile, which correlates with their microbiota signature (Franzosa et al., 2019). Since SCFA have been implicated with multiple aspects of the gut-brain axis (Cryan et al., 2019), we hypothesized that alterations in SCFA levels may be involved in the behavioural changes induced by the colitis-associated microbiota. While others have reported that SCFA have a strong correlation with the modulation of brain function in animal models (Braniste et al., 2014; Erny et al., 2015), we did not observe a correlation between the behavioural groups in our datasets. Future studies specifically designed to characterize the impact of the observed microbial changes on additional metabolites may provide further insight into the mechanism(s) driving transmissibility of behaviour seen in our studies.

In summary, we provide compelling evidence that inflammation-associated changes in the intestinal microbiota composition are sufficient to induce behavioural abnormalities upon MT into GF- and Abx-treated male mice. As we appreciate the sex differences regarding microbiota composition and mouse behavioural responses (Markle et al., 2013; Vicentini et al., 2021), we acknowledge that our study was limited because it lacks data in female mice. Although some reports suggest that psychiatric comorbidities in IBD patients are similar among males and females (Bernstein et al., 2019; Nahon et al., 2012), further studies are needed to better assess potentially relevant sex differences in microbiota-mediated behavioural changes. Experimental treatments targeting the intestinal microbiota have proven effective in different GI-related conditions associated with psychiatric conditions, both in rodents and humans (Bercik et al., 2011b; Emge et al., 2016; Pinto-Sanchez et al., 2017). With increasing interest in identifying novel treatments focused on manipulating the microbiota (Valencia et al., 2017), our work provides strong support for continued efforts to identify the key causative mechanism(s) that link the microbiota to behavioural comorbidities of IBD that will ultimately aid in the development of rational therapeutic approaches harnessing the power of the microbiota to achieve tangible improvements in patient quality of life.

5. Data availability statement.

Data are in NCBI BioProject ID: PRJNA755589.

Funding

This work was supported by grants from the Canadian Institutes of Health Research (SAH [PJT376341] and KAS [FDN148380]) and a Canadian Institutes of Health Research Team Grant: Health Challenges in Chronic Inflammation (QJP, MGS, SAH, KAS [THC135321]). Metabolomics data were acquired at the Calgary Metabolomics Research Facility (CMRF), supported by the International Microbiome Centre and the Canada Foundation for Innovation (CFI-JELF 34986). IAL is supported by an Alberta Innovates Translational Health Chair. DB is supported by Natural Sciences and Engineering Research Council (NSERC [DG 04547]). FAV was supported by the National Council for Scientific and Technological Development, Brazil.

Authors contributions

FAV, SAH, and KAS designed the studies; FAV, LG, and KN conducted the experiments and performed data analyses; JCS, LR, and MGS performed the microbiota sequencing and bioinformatic data analyses; DB and IAL performed the metabolomics experiments; FAV, SAH, and KAS drafted the manuscript. All authors had access to the study data and critically reviewed and approved the final manuscript for submission. QJP, MGS, SAH, and KAS obtained funding for the study.

Declaration of Competing Interest

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

Acknowledgements

The authors thank Dr. Kathy McCoy for advice and assistance with these studies. We are grateful to Catherine MacNaughton, Dr. Jean-Baptiste Cavin, Dr. Nina Cluny and Dr. Marija Drikic for their support and advice. We thank Dr. Kewir Nyuyki for assisting with the behavioural analyses, and Amanda Zucoloto for the crucial help with germ-free mouse experiments. We thank the University of Calgary Cumming School of Medicine Optogenetics Facility for providing support for behavioural analyses, the Snyder Institute Bioinstrumentational lab for infrastructural support for qPCR, and the International Microbiome Facility for providing the germ-free animals. MGS holds the Canada Research Chair (Tier 1) in Interdisciplinary Microbiome Research, at McMaster University. SAH holds the Canada Research Chair (Tier 2) in Host-microbe Interactions and Chronic Disease and the Dr. Lloyd Sutherland Investigatorship in Inflammatory Bowel Disease/GI Research, at the University of Calgary.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbi.2022.03.001.

References

Agrawal, S., Kumar, S., Sehgal, R., George, S., Gupta, R., Poddar, S., Jha, A., Pathak, S., 2019. El-MAVEN: a fast, robust, and user-friendly mass spectrometry data processing engine for metabolomics. Methods Mol. Biol. 1978, 301–321.

Ait-Belgnaoui, A., Durand, H., Cartier, C., Chaumaz, G., Eutamene, H., Ferrier, L., Houdeau, E., Fioramonti, J., Bueno, L., Theodorou, V., 2012. Prevention of gut leakiness by a probiotic treatment leads to attenuated HPA response to an acute psychological stress in rats. Psychoneuroendocrinology 37, 1885–1895.

Bartram, A.K., Lynch, M.D., Stearns, J.C., Moreno-Hagelsieb, G., Neufeld, J.D., 2011. Generation of multimillion-sequence 16S rRNA gene libraries from complex microbial communities by assembling paired-end illumina reads. Appl. Environ. Microbiol. 77, 3846–3852.

Bercik, P., Verdu, E.F., Foster, J.A., Macri, J., Potter, M., Huang, X., Malinowski, P., Jackson, W., Blennerhassett, P., Neufeld, K.A., Lu, J., Khan, W.I., Corthesy-Theulaz, I., Cherbut, C., Bergonzelli, G.E., Collins, S.M., 2010. Chronic gastrointestinal inflammation induces anxiety-like behavior and alters central nervous system biochemistry in mice. Gastroenterology 139, 2102–2112.e2101.

Bercik, P., Denou, E., Collins, J., Jackson, W., Lu, J., Jury, J., Deng, Y., Blennerhassett, P., Macri, J., McCoy, K.D., Verdu, E.F., Collins, S.M., 2011a. The intestinal microbiota affect central levels of brain-derived neurotropic factor and behavior in mice. Gastroenterology 141, 599–609, 609.e591-593.

Bercik, P., Park, A.J., Sinclair, D., Khoshdel, A., Lu, J., Huang, X., Deng, Y., Blennerhassett, P.A., Fahnestock, M., Moine, D., Berger, B., Huizinga, J.D., Kunze, W., McLean, P.G., Bergonzelli, G.E., Collins, S.M., Verdu, E.F., 2011b. The anxiolytic effect of Bifidobacterium longum NCC3001 involves vagal pathways for gut–brain communication. Neurogastroenterol. Motil. 23, 1132–1139.

Bernstein, C.N., 2018. Addressing mental health in persons with IBD. J. Can. Assoc. Gastroenterol. 1, 97–98.

Bernstein, C.N., Hitchon, C.A., Walld, R., Bolton, J.M., Sareen, J., Walker, J.R., Graff, L. A., Patten, S.B., Singer, A., Lix, L.M., El-Gabalawy, R., Katz, A., Fisk, J.D., Marrie, R. A., 2019. Increased burden of psychiatric disorders in inflammatory bowel disease. Inflamm. Bowel Dis. 25, 360–368.

Bihan, D., Rydzak, T., Wyss, M., Pittman, K., McCoy, K.D., Lewis, I.A., 2019. Method for absolute quantification of short chain fatty acids via reverse phase chromatography mass spectrometry. ChemRxiv. https://doi.org/10.26434/chemrxiv.9955553.

Blank, T., Detje, C.N., Spieß, A., Hagemeyer, N., Brendecke, S.M., Wolfart, J., Staszewski, O., Zöller, T., Papageorgiou, I., Schneider, J., Paricio-Montesinos, R., Eisel, U.L., Manahan-Vaughan, D., Jansen, S., Lienenklaus, S., Lu, B., Imai, Y., Müller, M., Goelz, S.E., Baker, D.P., Schwaninger, M., Kann, O., Heikenwalder, M., Kalinke, U., Prinz, M., 2016. Brain endothelial- and epithelial-specific interferon

receptor chain 1 drives virus-induced sickness behavior and cognitive impairment. Immunity 44, 901–912.
Braniste, V., Al-Asmakh, M., Kowal, C., Anuar, F., Abbaspour, A., Tóth, M., Korecka, A., Bakocevic, N., Ng, L.G., Kundu, P., Gulyás, B., Halldin, C., Hultenby, K., Nilsson, H., Hebert, H., Volpe, B.T., Diamond, B., Pettersson, S., 2014. The gut microbiota influences blood-brain barrier permeability in mice. Sci. Transl. Med. 6, 158–263.
Calhoon, G.G., Tye, K.M., 2015. Resolving the neural circuits of anxiety. Nat. Neurosci. 18, 1394–1404.
Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016. DADA2: High-resolution sample inference from Illumina amplicon data. Nat. Methods 13, 581–583.
Carabotti, M., Scirocco, A., Maselli, M.A., Severi, C., 2015. The gut-brain axis: interactions between enteric microbiota, central and enteric nervous systems. Ann. Gastroenterol. 28, 203–209.
Chassaing, B., Srinivasan, G., Delgado, M.A., Young, A.N., Gewirtz, A.T., Vijay-Kumar, M., 2012. Fecal lipocalin 2, a sensitive and broadly dynamic non-invasive biomarker for intestinal inflammation. PLoS ONE 7, e44328.
Chassaing, B., Aitken, J.D., Malleshappa, M., Vijay-Kumar, M., 2014. Dextran sulfate sodium (DSS)-induced colitis in mice. Curr. Protoc. Immunol. 104, 15.25.11-15.25.14.
Chen, J., Winston, J.H., Fu, Y., Guptarak, J., Jensen, K.L., Shi, X.Z., Green, T.A., Sarna, S. K., 2015. Genesis of anxiety, depression, and ongoing abdominal discomfort in ulcerative colitis-like colon inflammation. Am. J. Physiol. Regul. Integr. Comp. Physiol. 308, R18–27.
Chu, C., Murdock, M.H., Jing, D., Won, T.H., Chung, H., Kressel, A.M., Tsaava, T., Addorisio, M.E., Putzel, G.G., Zhou, L., Bessman, N.J., Yang, R., Moriyama, S., Parkhurst, C.N., Li, A., Meyer, H.C., Teng, F., Chavan, S.S., Tracey, K.J., Regev, A., Schroeder, F.C., Lee, F.S., Liston, C., Artis, D., 2019. The microbiota regulate neuronal function and fear extinction learning. Nature 574, 543–548.
Clarke, G., Grenham, S., Scully, P., Fitzgerald, P., Moloney, R.D., Shanahan, F., Dinan, T. G., Cryan, J.F., 2013. The microbiome-gut-brain axis during early life regulates the hippocampal serotonergic system in a sex-dependent manner. Mol. Psychiatry 18, 666–673.
Cluny, N.L., Keenan, C.M., Duncan, M., Fox, A., Lutz, B., Sharkey, K.A., 2010. Naphthalen-1-yl-(4-pentyloxynaphthalen-1-yl)methanone (SAB378), a peripherally restricted cannabinoid CB1/CB2 receptor agonist, inhibits gastrointestinal motility but has no effect on experimental colitis in mice. J. Pharmacol. Exp. Ther. 334, 973–980.
Collins, S.M., 2020. Interrogating the gut-brain axis in the context of inflammatory bowel disease: A translational approach. Inflamm. Bowel Dis. 26, 493–501.
Crumeyrolle-Arias, M., Jaglin, M., Bruneau, A., Vancassel, S., Cardona, A., Daugé, V., Naudon, L., Rabot, S., 2014. Absence of the gut microbiota enhances anxiety-like behavior and neuroendocrine response to acute stress in rats. Psychoneuroendocrinology 42, 207–217.
Cryan, J.F., O'Riordan, K.J., Cowan, C.S.M., Sandhu, K.V., Bastiaanssen, T.F.S., Boehme, M., Codagnone, M.G., Cussotto, S., Fulling, C., Golubeva, A.V., Guzzetta, K. E., Jaggar, M., Long-Smith, C.M., Lyte, J.M., Martin, J.A., Molinero-Perez, A., Moloney, G., Morelli, E., Morillas, E., O'Connor, R., Cruz-Pereira, J.S., Peterson, V.L., Rea, K., Ritz, N.L., Sherwin, E., Spichak, S., Teichman, E.M., van de Wouw, M., Ventura-Silva, A.P., Wallace-Fitzsimons, S.E., Hyland, N., Clarke, G., Dinan, T.G., 2019. The Microbiota-gut-brain axis. Physiol. Rev. 99, 1877–2013.
De Palma, G., Lynch, M.D., Lu, J., Dang, V.T., Deng, Y., Jury, J., Umeh, G., Miranda, P. M., Pigrau Pastor, M., Sidani, S., Pinto-Sanchez, M.I., Philip, V., McLean, P.G., Hagelsieb, M.G., Surette, M.G., Bergonzelli, G.E., Verdu, E.F., Britz-McKibbin, P., Neufeld, J.D., Collins, S.M., Bercik, P., 2017. Transplantation of fecal microbiota from patients with irritable bowel syndrome alters gut function and behavior in recipient mice. Sci. Transl. Med. 9, eaaf6397.
Dean, J., Keshavan, M., 2017. The neurobiology of depression: an integrated view. Asian J. Psychiatr. 27, 101–111.
Dempsey, E., Abautret-Daly, Á., Docherty, N.G., Medina, C., Harkin, A., 2019. Persistent central inflammation and region specific cellular activation accompany depression-and anxiety-like behaviours during the resolution phase of experimental colitis. Brain Behav. Immun. 80, 616–632.
Development Core Team, 2019. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
Dinan, T.G., Stanton, C., Cryan, J.F., 2013. Psychobiotics: a novel class of psychotropic. Biol. Psychiatry 74, 720–726.
D'Mello, C., Riazi, K., Le, T., Stevens, K.M., Wang, A., McKay, D.M., Pittman, Q.J., Swain, M.G., 2013. P-selectin-mediated monocyte-cerebral endothelium adhesive interactions link peripheral organ inflammation to sickness behaviors. J. Neurosci. 33, 14878–14888.
D'Mello, C., Ronaghan, N., Zaheer, R., Dicay, M., Le, T., MacNaughton, W.K., Surrette, M.G., Swain, M.G., 2015. Probiotics improve inflammation-associated sickness behavior by altering communication between the peripheral immune system and the brain. J. Neurosci. 35, 10821–10830.
Donaldson, G.P., Lee, S.M., Mazmanian, S.K., 2016. Gut biogeography of the bacterial microbiota. Nat. Rev. Microbiol. 14, 20–32.
Emge, J.R., Huynh, K., Miller, E.N., Kaur, M., Reardon, C., Barrett, K.E., Gareau, M.G., 2016. Modulation of the microbiota-gut-brain axis by probiotics in a murine model of inflammatory bowel disease. Am. J. Physiol.-Gastrointestinal Liver Physiol. 310, G989–G998.
Erny, D., Hrabě de Angelis, A.L., Jaitin, D., Wieghofer, P., Staszewski, O., David, E., Keren-Shaul, H., Mahlakoiv, T., Jakobshagen, K., Buch, T., Schwierzeck, V., Utermöhlen, O., Chun, E., Garrett, W.S., McCoy, K.D., Diefenbach, A., Staeheli, P., Stecher, B., Amit, I., Prinz, M., 2015. Host microbiota constantly control maturation and function of microglia in the CNS. Nat. Neurosci. 18, 965–977.
Franzosa, E.A., Sirota-Madi, A., Avila-Pacheco, J., Fornelos, N., Haiser, H.J., Reinker, S., Vatanen, T., Hall, A.B., Mallick, H., McIver, L.J., Sauk, J.S., Wilson, R.G., Stevens, B. W., Scott, J.M., Pierce, K., Deik, A.A., Bullock, K., Imhann, F., Porter, J.A., Zhernakova, A., Fu, J., Weersma, R.K., Wijmenga, C., Clish, C.B., Vlamakis, H., Huttenhower, C., Xavier, R.J., 2019. Gut microbiome structure and metabolic activity in inflammatory bowel disease. Nat. Microbiol. 4, 293–305.
Füzesi, T., Daviu, N., Wamsteeker Cusulin, J.I., Bonin, R.P., Bains, J.S., 2016. Hypothalamic CRH neurons orchestrate complex behaviours after stress. Nat. Commun. 7, 11937.
Gacias, M., Gaspari, S., Santos, P.M., Tamburini, S., Andrade, M., Zhang, F., Shen, N., Tolstikov, V., Kiebish, M.A., Dupree, J.L., Zachariou, V., Clemente, J.C., Casaccia, P., 2016. Microbiota-driven transcriptional changes in prefrontal cortex override genetic differences in social behavior. Elife 5, e13442.
Haj-Mirzaian, A., Amiri, S., Amini-Khoei, H., Hosseini, M.J., Haj-Mirzaian, A., Momeny, M., Rahimi-Balaei, M., Dehpour, A.R., 2017. Anxiety- and depressive-like behaviors are associated with altered hippocampal energy and inflammatory status in a mouse model of Crohn's disease. Neuroscience 366, 124–137.
Halfvarson, J., Brislawn, C.J., Lamendella, R., Vázquez-Baeza, Y., Walters, W.A., Bramer, L.M., D'Amato, M., Bonfiglio, F., McDonald, D., Gonzalez, A., McClure, E.E., Dunklebarger, M.F., Knight, R., Jansson, J.K., 2017. Dynamics of the human gut microbiome in inflammatory bowel disease. Nat. Microbiol. 2, 17004.
Han, Y., Zhao, T., Cheng, X., Zhao, M., Gong, S.H., Zhao, Y.Q., Wu, H.T., Fan, M., Zhu, L. L., 2018. Cortical inflammation is increased in a DSS-induced colitis mouse model. Neurosci. Bull. 34, 1058–1066.
Hassan, A.M., Jain, P., Reichmann, F., Mayerhofer, R., Farzi, A., Schuligoi, R., Holzer, P., 2014. Repeated predictable stress causes resilience against colitis-induced behavioral changes in mice. Front. Behav. Neurosci. 8, 386.
Heijtz, R.D., Wang, S., Anuar, F., Qian, Y., Björkholm, B., Samuelsson, A., Hibberd, M.L., Forssberg, H., Pettersson, S., 2011. Normal gut microbiota modulates brain development and behavior. Proc. Natl. Acad. Sci. U. S. A. 108, 3047–3052.
Henry, C.J., Huang, Y., Wynne, A., Hanke, M., Himler, J., Bailey, M.T., Sheridan, J.F., Godbout, J.P., 2008. Minocycline attenuates lipopolysaccharide (LPS)-induced neuroinflammation, sickness behavior, and anhedonia. J. Neuroinflammation 5, 15.
Heydarpour, P., Rahimian, R., Fakhfouri, G., Khoshkish, S., Fakhraei, N., Salehi-Sadaghiani, M., Wang, H., Abbasi, A., Dehpour, A.R., Ghia, J.-E., 2016. Behavioral despair associated with a mouse model of Crohn's disease: Role of nitric oxide pathway. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 64, 131–141.
Honda, K., Littman, D.R., 2016. The microbiota in adaptive immune homeostasis and disease. Nature 535, 75–84.
Jang, H.M., Kim, J.K., Joo, M.K., Shin, Y.J., Lee, C.K., Kim, H.J., Kim, D.H., 2021. Transplantation of fecal microbiota from patients with inflammatory bowel disease and depression alters immune response and behavior in recipient mice. Sci. Rep. 11, 20406.
Jiang, H., Ling, Z., Zhang, Y., Mao, H., Ma, Z., Yin, Y., Wang, W., Tang, W., Tan, Z., Shi, J., Li, L., Ruan, B., 2015. Altered fecal microbiota composition in patients with major depressive disorder. Brain Behav. Immun. 48, 186–194.
Kelly, J.R., Borre, Y., C, O.B., Patterson, E., El Aidy, S., Deane, J., Kennedy, P.J., Beers, S., Scott, K., Moloney, G., Hoban, A.E., Scott, L., Fitzgerald, P., Ross, P., Stanton, C., Clarke, G., Cryan, J.F., Dinan, T.G., 2016. Transferring the blues: Depression-associated gut microbiota induces neurobehavioural changes in the rat. J. Psychiatr. Res. 82, 109-118.
Krishnan, V., Nestler, E.J., 2008. The molecular neurobiology of depression. Nature 455, 894–902.
Lamers, F., van Oppen, P., Comijs, H.C., Smit, J.H., Spinhoven, P., van Balkom, A.J., Nolen, W.A., Zitman, F.G., Beekman, A.T., Penninx, B.W., 2011. Comorbidity patterns of anxiety and depressive disorders in a large cohort study: the Netherlands Study of Depression and Anxiety (NESDA). J. Clin. Psychiatry 72, 341–348.
Li, N., Wang, Q., Wang, Y., Sun, A., Lin, Y., Jin, Y., Li, X., 2019. Fecal microbiota transplantation from chronic unpredictable mild stress mice donors affects anxiety-like and depression-like behavior in recipient mice via the gut microbiota-inflammation-brain axis. Stress 22, 592–602.
Lopes, F., Vicentini, F.A., Cluny, N.L., Mathews, A.J., Lee, B.H., Almishri, W.A., Griffin, L., Gonçalves, W., Pinho, V., McKay, D.M., Hirota, S.A., Swain, M.G., Pittman, Q.J., Sharkey, K.A., 2020. Brain TNF drives post-inflammation depression-like behavior and persistent pain in experimental arthritis. Brain Behav. Immun. 89, 224–232.
Love, M.I., Huber, W., Anders, S., 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 15, 550.
Lu, B., Nagappan, G., Guan, X., Nathan, P.J., Wren, P., 2013. BDNF-based synaptic repair as a disease-modifying strategy for neurodegenerative diseases. Nat. Rev. Neurosci. 14, 401–416.
Mager, L.F., Burkhard, R., Pett, N., Cooke, N.C.A., Brown, K., Ramay, H., Paik, S., Stagg, J., Groves, R.A., Gallo, M., Lewis, I.A., Geuking, M.B., McCoy, K.D., 2020. Microbiome-derived inosine modulates response to checkpoint inhibitor immunotherapy. Science 369, 1481–1489.
Mandal, S., Van Treuren, W., White, R.A., Eggesbø, M., Knight, R., Peddada, S.D., 2015. Analysis of composition of microbiomes: a novel method for studying microbial composition. Microb. Ecol. Health Dis. 26, 27663.
Markle, J.G., Frank, D.N., Mortin-Toth, S., Robertson, C.E., Feazel, L.M., Rolle-Kampczyk, U., von Bergen, M., McCoy, K.D., Macpherson, A.J., Danska, J.S., 2013. Sex differences in the gut microbiome drive hormone-dependent regulation of autoimmunity. Science 339, 1084–1088.
Marrie, R.A., Walld, R., Bolton, J.M., Sareen, J., Walker, J.R., Patten, S.B., Singer, A., Lix, L.M., Hitchon, C.A., El-Gabalawy, R., Katz, A., Fisk, J.D., Bernstein, C.N., 2017. Increased incidence of psychiatric disorders in immune-mediated inflammatory disease. J. Psychosom. Res. 101, 17–23.

Martin, M., 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet.journal 17, 3.
Matisz, C.E., Vicentini, F.A., Hirota, S.A., Sharkey, K.A., Gruber, A.J., 2020. Behavioral adaptations in a relapsing mouse model of colitis. Physiol. Behav. 216, 112802.
McMurdie, P.J., Holmes, S., 2013. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS ONE 8, e61217.
Messaoudi, M., Lalonde, R., Violle, N., Javelot, H., Desor, D., Nejdi, A., Bisson, J.F., Rougeot, C., Pichelin, M., Cazaubiel, M., Cazaubiel, J.M., 2011. Assessment of psychotropic-like properties of a probiotic formulation (Lactobacillus helveticus R0052 and Bifidobacterium longum R0175) in rats and human subjects. Br. J. Nutr. 105, 755–764.
Mikocka-Walus, A., Knowles, S.R., Keefer, L., Graff, L., 2016a. Controversies revisited: a systematic review of the comorbidity of depression and anxiety with inflammatory bowel diseases. Inflamm. Bowel Dis. 22, 752–762.
Mikocka-Walus, A., Pittet, V., Rossel, J.B., von Känel, R., 2016b. Symptoms of depression and anxiety are independently associated with clinical recurrence of inflammatory bowel disease. Clin. Gastroenterol. Hepatol. 14, 829–835.e821.
Miller, K.A., Vicentini, F.A., Hirota, S.A., Sharkey, K.A., Wieser, M.E., 2019. Antibiotic treatment affects the expression levels of copper transporters and the isotopic composition of copper in the colon of mice. Proc. Natl. Acad. Sci. U. S. A. 116, 5955–5960.
Nahon, S., Lahmek, P., Durance, C., Olympie, A., Lesgourgues, B., Colombel, J.F., Gendre, J.P., 2012. Risk factors of anxiety and depression in inflammatory bowel disease. Inflamm. Bowel Dis. 18, 2086–2091.
Ng, S.C., Shi, H.Y., Hamidi, N., Underwood, F.E., Tang, W., Benchimol, E.I., Panaccione, R., Ghosh, S., Wu, J.C.Y., Chan, F.K.L., Sung, J.J.Y., Kaplan, G.G., 2018. Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies. Lancet 390, 2769–2778.
Nikolova, V.L., Hall, M.R.B., Hall, L.J., Cleare, A.J., Stone, J.M., Young, A.H., 2021. Perturbations in gut microbiota composition in psychiatric disorders: a review and meta-analysis. JAMA Psychiatry 78, 1343–1354.
Nyuyki, K.D., Cluny, N.L., Swain, M.G., Sharkey, K.A., Pittman, Q.J., 2018. Altered brain excitability and increased anxiety in mice with experimental colitis: consideration of hyperalgesia and sex differences. Front. Behav. Neurosci. 12, 58.
O'Connor, J.C., Lawson, M.A., André, C., Moreau, M., Lestage, J., Castanon, N., Kelley, K. W., Dantzer, R., 2009. Lipopolysaccharide-induced depressive-like behavior is mediated by indoleamine 2,3-dioxygenase activation in mice. Mol. Psychiatry 14, 511–522.
Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., 2019. Package 'vegan': community ecology package. R package version 2.5-6. https://CRAN.R-project.org/package=vegan.
Painsipp, E., Herzog, H., Sperk, G., Holzer, P., 2011. Sex-dependent control of murine emotional-affective behaviour in health and colitis by peptide YY and neuropeptide Y. Br. J. Pharmacol. 163, 1302–1314.
Peter, J., Fournier, C., Durdevic, M., Knoblich, L., Keip, B., Dejaco, C., Trauner, M., Moser, G., 2018. A microbial signature of psychological distress in Irritable Bowel Syndrome. Psychosom. Med. 80, 698–709.
Pinto-Sanchez, M.I., Hall, G.B., Ghajar, K., Nardelli, A., Bolino, C., Lau, J.T., Martin, F.-P., Cominetti, O., Welsh, C., Rieder, A., Traynor, J., Gregory, C., De Palma, G., Pigrau, M., Ford, A.C., Macri, J., Berger, B., Bergonzelli, G., Surette, M.G., Collins, S. M., Moayyedi, P., Bercik, P., 2017. Probiotic Bifidobacterium longum NCC3001 reduces depression scores and alters brain activity: a pilot study in patients with irritable bowel syndrome. Gastroenterology 153, 448–459.e448.
Planchez, B., Surget, A., Belzung, C., 2019. Animal models of major depression: drawbacks and challenges. J. Neural Transm. 126, 1383–1408.
Pulakazhi Venu, V.K., Alston, L., Iftinca, M., Tsai, Y.C., Stephens, M., Warriyar, K.V.V., Rehal, S., Hudson, G., Szczepanski, H., von der Weid, P.Y., Altier, C., Hirota, S.A., 2021. Nr4A1 modulates inflammation-associated intestinal fibrosis and dampens fibrogenic signaling in myofibroblasts. Am. J. Physiol. Gastrointest. Liver Physiol. 321, G280–g297.
Rakoff-Nahoum, S., Paglino, J., Eslami-Varzaneh, F., Edberg, S., Medzhitov, R., 2004. Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. Cell 118, 229–241.
Reichmann, F., Hassan, A.M., Farzi, A., Jain, P., Schuligoi, R., Holzer, P., 2015. Dextran sulfate sodium-induced colitis alters stress-associated behaviour and neuropeptide gene expression in the amygdala-hippocampus network of mice. Sci. Rep. 5, 9970.
Riazi, K., Galic, M.A., Kuzmiski, J.B., Ho, W., Sharkey, K.A., Pittman, Q.J., 2008. Microglial activation and TNFalpha production mediate altered CNS excitability following peripheral inflammation. Proc. Natl. Acad. Sci. U. S. A. 105, 17151–17156.
Riazi, K., Galic, M.A., Kentner, A.C., Reid, A.Y., Sharkey, K.A., Pittman, Q.J., 2015. Microglia-dependent alteration of glutamatergic synaptic transmission and plasticity in the hippocampus during peripheral inflammation. J. Neurosci. 35, 4942–4952.
Ryan, F.J., Ahern, A.M., Fitzgerald, R.S., Laserna-Mendieta, E.J., Power, E.M., Clooney, A.G., O'Donoghue, K.W., McMurdie, P.J., Iwai, S., Crits-Christoph, A., Sheehan, D., Moran, C., Flemer, B., Zomer, A.L., Fanning, A., O'Callaghan, J., Walton, J., Temko, A., Stack, W., Jackson, L., Joyce, S.A., Melgar, S., DeSantis, T.Z., Bell, J.T., Shanahan, F., Claesson, M.J., 2020. Colonic microbiota is associated with inflammation and host epigenomic alterations in inflammatory bowel disease. Nat. Commun. 11, 1512.
Salvo, E., Stokes, P., Keogh, C.E., Brust-Mascher, I., Hennessey, C., Knotts, T.A., Sladek, J. A., Rude, K.M., Swedek, M., Rabasa, G., Gareau, M.G., 2020. A murine model of pediatric inflammatory bowel disease causes microbiota-gut-brain axis deficits in adulthood. Am. J. Physiol. Gastrointest. Liver Physiol. 319, G361–g374.
Simpson, C.A., Diaz-Arteche, C., Eliby, D., Schwartz, O.S., Simmons, J.G., Cowan, C.S.M., 2021. The gut microbiota in anxiety and depression - A systematic review. Clin. Psychol. Rev. 83, 101943.
Sroor, H.M., Hassan, A.M., Zenz, G., Valadez-Cosmes, P., Farzi, A., Holzer, P., El-Sharif, A., Gomaa, F.-A.-Z.-M., Kargl, J., Reichmann, F., 2019. Experimental colitis reduces microglial cell activation in the mouse brain without affecting microglial cell numbers. Sci. Rep. 9, 20217.
Stearns, J.C., Davidson, C.J., McKeon, S., Whelan, F.J., Fontes, M.E., Schryvers, A.B., Bowdish, D.M., Kellner, J.D., Surette, M.G., 2015. Culture and molecular-based profiles show shifts in bacterial communities of the upper respiratory tract that occur with age. ISME J. 9, 1246–1259.
Su, G.L., Ko, C.W., Bercik, P., Falck-Ytter, Y., Sultan, S., Weizman, A.V., Morgan, R.L., 2020. AGA clinical practice guidelines on the role of probiotics in the management of gastrointestinal disorders. Gastroenterology 159, 697–705.
Sudo, N., Chida, Y., Aiba, Y., Sonoda, J., Oyama, N., Yu, X.-N., Kubo, C., Koga, Y., 2004. Postnatal microbial colonization programs the hypothalamic-pituitary-adrenal system for stress response in mice. J. Physiol. 558, 263–275.
Süß, P., Hoffmann, A., Rothe, T., Ouyang, Z., Baum, W., Staszewski, O., Schett, G., Prinz, M., Krönke, G., Glass, C.K., Winkler, J., Schlachetzki, J.C.M., 2020. Chronic peripheral inflammation causes a region-specific myeloid response in the central nervous system. Cell Rep. 30, 4082–4095.e4086.
Szigethy, E., Murphy, S.M., Ehrlich, O.G., Engel-Nitz, N.M., Heller, C.A., Henrichsen, K., Lawton, R., Meadows, P., Allen, J.I., 2021. Mental health costs of Inflammatory Bowel Diseases. Inflamm. Bowel Dis. 27, 40–48.
Takahashi, K., Nakagawasai, O., Nemoto, W., Odaira, T., Sakuma, W., Onogi, H., Nishijima, H., Furihata, R., Nemoto, Y., Iwasa, H., Tan-No, K., Tadano, T., 2019. Effect of Enterococcus faecalis 2001 on colitis and depressive-like behavior in dextran sulfate sodium-treated mice: involvement of the brain–gut axis. J. Neuroinflamm. 16, 201.
Thomann, A.K., Mak, J.W.Y., Zhang, J.W., Wuestenberg, T., Ebert, M.P., Sung, J.J.Y., Bernstein, Ç.N., Reindl, W., Ng, S.C., 2020. Review article: bugs, inflammation and mood-a microbiota-based approach to psychiatric symptoms in inflammatory bowel diseases. Aliment. Pharmacol. Ther. 52, 247–266.
Tillisch, K., Labus, J., Kilpatrick, L., Jiang, Z., Stains, J., Ebrat, B., Guyonnet, D., Legrain-Raspaud, S., Trotin, B., Naliboff, B., Mayer, E.A., 2013. Consumption of fermented milk product with probiotic modulates brain activity. Gastroenterology 144, 1394–1401.e1394.
Valencia, P.M., Richard, M., Brock, J., Boglioli, E., 2017. The human microbiome: opportunity or hype? Nat. Rev. Drug Discov. 16, 823–824.
Vicentini, F.A., Mathews, A.J., Pittman, Q.J., Swain, M.G., Sharkey, K.A., Hirota, S.A., 2021. Behavioural adaptations after antibiotic treatment in male mice are reversed by activation of the aryl hydrocarbon receptor. Brain Behav. Immun. 98, 317–329.
Vojinovic, D., Radjabzadeh, D., Kurilshikov, A., Amin, N., Wijmenga, C., Franke, L., Ikram, M.A., Uitterlinden, A.G., Zhernakova, A., Fu, J., Kraaij, R., van Duijn, C.M., 2019. Relationship between gut microbiota and circulating metabolites in population-based cohorts. Nat. Commun. 10, 5813.
Vuong, H.E., Pronovost, G.N., Williams, D.W., Coley, E.J.L., Siegler, E.L., Qiu, A., Kazantsev, M., Wilson, C.J., Rendon, T., Hsiao, E.Y., 2020. The maternal microbiome modulates fetal neurodevelopment in mice. Nature 586, 281–286.
Walf, A.A., Frye, C.A., 2007. The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. Nat. Protoc. 2, 322–328.
Walker, A.K., Kavelaars, A., Heijnen, C.J., Dantzer, R., 2014. Neuroinflammation and comorbidity of pain and depression. Pharmacol. Rev. 66, 80–101.
Wickham, H., 2016. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag, New York.
Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L.D., François, R., Grolemund, G., Hayes, A., Henry, L., Hester, J., Kuhn, M., Pedersen, T.L., Miller, E., Bache, S.M., Müller, K., Ooms, J., Robinson, D., Seidel, D.P., Spinu, V., Takahashi, K., Vaughan, D., Wilke, C., Woo, K.H.Y., 2019. Welcome to the tidyverse. J. Open Source Software 4, 1686.
Wikoff, W.R., Anfora, A.T., Liu, J., Schultz, P.G., Lesley, S.A., Peters, E.C., Siuzdak, G., 2009. Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites. Proc. Natl. Acad. Sci. U. S. A. 106, 3698–3703.
Wright, E.S., 2016. Using DECIPHER v2.0 to analyze big biological sequence data in R. R J. 8, 352–359.
Yang, J., Zheng, P., Li, Y., Wu, J., Tan, X., Zhou, J., Sun, Z., Chen, X., Zhang, G., Zhang, H., Huang, Y., Chai, T., Duan, J., Liang, W., Yin, B., Lai, J., Huang, T., Du, Y., Zhang, P., Jiang, J., Xi, C., Wu, L., Lu, J., Mou, T., Xu, Y., Perry, S.W., Wong, M.-L., Licinio, J., Hu, S., Wang, G., Xie, P., 2020. Landscapes of bacterial and metabolic signatures and their interaction in major depressive disorders. Sci. Adv. 6, eaba8555.
Zheng, P., Zeng, B., Zhou, C., Liu, M., Fang, Z., Xu, X., Zeng, L., Chen, J., Fan, S., Du, X., Zhang, X., Yang, D., Yang, Y., Meng, H., Li, W., Melgiri, N.D., Licinio, J., Wei, H., Xie, P., 2016. Gut microbiome remodeling induces depressive-like behaviors through a pathway mediated by the host's metabolism. Mol. Psychiatry 21, 786–796.
Zhu, L.J., Liu, M.Y., Li, H., Liu, X., Chen, C., Han, Z., Wu, H.Y., Jing, X., Zhou, H.H., Suh, H., Zhu, D.Y., Zhou, Q.G., 2014. The different roles of glucocorticoids in the hippocampus and hypothalamus in chronic stress-induced HPA axis hyperactivity. PLoS ONE 9, e97689.