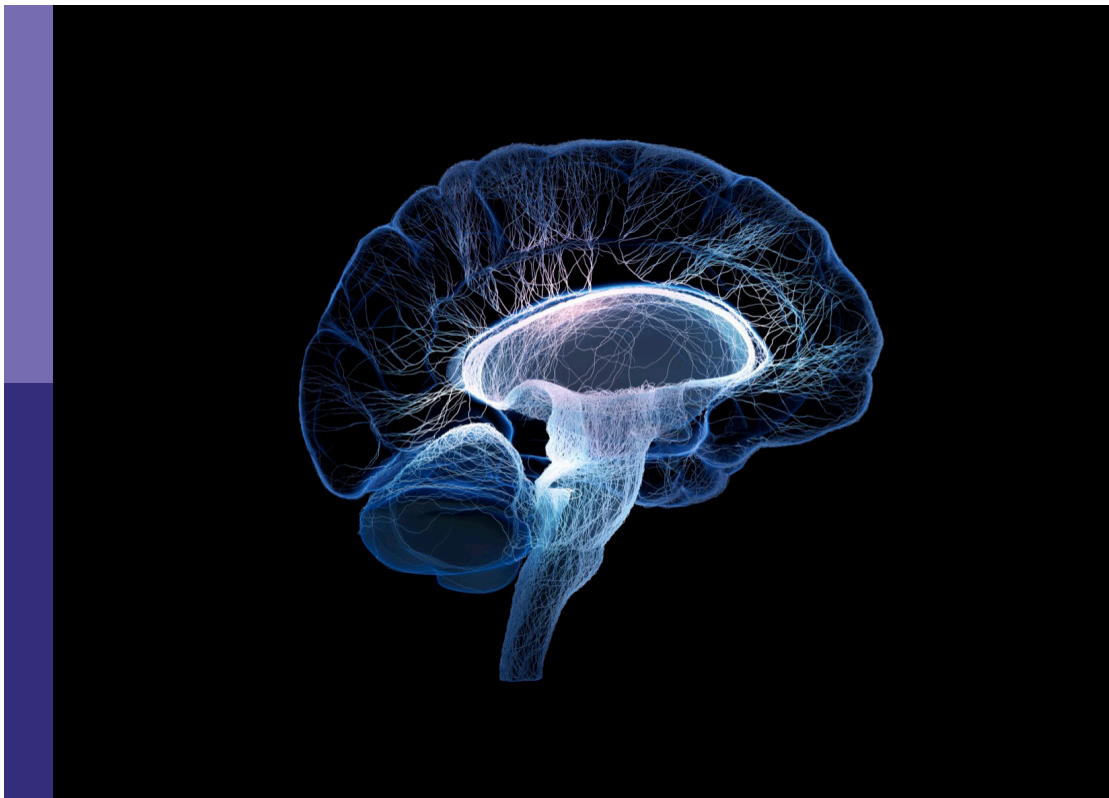
frontiers | Research Topics

Reviews in gut-brain axis: stress, dysregulation in gut-brain axis function and stress related disorders

Edited by
Zuleide Maria Ignácio, Avinash Veerappa, Jonathan B. Clayton and Margarete Bagatini

Published in
Frontiers in Neuroscience



ISSN 1664-8714
ISBN 978-2-8325-6867-5
DOI 10.3389/978-2-8325-6867-5

Generative AI statement
Any alternative text (Alt text) provided alongside figures in the articles in this ebook has been generated by Frontiers with the support of artificial intelligence and reasonable efforts have been made to ensure accuracy, including review by the authors wherever possible. If you identify any issues, please contact us.

About Frontiers

Frontiers is more than just an open access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers journal series

The Frontiers journal series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the *Frontiers journal series* operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews. Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the *Frontiers journals series*: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area.

Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers editorial office: frontiersin.org/about/contact

Reviews in gut-brain axis: stress, dysregulation in gut-brain axis function and stress related disorders

Topic editors

Zuleide Maria Ignácio — Federal University of the Southern Frontier, Brazil
Avinash Veerappa — University of Nebraska Medical Center, United States
Jonathan B. Clayton — University of Nebraska Omaha, United States
Margarete Bagatini — Federal University of the Southern Frontier, Brazil

Citation

Ignácio, Z. M., Veerappa, A., Clayton, J. B., Bagatini, M., eds. (2025). *Reviews in gut-brain axis: stress, dysregulation in gut-brain axis function and stress related disorders*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-8325-6867-5

Table of contents

05 Editorial: Reviews in gut-brain axis: stress, dysregulation in gut-brain axis function and stress related disorders
Zuleide Maria Ignácio, Jonathan B. Clayton, Margarete Dulce Bagatini and Avinash Veerappa

07 Antibiotic-induced gut dysbiosis and cognitive, emotional, and behavioral changes in rodents: a systematic review and meta-analysis
Shivdeep S. Hayer, Soonjo Hwang and Jonathan B. Clayton

27 Causal associations between gut microbiota and regional cortical structure: a Mendelian randomization study
Maochao Zhou, Song Chen, Yan Chen, Chunhua Wang and Chunmei Chen

36 Depression-associated gut microbes, metabolites and clinical trials
Meiling Wang, Zhaoqi Song, Shirong Lai, Furong Tang, Lijun Dou and Fenglong Yang

53 Bipolar disorder and the gut microbiota: a bibliometric analysis
Xiaoxiao Lin, Jinyu Huang, Shuai Wang and Kai Zhang

64 Causal links between gut microbiomes, cytokines and risk of different subtypes of epilepsy: a Mendelian randomization study
Youjia Qiu, Bingyi Song, Minjia Xie, Yuchen Tao, Ziqian Yin, Menghan Wang, Chao Ma, Zhouqing Chen and Zhong Wang

75 Aberrant intrinsic brain activities in functional gastrointestinal disorders revealed by seed-based d mapping with permutation of subject images
Yibin Shuai, Benhong Wang, Xiaomei Zhang, Zhongxia Shen, Shengbo Han and Cong Zhou

87 Mechanisms of microbiota-gut-brain axis communication in anxiety disorders
Min Jiang, Li Kang, Ya-Li Wang, Bin Zhou, Hong-Yi Li, Qiang Yan and Zhi-Gang Liu

99 Hypothalamus-pituitary-adrenal and gut-brain axes in biological interaction pathway of the depression
Amanda Gollo Bertollo, Camila Ferreira Santos, Margarete Dulce Bagatini and Zuleide Maria Ignácio

112 The brain-gut axis and chronic pain: mechanisms and therapeutic opportunities
Tim Ho, Ömer Elma, Lucy Kocanda, Katherine Brain, Thao Lam, Tejas Kanhere and Huan-Ji Dong

120 From the gut to the brain, mechanisms and clinical applications of γ-aminobutyric acid (GABA) on the treatment of anxiety and insomnia
Chengji Jiang, You Chen and Tao Sun

133 Exploring the role of gut microbiota in Parkinson's disease: insights from fecal microbiota transplantation
Mengqi Guo, Han Gao, Yuan Wang and Yuanyuan Xiang

frontiers | Frontiers in Neuroscience

TYPE Editorial
PUBLISHED 03 September 2025
DOI 10.3389/fnins.2025.1665282

OPEN ACCESS

EDITED AND REVIEWED BY
Rajnikant Dixit,
National Institute of Malaria Research (ICMR), India

*CORRESPONDENCE
Avinash Veerappa
avinash.vins@gmail.com

RECEIVED 13 July 2025
ACCEPTED 22 August 2025
PUBLISHED 03 September 2025

CITATION
Ignácio ZM, Clayton JB, Bagatini MD and Veerappa A (2025) Editorial: Reviews in gut-brain axis: stress, dysregulation in gut-brain axis function and stress related disorders. *Front. Neurosci.* 19:1665282. doi: 10.3389/fnins.2025.1665282

Editorial: Reviews in gut-brain axis: stress, dysregulation in gut-brain axis function and stress related disorders

Zuleide Maria Ignácio[1], Jonathan B. Clayton[2,3,4,5], Margarete Dulce Bagatini[1] and Avinash Veerappa[6]*

[1]Federal University of Fronteira Sul, Graduate Program in Biomedical Sciences, Chapecó, SC, Brazil, [2]University of Nebraska Omaha, Omaha, NE, United States, [3]Nebraska Food for Health Center, Lincoln, NE, United States, [4]University of Nebraska-Lincoln, Lincoln, NE, United States, [5]University of Nebraska Medical Center, Omaha, NE, United States, [6]Department of Biology, University of Nebraska at Omaha, Omaha, NE, United States

KEYWORDS

gut-brain axis, stress, brain function, inflammation, gastrointestinal

Editorial on the Research Topic
Reviews in gut-brain axis: stress, dysregulation in gut-brain axis function and stress related disorders

Emerging evidence underscores the important role of the gut-brain axis across a spectrum of neuropsychiatric, neurological, and gastrointestinal disorders. Understanding how gut microbiota influence brain function and behavior continues to interest alike. Recognizing the complexity and interdisciplinary nature of this field, we invited studies spanning diverse methodologies from bibliometric analyses and meta-analyses to Mendelian randomization (MR) approaches and clinical trial reviews. This editorial synthesizes recent findings from these varied approaches, highlighting both their innovative contributions and the methodological challenges that persist in gut-brain axis research.

Lin et al. employed bibliometric analysis to elucidate the growing interest and current hotspots surrounding bipolar disorder (BD) and gut microbiota. Their findings emphasized microbiome diversity, inflammation, and probiotics as critical areas of focus, while noting the necessity of more robust clinical trials to validate potential interventions (Lin et al.). In parallel, Wang et al. provided a comprehensive review of depression-associated gut microbes and metabolites, illustrating clear links between altered microbiota profiles, such as increased lactobacilli, and depressive symptoms. However, they cautioned the clinical community regarding inconsistent efficacy in microbiome-targeted treatments, underscoring the need for personalized, systems-level approaches to therapy (Wang et al.).

Extending beyond mood disorders, Zhou et al. used MR to explore causal relationships between gut microbiota and cortical structures implicated in neuropsychiatric conditions. Their findings notably revealed associations between gut taxa, such as Mollicutes and Tenericutes, and orbitofrontal cortical morphology, thereby proposing a biological substrate underpinning gut-brain interactions (Zhou et al.). Similarly, Qiu et al.'s MR study robustly linked the gut microbiome, specifically the family Veillonellaceae, to epilepsy subtypes, opening new avenues for targeted microbiome interventions despite unclear mechanistic pathways (Qiu et al.).

Further emphasizing neurological implications, Guo et al. reviewed the role of gut microbiota in Parkinson's disease (PD), highlighting fecal microbiota transplantation (FMT) as a promising therapeutic strategy. They detailed how gut dysbiosis exacerbates PD pathology through mechanisms like increased intestinal permeability, α-synuclein aggregation, and neuroinflammation, while also urging the need for rigorous clinical validation to substantiate therapeutic claims (Guo et al.).

Shifting focus to functional gastrointestinal disorders (FGIDs), Shuai et al. applied meta-analysis of resting-state fMRI studies, demonstrating altered brain activities, particularly in regions such as the insula and anterior cingulate cortex, among FGID patients. These findings underscore the complex interplay between gastrointestinal symptoms and brain networks, suggesting neurological targets for potential intervention (Shuai et al.).

Jiang C. et al. explored γ-aminobutyric acid (GABA) as a gut-derived therapeutic candidate for anxiety and insomnia, highlighting its neuroactive potential and advocating for engineered probiotics to enhance therapeutic efficacy. Nonetheless, they acknowledged significant gaps in validating clinical safety and effectiveness (Jiang C. et al.).

In reviewing chronic pain, Ho et al. elucidated how the brain-gut axis, mediated through microbiome dysbiosis and vagal dysfunction, significantly contributes to chronic pain mechanisms. Their narrative review proposed innovative therapeutic strategies including microbiome restoration and vagus nerve modulation, yet stressed the urgency for clinical trials to ascertain effectiveness and safety (Ho et al.).

Jiang M. et al. reviewed the microbiota-gut-brain axis's intricate role in anxiety disorders, detailing neuroimmune, endocrine, and neural signaling pathways implicated in anxiety pathophysiology. Despite promising preliminary findings, they pointed out considerable translational hurdles in moving microbiota-targeted therapies into clinical practice (Jiang M. et al.).

Additionally, Hayer et al. provided a systematic review and meta-analysis focusing on antibiotic-induced gut dysbiosis and its associations with cognitive, emotional, and behavioral changes in rodents. They reported significant associations between antibiotic intake and increased anxiety- and depression-like behaviors, as well as impaired spatial cognition. Although the findings indicate a potential causal relationship, the considerable heterogeneity in experimental designs and methodologies used across studies emphasizes the necessity for standardized approaches to enhance the reliability and translational potential of these findings (Hayer et al.).

Finally, Bertollo et al. concluded that there is an intricate interplay between the hypothalamus-pituitary-adrenal (HPA) axis and the gut-brain axis in the pathophysiology of depression. Dysregulation of the HPA axis, triggered by chronic stress, leads to elevated cortisol levels and neuronal damage in brain regions involved in mood regulation. Simultaneously, alterations in gut microbiota composition can impair gut-brain communication, promote systemic inflammation, and compromise serotonin production—factors closely linked to depressive symptoms. These interconnected pathways underscore the multifactorial nature of depression and suggest the potential of integrated therapeutic strategies targeting both neuroendocrine and microbiota-related mechanisms (Bertollo et al.).

Collectively, these studies represent groundbreaking efforts toward unraveling the complexities of the gut-brain axis across various disorders. Nevertheless, the heterogeneity of findings, coupled with methodological challenges such as inconsistent approaches, limited causal evidence, and translation gaps, highlight the necessity for integrated, interdisciplinary research frameworks. Future studies leveraging multi-omics platforms, bioinformatics, and artificial intelligence will be crucial in advancing this rapidly evolving field toward robust clinical application.

Author contributions

ZI: Writing – review & editing. JC: Writing – review & editing. MB: Conceptualization, Writing – review & editing. AV: Conceptualization, Supervision, Writing – original draft, Writing – review & editing.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Generative AI statement

The author(s) declare that no Gen AI was used in the creation of this manuscript.

Any alternative text (alt text) provided alongside figures in this article has been generated by Frontiers with the support of artificial intelligence and reasonable efforts have been made to ensure accuracy, including review by the authors wherever possible. If you identify any issues, please contact us.

Publisher's note

frontiers | Frontiers in Neuroscience

TYPE Systematic Review
PUBLISHED 01 Sepetember 2023
DOI 10.3389/fnins.2023.1237177

OPEN ACCESS

EDITED BY
David Vauzour,
University of East Anglia, United Kingdom

REVIEWED BY
Avinash Veerappa,
University of Nebraska Medical Center, United States
Eleonore Fröhlich,
Medical University of Graz, Austria

*CORRESPONDENCE
Jonathan B. Clayton
✉ jclayton@unomaha.edu

RECEIVED 09 June 2023
ACCEPTED 18 August 2023
PUBLISHED 01 Sepetember 2023

CITATION
Hayer SS, Hwang S and Clayton JB (2023) Antibiotic-induced gut dysbiosis and cognitive, emotional, and behavioral changes in rodents: a systematic review and meta-analysis. *Front. Neurosci.* 17:1237177. doi: 10.3389/fnins.2023.1237177

Antibiotic-induced gut dysbiosis and cognitive, emotional, and behavioral changes in rodents: a systematic review and meta-analysis

Shivdeep S. Hayer[1,2,3,4], Soonjo Hwang[5] and Jonathan B. Clayton[1,2,3,6,7,8]*

[1]Department of Biology, University of Nebraska at Omaha, Omaha, NE, United States, [2]Callitrichid Research Center, University of Nebraska at Omaha, Omaha, NE, United States, [3]Nebraska Food for Health Center, University of Nebraska-Lincoln, Lincoln, NE, United States, [4]Department of Population Medicine, University of Guelph, Guelph, ON, Canada, [5]Department of Psychiatry, University of Nebraska Medical Center, Omaha, NE, United States, [6]Department of Food Science and Technology, University of Nebraska-Lincoln, Lincoln, NE, United States, [7]Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, NE, United States, [8]Primate Microbiome Project, University of Nebraska-Lincoln, Lincoln, NE, United States

There are previous epidemiological studies reporting associations between antibiotic use and psychiatric symptoms. Antibiotic-induced gut dysbiosis and alteration of microbiota-gut-brain axis communication has been proposed to play a role in this association. In this systematic review and meta-analysis, we reviewed published articles that have presented results on changes in cognition, emotion, and behavior in rodents (rats and mice) after antibiotic-induced gut dysbiosis. We searched three databases—PubMed, Web of Science, and SCOPUS to identify such articles using dedicated search strings and extracted data from 48 articles. Increase in anxiety and depression-like behavior was reported in 32.7 and 40.7 percent of the study-populations, respectively. Decrease in sociability, social novelty preference, recognition memory and spatial cognition was found in 18.1, 35.3, 26.1, and 62.5 percent of the study-populations, respectively. Only one bacterial taxon (increase in gut *Proteobacteria*) showed statistically significant association with behavioral changes (increase in anxiety). There were no consistent findings with statistical significance for the potential biomarkers [Brain-derived neurotrophic factor (BDNF) expression in the hippocampus, serum corticosterone and circulating IL-6 and IL-1β levels]. Results of the meta-analysis revealed a significant association between symptoms of negative valence system (including anxiety and depression) and cognitive system (decreased spatial cognition) with antibiotic intake ($p < 0.05$). However, between-study heterogeneity and publication bias were statistically significant ($p < 0.05$). Risk of bias was evaluated to be high in the majority of the studies. We identified and discussed several reasons that could contribute to the heterogeneity between the results of the studies examined. The results of the meta-analysis provide promising evidence that there is indeed an association between antibiotic-induced gut dysbiosis and psychopathologies. However, inconsistencies in the implemented methodologies make generalizing these results difficult. Gut microbiota depletion using antibiotics may be a useful strategy to evaluate if and how gut microbes influence cognition, emotion, and behavior, but the heterogeneity in methodologies used precludes any definitive interpretations for a translational impact on clinical practice.

KEYWORDS

gut microbiota, behavior, anxiety, depression, social, antibiotics, anhedonia, microbiota-gut-brain axis

Introduction

Antibiotics are "miracle" drugs that have saved the lives of countless humans suffering from infectious diseases. However, the side-effects of these drugs are not fully known. In particular, the relationship between antibiotic intake and onset of neuro-psychiatric symptoms/syndromes such as depression and anxiety has been postulated based on large scale epidemiological studies in humans. An association between antibiotic use and higher risk for depression and anxiety was found in a nested case–control study in the United Kingdom (Lurie et al., 2015). A study showed a correlation between the high concentrations of antibiotics in urine and presence of depression in a cohort of elderly people in China (Liu et al., 2021). Mental health problems such as peer problems, hyperactivity and conduct issues were also associated with ciprofloxacin use in children in China (Zhang J. et al., 2021).

The gut microbiome is comprised of all the microorganisms in the gut including bacteria, viruses, and fungi and the bi-directional communication between the gut and brain mediated via actions of bacteria is referred to as the "microbiota-gut-brain axis" (Olavarría-Ramírez et al., 2023). Gut dysbiosis has been implicated in the pathogenesis of numerous human diseases including autoimmune diseases, metabolic disorders, cancer, and pertaining to our study, psychiatric disorders (Duvallet et al., 2017; Sherwin et al., 2019; Simpson et al., 2020). On the other hand, microbes (particularly probiotics) have also been studied for their beneficial effects in reducing stress and anxiety (Amirani et al., 2020; Zhang et al., 2020; Ma et al., 2021).

Antibiotics could potentially create the most potent disruption of the microbiota-gut-brain axis, which has significant implications in both basic and translational research (Ramirez et al., 2020). The mechanistic links between antibiotic-mediated gut dysbiosis and induction of cognitive, emotional, and behavioral changes have been studied in numerous rodent studies and recently reviewed (Hao et al., 2020). Briefly, these mechanisms include changes in circulating inflammatory cytokine levels, oxidative stress, inhibition of brain-derived neurotrophic factor (BDNF) expression, decrease in neurotransmitter concentrations in brain tissues and blood, changes in short chain fatty acid levels in gut, and disruption of vagal nerve activity (Hao et al., 2020).

There has been a spurt of articles linking antibiotics with cognitive, emotional, and behavioral changes in rodents in recent years. However, the results reported in these studies lack consensus and are often contradictory (Olavarría-Ramírez et al., 2023). In this systematic review and meta-analysis, we critically reviewed all the published articles that have presented results on changes in cognition, emotion, and behavior in rodents (rats and mice) after antibiotic-induced gut dysbiosis.

Methods

Narrative systematic review

A dedicated search string was created for three different databases (Web of Science, PubMed, and Scopus; Supplementary material 1). These search strings consisted of words that describe the four major items of this review: antibiotics, rodents, behaviors, and gut microbiome. The inclusion criteria included studies that contrasted changes in behavior between antibiotic-treated and vehicle-treated groups and described changes in gut microbiome in rats and mice. The articles were excluded if:

1. Either of the above items were missing from the article.
2. Strains of rodents used were created to model a specific disease (e.g., NOD mice for diabetes, *15qdup* for autism spectrum disorder).
3. There were other confounding factors such as probiotic administration, presence of a stressor, fecal microbiota transplantation, etc.
4. Behaviors other than those of interest (Table 1) were evaluated.
5. Articles were written in a language other than English.

The search was initially conducted on January 3, 2022 and revised on January 20, 2023. The details of the literature search conducted are presented in Figure 1. We extracted the following data:

A. Name of the last author and title of the publication.
B. Methodology to study gut bacterial population, bacterial taxa (phyla and genera level).
C. Details of the behavioral outcomes.
D. Molecular mechanisms potentially associated with changes in behavior after antibiotic-induced gut dysbiosis (BDNF in brain tissues, cytokines in blood and brain, and serum corticosterone).
E. Antibiotics used, route and duration of antibiotic administration, time between antibiotic administration, and behavioral assessment.
F. Rodent species, strain, sex, and age.

Some studies described results of experiments conducted on multiple populations such as those differentiated by mice strains, sexes, age-groups or antibiotics used within the same study. Hence, for the purpose of this review, the unit of analysis is the "population" within an article.

The tests included in this review are open field test (OFT), elevated plus maze test (EPM), light–dark box test (L/D), forced swim test (FST), tail suspension test (TST), Morris water maze test (MWM), novel object recognition test (NOR), three-chambered sociability test (sociability and social novelty), and sucrose preference test (SPT). We have provided a brief description of the tests (Table 1) and their

TABLE 1 Brief description of the behavioral tests included in this review.

Behavioral test	Brief synopsis	Behavioral assessment	Behavioral phenotype
Open field test (OFT)	Anxious mice are less likely to explore open spaces and will stick closer to walls	Time spent in center	Anxiety
Elevated plus maze test (EPM)	Anxious rodents are less likely to explore the open arms of an elevated platform	Time spent in open	Anxiety
Light/Dark box test (L/D box)	Anxious rodents are less likely to explore the illuminated chamber	Time spent in light	Anxiety
Morris water maze test (MWM)	After training for a few days, rodents would remember the quadrant where the platform is located in the water cylinder	Time in target quadrant	Spatial cognition
Morris water maze test (MWM)	Time taken for rodents to identify hidden platform in water and escape	Escape latency	Spatial cognition
Novel object recognition test (NOR)	Rodents tend to interact more with a novel object	Discrimination index	Recognition memory
Forced swimming test (FST)	Depressed rodents spend less time swimming as a coping mechanism	Immobility time	Depression
Tail suspension test (TST)	Depressed rodents spend less time struggling to escape when suspended from tail	Immobility time	Depression
Three-chambered sociability test (3-SC)	Rodents spend more time with a rodent than an object	Time spend interacting with rodent	Sociability
Three-chambered sociability test (3-SC)	Rodents spend more time with a new rodent as compared to a familiar rodent	Time spend interacting with new rodent	Social novelty
Sucrose preference test (SPT)	Rodents prefer sugary water over unflavored water	Ratio of sugar water intake and total water intake	Anhedonia

relevance to Research Domain Criteria (Morris and Cuthbert, 2012) in Table 2.

We generated a behavioral phenotype (e.g., decrease in anxiety) after assessing each behavioral outcome carefully for each study population. Some of the studies utilized multiple tests to study the same phenotype. For example, OFT, EPM, and L/D box test the unconditioned, exploratory behavior of the rodents, which is used to assess "anxiety-like" behavior. If a population was evaluated for the same phenotype using multiple tests, then such population was considered to be positive for said phenotype even if only one of the tests were positive.

Risk of bias assessment was conducted by modifying the SYRCLE's risk of bias tool (Hooijmans et al., 2014). The following items were evaluated for this assessment: randomization, baseline characteristics, blinding, husbandry characteristics, attrition bias, selective outcome reporting of behavioral assessment, and microbiome analysis.

Meta-analysis

Summary statistics of the behavioral assessments were usually not provided in the articles selected. We extracted the mean and variance estimates (standard deviation and standard error) from the figures using WebPlotDigitizer version 4.6 online (Rohatgi, 2014). Data was extracted for the following behavioral outcomes: time spent in center (OFT), immobility time (FST), immobility time (TST), time spent in light (L/D box), discrimination index (NOR), time spent in open arm (EPM), time in target quadrant (MWM), and time to latency (MWM). Since different studies used different time scales, we first estimated standardized mean differences. Hierarchical random effects models were built for each behavioral outcome separately to estimate pooled standardized mean differences using the package "metafor" (version 2.4–0) in R version 2022.12 (Viechtbauer, 2010). The model specifications included selecting t-distribution to model test statistics, restricted maximum likelihood estimation for determining the variance and the article as a random effect. Two types of models were built for each behavioral outcome separately: Model A consisting of all the available data and Model B consisting of data from male mice administered antibiotics orally. The latter was chosen as this was by far the most common population characteristic in the studies selected. These models also provided a test of heterogeneity as part of the default output.

Subgroup analysis was conducted to evaluate the impact of age at which antibiotic administration was initiated (pre-natal and early post-natal vs. adolescent-adult), duration of antibiotic treatment (less than 14 days vs. more than 14 days), number of antibiotics administered (single vs. multiple), and time between antibiotic treatment and behavioral assessment (immediate vs. several days). Publication bias was assessed using Egger's test (Egger et al., 1997).

Formal meta-analysis of microbiome data was not conducted because the data were not extractable from the graphs reported in the articles and raw reads were often not available in the public repositories. Hence, we conducted a semi-quantitative analysis to check if the direction of significant changes in gut microbiome (increase, decrease, or no change) were associated with behavioral changes (increase, decrease, or no change) using Fisher's exact test (2×2 tables) or Freeman–Halton extension of Fischer's exact test (3×3 or 3×2 tables). A meta-analysis of molecular mechanisms was not

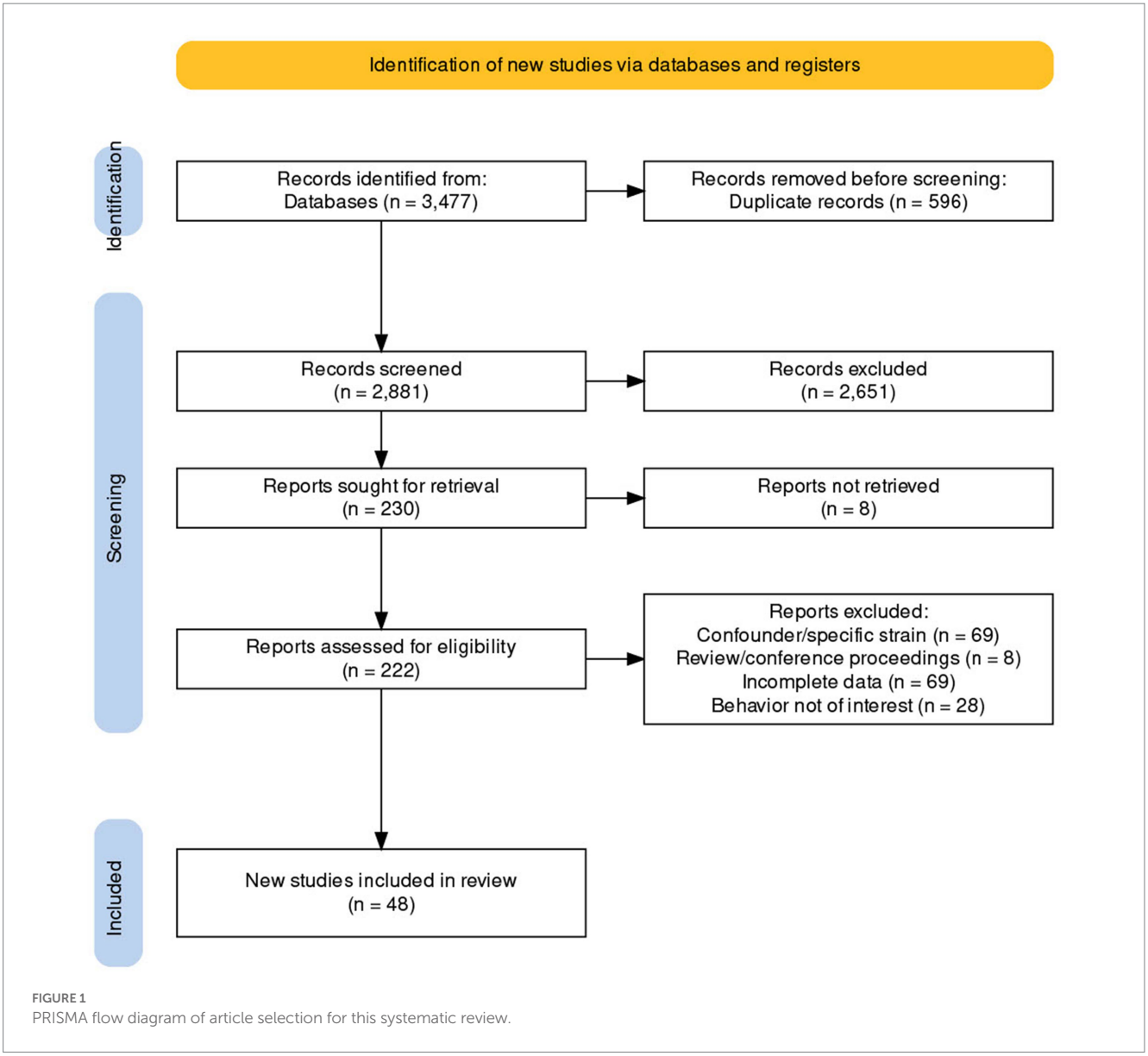


FIGURE 1
PRISMA flow diagram of article selection for this systematic review.

conducted because these were secondary outcomes and were not part of the search strings.

Results

Description of the articles

The details of the literature search conducted are presented in Figure 1. The complete data extracted are available in Supplementary material 2. We eventually included 48 articles in this review. Seventy-three different "populations" of animals were experimented on in these 48 studies. Thirty-three studies had data on only one population and 15 studies had data on multiple populations.

Mice and rats made up the subjects in 66 (90.4%) and 7 (9.6%) of these populations, respectively. C57BL/6J was the most common strain of mice used (34 populations), followed by BALB/C (13 populations). Cocktails consisting of several antibiotics were tested on 42 (57.5%) populations and the rest of the populations ($n = 31$, 42.5%) were tested with a single antibiotic. Oral route of antibiotic administration was by far the most common route of antibiotic administration (69 populations, 94.5%). Fifty-seven (78.1%) of these populations were comprised of male rodents only, and 10 (13.7%) and six (8.2%) populations were made up of female rodents only and rodents of both sexes, respectively. Antibiotics were administered on an average for 22.4 days (range: 1–150 days). The average time between the last day of antibiotic administration and start of behavioral testing was 14.3 days (range: 0–98 days). None of the articles reported an increase in anhedonia after antibiotic administration (based on SPT; Wang et al., 2020a,b; Li et al., 2021; Hassan et al., 2022; Yao et al., 2022; Yu et al., 2022). Anhedonia will not be discussed further in the subsequent paragraphs.

Risk of bias

The average risk of bias score was 3.67 (range: 2–7) out of a possible highest score of 7. Behavioral outcomes and husbandry

TABLE 2 Interpretation of the behavioral tests in the context of research domain criteria.

Behavioral test	Behavioral domain in research domain criteria
Open field test	Negative valence system: potential threat
	Positive valence system: reward responsiveness – reward anticipation
	Sensorimotor system: motor actions – initiation, execution, and inhibition/termination
Elevated plus maze test	Negative valence system: potential threat
	Positive valence system: reward responsiveness – reward anticipation
	Sensorimotor system: motor actions – initiation, execution, and inhibition/termination
Light/Dark box test	Negative valence system: acute threat and potential threat
Morris water maze test	Cognitive system: working memory – active maintenance
	Cognitive system: working memory – flexible updating
	Cognitive system: working memory – interference control
	Sensorimotor system: motor actions – execution
Novel object recognition test	Cognitive system: working memory – active maintenance
	Cognitive system: working memory – flexible updating
	Cognitive system: working memory – interference control
	Sensorimotor system: motor actions – execution
Forced swimming test	Negative valence system: acute threat
	Negative valence system: sustained threat
	Negative valence system: frustrative nonreward
	Sensorimotor system: motor actions – initiation
	Sensorimotor system: motor actions – execution
Tail suspension test	Negative valence system: acute threat
	Negative valence system: sustained threat
	Negative valence system: frustrative nonreward
	Sensorimotor system: motor actions – initiation
	Sensorimotor system: motor actions – execution
Three-chambered sociability test	Systems for social processes: affiliation and attachment
	Systems for social processes: social communication
	Systems for social processes: perception and understanding of others
Sucrose preference test	Positive valence system: reward anticipation
	Positive valence system: initial response to reward
	Positive valence system: reward satiation
	Positive valence system: reward valuation – effort

descriptions (including environmental conditions, housing, and feeding) were adequately reported in 48 (100%) and 46 (95.8%) articles, respectively. Randomization, microbiome data availability, baseline characteristics, blinding, and attrition bias were explained in only 54.2, 43.8, 12.5, 29.2, and 31.3% of the articles, respectively. Fecal and cecal contents were sampled in 32 (66.7%) and 16 (33.3%) of the articles, respectively. Storage temperatures or time between sample collection and DNA extraction of fecal/cecal contents was not provided in 25 (52.1%) articles. When provided, fecal samples were stored at −20 or −80°C without any additives.

General results of the studies

Decrease in sociability and social novelty preference was reported in 18.1% (4/22) and 35.3% (6/17) populations, respectively. Increase in anxiety was reported in 32.7% (19/58) populations. Increase in depression-like behavior was reported in 40.7% (11/27) populations. Decreased recognition memory and spatial cognition was observed in 26.1% (6/23) and 62.5% (10/16) populations based on NOR and MWM tests, respectively. Interestingly, a decrease in anxiety was also reported in 17.2% (10/58) of the populations.

Changes in behavior in mice at different life stages

Early postnatal phase

Antibiotics were administered to mice in the early postnatal phase of life in seven articles (16 populations; Table 3). Kayyal et al. (2020) reported that oral penicillin administration led to a decrease in sociability but not anxiety in male mice without any behavioral

TABLE 3 Changes in behaviors in rodents in early postnatal stage of life after antibiotic administration.

Article	Strain	Sex	Antibiotics	Duration of treatment	Time between treatment and behavioral testing	Sociability	Social novelty	Spatial cognition	Recognition memory	Depression	Anxiety
Desbonnet et al. (2015)	NIH Swiss	M	Ampicillin, vancomycin, neomycin, and metronidazole	24 days	None				Decrease		Decrease
Keogh et al. (2021)	C57BL/6 J	B	Ampicillin, neomycin, and vancomycin	16 days	28 days				Decrease		Decrease
Kayyal et al. (2020)	BALB/C	B	Penicillin V	7 days	21 days	±					NS
Li et al. (2022)	KM	M	Ampicillin, vancomycin, neomycin, bacitracin, and imipenem	21–84 days	0–63 days			±		±	Increase
Zhang et al. (2022)	KM	M	Ampicillin, vancomycin, neomycin, bacitracin, and imipenem	75 days	None			Decrease		Increase	NS
Lynch et al. (2023)	NIH Swiss	B	Ampicillin, gentamicin, vancomycin, and imipenem	7 days	15–35 days	NS	NS		NS	NS	±
Liu et al. (2022)	C57BL/6 J	B	Ampicillin or vancomycin	28 days	14 days	±	Decrease	Decrease			Increase

"M," male; "B," both sexes; NS, no change in behavior; ±, different changes in behavior across populations within a study.

10.3389/fnins.2023.1237177

changes in female mice 21 days post-antibiotic intake. Orally administered ampicillin and vancomycin alone caused significant increase in anxiety and decrease in social novelty, spatial cognition and sociability (except in ampicillin-treated mice) 14 days after cessation of treatments (Liu et al., 2022).

Li et al. (2022), Zhang et al. (2022), Desbonnet et al. (2015), and Keogh et al. (2021) observed decreased spatial cognition and recognition memory after oral intake of solution containing ampicillin, vancomycin, and other antibiotics. However, Li et al. (2022) and Zhang et al. (2022) found an increase, and Desbonnet et al. (2015) and Keogh et al. (2021) found a decrease in anxiety in these mice. Finally, Lynch et al. (2023) delivered a cocktail containing ampicillin, vancomycin, and other antibiotics to mice of both sexes and did not observe changes in sociability, social novelty, recognition memory, depressive-signs, and anxiety (with the exception of two of the six populations evaluated in this study).

Adolescent phase

Antibiotics were administered to mice in the adolescent phase of life in 23 articles (30 populations; Table 4). None and only one of the study populations displayed changes in sociability and social novelty, respectively after antibiotic administration regardless of sex, strain, or duration between treatments and behavioral observations (Table 4; Gacias et al., 2016; Guida et al., 2018; Lach et al., 2020; Zhang Z. et al., 2021). Similarly, recognition memory decreased in only one of the study populations tested (Guida et al., 2018; Lach et al., 2020; Saunders et al., 2020; Arslanova et al., 2021; Luo et al., 2022). Recognition memory was decreased in male mice administered a cocktail of four antibiotics intraperitoneally for 14 days and immediately followed by behavioral testing (Arslanova et al., 2021). Spatial cognition decreased in only two of the five study populations (Wang et al., 2021; Zheng et al., 2021; Li et al., 2022). These two study populations consisted of C57BL/6 J administered vancomycin for 77 days (Zheng et al., 2021) and ampicillin, streptomycin, and clindamycin for 21 days (Wang et al., 2021).

Unlike the results described above, antibiotics were able to induce depression-like behavior-and anxiety with more consistency. Oral intake of ampicillin or ceftriaxone alone was sufficient to increase depression-like behavior after 10 and 77 days of intake, respectively (Zhao et al., 2020; Hao et al., 2021). Ray et al. (2021) reported an increase in depression-like behavior in C57Bl/6 J mice after oral vancomycin administration for 6 days, but not in BALB/C mice. A combination of ampicillin, streptomycin, and clindamycin administered orally for 14–21 days led to an increase in depression-like behavior even after 14 days of last treatment (Guida et al., 2018; Wang et al., 2021; Sun et al., 2023). Interestingly, administration of combination of ampicillin, neomycin and/or vancomycin did not lead to changes in depression-like behavior regardless of strain, route of treatment, treatment duration, and duration between treatments and behavioral observations (Gacias et al., 2016; Wang et al., 2020b; Li et al., 2022).

Oral intake of ampicillin, ceftriaxone, vancomycin, or azithromycin alone was sufficient to induce anxiety (Jang et al., 2018; Zhao et al., 2020; Hao et al., 2021; Ray et al., 2021; Tang et al., 2022). However, in one experiment, oral ampicillin or neomycin alone did not induce anxiety in mice (Septyaningtrias et al., 2020). A cocktail of ampicillin, streptomycin, and clindamycin administered orally led to increased anxiety in two separate experiments (Wang et al., 2021; Sun et al., 2023). Impact of the oral administration of other antibiotic combinations on the anxiety of mice was ambiguous, with an increase and decrease in anxiety reported in 2 and 1 of the seven articles, respectively (Table 4). Finally, intraperitoneal administration of ampicillin, vancomycin, metronidazole, and neomycin for 14 days led to an increase in anxiety (Arslanova et al., 2021) but not when the same cocktail was administered subcutaneously for the same duration (Gacias et al., 2016).

Adult phase

Ten studies evaluated behavioral changes after antibiotic administration in 13 populations of adult mice (Table 5). Oral administration of ampicillin alone for 14 days was sufficient to cause decrease in spatial cognition, recognition memory, and anxiety (Roy Sarkar et al., 2020; Sarkar et al., 2022). Morozova et al. (2022) reported decrease in anxiety after oral vancomycin and rifampin administration. Fröhlich et al. (2016) found a decrease in recognition memory without changes in depressive-signs and anxiety after oral administration of an antibiotic cocktail for 7 days. Mezö et al. (2020) found no changes in recognition memory even after administering a cocktail of antibiotics for 60 days.

Geary et al. (2021) and Bercik et al. (2011) used an identical oral antibiotic cocktail for varying durations to evaluate changes in anxiety with mixed results. Geary et al. (2021) reported a decrease in anxiety in males but not in females. A decrease in anxiety was observed in male mice evaluated for behavior immediately after cessation of oral antibiotic intake, but not if behavior was evaluated 2 weeks after last dose of oral antibiotics or if the antibiotics were administered intraperitoneally (Bercik et al., 2011; Geary et al., 2021). Finally, no changes in behavior was observed by Yao et al. (2022), Lach et al. (2020), or Luo et al. (2021) after oral minocycline, oral antibiotic cocktail and intraperitoneal administration of cefazolin, respectively (Lach et al., 2020; Luo et al., 2021; Yao et al., 2022; Table 5).

Changes in behavior in offspring born to pregnant mice administered antibiotics

We identified five studies which evaluated the impact of microbiome changes in pregnant mice administered antibiotics on the behavior of offspring hence born. No changes in recognition memory were reported in newborn male offspring born to mothers given ampicillin, metronidazole, vancomycin, and neomycin (Bello-Medina et al., 2022). Studies also reported behavioral changes in offspring born to mothers given antibiotic cocktails but the results were inconsistent and cannot be generalized. A decrease in social novelty preference without change in sociability was observed in 98 days-old mice of either sex born to mothers given neomycin and vancomycin (Zhang Z. et al., 2021). Tochitani et al. (2016) reported an increase in anxiety without changes in sociability in 28 day-old mice of either sex born to mothers administered neomycin, bacitracin, and pimaricin.

Leclercq et al. (2017) and Champagne-Jorgensen et al. (2020) used nearly identical experimental protocols but found conflicting results. Pregnant BALB/C mice were fed low-dose penicillin V and behavior was evaluated in 42 day-old male and female offspring. Leclercq et al. (2017) reported decreased sociability and social novelty preference in male and female offspring but these changes were not reported by Champagne-Jorgensen et al. (2020). Both of these studies reported no

TABLE 4 Changes in behaviors in rodents in adolescent stage of life after antibiotic administration.

Article	Strain	Sex	Antibiotics	Rx duration	Time between rx and behavioral testing	Sociability	Social novelty	Spatial cognition	Recognition memory	Depression	Anxiety
Septyaningtrias et al. (2020)	C57BL/6J	M	amp or neo	28	None						NS
Hassan et al. (2022)	C57BL/6J	M	neo, van, and mer	28	None						Decrease
Reyes et al. (2020)	C57BL/6J	M	bac, neo, van, and pim	14	None						NS
Guida et al. (2018)	C57BL/6J	M	amp, str, and cli	14	14	NS	Decrease		NS	Increase	
Wang et al. (2021)	C57BL/6J	M	amp, str, and cli	21	None			Decrease		Increase	Increase
Zheng et al. (2021)	C57BL/6J	M	van	21–77	None			±			
Lach et al. (2020)	C57BL/6J	M	amp, van, cip, imi, and met	21	24	NS	NS		NS		Increase
Jang et al. (2018)	C57BL/6J	M	amp	2	10						Increase
Saunders et al. (2020)	CD1	M	str	1	77				NS		
Wang et al. (2020a)	C57BL/6J	M	amp, neo, and met	14	14					NS	
Zhao et al. (2020)	BALB/C	M	axo	77	None					Increase	Increase
Grant et al. (2021)	C57BL/6J	F	neo, van, and met	8	None						Increase
Gacias et al. (2016)	C57BL/6J	M	neo, van, met, and amp (SC, oral)	14	None	NS				NS	NS
Arslanova et al. (2021)	Unknown	M	amp, van, neo, and met (IP)	14	None				Decrease		Increase
Ray et al. (2021)	C57BL/6J or BALB/C	M	van	6	None-54					±	±
Hao et al. (2021)	C57BL/6J	M	amp	10	None					Increase	Increase
Zhang Z. et al. (2021)	KM	F	neo, van	21	21	NS	NS				
Lai et al. (2022)	C57BL/6J	M	amp, bac, neo, and van	14	None						NS
Li et al. (2022)	KM	M	amp, van, neo, bac, and imi	28	None			NS		NS	NS
Luo et al. (2022)	C57BL/6J	M	amp, van, neo, gen, and ery	28	28				NS		
Tang et al. (2022)	BALB/C	B	azi	63	7						Increase
Sun et al. (2023)	C57BL/6J	M	amp, str, and cli	21	14					Increase	Increase

"M," male; "B," both sexes; "F," female; NS, no change in behavior; ±, different changes in behavior across populations within a study; amp, ampicillin; neo, neomycin; van, vancomycin; mer, meropenem; bac, bacitracin; pim, pimaricin; str, streptomycin; cli, clindamycin; cip, ciprofloxacin; imi, imipenem; met, metronidazole; axo, ceftriaxone; gen, gentamicin; ery, erythromycin; azi, azithromycin rx-treatment. Durations and times were measured in days.

10.3389/fnins.2023.1237177

TABLE 5 Changes in behaviors in rodents in adult stage of life after antibiotic administration.

Article	Strain	Sex	Antibiotics	Duration of treatment	Time between treatment and behavioral testing	Sociability	Social novelty	Spatial cognition	Recognition memory	Depression	Anxiety
Luo et al. (2021)	C57BL/6J	M	Cefazolin (IP)	5 days	3 days			NS			
Fröhlich et al. (2016)	C57BL/6J	M	Ampicillin, bacitracin, meropenem, neomycin, vancomycin	7 days	None				Decrease	NS	NS
Mezö et al. (2020)	C57BL/6J	M	Vancomycin, Cefoxitin, Gentamicin, Metronidazole	60 days	None				NS		
Lach et al. (2020)	C57BL/6J	M	Ampicillin, Vancomycin, Ciprofloxacin, Imipenem, Metronidazole	21 days	24 days	NS	NS		NS		NS
Geary et al. (2021)	C57BL/6J	B	Neomycin, Bacitracin, Pimaricin	5 days	None						±
Bercik et al. (2011)	BALB/C	M	Neomycin, Bacitracin, Pimaricin (oral, IP)	7 days	None-14 days						±
Sarkar et al. (2022)	Swiss Albino	M	Ampicillin	14 days	None			Decrease	Decrease		Decrease
Roy Sarkar et al. (2020)	Swiss Albino	M	Ampicillin	14 days	7 days			Decrease	Decrease		Decrease
Morozova et al. (2022)	C57BL/6J	M	Vancomycin, rifampicin	14 days	None						Decrease
Yao et al. (2022)	C57BL/6J	M	Minocycline	14 days	None					NS	NS

"M," male; "B," both sexes; NS, no change in behavior; ±, different changes in behavior across populations within a study.

10.3389/fnins.2023.1237177

changes in anxiety. However, Leclercq et al. (2017) and Champagne-Jorgensen et al. (2020) measured a decrease in anxiety in male offspring and female offspring, respectively. This might also indicate the presence of differences in behavioral responses to gut-brain axis perturbations based on sex.

Changes in behavior in rats

Six studies evaluated changes in behavior in male rats after antibiotic administration. Hoban et al. (2016) and Ruiz-González et al. (2022) administered a cocktail of multiple antibiotics to 8–10 weeks old rats for 4–7 weeks and reported an increase in depression. Hoban et al. (2016) also reported decreased spatial cognition in these subjects but found no changes in anxiety and recognition memory. Cho et al. (2020), Leigh et al. (2020), Li et al. (2021), and Yu et al. (2022) administered a single antibiotic (penicillin G, minocycline, rifaximin, and imipenem, respectively) to rats and reported no changes in anxiety, anhedonia, spatial cognition, and recognition memory (not all of these behaviors were tested in each study). The context of these studies ranged from administering antibiotics to pregnant females and evaluating behavior in offspring of either sex (Cho et al., 2020), to adolescent (Li et al., 2021), and adult rats (Leigh et al., 2020; Yu et al., 2022).

Associations between gut microbiome and behavioral changes

Overall, 180 bacterial taxa were reported in the articles (Supplementary material 2). Not all the taxa were reported in every article. These included 22 bacterial phyla and 158 genera. Of these, 12 phyla and 55 genera were not correlated with any behavioral changes. *Lactobacillus*, *Bacteroides*, *Escherichia*, *Parabacteroides*, and *Odoribacter* were the top five most reported bacterial genera correlated with changes in behavior; and changes in these genera were associated with changes in 23, 18, 14, 13, and 13 behaviors across the different study populations, respectively (Table 6). Similarly, *Firmicutes*, *Proteobacteria*, *Bacteroidetes*, *Actinobacteria*, and *Verrucomicrobia* were the most reported bacterial phyla (Table 6). The details for other taxa-behavior correlations are available in Supplementary material 2.

Notably, the data at a behavior-taxa combination were very scant. However, some patterns were still evident. For example, levels of gut *Firmicutes* emerged as a "positive" indicator of behavioral status, and a decrease in *Firmicutes* was correlated with an increase in anxiety, depression-like behavior and decrease in spatial cognition (Table 6). Conversely, increase in *Proteobacteria* correlated with increased anxiety, depression-like behavior and decreased interest in novel mice and spatial cognition (Table 6). At a bacterial genera level, increase in gut *Lactobacillus* and *Bacteroides* levels were associated with increase in anxiety (Table 6). However, results of such associations were often conflicting at a descriptive level (Table 6). There was a statistically significant association between increase in gut *Proteobacteria* and increase in anxiety in rodents (Fischer's exact test, $p < 0.05$). However, the data was usually too scant to evaluate associations between bacterial taxa and changes in behavior.

The changes in gut microbiome after administration of the same antibiotic or antibiotic combinations were not consistent across studies. Hao et al. (2021) and Sarkar et al. (2022) administered ampicillin to mice for 10 days, but only the latter reported a decrease in *Lactobacillus* spp. However, these studies differed in the dosage of ampicillin used. Gut *Firmicutes* levels increased after penicillin V administration only in 14 days old male mice, but not in females (Kayyal et al., 2020). Sex-related differences in response to the same antibiotics were also reported by Lynch et al. (2023). Gut microbiome changes in response to neomycin and recovery of gut microbiome also differed by the strain of the mice used (Ray et al., 2021). Lach et al. (2020) observed drastically different changes in gut microbiome after oral administration of antibiotics in adolescent and adult mice. Number of days between antibiotic intake and evaluation of gut microbiome (Bercik et al., 2011), and route of administration were

TABLE 6 Most commonly bacterial taxa reported in the studies included in the review and the direction of changes in the relative abundance of these taxa relative to direction of changes in rodent behavior.

Taxa	↑ Anxiety	↑ Depression	↓ Recognition memory	↓ Spatial cognition	↓ Social novelty	↓ Sociability
Phylum						
Firmicutes	↑3, ↓10	↓7	↓3, ↑1	↓7	↓4, ↑2	↓1, ↑3
Proteobacteria	↑9, ↓4	↑6	↑3	↑5	↑6	↑3
Bacteroidetes	↑6, ↓2	↓4	↓2	↓2	↓4	↓2
Actinobacteria	↑1, ↓3	↑1, ↓1	↓1	-	↑1	-
Verrucomicrobia	↑2, ↓1	↑1	↑1	↑1	-	-
Genus						
Lactobacillus	↑4, ↓6	↓4	↓3, ↑1	↓4, ↑1	↓1	-
Bacteroides	↑8, ↓1	↑1, ↓1	↓1	↑3	↑2	↑1
Escherichia/Shigella	↑7, ↓2	↑2, ↓1	-	↓1, ↑1	-	-
Parabacteroides	↑3, ↓4	↑1	-	↑2	↑2	↑1
Odoribacter	↑2, ↓4	-	↓1	↓2	↓3	↓1

↑, increase; ↓, decrease. For example, increase in anxiety was associated with decrease in Firmicutes in 10 populations.

TABLE 7 Changes in key molecular biomarkers of behavioral changes (BDNF), endocrine signaling (corticosterone), and systemic inflammation (interleukins) after antibiotic administration.

Molecular mechanism	Direction of change	Number of populations	Article
IL-6 (circulating)	Increase	3	Leclercq et al. (2017); Jang et al. (2018); Zhao et al. (2020)
	Decrease	1	Leclercq et al. (2017)
	No change	10	Fröhlich et al. (2016); Kayyal et al. (2020); Wang et al. (2020a,b); Li et al. (2022); Zhang et al. (2022)
IL-1β (circulating)	Increase	2	Jang et al. (2018); Hao et al. (2021)
	Decrease	1	Li et al. (2022)
	No change	10	Fröhlich et al. (2016); Leclercq et al. (2017); Guida et al. (2018); Kayyal et al. (2020); Zhang et al. (2022)
BDNF expression (hippocampus)	Increase	1	Bercik et al. (2011)
	Decrease	6	Desbonnet et al. (2015); Guida et al. (2018); Jang et al. (2018); Kayyal et al. (2020); Ray et al. (2021)
	No change	15	Hoban et al. (2016); Champagne-Jorgensen et al. (2020); Kayyal et al. (2020); Leigh et al. (2020); Zhao et al. (2020); Keogh et al. (2021); Ray et al. (2021); Li et al. (2022); Zhang et al. (2022); Sun et al. (2023)
Corticosterone (circulating)	Increase	5	Jang et al. (2018); Zhao et al. (2020); Ruiz-González et al. (2022); Sarkar et al. (2022); Sun et al. (2023)
	Decrease	2	Li et al. (2022); Zhang et al. (2022)
	No change	6	Desbonnet et al. (2015); Hoban et al. (2016); Li et al. (2022); Yu et al. (2022)

other notable factors that could influence the gut bacterial populations (Bercik et al., 2011; Gacias et al., 2016).

Changes in molecular mechanisms

Brain-derived neurotrophic factor (BDNF) gene expression in the hippocampus, circulating levels of IL-6 and IL-1β and serum corticosterone were the three most common molecular mechanisms studied (22, 14, 13, and 13 populations, respectively; Table 7). Results of other molecular mechanisms are presented in the Supplementary material 2. Similar to the results described in the preceding sections, changes in the expression or concentrations of these molecules were not consistent. BDNF gene expression in the hippocampus decreased in 27.3% but did not change in 68.2% of the study populations after antibiotic administration (Table 7). Post antibiotic administration, circulating levels of IL-6 and IL-1β did not change in 71.4 and 76.9 percent of the study populations, respectively (Table 7). Serum corticosterone levels increased in 38.5% but did not change in 46.2% of the study populations after antibiotic administration, respectively. Other factors such as age, sex, duration of antibiotic treatment, and time between last antibiotic and sample collection might have influenced the changes in these molecular mechanisms, but the results were not generalizable.

Meta-analysis of behavioral changes

Decrease in time spent in center (OFT), increased immobility time during FST and TST and increased escape latency (MWM) were significantly associated with antibiotic administration ($p < 0.05$; Figures 2–5; Table 8). Results of other tests were not associated with antibiotic consumption, but there was a trend ($p < 0.10$) toward statistical significance in case of time spent in open arm (EPM), and discrimination index (NOR). Sensitivity analysis was conducted by removing outliers but the results of OFT, FST, and TST remained statistically significant. Meta-analysis on the results of male mice administered orally revealed that time in center (OFT), immobility time (TST), and escape latency (MWM) remained significantly associated with changes in behavior, but the results of FST were not significant.

Based on subgroup analysis, number of antibiotics (single vs. multiple), age (pre-natal exposure and early infant vs. adolescent-adult), days of antibiotic administration (14 days vs. more than 14 days), and time between antibiotic administration and behavioral analysis (none vs. several days) did not influence associations between changes in behavior during OFT, FST, and TST and antibiotic consumption. Significant between-study heterogeneity ($p < 0.05$) was reported in all the meta-analytic models. There was a significant publication bias ($p < 0.05$) in the meta-analysis of all the behaviors tested, with the exception of escape latency in MWM.

Discussion

We conducted a detailed systematic review to compile the results from articles that have described associations between antibiotic administration, gut microbiome changes, and changes in cognition, emotion, and behavior in rats and mice. This review highlights the uncertainties and inconsistencies in the current state of knowledge regarding aspects of the gut-brain-behavior axis, especially in the context of antibiotic-induced dysbiosis. Despite the large variation in behavioral tests applied, antibiotics used, bacterial taxa studied, and other factors, we were still able to identify some key patterns.

Significant increase in anxiety-like (OFT), depression-like behavior (FST and TST), and decrease in spatial cognition (MWM) after antibiotic use were the most outstanding results of this meta-analysis. These results should aid in strengthening the hypothesis that antibiotic-induced dysbiosis can manifest as cognitive and behavioral disturbances in rodents. Meta-analysis of the results of other behavioral tests, although not significant, also favored the hypothesis that antibiotic use can have deleterious effects on cognitive and behavioral functions in rodents. The discrepancy between the results of narrative review and meta-analysis can be explained by the likelihood that the studies were under-powered and there was a high chance of committing type II errors (Van Voorhis and Morgan, 2007), leading to the experimenters assigning a lack of statistical significance to a study despite the effect size and direction favoring the hypothesis. Inadequate sampling size in rodent studies has been acknowledged as one of the reasons why preclinical research often does not translate to human clinical applications (Bracken, 2009). One of the benefits of conducting a meta-analysis is the increase in the power to detect effects across studies (Der Simonian and Laird, 2015). The results of our meta-analysis additionally serve as an example of how meta-analysis of preclinical animal studies can aid in improving the power to detect effects in rodent studies. None of the studies provided a sample size justification for the number of animals used in these experiments. Considering that there is a preponderance of articles published on this topic, sample sizes should be calibrated to decrease the chance of committing type II errors.

Although these results are promising, caution should be exercised while interpreting these results. A significant publication bias indicates that results were more likely to be published if they were significant. This is consistent with the results of other systematic reviews published on rodent studies (Bonapersona et al., 2019; Wang et al., 2020; Theofilis et al., 2022). Under-representation of null or negative results can severely affect the overall conclusions and generalizability of any findings presented in this article.

There was a significant statistical between-study heterogeneity in the case of all except one behavioral test, which indicates that the results might not be generalizable. Multiple factors such as rodent species, strain, age, sex, antibiotics used, route of administration, and the duration of treatment could have played a role in this heterogeneity. However, the results of the meta-analysis still hold true after conducting analysis only on data collected from populations with a more homogenous background (i.e., only male mice administered oral antibiotics; Table 8). This reaffirms the evidence that antibiotic mediated gut microbiome changes can indeed cause cognitive and behavioral changes despite the differences in study designs and populations. Finally, several studies did not report whether investigators were blinded during different phases of the experiments. Several systematic reviews have found that the intervention effect is overestimated if investigators are not blinded during the conduct of experiment and assessment of outcomes (Karp et al., 2022).

The correlation between increase in Phylum *Proteobacteria* in the gut after antibiotic intake and an increase in anxiety was the most significant association between gut bacteria and behavioral changes. Jeong et al. (2019) reported that a high-fat diet can potentially cause an increase in anxiety in mice by altering gut microbiota, especially by increasing relative abundance of *Proteobacteria* (Jeong et al., 2019). Simpson et al. (2020) conducted a systematic review of human and animal trials and found an association between inflammatory bowel disease, high gut levels of *Proteobacteria*, and anxiety/depression (Simpson et al., 2020). Hence, the results of our review are in line with existing scientific literature. Lipopolysaccharides secreted by *Proteobacteria* are known to be highly pro-inflammatory (Lin et al., 2020) and have been associated with pathogenesis of anxiety (Depino, 2015; van Eeden et al., 2021).

Despite the above results, we were not able to identify study designs and populations that can elicit behavioral changes post-antibiotic administration consistently. The following factors might have played a role in this lack of consistency:

Study subjects

Rats and mice have inherently different characteristics that can alter their responses to specific stimuli. For example, rats are more habituated to human handling as compared to mice and this can reduce certain erratic behaviors during the conduct of anxiety tests (Wang et al., 2020). Rats and mice diverged around 15–20 million years ago and from an evolutionary perspective, they differ in serotonergic innervation and levels of neurogenesis (Ellenbroek and Youn, 2016). Rats and mice exhibit different behaviors during swimming leading to discrepancies in read-outs of tests such as MWM (Othman et al., 2022).

Differences in performances during behavioral paradigms have also been observed at a strain level. For example, van Gaalen and Steckler (2000) compared the differences in locomotion and anxiety-like behavior using L/D box and EPM, and found significant differences in the performances of four different strains of mice. C57BL mice consistently develop spatial memory in MWM, whereas BALB mice have been reported to perform poorly by some scientists (Van Voorhis and Morgan, 2007). Even the same strains of rats obtained from different vendors have been reported to differ in anxiety levels based on FST (Porsolt et al., 1978; Bogdanova et al., 2013). Age and sex also play a role in the behavioral phenotypes observed during FST (Bogdanova et al., 2013), OFT (Seliger, 1977; Börchers et al., 2022), EPM (Börchers et al., 2022), and NOR (Sutcliffe et al., 2007).

Results generated from rodent models should only be generalized to human populations after exercising great caution. We also considered studies from other animal models such as non-human primates, and humans at the inception of this review. However, we were not able to find any studies that involved non-human primates and were within the scope of this review. Non-human primates are evolutionarily closer to humans than rodents and are the best models to study psychiatric symptoms and behavioral paradigms. These models can be used to study the impact of antibiotic-induced gut dysbiosis on complex human behaviors and psychiatric disorders such as addiction, sociability, response to stress, etc. Studies similar to those included in this review can be conducted on non-human primates in order to generate further pre-clinical evidence that antibiotics can indeed lead to behavioral changes by altering the gut microbiome.

Antibiotics

The choice and combination of antibiotics, route of administration, and duration between antibiotic administration and behavioral testing

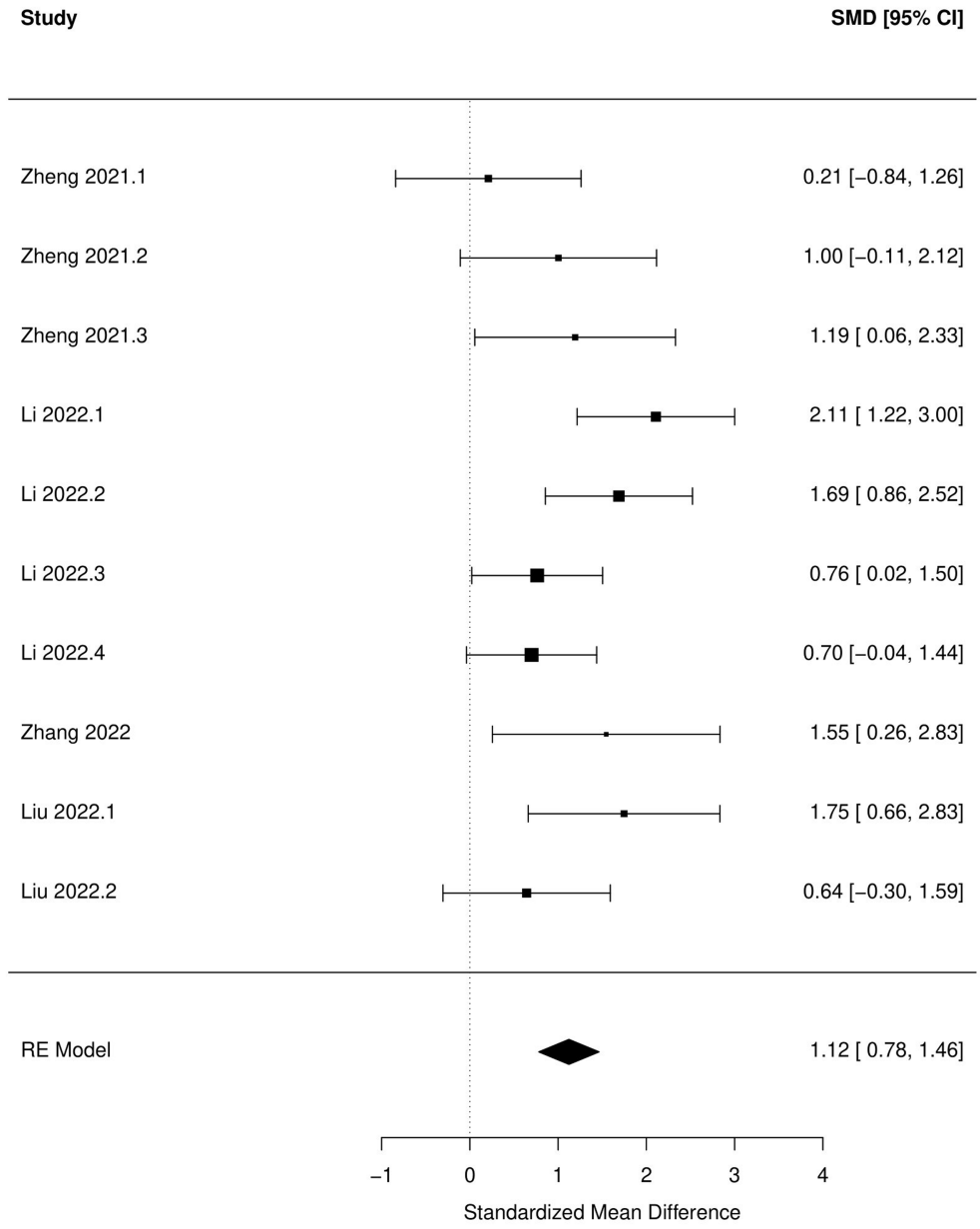


FIGURE 2
Forest plot of the random effects meta-analysis conducted using standardized mean differences of the latent time spend during Morris water maze test. "Study" is cross-referenced with the column "study population" in meta-analysis sheet in Supplementary material 2. Meta-data for each study can be assessed from this file. "SMD," standardized mean difference; "RE," random-effects model.

can all play a major role in determining the behavioral outcomes in rodents. Ampicillin, vancomycin, and penicillin V were the three most common antibiotics that were administered individually. Ampicillin is a broad-spectrum antibiotic and effective against both Gram-positive and-negative bacteria, whereas vancomycin and penicillin V are narrow spectrum antibiotics efficacious against only Gram-positive bacteria (Kahn and Line, 2010). Using antibiotics with differing modes of action and spectrum of action leads to differences in the gut dysbiosis induced (Septyaningtrias et al., 2020; Liu et al., 2022). It should also be noted that not all antibiotics have a potentially depressogenic effect. For example, minocycline (a broad-spectrum antibiotic) was used in one of the experiments to test its anxiolytic effect (Yao et al., 2022).

It can be hypothesized that using a cocktail of antibiotic classes can induce a broader disruption of the gut microbiome as compared to a single antibiotic. In the studies included in this review, a combination of ampicillin, neomycin, or vancomycin with other antibiotics was the most common cocktail used and should have hypothetically induced a greater disruption and hence, bigger changes in behaviors recorded. Surprisingly, this was not the case based on the narrative review and subgroup analysis. On the flip side, testing a single antibiotic for behavioral changes might have far more direct translational potential for clinical practice because single antibiotics are more commonly prescribed in clinical practice and a combination of antibiotics are reserved for more complicated cases (Leekha et al., 2011).

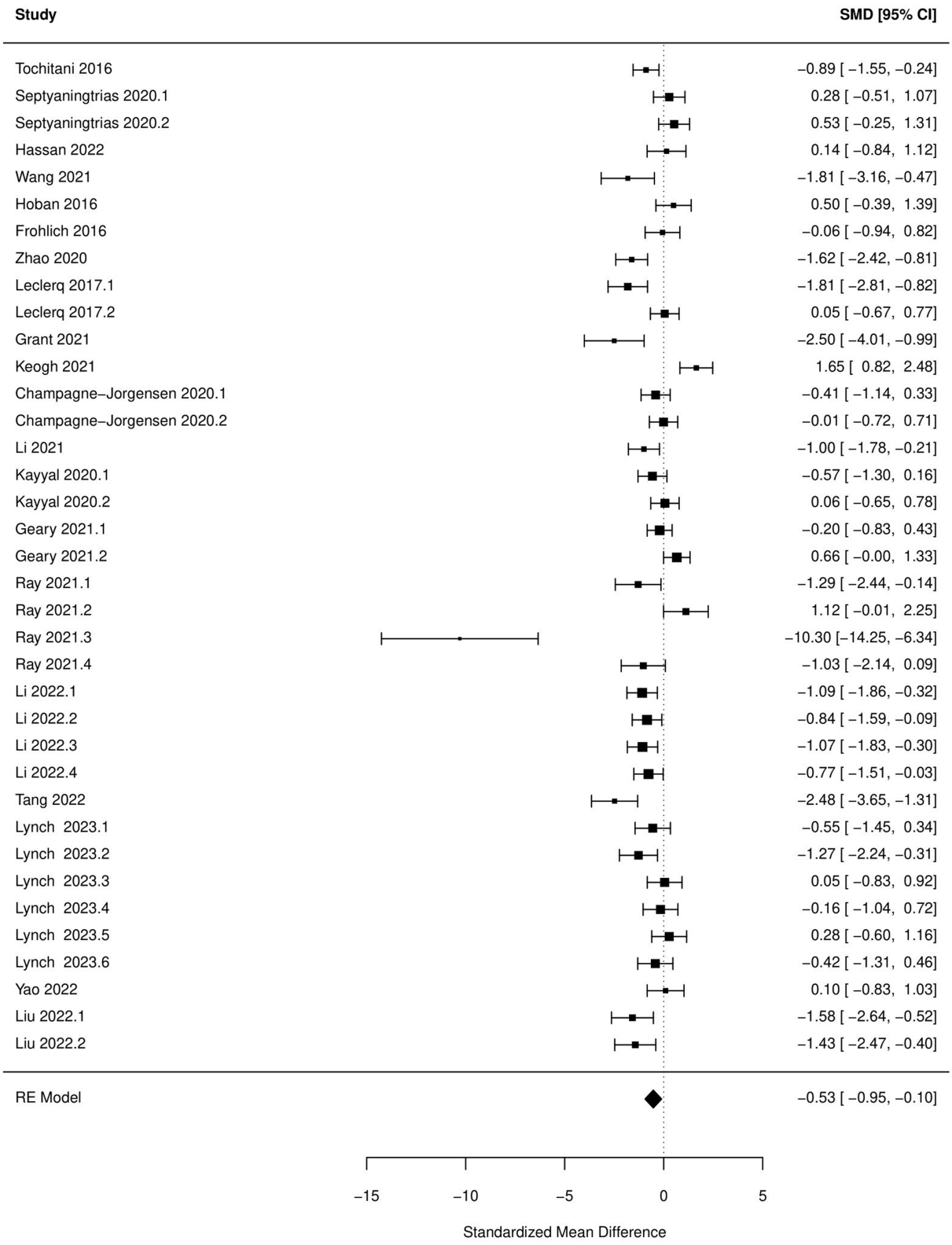


FIGURE 3
Forest plot of the random effects meta-analysis conducted using standardized mean differences of the time spend in center during open field test. "Study" is cross-referenced with the column "study population" in meta-analysis sheet in Supplementary material 2. Meta-data for each study can be assessed from this file. "SMD," standardized mean difference; "RE," random-effects model.

Some of the antibiotics used in these studies, such as metronidazole, ciprofloxacin, clindamycin etc. are easily absorbable from intestines (Hackam et al., 1998; Kasten, 1999). Systematic effects of these antibiotics after oral absorption can potentially confound the true relation between gut dysbiosis and behavioral alterations, especially if they cross blood–brain barrier. For example,

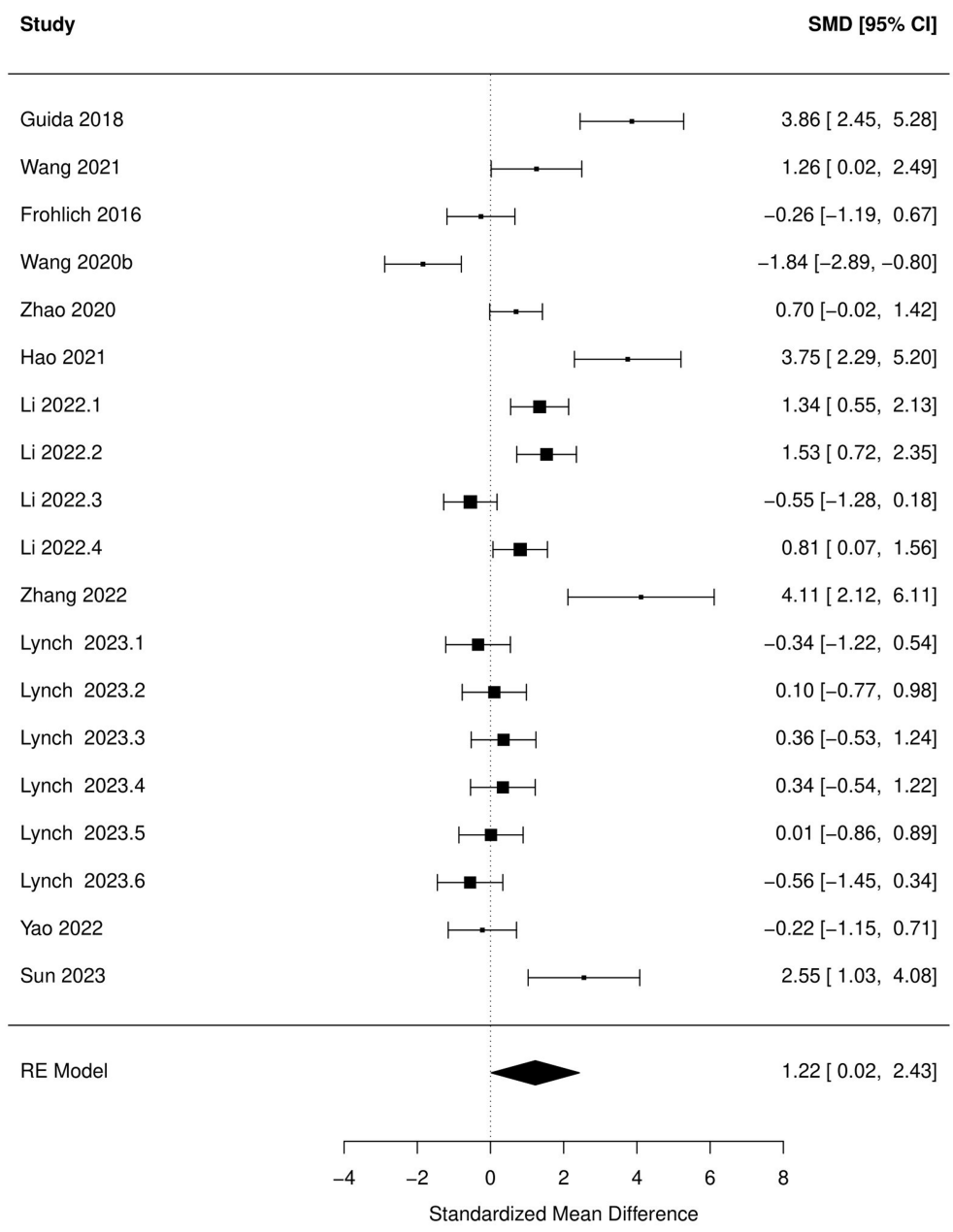


FIGURE 4
Forest plot of the random effects meta-analysis conducted using standardized mean differences of the immobile time during tail suspension test. "Study" is cross-referenced with the column "study population" in meta-analysis sheet in Supplementary material 2. Meta-data for each study can be assessed from this file. "SMD," standardized mean difference; "RE," random-effects model.

metronidazole can easily cross the blood–brain barrier (Tsai and Chen, 2003; Nau et al., 2010) and have been known to occasionally cause neuropathy and encephalopathy in humans (Sørensen et al., 2020). Ciprofloxacin has also been known to be directly toxic to the central nervous system (Ilgin et al., 2015).

Route of administration and time between antibiotic administration and evaluation of gut microbial populations are other factors that can bias the interpretation of the studies included in this review. Antibiotics given orally are far more likely to cause disruptions in gut bacterial populations as compared to parenteral route (Kelly et al., 2020; Zhou et al., 2020). In a very limited number of studies included in this review, rodents administered antibiotics parenterally displayed less changes in behavior and gut dysbiosis as compared to rodents given antibiotic orally. The impact of antibiotics on the disruption of gut microbiome is usually reversible, although the time taken to full recovery is still a question of research. Ray et al. (2021) demonstrated how the gut microbiome gradually returned to near normalcy within 60 days of vancomycin treatment in BALB/C mice. This was accompanied by a return to normal behavior. Baseline structure of gut microbial populations can also influence the response to antibiotics (Zhou et al., 2020). Unfortunately, such baseline data were missing from a vast majority of the studies.

Test setup

We did not include the reported testing methodology in our risk-of-bias assessment because each test was not conducted in every

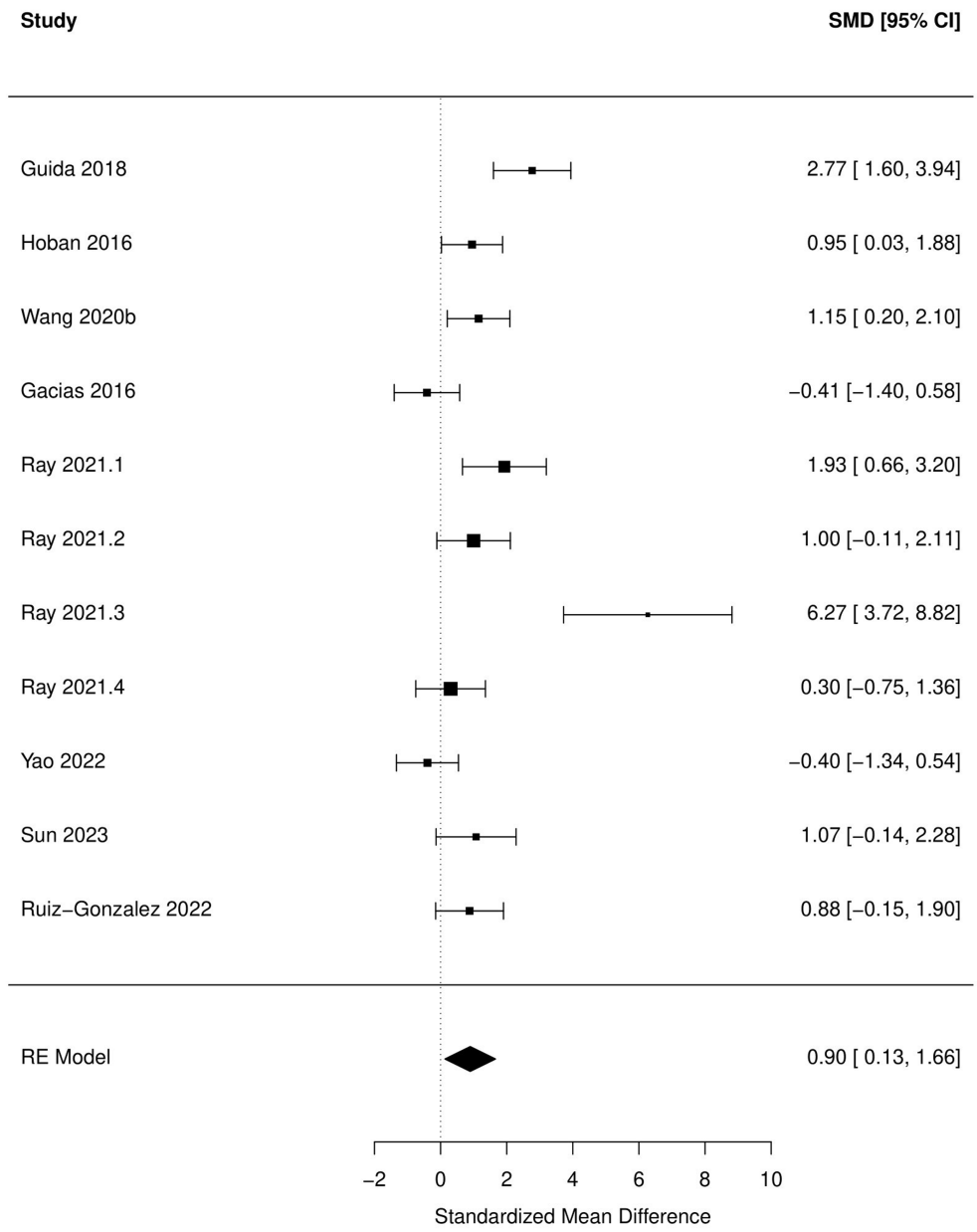


FIGURE 5
Forest plot of the random effects meta-analysis conducted on standardized mean differences of the immobile time during forced swim test. "Study" is cross-referenced with the column "study population" in meta-analysis sheet in Supplementary material 2. Meta-data for each study can be assessed from this file. "SMD," standardized mean difference; "RE," random-effects model.

study. The variability in how the tests were conducted might be one of the biggest factors influencing the results of behavioral outcomes. The detailed testing methodology was often not provided by the authors. Even when reported, there were huge discrepancies in testing methodology. For example, the trial time in OFT ranged from 3 min (Arslanova et al., 2021) to 2 h (Tochitani et al., 2016). This can have a significant effect on the exploratory behavior because the tendency to explore might decrease over time (Tochitani et al., 2016). Similarly, Septyaningtrias et al. (2020) and Hassan et al. (2022) considered the central 60 and 72% of the arena as the "central zone" in OFT, respectively. The designation of central area can have a direct impact on evaluation of the time spent in center, which is the main OFT metric used by several studies (Tochitani et al., 2016). The time between acquisition phase and testing phase (inter-trial period) also varied from 1 h (Fröhlich et al., 2016; Hoban et al., 2016; Lach et al., 2020) to 1 day (Saunders et al., 2020) across the studies. Sutcliffe et al. (2007) have demonstrated that the ability of male rats to distinguish between familiar and novel objects as well as overall object exploration time decreases drastically after 1 h of inter-trial period. We have highlighted only a few discrepancies that were reported in these studies. There are a huge range of factors that can influence the results of behavioral tests including dimensions of the apparatus, ambient temperature and humidity, ambient light, human handling, and habituation to a procedure (Rodgers and Dalvi, 1997;

TABLE 8 Brief results of the meta-analysis conducted for some common behaviors tested in rodents administered antibiotics.

	Model A			Model B		
Behavioral phenotype	*N*	Pooled SMD (95% CI)	*p* value	*N*	Pooled SMD (95% CI)	*p* value
EPM-open arm time	22	−0.96 (−1.96, 0.03)	0.06	14	−1.08 (−2.44, 0.28)	0.11
FST-immobile time	11	0.90 (0.13, 1.66)	0.03	9	0.90 (−0.20, 1.99)	0.10
L/D-time in light	10	−0.28 (−2.59, 2.03)	0.79	8	−0.63 (−3.43, 2.16)	0.61
MWM-time in target quadrant	9	−2.14 (−4.88, 0.59)	0.11	5	−4.07 (−10.6, 2.47)	0.16
MWM-escape latency	10	1.12 (0.78, 1.46)	<0.01	8	1.12 (0.72, 1.52)	<0.01
NOR-discrimination index	17	−1.07 (−2.23, 0.10)	0.07	11	−1.02 (−2.22, 0.18)	0.09
OFT-time center	37	−0.53 (−0.95, −0.10)	<0.01	22	−0.52 (−0.92, −0.13)	0.01
TST-immobile time	19	1.22 (0.02, 2.43)	0.05	16	1.23 (0.02, 2.45)	0.05

Model A used data from all the study populations and Model B used data from populations with homogenous characteristics (male mice administered antibiotics orally). "N," number of study populations; "SMD," standardized mean difference calculated from random-effects meta-analytic model; and "CI," confidence interval.

Kaidanovich-Beilin et al., 2011; Antunes and Biala, 2012; Bogdanova et al., 2013; Seibenhener and Wooten, 2015). We realize that there are no standardized experimental protocols to conduct these behavioral tests that have been uniformly adopted by the scientific community. But the authors can still provide details on the testing methodology in the supplementary material so that the behavioral outcomes can be compared across the studies.

Analysis of the gut microbiome

Although nearly all of the studies used 16S rRNA sequencing as a tool to study gut microbiome, the methodologies used to generate, process and analyze the sequencing data varied widely. Varying methodological considerations include the target regions sequenced, bioinformatic pipelines used, databases used for classification of bacterial taxa and statistical methods used. Comparison of performances between commonly used pipelines have revealed significant differences in the structure of bacterial populations and these differences were further dependent on the gene databases used (Almeida et al., 2018; López-García et al., 2018). Statistical methods, such as ANCOM-BC, LefSe, and Aldex2 used to identify differentially abundant taxa across experimental groups can produce discordant results to varying degrees (Wallen, 2021; Nearing et al., 2022). These are some of the challenges in generalizing the results of microbiome studies and are not limited to articles included here. Moreover, the results of 16S rRNA and shotgun sequencing can yield tens to hundreds of different bacterial taxa and it is not feasible for scientists to report on each bacterial taxon detected. However, authors should at the very least share the taxa tables generated by the bioinformatic pipelines, share the codes used using a public repository, and upload the raw reads on a gene repository (such as GenBank). This way adequate data will be available for the scientific community to compare the results across different studies. Direct correlations between behavior and abundances of bacterial taxa were seldomly reported in these studies. Availability of genomic data might have also allowed for an estimation of behavioral changes and bacterial taxa using a meta-analytic approach. Finally, differences in sampling and storage procedures can further contribute to the heterogeneity of the results.

Future direction—research domain criteria approach

To provide a better framework for conduction of psychiatric research, National Institute of Mental Health (NIMH) launched the Research Domain Criteria (RDoC) in 2013 (Cuthbert and Insel, 2013). The RDoC agenda strives to explicate fundamental bio-behavioral dimensions that span multiple current heterogeneous disorder categories (Insel et al., 2010). Since the RDoC domains are mainly constructed by basic neuroscience work including animal models, the animal model studies will have a much better opportunity to be aligned well with the RDoC frame. This can potentially lead to addressing fundamental questions on establishing neurobiological models for mental health disorders (Anderzhanova et al., 2017). In this regard, future studies of microbiota-gut-brain axis using animal models may seriously consider implementing the RDoC approach, by focusing on dimensional psychopathologies and underlying neurobiological mechanisms, avoiding focusing on categorical psychiatric diagnoses. In this regard, commonly used animal experiments to elicit behavioral phenotypes, can be considered within the RDoC framework as well (Table 2), which should be carefully considered when designing studies of microbiota-gut-brain axis.

Limitations

This study was limited by the various methodological variations, biases, incomplete reporting, and heterogeneity of the rodent populations studied. We limited search to the English language and did not search gray literature such as dissertations or preprint repositories, which might have led to the exclusion of some studies. We did not conduct a meta-analysis of the molecular mechanisms because these were not part of the original search string. Some of the terms such as anxiety or depression might not have direct translational value and this topic is debated among neuroscientists (Molendijk and de Kloet, 2019). However, we used these terms because of their simple interpretations and these are widely used in the existing literature, including the studies we collected the data from. We combined the results of different tests to form a behavioral phenotype. These tests might evaluate the same behavioral phenotype under different conditions and the results

might differ slightly (Steimer, 2011). This was done to simplify the narrative review and we have provided details for individual tests in the Supplementary material 2. We would also like to reiterate that significant heterogeneity (due to confounding factors such as sex, strain, age, antibiotics used, and different husbandry between studies and methodological variations), publication bias, small sample sizes, and inadequate data reporting limits the potential generalizability of the results of the meta-analysis.

Conclusion

The results of this systematic review meta-analysis provide some evidence that antibiotic-induced gut dysbiosis can indeed cause some behavioral changes in rodents. However, the heterogeneity between study populations, behavioral testing and microbiome evaluation makes it difficult to generalize these results. We are still far from establishing a model of antibiotic-induced gut dysbiosis and behavioral change in rodent models that can consistently reproduce the same results.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

SHa: conceptualization, conducting review, data analysis, and writing. SHw: writing. JC: conceptualization and writing. All authors contributed to the article and approved the submitted version.

Funding

This research was supported by startup funds provided by the University of Nebraska at Omaha and the Nebraska Food for Health Center to JC. Research reported in this publication was supported by the Office of the Director, National Institutes of Health of the National Institutes of Health under Award Number K01OD030514 awarded to JC. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnins.2023.1237177/full#supplementary-material

References

Almeida, A., Mitchell, A. L., Tarkowska, A., and Finn, R. D. (2018). Benchmarking taxonomic assignments based on 16S rRNA gene profiling of the microbiota from commonly sampled environments. *Gigascience* 7. doi: 10.1093/gigascience/giy054

Amirani, E., Milajerdi, A., Mirzaei, H., Jamilian, H., Mansournia, M. A., Hallajzadeh, J., et al. (2020). The effects of probiotic supplementation on mental health, biomarkers of inflammation and oxidative stress in patients with psychiatric disorders: a systematic review and meta-analysis of randomized controlled trials. *Complement. Ther. Med.* 49:102361. doi: 10.1016/j.ctim.2020.102361

Anderzhanova, E., Kirmeier, T., and Wotjak, C. T. (2017). Animal models in psychiatric research: the RDoC system as a new framework for endophenotype-oriented translational neuroscience. *Neurobiol. Stress* 7, 47–56. doi: 10.1016/j.ynstr.2017.03.003

Antunes, M., and Biala, G. (2012). The novel object recognition memory: neurobiology, test procedure, and its modifications. *Cogn. Process.* 13, 93–110. doi: 10.1007/s10339-011-0430-z

Arslanova, A., Tarasova, A., Alexandrova, A., Novoselova, V., Shaidullov, I., Khusnutdinova, D., et al. (2021). Protective effects of probiotics on cognitive and motor functions, anxiety level, visceral sensitivity, oxidative stress and microbiota in mice with antibiotic-induced Dysbiosis. *Lifestyles* 11:764. doi: 10.3390/life11080764

Bello-Medina, P. C., Corona-Cervantes, K., Zavala Torres, N. G., González, A., Pérez-Morales, M., González-Franco, D. A., et al. (2022). Chronic-antibiotics induced gut microbiota Dysbiosis rescues memory impairment and reduces β-amyloid aggregation in a preclinical Alzheimer's disease model. *Int. J. Mol. Sci.* 23, 1–25. doi: 10.3390/ijms23158209

Bercik, P., Denou, E., Collins, J., Jackson, W., Lu, J., Jury, J., et al. (2011). The intestinal microbiota affect central levels of brain-derived neurotropic factor and behavior in mice. *Gastroenterology* 141, 599–593. doi: 10.1053/j.gastro.2011.04.052

Bogdanova, O. V., Kanekar, S., D'Anci, K. E., and Renshaw, P. F. (2013). Factors influencing behavior in the forced swim test. *Physiol. Behav.* 118, 227–239. doi: 10.1016/j.physbeh.2013.05.012

Bonapersona, V., Kentrop, J., Van Lissa, C. J., van der Veen, R., Joëls, M., and Sarabdjitsingh, R. A. (2019). The behavioral phenotype of early life adversity: a 3-level meta-analysis of rodent studies. *Neurosci. Biobehav. Rev.* 102, 299–307. doi: 10.1016/j.neubiorev.2019.04.021

Börchers, S., Krieger, J.-P., Asker, M., Maric, I., and Skibicka, K. P. (2022). Commonly-used rodent tests of anxiety-like behavior lack predictive validity for human sex differences. *Psychoneuroendocrinology* 141:105733. doi: 10.1016/j.psyneuen.2022.105733

Bracken, M. B. (2009). Why animal studies are often poor predictors of human reactions to exposure. *J. R. Soc. Med.* 102, 120–122. doi: 10.1258/jrsm.2008.08k033

Champagne-Jorgensen, K., Mian, M. F., Kay, S., Hanani, H., Ziv, O., McVey Neufeld, K.-A., et al. (2020). Prenatal low-dose penicillin results in long-term sex-specific changes to murine behaviour, immune regulation, and gut microbiota. *Brain Behav. Immun.* 84, 154–163. doi: 10.1016/j.bbi.2019.11.020

Cho, N. A., Nicolucci, A. C., Klancic, T., Wang, W., Sharkey, K. A., Mychasiuk, R., et al. (2020). Impaired hypothalamic microglial activation in offspring of antibiotic-treated pregnant/lactating rats is attenuated by prebiotic Oligofructose co-administration. *Microorganisms* 8, 1–24. doi: 10.3390/microorganisms8071085

Cuthbert, B. N., and Insel, T. R. (2013). Toward the future of psychiatric diagnosis: the seven pillars of RDoC. *BMC Med.* 11:126. doi: 10.1186/1741-7015-11-126

Depino, A. M. (2015). Early prenatal exposure to LPS results in anxiety-and depression-related behaviors in adulthood. *Neuroscience* 299, 56–65. doi: 10.1016/j.neuroscience.2015.04.065

Der Simonian, R., and Laird, N. (2015). Meta-analysis in clinical trials revisited. *Contemp. Clin. Trials* 45, 139–145. doi: 10.1016/j.cct.2015.09.002

Desbonnet, L., Clarke, G., Traplin, A., O'Sullivan, O., Crispie, F., Moloney, R. D., et al. (2015). Gut microbiota depletion from early adolescence in mice: implications for brain and behaviour. *Brain Behav. Immun.* 48, 165–173. doi: 10.1016/j.bbi.2015.04.004

Duvallet, C., Gibbons, S. M., Gurry, T., Irizarry, R. A., and Alm, E. J. (2017). Meta-analysis of gut microbiome studies identifies disease-specific and shared responses. *Nat. Commun.* 8:1784. doi: 10.1038/s41467-017-01973-8

Egger, M., Davey Smith, G., Schneider, M., and Minder, C. (1997). Bias in meta-analysis detected by a simple, graphical test. *BMJ* 315, 629–634. doi: 10.1136/bmj.315.7109.629

Ellenbroek, B., and Youn, J. (2016). Rodent models in neuroscience research: is it a rat race? *Dis. Model. Mech.* 9, 1079–1087. doi: 10.1242/dmm.026120

Fröhlich, E. E., Farzi, A., Mayerhofer, R., Reichmann, F., Jačan, A., Wagner, B., et al. (2016). Cognitive impairment by antibiotic-induced gut dysbiosis: analysis of gut microbiota-brain communication. *Brain Behav. Immun.* 56, 140–155. doi: 10.1016/j.bbi.2016.02.020

Gacias, M., Gaspari, S., Santos, P.-M. G., Tamburini, S., Andrade, M., Zhang, F., et al. (2016). Microbiota-driven transcriptional changes in prefrontal cortex override genetic differences in social behavior. *elife* 5, 1–27. doi: 10.7554/eLife.13442

Geary, C. G., Wilk, V. C., Barton, K. L., Jefferson, P. O., Binder, T., Bhutani, V., et al. (2021). Sex differences in gut microbiota modulation of aversive conditioning, open field activity, and basolateral amygdala dendritic spine density. *J. Neurosci. Res.* 99, 1780–1801. doi: 10.1002/jnr.24848

Grant, C. V., Loman, B. R., Bailey, M. T., and Pyter, L. M. (2021). Manipulations of the gut microbiome alter chemotherapy-induced inflammation and behavioral side effects in female mice. *Brain Behav. Immun.* 95, 401–412. doi: 10.1016/j.bbi.2021.04.014

Guida, F., Turco, F., Iannotta, M., De Gregorio, D., Palumbo, I., Sarnelli, G., et al. (2018). Antibiotic-induced microbiota perturbation causes gut endocannabinoidome changes, hippocampal neuroglial reorganization and depression in mice. *Brain Behav. Immun.* 67, 230–245. doi: 10.1016/j.bbi.2017.09.001

Hackam, D. J., Christou, N., Khaliq, Y., Duffy, D. R., Vaughan, D., Marshall, J. C., et al. (1998). Bioavailability of oral ciprofloxacin in early postsurgical patients. *Arch. Surg.* 133, 1221–1225. doi: 10.1001/archsurg.133.11.1221

Hao, W.-Z., Li, X.-J., Zhang, P.-W., and Chen, J.-X. (2020). A review of antibiotics, depression, and the gut microbiome. *Psychiatry Res.* 284:112691. doi: 10.1016/j.psychres.2019.112691

Hao, W., Wu, J., Yuan, N., Gong, L., Huang, J., Ma, Q., et al. (2021). Xiaoyaosan improves antibiotic-induced depressive-like and anxiety-like behavior in mice through modulating the gut microbiota and regulating the NLRP3 Inflammasome in the Colon. *Front. Pharmacol.* 12:619103. doi: 10.3389/fphar.2021.619103

Hassan, A. M., Mancano, G., Kashofer, K., Liebisch, G., Farzi, A., Zenz, G., et al. (2022). Anhedonia induced by high-fat diet in mice depends on gut microbiota and leptin. *Nutr. Neurosci.* 25, 299–312. doi: 10.1080/1028415X.2020.1751508

Hoban, A. E., Moloney, R. D., Golubeva, A. V., McVey Neufeld, K. A., O'Sullivan, O., Patterson, E., et al. (2016). Behavioural and neurochemical consequences of chronic gut microbiota depletion during adulthood in the rat. *Neuroscience* 339, 463–477. doi: 10.1016/j.neuroscience.2016.10.003

Hooijmans, C. R., Rovers, M. M., de Vries, R. B. M., Leenaars, M., Ritskes-Hoitinga, M., and Langendam, M. W. (2014). SYRCLE's risk of bias tool for animal studies. *BMC Med. Res. Methodol.* 14:43. doi: 10.1186/1471-2288-14-43

Ilgin, S., Can, O. D., Atli, O., Ucel, U. I., Sener, E., and Guven, I. (2015). Ciprofloxacin-induced neurotoxicity: evaluation of possible underlying mechanisms. *Toxicol. Mech. Methods* 25, 374–381. doi: 10.3109/15376516.2015.1026008

Insel, T., Cuthbert, B., Garvey, M., Heinssen, R., Pine, D. S., Quinn, K., et al. (2010). Research domain criteria (RDoC): toward a new classification framework for research on mental disorders. *Am. J. Psychiatry* 167, 748–751. doi: 10.1176/appi.ajp.2010.09091379

Jang, H.-M., Lee, H.-J., Jang, S.-E., Han, M. J., and Kim, D.-H. (2018). Evidence for interplay among antibacterial-induced gut microbiota disturbance, neuro-inflammation, and anxiety in mice. *Mucosal Immunol.* 11, 1386–1397. doi: 10.1038/s41385-018-0042-3

Jeong, M.-Y., Jang, H.-M., and Kim, D.-H. (2019). High-fat diet causes psychiatric disorders in mice by increasing Proteobacteria population. *Neurosci. Lett.* 698, 51–57. doi: 10.1016/j.neulet.2019.01.006

Kahn, C. M., and Line, S. (2010). *The Merck Veterinary Manual*. NJ: Merck Kenilworth

Kaidanovich-Beilin, O., Lipina, T., Vukobradovic, I., Roder, J., and Woodgett, J. R. (2011). Assessment of social interaction behaviors. *J. Vis. Exp.* doi: 10.3791/2473

Karp, N. A., Pearl, E. J., Stringer, E. J., Barkus, C., Ulrichsen, J. C., and du Sert, N. P. (2022). A qualitative study of the barriers to using blinding in in vivo experiments and suggestions for improvement. *PLoS Biol.* 20:e3001873. doi: 10.1371/journal.pbio.3001873

Kasten, M. J. (1999). Clindamycin, metronidazole, and chloramphenicol. *Mayo Clin. Proc.* 74, 825–833. doi: 10.4065/74.8.825

Kayyal, M., Javkar, T., Firoz Mian, M., Binyamin, D., Koren, O., McVey Neufeld, K.-A., et al. (2020). Publisher correction: sex dependent effects of post-natal penicillin on brain, behavior and immune regulation are prevented by concurrent probiotic treatment. *Sci. Rep.* 10:18355. doi: 10.1038/s41598-020-75606-4

Kelly, S. A., Rodgers, A. M., O'Brien, S. C., Donnelly, R. F., and Gilmore, B. F. (2020). Gut check time: antibiotic delivery strategies to reduce antimicrobial resistance. *Trends Biotechnol.* 38, 447–462. doi: 10.1016/j.tibtech.2019.10.008

Keogh, C. E., Kim, D. H. J., Pusceddu, M. M., Knotts, T. A., Rabasa, G., Sladek, J. A., et al. (2021). Myelin as a regulator of development of the microbiota-gut-brain axis. *Brain Behav. Immun.* 91, 437–450. doi: 10.1016/j.bbi.2020.11.001

Lach, G., Fülling, C., Bastiaanssen, T. F. S., Fouhy, F., Donovan, A. N. O., Ventura-Silva, A. P., et al. (2020). Enduring neurobehavioral effects induced by microbiota depletion during the adolescent period. *Transl. Psychiatry* 10:382. doi: 10.1038/s41398-020-01073-0

Lai, S., Wang, J., Wang, B., Wang, R., Li, G., Jia, Y., et al. (2022). Alterations in gut microbiota affect behavioral and inflammatory responses to methamphetamine in mice. *Psychopharmacology* 239, 1–16. doi: 10.1007/s00213-022-06154-0

Leclercq, S., Mian, F. M., Stanisz, A. M., Bindels, L. B., Cambier, E., Ben-Amram, H., et al. (2017). Low-dose penicillin in early life induces long-term changes in murine gut microbiota, brain cytokines and behavior. *Nat. Commun.* 8:15062. doi: 10.1038/ncomms15062

Leekha, S., Terrell, C. L., and Edson, R. S. (2011). General principles of antimicrobial therapy. *Mayo Clin. Proc.* 86, 156–167. doi: 10.4065/mcp.2010.0639

Leigh, S.-J., Kaakoush, N. O., Westbrook, R. F., and Morris, M. J. (2020). Minocycline-induced microbiome alterations predict cafeteria diet-induced spatial recognition memory impairments in rats. *Transl. Psychiatry* 10:92. doi: 10.1038/s41398-020-0774-1

Li, J., Pu, F., Peng, C., Wang, Y., Zhang, Y., Wu, S., et al. (2022). Antibiotic cocktail-induced gut microbiota depletion in different stages could cause host cognitive impairment and emotional disorders in adulthood in different manners. *Neurobiol. Dis.* 170:105757. doi: 10.1016/j.nbd.2022.105757

Li, H., Xiang, Y., Zhu, Z., Wang, W., Jiang, Z., Zhao, M., et al. (2021). Rifaximin-mediated gut microbiota regulation modulates the function of microglia and protects against CUMS-induced depression-like behaviors in adolescent rat. *J. Neuroinflammation* 18:254. doi: 10.1186/s12974-021-02303-y

Lin, T.-L., Shu, C.-C., Chen, Y.-M., Lu, J.-J., Wu, T.-S., Lai, W.-F., et al. (2020). Like cures like: pharmacological activity of anti-inflammatory lipopolysaccharides from gut microbiome. *Front. Pharmacol.* 11:554. doi: 10.3389/fphar.2020.00554

Liu, G., Yu, Q., Tan, B., Ke, X., Zhang, C., Li, H., et al. (2022). Gut dysbiosis impairs hippocampal plasticity and behaviors by remodeling serum metabolome. *Gut Microbes* 14:2104089. doi: 10.1080/19490976.2022.2104089

Liu, X., Zhang, J., Sang, Y., Liu, K., Zhu, Y., Yang, L., et al. (2021). Antibiotic exposure and potential risk of depression in the Chinese elderly: a biomonitoring-based population study. *Environ. Sci. Pollut. Res. Int.* 28, 26794–26806. doi: 10.1007/s11356-021-12560-2

López-García, A., Pineda-Quiroga, C., Atxaerandio, R., Pérez, A., Hernández, I., García-Rodríguez, A., et al. (2018). Comparison of Mothur and QIIME for the analysis of rumen microbiota composition based on 16S rRNA amplicon sequences. *Front. Microbiol.* 9:3010. doi: 10.3389/fmicb.2018.03010

Luo, A., Li, S., Wang, X., Xie, Z., Li, S., and Hua, D. (2021). Cefazolin improves anesthesia and surgery-induced cognitive impairments by modulating blood-brain barrier function, gut Bacteria and short chain fatty acids. *Front. Aging Neurosci.* 13:748637. doi: 10.3389/fnagi.2021.748637

Luo, N., Zhu, W., Li, X., Fu, M., Peng, X., Yang, F., et al. (2022). Impact of gut microbiota on radiation-associated cognitive dysfunction and Neuroinflammation in mice. *Radiat. Res.* 197, 350–364. doi: 10.1667/RADE-21-00006.1

Lurie, I., Yang, Y.-X., Haynes, K., Mamtani, R., and Boursi, B. (2015). Antibiotic exposure and the risk for depression, anxiety, or psychosis: a nested case-control study. *J. Clin. Psychiatry* 76, 1522–1528. doi: 10.4088/JCP.15m09961

Lynch, C. M. K., Cowan, C. S. M., Bastiaanssen, T. F. S., Moloney, G. M., Theune, N., van de Wouw, M., et al. (2023). Critical windows of early-life microbiota disruption on behaviour, neuroimmune function, and neurodevelopment. *Brain Behav. Immun.* 108, 309–327. doi: 10.1016/j.bbi.2022.12.008

Ma, T., Jin, H., Kwok, L.-Y., Sun, Z., Liong, M.-T., and Zhang, H. (2021). Probiotic consumption relieved human stress and anxiety symptoms possibly via modulating the neuroactive potential of the gut microbiota. *Neurobiol. Stress* 14:100294. doi: 10.1016/j.ynstr.2021.100294

Mezö, C., Dokalis, N., Mossad, O., Staszewski, O., Neuber, J., Yilmaz, B., et al. (2020). Different effects of constitutive and induced microbiota modulation on microglia in a mouse model of Alzheimer's disease. *Acta Neuropathol. Commun.* 8:119. doi: 10.1186/s40478-020-00988-5

Molendijk, M. L., and de Kloet, E. R. (2019). Coping with the forced swim stressor: current state-of-the-art. *Behav. Brain Res.* 364, 1–10. doi: 10.1016/j.bbr.2019.02.005

Morozova, M. V., Borisova, M. A., Snytnikova, O. A., Achasova, K. M., Litvinova, E. A., Tsentalovich, Y. P., et al. (2022). Colitis-associated intestinal microbiota regulates brain glycine and host behavior in mice. *Sci. Rep.* 12:16345. doi: 10.1038/s41598-022-19219-z

Morris, S. E., and Cuthbert, B. N. (2012). Research domain criteria: cognitive systems, neural circuits, and dimensions of behavior. *Dialogues Clin. Neurosci.* 14, 29–37. doi: 10.31887/DCNS.2012.14.1/smorris

Nau, R., Sörgel, F., and Eiffert, H. (2010). Penetration of drugs through the blood-cerebrospinal fluid/blood-brain barrier for treatment of central nervous system infections. *Clin. Microbiol. Rev.* 23, 858–883. doi: 10.1128/CMR.00007-10

Nearing, J. T., Douglas, G. M., Hayes, M. G., Mac Donald, J., Desai, D. K., Allward, N., et al. (2022). Microbiome differential abundance methods produce different results across 38 datasets. *Nat. Commun.* 13:342. doi: 10.1038/s41467-022-28034-z

Olavarría-Ramírez, L., Cooney-Quane, J., Murphy, G., McCafferty, C. P., Cryan, J. F., and Dockray, S. (2023). A systematic review of the effects of gut microbiota depletion on social and anxiety-related behaviours in adult rodents: implications for translational research. *Neurosci. Biobehav. Rev.* 145:105013. doi: 10.1016/j.neubiorev.2022.105013

Othman, M. Z., Hassan, Z., and Che Has, A. T. (2022). Morris water maze: a versatile and pertinent tool for assessing spatial learning and memory. *Exp. Anim.* 71, 264–280. doi: 10.1538/expanim.21-0120

Porsolt, R. D., Anton, G., Blavet, N., and Jalfre, M. (1978). Behavioural despair in rats: a new model sensitive to antidepressant treatments. *Eur. J. Pharmacol.* 47, 379–391. doi: 10.1016/0014-2999(78)90118-8

Ramirez, J., Guarner, F., Bustos Fernandez, L., Maruy, A., Sdepanian, V. L., and Cohen, H. (2020). Antibiotics as major disruptors of gut microbiota. *Front. Cell. Infect. Microbiol.* 10:572912. doi: 10.3389/fcimb.2020.572912

Ray, P., Pandey, U., Das, D., and Aich, P. (2021). Vancomycin-induced changes in host immunity and behavior: comparative genomic and metagenomic analysis in C57BL/6 and BALB/c mice. *Dig. Dis. Sci.* 66, 3776–3791. doi: 10.1007/s10620-020-06729-x

Reyes, R. E. N., Al Omran, A. J., Davies, D. L., and Asatryan, L. (2020). Antibiotic-induced disruption of commensal microbiome linked to increases in binge-like ethanol consumption behavior. *Brain Res.* 1747:147067. doi: 10.1016/j.brainres.2020.147067

Rodgers, R. J., and Dalvi, A. (1997). Anxiety, defence and the elevated plus-maze. *Neurosci. Biobehav. Rev.* 21, 801–810. doi: 10.1016/S0149-7634(96)00058-9

Rohatgi, A. (2014). Web PlotDigitizer user manual version 3.4. Available at: http://arohatgi.info/WebPlotDigitizer/app, 1–18

Roy Sarkar, S., Mitra Mazumder, P., and Banerjee, S. (2020). Probiotics protect against gut dysbiosis associated decline in learning and memory. *J. Neuroimmunol.* 348:577390. doi: 10.1016/j.jneuroim.2020.577390

Ruiz-González, R., Lajud, N., Tejeda-Martínez, A. R., Flores-Soto, M. E., Valdez-Alarcón, J. J., Tellez, L. A., et al. (2022). Antibiotic-induced microbiota depletion in normally-reared adult rats mimics the neuroendocrine effects of early life stress. *Brain Res.* 1793:148055. doi: 10.1016/j.brainres.2022.148055

Sarkar, S. R., Mazumder, P. M., and Banerjee, S. (2022). Oligosaccharide and Flavanoid mediated prebiotic interventions to treat gut Dysbiosis associated cognitive decline. *J. NeuroImmune Pharmacol.* 17, 94–110. doi: 10.1007/s11481-021-10041-4

Saunders, J. M., Moreno, J. L., Ibi, D., Sikaroodi, M., Kang, D. J., Muñoz-Moreno, R., et al. (2020). Gut microbiota manipulation during the prepubertal period shapes behavioral abnormalities in a mouse neurodevelopmental disorder model. *Sci. Rep.* 10:4697. doi: 10.1038/s41598-020-61635-6

Seibenhener, M. L., and Wooten, M. C. (2015). Use of the open field maze to measure locomotor and anxiety-like behavior in mice. *J. Vis. Exp.* 1–6. doi: 10.3791/52434

Seliger, D. L. (1977). Effects of age, sex, and brightness of riel on open-field behaviors of rats. *Percept. Mot. Skills* 45, 1059–1067. doi: 10.2466/pms.1977.45.3f.1059

Septyaningtrias, D. E., Lin, C.-W., Ouchida, R., Nakai, N., Suda, W., Hattori, M., et al. (2020). Altered microbiota composition reflects enhanced communication in 15q11-13 CNV mice. *Neurosci. Res.* 161, 59–67. doi: 10.1016/j.neures.2019.12.010

Sherwin, E., Bordenstein, S. R., Quinn, J. L., Dinan, T. G., and Cryan, J. F. (2019). Microbiota and the social brain. *Science* 366, 1–15. doi: 10.1126/science.aar2016

Simpson, C. A., Mu, A., Haslam, N., Schwartz, O. S., and Simmons, J. G. (2020). Feeling down? A systematic review of the gut microbiota in anxiety/depression and irritable bowel syndrome. *J. Affect. Disord.* 266, 429–446. doi: 10.1016/j.jad.2020.01.124

Sørensen, C. G., Karlsson, W. K., Amin, F. M., and Lindelof, M. (2020). Metronidazole-induced encephalopathy: a systematic review. *J. Neurol.* 267, 1–13. doi: 10.1007/s00415-018-9147-6

Steimer, T. (2011). Animal models of anxiety disorders in rats and mice: some conceptual issues. *Dialogues Clin. Neurosci.* 13, 495–506. doi: 10.31887/DCNS.2011.13.4/tsteimer

Sun, Y., Zhu, H., Cheng, R., Tang, Z., and Zhang, M. (2023). Outer membrane protein Amuc_1100 of *Akkermansia muciniphila* alleviates antibiotic-induced anxiety and depression-like behavior in mice. *Physiol. Behav.* 258:114023. doi: 10.1016/j.physbeh.2022.114023

Sutcliffe, J. S., Marshall, K. M., and Neill, J. C. (2007). Influence of gender on working and spatial memory in the novel object recognition task in the rat. *Behav. Brain Res.* 177, 117–125. doi: 10.1016/j.bbr.2006.10.029

Tang, Q., Li, S., Fang, C., and Yu, H. (2022). Evaluating the reparative effects and the mechanism of action of docosahexaenoic acid on azithromycin-induced lipid metabolism dysfunction. *Food Chem. Toxicol.* 159:112699. doi: 10.1016/j.fct.2021.112699

Theofilis, P., Sagris, M., Oikonomou, E., Antonopoulos, A. S., Siasos, G., Tsioufis, K., et al. (2022). The impact of SGLT2 inhibitors on inflammation: a systematic review and meta-analysis of studies in rodents. *Int. Immunopharmacol.* 111:109080. doi: 10.1016/j.intimp.2022.109080

Tochitani, S., Ikeno, T., Ito, T., Sakurai, A., Yamauchi, T., and Matsuzaki, H. (2016). Administration of non-Absorbable Antibiotics to pregnant mice to perturb the maternal gut microbiota is associated with alterations in offspring behavior. *PLoS One* 11:e0138293. doi: 10.1371/journal.pone.0138293

Tsai, T.-H., and Chen, Y.-F. (2003). Pharmacokinetics of metronidazole in rat blood, brain and bile studied by microdialysis coupled to microbore liquid chromatography. *J. Chromatogr. A* 987, 277–282. doi: 10.1016/S0021-9673(02)01454-1

van Eeden, W. A., El Filali, E., van Hemert, A. M., Carlier, I. V. E., Penninx, B. W. J. H., Lamers, F., et al. (2021). Basal and LPS-stimulated inflammatory markers and the course of anxiety symptoms. *Brain Behav. Immun.* 98, 378–387. doi: 10.1016/j.bbi.2021.09.001

van Gaalen, M. M., and Steckler, T. (2000). Behavioural analysis of four mouse strains in an anxiety test battery. *Behav. Brain Res.* 115, 95–106. doi: 10.1016/S0166-4328(00)00240-0

Van Voorhis, C. R. W., and Morgan, B. L., and Others (2007). Understanding power and rules of thumb for determining sample sizes. *Tutor. Quant. Methods Psychol.* 3, 43–50. doi: 10.20982/tqmp.03.2.p043

Viechtbauer, W. (2010). Conducting Meta-analyses in Rwith themetafor package. *J. Stat. Softw.* 36, 1–48. doi: 10.18637/jss.v036.i03

Wallen, Z. D. (2021). Comparison study of differential abundance testing methods using two large Parkinson disease gut microbiome datasets derived from 16S amplicon sequencing. *BMC Bioinformatics* 22:265. doi: 10.1186/s12859-021-04193-6

Wang, S., Ishima, T., Zhang, J., Qu, Y., Chang, L., Pu, Y., et al. (2020a). Ingestion of Lactobacillus intestinalis and *Lactobacillus reuteri* causes depression-and anhedonia-like phenotypes in antibiotic-treated mice via the vagus nerve. *J. Neuroinflammation* 17:241. doi: 10.1186/s12974-020-01916-z

Wang, D., Levine, J. L. S., Avila-Quintero, V., Bloch, M., and Kaffman, A. (2020). Systematic review and meta-analysis: effects of maternal separation on anxiety-like behavior in rodents. *Transl. Psychiatry* 10:174. doi: 10.1038/s41398-020-0856-0

Wang, S., Qu, Y., Chang, L., Pu, Y., Zhang, K., and Hashimoto, K. (2020b). Antibiotic-induced microbiome depletion is associated with resilience in mice after chronic social defeat stress. *J. Affect. Disord.* 260, 448–457. doi: 10.1016/j.jad.2019.09.064

Wang, P., Tu, K., Cao, P., Yang, Y., Zhang, H., Qiu, X.-T., et al. (2021). Correction to: antibiotics-induced intestinal dysbacteriosis caused behavioral alternations and neuronal activation in different brain regions in mice. *Mol. Brain* 14:69. doi: 10.1186/s13041-021-00781-y

Yao, H., Zhang, D., Yu, H., Shen, H., Lan, X., Liu, H., et al. (2022). Chronic ethanol exposure induced anxiety-like behaviour by altering gut microbiota and GABA system. *Addict. Biol.* 27:e13203. doi: 10.1111/adb.13203

Yu, M., Jia, H.-M., Qin, L.-L., and Zou, Z.-M. (2022). Gut microbiota and gut tissue metabolites involved in development and prevention of depression. *J. Affect. Disord.* 297, 8–17. doi: 10.1016/j.jad.2021.10.016

Zhang, Y., Liang, H., Wang, Y., Cheng, R., Pu, F., Yang, Y., et al. (2022). Heat-inactivated Lacticaseibacillus paracasei N1115 alleviates the damage due to brain function caused by long-term antibiotic cocktail exposure in mice. *BMC Neurosci.* 23:38. doi: 10.1186/s12868-022-00724-w

Zhang, J., Liu, K., Sun, L., Yang, L., Liu, X., Zhu, Y., et al. (2021). Exposure to antibiotics and mental disorders in children: a community-based cross-sectional study. *Environ. Geochem. Health* 43, 3237–3253. doi: 10.1007/s10653-021-00840-2

Zhang, Z., Xue, C., Ju, M., Guo, J., Wang, M., Yi, S., et al. (2021). Maternal gut Dysbiosis alters offspring microbiota and social interactions. *Microorganisms* 9, 1–14. doi: 10.3390/microorganisms9081742

Zhang, N., Zhang, Y., Li, M., Wang, W., Liu, Z., Xi, C., et al. (2020). Efficacy of probiotics on stress in healthy volunteers: a systematic review and meta-analysis based on randomized controlled trials. *Brain Behav.* 10:e01699. doi: 10.1002/brb3.1699

Zhao, Z., Wang, B., Mu, L., Wang, H., Luo, J., Yang, Y., et al. (2020). Long-term exposure to ceftriaxone sodium induces alteration of gut microbiota accompanied by abnormal behaviors in mice. *Front. Cell. Infect. Microbiol.* 10:258. doi: 10.3389/fcimb.2020.00258

Zheng, H., Xu, P., Jiang, Q., Xu, Q., Zheng, Y., Yan, J., et al. (2021). Correction to: depletion of acetate-producing bacteria from the gut microbiota facilitates cognitive impairment through the gut-brain neural mechanism in diabetic mice. *Microbiome* 9:164. doi: 10.1186/s40168-021-01127-5

Zhou, Y., Li, Y., Zhang, L., Wu, Z., Huang, Y., Yan, H., et al. (2020). Antibiotic administration routes and Oral exposure to antibiotic resistant Bacteria as key drivers for gut microbiota disruption and Resistome in poultry. *Front. Microbiol.* 11:1319. doi: 10.3389/fmicb.2020.01319

frontiers | Frontiers in Neuroscience

TYPE Original Research
PUBLISHED 22 December 2023
DOI 10.3389/fnins.2023.1296145

OPEN ACCESS

EDITED BY
Zuleide Maria Ignácio,
Federal University of the Southern Frontier, Brazil

REVIEWED BY
Benneth Ben-Azu,
Delta State University, Abraka, Nigeria
Jiangtao Yu,
Fuyang City People's Hospital, China

*CORRESPONDENCE
Chunmei Chen
✉ cmchen2009@sina.com

†These authors share first authorship

RECEIVED 18 September 2023
ACCEPTED 05 December 2023
PUBLISHED 22 December 2023

CITATION
Zhou M, Chen S, Chen Y, Wang C and Chen C (2023) Causal associations between gut microbiota and regional cortical structure: a Mendelian randomization study. *Front. Neurosci.* 17:1296145. doi: 10.3389/fnins.2023.1296145

Causal associations between gut microbiota and regional cortical structure: a Mendelian randomization study

Maochao Zhou[1†], Song Chen[1†], Yan Chen[1], Chunhua Wang[2] and Chunmei Chen[1*]

[1]Department of Neurosurgery, Fujian Medical University Union Hospital, Fuzhou, China, [2]Fujian Institute of Neurosurgery, Fuzhou, China

Introduction: Observational studies have reported associations between gut microbiota composition and central nervous system diseases. However, the potential causal relationships and underlying mechanisms remain unclear. Here, we applied Mendelian randomization (MR) to investigate the causal effects of gut microbiota on cortical surface area (SA) and thickness (TH) in the brain.

Methods: We used genome-wide association study summary statistics of gut microbiota abundance in 18,340 individuals from the MiBioGen Consortium to identify genetic instruments for 196 gut microbial taxa. We then analyzed data from 56,761 individuals from the ENIGMA Consortium to examine associations of genetically predicted gut microbiota with alterations in cortical SA and TH globally and across 34 functional brain regions. Inverse-variance weighted analysis was used as the primary MR method, with MR Egger regression, MR-PRESSO, Cochran's *Q* test, and leave-one-out analysis to assess heterogeneity and pleiotropy.

Results: At the functional region level, genetically predicted higher abundance of class Mollicutes was associated with greater SA of the medial orbitofrontal cortex (β = 8.39 mm^2, 95% CI: 3.08–13.70 mm^2, p = 0.002), as was higher abundance of phylum Tenericutes (β = 8.39 mm^2, 95% CI: 3.08–13.70 mm^2, p = 0.002). Additionally, higher abundance of phylum Tenericutes was associated with greater SA of the lateral orbitofrontal cortex (β = 10.51 mm^2, 95% CI: 3.24–17.79 mm^2, p = 0.0046). No evidence of heterogeneity or pleiotropy was detected.

Conclusion: Specific gut microbiota may causally influence cortical structure in brain regions involved in neuropsychiatric disorders. The findings provide evidence for a gut-brain axis influencing cortical development, particularly in the orbitofrontal cortex during adolescence.

KEYWORDS

gut microbiota, brain cortical structure, Mendelian randomization study, gut-brain axis, neuropsychiatric disorders

1 Introduction

The gut microbiota, containing trillions of microbes, influences body function through critical pathways involving the enteric nervous system that connects to the central nervous system (Dupont et al., 2020). From an evolutionary perspective, the hologenome theory posits that genetic variation in the holobiont arises from both the host genome and microbiome (Cryan et al., 2019), underscoring the essential role of the gut microbiota in modulating brain function. 16S rRNA gene sequencing enables large-scale profiling of microbial abundances in a cost-effective manner. Accumulating evidence suggests gut dysbiosis contributes to various central nervous system conditions like Alzheimer's, Parkinson's, depression, and anxiety (Aho et al., 2019; Yang et al., 2020; Qian et al., 2021; Simpson et al., 2021).

The cerebral cortex, vital for cognitive abilities, can be measured *in vivo* using magnetic resonance imaging (MRI) to assess cortical surface area (SA) and thickness (TH) associated with neurological, psychological, and behavioral traits (Thompson et al., 2020). Hence, it's feasible that using the cortical SA and TH as parameters for brain functions and structure alteration. Attention on the gut-brain axis, describing bidirectional signaling between the gut and brain (Cryan et al., 2019), has surged given demonstrations that the gut microbiota influences brain function through immune, endocrine, vagus nerve, and inflammatory pathways (Bravo et al., 2011; Cussotto et al., 2018; Matheoud et al., 2019).

MRI has provided an ideal approach for studying gut-brain interactions preclinically and clinically. A preclinical study using diffusion tensor imaging identified diet-dependent changes in white matter integrity associated with gut microbiome alterations in rats (Ong et al., 2018). Clinically, consuming fermented milk affected resting brain activity in healthy women, particularly in the periaqueductal gray, prefrontal cortex, basal ganglia, precuneus, and parahippocampal gyrus (Tillisch et al., 2013). Studies have uncovered links between irritable bowel syndrome, microbiota shifts, and brain changes (Labus et al., 2017; Pinto-Sanchez et al., 2017). A *Bifidobacterium longum* strain reduced amygdala and frontal-limbic responses to negative stimuli (Labus et al., 2017). Prevotella abundance associated with right hippocampal differences in emotional processing compared to Bacteroides (Pinto-Sanchez et al., 2017). Emerging evidence also connects the gut microbiota to neuropsychiatric disorders. One study linked schizophrenia prodrome to altered gut microbiota and elevated choline in the anterior cingulate cortex, potentially from increased short-chain fatty acid production (He et al., 2018). The gut microbiota also affects early brain development, with higher alpha diversity correlating to slower development but minimal impact on regional brain volumes at age two (Carlson et al., 2018).

While studies increasingly demonstrate the gut microbiota shapes brain structure and function, limitations exist due to insufficient statistical power from small samples, inconsistent interpretations, and confounders in observational studies. Mendelian randomization (MR) utilizes genetic variants as proxies for exposures, enabling investigation of causal relationships while controlling biases (Ong and Mac, 2019). Despite associations between the gut microbiota and brain structure (Tillisch et al., 2013; Labus et al., 2017; Pinto-Sanchez et al., 2017; Carlson et al., 2018; He et al., 2018), causality remains undetermined. Using large GWAS datasets, we conducted two-sample MR to explore the causal effect of the gut microbiota on cortical structure. We leveraged human genetics within the MR framework to elucidate the impact of the gut microbiota on SA and TH (Figure 1), including regional analyses. Our findings provide valuable insight into the gut-brain axis.

2 Method

2.1 Data source for gut microbiota

Summary statistics for gut microbiota were from a meta-analysis of GWAS in 18,340 multi-ethnic participants from the MinGen Consortium (23 European ancestry cohorts, >78% European ancestry, cohort details in Supplementary Table S11) (Kurilshikov et al., 2021). The genetic instrumental variables of each bacterial taxon were analyzed separately. The genome-wide association study of the gut microbiome adjusted for age, sex, technical factors, and principal components. After excluding unknown taxa (3 families, 12 genera), 196 instruments categorized by taxonomy were obtained - 9 phyla, 16 classes, 20 orders, 32 families, 119 genera - for Mendelian randomization.

2.2 Data source for brain cortex SA and TH

Brain cortical structure GWAS summary statistics were from the ENIGMA Consortium meta-analysis of MRI data from 51,655 individuals of predominantly European ancestry (60 global cohorts) (Grasby et al., 2020). Cortical TH and SA were measured. We utilized European ancestry results (cohort details in Supplementary Table S10). The Desikan-Killiany atlas parcellates the cortex into 34 regions bounded by gyral/sulcal anatomy (Desikan et al., 2006). Average TH and SA were measured for each region across hemispheres. We performed Mendelian randomization for the whole cortex and each region, analyzing 196 taxa per outcome. In total, 70 outcomes were obtained – global and regional TH and SA. Global analyses were weighted.

2.3 Selection of genetic instrument

Gut microbiota SNPs underwent the following quality control: (1) To obtain a sufficient number of SNPS, we chose a threshold of ($p < 1 \times 10–5$) for SNP selection (Sanna et al., 2019). (2) Linkage disequilibrium pruning (LD $r^2 < 0.001$, clump_kb = 10,000 bp) was performed in TwoSampleMR (v0.5.7) with EUR reference panel (Burgess and Thompson, 2011). (3) SNPs with F-statistic <10 were excluded to avoid weak instrument bias, calculated as $(n-2) \times R2/(1-R2)$ where $R2 = 2 \times MAF \times (1-MAF) \times \beta 2$. (4) Palindromic SNPs and those with intermediate allele frequencies were removed during harmonization. (5) SNPs reversing the overall direction in leave-one-out analysis were eliminated to ensure reliability.

We selected genetic instruments with F statistics >10 as robust variables. We verified results through Phenoscanncer to ensure confounders did not violate findings. We thoroughly assessed directional pleiotropy using MR-Egger intercepts and conducted leave-one-out analyses to evaluate SNP-driven bias. These steps aimed

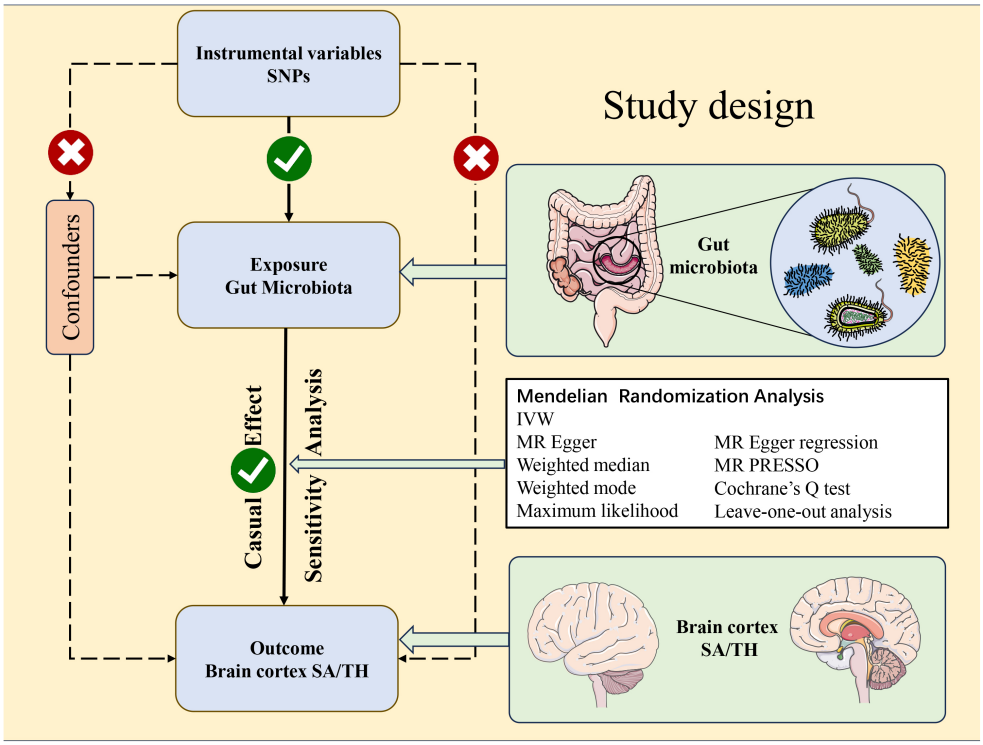


FIGURE 1
Study design of the Mendelian randomization study between gut microbiota taxa and the brain cortical structure as defined using magnetic resonance imaging-measured brain cortical surficial area and thickness.

to achieve robust Mendelian randomization, meeting assumptions that: (i) instruments exhibit strong exposure associations; (ii) instruments are independent of confounders; and (iii) instruments affect outcomes only through the exposure.

2.4 Mendelian randomization analysis and sensitivity analysis

To assess the potential causal relationship between gut microbiota and brain cortex structure, we primarily utilized the inverse-variance weighted (IVW) Mendelian randomization method. The weighted median, MR-Egger regression, weighted mode, and maximum likelihood were also performed for validation and to corroborate IVW results.

To evaluate heterogeneity, we calculated Cochran's *Q* statistic, performed MR-Egger intercept testing, and leave-one-out analysis to assess pleiotropy. The MR-PRESSO test was applied to detect and correct for outliers and heterogeneity when significant associations were identified.

To account for confounding, SNPs with significant MR estimates were assessed in PhenoScanner[1] for associations with central nervous system disease risk factors (e.g., Alzheimer's, depression, cerebrovascular events). Significant hits were excluded and MR re-run to evaluate robustness. Sample overlap between the exposure and outcome datasets was assessed using an online tool[2] to evaluate bias and type I error rates (Burgess et al., 2016).

2.5 Statistical sets

Analyses were conducted in R v4.2.2 using TwoSampleMR v0.5.7. A global significance level of 0.05 was set. Bonferroni correction established value of *p* thresholds for each taxonomic level based on the number of taxa – phylum ($n = 9$): 0.05/9; class ($n = 16$): 0.05/16; order ($n = 20$): 0.05/20; family ($n = 32$): 0.05/32; genus ($n = 119$): 0.05/119. MR results below these thresholds were significant. Results between the threshold and 0.05 were nominally significant. An online tool[3] estimated statistical power. Power ≥ 0.8 was considered sufficient, allowing rejection of $\geq 4/5$ false null hypotheses (Fox and Mathers, 1997; Burgess, 2014).

3 Ethics statement

This study utilized publicly available deidentified data from participant studies that were approved by an ethical standards committee with respect to human experimentation. No separate ethical approval was required for this study.

1 http://www.phenoscanner.medschl.cam.ac.uk

2 https://sb452.shinyapps.io/overlap/

3 https://shiny.cnsgenomics.com/mRnd/

4 Role of funding source

The funders had no role in study design, data collection, data analysis, interpretation, or writing of the report. The corresponding author had full access to all data in this study and was ultimately responsible for the decision to submit for publication.

5 Result

In brief, SNPs were selected to genetically predict gut microbiota's causal effect on 34 functional brain areas, the number of SNP for each MR analysis is ranged from 26 to 67. The F statistics value for each genetic instrument was larger than 10, indicating strong instruments (Pierce et al., 2011).

We conducted a comprehensive MR study examining the causal impact of genetically predicted 196 gut taxa on SA/TH of global and 34 brain functional areas. The heatmap showed IVW-derived *p*-values from all potential causal relationships between gut microbiota on brain cortex regions (Figure 2). Finally, we identified several significant or nominal significant gyrus influenced by gut microbiota. At the global level, four taxa, family Methanobacteriaceae, class Methanobacteria and order Methanobacteriales, were found to decrease SA and genus Turicibacter increased SA with a *p* value of IVW method <0.05 but not meeting Bonferroni corrected threshold. Another four taxa, genus familyXIIIAD3011group, family Defluviitaleaceae and Genus DefluviitaleaceaeUCG011 were found to increase TH and genus Tyzzerella3 decrease TH with p value of IVW method <0.05 but not meet Bonferroni corrected threshold. Gut microbiota had no causal relationship with the global SA and TH by strict value of *p* threshold.

At the brain functional region-level analysis, we found that 166 taxa had a value of p of the IVW method less than 0.05 on SA and 154 taxa on TH (Supplementary Tables S1–8). To be exact, three taxa, class Mollicutes on medial orbitofrontal (β = 8.39 mm^2, 95% CI: 3.08–13.70 mm^2, p = 0.0002), phylum Tenericutes on medial orbitofrontal (β = 8.39 mm^2, 95% CI: 3.08–13.70 mm^2, p = 0.0002) and phylum Tenericutes on lateral orbitofrontal (β = 10.51 mm^2, 95% CI: 3.24–17.79 mm^2, p = 0.0046), significantly increased SA (Table 1) (Supplementary Table S12). And the causal relationship trend of significant SA result is depicted as shown in the scatter plot (Figure 3). Also, two taxa, order Gastranaerophilales on parsopercularis (β = 0.0049 mm, 95% CI: 0.0017–0.0081 mm, p = 0.0016) and class Melainabacteria on parsopercularis (β = 0.0049 mm, 95% CI: 0.0017–0.0080 mm, p = 0.0016), significantly increase TH (Supplementary Table S13). And the scatter plot (Supplementary Figure S1) and forest plot (Supplementary Figure S2) shown the causal relationship trend of significant TH result. The rest of the taxa on specific gyrus had nominal significant *p* values. Given that the global TH of the brain cortex was 2.45 mm (SD = 0.11 mm) (Grasby et al., 2020), the alteration of gut microbiota causing changes in TH is not considered to reach clinically significant levels. No pleiotropy or heterogeneity was detected. Details are presented in the Supplementary data. To test whether the significant estimate was biased by risk factors, we conducted SNPs lookup in Phenoscanner (Supplementary Table S19). No significant biased SNPs were found.

For both statistically significant and nominally significant estimates, we employed Cochran's *Q* test (Table 1), MR-Egger intercept test (Table 1), leave-one-out analyses (Figure 4 and Supplementary Figure S4), and funnel plots (Supplementary Figure S3) to evaluate horizontal pleiotropy (Supplementary Table S17). All *p*-values from the MR-Egger intercept tests and Cochran's *Q* test were greater than 0.05, indicating no horizontal pleiotropy (Supplementary Table S18). MR-PRESSO results also found no heterogeneity (Supplementary Table S16). The estimates were not affected by individual single nucleotide polymorphisms (SNPs), indicating the robustness of the estimates. And power calculations shown the reliability of the results (Supplementary Table S14).

6 Discussion

To our knowledge, this is the first large-scale analysis that comprehensively investigates the causal relationship between gut microbiota and brain cortical structure using Mendelian randomization analysis. In this study, we systematically assessed the causal relationship between genetically predicted gut microbiota taxa and cortical structure across the brain. Our findings indicate that alterations in the abundance of specific microbial taxa can specifically impact cortical morphology, thereby providing causal evidence for earlier observational studies suggesting potential pathophysiological interactions between the gut microbiota and brain. These results substantiate and extend previous correlative research, emphasizing the presence of bidirectional signaling along the gut-brain axis.

Since the early 2000s, MRI has emerged as a prevalent technique for *in vivo* brain imaging. Consequently, it now presents an optimal approach for investigating gut-brain interactions within living organisms (Mayer et al., 2015). Previous observational studies have demonstrated that gut microbiota composition can alter brain structure and function. One study concluded the impact of fermented milk consumption on brain function in healthy women (Tillisch et al., 2013). Another two separate studies have proved the connection between irritable bowel syndrome (IBS), microbiota alterations, and related brain structure changes (Labus et al., 2017; Pinto-Sanchez et al., 2017). And studies have found that gut microbiota is associated with schizophrenia and affects children's nervous system development (Carlson et al., 2018; He et al., 2018). These observational studies conclude an association between the gut microbiota and brain structure alteration detecting by MRI or neurological diseases, but cannot prove causality. Our study fills this limitation and provides a new perspective on the causal relationship between gut microbiota and cerebral cortex. As for the gut-brain axis causal findings, Crohn's disease significantly decreased the TH of pars orbitalis. And IL-6 was observed to reduce the SA of middle temporal and increase the TH of fusiform and pars opercularis. Furthermore, a causal relationship between IL-6Rα and changes in the superior frontal and supramarginal (Liu et al., 2023). The brain structure alteration interacts cortical structure and Alzheimer's disease, the study found that associations of the decreased SA of the temporal pole and decreased TH of cuneus with higher Alzheimer's risk (Wu et al., 2021). These researches proved the casual relationship between inflammatory bowel disease or Alzheimer's disease and changes in brain cortex. These results indicate that intestinal diseases and cerebral cortical changes, as well as cerebral cortical changes and brain function damage are cause-effect relationships. It established the link between intestinal diseases and brain diseases through cortical changes, which support our findings in this study. However, the above studies on causality are limited to the effects of specific diseases on functional areas, while this study focuses on a comprehensive summary of broad-spectrum gut microbiota and whole brain functional areas.

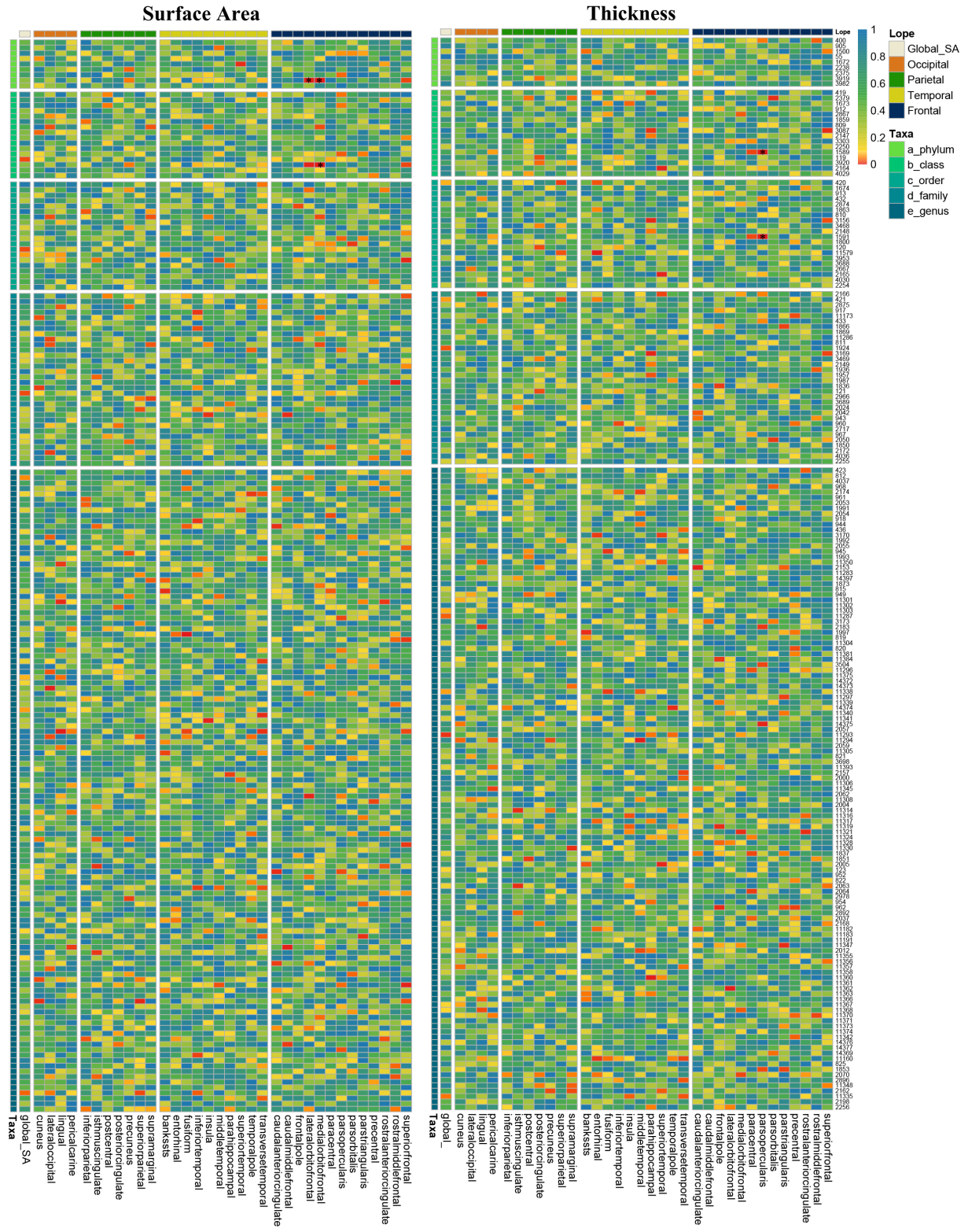


FIGURE 2
Heatmap of IVW estimates from196 gut microbiota taxa on global and 34 regions of brain cortical structure using MRI-measured surface area and thickness. The color of each cell represents the IVW-derived *p*-values of every MR analysis.

Overall, the gut microbiota has widespread effects on brain structure and function. However, the causal relationships between the gut microbiota and cortical structure or neuropsychiatric disorders have yet to be fully elucidated. Cerebral cortical SA and TH are regarded as neuroimaging biomarkers that can predict cognitive ability and higher-order brain functions (Desikan et al.,

TABLE 1 Significant Mendelian randomization estimates from gut microbiota on genetically predicted cortical structure in SA.

Exposure	Outcome	IVW *P*-value	β (95% Confidence intervals)	Cochran's *Q* *P*-value	MR-Egger intercept *P*-value	N snp
Class.Mollicutes.id.3920	Medialorbitofrontal SA	0.0020	8.39 mm^2(3.08mm^2-13.70mm^2)	0.95	0.15	42
Phylum.Tenericutes.id.3919	Medialorbitofrontal SA	0.0020	8.39 mm^2(3.08mm^2-13.70mm^2)	0.95	0.15	42
Phylum.Tenericutes.id.3919	Lateralorbitofrontal SA	0.0046	10.51 mm^2(3.24mm^2-17.79mm^2)	0.79	0.66	40

Cochran's *Q* *P*-value and MR-Egger intercept *P*-value < 0.05 is significant. IVW, Inverseb variance weighted; SA, cortical surficial area.

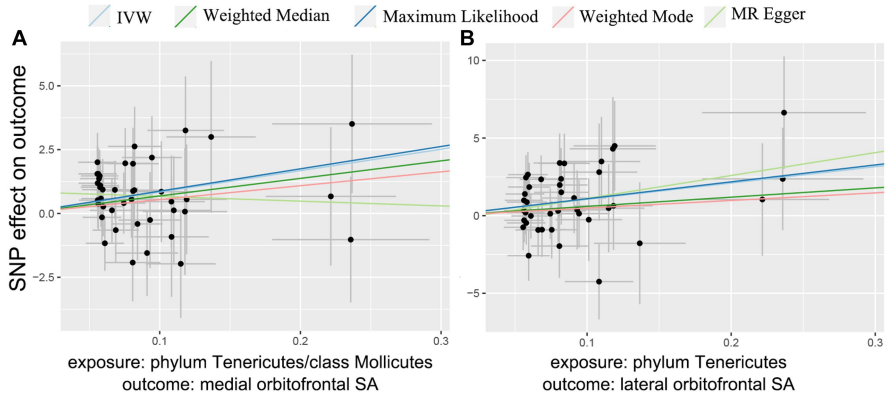


FIGURE 3
MR scatter plots of significant gut microbiota taxa on orbitofrontal SA.

2006; Huang et al., 2023; Zhang et al., 2023). MRI can reveal causal associations between the gut microbiota and neuropsychiatric disorders, provided disease heterogeneity and severity are adequately addressed. Furthermore, MRI provides an objective method to evaluate the causal effects of the gut microbiota on SA and cortical TH alterations across the brain. Changes in brain structure may signify functional alterations and disease pathogenesis in neuropsychiatric disorders. In this study, we performed a comprehensive Mendelian randomization analysis to estimate the causal effects between gut microbiota taxa and cortical structural changes in the brain.

The main finding of our study is that orbitofrontal cortical SA is significantly influenced by the gut microbiota, including both the lateral and medial orbitofrontal regions. Moreover, the gut microbiota significantly affects cortical TH in the pars opercularis, although the small effect size limits potential clinical translation. The orbitofrontal cortex (OFC) plays a vital role in cognitive and behavioral regulation. OFC alterations have been associated with neuropsychiatric disorders including depression, schizophrenia, and addictive behaviors, as well as cognitive-motivational impairments (Olausson et al., 2007; Huang et al., 2023; Rompala et al., 2023; Wang et al., 2023). Furthermore, gut microbiota dysbiosis can increase gut permeability, thereby influencing hypothalamic–pituitary–adrenal (HPA) axis function and activity (Cussotto et al., 2018). The HPA axis regulates neurotransmitter release, brain-derived neurotrophic factor abundance, immune responses, and inflammation; all of which have been linked to depression pathogenesis (Du et al., 2020). Interestingly, the class Mollicutes belongs to the phylum Tenericutes. Research on these two microbial taxa is limited. However, studies have found Tenericutes and Mollicutes to be associated with intrahepatic cholestasis of pregnancy (Li et al., 2023), reduced risk of atopic dermatitis (Xue et al., 2023), and increased abundance in ulcerative colitis (Scaldaferri et al., 2023). Additionally, a positive correlation between Tenericutes and plasma leptin has been reported, which may relate to obesity pathophysiology (Cha et al., 2023).

Further investigations are warranted to elucidate the mechanisms underlying orbitofrontal cortex alterations and their associations with neuropsychiatric disorders. The role of pars opercularis cortical TH changes also merits exploration given this region's importance for language and observations of structural alterations in multiple sclerosis (Cohen-Adad et al., 2011; Lindsay et al., 2020). Future studies should investigate whether gut microbiota-mediated changes in cortical SA or TH of the orbitofrontal and pars opercularis cortices influence brain function and contribute to the pathogenesis of disorders like depression and multiple sclerosis.

Estévez-López et al. retrospectively analyzed 12,286 prepubertal children and found that somatic symptoms were associated with reduced lateral orbitofrontal and parstriangularis cortical SA (Estévez-López et al., 2023). Since somatic complaints commonly manifest in depression, these findings further demonstrate that the gut microbiota may influence depressive disorder development by altering orbitofrontal cortex SA. Our

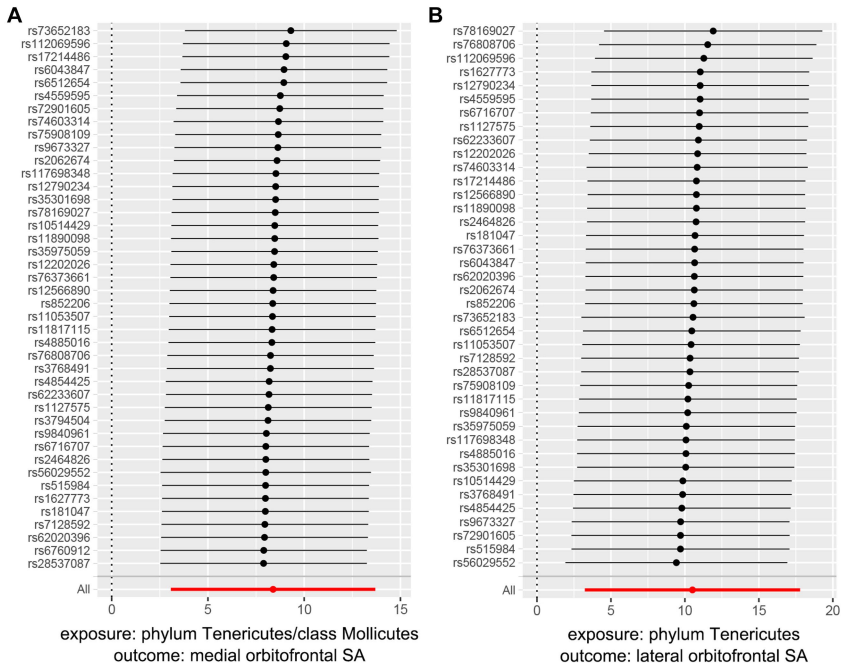


FIGURE 4
MR leave one out analysis plots of significant gut microbiota taxa on orbitofrontal SA.

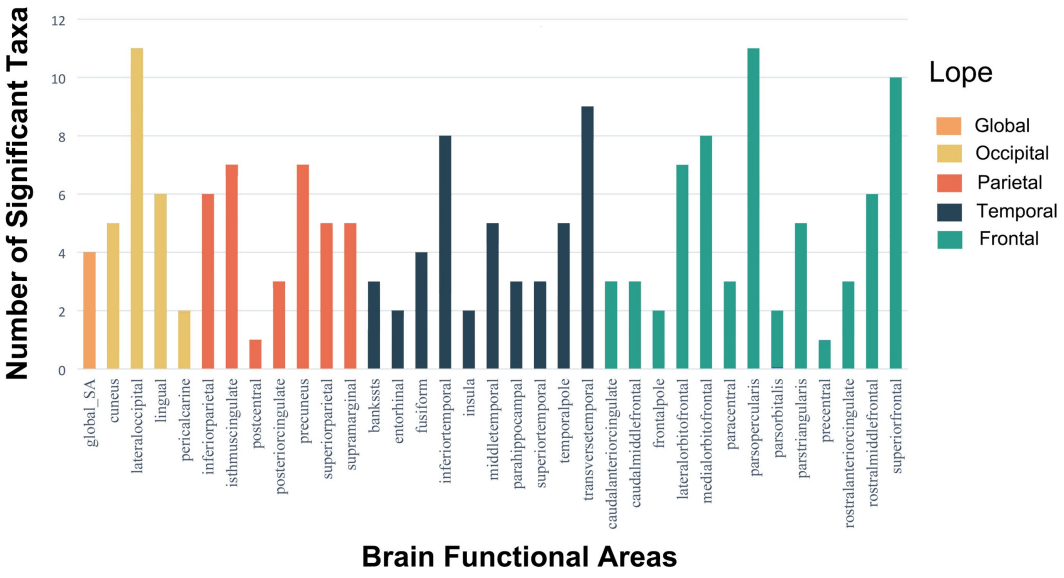


FIGURE 5
Histograms of the distribution of meaningful gut microbiota with p-values <0.05 in SA of global and 34 brain functional areas.

estimates suggest the gut microbiota causally reduces orbitofrontal cortex SA. However, further research is needed to determine whether the gut microbiota contributes to depression by disrupting structural integrity of the orbitofrontal cortex.

Although only five estimate passed Bonferroni correction in SA analysis, other estimates with IVW-derived $p < 0.05$ should also be interpreted cautiously. Figure 5 shows the gut microbiota significantly influenced the lateral occipital, parsopercularis, and superior frontal regions in SA based on microbiota taxa distribution across 34 brain regions at $p < 0.05$ (Supplementary Table S9). Previous research found a significant negative genetic correlation between total SA and average cortical TH (rG = −0.32, SE = 0.05, $p = 6.50 \times 10–12$) (Chen et al., 2021). That study revealed SA was influenced by genetic variants altering neural progenitor cell gene

regulation during fetal development, while active regulatory elements in the adult brain influenced TH. As the cerebral cortex develops continuously from fetal life through young adulthood, and the microbiota begins impacting fetal brain at birth, our results elucidate significant microbiota effects on SA, likely originating postnatally during cortical maturation. A growing number of preclinical studies demonstrate a consensus that gut microbes significantly impact brain structure/function in early life (Codagnone et al., 2019). This elucidates the microbiota's effects on SA, implying influences on SA originate after birth through cortical maturation.

Numerous observational studies reveal microbiota-brain correlations, but we explored causal relationships between specific microbiota and structural (SA) and functional (TH) cortical changes across 34 regions. This provides new insights into the gut-brain axis concept.

Our study has several limitations. First, the enrolled patients were all European, so the causal relationship between gut microbiota and brain cortical structure in other populations remains unknown. Second, our findings only report alterations in the brain cortical structure of gut microbiota, but the underlying mechanisms warrant further investigation. Third, there is an overlap of 3 cohorts between the MiBioGen consortium and ENIGMA, with a total of less than 2,180 overlapped participants (11.89% overlap rate) in the MiBioGen consortium and 2,331 (4.11%) overlapped participants in the ENIGMA consortium. Given that it is difficult to achieve complete non-overlap summary data publicly. Our study's proportion of overlapped participants is less than 11.89%. Based on Burgess's simulation, the expected bias is less than 0.001, and the type I error is less than 0.05 (Supplementary Table S15). Four, the lack of heritability in gut microbiota GWAS data suggests that these associations may be part of a larger spectrum that was undetectable in the current GWAS sample size. This guarantees that future studies should utilize larger sample sizes, harmonized protocols, and more advanced approaches to microbiome analysis, including metagenomic sequencing, rather than 16S analysis and quantification of bacterial cell counts.

Our research results provide a new perspective to explore the causal relationship between gut microbiota and changes in SA and TH of the brain cortex, as well as the resulting neuropsychiatric disorders. The specific mechanisms through which gut microbiota influence changes in the brain cortex's SA and TH require further investigation to explore the prevention and treatment of neuropsychiatric diseases resulting from these alterations.

7 Conclusion

This study is the first comprehensive MR analysis that reveals gut microbiota's potential causal effect on the brain cortex structure, comprising 196 gut taxa and 34 functional brain regions. Our findings indicate that class Mollicutes and phylum Tenericutes causally increased SA of the orbitofrontal cortex. Clinical doctors must proceed with caution when encountering dysbiosis in a patient's gut microbiota, especially when observing disturbances in the class Mollicutes and phylum Tenericutes.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

MZ: Software, Validation, Writing – review & editing, Writing – original draft. SC: Formal analysis, Writing – original draft. YC: Conceptualization, Data curation, Investigation, Writing – review & editing. CW: Formal Analysis, Methodology, Validation, Writing – review & editing. CC: Conceptualization, Project administration, Validation, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This study was supported by Fujian Provincial Clinical Key Specialty Construction Program of Neurosurgery (grant no. 05029002).

Acknowledgments

We thank MiBioGen Consortium and the ENIGMA Consortium for providing GWAS data. The Figure 1 was partly generated using Servier Medical Art, provided by Servier, licensed under a Creative Commons Attribution 3.0 unported license.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnins.2023.1296145/full#supplementary-material

References

Aho, V. T. E., Pereira, P. A. B., Voutilainen, S., Paulin, L., Pekkonen, E., Auvinen, P., et al. (2019). Gut microbiota in Parkinson's disease: temporal stability and relations to disease progression. *EBioMedicine* 44, 691–707. doi: 10.1016/j.ebiom.2019.05.064

Bravo, J. A., Forsythe, P., Chew, M. V., Escaravage, E., Savignac, H. M., Dinan, T. G., et al. (2011). Ingestion of Lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. *Proc. Natl. Acad. Sci. U. S. A.* 108, 16050–16055. doi: 10.1073/pnas.1102999108

Burgess, S. (2014). Sample size and power calculations in Mendelian randomization with a single instrumental variable and a binary outcome. *Int. J. Epidemiol.* 43, 922–929. doi: 10.1093/ije/dyu005

Burgess, S., Davies, N. M., and Thompson, S. G. (2016). Bias due to participant overlap in two-sample Mendelian randomization. *Genet. Epidemiol.* 40, 597–608. doi: 10.1002/gepi.21998

Burgess, S., and Thompson, S. G. (2011). Avoiding bias from weak instruments in Mendelian randomization studies. *Int. J. Epidemiol.* 40, 755–764. doi: 10.1093/ije/dyr036

Carlson, A. L., Xia, K., Azcarate-Peril, M. A., Goldman, B. D., Ahn, M., Styner, M. A., et al. (2018). Infant gut microbiome associated with cognitive development. *Biol. Psychiatry* 83, 148–159. doi: 10.1016/j.biopsych.2017.06.021

Cha, Y. J., Chang, I. A., Jin, E.-H., et al. (2023). Association between LEPR genotype and gut microbiome in healthy non-obese Korean adults. *Biomol Ther.* doi: 10.4062/biomolther.2023.116

Chen, X., Kong, J., Pan, J., Huang, K., Zhou, W., Diao, X., et al. (2021). Kidney damage causally affects the brain cortical structure: a Mendelian randomization study. *EBioMedicine* 72:103592. doi: 10.1016/j.ebiom.2021.103592

Codagnone, M. G., Spichak, S., O'Mahony, S. M., O'Leary, O. F., Clarke, G., Stanton, C., et al. (2019). Programming bugs: microbiota and the developmental origins of brain health and disease. *Biol. Psychiatry* 85, 150–163. doi: 10.1016/j.biopsych.2018.06.014

Cohen-Adad, J., Benner, T., Greve, D., Kinkel, R. P., Radding, A., Fischl, B., et al. (2011). In vivo evidence of disseminated subpial T2* signal changes in multiple sclerosis at 7 T: a surface-based analysis. *NeuroImage* 57, 55–62. doi: 10.1016/j.neuroimage.2011.04.009

Cryan, J. F., O'Riordan, K. J., Cowan, C. S. M., Sandhu, K. V., Bastiaanssen, T. F. S., Boehme, M., et al. (2019). The microbiota-gut-brain Axis. *Physiol. Rev.* 99, 1877–2013. doi: 10.1152/physrev.00018.2018

Cussotto, S., Sandhu, K. V., Dinan, T. G., and Cryan, J. F. (2018). The neuroendocrinology of the microbiota-gut-brain Axis: a Behavioural perspective. *Front. Neuroendocrinol.* 51, 80–101. doi: 10.1016/j.yfrne.2018.04.002

Desikan, R. S., Ségonne, F., Fischl, B., Quinn, B. T., Dickerson, B. C., Blacker, D., et al. (2006). An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. *NeuroImage* 31, 968–980. doi: 10.1016/j.neuroimage.2006.01.021

Du, Y., Gao, X.-R., Peng, L., and Ge, J.-F. (2020). Crosstalk between the microbiota-gut-brain axis and depression. *Heliyon* 6:e04097. doi: 10.1016/j.heliyon.2020.e04097

Dupont, H. L., Jiang, Z.-D., Dupont, A. W., and Utay, N. S. (2020). The intestinal microbiome in human health and disease. *Trans. Am. Clin. Climatol. Assoc.* 131, 178–197.

Estévez-López, F., Kim, H. H., López-Vicente, M., Legerstee, J. S., Hillegers, M. H. J., Tiemeier, H., et al. (2023). Physical symptoms and brain morphology: a population neuroimaging study in 12, 286 pre-adolescents. *Transl. Psychiatry* 13:254. doi: 10.1038/s41398-023-02528-w

Fox, N., and Mathers, N. (1997). Empowering research: statistical power in general practice research. *Fam. Pract.* 14, 324–329. doi: 10.1093/fampra/14.4.324

Grasby, K. L., Jahanshad, N., Painter, J. N., Colodro-Conde, L., Bralten, J., Hibar, D. P., et al. (2020). The genetic architecture of the human cerebral cortex. *Science* 367. doi: 10.1126/science.aay6690

He, Y., Kosciolek, T., Tang, J., Zhou, Y., Li, Z., Ma, X., et al. (2018). Gut microbiome and magnetic resonance spectroscopy study of subjects at ultra-high risk for psychosis may support the membrane hypothesis. *Eur. Psychiatry* 53, 37–45. doi: 10.1016/j.eurpsy.2018.05.011

Huang, C.-C., Chen, P.-H., Tsai, C.-C., Chiang, H. F., Hsieh, C. C., Chen, T. L., et al. (2023). Diffusion and structural MRI as potential biomarkers in people with Parkinson's disease and cognitive impairment. *Eur. Radiol.* doi: 10.1007/s00330-023-10012-8

Huang, H., Rong, B., Chen, C., Wan, Q., Liu, Z., Zhou, Y., et al. (2023). Common and distinct functional connectivity of the orbitofrontal cortex in depression and schizophrenia. *Brain Sci.* 13. doi: 10.3390/brainsci13070997

Kurilshikov, A., Medina-Gomez, C., Bacigalupe, R., Radjabzadeh, D., Wang, J., Demirkan, A., et al. (2021). Large-scale association analyses identify host factors influencing human gut microbiome composition. *Nat. Genet.* 53, 156–165. doi: 10.1038/s41588-020-00763-1

Labus, J. S., Hollister, E. B., Jacobs, J., Kirbach, K., Oezguen, N., Gupta, A., et al. (2017). Differences in gut microbial composition correlate with regional brain volumes in irritable bowel syndrome. *Microbiome* 5:49. doi: 10.1186/s40168-017-0260-z

Li, C., Li, N., Liu, C., and Yin, S. (2023). Causal association between gut microbiota and intrahepatic cholestasis of pregnancy: mendelian randomization study. *BMC Pregnancy Childbirth* 23:568. doi: 10.1186/s12884-023-05889-8

Lindsay, E. C., Metcalfe, N. B., and Llewellyn, M. S. (2020). The potential role of the gut microbiota in shaping host energetics and metabolic rate. *J. Anim. Ecol.* 89, 2415–2426. doi: 10.1111/1365-2656.13327

Liu, C., Zhu, S., Zhang, J., Ren, K., Li, K., and Yu, J. (2023). Inflammatory bowel diseases, interleukin-6 and interleukin-6 receptor subunit alpha in causal association with cerebral cortical structure: a Mendelian randomization analysis. *Front. Immunol.* 14:1154746. doi: 10.3389/fimmu.2023.1154746

Matheoud, D., Cannon, T., Voisin, A., Penttinen, A. M., Ramet, L., Fahmy, A. M., et al. (2019). Intestinal infection triggers Parkinson's disease-like symptoms in pink 1−/− mice. *Nature* 571, 565–569. doi: 10.1038/s41586-019-1405-y

Mayer, E. A., Tillisch, K., and Gupta, A. (2015). Gut/brain axis and the microbiota. *J. Clin. Invest.* 125, 926–938. doi: 10.1172/JCI76304

Olausson, P., Jentsch, J. D., Krueger, D. D., Tronson, N. C., Nairn, A. C., and Taylor, J. R. (2007). Orbitofrontal cortex and cognitive-motivational impairments in psychostimulant addiction: evidence from experiments in the non-human primate. *Ann. N. Y. Acad. Sci.* 1121, 610–638. doi: 10.1196/annals.1401.016

Ong, I. M., Gonzalez, J. G., McIlwain, S. J., Sawin, E. A., Schoen, A. J., Adluru, N., et al. (2018). Gut microbiome populations are associated with structure-specific changes in white matter architecture. *Transl. Psychiatry* 8:6. doi: 10.1038/s41398-017-0022-5

Ong, J.-S., and Mac, G. S. (2019). Implementing MR-PRESSO and GCTA-GSMR for pleiotropy assessment in Mendelian randomization studies from a practitioner's perspective. *Genet. Epidemiol.* 43, 609–616. doi: 10.1002/gepi.22207

Pierce, B. L., Ahsan, H., and Vanderweele, T. J. (2011). Power and instrument strength requirements for Mendelian randomization studies using multiple genetic variants. *Int. J. Epidemiol.* 40, 740–752. doi: 10.1093/ije/dyq151

Pinto-Sanchez, M. I., Hall, G. B., Ghajar, K., Nardelli, A., Bolino, C., Lau, J. T., et al. (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.e8. doi: 10.1053/j.gastro.2017.05.003

Qian, X.-H., Song, X.-X., Liu, X.-L., Chen, S.-D., and Tang, H.-D. (2021). Inflammatory pathways in Alzheimer's disease mediated by gut microbiota. *Ageing Res. Rev.* 68:101317. doi: 10.1016/j.arr.2021.101317

Rompala, G., Nagamatsu, S. T., Martínez-Magaña, J. J., Nuñez-Ríos, D. L., Wang, J., Girgenti, M. J., et al. (2023). Profiling neuronal methylome and hydroxymethylome of opioid use disorder in the human orbitofrontal cortex. *Nat. Commun.* 14:4544. doi: 10.1038/s41467-023-40285-y

Sanna, S., van Zuydam, N. R., Mahajan, A., Kurilshikov, A., Vich Vila, A., Võsa, U., et al. (2019). Causal relationships among the gut microbiome, short-chain fatty acids and metabolic diseases. *Nat. Genet.* 51, 600–605. doi: 10.1038/s41588-019-0350-x

Scaldaferri, F., D'Onofrio, A. M., Calia, R., di Vincenzo, F., Ferrajoli, G. F., Petito, V., et al. (2023). Gut microbiota signatures are associated with psychopathological profiles in patients with ulcerative colitis: results from an Italian tertiary IBD center. *Inflamm. Bowel Dis.* 29, 1805–1818. doi: 10.1093/ibd/izad091

Simpson, C. A., Diaz-Arteche, C., Eliby, D., Schwartz, O. S., Simmons, J. G., and Cowan, C. S. M. (2021). The gut microbiota in anxiety and depression - a systematic review. *Clin. Psychol. Rev.* 83:101943. doi: 10.1016/j.cpr.2020.101943

Thompson, P. M., Jahanshad, N., Ching, C. R. K., Salminen, L. E., Thomopoulos, S. I., Bright, J., et al. (2020). ENIGMA and global neuroscience: a decade of large-scale studies of the brain in health and disease across more than 40 countries. *Transl. Psychiatry* 10:100. doi: 10.1038/s41398-020-0705-1

Tillisch, K., Labus, J., Kilpatrick, L., Jiang, Z., Stains, J., Ebrat, B., et al. (2013). Consumption of fermented milk product with probiotic modulates brain activity. *Gastroenterology* 144, 1394–1401.e4. doi: 10.1053/j.gastro.2013.02.043

Wang, B. A., Veismann, M., Banerjee, A., and Pleger, B. (2023). Human orbitofrontal cortex signals decision outcomes to sensory cortex during behavioral adaptations. *Nat. Commun.* 14:3552. doi: 10.1038/s41467-023-38671-7

Wu, B.-S., Zhang, Y.-R., Li, H.-Q., Kuo, K., Chen, S. D., Dong, Q., et al. (2021). Cortical structure and the risk for Alzheimer's disease: a bidirectional Mendelian randomization study. *Transl. Psychiatry* 11:476. doi: 10.1038/s41398-021-01599-x

Xue, Y., Zhang, L., Chen, Y., Wang, H., and Xie, J. (2023). Gut microbiota and atopic dermatitis: a two-sample Mendelian randomization study. *Front Med (Lausanne)* 10:1174331. doi: 10.3389/fmed.2023.1174331

Yang, Z., Li, J., Gui, X., Shi, X., Bao, Z., Han, H., et al. (2020). Updated review of research on the gut microbiota and their relation to depression in animals and human beings. *Mol. Psychiatry* 25, 2759–2772. doi: 10.1038/s41380-020-0729-1

Zhang, S., She, S., Qiu, Y., Li, Z., Wu, X., Hu, H., et al. (2023). Multi-modal MRI measures reveal sensory abnormalities in major depressive disorder patients: a surface-based study. *Neuroimage Clin.* 39:103468. doi: 10.1016/j.nicl.2023.103468

frontiers | Frontiers in Microbiology

TYPE Review
PUBLISHED 31 January 2024
DOI 10.3389/fmicb.2024.1292004

OPEN ACCESS

EDITED BY
Avinash Veerappa,
University of Nebraska Medical Center,
United States

REVIEWED BY
Yingli Jing,
Capital Medical University, China
Sankarasubramanian Jagadesan,
University of Nebraska Medical Center,
United States
Nazia Saiyed,
Beaumont Hospital,
Beaumont Health, United States
Nagavardhini Avuthu,
University of Nebraska Medical Center,
United States

*CORRESPONDENCE
Fenglong Yang
✉ yangfenglong@fjmu.edu.cn
Lijun Dou
✉ doul2@ccf.org

†These authors have contributed equally to this work and share first authorship

RECEIVED 10 September 2023
ACCEPTED 16 January 2024
PUBLISHED 31 January 2024

CITATION
Wang M, Song Z, Lai S, Tang F, Dou L and Yang F (2024) Depression-associated gut microbes, metabolites and clinical trials. *Front. Microbiol.* 15:1292004. doi: 10.3389/fmicb.2024.1292004

Depression-associated gut microbes, metabolites and clinical trials

Meiling Wang[1†], Zhaoqi Song[1†], Shirong Lai[1], Furong Tang[2], Lijun Dou[3*] and Fenglong Yang[1,4*]

[1]Department of Bioinformatics, Fujian Key Laboratory of Medical Bioinformatics, School of Medical Technology and Engineering, Fujian Medical University, Fuzhou, China, [2]Department of Basic Medical Sciences, School of Medicine, Tsinghua University, Beijing, China, [3]Genomic Medicine Institute, Lerner Research Institute, Cleveland, OH, United States, [4]Key Laboratory of Ministry of Education for Gastrointestinal Cancer, School of Basic Medical Sciences, Fujian Medical University, Fuzhou, China

Depression is one of the most prevalent mental disorders today. Over the past decade, there has been considerable attention given to the field of gut microbiota associated with depression. A substantial body of research indicates a bidirectional communication pathway between gut microbiota and the brain. In this review, we extensively detail the correlation between gut microbiota, including *Lactobacillus acidophilus* and *Bifidobacterium longum*, and metabolites such as short-chain fatty acids (SCFAs) and 5-hydroxytryptamine (5-HT) concerning depression. Furthermore, we delve into the potential health benefits of microbiome-targeted therapies, encompassing probiotics, prebiotics, and synbiotics, in alleviating depression. Lastly, we underscore the importance of employing a constraint-based modeling framework in the era of systems medicine to contextualize metabolomic measurements and integrate multi-omics data. This approach can offer valuable insights into the complex metabolic host-microbiota interactions, enabling personalized recommendations for potential biomarkers, novel drugs, and treatments for depression.

KEYWORDS

depression, gut microbiota, metabolites, pathogenesis, clinical trials

Highlights

- This paper reviews reported microbiota-based animal studies related to depression and microbiome-based clinical trials in depression. We also summarized the different combinations of therapies and the resulting efficacy, and explored possible reasons for the poor efficacy.
- We briefly describe certain gut microbial metabolites that influence the onset and progression of depression via MGB axis, including 5-HT, SCFAs, GABA, GP, choline, lactate, BAs and vitamin (folate).
- This paper proposes metabolic models as a means of testing new hypotheses and exploring new microbial-based therapies for depression.

Introduction

Depression is the most common mental disorder today, characterized by persistent and prolonged feelings of sadness as its main clinical feature. It has a high prevalence, high recurrence rate, and a tendency towards suicide (Belmaker and Agam, 2008), making it one of the most significant types of psychological disorders in modern society. Epidemiological studies from various countries indicate a depression prevalence rate of approximately 7% in the general population (For Anxiety and Depression, R. G. for Treatment, 2017), and up to 10% of individuals with depression may attempt suicide (Bachmann, 2018). Major depressive disorder (MDD) has a devastating impact on global public health, not only causing social and economic burdens but also being a leading cause of severe disability (World Health Organization, 2008). The World Health Organization has identified depression as one of the top public health priorities, estimating that by 2030, it will be the leading cause of death and disability in Western countries (For Anxiety and Depression, R. G. for Treatment, 2017).

Over the past decade, there has been a growing interest in the relationship between gut microbiota and mental disorders (Sanada et al., 2020). The gut microbiota ecosystem contains approximately 1 kg of bacteria, encompassing all microorganisms, their genes, encoded proteins, and metabolites (Eckburg et al., 2005; Korecka and Arulampalam, 2012). Additionally, the gut houses a large number of neurons, second only to the brain, earning the nickname "the second brain" (Ridaura and Belkaid, 2015). Extensive research has established the microbiota-gut-brain (MGB) axis (Bercik, 2011; Camilleri et al., 2012). Along this axis, the microbial community in the gut influences brain function through three bidirectional signaling pathways (Macpherson and Harris, 2004; Bäckhed et al., 2005; Heijtz et al., 2011; Cryan and Dinan, 2012; Breit et al., 2018). Alterations in these signaling pathways may contribute to mental health problems. Therefore, investigating the MGB axis provides a novel approach to exploring the pathogenesis of depression and developing appropriate therapeutic strategies.

In this review, we will summarize the recent advances in gut microbiota research related to depression, focusing on the relationship and role of the MGB axis in depression. We aim to explore the value and potential of the MGB axis in the diagnosis of depression.

Gut microbes in depression

There are several pathophysiological hypotheses explaining depression, including the monoamine, brain-derived neurotrophic factor (BDNF), and cytokine hypotheses (Boku et al., 2018). However, these hypotheses have their limitations. Increasing evidence suggests that the gut microbiota may play a role in depression. In a study by McGuinness et al. (2022), it was found that the α-diversity did not significantly differ between the majority of MDD cases and the control group, with only a few reports indicating higher or lower α-diversity between the two groups. However, when statistically tested using β-diversity analysis, 87% of MDD cases showed differences in gut microbiota composition compared to the control group. The study identified 21 bacterial genera with differential abundance at the genus level. Higher abundances of *Alistipes*, *Parabacteroides*, *Streptococcus*, *Veillonella*, *Enterococcus*, *Flavonifractor*, *Eggerthella*, *Escherichia*, and lower abundances of *Coprococcus*, *Prevotella*, *Faecalibacterium*, and *Ruminococcus* were observed in MDD cases. Among them, higher proportions of *lactobacilli* and lactic acid-producing bacteria, which are generally considered beneficial to the host, were found. These bacteria promote gut microbiota balance (Shi et al., 2017), maintain a normal microbial environment, and have immunomodulatory effects (Pessione, 2012; George et al., 2018). However, in certain circumstances, the production and utilization of lactate can also have detrimental effects on host health. Lactate can accumulate in the gut and cross the blood–brain barrier (Proia et al., 2016), potentially leading to acidosis, arrhythmias, and neurotoxicity (Duncan et al., 2004; Pham et al., 2017). Many psychiatric disorders are also associated with mitochondrial energy dysfunction (Regenold et al., 2009), indicated by increased lactate and decreased pH in the brain. Elevated levels of lactate have been observed in the brains of MDD patients (Ernst et al., 2017), suggesting that an increase in the abundance of lactate-producing bacteria and subsequent lactate accumulation may contribute to the pathophysiology of depression. Additionally, there is evidence suggesting an association between *Clostridium difficile* and the onset of depression. In a study conducted by Fondden et al. and published in the journal "Nutrients" in 2020, a significant finding indicated that an elevated presence of *C. difficile* is associated with an increased risk of depression. The study compared individuals with depression to healthy individuals and discovered a 36% higher abundance of *C. difficile* in those with depression. However, it was also observed that fecal microbiota transplantation proved to be an effective method in reducing the levels of *C. difficile*, thereby inhibiting the occurrence of depression (van Nood et al., 2013; Austin et al., 2014; Cammarota et al., 2015; Li et al., 2016; Hocquart et al., 2018; Fond et al., 2020). The recent study published in 'Nature Communications' provide some of the most compelling evidence to date regarding the relationship between depression and gut microbiota (Radjabzadeh et al., 2022). One study, known as HELIUS, specifically examined health disparities among individuals of different racial backgrounds living in the same urban environment, with the primary aim of investigating the general association between the microbiome and depression. Interestingly, one of the studies within this research did indeed uncover variations in depression risk among different racial groups, but these differences could be explained by individual variations in the composition of one's microbiome. Overall, the study found a consistent association between overall microbial diversity and depression, transcending racial boundaries. The second study delved more specifically into the types of gut bacteria that may be linked to depression. In a meticulous analysis of fecal samples from approximately 1,000 participants in an ongoing population health study in Rotterdam, 13 microbial species were directly associated with symptoms of depression. The most significant new discovery in this research was the connection between *Sellimonas* and depression symptoms. Bacterial species belonging to the *Sellimonas* genus are involved in various inflammatory diseases, potentially linking them to inflammation in individuals with depression. These findings suggest that a causal relationship between the microbiome and depression is entirely plausible, and it is reasonable to consider that depression may lead to other physiological changes, subsequently altering the microbiome (Radjabzadeh et al., 2022).

Multiple studies have demonstrated the involvement of the gut microbiota in the occurrence and development of psychiatric disorders, including depression (Desbonnet et al., 2010; Zheng et al.,

2016; Rincel et al., 2019; Pearson-Leary et al., 2020). Transplanting microbiota from depressed patients into normal animals has been found to induce depression-like behaviors (Bravo et al., 2011; Savignac et al., 2014; Liang et al., 2015; Campos et al., 2016; Gacias et al., 2016; Kelly et al., 2016; Zheng et al., 2016). Rats receiving fecal microbiota transplantation from depressed patients displayed anhedonia-like behavior in a sucrose preference test (Kelly et al., 2016). Germ-free mice colonized with microbiota from depressed patients showed increased immobility time in tail suspension and forced swim tests, along with an increased abundance of Actinobacteria, compared to mice colonized with microbiota from healthy individuals (Zheng et al., 2016). These findings, supported by a substantial body of evidence, suggest that alterations in the gut microbiota can contribute to the onset of depression.

While changes in the gut microbiota can contribute to the onset of depression, the gut microbiota also has the potential to improve depressive symptoms. Animal studies (Figure 1) have shown that both antibiotics (Gacias et al., 2016; Guida et al., 2018) and probiotics (Bravo et al., 2011) can significantly alter depression-like behaviors in rats and mice, demonstrating the beneficial effects of gut microbiota as probiotics in alleviating depressive symptoms (Table 1). For instance, in rat studies, a combination therapy of eight probiotic strains (*B. bifidum* W23, *B. lactis* W52, *L. acidophilus* W37, *L. brevis* W63, *L. casei* W56, *L. salivarius* W24, *Lactococcus lactis* W19, *Lactococcus lactis* W58) significantly reduced diet-independent depression-like behaviors (Abildgaard et al., 2017). In mouse studies, supplementation of *L. helveticus* MCC1848 significantly increased interaction time in the social interaction test and sucrose preference ratio in the sucrose preference test (Maehata et al., 2019). Oral administration of *L. kefiranofaciens* ZW3 improved depression-like behaviors and independent exploration ability, regulating biochemical disorders in the hypothalamic–pituitary–adrenal axis, immune system, and tryptophan metabolism (Sun et al., 2019). *Clostridioides butyricum* demonstrated significant effects by increasing 5-HT and glucagon-like peptide-1 (GLP-1), upregulating BDNF expression, and promoting GLP-1 secretion and GLP-1 receptor expression (Sun et al., 2018) and GLP-1 has been reported to possess the potential to alleviate depression by regulating neuroinflammation, neurotransmitters, neurogenesis, and synaptic function (Kim et al., 2020). Furthermore, treatment with a multi-strain probiotics approach (*L. helveticus* R0052, *L. plantarum* R1012, and *B. longum* R0175) attenuated anxiety and depression-like behaviors induced by chronic mild stress, significantly increased *Lactobacillus* abundance, and reversed immune changes in the hippocampus induced by chronic mild stress (Li et al., 2018). Mice subjected to a series of stress stimuli exhibited depression-like behaviors and dysbiosis of the microbiota, but this condition could be reversed by probiotic administration, supplementation with gut bacteria, or antibiotic treatment. Mice subjected to chronic social defeat stress and treated with prebiotics or *Bifidobacterium* orally showed a reduction in depression-like behaviors in tests such as tail suspension and forced swim (Burokas et al., 2017; Yang et al., 2017). Similarly, mice and rats subjected to chronic restraint stress and treated with minocycline or oral *L. helveticus* NS8, respectively, reversed the increased depression-like behaviors and altered gut microbiota induced by chronic restraint stress (Liang et al., 2015; Wong et al., 2016). Studies involving mice and rats in models of unpredictable chronic mild stress (Marin et al., 2017), learned helplessness (Mika et al., 2017; Takajo et al., 2019), and maternal separation (Zheng et al., 2016) observed a reduction in depression-like behaviors following treatment with probiotics and supplementation with gut bacteria, while untreated mice and rats showed changes in fecal metabolomic profiles associated with depression-like behaviors (O'Mahony et al., 2009; Jianguo et al., 2019; Zhang et al., 2019). These findings from animal models of depression collectively emphasize the significant role of the gut microbiota and underscore the importance of animal models in microbiota research.

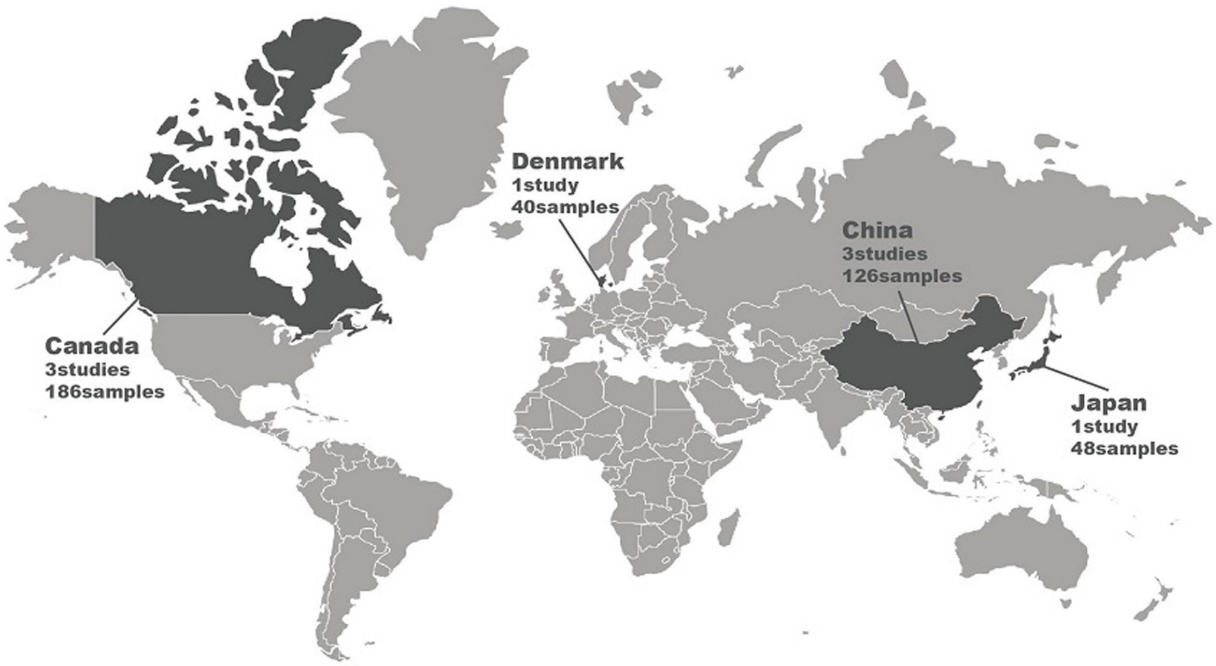
Metabolites in depression

Certain gut microbial metabolites have been shown to influence the occurrence and progression of depression through the MGB axis (Figure 2).

Tryptophan metabolism

One important factor in depression is tryptophan, and a study focusing on the impact of dietary tryptophan on mood disorders emphasizes that a diet rich in tryptophan helps reduce depressive symptoms and improve an individual's emotional state. Conversely, a low tryptophan diet can lead to irritability and anxiety (Lindseth et al., 2015). Tryptophan can be metabolized in two crucial pathways of depression: the 5-HT and kynurenine pathways (Comai et al., 2020).

As a key modulator of the gut-brain axis, the neurotransmitter 5-HT plays a crucial role in the communication between the gut and the brain in the signaling of the MGB axis. Recently, an increasing number of studies have indicated the significant role of 5-HT, including its precursor 5-hydroxytryptophan (5-HTP), in the development of depression. Tryptophan hydroxylase (TPH) enzyme plays an important role in various psychiatric disorders, including depression. Research suggests that stress suppresses the expression of this enzyme, thereby reducing the levels of 5-HT (Chen et al., 2017b). Peripheral cells involved in the production of 5-HT exhibit TPH1 dysfunction, leading to insufficient levels of 5-HT in the brain, which in turn triggers a homeostatic response of TPH2, an enzyme that is overexpressed in individuals with suicidal tendencies, in response to low 5-HT levels (Bach-Mizrachi et al., 2006; Fukuda, 2014). This suggests that low 5-HT may contribute to the development of depression. The gut microbiota, in turn, influences depression by regulating the levels of 5-HT. Antibiotic-treated or germ-free mice exhibit reduced synthesis of 5-HT, which can be reversed by the colonization of spore-forming bacteria (Yano et al., 2015). Specific spore-forming bacteria from humans and mice increase the levels of 5-HT in the colon and serum of germ-free mice through the production of SCFAs, thereby upregulating the expression of TPH1 in ECCs and enhancing 5-HT production (Reigstad et al., 2015; Yano et al., 2015). This also improves gut motility disorders associated with germ-free conditions (Furusawa et al., 2013). The communication between ECCs-released 5-HT and the gut microbiota *Turicibacter sanguinis*, which possesses a 5-HT uptake mechanism, is involved in its colonization and host physiology (Fung et al., 2019). The most common antidepressant medications are based on blocking the reuptake of 5-HT, thereby increasing its levels in the synaptic cleft and promoting antidepressant responses (Cowen and Browning, 2015; Vahid-Ansari and Albert, 2021). This explains the bidirectional effects



Model animals	Study	Country	Male	Female	Temperature	PMID
Mice	Neufeld,2018	Canada	n=82	n=0	21 ± 1°C	29867313
	Maehata,2019	Japan	n=48	n=0	NA	30898081
	Sun,2019	China	n=48	n=0	25°C	30698577
	Li,2018	China	n=48	n=0	21 ± 1°C	30459574
	Sun,2018	China	n=30	n=0	22 ± 1°C	30040410
Rat	Abildgaard,2017	Denmark	n=40	n=0	20°C	28259042
	Gilbert,2012	Canada	n=64	n=0	21 – 22°C	23068715
	Bre ´ard,2011	Canada	n=40	n=0	21 – 22°C	21933458

FIGURE 1
Geographical locations and sample metadata of different animal studies related to depression.

observed between certain psychotropic drugs, including selective serotonin reuptake inhibitors, and the gut microbiota (Cussotto et al., 2019), suggesting the facilitatory role of high concentrations of 5-HT in antidepressant effects. Furthermore, depression patients show inadequate transport of 5-HTP to the brain (Ryan, 1992; Maffei, 2020), and another study also suggests that the combination of 5-HTP with niacinamide is more effective in combating depression compared to niacinamide alone (López-Ibor Aliño et al., 1976; Maffei, 2020), highlighting the impact of 5-HTP on depression.

Another pathway of tryptophan metabolism is the kynurenine pathway. Excessive pro-inflammatory cytokines produced in depression over activate the enzymes indoleamine 2,3-dioxygnease (IDO) and tryptophan 2,3-dioxygenase (TDO), promoting the kynurenine pathway and consequently reducing the activation of the 5-HT pathway and decreasing the production of 5-HT (Miura et al., 2008). It is worth mentioning that IDO and TDO inhibitors, by inhibiting the activation of IDO and TDO enzymes, can serve as potential drugs for the treatment of depression (Qin et al., 2018). Studies on germ-free mice have shown that the availability of tryptophan increases due to reduced activation of the peripheral kynurenine pathway (Clarke et al., 2013). Moreover, in a rodent model of chronic unpredictable stress, the decrease in stress-induced *lactobacillus* abundance weakens the inhibition of IDO 1 mediated by hydrogen peroxide. This inhibition leads to an increase in the conversion of tryptophan to kynurenine, resulting in behavioral changes resembling depression in mice exposed to chronic stress (Marin et al., 2017). In contrast to 5-HT, kynurenine can cross the blood–brain barrier and negatively impact brain health through the

TABLE 1 Antidepressant effects of probiotics in animal studies.

Model animals	Probiotics	Administration form	Finding	PMID
Mice	*Lactobacillus (L.) rhamnosus* JB-1™	Oral administration via drinking water	Adult male BALB/c mice responded with greater antidepressive-like behavior to probiotic while SW mice did not.	29867313
	L. helveticus strain MCC1848	Oral intake of heat-killed probiotics	MCC1848 supplementation significantly enhanced interaction time in social interaction test and sucrose preference ratio in the sucrose preference test.	30898081
	L. kefiranofaciens ZW3	Oral administration	ZW3 improved depression-like behavior and independent exploration ability, regulated biochemical disorders in the hypothalamic–pituitary–adrenal axis, immune system, and tryptophan metabolism. Probiotic strain stayed in intestine 7 days after intervention ceased.	30698577
	L. helveticus R0052, *L. plantarum* R1012, *and Bifidobacterium (B.) longum* R0175	Oral administration	Probiotics attenuated CMS-induced anxiety-and depressive-like behaviors, significantly increased Lactobacillus abundance, and reversed the CMS-induced immune changes in the hippocampus.	30459574
	Clostridioides butyricum	Gavage administration	Clostridioides butyricum exhibited prominent effects, increasing 5-HT and GLP-1 and upregulating BDNF expression, and secretion of GLP-1 and upregulated GLP-1R expression.	30040410
Rat	*B. bifidum* W23, *B. lactis* W52, *L. acidophilus* W37, *L. brevis* W63, *L. casei* W56, *L. salivarius* W24, *Lactococcus lactis* W19, *Lactococcus lactis* W58	Oral administration via drinking water	Multispecies probiotics treatment markedly reduced depressive-like behavior independently of diet.	28259042
	L. helveticus R0052 and *B. longum* R0175	Gavage administration	Probiotics attenuated post-myocardial infarction depression as well as n-3 fatty acids did.	23068715
	L. helveticus R0052 and *B. longum* R0175	Oral administration via drinking water	Probiotics interferes with the development of post-MI depressive behavior and restores intestinal barrier integrity in MI rats.	21933458

Details of the samples used in theses studies can be found in Figure 1.

induction of neuroinflammation and neurodegenerative changes (Kennedy et al., 2017). Therefore, the study of 5-HT and kynurenine metabolism in tryptophan metabolism holds significant importance in depression research, and the balance between the two is closely linked to the physiopathology of depression.

SCFAs

In the gut, short-chain fatty acids (SCFAs) serve as common microbial metabolites and are closely associated with depression. Reports indicate that SCFAs are depleted in patients with MDD

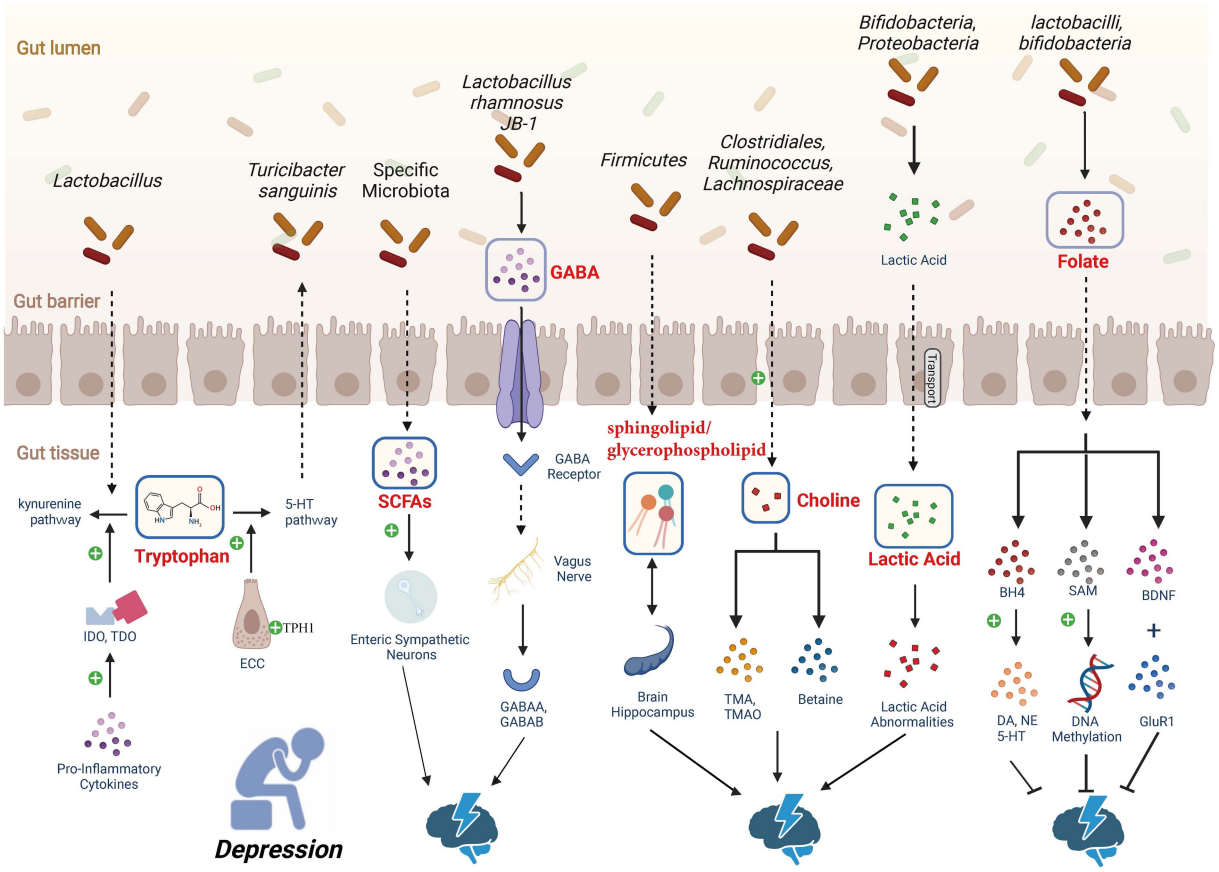


FIGURE 2
Depression-associated metabolic pathways. Gut microbiota regulates the levels of 5-HT to influence depression. Through dietary or drug interventions, it upregulates the expression of tryptophan hydroxylase 1 (TPH1) in enterochromaffin cells (ECCs), enhancing the production of 5-HT. SCFAs have been shown to affect the production of 5-HT in the gut. An increase in dietary tryptophan induces the synthesis of SCFAs by gut microbiota, leading to an increase in 5-HT production and release in ECCs. Gut microbiota has the ability to increase γ-aminobutyric acid (GABA) levels in the central nervous system (CNS) of mice. GABA crosses the intestinal barrier and is sensed by the vagus nerve, transmitting signals to paraventricular nucleus (PVN) neurons, thereby initiating hypothalamic–pituitary–adrenal (HPA) axis activity and producing an antidepressant effect. In the human body, choline positively influences emotions through promoting SAM-dependent DNA methylation. However, oral choline increases the concentration of acetylcholine in the brain, promoting depression-like behavior. Choline deficiency or excess may both affect depression, highlighting the complexity of the relationship between choline metabolism and depressive behavior. Lactic acid can pass through the blood–brain barrier, and studies in rodents and humans have found a connection between depression and lactic acid abnormalities. About one-third of depression patients exhibit folate deficiency. Folate supplementation has demonstrated its antidepressant effects, but clinical trials have not yet provided strong evidence to support folate as an advantageous adjunctive strategy for depression. The signaling pathway of bile acid metabolism within the gut–liver axis (not depicted in the figure), subject to structural modifications by the gut microbiota, can elicit either severe depression or manifest antidepressant effects based on the specific receptors involved. Created with BioRender.com.

(Zheng et al., 2016; Skonieczna-Żydecka et al., 2018). However, administration of SCFAs, especially butyrate, has been shown to improve depression-related gut permeability and HPA axis reactivity, resulting in antidepressant effects (Van De Wouw et al., 2018; Caspani et al., 2019). Research related to depression suggests that the expression of BDNF, can be altered by exogenous SCFAs. Long-term administration of exogenous sodium butyrate in mice has significantly reduced depressive-like behavior, indicating that SCFAs may influence the occurrence and development of depression through their effects on the brain (Schroeder et al., 2007).

SCFAs impact the activity of the enteric nervous system (ENS) and regulate intestinal motility in rodents through free fatty acid receptors present on epithelial cells, enteroendocrine cells (EECs), ECCs, immune cells, and endogenous and exogenous neurons (Muller et al., 2020). These pathways modulate local neuronal cells in the metabolism and/or ENS and the afferent pathway of the vagus nerve, which directly signals to the brain (Morais et al., 2021). A study showed that germ-free mice, lacking gut microbiota, exhibited increased activation of extrinsic neurons connecting the brainstem sensory and enteric sympathetic neurons. However, the activation of these neurons was inhibited by the administration of gut microbiota that produces SCFAs. These findings suggest that gut microbiota can regulate the gut-brain axis neuronal pathway through SCFAs. Additionally, SCFAs have been shown to affect the production of intestinal 5-HT (Bonaz et al., 2018). In humans and mice, increased dietary tryptophan induces the synthesis of SCFAs by gut microbiota, leading to increased production and release of 5-HT in ECCs and enhanced gastrointestinal motility (Reigstad et al., 2015; Yano et al., 2015; Agus et al., 2018; Yu et al., 2019).

However, SCFAs have a relatively short half-life (25 min to 3 h), and further research is needed to determine the extent of the influence of physiologically relevant concentrations of SCFAs on the brain (Margolis et al., 2021).

GABA

γ-aminobutyric acid (GABA) is a naturally occurring amino acid found widely in vertebrates, plants, and microorganisms. It is an important inhibitory neurotransmitter in the central nervous system (Rashmi et al., 2018). In recent probiotic research, it has been reported that GABA can be produced by gut microbiota, following a synthesis pathway similar to that in the central nervous system (Janik et al., 2016), and has been shown to alleviate depressive-like behavior in mice (Bravo et al., 2011).

Studies have indicated that certain gut microbiota have the ability to increase GABA levels in the central nervous system of mice, thereby modulating depressive-like behavior. One such microorganism is *L. rhamnosus* JB-1 (Yunes et al., 2016; Strandwitz et al., 2018), which produces GABA that can cross the intestinal barrier via the proton-coupled amino acid transporter hPAT1 (Nielsen et al., 2012; Bonaz et al., 2017; Yong et al., 2020) and is sensed by the vagus nerve (Bonaz et al., 2017). The vagus nerve activates the GABA signaling pathway to regulate the expression of GABAA and GABAB receptors (Yunes et al., 2016; Strandwitz et al., 2018), allowing the GABA produced by the microbiota to interact with the widely expressed GABA receptors and transporters on the afferent neurons of the vagus nerve (Nielsen et al., 2012; Yong et al., 2020). Additionally, the vagus nerve initiates neural activation in the nucleus tractus solitarius (NTS) of the central nervous system. Sensory gut information transmitted to the NTS is then integrated into its extensive projections, such as the PVN of the hypothalamus, where PVN neurons are responsible for initiating HPA axis activity and producing antidepressant effects (Bravo et al., 2011; Janik et al., 2016).

Glycerophospholipids

Some evidence suggests that the host's lipid metabolism is influenced by the gut microbiota (Blaszczak et al., 2019). Lipids play a crucial role in neuronal function, and the lipid composition of the brain may impact perception and emotional behavior, potentially leading to depression and anxiety (Adibhatla and Hatcher, 2008; Yadav and Tiwari, 2014; Kornhuber et al., 2015). Glycerophospholipids (GP) are major structural lipid components of eukaryotic cell membranes and are involved in numerous cellular processes. Disruption of the gut microbiota, as observed in germ-free mouse experiments, may induce depressive-like behavior by modulating host metabolism (Zheng et al., 2016). Further research has revealed that the gut microbiota primarily influences host GP metabolism (Tian et al., 2022). An experiment found that the hippocampus exhibited the highest degree of disruption in lipid metabolic pathways. The differentially metabolized compounds in the hippocampus were mainly enriched in GP metabolites, with a small proportion belonging to sphingolipid metabolism. Compared to the healthy control (HC) group, the depressive-like (DL) group showed upregulation of most hippocampal metabolites involved in GP metabolism. Furthermore, two metabolites involved in sphingolipid metabolism (dihydroceramide and ceramide-1-phosphate) were significantly decreased in the DL group compared to the HC group. In a chronic unpredictable mild stress rat model of depression, a decrease in lipid metabolism-related enzymes associated with fatty acid synthesis and metabolism, as well as GP metabolism, was observed (Oliveira et al., 2016). These findings indicate an imbalance in hippocampal sphingolipid and GP metabolism associated with depressive-like behavior (Zheng et al., 2021).

Consistent results from studies on humans and non-human primates indicate that dysbiosis of the bacterial phylum Firmicutes may be a hallmark of depression. Zheng et al. found that alterations in microbial and metabolic modules related to fatty acyl, sphingolipid, and GP metabolism were highly correlated with depressive-like behavior (Zheng et al., 2021). Within these microbial modules, several microbial genes involved in fatty acyl, sphingolipid, and GP metabolism were identified, suggesting that the gut microbiota and their regulated host metabolites may play a crucial role in the pathophysiology of depression. Interestingly, most of the unsaturated fatty acids used for the synthesis of brain neuronal membrane GP originate from the gastrointestinal tract rather than the central nervous system, indicating that GP metabolism via the gut-brain axis interferes with depression.

Choline metabolites

Choline is a constituent of all biological membranes and a precursor of acetylcholine in cholinergic neurons. The acquisition of choline in the body occurs through food sources such as liver and eggs, primarily in the form of phosphatidylcholine (PC), or from endogenously synthesized PC through a continuous methylation process of phosphatidylethanolamine (PE). Choline itself is not a product of bacteria, but under the influence of the gut microbiota, choline can be metabolized into a series of compounds, including trimethylglycine (betaine) and trimethylamine (TMA). In the liver, TMA is converted to trimethylamine N-oxide (TMAO) by flavin monooxygenases (Dumas et al., 2006). Studies have found that the levels of TMA and TMAO in mouse plasma are positively correlated with *Clostridiales*, *Ruminococcus*, and *Lachnospiraceae* in the gut, while negatively correlated with the proportions of S24-7, an abundant family from *Bacteroidetes* (Wang et al., 2015). In the findings by Romano et al. (2017), choline and its metabolites were found to affect emotional behavior through DNA methylation. Choline regulates the production of the methyl donor S-adenosylmethionine (SAM) to promote DNA methylation. Bacterial consumption of choline reduces the availability of methyl donors, which is consistent with reports of decreased hippocampal DNA methylation and abnormal neurodevelopment in offspring due to maternal choline deficiency (Mellott et al., 2007). In the human body, betaine has a positive impact on emotions by promoting SAM-dependent DNA methylation (Di Pierro and Settembre, 2015). In a rat model of stress, supplementation of choline, betaine, and other methyl donors successfully reversed depressive-like behavior (Paternain et al., 2016).

However, oral administration of choline may promote depressive-like behavior. Choline can actively cross the blood–brain barrier (Sawada et al., 2010), and oral choline increases the concentration of acetylcholine in the brain (Babb et al., 2004), indicating that abnormal choline metabolism may promote depressive-like behavior by altering

the choline concentration used for acetylcholine synthesis. Studies have shown that the concentration of the neurotransmitter acetylcholine is significantly higher in patients with MDD than in healthy subjects (Mineur and Picciotto, 2010). All of these findings indicate that choline and its metabolites have a significant impact on emotions through the gut microbiota. Choline deficiency can impair mental health, while excessive choline intake may lead to excessive synthesis of acetylcholine and result in depressive-like behavior. This also suggests the complexity of the relationship between choline metabolism and depressive behavior.

Lactate

Lactic acid is produced through the fermentation of dietary fiber by mammalian hosts and specific bacteria such as lactic acid bacteria, *Bifidobacteria*, and *Proteobacteria* (Ríos-Covián et al., 2016). Although the concentration of lactate in the intestine is low, it can be absorbed into the bloodstream (Tahara et al., 2018) and can cross the blood–brain barrier (Knudsen et al., 1991). Studies in rodents and humans have indicated a connection between depression and lactate abnormalities (Carrard et al., 2018; Karnib et al., 2019), with increased urinary lactate levels observed in severe MDD patients (Chen et al., 2017a). In germ-free mice, elevated lactate concentrations have been observed in the hippocampus, while germ-free rats exhibit increased lactate concentrations in the frontal lobe (Swann et al., 2017).

Karnib et al. (2019) found that lactate salts have a protective and reversing effect on depression. Mice treated with lactate showed increased levels and activity of HDAC2/3 in the hippocampus, while mice not receiving lactate exhibited depressive-like behavior. The efficient exchange of lactate between the peripheral and central nervous systems (Knudsen et al., 1991) suggests the crucial role of the gut microbiota in mediating the antidepressant effects of lactate salts.

Bile acids

Bile acids (BAs), synthesized from liver cholesterol, are pivotal end-products in cholesterol metabolism, constituting essential components of bile and primarily existing in the enterohepatic circulation system. In humans and rats, the main BAs are cholic acid (CA) and chenodeoxycholic acid (CDCA), subject to structural modifications by gut microbiota, leading to the formation of secondary and tertiary BAs (Russell, 2003). Higher levels of cytotoxic secondary BAs, derived from the primary bile acid CDCA by bacterial modifications, have been extensively reviewed in correlation with the severity of anxiety symptoms (Guzior and Quinn, 2021; Mahmoudian Dehkordi et al., 2022; Qu et al., 2022; Sun et al., 2022).

The signaling pathway of BAs is initiated by their binding to farnesoid X receptor (FXR) and Takeda G protein-coupled receptor 5 (Mertens et al., 2017). FXR is involved in bile acid synthesis, secretion, transport, and regulation of cAMP-response element binding protein (CREB) activity. By inhibiting the transcription factor CREB, BAs can suppress the transcription of BDNF, suggesting a potential influence of BAs on depression. Reports have indicated that the abnormal activity of BDNF in individuals with depression may be partially caused by changes in bile acid activity. In the chronic unpredictable mild stress (CUMS) rodent model of depression, an overexpression of FXR in the hippocampus has been observed. Conversely, overexpression of FXR in the rat hippocampus induces depression-like behavior, while the deletion of the FXR gene in juvenile rats inhibits the occurrence of depression-like behavior (Chen et al., 2018). An independent study has also confirmed the antidepressant effect of FXR gene deletion (Huang et al., 2015). Additionally, BAs may disrupt tight junction expression, leading to increased permeability of the intestinal and central epithelial cells, which can result in severe depression (Quinn et al., 2014). However, some BAs, such as ursodeoxycholic acid, have shown good antidepressant effects (Moore et al., 2000; Spedding, 2014), indicating that the impact of BAs on depression-like behavior may depend on specific receptors involved.

Vitamin (folate)

Humans heavily rely on the gut microbiota, such as *lactobacilli* and *bifidobacteria*, to produce vitamins (Gu and Li, 2016), which play important roles in the human body. In the central nervous system, vitamins can influence neurotransmitter production (Kennedy, 2016), thereby impacting neuronal function.

Folate, a microbiota-derived vitamin, has been widely implicated in depression research (Brocardo et al., 2008, 2009; Molina-Hernández et al., 2011; McCoy et al., 2016; Paternain et al., 2016; Gao et al., 2017), with approximately one-third of individuals with depression showing folate deficiency (Miller, 2008). Administration of folate has demonstrated antidepressant effects in animal models of depression (Brocardo et al., 2008; Molina-Hernández et al., 2011; Gao et al., 2017), and some clinical studies have indicated its potential as an adjunctive therapy for depression in humans (Coppen et al., 1986; Coppen and Bailey, 2000). Folate can synthesize tetrahydrobiopterin, which acts as a cofactor for the conversion of phenylalanine and tryptophan into neurotransmitters dopamine, nzorepinephrine and 5-HT (Wolf et al., 1991), thereby enhancing serotonergic and noradrenergic activity in mice and exerting antidepressant effects. Furthermore, in addition to increasing central 5-HT, folate can induce increased expression of BDNF and glutamate receptor 1 in the hippocampus and associated cortex. The active metabolite of folate, 5-methyltetrahydrofolate, converts homocysteine into methionine, which is used as a methyl donor to produce S-adenosylmethionine (SAM). SAM has been shown to exert antidepressant effects through DNA methylation of phospholipids (Young and Ghadirian, 1989; Kagan et al., 1990; Bottiglieri et al., 1994). Although significant improvements in depression-like behavior have been observed in animal studies, clinical trials have not provided strong evidence supporting the advantage of folate as an adjunctive strategy for depression (Roberts et al., 2018).

The effects of the above metabolites on depression, relevant mechanisms, and reference information have been summarized in Table 2.

Microbiome-based clinical trials in depression

So far, probiotics have received significant attention as potential therapies for mood disorders and MDD (Figure 3; Table 3). The occurrence of different probiotics across various clinical trials was

TABLE 2 Impact of metabolites on depression.

Metabolites	Impacts on depression	Mechanism	PMID
Tryptophan	A diet rich in tryptophan helps reduce depressive symptoms and improve an individual's emotional state. A low tryptophan diet can lead to irritability and anxiety.	Tryptophan hydroxylase (TPH) enzyme plays an important role in various psychiatric disorders, including depression. The peripheral cells involved in the production of 5-HT showed TPH1 dysfunction, leading to insufficient levels of 5-HT in the brain, which in turn triggers a homeostatic response of TPH2. Excessive pro-inflammatory cytokines produced in depression over-activate the enzymes IDO and TDO, promoting the kynurenine pathway, thereby reducing the activation of the 5-HT pathway. Kynurenine can cross the blood–brain barrier and negatively impact brain health by inducing neuroinflammation and neurodegenerative changes.	25858202 28968985 16192985 25540092 25860609 25550456 18465467 22688187 27392632
SCFAs	Short-chain fatty acids shown to have antidepressant effects.	Butyrate administration can improve depression-related intestinal permeability and responsiveness of the HPA axis, resulting in an antidepressant effect. In humans and mice, an increase in dietary tryptophan induces the synthesis of SCFAs by the gut microbiota, resulting in increased production and release of 5-HT in ECs.	31646148 30066368 16945350 25860609 25550456 31216174 29902437
GABA	GABA has been shown to alleviate depressive-like behavior in mice.	JB-1 produce GABA, which crosses the intestinal barrier and is sensed by the vagus nerve, which activates the GABA signaling pathway, regulates the expression of GABAA and GABAB receptors and interacts with GABA. At the same time, the vagus nerve initiates neural activation, and PVN neurons initiate HPA axis activity to produce antidepressant effects.	21876150 27794467 30531975 22452873 32009871 29163522 26577887
Glycerophospholipid	An imbalance in hippocampal sphingolipid and glycerophospholipid metabolism associated with depressive-like behavior.	In a chronic unpredictable mild stress rat model of depression, a decrease in lipid metabolism-related enzymes associated with fatty acid synthesis and metabolism and glycerophospholipid metabolism was observed, indicating an imbalance in hippocampal sphingolipid and glycerophospholipid metabolism associated with depression-like behavior.	25754084 32376998 25803076 18755070 24590317
Choline	Choline deficiency can impair mental health, while excessive choline intake may lead to excessive synthesis of acetylcholine and result in depressive-like behavior.	Choline and its metabolites affect emotional behavior through DNA methylation. Supplementation with choline, betaine, and other methyl donors successfully reversed depression-like behavior. At the same time, cholinergic passage through the blood–brain barrier increases the concentration of acetylcholine in the brain, and abnormal choline metabolism may promote depression-like behavior.	28844887 25678811 26628207 10467961 14972364
Lactate	Lactate salts have a protective and reversing effect on depression.	Lactate can cross the blood–brain barrier and elevated levels of lactate have been observed in both germ-free rodents and MDD patients. Depression-like behavior was reversed by lactate administration.	2050746 30647450 28624318 28595107
Bile acids	Bile acid can cause severe depression, but some Bile acid, such as Ursodeoxycholic acid, show good antidepressant effect.	FXR is involved in bile acid synthesis, secretion, transport and regulation of CREB activity. By inhibiting CREB, bile acid can inhibit the transcription of BDNF, resulting in abnormal BDNF activity in patients with depression.	29163019 29677620 25870546 24629820
Vitamin (folate)	About one-third of depression patients exhibit folate deficiency, and administering folate in animal models of depression shows antidepressant effects.	Folate can synthesize BH4, which converts phenylalanine and tryptophan into the neurotransmitters dopamine, norepinephrine and 5-HT, thereby exerting an antidepressant effect. BDNF and GluR1 expression was also induced in the hippocampus and associated cortex.	18078962 20816716 10967371 2939126 1716662

Study	Country	Probiotics	placebo	Male	Female	PMID/DOI
Pinto-Sanchez,2017	Canada	n=22	n=22	NA	NA	28483500
Rao,2009	Canada	n=19	n=16	n=8	n=27	19338686
Mohammadi,2016	Iran	n=50	n=20	n=36	n=34	25879690
Akkasheh,2015	Iran	n=20	n=20	n=6	n=34	26706022
Shinkai,2012	Japan	n=285	n=93	NA	NA	22947249
Nishihira,2014	Japan	n=115	n=109	n=69	n=155	10.1016/j.jff.2014.09.002
Östlund-Lagerström,2016	Sweden	n=125	n=124	n=96	n=153	27612653
Steenbergen,2015	Netherlands	n=20	n=20	n=8	n=32	25862297
Bambling,2017	Australia	NA	NA	n=4	n=8	28155119
Chung,2014	Korea	n=26	n=10	n=20	n=16	10.1016/j.jff.2014.07.007
Messaoudi,2011	France	n=10	n=15	NA	NA	21983070
Romijn,2017	New Zealand	n=40	n=39	n=17	n=62	28068788

FIGURE 3
Geographical locations and sample metadata of different clinical trials related to depression.

explored (Figure 4). *L. acidophilus* and *B. longum* are the most widely used probiotics in clinical trials. In healthy individuals, probiotic treatment with *L. helveticus* R0052 and *B. longum* R0175 for 30 days has been shown to reduce depression scores on the Hospital Anxiety and Depression (HAD) scale (Messaoudi et al., 2011). In patients with mild to moderate depression, a four-week treatment with a variety of probiotics (Bio-Kult Advanced®) significantly decreased PHQ9 scores (Baião et al., 2023). However, some studies have indicated that probiotic interventions may not effectively alleviate depressive symptoms. In individuals with moderate to severe depression, an eight-week administration of a probiotic mixture containing *L. helveticus* R0052 and *B. longum* R0175 as an adjunctive therapy did not improve depressive symptoms (Romijn et al., 2017). This study had a larger sample size and a longer treatment duration compared to previous successful trials of the same probiotic mixture in healthy subjects (Messaoudi et al., 2011). Probiotics are microbial preparations

TABLE 3 Antidepressant effects of probiotics observed in clinical trials.

Probiotics	Administration form	Findings	Participant type	PMID/DOI
B. longum NCC3001 (BL)	Oral administration	BL reduces depression but not anxiety scores and increases quality of life in patients with IBS.	Adults with IBS and diarrhea or a mixed-stool pattern (based on Rome III criteria) and mild to moderate anxiety and/or depression.	28483500
L. casei strain Shirota (LcS)	Oral administration	Lactobacillus and Bifidobacteria significantly increased and symptoms of anxiety decreased in those taking LcS.	CFS patients	19338686
Probiotic yogurt contained *L. acidophilus* LA5 and *B. lactis* BB12. Conventional yogurt contained *S. thermophilus* and *L. bulgaricus*. Probiotic capsule contained *Lactobacillus casei*, *L. acidophilus*, *L. rhamnosus*, *L. bulgaricus*, *B. breve*, *B. longum*, and *S. thermophilus*	Oral administration of probiotic yogurt or probiotic capsule	After 6 weeks of intervention, a significant improvement of GHQ was observed in the probiotic yogurt and in the probiotic capsule group, as well as a significant improvement in DASS scores in the probiotic yogurt and the probiotic capsule group.	Petrochemical workers	25,879,690
L. acidophilus, *L. casei*, and *B. bifidum*	Oral administration of probiotic capsule with three viable and freeze-dried strains	8-week intervention decreased Beck Depression Inventory total scores, serum insulin concentration, homeostasis model assessment of insulin resistance, and serum hs-CRP concentration. Plasma total glutathione concentration was elevated.	Aged 20 to 55 with major depressive disorder (MDD).	26706022
L. pentosus strain b240	Oral administration of heat-killed probiotics	Oral probiotics significantly reduced the incidence of the common cold in elderly adults.	Elderly adults aged 65 and above	22947249
L. gasseri SBT2055 and *B. longum* SBT2928	Oral administration of yogurt containing two different probiotics	Probiotics enhanced immunity and alleviated stress.	Healthy adults aged 32 to 76.	10.1016/j.jff.2014.09.002
L. reuteri DSM17938	Oral administration	No persistent significant effects were observed on the primary or secondary outcome measures of the study.	General elderly	27612653
Bifidobacterium (B.) bifidum W23, *B. lactis* W52, *Lactobacillus (L.) acidophilus* W37, *L. brevis* W63, *L. casei* W56, *L. salivarius* W24, and *Lactococcus lactis* (W19 and W58)	Oral administration of freeze-dried powder of the probiotic mixture	Consumption of multiple probiotics for 4 weeks significantly reduced overall cognitive responses to depression, especially aggression and rumination.	No psychiatric or neurological disorders, no personal or family history of depression or migraines.	25862297
L. acidophilus, B. bifidum, Streptoccocus (S.) thermophiles	Probiotics/magnesium orotate formulation adjuvant administered with SSRIs	At the end of an 8-week intervention mean changes for depression scores and quality of life in the group was clinically significantly improved. The participants who responded to treatment reported a subjective increase in well-being and improved energy levels.	Meets criteria for resistant depression, is currently taking antidepressants, and has a history of multiple depressive episodes that have poor response to treatment.	28155119
L. helveticus IDCC3801	Oral administration of fermentation of milk using probiotic Lactobacillus suis IDCC3801 (LHFM)	Cognitive tests improved after 12 weeks of LHFM administration.	Healthy elderly people aged 60 to 75	10.1016/j.jff.2014.07.007
L. helveticus R0052 and *B. longum* R0175	Oral administration	*Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175 (PF) taken in combination for 30 days decreased the global scores of hospital anxiety and depression scale (HADs), and the global severity index of the Hopkins symptoms checklist (HSCL90).	General population	21983070
L. helveticus, B. longum	Oral administration	No significant evidence was found effectively treating low mood or modcrating the levels of inflammatory and other biomarker.	Not currently taking psychotropic drugs and scoring at least moderate on a self-reported emotional measurement.	28068788

Details of the samples used in these studies can be found in Figure 2.

10.3389/fmicb.2024.1292004

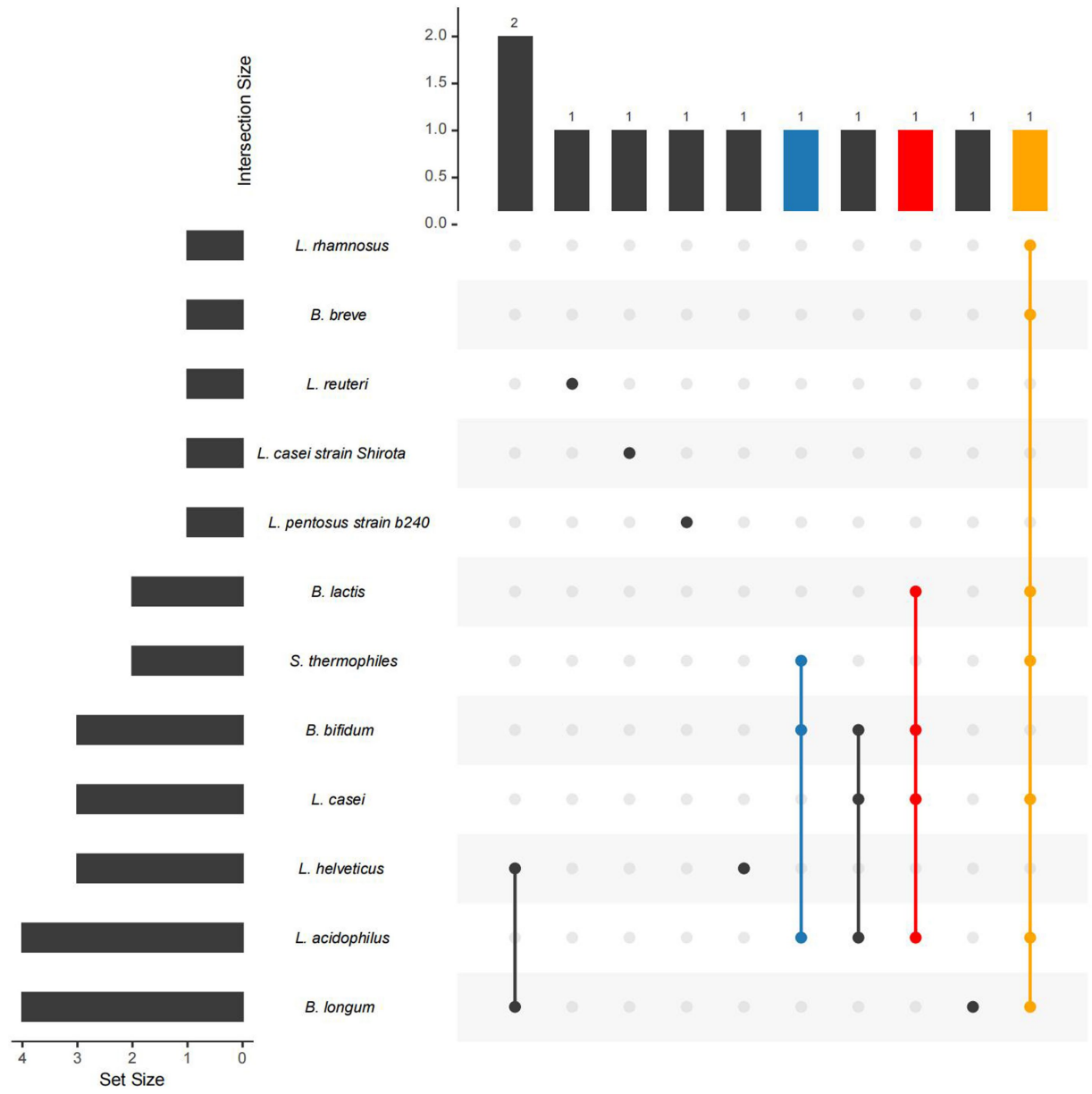


FIGURE 4
The occurrence of different probiotics across various studies. The left bar in the Upset Plot indicating the number of studies related to each microbe. Higher bars signify stronger evidence linking the microbe to depression. The upper bar displays the number of studies in which several connected microbes simultaneously used. A higher bar indicates that these microbes are found in a greater number of studies.

that, when ingested, can improve the balance of gut microbiota (Gibson and Roberfroid, 1995).

Prebiotics are compounds that are not broken down, absorbed, or utilized by the human body but promote the growth of gut microbiota, ultimately benefiting the host (Valcheva and Dieleman, 2016). Compounds evaluated in prebiotic trials include Bimuno®-galactooligosaccharide (B-GOS), fructooligosaccharide (FOS), GOS, and short-chain FOS (scFOS). None of the five depression prebiotic trials (Smith, 2005; Silk et al., 2009; Smith et al., 2015; Azpiroz et al., 2017; Kazemi et al., 2019) demonstrated a significant impact on depressive symptoms. Two studies examining the benefits of prebiotic treatment also did not observe a decrease in depression symptom scores during the eight-week follow-up period (Heidarzadeh-Rad et al., 2020; Vaghef-Mehrabany et al., 2021). When studied as standalone therapies, prebiotics yielded non-statistically significant results (Akkasheh et al., 2016; Kazemi et al., 2019; Rudzki et al., 2019). However, no studies have indicated any negative effects of probiotic/prebiotic interventions on depressive symptoms.

The combination of probiotics and prebiotics is known as synbiotics (Sorbara and Pamer, 2022). In a single synbiotic study by Ghorbani et al., a reduction in depressive symptoms was observed after 8 weeks of synbiotic treatment (Ghorbani et al., 2018). In a recent

systematic review and meta-analysis by Hofmeister et al., evidence from 50 randomized controlled trials (RCTs) evaluating probiotics, prebiotics, synbiotics, postbiotics, or fecal microbiota transplantation interventions in adult populations was synthesized. Improvement in depressive symptoms was reported based on the Beck Depression Inventory (BDI) and the depression subscale of the Hospital Anxiety and Depression Scale (Hofmeister et al., 2021).

Current evidence suggests that prebiotics as standalone therapies are unlikely to be effective for depression (Akkasheh et al., 2016; Ghorbani et al., 2018; Majeed et al., 2018; Miyaoka et al., 2018; Kazemi et al., 2019). While probiotics and synbiotics appear to be effective in alleviating depressive symptoms, the evidence supporting this observation is mixed. One possible explanation for these mixed findings is that individuals with mild depression may derive more benefits from probiotic and synbiotic treatments compared to those with chronic treatment-resistant depression (Wallace and Milev, 2021). Further studies focusing on different levels of depression severity would help clarify the benefits of these treatments. Additionally, the use of prebiotic and synbiotic therapies for depression is largely understudied, and the evidence is not as specific, necessitating multiple studies analyzing each compound.

Prospects and summary

The gut microbiota plays a crucial role in regulating human health. Extensive research has shown that the gut microbiota can influence the occurrence and development of depression through the MGB axis, involving neural, immune, and especially metabolic pathways. In reported studies, the gut microbiota has been found to play a significant role in the onset and progression of mental disorders, including depression. For example, when the microbiota from individuals with depression is transplanted into healthy animals, it can induce depression-like behaviors. At the same time, the microbiota can also improve depression-like behaviors. In animal studies, the administration of probiotics has been shown to significantly improve depression-like behaviors in both rats and mice.

Diagnosis and treatment of depression based on the gut microbiota is considered a future research direction. In human studies, administration of probiotics has shown some degree of effectiveness in alleviating depression symptoms. However, prebiotics as a standalone therapy for depression are unlikely to be effective, and the combination of probiotics and prebiotics has not demonstrated significant symptom relief. Yet, there is still limited research on the use of prebiotics and synbiotics in the treatment of depression, and exploring these therapies may uncover beneficial effects.

The CRISPR/Cas9 system is a potent genome editing tool widely utilized in basic, preclinical, and clinical studies as extensively reviewed for genetic disorders (Zhang et al., 2023). While limited studies have employed CRISPR/Cas9 in depression-associated research, there are already CRISPR/Cas-based genome editing tools for *Bacteroides* (Zheng et al., 2022). These tools significantly aid mechanistic studies of gut commensals and the development of engineered live biotherapeutics.

Constrained-based modeling (Heinken and Thiele, 2015) allows for the versatility needed to simulate bacterial communities under various conditions that cannot be replicated *in vivo*, such as *C. difficile* infection. Thus, by employing computational modeling of microbial metabolism using software like MICOM (Diener et al., 2020), a framework is provided to infer the growth rates of selected bacteria and the metabolic interactions within the gut microbiota. Additionally, it offers a high-throughput platform for generating mechanistic hypotheses and testing them in clinical analyses. We believe that the most valuable application of metabolic models in bacterial communities is to provide detailed functional metabolic inferences as a means of testing new hypotheses, thereby laying the groundwork for the development of more accurate models. While these computational approaches have been applied to the study of the gut microbiota, their use in exploring the metabolic consequences of depression is groundbreaking. The outcomes of this approach include the development of more precise models by incorporating information about the ecological relationships between the gut microbiota and its host. From a metabolic perspective, integrating individual differences in genetics and gut microbiota holds promise for personalized recommendations of effective therapies for depression.

Author contributions

MW: Writing – original draft. ZS: Writing – original draft. SL: Writing – original draft, Data curation, Validation, Visualization. FT: Writing – review & editing, Methodology, Supervision, Validation, Investigation. LD: Funding acquisition, Writing – review & editing. FY: Conceptualization, Funding acquisition, Project administration, Writing – review & editing, Writing – original draft.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This research was financially supported by the National Natural Science Foundation of China [62102065, 62002242, 62271353, 62001311], joint funds for the Innovation of Science and Technology, Fujian Province (grant number: 2022J05055), Fujian Medical University Research Foundation of Talented Scholars [XRCZX2022003] and Natural Science Foundation of Sichuan Province (2022NSFSC0926).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

Abildgaard, A., Elfving, B., Hokland, M., Wegener, G., and Lund, S. (2017). Probiotic treatment reduces depressive-like behaviour in rats independently of diet. *Psychoneuroendocrinology* 79, 40–48. doi: 10.1016/j.psyneuen.2017.02.014

Adibhatla, R. M., and Hatcher, J. F. (2008). Phospholipase a $_2$, reactive oxygen species, and lipid peroxidation in CNS pathologies. *BMB Rep.* 41, 560–567. doi: 10.5483/BMBRep.2008.41.8.560

Agus, A., Planchais, J., and Sokol, H. (2018). Gut microbiota regulation of tryptophan metabolism in health and disease. *Cell Host Microbe* 23, 716–724. doi: 10.1016/j.chom.2018.05.003

Akkasheh, G., Kashani-Poor, Z., Tajabadi-Ebrahimi, M., Jafari, P., Akbari, H., Taghizadeh, M., et al. (2016). Clinical and metabolic response to probiotic administration in patients with major depressive disorder: a randomized, double-blind, placebo-controlled trial. *Nutrition* 32, 315–320. doi: 10.1016/j.nut.2015.09.003

Austin, M., Mellow, M., and Tierney, W. M. (2014). Fecal microbiota transplantation in the treatment of *Clostridium difficile* infections. *Am. J. Med.* 127, 479–483. doi: 10.1016/j.amjmed.2014.02.017

Azpiroz, F., Dubray, C., Bernalier-Donadille, A., Cardot, J.-M., Accarino, A., Serra, J., et al. (2017). Effects of scFOS on the composition of fecal microbiota and anxiety in patients with irritable bowel syndrome: a randomized, double blind, placebo controlled study. *Neurogastroenterol. Motil.* 29:e12911. doi: 10.1111/nmo.12911

Babb, S. M., Ke, Y., Lange, N., Kaufman, M. J., Renshaw, P. F., and Cohen, B. M. (2004). Oral choline increases choline metabolites in human brain. *Psychiatry Res. Neuroimaging* 130, 1–9. doi: 10.1016/S0925-4927(03)00104-5

Bachmann, S. (2018). Epidemiology of suicide and the psychiatric perspective. *Int. J. Environ. Res. Public Health* 15:1425. doi: 10.3390/ijerph15071425

Bach-Mizrachi, H., Underwood, M. D., Kassir, S. A., Bakalian, M. J., Sibille, E., Tamir, H., et al. (2006). Neuronal tryptophan hydroxylase mRNA expression in the human dorsal and median raphe nuclei: major Depression and suicide. *Neuropsychopharmacology* 31, 814–824. doi: 10.1038/sj.npp.1300897

Bäckhed, F., Ley, R. E., Sonnenburg, J. L., Peterson, D. A., and Gordon, J. I. (2005). Host-bacterial mutualism in the human intestine. *Science* 307, 1915–1920. doi: 10.1126/science.1104816

Baião, R., Capitão, L. P., Higgins, C., Browning, M., Harmer, C. J., and Burnet, P. W. J. (2023). Multispecies probiotic administration reduces emotional salience and improves mood in subjects with moderate depression: a randomised, double-blind, placebo-controlled study. *Psychol. Med.* 53, 3437–3447. doi: 10.1017/S003329172100550X

Belmaker, R. H., and Agam, G. (2008). Major Depressive Disorder. *N. Engl. J. Med.* 358, 55–68. doi: 10.1056/NEJMra073096

Bercik, P. (2011). The microbiota-gut-brain axis: learning from intestinal bacteria? *Gut* 60, 288–289. doi: 10.1136/gut.2010.226779

Blaszczak, A. M., Dunaway, S., Ali Alikhan, M., Kelly, S., Levin, D., and Kaffenberger, J. (2019). Safety of systemic therapies in the treatment of psoriasis with concomitant cirrhosis: a retrospective study. *Int. J. Dermatol.* 58, e228–e229. doi: 10.1111/ijd.14556

Boku, S., Nakagawa, S., Toda, H., and Hishimoto, A. (2018). Neural basis of major depressive disorder: beyond monoamine hypothesis: neural basis of major depressive disorder. *Psychiatry Clin. Neurosci.* 72, 3–12. doi: 10.1111/pcn.12604

Bonaz, B., Bazin, T., and Pellissier, S. (2018). The Vagus nerve at the Interface of the microbiota-gut-brain Axis. *Front. Neurosci.* 12:49. doi: 10.3389/fnins.2018.00049

Bonaz, B., Sinniger, V., and Pellissier, S. (2017). The Vagus nerve in the neuro-immune Axis: implications in the pathology of the gastrointestinal tract. *Front. Immunol.* 8:1452. doi: 10.3389/fimmu.2017.01452

Bottiglieri, T., Hyland, K., and Reynolds, E. H. (1994). The clinical potential of ademetionine (S-Adenosylmethionine) in neurological disorders. *Drugs* 48, 137–152. doi: 10.2165/00003495-199448020-00002

Bravo, J. A., Forsythe, P., Chew, M. V., Escaravage, E., Savignac, H. M., Dinan, T. G., et al. (2011). Ingestion of Lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. *Proc. Natl. Acad. Sci.* 108, 16050–16055. doi: 10.1073/pnas.1102999108

Breit, S., Kupferberg, A., Rogler, G., and Hasler, G. (2018). Vagus nerve as modulator of the brain-gut Axis in psychiatric and inflammatory disorders. *Front. Psych.* 9:44. doi: 10.3389/fpsyt.2018.00044

Brocardo, P. S., Budni, J., Kaster, M. P., Santos, A. R. S., and Rodrigues, A. L. S. (2008). Folic acid administration produces an antidepressant-like effect in mice: evidence for the involvement of the serotonergic and noradrenergic systems. *Neuropharmacology* 54, 464–473. doi: 10.1016/j.neuropharm.2007.10.016

Brocardo, P. S., Budni, J., Lobato, K. R., Santos, A. R. S., and Rodrigues, A. L. S. (2009). Evidence for the involvement of the opioid system in the antidepressant-like effect of folic acid in the mouse forced swimming test. *Behav. Brain Res.* 200, 122–127. doi: 10.1016/j.bbr.2009.01.004

Burokas, A., Arboleya, S., Moloney, R. D., Peterson, V. L., Murphy, K., Clarke, G., et al. (2017). Targeting the microbiota-gut-brain Axis: prebiotics have anxiolytic and antidepressant-like effects and reverse the impact of chronic stress in mice. *Biol. Psychiatry* 82, 472–487. doi: 10.1016/j.biopsych.2016.12.031

Camilleri, M., Madsen, K., Spiller, R., Van Meerveld, B. G., and Verne, G. N. (2012). Intestinal barrier function in health and gastrointestinal disease: intestinal barrier function. *Neurogastroenterol. Motil.* 24, 503–512. doi: 10.1111/j.1365-2982.2012.01921.x

Cammarota, G., Masucci, L., Ianiro, G., Bibbò, S., Dinoi, G., Costamagna, G., et al. (2015). Randomised clinical trial: Faecal microbiota transplantation by colonoscopy vs. vancomycin for the treatment of recurrent *Clostridium difficile* infection. *Aliment. Pharmacol. Ther.* 41, 835–843. doi: 10.1111/apt.13144

Campos, A. C., Rocha, N. P., Nicoli, J. R., Vieira, L. Q., Teixeira, M. M., and Teixeira, A. L. (2016). Absence of gut microbiota influences lipopolysaccharide-induced behavioral changes in mice. *Behav. Brain Res.* 312, 186–194. doi: 10.1016/j.bbr.2016.06.027

Carrard, A., Elsayed, M., Margineanu, M., Boury-Jamot, B., Fragnière, L., Meylan, E. M., et al. (2018). Peripheral administration of lactate produces antidepressant-like effects. *Mol. Psychiatry* 23, 392–399. doi: 10.1038/mp.2016.179

Caspani, G., Kennedy, S., Foster, J. A., and Swann, J. (2019). Gut microbial metabolites in depression: understanding the biochemical mechanisms. *Microbial Cell* 6, 454–481. doi: 10.15698/mic2019.10.693

Chen, Y., Xu, H., Zhu, M., Liu, K., Lin, B., Luo, R., et al. (2017b). Stress inhibits tryptophan hydroxylase expression in a rat model of depression. *Oncotarget* 8, 63247–63257. doi: 10.18632/oncotarget.18780

Chen, W., Zheng, J., Xu, X., Hu, Y., and Ma, Y. (2018). Hippocampal FXR plays a role in the pathogenesis of depression: a preliminary study based on lentiviral gene modulation. *Psychiatry Res.* 264, 374–379. doi: 10.1016/j.psychres.2018.04.025

Chen, J., Zhou, C., Zheng, P., Cheng, K., Wang, H., Li, J., et al. (2017a). Differential urinary metabolites related with the severity of major depressive disorder. *Behav. Brain Res.* 332, 280–287. doi: 10.1016/j.bbr.2017.06.012

Clarke, G., Grenham, S., Scully, P., Fitzgerald, P., Moloney, R. D., Shanahan, F., et al. (2013). The microbiome-gut-brain axis during early life regulates the hippocampal serotonergic system in a sex-dependent manner. *Mol. Psychiatry* 18, 666–673. doi: 10.1038/mp.2012.77

Comai, S., Bertazzo, A., Brughera, M., and Crotti, S. (2020). Tryptophan in health and disease. *Adv. Clin. Chem* 95, 165–218. doi: 10.1016/bs.acc.2019.08.005

Coppen, A., and Bailey, J. (2000). Enhancement of the antidepressant action of fluoxetine by folic acid: a randomised, placebo controlled trial. *J. Affect. Disord.* 60, 121–130. doi: 10.1016/S0165-0327(00)00153-1

Coppen, A., Chaudhry, S., and Swade, C. (1986). Folic acid enhances lithium prophylaxis. *J. Affect. Disord.* 10, 9–13. doi: 10.1016/0165-0327(86)90043-1

Cowen, P. J., and Browning, M. (2015). What has serotonin to do with depression? *World Psychiatry* 14, 158–160. doi: 10.1002/wps.20229

Cryan, J. F., and Dinan, T. G. (2012). Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. *Nat. Rev. Neurosci.* 13, 701–712. doi: 10.1038/nrn3346

Cussotto, S., Clarke, G., Dinan, T. G., and Cryan, J. F. (2019). Psychotropics and the microbiome: a chamber of secrets …. *Psychopharmacology* 236, 1411–1432. doi: 10.1007/s00213-019-5185-8

Desbonnet, L., Garrett, L., Clarke, G., Kiely, B., Cryan, J. F., and Dinan, T. G. (2010). Effects of the probiotic *Bifidobacterium infantis* in the maternal separation model of depression. *Neuroscience* 170, 1179–1188. doi: 10.1016/j.neuroscience.2010.08.005

Di Pierro, F., and Settembre, R. (2015). Preliminary results of a randomized controlled trial carried out with a fixed combination of S-adenosyl-L-methionine and betaine versus amitriptyline in patients with mild depression. *Int. J. Gen. Med.* 8, 73–78. doi: 10.2147/IJGM.S79518

Diener, C., Gibbons, S. M., and Resendis-Antonio, O. (2020). MICOM: metagenome-scale modeling to infer metabolic interactions in the gut microbiota. *mSystems* 5, e00606–e00619. doi: 10.1128/mSystems.00606-19

Dumas, M.-E., Barton, R. H., Toye, A., Cloarec, O., Blancher, C., Rothwell, A., et al. (2006). Metabolic profiling reveals a contribution of gut microbiota to fatty liver phenotype in insulin-resistant mice. *Proc. Natl. Acad. Sci.* 103, 12511–12516. doi: 10.1073/pnas.0601056103

Duncan, S. H., Louis, P., and Flint, H. J. (2004). Lactate-utilizing Bacteria, isolated from human feces, that produce butyrate as a major fermentation product. *Appl. Environ. Microbiol.* 70, 5810–5817. doi: 10.1128/AEM.70.10.5810-5817.2004

Eckburg, P. B., Bik, E. M., Bernstein, C. N., Purdom, E., Dethlefsen, L., Sargent, M., et al. (2005). Diversity of the human intestinal microbial Flora. *Science* 308, 1635–1638. doi: 10.1126/science.1110591

Ernst, J., Hock, A., Henning, A., Seifritz, E., Boeker, H., and Grimm, S. (2017). Increased pregenual anterior cingulate glucose and lactate concentrations in major depressive disorder. *Mol. Psychiatry* 22, 113–119. doi: 10.1038/mp.2016.73

Fond, G. B., Lagier, J.-C., Honore, S., Lancon, C., Korchia, T., Verville, P.-L. S. D., et al. (2020). Microbiota-orientated treatments for major Depression and schizophrenia. *Nutrients* 12:1024. doi: 10.3390/nu12041024

For Anxiety and Depression, R. G. for Treatment (2017). Psychotherapies for anxiety and depression: benefits and costs. *Res. Psychother.* 20:284. doi: 10.4081/ripppo.2017.284

Fukuda, K. (2014). Etiological classification of depression based on the enzymes of tryptophan metabolism. *BMC Psychiatry* 14:372. doi: 10.1186/s12888-014-0372-y

Fung, T. C., Vuong, H. E., Luna, C. D. G., Pronovost, G. N., Aleksandrova, A. A., Riley, N. G., et al. (2019). Intestinal serotonin and fluoxetine exposure modulate bacterial colonization in the gut. *Nat. Microbiol.* 4, 2064–2073. doi: 10.1038/s41564-019-0540-4

Furusawa, Y., Obata, Y., Fukuda, S., Endo, T. A., Nakato, G., Takahashi, D., et al. (2013). Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. *Nature* 504, 446–450. doi: 10.1038/nature12721

Gacias, M., Gaspari, S., Santos, P.-M. G., Tamburini, S., Andrade, M., Zhang, F., et al. (2016). Microbiota-driven transcriptional changes in prefrontal cortex override genetic differences in social behavior. *elife* 5:e13442. doi: 10.7554/eLife.13442

Gao, L., Liu, X., Yu, L., Wu, J., Xu, M., and Liu, Y. (2017). Folic acid exerts antidepressant effects by upregulating brain-derived neurotrophic factor and glutamate receptor 1 expression in brain. *Neuro Report* 28, 1078–1084. doi: 10.1097/WNR.0000000000000887

George, F., Daniel, C., Thomas, M., Singer, E., Guilbaud, A., Tessier, F. J., et al. (2018). Occurrence and dynamism of lactic acid Bacteria in distinct ecological niches: a multifaceted functional health perspective. *Front. Microbiol.* 9:2899. doi: 10.3389/fmicb.2018.02899

Ghorbani, Z., Nazari, S., Etesam, F., Nourimajd, S., Ahmadpanah, M., and Razeghi Jahromi, S. (2018). The effect of Synbiotic as an adjuvant therapy to fluoxetine in moderate depression: a randomized multicenter trial. *Arch. Neurosci.* 5:e60507. doi: 10.5812/archneurosci.60507

Gibson, G. R., and Roberfroid, M. B. (1995). Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *J. Nutr.* 125, 1401–1412. doi: 10.1093/jn/125.6.1401

Gu, Q., and Li, P. (2016). Biosynthesis of vitamins by probiotic bacteria. *Probiotics and Prebiotics in Human Nutrition and Health*. 135–48. doi: 10.5772/63117

Guida, F., Turco, F., Iannotta, M., De Gregorio, D., Palumbo, I., Sarnelli, G., et al. (2018). Antibiotic-induced microbiota perturbation causes gut endocannabinoidome changes, hippocampal neuroglial reorganization and depression in mice. *Brain Behav. Immun.* 67, 230–245. doi: 10.1016/j.bbi.2017.09.001

Guzior, D. V., and Quinn, R. A. (2021). Review: microbial transformations of human bile acids. *Microbiome* 9:140. doi: 10.1186/s40168-021-01101-1

Heidarzadeh-Rad, N., Gökmen-Özel, H., Kazemi, A., Almasi, N., and Djafarian, K. (2020). Effects of a Psychobiotic supplement on serum brain-derived neurotrophic factor levels in depressive patients: a post hoc analysis of a randomized clinical trial. *J. Neurogastroenterol. Motil.* 26, 486–495. doi: 10.5056/jnm20079

Heijtz, R. D., Wang, S., Anuar, F., Qian, Y., Björkholm, B., Samuelsson, A., et al. (2011). Normal gut microbiota modulates brain development and behavior. *Proc. Natl. Acad. Sci.* 108, 3047–3052. doi: 10.1073/pnas.1010529108

Heinken, A., and Thiele, I. (2015). Systematic prediction of health-relevant human-microbial co-metabolism through a computational framework. *Gut Microbes* 6, 120–130. doi: 10.1080/19490976.2015.1023494

Hocquart, M., Lagier, J.-C., Cassir, N., Saidani, N., Eldin, C., Kerbaj, J., et al. (2018). Early fecal microbiota transplantation improves survival in severe *Clostridium difficile* infections. *Clin. Infect. Dis.* 66, 645–650. doi: 10.1093/cid/cix762

Hofmeister, M., Clement, F., Patten, S., Li, J., Dowsett, L. E., Farkas, B., et al. (2021). The effect of interventions targeting gut microbiota on depressive symptoms: a systematic review and meta-analysis. *CMAJ Open* 9, E1195–E1204. doi: 10.9778/cmajo.20200283

Huang, F., Wang, T., Lan, Y., Yang, L., Pan, W., Zhu, Y., et al. (2015). Deletion of mouse FXR gene disturbs multiple neurotransmitter systems and alters neurobehavior. *Front. Behav. Neurosci.* 9:70. doi: 10.3389/fnbeh.2015.00070

Janik, R., Thomason, L. A. M., Stanisz, A. M., Forsythe, P., Bienenstock, J., and Stanisz, G. J. (2016). Magnetic resonance spectroscopy reveals oral Lactobacillus promotion of increases in brain GABA, N-acetyl aspartate and glutamate. *Neuro Image* 125, 988–995. doi: 10.1016/j.neuroimage.2015.11.018

Jianguo, L., Xueyang, J., Cui, W., Changxin, W., and Xuemei, Q. (2019). Altered gut metabolome contributes to depression-like behaviors in rats exposed to chronic unpredictable mild stress. *Transl. Psychiatry* 9:40. doi: 10.1038/s41398-019-0391-z

Kagan, B. L., Sultzer, D. L., Rosenlicht, N., and Gerner, R. H. (1990). Oral S-adenosylmethionine in depression: a randomized, double-blind, placebo-controlled trial. *Am. J. Psychiatr.* 147, 591–595. doi: 10.1176/ajp.147.5.591

Karnib, N., El-Ghandour, R., El Hayek, L., Nasrallah, P., Khalifeh, M., Barmo, N., et al. (2019). Lactate is an antidepressant that mediates resilience to stress by modulating the hippocampal levels and activity of histone deacetylases. *Neuropsychopharmacology* 44, 1152–1162. doi: 10.1038/s41386-019-0313-z

Kazemi, A., Noorbala, A. A., Azam, K., Eskandari, M. H., and Djafarian, K. (2019). Effect of probiotic and prebiotic vs placebo on psychological outcomes in patients with major depressive disorder: a randomized clinical trial. *Clin. Nut.* 38, 522–528. doi: 10.1016/j.clnu.2018.04.010

Kelly, J. R., Borre, Y., O'Brien, C., Patterson, E., El Aidy, S., Deane, J., et al. (2016). Transferring the blues: Depression-associated gut microbiota induces neurobehavioural changes in the rat. *J. Psychiatr. Res.* 82, 109–118. doi: 10.1016/j.jpsychires.2016.07.019

Kennedy, D. (2016). B vitamins and the brain: mechanisms, dose and efficacy—a review. *Nutrients* 8:68. doi: 10.3390/nu8020068

Kennedy, P. J., Cryan, J. F., Dinan, T. G., and Clarke, G. (2017). Kynurenine pathway metabolism and the microbiota-gut-brain axis. *Neuropharmacology* 112, 399–412. doi: 10.1016/j.neuropharm.2016.07.002

Kim, Y.-K., Kim, O. Y., and Song, J. (2020). Alleviation of Depression by glucagon-like peptide 1 through the regulation of Neuroinflammation, neurotransmitters, neurogenesis, and synaptic function. *Front. Pharmacol.* 11:1270. doi: 10.3389/fphar.2020.01270

Knudsen, G. M., Paulson, O. B., and Hertz, M. M. (1991). Kinetic analysis of the human blood-brain barrier transport of lactate and its influence by hypercapnia. *J. Cereb. Blood Flow Metab.* 11, 581–586. doi: 10.1038/jcbfm.1991.107

Korecka, A., and Arulampalam, V. (2012). The gut microbiome: scourge, sentinel or spectator? *J. Oral Microbiol.* 4:9367. doi: 10.3402/jom.v4i0.9367

Kornhuber, J., Rhein, C., Müller, C. P., and Mühle, C. (2015). Secretory sphingomyelinase in health and disease. *Biol. Chem.* 396, 707–736. doi: 10.1515/hsz-2015-0109

Li, Y.-T., Cai, H.-F., Wang, Z.-H., Xu, J., and Fang, J.-Y. (2016). Systematic review with meta-analysis: long-term outcomes of faecal microbiota transplantation for *Clostridium difficile* infection. *Aliment. Pharmacol. Ther.* 43, 445–457. doi: 10.1111/apt.13492

Li, N., Wang, Q., Wang, Y., Sun, A., Lin, Y., Jin, Y., et al. (2018). Oral probiotics ameliorate the behavioral deficits induced by chronic mild stress in mice via the gut microbiota-inflammation Axis. *Front. Behav. Neurosci.* 12:266. doi: 10.3389/fnbeh.2018.00266

Liang, S., Wang, T., Hu, X., Luo, J., Li, W., Wu, X., et al. (2015). Administration of *Lactobacillus helveticus* NS8 improves behavioral, cognitive, and biochemical aberrations caused by chronic restraint stress. *Neuroscience* 310, 561–577. doi: 10.1016/j.neuroscience.2015.09.033

Lindseth, G., Helland, B., and Caspers, J. (2015). The effects of dietary tryptophan on affective disorders. *Arch. Psychiatr. Nurs.* 29, 102–107. doi: 10.1016/j.apnu.2014.11.008

López-Ibor Aliño, J. J., Ayuso Gutierrez, J. L., and Montejo Iglesias, M. L. (1976). 5-Hydroxytryptophan (5-HTP) and a MAOI (Nialamide) in the treatment of depressions. *Int. Pharmacopsychiatry* 11, 8–15. doi: 10.1159/000468207

Macpherson, A. J., and Harris, N. L. (2004). Interactions between commensal intestinal bacteria and the immune system. *Nat. Rev. Immunol.* 4, 478–485. doi: 10.1038/nri1373

Maehata, H., Kobayashi, Y., Mitsuyama, E., Kawase, T., Kuhara, T., Xiao, J.-Z., et al. (2019). Heat-killed *Lactobacillus helveticus* strain MCC1848 confers resilience to anxiety or depression-like symptoms caused by subchronic social defeat stress in mice. *Biosci. Biotechnol. Biochem.* 83, 1239–1247. doi: 10.1080/09168451.2019.1591263

Maffei, M. E. (2020). 5-Hydroxytryptophan (5-HTP): natural occurrence, analysis, biosynthesis, biotechnology, physiology and toxicology. *Int. J. Mol. Sci.* 22:181. doi: 10.3390/ijms22010181

Mahmoudian Dehkordi, S., Bhattacharyya, S., Brydges, C. R., Jia, W., Fiehn, O., Rush, A. J., et al. (2022). Gut microbiome-linked metabolites in the pathobiology of major Depression with or without anxiety—a role for bile acids. *Front. Neurosci.* 16:937906. doi: 10.3389/fnins.2022.937906

Majeed, M., Nagabhushanam, K., Arumugam, S., Majeed, S., and Ali, F. (2018). *Bacillus coagulans* MTCC 5856 for the management of major depression with irritable bowel syndrome: a randomised, double-blind, placebo controlled, multi-Centre, pilot clinical study. *Food Nutr. Res.* 62:1218. doi: 10.29219/fnr.v62.1218

Margolis, K. G., Cryan, J. F., and Mayer, E. A. (2021). The microbiota-gut-brain Axis: from motility to mood. *Gastroenterology* 160, 1486–1501. doi: 10.1053/j.gastro.2020.10.066

Marin, I. A., Goertz, J. E., Ren, T., Rich, S. S., Onengut-Gumuscu, S., Farber, E., et al. (2017). Microbiota alteration is associated with the development of stress-induced despair behavior. *Sci. Rep.* 7:43859. doi: 10.1038/srep43859

McCoy, C. R., Rana, S., Stringfellow, S. A., Day, J. J., Wyss, J. M., Clinton, S. M., et al. (2016). Neonatal maternal separation stress elicits lasting DNA methylation changes in the hippocampus of stress-reactive Wistar Kyoto rats. *Eur. J. Neurosci.* 44, 2829–2845. doi: 10.1111/ejn.13404

McGuinness, A. J., Davis, J. A., Dawson, S. L., Loughman, A., Collier, F., O'Hely, M., et al. (2022). A systematic review of gut microbiota composition in observational studies of major depressive disorder, bipolar disorder and schizophrenia. *Mol. Psychiatry* 27, 1920–1935. doi: 10.1038/s41380-022-01456-3

Mellott, T. J., Kowall, N. W., Lopez-Coviella, I., and Blusztajn, J. K. (2007). Prenatal choline deficiency increases choline transporter expression in the septum and hippocampus during postnatal development and in adulthood in rats. *Brain Res.* 1151, 1–11. doi: 10.1016/j.brainres.2007.03.004

Mertens, K. L., Kalsbeek, A., Soeters, M. R., and Eggink, H. M. (2017). Bile acid signaling pathways from the enterohepatic circulation to the central nervous system. *Front. Neurosci.* 11:617. doi: 10.3389/fnins.2017.00617

Messaoudi, M., Lalonde, R., Violle, N., Javelot, H., Desor, D., Nejdi, A., et al. (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. doi: 10.1017/S0007114510004319

Mika, A., Day, H. E. W., Martinez, A., Rumian, N. L., Greenwood, B. N., Chichlowski, M., et al. (2017). Early life diets with prebiotics and bioactive milk fractions attenuate the impact of stress on learned helplessness behaviours and alter gene expression within neural circuits important for stress resistance. *Eur. J. Neurosci.* 45, 342–357. doi: 10.1111/ejn.13444

Miller, A. L. (2008). The methylation, neurotransmitter, and antioxidant connections between folate and depression. *Altern. Med. Rev.* 13, 216–226.

Mineur, Y. S., and Picciotto, M. R. (2010). Nicotine receptors and depression: revisiting and revising the cholinergic hypothesis. *Trends Pharmacol. Sci.* 31, 580–586. doi: 10.1016/j.tips.2010.09.004

Miura, H., Ozaki, N., Sawada, M., Isobe, K., Ohta, T., and Nagatsu, T. (2008). A link between stress and depression: shifts in the balance between the kynurenine and serotonin pathways of tryptophan metabolism and the etiology and pathophysiology of depression. *Stress* 11, 198–209. doi: 10.1080/10253890701754068

Miyaoka, T., Kanayama, M., Wake, R., Hashioka, S., Hayashida, M., Nagahama, M., et al. (2018). *Clostridium butyricum* MIYAIRI 588 as adjunctive therapy for treatment-resistant major depressive disorder: a prospective open-label trial. *Clin. Neuropharmacol.* 41, 151–155. doi: 10.1097/WNF.0000000000000299

Molina-Hernández, M., Téllez-Alcántara, N. P., Olivera-López, J. I., and Jaramillo, M. T. (2011). The folic acid combined with 17-β estradiol produces antidepressant-like actions in ovariectomized rats forced to swim. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* 35, 60–66. doi: 10.1016/j.pnpbp.2010.08.022

Moore, L. B., Goodwin, B., Jones, S. A., Wisely, G. B., Serabjit-Singh, C. J., Willson, T. M., et al. (2000). St. John's wort induces hepatic drug metabolism through activation of the pregnane X receptor. *Proc. Natl. Acad. Sci.* 97, 7500–7502. doi: 10.1073/pnas.130155097

Morais, L. H., Schreiber, H. L., and Mazmanian, S. K. (2021). The gut microbiota–brain axis in behaviour and brain disorders. *Nat. Rev. Microbiol.* 19, 241–255. doi: 10.1038/s41579-020-00460-0

Muller, P. A., Schneeberger, M., Matheis, F., Wang, P., Kerner, Z., Ilanges, A., et al. (2020). Microbiota modulate sympathetic neurons via a gut–brain circuit. *Nature* 583, 441–446. doi: 10.1038/s41586-020-2474-7

Nielsen, C. U., Carstensen, M., and Brodin, B. (2012). Carrier-mediated γ-aminobutyric acid transport across the basolateral membrane of human intestinal Caco-2 cell monolayers. *Eur. J. Pharm. Biopharm.* 81, 458–462. doi: 10.1016/j.ejpb.2012.03.007

O'Mahony, S. M., Marchesi, J. R., Scully, P., Codling, C., Ceolho, A.-M., Quigley, E. M. M., et al. (2009). Early life stress alters behavior, immunity, and microbiota in rats: implications for irritable bowel syndrome and psychiatric illnesses. *Biol. Psychiatry* 65, 263–267. doi: 10.1016/j.biopsych.2008.06.026

Oliveira, T. G., Chan, R. B., Bravo, F. V., Miranda, A., Silva, R. R., Zhou, B., et al. (2016). The impact of chronic stress on the rat brain lipidome. *Mol. Psychiatry* 21, 80–88. doi: 10.1038/mp.2015.14

Paternain, L., Martisova, E., Campión, J., Martínez, J. A., Ramírez, M. J., and Milagro, F. I. (2016). Methyl donor supplementation in rats reverses the deleterious effect of maternal separation on depression-like behaviour. *Behav. Brain Res.* 299, 51–58. doi: 10.1016/j.bbr.2015.11.031

Pearson-Leary, J., Zhao, C., Bittinger, K., Eacret, D., Luz, S., Vigderman, A. S., et al. (2020). The gut microbiome regulates the increases in depressive-type behaviors and in inflammatory processes in the ventral hippocampus of stress vulnerable rats. *Mol. Psychiatry* 25, 1068–1079. doi: 10.1038/s41380-019-0380-x

Pessione, E. (2012). Lactic acid bacteria contribution to gut microbiota complexity: lights and shadows. *Front. Cell. Infect. Microbiol.* 2:86. doi: 10.3389/fcimb.2012.00086

Pham, V. T., Lacroix, C., Braegger, C. P., and Chassard, C. (2017). Lactate-utilizing community is associated with gut microbiota dysbiosis in colicky infants. *Sci. Rep.* 7:11176. doi: 10.1038/s41598-017-11509-1

Proia, P., Di Liegro, C., Schiera, G., Fricano, A., and Di Liegro, I. (2016). Lactate as a metabolite and a regulator in the central nervous system. *Int. J. Mol. Sci.* 17:1450. doi: 10.3390/ijms17091450

Qin, Y., Wang, N., Zhang, X., Han, X., Zhai, X., and Lu, Y. (2018). IDO and TDO as a potential therapeutic target in different types of depression. *Metab. Brain Dis.* 33, 1787–1800. doi: 10.1007/s11011-018-0290-7

Qu, Y., Su, C., Zhao, Q., Shi, A., Zhao, F., Tang, L., et al. (2022). Gut microbiota-mediated elevated production of secondary bile acids in chronic unpredictable mild stress. *Front. Pharmacol.* 13:837543. doi: 10.3389/fphar.2022.837543

Quinn, M., McMillin, M., Galindo, C., Frampton, G., Pae, H. Y., and DeMorrow, S. (2014). Bile acids permeabilize the blood brain barrier after bile duct ligation in rats via Rac 1-dependent mechanisms. *Dig. Liver Dis.* 46, 527–534. doi: 10.1016/j.dld.2014.01.159

Radjabzadeh, D., Bosch, J. A., Uitterlinden, A. G., Zwinderman, A. H., Ikram, M. A., van Meurs, J. B. J., et al. (2022). Gut microbiome-wide association study of depressive symptoms. *Nat. Commun.* 13:7128. doi: 10.1038/s41467-022-34502-3

Rashmi, D., Zanan, R., John, S., Khandagale, K., and Nadaf, A. (2018). γ-Aminobutyric acid (GABA): biosynthesis, role, commercial production, and applications. *Stud. Nat. Prod. Chem* 57, 413–452. doi: 10.1016/B978-0-444-64057-4.00013-2

Regenold, W. T., Phatak, P., Marano, C. M., Sassan, A., Conley, R. R., and Kling, M. A. (2009). Elevated cerebrospinal fluid lactate concentrations in patients with bipolar disorder and schizophrenia: implications for the mitochondrial dysfunction hypothesis. *Biol. Psychiatry* 65, 489–494. doi: 10.1016/j.biopsych.2008.11.010

Reigstad, C. S., Salmonson, C. E., Iii, J. F. R., Szurszewski, J. H., Linden, D. R., Sonnenburg, J. L., et al. (2015). Gut microbes promote colonic serotonin production through an effect of short-chain fatty acids on enterochromaffin cells. *FASEB J.* 29, 1395–1403. doi: 10.1096/fj.14-259598

Ridaura, V., and Belkaid, Y. (2015). Gut microbiota: the link to your second brain. *Cell* 161, 193–194. doi: 10.1016/j.cell.2015.03.033

Rincel, M., Aubert, P., Chevalier, J., Grohard, P.-A., Basso, L., Monchaux de Oliveira, C., et al. (2019). Multi-hit early life adversity affects gut microbiota, brain and behavior in a sex-dependent manner. *Brain Behav. Immun.* 80, 179–192. doi: 10.1016/j.bbi.2019.03.006

Ríos-Covián, D., Ruas-Madiedo, P., Margolles, A., Gueimonde, M., De Los Reyes-Gavilán, C. G., and Salazar, N. (2016). Intestinal short chain fatty acids and their link with diet and human health. *Front. Microbiol.* 7:185. doi: 10.3389/fmicb.2016.00185

Roberts, E., Carter, B., and Young, A. H. (2018). Caveat emptor: folate in unipolar depressive illness, a systematic review and meta-analysis. *J. Psychopharmacol.* 32, 377–384. doi: 10.1177/0269881118756060

Romano, K. A., Martinez-del Campo, A., Kasahara, K., Chittim, C. L., Vivas, E. I., Amador-Noguez, D., et al. (2017). Metabolic, epigenetic, and transgenerational effects of gut bacterial choline consumption. *Cell Host Microbe* 22, 279–290.e7. doi: 10.1016/j.chom.2017.07.021

Romijn, A. R., Rucklidge, J. J., Kuijer, R. G., and Frampton, C. (2017). A double-blind, randomized, placebo-controlled trial of Lactobacillus helveticus and *Bifidobacterium longum* for the symptoms of depression. *Aust. N. Z. J. Psychiatry* 51, 810–821. doi: 10.1177/0004867416686694

Rudzki, L., Ostrowska, L., Pawlak, D., Małus, A., Pawlak, K., Waszkiewicz, N., et al. (2019). Probiotic *Lactobacillus Plantarum* 299v decreases kynurenine concentration and improves cognitive functions in patients with major depression: a double-blind, randomized, placebo controlled study. *Psychoneuroendocrinology* 100, 213–222. doi: 10.1016/j.psyneuen.2018.10.010

Russell, D. W. (2003). The enzymes, regulation, and genetics of bile acid synthesis. *Annu. Rev. Biochem.* 72, 137–174. doi: 10.1146/annurev.biochem.72.121801.161712

Ryan, N. D. (1992). Neuroendocrine response to L-5-Hydroxytryptophan challenge in Prepubertal major Depression: depressed vs Normal children. *Arch. Gen. Psychiatry* 49, 843–851. doi: 10.1001/archpsyc.1992.01820110007001

Sanada, K., Nakajima, S., Kurokawa, S., Barceló-Soler, A., Ikuse, D., Hirata, A., et al. (2020). Gut microbiota and major depressive disorder: a systematic review and meta-analysis. *J. Affect. Disord.* 266, 1–13. doi: 10.1016/j.jad.2020.01.102

Savignac, H. M., Kiely, B., Dinan, T. G., and Cryan, J. F. (2014). Bifidobacteria exert strain-specific effects on stress-related behavior and physiology in BALB/c mice. *Neurogastroenterol. Motil.* 26, 1615–1627. doi: 10.1111/nmo.12427

Sawada, N., Takanaga, H., Matsuo, H., Naito, M., Tsuruo, T., and Sawada, Y. (2010). Choline uptake by mouse brain capillary endothelial cells in culture. *J. Pharm. Pharmacol.* 51, 847–852. doi: 10.1211/0022357991773050

Schroeder, F. A., Lin, C. L., Crusio, W. E., and Akbarian, S. (2007). Antidepressant-like effects of the histone deacetylase inhibitor, sodium butyrate, in the mouse. *Biol. Psychiatry* 62, 55–64. doi: 10.1016/j.biopsych.2006.06.036

Shi, Y., Zhai, Q., Li, D., Mao, B., Liu, X., Zhao, J., et al. (2017). Restoration of cefixime-induced gut microbiota changes by Lactobacillus cocktails and fructooligosaccharides in a mouse model. *Microbiol. Res.* 200, 14–24. doi: 10.1016/j.micres.2017.04.001

Silk, D. B. A., Davis, A., Vulevic, J., Tzortzis, G., and Gibson, G. R. (2009). Clinical trial: the effects of a trans-galactooligosaccharide prebiotic on faecal microbiota and symptoms in irritable bowel syndrome. *Aliment. Pharmacol. Ther.* 29, 508–518. doi: 10.1111/j.1365-2036.2008.03911.x

Skonieczna-Żydecka, K., Grochans, E., Maciejewska, D., Szkup, M., Schneider-Matyka, D., Jurczak, A., et al. (2018). Faecal short chain fatty acids profile is changed in polish depressive women. *Nutrients* 10:1939. doi: 10.3390/nu10121939

Smith, A. (2005). The concept of well-being: relevance to nutrition research. *Arthritis Res. Ther.* 7:S1. doi: 10.1186/ar1506

Smith, A. P., Sutherland, D., and Hewlett, P. (2015). An investigation of the acute effects of Oligofructose-enriched inulin on subjective wellbeing, mood cognitive performance. *Nutrients* 7, 8887–8896. doi: 10.3390/nu7115441

Sorbara, M. T., and Pamer, E. G. (2022). Microbiome-based therapeutics. Nature reviews. *Microbiology* 20, 365–380. doi: 10.1038/s41579-021-00667-9

Spedding, S. (2014). Vitamin D and Depression: a systematic review and Meta-analysis comparing studies with and without biological flaws. *Nutrients* 6, 1501–1518. doi: 10.3390/nu6041501

Strandwitz, P., Kim, K. H., Terekhova, D., Liu, J. K., Sharma, A., Levering, J., et al. (2018). GABA-modulating bacteria of the human gut microbiota. *Nat. Microbiol.* 4, 396–403. doi: 10.1038/s41564-018-0307-3

Sun, Y., Geng, W., Pan, Y., Wang, J., Xiao, P., and Wang, Y. (2019). Supplementation with *Lactobacillus kefiranofaciens* ZW3 from Tibetan kefir improves depression-like behavior in stressed mice by modulating the gut microbiota. *Food Funct.* 10, 925–937. doi: 10.1039/C8FO02096E

Sun, J., Wang, F., Hu, X., Yang, C., Xu, H., Yao, Y., et al. (2018). *Clostridium butyricum* attenuates chronic unpredictable mild stress-induced depressive-like behavior in mice via the gut-brain Axis. *J. Agric. Food Chem.* 66, 8415–8421. doi: 10.1021/acs.jafc.8b02462

Sun, N., Zhang, J., Wang, J., Liu, Z., Wang, X., Kang, P., et al. (2022). Abnormal gut microbiota and bile acids in patients with first-episode major depressive disorder and correlation analysis. *Psychiatry Clin. Neurosci.* 76, 321–328. doi: 10.1111/pcn.13368

Swann, J. R., Garcia-Perez, I., Braniste, V., Wilson, I. D., Sidaway, J. E., Nicholson, J. K., et al. (2017). Application of 1 H NMR spectroscopy to the metabolic phenotyping of rodent brain extracts: a metabonomic study of gut microbial influence on host brain metabolism. *J. Pharm. Biomed. Anal.* 143, 141–146. doi: 10.1016/j.jpba.2017.05.040

Tahara, Y., Yamazaki, M., Sukigara, H., Motohashi, H., Sasaki, H., Miyakawa, H., et al. (2018). Gut microbiota-derived short chain fatty acids induce circadian clock entrainment in mouse peripheral tissue. *Sci. Rep.* 8:1395. doi: 10.1038/s41598-018-19836-7

Takajo, T., Tomita, K., Tsuchihashi, H., Enomoto, S., Tanichi, M., Toda, H., et al. (2019). Depression promotes the onset of irritable bowel syndrome through unique Dysbiosis in rats. *Gut Liver* 13, 325–332. doi: 10.5009/gnl18296

Tian, T., Mao, Q., Xie, J., Wang, Y., Shao, W., Zhong, Q., et al. (2022). Multi-omics data reveals the disturbance of glycerophospholipid metabolism caused by disordered gut microbiota in depressed mice. *J. Adv. Res.* 39, 135–145. doi: 10.1016/j.jare.2021.10.002

Vaghef-Mehrabany, E., Ranjbar, F., Asghari-Jafarabadi, M., Hosseinpour-Arjmand, S., and Ebrahimi-Mameghani, M. (2021). Calorie restriction in combination with prebiotic supplementation in obese women with depression: effects on metabolic and clinical response. *Nutr. Neurosci.* 24, 339–353. doi: 10.1080/1028415X.2019.1630985

Vahid-Ansari, F., and Albert, P. R. (2021). Rewiring of the serotonin system in major Depression. *Front. Psych.* 12:802581. doi: 10.3389/fpsyt.2021.802581

Valcheva, R., and Dieleman, L. A. (2016). Prebiotics: definition and protective mechanisms. *Best Pract. Res. Clin. Gastroenterol.* 30, 27–37. doi: 10.1016/j.bpg.2016.02.008

Van De Wouw, M., Boehme, M., Lyte, J. M., Wiley, N., Strain, C., O'Sullivan, O., et al. (2018). Short-chain fatty acids: microbial metabolites that alleviate stress-induced brain-gut axis alterations: SCFAs alleviate stress-induced brain-gut axis alterations. *J. Physiol.* 596, 4923–4944. doi: 10.1113/JP276431

van Nood, E., Vrieze, A., Nieuwdorp, M., Fuentes, S., Zoetendal, E. G., de Vos, W. M., et al. (2013). Duodenal infusion of donor feces for recurrent *Clostridium difficile*. *N. Engl. J. Med.* 368, 407–415. doi: 10.1056/NEJMoa1205037

Wallace, C. J. K., and Milev, R. V. (2021). The efficacy, safety, and tolerability of probiotics on Depression: clinical results from an open-label pilot study. *Front. Psych.* 12:618279. doi: 10.3389/fpsyt.2021.618279

Wang, Z., Roberts, A. B., Buffa, J. A., Levison, B. S., Zhu, W., Org, E., et al. (2015). Non-lethal inhibition of gut microbial trimethylamine production for the treatment of atherosclerosis. *Cell* 163, 1585–1595. doi: 10.1016/j.cell.2015.11.055

Wolf, W. A., Ziaja, E., Arthur, R. A., Anastasiadis, P. Z., Levine, R. A., and Kuhn, D. M. (1991). Effect of tetrahydrobiopterin on serotonin synthesis, release, and metabolism in superfused hippocampal slices. *J. Neurochem.* 57, 1191–1197. doi: 10.1111/j.1471-4159.1991.tb08279.x

Wong, M.-L., Inserra, A., Lewis, M. D., Mastronardi, C. A., Leong, L., Choo, J., et al. (2016). Inflammasome signaling affects anxiety-and depressive-like behavior and gut microbiome composition. *Mol. Psychiatry* 21, 797–805. doi: 10.1038/mp.2016.46

World Health Organization. (2008). *The Global Burden of Disease: 2004 Update*. Geneva, Switzerland: World Health Organization 146.

Yadav, R. S., and Tiwari, N. K. (2014). Lipid integration in neurodegeneration: an overview of Alzheimer's disease. *Mol. Neurobiol.* 50, 168–176. doi: 10.1007/s12035-014-8661-5

Yang, C., Fujita, Y., Ren, Q., Ma, M., Dong, C., and Hashimoto, K. (2017). Bifidobacterium in the gut microbiota confer resilience to chronic social defeat stress in mice. *Sci. Rep.* 7:45942. doi: 10.1038/srep45942

Yano, J. M., Yu, K., Donaldson, G. P., Shastri, G. G., Ann, P., Ma, L., et al. (2015). Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. *Cell* 161, 264–276. doi: 10.1016/j.cell.2015.02.047

Yong, S. J., Tong, T., Chew, J., and Lim, W. L. (2020). Antidepressive mechanisms of probiotics and their therapeutic potential. *Front. Neurosci.* 13:1361. doi: 10.3389/fnins.2019.01361

Young, S. N., and Ghadirian, A. M. (1989). Folic acid and psychopathology. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* 13, 841–863. doi: 10.1016/0278-5846(89)90037-7

Yu, Y., Villalobos-Hernandez, E. C., Pradhananga, S., Baker, C. C., Keating, C., Grundy, D., et al. (2019). Deoxycholic acid activates colonic afferent nerves via 5-HT $_3$ receptor-dependent and-independent mechanisms. American journal of physiology-gastrointestinal and liver. *Physiology* 317, G275–G284. doi: 10.1152/ajpgi.00016.2019

Yunes, R. A., Poluektova, E. U., Dyachkova, M. S., Klimina, K. M., Kovtun, A. S., Averina, O. V., et al. (2016). GABA production and structure of gadB/gadC genes in Lactobacillus and Bifidobacterium strains from human microbiota. *Anaerobe* 42, 197–204. doi: 10.1016/j.anaerobe.2016.10.011

Zhang, K., Fujita, Y., Chang, L., Qu, Y., Pu, Y., Wang, S., et al. (2019). Abnormal composition of gut microbiota is associated with resilience versus susceptibility to inescapable electric stress. *Transl. Psychiatry* 9:231. doi: 10.1038/s41398-019-0571-x

Zhang, S., Wang, Y., Mao, D., Wang, Y., Zhang, H., Pan, Y., et al. (2023). Current trends of clinical trials involving CRISPR/Cas systems. *Front. Med.* 10:1292452. doi: 10.3389/fmed.2023.1292452

Zheng, L., Tan, Y., Hu, Y., Shen, J., Qu, Z., Chen, X., et al. (2022). CRISPR/Cas-based genome editing for human gut commensal Bacteroides species. *ACS Synth. Biol.* 11, 464–472. doi: 10.1021/acssynbio.1c00543

Zheng, P., Wu, J., Zhang, H., Perry, S. W., Yin, B., Tan, X., et al. (2021). The gut microbiome modulates gut–brain axis glycerophospholipid metabolism in a region-specific manner in a nonhuman primate model of depression. *Mol. Psychiatry* 26, 2380–2392. doi: 10.1038/s41380-020-0744-2

Zheng, P., Zeng, B., Zhou, C., Liu, M., Fang, Z., Xu, X., et al. (2016). Gut microbiome remodeling induces depressive-like behaviors through a pathway mediated by the host's metabolism. *Mol. Psychiatry* 21, 786–796. doi: 10.1038/mp.2016.44

frontiers | Frontiers in Neuroscience

TYPE Systematic Review
PUBLISHED 21 March 2024
DOI 10.3389/fnins.2024.1290826

OPEN ACCESS

EDITED BY
Mingbang Wang,
South China Hospital of Shenzhen University, China

REVIEWED BY
Kiran Veer Sandhu,
University College Cork, Ireland
Shaghayegh Baradaran Ghavami,
Shahid Beheshti University of Medical Sciences, Iran

*CORRESPONDENCE
Xiaoxiao Lin
✉ linxiaoxiao@zcmu.edu.cn
Shuai Wang
✉ drwangshuai@zju.edu.cn
Kai Zhang
✉ zhangkai@ahmu.edu.cn

RECEIVED 15 September 2023
ACCEPTED 18 January 2024
PUBLISHED 21 March 2024

CITATION
Lin X, Huang J, Wang S and Zhang K (2024) Bipolar disorder and the gut microbiota: a bibliometric analysis.
Front. Neurosci. 18:1290826.
doi: 10.3389/fnins.2024.1290826

Bipolar disorder and the gut microbiota: a bibliometric analysis

Xiaoxiao Lin[1]*, Jinyu Huang[1], Shuai Wang[1]* and Kai Zhang[2,3]*

[1]Hangzhou First People's Hospital, Hangzhou, China, [2]Department of Psychiatry, Chaohu Hospital of Anhui Medical University, Hefei, China, [3]School of Mental Health and Psychological Sciences, Anhui Medical University, Hefei, China

Background: Previous studies have explored the relationship between bipolar disorder and gut microbiota. However, there has been no bibliometric analysis to summarize and analyze these publications. Our objective was to perform a bibliometric analysis to investigate the current status and frontiers of the publications in the field of the association between bipolar disorder and the gut microbiota.

Methods: We retrieved publications concerning the interplay between the gut microbiota and bipolar disorder from the Web of Science Core Collection (WoSCC). The analysis was executed using WoSCC's literature analysis tool and VOSviewer 1.6.16.

Results: In total, we identified 177 publications originating from 362 institutions across 39 countries/regions, and these articles were disseminated in 104 different journals. The most productive institutions, authors, countries/regions, and journals were Zhejiang University contributing 18 publications, Shaohua Hu authoring 12 publications, China with 53 publications, and *Frontiers in Psychiatry* with 11 publications. The first high-cited document was published in the Journal of Psychiatric Research in 2017, and authored by Evans. In this article, they found gut microbiome composition was associated with BD and its illness severity, and they concluded that targeting the gut microbiota may be helpful to develop the effective treatment for bipolar disorder. The top 5 keywords with the highest frequency except for bipolar disorder and gut microbiota were as follows: depression, inflammation, probiotic, gut-brain axis, and anxiety.

Conclusion: In conclusion, this is the first bibliometric analysis to explore the publications in the field of the association between bipolar disorder and the gut microbiota. The main research hotspots regarding this field were the characteristics, abundance, and diversity of gut microbiome in bipolar disorder, the role of treatment and gut microbiome in bipolar disorder, microbiome-brain connections in bipolar disorder, and interventions for bipolar disorder based on microbiota composition modification. The number of studies about the association between gut microbiota and bipolar disorder is relatively small, and more studies are needed to expand our understanding the association between gut microbiota and bipolar disorder.

KEYWORDS

bipolar disorder, gut microbiota, a bibliometric analysis, gut-brain axis, interventions

Introduction

Bipolar disorder is a severe psychiatric disorder which is characterized by hypomanic states, manic episodes, and the interweaving or alternating occurrences of depressive episodes (Craddock and Sklar, 2013; Grande et al., 2016; Scott et al., 2017; Carvalho et al., 2020; McIntyre et al., 2020; Goes, 2023). It is estimated that the 12-month and lifetime prevalence for bipolar disorder is 1.5 and 2.4%, respectively. It was reported 6 to 7% of patients with bipolar disorder committed suicide (Carvalho et al., 2020). The treatment for bipolar disorder consists two aspects of acute management and long-term management (Goes, 2023). In the phase of acute management, antipsychotics and mood stabilizers are the mainstay of depression and bipolar mania. In addition, electroconvulsive therapy (ECT) is effective for treatment-resistant patients, particularly those with catatonic features or psychotic. For the phase of long-term management, the combinations of pharmacological, psychological, and lifestyle interventions should be used (Carvalho et al., 2020). More strategies should be explored to prevent and treat the bipolar disorder.

In recent years, the studies about bipolar disorder and the gut microbiome become more and more popular (Evans et al., 2017; Zheng et al., 2020; Li et al., 2022). The gut microbiota is a rapidly advancing biomedical frontier, which is associated with psychiatric diseases including schizophrenia (Samochowiec and Misiak, 2021; Yang et al., 2022), bipolar disorder (Li et al., 2022), depression (Zhu et al., 2021; Liu et al., 2023), and autism (Sharon et al., 2019; Lin et al., 2022). Gut microbiota, act as "metabolic machinery," can influence many aspects of physiology through immunological hormonal, and neural pathways (Hu et al., 2019). The gut microbiota can influence host metabolism, and interact with the central nervous system by gut-brain axis (Basiji et al., 2023). Gut microbiota may influence the pathophysiology and etiology of bipolar disorder by disrupting homeostatic regulation (Li et al., 2022). Understanding the association between gut microbiota in bipolar disorder (BD) will be helpful to find new effective disease markers and treatment strategies (Fond et al., 2015; Nguyen et al., 2018). Bibliometric analysis is the most frequently used method to summarize the current status and predict developmental trends by analysis of many components such as authors, countries, institutions, and citations for overall studies in a specific field. By this method, previous studies have explored the association between gut microbiota and many psychiatric conditions including schizophrenia (Yang et al., 2022), depression (Zhu et al., 2021), and autism (Lin et al., 2022). However, there was no bibliometric study to explore the association between gut microbiota and bipolar disorder. Bibliometric analysis is a methodological approach used to quantitatively assess academic literature, providing a comprehensive overview of research trends, patterns, and networks in a specific field. It involves statistical analysis of various aspects of scientific publications, such as publication and citation counts, to gauge the impact and influence of research works, authors, or journals. This analysis also includes examining the relationships and collaborations between authors, institutions, and countries, as well as identifying dominant themes, emerging trends, and potential gaps in the research through content analysis. Conducting a bibliometric analysis of the relationship between BD and gut microbiota is crucial can provide a comprehensive overview of the research landscape, identifying pivotal studies, trends, and gaps in this emerging field. Therefore, our study aims to conduct a bibliometric analysis for the publications in the field of the association between gut microbiota and bipolar disorder, to determine the current status and frontiers in this field.

Materials and methods

In our study, all relevant publications were extracted from Web of Science Core Collection (WoSCC). The search terms were: (microbiome* OR microbiot* OR microflora OR microbiota OR microbiome) AND (intestin* OR intestinal OR gut OR gastro-intestin* OR gastrointestinal*) AND ("bipolar disorder" or "bipolar depression" or "bipolar disorders" or mania or "mood disorder" or "affective disorder" or "mood disorders" or "affective disorders") from inception to 1 June 2023. Two reviewers (Lin and Wang) investigated each study according to inclusion criteria by screening the titles, abstracts, and full-text of publications. They discussed with the third author (Huang) if they could not reach an agreement about some publications.

After the selection process, we downloaded and imported the TXT format containing "Full Record and Cited References" into the VOSviewer software. Our analysis encompassed two stages, utilizing both the WoSCC literature analysis system and the VOSviewer 1.6.16 software. The WoSCC literature analysis system was used to analysis categories, publication years, document types, *h*-index and the distribution of institutions, countries/regions and authors. We summarized the leading 10 authors, institutions, countries/regions, and journals in terms of the number of publications, and provided the information of the 20 most highly cited publications. VOSviewer1.6.16 software was used to perform co-authorship of authors, organizations, and countries, co-occurrence of all keywords and co-citation of cited references. For co-occurrence analysis of keywords, we merge the synonyms of "gut microbiota," "gut microbiome," "fecal microbiota," "gut-microbiota," "intestinal microbiota," "microbiota," and "microbiome" into the term "gut microbiota"; "major depressive disorder" and "depression" into the term "depression."

Results

Literature search and trends analysis

A total of 436 records were yielded in our primary database search. After screening titles, abstracts, and full text, a total of 177 publications were included in the analysis, which was shown in **Figure 1**. The total number of publications per year was listed in **Figure 2**. The documents can be classified into two phases: documents published before 2017 were in the first phase. In this phase, the number of documents was small annually. The second phase was 2017–2023, and in this phase, the number of documents was all beyond 10 publications per year. For subject area of documents, the top two subject categories were the psychiatry and neurosciences, accounting for 79 and 56 publications, respectively.

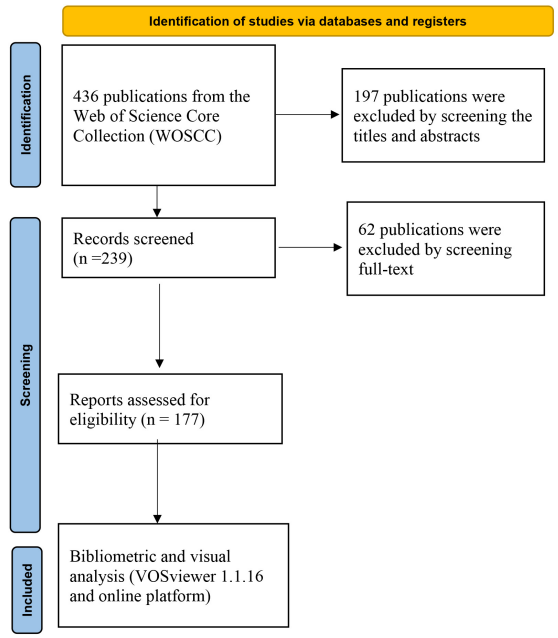


FIGURE 1
Search flowchart.

Country/region, institution and author analysis

In total, 394 institutions across 46 countries/regions were included. The most productive institution was Zhejiang University contributing 18 publications, which was followed by University of Toronto contributing 11 publications, and Baylor College of Medicine contributing 9 publications. The top 3 productive countries were China with 53 publications, USA with 42 publications, and Canada with 19 publications. For most productive authors, Shaohua Hu contributed the most publications with 12 documents, and Peifen Zhang, Caixi Xi, Jianbo Lai with 9 publications following him. The top 10 most productive organizations, authors, and countries/regions were shown in **Table 1**. In addition, the network visualization maps of co-authorship of countries, authors, and institutions were displayed in **Figure 3**, and the top 3 cooperative authors were Shaohua Hu (TLS = 118), Jianbo Lai (TLS = 113), and Caixi Xi (TLS = 89). For organizations, they were Zhejiang University (TLS = 47), Melbourne University (TLS = 30), and Deakin University (TLS = 30). For countries/regions, they were USA (TLS = 34), Australia (TLS = 25) and Canada (TLS = 22).

Journal and high-cited publication analysis

All publications were distributed in 112 journals. Among them, *Frontiers in Psychiatry* was the most productive journal with 11 publications, and followed by *Progress in Neuro-Psychopharmacology & Biological Psychiatry* with 8 publications, and *Journal of Affective Disorders* with 5 publications. The top 10 most productive journals were summarized in **Table 2**. For the citation of publications, the first high-cited document was in *Journal of Psychiatric Research* in 2017, and authored by Evans et al. (2017). In this study, 115 patients with bipolar disorder and 64 control subjects were included and the stool microbiome were measured by the stool microbiome. They found gut microbiome compositions were associated with BD and its illness severity, and they concluded that targeting the gut microbiota may be helpful to develop effective treatment for bipolar disorder. The second high-cited document was in *Brain Behavior and Immunity* in 2017, and authored by Dickerson et al. (2017). In their article, they believed that the successful development of therapeutic agents which can alter gut microbiome and gastrointestinal inflammation in bipolar disorder will be helpful to develop novel effective strategies to prevent and treat it. The third high-cited document was in *JAMA Psychiatry* in 2021, and authored by Nikolova et al. (2021). In this article, they found that gut microbiome perturbations were related to the transdiagnostic pattern with bipolar disorder. The characteristics of top 20 most high-cited publications (Hornig, 2013; Fond et al., 2015; Alam et al., 2017; Dickerson et al., 2017; Evans et al., 2017; Flowers et al., 2017; Jacka, 2017; Rosenblat and McIntyre, 2017; Kim and Shin, 2018; Nguyen et al., 2018; Coello et al., 2019; Fries et al., 2019; Hu et al., 2019; Huang et al., 2019; Painold et al., 2019; Rong et al., 2019; Misiak et al., 2020; Zheng et al., 2020; Nikolova et al., 2021; McGuinness et al., 2022) were displayed in **Table 3**. The network visualization maps of citations of journals and documents were summarized in **Figure 4**. In addition, **Figure 5** displayed the co-cited references, which are cited by

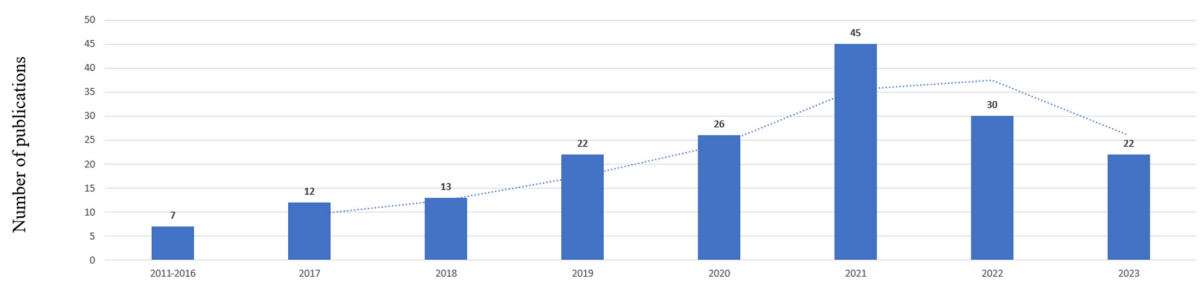


FIGURE 2
Annual publications quantitative distribution.

TABLE 1 The top 10 productive authors, institutions and countries based on publications.

Ranking	Country	Number	Citations	H-index
Publications				
1	China	53	897	17
2	USA	42	1,607	20
3	Canada	18	404	10
4	Australia	15	520	11
5	Austria	10	250	6
6	Brazil	10	268	7
7	Italy	10	93	5
8	Poland	9	255	8
9	Denmark	8	197	7
10	Netherlands	8	233	7
1	Zhejiang University	18	421	8
2	University of Toronto	11	284	7
3	Baylor College of Medicine	9	173	5
4	University of Texas System	9	213	6
5	University of Copenhagen	8	197	7
6	University of Melbourne	8	382	6
7	University of Michigan	8	377	4
8	Chinese Academy of Sciences	7	148	5
9	Deakin University	7	307	6
10	Medical University of Graz	7	189	4
1	Shaohua Hu	12	284	6
2	Pei fen Zhang	9	130	5
3	Caixi Xi	9	94	5
4	Jianbo Lai	9	130	5
5	Jiajun Jiang	8	94	5
6	Sabrina Moerkl	7	188	4
7	E. Z Reininghaus	6	219	5
8	Martina Platzer	6	197	4
9	Nina Dalkner	6	219	5
10	Antonio L Teixeira	6	185	6

more than one article in these 177 documents, and the top 3 co-cited references were consistent with the top three high-cited publications.

Keyword analysis

The map of the co-occurrence of keywords was displayed in Figure 6, and there were four research directions are also shown. The green cluster includes inflammation and brain. The red cluster includes gut microbiota, depression, and anxiety. The blue cluster includes bipolar disorder, schizophrenia, and probiotic. The yellow cluster includes gut-brain axis and stress. The top 5 keywords with the highest frequency except for bipolar disorder and gut microbiota were as follows: depression (N = 62), schizophrenia (n = 50), inflammation (N = 39), gut-brain axis (N = 25), and brain (N = 24).

Discussion

General information

To the best of knowledge, it is the first bibliometric analysis of publications in the field of bipolar disorder and the gut microbiome. There were 177 documents from 362 organizations across 39 countries/regions, which were published in 104 journals. The most productive organizations, authors, countries/regions, and journals were Zhejiang University contributing 18 publications, Shaohua Hu authoring 12 publications, China with 53 publications, and *Frontiers in Psychiatry* with 11 publications. The first high-cited document was in *Journal of Psychiatric Research* in 2017, and authored by Evans et al. (2017). In this article, they found gut microbiome composition were associated with BD and its illness severity, and they concluded that targeting the gut microbiota may be helpful to develop effective treatment for bipolar disorder. The top 5 keywords with the highest frequency except for bipolar disorder and gut microbiota were as follows: depression, inflammation, probiotic, gut-brain axis, and anxiety. Most of the included publications were from China and USA (95/177, 53.7%), and the number of publications from other countries/regions should be improved.

Hotspots and frontiers

According to top 20 most high-cited documents and the core keywords, the research hotspots and frontiers were summarized as follows:

(1) The characteristics, abundance, and diversity of gut microbiome in bipolar disorder. In the top 20 most high-cited references, 11 publications (Evans et al., 2017; Flowers et al., 2017; Nguyen et al., 2018; Coello et al., 2019; Hu et al., 2019; Huang et al., 2019; Painold et al., 2019; Rong et al., 2019; Zheng et al., 2020; Nikolova et al., 2021; McGuinness et al., 2022) explored the characteristics abundance, and diversity of gut microbiome in bipolar disorder. Some systematic review and meta-analysis (Nguyen et al., 2021; Sublette et al., 2021; Vindegaard et al., 2021; McGuinness et al., 2022; Obi-Azuike et al., 2023) explored this topic, and they were summarized in Table 4. More and more studies have demonstrated disparities in gut microbiome, abundance, and diversity in BD (Chang et al., 2014; Evans et al., 2017; Flowers et al., 2017; Bengesser et al., 2018; Aizawa et al., 2019; Coello et al., 2019;

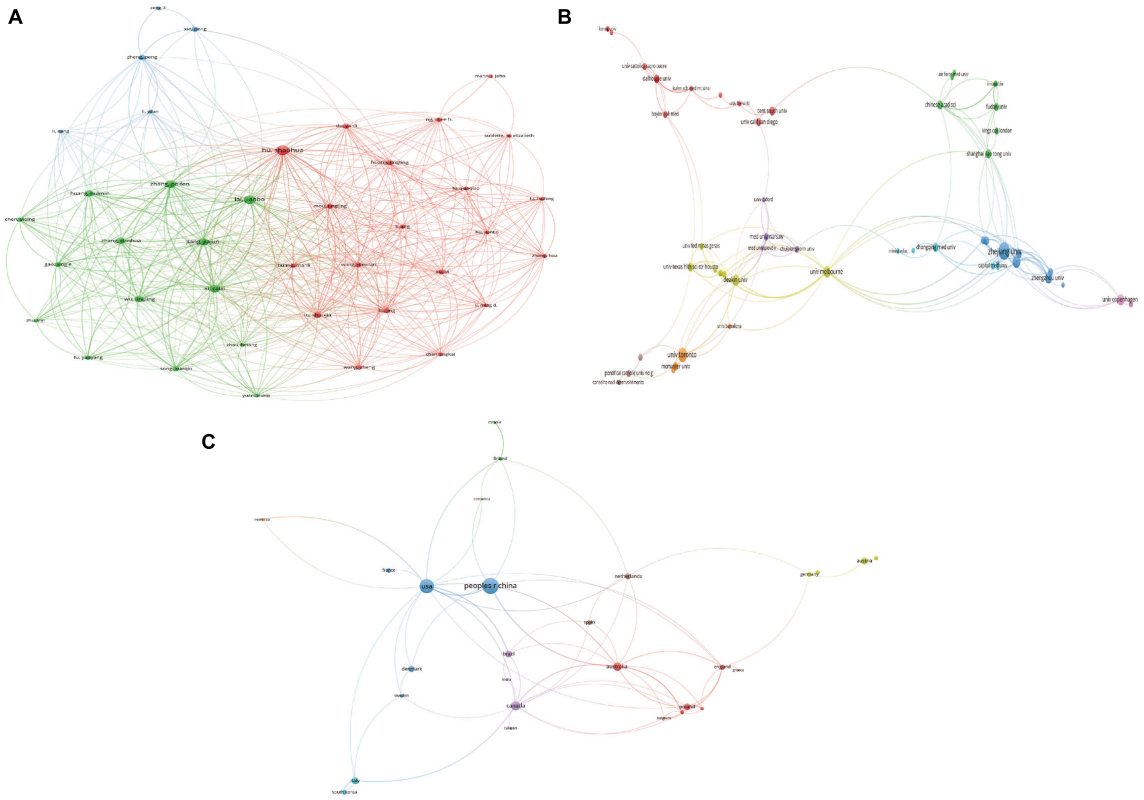


FIGURE 3
Visualization knowledge maps of authors, institutions, and countries/regions **(A)** The co-authorship map of authors **(B)** the co-authorship map of institutions **(C)** the co-authorship map of countries/regions.

TABLE 2 **The top 10 productive journals based on publications.**

Ranking	Journal name	Country	Counts	Citation
1	*Frontiers in Psychiatry*	Switzerland	11	235
2	*Progress in Neuro-Psychopharmacology & Biological Psychiatry*	England	8	197
3	*Journal of Affective Disorders*	Netherlands	5	50
4	*Neuro-psychobiology*	Switzerland	5	85
5	*Brain Behavior and Immunity*	USA	4	258
6	*Journal of Psychiatric Research*	England	4	373
7	*Molecular Psychiatry*	England	4	144
8	*Nutrients*	Switzerland	4	61
9	*CNS Neuroscience Therapeutics*	China	3	5
10	*Frontiers in Pharmacology*	Switzerland	3	23

Hu et al., 2019; Jiang et al., 2019; Lu et al., 2019; Painold et al., 2019; Rong et al., 2019; Vinberg et al., 2019; Lai et al., 2021; McIntyre et al., 2021). According to these systematic reviews (Nguyen et al., 2021; Sublette et al., 2021; Vindegaard et al., 2021; McGuinness et al., 2022; Obi-Azuike et al., 2023), it may be confirmed that there were reductions in overall microbial richness in BD. The findings about the α-diversity metrics including microbial richness were controversial. It seems that alterations in *Ruminococcaceae*, *Faecalibacterium*, *Actinobacteria*, *Ruminococcus*, *Lachnospiraceae*, and *Bacteroidetes* were most represented. In addition, the causation of alterations in the gut microbiota and bipolar disorder cannot be determined based on current studies since most of them are observational.

(2) The role of treatment and gut microbiome in bipolar disorder. A few studies explored the association between medication treatment for bipolar disorder and gut microbiome. For example, Flowers et al. (2017) found that the treatment of atypical antipsychotic (AAP) was associated with specific representation of gut bacterial families, and it is also is related to decreased species richness in female. Lai et al. (2022) focused on understanding the gut microbiota changes in bipolar disorder (BD) patients experiencing depressive episodes and the effects of quetiapine

TABLE 3 The top 20 most high-cited references.

Rank	Title	Journal	Total citations	Year	First author
1	The gut microbiome composition associates with bipolar disorder and illness severity	*Journal of Psychiatric Research*	161	2017	Simon Evans
2	The microbiome, immunity, and schizophrenia and bipolar disorder	*Brain Behavior and Immunity*	155	2017	Faith Dickerson
3	Perturbations in gut microbiota composition in psychiatric disorders a review and meta-analysis	*JAMA Psychiatry*	146	2021	Yang Yang
4	Bipolar disorder and immune dysfunction: epidemiological findings, proposed pathophysiology and clinical implications	*Brain Sciences*	139	2017	Gregory H. Jones
5	The "psychomicrobiotic": targeting microbiota in major psychiatric disorders: a systematic review	*Pathologie Biologie*	139	2015	Duygu Agagunduz
6	Interaction between atypical antipsychotics and the gut microbiome in a bipolar disease cohort	*Pathologie Biologie*	137	2017	Li Huang
7	Microbiome, inflammation, epigenetic alterations, and mental diseases	*American Journal of Medical Genetics Part B-Neuropsychiatric Genetics*	126	2017	Ting-Ting Huang
8	Nutritional psychiatry: where to next?	*Ebiomedicine*	121	2017	Crystal Obi-Azuike
9	Current understanding of gut microbiota in mood disorders: an update of human studies	*Frontiers in Genetics*	117	2019	Cherise R. Chin Fatt
10	Overview and systematic review of studies of microbiome in schizophrenia and bipolar disorder	*Journal of Psychiatric Research*	117	2018	Jianzhao Zhang
11	A step ahead: exploring the gut microbiota in inpatients with bipolar disorder during a depressive episode	*Bipolar Disorders*	104	2019	Kan Yu
12	The microbiota-gut-brain axis in neuropsychiatric disorders: patho-physiological mechanisms and novel treatments	*Current Neuropharmacology*	103	2018	Andrea Schneider
13	The role of microbes and autoimmunity in the pathogenesis of neuropsychiatric illness	*Current Opinion in Rheumatology*	99	2013	Hamid Mostafavi Abdolmaleky
14	The HPA axis dysregulation in severe mental illness: can we shift the blame to gut microbiota?	*Progress in Neuro-Psychopharmacology & Biological Psychiatry*	86	2020	Wujie Ye
15	A systematic review of gut microbiota composition in observational studies of major depressive disorder, bipolar disorder and schizophrenia	*Molecular Psychiatry*	83	2022	Ilya Smolensky
16	Gut microbiota changes in patients with bipolar depression	*Advanced Science*	83	2019	Antonina Kurowska
17	Similarly in depression, nuances of gut microbiota: evidences from a shotgun metagenomics sequencing study on major depressive disorder versus bipolar disorder with current major depressive episode patients	*Journal of Psychiatric Research*	80	2019	James Melrose
18	Gut microbiota composition in patients with newly diagnosed bipolar disorder and their unaffected first-degree relatives	*Brain Behavior and Immunity*	79	2019	Weiming Gong
19	Revisiting inflammation in bipolar disorder	*Pharmacology Biochemistry and Behavior*	73	2019	Yaning Zang
20	Gut microbial signatures can discriminate unipolar from bipolar depression	*Advanced Science*	72	2020	Peifen Zhang

monotherapy. The research involved 62 BD patients and 60 healthy individuals, with fecal samples collected for metagenomic sequencing. The study found that BD patients had specific alterations in gut microbial diversity and composition, which were notably modified after 1 month of quetiapine treatment. A significant finding was the correlation of *Clostridium bartlettii*

TABLE 4 The systematic review and meta-analysis of the characteristics, abundance, and diversity of gut microbiome in bipolar disorder.

References	Included studies of BD	Included participants	The main findings
Obi-Azuike et al., 2023	12 articles	613 BD patients	There was overall difference in gut microbiota composition, but the alterations found were not consistent. Differences in *Lactobacillus*, *Faecalibacterium*, and *Ruminococcus* abundance was found to be the most consistent. Probiotic supplementation can lower patient rehospitalizations and improve cognitive impairments and depressive symptoms significantly.
Vindegaard et al., 2021	4 studies	299 BD cases and 209 non-BD controls	There was higher abundance of *Actinobacteria*, and lower abundance of *Firmicutes*, *Lachnospiraceae*, *Faecalibacterium*
Nguyen et al., 2021	7 studies	520 BD cases	*Ruminococcaceae* and *Faecalibacterium* were relatively decreased in BD
Sublette et al., 2021	13 studies	474 patients with BD and 285 non-BD controls	The low α-diversity and dysbiosis of abundance of *Faecalibacterium* and *Bacteroides* may characterize BD
McGuinness et al., 2022	7 studies	527 BD cases and 477 non-BD controls	There were differences in overall community composition (β-diversity), but no strong evidence for a difference in the number or distribution (α-diversity)

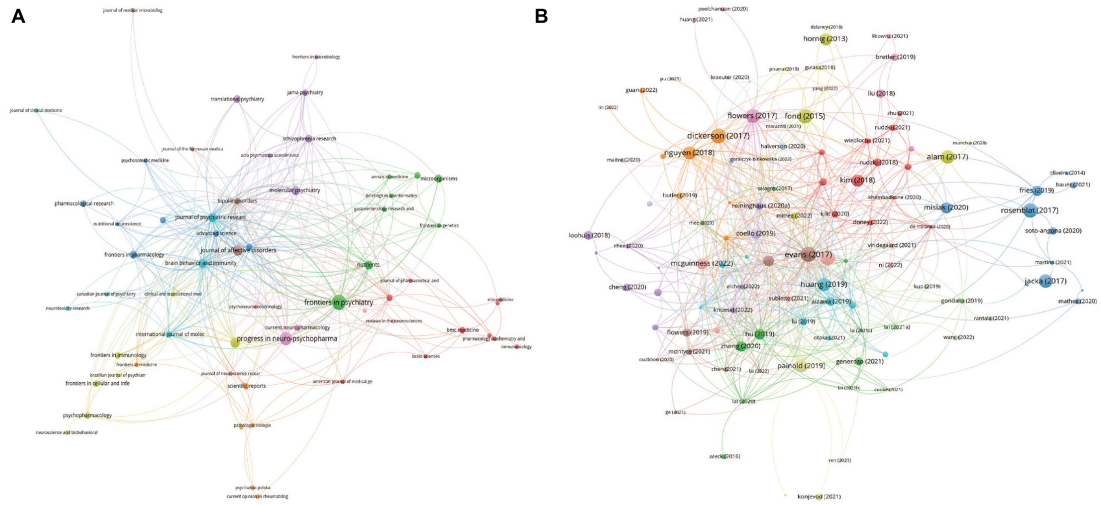


FIGURE 4
Visualization knowledge maps of journals and references **(A)** citation of journal; **(B)** citation of references.

abundance with factors like patient age, baseline depression severity, and brain function, particularly in the hippocampus. The study also developed random forest models based on bacterial species, achieving reasonable accuracy in distinguishing between patients and controls, and between treatment responders and non-responders. These results suggest that gut microbiota alterations could serve as potential biomarkers for diagnosing BD and predicting treatment outcomes.

(3) Microbiome-brain connections in bipolar disorder. In the top 20 most high-cited references, 7 publications (Hornig, 2013; Alam et al., 2017; Dickerson et al., 2017; Rosenblat and McIntyre, 2017; Kim and Shin, 2018; Fries et al., 2019; Misiak et al., 2020) explored the microbiome-brain connections in bipolar disorder. For core keywords, inflammation in the green cluster, and gut-brain axis in the yellow cluster were related to this topic. Microbiome-brain connections in bipolar disorder may be associated with inflammation (Yuan et al., 2019; Jones et al., 2021; Lai et al., 2021), tryptophan metabolism (Barbuti et al., 2017; Wang and Miller, 2018; Wang et al., 2019), microglia (Cenit et al., 2017; Morais et al., 2021), the aryl hydrocarbon receptor (Reiter et al., 2018; Neamah et al., 2020; Tischkau, 2020; Barroso et al., 2021), and endocrine function (Perry et al., 2019; Anderson and Maes, 2020). A recent study (Li et al., 2022) used multi-omics analyses to explore microbiome-brain connections, and in this study, 109 unmedicated patients with BD and 40 controls were included. The serum metabolomics, fecal metagenomic, and neuroimaging were used to explore the characteristics of microbial-gut-brain axis in BD. The findings revealed the identification of BD-associated neuroactive microbes and metabolites, which emerged as potential markers linked to distinct features of brain network functional connectivity in BD. These markers suggested possible implications for disrupted cognitive functioning and emotional regulation. In a comprehensive analysis of over 12,000 measured metabolic features, a substantial divergence (73.54%) in serum metabolome profiles was observed between BD patients and healthy controls. This divergence pinpointed distinctively abundant microbial-derived neuroactive metabolites, encompassing various gamma-aminobutyric acid, kynurenic acid, B vitamins, and short-chain

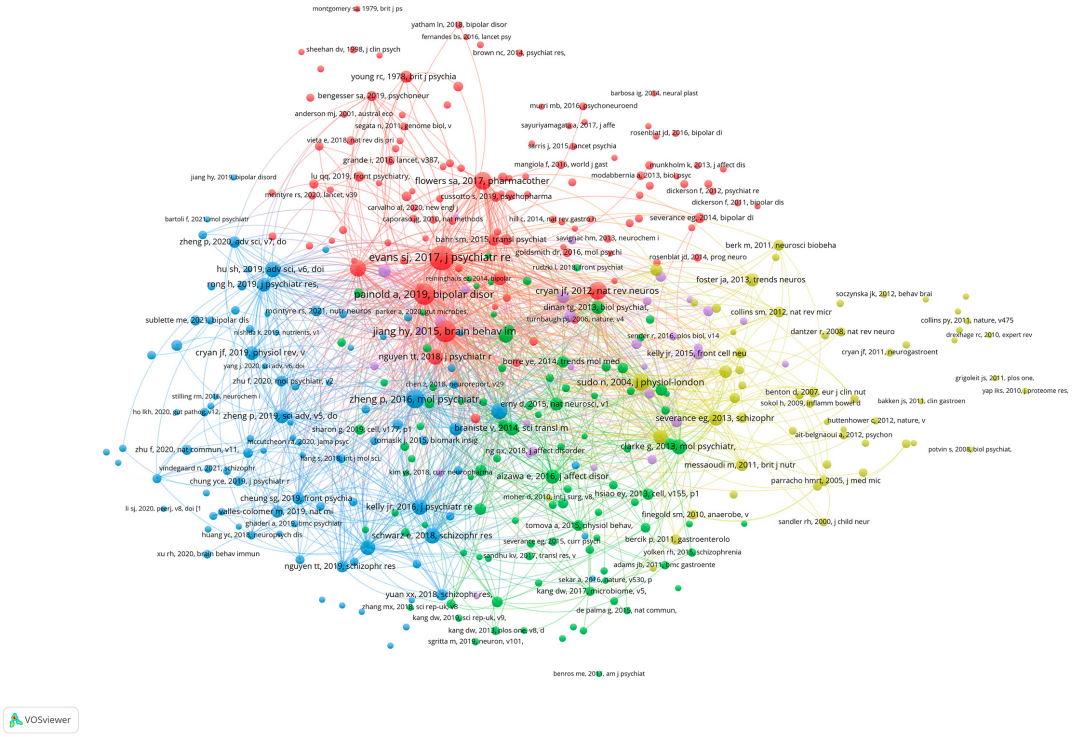


FIGURE 5
The network visualization map of co-citation of references.

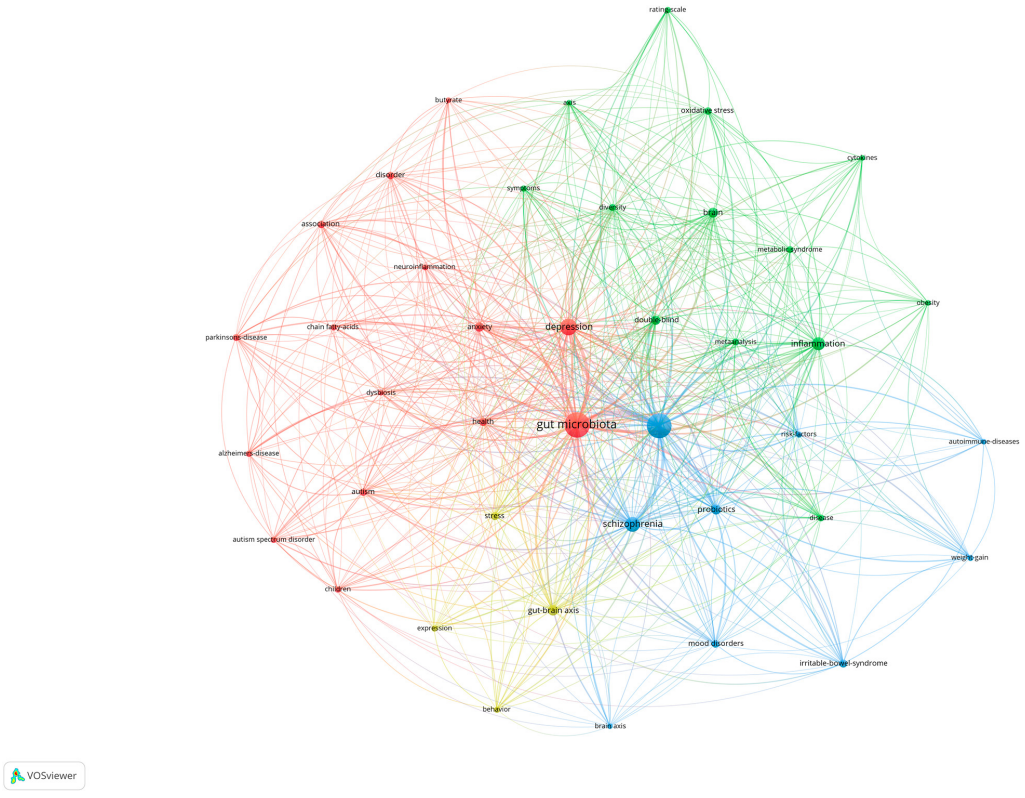


FIGURE 6
Visualization of keyword co-occurrence analysis.

fatty acids. These identified metabolites demonstrated potential connections with the prevalence of specific gut microbiota species, each with corresponding biosynthetic capabilities. Notable among them were *Akkermansia muciniphila*, *Citrobacter* spp, *Phascolarctobacterium* spp, *Yersinia* spp, *Enterobacter* spp, and *Flavobacterium* spp. Some bacteria may play an important role as "psychobiotic" in the gut-brain axis connection between bipolar disorder and gut microbiome. This approach unveiled possible signaling pathways connecting the gut, the microbiome, and the brain, suggesting a potential contribution to the underlying mechanisms of BD.

(4) Interventions for bipolar disorder based on microbiota composition modification. In the top 20 most high-cited references, 5 publications mentioned the microbial-based interventions for bipolar disorder (Fond et al., 2015; Dickerson et al., 2017; Evans et al., 2017; Jacka, 2017; Rosenblat and McIntyre, 2017). For core words, the probiotic in the blue cluster was related to this topic. It may be a promising avenue to combine microbial-based interventions and standard therapy for improving certain parameters like hospitalization length, cognition, and metabolic side effects in bipolar disorder. For example, Reininghaus et al. (2018) conducted pilot study to analyze the effect of probiotic supplements on cognitive parameters in patients with bipolar disorder, and found that probiotic supplement might be helpful to improve the cognitive function in patients with bipolar disorder. Dickerson et al. (2018) conducted a trial to investigate whether the administration of probiotic supplement can prevent psychiatric rehospitalizations in individuals with BD, and in their study, 66 patients with BD were randomized to receive 6 months of adjunctive probiotics or adjunctive placebo. The results demonstrated that 8 rehospitalizations in the 33 patients who received the probiotics while 24 rehospitalizations in the 33 patients who received placebo (P = 0.009), and what's more, probiotics was associated with a significant advantage (P = 0.007). They concluded that Probiotic supplementation may be associated with a lower rate of rehospitalization. A systematic review (Sublette et al., 2021) demonstrated that probiotic supplementation can lower patient rehospitalizations and improve cognitive impairments and depressive symptoms significantly in patients with BD. In addition, fecal microbial transplantation (FMT) may be a potentially effective strategy for the treatment of bipolar disorder.

Our study has some limitations that should be acknowledged. Firstly, we solely utilized the WoSCC database due to the constraints of the VOSviewer software, which prevented the analysis and visualization of co-citation maps from other databases such as Embase and PubMed. Secondly, the inclusion of publications from the year 2023 was incomplete due to our study's cutoff date. In addition, there is a distinct role of gender in bipolar disorder. However, only few studies explored the gender differences between bipolar disorder and the gut microbiota (Flowers et al., 2019; Nikolova et al., 2021). Furthermore, the total number of publications in this specific domain remains relatively small, underscoring the necessity for further investigations to expand our understanding of the association between gut microbiota and bipolar disorder.

In conclusion, this is the first bibliometric analysis to investigate the publications in the field of the association between bipolar disorder and the gut microbiota. The main research hotspots regarding this field were the characteristics, abundance, and diversity of gut microbiome in bipolar disorder, the role of treatment and gut microbiome in bipolar disorder, microbiome-brain connections in bipolar disorder, and interventions for bipolar disorder based on microbiota composition modification. The number of studies about the association between gut microbiota and bipolar disorder is relatively small, and more studies are needed to expand our understanding of the link between gut microbiota and bipolar disorder.

Data availability statement

The original contributions presented in this study are included in this article/supplementary material, further inquiries can be directed to the corresponding authors.

Author contributions

XL: Data curation, Formal Analysis, Methodology, Project administration, Supervision, Writing – original draft. SW: Data curation, Funding acquisition, Methodology, Resources, Supervision, Writing – original draft, Writing – review & editing. JH: Formal Analysis, Funding acquisition, Project administration, Resources, Visualization, Writing – original draft, Writing – review & editing. KZ: Data curation, Supervision, Writing – original draft, Writing – review & editing.

Funding

The authors declare that no financial support was received for the research, authorship, and/or publication of this article.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

Aizawa, E., Tsuji, H., Asahara, T., Takahashi, T., Teraishi, T., Yoshida, S., et al. (2019). Bifidobacterium and Lactobacillus counts in the gut Microbiota of patients with bipolar disorder and healthy controls. *Front. Psychiatry* 9:730. doi: 10.3389/fpsyt.2018.00730

Alam, R., Abdolmaleky, H., and Zhou, J. (2017). Microbiome, inflammation, epigenetic alterations, and mental diseases. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* 174, 651–660.

Anderson, G., and Maes, M. (2020). Gut dysbiosis dysregulates central and systemic homeostasis via suboptimal mitochondrial function: assessment, treatment and classification implications. *Curr. Top. Med. Chem.* 20, 524–539. doi: 10.2174/1568026620666200131094445

Barbuti, M., Carvalho, A., Köhler, C., Murru, A., Verdolini, N., Guiso, G., et al. (2017). Thyroid autoimmunity in bipolar disorder: a systematic review. *J. Affect. Disord.* 221, 97–106.

Barroso, A., Mahler, J., Fonseca-Castro, P., and Quintana, F. (2021). The aryl hydrocarbon receptor and the gut-brain axis. *Cell Mol. Immunol.* 18, 259–268.

Basiji, K., Sendani, A., Ghavami, S., Farmani, M., Kazemifard, N., Sadeghi, A., et al. (2023). The critical role of gut-brain axis microbiome in mental disorders. *Metab. Brain Dis.* 38, 2547–2561.

Bengesser, S., Reininghaus, E., Lackner, N., Birner, A., Fellendorf, F., Platzer, M., et al. (2018). Is the molecular clock ticking differently in bipolar disorder? Methylation analysis of the clock gene ARNTL. *World J. Biol. Psychiatry* 19, S21–S29. doi: 10.1080/15622975.2016.1231421

Carvalho, A., Firth, J., and Vieta, E. (2020). Bipolar disorder. *N. Engl. J. Med.* 383, 58–66.

Cenit, M., Sanz, Y., and Codoñer-Franch, P. (2017). Influence of gut microbiota on neuropsychiatric disorders. *World J. Gastroenterol.* 23, 5486–5498.

Chang, P., Hao, L., Offermanns, S., and Medzhitov, R. (2014). The microbial metabolite butyrate regulates intestinal macrophage function via histone deacetylase inhibition. *Proc. Natl. Acad. Sci. U S A.* 111, 2247–2252.

Coello, K., Hansen, T., Sørensen, N., Munkholm, K., Kessing, L., Pedersen, O., et al. (2019). Gut microbiota composition in patients with newly diagnosed bipolar disorder and their unaffected first-degree relatives. *Brain Behav. Immun.* 75, 112–118. doi: 10.1016/j.bbi.2018.09.026

Craddock, N., and Sklar, P. (2013). Genetics of bipolar disorder. *Lancet* 381, 1654–1662.

Dickerson, F., Adamos, M., Katsafanas, E., Khushalani, S., Origoni, A., Savage, C., et al. (2018). Adjunctive probiotic microorganisms to prevent rehospitalization in patients with acute mania: a randomized controlled trial. *Bipolar. Disord.* 20, 614–621. doi: 10.1111/bdi.12652

Dickerson, F., Severance, E., and Yolken, R. (2017). The microbiome, immunity, and schizophrenia and bipolar disorder. *Brain Behav. Immun.* 62, 46–52.

Evans, S., Bassis, C., Hein, R., Assari, S., Flowers, S., Kelly, M., et al. (2017). The gut microbiome composition associates with bipolar disorder and illness severity. *J. Psychiatr Res.* 87, 23–29. doi: 10.1016/j.jpsychires.2016.12.007

Flowers, S., Baxter, N., Ward, K., Kraal, A., McInnis, M., Schmidt, T., et al. (2019). Effects of atypical antipsychotic treatment and resistant starch supplementation on gut microbiome composition in a cohort of patients with bipolar disorder or Schizophrenia. *Pharmacotherapy* 39, 161–170.

Flowers, S., Evans, S., Ward, K., McInnis, M., and Ellingrod, V. (2017). Interaction between atypical antipsychotics and the gut microbiome in a bipolar disease cohort. *Pharmacotherapy* 37, 261–267. doi: 10.1002/phar.1890

Fond, G., Boukouaci, W., Chevalier, G., Regnault, A., Eberl, G., Hamdani, N., et al. (2015). The "psychomicrobiotic": targeting microbiota in major psychiatric disorders: a systematic review. *Pathol. Biol.* 63, 35–42.

Fries, G., Walss-Bass, C., Bauer, M., and Teixeira, A. (2019). Revisiting inflammation in bipolar disorder. *Pharmacol. Biochem. Behav.* 177, 12–19.

Goes, F. (2023). Diagnosis and management of bipolar disorders. *BMJ* 381:e073591.

Grande, I., Berk, M., Birmaher, B., and Vieta, E. (2016). Bipolar disorder. *Lancet* 387, 1561–1572.

Hornig, M. (2013). The role of microbes and autoimmunity in the pathogenesis of neuropsychiatric illness. *Curr. Opin. Rheumatol.* 25, 488–795.

Hu, S., Li, A., Huang, T., Lai, J., Li, J., Sublette, M., et al. (2019). Gut microbiota changes in patients with bipolar depression. *Adv. Sci.* 6:1900752.

Huang, T., Lai, J., Du, Y., Xu, Y., Ruan, L., and Hu, S. (2019). Current understanding of gut microbiota in mood disorders: an update of human studies. *Front. Genet.* 10:98. doi: 10.3389/fgene.2019.00098

Jacka, F. (2017). Nutritional psychiatry: where to next? *EBioMedicine* 17, 24–29.

Jiang, H., Xu, L., Zhang, X., Zhang, Z., and Ruan, B. (2019). The microbiome in bipolar depression: a longitudinal study of one pair of monozygotic twins. *Bipolar. Disord.* 21, 93–97. doi: 10.1111/bdi.12736

Jones, G., Vecera, C., Pinjari, O., and Machado-Vieira, R. (2021). Inflammatory signaling mechanisms in bipolar disorder. *J. Biomed. Sci.* 28:45.

Kim, Y., and Shin, C. (2018). The microbiota-gut-brain axis in neuropsychiatric disorders: pathophysiological mechanisms and novel treatments. *Curr. Neuropharmacol.* 16, 559–573.

Lai, J., Li, A., Jiang, J., Yuan, X., Zhang, P., Xi, C., et al. (2022). Metagenomic analysis reveals gut bacterial signatures for diagnosis and treatment outcome prediction in bipolar depression. *Psychiatry Res.* 307:114326. doi: 10.1016/j.psychres.2021.114326

Lai, W., Zhao, J., Xu, S., Deng, W., Xu, D., Wang, M., et al. (2021). Shotgun metagenomics reveals both taxonomic and tryptophan pathway differences of gut microbiota in bipolar disorder with current major depressive episode patients. *J. Affect. Disord.* 278, 311–319. doi: 10.1016/j.jad.2020.09.010

Li, Z., Lai, J., Zhang, P., Ding, J., Jiang, J., Liu, C., et al. (2022). Multi-omics analyses of serum metabolome, gut microbiome and brain function reveal dysregulated microbiota-gut-brain axis in bipolar depression. *Mol. Psychiatry* 27, 4123–4135. doi: 10.1038/s41380-022-01569-9

Lin, X., Zhou, R., Liang, D., Xia, L., Zeng, L., and Chen, X. (2022). The role of microbiota in autism spectrum disorder: a bibliometric analysis based on original articles. *Front. Psychiatry* 13:976827. doi: 10.3389/fpsyt.2022.976827

Liu, L., Wang, H., Chen, X., Zhang, Y., Zhang, H., and Xie, P. (2023). Gut microbiota and its metabolites in depression: from pathogenesis to treatment. *EBioMedicine* 90:104527.

Lu, Q., Lai, J., Lu, H., Ng, C., Huang, T., Zhang, H., et al. (2019). Gut microbiota in bipolar depression and its relationship to brain function: an advanced exploration. *Front. Psychiatry* 10:784. doi: 10.3389/fpsyt.2019.00784

McGuinness, A., Davis, J., Dawson, S., Loughman, A., Collier, F., O'Hely, M., et al. (2022). A systematic review of gut microbiota composition in observational studies of major depressive disorder, bipolar disorder and schizophrenia. *Mol. Psychiatry* 27, 1920–1935. doi: 10.1038/s41380-022-01456-3

McIntyre, R., Berk, M., Brietzke, E., Goldstein, B., López-Jaramillo, C., Kessing, L., et al. (2020). Bipolar disorders. *Lancet* 396, 1841–1856.

McIntyre, R., Subramaniapillai, M., Shekotikhina, M., Carmona, N., Lee, Y., Mansur, R., et al. (2021). Characterizing the gut microbiota in adults with bipolar disorder: a pilot study. *Nutr. Neurosci.* 24, 173–180. doi: 10.1080/1028415X.2019.1612555

Misiak, B., Łoniewski, I., Marlicz, W., Frydecka, D., Szulc, A., Rudzki, L., et al. (2020). The HPA axis dysregulation in severe mental illness: can we shift the blame to gut microbiota? *Prog Neuropsychopharmacol Biol. Psychiatry* 102:109951. doi: 10.1016/j.pnpbp.2020.109951

Morais, L., Schreiber, H., and Mazmanian, S. (2021). The gut microbiota-brain axis in behaviour and brain disorders. *Nat. Rev. Microbiol.* 19, 241–255.

Neamah, W., Busbee, P., Alghetaa, H., Abdulla, O., Nagarkatti, M., and Nagarkatti, P. (2020). AhR activation leads to alterations in the gut microbiome with consequent effect on induction of myeloid derived suppressor cells in a CXCR2-dependent manner. *Int. J. Mol. Sci.* 21:9613. doi: 10.3390/ijms21249613

Nguyen, T., Hathaway, H., Kosciolek, T., Knight, R., and Jeste, D. (2021). Gut microbiome in serious mental illnesses: a systematic review and critical evaluation. *Schizophr. Res.* 234, 24–40. doi: 10.1016/j.schres.2019.08.026

Nguyen, T., Kosciolek, T., Eyler, L., Knight, R., and Jeste, D. (2018). Overview and systematic review of studies of microbiome in schizophrenia and bipolar disorder. *J. Psychiatr Res.* 99, 50–61. doi: 10.1016/j.jpsychires.2018.01.013

Nikolova, V., Smith, M., Hall, L., Cleare, A., Stone, J., and Young, A. (2021). Perturbations in gut microbiota composition in psychiatric disorders: a review and meta-analysis. *JAMA Psychiatry* 78, 1343–1354. doi: 10.1001/jamapsychiatry.2021.2573

Obi-Azuike, C., Ebiai, R., Gibson, T., Hernandez, A., Khan, A., Anugwom, G., et al. (2023). A systematic review on gut-brain axis aberrations in bipolar disorder and methods of balancing the gut microbiota. *Brain Behav.* 13:e3037. doi: 10.1002/brb3.3037

Painold, A., Mörkl, S., Kashofer, K., Halwachs, B., Dalkner, N., Bengesser, S., et al. (2019). A step ahead: exploring the gut microbiota in inpatients with bipolar disorder during a depressive episode. *Bipolar Disord.* 21, 40–49. doi: 10.1111/bdi.12682

Perry, A., Roberts, G., Mitchell, P., and Breakspear, M. (2019). Connectomics of bipolar disorder: a critical review, and evidence for dynamic instabilities within interoceptive networks. *Mol. Psychiatry* 24, 1296–1318.

Reininghaus, E., Wetzlmair, L., Fellendorf, F., Platzer, M., Queissner, R., Birner, A., et al. (2018). The impact of probiotic supplements on cognitive parameters in euthymic individuals with bipolar disorder: a pilot study. *Neuropsychobiology* Online ahead of print. doi: 10.1159/000492537

Reiter, R., Tan, D., Rosales-Corral, S., Galano, A., Zhou, X., and Xu, B. (2018). Mitochondria: central organelles for melatonin's antioxidant and anti-aging actions. *Molecules* 23:509. doi: 10.3390/molecules23020509

Rong, H., Xie, X., Zhao, J., Lai, W., Wang, M., Xu, D., et al. (2019). Similarly in depression, nuances of gut microbiota: evidences from a shotgun metagenomics sequencing study on major depressive disorder versus bipolar disorder with current major depressive episode patients. *J. Psychiatr. Res.* 113, 90–99. doi: 10.1016/j.jpsychires.2019.03.017

Rosenblat, J., and McIntyre, R. (2017). Bipolar disorder and immune dysfunction: epidemiological findings, proposed pathophysiology and clinical implications. *Brain Sci.* 7:144. doi: 10.3390/brainsci7110144

Samochowiec, J., and Misiak, B. (2021). Gut microbiota and microbiome in schizophrenia. *Curr. Opin. Psychiatry* 34, 503–507.

Scott, J., Murray, G., Henry, C., Morken, G., Scott, E., Angst, J., et al. (2017). Activation in bipolar disorders: a systematic review. *JAMA Psychiatry* 74, 189–196.

Sharon, G., Cruz, N., Kang, D., Gandal, M., Wang, B., Kim, Y., et al. (2019). Human gut microbiota from autism spectrum disorder promote behavioral symptoms in mice. *Cell* 177, 1600–1618.e17. doi: 10.1016/j.cell.2019.05.004

Sublette, M., Cheung, S., Lieberman, E., Hu, S., Mann, J., Uhlemann, A., et al. (2021). Bipolar disorder and the gut microbiome: a systematic review. *Bipolar. Disord.* 23, 544–564.

Tischkau, S. (2020). Mechanisms of circadian clock interactions with aryl hydrocarbon receptor signalling. *Eur. J. Neurosci.* 51, 379–395.

Vinberg, M., Ottesen, N., Meluken, I., Sørensen, N., Pedersen, O., Kessing, L., et al. (2019). Remitted affective disorders and high familial risk of affective disorders associate with aberrant intestinal microbiota. *Acta Psychiatr. Scand.* 139, 174–184. doi: 10.1111/acps.12976

Vindegaard, N., Speyer, H., Nordentoft, M., Rasmussen, S., and Benros, M. (2021). Gut microbial changes of patients with psychotic and affective disorders: a systematic review. *Schizophr. Res.* 234, 1–10. doi: 10.1016/j.schres.2019.12.014

Wang, A., and Miller, B. (2018). Meta-analysis of cerebrospinal fluid cytokine and tryptophan catabolite alterations in psychiatric patients: comparisons between Schizophrenia, bipolar disorder, and depression. *Schizophr. Bull.* 44, 75–83. doi: 10.1093/schbul/sbx035

Wang, X., Sun, G., Feng, T., Zhang, J., Huang, X., Wang, T., et al. (2019). Sodium oligomannate therapeutically remodels gut microbiota and suppresses gut bacterial amino acids-shaped neuroinflammation to inhibit Alzheimer's disease progression. *Cell Res.* 29, 787–803. doi: 10.1038/s41422-019-0216-x

Yang, C., Lin, X., Wang, X., Liu, H., Huang, J., and Wang, S. (2022). The schizophrenia and gut microbiota: a bibliometric and visual analysis. *Front. Psychiatry* 13:1022472.

Yuan, N., Chen, Y., Xia, Y., Dai, J., and Liu, C. (2019). Inflammation-related biomarkers in major psychiatric disorders: a cross-disorder assessment of reproducibility and specificity in 43 meta-analyses. *Transl. Psychiatry* 9:233. doi: 10.1038/s41398-019-0570-y

Zheng, P., Yang, J., Li, Y., Wu, J., Liang, W., Yin, B., et al. (2020). Gut microbial signatures can discriminate unipolar from bipolar depression. *Adv. Sci.* 7:1902862. doi: 10.1002/advs.201902862

Zhu, X., Hu, J., Deng, S., Tan, Y., Qiu, C., Zhang, M., et al. (2021). Bibliometric and visual analysis of research on the links between the gut microbiota and depression from 1999 to 2019. *Front. Psychiatry* 11:587670. doi: 10.3389/fpsyt.2020.587670

frontiers | Frontiers in Neuroscience

TYPE Original Research
PUBLISHED 24 May 2024
DOI 10.3389/fnins.2024.1397430

OPEN ACCESS

EDITED BY
Avinash Veerappa,
University of Nebraska Medical Center,
United States

REVIEWED BY
Jai Chand Patel,
University of Nebraska Medical Center,
United States
Di Xie,
Shanghai Jiao Tong University, China

*CORRESPONDENCE
Zhong Wang
✉ wangzhong761@163.com
Zhouqing Chen
✉ zqchen6@163.com

†These authors have contributed equally to this work

RECEIVED 07 March 2024
ACCEPTED 13 May 2024
PUBLISHED 24 May 2024

CITATION
Qiu Y, Song B, Xie M, Tao Y, Yin Z, Wang M, Ma C, Chen Z and Wang Z (2024) Causal links between gut microbiomes, cytokines and risk of different subtypes of epilepsy: a Mendelian randomization study.
Front. Neurosci. 18:1397430.
doi: 10.3389/fnins.2024.1397430

Causal links between gut microbiomes, cytokines and risk of different subtypes of epilepsy: a Mendelian randomization study

Youjia Qiu[1†], Bingyi Song[1†], Minjia Xie[1], Yuchen Tao[2], Ziqian Yin[1], Menghan Wang[1], Chao Ma[1], Zhouqing Chen[1*] and Zhong Wang[1*]

[1]Department of Neurosurgery, The First Affiliated Hospital of Soochow University, Suzhou, China, [2]Suzhou Medical College of Soochow University, Suzhou, China

Objective: Recent research suggests a potential link between the gut microbiome (GM) and epilepsy. We undertook a Mendelian randomization (MR) study to determine the possible causal influence of GM on epilepsy and its various subtypes, and explore whether cytokines act as mediators.

Methods: We utilized Genome-Wide Association Study (GWAS) summary statistics to examine the causal relationships between GM, cytokines, and four epilepsy subtypes. Furthermore, we assessed whether cytokines mediate the relationship between GM and epilepsy. Significant GMs were further investigated using transcriptomic MR analysis with genes mapped from the FUMA GWAS. Sensitivity analyses and reverse MR were conducted for validation, and false discovery rate (FDR) correction was applied for multiple comparisons.

Results: We pinpointed causal relationships between 30 GMs and various epilepsy subtypes. Notably, the Family Veillonellaceae (OR:1.03, 95%CI:1.02–1.05, $p = 0.0003$) consistently showed a strong positive association with child absence epilepsy, and this causal association endured even after FDR correction (p-FDR < 0.05). Seven cytokines were significantly associated with epilepsy and its subtypes. A mediating role for cytokines has not been demonstrated. Sensitivity tests validated the primary MR analysis outcomes. Additionally, no reverse causality was detected between significant GMs and epilepsy. Of the mapped genes of notable GMs, genes like BLK, FDFT1, DOK2, FAM167A, ZSCAN9, RNGTT, RBM47, DNAJC21, SUMF1, TCF20, GLO1, TMTC1, VAV2, and RNF14 exhibited a profound correlation with the risk factors of epilepsy subtypes.

Conclusion: Our research validates the causal role of GMs and cytokines in various epilepsy subtypes, and there has been no evidence that cytokines play a mediating role between GM and epilepsy. This could provide fresh perspectives for the prevention and treatment of epilepsy.

KEYWORDS

gut microbiome, subtype of epilepsy, cytokines, Mendelian randomization, mapped genes, FUMA

1 Introduction

Epilepsy is a widespread and severe neurological disorder marked by sudden, simultaneous abnormal neuron activity in the brain. The rate of active epilepsy typically ranges between 4 and 12 per 1,000 (Thijs et al., 2019). Epilepsy's incidence shows a bimodal distribution, with the highest risk seen in infants and the elderly (Thijs et al., 2019). Epilepsy remains a significant cause of disability and death, posing a considerable global societal burden. It can be categorized into focal epilepsy (FE) and generalized epilepsy (GE). The latter is subdivided into motor and non-motor (absence) epilepsy. In GE, the epileptogenic networks are widespread, covering thalamocortical structures bilaterally (Asadi-Pooya, 2019). By contrast, FE networks are limited to a single hemisphere, often involving limbic or neocortical regions (Allers et al., 2015). Both childhood absence epilepsy (CAE) and juvenile absence epilepsy (JAE) are part of an epileptic syndrome, commonly presenting with brief staring, rhythmic blinking, or motor automatisms and changes in the electroencephalogram (Kessler and McGinnis, 2019). While 65% of epilepsy patients find relief with antiepileptic medication (Kwan et al., 2011), about a third face drug resistance (Perucca et al., 2023). The frequent seizures, cognitive challenges, psychological disorders, and drug side effects profoundly impact these patients' quality of life (Wang et al., 2023). Therefore, further exploring the etiology of epilepsy and new therapeutic options for patients has become necessary.

Recently, mounting evidence indicates a strong link between gut microbiomes (GMs) and human health (Järbrink-Sehgal and Andreasson, 2020). Research reveals that GM affects the central nervous system's physiology, neurochemistry, behavior, and cognitive growth. The theory suggests that communication between the GM and the brain is facilitated through diverse channels, including the central and enteric nervous systems and endocrine and immune pathways (Grenham et al., 2011). This intricate relationship, the gut-brain axis, is maintained through multiple signaling pathways and connectivity networks. Studies have highlighted the role of GM and the gut-brain axis in neurological disorders such as depression, Alzheimer's disease, and Parkinson's disease. The hypothesis has been advised that GM dysbiosis is linked to development of epilepsy (Sorboni et al., 2022). The composition of GM in individuals with epilepsy is different from that of healthy control subjects, both before and after treatment (Gong et al., 2022). Additionally, disturbances at the level of specific GM taxa can influence the activity of neurons associated with epilepsy (Darch and McCafferty, 2022). The ketogenic diet (KD), a non-pharmacological option for those with treatment-resistant epilepsy (Socała et al., 2021), can reduce seizure attacks by 50%, as observed in 22–55% of adults and 60–75% of children (Özcan et al., 2022). Yet, whether alteration in GMs induced by KD is protective or detrimental remains debatable.

In general, neuroinflammation is a normal response that helps maintain homeostasis. However, excessive or persistent neuroinflammation can lead to cellular dysfunction (Xanthos and Sandkühler, 2014). Related studies have shown that neuroinflammatory responses are not only the result of epileptic seizures or brain neuropathology but are involved in their production (Prabowo et al., 2013; Pauletti et al., 2019). Activated microglia and astrocytes are the major cell types contributing to neuroinflammation (Vezzani et al., 2011, 2015). Several bioactive products from the GM can trigger the release of inflammatory factors by activating microglia (Kim et al., 2012). Therefore, we hypothesized that cytokines may be mediators in the pathway from GM to epilepsy. Cytokines are inflammatory regulators and, therefore, important intermediate phenotypes in inflammatory diseases. The pro-inflammatory cytokines IL-1β and IL-6 are important contributors to the inflammatory response in the brain. There is evidence that epilepsy is associated with elevated levels of pro-inflammatory cytokines (Vezzani et al., 2013). Moreover, microglia may, with the help of the above cytokines, play a role in the pathology of epilepsy through the IL-1 receptor/Toll-like receptor signaling, COX-2, and the TGF-β signaling process (Vezzani et al., 2016; Alvim et al., 2021; Soltani Khaboushan et al., 2022).

Robust evidence from randomized controlled trials (RCTs) verifies the connection between GM dysfunction and epilepsy onset. However, the nature of this relationship, whether causal or not, requires further study. Mendelian randomization (MR) is an advanced statistical method designed to explore causative associations between variables. It uses single nucleotide polymorphisms (SNPs) as instrumental variables. This method can help negate reverse causality and confounding factor effects. Still, no MR studies have focused on children with juvenile absence epilepsy, who often exhibit higher drug resistance (Symonds et al., 2021). Thus, our research employed a MR analysis using data from a published Genome-Wide Association Study (GWAS) to explore the potential causal link between GM and various epilepsy subtypes.

2 Materials and methods

2.1 Ethical approval

The GWAS data utilized in this study were publicly available and lacked identifiable details. The data had already been approved by an ethics committee, eliminating the need for additional ethical clearance for our research.

2.2 Study design

We employed two-sample MR to explore the relationship between GM and various epilepsy subtypes such as FE, GE, JAE, and CAE. Our research follows STROBE-MR guidance (Supplementary Table S1). The study's flowchart is depicted in Figure 1. For reliable MR study outcomes, three criteria must be met: (1) There must be a significant association between genetic variants and the specific exposure. (2) Genetic variants must not be linked to confounding factors associated with the outcomes. (3) Genetic variants should only affect the outcomes through exposure factors and no alternative pathways. IVs satisfying these criteria are deemed fit for MR analysis, ensuring accurate causal inference.

2.3 Data source

Summary statistics for GMs were sourced from an extensive GWAS by the MiBioGen consortium, which included 18,340 individuals of European ethnic backgrounds across 11 countries and analyzed 122,110 genetic variation loci (Kurilshikov et al., 2021). We accessed data on 211 gut microbial taxa, covering five taxonomic

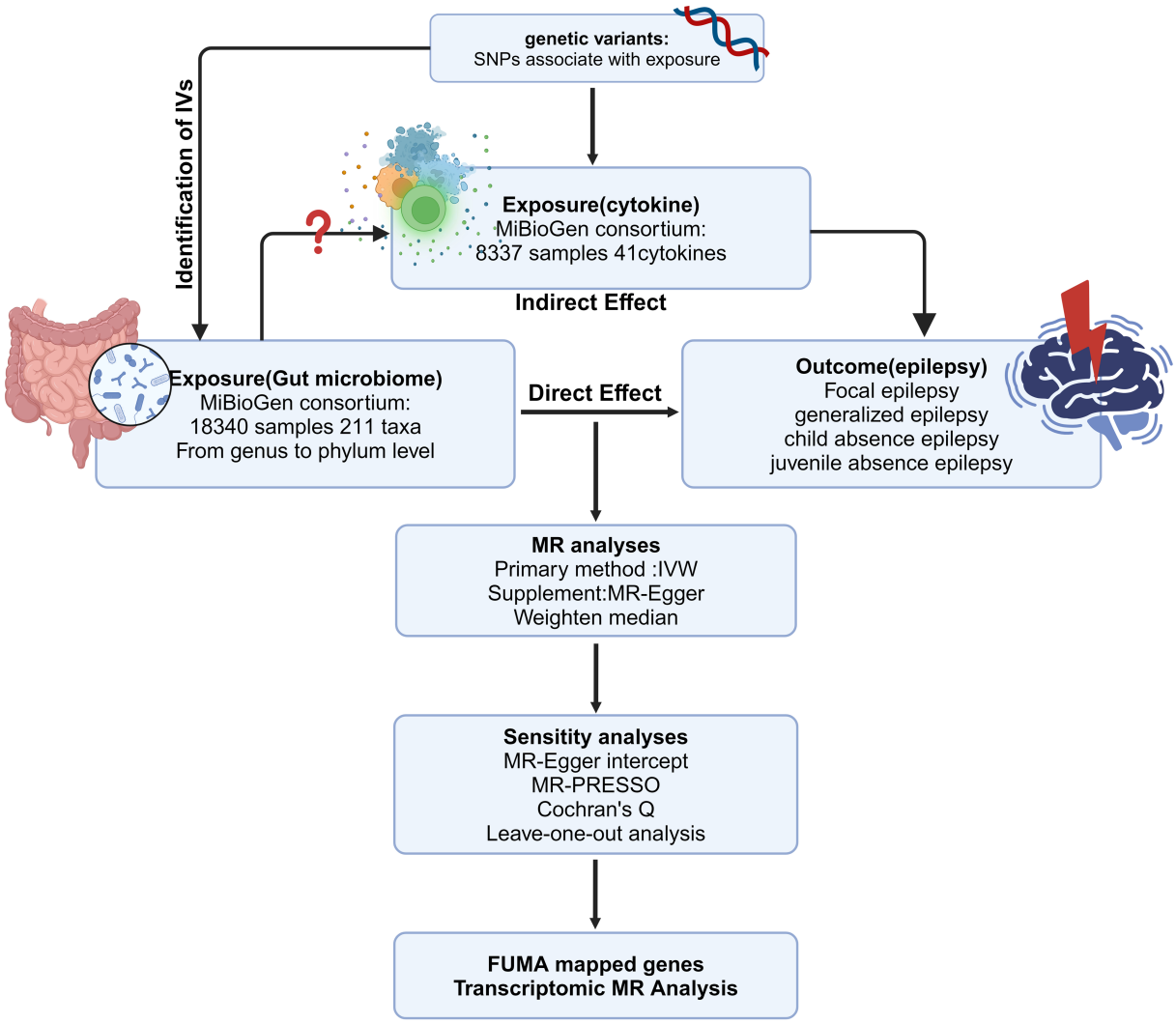


FIGURE 1
The overview of the MR analysis design (Created with BioRender.com). IV, instrumental variable; MR, Mendelian randomization; SNPs, single-nucleotide polymorphisms; IVW, inverse-variance weighted; MR-PRESSO, MR pleiotropy residual sum and outlier.

levels from genus to phylum. The adjustment was made for sex, age, study-specific covariates, and the primary genetic principal components reflecting population stratification (Kurilshikov et al., 2021). GWAS data for FE and GE were procured from FinnGen Research (https://r9.finngen.fi/) and released in September 2022. Epilepsy diagnoses in the FinnGen dataset used the G40 code from the 10th edition of the International Classification of Diseases (ICD). The summary data for CAE and JAE came from the IEU open GAWS project (https://gwas.mrcieu.ac.uk/). As data on GMs and different subtypes of epilepsy were obtained from different databases, the populations with GMs and epilepsy did not overlap. Genetic data for cytokines were from a previous GWAS (8,337 individuals) and included 41 inflammatory cytokines (Ahola-Olli et al., 2017). This study amalgamated findings from both The Cardiovascular Risk in Young Finns Study and FINRISK surveys, wherein participants averaged 37 years and 60 years, respectively. Under the framework of our MR design, a comprehensive summary of the exposures and outcomes subjected to analysis is provided in Table 1.

2.4 Identification of IVs

To enhance the precision of our results, we conducted a thorough data screening process for the information extracted from MiBioGen. Owing to the limited number of IVs meeting a stringent threshold of $p < 5 \times 10^{-8}$, we adjusted the threshold to $p < 1 \times 10^{-5}$ to obtain a more substantial number of IVs derived from GMs for our analysis. By increasing the number of IVs included, the efficacy of the statistical analyses can be improved. Similarly, we set the threshold to $p < 5 \times 10^{-6}$ to expand the number of available IVs for each cytokine. Additionally, we set r^2 to 0.01 and the clumping window width kb = 10,000 to ensure no linkage disequilibrium among IVs. Further refinements to our IVs included removing palindromic SNPs and any not present in the results, allowing us to assess whether the selected IVs had any associations with potential confounding risk factors. Lastly, the F-statistics were calculated using the subsequent formula to evaluate the potential bias arising from weak instruments: ($F = \text{beta}^2/\text{se}^2$).

TABLE 1 Details of the exposure and outcome.

Trait	Consortium	Samples	Case	Control
Exposure				
211 GM taxa	MiBioGen	18,340	/	/
41 cytokines	Ahola-Olli et al.	8,337	/	/
Outcome				
Generalized epilepsy	FinnGen (R9)		2,632	300,631
Focal epilepsy	FinnGen (R9)		1,297	300,631
Child absence epilepsy	IEU OpenGWAS project	30,470	793	29,677
Juvenile absence epilepsy	IEU OpenGWAS project	30,092	415	29,677

GM, gut microbiome; GWAS, genome-wide association study.

2.5 Primary analyses

We used the inverse variance weighted (IVW) method as our primary means to evaluate the causal relationship. Other methods, including MR-Egger, weighted mode, weighted median, and simple mode, were also employed. MR-Egger detects and adjusts for possible horizontal pleiotropy, but its results can be skewed by the presence of outlying genetic variables (Sun et al., 2023). The weighted median method ensures causality estimate stability by correcting potential errors when up to 50% of the IVs might be invalid and may provide better causality detection than the MR-Egger under certain conditions (Morris et al., 2022). In addition, reverse MR was conducted to rule out reverse causality. The odds ratio (OR) with its 95% confidence interval (95% CI) indicates GM's causal effect on epilepsy. We adjusted the *p*-value using the false discovery rate (FDR) procedure for multiple comparisons (Xiang et al., 2021).

2.6 Mediation analysis

Mediation analysis aims to evaluate the pathway from exposure to outcome through a mediator, which helps explore the potential mechanisms by which exposure affects outcome (Carter et al., 2021). After assessing causal effects using two-sample analysis, the selected GM and cytokines with significant causal impact on epilepsy will be included in the mediation analysis. If GM has a causal effect on cytokines, we will explore whether cytokines are mediators in the pathway from GM to epilepsy. Specifically, a two-step MR analysis was conducted to examine the mediating pathways from GM to epilepsy. In the first step, the IV of GM was used to estimate the causal effect of exposure on potential mediating variables. In the second step, the causal effect of mediating variables on epilepsy risk was estimated. Finally, the indirect effect of GM on epilepsy was investigated by cytokines. The two-step MR is similar to the product of coefficient methods. Two MR estimates were calculated: β1: causal effect of exposure on the mediator; β2: causal effect of the mediator on the outcome; β3: causal effect of exposure on the outcome (Relton and Davey Smith, 2012; Burgess et al., 2015). These two estimates can be multiplied to estimate indirect effects, and the percentage of mediation was calculated by applying the following formula: $(\beta1 \times \beta2)/(\beta3)$.

2.7 Sensitivity analyses

Sensitivity analyses were performed to evaluate the robustness of MR estimates. We assessed the heterogeneity of the IVs through the calculation of I^2 statistics and the application of Cochran's Q test. The MR-Egger intercept and MR pleiotropy residual sum and outlier (MR-PRESSO) tests were employed to pinpoint pleiotropy and to identify potential outliers with heterogeneity among the IVs (Li et al., 2023). Furthermore, a leave-one-out analysis determined if a single SNP influenced the MR findings. This approach was carried out to assess whether the estimates were affected by biases or driven by outliers (Li et al., 2023). All MR calculations were conducted using R software version 4.3.0 and the R package MendelR (7.8.0).

2.8 Mapping SNPs to genes

To better understand GMs' effect on epilepsy, we integrated significant SNPs from each GM as primary SNPs in the FUMA GWAS (Watanabe et al., 2017). These SNPs were then related to genes using FUMA integrated SNPGENE tool. A protein–protein interaction (PPI) network was created using the STRING database, with a recommended minimum interaction score of 0.4 and default settings for other parameters (Szklarczyk et al., 2021). The PPI network was visualized in Cytoscape (V3.9.1).

2.9 Transcriptomic MR analysis

To delve deeper into the role of genes derived from the positive GMs and CAE GWAS, we executed a transcriptomic MR analysis. Cis-expression quantitative trait loci (cis-eQTL) data related to these genes were sourced from the eQTLGen Consortium (https://eqtlgen.org/), comprising 16,987 genes from an extensive pool of 31,684 blood samples, mainly from a healthy European population (Võsa et al., 2021). For cis-eQTL MR, using a very stringent correlation criterion could exclude causal variants. Hence, these expression quantitative trait loci (eQTLs) underwent clumping based on pairwise linkage disequilibrium (LD) threshold, with an r-squared (r^2) value threshold of less than 0.1. Subsequently, the SNPs linked to these genes were used as IVs for MR between these IVs and epilepsy. We used FDR correction for multiple comparisons, and a result of p-FDR < 0.05 indicated statistical significance.

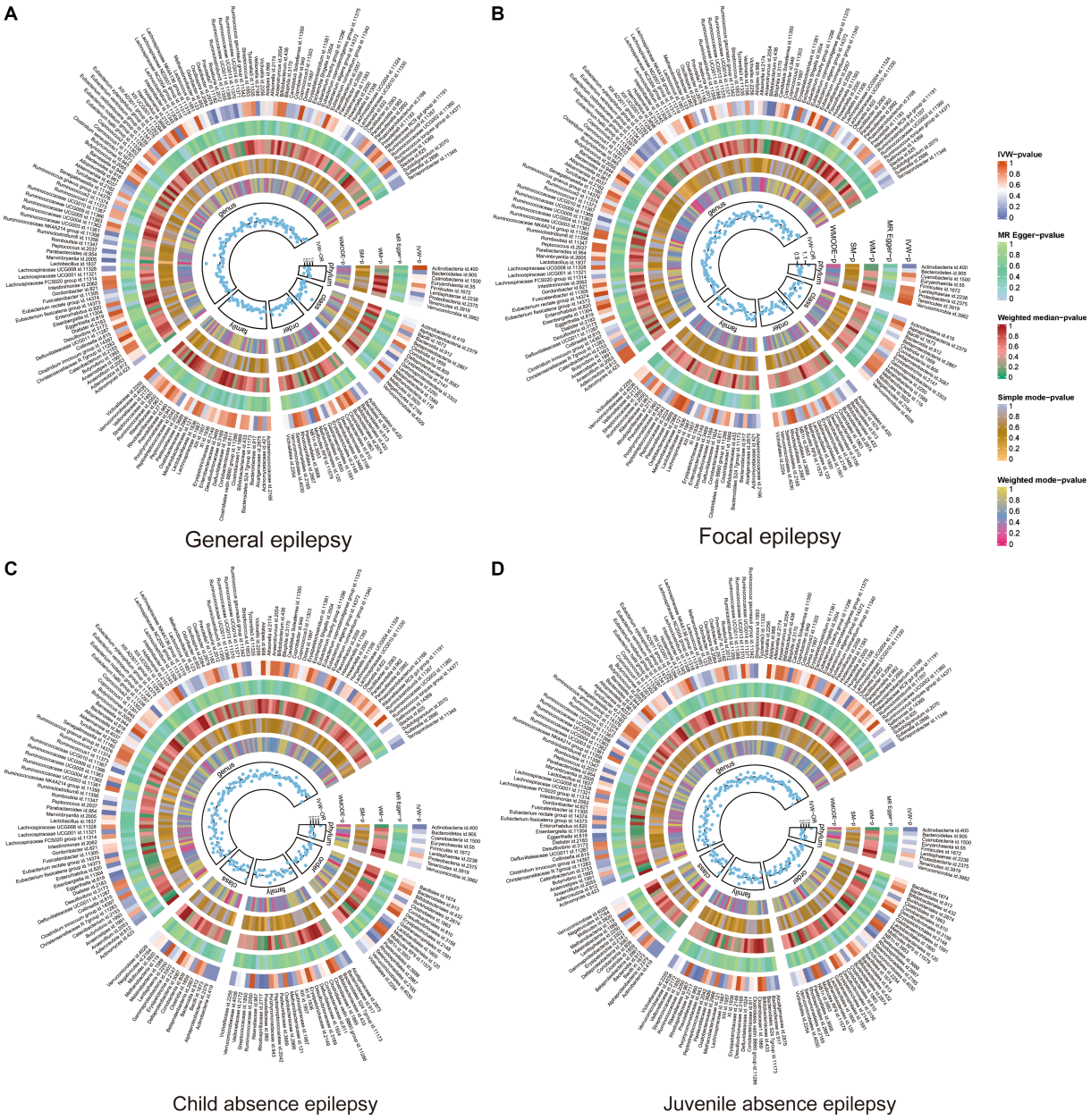


FIGURE 2
Heat map of GM taxa causally associated with subtypes of epilepsy. Red represents risk factors, while blue represents protective factors. **(A)** Heat map of GM taxa causally associated with GE; **(B)** Heat map of GM taxa causally associated with FE; **(C)** Heat map of GM taxa causally associated with CAE; **(D)** Heat map of GM taxa causally associated with JAE. GM, gut microbiome; IVW, inverse-variance weighted method; WM, weighted median; OR, odds ratios; SM, simple mode; WMODE, weighted mode.

3 Results

3.1 Details of GMs

A total of 211 GMs spanning five biological levels were included in this study. From these, 15 bacterial traits with unspecified characteristics were excluded. Consequently, 196 bacterial traits from the IEU and FinnGen datasets were incorporated into the MR analysis. The *F* value for all IVs exceeded 10, signifying no bias from weak IVs. Details about IVs can be found in Supplementary Tables S2, S3, and the MR results for the 196 GMs are presented in Figure 2 and Supplementary Table S4. Supplementary Table S5 shows the whole results of MR analysis between cytokines and epilepsy.

3.2 MR estimates

In FE, the genera *Bilophila* (OR: 1.24, 95% CI: 1.04–1.48, $p = 0.02$), *Bifidobacterium* (OR: 1.26, 95% CI: 1.04–1.51, $p = 0.02$), *Eubacterium nodatum group* (OR: 1.10, 95% CI: 1.01–1.21,

$p = 0.04$), and *Erysipelatoclostridium* (OR:1.14, 95% CI:1.002–1.30, $p < 0.05$) were positively associated with FE, while *Flavonifractor* (OR:0.78, 95% CI:0.62–0.99 $p = 0.04$) was negatively correlated with FE. In GE, six GMs were identified. Of these, class *Gammaproteobacteria* (OR: 2.05, 95% CI: 1.02–4.14, $p < 0.05$), genus *Marvinbryantia* (OR: 2.04, 95% CI: 1.35–3.10, $p = 0.001$), genus *Oxalobacter* (OR: 1.30, 95% CI: 1.04–1.64, $p = 0.02$), genus *Ruminococcaceae Ucg013* (OR: 1.59, 95% CI: 1.02–2.45, $p = 0.04$), and order *Mollicutes RF9* (OR: 1.48, 95% CI:1.03–2.13, $p = 0.03$) were associated with an increased risk of GE; however, the genus *Phascolarctobacterium* (OR: 0.58, 95% CI: 0.37–0.91, $p = 0.02$) was associated with a risk reduction of GE.

Nine GMs were identified in CAE. The family *Veillonellaceae* (OR: 1.03, 95% CI: 1.02–1.05, $p < 0.001$), genus *Desulfovibrio* (OR: 1.03, 95% CI: 1.00–1.06, $p = 0.03$), genus *Oscillibacter* (OR: 1.02, 95% CI: 1.001–1.04, $p = 0.04$), genus *Anaerostipes* (OR: 1.03, 95% CI:1.0004–1.06, $p < 0.05$), and phylum *Verrucomicrobia* (OR:1.02, 95% CI: 1.003–1.039, $p = 0.03$) were associated with an increased risk of CAE, while the class *Bacteroidia* (OR:0.97, 95% CI:0.95–0.995, $p = 0.02$), genus *Ruminococcaceae NK4A214 group* (OR: 0.97, 95% CI:0.94–0.996, $p = 0.03$), phylum *Bacteroidetes* (OR: 0.97, 95% CI: 0.95–0.99, $p = 0.01$), and order *Bacteroidales* (OR: 0.97, 95% CI: 0.95–0.995, $p = 0.02$) were associated with a reduced risk of CAE. For JAE, 10 GMs were identified to have a causal association. Of these, the genus *Ruminococcaceae UCG004* (OR: 1.02, 95% CI: 1.003–1.03, $p = 0.02$), genus *Candidatus soleaferrea* (OR: 1.01, 95% CI: 1.001–1.02, $p = 0.03$), and genus *Lachnospiraceae UCG010* (OR: 1.02, 95% CI: 1.001–1.03, $p = 0.04$) were positively correlated with JAE; while family *Rhodospirillaceae* (OR: 0.99, 95% CI: 0.98–0.996, $p = 0.007$), family *Prevotellaceae* (OR: 0.99, 95% CI: 0.97–0.999, $p = 0.03$), genus *Parabacteroides* (OR: 0.97, 95% CI: 0.95–0.99, $p = 0.01$), genus *Ruminococcaceae UCG010* (OR: 0.98, 95% CI: 0.95–0.998, $p = 0.03$), genus *Eggerthella* (OR: 0.99, 95% CI: 0.97–0.999, $p = 0.03$), genus *Eubacterium nodatum group* (OR:0.99, 95% CI: 0.99–0.9998, $p = 0.04$), and order *Rhodospirillales* (OR: 0.99, 95% CI: 0.98–0.998, $p = 0.02$) were negatively associated with JAE. Of 30 GMs, only family *Veillonellaceae* in CAE passed FDR correction (p-FDR < 0.05). The MR results of positive GMs in different epilepsy subtypes are shown in Supplementary Table S6.

In exploring the causal relationship between cytokines and epilepsy, we found that Interleukin-1-receptor antagonist (OR: 1.03, 95% CI: 1.01–1.04, $p < 0.001$) and Vascular endothelial growth factor (OR: 1.18, 95% CI: 1.03–1.35, $p = 0.02$) have a protective effect on epilepsy. Besides, Fibroblast growth factor basic (OR: 0.80, 95% CI:0.67–0.97, $p = 0.02$) can increase the risk of FE (Figure 3).

3.3 Mediation analysis

In this study, both GM and cytokines were causally associated with epilepsy. This seems to indicate that cytokines play a mediating role in the pathway between GM and dementia. This mediation analysis was based on the significant correlation between GM and cytokines. However, we did not observe a causal relationship between GM and cytokines significantly associated with epilepsy (Supplementary Table S7). Therefore, there is insufficient evidence to prove that GM has an indirect effect on epilepsy through cytokines.

3.4 Sensitivity analyses

The MR-PRESSO and MR-Egger intercept analysis confirmed the absence of potential horizontal pleiotropy, as detailed in Table 2 and Supplementary Table S8. Based on the Cochrane Q test and I^2 statistics, no evidence of heterogeneity was found among the selected IVs and their relationship with epilepsy. Results from the scatter plot are provided in the Supplementary Figure S1. The leave-one-out analysis did not identify any outlier SNPs (Supplementary Figure S2). In conclusion, these findings underline a consistent and robust causal link between GM and epilepsy.

3.5 Reverse Mendelian randomization

1To examine reverse causality, we considered epilepsy subtypes as exposure and significant GMs as outcomes. After adjusting for linkage disequilibrium, we sourced 17, 11, 14, and 8 SNPs associated with CAE, JAE, FE, and GE from the GWAS database. As demonstrated in Supplementary Table S9, no reverse causality between epilepsy subtypes and GMs was evident ($p > 0.05$).

3.6 Mapping SNPs to genes

To delve deeper into biologically significant findings, the IVs, used as genetic variants, were functionally annotated using the FUMA GWAS tool. The identified genes are listed in Supplementary Table S10. Using STRING, we constructed a PPI network for these mapped genes across different epilepsy subtypes, with outcomes displayed in Supplementary Figure S3.

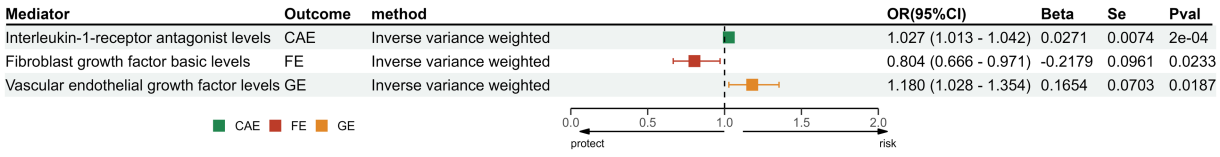


FIGURE 3
The positive results of MR analysis between cytokines and different subtypes of epilepsy. IL-1Ra, Interleukin-1-receptor antagonist levels; FGF-b, Fibroblast growth factor basic levels; VEGF, Vascular endothelial growth factor levels; SNP, single-nucleotide polymorphisms; OR, odds ratios; CI, confidence interval.

TABLE 2 Horizontal pleiotropy analysis for IVs of 30 GM taxa associated with epilepsy.

Exposure	Outcome	MR-Egger intercept test		MR-PRESSO global test	
		Egger_ intercept	*p*-value	RSS obs	*p*-value
Genus Phascolarctobacterium id.2168	GE	5.68	0.68	7.76	0.50
Genus Oxalobacter id.2978	GE	8.16	0.52	10.06	0.57
Genus Ruminococcaceae UCG013 id.11370	GE	10.55	0.31	11.04	0.54
Genus Marvinbryantia id.2005	GE	5.76	0.33	12.50	0.43
Order Mollicutes RF9 id.11579	GE	12.72	0.24	15.28	0.34
Class Gammaproteobacteria id.3303	GE	5.61	0.23	10.28	0.26
Genus *Eubacterium nodatum* group id.11297	FE	0.03	0.41	6.44	0.88
Genus Bifidobacterium id.436	FE	−0.01	0.47	19.22	0.17
Genus Bilophila id.3170	FE	0.02	0.60	15.34	0.40
Genus Erysipelatoclostridium id.11381	FE	−0.01	0.77	10.49	0.83
Genus Flavonifractor id.2059	FE	0.02	0.04	3.71	0.69
Family Veillonellaceae id.2172	CAE	0.00	0.69	5.29	0.94
Phylum Bacteroidetes id.905	CAE	0.00	0.67	6.41	0.70
Phylum Verrucomicrobia id.3982	CAE	0.00	0.48	4.09	0.83
Class Bacteroidia id.912	CAE	0.00	0.30	5.84	0.75
Order Bacteroidales id.913	CAE	0.00	0.30	5.84	0.76
Genus Anaerostipes id.1991	CAE	−0.01	0.37	4.94	0.48
Genus Desulfovibrio id.3173	CAE	0.04	0.40	NA	NA
Genus Ruminococcaceae NK4A214 group id.11358	CAE	0.00	0.96	1.11	0.90
Genus Oscillibacter id.2063	CAE	0.00	0.93	12.74	0.19
Family Prevotellaceae id.960	JAE	−0.01	0.09	7.96	0.71
Family Rhodospirillaceae id.2717	JAE	0.00	0.55	12.10	0.55
Order Rhodospirillales id.2667	JAE	0.00	0.72	12.79	0.48
Genus Eggerthella id.819	JAE	0.00	0.44	1.81	0.83
Genus Parabacteroides id.954	JAE	0.00	0.79	1.92	0.81
Genus Ruminococcaceae UCG004 id.11362	JAE	0.00	0.67	5.43	0.63
Genus Ruminococcaceae UCG010 id.11367	JAE	−0.01	0.42	NA	NA
Genus *Eubacterium nodatum* group id.11297	JAE	0.00	0.29	5.86	0.74
Genus Candidatus Soleaferrea id.11350	JAE	0.01	0.47	5.89	0.57
Genus Lachnospiraceae UCG010 id.11330	JAE	0.00	0.62	3.51	0.73

GE, generalized epilepsy; FE, focal epilepsy; CAE, child absence epilepsy; JAE, juvenile absence epilepsy; MR-PRESSO, MR pleiotropy residual sum and outlier.

3.7 Transcriptomic MR analysis

We sourced SNPs linked to gene expression (eQTLs) from the eQTLGen consortium. Transcriptomic MR analysis of the family *Veillonellaceae* and CAE remained significant post-FDR correction. Of the 631 mapped genes, 501 were derived from cis-eQTL summary statistics. Post FDR correction, genes like FDFT1, DOK2, FAM167A, and ZSCAN9 in the genus *Oscillibacter* showed a positive correlation with CAE risk, while BLK indicated a negative correlation. For JAE, RNGTT from the family *Rhodospirillaceae* and RBM47 from the genus of *Ruminococcaceae UCG010* showed positive associations. DNAJC in genus *Erysipelatoclostridium* was associated with FE risk. In the case of GE, genes such as SUMF1 and TCF20 from order *Mollicutes RF9* and VAV2 from the genus *Ruminococcaceae UCG13* were positively associated; conversely, GLO1 from the genus *Oxalobacter* and TMTC from the genus *Ruminococcaceae UCG13* indicated a reduced risk for GE. Comprehensive results from the five methods are consolidated in Supplementary Table S11; the directionality across these methods was consistent (Figure 4). Heterogeneity testing showed nonsignificant differences, suggesting no notable heterogeneity in the MR estimates (Supplementary Table S12).

4 Discussion

In this study, we conducted MR analyses to investigate the potential causal relationship between GMs and different epilepsy subtypes. We found that 30 GMs were associated with four epilepsy subtypes using extensive GWAS statistics, and no reverse causality was identified. Notably, the family *Veillonellaceae* in

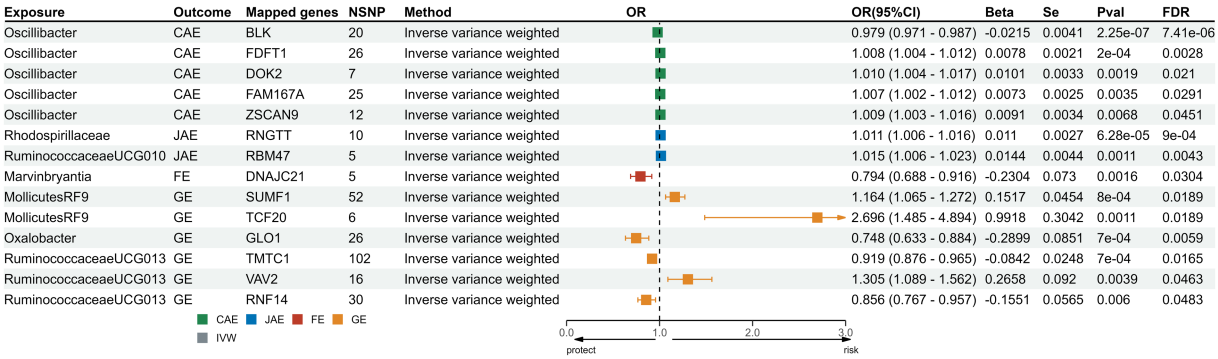


FIGURE 4
Forest plots for transcriptomic MR analysis of significant mapped genes. CAE, child absence epilepsy; JAE, juvenile absence epilepsy; FE, focal epilepsy; GE, generalized epilepsy; IVW, inverse-variance weighted method; OR, odds ratios; CI, confidence interval; FDR: false discovery rate correction.

CAE displayed a significant causal association after FDR correction. Three types of cytokines have significant effects on epilepsy. These results could provide insights into the therapeutic role of epilepsy.

The role of GM in maintaining the state of epilepsy has gained increasing recognition. Yet, due to limited clinical trials examining the differences in the metagenome/metabolome between epilepsy patients and healthy individuals, the influence of GM on epilepsy remains elusive. In our conclusions, no reverse causality was found for epilepsy on GM. Considering modifications related to several external factors (e.g., diet and age), it is not easy to conduct relevant animal or clinical studies. Although some studies have found increased alpha diversity and abundance of thick-walled phyla in patients with drug-resistant epilepsy and altered abundance of *Prevotella*, *Ruminococcus*, and other flora in patients with cerebral palsy with epilepsy (Peng et al., 2018; Huang et al., 2019). The results of the available studies are not uniform, and it is therefore difficult to define relevant criteria (Russo, 2022). Previous research has highlighted the importance of *Veillonellaceae* in developing the intestinal microecosystem during early life (Lou et al., 2022). While direct evidence connecting *Veillonellaceae* and epilepsy is scarce, its influence on normal brain functions is documented. Furthermore, *Veillonellaceae*, part of the phylum *Firmicutes*, shows increased abundance in patients with drug-resistant epilepsy, which also decreases after antiepileptic treatment (Cheraghmakani et al., 2021). Research has identified a prevalent genus, *Marvinbryantia*, in epileptic animals, which correlates positively with excitatory neurotransmitters (Cheraghmakani et al., 2021). Our findings show that a positive causal relationship between the genus *Marvinbryantia* and GE aligns with these experimental results. Oliveira et al. observed a negative correlation between *Marvinbryantia* and d-glucose and lactate. This negative association may disrupt carbohydrate metabolism, possibly contributing to the mechanisms underlying epilepsy (Fei et al., 2020). Additionally, *Marvinbryantia* is a pro-inflammatory bacterium, possibly exacerbating inflammation and precipitating epileptic conditions (Oliveira et al., 2022). Our MR analysis highlighted *Parabacteroides*, belonging to the family *Tannerellaceae*, as a potential protective agent for JAE. Previously linked to increased ketosis and metabolic enhancement in humans (David et al., 2014), this bacterium might explain the seizure control seen with the KD (Bough and Rho, 2007). Olson et al. posited that the KD's anti-seizure effects arise from increasing specific bacterial species, particularly *Parabacteroides merdae*. Together, these bacteria reduce the γ-glutamylation of amino acids and boost the hippocampus's gamma-aminobutyric acid/glutamate ratio, preventing epilepsy (Olson et al., 2018).

Interestingly, 28 other GMs with nominal causal associations, such as Ruminococcus, echoed past findings. Similar to *Veillonellaceae*, *Ruminococcus*, another microbiome from phylum *Firmicutes*, increases in individuals with autism spectrum disorders (Wang et al., 2013). *Ruminococcus* causes reduced levels of N-acetyl aspartate, a marker of neuronal health, and is typically diminished in individuals diagnosed with epilepsy (Zhang et al., 2016; Mudd et al., 2017). The presence of this microorganism also correlates positively with glutamate and glutamine, both closely associated with the pathogenesis of epilepsy (Sun et al., 2016). Furthermore, *Ruminococcus* is associated with reduced levels of 5-hydroxytryptophan, which inhibits T-type calcium channels and subsequently reduces epileptiform discharges (Petersen et al., 2017). Additionally, the abundance of the phylum *Verrucomicrobia*, *Bacteroides*, and its related genus (including *Bacteroides* and *Barnesiella*) was observed to be higher in the drug-resistant epilepsy group than in the control group, aligning with our findings (Peng et al., 2018). Notably, subjects with cerebral palsy and epilepsy, potential risk factors for FE and GE, exhibited an elevated presence of *Eubacterium* (Peng et al., 2023). Moreover, *Anaerostipes* was found to be more prevalent in epilepsy patients (Gong et al., 2021). However, it is noteworthy that while *Lachnospiraceae* is more abundant in healthy individuals (Ding et al., 2021), a reduced presence was noted in temporal lobe epilepsy patients (Wei et al., 2023). This observation contrasts with our MR findings and necessitates further exploration.

Currently, the exact mechanism by which the GM contributes to epilepsy has not been determined. Although the available data do not confirm that cytokines are mediators between GM and epilepsy, we observed many results that are consistent with previous studies. As a protective factor in epilepsy, the IL-1 receptor antagonist anakinra can be used as a treatment for refractory epilepsy (Yamanaka et al., 2021), and vascular endothelial growth factor is considered an attractive target for epilepsy treatment (Lange et al., 2016). On the other hand,

we have found that IL-2 increases the risk of epilepsy. As a factor that plays an important immune role, elevated levels of IL-2 over-activate signaling pathways, leading to a worse prognosis in temporal lobe epilepsy (Mazumder et al., 2019).

The precise mechanisms driving the relationship between GMs and epilepsy are not yet fully understood, but emerging evidence provides some initial insights into possible mechanisms. Various bioactive products from GMs have the potential to influence the brain either directly or indirectly. As mentioned above, GM can mediate neuroinflammation, and pro-inflammatory cytokines may contribute to epilepsy by triggering oxidative stress, increased PIC secretion and BBB disruption (Vezzani et al., 2013). For instance, lipopolysaccharides can directly affect the central nervous system by activating Toll-like receptors on microglia and triggering the release of inflammatory factors (Kim et al., 2012). In addition, enteroendocrine signaling and microbial metabolism are among the mechanisms that may contribute to epilepsy. By increasing the release of excitatory neurotransmitters such as glutamate and decreasing inhibitory neurotransmitters such as GABA, neurotransmission can be disrupted (Su et al., 2015; Rana and Musto, 2018). SCFAs are also thought to be protective against epilepsy (Ding et al., 2021). From an autoimmune perspective, GM can regulate microglia maturation as well as astrocyte activation (de Theije et al., 2011). This implies that GM can regulate epilepsy development by modulating innate immunity, adaptive immunity and inflammatory mechanisms (Ding et al., 2021). Notably, *Bacteroidetes* have been linked to conditions such as encephalitis and autoimmunity (Xu et al., 2020), which could play a role in epilepsy development. A theory also suggests that GMs might affect the hypothalamic–pituitary–adrenal axis, thereby increasing the likelihood of epilepsy (Sudo et al., 2004).

Combined with existing theories, our study has profound clinical implications and provides new ideas for the clinical management of epilepsy. The risk of epilepsy can be reduced by the use of probiotics. In the field of pharmacological microbiology, GMs and their secreted cytokines can be used as drug targets. He et al. used FMT to improve epilepsy in a 22-year-old girl (He et al., 2017). Additionally, GMs may serve as promising biomarkers for identifying the prognosis of epilepsy patients. For instance, a smaller Bacteroidetes/Firmicutes ratio may increase the risk of seizures and lead to poor prognosis (Citraro et al., 2021). The different abundance composition of GMs in the intestine can also be an indicator for evaluating the efficacy of KD (Xie et al., 2017). Furthermore, this study also has several strengths. First, it comprehensively examines the causal relationship between GM and various epilepsy subtypes. Unlike observational studies, this analysis is less vulnerable to confounding factors and reverse causality. Second, the paper's statistical robustness is evident with a substantial GWAS data sample size for exposure and outcome and significant estimated effects for each genetic variable (*F*-value >10). Besides, a rigorous FDR correction was applied in the MR analysis to minimize type I errors. Third, we included cytokines to analyze possible mediating factors from GM to epilepsy and annotated genetic variants through FUMA. However, certain limitations warrant consideration. The GWAS data on GMs encompass various diseases and age groups without specific gender and age stratification. We included only individuals of European descent due to the absence of large-scale GWAS data for other ancestries, which might make these findings less generalizable. Lastly, while the MR analysis parallels the insights from RCT studies, further validation through animal experiments remains essential.

5 Conclusion

In conclusion, our MR study underscores potential causal connections between GMs and various epilepsy subtypes (GE, FE, JAE, and CAE), suggesting that the dysbiosis of the family *Veillonellaceae* might play a role in CAE onset. A mediating role of the cytokines has not been shown to exist between GM and epilepsy. Further studies are essential to understand the potential mechanisms by which GM might influence epilepsy treatment.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding authors.

Author contributions

YQ: Conceptualization, Formal analysis, Writing – original draft. BS: Conceptualization, Formal analysis, Writing – original draft. MX: Data curation, Writing – original draft. YT: Data curation, Writing – original draft. ZY: Methodology, Software, Writing – original draft. MW: Methodology, Software, Writing – original draft. CM: Supervision, Validation, Writing – review & editing. ZC: Supervision, Validation, Writing – review & editing. ZW: Funding acquisition, Resources, Supervision, Writing – review & editing.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. This work was supported by the National Natural Science Foundation of China (Grant No. 82171309).

Acknowledgments

We want to acknowledge the MiBioGen consortium for providing GM-related GWAS summary data. We wish to acknowledge the participants and investigators of the FinnGen study. We want to acknowledge Ahola-Olli et al. for providing cytokine-related GWAS summary data. We want to acknowledge the eQTLGen Consortium for the cis-expression quantitative trait loci data.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnins.2024.1397430/full#supplementary-material

References

Ahola-Olli, A. V., Würtz, P., Havulinna, A. S., Aalto, K., Pitkänen, N., Lehtimäki, T., et al. (2017). Genome-wide association study identifies 27 loci influencing concentrations of circulating cytokines and growth factors. *Am. J. Hum. Genet.* 100, 40–50. doi: 10.1016/j.ajhg.2016.11.007

Allers, K., Essue, B. M., Hackett, M. L., Muhunthan, J., Anderson, C. S., Pickles, K., et al. (2015). The economic impact of epilepsy: a systematic review. *BMC Neurol.* 15:245. doi: 10.1186/s12883-015-0494-y

Alvim, M. K. M., Morita-Sherman, M. E., Yasuda, C. L., Rocha, N. P., Vieira, É., Pimentel-Silva, L. R., et al. (2021). Inflammatory and neurotrophic factor plasma levels are related to epilepsy independently of etiology. *Epilepsia* 62, 2385–2394. doi: 10.1111/epi.17023

Asadi-Pooya, A. A. (2019). Semiological classification of psychogenic nonepileptic seizures: a systematic review and a new proposal. *Epilepsy Behav.* 100:106412. doi: 10.1016/j.yebeh.2019.07.013

Bough, K. J., and Rho, J. M. (2007). Anticonvulsant mechanisms of the ketogenic diet. *Epilepsia* 48, 43–58. doi: 10.1111/j.1528-1167.2007.00915.x

Burgess, S., Daniel, R. M., Butterworth, A. S., and Thompson, S. G. (2015). Network Mendelian randomization: using genetic variants as instrumental variables to investigate mediation in causal pathways. *Int. J. Epidemiol.* 44, 484–495. doi: 10.1093/ije/dyu176

Carter, A. R., Sanderson, E., Hammerton, G., Richmond, R. C., Davey Smith, G., Heron, J., et al. (2021). Mendelian randomisation for mediation analysis: current methods and challenges for implementation. *Eur. J. Epidemiol.* 36, 465–478. doi: 10.1007/s10654-021-00757-1

Cheraghmakani, H., Rezai, M. S., Valadan, R., Rahimzadeh, G., Moradi, M., Jahanfekr, V., et al. (2021). Ciprofloxacin for treatment of drug-resistant epilepsy. *Epilepsy Res.* 176:106742. doi: 10.1016/j.eplepsyres.2021.106742

Citraro, R., Lembo, F., De Caro, C., Tallarico, M., Coretti, L., Iannone, L. F., et al. (2021). First evidence of altered microbiota and intestinal damage and their link to absence epilepsy in a genetic animal model, the WAG/Rij rat. *Epilepsia* 62, 529–541. doi: 10.1111/epi.16813

Darch, H., and McCafferty, C. P. (2022). Gut microbiome effects on neuronal excitability & activity: implications for epilepsy. *Neurobiol. Dis.* 165:105629. doi: 10.1016/j.nbd.2022.105629

David, L. A., Maurice, C. F., Carmody, R. N., Gootenberg, D. B., Button, J. E., Wolfe, B. E., et al. (2014). Diet rapidly and reproducibly alters the human gut microbiome. *Nature* 505, 559–563. doi: 10.1038/nature12820

de Theije, C. G., Wu, J., da Silva, S. L., Kamphuis, P. J., Garssen, J., Korte, S. M., et al. (2011). Pathways underlying the gut-to-brain connection in autism spectrum disorders as future targets for disease management. *Eur. J. Pharmacol.* 668, S70–S80. doi: 10.1016/j.ejphar.2011.07.013

Ding, M., Lang, Y., Shu, H., Shao, J., and Cui, L. (2021). Microbiota-gut-brain Axis and epilepsy: a review on mechanisms and potential therapeutics. *Front. Immunol.* 12:742449. doi: 10.3389/fimmu.2021.742449

Fei, Y., Shi, R., Song, Z., and Wu, J. (2020). Metabolic control of epilepsy: a promising therapeutic target for epilepsy. *Front. Neurol.* 11:592514. doi: 10.3389/fneur.2020.592514

Gong, X., Cai, Q., Liu, X., An, D., Zhou, D., Luo, R., et al. (2021). Gut flora and metabolism are altered in epilepsy and partially restored after ketogenic diets. *Microb. Pathog.* 155:104899. doi: 10.1016/j.micpath.2021.104899

Gong, S. Z., Qiu, J., Wu, L. W., and Tan, L. H. (2022). Change in intestinal flora after treatment in children with focal epilepsy. *Zhongguo Dang Dai Er Ke Za Zhi* 24, 290–296. doi: 10.7499/j.issn.1008-8830.2109134

Grenham, S., Clarke, G., Cryan, J. F., and Dinan, T. G. (2011). Brain-gut-microbe communication in health and disease. *Front. Physiol.* 2:94. doi: 10.3389/fphys.2011.00094

He, Z., Cui, B. T., Zhang, T., Li, P., Long, C. Y., Ji, G. Z., et al. (2017). Fecal microbiota transplantation cured epilepsy in a case with Crohn's disease: the first report. *World J. Gastroenterol.* 23, 3565–3568. doi: 10.3748/wjg.v23.i19.3565

Huang, C., Li, Y., Feng, X., Li, D., Li, X., Ouyang, Q., et al. (2019). Distinct gut microbiota composition and functional category in children with cerebral palsy and epilepsy. *Front. Pediatr.* 7:394. doi: 10.3389/fped.2019.00394

Järbrink-Sehgal, E., and Andreasson, A. (2020). The gut microbiota and mental health in adults. *Curr. Opin. Neurobiol.* 62, 102–114. doi: 10.1016/j.conb.2020.01.016

Kessler, S. K., and McGinnis, E. (2019). A practical guide to treatment of childhood absence epilepsy. *Paediatr. Drugs* 21, 15–24. doi: 10.1007/s40272-019-00325-x

Kim, K. A., Gu, W., Lee, I. A., Joh, E. H., and Kim, D. H. (2012). High fat diet-induced gut microbiota exacerbates inflammation and obesity in mice via the TLR4 signaling pathway. *PLoS One* 7:e47713. doi: 10.1371/journal.pone.0047713

Kurilshikov, A., Medina-Gomez, C., Bacigalupe, R., Radjabzadeh, D., Wang, J., Demirkan, A., et al. (2021). Large-scale association analyses identify host factors influencing human gut microbiome composition. *Nat. Genet.* 53, 156–165. doi: 10.1038/s41588-020-00763-1

Kwan, P., Schachter, S. C., and Brodie, M. J. (2011). Drug-resistant epilepsy. *N. Engl. J. Med.* 365, 919–926. doi: 10.1056/NEJMra1004418

Lange, C., Storkebaum, E., de Almodóvar, C. R., Dewerchin, M., and Carmeliet, P. (2016). Vascular endothelial growth factor: a neurovascular target in neurological diseases. *Nat. Rev. Neurol.* 12, 439–454. doi: 10.1038/nrneurol.2016.88

Li, W., Ren, A., Qin, Q., Zhao, L., Peng, Q., Ma, R., et al. (2023). Causal associations between human gut microbiota and cholelithiasis: a mendelian randomization study. *Front. Cell. Infect. Microbiol.* 13:1169119. doi: 10.3389/fcimb.2023.1169119

Lou, M., Cao, A., Jin, C., Mi, K., Xiong, X., Zeng, Z., et al. (2022). Deviated and early unsustainable stunted development of gut microbiota in children with autism spectrum disorder. *Gut* 71, gutjnl-2021-325115–gutjnl-2021-321599. doi: 10.1136/gutjnl-2021-325115

Mazumder, A. G., Patial, V., and Singh, D. (2019). Mycophenolate mofetil contributes to downregulation of the hippocampal interleukin type 2 and 1β mediated PI3K/AKT/mTOR pathway hyperactivation and attenuates neurobehavioral comorbidities in a rat model of temporal lobe epilepsy. *Brain Behav. Immun.* 75, 84–93. doi: 10.1016/j.bbi.2018.09.020

Morris, D. R., Jones, G. T., Holmes, M. V., Bown, M. J., Bulbulia, R., Singh, T. P., et al. (2022). Genetic predisposition to diabetes and abdominal aortic aneurysm: a two stage Mendelian randomisation study. *Eur. J. Vasc. Endovasc. Surg.* 63, 512–519. doi: 10.1016/j.ejvs.2021.10.038

Mudd, A. T., Berding, K., Wang, M., Donovan, S. M., and Dilger, R. N. (2017). Serum cortisol mediates the relationship between fecal Ruminococcus and brain N-acetylaspartate in the young pig. *Gut Microbes* 8, 589–600. doi: 10.1080/19490976.2017.1353849

Oliveira, M. E. T., Paulino, G. V. B., Dos Santos Júnior, E. D., da Silva Oliveira, F. A., Melo, V. M. M., Ursulino, J. S., et al. (2022). Multi-omic analysis of the gut microbiome in rats with Lithium-pilocarpine-induced temporal lobe epilepsy. *Mol. Neurobiol.* 59, 6429–6446. doi: 10.1007/s12035-022-02984-3

Olson, C. A., Vuong, H. E., Yano, J. M., Liang, Q. Y., Nusbaum, D. J., and Hsiao, E. Y. (2018). The gut microbiota mediates the anti-seizure effects of the ketogenic diet. *Cell* 173, 1728–1741.e13. doi: 10.1016/j.cell.2018.04.027

Özcan, E., Lum, G. R., and Hsiao, E. Y. (2022). Interactions between the gut microbiome and ketogenic diet in refractory epilepsy. *Int. Rev. Neurobiol.* 167, 217–249. doi: 10.1016/bs.irn.2022.06.002

Pauletti, A., Terrone, G., Shekh-Ahmad, T., Salamone, A., Ravizza, T., Rizzi, M., et al. (2019). Targeting oxidative stress improves disease outcomes in a rat model of acquired epilepsy. *Brain* 142:e39. doi: 10.1093/brain/awz130

Peng, Y., Chiu, A. T. G., Li, V. W. Y., Zhang, X., Yeung, W. L., Chan, S. H. S., et al. (2023). The role of the gut-microbiome-brain axis in metabolic remodeling amongst children with cerebral palsy and epilepsy. *Front. Neurol.* 14:1109469. doi: 10.3389/fneur.2023.1109469

Peng, A., Qiu, X., Lai, W., Li, W., Zhang, L., Zhu, X., et al. (2018). Altered composition of the gut microbiome in patients with drug-resistant epilepsy. *Epilepsy Res.* 147, 102–107. doi: 10.1016/j.eplepsyres.2018.09.013

Perucca, E., Perucca, P., White, H. S., and Wirrell, E. C. (2023). Drug resistance in epilepsy. *Lancet Neurol.* 22, 723–734. doi: 10.1016/s1474-4422(23)00151-5

Petersen, A. V., Jensen, C. S., Crépel, V., Falkerslev, M., and Perrier, J. F. (2017). Serotonin regulates the firing of principal cells of the subiculum by inhibiting a T-type ca(2+) current. *Front. Cell. Neurosci.* 11:60. doi: 10.3389/fncel.2017.00060

Prabowo, A. S., Anink, J. J., Lammens, M., Nellist, M., van den Ouweland, A. M., Adle-Biassette, H., et al. (2013). Fetal brain lesions in tuberous sclerosis complex: TORC1 activation and inflammation. *Brain Pathol.* 23, 45–59. doi: 10.1111/j.1750-3639.2012.00616.x

Rana, A., and Musto, A. E. (2018). The role of inflammation in the development of epilepsy. *J. Neuroinflammation* 15:144. doi: 10.1186/s12974-018-1192-7

Relton, C. L., and Davey Smith, G. (2012). Two-step epigenetic Mendelian randomization: a strategy for establishing the causal role of epigenetic processes in pathways to disease. *Int. J. Epidemiol.* 41, 161–176. doi: 10.1093/ije/dyr233

Russo, E. (2022). The gut microbiota as a biomarker in epilepsy. *Neurobiol. Dis.* 163:105598. doi: 10.1016/j.nbd.2021.105598

Socała, K., Doboszewska, U., Szopa, A., Serefko, A., Włodarczyk, M., Zielińska, A., et al. (2021). The role of microbiota-gut-brain axis in neuropsychiatric and neurological disorders. *Pharmacol. Res.* 172:105840. doi: 10.1016/j.phrs.2021.105840

Soltani Khaboushan, A., Yazdanpanah, N., and Rezaei, N. (2022). Neuroinflammation and Proinflammatory cytokines in Epileptogenesis. *Mol. Neurobiol.* 59, 1724–1743. doi: 10.1007/s12035-022-02725-6

Sorboni, S. G., Moghaddam, H. S., Jafarzadeh-Esfehani, R., and Soleimanpour, S. (2022). A comprehensive review on the role of the gut microbiome in human neurological disorders. *Clin. Microbiol. Rev.* 35:e0033820. doi: 10.1128/cmr.00338-20

Su, J., Yin, J., Qin, W., Sha, S., Xu, J., and Jiang, C. (2015). Role for pro-inflammatory cytokines in regulating expression of GABA transporter type 1 and 3 in specific brain regions of kainic acid-induced status epilepticus. *Neurochem. Res.* 40, 621–627. doi: 10.1007/s11064-014-1504-y

Sudo, N., Chida, Y., Aiba, Y., Sonoda, J., Oyama, N., Yu, X. N., et al. (2004). Postnatal microbial colonization programs the hypothalamic-pituitary-adrenal system for stress response in mice. *J. Physiol.* 558, 263–275. doi: 10.1113/jphysiol.2004.063388

Sun, Y., Jin, M., Yu, T., and Zhang, J. (2023). Cardiovascular risk factors mediating the protective effect of education on cervical spondylosis risk. *Sci. Rep.* 13:936. doi: 10.1038/s41598-023-28153-7

Sun, Y., Su, Y., and Zhu, W. (2016). Microbiome-metabolome responses in the cecum and Colon of pig to a high resistant starch diet. *Front. Microbiol.* 7:779. doi: 10.3389/fmicb.2016.00779

Symonds, J. D., Elliott, K. S., Shetty, J., Armstrong, M., Brunklaus, A., Cutcutache, I., et al. (2021). Early childhood epilepsies: epidemiology, classification, aetiology, and socio-economic determinants. *Brain* 144, 2879–2891. doi: 10.1093/brain/awab162

Szklarczyk, D., Gable, A. L., Nastou, K. C., Lyon, D., Kirsch, R., Pyysalo, S., et al. (2021). The STRING database in 2021: customizable protein-protein networks, and functional characterization of user-uploaded gene/measurement sets. *Nucleic Acids Res.* 49, D605–d612. doi: 10.1093/nar/gkaa1074

Thijs, R. D., Surges, R., O'Brien, T. J., and Sander, J. W. (2019). Epilepsy in adults. *Lancet* 393, 689–701. doi: 10.1016/s0140-6736(18)32596-0

Vezzani, A., French, J., Bartfai, T., and Baram, T. Z. (2011). The role of inflammation in epilepsy. *Nat. Rev. Neurol.* 7, 31–40. doi: 10.1038/nrneurol.2010.178

Vezzani, A., Friedman, A., and Dingledine, R. J. (2013). The role of inflammation in epileptogenesis. *Neuropharmacology* 69, 16–24. doi: 10.1016/j.neuropharm.2012.04.004

Vezzani, A., Fujinami, R. S., White, H. S., Preux, P. M., Blümcke, I., Sander, J. W., et al. (2016). Infections, inflammation and epilepsy. *Acta Neuropathol.* 131, 211–234. doi: 10.1007/s00401-015-1481-5

Vezzani, A., Lang, B., and Aronica, E. (2015). Immunity and inflammation in epilepsy. *Cold Spring Harb. Perspect. Med.* 6:a022699. doi: 10.1101/cshperspect.a022699

Võsa, U., Claringbould, A., Westra, H. J., Bonder, M. J., Deelen, P., Zeng, B., et al. (2021). Large-scale cis- and trans-eQTL analyses identify thousands of genetic loci and polygenic scores that regulate blood gene expression. *Nat. Genet.* 53, 1300–1310. doi: 10.1038/s41588-021-00913-z

Wang, L., Christophersen, C. T., Sorich, M. J., Gerber, J. P., Angley, M. T., and Conlon, M. A. (2013). Increased abundance of Sutterella spp. and *Ruminococcus torques* in feces of children with autism spectrum disorder. *Mol. Autism.* 4:42. doi: 10.1186/2040-2392-4-42

Wang, Y., Zhuo, Z., and Wang, H. (2023). Epilepsy, gut microbiota, and circadian rhythm. *Front. Neurol.* 14:1157358. doi: 10.3389/fneur.2023.1157358

Watanabe, K., Taskesen, E., van Bochoven, A., and Posthuma, D. (2017). Functional mapping and annotation of genetic associations with FUMA. *Nat. Commun.* 8:1826. doi: 10.1038/s41467-017-01261-5

Wei, S., Mai, Y., Hu, L., Zheng, R., Zheng, D., Chen, W., et al. (2023). Altered gut microbiota in temporal lobe epilepsy with anxiety disorders. *Front. Microbiol.* 14:1165787. doi: 10.3389/fmicb.2023.1165787

Xanthos, D. N., and Sandkühler, J. (2014). Neurogenic neuroinflammation: inflammatory CNS reactions in response to neuronal activity. *Nat. Rev. Neurosci.* 15, 43–53. doi: 10.1038/nrn3617

Xiang, K., Wang, P., Xu, Z., Hu, Y. Q., He, Y. S., Chen, Y., et al. (2021). Causal effects of gut microbiome on systemic lupus erythematosus: a two-sample Mendelian randomization study. *Front. Immunol.* 12:667097. doi: 10.3389/fimmu.2021.667097

Xie, G., Zhou, Q., Qiu, C. Z., Dai, W. K., Wang, H. P., Li, Y. H., et al. (2017). Ketogenic diet poses a significant effect on imbalanced gut microbiota in infants with refractory epilepsy. *World J. Gastroenterol.* 23, 6164–6171. doi: 10.3748/wjg.v23.i33.6164

Xu, R., Tan, C., He, Y., Wu, Q., Wang, H., and Yin, J. (2020). Dysbiosis of gut microbiota and short-chain fatty acids in encephalitis: a Chinese pilot study. *Front. Immunol.* 11:1994. doi: 10.3389/fimmu.2020.01994

Yamanaka, G., Ishida, Y., Kanou, K., Suzuki, S., Watanabe, Y., Takamatsu, T., et al. (2021). Towards a treatment for Neuroinflammation in epilepsy: Interleukin-1 receptor antagonist, Anakinra, as a potential treatment in intractable epilepsy. *Int. J. Mol. Sci.* 22:6282. doi: 10.3390/ijms22126282

Zhang, L., Li, H., Hong, P., and Zou, X. (2016). Proton magnetic resonance spectroscopy in juvenile myoclonic epilepsy: a systematic review and meta-analysis. *Epilepsy Res.* 121, 33–38. doi: 10.1016/j.eplepsyres.2016.01.004

frontiers | Frontiers in Neuroscience

TYPE Review
PUBLISHED 15 November 2024
DOI 10.3389/fnins.2024.1452216

OPEN ACCESS

EDITED BY
Avinash Veerappa,
University of Nebraska Medical Center,
United States

REVIEWED BY
Atchariya Chanpong,
University College London, United Kingdom
Mladenka Tkalcic,
University of Rijeka, Croatia
Jai Chand Patel,
University of Nebraska Medical Center,
United States

*CORRESPONDENCE
Cong Zhou
✉ doctorzhoucong@163.com

RECEIVED 20 June 2024
ACCEPTED 29 October 2024
PUBLISHED 15 November 2024

CITATION
Shuai Y, Wang B, Zhang X, Shen Z, Han S and Zhou C (2024) Aberrant intrinsic brain activities in functional gastrointestinal disorders revealed by seed-based d mapping with permutation of subject images. *Front. Neurosci.* 18:1452216. doi: 10.3389/fnins.2024.1452216

Aberrant intrinsic brain activities in functional gastrointestinal disorders revealed by seed-based d mapping with permutation of subject images

Yibin Shuai[1], Benhong Wang[1], Xiaomei Zhang[1], Zhongxia Shen[1], Shengbo Han[2] and Cong Zhou[3,4]*

[1]Sleep Medical Center, Huzhou Third Municipal Hospital, The Affiliated Hospital of Huzhou University, Huzhou, China, [2]School of Clinical Medicine, Jining Medical University, Jining, China, [3]School of Mental Health, Jining Medical University, Jining, China, [4]Department of Psychology, Affiliated Hospital of Jining Medical University, Jining, China

Functional gastrointestinal disorders (FGIDs) are characterized by complex interactions between the gut and brain, leading to altered brain function and symptom manifestation. We used neuroimaging meta-analytic techniques in order to analyze the correlation between FGIDs and aberrant brain activity. A systematic review was performed to ascertain resting-state functional magnetic resonance imaging (rs-fMRI) studies examining brain function in FGIDs. Pooled meta-analyses by seed-based d mapping with permutation of subject images (SDM-PSI) were performed to assess variations in regional brain activity, and sensitivity analyses were applied to evaluate the robustness of findings. Meta-regression analyses were then carried out to examine possible links between demographic factors and neuroimaging changes. Our meta-analysis revealed significant changes in regional brain activities among FGIDs patients compared to healthy controls (HC). Increased brain activation was observed in several regions including the postcentral gyrus, calcarine fissure/surrounding cortex, superior frontal gyrus, and insula, while decreased activity was noted in the left posterior cingulate gyrus, right median cingulate/paracingulate gyri, and the left caudate nucleus. Furthermore, meta-regression analyses indicated negative associations between disease duration and alterations in specific brain regions. These findings underscored the intricate interplay between gut dysfunction and aberrant brain activity in FGIDs. Early intervention and multidisciplinary approaches addressing both gastrointestinal symptoms and associated emotional distress are crucial for improving the quality of life of the patients.

KEYWORDS

functional gastrointestinal disorders, resting-state fMRI, irritable bowel syndrome, functional dyspepsia, functional constipation, seed-based d mapping

Introduction

Functional gastrointestinal disorders (FGIDs) encompass a cluster of chronic conditions affecting the alimentary system, characterized by recurrent symptoms without identifiable structural or biochemical abnormalities. Common FGIDs include irritable bowel syndrome (IBS), functional dyspepsia (FD), and functional constipation (FC) (Black et al., 2020). According to global epidemiological studies, FGIDs affect

approximately 10–20% of the world's population (Suares and Ford, 2011; Lovell and Ford, 2012; Aro et al., 2015; Kano et al., 2018; Sperber et al., 2021), leading to substantial healthcare utilization, impaired quality of life, and significant economic burden. IBS presents with abdominal pain and altered bowel habits, FC is characterized by infrequent bowel movements and difficulty in defecation, while FD involves persistent upper abdominal pain or discomfort without an identifiable organic cause. These disorders manifest with symptoms such as abdominal pain, bloating, altered bowel habits, and indigestion, often resulting in reduced productivity and psychological distress among patients (Black et al., 2020; Sperber et al., 2021). Understanding the prevalence and impact of FGIDs is crucial for effective management and public health interventions to alleviate the burden on individuals and healthcare systems.

The FGIDs are closely associated with psychological and emotional states, often overlapping with conditions such as depression and anxiety. Psychological factors such as stress and negative life events can influence gut motility, visceral sensitivity, and immune function, contributing to the onset and exacerbation of FGIDs (Black et al., 2020). Research has shown that individuals with FGIDs frequently experience comorbid psychiatric conditions. For example, a longitudinal study by Aro et al. (2015) found that anxiety was related to new-found indigestion in the population of Sweden, suggesting a potential causal relationship between psychological distress and gastrointestinal symptoms. Similarly, a meta-analysis by Lovell and Ford (2012) revealed a higher prevalence of anxiety and depression in patients with IBS compared to the general population. The gut-brain axis is recognized as pivotal in the development of FGIDs, facilitating bidirectional communication between the central nervous system and the enteric nervous system (Mayer et al., 2022). This axis plays a critical role in modulating both gastrointestinal function and emotional responses (Mayer et al., 2022; Gong et al., 2023). Dysregulation of the gut-brain axis, characterized by altered neurotransmitter signaling, immune activation, and changes in gut microbiota composition, has been implicated in the pathophysiology of FGIDs (Mayer et al., 2015a; Mayer et al., 2015b). For example, stress-induced alterations in gut microbiota composition can impact gut permeability and immune function, contributing to intestinal inflammation and visceral hypersensitivity observed in FGIDs (Mayer et al., 2015b), which might underlie the patients' gastrointestinal symptoms.

Recent advances in neuroimaging technology have facilitated the exploration of brain function through non-invasive methods, with resting-state functional magnetic resonance imaging (rs-fMRI) emerging as a prominent technique. Among this, regional homogeneity (ReHo), amplitude of low-frequency fluctuations (ALFF), and fractional ALFF (fALFF) are commonly utilized approaches for exhibiting local spontaneous activity in rs-fMRI data (Zang et al., 2015). Each of these metrics offers unique insights into regional spontaneous brain activities, collectively enhancing our understanding of brain function in health and disease (Zang et al., 2015; Salvia et al., 2019). Numerous neuroimaging studies have revealed abnormal brain structure and function in individuals with FGIDs, emphasizing the significance of the gut-brain axis in the development of these disorders (Kano et al., 2018). However, current findings are inconsistent, possibly due to the heterogeneity in samples and imaging methodologies.

Meta-analysis serves as a robust method to consolidate neuroimaging observations from diverse researches, offering an all-sided synthesis of regional alterations. This method not only addresses discrepancies among neuroimaging studies but also distinguishes between spurious results and reproducible findings. By aggregating data across studies, meta-analysis provides a unified perspective and facilitates the integration of vast amounts of information (Muller et al., 2018). Among these techniques, the seed-based d mapping with permutation of subject images (SDM-PSI) is a notable progressive statistical method for coordinate-based meta-analysis (CBMA) (Radua et al., 2012). SDM-PSI allows for the objective and quantitative integration of diverse neuroimaging findings. One former meta-analysis investigated irregular local spontaneous functional activity during resting states in IBS patients, identifying transformations in brain regions associated with emotional management and somatic sensation (Su et al., 2022). Similarly, a systematic review by Yu et al. (2022) highlighted altered resting brain functions in IBS patients, implicating disruptions in neural networks involved in pain modulation and emotional regulation. Furthermore, another meta-analysis examined alterations in default mode network functionality and gray matter structure in IBS patients, emphasizing abnormalities in brain regions responsible pain perception, transmission and interpretation (Zhao et al., 2023). Besides, a CBMA found changes in brain regions responsible for visceral sensation, pain modulation, and emotion regulation in FD patients (Mao et al., 2023). One recent review indicated that FC is linked to changes in brain function and structure, especially in regions and networks related to emotion regulation, motor control, somatic sensation, and self-referential processing (Feng et al., 2023). These studies provided valuable insights into the neuropathological characteristics of FGIDs and underscored the importance of understanding the brain-gut axis in these disorders. However, these studies conducted meta-analysis focusing on a specific disorder, which might contain certain limitations.

This present study aims to enhance CBMA across various FGIDs, focusing on consolidating regional aberrations in brain activity utilizing ReHo, ALFF, and fALFF metrics. Additionally, with the approach of meta-regression, we seek to explore the latent influence of demographics and clinical parameters on brain functions, such as age and disease duration. We expect these findings will provide valuable insights to advance the diagnosis and treatment of FGIDs in clinical practice. Based on previous research findings, we hypothesize that FGIDs patients may manifest altered brain activations in regions associated with emotion regulation, pain perception, and sensory processing such as the frontal gyrus and insula.

Methods

Literature search strategy

The protocol for this CBMA was duly registered with PROSPERO (registration number: CRD42024536106; accessible at http://www.crd.york.ac.uk/PROSPERO), underscoring our commitment to methodological rigor and transparency. The current meta-analysis adhered strictly to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Liberati et al., 2009; Moher et al., 2009a,b; Page et al., 2021) as well as the rules for neuroimaging meta-analysis (Muller et al., 2018) to ensure

transparency and reliability in our methods. Relevant literature was systematically sourced through comprehensive searches of the PubMed, Web of Science, and Cochrane Library databases, encompassing publications up to January 31, 2024. The search strategy involved keywords including ("functional gastrointestinal disorders" or "FGIDs" or "irritable bowel syndrome" or "IBS" or "functional constipation" or "functional dyspepsia") in conjunction with [("resting state" or "resting-state" or "at rest" or "resting") or ("amplitude of low frequency fluctuation" or "fractional amplitude of low frequency fluctuation" or "ALFF" or "fALFF") or ("regional homogeneity" or "ReHo" or "local connectivity" or "coherence")]. Moreover, for the purpose of preventing oversight, we conducted a manual examination of the reference lists of approved studies and correlated reviews.

Study selection

The criteria for the selection of studies were delineated as follows: (1) Studies comparing ReHo, ALFF, or fALFF values between patients with FGIDs and HC through whole-brain analyses were included. (2) Results needed to be provided in either Talairach or Montreal Neurological Institute (MNI) coordinates. (3) Utilization of a significance threshold; (4) publication in peer-reviewed journals and composed in the English language. The criteria for exclusion were as below: (1) meta-analyses, reviews, and case reports were excluded. (2) Studies that did not include direct between-group comparisons were excluded. (3) Studies were excluded if the peak coordinates or parametric maps were inaccessible.

Quality assessment and data extraction

The process of quality assessment and data extraction was executed by two authors (S.Y. and W.B.) respectively. They performed literature searches, evaluated the quality of retrieved articles, and extracted and cross-validated data from eligible articles. Additionally, both authors independently assessed the quality of the final studies in accordance with guidelines for neuroimaging meta-analyses (Muller et al., 2018). We documented the following parameters: lead author, sample size, participant characteristics (such as age and gender), criteria for diagnosis, illness duration, imaging protocols, methods of data processing, and the statistical thresholds applied in individual studies.

Meta-analysis

We performed meta-analyses using SDM software (Radua and Mataix-Cols, 2009; Albajes-Eizagirre et al., 2019) to investigate discrepancies in local brain activation between FGIDs patients and HC. The SDM-PSI technique, known for its robust statistical approach, utilizes peak coordinates to evaluate disparities in cerebral activity (Radua et al., 2012). The detailed procedures of SDM-PSI, outlined extensively elsewhere (Duan et al., 2022; Liu et al., 2022; Wang et al., 2022), and are succinctly summarized as follows: (1) the software generated effect-size maps illustrating differences in regional activities between patients and HC for each study, based on peak coordinates of effects and associated statistics, such as *t*-statistics. Significant cluster *Z*- or *p*-values were transformed to t-statistics using the SDM online converter. (2) Peak coordinates for each study were reconstructed utilizing a standard MNI map of effect size for group differences in neuroimaging, employing an anisotropic Gaussian kernel (Radua et al., 2014). (3) A comprehensive meta-analysis was performed to generate a mean map through voxel-wise computation of the random-effects mean of the study maps.

Following the methodology established by Radua et al. (2012), we adopted $p = 0.005$ in SDM-PSI analyses. Furthermore, we employed a peak height threshold of $Z = 1.00$ and a cluster size threshold of 10 voxels to ensure robustness in our findings.

Sensitivity analyses

We then conducted sensitivity analyses to assess the repeatability of our findings. If a particular brain region consistently demonstrated significance across the majority or all combinations of studies during the jackknife sensitivity analysis, it was considered highly replicable (Radua and Mataix-Cols, 2009).

Subgroup meta-analyses

We conducted subgroup meta-analyses focusing exclusively on each subtype of the FGIDs (IBS, FD, and FC).

Meta-regression analyses

Meta-regression analyses were performed in each patient group to explore potential demographic variables on neuroimaging changes. We used $p < 0.0005$ to serve as a threshold for significance (Radua and Mataix-Cols, 2009). We only considered brain regions identified in the main effect.

Results

Sample characteristics of included studies

Figure 1 depicts a flow diagram outlining the process of including studies related to FGIDs. Table 1 summarizes the demographic characteristics and neuroimaging methodologies utilized in each subgroup corresponding to the studied diseases. We identified 1895 studies under the search strategy, with 12 fitting the predefined inclusion criteria (Zhou et al., 2013; Nan et al., 2014; Ke et al., 2015; Ma et al., 2015; Qi et al., 2016; Lee et al., 2018; Jin et al., 2019; Qi et al., 2020; Ao et al., 2021; Chen et al., 2021; Li et al., 2021; Cai et al., 2024). Notably, the study by Qi et al. (2020) included two subgroups of FD, while the study by Li et al. (2021) encompassed two subgroups of functional constipation FC patients. Additionally, Chen et al. (2021) conducted both ALFF and ReHo analyses. The final sample comprised 463 patients and 469 healthy controls, with 99 coordinates extracted from 15 datasets.

Meta-analyses findings

The primary meta-analysis unveiled that FGIDs patients displayed significantly heightened brain activities across five clusters, notably

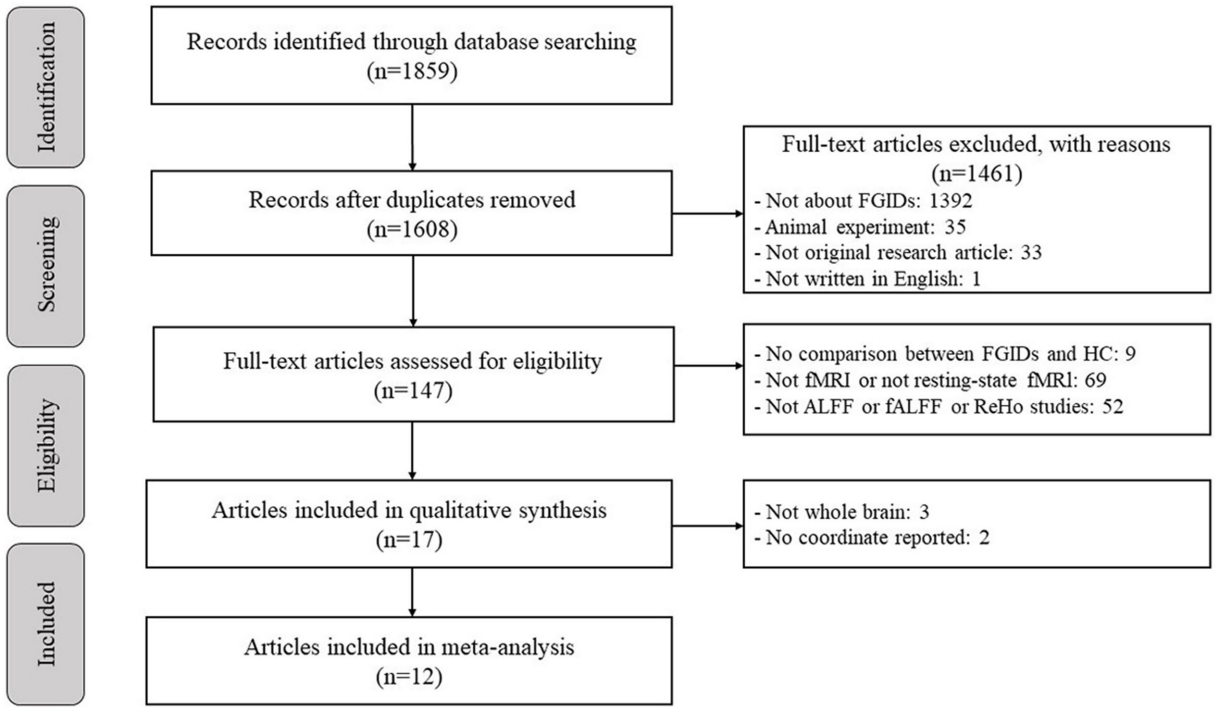


FIGURE 1
Flow diagram for the identification and exclusion of studies.

encompassing the right postcentral gyrus, left calcarine fissure/surrounding cortex, right superior frontal gyrus (medial), right insula, and the left superior frontal gyrus (orbital part). Additionally, three clusters exhibited decreased brain activities, including the left posterior cingulate gyrus, right median cingulate/paracingulate gyri, and the left caudate nucleus. These results are visually shown in Figure 2 and thoroughly summarized in Table 2.

Sensitivity analysis

The sensitivity analysis, conducted through whole-brain jackknife analyses, demonstrated high reproducibility of the results. Specifically, consistently significant findings across all dataset combinations included increased brain activity in the right postcentral gyrus and reduced activity in the right median cingulate/paracingulate gyri. Likewise, heightened brain activity in the right superior frontal gyrus (medial) and reduced brain activity in the left caudate nucleus remained consistently significant across all but one dataset combination. Additionally, the remaining clusters retained significance in all but three dataset combinations. Detailed information can be found in Table 2.

Subgroup analysis

Despite the inclusion of a sample size below the recommended minimum of 10 datasets for SDM meta-analyses, we conducted an exploratory analysis of different subtypes of FGIDs. Detailed results of the subtypes of FGIDs are provided in Supplementary Table S1. The findings from subgroups partially aligned with the pooled meta-analysis.

Meta-regression analysis

Meta-regression analysis unveiled a negative correlation between the duration of FGIDs in patients and brain activity changes, particularly within the right median cingulate/paracingulate gyri and the left caudate nucleus (Table 3).

Discussion

Consistent with prior studies, our study revealed significantly changed regional brain functions in several regions including increased brain activities in the postcentral gyrus, calcarine fissure/surrounding cortex, superior frontal gyrus, and insula, as well as lower activation in the left posterior cingulate gyrus, right median cingulate/paracingulate gyri, and the left caudate nucleus. These findings reflected the complex interplay between the gut and brain in FGIDs. The observed negative association between the duration of FGIDs and alterations in specific brain regions suggested potential disease progression effects on neural function, highlighting the dynamic nature of gut-brain interactions in FGIDs patients.

The identified alterations in brain function observed in various regions implicated in sensory processing, emotional regulation, and cognitive control provide valuable insights into the pathophysiology of FGIDs (Yu et al., 2022; Mao et al., 2023). The increased brain activities detected in the postcentral gyrus, calcarine fissure/

TABLE 1 Demographic and clinical characteristics and the neuroimaging approaches of the participants in the studies included in the meta-analyses.

	Sample size (female)		Mean age (SD)		Diagnostic criteria	Duration (month)	Scanner	Indices	Statistical threshold	Number of coordinates
	Patients	HC	Patients	HC						
IBS studies										
Ke et al. (2015)	31 (6)	32 (7)	29.2	27.5	Rome III criteria	32.7	3.0T	ReHo	$p < 0.05$, FDR corrected	19
Ma et al. (2015)	21 (7)	21 (10)	41.8	35.9	Rome III criteria	59.0	1.5T	ALFF	$P < 0.05$, Alphasim corrected	8
Qi et al. (2016)	30 (6)	31 (7)	28.9	26.9	Rome III criteria	20.4	3.0T	ALFF	$P < 0.05$, Alphasim corrected	11
Ao et al. (2021)	13 (5)	14 (6)	32.2	29.1	Rome III criteria	16.6	3.0T	fALFF	$p < 0.005$, uncorrected	3
Chen et al. (2021)	36 (20)	36 (26)	34.4	31.7	Rome III criteria	19.1	3.0T	ALFF	$P < 0.05$, FWE corrected	9
Chen et al. (2021)	36 (20)	36 (26)	34.4	31.7	Rome III criteria	19.1	3.0T	ReHo	$P < 0.05$, FWE corrected	6
FD studies										
Zhou et al. (2013)	29 (16)	16 (10)	22.4	21.9	Rome III criteria	33.7	3.0T	fALFF	$P < 0.05$, TFCE corrected	5
Nan et al. (2014)	40 (29)	20 (12)	22.3	22.1	Rome III criteria	37.2	3.0T	ReHo	$P < 0.05$, FDR corrected	7
Lee et al. (2018)	12 (7)	14 (9)	46.5	45.8	Rome III criteria	156.0	3.0T	ALFF	$P < 0.05$, FWE corrected	6
Qi et al. (2020)	18 (7)	22 (13)	43.8	41.4	Rome III criteria	48.9	3.0T	ALFF	$P < 0.05$, RFT corrected	7
Qi et al. (2020)	13 (6)	22 (13)	41.8	41.4	Rome III criteria	54.0	3.0T	ALFF	$P < 0.05$, RFT corrected	6
FC studies										
Jin et al. (2019)	46 (34)	53 (35)	40.0	40.8	Rome IV criteria	120.8	1.5T	ALFF	$P < 0.05$, FWE corrected	1
Li et al. (2021)	37 (29)	42 (25)	40.9	40.6	Rome IV criteria	105.6	1.5T	fALFF	$P < 0.05$, FWE corrected	5
Li et al. (2021)	28 (19)	42 (25)	43.0	40.6	Rome IV criteria	112.8	1.5T	fALFF	$P < 0.05$, FWE corrected	2
Cai et al. (2024)	73 (53)	68 (45)	51.1	53.2	Rome IV criteria	N/A	3.0T	ReHo	$P < 0.05$, FWE corrected	3

ALFF, amplitude of low-frequency fluctuation; fALFF, fractional amplitude of low-frequency fluctuation; FC, functional constipation; FD, functional dyspepsia; FDR, false discovery rate; FWE, family-wise error; HC, healthy controls; IBS, irritable bowel syndrome; N/A, not available; ReHo, regional homogeneity; RFT, random-field theory; T, Tesla; TFCE, threshold-free cluster enhancement.

10.3389/fnins.2024.1452216

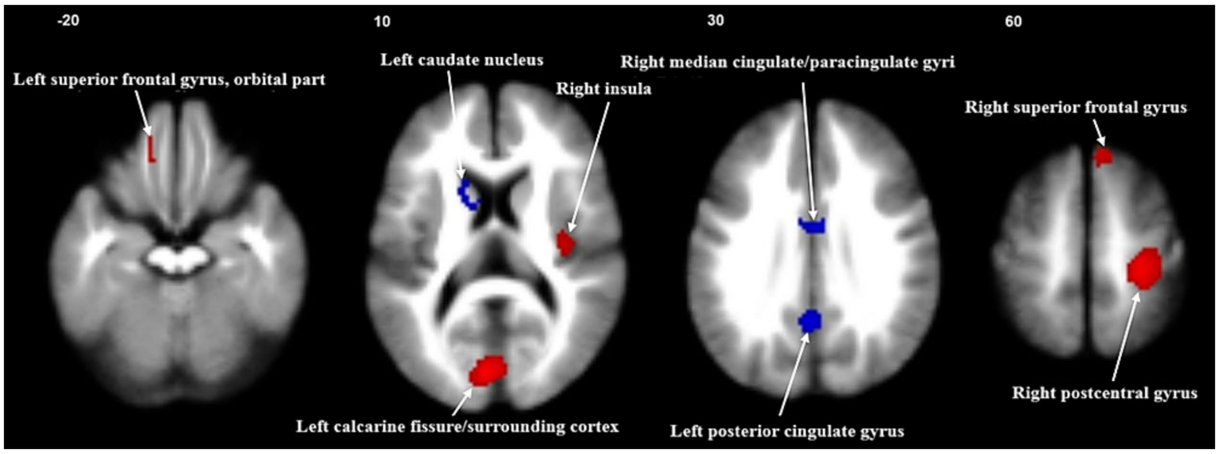


FIGURE 2
Meta-analysis of regional abnormal resting-state brain activities in functional gastrointestinal disorders. Significant clusters are overlaid on MRIcron template for Windows for display purposes only.

surrounding cortex, superior frontal gyrus, and insula are indicative of heightened sensory processing and visceral sensation perception (Hong et al., 2013). The postcentral gyrus, known for its role in somatosensory processing, may contribute to the heightened perception of visceral pain commonly reported by FGIDs patients (Dong et al., 2022). Similarly, the increased activity in the insula, a key region involved in interoception and emotional processing, suggests dysregulated visceral sensation and emotional responses in FGIDs patients (Zhang et al., 2023). Notably, one previous fMRI study demonstrated that major depressive disorder (MDD) patients with gastrointestinal symptoms showed increased functional asymmetry than those without gastrointestinal symptoms (Fu et al., 2021), this suggests an imbalance in the regulation of brain-gastrointestinal function, emphasizing the involvement of the frontal gyrus in FGIDs.

On the other hand, decreased activation in regions such as the left posterior cingulate gyrus, right median cingulate/paracingulate gyri, and the left caudate nucleus underscored the multifaceted nature of FGIDs (Mayer et al., 2015a). The left posterior cingulate gyrus, known for its involvement in self-awareness, cognition, and emotion regulation, exhibits reduced activity in FGIDs patients compared to healthy controls (Labus et al., 2013). This diminishment in activity might reflect abnormalities in emotional regulation and self-awareness commonly observed in FGIDs patients, which are often linked to anxiety and depression. Additionally, the posterior cingulate gyrus is implicated in pain processing, suggesting its involvement in the altered perception of pain experienced by FGIDs patients (Hong et al., 2014). The cingulate cortex plays crucial roles in emotional regulation and autonomic nervous system function (Berman et al., 2008). The decreased activation observed in right median cingulate/paracingulate gyri might contribute to the emotional dysregulation and autonomic dysfunction often observed in FGIDs patients. Prospective studies have highlighted heightened stress responses and autonomic nervous system dysfunction in FGIDs patients, aligning with the observed reduction in activity in these brain regions (Kano et al., 2018). The caudate nucleus, associated with motor control and reward processing, exhibits decreased activation in IBS patients (Weng et al., 2017). This reduction in activity may be related to the motor dysfunction and altered reward processing observed in FGIDs patients, including changes in gastrointestinal motility and appetite regulation (Kano et al., 2018). It is noteworthy that these brain regions exhibiting aberrant intrinsic brain activities were commonly implicated in emotional disorders such as anxiety and depression (Elsenbruch et al., 2010; Labus et al., 2013), providing microscopic evidence for the frequent comorbidity of FGIDs with mood disturbances. This indicated the intricate interplay between gut-brain interactions and emotional regulation in FGIDs, highlighting the need for comprehensive therapeutic interventions addressing both gastrointestinal symptoms and associated emotional distress. Taken together, the identified alterations in brain activation patterns highlighted the widespread impact of gut dysfunction on brain function in FGIDs. These findings provided insights for further research to elucidate the precise mechanisms underlying these alterations and seek potential interventions targeting these brain regions to relieve symptoms and improve quality of life in FGIDs patients. We propose that future treatments for FGIDs might benefit from targeted interventions aimed at the brain regions implicated in our study, potentially through neuromodulation therapies or cognitive-behavioral strategies.

The gut-brain axis serves as a crucial interface between the gastrointestinal system and the central nervous system, playing a pivotal role in the pathophysiology of FGIDs (Mayer et al., 2022). In line with the predictive coding theory, our findings of altered brain activity in FGIDs may reflect disruptions in the brain's predictive mechanisms, particularly in processing interoceptive signals from the gut. Dysfunctional gut-brain communication, influenced by factors such as gut microbiota, neuroendocrine signaling, and immune responses, contributes to the onset, progression, and perpetuation of FGIDs symptoms (Mayer et al., 2015a). Reflecting in neuroimaging, our findings demonstrated the significant alterations in brain function observed in FGID patients, highlighted the multifaceted influence of the gut-brain axis on neural processing, and indicated a disrupted homeostatic balance between the gut and brain (Al Omran and Aziz, 2014). In addition, a compelling correlation emerged between the duration of FGIDs and specific alterations in brain regions, suggesting a progressive impact of the disease on neural function. Therefore, it emphasizes the importance of implementing effective treatments and

TABLE 2 Regional brain spontaneous activity changes in functional gastrointestinal disorders.

Regions	Maximum					Cluster		Jackknife sensitivity analysis
	MNI coordinates			SDM Value	*P*	Number of voxels*	Breakdown (number of voxels)	
	X	*Y*	*Z*					
FGIDs>HC								
Right postcentral gyrus, BA 4	24	−30	62	1.946	0.000098050	631	Right precentral gyrus, BA 6 (186) Right postcentral gyrus, BA 4 (118) Right precentral gyrus, BA 4 (112) Right postcentral gyrus, BA 3 (101) Right postcentral gyrus, BA 6 (31) Corpus callosum (25) Right hand superior U tract (24) Right postcentral gyrus, BA 2 (14) Right precentral gyrus, BA 3 (6) Right precentral gyrus (5) Right postcentral gyrus (5) Right superior longitudinal fasciculus II (4)	15/15
Left calcarine fissure/ surrounding cortex, BA 18	−4	−82	12	1.948	0.000098050	533	Left calcarine fissure/surrounding cortex, BA 17 (150) Corpus callosum (127) Right calcarine fissure/surrounding cortex, BA 17 (65) Left lingual gyrus, BA 17 (48) Left calcarine fissure/surrounding cortex, BA 18 (42) Left cuneus cortex, BA 18 (38) Right inferior network, inferior longitudinal fasciculus (25) Right lingual gyrus, BA 17 (22) Left calcarine fissure/surrounding cortex (11) Right calcarine fissure/surrounding cortex, BA 18 (2) Left inferior network, inferior longitudinal fasciculus (2) Left lingual gyrus (1)	13/15
Right superior frontal gyrus, medial, BA 8	4	26	62	1.558	0.001661777	92	Right superior frontal gyrus, medial, BA 8 (83) Right supplementary motor area, BA 8 (7) Right superior frontal gyrus, medial (1) Right superior frontal gyrus, dorsolateral, BA 8 (1)	14/15

(Continued)

10.3389/fnins.2024.1452216

TABLE 2 (Continued)

Regions	Maximum					Cluster		Jackknife sensitivity analysis
	MNI coordinates			SDM Value	*P*	Number of voxels*	Breakdown (number of voxels)	
	X	*Y*	*Z*					
Right insula, BA 48	36	−14	12	1.464	0.002915859	95	Right insula, BA 48 (92) Right rolandic operculum, BA 48 (2) Right fronto-insular tract 5 (1)	12/15
Left superior frontal gyrus, orbital part, BA 11	−14	34	−20	1.588	0.001388252	35	Left superior frontal gyrus, orbital part, BA 11 (20) Corpus callosum (8) Left striatum (3) Left gyrus rectus, BA 11 (2) Left inferior network, uncinate fasciculus (1) Left superior frontal gyrus, orbital part (1)	13/15
FGIDs>HC								
Left posterior cingulate gyrus, BA 23	−2	−54	26	−1.854	0.000438690	295	Left precuneus, BA 23 (68) Left posterior cingulate gyrus, BA 23 (39) Right precuneus, BA 23 (36) Left precuneus (33) Left posterior cingulate gyrus (21) Left precuneus, BA 30 (21) Left posterior cingulate gyrus, BA 30 (17) Right posterior cingulate gyrus, BA 23 (11) Left median network, cingulum (7) Right median cingulate/paracingulate gyri, BA 23 (7) Right posterior cingulate gyrus, BA 30 (7) Right precuneus (7) Right precuneus, BA 30 (6) Right posterior cingulate gyrus (2) Left posterior cingulate gyrus, BA 26 (2) Right posterior cingulate gyrus, BA 26 (1) (undefined) (8) (undefined), BA 30 (2)	13/15

(Continued)

10.3389/fnins.2024.1452216

TABLE 2 (Continued)

Regions	Maximum					Cluster		Jackknife sensitivity analysis
	MNI coordinates			SDM Value	*P*	Number of voxels*	Breakdown (number of voxels)	
	X	*Y*	*Z*					
Right median cingulate/paracingulate gyri, BA 24	8	2	42	−2.019	0.000154853	244	Right median cingulate/paracingulate gyri, BA 24 (43) Right median cingulate/paracingulate gyri, BA 32 (39) Right median cingulate/paracingulate gyri (25) Left median cingulate/paracingulate gyri, BA 24 (19) Right median network, cingulum (14) Right supplementary motor area, BA 24 (12) Corpus callosum (12) Right median cingulate/paracingulate gyri, BA 23 (11) Left median network, cingulum (11) Right supplementary motor area, BA 32 (10) Right supplementary motor area (10) Left supplementary motor area, BA 32 (8) Left median cingulate/paracingulate gyri, BA 23 (7) Left supplementary motor area (6) Left median cingulate/paracingulate gyri (5) Left anterior cingulate/paracingulate gyri (4) Left supplementary motor area, BA 24 (2) Right supplementary motor area, BA 6 (2) (undefined) (4)	15/15
Left caudate nucleus	−12	16	14	−1.749	0.000856698	80	Left anterior thalamic projections (51) Left caudate nucleus (19) Left striatum (7) Corpus callosum (1) Left caudate nucleus, BA 25 (1) (undefined) (1)	14/15

*All voxels with $P < 0.005$ uncorrected. BA, Brodmann area; FGIDs, functional gastrointestinal disorders; HC, healthy controls; MNI, Montreal Neurological Institute; SDM, seed-based d mapping.

10.3389/fnins.2024.1452216

TABLE 3 Negative correlations between duration and regional brain activity alterations in FGIDs patients revealed by meta-regression analysis.

		MNI coordinates					
Factor	Anatomic label	*X*	*Y*	*Z*	SDM Value	*P*	Number of voxels
Duration	Right median cingulate/paracingulate gyri, BA 24	6	4	42	−3.982	~0	758
	Left caudate nucleus	−14	16	12	−2.764	0.000294149	18

FGIDs, functional gastrointestinal disorders; MNI, Montreal Neurological Institute; SDM, seed-based d mapping.

interventions for FGIDs as early as possible. Moreover, in contrast to previous studies, this research conducted an analysis by amalgamating all FGIDs and solely focusing on local brain functional metrics based on ReHo, ALFF, and fALFF, without incorporating other imaging modalities such as positron emission computed tomography (PET) and functional connectivity metrics of fMRI. Subgroup analyses concerning different disorders revealed that FGIDs constitute a complex spectrum of conditions, with IBS, FD, and FC each exhibiting distinct neurobiological mechanisms. However, these findings warrant cautious interpretation given the limited sample size.

The study acknowledges several limitations that should be taken into consideration. Firstly, the heterogeneous nature of data acquisition parameters and clinical variables across the included studies introduced potential biases not fully addressed by statistical methods alone. Secondly, the absence of longitudinal studies in both our meta-analysis and the literature reviewed herein restricted our ability to explore the dynamicity and reversibility of neural activities associated with FGIDs. Longitudinal studies and task-based functional MRI studies are crucial for elucidating the temporal dynamics of brain functions in FGIDs. These studies are especially crucial in examining the temporal interplay between brain activity and the progression of FGID symptoms. Future studies might usefully adopt methodologies that either track these changes longitudinally or involve the presentation of controlled gut-related stimuli to participants. Thirdly, our meta-analysis exclusively focused on changes in resting-state regional spontaneous brain activity in FGIDs, neglecting other valuable aspects such as functional connectivity, graph theory, independent component analysis (ICA), and task-based fMRI studies. Incorporating these methodologies in future investigations could offer a more comprehensive understanding of the functional patterns associated with FGIDs. Lastly, methodological constraints precluded direct comparisons between different subtypes of FGIDs in this study. Future research efforts should strive to overcome these limitations through advancements in analytical techniques.

Conclusion

In conclusion, our study shed light on the intricate relationship between FGIDs and aberrant brain activity, providing valuable insights into the underlying pathophysiology of these conditions. Our findings revealed significant regional abnormalities in brain activities, implicating areas involved in sensory processing, emotional regulation, and cognitive control. Notably, heightened brain activity in sensory processing regions and reduced activation in areas associated with emotional regulation highlighted the complex interplay between gut dysfunction and brain function in FGIDs.

Author contributions

YS: Investigation, Methodology, Software, Writing – original draft, Data curation. BW: Data curation, Formal analysis, Project administration, Writing – review & editing. XZ: Data curation, Methodology, Validation, Writing – review & editing. ZS: Project administration, Resources, Visualization, Writing – review & editing. SH: Formal analysis, Investigation, Project administration, Writing – review & editing. CZ: Formal analysis, Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing, Conceptualization.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. This work was supported by the Medical and Health Science and Technology Development Plan of Shandong Province (202304011343), the Ministry of Education Industry-University Cooperative Education Project (220900242232529), the Key Research and Development Plan of Jining City (2023JNZC141), and the Cultivation Plan of High-level Scientific Research Projects of Jining Medical University (JYGC2021KJ006).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnins.2024.1452216/full#supplementary-material

References

Al Omran, Y., and Aziz, Q. (2014). Functional brain imaging in gastroenterology: to new beginnings. *Nat. Rev. Gastroenterol. Hepatol.* 11, 565–576. doi: 10.1038/nrgastro.2014.89

Albajes-Eizagirre, A., Solanes, A., Vieta, E., and Radua, J. (2019). Voxel-based meta-analysis via permutation of subject images (PSI): theory and implementation for SDM. *NeuroImage* 186, 174–184. doi: 10.1016/j.neuroimage.2018.10.077

Ao, W., Cheng, Y., Chen, M., Wei, F., Yang, G., An, Y., et al. (2021). Intrinsic brain abnormalities of irritable bowel syndrome with diarrhea: a preliminary resting-state functional magnetic resonance imaging study. *BMC Med. Imaging* 21:4. doi: 10.1186/s12880-020-00541-9

Aro, P., Talley, N. J., Johansson, S. E., Agreus, L., and Ronkainen, J. (2015). Anxiety is linked to new-onset dyspepsia in the Swedish population: a 10-year follow-up study. *Gastroenterology* 148, 928–937. doi: 10.1053/j.gastro.2015.01.039

Berman, S. M., Naliboff, B. D., Suyenobu, B., Labus, J. S., Stains, J., Ohning, G., et al. (2008). Reduced brainstem inhibition during anticipated pelvic visceral pain correlates with enhanced brain response to the visceral stimulus in women with irritable bowel syndrome. *J. Neurosci.* 28, 349–359. doi: 10.1523/JNEUROSCI.2500-07.2008

Black, C. J., Drossman, D. A., Talley, N. J., Ruddy, J., and Ford, A. C. (2020). Functional gastrointestinal disorders: advances in understanding and management. *Lancet* 396, 1664–1674. doi: 10.1016/s0140-6736(20)32115-2

Cai, W., Tian, H., Sun, P., Hua, T., Gong, J., Zhang, R., et al. (2024). Regional homogeneity alterations in patients with functional constipation and their associations with gene expression profiles. *Cereb. Cortex* 34:403. doi: 10.1093/cercor/bhad403

Chen, X. F., Guo, Y., Lu, X. Q., Qi, L., Xu, K. H., Chen, Y., et al. (2021). Aberrant intraregional brain activity and functional connectivity in patients with diarrhea-predominant irritable bowel syndrome. *Front. Neurosci.* 15:721822. doi: 10.3389/fnins.2021.721822

Dong, X., Yin, T., Yu, S., He, Z., Chen, Y., Ma, P., et al. (2022). Neural responses of acupuncture for treating functional dyspepsia: An fMRI study. *Front. Neurosci.* 16:819310. doi: 10.3389/fnins.2022.819310

Duan, W., Liu, X., Ping, L., Jin, S., Yu, H., Dong, M., et al. (2022). Distinct functional brain abnormalities in insomnia disorder and obstructive sleep apnea. *Eur. Arch. Psychiatry Clin. Neurosci.* 273, 493–509. doi: 10.1007/s00406-022-01485-7

Elsenbruch, S., Rosenberger, C., Bingel, U., Forsting, M., Schedlowski, M., and Gizewski, E. R. (2010). Patients with irritable bowel syndrome have altered emotional modulation of neural responses to visceral stimuli. *Gastroenterology* 139, 1310–1319.e4. doi: 10.1053/j.gastro.2010.06.054

Feng, Y., Li, G., Hu, Y., Zhang, W., Ji, W., Zhou, H., et al. (2023). Magnetic resonance imaging and functional constipation. *J. Clin. Basic Psychosomat.* 2:1463. doi: 10.36922/jcbp.1463

Fu, X., Ding, Y., Chen, J., Liu, F., Li, H., Zhao, J., et al. (2021). Altered brain functional asymmetry in patients with major depressive disorder related to gastrointestinal symptoms. *Front. Neurosci.* 15:797598. doi: 10.3389/fnins.2021.797598

Gong, W., Guo, P., Li, Y., Liu, L., Yan, R., Liu, S., et al. (2023). Role of the gut-brain Axis in the shared genetic etiology between gastrointestinal tract diseases and psychiatric disorders. *JAMA Psychiatry* 80, 360–370. doi: 10.1001/jamapsychiatry.2022.4974

Hong, J. Y., Kilpatrick, L. A., Labus, J., Gupta, A., Jiang, Z., Ashe-McNalley, C., et al. (2013). Patients with chronic visceral pain show sex-related alterations in intrinsic oscillations of the resting brain. *J. Neurosci.* 33, 11994–12002. doi: 10.1523/JNEUROSCI.5733-12.2013

Hong, J. Y., Labus, J. S., Jiang, Z., Ashe-Mcnalley, C., Dinov, I., Gupta, A., et al. (2014). Regional neuroplastic brain changes in patients with chronic inflammatory and non-inflammatory visceral pain. *PLoS One* 9:e84564. doi: 10.1371/journal.pone.0084564

Jin, Q., Duan, S., Li, G., Sun, L., Hu, Y., Hu, C., et al. (2019). Sex-related differences in resting-state brain activity and connectivity in the orbital frontal cortex and insula in patients with functional constipation. *Neurogastroenterol. Motil.* 31:e13566. doi: 10.1111/nmo.13566

Kano, M., Dupont, P., Aziz, Q., and Fukudo, S. (2018). Understanding neurogastroenterology from neuroimaging perspective: a comprehensive review of functional and structural brain imaging in functional gastrointestinal disorders. *J Neurogastroenterol Motil* 24, 512–527. doi: 10.5056/jnm18072

Ke, J., Qi, R., Liu, C., Xu, Q., Wang, F., Zhang, L., et al. (2015). Abnormal regional homogeneity in patients with irritable bowel syndrome: a resting-state functional MRI study. *Neurogastroenterol. Motility* 27, 1796–1803. doi: 10.1111/nmo.12692

Labus, J. S., Hubbard, C. S., Bueller, J., Ebrat, B., Tillisch, K., Chen, M., et al. (2013). Impaired emotional learning and involvement of the corticotropin-releasing factor signaling system in patients with irritable bowel syndrome. *Gastroenterology* 145, 1253–1261. doi: 10.1053/j.gastro.2013.08.016

Lee, I.-S., Kullmann, S., Scheffler, K., Preissl, H., and Enck, P. (2018). Fat label compared with fat content: gastrointestinal symptoms and brain activity in functional dyspepsia patients and healthy controls. *Am. J. Clin. Nutr.* 108, 127–135. doi: 10.1093/ajcn/nqy077

Li, G., Zhang, W., Hu, Y., Wang, J., Li, J., Jia, Z., et al. (2021). Distinct basal brain functional activity and connectivity in the emotional-arousal network and thalamus in patients with functional constipation associated with anxiety and/or depressive disorders. *Psychosom. Med.* 83, 707–714. doi: 10.1097/PSY.0000000000000958

Liberati, A., Altman, D. G., Tetzlaff, J., Mulrow, C., Gotzsche, P. C., Ioannidis, J. P. A., et al. (2009). The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration. *BMJ* 339:b2700. doi: 10.1136/bmj.b2700

Liu, J., Cao, L., Li, H., Gao, Y., Bu, X., Liang, K., et al. (2022). Abnormal resting-state functional connectivity in patients with obsessive-compulsive disorder: a systematic review and meta-analysis. *Neurosci. Biobehav. Rev.* 135:104574. doi: 10.1016/j.neubiorev.2022.104574

Lovell, R. M., and Ford, A. C. (2012). Global prevalence of and risk factors for irritable bowel syndrome: a meta-analysis. *Clin. Gastroenterol. Hepatol.* 10, 712–721.e4. doi: 10.1016/j.cgh.2012.02.029

Ma, X., Li, S., Tian, J., Jiang, G., Wen, H., Wang, T., et al. (2015). Altered brain spontaneous activity and connectivity network in irritable bowel syndrome patients: a resting-state fMRI study. *Clin. Neurophysiol.* 126, 1190–1197. doi: 10.1016/j.clinph.2014.10.004

Mao, Y., Zhang, P., Sun, R., Zhang, X., He, Y., Li, S., et al. (2023). Altered resting-state brain activity in functional dyspepsia patients: a coordinate-based meta-analysis. *Front. Neurosci.* 17:4287. doi: 10.3389/fnins.2023.1174287

Mayer, E. A., Labus, J. S., Tillisch, K., Cole, S. W., and Baldi, P. (2015a). Towards a systems view of IBS. *Nat. Rev. Gastroenterol. Hepatol.* 12, 592–605. doi: 10.1038/nrgastro.2015.121

Mayer, E. A., Nance, K., and Chen, S. (2022). The gut–brain Axis. *Annu. Rev. Med.* 73, 439–453. doi: 10.1146/annurev-med-042320-014032

Mayer, E. A., Tillisch, K., and Gupta, A. (2015b). Gut/brain axis and the microbiota. *J. Clin. Invest.* 125, 926–938. doi: 10.1172/JCI76304

Moher, D., Liberati, A., Tetzlaff, J., and Altman, D. G.Group, P (2009a). Preferred reporting items for systematic reviews and Meta-analyses: the PRISMA statement. *PLoS Med.* 6:e1000097. doi: 10.1371/journal.pmed.1000097

Moher, D., Liberati, A., Tetzlaff, J., and Altman, D. G.Group, P (2009b). Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *J. Clin. Epidemiol.* 62, 1006–1012. doi: 10.1016/j.jclinepi.2009.06.005

Muller, V. I., Cieslik, E. C., Laird, A. R., Fox, P. T., Radua, J., Mataix-Cols, D., et al. (2018). Ten simple rules for neuroimaging meta-analysis. *Neurosci. Biobehav. Rev.* 84, 151–161. doi: 10.1016/j.neubiorev.2017.11.012

Nan, J., Liu, J., Zhang, D., Yang, Y., Yan, X., Yin, Q., et al. (2014). Altered intrinsic regional activity and corresponding brain pathways reflect the symptom severity of functional dyspepsia. *Neurogastroenterol. Motil.* 26, 660–669. doi: 10.1111/nmo.12311

Page, M. J., Moher, D., Bossuyt, P. M., Boutron, I., Hoffmann, T. C., Mulrow, C. D., et al. (2021). PRISMA 2020 explanation and elaboration: updated guidance and exemplars for reporting systematic reviews. *BMJ* 372:n160. doi: 10.1136/bmj.n160

Qi, R., Liu, C., Ke, J., Xu, Q., Zhong, J., Wang, F., et al. (2016). Intrinsic brain abnormalities in irritable bowel syndrome and effect of anxiety and depression. *Brain Imaging Behav.* 10, 1127–1134. doi: 10.1007/s11682-015-9478-1

Qi, R., Shi, Z., Weng, Y., Yang, Y., Zhou, Y., Surento, W., et al. (2020). Similarity and diversity of spontaneous brain activity in functional dyspepsia subtypes. *Acta Radiol.* 61, 927–935. doi: 10.1177/0284185119883391

Radua, J., and Mataix-Cols, D. (2009). Voxel-wise meta-analysis of grey matter changes in obsessive-compulsive disorder. *Br. J. Psychiatry* 195, 393–402. doi: 10.1192/bjp.bp.108.055046

Radua, J., Mataix-Cols, D., Phillips, M. L., El-Hage, W., Kronhaus, D. M., Cardoner, N., et al. (2012). A new meta-analytic method for neuroimaging studies that combines reported peak coordinates and statistical parametric maps. *Eur. Psychiatry* 27, 605–611. doi: 10.1016/j.eurpsy.2011.04.001

Radua, J., Rubia, K., Canales-Rodriguez, E. J., Pomarol-Clotet, E., Fusar-Poli, P., and Mataix-Cols, D. (2014). Anisotropic kernels for coordinate-based meta-analyses of neuroimaging studies. *Front. Psych.* 5:13. doi: 10.3389/fpsyt.2014.00013

Salvia, E., Tissier, C., Charron, S., Herent, P., Vidal, J., Lion, S., et al. (2019). The local properties of bold signal fluctuations at rest monitor inhibitory control training in adolescents. *Dev. Cogn. Neurosci.* 38:100664. doi: 10.1016/j.dcn.2019.100664

Sperber, A. D., Bangdiwala, S. I., Drossman, D. A., Ghoshal, U. C., Simren, M., Tack, J., et al. (2021). Worldwide prevalence and burden of functional gastrointestinal disorders, results of Rome foundation global study. *Gastroenterology* 160, 99–114.e3. doi: 10.1053/j.gastro.2020.04.014

Su, C., Liu, W., Wang, Q., Qiu, S., Li, M., Lv, Y., et al. (2022). Abnormal resting-state local spontaneous functional activity in irritable bowel syndrome patients: a meta-analysis. *J. Affect. Disord.* 302, 177–184. doi: 10.1016/j.jad.2022.01.075

Suares, N. C., and Ford, A. C. (2011). Prevalence of, and risk factors for, chronic idiopathic constipation in the community: systematic review and meta-analysis. *Am. J. Gastroenterol.* 106, 1582–1591. doi: 10.1038/ajg.2011.164

Wang, Q., Wang, C., Deng, Q., Zhan, L., Tang, Y., Li, H., et al. (2022). Alterations of regional spontaneous brain activities in anxiety disorders: a meta-analysis. *J. Affect. Disord.* 296, 233–240. doi: 10.1016/j.jad.2021.09.062

Weng, Y., Qi, R., Liu, C., Ke, J., Xu, Q., Wang, F., et al. (2017). Disrupted functional connectivity density in irritable bowel syndrome patients. *Brain Imaging Behav.* 11, 1812–1822. doi: 10.1007/s11682-016-9653-z

Yu, Z., Liu, L.-Y., Lai, Y.-Y., Tian, Z.-L., Yang, L., Zhang, Q., et al. (2022). Altered resting brain functions in patients with irritable bowel syndrome: a systematic review. *Front. Hum. Neurosci.* 16:851586. doi: 10.3389/fnhum.2022.851586

Zang, Y. F., Zuo, X. N., Milham, M., and Hallett, M. (2015). Toward a Meta-analytic synthesis of the resting-state fMRI literature for clinical populations. *Biomed. Res. Int.* 2015:435265, 1–3. doi: 10.1155/2015/435265

Zhang, N., Teng, B., Lu, X., Shi, L., Liu, L., Zhou, F., et al. (2023). Exploring the neural mechanisms underlying achalasia: a study of functional connectivity and regional brain activity. *NeuroImage* 284:120447. doi: 10.1016/j.neuroimage.2023.120447

Zhao, M., Hao, Z., Li, M., Xi, H., Hu, S., Wen, J., et al. (2023). Functional changes of default mode network and structural alterations of gray matter in patients with irritable bowel syndrome: a meta-analysis of whole-brain studies. *Front. Neurosci.* 17:6069. doi: 10.3389/fnins.2023.1236069

Zhou, G., Liu, P., Wang, J., Wen, H., Zhu, M., Zhao, R., et al. (2013). Fractional amplitude of low-frequency fluctuation changes in functional dyspepsia: a resting-state fMRI study. *Magn. Reson. Imaging* 31, 996–1000. doi: 10.1016/j.mri.2013.03.019

frontiers | Frontiers in Neuroscience

TYPE Review
PUBLISHED 09 December 2024
DOI 10.3389/fnins.2024.1501134

OPEN ACCESS

EDITED BY
Zuleide Maria Ignácio,
Federal University of the Southern Frontier, Brazil

REVIEWED BY
Phakkharawat Sittiprapaporn,
Mae Fah Luang University, Thailand
An Zhou,
Army Medical University, China

*CORRESPONDENCE
Min Jiang
✉ 18582688810@163.com

RECEIVED 24 September 2024
ACCEPTED 26 November 2024
PUBLISHED 09 December 2024

CITATION
Jiang M, Kang L, Wang Y-L, Zhou B, Li H-Y, Yan Q and Liu Z-G (2024) Mechanisms of microbiota-gut-brain axis communication in anxiety disorders.
Front. Neurosci. 18:1501134.
doi: 10.3389/fnins.2024.1501134

Mechanisms of microbiota-gut-brain axis communication in anxiety disorders

Min Jiang[1]*, Li Kang[2], Ya-Li Wang[3], Bin Zhou[3], Hong-Yi Li[3], Qiang Yan[1] and Zhi-Gang Liu[1]

[1]Department of Clinical Laboratory, Neijiang Central District People's Hospital, Neijiang, Sichuan, China, [2]Department of Anesthesiology, The First People's Hospital of Neijiang, Neijiang, Sichuan, China, [3]Department of Neurology, Neijiang Central District People's Hospital, Neijiang, Sichuan, China

Anxiety disorders, prevalent mental health conditions, receive significant attention globally due to their intricate etiology and the suboptimal effectiveness of existing therapies. Research is increasingly recognizing that the genesis of anxiety involves not only neurochemical brain alterations but also changes in gut microbiota. The microbiota-gut-brain axis (MGBA), serving as a bidirectional communication pathway between the gut microbiota and the central nervous system (CNS), is at the forefront of novel approaches to deciphering the complex pathophysiology of anxiety disorders. This review scrutinizes the role and recent advancements in the MGBA concerning anxiety disorders through a review of the literature, emphasizing mechanisms via neural signals, endocrine pathways, and immune responses. The evidence robustly supports the critical influence of MGBA in both the development and progression of these disorders. Furthermore, this discussion explores potential therapeutic avenues stemming from these insights, alongside the challenges and issues present in this realm. Collectively, our findings aim to enhance understanding of the pathological mechanisms and foster improved preventative and therapeutic strategies for anxiety disorders.

KEYWORDS

anxiety disorders, gut microbiota, MGBA, neural signal, endocrine pathways, immune pathways

1 Introduction

Characterized by persistent unease, fear, and cognitive disturbances, anxiety disorders are highly prevalent, with substantial recurrence rates post-treatment, posing significant public health challenges and affecting socio-economic health (Scholten et al., 2021). For an extended period, the etiology of anxiety disorders has been primarily attributed to neurochemical factors, brain structure, and function, as well as environmental, genetic, and psychological influences. These areas have thus become the main focus of scholarly research. Despite extensive studies, effective treatment outcomes remain elusive. Current treatments frequently result in insufficient symptom relief or significant side effects for many patients. This issue stems from the underappreciated complexity of the mechanisms behind anxiety disorders. Moreover, the development of new therapeutic strategies is hampered by a limited understanding of these mechanisms. Therefore, probing the complex mechanisms underlying these disorders is crucial for devising more potent therapeutic strategies.

Recent interdisciplinary research in neuroscience and microbiology is revealing the complex interactions between the gut and the CNS. The gut microbiota, comprising trillions

of microorganisms, is essential for digestion, immune regulation, and metabolism. It also participates in bidirectional communication with the brain via the MGBA, influencing human behavior and psychological states (Cryan et al., 2019). The MGBA is a comprehensive system that includes gut microorganisms, the gastrointestinal tract, the vagus nerve (VN), and endocrine and immune components. It acts as a vital link between the gut microbiome and the brain. Evidence shows that gut-derived stimuli can directly affect brain activity via the VN. Additionally, the metabolic products of gut microbiota can alter endocrine balance and neuroinflammation states, thus affecting the brain's regulation of cognition and emotion (Generoso et al., 2021).

The role of gut microbiota in regulating emotions and behavior, especially via the CNS, is becoming more apparent as research advances. Numerous studies highlight that the MGBA is the primary communication pathway for this interaction. Factors such as neural signaling, endocrine mechanisms, and immune regulation significantly influence the functioning of this axis. These insights open new avenues for understanding and treating anxiety and other mental disorders, suggesting novel treatment strategies that include manipulating gut microbiota. Techniques such as the use of probiotics, prebiotics, and fecal microbiota transplantation (FMT) can modify the composition of gut microbiota and rebuild the gut environment, improving the psychological well-being of individuals (Baske et al., 2024; Chudzik et al., 2021; Noonan et al., 2020).

This review systematically elucidates the mechanisms through which the MGBA mediates anxiety disorders, focusing on recent advances in neural, endocrine, and immune pathways. Drawing on these findings, we discuss currently available therapeutic strategies, as well as unresolved questions and challenges within this research domain.

2 Gut microbiota

The gut microbiota comprises a vast community of microorganisms, including bacteria, archaea, and fungi, inhabiting the human gastrointestinal tract. This microbial population is enormous, vastly outnumbering human cells and boasting a vast genetic diversity that represents about 99% of the genes in our bodies. The gut microbiota evolves alongside the host, mutually influencing each other and performing functions akin to those of the nervous, endocrine, and immune systems (Chen et al., 2021; Cryan et al., 2019). The gut is a stable, resilient ecosystem. Maintaining the balance of this microenvironment is vital for human health, involving microbial population dynamics and changes in the synthesis and metabolism of products that alter intestinal pH, stress levels, and hormone secretion. Disruptions in this stable microenvironment, beyond its self-repair capacity, can lead to diseases, including mental disorders like anxiety. One human study has shown that, compared to healthy individuals, patients with generalized anxiety disorder have a significantly lower abundance and diversity of gut microbiota, indicating a pronounced state of dysbiosis (Jiang et al., 2018). Xiong et al. (2023) have cataloged over 30 basic and epidemiological studies demonstrating the relationship between the gut microbiota and a range of mental disorders, including anxiety. Current research indicates that gut microbiota metabolizes dietary fiber into bioactive components, enhancing digestion and nutrient absorption. Additionally, it produces various antimicrobial substances and other metabolic products through interaction with the host, which may protect beneficial microbiota and the host, and influence mental states and behaviors associated with anxiety disorders (Antushevich, 2020; Gomaa, 2020).

3 Anxiety disorders

Anxiety disorders, also known as anxiety neurosis, represent the most prevalent type of neurosis, stemming from the interaction of genetic and environmental factors. They manifest as episodic or persistent anxiety, accompanied by symptoms of autonomic nervous system dysregulation. Beyond emotional disturbances, patients may experience compromised executive functions and cognitive processes. The pathogenesis of anxiety disorders, involving complex dysregulation across multiple systems, remains incompletely understood. Contemporary medical research on the pathogenesis of anxiety focuses on neurotransmitter dysregulation, endocrine imbalances, and immune system dysfunctions. Communication within the CNS depends heavily on chemical neurotransmitters. Research has identified that abnormal concentrations of various neurotransmitters in synaptic clefts can precipitate anxiety symptoms. The neurotransmitters implicated, such as γ-aminobutyric acid (GABA), serotonin (5-hydroxytryptamine, 5-HT), dopamine (DA), and norepinephrine (NE), all common in brain regions involved in emotional regulation (Hakamata et al., 2022; Nguyen et al., 2021; Ren, 2016; Xia et al., 2021). Endocrine dysfunctions can lead to psychological and behavioral changes, often triggered by external stimuli affecting the endocrine system, especially the hypothalamic–pituitary–adrenal (HPA) axis (Tafet and Nemeroff, 2020). Additionally, studies indicate that anxiety patients often display immune dysfunctions (Hou et al., 2017). Current pharmacological treatments primarily include first-line medications like selective serotonin reuptake inhibitors (SSRIs) and serotonin-norepinephrine reuptake inhibitors (SNRIs), alongside second-line drugs such as beta-blockers and benzodiazepines. While these drugs are effective in reducing anxiety, they also have drawbacks including delayed onset, variable effectiveness, withdrawal symptoms, and side effects such as headaches, nausea, and sexual dysfunction. Therefore, exploring the complex mechanisms underlying anxiety is critical to developing novel therapeutic strategies (Szuhany and Simon, 2022).

4 Gut MGBA-mediated pathways in anxiety disorders

4.1 Neural signal pathways

Neural signaling pathways consist of multiple neurons and synapses that conduct information in various directions to facilitate physiological activities. The VN, containing extensive visceral sensory and motor fibers, is the primary bidirectional regulatory channel connecting visceral organs with the CNS. Gut microbiota metabolize substances including short-chain fatty acids (SCFAs), vitamins, and lipopolysaccharides (LPS), and also synthesize and utilize neurotransmitters such as GABA, DA, NE, and 5-HT (Dicks, 2022; Strandwitz, 2018). These compounds can stimulate the enteric nervous system (ENS) within the gastrointestinal tract wall, converting

chemical signals from the gut into neural impulses that are transmitted to the VN and CNS. Additionally, they may cross the blood–brain barrier (BBB) to impact specific brain regions directly. This interaction helps maintain the balance of the gut microbiota and its metabolic products, affecting related neural pathways and influencing behaviors and emotions (Figure 1).

The VN, one of the longest cranial nerves, originates from the medulla oblongata and extends to the thoracic and abdominal regions, terminating beneath the intestinal epithelium. This nerve detects various stimuli from the gut and conveys them to the CNS, influencing behavioral, cognitive, and emotional regulation. The gut microbiota and its metabolic products act as major sources of stimulation, forming a bidirectional connection with the brain via the VN and participating in the modulation of anxiety.

Research by Klarer et al. (2014) demonstrated that subdiaphragmatic vagotomy blocks afferent signals from the gut, resulting in changes in noradrenergic and GABAergic neurons within the ventral prefrontal cortex (vPFC) and nucleus accumbens (NAc), significantly reducing anxiety-like behaviors. Similarly, chronic lesioning of gut-innervating vagal afferent neurons has been found to disrupt GABAergic gene networks in the male rat amygdala, impacting anxiety states (Krieger et al., 2022). These findings highlight the critical role of VN communication in maintaining neurotransmitter system balance essential for anxiety regulation. Other studies show that afferent vagal neuron cell bodies are located in the nodose ganglion (NG) and extend to various visceral organs, including the gastrointestinal tract. They respond to gut stimuli and transmit signals to the nucleus tractus solitarius (NTS) via ascending pathways. Regulation of the locus coeruleus (LC)-NE system in the basolateral amygdala (BLA) via the NTS can mediate anxiety responses, while bilateral ablation of vagal afferents from the gastrointestinal tract can prevent the development of negative emotions (Chen et al., 2023; Cordner et al., 2021). These details underscore the extensive role of the VN and the LC-NE system as a key mediator in transforming gut signals into emotional responses, enhancing our understanding of how visceral information influences CNS activity. However, the mechanisms by which neural networks acquire and integrate these signals are not fully understood. There are indications that harmful metabolic products from the gut microbiota may induce local or systemic chronic inflammation, potentially affecting sensory neurons in the NG, and thus altering vagal nerve function. Although the data is incomplete, these insights are crucial for understanding the importance of gut microbiota-VN communication in the etiology and progression of disorders such as anxiety disorders (Cawthon and de La Serre, 2018).

Furthermore, neurotransmitters including GABA, DA, NE, and 5-HT, metabolized by gut microbiota, may cross the BBB under specific conditions like inflammation, influencing brain regions that regulate anxiety emotions (Strandwitz, 2018). This translocation highlights another pathway through which the gut microbiota can influence the emotional regulation via endogenous neurotransmitters.

γ-aminobutyric acid, an inhibitory neurotransmitter within the CNS, plays a crucial role in regulating physiological and psychological responses, especially emotional regulation. *Bifidobacterium* and *Lactobacillus* in the gut microbiota can ferment and produce GABA. This neurotransmitter activates the TrkB receptor for brain-derived neurotrophic factor (BDNF), supporting the integrity and function of the hippocampus. Such activation may alleviate symptoms of stress-induced anxiety and depression while enhancing 5-HT and DA levels (Kim et al., 2024).

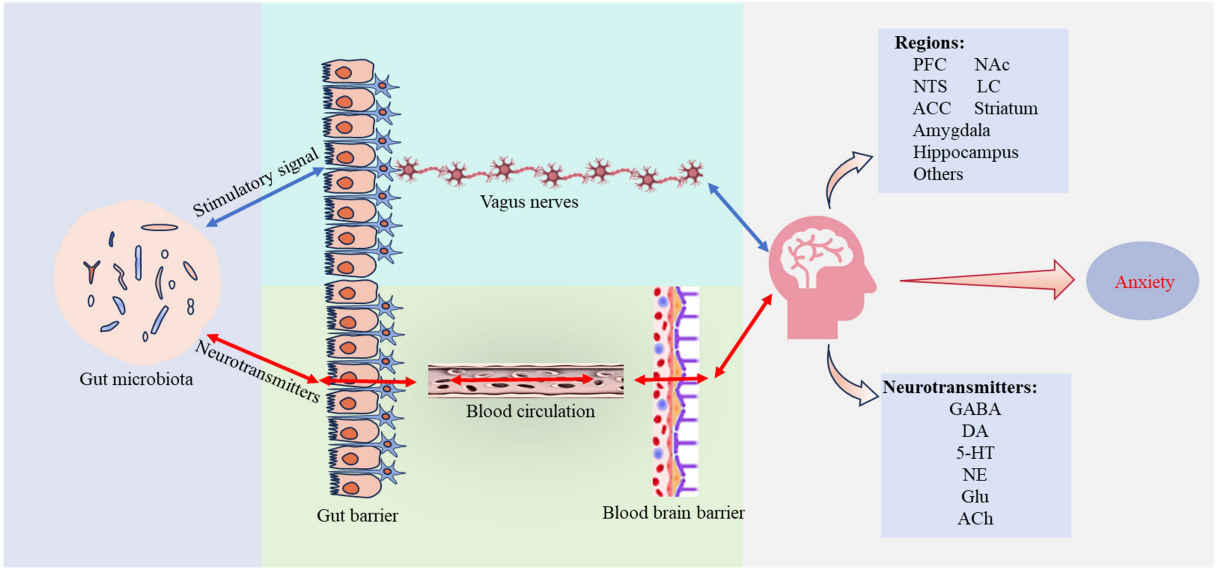


FIGURE 1
Neural signaling pathways. Gut microbiota contribute to anxiety regulation through two distinct neural pathways: one pathway transmits sensory signals from the intestinal wall to brain regions involved in emotional regulation through the vagus nerve (VN); the other pathway involves neurotransmitters produced by microbial metabolism that traverse the gut and blood–brain barrier (BBB), altering the equilibrium of original neurotransmitters. Both pathways result in altered neurotransmitter levels and changes in the function of brain regions involved in emotional regulation, ultimately affecting the development and progression of anxiety. Supplementary explanation of acronyms used in figure: 5-HT, 5-hydroxytryptamine; ACC, anterior cingulate cortex; Ach, acetylcholine; DA, dopamine; GABA, y-aminobutyric acid; Glu, glutamate; LC, locus coeruleus; Nac, nucleus accumbens; NE, norepinephrine; NTS, nucleus tractus solitarius; PFC, prefrontal cortex.

5-HT, another inhibitory neurotransmitter, regulates emotions and stress responses by regulating neuronal activity in the amygdala and prefrontal cortex (PFC). Approximately 95% of 5-HT is produced in the gut, with enterochromaffin cells (ECL) as its main source of synthesis (Koopman et al., 2021). These cells produce 5-HT by ingesting cellular debris, microbial entities, and tryptophan (Trp) present in bodily fluids. Moreover, bacterial genera such as Enterococcus, Escherichia, and Lactobacillus can generate 5-HT through the metabolic conversion of Trp, underscoring the gut's crucial role in regulating body 5-HT levels.

DA is fundamental in regulating brain reward, attention, and motivation, influencing emotional states based on the organism's perception of reward. Research shows the involvement of various bacterial genera in the gut in regulating DA levels, including *Bacillus*, *Lactobacillus*, and *Enterococcus*. For instance, *Enterococcus faecium* can produce levodopa (L-DOPA), which, upon crossing the BBB, is converted into DA within the brain, potentially at dopaminergic, adrenergic, and serotonergic nerve terminals (Wang et al., 2021). *Bacillus licheniformis* may decrease the levels of Trp, DA, and GABA by modifying the gut's microenvironment and boosting the production of colonic SCFAs, alleviating symptoms associated with anxiety and depression (Feng et al., 2023). A recent study has also identified a novel mechanism by which gut bacteria regulate DA levels after the methylation of DA by host catechol *O*-methyltransferase (COMT), enabling the conversion of 3-methoxytyramine (3MT) back to DA (Rich et al., 2022). DA also serves as a precursor for the synthesis of NE.

The NE is a key neurotransmitter studied in the context of anxiety disorder pathogenesis. Its levels are affected not only by synthetic precursors like DA but also by certain gut microbiota. For example, heat-killed *Enterococcus faecalis* (EC-12) can boost the concentration of central adrenoceptor b3 (Adrb3) activation in the prefrontal cortex of male mice, enhancing the release of NE. Additionally, Bifidobacterium CECT 7765 has been found to decrease NE levels in the hypothalamus of male mice (Huang and Wu, 2021). These findings support the role of gut microbiota in influencing the synthesis and release of NE. The CNS's ascending projections of NE originate in the LC of the brainstem and reach multiple brain regions, regulating emotions, arousal, and cognitive functions. For example, activation of the LC-NE system, particularly projections to the anterior cingulate cortex (ACC), is linked to increased anxiety-like behavior in the context of chronic pain (Suárez-Pereira et al., 2022). NE also affects the cardiovascular system by increasing heart rate and vasoconstriction, potentially exacerbating anxiety perceptions.

The cholinergic neuronal system is involved in emotional and affective regulation, and abnormalities may lead to emotional disorders, including depression and anxiety. Acetylcholine (ACh) is secreted by intestinal tuft cells and microbes such as *Lactobacillus*. Most of this secreted ACh is transported via the bloodstream to various tissues and organs, while a small amount enters brain tissue through mechanisms like choline transporters. This suggests that cholinergic signaling may represent another pathway through which gut microbiota influence brain function and emotional regulation, although further empirical evidence is needed to confirm (Hendel et al., 2022; Wiley et al., 2021).

Glutamate (Glu) is an excitatory neurotransmitter critical to the normal functioning of the CNS. The role of Glu in anxiety disorders is still debated, with no clear consensus yet reached. Some researchers believe that abnormalities in Glu metabolism may disrupt the balance of other neurotransmitters, affecting emotional regulation. *Bifidobacterium* and *Lactobacillus* can transform Glu to GABA, influencing the synthesis and release of GABA, and thereby indirectly impacting anxiety levels (Clos-Garcia et al., 2019; Nasir et al., 2020). Additionally, Glu's interaction with *N*-methyl D-aspartate (NMDA) receptors may regulate neurotransmission and neuronal excitability, contributing to the development and progression of anxiety disorders (Riaza Bermudo-Soriano et al., 2012). Although conclusive data are lacking, existing findings suggest that Glu might be a significant mediator of anxiety affected by gut microbiota.

It is crucial to understand that the array of neural output signals is governed not by a single neurotransmitter but by the interactions of various neurotransmitters within complex neural networks. For example, GABA has an indirect inhibitory effect on the release of DA, 5-HT, and NE, while also reducing Glu excitability. Conversely, 5-HT can modulate GABA release through its receptors. The 5-HT and DA systems maintain a mutual regulatory relationship, affecting DA synthesis, release, and reuptake, especially in areas like the PFC and striatum. DA can either suppress GABA secretion or stimulate Glu release through its receptors. ACh can also inhibit GABA release and enhance DA secretion through interactions with other neurotransmitters, however, the effectiveness of this action depends on the specific brain regions and receptor subtypes involved. In summary, this represents a highly complex sum of effects involving multiple receptors, regions, and signal transduction pathways.

4.2 Endocrine pathways

The endocrine system, a complex network of tissues and glands, synthesizes, stores, and release hormones, neurotransmitters, and cytokines. It plays a key role in regulating growth and development, metabolism, immune response, and emotional changes. Some scholars refer to the human colonizing microbiota as a "virtual endocrine organ," noting that its synthesis and metabolic products emit chemical signals similar to those of hormones (O'Callaghan et al., 2016). The gut microbiota, the largest reservoir of microorganisms in the human body, participates in regulating anxiety through various endocrine pathways (Figure 2).

The HPA axis is the body's principal neuroendocrine system and a major bidirectional regulatory pathway of the MGBA. Studies show that gut microbiota can disrupt the normal development of the HPA axis, influencing stress responses, emotions, and cognitive functions. Activation of the HPA axis initiates the synthesis and secretion of glucocorticoids (GCs) from the adrenal glands. These hormones interact with receptors in the hypothalamic paraventricular nucleus (PVN) and the pituitary, reducing the production and release of corticotropin-releasing hormone (CRH) and adrenocorticotropic hormone (ACTH), creating a negative feedback loop. GCs in the hippocampus can suppress HPA axis activity by acting on GABAergic neurons in the PVN (Leistner and Menke, 2020). Research by Frankiensztajn et al. (2020) has shown that mice subjected to restraint stress exhibit increased blood corticosterone levels, decreased BDNF expression in the hippocampus, activated NF-κB, and elevated monocyte recruitment to the colon. Furthermore, the relative abundance of Proteobacteria and *Escherichia coli* increased, while the

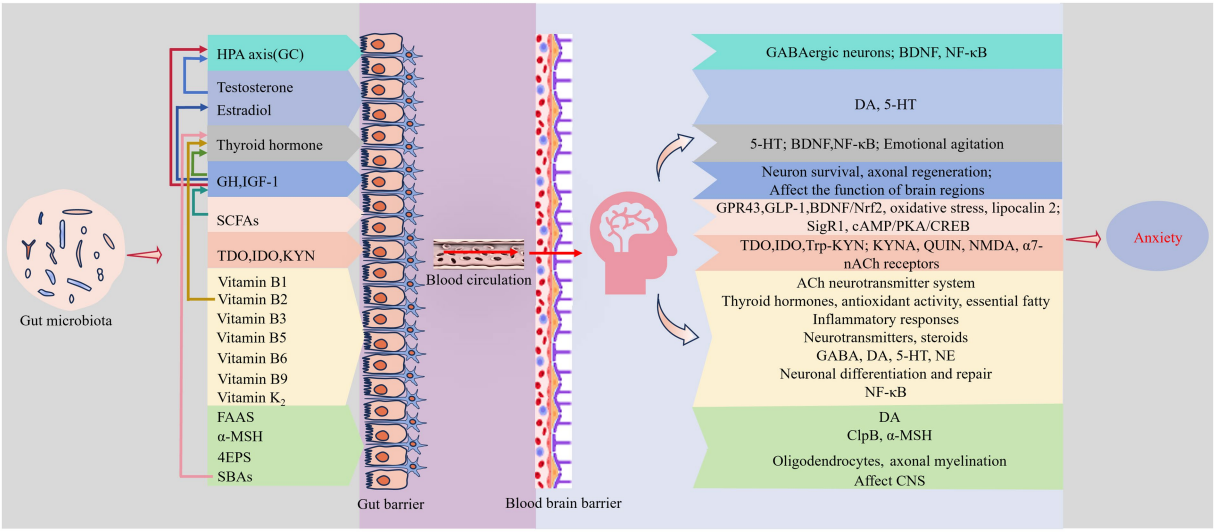


FIGURE 2
Endocrine pathways. Gut microbiota metabolites, including glucocorticoids (GCs), sex hormones, thyroid hormones, growth hormones, short-chain fatty acids (SCFAs), Trp-2,3-dioxygenase (TDO), indoleamine-2,3-dioxygenase (IDO), kynurenine (KYN), vitamin K2, water-soluble B vitamins, fatty acid amides (FAAs), a-melanocyte-stimulating hormone (a-MSH), 4-ethylphenylsulfate (4EPS), and secondary bile acids (SBAs), can cross the gut barrier and the BBB into specific regions of the brain associated with mood regulation under certain conditions. These metabolites mediate anxiety through diverse mechanisms, and their interactions create a complex and layered system that collectively influences the development and management of anxiety. Supplementary explanation of acronyms used in figure: BDNF, brain-derived neurotrophic factor; ClpB, caseinolytic protease B; CNS, central nervous system; GH, growth hormone; GLP-1, glucagon-like peptide-1; HPA, hypothalamic–pituitary–adrenal; IGF-1, insulin-like growth factor-1; KYNA, kynurenic acid; NMDA, *N*-methyl D-aspartate; QUIN, quinolinic acid; SigR1, Sigmar-1 receptor; a7-nACh, a7-nicotinic ACh.

abundance of *Lactobacillus* significantly decreased, feeding symbiotic *Lactobacillus* significantly improved these anxiety-like behaviors and reduced associated biomarkers (Frankiensztajn et al., 2020). Furthermore, studies on animals indicate that FMT can lower arachidonic acid and raise corticosterone in rats affected by high-salt-induced hypertension (Yan et al., 2020). It is critical to acknowledge that the effects of such transplantation are not immediate. Investigations reveal that germ-free (GF) mice, after receiving gut microbiota from specific pathogen-free (SPF) mice at week six, did not exhibit altered HPA axis responses to stress until week 14, suggesting a critical timeframe for these effects (Neufeld et al., 2011). These findings underscore the complex interplay between the gut microbiota and the HPA axis in regulating stress responses and anxiety-like behaviors.

Extensive research suggests that gut microbiota can impact host sex hormone levels, which may modify anxiety emotions (Liu et al., 2022; Matsushita et al., 2022; Santos-Marcos et al., 2023). *Escherichia coli* in the gut can synthesize 3β-hydroxysteroid dehydrogenase (3β-HSD), facilitating testosterone degradation. Administering this bacterium to rats significantly reduced testosterone levels in both blood and brain, triggering negative emotions like anxiety and depression (Li et al., 2022). Moreover, testosterone from peripheral blood is known to cross the BBB, enhancing DA and 5-HT release in the striatum and NAc, thereby attenuating anxiety-like emotions and indicating another pathway through which gut microbes might regulate emotions via testosterone (McHenry et al., 2014). The analysis of clinical cases revealed that women are approximately twice as likely as men to experience anxiety disorders. Although women are more sensitive to testosterone, they produce it at rates approximately one-tenth those of men, potentially explaining the observed gender disparities in anxiety disorder prevalence. Additionally, *Escherichia coli* and other bacteria can metabolize and produce β-glucuronidase (GUS), enzymes that influence estrogen levels by promoting the dissociation and hydroxylation of estrogen in the gut (Hu et al., 2023). Fluctuations in estrogen levels are also closely linked to anxiety, as estrogen withdrawal can induce neural plasticity in hypothalamic and dorsal raphe nucleus neurons, increasing anxiety-like behaviors (Hedges et al., 2021). Another study demonstrated that middle-aged female rats displayed anxiety-like states post-ovariectomy, which significantly improved following estradiol supplementation (Renczés et al., 2020). Furthermore, estrogen has been shown to elevate HPA axis activity, further elucidating the gender differences in anxiety disorder prevalence and highlighting the need for personalized treatment strategies based on hormonal status, thereby suggesting that sex hormones may play a role in regulating anxiety through modulation of the HPA axis (Peirce and Alviña, 2019).

A two-sample Mendelian randomization study identified that 34 gut microbiota taxa are involved in regulating thyroid function, thereby affecting thyroid hormone levels (Xie et al., 2023). There is a close link between thyroid hormone levels and the development of anxiety disorders. Studies in animals have demonstrated that levothyroxine-induced hyperthyroidism in rats significantly elevates serum FT3 and FT4 levels, increases brain 5-HT expression, and reduces hippocampal BDNF content, leading to pronounced anxiety-like behaviors. Conversely, hypothyroid rats induced by ^{131}I injection exhibited opposite effects (Yu et al., 2015). Clinical cases also demonstrate that an excess of thyroid hormones can cause increased metabolic activity, higher heart rates, and emotional agitation, potentially precipitating or exacerbating anxiety behaviors. Interestingly, some reports indicate that hypothyroidism can also result in symptoms of anxiety. Buras et al. (2014) reported that hypothyroidism may diminish BDNF

signaling in hippocampal neuron dendritic spines, resulting in decreased dendritic spine density, which induces anxiety. These conflicting results might relate to the varied impacts of thyroid hormone levels and the state of the thyroid axis on neurotransmitter systems and neural plasticity. While definitive evidence is yet to be established, these findings adequately illustrate the bidirectional nature of thyroid dysfunction and its influence on emotional states, with gut microbiota acting as a potential mediator of this dynamic.

Recent advances in microbial endocrinology have revealed that gut microbiota and their metabolic byproducts play significant roles in the regulation of growth hormone (GH) and insulin-like growth factor-1 (IGF-1) via multiple pathways (Jensen et al., 2020). Research links these hormones closely with anxiety, as demonstrated in both human and animal studies. For example, children and adolescents deficient in GH show a higher prevalence of anxiety, which improves with the administration of exogenous GH (Karachaliou et al., 2021). In mice with anxiety resulting from hypothalamic axon damage, GH treatment enhanced IGF-1 and its receptor expression, supporting the survival and regeneration of hypothalamic neurons after injury, thus reducing anxiety symptoms (Li et al., 2023). Although the underlying mechanisms are intricate and not fully elucidated, two primary explanatory approaches can be identified: First, GH receptors located in key brain regions such as the hypothalamus, amygdala, and bed nucleus of the stria terminalis may influence anxiety directly by altering the function of these areas (dos Santos et al., 2023). Additionally, GH and IGF-1 interact with CRH, its downstream hormones, thyroid hormones and their receptors, and sex hormones, potentially participating in anxiety regulation indirectly through these pathways (Fernández-Pérez et al., 2016; Kucharska et al., 2021; Quaresma et al., 2020).

Short-chain fatty acids, crucial fermentation byproducts of carbohydrate metabolism by gut microbiota, notably include acetate, propionate, and butyrate, which constitute approximately 95% of SCFAs. These compounds are essential for the bidirectional gut-brain communication and act as bacterial signaling molecules influencing cellular activities via G protein-coupled receptors on cell surfaces (Parada Venegas et al., 2019). For instance, SCFAs can promote the release of glucagon-like peptide-1 (GLP-1) by activating GPR43 on intestinal L-cells or serving as endogenous ligands for free fatty acid receptors 2 (FFAR2) and 3 (FFAR3). Given that GLP-1 receptors are prevalent in brain regions like the amygdala and limbic system, linked with anxiety, they represent a promising research area. Agonists of GLP-1 receptors have been shown to reduce anxiety-like behaviors in rodent models, possibly by maintaining BDNF and Nrf2 levels and reducing oxidative stress and lipocalin 2 in the hippocampus (Cawthon and de La Serre, 2018; Sağlam et al., 2022). Simultaneously, SCFAs derived from microbes can also enhance the secretion of mature BDNF by activating the Sigmar-1 receptor (SigR1), thereby ameliorating anxiety and depression-like behaviors (Zhang et al., 2023). Based on the discussion of BDNF's role in anxiety within this article, it is speculated that BDNF is a pivotal target in the anxiety-regulation pathways mediated by gut microbiota. Additionally, SCFAs may influence GH regulation through the cAMP/PKA/CREB pathway, suggesting another potential mechanism through which SCFAs modulate anxiety (Wang et al., 2013).

Specific gut bacteria, such as *Pseudomonas aeruginosa*, synthesize enzymes including Trp-2,3-dioxygenase (TDO) and indoleamine-2,3-dioxygenase (IDO), which are involved in the Trp-kynurenine (KYN) metabolism pathway. This pathway impacts Trp degradation, thus affecting the synthesis and release of neurotransmitters such as 5-HT, DA, and GABA, which are integral to anxiety regulation (Roager and Licht, 2018). It is noteworthy that comparisons of gender differences reveal heightened IDO activation in females, which may be another key factor contributing to the observed gender disparities in the prevalence of anxiety disorders (Songtachalert et al., 2018). Trp can also be converted to KYN by IDO and TDO enzymes, and various CNS cells metabolize KYN into neuroactive substances. For example, astrocytes convert KYN into neuroprotective kynurenic acid (KYNA), whereas microglia generate neurotoxic quinolinic acid (QUIN). These metabolites significantly influence anxiety-related emotions by activating NMDA and α7-nicotinic ACh (α7-nACh) receptors, affecting multiple nervous systems, including the ENS (Evrensel et al., 2020; Kim and Jeon, 2018; Lim et al., 2017).

Vitamins are crucial nutrients for human health. In addition to external sources, specific gut microorganisms are capable of metabolizing and synthesizing certain vitamins, influencing their absorption and conversion. Metagenomic analysis of human feces has shown that gut bacteria can produce vitamin K_2 and water-soluble B vitamins (Das et al., 2019). These vitamins traverse the intestinal mucosa and are transported into the brain via specialized mechanisms across the BBB and choroid plexus, playing roles in regulating various biological functions including anxiety. For instance, vitamin B1 is involved in regulating the ACh neurotransmitter system. Vitamin B2 plays a role in the regulation of thyroid hormones, antioxidant activity, and the metabolism of essential fatty acids in brain lipids. Vitamin B3 affects brain inflammatory responses, and vitamin B5 is crucial in synthesizing various neurotransmitters and steroids. Vitamin B6 acts as a limiting cofactor in the synthesis of neurotransmitters such as GABA, DA, 5-HT, and NE. Vitamin B9 deficiency can alter DNA stability, hinder neuronal differentiation and repair, and affect the levels of monoamine neurotransmitters such as DA, NE, and 5-HT by regulating the synthesis of tetrahydrobiopterin (BH4). Certain forms of vitamin K_2 have been shown to mitigate LPS-induced microglial inflammatory responses by inhibiting the NF-κB signaling pathway (Kennedy, 2016). Although direct connections between vitamins and anxiety have not been conclusively established, the pathways they influence significantly overlap with those involved in the pathogenesis of anxiety disorders, offering new perspectives on the gut microbiota-mediated mechanisms of anxiety and underscoring the importance of gut microbiota in nutritional psychiatry.

Additionally, additional metabolites produced by the gut microbiota are implicated in anxiety regulation. For example, certain Gram-negative bacilli in the gut can produce fatty acid amides (FAAs), which interact with the endogenous cannabinoid receptor CB1, activate sensory nerves in the intestinal wall, and transmit signals to the brain, thereby increasing DA levels in the ventral striatum and influencing anxiety-related emotions (Dohnalová et al., 2022). *α*-melanocyte-stimulating hormone (α-MSH), critical in satiety and anxiety signal transduction, is influenced by caseinolytic protease B (ClpB) produced by *Escherichia coli* and other *Enterobacteriaceae*, highlighting ClpB as a potential mediator in anxiety regulation through gut microbiota (Navarro-Tapia et al., 2021). Animal experiments have found that some gut bacteria in mice can produce 4-ethylphenylsulfate (4EPS), a compound that damages

oligodendrocytes, inhibits their maturation, and reduces axonal myelination, leading to anxiety-like behaviors. Treatments that foster oligodendrocyte differentiation may alleviate these negative emotions (Needham et al., 2022). In the enterohepatic circulation, about 5% of bile acids (BAs) are unabsorbed and enter the gut where bacteria transform these primary BAs into secondary bile acids (SBAs) through processes like early deconjugation and 7α-dehydroxylation. These secondary products may enter the CNS via circulation and affect anxiety regulation (MahmoudianDehkordi et al., 2022; Xing et al., 2023). Reports also suggest that SBAs can facilitate the activation of thyroid hormones via the G protein-coupled bile acid receptor 1 (GPBAR1), proposing an additional pathway for their role in anxiety regulation. While the precise mechanisms of these correlations remain to be fully elucidated, these findings affirm the active involvement of gut microbiota in the cerebral regulation of emotional states through their metabolic products (Sasaki et al., 2018). Moreover, new gut microbiota metabolites related to anxiety continue to be discovered and elucidated.

4.3 Immune pathways

The gut, a major organ in contact with the external environment, exhibits distinct regional immune characteristics due to its continuous exposure to diverse antigens and microorganisms. The proper functioning of this system relies on the homeostasis of the gut microenvironment, which comprises intestinal epithelial cells (IECs), mucosa, immune cells, and microbiota. Disturbances in this environment can lead to structural and functional immune disorders. Such disruptions are known to influence brain regions linked with anxiety (Felger, 2018). Historically considered a protective barrier, the BBB was thought to shield the brain from peripheral inflammation. However, recent findings suggest that peripheral inflammation can interact with the brain via signaling mechanisms involving the VN, brain endothelial cells, circumventricular organs (CVOs), and peripheral immune cells. This interaction can trigger neuroinflammation, consequently affecting the brain's regulatory functions over behavior, cognition, and anxiety (D'Mello and Swain, 2016) (Figure 3).

Lipopolysaccharides, derived from the cell walls of Gram-negative bacteria, are potent pro-inflammatory agents prevalent in the human gut. They are implicated in numerous immune pathways critical to the development and progression of anxiety disorders, primarily through the activation of Toll-like receptors (TLRs), particularly TLR4. TLR4 is expressed extensively across gastrointestinal epithelial cells, immune cells, and neurons. Upon binding of LPS to TLR4, downstream signaling pathways such as MyD88 and NF-κB are activated, contributing directly or indirectly to neuroinflammation in the brain (Carloni and Rescigno, 2023; Peng et al., 2021; Vargas-Caraveo et al., 2017). Research by Yang et al. (2023) has demonstrated that inhibiting the TLR4/MyD88/NF-κB signaling pathway reduces inflammation, protects hippocampal neurons, and significantly alleviates anxiety-like behaviors induced by methyl methanesulfonate in male C57BL/6 mice. This highlights the pivotal role of TLR4-mediated signaling in controlling neuroinflammation and its potential as a target for treating anxiety disorders.

Meanwhile, increased intestinal permeability allows LPS to directly cross the gut mucosa into the bloodstream. This translocation can compromise the BBB, induce neuroinflammation, and affect brain regions responsible for emotional regulation. Accompanying this are

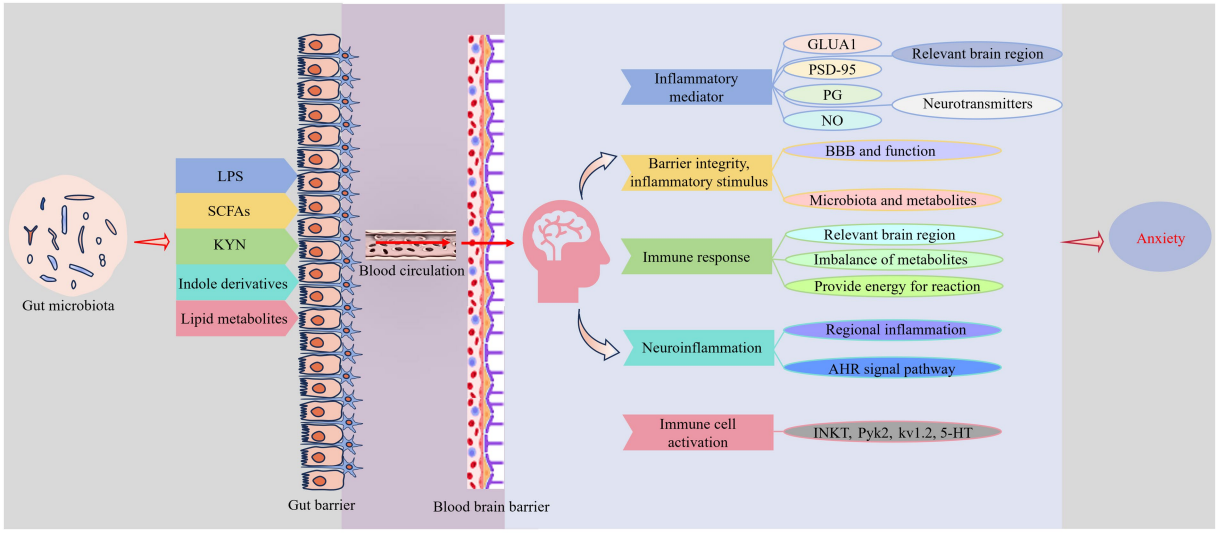


FIGURE 3
Immune pathways. Pro-inflammatory and anti-inflammatory metabolites produced by gut microbiota are crucial in modulating anxiety emotions through immune pathways. Inflammatory mediators, including lipopolysaccharides (LPS), indole derivatives, and lipid metabolites, can compromise the integrity of the intestinal mucosal barrier, facilitating their entry into the bloodstream and crossing the BBB, thereby initiating neuroinflammation. Conversely, anti-inflammatory agents like SCFAs may counter these effects. The cumulative immune responses of these metabolites affect the function of brain regions and neurotransmitter levels involved in mood regulation, thereby influencing the brain's ability to modulate anxiety emotions. Supplementary explanation of acronyms used in figure: AHR, aryl hydrocarbon receptor; GluAl, a-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor A1; INKT, invariant natural killer T; Kv1.2, voltage-gated potassium channel subfamily A member 2; NO, nitric oxide; PG, prostaglandin; PSD-95, postsynaptic density-95; Pyk2, proline-rich tyrosine kinase 2.

releases of pro-inflammatory cytokines, including IL-1β, IL-6, and TNF-α. These cytokines are recognized by macrophages and the afferent VN, which activate ascending neural signals to brain areas that manage emotions, precipitating anxiety-like states (D'Mello and Swain, 2016). Additionally, these pro-inflammatory cytokines can downregulate levels of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor A1 (GluA1) and postsynaptic density-95 (PSD-95) in the medial PFC via the subdiaphragmatic VN (Zhang et al., 2020). The regulation of excitability in the PFC through the *N*-cadherin-GluA1 pathway has been observed to mediate anxiety-like behavior in male offspring following prenatal stress (Shao et al., 2021). PSD-95, a critical structural component of the postsynaptic membrane in neurons, is essential for anchoring Glu receptors and maintaining synaptic stability and plasticity. By interacting with relevant receptors, ion channels, and signaling proteins, PSD-95 influences the nervous system's control over learning, memory, and anxiety (Fitzgerald et al., 2014; Liu et al., 2017).

Receptors for inflammatory factors like TNF-α, IL-1β, and IL-6 are also found in brain endothelial cells, neurons, and glial cells. These can bind directly to cytokines in CVOs, which lack a complete BBB, thereby inducing neuroinflammation and subsequent anxiety-like behaviors. Additionally, these pathways can lead to increased production of nitric oxide (NO) and prostaglandins (PGs) in the brain (D'Mello and Swain, 2016). NO can upregulate ACh levels in the NAc, stimulate hippocampal release of NE and Glu, and alter GABA levels depending on its concentration. It also prompts the medial preoptic area to release DA and 5-HT, contributing to anxiety regulation (Gulati et al., 2017). Meanwhile, elevated levels of PGs in regions such as the hypothalamus, hippocampus, and PFC have also been proven to induce anxiety symptoms (Yin et al., 2022). Furthermore, inflammation-induced damage to the BBB can disrupt its regulation and restriction of peripheral neurotransmitters like DA, 5-HT, and NE, thereby affecting anxiety modulation through neural pathways.

The SCFAs serve as energy sources for IECs and microbiota, and in conjunction with thyroid hormones, they reinforce the tight junctions of gut wall cells. This reinforcement potentially affects the gut microenvironment and the integrity of the mucosal barrier. SCFAs also attenuate inflammation by enhancing beneficial gut bacteria and blocking detrimental stimuli like LPS from accessing the systemic circulation (Knezevic et al., 2020; Taylor and Holscher, 2018). Furthermore, SCFAs can traverse the BBB and impact the integrity and function of this barrier. Their receptors are expressed in various cell types, including brain endothelial cells, neutrophils, and lymphocytes. These fatty acids actively participate in anti-inflammatory responses and diminish the production of inflammatory cytokines such as IL-6 and TNF-α by inhibiting NF-κB activity, thereby improving anxiety symptoms (Franzosa et al., 2018; O'Riordan et al., 2022). Based on our previous discussions regarding SCFAs, it is evident that SCFAs can regulate anxiety-related emotions through multiple important pathways. This suggests their potential as effective candidate therapeutics aimed at alleviating anxiety symptoms.

In the previous section, we explored how KYN modulates anxiety through the endocrine system. Intriguingly, systemic inflammation triggered by LPS also appears to maintain a complex balance with KYN and its derivatives, which might influence anxiety via the immune system. Many enzymes in the KYN pathway, such as IDO, exhibit immunoreactivity. Pro-inflammatory cytokines including TNF-α, IL-1β, and IFN-γ can upregulate IDO expression, facilitating the metabolism of Trp to KYN. TLR4 also activates this pathway, increasing KYN transport to the brain during systemic inflammation. However, some studies suggest that this transport is unrelated to IDO activity. We hypothesize that this effect may result from enhanced permeability of the BBB during inflammation, facilitating the influx of KYN and its metabolites into the brain (Gao et al., 2020; Savitz, 2019). Additionally, certain metabolites in the KYN pathway, such as KYNA and QUIN, have contrasting effects on neurotransmitters. Inflammation might alter their levels, affecting anxiety symptoms. Notably, QUIN can also convert to nicotinamide adenine dinucleotide (NAD+), involved in cellular energy metabolism, potentially influencing immune responses that demand substantial energy, thus connecting gut microbiota with anxiety regulation through an immunological pathway (Platten et al., 2019).

Trp metabolism by gut microbiota produces indole derivatives such as indole-3-acetic acid (IAA) and indole-3-propionic acid (IPA), which may regulate anxiety via immune mechanisms. Studies by Jennis et al. (2017) showed that IPA reduces intestinal permeability exacerbated by a high-fat diet in mice. *In vitro* tests indicate that IPA decreases cytokine-induced permeability in T84 cell monolayers (Jennis et al., 2017). These effects suggest that IPA enhances gut barrier function, limiting harmful substances like LPS from entering the bloodstream and reducing systemic inflammation, thereby improving anxiety symptoms. Furthermore, the aryl hydrocarbon receptor (AHR), involved in Trp metabolism, is expressed in microglia, astrocytes, and neurons in the brain. Indole derivatives like IAA and IPA, as AHR ligands, can cross the BBB and activate AHR signaling in astrocytes, inhibiting neuroinflammation and potentially regulating anxiety (Gao et al., 2020).

Lipid metabolites produced by gut microbiota may regulate anxiety via immune pathways. Recent research suggests that these metabolites are recognized by CD1d molecules on IECs, activating invariant natural killer T (iNKT) cells, which in turn trigger proline-rich tyrosine kinase 2 (Pyk2). Pyk2 activation influences voltage-gated potassium channel subfamily A member 2 (Kv1.2) through tyrosine phosphorylation, reducing K^+ conductance and increasing Ca^{2+} influx, thereby promoting 5-HT release. Meanwhile, inflammatory states in the gut increase gut barrier permeability, enhancing the translocation of 5-HT into the bloodstream and its entry into specific brain regions involved in anxiety regulation Although further studies are needed to confirm these observations, they introduce a novel perspective for understanding how gut microbiota might control anxiety-related emotions through immune pathways (Luo et al., 2023).

5 Current treatment and prevention strategies

The MGBA is an essential pathway through which gut microbiota influence anxiety-related emotions. The initial step in this axis involves the gut microbiota, which normalize the axis by sustaining a stable intestinal microenvironment through appropriate quantities, proportions, and metabolites. Various therapeutic approaches have been employed for the reconstruction of the gut microenvironment, potentially serving as effective strategies to ameliorate anxiety.

Probiotics, which are beneficial bacteria for the gut microbiota, can be administered through oral supplements or included in dietary choices. Popular probiotics include strains of lactic acid bacteria,

bifidobacteria, and yeast. These organisms colonize the gastrointestinal tract, engage in interactions with other microbes, augment populations of beneficial bacteria, and suppress deleterious bacteria, thereby promoting a balanced gut microbiota and enhancing the overall gut environment. Prebiotics are food components utilized by gut microorganisms to promote the growth and activity of beneficial bacteria. Typical prebiotics include fructans and glucose-derived oligosaccharides. Although not absorbed by the human body, prebiotics are fermented by beneficial gut bacteria, producing advantageous metabolites like SCFAs, which are crucial for the growth and vitality of beneficial microbes, thus maintaining microbial equilibrium in the gut (Kim et al., 2019; Rau et al., 2024). Probiotics and prebiotics exhibit a synergistic effect and often show greater therapeutic effects when used together. It should be noted that different probiotics and prebiotics may have variable effects among individuals, therefore, individualized selection and use are necessary.

A broad analysis of studies connecting nutrition to mental health disorders reveals that dietary interventions can exert therapeutic effects on chronic neurological conditions. The gut microenvironment serves as a crucial link between the two. Dietary influences on gut microbiota ecological balance primarily involve the composition of microbial communities, gut permeability, inflammatory responses, and the metabolites produced by different microbial populations (Navarro-Tapia et al., 2021; Ross et al., 2024; Schnorr and Bachner, 2016). For instance, diets rich in omega-3 fatty acids, such as those including fish oil, can enhance beneficial microbial communities, bolster gut barrier function, and decrease levels of circulating LPS, thus mitigating systemic inflammation. Additionally, the levels of trace and macronutrients in food can impact the gut microenvironment and microbial composition. A magnesium-deficient diet may reduce beneficial gut bacteria and increase negative emotions, while a high-fat diet can alter gut microbiota composition, affecting metabolism, gut permeability, and inflammation. Moreover, food additives significantly impact the composition of gut microbiota; for instance, high-salt diets may reduce populations of beneficial bacteria such as bifidobacteria, and emulsifiers may increase the pro-inflammatory potential of certain gut microbes (Bear et al., 2020). It is crucial to recognize that these relationships are typically non-linear, thus necessitating careful exploration of optimal dosages when employing beneficial supplements for treatment purposes.

The FMT is a technique employed to reconstruct the gut microenvironment by transferring microbiota from the feces of healthy donors into the patient's gastrointestinal system. This method helps restore microbial diversity and balance, promotes the growth of beneficial bacteria, and inhibits harmful bacteria. FMT has proven effective for treating recurrent *Clostridium difficile* infection (D and Venkatesh, 2023; Minkoff et al., 2023). However, further research are required to verify its safety and effectiveness in treating other conditions, including anxiety disorders, and to resolve associated technical and procedural challenges.

The reconstruction of the gut microenvironment is a prolonged process, and maintaining reasonable lifestyle habits is necessary for both prevention and during reconstruction. For example, the adverse effects of stress on the gut microbiota can be alleviated by ensuring sufficient rest, engaging in relaxation training, and receiving psychological counseling. Moderate physical activity enhances intestinal motility and improves the gut microenvironment. Furthermore, avoiding the prolonged, frequent, and inappropriate use of antibiotics can minimize their negative impacts on gut microorganisms. Additionally, reducing the use of germicides and excessive disinfection, maintaining moderate hygiene standards, and avoiding the destruction of beneficial microbes are also vital for sustaining a healthy gut microbiota (Fishbein et al., 2023; Gubert et al., 2020; Sciurba et al., 2021).

6 Unresolved issues, challenges, and future research priorities

While considerable advancements have been made in the study of the MGBA, numerous challenges persist in this area. The diversity and individual variability of gut microbiota complicate the identification of specific microbial profiles linked to anxiety. Concurrently, most evidence comes from animal studies, and translating these findings to humans requires careful consideration and validation. The precise mechanisms underlying the interactions between microbiota, the gut, and the brain are still not entirely comprehended, and clarifying the directionality of these interactions remains a significant task. Despite these challenges, the active nature of this research field continues to yield new insights, providing hope for further clarification of the complex relationships between gut microbiota and anxiety.

Future research should focus on human clinical trials to validate phenomena observed in animal models, enhance the clinical relevance of findings, and establish causal relationships between gut microbiota and anxiety. Further research is also needed to understand how dietary choices, antibiotics, prebiotics, and probiotics influence gut microbiota and impact the gut-brain axis. Investigating individual differences in gut microbiota composition, influenced by genetic factors and early-life experiences, may offer personalized strategies for managing anxiety through gut microbiota modulation. Additionally, the development of new therapies targeting the MGBA, including psychobiotics, will require rigorous clinical trials to assess their efficacy and safety in treating anxiety disorders. Addressing these challenges will be crucial for leveraging the potential of the MGBA in the prevention and treatment of anxiety and other neuropsychiatric disorders.

7 Conclusion

Research increasingly confirms that the MGBA constitutes a bidirectional communication network crucial for maintaining gut physiological homeostasis and influencing brain function and behavior. Neurologically, gut microbiota and their metabolites directly interact with the brain through the VN or indirectly affect emotion-regulating brain regions by crossing the BBB, mediated by neurotransmitters and their precursors derived from bacterial metabolism. In the endocrine pathway, gut microbiota play a pivotal role in the regulation of various hormones and other substances that contribute to stress response and emotional regulation. Immunologically, disruptions in the gut microenvironment can enhance intestinal permeability, permitting the entry of bacterial components and detrimental metabolites into the bloodstream. This influx triggers systemic inflammation, which in turn impacts the nervous system's role in managing anxiety-related emotions. These insights provide a refined and comprehensive understanding of the

pathological mechanisms that underlie anxiety disorders, thereby underscoring potential therapeutic targets and furnishing a more detailed framework for grasping the origins of anxiety and identifying prospective treatment options.

It is important to note that the neuro, endocrine, and immune pathways within the MGBA interact in a complex and intertwined system. The CNS reacts to gut-derived stimuli by activating the endocrine system, which in turn alters hormone levels and other substances, affecting emotional states. Conversely, changes in the endocrine environment can modulate neural activity, influencing neurotransmitter dynamics and emotional regulation. Additionally, systemic inflammation within the immune system can modify neural signaling and endocrine functions, ultimately creating a feedback loop that impacts anxiety responses. This complex interplay suggests that modifications in one pathway can trigger cascading effects on others, underscoring the need for therapeutic strategies that consider the overall effects.

In summary, this study focuses on the unique and interconnected complex mechanisms of the MGBA in the pathogenesis and progression of anxiety disorders, offering new insights into the pathophysiological mechanisms of anxiety and the development of novel therapeutic strategies.

Author contributions

MJ: Conceptualization, Project administration, Resources, Visualization, Writing – original draft, Writing – review & editing, Funding acquisition, Supervision. LK: Resources, Writing – original draft, Writing – review & editing. Y-LW: Funding acquisition, Resources, Writing – original draft. BZ: Funding acquisition, Resources, Writing – original draft. H-YL: Funding acquisition, Resources, Writing – original draft. QY: Resources, Writing – original draft. Z-GL: Resources, Writing – original draft.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. This study was supported by the Fundamental and Applied Basic Research Funds of Neijiang (2024NJJCYJSZQ002).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

Antushevich, H. (2020). Fecal microbiota transplantation in disease therapy. *Clin. Chim. Acta*. 503, 90–98. doi: 10.1016/j.cca.2019.12.010

Baske, M. M., Timmerman, K. C., Garmo, L. G., Freitas, M. N., McCollum, K. A., and Ren, T. Y. (2024). Fecal microbiota transplant on Escherichia-Shigella gut composition and its potential role in the treatment of generalized anxiety disorder: a systematic review. *J. Affect. Disord.* 354, 309–317. doi: 10.1016/j.jad.2024.03.088

Bear, T. L. K., Dalziel, J. E., Coad, J., Roy, N. C., Butts, C. A., and Gopal, P. K. (2020). The role of the gut microbiota in dietary interventions for depression and anxiety. *Adv. Nutr.* 11, 890–907. doi: 10.1093/advances/nmaa016

Buras, A., Battle, L., Landers, E., Nguyen, T., and Vasudevan, N. (2014). Thyroid hormones regulate anxiety in the male mouse. *Horm. Behav.* 65, 88–96. doi: 10.1016/j.yhbeh.2013.11.008

Carloni, S., and Rescigno, M. (2023). The gut-brain vascular axis in neuroinflammation. *Semin. Immunol.* 69:101802. doi: 10.1016/j.smim.2023.101802

Cawthon, C. R., and de La Serre, C. B. (2018). Gut bacteria interaction with vagal afferents. *Brain Res.* 1693, 134–139. doi: 10.1016/j.brainres.2018.01.012

Chen, C.-H., Tsai, T.-C., Wu, Y.-J., and Hsu, K.-S. (2023). Gastric vagal afferent signaling to the basolateral amygdala mediates anxiety-like behaviors in experimental colitis mice. *JCI Insight*. 8:1874. doi: 10.1172/jci.insight.161874

Chen, Y., Zhou, J., and Wang, L. (2021). Role and mechanism of gut microbiota in human disease. *Front. Cell. Infect. Microbiol.* 11:625913. doi: 10.3389/fcimb.2021.625913

Chudzik, A., Orzyłowska, A., Rola, R., and Stanisz, G. J. (2021). Probiotics, prebiotics and Postbiotics on mitigation of depression symptoms: modulation of the brain–gut–microbiome Axis. *Biomol. Ther.* 11:1000. doi: 10.3390/biom11071000

Clos-Garcia, M., Andrés-Marin, N., Fernández-Eulate, G., Abecia, L., Lavín, J. L., van Liempd, S., et al. (2019). Gut microbiome and serum metabolome analyses identify molecular biomarkers and altered glutamate metabolism in fibromyalgia. *EBioMedicine*. 46, 499–511. doi: 10.1016/j.ebiom.2019.07.031

Cordner, Z. A., Li, Q., Liu, L., Tamashiro, K. L., Bhargava, A., Moran, T. H., et al. (2021). Vagal gut-brain signaling mediates amygdaloid plasticity, affect, and pain in a functional dyspepsia model. *JCI Insight*. 6:4046. doi: 10.1172/jci.insight.144046

Cryan, J. F., O'Riordan, K. J., Cowan, C. S. M., Sandhu, K. V., Bastiaanssen, T. F. S., Boehme, M., et al. (2019). The microbiota-gut-brain Axis. *Physiol. Rev.* 99, 1877–2013. doi: 10.1152/physrev.00018.2018

D, T., and Venkatesh, M. P. (2023). Fecal microbiota transplantation: history, procedure and regulatory considerations. *Presse Med.* 52:104204. doi: 10.1016/j.lpm.2023.104204

D'Mello, C., and Swain, M. G. (2016). Immune-to-brain communication pathways in inflammation-associated sickness and depression. *Curr. Top. Behav. Neurosci.* 31, 73–94. doi: 10.1007/7854-2016-37

Das, P., Babaei, P., and Nielsen, J. (2019). Metagenomic analysis of microbe-mediated vitamin metabolism in the human gut microbiome. *BMC Genomics*. 20:208. doi: 10.1186/s12864-019-5591-7

Dicks, L. M. T. (2022). Gut Bacteria and neurotransmitters. *Microorganisms*. 10:1838. doi: 10.3390/microorganisms10091838

Dohnalová, L., Lundgren, P., Carty, J. R. E., Goldstein, N., Wenski, S. L., Nanudorn, P., et al. (2022). A microbiome-dependent gut–brain pathway regulates motivation for exercise. *Nature*. 612, 739–747. doi: 10.1038/s41586-022-05525-z

dos Santos, W. O., Juliano, V. A. L., Chaves, F. M., Vieira, H. R., Frazao, R., List, E. O., et al. (2023). Growth hormone action in somatostatin neurons regulates anxiety and fear memory. *J. Neurosci.* 43, 6816–6829. doi: 10.1523/jneurosci.0254-23.2023

Evrensel, A., Ünsalver, B. Ö., and Ceylan, M. E. (2020). Immune-kynurenine pathways and the gut microbiota-brain Axis in anxiety disorders. *Adv. Exp. Med. Biol.* 1191, 155–167. doi: 10.1007/978-981-32-9705-0_10

Felger, J. C. (2018). Imaging the role of inflammation in mood and anxiety-related disorders. *Curr. Neuropharmacol.* 16, 533–558. doi: 10.2174/1570159x15666171123201142

Feng, S., Meng, C., Liu, Y., Yi, Y., Liang, A., Zhang, Y., et al. (2023). *Bacillus licheniformis* prevents and reduces anxiety-like and depression-like behaviours. *Appl. Microbiol. Biotechnol.* 107, 4355–4368. doi: 10.1007/s00253-023-12580-7

Fernández-Pérez, L., de Mirecki-Garrido, M., Guerra, B., Díaz, M., and Díaz-Chico, J. C. (2016). Sex steroids and growth hormone interactions. *Endocrinol. Nutr.* 63, 171–180. doi: 10.1016/j.endonu.2015.11.004

Fishbein, S. R. S., Mahmud, B., and Dantas, G. (2023). Antibiotic perturbations to the gut microbiome. *Nat. Rev. Microbiol.* 21, 772–788. doi: 10.1038/s41579-023-00933-y

Fitzgerald, P. J., Pinard, C. R., Camp, M. C., Feyder, M., Sah, A., Bergstrom, H. C., et al. (2014). Durable fear memories require PSD-95. *Mol. Psychiatry*. 20, 901–912. doi: 10.1038/mp.2014.161

Frankiensztajn, L. M., Elliott, E., and Koren, O. (2020). The microbiota and the hypothalamus-pituitary-adrenocortical (HPA) axis, implications for anxiety and stress disorders. *Curr. Opin. Neurobiol.* 62, 76–82. doi: 10.1016/j.conb.2019.12.003

Franzosa, E. A., Sirota-Madi, A., Avila-Pacheco, J., Fornelos, N., Haiser, H. J., Reinker, S., et al. (2018). Gut microbiome structure and metabolic activity in inflammatory bowel disease. *Nat. Microbiol.* 4, 293–305. doi: 10.1038/s41564-018-0306-4

Gao, K., Mu, C.-L., Farzi, A., and Zhu, W.-Y. (2020). Tryptophan metabolism: a link between the gut microbiota and brain. *Adv. Nutr.* 11, 709–723. doi: 10.1093/advances/nmz127

Generoso, J. S., Giridharan, V. V., Lee, J., Macedo, D., and Barichello, T. (2021). The role of the microbiota-gut-brain axis in neuropsychiatric disorders. *Brazilian J. Psychiatry*. 43, 293–305. doi: 10.1590/1516-4446-2020-0987

Gomaa, E. Z. (2020). Human gut microbiota/microbiome in health and diseases: a review. *Antonie Van Leeuwenhoek*. 113, 2019–2040. doi: 10.1007/s10482-020-01474-7

Gubert, C., Kong, G., Renoir, T., and Hannan, A. J. (2020). Exercise, diet and stress as modulators of gut microbiota: implications for neurodegenerative diseases. *Neurobiol. Dis.* 134:104621. doi: 10.1016/j.nbd.2019.104621

Gulati, K., Rai, N., and Ray, A. (2017). Nitric Oxide and Anxiety. *Vitamins and hormones*. 103, 169–192. doi: 10.1016/bs.vh.2016.09.001

Hakamata, Y., Mizukami, S., Izawa, S., Okamura, H., Mihara, K., Marusak, H., et al. (2022). Implicit and explicit emotional memory recall in anxiety and depression: role of basolateral amygdala and cortisol-norepinephrine interaction. *Psychoneuroendocrinology*. 136:105598. doi: 10.1016/j.psyneuen.2021.105598

Hedges, V. L., Heaton, E. C., Amaral, C., Benedetto, L. E., Bodie, C. L., D'Antonio, B. I., et al. (2021). Estrogen withdrawal increases postpartum anxiety via oxytocin plasticity in the paraventricular hypothalamus and dorsal raphe nucleus. *Biol. Psychiatry*. 89, 929–938. doi: 10.1016/j.biopsych.2020.11.016

Hendel, S. K., Kellermann, L., Hausmann, A., Bindslev, N., Jensen, K. B., and Nielsen, O. H. (2022). Tuft cells and their role in intestinal diseases. *Front. Immunol.* 13:2867. doi: 10.3389/fimmu.2022.822867

Hou, R., Garner, M., Holmes, C., Osmond, C., Teeling, J., Lau, L., et al. (2017). Peripheral inflammatory cytokines and immune balance in generalised anxiety disorder: case-controlled study. *Brain Behav. Immun.* 62, 212–218. doi: 10.1016/j.bbi.2017.01.021

Hu, S., Ding, Q., Zhang, W., Kang, M., Ma, J., and Zhao, L. (2023). Gut microbial beta-glucuronidase: a vital regulator in female estrogen metabolism. *Gut Microbes*. 15:6749. doi: 10.1080/19490976.2023.2236749

Huang, F., and Wu, X. (2021). Brain neurotransmitter modulation by gut microbiota in anxiety and depression. *Front. Cell Dev. Biol.* 9:649103. doi: 10.3389/fcell.2021.649103

Jennis, M., Cavanaugh, C. R., Leo, G. C., Mabus, J. R., Lenhard, J., and Hornby, P. J. (2017). Microbiota-derived tryptophan indoles increase after gastric bypass surgery and reduce intestinal permeability in vitro and in vivo. *Neurogastroenterol. Motility*. 30:3178. doi: 10.1111/nmo.13178

Jensen, E. A., Young, J. A., Mathes, S. C., List, E. O., Carroll, R. K., Kuhn, J., et al. (2020). Crosstalk between the growth hormone/insulin-like growth factor-1 axis and the gut microbiome: a new frontier for microbial endocrinology. *Growth Hormon. IGF Res.* 53-54:101333. doi: 10.1016/j.ghir.2020.101333

Jiang, H. Y., Zhang, X., Yu, Z. H., Zhang, Z., Deng, M., Zhao, J. H., et al. (2018). Altered gut microbiota profile in patients with generalized anxiety disorder. *J. Psychiatr. Res.* 104, 130–136. doi: 10.1016/j.jpsychires.2018.07.007

Karachaliou, F.-H., Karavanaki, K., Simatou, A., Tsintzou, E., Skarakis, N. S., and Kanaka-Gatenbein, C. (2021). Association of growth hormone deficiency (GHD) with anxiety and depression: experimental data and evidence from GHD children and adolescents. *Hormones*. 20, 679–689. doi: 10.1007/s42000-021-00306-1

Kennedy, D. (2016). B vitamins and the brain: mechanisms, dose and efficacy—a review. *Nutrients*. 8:68. doi: 10.3390/nu8020068

Kim, S.-K., Guevarra, R. B., Kim, Y.-T., Kwon, J., Kim, H., Cho, J. H., et al. (2019). Role of probiotics in human gut microbiome-associated diseases. *J. Microbiol. Biotechnol.* 29, 1335–1340. doi: 10.4014/jmb.1906.06064

Kim, Y.-K., and Jeon, S. W. (2018). Neuroinflammation and the immune-kynurenine pathway in anxiety disorders. *Curr. Neuropharmacol.* 16, 574–582. doi: 10.2174/1570159x15666170913110426

Kim, H., Kim, H., Suh, H. J., and Choi, H.-S. (2024). *Lactobacillus brevis*-fermented gamma-aminobutyric acid ameliorates depression- and anxiety-like behaviors by activating the brain-derived neurotrophic factor-tropomyosin receptor kinase B signaling pathway in BALB/C mice. *J. Agric. Food Chem.* 72, 2977–2988. doi: 10.1021/acs.jafc.3c07260

Klarer, M., Arnold, M., Günther, L., Winter, C., Langhans, W., and Meyer, U. (2014). Gut vagal afferents differentially modulate innate anxiety and learned fear. *J. Neurosci.* 34, 7067–7076. doi: 10.1523/jneurosci.0252-14.2014

Knezevic, J., Starchl, C., Tmava Berisha, A., and Amrein, K. (2020). Thyroid-gut-Axis: how does the microbiota influence thyroid function? *Nutrients*. 12:1769. doi: 10.3390/nu12061769

Koopman, N., Katsavelis, D., Ten Hove, A., Brul, S., de Jonge, W., and Seppen, J. (2021). The multifaceted role of serotonin in intestinal homeostasis. *Int. J. Mol. Sci.* 22:9487. doi: 10.3390/ijms22179487

Krieger, J.-P., Asker, M., van der Velden, P., Börchers, S., Richard, J. E., Maric, I., et al. (2022). Neural pathway for gut feelings: vagal interoceptive feedback from the gastrointestinal tract is a critical modulator of anxiety-like behavior. *Biol. Psychiatry*. 92, 709–721. doi: 10.1016/j.biopsych.2022.04.020

Kucharska, A. M., Witkowska-Sędek, E., Rumińska, M., and Pyrżak, B. (2021). Thyroid hormone changes related to growth hormone therapy in growth hormone deficient patients. *J. Clin. Med.* 10:5354. doi: 10.3390/jcm10225354

Leistner, C., and Menke, A. (2020). Hypothalamic-pituitary-adrenal axis and stress. *Handb. Clin. Neurol.* 175, 55–64. doi: 10.1016/B978-0-444-64123-6.00004-7

Li, K., Feng, Z., Xiong, Z., Pan, J., Zhou, M., Li, W., et al. (2023). Growth hormone promotes the reconstruction of injured axons in the hypothalamo-neurohypophyseal system. *Neural Regen. Res.* 19, 2249–2258. doi: 10.4103/1673-5374.389358

Li, D., Liu, R., Wang, M., Peng, R., Fu, S., Fu, A., et al. (2022). 3β-hydroxysteroid dehydrogenase expressed by gut microbes degrades testosterone and is linked to depression in males. *Cell Host Microbe*. 30, 329–339.e5. doi: 10.1016/j.chom.2022.01.001

Lim, C. K., Fernández-Gomez, F. J., Braidy, N., Estrada, C., Costa, C., Costa, S., et al. (2017). Involvement of the kynurenine pathway in the pathogenesis of Parkinson's disease. *Prog. Neurobiol.* 155, 76–95. doi: 10.1016/j.pneurobio.2015.12.009

Liu, S., Cao, R., Liu, L., Lv, Y., Qi, X., Yuan, Z., et al. (2022). Correlation between gut microbiota and testosterone in male patients with type 2 diabetes mellitus. *Front. Endocrinol.* 13:836485. doi: 10.3389/fendo.2022.836485

Liu, C., Zhang, L., Wu, J., Sui, X., Xu, Y., Huang, L., et al. (2017). AnkG hemizygous mice present cognitive impairment and elevated anxiety/depressive-like traits associated with decreased expression of GABA receptors and postsynaptic density protein. *Exp. Brain Res.* 235, 3375–3390. doi: 10.1007/s00221-017-5056-7

Luo, J., Chen, Z., Castellano, D., Bao, B., Han, W., Li, J., et al. (2023). Lipids regulate peripheral serotonin release via gut CD1d. *Immunity*. 56, 1533–1547.e7. doi: 10.1016/j.immuni.2023.06.001

MahmoudianDehkordi, S., Bhattacharyya, S., Brydges, C. R., Jia, W., Fiehn, O., Rush, A. J., et al. (2022). Gut microbiome-linked metabolites in the pathobiology of major depression with or without anxiety—a role for bile acids. *Front. Neurosci.* 16:7906. doi: 10.3389/fnins.2022.937906

Matsushita, M., Fujita, K., Motooka, D., Hatano, K., Hata, J., Nishimoto, M., et al. (2022). Firmicutes in gut microbiota correlate with blood testosterone levels in elderly men. *World J. Mens Health*. 40, 517–525. doi: 10.5534/wjmh.210190

McHenry, J., Carrier, N., Hull, E., and Kabbaj, M. (2014). Sex differences in anxiety and depression: role of testosterone. *Front. Neuroendocrinol.* 35, 42–57. doi: 10.1016/j.yfrne.2013.09.001

Minkoff, N. Z., Aslam, S., Medina, M., Tanner-Smith, E. E., Zackular, J. P., Acra, S., et al. (2023). Fecal microbiota transplantation for the treatment of recurrent Clostridioides difficile (*Clostridium difficile*). *Cochrane Database Syst. Rev.* 4:CD013871. doi: 10.1002/14651858.CD013871.pub2

Nasir, M., Trujillo, D., Levine, J., Dwyer, J. B., Rupp, Z. W., and Bloch, M. H. (2020). Glutamate systems in DSM-5 anxiety disorders: their role and a review of glutamate and GABA psychopharmacology. *Front. Psych.* 11:548505. doi: 10.3389/fpsyt.2020.548505

Navarro-Tapia, E., Almeida-Toledano, L., Sebastiani, G., Serra-Delgado, M., García-Algar, Ó., and Andreu-Fernández, V. (2021). Effects of microbiota imbalance in anxiety and eating disorders: probiotics as novel therapeutic approaches. *Int. J. Mol. Sci.* 22:2351. doi: 10.3390/ijms22052351

Needham, B. D., Funabashi, M., Adame, M. D., Wang, Z., Boktor, J. C., Haney, J., et al. (2022). A gut-derived metabolite alters brain activity and anxiety behaviour in mice. *Nature*. 602, 647–653. doi: 10.1038/s41586-022-04396-8

Neufeld, K. A., Kang, N., Bienenstock, J., and Foster, J. A. (2011). Effects of intestinal microbiota on anxiety-like behavior. *Commun Integr Biol.* 4, 492–494. doi: 10.4161/cib.15702

Nguyen, C., Mondoloni, S., Le Borgne, T., Centeno, I., Come, M., Jehl, J., et al. (2021). Nicotine inhibits the VTA-to-amygdala dopamine pathway to promote anxiety. *Neuron*. 109, 2604–2615.e9. doi: 10.1016/j.neuron.2021.06.013

Noonan, S., Zaveri, M., Macaninch, E., and Martyn, K. (2020). Food and mood: a review of supplementary prebiotic and probiotic interventions in the treatment of anxiety and depression in adults. *BMJ Nutr. Prevent Health*. 3, 351–362. doi: 10.1136/bmjnph-2019-000053

O'Callaghan, T. F., Ross, R. P., Stanton, C., and Clarke, G. (2016). The gut microbiome as a virtual endocrine organ with implications for farm and domestic animal endocrinology. *Domest. Anim. Endocrinol.* 56, S44–S55. doi: 10.1016/j.domaniend.2016.05.003

O'Riordan, K. J., Collins, M. K., Moloney, G. M., Knox, E. G., Aburto, M. R., Fülling, C., et al. (2022). Short chain fatty acids: microbial metabolites for gut-brain axis signalling. *Mol. Cell. Endocrinol.* 546:111572. doi: 10.1016/j.mce.2022.111572

Parada Venegas, D., De la Fuente, M. K., Landskron, G., González, M. J., Quera, R., Dijkstra, G., et al. (2019). Short chain fatty acids (SCFAs)-mediated gut epithelial and immune regulation and its relevance for inflammatory bowel diseases. *Front. Immunol.* 10:277. doi: 10.3389/fimmu.2019.00277

Peirce, J. M., and Alviña, K. (2019). The role of inflammation and the gut microbiome in depression and anxiety. *J. Neurosci. Res.* 97, 1223–1241. doi: 10.1002/jnr.24476

Peng, X., Luo, Z., He, S., Zhang, L., and Li, Y. (2021). Blood-brain barrier disruption by lipopolysaccharide and Sepsis-associated encephalopathy. *Front. Cell. Infect. Microbiol.* 11:768108. doi: 10.3389/fcimb.2021.768108

Platten, M., Nollen, E. A. A., Röhrig, U. F., Fallarino, F., and Opitz, C. A. (2019). Tryptophan metabolism as a common therapeutic target in cancer, neurodegeneration and beyond. *Nat. Rev. Drug Discov.* 18, 379–401. doi: 10.1038/s41573-019-0016-5

Quaresma, P. G. F., dos Santos, W. O., Wasinski, F., Metzger, M., and Donato, J. (2020). Neurochemical phenotype of growth hormone-responsive cells in the mouse paraventricular nucleus of the hypothalamus. *J. Comp. Neurol.* 529, 1228–1239. doi: 10.1002/cne.25017

Rau, S., Gregg, A., Yaceczko, S., and Limketkai, B. (2024). Prebiotics and probiotics for gastrointestinal disorders. *Nutrients*. 16:778. doi: 10.3390/nu16060778

Ren, Y. (2016). Research Progress of the antidepressants targeting the Monomine receptors and glutamate system. *Pharmacy Informat.* 5, 38–44. doi: 10.12677/pi.2016.52007

Renczés, E., Borbélyová, V., Steinhardt, M., Höpfner, T., Stehle, T., Ostatníková, D., et al. (2020). The role of estrogen in anxiety-like behavior and memory of middle-aged female rats. *Front. Endocrinol.* 11:570560. doi: 10.3389/fendo.2020.570560

Riaza Bermudo-Soriano, C., Perez-Rodriguez, M. M., Vaquero-Lorenzo, C., and Baca-Garcia, E. (2012). New perspectives in glutamate and anxiety. *Pharmacol. Biochem. Behav.* 100, 752–774. doi: 10.1016/j.pbb.2011.04.010

Rich, B. E., Jackson, J. C., de Ora, L. O., Long, Z. G., Uyeda, K. S., and Bess, E. N. (2022). Alternative pathway for dopamine production by acetogenic gut bacteria that O-demethylate 3-methoxytyramine, a metabolite of catechol O-methyltransferase. *J. Appl. Microbiol.* 133, 1697–1708. doi: 10.1111/jam.15682

Roager, H. M., and Licht, T. R. (2018). Microbial tryptophan catabolites in health and disease. *Nat. Commun.* 9:3294. doi: 10.1038/s41467-018-05470-4

Ross, F. C., Mayer, D. E., Gupta, A., Gill, C. I. R., Del Rio, D., Cryan, J. F., et al. (2024). Existing and future strategies to manipulate the gut microbiota with diet as a potential adjuvant treatment for psychiatric disorders. *Biol. Psychiatry*. 95, 348–360. doi: 10.1016/j.biopsych.2023.10.018

Sağlam, C., Turan, İ., and Özaçmak, H. S. (2022). The effect of glucagon like peptide-1 receptor agonist on behavioral despair and anxiety-like behavior in ovariectomized rats: modulation of BDNF/CREB, Nrf2 and lipocalin 2. *Behav. Brain Res.* 435:114053. doi: 10.1016/j.bbr.2022.114053

Santos-Marcos, J. A., Mora-Ortiz, M., Tena-Sempere, M., Lopez-Miranda, J., and Camargo, A. (2023). Interaction between gut microbiota and sex hormones and their relation to sexual dimorphism in metabolic diseases. *Biol. Sex Differ.* 14:4. doi: 10.1186/s13293-023-00490-2

Sasaki, T., Kuboyama, A., Mita, M., Murata, S., Shimizu, M., Inoue, J., et al. (2018). The exercise-inducible bile acid receptor Tgr5 improves skeletal muscle function in mice. *J. Biol. Chem.* 293, 10322–10332. doi: 10.1074/jbc.RA118.002733

Savitz, J. (2019). The kynurenine pathway: a finger in every pie. *Mol. Psychiatry*. 25, 131–147. doi: 10.1038/s41380-019-0414-4

Schnorr, S. L., and Bachner, H. A. (2016). Integrative therapies in anxiety treatment with special emphasis on the gut microbiome. *Yale J. Biol. Med.* 89, 397–422

Scholten, W., ten Have, M., van Geel, C., van Balkom, A., de Graaf, R., and Batelaan, N. (2021). Recurrence of anxiety disorders and its predictors in the general population. *Psychol. Med.* 53, 1334–1342. doi: 10.1017/s0033291721002877

Sciurba, J. D., Chlipala, G. E., Green, S. J., Delaney, M. A., Fortman, J. D., and Purcell, J. E. (2021). Evaluation of effects of laboratory disinfectants on mouse gut microbiota. *Comp. Med.* 71, 492–501. doi: 10.30802/aalas-cm-21-000051

Shao, S., Li, J., Chen, S., Dong, Y., Wang, S., Zhu, Z., et al. (2021). Sex-dependent expression of N-cadherin-GluA1 pathway-related molecules in the prefrontal cortex mediates anxiety-like behavior in male offspring following prenatal stress. *Stress*. 24, 612–620. doi: 10.1080/10253890.2021.1942829

Songtachalert, T., Roomruangwong, C., Carvalho, A. F., Bourin, M., and Maes, M. (2018). Anxiety disorders: sex differences in serotonin and tryptophan metabolism. *Curr. Top. Med. Chem.* 18, 1704–1715. doi: 10.2174/1568026618666181115093136

Strandwitz, P. (2018). Neurotransmitter modulation by the gut microbiota. *Brain Res.* 1693, 128–133. doi: 10.1016/j.brainres.2018.03.015

Suárez-Pereira, I., Llorca-Torralba, M., Bravo, L., Camarena-Delgado, C., Soriano-Mas, C., and Berrocoso, E. (2022). The role of the locus Coeruleus in pain and associated stress-related disorders. *Biol. Psychiatry*. 91, 786–797. doi: 10.1016/j.biopsych.2021.11.023

Szuhany, K. L., and Simon, N. M. (2022). Anxiety Disorders. *JAMA*. 328:2431. doi: 10.1001/jama.2022.22744

Tafet, G. E., and Nemeroff, C. B. (2020). Pharmacological treatment of anxiety disorders: the role of the HPA Axis. *Front. Psych.* 11:443. doi: 10.3389/fpsyt.2020.00443

Taylor, A. M., and Holscher, H. D. (2018). A review of dietary and microbial connections to depression, anxiety, and stress. *Nutr. Neurosci.* 23, 237–250. doi: 10.1080/1028415x.2018.1493808

Vargas-Caraveo, A., Sayd, A., Maus, S. R., Caso, J. R., Madrigal, J. L. M., García-Bueno, B., et al. (2017). Lipopolysaccharide enters the rat brain by a lipoprotein-mediated transport mechanism in physiological conditions. *Sci. Rep.* 7:13113. doi: 10.1038/s41598-017-13302-6

Wang, J.-F., Fu, S.-P., Li, S.-N., Hu, Z.-M., Xue, W.-J., Li, Z.-Q., et al. (2013). Short-chain fatty acids inhibit growth hormone and prolactin gene transcription via cAMP/PKA/CREB signaling pathway in dairy cow anterior pituitary cells. *Int. J. Mol. Sci.* 14, 21474–21488. doi: 10.3390/ijms141121474

Wang, Y., Tong, Q., Ma, S.-R., Zhao, Z.-X., Pan, L.-B., Cong, L., et al. (2021). Oral berberine improves brain dopa/dopamine levels to ameliorate Parkinson's disease by regulating gut microbiota. *Signal Transduct. Target. Ther.* 6:77. doi: 10.1038/s41392-020-00456-5

Wiley, N. C., Cryan, J. F., Dinan, T. G., Ross, R. P., and Stanton, C. (2021). Production of psychoactive metabolites by gut bacteria. *Mod. trends psychiatry*. 32, 74–99. doi: 10.1159/000510419

Xia, G., Han, Y., Meng, F., He, Y., Srisai, D., Farias, M., et al. (2021). Reciprocal control of obesity and anxiety–depressive disorder via a GABA and serotonin neural circuit. *Mol. Psychiatry.* 26, 2837–2853. doi: 10.1038/s41380-021-01053-w

Xie, L., Zhao, H., and Chen, W. (2023). Relationship between gut microbiota and thyroid function: a two-sample Mendelian randomization study. *Front. Endocrinol.* 14:1240752. doi: 10.3389/fendo.2023.1240752

Xing, C., Huang, X., Wang, D., Yu, D., Hou, S., Cui, H., et al. (2023). Roles of bile acids signaling in neuromodulation under physiological and pathological conditions. *Cell Biosci.* 13:106. doi: 10.1186/s13578-023-01053-z

Xiong, R. G., Li, J., Cheng, J., Zhou, D. D., Wu, S. X., Huang, S. Y., et al. (2023). The role of gut microbiota in anxiety, depression, and other mental disorders as well as the protective effects of dietary components. *Nutrients*. 15:3258. doi: 10.3390/nu15143258

Yan, X., Jin, J., Su, X., Yin, X., Gao, J., Wang, X., et al. (2020). Intestinal Flora modulates blood pressure by regulating the synthesis of intestinal-derived corticosterone in high salt-induced hypertension. *Circ. Res.* 126, 839–853. doi: 10.1161/circresaha.119.316394

Yang, J.-Z., Zhang, K.-K., He, J.-T., Chen, L.-J., Ding, J.-F., Liu, J.-L., et al. (2023). Obeticholic acid protects against methamphetamine-induced anxiety-like behavior by ameliorating microbiota-mediated intestinal barrier impairment. *Toxicology.* 486:153447. doi: 10.1016/j.tox.2023.153447

Yin, W., Swanson, S. P., Biltz, R. G., Goodman, E. J., Gallagher, N. R., Sheridan, J. F., et al. (2022). Unique brain endothelial profiles activated by social stress promote cell adhesion, prostaglandin E2 signaling, hypothalamic–pituitary–adrenal axis modulation, and anxiety. *Neuropsychopharmacology*. 47, 2271–2282. doi: 10.1038/s41386-022-01434-x

Yu, D., Zhou, H., Yang, Y., Jiang, Y., Wang, T., Lv, L., et al. (2015). The bidirectional effects of hypothyroidism and hyperthyroidism on anxiety- and depression-like behaviors in rats. *Horm. Behav.* 69, 106–115. doi: 10.1016/j.yhbeh.2015.01.003

Zhang, K., Chen, L., Yang, J., Liu, J., Li, J., Liu, Y., et al. (2023). Gut microbiota-derived short-chain fatty acids ameliorate methamphetamine-induced depression- and anxiety-like behaviors in a Sigmar-1 receptor-dependent manner. *Acta Pharm. Sin. B* 13, 4801–4822. doi: 10.1016/j.apsb.2023.09.010

Zhang, J., Ma, L., Chang, L., Pu, Y., Qu, Y., and Hashimoto, K. (2020). A key role of the subdiaphragmatic vagus nerve in the depression-like phenotype and abnormal composition of gut microbiota in mice after lipopolysaccharide administration. *Transl. Psychiatry*. 10:186. doi: 10.1038/s41398-020-00878-3

frontiers | Frontiers in Neuroscience

TYPE Review
PUBLISHED 06 February 2025
DOI 10.3389/fnins.2025.1541075

OPEN ACCESS

EDITED BY
Sudeepa Bhattacharyya,
Arkansas State University, United States

REVIEWED BY
Qiuyue Xu,
Nanjing University of Chinese Medicine, China
Ameer Luqman,
Chongqing University, China

*CORRESPONDENCE
Zuleide Maria Ignácio
✉ zuleideignacio@gmail.com

RECEIVED 06 December 2024
ACCEPTED 16 January 2025
PUBLISHED 06 February 2025

CITATION
Bertollo AG, Santos CF, Bagatini MD and Ignácio ZM (2025) Hypothalamus-pituitary-adrenal and gut-brain axes in biological interaction pathway of the depression.
Front. Neurosci. 19:1541075.
doi: 10.3389/fnins.2025.1541075

Hypothalamus-pituitary-adrenal and gut-brain axes in biological interaction pathway of the depression

Amanda Gollo Bertollo, Camila Ferreira Santos, Margarete Dulce Bagatini and Zuleide Maria Ignácio *

Laboratory of Physiology Pharmacology and Psychopathology, Graduate Program in Biomedical Sciences, Federal University of Fronteira Sul, Chapecó, Brazil

The hypothalamus-pituitary-adrenal (HPA) and gut-brain axes are vital biological pathways in depression. The HPA axis regulates the body's stress response, and chronic stress can lead to overactivation of the HPA axis, resulting in elevated cortisol levels that contribute to neuronal damage, particularly in regions such as the hippocampus and prefrontal cortex, both of which are involved in mood regulation and mental disorders. In parallel, the gut-brain axis, a bidirectional communication network between the gut microbiota and the central nervous system, influences emotional and cognitive functions. Imbalances in gut microbiota can affect the HPA axis, promoting inflammation and increasing gut permeability. This allows endotoxins to enter the bloodstream, contributing to neuroinflammation and altering neurotransmitter production, including serotonin. Since the majority of serotonin is produced in the gut, disruptions in this pathway may be linked to depressive symptoms. This review explores the interplay between the HPA axis and the gut-brain axis in the context of depression.

KEYWORDS

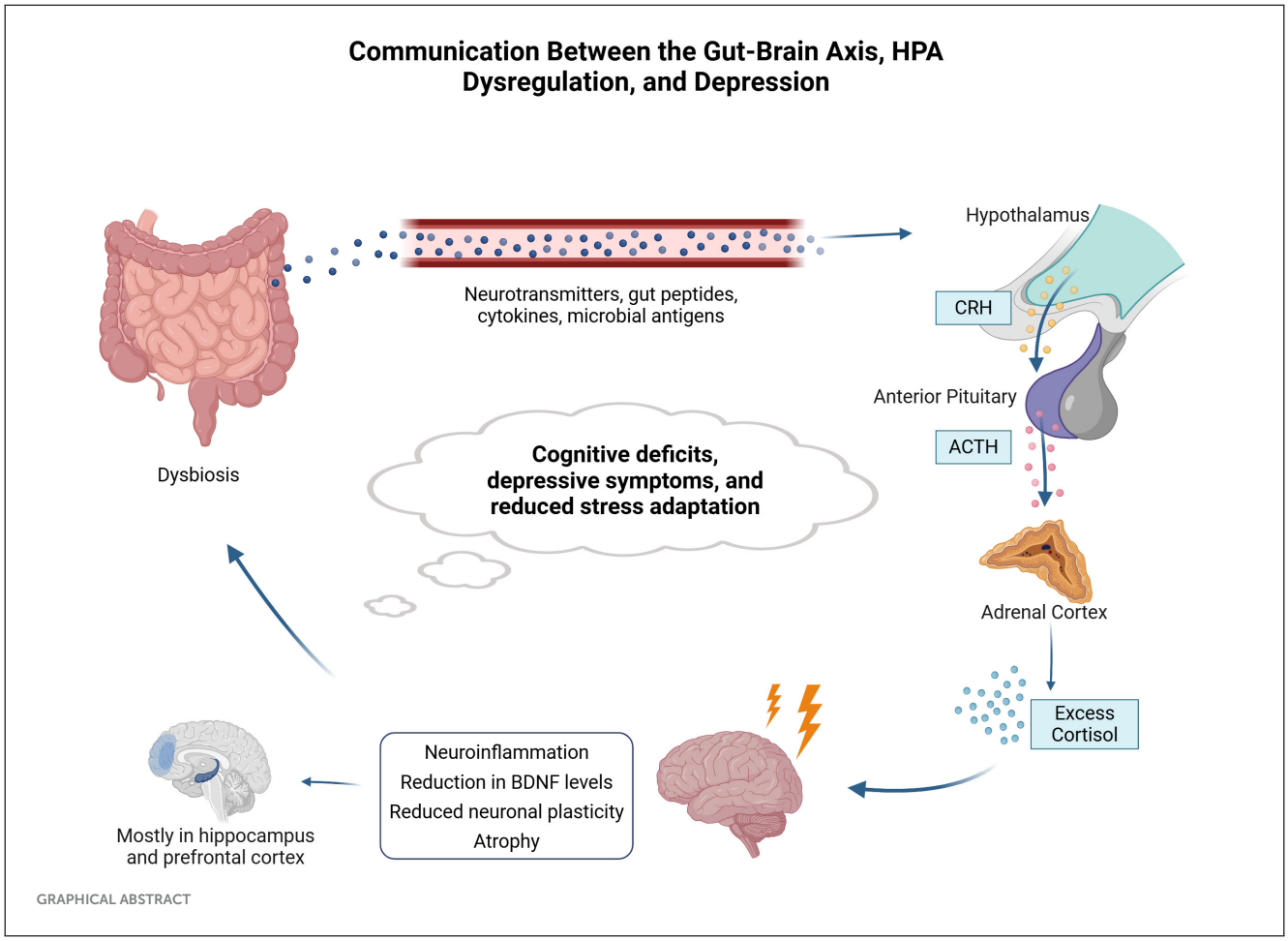
hypothalamus-pituitary-adrenal axis, gut-brain axis, gut microbiota, depression, stress response

Introduction

Major Depressive Disorder (MDD) is a complex psychiatric condition that affects millions of people worldwide, with a significant impact on social and occupational functioning (Kessler and Bromet, 2013). It is characterized by a profound sense of sadness, a marked loss of interest or pleasure in previously moderate activities, and a range of cognitive and physical symptoms, such as fatigue, feelings of worthlessness, difficulty concentrating, and changes in appetite and sleep (American Psychiatric Association, 2022). These symptoms lead to severe impairments in quality of life, making MDD a leading cause of disability globally (World Health Organization, 2023).

Recent advances have expanded our understanding of MDD beyond the traditional focus on neurotransmitter imbalances, particularly dysregulation of serotonin and dopamine. The modern view includes a broader analysis of the gut-brain axis (GBA) and the hypothalamic-pituitary-adrenal (HPA) axis, as well as the mechanisms underlying the inflammatory response that may contribute to the development and persistence of depressive symptoms (Otte et al., 2016).

The HPA axis plays a crucial role in the body's stress response by regulating cortisol release. Chronic dysregulation of the HPA axis, often seen in individuals with MDD,



results in prolonged exposure to cortisol, leading to atrophy of the hippocampus, a brain region involved in mood regulation (Pariante and Lightman, 2008). This neuroendocrine pathway has been shown to interact with inflammatory processes and oxidative stress, further exacerbating the depressive symptoms and cognitive impairments associated with MDD (Zunszain et al., 2013).

In patients with MDD, hyperactivity of the HPA axis is frequently observed, resulting in elevated and persistent cortisol levels. While cortisol is necessary for an adaptive stress response, its chronic and excessive production can have neurotoxic effects, including reduced hippocampal volume, a brain area crucial for emotional regulation and memory. The hippocampus is a central brain structure for emotional regulation and memory, and its atrophy is associated with impairing these functions in people with depression (Videbech and Ravnkilde, 2004; Knezevic et al., 2023).

Furthermore, under normal conditions, high cortisol levels should activate harmful feedback mechanisms to inhibit further production of this hormone. However, in individuals with MDD, there is evidence that this negative feedback is altered, resulting in sustained activation of the HPA axis and perpetuation of the hypercortisolism state (Holsen et al., 2011). This prolonged state of HPA axis activation contributes to a series of physiological consequences, including amplifying the inflammatory response, which, as previously mentioned, affects neuroplasticity and exacerbates depressive symptoms (Kinlein et al., 2015).

The GBA is a bidirectional communication pathway between the gastrointestinal tract and the central nervous system (CNS). This axis involves a complex network of neurochemical, immunological, and hormonal interactions that allow the gut and brain to communicate continuously and dynamically. Gut dysbiosis, an imbalance in the microbial composition in the gut (Sorboni et al., 2022), has been linked to altered neurotransmitter production and increased inflammation, which have been implicated in depressive symptoms (Kelly et al., 2016). Furthermore, changes in the gut microbiota can activate the immune system, increasing pro-inflammatory cytokines that may disrupt normal brain function and contribute to MDD (Cryan and Dinan, 2012).

The gut microbiome plays a fundamental role in GBA interaction, influencing digestive health, brain function, and human behavior. Recent studies indicate that the gut microbiome, composed of trillions of microorganisms, not only participates in the digestion of food but also synthesizes neurotransmitters such as serotonin, dopamine, and GABA (gamma-aminobutyric acid), which are crucial for regulating mood and anxiety, two aspects closely related to MDD (Dinan and Cryan, 2017). For example, about 90% of the body's serotonin is produced in the gut, highlighting the importance of this pathway for mental health (Yano et al., 2015).

Intestinal dysbiosis, an imbalance in the composition of gut bacteria, has been consistently associated with the development

of mood disorders, including MDD (Sampson and Mazmanian, 2015). This imbalance can increase intestinal permeability, allowing lipopolysaccharides (LPS), components of bacterial cell walls, to enter the systemic circulation. This triggers a chronic inflammatory response that can have deleterious effects on the CNS. Pro-inflammatory cytokines such as interleukin (IL)-6 and tumor necrosis factor-alpha (TNF-α) can cross the blood-brain barrier, negatively influencing neuroplasticity and neuronal function, thereby contributing to depressive symptomatology (Kouba et al., 2024; Liu F. et al., 2024).

The GBA and HPA axes do not function in isolation. On the contrary, they are interconnected, and their interaction is central to understanding MDD. Chronic inflammation, promoted by intestinal dysbiosis and prolonged HPA axis activation, plays a central role in the pathophysiology of MDD. This systemic inflammation can promote neuroinflammation, negatively affecting the neural networks involved in mood regulation, memory, and cognition (Miller and Raison, 2016). Inflammation can also compromise the integrity of the blood-brain barrier, allowing inflammatory molecules, such as cytokines, to enter the brain and directly impact neurons and glial cells (Varatharaj and Galea, 2017).

Thus, the complex interaction between the GBA and HPA axes and the underlying mechanisms offers a more holistic view of the MDD pathophysiology. This expanded understanding suggests integrative therapeutic approaches may be more effective, focusing on the CNS and considering gut health and stress regulation. Interventions such as modulation of the gut microbiome through specific diets, probiotics, and prebiotics, as well as strategies to normalize HPA axis function, such as stress reduction techniques and cognitive-behavioral therapy, offer new possibilities for treating and preventing MDD. Thus, a multidisciplinary approach that considers the complex interactions between body and mind may be the key to effectively managing this debilitating condition.

Major depressive disorder

MDD is a debilitating psychiatric condition that affects a significant portion of the world's population and is considered one of the leading causes of disability and reduced quality of life (Ferrari et al., 2013). In addition to symptoms such as intense sadness, lack of interest in previously pleasurable activities, fatigue, and changes in appetite and sleep, depression can lead to severe cognitive and socioeconomic impairments. The public health impacts of MDD are broad, including increased use of health services, absenteeism, loss of productivity, and an elevated risk of suicide, especially among young adults (Siu et al., 2016).

WHO projections indicate that, by 2030, depression could become the most prevalent disease globally (Liu et al., 2020). In 2015, more than 80% of depression-related deaths occurred in low- and middle-income countries, where MDD accounted for 25.3 and 33.5% of years lost due to disability, respectively (Corea Del Cid, 2021). This scenario underscores the importance of effective and accessible interventions, especially in regions with limited resources.

In addition to directly impacting individuals' health, MDD imposes a substantial economic burden on society. Direct costs include medical treatment and prolonged care, while indirect costs are associated with loss of productivity and absenteeism. The global economic impact of depression is estimated at trillions of dollars per year (Corea Del Cid, 2021), highlighting the urgent need to develop and implement more effective prevention and treatment strategies.

The understanding of MDD's pathophysiological mechanisms has evolved considerably, highlighting a multifactorial etiology involving the interaction of genetic, neurobiological, psychosocial, and environmental factors. Traditionally, depression has been associated with imbalances in brain neurotransmitters such as serotonin, norepinephrine, and dopamine, which are essential for regulating mood, sleep, and appetite. However, the variability in response to antidepressant treatment that modulates these neurotransmitters suggests that other biological pathways also play a crucial role in the development of MDD. Freudian psychoanalysis, for example, conceptualizes depression as a state akin to mourning, characterized by a reduction in self-esteem, disinterest in the external world, loss of the capacity to love, and inhibition of productivity (Corea Del Cid, 2021).

One of the most widely accepted theories today is that MDD is related to alterations in synaptic plasticity, or the brain's ability to strengthen or weaken connections between neurons in response to experiences. Neuroplasticity is fundamental for emotional adaptation and learning. Chronic stress, a known risk factor for depression, can compromise this plasticity by reducing the levels of neurotrophic factors such as brain-derived neurotrophic factor (BDNF). Studies indicate that low BDNF levels are associated with hippocampal atrophy, a brain region crucial for emotional regulation and memory, in people with MDD (Duman et al., 2016).

Another important aspect of MDD pathophysiology involves systemic inflammation and neuroinflammation. Patients with MDD often present elevated levels of inflammatory markers such as C-reactive protein (CRP), IL-6, and TNF-α. Chronic inflammation can affect the brain by crossing the blood-brain barrier and inducing an inflammatory response within the CNS. This neuroinflammation can damage neurons, compromise synaptic plasticity, and alter neurotransmitter function, exacerbating depressive symptoms (Miller and Raison, 2016).

Oxidative stress also plays a crucial role in the pathophysiology of MDD. It results from an imbalance between reactive oxygen species (ROS) production and the body's ability to neutralize them with antioxidants. The excessive accumulation of ROS can damage lipids, proteins, and DNA in brain cells, contributing to neuronal dysfunction and cell death, processes strongly associated with depressive symptoms (Maes et al., 2011).

These advances in understanding the underlying mechanisms of MDD expand scientific knowledge and point to potential therapeutic targets for developing new treatment approaches that may be more effective and tailored to the needs of affected individuals.

Hypothalamus-pituitary-adrenal axis

The HPA axis plays a central role in the body's stress response and mood regulation, fundamental for understanding the biological mechanisms involved in depression. The HPA axis is

activated when the hypothalamus releases corticotropin-releasing hormone (CRH) in response to stress. This hormone stimulates the pituitary gland to secrete adrenocorticotropic hormone (ACTH), which, when circulating through the blood system, stimulates the adrenal glands to release glucocorticoids, such as cortisol, the main stress hormone in humans (Buttenschøn et al., 2017).

A key component of HPA axis regulation is negative feedback, in which cortisol inhibits the production of CRH and ACTH, modulating their levels. Under conditions of chronic stress, this mechanism can break down, generating hyperactivity of the HPA axis, often associated with depression and contributing to symptoms such as apathy, demotivation, and fatigue (Dedovic et al., 2009). Studies suggest that genetic factors may influence susceptibility to HPA axis dysfunction, increasing the predisposition to depression (Buttenschøn et al., 2017).

Prolonged exposure to cortisol can damage structural regions such as the hippocampus, which is critical for memory and learning, and the prefrontal cortex, which is responsible for emotional control and decision-making. Furthermore, the amygdala, which is involved in emotional processing, can become hyperactive, exacerbating symptoms of anxiety and emotional reactivity (Mello et al., 2003; Geerlings and Gerritsen, 2017).

Another critical aspect of HPA axis dysfunction in MDD is the desensitization of glucocorticoid receptors (GR) in target tissues, including the brain. GRs are responsible for mediating the effects of cortisol and play a vital role in the negative feedback of the HPA axis. Desensitization of these receptors can result in an inadequate response to cortisol, further increasing the secretion of this hormone and exacerbating systemic inflammation and neuroinflammation (Pariante, 2004). The dysfunction of the HPA axis can affect several biological mechanisms crucial for mental and physical health. One of the main ones is neurogenesis, the process of forming new neurons in the brain. Elevated cortisol has been associated with suppressing neurogenesis, particularly in the hippocampus. Studies suggest that reduced neurogenesis may contribute to the cognitive and memory deficits often observed in patients with MDD (Lucassen et al., 2010).

Additionally, elevated cortisol can promote inflammation by inducing the production of pro-inflammatory cytokines such as IL-1, IL-6, and TNF-α. These cytokines can cross the blood-brain barrier and contribute to neuroinflammation, which is implicated in the pathogenesis of depression and other neuropsychiatric conditions (Miller and Raison, 2016).

Another significant biological effect of HPA axis dysfunction is the alteration of synaptic plasticity, which is essential for emotional adaptation and learning. Elevated cortisol can interfere with the signaling of BDNF, a protein crucial for the survival and growth of neurons. Reduced levels of BDNF and the consequent decrease in synaptic plasticity have been associated with the severity of depressive symptoms and resistance to antidepressant treatment (Duman and Monteggia, 2006).

The relationship between the HPA axis and the GBA is complex and bidirectional, with significant implications for understanding the pathophysiology of MDD. By activating the HPA axis, chronic stress can lead to alterations in the gut microbiome, promoting dysbiosis and an imbalance in the composition of gut bacteria. Dysbiosis, in turn, can increase intestinal permeability, allowing bacterial components such as LPS to enter the systemic circulation and induce a chronic inflammatory response (Foster and McVey Neufeld, 2013). This systemic inflammation can exacerbate HPA axis dysfunction by increasing the production of pro-inflammatory cytokines, which can cross the blood-brain barrier and directly affect the brain, contributing to neuroinflammation and the perpetuation of depressive symptoms. Moreover, the gut microbiome influences the production of neurotransmitters and other neuroactive substances, such as serotonin, which plays a crucial role in mood regulation and can be modulated by cortisol and inflammatory cytokines (Moreira et al., 2023).

Therefore, HPA axis dysfunction can initiate a negative feedback loop involving both systemic inflammation and alterations in the gut microbiome, exacerbating MDD symptoms and contributing to treatment resistance. Understanding this complex interaction suggests that therapeutic approaches targeting both HPA axis regulation and gut microbiome modulation may be essential to effectively treat MDD.

Gut-brain axis—GBA

The GBA is a bidirectional communication network connecting the gastrointestinal and CNS (Figure 1). It allows for exchanging information and coordinating various physiological processes (Hattori and Yamashiro, 2021). The gut microbiota, the trillions of microorganisms in the human gastrointestinal tract, plays a crucial role in the GBA (Tan, 2023). Healthy gut function has been linked to normal CNS function, as hormones, neurotransmitters, and immunological factors released from the gut can directly or indirectly send signals to the brain via the autonomic nervous system (ANS) (Clapp et al., 2017).

The existence of the GBA was first demonstrated in a landmark study that found impaired stress response in germ-free mice, suggesting that the gut microbiota is essential for developing a normal stress response (Clapp et al., 2017). This axis involves gastrointestinal motor and sensory components sending messages to the CNS, which can influence brain development and function (Hattori and Yamashiro, 2021).

The axis acts through neural, immunological, and endocrine pathways. In the neural pathway, the enteric nervous system (ENS) and the vagus nerve (VN) facilitate communication between the gut and the CNS (Zhu et al., 2017). Additionally, the microbiome can impact the production of neurochemicals such as serotonin, which has been linked to conditions like irritable bowel syndrome (Aszalós, 2008).

Gut bacteria are involved in the production of neurotransmitters like serotonin and GABA. Approximately 90–95% of the body's serotonin is produced in the gastrointestinal tract, primarily by enterochromaffin cells, and gut bacteria can influence this production (Yano et al., 2015). Certain bacteria, such as *Lactobacillus* and *Bifidobacterium*, produce GABA, which helps modulate anxiety and mood (Dinan and Cryan, 2017). Studies have shown that the gut microbiota can modulate the expression and sensitivity of neurotransmitter receptors, impacting emotional responses and stress management (Bruce-Keller et al., 2015). Moreover, the gut microbiota can influence the CNS by producing short-chain fatty acids (SCFAs), which affect neurotransmitter synthesis and receptor activity. SCFAs such as butyrate can enhance

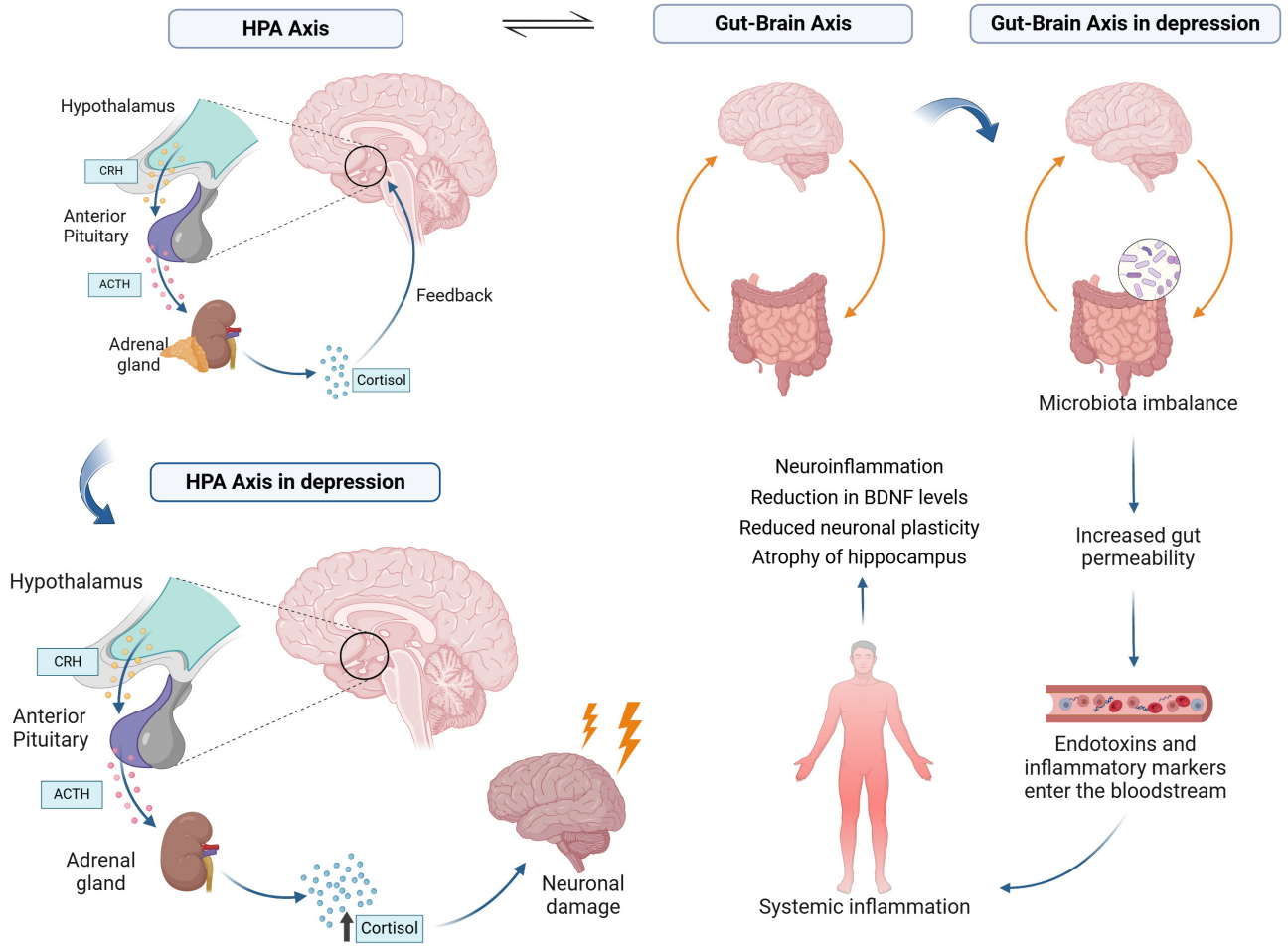


FIGURE 1
The gut-brain axis (GBA) involves bidirectional communication via neural, immune, and endocrine pathways. The neural pathway operates through the vagus nerve and enteric nervous system, influencing brain function via neurotransmitters like serotonin and GABA. The immune pathway involves cytokine signaling from gut microbiota interactions with immune cells. The endocrine pathway includes hormones like ghrelin, leptin, and SCFAs (short-chain fatty acids) affecting mood and cognition. External factors such as diet, stress, and antibiotics modulate these pathways by altering gut microbiota composition.

serotonin production and influence receptor expression (Silva et al., 2020).

Gut peptides such as ghrelin and leptin, which are involved in hunger regulation, also signal the brain. These peptides can influence cognitive functions and mood. Leptin can modulate depressive behavior by crossing the blood-brain barrier to interact with neurons in the hypothalamic arcuate nucleus, affecting the secretion of neuropeptides such as proopiomelanocortin (POMC) and neuropeptide Y (NPY), which play significant roles in mood regulation (Zarouna, 2015). Ghrelin administration in male rats improved mood and cognitive function (Jackson et al., 2019), increasing serotonin activity and receptor expression in key brain areas such as the amygdala and dorsal raphe (Hansson et al., 2014).

The immune system also plays a crucial role, with gut microbiota influencing inflammatory processes in the brain (Giau et al., 2018). Studies demonstrate alterations in the immune system and GBA function in association with various neurological and psychiatric disorders, including neurodevelopmental conditions, neurodegenerative diseases, and psychiatric illnesses (Long-Smith et al., 2020).

Disruptions in this axis, caused by poor diet, excessive antibiotic use, chronic stress, and gastrointestinal diseases, can exacerbate inflammation and depressive symptoms (Zhu et al., 2017; Long-Smith et al., 2020; Tan, 2023). A diet high in fat and sugar can negatively impact the gut microbiota, leading to increased inflammation and changes in brain function. A study by Bruce-Keller et al. found that mice fed a high-fat diet showed signs of inflammation and behavioral changes similar to depression. The research utilized a mouse model, where groups were fed either high-fat or control diets. The study's methodology involved microbiota transplantation from obese donors into germ-free mice and assessments of inflammation and behavioral changes using validated neurobehavioral tests. While not explicitly stated, the sample size likely followed standard experimental protocols for murine studies. The outcomes demonstrated that the high-fat diet induced significant inflammation and depressive-like behaviors, suggesting a gut-brain axis connection (Bruce-Keller et al., 2015).

Another research, by Korpela et al. demonstrated that children who received multiple courses of antibiotics had an increased risk of developing mental health issues. The authors examined the relationship between the intestinal microbiome and lifetime antibiotic use in Finnish preschool children. This research included a cohort of 142 children, with data collected longitudinally. Stool samples were analyzed using high-throughput sequencing to characterize the microbiome composition, while antibiotic usage was tracked through medical records. The findings revealed that children with multiple antibiotic courses exhibited an altered microbiome composition and an elevated risk of mental health issues, including anxiety and depression (Korpela et al., 2016).

The endocrine system and the gut microbiome are intricately linked, with the microbiome considered a full-fledged endocrine organ due to its various effects on the intestinal environment, which influences distant organs and pathways. The microbiota plays a crucial role in the reproductive endocrine system throughout a woman's lifetime by interacting with hormones such as estrogen, androgens, and insulin. Imbalances in gut microbiota composition can lead to several diseases and conditions, including pregnancy complications, adverse pregnancy outcomes, polycystic ovary syndrome (PCOS), endometriosis, and cancer (Qi et al., 2021). The relation between brain function and behavior and the endocrine system is actively being investigated. The emerging evidence underscores the importance of the GBA in regulating physiological and behavioral responses, particularly in stress (Foster et al., 2017; Long-Smith et al., 2020).

Gut microbiota and depression

The GBA is crucial in maintaining bodily homeostasis and mental health. This axis is essential for the interaction between the gastrointestinal system and the brain, and imbalances in the gut microbiota, chronic inflammation, and stress can negatively affect brain function and mood (Du et al., 2020). Several studies have found that changes in the GBA modulate depressive symptoms. One of its related mechanisms is its role in synthesizing and metabolizing key neurotransmitters, including serotonin and dopamine, necessary for mood regulation and mental health (Huang and Wu, 2021).

Approximately 90% of the body's serotonin is synthesized in the gut by enterochromaffin cells, with gut bacteria influencing its production through various mechanisms. For instance, specific bacterial strains, such as *Lactobacillus* and *Bifidobacterium*, promote the synthesis of serotonin by modulating the availability of tryptophan, a precursor of serotonin (Yano et al., 2015; Valles-Colomer et al., 2019). Alterations in the GBA, such as those caused by dysbiosis, can disrupt serotonin levels, contributing to the development of depressive symptoms (Kelly et al., 2015).

Dopamine metabolism is also impacted by gut microbiota composition. Dopamine synthesis begins with the amino acid tyrosine, converted into L-DOPA by tyrosine hydroxylase. Certain gut bacteria, such as *Escherichia coli*, produce enzymes that influence dopamine metabolism. Dysbiosis can alter dopamine signaling by affecting the levels of its precursors and metabolites, leading to changes in reward-related behaviors and mood regulation (Cryan et al., 2019; Hamamah et al., 2022). These disruptions are increasingly recognized as potential contributors to the onset and progression of depression.

The GBA provides a bidirectional communication pathway through which gut microbiota regulate neurotransmitter production and vice versa. Influenced by gut bacteria, tryptophan metabolism plays a pivotal role in balancing the kynurenine and serotonin pathways. Chronic inflammation and gut dysbiosis have been shown to shift tryptophan metabolism toward the kynurenine pathway, increasing metabolites with neurotoxic properties, which is associated with neuroinflammation and depressive symptoms (Lukić et al., 2022; Mingoti et al., 2023).

Other changes in the gut microbiota have been associated with anxiety and depressive-like behaviors in humans (Tian et al., 2022; Zhu et al., 2023) and animal models (Guo et al., 2019; Cao et al., 2020). Evidence indicates that the microbiota of mice exposed to chronic stress increases neuroinflammation and contributes to these behaviors by altering bacterial composition and inflammatory cytokine activity in the brain (Li et al., 2019).

In humans, administration of the probiotic *Lactobacillus plantarum* JYLP-326 (Zhu et al., 2023) and *Bifidobacterium breve* CCFM1025 (Tian et al., 2022) showed significant antidepressant effects in patients with MDD. These effects were associated with modifications in the intestinal microbiota (Zhu et al., 2023) and in tryptophan metabolism, resulting in changes in the levels of serotonin and other relevant metabolites in serum, reflecting an improvement in the regulation of the GBA (Tian et al., 2022).

Emerging evidence suggests that alterations in the gut microbiome, known as dysbiosis, may contribute to depression (Rudzki and Szulc, 2018; Long-Smith et al., 2020). Dysbiosis can lead to increased intestinal permeability, which allows for the passage of inflammatory molecules and pathogens into the bloodstream, triggering an immune response that can adversely affect the brain (De Palma et al., 2015; Rudzki and Szulc, 2018). Chronic stress, a significant risk factor for depression, has also been shown to disrupt the gut microbiome, further exacerbating the problem (Tan, 2023).

While chronic stress may contribute to exacerbating inflammation associated with depression, the composition of the gut microbiota also plays a crucial role in this process, influencing both inflammatory factors and symptom severity. Studies show that stress, central and peripheral inflammation, and alterations in the gut microbiota are important risk factors for MDD (Cruz-Pereira et al., 2020; Pearson-Leary et al., 2020).

The gut microbiota plays a crucial role in regulating intestinal permeability, which may contribute to the chronic low-grade inflammation observed in disorders such as depression. Stress and depression can alter the composition of gut bacteria, which may influence eating behavior and mood. These changes in the intestinal microbiota may, in turn, increase the risk of developing depression, highlighting the connection between intestinal health and psychiatric disorders (Kelly et al., 2015; Madison and Kiecolt-Glaser, 2019).

Inflammatory depression, a treatment-resistant subtype, is associated with an altered composition of the gut microbiota, including higher levels of Bacteroides and lower levels of *Clostridium*, as well as an increase in SCFA-producing species

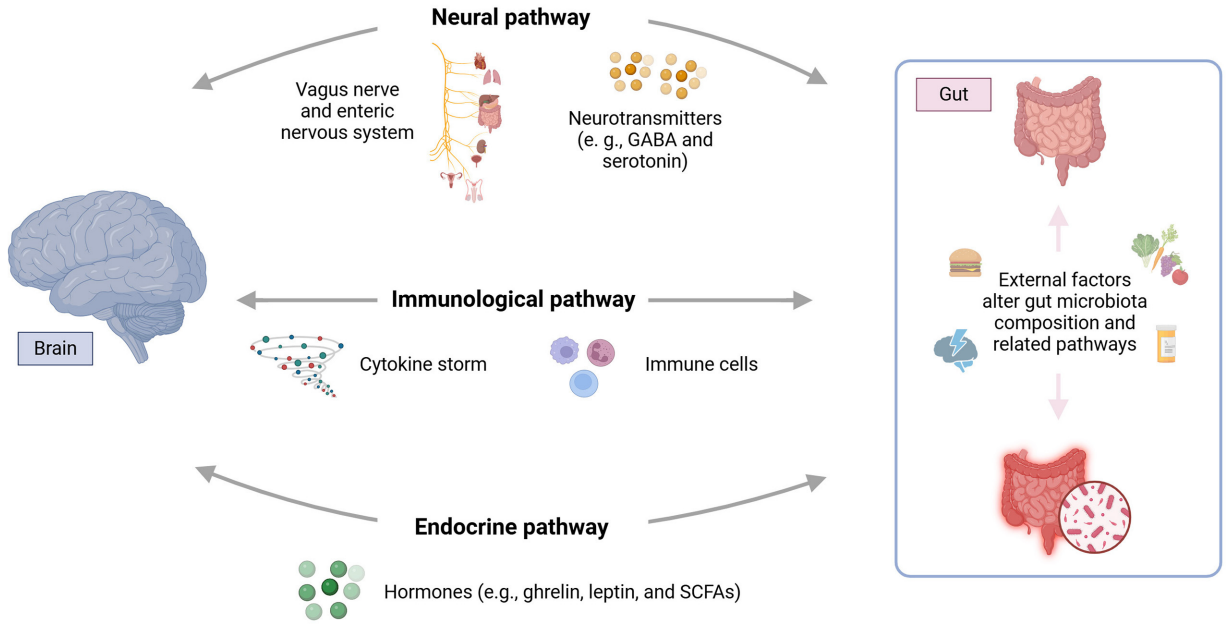


FIGURE 2
Bidirectional interaction between the HPA axis and the gut-brain axis (GBA). The HPA axis responds to stress by releasing glucocorticoids, such as cortisol, which modulate the intestinal barrier, IgA production, and gut microbiota. The intestinal barrier, composed of epithelial cells and tight junctions, is crucial to preventing the translocation of pathogens and toxins. Microbiota can influence the HPA axis through immune molecules, hormones and neurotransmitters.

with abnormal butanoate metabolism. Supplementation with *Clostridium butyricum* has been shown to normalize the gut microbiota, reduce inflammatory factors, and exhibit antidepressant effects in a murine model of inflammatory depression, suggesting that inflammatory processes derived from the gut microbiota may be involved in the neuroinflammation associated with inflammatory depression (Liu P. et al., 2024).

In animals, chronic stress disrupts the balance of the intestinal microbiota. It stimulates inflammatory mechanisms in the brain, leading to anxiety disorders and MDD. At the same time, *Bifidobacterium adolescentis* demonstrates anxiolytic and antidepressant effects by reducing the inflammatory cytokines IL-1β and TNF-α, in addition to decreasing the expression of p-nuclear factor-kappa B (NF-κB) p65 and Ionized calcium-binding adaptor molecule 1 (Iba1), and by rebalancing the intestinal microbiota (Guo et al., 2019).

Another substance with potential antidepressant effects by regulating the GBA, altering the intestinal microbiota, and reducing LPS and inflammatory cytokines is Kai-Xin-San (KXS). In mice with chronic stress-induced depression, KXS increased the expression of tight junction proteins in the gut barrier and blood-brain barrier and decreased stress-related hormones in the CNS. Antibiotic administration attenuated the antidepressant effects of KXS, indicating that its benefits are linked to the modulation of inflammation and regulation of the gut microbiota (Cao et al., 2020).

The HPA axis and the GBA

The HPA axis is activated in response to stress, releasing hormones such as cortisol, which help the body cope with adverse situations. On the other hand, the GBA regulates the bidirectional communication between the brain and the gut, involving the CNS, the enteric nervous system, and the gut microbiota (Figure 2). Studies show that stress can alter the intestinal microbial composition, while changes in the microbiota can, in turn, influence HPA axis activity (Foster et al., 2017).

The HPA axis, by releasing glucocorticoids in response to internal and external stimuli, directly influences the production of immunoglobulin A (IgA) in the gastrointestinal tract. This immunoglobulin is essential for protecting the intestinal mucosa, and neutralizing pathogens and toxins while preserving microbiota homeostasis. Studies highlight that alterations in HPA axis activity, as seen in specific stress situations, can reduce IgA levels, compromising the body's ability to preventively respond to external threats and destabilizing microbial balance (Rea et al., 2020).

Furthermore, the intestinal barrier, composed of a layer of epithelial cells interconnected by tight junctions, is a critical structure for separating the intestinal lumen from the circulatory system (Rea et al., 2020). The glucocorticoids released by the HPA axis can modulate the expression of tight junction proteins, such as occludin and claudin (Rusch et al., 2023). In conditions of HPA axis hyperactivity, observed in inflammatory or infectious contexts,

this regulation is disrupted, resulting in increased intestinal permeability. This facilitates the translocation of microorganisms, toxins, and pro-inflammatory molecules into the bloodstream, activating systemic inflammatory responses (Rusch et al., 2023).

The activation of the HPA axis during infections alters the composition of the gut microbiota and the production of antimicrobial metabolites (Rusch et al., 2023). This interaction is essential for modulating the host immune response and maintaining microbiota functionality during infectious episodes. For example, during bacterial infections, the HPA axis response can stimulate the production of specific metabolites that contain invasive pathogens while preserving beneficial microbiota species (Thaiss et al., 2016).

Diet and lifestyle modulate the GBA and HPA axis. A balanced diet rich in fiber and low in processed foods, combined with healthy lifestyle practices, can promote a healthy gut microbiota and stable HPA axis, potentially reducing the risk of chronic diseases and stress-related disorders (Rusch et al., 2023).

Diet, particularly macronutrients like fibers, proteins, and fats, play a major role in gut microbiota shaping. High-fiber diets promote beneficial bacteria and the production of short-chain fatty acids, which are important for gut health and reducing inflammation (Conlon and Bird, 2014; De Angelis et al., 2019; Hills et al., 2022). Conversely, Western diets high in fats and proteins and low in fibers can lead to dysbiosis, promoting inflammation and metabolic dysfunction (Moschen et al., 2012; Rinninella et al., 2019; Barber et al., 2021). The gut microbiota communicates with the brain through the GBA, influencing the HPA axis (Cryan et al., 2019; Simkin, 2019; Barber et al., 2021). For instance, diets rich in plant polysaccharides can enhance microbiota diversity and functionality, potentially stabilizing the HPA axis and reducing stress-related symptoms (Cryan et al., 2019; Hills et al., 2022).

Lifestyle elements such as stress, sleep, and exercise also affect gut microbiota. Stress, particularly in athletes, can alter gut microbiota composition, impact immune function, and potentially lead to gastrointestinal issues (Clark et al., 2016; Redondo-Useros et al., 2020). Modern lifestyle habits, including processed food consumption and lack of sleep, contribute to gut dysbiosis and related health issues (Simkin, 2019; Redondo-Useros et al., 2020).

Stress profoundly impacts the bidirectional communication between the HPA axis and the gut microbiota through the GBA. Activation of the HPA axis during stress increases glucocorticoid levels, such as cortisol, which can disrupt gut microbiota composition and compromise intestinal barrier integrity. This allows microbial components like LPS to enter circulation, promoting systemic inflammation and worsening gut dysbiosis (Sudo et al., 2004).

Conversely, dysbiotic gut microbiota can influence the HPA axis by reducing the production of metabolites such as SCFAs, which are essential for neuroimmune signaling and stress regulation (Moloney et al., 2014). Dysbiosis also affects neurotransmitter synthesis, such as GABA, intensifying the stress response (Cryan and Dinan, 2012). Strategies such as probiotics and dietary changes have shown the potential to modulate this communication. Probiotic strains like *Lactobacillus* and *Bifidobacterium* can help restore microbiota balance, reduce cortisol levels, and support HPA axis stability (Messaoudi et al., 2011).

Interaction between the HPA axis, gut-brain and biological mechanisms involved in depression

The bidirectional communication of the GBA, a pivotal regulator of the HPA axis, is deeply involved in the development of MDD (Figure 3; Makris et al., 2021). The gut microbiota, through its influence on the HPA axis or alteration of its composition, potentially via neurotransmitters, gut peptides, and immune system activation, significantly affects this process (Młynarska et al., 2022). Furthermore, the impact of mediators from the gut microbiota that cross the blood-brain barrier on HPA axis activity and gut-brain communication, particularly in severe mental disorders, is a significant area of study (Misiak et al., 2020).

The increased activity of the HPA axis leads to greater secretion of cortisol, which contributes to the elevation of inflammatory biomarkers. One explanation for the cause of these disorders is that, with chronic stress and MDD, there is a dysfunction of glucocorticoid receptors that impairs the negative feedback of the HPA axis. This affects the glucocorticoid receptors in the hypothalamus and pituitary gland (Faria and Longui, 2006), potentially resulting in glucocorticoid resistance (Neufeld et al., 2011; Nikkheslat et al., 2020).

Glucocorticoids are steroid hormones secreted by the adrenal cortex and are also related to the circadian cycle (Nikkheslat et al., 2020). Hormonal secretion triggers different responses in the body. In memory consolidation and learning processes, glucocorticoids, in conjunction with their receptors, modulate brain areas involved in these processes, including the hippocampus, amygdala, and prefrontal cortex (Groeneweg et al., 2012).

Glucocorticoids are involved in fundamental metabolic processes in the human body and are regulated through the HPA axis (Nicolaides et al., 2010). Glucocorticoids also control and maintain basal homeostasis in stressful situations (Galon et al., 2002; Faria and Longui, 2006). Approximately 20% of the genes expressed in leukocytes are positively or negatively regulated by glucocorticoids in humans (Galon et al., 2002).

When there is intense or chronic exposure to stress, brain homeostasis, particularly in those brain regions closely related to glucocorticoids, can be affected, resulting in deficits in neuronal neuroplasticity that disadvantage the affected individual in dealing with new stressful situations (McEwen and Gianaros, 2010). Moreover, the chronic elevation of glucocorticoids desensitizes the activation of their receptors, rendering them resistant in patients with a recurrent and immature history of stress associated with MDD (Fernández-Guasti et al., 2012).

Sustained activation of the HPA axis leads to excessive release of glucocorticoids, which under normal conditions have an anti-inflammatory effect. However, the chronic inflammatory state induces glucocorticoid resistance, resulting in the perpetuation of the inflammatory response and regulatory dysfunction of the HPA axis (Knezevic et al., 2023).

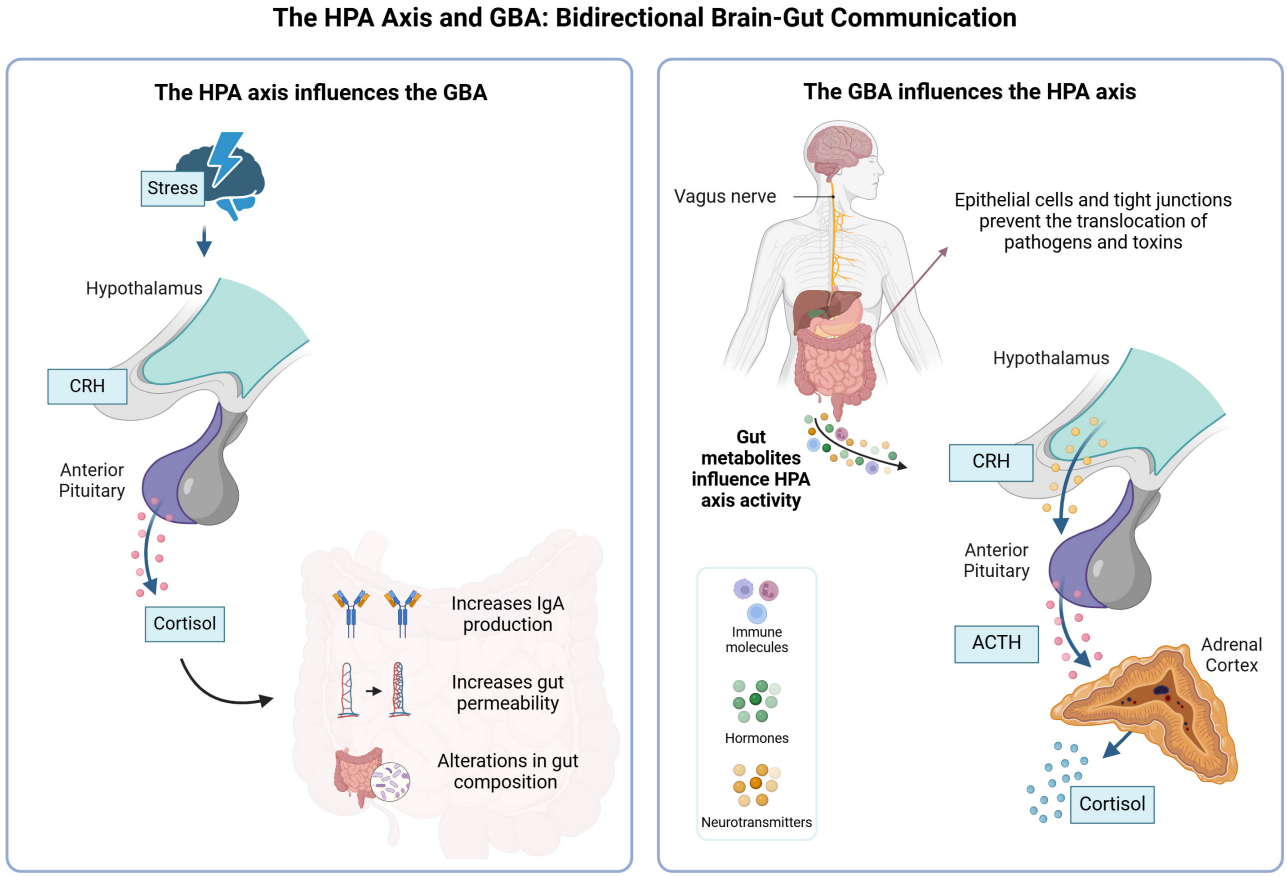


FIGURE 3
Communication between the gut-brain axis, HPA dysregulation, and depression. Gut dysbiosis contributes to changes in the release of neurotransmitters, gut peptides, cytokines, and microbial antigens, which influence the hypothalamic-pituitary-adrenal (HPA) axis. The hypothalamus releases corticotropin-releasing hormone (CRH), stimulating the anterior pituitary to produce adrenocorticotropic hormone (ACTH), which stimulates the release of cortisol by the adrenal cortex. Dysbiosis can result in chronic alterations in the HPA axis, impairing axis regulation and chronic excess cortisol in the circulation. Excess cortisol can culminate in neuroinflammation and brain changes, such as reduced neuronal plasticity, decreased brain-derived neurotrophic factor (BDNF) levels, and atrophy. All these changes are mostly present in the hippocampus and prefrontal cortex and contribute to cognitive deficits, depressive symptoms, and impaired adaptation to stress. Chronic stress and HPA dysregulation further exacerbate gut dysbiosis, creating a pathological feedback loop. The blue arrows indicate the communication pathways between the different components of the gut-brain axis and the HPA axis. The granules represent indirect or molecule-mediated influences, such as neurotransmitters, gut peptides, and cytokines. The light blue circular area at the bottom represents the pathological feedback loop between gut dysbiosis and HPA axis dysregulation.

Chronic inflammatory stimuli induce epigenetic modifications, such as methylation of inflammatory genes and alterations in histone acetylation. These changes stabilize pro-inflammatory states and sensitize the immune and nervous systems to new stressors, increasing the risk of depressive episodes (Stankiewicz et al., 2013).

Stress can lead to the chronic sensitization of an individual to the secretion of hormones such as CRH, which in turn influences the increase of pituitary ACTH. The release of these hormones triggers the biosynthesis and release of glucocorticoids in the adrenal cortex (Fernández-Guasti et al., 2012).

Boyle et al. (2005) reinforce this hypothesis by finding that rodents with glucocorticoid receptor dysfunction exhibited increased HPA activity and behaviors related to depressive and anxious states. Additionally, the interaction between the HPA axis and the GBA is crucial in modulating inflammation and impacting mental health. Hyperactivity of the HPA axis in response to chronic stress can result in resistance to the effects of cortisol. Combined with an imbalanced gut microbiota that promotes systemic and local inflammation, this can amplify neuroinflammation in the brain, exacerbating depressive symptoms (Black and Garbutt, 2002; Dantzer et al., 2008; Mayer et al., 2015).

Similarly, gut dysbiosis can influence HPA axis activity and, consequently, the production of inflammatory mediators, exacerbating depressive symptoms. The gut microbiota can activate the HPA axis through mediators such as microbial antigens, cytokines, prostaglandins, neurotransmitters, gut peptides, and immune system activation, impacting HPA axis activity and intestinal permeability in severe mental disorders. Biological mechanisms, such as altered intestinal permeability and systemic inflammatory response, have been associated with greater susceptibility to emotional disorders (Misiak et al., 2020; Młynarska et al., 2022).

Increased cortisol levels due to HPA axis hyperactivity can negatively affect the production and availability of neurotransmitters such as serotonin and glutamate.

Simultaneously, changes in the intestinal microbiota can alter the synthesis and metabolism of neurotransmitters (Holsboer, 2000; Foster and McVey Neufeld, 2013), such as by reducing the availability of tryptophan, an essential precursor for the production of serotonin (Stephens and Wand, 2012), in addition to influencing the function of glutamate receptors and influencing the release and uptake of this neurotransmitter (McEwen, 2007), contributing to a depressive state.

Therefore, systemic inflammation alters the metabolism of tryptophan by activating the enzyme indoleamine 2,3-dioxygenase (IDO). This process diverts tryptophan to the kynurenine pathway, which is responsible for the synthesis of serotonin. Neurotoxic metabolites, such as quinolinic acid, are offered and reduced for excitotoxicity and oxidative stress, worsening depressive symptoms (Pariante and Lightman, 2008).

Chronic stress and hyperactivity of the HPA axis can induce atrophy of brain regions crucial for neuronal plasticity, such as the hippocampus. Studies show that excess cortisol associated with prolonged stress causes a reduction in neurogenesis and dendrite density in the hippocampus, contributing to cognitive deficits and depressive symptoms (McEwen, 2007). Furthermore, alterations in the intestinal microbiota can amplify this neuronal dysfunction. Intestinal dysbiosis can increase systemic and neuronal inflammation, affecting the production and availability of neurotransmitters, such as serotonin and glutamate, and exacerbating the neuronal dysfunction associated with depression (Cryan and Dinan, 2012).

BDNF is essential for neurons' survival, growth, and plasticity, with higher levels associated with better mental health and reduced vulnerability to depression. Decreased BDNF levels, often observed in individuals with MDD, are related to atrophy of brain areas such as the hippocampus (Duman and Monteggia, 2006). The HPA axis regulates the stress response and, when overactive, elevates cortisol levels, which can cause atrophy of brain regions crucial for neuronal plasticity and reduce the availability of BDNF, exacerbating depression (De Kloet et al., 2005). The gut microbiota, which communicates with the brain through the GBA, can, when altered (dysbiosis), increase systemic and neuronal inflammation, affect neurotransmitter production, and negatively impact BDNF function. Gut dysbiosis can also contribute to HPA axis dysfunction, increasing vulnerability to depression (Dinan and Cryan, 2017).

Specific bacteria and their metabolites significantly influence the HPA axis and neurotransmitter levels through multiple mechanisms. Microbial components, such as LPS, can activate the HPA axis by triggering inflammatory responses mediated by cytokines like IL-6 and TNF-α, which cross the blood-brain barrier and stimulate the release of stress hormones such as cortisol or corticosterone (Zimomra et al., 2011; Misiak et al., 2020). Stress-induced bacterial translocation, often involving pathogens like *Salmonella*, further exacerbates HPA axis activation by increasing corticosterone levels (Ando et al., 2000). Prostaglandins produced during bacterial challenges facilitate the initial rise in corticosterone, while cytokines maintain this response over time (Zimomra et al., 2011).

In addition to their effects on the HPA axis, specific bacteria influence neurotransmitter levels through direct and indirect pathways. Certain strains, including *Lactobacillus* and *Bifidobacterium*, produce neurotransmitters such as GABA and serotonin, which modulate neural signaling and mood (Misiak et al., 2020; Horvath et al., 2021). LPS from bacterial origin can also alter the metabolism of serotonin and catecholamines in the CNS, impacting behavior and emotional responses (Linthorst et al., 1995; Ando et al., 2000). Furthermore, bacteria like *Bacteroides* modulate intestinal neurotransmitter levels, communicating with the brain via the vagus nerve and shaping cognitive and emotional processes (Horvath et al., 2021).

HPA axis dysfunction, combined with alterations in the gut microbiota, can create an environment conducive to dysregulated stress responses. Chronic stress can amplify the effects of an imbalanced microbiota, potentiating inflammation and negatively affecting neurotransmitter function. Furthermore, this combination can lead to atrophy of brain tissues, such as the hippocampus, further impairing neuronal plasticity. The interaction between prolonged stress, gut dysbiosis, and brain atrophy can decrease levels of BDNF, a crucial factor for neuron survival and growth, exacerbating the inflammatory and neurotransmitter response and significantly increasing vulnerability to MDD.

To apply the findings to clinical practice, it is recommended that clinicians consider both the HPA and gut-brain axes as essential components in the treatment of depression. Interventions focused on reducing chronic stress and modulating inflammation, such as mindfulness-based stress reduction, anti-inflammatory treatments, and nutritional strategies (including the use of probiotics, prebiotics, and fiber-rich diets), should be integrated into therapeutic approaches (Rudzki and Szulc, 2018; Tian et al., 2022; Zhu et al., 2023). These strategies can help regulate cortisol levels, support gut microbiota balance, and enhance neurotransmitter production, particularly serotonin (Cao et al., 2020; Li et al., 2019). Additionally, exploring emerging therapies like fecal microbiota transplantation (FMT) may provide valuable options for patients with treatment-resistant depression (Cao et al., 2020; Long-Smith et al., 2020). Addressing these interconnected pathways can more effectively target the biological mechanisms of depression and improve patient outcomes.

Considerations and future directions

The interaction between the HPA axis and the GBA plays a significant role in the biological pathways that contribute to depression. Research indicates that imbalances in the gut microbiota, chronic inflammation, and stress can profoundly affect brain function and mood regulation. Studies show that alterations in gut microbiota composition are linked to anxiety and depression-like behaviors in both humans and animal models. Dysbiosis, marked by changes in gut microbiota, has been connected to increased intestinal permeability, allowing inflammatory molecules to enter the bloodstream and trigger immune responses that harm the brain. Chronic stress worsens these effects by disrupting gut microbiota, further contributing to the onset and persistence of depression.

Gut microbiota's role in regulating intestinal permeability and inflammation helps explain its impact on mental health. For example, changes in microbiota diversity and imbalances in key

bacterial species have been identified in inflammatory depression, which is often resistant to conventional treatments. Emerging therapies targeting the gut microbiota, including probiotics and microbiota-modulating compounds, have shown promise in alleviating depressive symptoms by enhancing gut barrier function, reducing inflammation, and regulating neurotransmitter synthesis. Specific probiotic strains have been shown to alleviate depressive symptoms by influencing gut microbiota and tryptophan metabolism, which directly impacts serotonin levels.

Further exploring the connection between the HPA axis and gut microbiota could enhance understanding of stress regulation and its influence on mental health. Studying individual differences in microbiota composition and stress responses could pave the way for developing personalized treatment approaches. Emerging technologies such as FMT and precision medicine offer promising avenues for addressing depression by effectively targeting the gut microbiota and HPA axis. Additionally, developing novel probiotics, tailored to modulate the gut microbiota more precisely, may lead to more targeted interventions for depression.

Collaboration among neuroscience, psychiatry, and microbiology is essential for advancing knowledge. Continued research could lead to more effective and comprehensive treatments, ultimately improving outcomes for individuals suffering from depression.

Author contributions

AB: Writing – original draft, Writing – review & editing. CS: Writing – original draft. MB: Supervision, Writing – review & editing. ZI: Conceptualization, Supervision, Writing – review & editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare that no Gen AI was used in the creation of this manuscript.

Publisher's note

References

American Psychiatric Association (2022). *Diagnostic and Statistical Manual of Mental Disorders, DSM-5-TR. American Psychiatric Association Publishing.* doi: 10.1176/appi.books.9780890425787

Ando, T., Brown, R. F., Berg, R. D., and Dunn, A. J. (2000). Bacterial translocation can increase plasma corticosterone and brain catecholamine and indoleamine metabolism. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 279, R2164–R2172. doi: 10.1152/ajpregu.2000.279.6.R2164

Aszalós, Z. (2008). Neurological and psychiatric aspects of some gastrointestinal diseases. *Orv. Hetil.* 149, 2079–2086. doi: 10.1556/oh.2008.28480

Barber, T. M., Valsamakis, G., Mastorakos, G., Hanson, P., Kyrou, I., Randeva, H. S., et al. (2021). Dietary influences on the microbiota-gut-brain axis. *Int. J. Mol. Sci.* 22:3502. doi: 10.3390/ijms22073502

Black, P. H., and Garbutt, L. D. (2002). Stress, inflammation and cardiovascular disease. *J. Psychosom. Res.* 52, 1–23. doi: 10.1016/S0022-3999(01)00302-6

Boyle, M. P., Brewer, J. A., Funatsu, M., Wozniak, D. F., Tsien, J. Z., Izumi, Y., et al. (2005). Acquired deficit of forebrain glucocorticoid receptor produces depression-like changes in adrenal axis regulation and behavior. *Proc. Natl. Acad. Sci.* 102, 473–478. doi: 10.1073/pnas.0406458102

Bruce-Keller, A. J., Salbaum, J. M., Luo, M., Blanchard, E., Taylor, C. M., Welsh, D. A., et al. (2015). Obese-type gut microbiota induce neurobehavioral changes in the absence of obesity. *Biol. Psychiatry* 77, 607–615. doi: 10.1016/j.biopsych.2014.07.012

Buttenschøn, H. N., Krogh, J., Nielsen, M. N., Kaerlev, L., Nordentoft, M., and Mors, O. (2017). Association analyses of depression and genes in the hypothalamus-pituitary-adrenal axis. *Acta Neuropsychiatr.* 29, 59–64. doi: 10.1017/neu.2016.26

Cao, C., Liu, M., Qu, S., Huang, R., Qi, M., Zhu, Z., et al. (2020). Chinese medicine formula Kai-Xin-San ameliorates depression-like behaviours in chronic unpredictable mild stressed mice by regulating gut microbiota-inflammation-stress system. *J. Ethnopharmacol.* 261:113055. doi: 10.1016/j.jep.2020.113055

Clapp, M., Aurora, N., Herrera, L., Bhatia, M., Wilen, E., and Wakefield, S. (2017). Gut microbiota's effect on mental health: the gut-brain axis. *Clin. Pract.* 7:987. doi: 10.4081/cp.2017.987

Clark, O., Yen, K., and Mellinghoff, I. K. (2016). Molecular pathways: isocitrate dehydrogenase mutations in cancer. *Clin. Cancer Res.* 22, 1837–1842. doi: 10.1158/1078-0432.CCR-13-1333

Conlon, M., and Bird, A. (2014). The impact of diet and lifestyle on gut microbiota and human health. *Nutrients* 7, 17–44. doi: 10.3390/nu7010017

Corea Del Cid, M. T. (2021). La depresión y su impacto en la salud pública. *Rev. Méd. Hondureña* 89, 46–52. doi: 10.5377/rmh.v89iSupl.1.12047

Cruz-Pereira, J. S., Rea, K., Nolan, Y. M., O'Leary, O. F., Dinan, T. G., and Cryan, J. F. (2020). Depression's unholy trinity: dysregulated stress, immunity, and the microbiome. *Ann. Rev. Psychol.* 71, 49–78. doi: 10.1146/annurev-psych-122216-011613

Cryan, J. F., and Dinan, T. G. (2012). Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. *Nat. Rev. Neurosci.* 13, 701–712. doi: 10.1038/nrn3346

Cryan, J. F., O'Riordan, K. J., Cowan, C. S. M., Sandhu, K. V., Bastiaanssen, T. F. S., Boehme, M., et al. (2019). The microbiota-gut-brain axis. *Physiol. Rev.* 99, 1877–2013. doi: 10.1152/physrev.00018.2018

Dantzer, R., O'Connor, J. C., Freund, G. G., Johnson, R. W., and Kelley, K. W. (2008). From inflammation to sickness and depression: when the immune system subjugates the brain. *Nat. Rev. Neurosci.* 9, 46–56. doi: 10.1038/nrn2297

De Angelis, M., Garruti, G., Minervini, F., Bonfrate, L., Portincasa, P., and Gobbetti, M. (2019). The food-gut human axis: the effects of diet on gut microbiota and metabolome. *Curr. Med. Chem.* 26, 3567–3583. doi: 10.2174/0929867324666170428103848

De Kloet, E. R., Joëls, M., and Holsboer, F. (2005). Stress and the brain: from adaptation to disease. *Nat. Rev. Neurosci.* 6, 463–475. doi: 10.1038/nrn1683

De Palma, G., Blennerhassett, P., Lu, J., Deng, Y., Park, A. J., Green, W., et al. (2015). Microbiota and host determinants of behavioural phenotype in maternally separated mice. *Nat. Commun*. 6:7735. doi: 10.1038/ncomms8735

Dedovic, K., Duchesne, A., Andrews, J., Engert, V., and Pruessner, J. C. (2009). The brain and the stress axis: the neural correlates of cortisol regulation in response to stress. *NeuroImage* 47, 864–871. doi: 10.1016/j.neuroimage.2009.05.074

Dinan, T. G., and Cryan, J. F. (2017). The microbiome-gut-brain axis in health and disease. *Gastroenterol. Clin. North Am*. 46, 77–89. doi: 10.1016/j.gtc.2016.09.007

Du, Y., Gao, X.-R., Peng, L., and Ge, J.-F. (2020). Crosstalk between the microbiota-gut-brain axis and depression. *Heliyon* 6:e04097. doi: 10.1016/j.heliyon.2020.e04097

Duman, R. S., Aghajanian, G. K., Sanacora, G., and Krystal, J. H. (2016). Synaptic plasticity and depression: new insights from stress and rapid-acting antidepressants. *Nat. Med*. 22, 238–249. doi: 10.1038/nm.4050

Duman, R. S., and Monteggia, L. M. (2006). A neurotrophic model for stress-related mood disorders. *Biol. Psychiatry* 59, 1116–1127. doi: 10.1016/j.biopsych.2006.02.013

Faria, C. D. C., and Longui, C. A. (2006). Aspectos moleculares da sensibilidade aos glicocorticóides. *Arq. Bras. Endocrinol. Metabol*. 50, 983–995. doi: 10.1590/S0004-27302006000600003

Fernández-Guasti, A., Fiedler, J., Herrera, L., and Handa, R. (2012). Sex, stress, and mood disorders: at the intersection of adrenal and gonadal hormones. *Horm. Metab. Res*. 44, 607–618. doi: 10.1055/s-0032-1312592

Ferrari, A. J., Charlson, F. J., Norman, R. E., Patten, S. B., Freedman, G., Murray, C. J. L., et al. (2013). Burden of depressive disorders by country, sex, age, and year: findings from the global burden of disease study 2010. *PLoS Med*. 10:e1001547. doi: 10.1371/journal.pmed.1001547

Foster, J. A., and McVey Neufeld, K.-A. (2013). Gut-brain axis: how the microbiome influences anxiety and depression. *Trends Neurosci*. 36, 305–312. doi: 10.1016/j.tins.2013.01.005

Foster, J. A., Rinaman, L., and Cryan, J. F. (2017). Stress & the gut-brain axis: regulation by the microbiome. *Neurobiol. Stress* 7, 124–136. doi: 10.1016/j.ynstr.2017.03.001

Galon, J., Franchimont, D., Hiroi, N., Frey, G., Boettner, A., Ehrhart-Bornstein, M., et al. (2002). Gene profiling reveals unknown enhancing and suppressive actions of glucocorticoids on immune cells. *FASEB J*. 16, 61–71. doi: 10.1096/fj.01-0245com

Geerlings, M. I., and Gerritsen, L. (2017). Late-life depression, hippocampal volumes, and hypothalamic-pituitary-adrenal axis regulation: a systematic review and meta-analysis. *Biol. Psychiatry* 82, 339–350. doi: 10.1016/j.biopsych.2016.12.032

Giau, V., Wu, S., Jamerlan, A., An, S., Kim, S., and Hulme, J. (2018). Gut microbiota and their neuroinflammatory implications in alzheimer's disease. *Nutrients* 10:1765. doi: 10.3390/nu10111765

Groeneweg, F. L., Karst, H., De Kloet, E. R., and Joëls, M. (2012). Mineralocorticoid and glucocorticoid receptors at the neuronal membrane, regulators of nongenomic corticosteroid signalling. *Mol. Cell. Endocrinol*. 350, 299–309. doi: 10.1016/j.mce.2011.06.020

Guo, Y., Xie, J.-P., Deng, K., Li, X., Yuan, Y., Xuan, Q., et al. (2019). Prophylactic effects of *Bifidobacterium adolescentis* on anxiety and depression-like phenotypes after chronic stress: a role of the gut microbiota-inflammation axis. *Front. Behav. Neurosci*. 13:126. doi: 10.3389/fnbeh.2019.00126

Hamamah, S., Aghazarian, A., Nazaryan, A., Hajnal, A., and Covasa, M. (2022). Role of microbiota-gut-brain axis in regulating dopaminergic signaling. *Biomedicines* 10:436. doi: 10.3390/biomedicines10020436

Hansson, C., Alvarez-Crespo, M., Taube, M., Skibicka, K. P., Schmidt, L., Karlsson-Lindahl, L., et al. (2014). Influence of ghrelin on the central serotonergic signaling system in mice. *Neuropharmacology* 79, 498–505. doi: 10.1016/j.neuropharm.2013.12.012

Hattori, N., and Yamashiro, Y. (2021). The gut-brain axis. *Ann. Nutr. Metab*. 77, 1–3. doi: 10.1159/000512226

Hills Jr., R. D., Pontefract, B. A., Mishcon, H. R., Black, C. A., Sutton, S. C., et al. (2022). Gut microbiome: profound implications for diet and disease. *Kompass Nutr. Diet*. 2, 1–16. doi: 10.1159/000523712

Holsboer, F. (2000). The corticosteroid receptor hypothesis of depression. *Neuropsychopharmacology* 23, 477–501. doi: 10.1016/S0893-133X(00)00159-7

Holsen, L. M., Spaeth, S. B., Lee, J.-H., Ogden, L. A., Klibanski, A., Whitfield-Gabrieli, S., et al. (2011). Stress response circuitry hypoactivation related to hormonal dysfunction in women with major depression. *J. Affect. Disord*. 131, 379–387. doi: 10.1016/j.jad.2010.11.024

Horvath, T., Ihekweazu, F., Haidacher, S., Engevik, K., Fultz, R., Hoch, K., et al. (2021). *Bacteroides ovatus* influences the levels of intestinal neurotransmitters in a gnotobiotic model. *FASEB J*. 35:fasebj.2021.35.S1.03956. doi: 10.1096/fasebj.2021.35.S1.03956

Huang, F., and Wu, X. (2021). Brain neurotransmitter modulation by gut microbiota in anxiety and depression. *Front. Cell Dev. Biol*. 9:649103. doi: 10.3389/fcell.2021.649103

Jackson, T. M., Ostrowski, T. D., and Middlemas, D. S. (2019). Intracerebroventricular ghrelin administration increases depressive-like behavior in male juvenile rats. *Front. Behav. Neurosci*. 13:77. doi: 10.3389/fnbeh.2019.00077

Kelly, J. R., Borre, Y., O' Brien, C., Patterson, E., El Aidy, S., Deane, J., et al. (2016). Transferring the blues: depression-associated gut microbiota induces neurobehavioural changes in the rat. *J. Psychiatr. Res*. 82, 109–118. doi: 10.1016/j.jpsychires.2016.07.019

Kelly, J. R., Kennedy, P. J., Cryan, J. F., Dinan, T. G., Clarke, G., and Hyland, N. P. (2015). Breaking down the barriers: the gut microbiome, intestinal permeability and stress-related psychiatric disorders. *Front. Cell. Neurosci*. 9:392. doi: 10.3389/fncel.2015.00392

Kessler, R. C., and Bromet, E. J. (2013). The epidemiology of depression across cultures. *Annu. Rev. Public Health* 34, 119–138. doi: 10.1146/annurev-publhealth-031912-114409

Kinlein, S. A., Wilson, C. D., and Karatsoreos, I. N. (2015). Dysregulated hypothalamic—pituitary—adrenal axis function contributes to altered endocrine and neurobehavioral responses to acute stress. *Front. Psychiatry* 6:31. doi: 10.3389/fpsyt.2015.00031

Knezevic, E., Nenic, K., Milanovic, V., and Knezevic, N. N. (2023). The role of cortisol in chronic stress, neurodegenerative diseases, and psychological disorders. *Cells* 12:2726. doi: 10.3390/cells12232726

Korpela, K., Salonen, A., Virta, L. J., Kekkonen, R. A., Forslund, K., Bork, P., et al. (2016). Intestinal microbiome is related to lifetime antibiotic use in Finnish pre-school children. *Nat. Commun*. 7:10410. doi: 10.1038/ncomms10410

Kouba, B. R., De Araujo Borba, L., Borges De Souza, P., Gil-Mohapel, J., and Rodrigues, A. L. S. (2024). Role of inflammatory mechanisms in major depressive disorder: from etiology to potential pharmacological targets. *Cells* 13:423. doi: 10.3390/cells13050423

Li, N., Wang, Q., Wang, Y., Sun, A., Lin, Y., Jin, Y., et al. (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. doi: 10.1080/10253890.2019.1617267

Linthorst, A., Flachskamm, C., Muller-Preuss, P., Holsboer, F., and Reul, J. (1995). Effect of bacterial endotoxin and interleukin-1 beta on hippocampal serotonergic neurotransmission, behavioral activity, and free corticosterone levels: an *in vivo* microdialysis study. *J. Neurosci*. 15, 2920–2934. doi: 10.1523/JNEUROSCI.15-04-02920.1995

Liu, F., Yang, Y., Fan, X.-W., Zhang, N., Wang, S., Shi, Y.-J., et al. (2024). Impacts of inflammatory cytokines on depression: a cohort study. *BMC Psychiatry* 24:195. doi: 10.1186/s12888-024-05639-w

Liu, P., Liu, Z., Wang, J., Wang, J., Gao, M., Zhang, Y., et al. (2024). Immunoregulatory role of the gut microbiota in inflammatory depression. *Nat. Commun*. 15:3003. doi: 10.1038/s41467-024-47273-w

Liu, Q., He, H., Yang, J., Feng, X., Zhao, F., and Lyu, J. (2020). Changes in the global burden of depression from 1990 to 2017: findings from the global burden of disease study. *J. Psychiatr. Res*. 126, 134–140. doi: 10.1016/j.jpsychires.2019.08.002

Long-Smith, C., O'Riordan, K. J., Clarke, G., Stanton, C., Dinan, T. G., and Cryan, J. F. (2020). Microbiota-gut-brain axis: new therapeutic opportunities. *Annu. Rev. Pharmacol. Toxicol*. 60, 477–502. doi: 10.1146/annurev-pharmtox-010919-023628

Lucassen, P. J., Meerlo, P., Naylor, A. S., Van Dam, A. M., Dayer, A. G., Fuchs, E., et al. (2010). Regulation of adult neurogenesis by stress, sleep disruption, exercise and inflammation: implications for depression and antidepressant action. *Eur. Neuropsychopharmacol*. 20, 1–17. doi: 10.1016/j.euroneuro.2009.08.003

Lukić, I., Ivković, S., Mitić, M., and AdŽić, M. (2022). Tryptophan metabolites in depression: modulation by gut microbiota. *Front. Behav. Neurosci*. 16:987697. doi: 10.3389/fnbeh.2022.987697

Madison, A., and Kiecolt-Glaser, J. K. (2019). Stress, depression, diet, and the gut microbiota: human-bacteria interactions at the core of psychoneuroimmunology and nutrition. *Curr. Opin. Behav. Sci*. 28, 105–110. doi: 10.1016/j.cobeha.2019.01.011

Maes, M., Leonard, B. E., Myint, A. M., Kubera, M., and Verkerk, R. (2011). The new '5-HT' hypothesis of depression: cell-mediated immune activation induces indoleamine 2,3-dioxygenase, which leads to lower plasma tryptophan and an increased synthesis of detrimental tryptophan catabolites (TRYCATs), both of which contribute to the onset of depression. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 35, 702–721. doi: 10.1016/j.pnpbp.2010.12.017

Makris, A. P., Karianaki, M., Tsamis, K. I., and Paschou, S. A. (2021). The role of the gut-brain axis in depression: endocrine, neural, and immune pathways. *Hormones* 20, 1–12. doi: 10.1007/s42000-020-00236-4

Mayer, E. A., Tillisch, K., and Gupta, A. (2015). Gut/brain axis and the microbiota. *J. Clin. Invest*. 125, 926–938. doi: 10.1172/JCI76304

McEwen, B. S. (2007). Physiology and neurobiology of stress and adaptation: central role of the brain. *Physiol. Rev*. 87, 873–904. doi: 10.1152/physrev.00041.2006

McEwen, B. S., and Gianaros, P. J. (2010). Central role of the brain in stress and adaptation: links to socioeconomic status, health, and disease. *Ann. N. Y. Acad. Sci*. 1186, 190–222. doi: 10.1111/j.1749-6632.2009.05331.x

Mello, A. D. A. F. D., Mello, M. F. D., Carpenter, L. L., and Price, L. H. (2003). Update on stress and depression: the role of the hypothalamic-pituitary-adrenal (HPA) axis. *Rev. Bras. Psiquiatr*. 25, 231–238. doi: 10.1590/S1516-44462003000400010

Messaoudi, M., Lalonde, R., Violle, N., Javelot, H., Desor, D., Nejdi, A., et al. (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. doi: 10.1017/S0007114510004319

Miller, A. H., and Raison, C. L. (2016). The role of inflammation in depression: from evolutionary imperative to modern treatment target. *Nat. Rev. Immunol*. 16, 22–34. doi: 10.1038/nri.2015.5

Mingoti, M. E. D., Bertollo, A. G., De Oliveira, T., and Ignácio, Z. M. (2023). "Stress and kynurenine-inflammation pathway in major depressive disorder," in *Neuroinflammation, Gut-Brain Axis and Immunity in Neuropsychiatric Disorders*, ed. *Y.-K. Kim (Singapore: Springer Nature Singapore)*, 163–190. doi: 10.1007/978-981-19-7376-5_8

Misiak, B., Łoniewski, I., Marlicz, W., Frydecka, D., Szulc, A., Rudzki, L., et al. (2020). The HPA axis dysregulation in severe mental illness: can we shift the blame to gut microbiota? *Prog. Neuropsychopharmacol. Biol. Psychiatry* 102:109951. doi: 10.1016/j.pnpbp.2020.109951

Młynarska, E., Gadzinowska, J., Tokarek, J., Forycka, J., Szuman, A., Franczyk, B., et al. (2022). The role of the microbiome-brain-gut axis in the pathogenesis of depressive disorder. *Nutrients* 14:1921. doi: 10.3390/nu14091921

Moloney, R. D., Desbonnet, L., Clarke, G., Dinan, T. G., and Cryan, J. F. (2014). The microbiome: stress, health and disease. *Mamm. Genome* 25, 49–74. doi: 10.1007/s00335-013-9488-5

Moreira, N. R. F., Tecchio, J. V. S., Calderari, M. R. D. C. M., Barboza, K. R. M., Costa, C. N. L. D., Favacho, M. C. C., et al. (2023). A ligação entre o intestino e a depressão: uma revisão sistemática dos mecanismos envolvidos no eixo intestino-cérebro. *Rev. Ibero-Am. Humanidades Ciênc. E Educ*. 9, 2475–2487. doi: 10.51891/rease.v9i9.11320

Moschen, A. R., Wieser, V., and Tilg, H. (2012). Dietary factors: major regulators of the gut's microbiota. *Gut Liver* 6, 411–416. doi: 10.5009/gnl.2012.6.4.411

Neufeld, K. M., Kang, N., Bienenstock, J., and Foster, J. A. (2011). Reduced anxiety-like behavior and central neurochemical change in germ-free mice: behavior in germ-free mice. *Neurogastroenterol. Motil*. 23, 255–e119. doi: 10.1111/j.1365-2982.2010.01620.x

Nicolaides, N. C., Galata, Z., Kino, T., Chrousos, G. P., and Charmandari, E. (2010). The human glucocorticoid receptor: molecular basis of biologic function. *Steroids* 75, 1–12. doi: 10.1016/j.steroids.2009.09.002

Nikkheslat, N., McLaughlin, A. P., Hastings, C., Zajkowska, Z., Nettis, M. A., Mariani, N., et al. (2020). Childhood trauma, HPA axis activity and antidepressant response in patients with depression. *Brain. Behav. Immun*. 87, 229–237. doi: 10.1016/j.bbi.2019.11.024

Otte, C., Gold, S. M., Penninx, B. W., Pariante, C. M., Etkin, A., Fava, M., et al. (2016). Major depressive disorder. *Nat. Rev. Dis. Primer* 2:16065. doi: 10.1038/nrdp.2016.65

Pariante, C. M. (2004). Glucocorticoid receptor function *in vitro* in patients with major depression. *Stress* 7, 209–219. doi: 10.1080/10253890500069650

Pariante, C. M., and Lightman, S. L. (2008). The HPA axis in major depression: classical theories and new developments. *Trends Neurosci*. 31, 464–468. doi: 10.1016/j.tins.2008.06.006

Pearson-Leary, J., Zhao, C., Bittinger, K., Eacret, D., Luz, S., Vigderman, A. S., et al. (2020). The gut microbiome regulates the increases in depressive-type behaviors and in inflammatory processes in the ventral hippocampus of stress vulnerable rats. *Mol. Psychiatry* 25, 1068–1079. doi: 10.1038/s41380-019-0380-x

Qi, X., Yun, C., Pang, Y., and Qiao, J. (2021). The impact of the gut microbiota on the reproductive and metabolic endocrine system. *Gut Microbes* 13:1894070. doi: 10.1080/19490976.2021.1894070

Rea, K., Dinan, T. G., and Cryan, J. F. (2020). Gut microbiota: a perspective for psychiatrists. *Neuropsychobiology* 79, 50–62. doi: 10.1159/000504495

Redondo-Useros, N., Nova, E., González-Zancada, N., Díaz, L. E., Gómez-Martínez, S., and Marcos, A. (2020). Microbiota and lifestyle: a special focus on diet. *Nutrients* 12:1776. doi: 10.3390/nu12061776

Rinninella, E., Cintoni, M., Raoul, P., Lopetuso, L. R., Scaldaferri, F., Pulcini, G., et al. (2019). Food components and dietary habits: keys for a healthy gut microbiota composition. *Nutrients* 11:2393. doi: 10.3390/nu11102393

Rudzki, L., and Szulc, A. (2018). "Immune gate" of psychopathology-the role of gut derived immune activation in major psychiatric disorders. *Front. Psychiatry* 9:205. doi: 10.3389/fpsyt.2018.00205

Rusch, J. A., Layden, B. T., and Dugas, L. R. (2023). Signalling cognition: the gut microbiota and hypothalamic-pituitary-adrenal axis. *Front. Endocrinol*. 14:1130689. doi: 10.3389/fendo.2023.1130689

Sampson, T. R., and Mazmanian, S. K. (2015). Control of brain development, function, and behavior by the microbiome. *Cell Host Microbe* 17, 565–576. doi: 10.1016/j.chom.2015.04.011

Silva, Y. P., Bernardi, A., and Frozza, R. L. (2020). The role of short-chain fatty acids from gut microbiota in gut-brain communication. *Front. Endocrinol*. 11:25. doi: 10.3389/fendo.2020.00025

Simkin, D. R. (2019). Microbiome and mental health, specifically as it relates to adolescents. *Curr. Psychiatry Rep*. 21:93. doi: 10.1007/s11920-019-1075-3

Siu, A. L., Bibbins-Domingo, K., Grossman, D. C., Baumann, L. C., Davidson, K. W., and the US Preventive Services Task Force (USPSTF)., et al. (2016). Screening for depression in adults: US preventive services task force recommendation statement. *JAMA* 315:380. doi: 10.1001/jama.2015.18392

Sorboni, S. G., Moghaddam, H. S., Jafarzadeh-Esfehani, R., and Soleimanpour, S. (2022). A comprehensive review on the role of the gut microbiome in human neurological disorders. *Clin. Microbiol. Rev*. 35, e00338–20. doi: 10.1128/CMR.00338-20

Stankiewicz, A. M., Swiergiel, A. H., and Lisowski, P. (2013). Epigenetics of stress adaptations in the brain. *Brain Res. Bull*. 98, 76–92. doi: 10.1016/j.brainresbull.2013.07.003

Stephens, M. A. C., and Wand, G. (2012). Stress and the HPA axis: role of glucocorticoids in alcohol dependence. *Alcohol Res. Curr. Rev*. 34, 468–483.

Sudo, N., Chida, Y., Aiba, Y., Sonoda, J., Oyama, N., Yu, X., et al. (2004). Postnatal microbial colonization programs the hypothalamic-pituitary-adrenal system for stress response in mice. *J. Physiol*. 558, 263–275. doi: 10.1113/jphysiol.2004.063388

Tan, H.-E. (2023). The microbiota-gut-brain axis in stress and depression. *Front. Neurosci*. 17:1151478. doi: 10.3389/fnins.2023.1151478

Thaiss, C. A., Zmora, N., Levy, M., and Elinav, E. (2016). The microbiome and innate immunity. *Nature* 535, 65–74. doi: 10.1038/nature18847

Tian, P., Chen, Y., Zhu, H., Wang, L., Qian, X., Zou, R., et al. (2022). *Bifidobacterium breve* CCFM1025 attenuates major depression disorder via regulating gut microbiome and tryptophan metabolism: a randomized clinical trial. *Brain. Behav. Immun*. 100, 233–241. doi: 10.1016/j.bbi.2021.11.023

Valles-Colomer, M., Falony, G., Darzi, Y., Tigchelaar, E. F., Wang, J., Tito, R. Y., et al. (2019). The neuroactive potential of the human gut microbiota in quality of life and depression. *Nat. Microbiol*. 4, 623–632. doi: 10.1038/s41564-018-0337-x

Varatharaj, A., and Galea, I. (2017). The blood-brain barrier in systemic inflammation. *Brain. Behav. Immun*. 60, 1–12. doi: 10.1016/j.bbi.2016.03.010

Videbech, P., and Ravnkilde, B. (2004). Hippocampal volume and depression: a meta-analysis of MRI studies. *Am. J. Psychiatry* 161, 1957–1966. doi: 10.1176/appi.ajp.161.11.1957

World Health Organization (2023). *Depressive disorder (depression)*. Available at: https://www.who.int/news-room/fact-sheets/detail/depression (accessed December 4, 2024).

Yano, J. M., Yu, K., Donaldson, G. P., Shastri, G. G., Ann, P., Ma, L., et al. (2015). Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. *Cell* 161, 264–276. doi: 10.1016/j.cell.2015.02.047

Zarouna, S. (2015). Mood disorders: a potential link between ghrelin and leptin on human body? *World J. Exp. Med*. 5:103. doi: 10.5493/wjem.v5.i2.103

Zhu, R., Fang, Y., Li, H., Liu, Y., Wei, J., Zhang, S., et al. (2023). Psychobiotic *Lactobacillus plantarum* JYLP-326 relieves anxiety, depression, and insomnia symptoms in test anxious college via modulating the gut microbiota and its metabolism. *Front. Immunol*. 14:1158137. doi: 10.3389/fimmu.2023.1158137

Zhu, X., Han, Y., Du, J., Liu, R., Jin, K., and Yi, W. (2017). Microbiota-gut-brain axis and the central nervous system. *Oncotarget* 8, 53829–53838. doi: 10.18632/oncotarget.17754

Zimomra, Z. R., Porterfield, V. M., Camp, R. M., and Johnson, J. D. (2011). Time-dependent mediators of HPA axis activation following live *Escherichia coli*. *Am. J. Physiol. Regul. Integr. Comp. Physiol*. 301, R1648–R1657. doi: 10.1152/ajpregu.00301.2011

Zunszain, P. A., Hepgul, N., and Pariante, C. M. (2013). Inflammation and depression. *Curr. Top. Behav. Neurosci*. 14, 135–151. doi: 10.1007/7854_2012_211

frontiers | Frontiers in Neuroscience

TYPE Mini Review
PUBLISHED 14 February 2025
DOI 10.3389/fnins.2025.1545997

OPEN ACCESS

EDITED BY
Avinash Veerappa,
University of Nebraska Medical Center,
United States

REVIEWED BY
Ran Tao,
Texas A&M University Baylor College
of Dentistry, United States

*CORRESPONDENCE
Tim Ho
✉ timcth@hotmail.com

RECEIVED 16 December 2024
ACCEPTED 29 January 2025
PUBLISHED 14 February 2025

CITATION
Ho T, Elma Ö, Kocanda L, Brain K, Lam T, Kanhere T and Dong H-J (2025) The brain-gut axis and chronic pain: mechanisms and therapeutic opportunities. *Front. Neurosci.* 19:1545997. doi: 10.3389/fnins.2025.1545997

The brain-gut axis and chronic pain: mechanisms and therapeutic opportunities

Tim Ho[1]*, Ömer Elma[2,3], Lucy Kocanda[4,5,6], Katherine Brain[6,7,8], Thao Lam[1], Tejas Kanhere[9] and Huan-Ji Dong[10]

[1]Faculty of Medicine and Health, Sydney Medical School, Westmead Clinical School, The University of Sydney, Sydney, Australia, [2]Unit of Physiotherapy, Department of Rehabilitation and Sport Sciences, Faculty of Health and Social Sciences, Bournemouth University, Bournemouth, United Kingdom, [3]Pain in Motion International Research Group, Vrije Universiteit Brussels, Brussels, Belgium, [4]Department of Rural Health, College of Health, Medicine and Wellbeing, University of Newcastle, Tamworth, NSW, Australia, [5]Tamworth Integrated Pain Service, Hunter New England Local Health District, Tamworth, NSW, Australia, [6]Food and Nutrition Research Program, Hunter Medical Research Institute, New Lambton Heights, NSW, Australia, [7]Hunter Integrated Pain Service, Hunter New England Local Health District, New Lambton Heights, NSW, Australia, [8]School of Health Sciences, College of Health, Medicine and Wellbeing, University of Newcastle, Callaghan, NSW, Australia, [9]Faculty of Medicine and Health, University of New South Wales, Sydney, Australia, [10]Pain and Rehabilitation Centre, and Department of Health, Medicine and Caring Sciences, Linköping University, Linköping, Sweden

The brain-gut axis (BGA) is emerging as a critical mediator in chronic pain, involving bidirectional communication between the central nervous system and the gastrointestinal system. The "Pain Matrix" is associated with microbial dysbiosis, vagus nerve dysfunction, and hypothalamic-pituitary-adrenal (HPA) axis dysregulation, driving neuroinflammation and central sensitization. Key mechanisms include microbial diversity loss, leaky gut, and altered neuroactive signaling via short-chain fatty acids (SCFAs) and vagal pathways. This narrative review explores the intricate interplay between BGA mechanisms and chronic pain, highlighting therapeutic opportunities such as restoring dysbiosis, modulating vagus nerve activity, and regulating endocrine pathways. These interventions target inflammation, autonomic balance, and stress/reward pathway modulation, offering a promising path toward integrative pain management. Further research is required to validate these strategies and improve patient outcomes.

KEYWORDS

chronic pain, brain-gut axis, microbiome, vagus nerve, HPA–hypothalamic-pituitary-adrenal

1 Introduction

Chronic pain is defined as pain persisting or recurring for longer than 3 months (Raffaeli et al., 2021). It is conceptualized as a long-term condition influenced by central nervous system mechanisms, as well as psychosocial factors (Gatchel et al., 2007). Chronic pain management requires an integrative, multidisciplinary approach, including pharmacological treatments, psychological support, physical rehabilitation, and lifestyle modifications to address its complex pathophysiology and common comorbidities. This complexity results in the limited effectiveness of pharmacological therapies, with side effects being extremely common (Martel et al., 2015). Specifically, the chronic primary

pain discussed in this paper refers to pain that persists for more than three months, and is associated with significant emotional distress or functional disability, and cannot be better explained by another condition associated according to the International Association Study of Pain classification (Nicholas et al., 2019).

It is well-known that the gut and brain have a bidirectional communication pathway, and brain-gut interactions play an important role in regulating vital functions in the human body (Mayer and Tillisch, 2011). The gut has been called the "second brain" and connects the vagus nerve and brainstem, via spinal afferents in the spinal cord. Research over the past decade has significantly advanced our understanding of brain-gut interactions in gastrointestinal diseases and abdominal pain syndromes (e.g., irritable bowel syndrome and functional dyspepsia) (Guo et al., 2019). Evidence shows that sensitization mechanisms play a role in chronic pain conditions such as fibromyalgia, headaches, and neuropathic pain. These mechanisms are closely linked to brain-gut axis dysregulation, highlighting the need for targeted treatments (Guo et al., 2019). Several informative reviews have provided extensive knowledge on animal studies (Li et al., 2019; Pak et al., 2024; Rusch et al., 2023).

This narrative review focuses on the understanding of the factors affecting alterations in brain-gut axis (BGA) related to peripheral and central sensitization. Understanding these underlying mechanisms is essential for developing therapeutic approaches in integrative pain management.

2 Materials and methods

2.1 Research question

This narrative review addressed the following question: What are the underlying mechanisms of interaction between the brain-gut axis (BGA) and chronic primary pain?

2.2 Eligibility criteria

Studies eligible for inclusion in the review included:

- Populations with chronic primary pain conditions based on International Classification of Diseases (ICD) 11 (e.g., fibromyalgia, irritable bowel syndrome [IBS], chronic pelvic pain, other nociplastic pain) in human subjects. Animal studies were excluded.
- Examination of mechanistic insights into the BGA, including microbial diversity, inflammation, gut barrier integrity, neuroendocrine modulation, vagal nerve activation, and autonomic nervous system (ANS) or hypothalamic-pituitary-adrenal (HPA) axis involvement. Pharmacological intervention studies were excluded.
- Systematic reviews, narrative reviews, clinical research (e.g., observational studies), and basic science investigations. Non-peer-reviewed materials (e.g., opinion articles, letters, and conference abstracts) and articles published before 2000 or in languages other than English were excluded.

2.3 Data sources

The literature search included the following databases to ensure comprehensive coverage:

- PubMed
- Web of Science
- Google Scholar
- EBSCO

2.4 Search strategy

A structured search strategy was employed to identify relevant studies. The search terms included combinations of the following keywords (Table 1):

TABLE 1 Search terms for PICO framework.

PICO component	Search terms
Population (P)	"chronic pain," "chronic primary pain," "fibromyalgia," "IBS," "chronic pelvic pain," "nociplastic pain"
Intervention (I)	"gut-brain axis," "microbiome diversity," "gut barrier," "vagal nerve," "HPA axis," "SCFAs," "dysbiosis"
Comparison (C)	"healthy controls," "normal microbiota," "placebo," "sham interventions"
Outcome (O)	"pain modulation," "neuroinflammation," "dysbiosis," "autonomic imbalance"

Boolean operators (AND, OR) were used to combine search terms, and the search strategy was adapted for each database to optimize retrieval of relevant literature.

2.5 Study selection

The identified studies underwent a structured screening process to ensure relevance:

- Initial screening: Titles and abstracts were reviewed against the eligibility criteria to exclude irrelevant studies.
- Full-text review: Studies that passed the initial screening were reviewed in full to confirm inclusion. Conflicts during the screening process were resolved through discussion or by consulting a third reviewer.

2.6 Data organization and presentation

Findings from the included studies were synthesized narratively, with a focus on thematic organization. Key mechanisms

TABLE 2 Key microbiota species identified in chronic pain conditions and conditions associated with chronic pain symptoms.

Microbiota species (Freidin et al., 2020; Goudman et al., 2024)	Observation in chronic pain	Potential mechanism
Faecalibacterium prausnitzii	Decreased in migraine, fibromyalgia, and ME/CFS patients.	Anti-inflammatory via butyrate production; supports gut barrier integrity.
Coprococcus catus	Reduced in ME/CFS patients.	SCFA producer; supports gut and CNS homeostasis.
Coprococcus comes	Reduced in chronic pain conditions like ME/CFS.	SCFA producer; contributes to anti-inflammatory signaling.
Ruminococcus obeum	Reduced in ME/CFS and migraine patients.	SCFA producer; role in maintaining gut health.
Odoribacter splanchnicus	Reduced in bladder pain syndrome, migraine, and ME/CFS.	Produces SCFAs; modulates gut immune responses.
Roseburia inulinivorans	Decreased in fibromyalgia and chronic fatigue syndrome.	Produces butyrate; enhances gut epithelial integrity.
Eggerthella lenta	Increased in migraine and ME/CFS patients.	Pro-inflammatory activity; alters gut-brain communication.
Clostridium symbiosum	Increased in migraine and ME/CFS patients.	Produces metabolites impacting gut-brain signaling.

underlying the interaction between the brain-gut axis (BGA) and chronic primary pain were highlighted, including:

- The role of microbial diversity, inflammation, and gut barrier integrity.
- Neuroendocrine modulation, vagal nerve activation, and autonomic nervous system (ANS) involvement.
- The hypothalamic-pituitary-adrenal (HPA) axis and its contribution to chronic pain mechanisms.

3 Microbiome diversity and chronic pain: connected via brain-gut axis

Understanding microbial diversity within the brain-gut axis is essential for grasping its implications for chronic pain. Microbial diversity is generally assessed through alpha and beta diversity indices. Alpha diversity evaluates within-individual diversity using metrics like Shannon, Simpson, and Faith's phylogenetic diversity, while beta diversity examines differences across individuals or groups (Freidin et al., 2020; Goudman et al., 2024; Li et al., 2022; Liu et al., 2023).

3.1 Alpha diversity, species-level diversity and pain pathways

In the context of chronic pain, alpha diversity metrics—especially Shannon and Faith's indices—have been associated with neuroinflammatory processes. Although not all studies target chronic pain specifically, reduced alpha diversity is linked to neuroinflammatory and neurological conditions, suggesting parallels with pain sensitization mechanisms (Goudman et al., 2024). Certain taxa, such as *Coprococcus comes* and *Faecalibacterium prausnitzii*, known for anti-inflammatory roles, are often depleted in chronic pain. Additionally, butyrate-producing bacteria like those in the Lachnospiraceae family support gut health and inflammation modulation, indicating their potential impact on pain pathways (Freidin et al., 2020; Goudman et al., 2024).

While broader taxonomic diversity offers insights into overall composition, species-level diversity—such as *Faecalibacterium prausnitzii* and *Odoribacter splanchnicus* abundance—provides specific relevance to chronic pain. These bacteria produce anti-inflammatory metabolites, such as butyrate, which enhance gut barrier integrity, mitigate systemic inflammation, and modulate neuroinflammatory pathways implicated in pain sensitization. However, current data limitations underscore the need for further species-level research to clarify these associations with neuroinflammatory outcomes (Freidin et al., 2020; Liu et al., 2023).

3.2 Mechanisms of communication with the central nervous system

Communication of microbial diversity changes to the central nervous system (CNS) occurs through gut barrier integrity, immune signaling, metabolites, and neurotransmitters. Increased intestinal permeability, or "leaky gut," permits microbial products like lipopolysaccharides (LPS) into circulation, activating gut-associated lymphoid tissue (GALT) and leading to neuroinflammation that may heighten pain sensitivity. Microbial metabolites, especially short-chain fatty acids (SCFAs) like butyrate, fortify gut barrier integrity and reduce pro-inflammatory cytokines, indirectly protecting the CNS. Microbial neurotransmitters, including GABA and serotonin, interact with the CNS via the vagus nerve, influencing mood and pain perception. Dysbiosis in these pathways reinforces the brain-gut axis as a significant target for chronic pain management (Freidin et al., 2020; Li et al., 2022; Liu et al., 2023).

Table 2 outlines the key microbiota species identified in chronic pain conditions, highlighting observed changes in their abundance and their potential mechanistic roles. The table categorizes microbiota at the species level, showing reductions or increases in specific taxa across various chronic pain disorders, such as migraine, fibromyalgia, and bladder pain syndrome. The mechanisms discussed include their roles in anti-inflammatory processes (e.g., butyrate production), gut barrier maintenance, and modulation of neuroinflammatory pathways, illustrating their relevance as potential targets for intervention in chronic pain management.

4 The role of the vagus nerve in brain-gut axis and pain modulation

The vagus nerve, the tenth and longest cranial nerve, plays a pivotal role in the modulation of various physiological processes, including pain perception, inflammation, and homeostasis. With its extensive afferent and efferent projections connecting the brainstem to visceral organs, the vagus nerve is a critical component of the brain-gut axis and the autonomic nervous system (ANS).

4.1 Neural pathways connecting the vagus nerve to central pain networks

The vagus nerve connects to central pain networks primarily through its afferent fibers, which transmit sensory information from visceral organs to the brainstem. The nucleus tractus solitarius (NTS) serves as a primary relay station, where afferent vagal signals are integrated and relayed to other brain regions, including the locus coeruleus, hypothalamus, and amygdala. These areas are involved in the modulation of pain, emotional responses, and autonomic functions (Bonaz et al., 2017).

Through its connections with the locus coeruleus, the vagus nerve can influence the release of norepinephrine, which plays a key role in the descending pain inhibitory pathways. Additionally, projections from the NTS to the periaqueductal gray (PAG) and raphe nuclei activate serotonergic and opioid pathways, further contributing to pain inhibition (Morris et al., 2020).

This intricate network underscores the vagus nerve's capacity to modulate pain perception both directly and indirectly.

4.2 Chronic pain and plastic changes in the vagus nerve

Chronic pain is associated with significant alterations in the neural pathways and structures involved in pain perception and modulation. The vagus nerve, due to its extensive connections with the brainstem and spinal cord, undergoes plastic changes in response to chronic pain (Wu et al., 2024). These changes involve the sensitization of nociceptive pathways and the alteration of autonomic regulation. Specifically, chronic pain enhances the activity of the NTS, (Shao et al., 2023) which is a critical hub for afferent vagal fibers. This heightened activity can lead to increased sympathetic output and altered parasympathetic tone, further perpetuating pain and stress responses.

The implications of these plastic changes are profound. By modulating the vagus nerve, it may be possible to reverse or attenuate these maladaptive alterations, thereby reducing pain perception and improving autonomic balance. For instance, vagus nerve stimulation (VNS) has been shown to decrease the activity of nociceptive neurons in the spinal cord and brainstem, potentially reversing the hyperexcitability associated with chronic pain (Shao et al., 2023).

4.3 Neuroactive molecules, short-chain fatty acids and vagus nerve activity in chronic primary pain conditions

In patients with chronic primary pain conditions (e.g., fibromyalgia, low back pain, etc.), levels of neuroactive molecules related to vagus nerve activity, such as norepinephrine, serotonin, and acetylcholine, are often dysregulated. Studies have shown that these patients exhibit altered levels of these molecules compared to healthy controls, and these alterations correlate with pain severity and brain connectivity (Yang and Chang, 2019).

Short-chain fatty acids (SCFAs), particularly acetate, propionate, and butyrate, are produced by gut microbiota through the fermentation of dietary fibers. These SCFAs play a crucial role in communicating with the brain via the vagus nerve, forming an essential part of the gut-brain axis. SCFAs can directly activate the vagus nerve, with butyrate shown to increase the firing rate of vagal afferent neurons. The SCFA receptor FFAR3, expressed on vagal afferents, is shown to be important for this communication (Mansuy-Aubert and Ravussin, 2023).

In addition, other receptors such as GPR109A and OR51E2 mediate SCFA effects. GPR109A, expressed on immune cells like macrophages and dendritic cells, is activated by butyrate and plays a key role in anti-inflammatory processes and metabolic regulation. While there is no direct evidence that GPR109A regulates the vagus nerve, its role in reducing inflammation and maintaining gut homeostasis may indirectly influence vagal activity through the gut-brain axis. Similarly, OR51E2, another SCFA receptor expressed on immune cells, senses acetate and propionate and indirectly affects vagus nerve activity by modulating immune responses (Mansuy-Aubert and Ravussin, 2023; O'Riordan et al., 2022).

4.4 Vagus nerve modulation of inflammation via the gut barrier and spleen

The vagus nerve has an efferent activity that may modulate inflammation through its interactions with the gut barrier and the spleen. This modulation is primarily mediated by the cholinergic anti-inflammatory pathway (CAIP), which involves the release of acetylcholine from efferent vagal fibers. Acetylcholine binds to receptors on macrophages in the spleen, inhibiting the release of pro-inflammatory cytokines such as TNF-α, IL-1, and IL-6. In addition to its direct anti-inflammatory effects, the vagus nerve influences the integrity of the gut barrier. By modulating gastrointestinal motility and secretion, the vagus nerve helps maintain the balance of the gut microbiota, which is critical for preventing the translocation of pathogens and endotoxins into the bloodstream. This gut-brain communication is essential for controlling systemic inflammation and maintaining immune homeostasis (Breit et al., 2018).

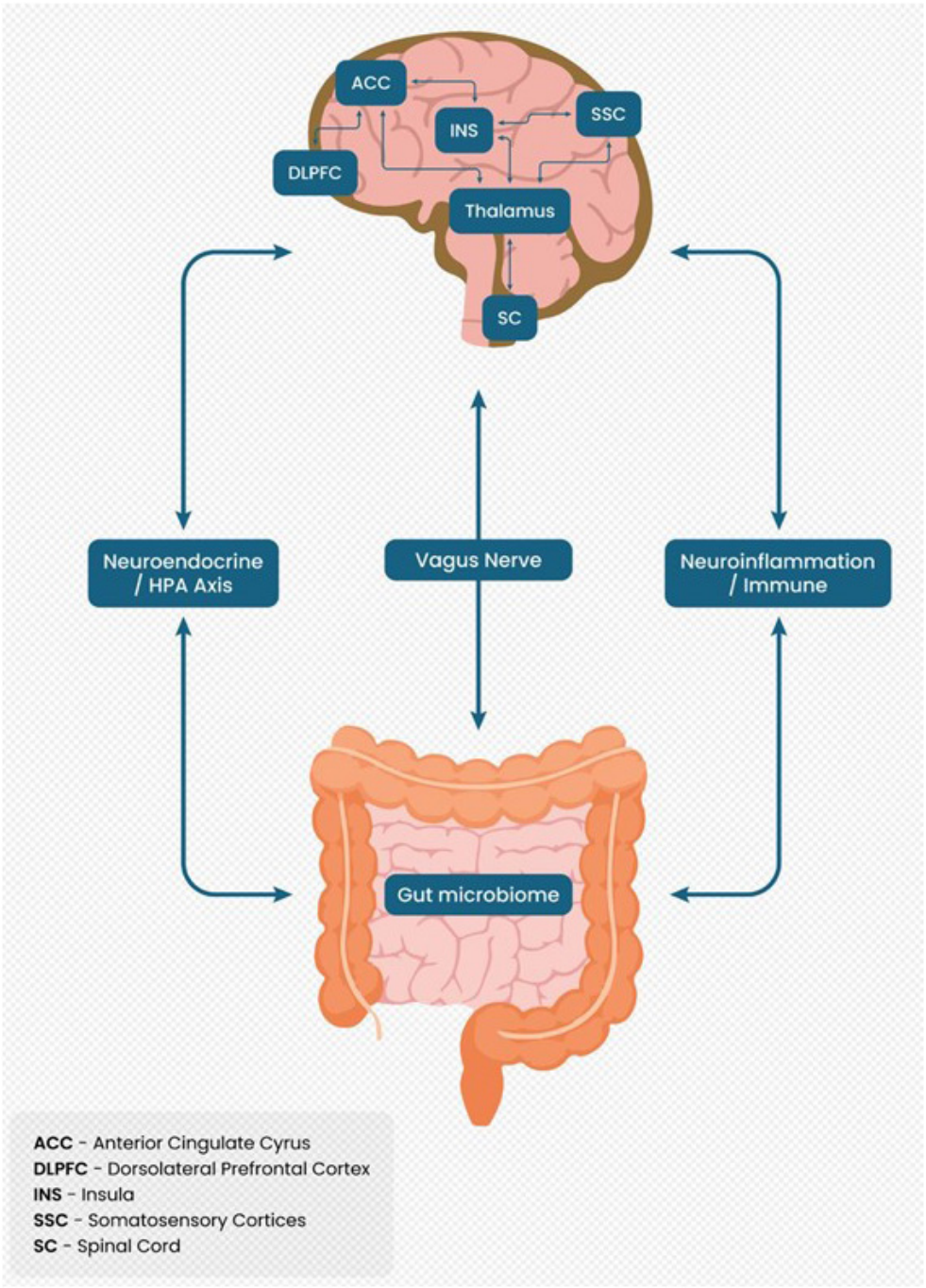


FIGURE 1
Brain-gut axis as a bidirectional communication system in chronic pain.

5 Endocrine pathways in the gut brain axis

The gut-brain axis also includes bidirectional hormonal signaling pathways aimed at relaying information about ingested nutrients during a meal to the brain, which integrates these signals to coordinate the regulation of food intake, energy expenditure and glucose homeostasis via enteroendocrine cells within the intestinal epithelium secreting hormones such as GLP-1 and cholecystokinin.

5.1 Role of GLP-1 in pain modulation

Indeed, GLP-1 has been shown in pre-clinical models to modulate chronic pain by reducing neuroinflammation, enhancing anti-inflammatory mediators (e.g., IL-10, β-endorphins), and regulating pain signaling in the dorsal horn. In preclinical studies, GLP-1 also modulates brain circuitry by reducing neuroinflammation, altering dopaminergic activity, and decreasing reward sensitivity in regions like the nucleus accumbens and

ventral tegmental area (Halloum et al., 2024; Martinelli et al., 2024; Wachsmuth et al., 2022).

5.2 The hypothalamic-pituitary-adrenal axis and chronic cortisol dysregulation

The hypothalamic-pituitary-adrenal (HPA) is a major hormonal system within the body linked with stress response and is thought to play a key role in regulating the brain-gut axis (Dinan and Cryan, 2016). Hormones within the HPA axis include Corticotrophin Releasing Hormone (CRH) released by the hypothalamus, which stimulates the pituitary gland to release Adrenocorticotropic Hormone (ACTH), which then triggers the adrenal glands to produce cortisol. Elevated cortisol levels are associated with alteration of gut microbiota composition as well as increased gut permeability and this has been linked to impaired neural activity and connectivity, often resulting in cognitive, emotional, and stress regulation deficits (Rusch et al., 2023). ACTH is also thought to modulate the inhibitory controls of endogenous opioid peptides, which can influence pain processing (Hannibal and Bishop, 2014).

The HPA axis, a key regulator of the stress response, is frequently dysregulated in chronic pain, marked by sustained cortisol release due to impaired negative feedback in the hypothalamus and pituitary. This prolonged cortisol elevation perpetuates a maladaptive stress response, contributing to persistent pain. Chronic high cortisol levels are linked to reduced hippocampal volume, amplifying stress reactivity and reinforcing pain sensitivity, creating an allostatic load that perpetuates a harmful feedback loop in pain perception and stress response (Rusch et al., 2023; Vachon-Presseau et al., 2013).

Unlike the temporary anti-inflammatory effects of acute cortisol release, chronic cortisol elevation induces a pro-inflammatory state. Tissues develop resistance to cortisol, blunting its anti-inflammatory role and leading to cytokine release that sensitizes pain pathways. In the CNS, this promotes neuroinflammation, particularly in areas like the parahippocampal gyrus, intensifying pain and anxiety-related response (Hannibal and Bishop, 2014; Rusch et al., 2023).

5.3 Impact on gut integrity and systemic inflammation

HPA axis dysregulation impacts gut integrity by increasing gut permeability ("leaky gut"), allowing microbial products to enter the bloodstream and trigger systemic inflammation. This inflammation can further activate the CNS, linking gut health with pain perception and explaining the association of chronic pain with gastrointestinal symptoms, such as irritable bowel syndrome (IBS) (Rusch et al., 2023).

6 Future directions and emerging research in brain-gut axis modulation for chronic pain management

Emerging research on the brain-gut axis (BGA) offers promising avenues for innovative chronic pain therapies (Mayer and Tillisch, 2011; Pak et al., 2024). This section highlights several key areas for future investigation, focusing on microbiome communication with the brain, vagus nerve function and its modulation, and endocrine pathways relating to BGA. By advancing research across these domains, we can deepen our understanding of the brain-gut axis and its role in chronic pain, moving toward holistic, innovative treatments.

6.1 Understanding microbiome communication with the brain in pain modulation

Microbial diversity and its communication with the central nervous system (CNS) through metabolites and

TABLE 3 Mechanisms and therapeutic targets in the brain-gut axis for chronic pain.

Category	Key mechanisms	Potential targets for future research
Microbiome	Reduced diversity (e.g., Faecalibacterium depletion) linked to neuroinflammation.	Probiotics, dietary interventions, FMT to enhance microbial diversity.
	SCFAs (e.g., butyrate) support gut barrier and reduce inflammation.	SCFA supplementation or microbiota modulation therapies.
	Dysbiosis alters neurotransmitter signaling (e.g., GABA, serotonin).	Microbiota-targeting interventions to influence CNS signaling pathways.
Inflammation	Leaky gut and cytokines (e.g., TNF-α) drive neuroinflammatory sensitization.	Therapies to strengthen gut barrier integrity and reduce systemic cytokines.
Vagal nerve	Links gut to CNS pain centers (e.g., NTS, PAG).	Non-invasive and invasive VNS for pain and autonomic dysfunction.
	SCFAs activate vagal pathways, influencing pain and inflammation.	Enhancing SCFA signaling to leverage vagal modulation.
Endocrine/HPA axis	Dysregulated cortisol elevates neuroinflammation and gut permeability.	Strategies to rebalance the HPA axis and reduce chronic stress responses.
	GLP-1 reduces neuroinflammation and modulates pain signaling.	Development of GLP-1 receptor agonists for chronic pain management.

neurotransmitters presents an exciting research frontier. Reduced microbial diversity—especially a loss of anti-inflammatory bacteria like *Faecalibacterium prausnitzii*—is associated with neuroinflammation and heightened pain sensitivity (Freidin et al., 2020; Goudman et al., 2024). Future studies should expand species-level analyses to uncover microbial taxa that specifically influence pain pathways, as well as evaluate how microbial metabolites like butyrate interact with vagal and CNS receptors, such as FFAR3, which is implicated in gut-brain communication (Mansuy-Aubert and Ravussin, 2023). Longitudinal studies assessing the effects of probiotics, dietary interventions, and fecal microbiota transplantation (FMT) on microbial diversity and chronic pain outcomes could further validate microbiome modulation as a therapeutic approach.

6.2 The role of GLP-1 receptor agonists

Glucagon-like peptide-1 receptor (GLP-1R) agonists present a promising avenue for chronic pain management, as preclinical studies demonstrate their role in modulating neuroinflammation, central sensitization, and pain signaling pathways. These agents act on GLP-1Rs expressed in microglial cells and spinal neurons to attenuate inflammatory and neuropathic pain by reducing pro-inflammatory cytokines and promoting anti-inflammatory mediators such as interleukin-10. Additionally, GLP-1R agonists reduce hypersensitivity without inducing tolerance, offering potential for sustained analgesia (Halloum et al., 2024). Further research is needed to clarify mechanisms, validate efficacy in human trials, and explore combinations with other therapies to optimize outcomes in pain management.

6.3 Vagus nerve modulation and neuroplasticity

The vagus nerve's role in autonomic and inflammatory regulation highlights its potential as a therapeutic target for chronic pain management. Vagus nerve stimulation (VNS), both invasive and non-invasive, has shown promise in reducing pain and improving autonomic function (Shao et al., 2023). Future research should investigate how VNS impacts neuroplasticity in pain pathways and influences markers of autonomic function, such as heart rate variability (HRV). Elucidating the neural adaptations resulting from VNS may help refine treatment protocols for chronic pain conditions with autonomic dysregulation, like fibromyalgia and irritable bowel syndrome (IBS).

Figure 1 illustrates the BGA as a bidirectional communication system. It highlights connections between the gut microbiome, vagus nerve, neuroendocrine pathways (HPA axis), and CNS regions such as the anterior cingulate cyrus, insula, thalamus, and spinal cord, emphasizing their role in chronic pain mechanisms.

Table 3 details the mechanisms and potential therapeutic targets within the BGA. It covers categories such as the microbiome, inflammation, vagal nerve, and endocrine pathways, linking these mechanisms to emerging interventions like probiotics, SCFA supplementation, VNS, and GLP-1 receptor agonists for chronic pain management.

7 Conclusion

The BGA represents a vital framework for understanding the mechanisms underlying chronic pain, highlighting the complex interplay between the gut microbiome, vagus nerve, and neuroendocrine pathways. Dysbiosis, neuroinflammation, and HPA axis dysregulation are key drivers of chronic pain, impacting gut integrity, autonomic regulation, and central sensitization. Therapeutic opportunities such as dietary interventions, microbiome modification, vagus nerve stimulation, and neuroendocrine regulation offer promising pathways for integrative management, targeting neuroinflammation, autonomic balance, stress response, and reward circuitry regulation.

Future research should focus on validating these interventions in clinical settings, enhancing our understanding of species-specific microbial roles, and refining neuromodulation techniques. Leveraging these insights can lead to more effective, holistic approaches to chronic pain management, ultimately improving patient outcomes.

Author contributions

TH: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review and editing. ÖE: Writing – original draft, Writing – review and editing. LK: Writing – review and editing. KB: Writing – review and editing. TL: Writing – review and editing. TK: Visualization, Writing – original draft, Writing – review and editing. H-JD: Writing – original draft, Writing – review and editing.

Funding

The authors declare that no financial support was received for the research, authorship, and/or publication of this article.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The authors declare that no Generative AI was used in the creation of this manuscript.

Publisher's note

References

Bonaz, B., Sinniger, V., and Pellissier, S. (2017). The vagus nerve in the neuro-immune axis: Implications in the pathology of the gastrointestinal tract. *Front. Immunol.* 8:1452. doi: 10.3389/fimmu.2017.01452

Breit, S., Kupferberg, A., Rogler, G., and Hasler, G. (2018). Vagus nerve as modulator of the brain–gut axis in psychiatric and inflammatory disorders. *Front. Psychiatry* 9:44. doi: 10.3389/fpsyt.2018.00044

Dinan, T., and Cryan, J. (2016). Gut instincts: Microbiota as a key regulator of brain development, ageing and neurodegeneration. *J. Physiol.* 595, 489–503. doi: 10.1113/jp273106

Freidin, M., Stalteri, M., Wells, P., Lachance, G., Baleanu, A., Bowyer, R., et al. (2020). An association between chronic widespread pain and the gut microbiome. *Rheumatology* 60, 3727–3737. doi: 10.1093/rheumatology/keaa847

Gatchel, R., Peng, Y., Peters, M., Fuchs, P., and Turk, D. (2007). The biopsychosocial approach to chronic pain: Scientific advances and future directions. *Psychol. Bull.* 133, 581–624. doi: 10.1037/0033-2909.133.4.581

Goudman, L., Demuyser, T., Pilitsis, J., Billot, M., Roulaud, M., Rigoard, P., et al. (2024). Gut dysbiosis in patients with chronic pain: A systematic review and meta-analysis. *Front. Immunol.* 15:1342833. doi: 10.3389/fimmu.2024.1342833

Guo, R., Chen, L., Xing, C., and Liu, T. (2019). Pain regulation by gut microbiota: Molecular mechanisms and therapeutic potential. *Br. J. Anaesthesia* 123, 637–654. doi: 10.1016/j.bja.2019.07.026

Halloum, W., Dughem, Y., Beier, D., and Pellesi, L. (2024). Glucagon-like peptide-1 (GLP-1) receptor agonists for headache and pain disorders: A systematic review. *J. Headache Pain* 25:112. doi: 10.1186/s10194-024-01821-3

Hannibal, K., and Bishop, M. (2014). Chronic stress, cortisol dysfunction, and pain: A psychoneuroendocrine rationale for stress management in pain rehabilitation. *Phys. Therapy* 94, 1816–1825. doi: 10.2522/ptj.20130597

Li, S., Hua, D., Wang, Q., Yang, L., Wang, X., Luo, A., et al. (2019). The role of bacteria and its derived metabolites in chronic pain and depression: Recent findings and research progress. *Int. J. Neuropsychopharmacol.* 23, 26–41. doi: 10.1093/ijnp/pyz061

Li, Z., Zhou, J., Liang, H., Ye, L., Lan, L., Lu, F., et al. (2022). Differences in alpha diversity of gut microbiota in neurological diseases. *Front. Neurosci.* 16:879318. doi: 10.3389/fnins.2022.879318

Liu, J., Xu, K., Wu, T., Yao, L., Nguyen, T., Jeste, D., et al. (2023). Deciphering the 'gut–brain axis' through microbiome diversity. *General Psychiatry* 36:e101090. doi: 10.1136/gpsych-2023-101090

Mansuy-Aubert, V., and Ravussin, Y. (2023). Short chain fatty acids: The messengers from down below. *Front. Neurosci.* 17:1197759. doi: 10.3389/fnins.2023.1197759

Martel, M., Finan, P., Dolman, A., Subramanian, S., Edwards, R., Wasan, A., et al. (2015). Self-reports of medication side effects and pain-related activity interference in patients with chronic pain. *Pain* 156, 1092–1100. doi: 10.1097/j.pain.0000000000000154

Martinelli, S., Mazzotta, A., Longaroni, M., and Petrucciani, N. (2024). Potential role of glucagon-like peptide-1 (GLP-1) receptor agonists in substance use disorder: A systematic review of randomized trials. *Drug Alcohol Dependence* 264:112424. doi: 10.1016/j.drugalcdep.2024.112424

Mayer, E., and Tillisch, K. (2011). The brain-gut axis in abdominal pain syndromes. *Annu. Rev. Med.* 62, 381–396. doi: 10.1146/annurev-med-012309-103958

Morris, L., McCall, J., Charney, D., and Murrough, J. (2020). The role of the locus coeruleus in the generation of pathological anxiety. *Brain Neurosci. Adv.* 4, doi: 10.1177/2398212820930321

Nicholas, M., Vlaeyen, J., Rief, W., Barke, A., Aziz, Q., Benoliel, R., et al. (2019). The IASP classification of chronic pain for ICD-11: Chronic primary pain. *Pain* 160, 28–37. doi: 10.1097/j.pain.0000000000001390

O'Riordan, K. J., Collins, M. K., Moloney, G. M., Knox, E. G., Aburto, M. R., Fülling, C., et al. (2022). Short chain fatty acids: Microbial metabolites for gut-brain axis signalling. *Mol. Cell. Endocrinol.* 546:111572. doi: 10.1016/j.mce.2022.111572

Pak, R., Cho, M., Pride, K., and Elsayed, A. (2024). The gut microbiota and chronic pain. *Curr. Pain Headache Rep.* 28, 259–269. doi: 10.1007/s11916-024-01221-x

Raffaeli, W., Tenti, M., Corraro, A., Malafoglia, V., Ilari, S., Balzani, E., et al. (2021). Chronic pain: What does it mean? A review on the use of the term chronic pain in clinical practice. *J. Pain Res.* 14, 827–835. doi: 10.2147/jpr.s303186

Rusch, J., Layden, B., and Dugas, L. (2023). Signalling cognition: The gut microbiota and hypothalamic-pituitary-adrenal axis. *Front. Endocrinol.* 14:1130689. doi: 10.3389/fendo.2023.1130689

Shao, P., Li, H., Jiang, J., Guan, Y., Chen, X., Wang, Y., et al. (2023). Role of vagus nerve stimulation in the treatment of chronic pain. *Neuroimmunomodulation* 30, 167–183. doi: 10.1159/000531626

Vachon-Presseau, E., Roy, M., Martel, M., Caron, E., Marin, M., Chen, J., et al. (2013). The stress model of chronic pain: Evidence from basal cortisol and hippocampal structure and function in humans. *Brain* 136, 815–827. doi: 10.1093/brain/aws371

Wachsmuth, H., Weninger, S., and Duca, F. (2022). Role of the gut–brain axis in energy and glucose metabolism. *Exp. Mol. Med.* 54, 377–392. doi: 10.1038/s12276-021-00677-w

Wu, P., Caceres, A., Chen, J., Sokoloff, J., Huang, M., Baht, G., et al. (2024). Vagus nerve stimulation rescues persistent pain following orthopedic surgery in adult mice. *Pain* 165, e80–e92. doi: 10.1097/j.pain.0000000000003181

Yang, S., and Chang, M. (2019). Chronic pain: Structural and functional changes in brain structures and associated negative affective states. *Int. J. Mol. Sci.* 20:3130. doi: 10.3390/ijms20133130

frontiers | Frontiers in Neuroscience

TYPE Review
PUBLISHED 07 May 2025
DOI 10.3389/fnins.2025.1570173

OPEN ACCESS

EDITED BY
Avinash Veerappa,
University of Nebraska Medical Center,
United States

REVIEWED BY
Sushil Shakyawar,
University of Nebraska Medical Center,
United States
Nana Zhang,
Xiamen University, China

*CORRESPONDENCE
Tao Sun
✉ taosun@hqu.edu.cn

RECEIVED 03 February 2025
ACCEPTED 22 April 2025
PUBLISHED 07 May 2025

CITATION
Jiang C, Chen Y and Sun T (2025) From the gut to the brain, mechanisms and clinical applications of γ-aminobutyric acid (GABA) on the treatment of anxiety and insomnia. *Front. Neurosci.* 19:1570173. doi: 10.3389/fnins.2025.1570173

From the gut to the brain, mechanisms and clinical applications of γ-aminobutyric acid (GABA) on the treatment of anxiety and insomnia

Chengji Jiang[1], You Chen[2] and Tao Sun[1]*

[1]Center for Precision Medicine, School of Medicine and Biomedical Sciences, Huaqiao University, Xiamen, China, [2]GeneYoung Biopharmaceuticals, Shenzhen, China

Anxiety and insomnia are prevalent global mood disorders, and affect approximately 4 and 10 out of every 100 individuals, respectively. Common abnormal brain activity and altered neural circuitries are detected in patients with anxiety disorders and insomnia, suggesting overlapping pathogenesis in these two disorders. Promisingly, GABA from dietary supplements and GABA produced by gut microbiota have shown significant treatment effects in anxiety and insomnia. This review summarizes neurological mechanisms causing anxiety and insomnia, reveals cellular pathways transferring GABA from the gut to the brain, and delivers the therapeutic potential of gut derived GABA for anxiety and insomnia. Moreover, this review proposes emerging therapeutic strategies utilizing engineered GABA-producing bacteria to target anxiety and insomnia, and highlights the potential of live biotherapeutics as novel interventions for mood disorders.

KEYWORDS

GABA, anxiety, insomnia, gut microbiota, probiotics, genetic engineered bacteria

1 Introduction

The global prevalence of anxiety disorder is increasing, particularly in cities with greater levels of stress and fast-paced lifestyles. In 2024, nearly 275 million people are suffering from anxiety disorders, accounting for approximately 3.5% of the global population (Calm, 2024). Moreover, about 10% of adults are affected by insomnia disorders, and an additional 20% experiences occasional insomnia symptoms worldwide (Morin and Jarrin, 2022). Anxiety disorders and sleep disturbances exhibit a bidirectional relationship, where anxiety-induced hyperarousal disrupts sleep architecture, and sleep deprivation amplifies emotional vulnerability (Bragantini et al., 2019; Pillai et al., 2014; Sadeh et al., 2004). Specifically, sleep deprivation increases cortisol levels, amplifies emotional procession of the limbic system and the salience network involved in cognitive control (dorsal anterior cingulate cortex and anterior insula), and creates a vicious cycle of hyperarousal and anxious apprehension (Carlisi et al., 2017; Palmer and Alfano, 2020; Yoo et al., 2007). Conversely, anxiety disorders disrupt sleep architecture by reducing slow-wave sleep and increase nocturnal awakenings through noradrenergic hyperactivity in the locus coeruleus (Chellappa and Aeschbach, 2022; Gong et al., 2021).

Pathogenic studies have shown that anxiety and insomnia share similar biological mechanisms (Morris et al., 2020; Van Someren, 2021). The locus coeruleus (LC) can activate the amygdala by releasing norepinephrine (NE), thereby inducing anxiety and triggering the body's anxious response (Suárez-Pereira et al., 2022). The locus coeruleus also acts as the

control center for wakefulness. Its specialized nerve cells release norepinephrine when one is awake, like an internal "alarm system." During deep and slow-wave sleep, these cells quiet down significantly, and fall silent completely during rapid eye movement (REM) sleep, the stage mostly associated with vivid dreaming (Verret et al., 2006). Thus, the LC serves as a pivotal point where the two disorders converge.

Another potential pivotal point is the hypothalamic–pituitary–adrenal (HPA) axis. Anxiety and insomnia patients commonly exhibit activation of the HPA axis, characterized by elevated hormone levels such as cortisol (Juruena et al., 2020; Zhang et al., 2014). Elevated cortisol levels enhance the amygdala's sensitivity to threat signals (Roberts et al., 2022). Prolonged high cortisol exposure keeps it in "hyper-vigilance," causing continuous anxiety even without real threats. Moreover, high cortisol can activate the amygdala, and reduce slow-wave sleep (Buckley and Schatzberg, 2005; Wong et al., 2000). Additionally, increased cortisol damages prefrontal cortex neurons and weakens its inhibitory function on the amygdala, impairing one's emotion regulation ability (Ren et al., 2022).

Currently, besides behavioral interventions such as mental therapy and exercise, medication remains an effective treatment for anxiety and insomnia (Kandola and Stubbs, 2020; Riedel et al., 2024; Shaha, 2023; Vu and Conant-Norville, 2021). Many medications have been developed, with several already commercialized and available on the market. For example, classic medications such as barbiturates and benzodiazepines are known to possess sedative and hypnotic effects (Bormann, 1988; Bowery et al., 1979; Sigel and Ernst, 2018). However, the current medications for anxiety and insomnia often cause various side effects. For instance, benzodiazepines can cause sedation, slowed reaction time, and impaired memory and psychomotor function (Greenblatt et al., 2020). Therefore, the development of a more natural and less harmful medication holds significant promise for the treatment of anxiety and insomnia.

As a natural neurotransmitter, γ-aminobutyric acid (GABA) is promoted for its benefits in addressing anxiety and insomnia (Hepsomali et al., 2020; Ngo and Vo, 2019). The efficacy of orally administered GABA is supported by positive consumer reviews and clinical experiments, suggesting its promising therapeutic effects on anxiety and insomnia symptoms (Ngo and Vo, 2019). Dietary sources that are rich in GABA and GABA-producing probiotics also display significant therapeutic effects on anxiety and insomnia (Liwinski et al., 2023; Strandwitz, 2018).

Because the association of gut microbiota in human health and diseases is well recognized, it is timely to better understand how gut derived GABA may function in humans. Moreover, in this review, orally administered GABA, GABA from GABA-rich food, and GABA from GABA-producing probiotics are collectively referred to as "gut derived GABA." This review explores production, absorption and transmission of gut derived GABA, clarifies its potential mechanisms in alleviating anxiety and insomnia, and discusses future applications for optimized production and clinical use.

2 Anxiety and insomnia

2.1 Anxiety

Anxiety disorder represents a prevalent category of mood disorders (Byrd and Brook, 2014). While acute anxiety involves a sudden onset of intense fearful experiences, chronic anxiety is the most common presentation of anxiety disorders, with symptoms including persistent and unwarranted worries, fatigue, insomnia and profound distress (Nutt, 2005; Robinson et al., 2019). A variety of risk factors, including family issues, environmental exposures, and perceived threats, can contribute to the development of anxiety (Warner and Strawn, 2023).

The neural circuitry associated with anxiety has been studied. The amygdala plays a crucial role in emotion generation, recognition and regulation, as well as in controlling learning and memory, and fear response through receiving and responding information from the locus coeruleus (LC) and other sensory inputs (Krettek and Price, 1978; Suárez-Pereira et al., 2022). For transient panic, the basolateral amygdala (BLA) integrates sensory information from the environment and activates the central amygdala (CE). Subsequently, the central nucleus of the amygdala (CeA) triggers defensive responses by projecting to brain regions such as the ventral striatum, hippocampus, lateral hypothalamus (LH) and surrounding areas (LeDoux, 2000). For persistent anxiety, the bed nucleus of the stria terminalis (BNST), which is part of an extended amygdala, is more directly involved and closely coupled with the CE (Zhu et al., 2024).

Under high-pressure conditions, the LC enhances amygdala function while simultaneously attenuates the prefrontal cortex (PFC) function to facilitate fear learning. Conversely, under low-pressure conditions, the LC enhances PFC function, thereby increasing the inhibitory effect of the medial prefrontal cortex (mPFC) on the amygdala from top to bottom, and subsequently promoting fear extinction (Giustino and Maren, 2018).

Moreover, the hippocampus receives signals from the BLA and stores fear and long-term anxiety signals (Hajisoltani and Meftahi, 2024). These memories persist for a period and can be reactivated by a similar context, even if the individual is no longer directly in that situation (Chaaya et al., 2018). In addition to anxiety signals from the locus coeruleus, sensory information from visceral changes, such as heart rate, gastrointestinal responses and blood pressure, can be transmitted to the mPFC via the anterior insula, thereby contributing to the maintenance of anxiety (Vertes, 2004). Disrupted limbic-hypothalamic–pituitary–adrenal axis (LHPA), commonly referred to as the HPA axis, is also involved in anxiety disorders. Patients with anxiety exhibit elevated levels of plasma cortisol, which is released from the adrenal gland, potentially intensifying feelings of fatigue due to the acceleration of glycogen metabolism (Juruena et al., 2020).

2.2 Insomnia

Insomnia is the most prevalent sleep disorder in industrialized countries, characterized by symptoms such as dissatisfaction with sleep quality or duration, difficulty falling asleep at bedtime, waking up in the middle of the night or too early in the morning (Morin and Benca, 2012). Although symptoms of insomnia primarily occur at night, many individuals also experience daytime cognitive impairments, such as difficulties with attention, concentration and memory, as along with mood disturbances like irritability and dysphoria (Buysse et al., 2007). Even though a significant number of adults are suffering from severe insomnia, less than 15% of those receive proper treatment (Bhat et al., 2008).

Dysfunction of the wakefulness and sleep neural systems is the major cause of insomnia (Riemann et al., 2010). The wakefulness regulatory system comprises two pathways (Saper et al., 2005). One pathway involves NA neurons in the LC, serotonin (5-HT) neurons in the raphe nuclei, histaminergic (His) neurons in the tuberomammillary nucleus (TMN) and dopaminergic (DA) neurons in the ventral periaqueductal gray (vPAG) (John et al., 2004; Ko et al., 2003; Verret et al., 2006). This pathway receives signals from neurons in the LH, including orexin (ORX) and melanin-concentrating hormone (MCH), and acetylcholine (ACh) and GABA from neurons in the basal forebrain (BF), and leads to cortical activation and wakefulness. The second ascending arousal pathway includes cholinergic neurons in the pedunculopontine tegmental nucleus (PPT) and laterodorsal tegmental nucleus (LDT). Their activation stimulates thalamic relay neurons, resulting in cortical activation and maintenance of conscious wakefulness (Morin et al., 2015).

In addition, the sleep-promoting system includes the hypothalamic ventrolateral preoptic area, basal ganglia, cerebral cortex, limbic system, BF, and the brainstem and thalamus (Saper et al., 2005). Activation of GABAergic neurons in the ventrolateral preoptic nucleus (VLPO) and inhibition of NA activity mediated sleep are the core sleep-promoting cluster (Dubourget et al., 2017). Moreover, VLPO GABAergic neurons can inhibit the activation of wakefulness systems, including the LDT, dorsal raphe nucleus (DRN), and locus coeruleus (LC), causing a transition to sleep (Morin et al., 2015).

Sleep–wake homeostasis is maintained by the "Flip-Flop Switch" model, in which transition between sleep and arousal depends on interactions among the VLPO, monoaminergic cell groups (nuclei) and ORX neurons (Morin et al., 2015; Saper et al., 2005). Dysregulation of the arousal system, characterized by heightened physiological arousal during both sleep and wakefulness, results in a state known as hyperarousal, which is a significant contributor to insomnia (Riemann et al., 2010).

3 An association of GABA with anxiety and insomnia

Insomnia is considered to exacerbate anxiety, since inadequate sleep can increase negative emotions, diminish positive emotions, and alter adolescents' comprehension, expression and modulation of emotions (Blake et al., 2018; Palmer and Alfano, 2017). Conversely, anxiety can contribute to the onset of insomnia, and stress exposure disrupts sleep, leading to difficulties in both falling asleep and staying asleep (Kalmbach et al., 2018).

It appears that both anxiety disorder and insomnia are associated with decreased levels of GABA. Significantly reduced GABA levels have been observed in brains of individuals with anxiety disorders, particularly in the thalamus and the amygdala, as detected by Magnetic Resonance Spectroscopy (MRS) analysis (Babaev et al., 2018; Streeter et al., 2010). GABA levels in plasma of patients with anxiety have shown notable decreases (Lydiard, 2003; Shutta et al., 2021). Moreover, reduced expression levels of $GABA_A$ receptor α1 and α2 subunits have also been detected in the serum of insomnia patients (Xiang et al., 2023). On the other hand, the $GABA_A$ receptor agonist, such as benzodiazepine, can effectively alleviate symptoms of anxiety and treat insomnia, and the antagonist of $GABA_B$ and $GABA_C$ receptors, like CGP-36742, can reduce a patient's slow-wave sleep (Deschaux et al., 2006; Nutt, 2005; Sternbach, 1978).

4 Synthesis and functions of GABA

GABA is a prominent neurotransmitter within the brain, and also functions as a regulatory hormone in peripheral organs, which holds significant importance for the human body (Jin and Korol, 2023; Zhang et al., 2021). GABA, also known as 4-aminobutyric acid, is a compound represented by the chemical formula $C_4H_9NO_2$ (Figure 1A). It is an amino acid that is widely distributed among vertebrates, plants, and microorganisms (Sarasa et al., 2020). In living organisms, GABA is primarily synthesized by glutamate decarboxylase (GAD), an enzyme that uses glutamate (L-Glu) as the raw material in the presence of coenzyme pyridoxal phosphate and protons (Figure 1A) (Soghomonian and Martin, 1998).

In bacteria, GABA is primarily involved in the survival ability in an acidic environment. *Escherichia coli* and other enterobacteria utilize the H^+ consuming reaction catalyzed by gad to reduce the concentration of H^+ around the microorganism itself, and survive in the stomach acidity before reaching the intestine (Figure 1B) (Sarasa et al., 2020). In the vertebrates, GABA primarily acts on GABA receptors, triggering ion exchange across the neuronal membrane, altering current signals, and generating inhibitory potentials, thereby inhibiting neuronal excitation (Taylor et al., 2003). Particularly, GABA can act on ionotropic receptors such as $GABA_A$ and $GABA_C$ receptors, as well as the metabotropic receptor $GABA_B$ receptor in the brain (Figure 2) (Felice et al., 2022).

$GABA_A$ receptors are pentameric ligand-gated ion channels widely expressed throughout the central and peripheral nervous system (Luo and Balle, 2022). When GABA binds to the $GABA_A$ receptor, it causes the chloride ion channels on the receptor to open, and leads to either the influx or efflux of chloride ions, with the predominant movement being the influx (Figure 2A) (Kim and Hibbs, 2021). The opening of the chloride ion channels by GABA helps stabilize the resting potential of cells during the activation of excitatory receptors and makes it more difficult for neurons to generate excitatory action potentials and release neurotransmitters (Bormann, 1988; Kim and Hibbs, 2021).

$GABA_B$ receptors are G-protein coupled receptors and primarily exist in a heterodimeric form. Different from the $GABA_A$ ion channels, $GABA_B$ receptors can anchor to Ca^{2+} and K^+ channels on the cell membrane (Figure 2B) (Bormann, 1988). By dynamically regulating the closure of these ion channels, it inhibits the influx of Ca^{2+} and promotes the influx of K^+ to achieve an inhibitory hyperpolarized state of the membrane potential. Additionally, it can dynamically regulate the switches of the G-proteins it anchors to, realizing the activation of guanine nucleotide-binding proteins and indirectly producing the second messenger cAMP to exert its effects (Bettler et al., 2004).

The $GABA_C$ receptor is a subclass of the $GABA_A$ receptor, and is often referred to as the $GABA_A$ rho receptor with three different subtypes ρ1-3 (Olsen and Sieghart, 2008). Its structure and function are largely similar to those of $GABA_A$ receptors. It is categorized separately due to being insensitive to both bicuculline and baclofen (Johnston, 2013).

In the brain, GABA primarily plays a role in intermediate neurons, which are notably rich in the enzyme GAD. Experimental

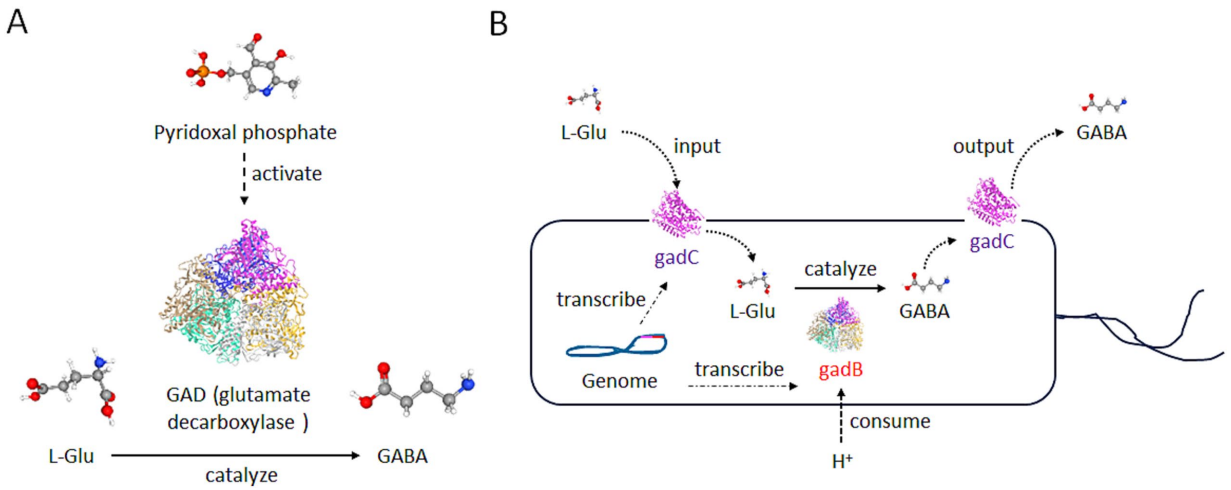


FIGURE 1
Structure and production of GABA. **(A)** The production diagram of GABA. With the assistance of pyridoxal phosphate, the glutamate decarboxylase (GAD) enzyme is transformed into an active structure and then converts L-Glu into GABA. L-Glu, GABA, and Pyridoxal phosphate are illustrated by PubChem. White ball: the hydrogen atom; red ball: the oxygen atom; gray ball: the carbon atom; blue ball: the nitrogen atom; yellow ball: the phosphorus atom. **(B)** The diagram of GABA's production by gut microbiota. The genome of many bacteria carries the gadB and gadC genes. The gadB is a type of GAD in **(A)**, and functions in the same manner as GAD. GABA produced by gadB can be transported extracellularly through gadC. Meanwhile, the raw material L-Glu can also enter the cell through gadC, and this process can consume H^+ ions outside the environment.

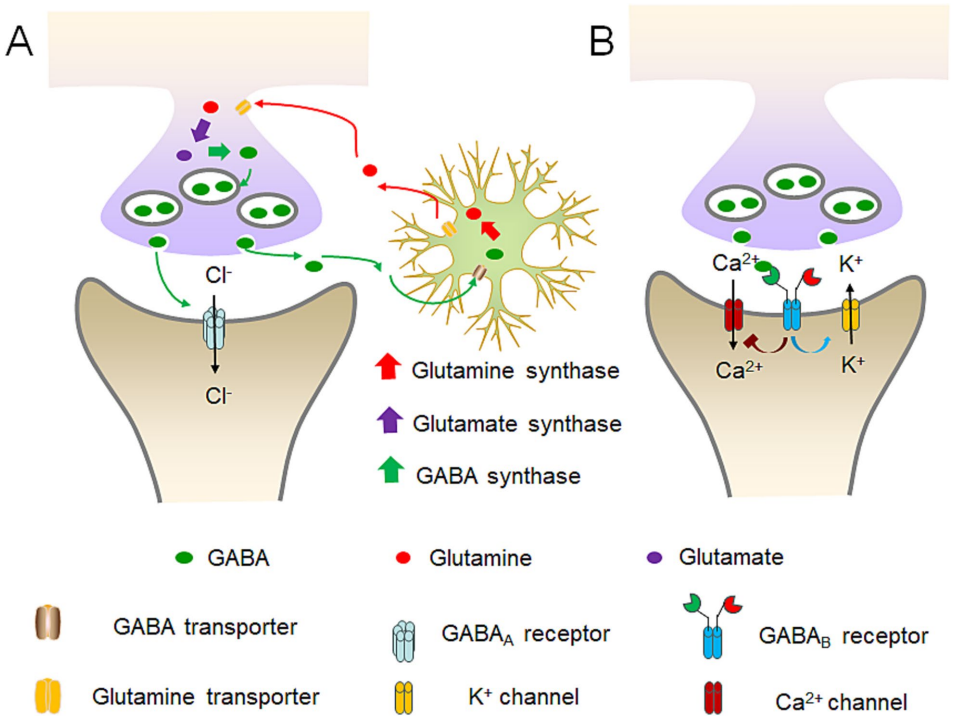


FIGURE 2
Diagram of GABA receptors' workflow and GABA's conversion balance. **(A)** $GABA_A$ receptors' workflow and the balance among GABA, glutamine and glutamate. GABA neurons can convert the absorbed glutamine into glutamate, and then into GABA. Subsequently, GABA is excreted and acts on the next neuron. Upon the binding of GABA to the $GABA_A$ receptor, it prompts the opening of chloride ion channels within the receptor, thereby giving rise to either the inward or outward flow of chloride ions. The predominant direction of movement is the inward flow. **(B)** $GABA_B$ receptors' workflow. $GABA_B$ receptors are capable of anchoring to Ca^{2+} and K^+ channels on the cell membrane. Through the dynamic modulation of the closure of these ion channels, it restricts the influx of Ca^{2+} and augments the influx of K^+, thereby attaining an inhibitory hyperpolarized state of the membrane potential.

findings have revealed that the removal of the GAD enzyme from the mouse brain tissue results in a significant reduction in detectable GABA levels (Ji et al., 1999).

In the synaptic cleft, any excess GABA that remains unabsorbed after transmission is re-uptaken by astrocytes, where it is converted into glutamine (Figure 2A). This glutamine is then utilized by glutamatergic neurons to synthesize glutamate. Subsequently, the released glutamate is taken up by GABAergic neurons to regenerate GABA. Through the coordinated activity of glutamatergic neurons, GABAergic neurons and astrocytes, GABA stays at a balanced level, and the neural network maintains a state of equilibrium, which is essential for the proper functioning of the brain (Mazzoli and Pessione, 2016; Steel et al., 2020).

In patients with anxiety and insomnia, signs of excessive consumption of GABA were detected. This may be related to the overactivity of GABA transaminase, which causes excessive conversion of GABA into succinic semialdehyde (SSA), reducing the neuroinhibitory effect of GABA (Liu M. Q. et al., 2022). Adding some GABA transaminase inhibitors can be effective in the treatment of anxiety and insomnia (Di Pierro et al., 2024; Liu M. Q. et al., 2022; Srichomphu et al., 2022).

5 Therapeutic effects of gut derived GABA on anxiety and insomnia

5.1 Anxiety

Gut derived GABA encompasses GABA obtained from dietary sources as well as GABA produced by the resident gut microbiota, and represents a significant source of GABA in the human body (Duranti et al., 2020; Liwinski et al., 2023). Direct oral GABA supplements and dietary food, for instance potatoes, tomatoes, rice, tea and beans, are primary sources of gut derived GABA (Tables 1, 2) (Cai et al., 2014; Hinton and Johnston, 2020; Kim et al., 2015; Lei et al., 2023; Liwinski et al., 2023; Vann et al., 2020; Yan et al., 2021). Moreover, bacteria can convert glutamate into GABA through a process that utilizes protons (Feehily and Karatzas, 2013). Probiotic bacteria such as *Bifidobacterium*, *Lactobacillus* and *Lactiplantibacillus* can produce GABA and significantly increase the GABA level in the intestines (Table 3) (Barrett et al., 2012; Duranti et al., 2020; Luck et al., 2021; Watanabe et al., 2022). Notably, studies have shown that gut derived GABA can treat anxiety and insomnia by exerting a widespread influence on hormone levels, secretion of signaling molecules, gene expression, and various other physiological changes (Abdou et al., 2006; Liwinski et al., 2023; Yamatsu et al., 2015).

Studies indicate that both the direct administration of 100 mg of GABA and the consumption of GABA-containing foods exhibit a certain therapeutic effect on anxiety-related behaviors (Table 1) (Barros-Santos et al., 2020; Faucher et al., 2022; Yoto et al., 2012). The administration of the GABA-producing strain *Bifidobacterium adolescentis IM38* significantly improved anxiety-like behaviors in mice, and this effect was blocked by flumazenil, a benzodiazepine receptor antagonist (Jang et al., 2018). This finding suggests that gut derived GABA exerts their influence through GABA-regulated sites, potentially acting on GABA receptors in the brain. Moreover, administering probiotics *Lactiplantibacillus plantarum SNK12*, which produces GABA, has been shown to alleviate anxiety and reduce salivary cortisol levels in humans (Watanabe et al., 2022). Similar phenomena are also observed in animal models (Cai et al., 2022).

5.2 Insomnia

Clinical trials have provided evidence that the administration of 100 mg of GABA significantly improves sleep quality, which is reflected by a reduction in sleep latency and an increase in the duration of non-REM sleep (Table 2) (Yamatsu et al., 2015; Yamatsu et al., 2016). Similar positive effects on sleep quality have been observed in clinical trials involving the consumption of GABA tea (Cheng and Tsai, 2009). In animal studies, a significant decrease in peripheral blood GABA concentration of sleep-deprived monkeys, accompanied by a substantial increase in peripheral blood NE and cortisol, both indicative of HPA axis upregulation (Zhao et al., 2022). Supplementation with probiotics producing GABA significantly reduced the levels of hormones associated with the HPA axis and increased peripheral blood GABA levels, suggesting a therapeutic effect. Similar findings were also reported in mouse experiments. Mice fed GABA black tea exhibited a significant decrease in sleep latency

TABLE 1 Gut derived GABA exhibits therapeutic effects in the treatment of anxiety.

Subject	Dosing	Frequency	Function	References
Human	100 mg	3 administrations, separated by 7-day intervals	Anxiety ↓, Brain alpha wave ↑, beta wave ↓, saliva IgA level ↑	Abdou et al. (2006)
Human	100 mg	Everyday for 8 weeks	Stress ↓, Alpha & beta band brain waves ↓	Yoto et al. (2012)
Human	0–50 mg (beverage)	Once a time	Psychological fatigue ↓, Salivary chromogranin A & cortisol levels ↓	Kanehira et al. (2011)
Human	28 mg GABA (chocolate)	Once a time	Psychological stress ↓, HRV (heart rate variability) ↑	Nakamura et al. (2009)
Human	Vegetable tablets containing GABA	Once a time	Sympathetic nervous activity ↓, blood pressure ↑	Okita et al. (2009)
Human	GABA-fortified oolong tea	Once a time	Chronic stress ↓, autonomic imbalance impact ↓, HRV ↑	Hinton et al. (2019)

TABLE 2 Gut derived GABA exhibits therapeutic effects in the treatment of insomnia.

Subject	Dosing	Dosing frequency	Function	References
Human	300 mg	Everyday for 4 weeks	Sleep latency ↓, sleep efficacy ↑	Byun et al. (2018)
Human	100 mg	30 min before going to bed, everyday	Sleep latency ↓, NREM sleep ↑	Yamatsu et al. (2015)
Human	100 mg	Every day for a week	Plasma GABA levels ↑, sleep latency ↓, total non-REM sleep duration ↑	Yamatsu et al. (2016)
Human	GABA tea	Everyday for a month	Sleep onset latency ↓, REM onset latency ↑	Cheng and Tsai (2009)

period induced by pentobarbital sodium, accompanied by a substantial increase in the duration of effective sleep (Zhao et al., 2015). Administration of 100 mg/kg GABA not only significantly reduced sleep latency and increased sleep duration, but also elevated the expression levels of GABA receptor mRNA in the mouse brain, proving that supplementing with gut derived GABA can indeed enhance GABAergic signaling in the brain and improve sleep (Chen et al., 2021; Kim et al., 2019; Liu D. et al., 2022).

5.3 Dosage and side effects

Studies indicated that a direct administration of 100 mg of GABA represents an appropriate dosage for the treatment of anxiety and insomnia. This dosage can exert positive effects in terms of alleviating anxiety, shortening sleep latency, and enhancing sleep quality (Abdou et al., 2006; Yamatsu et al., 2015; Yamatsu et al., 2016; Yoto et al., 2012). Research has demonstrated that even when a relatively high dose, such as 6 g of GABA per day, is administered, no obvious side effects are observed in volunteers, suggesting the favorable biosafety of GABA (Li et al., 2015). However, it is noteworthy that current studies have revealed that GABA is not conducive to the anti-tumor response. Multiple investigations have elucidated that GABA can inhibit dendritic cell mediated T-cell recruitment and activation (Huang et al., 2022; Zhang et al., 2021). Therefore, it is not advisable to use gut derived GABA as a drug treatment for cancer patients.

5.4 Probiotics and engineered bacteria

It is worth noting that in addition to the dietary and orally administered GABA presented in Tables 1, 2, the utilization of GABA-producing bacteria has emerged as an innovative therapeutic strategy for anxiety and insomnia (Table 3). A substantial body of research demonstrates that GABA-producing bacteria can effectively mitigate these disorders. These bacteria exert diverse effects on the body, such as diminishing stress factor levels, augmenting GABA concentrations, and modulating the levels of GABA receptors and their corresponding mRNAs in the brain (Janik et al., 2016; Jin et al., 2023; Liu G. et al., 2022; Zhou B. et al., 2022).

Unlike orally ingested GABA, which is rapidly eliminated from the body, GABA-producing gut bacteria can colonize the host's gastrointestinal tract for an extended period. This persistent colonization enables continuous GABA synthesis, thereby conferring prolonged therapeutic benefits (Pokusaeva et al., 2017). Additionally, the impact of these bacteria on the gut's ecological environment is persistent over time. Significantly, the employment of probiotic strains has the significant advantage of imposing a lower metabolic burden on organs such as the liver compared to traditional small molecule drugs (Li et al., 2015; Oketch-Rabah et al., 2021).

Moreover, through microbiome sequencing, it has been found that some bacteria can either promote or inhibit the growth of GABA-producing bacteria. Supplementation with *Limosilactobacillus fermentum L18* can significantly increase the abundance of *Bifidobacterium* and *Lactobacillus* (Kaur et al., 2023). This might be due to that these strains can optimize the acidic environment for GABA production by regulating the intestinal pH value and metabolite exchange. However, some strains, such as *Enterobacteriaceae*, can reduce the abundance of GABA-producing bacteria. This could be due to the production of substances like lipopolysaccharides by these strains, which hinder the growth of GABA-producing bacteria (Zeng et al., 2024). Thus, the supplementation of GABA-producing bacteria is still an effective method. In the future, the administration of combined bacterial agents could be considered (Zhao et al., 2022).

Apart from certain natural probiotic strains like *Bifidobacterium* and *Lactobacillus*, genetically engineered bacterial strains have also been harnessed in the treatment of anxiety. To date, numerous studies have endeavored to employ engineered probiotics to express GABA and address anxiety (Lebovich and Andrews, 2022, 2023; Pan et al., 2022; Pokusaeva et al., 2017). The advantage of using engineered bacteria for anxiety treatment resides in the precise regulation of GABA production, for instance treating anxious mice using an engineered *Lactococcus lactis* strain (Pan et al., 2022).

Moreover, the majority of these studies have adopted *Escherichia coli Nissle 1917* (*EcN*) (Lebovich and Andrews, 2022, 2023). *EcN* is a renowned probiotic strain of *Escherichia coli*. Owing to its probiotic properties and well-defined manipulable genetic background, it is currently extensively utilized as a vector in engineered bacteria therapy (Lynch et al., 2022). When engineered *EcN* is employed to produce GABA, the GABA yield can attain as high as 17.9 g/L (Lan et al., 2021). Another benefit of engineered bacteria is their proficiency in GABA production, as they can overexpress gadB and gadC proteins within the bacteria. Nevertheless, at present, research articles on the application of *EcN* that produces GABA to treat neurological diseases remain relatively scarce, presenting an area worthy of future exploration.

There are several issues regarding probiotics that need to be addressed. The introduction of gut microbiota can alter intestinal metabolic activities in the host, which may have adverse effects on a very small number of individuals. For example, the probiotics *L. acidophilus* and *Bifidobacterium* can convert primary bile salts into

TABLE 3 Summary of bacterial strains producing GABA.

Subject	Strain	Function	References
Human	*Bifidobacterium breve M-16V*	Mood and sleep scores ↑	Mutoh et al. (2024)
Human	*Lactobacillus brevis DPC6108*	Gut GABA capacity ↑	Barrett et al. (2012)
Mice	*Bifidobacterium adolescentis IM38*	Anti-anxiolytic-like effect ↑, Corticosterone and IL-6 blood levels ↓, Blood TNF-α level ↓	Jang et al. (2018)
Mice	*Bifidobacterium longum DD98*	Depression and anxiety-like behaviors ↓, Serotonin, GABA, NPY, BDNF expression ↑	Jin et al. (2023)
Mice	*Lactobacillus rhamnosus (JB-1)*	Anxiety and depression-related behaviors ↓, $GABA_{A,B}$ receptors sub-types in specific brain regions ↑	Janik et al. (2016)
Mice	*Lactobacillus plantarum 286 and 81*	Anti-depressant-like and anxiolytic-like effects ↑	Barros-Santos et al. (2020)
Mice	*Lactobacillus plantarum HJZW08*	Anxiety-like and depressive-like behaviors ↓, neuroactive molecules levels ↑	Wu et al. (2022)
Mice	*Lactobacillus rhamnosus GG*	Anxiety behaviors ↓, BDNF and GABA receptors levels in hippocampus and amygdala ↑	Zhou B. et al. (2022)
Mice	*Lactiplantibacillus plantarum LZU-J-TSL6*	Anxiety disorder ↓, hippocampal region GABA content ↑, some anxiety-related markers ↑	Liu G. et al. (2022)
Mice	*Lacticaseibacillus rhamnosus GG*	Anxiety and depressive-like phenotype ↓	Faucher et al. (2022)
Monkey	*Bifid Triple Viable Capsules* (*Bifidobacterium*, *Lactobacillus*, and *Enterococcus faecalis*.)	Plasma GABA levels ↑, stress responses and gut microbiota dysbiosis ↓	Zhao et al. (2022)

secondary bile salts (Ruseler-van Embden et al., 1995). Most secondary bile salts are reabsorbed to enter the liver for metabolism. If the liver function is abnormal and unable to metabolize secondary bile salts properly, it may lead to their accumulation in the liver, exerting toxic effects on liver cells and further aggravating liver damage (Zawistowska-Rojek and Tyski, 2018). Moreover, some probiotics may carry antibiotic-resistance genes, which poses a risk of potential transfer of these genes within the gut microbiota (Merenstein et al., 2023). These issues need to be considered and addressed in the future studies.

5.5 Gut microbiota

To optimize the application of gut derived GABA, it is crucial to summarize and investigate the underlying mechanisms of its effects. When GABA is taken orally and enters the intestinal tract, it will first be affected by the body's intestinal microbiota (Figure 3). GABA is reported to act as a critical growth factor for certain strains of intestinal bacteria in the human gut microbiota, leading to an increase of these specific microbial communities within the host (Strandwitz et al., 2019). GABA also serves as a carbon and nitrogen source for the gut microbiota. Within bacteria, GABA can be further converted into succinic semialdehyde (SSA) by GABA transaminase. Subsequently, SSA is transformed into succinate by the enzyme succinic semialdehyde dehydrogenase, which then enters the tricarboxylic acid (TCA) cycle for further metabolic processing (Sarasa et al., 2020).

5.6 GABA absorption

5.6.1 Small intestine epithelial cell transport: SLC family

The initial site of interaction between gut derived GABA and the human body is the intestinal tract. In the intestine, gut derived GABA is absorbed by intestine epithelial cells (Figure 4). The intestine normally employs passive absorption to transport small-molecule substances, such as amino acids (Nácher et al., 1994; Thwaites et al., 2000). GABA transporters in the intestines of both rodents and humans facilitate both active and passive absorption of GABA (Nácher et al., 1994; Thwaites et al., 2000). Proton-coupled amino acid transporter 1 (PAT1) has been confirmed as the GABA transporter responsible for transporting GABA across the apical membrane of intestinal epithelial cells into the cells (Figure 4B) (Chen et al., 2003).

It is speculated that small intestinal epithelial cells exclude GABA from the basal membrane into the extracellular space of the submucosal layer through one of the solute carrier transporters (SLC) family proteins (Figure 4B). SLC transporters comprise approximately 350 members belonging to 55 families, with some SLC proteins exhibit bidirectional transport functions (Kristensen et al., 2011). For example, SLC6A1 (GAT1) mediates the transport of GABA together with sodium and chloride ions, and is responsible for the reuptake of GABA from the synapse. The direction and magnitude of GABA transport are determined by

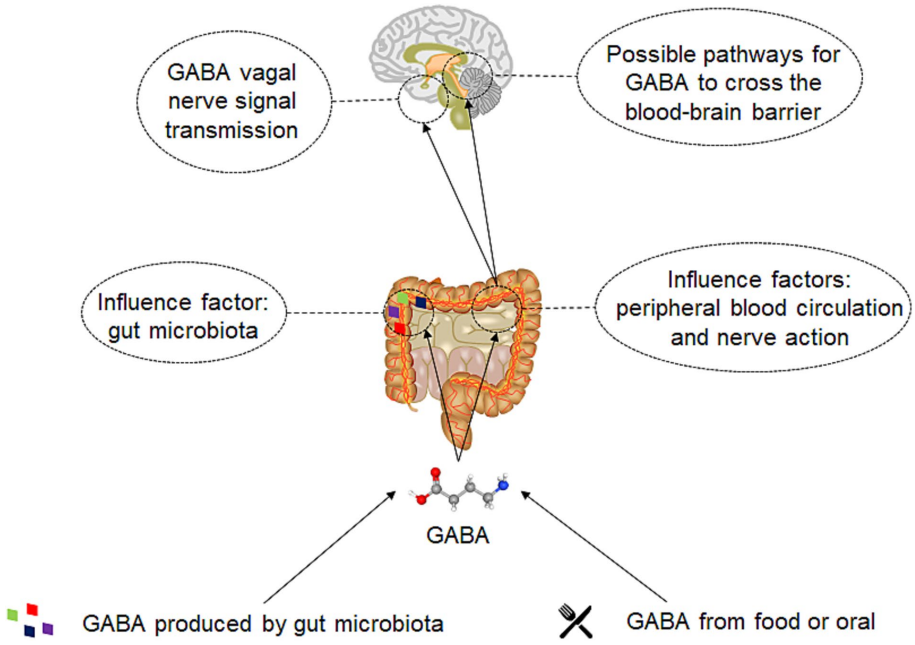


FIGURE 3
The mechanism of gut derived GABA on human body. Two sources gut derived GABA: one is obtained through direct oral intake or via food, and the other is generated by the gut microbiota. Subsequently, GABA will directly act on the gut microbiota and have a direct impact on its metabolism. A significant portion will also be absorbed by the gut, enter the bloodstream, or act on enteric neurons and the vagus nerve. GABA that acts on the enteric nerves and vagus nerve can be transmitted through neural signals and affect the brain. GABA that enters the bloodstream may eventually interact with the brain by crossing the blood–brain barrier.

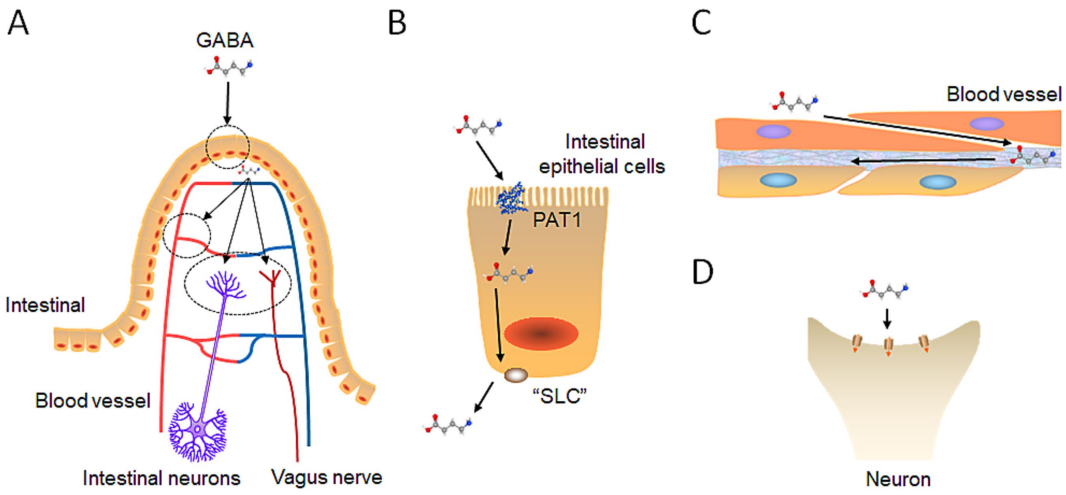


FIGURE 4
Exogenous GABA intestinal absorption pathway. **(A)** GABA intestinal absorption diagram: Initially, GABA is taken up by intestinal epithelial cells. Then, it diverges into three pathways: bloodstream entry, interaction with enteric neurons, and connection with the vagus nerve. **(B)** GABA intestinal transport: GABA crosses the small intestine membrane via the PAT protein on the basolateral side and exits through a probable SLC family protein on the apical side. **(C)** GABA integrates into the blood circulation: GABA penetrates the fenestrated capillaries within the intestinal lining to join the bloodstream. **(D)** GABA binds to receptors: GABA interacts with GABA receptors on intestinal neurons and the vagus nerve, as previously described, to exert its effects.

thermodynamic conditions, influenced by factors such as membrane potential and the concentrations of Na^+, Cl^-, and GABA (Mattison et al., 2018). SLC family proteins with bidirectional transport mechanisms or one that transports GABA from intracellular to extracellular spaces may exist at the basal membrane of small intestinal epithelial cells, facilitating the transport of GABA (Zafar and Jabeen, 2018). Further research is necessary to confirm this possibility.

5.6.2 Capillary absorption

When GABA is transported by small intestinal epithelial cells to the lamina propria, it partially enters the capillaries to participate in

blood circulation (Figure 4C). The capillaries in the lamina propria are fenestrated, called "fenestrated capillary," meaning that they have intracellular pores or "windows" with a diaphragm that penetrate the endothelial lining (Augustin and Koh, 2017). These pores facilitate the exchange of water and allow the passage of solutes, such as small peptides, between plasma and interstitial fluid. Fenestrated capillaries are found throughout various tissues, including the choroid plexus of the brain, certain endocrine organs, kidney filtration sites, and intestinal absorptive regions (Augustin and Koh, 2017). Given that GABA is significantly smaller than peptides, it can theoretically pass through the pores of fenestrated capillaries. Consequently, research has demonstrated that GABA derived from the gut can effectively elevate GABA levels in the bloodstream of both humans and animal models (Yamatsu et al., 2016; Zhao et al., 2022).

5.6.3 Vagus nerve absorption

GABA is transported by small intestinal epithelial cells to the lamina propria, where some enter the bloodstream, and some act on the intestinal nerve plexus, which causes intestinal muscles and glands to respond and transmit signals to other parts such as the brain through the vagus nerve (Figure 4D). Interestingly, administering *Lactobacillus rhamnosus (JB-1)*, a probiotic strain producing GABA, has been shown to improve anxiety-and depression-related behaviors in mice (Bravo et al., 2011). However, after surgically removing the vagus nerves in mice fed with the probiotic, the behavioral improvements were lost, indicating that the vagus nerve serves as a major modulatory constitutive communication pathway between gut-exposed bacteria and the brain.

5.6.4 Hypothalamus—hormone secretion

After receiving the signals of gut derived GABA, the afferent fibers of the vagus nerve in the gastrointestinal tract transmit the messages through neuronal synapses, converting them into cholinergic signals (Qu et al., 2024). These signals are then transmitted to the nucleus tractus solitarius (NTS) in the medulla oblongata. Subsequently, the NTS communicates with the locus coeruleus, which sends signals onward to either the amygdala or the thalamus (Breit et al., 2018). Additionally, the hypothalamus directly receives signals from GABA in the peripheral blood. Structurally, it is closely connected to the primary capillary plexus within the pituitary portal system (Ilgin, 2020). Studies have shown that injecting competitive antagonists of $GABA_A$ receptors into the PVN significantly increases stress-induced cortisol secretion, whereas selective GABA receptor antagonists notably reduce it (Cullinan et al., 2008). Moreover, supplementation with GABA-producing probiotics inhibits the activated HPA axis. Indeed, this indirectly provides evidence that GABA in peripheral blood can act on the hypothalamus to regulate the HPA axis (Cai et al., 2022; Watanabe et al., 2022).

5.7 Brain

5.7.1 The pathway of gut derived GABA entering the brain

The impact of gut derived GABA on the brain has been a contentious topic, with a central question being the permeability of the blood–brain barrier (BBB). While some studies suggest that GABA cannot penetrate the BBB in mammals, other research indicates that GABA can traverse it, albeit in modest quantities (Boonstra et al., 2015; Frey and Löscher, 1980; Knudsen et al., 1988; Toth and Lajtha, 1981; Van Gelder and Elliott, 1958). Analyzing the structural aspects of the BBB could provide a theoretical basis to support the potential passage of GABA through this barrier.

The BBB consists of adjacent capillary endothelial cells interconnected by impermeable tight junctions, requiring molecules to enter through active uptake by specialized transporter proteins or by diffusing into the BBB cells (Abbott et al., 2010; Brightman and Reese, 1969; Pardridge, 2005, 2007). Among these transporters, a crucial group is the SLC6 family, one of the largest SLC families comprising 20 genes that encode highly similar transporter proteins. The SLC6 family, abundantly expressed in the brain, includes GABA transporters such as GAT1 (SLC6A1), GAT2 (SLC6A13), GAT3 (SLC6A11), and BGT1 (SLC6A12). These transporters are responsible for the transport of neurotransmitters into and out of the brain, thereby regulating neurotransmitter homeostasis (Abbott et al., 2010). Studies have shown that GAT2/BGT-1 expressed in epithelial cells serve as GABA transporters at the BBB to transport GABA (Takanaga et al., 2001). In addition, GAT2/BGT-1 has been found to be widely expressed in the mouse brain. Notably, GAT2 is expressed on both the apical and basal membranes of epithelial cells, exhibiting bidirectional transport capability. In contrast, BGT1 is expressed solely on the basal membrane. GAT2 at the BBB facilitates the transport of GABA.

Extensive studies have observed increased concentrations of brain GABA and mRNA expression levels of GABA receptors following administration of gut derived GABA (Jin et al., 2023; Watanabe et al., 2022; Zhou H. et al., 2022). These findings collectively support that gut derived GABA can modulate the brain's GABAergic system, potentially alleviating neurological disorders by enhancing GABAergic signaling in the brain.

6 Future direction

Currently, a large portion of the global population is suffering from anxiety and insomnia, grappling with the challenges posed by these mental health disorders. The current sedatives and sleep aids available for treating anxiety and insomnia often come with side effects like physical dependence and withdrawal symptoms (Shyken et al., 2019). Hence, the development of novel interventions that address anxiety and insomnia without such drawbacks remains necessary.

GABA, as a natural neurotransmitter in the human body, poses minimal harm even in high doses (Li et al., 2015). The supplementation of GABA through gut derived sources holds great promise. Oral GABA, GABA-enriched diets, and probiotic strains producing GABA have demonstrated positive and effective outcomes in treating anxiety and insomnia. However, oral GABA has several limitations, which include: First, low blood–brain barrier permeability: Unlike benzodiazepines, GABA does not efficiently cross the blood–brain barrier, limiting its ability to exert strong pharmacological effects. Overall, the efficiency of GABA crossing the blood–brain barrier is not very high. Second, low

bioavailability: The bioavailability of GABA when ingested is relatively low, and it is quickly eliminated from the body, which makes it challenging to sustain therapeutic efficacy. Third, non-specific effects: GABA is widely distributed throughout the body, not only in the central nervous system but also in many other tissues and organs. This wide distribution may lead to non-specific effects rather than targeting specific diseases or symptoms. An increasing amount of research has found that GABA functions as a signal molecule in the immune system. Several studies have clarified that GABA is not beneficial for the anti-tumor response. So, it is not recommended to provide gut derived GABA as a drug treatment for cancer patients.

Probiotic and engineered bacteria capable of producing GABA present promising avenues for the treatment of anxiety and insomnia. These bacteria can colonize in the human gut and release GABA promptly. There remain several aspects that warrant further exploration. For instance, determining the optimal drug dosage of probiotics for human treatment and, notably, laying particular emphasis on considering the safety of engineered foreign genes. Future research efforts should be intensified to not only optimize the drug dosage regimens but also to conduct rigorous and systematic investigations into the safety of foreign genes. Only through such meticulous exploration can we hope to translate these promising approaches into reliable and effective therapeutic solutions, thereby offering a new ray of hope for the countless individuals suffering from anxiety and insomnia, and revolutionizing the landscape of mental health treatment.

Author contributions

CJ: Conceptualization, Writing – original draft, Writing – review & editing. YC: Writing – review & editing. TS: Conceptualization, Investigation, Project administration, Supervision, Writing – review & editing.

Funding

The author(s) declare that financial support was received for the research and/or publication of this article. This work was supported by the Scientific Research Funds of Huaqiao University (Z16Y0017, TS), and the National Natural Science Foundation of China (32271002, TS).

Acknowledgments

We thank members of the Sun laboratory and members of GeneYoung Biopharmaceuticals for their valuable discussions and advice.

Conflict of interest

YC is employed by GeneYoung Biopharmaceuticals.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The authors declare that Gen AI was used in the creation of this manuscript. Chengji Jiang polished the paper with ChatGPT.

Publisher's note

References

Abbott, N. J., Patabendige, A. A., Dolman, D. E., Yusof, S. R., and Begley, D. J. (2010). Structure and function of the blood-brain barrier. *Neurobiol. Dis.* 37, 13–25. doi: 10.1016/j.nbd.2009.07.030

Abdou, A. M., Higashiguchi, S., Horie, K., Kim, M., Hatta, H., and Yokogoshi, H. (2006). Relaxation and immunity enhancement effects of gamma-aminobutyric acid (GABA) administration in humans. *Biofactors* 26, 201–208. doi: 10.1002/biof.5520260305

Augustin, H. G., and Koh, G. Y. (2017). Organotypic vasculature: from descriptive heterogeneity to functional pathophysiology. *Science* 357:eaal2379. doi: 10.1126/science.aal2379

Babaev, O., Piletti Chatain, C., and Krueger-Burg, D. (2018). Inhibition in the amygdala anxiety circuitry. *Exp. Mol. Med.* 50, 1–16. doi: 10.1038/s12276-018-0063-8

Barrett, E., Ross, R. P., O'Toole, P. W., Fitzgerald, G. F., and Stanton, C. (2012). γ-Aminobutyric acid production by culturable bacteria from the human intestine. *J. Appl. Microbiol.* 113, 411–417. doi: 10.1111/j.1365-2672.2012.05344.x

Barros-Santos, T., Silva, K. S. O., Libarino-Santos, M., Elisangela Gouveia, C.-P., Reis, H. S., Tamura, E. K., et al. (2020). Effects of chronic treatment with new strains of *Lactobacillus plantarum* on cognitive, anxiety-and depressive-like behaviors in male mice. *PLoS One* 15:e0234037. doi: 10.1371/journal.pone.0234037

Bettler, B., Kaupmann, K., Mosbacher, J., and Gassmann, M. (2004). Molecular structure and physiological functions of GABA(B) receptors. *Physiol. Rev.* 84, 835–867. doi: 10.1152/physrev.00036.2003

Bhat, A., Shafi, F., and El Solh, A. A. (2008). Pharmacotherapy of insomnia. *Expert. Opin. Pharmacother.* 9, 351–362. doi: 10.1517/14656566.9.3.351

Blake, M. J., Trinder, J. A., and Allen, N. B. (2018). Mechanisms underlying the association between insomnia, anxiety, and depression in adolescence: implications for behavioral sleep interventions. *Clin. Psychol. Rev.* 63, 25–40. doi: 10.1016/j.cpr.2018.05.006

Boonstra, E., de Kleijn, R., Colzato, L. S., Alkemade, A., Forstmann, B. U., and Nieuwenhuis, S. (2015). Neurotransmitters as food supplements: the effects of GABA on brain and behavior. *Front. Psychol.* 6:1520. doi: 10.3389/fpsyg.2015.01520

Bormann, J. (1988). Electrophysiology of GABAA and GABAB receptor subtypes. *Trends Neurosci.* 11, 112–116. doi: 10.1016/0166-2236(88)90156-7

Bowery, N. G., Doble, A., Hill, D. R., Hudson, A. L., Shaw, J. S., and Turnbull, M. J. (1979). Baclofen: a selective agonist for a novel type of GABA receptor proceedings. *Br. J. Pharmacol.* 67, 444–445.

Bragantini, D., Sivertsen, B., Gehrman, P., Lydersen, S., and Güzey, I. C. (2019). Differences in anxiety levels among symptoms of insomnia. The HUNT study. *Sleep Health* 5, 370–375. doi: 10.1016/j.sleh.2019.01.002

Bravo, J. A., Forsythe, P., Chew, M. V., Escaravage, E., Savignac, H. M., Dinan, T. G., et al. (2011). Ingestion of Lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. *Proc. Natl. Acad. Sci. USA* 108, 16050–16055. doi: 10.1073/pnas.1102999108

Breit, S., Kupferberg, A., Rogler, G., and Hasler, G. (2018). Vagus nerve as modulator of the brain-gut Axis in psychiatric and inflammatory disorders. *Front. Psych.* 9:44. doi: 10.3389/fpsyt.2018.00044

Brightman, M. W., and Reese, T. S. (1969). Junctions between intimately apposed cell membranes in the vertebrate brain. *J. Cell Biol.* 40, 648–677. doi: 10.1083/jcb.40.3.648

Buckley, T. M., and Schatzberg, A. F. (2005). On the interactions of the hypothalamic-pituitary-adrenal (HPA) axis and sleep: normal HPA axis activity and circadian rhythm, exemplary sleep disorders. *J. Clin. Endocrinol. Metab.* 90, 3106–3114. doi: 10.1210/jc.2004-1056

Buysse, D. J., Thompson, W., Scott, J., Franzen, P. L., Germain, A., Hall, M., et al. (2007). Daytime symptoms in primary insomnia: a prospective analysis using ecological momentary assessment. *Sleep Med.* 8, 198–208. doi: 10.1016/j.sleep.2006.10.006

Byrd, J. B., and Brook, R. D. (2014). Anxiety in the "age of hypertension". *Curr. Hypertens. Rep.* 16:486. doi: 10.1007/s11906-014-0486-0

Byun, J. I., Shin, Y. Y., Chung, S. E., and Shin, W. C. (2018). Safety and efficacy of gamma-aminobutyric acid from fermented Rice germ in patients with insomnia symptoms: a randomized, double-blind trial. *J. Clin. Neurol.* 14, 291–295. doi: 10.3988/jcn.2018.14.3.291

Cai, S., Gao, F., Zhang, X., Wang, O., Wu, W., Zhu, S., et al. (2014). Evaluation of γ-aminobutyric acid, phytate and antioxidant activity of tempeh-like fermented oats (*Avena sativa* L.) prepared with different filamentous fungi. *J. Food Sci. Technol.* 51, 2544–2551. doi: 10.1007/s13197-012-0748-2

Cai, L., Tao, Q., Li, W., Zhu, X., and Cui, C. (2022). The anti-anxiety/depression effect of a combined complex of casein hydrolysate and γ-aminobutyric acid on C57BL/6 mice. *Front. Nutr.* 9:971853. doi: 10.3389/fnut.2022.971853

Calm, C. C. (2024). Mental health 2024 statistics: the growing impact of anxiety. Available online at: https://www.laurageftman.com/blog/mental-health-2024-statistics-anxiety.

Carlisi, C. O., Hilbert, K., Guyer, A. E., and Ernst, M. (2017). Sleep-amount differentially affects fear-processing neural circuitry in pediatric anxiety: a preliminary fMRI investigation. *Cogn. Affect. Behav. Neurosci.* 17, 1098–1113. doi: 10.3758/s13415-017-0535-7

Chaaya, N., Battle, A. R., and Johnson, L. R. (2018). An update on contextual fear memory mechanisms: transition between amygdala and Hippocampus. *Neurosci. Biobehav. Rev.* 92, 43–54. doi: 10.1016/j.neubiorev.2018.05.013

Chellappa, S. L., and Aeschbach, D. (2022). Sleep and anxiety: from mechanisms to interventions. *Sleep Med. Rev.* 61:101583. doi: 10.1016/j.smrv.2021.101583

Chen, Z., Fei, Y. J., Anderson, C. M., Wake, K. A., Miyauchi, S., Huang, W., et al. (2003). Structure, function and immunolocalization of a proton-coupled amino acid transporter (hPAT1) in the human intestinal cell line Caco-2. *J. Physiol.* 546, 349–361. doi: 10.1113/jphysiol.2002.026500

Chen, X., Jia, X., Zhang, Y., Zhao, Z., Hao, J., Li, H., et al. (2021). The combined use of gamma-aminobutyric acid and walnut peptide enhances sleep in mice. *Ann. Palliat. Med.* 10, 11074–11082. doi: 10.21037/apm-21-2798

Cheng, T. C., and Tsai, J. F. (2009). GABA tea helps sleep. *J. Altern. Complement. Med.* 15, 697–698. doi: 10.1089/acm.2009.0023

Cullinan, W. E., Ziegler, D. R., and Herman, J. P. (2008). Functional role of local GABAergic influences on the HPA axis. *Brain Struct. Funct.* 213, 63–72. doi: 10.1007/s00429-008-0192-2

Deschaux, O., Froestl, W., and Gottesmann, C. (2006). Influence of a GABA(B) and GABA(C) receptor antagonist on sleep-waking cycle in the rat. *Eur. J. Pharmacol.* 535, 177–181. doi: 10.1016/j.ejphar.2006.01.066

Di Pierro, F., Sisti, D., Rocchi, M., Belli, A., Bertuccioli, A., Cazzaniga, M., et al. (2024). Effects of *Melissa officinalis* Phytosome on sleep quality: results of a prospective, double-blind, placebo-controlled, and cross-over study. *Nutrients* 16:4199. doi: 10.3390/nu16234199

Dubourget, R., Sangare, A., Geoffroy, H., Gallopin, T., and Rancillac, A. (2017). Multiparametric characterization of neuronal subpopulations in the ventrolateral preoptic nucleus. *Brain Struct. Funct.* 222, 1153–1167. doi: 10.1007/s00429-016-1265-2

Duranti, S., Ruiz, L., Lugli, G. A., Tames, H., Milani, C., Mancabelli, L., et al. (2020). *Bifidobacterium adolescentis* as a key member of the human gut microbiota in the production of GABA. *Sci. Rep.* 10:14112. doi: 10.1038/s41598-020-70986-z

Faucher, P., Dries, A., Mousset, P. Y., Leboyer, M., Dore, J., and Beracochea, D. (2022). Synergistic effects of Lacticaseibacillus rhamnosus GG, glutamine, and curcumin on chronic unpredictable mild stress-induced depression in a mouse model. *Benef Microbes* 13, 253–264. doi: 10.3920/bm2021.0188

Feehily, C., and Karatzas, K. A. (2013). Role of glutamate metabolism in bacterial responses towards acid and other stresses. *J. Appl. Microbiol.* 114, 11–24. doi: 10.1111/j.1365-2672.2012.05434.x

Felice, D., Cryan, J. F., and O'Leary, O. F. (2022). GABA(B) receptors: anxiety and mood disorders. *Curr. Top. Behav. Neurosci.* 52, 241–265. doi: 10.1007/7854_2020_171

Frey, H. H., and Löscher, W. (1980). Cetyl GABA: effect on convulsant thresholds in mice and acute toxicity. *Neuropharmacology* 19, 217–220. doi: 10.1016/0028-3908(80)90141-0

Giustino, T. F., and Maren, S. (2018). Noradrenergic modulation of fear conditioning and extinction. *Front. Behav. Neurosci.* 12:43. doi: 10.3389/fnbeh.2018.00043

Gong, L., Shi, M., Wang, J., Xu, R., Yu, S., Liu, D., et al. (2021). The abnormal functional connectivity in the locus Coeruleus-norepinephrine system associated with anxiety symptom in chronic insomnia disorder. *Front. Neurosci.* 15:678465. doi: 10.3389/fnins.2021.678465

Greenblatt, D. J., Harmatz, J. S., and Shader, R. I. (2020). Diazepam in the elderly: looking Back, ahead, and at the evidence. *J. Clin. Psychopharmacol.* 40, 215–219. doi: 10.1097/jcp.0000000000001213

Hajisoltani, R., and Meftahi, G. H. (2024). Epinephrine injected into the basolateral amygdala affects anxiety-like behavior and memory performance in stressed rats. *Neurosci. Lett.* 819:137590. doi: 10.1016/j.neulet.2023.137590

Hepsomali, P., Groeger, J. A., Nishihira, J., and Scholey, A. (2020). Effects of Oral gamma-aminobutyric acid (GABA) administration on stress and sleep in humans: a systematic review. *Front. Neurosci.* 14:923. doi: 10.3389/fnins.2020.00923

Hinton, T., Jelinek, H. F., Viengkhou, V., Johnston, G. A., and Matthews, S. (2019). Effect of GABA-fortified oolong tea on reducing stress in a university student cohort. *Front. Nutr.* 6:27. doi: 10.3389/fnut.2019.00027

Hinton, T., and Johnston, G. A. R. (2020). GABA-enriched teas as neuro-nutraceuticals. *Neurochem. Int.* 141:104895. doi: 10.1016/j.neuint.2020.104895

Huang, D., Wang, Y., Thompson, J. W., Yin, T., Alexander, P. B., Qin, D., et al. (2022). Cancer-cell-derived GABA promotes β-catenin-mediated tumour growth and immunosuppression. *Nat. Cell Biol.* 24, 230–241. doi: 10.1038/s41556-021-00820-9

Ilgin, S. (2020). The adverse effects of psychotropic drugs as an endocrine disrupting chemicals on the hypothalamic-pituitary regulation in male. *Life Sci.* 253:117704. doi: 10.1016/j.lfs.2020.117704

Jang, H. M., Jang, S. E., Han, M. J., and Kim, D. H. (2018). Anxiolytic-like effect of *Bifidobacterium adolescentis* IM38 in mice with or without immobilisation stress. *Benef Microbes* 9, 123–132. doi: 10.3920/bm2016.0226

Janik, R., Thomason, L. A. M., Stanisz, A. M., Forsythe, P., Bienenstock, J., and Stanisz, G. J. (2016). Magnetic resonance spectroscopy reveals oral Lactobacillus promotion of increases in brain GABA, N-acetyl aspartate and glutamate. *NeuroImage* 125, 988–995. doi: 10.1016/j.neuroimage.2015.11.018

Ji, F., Kanbara, N., and Obata, K. (1999). GABA and histogenesis in fetal and neonatal mouse brain lacking both the isoforms of glutamic acid decarboxylase. *Neurosci. Res.* 33, 187–194. doi: 10.1016/s0168-0102(99)00011-5

Jin, X., Hu, Y., Lin, T., Gao, F., Xu, Z., Hou, X., et al. (2023). Selenium-enriched *Bifidobacterium longum* DD98 relieves irritable bowel syndrome induced by chronic unpredictable mild stress in mice. *Food Funct.* 14, 5355–5374. doi: 10.1039/d2fo03408e

Jin, Z., and Korol, S. V. (2023). GABA signalling in human pancreatic islets. *Front. Endocrinol. (Lausanne)* 14:1059110. doi: 10.3389/fendo.2023.1059110

John, J., Wu, M. F., Boehmer, L. N., and Siegel, J. M. (2004). Cataplexy-active neurons in the hypothalamus: implications for the role of histamine in sleep and waking behavior. *Neuron* 42, 619–634. doi: 10.1016/s0896-6273(04)00247-8

Johnston, G. A. (2013). Advantages of an antagonist: bicuculline and other GABA antagonists. *Br. J. Pharmacol.* 169, 328–336. doi: 10.1111/bph.12127

Juruena, M. F., Eror, F., Cleare, A. J., and Young, A. H. (2020). The role of early life stress in HPA Axis and anxiety. *Adv. Exp. Med. Biol.* 1191, 141–153. doi: 10.1007/978-981-32-9705-0_9

Kalmbach, D. A., Anderson, J. R., and Drake, C. L. (2018). The impact of stress on sleep: pathogenic sleep reactivity as a vulnerability to insomnia and circadian disorders. *J. Sleep Res.* 27:e12710. doi: 10.1111/jsr.12710

Kandola, A., and Stubbs, B. (2020). Exercise and anxiety. *Adv. Exp. Med. Biol.* 1228, 345–352. doi: 10.1007/978-981-15-1792-1_23

Kanehira, T., Nakamura, Y., Nakamura, K., Horie, K., Horie, N., Furugori, K., et al. (2011). Relieving occupational fatigue by consumption of a beverage containing γ-amino butyric acid. *J. Nutr. Sci. Vitaminol. (Tokyo)* 57, 9–15. doi: 10.3177/jnsv.57.9

Kaur, S., Sharma, P., Mayer, M. J., Neuert, S., Narbad, A., and Kaur, S. (2023). Beneficial effects of GABA-producing potential probiotic Limosilactobacillus fermentum L18 of human origin on intestinal permeability and human gut microbiota. *Microb. Cell Factories* 22:256. doi: 10.1186/s12934-023-02264-2

Kim, J. J., and Hibbs, R. E. (2021). Direct structural insights into GABA(a) receptor pharmacology. *Trends Biochem. Sci.* 46, 502–517. doi: 10.1016/j.tibs.2021.01.011

Kim, S., Jo, K., Hong, K. B., Han, S. H., and Suh, H. J. (2019). GABA and l-theanine mixture decreases sleep latency and improves NREM sleep. *Pharm. Biol.* 57, 65–73. doi: 10.1080/13880209.2018.1557698

Kim, H. S., Lee, E. J., Lim, S. T., and Han, J. A. (2015). Self-enhancement of GABA in rice bran using various stress treatments. *Food Chem.* 172, 657–662. doi: 10.1016/j.foodchem.2014.09.107

Knudsen, G. M., Poulsen, H. E., and Paulson, O. B. (1988). Blood-brain barrier permeability in galactosamine-induced hepatic encephalopathy. No evidence for increased GABA-transport. *J. Hepatol.* 6, 187–192. doi: 10.1016/s0168-8278(88)80030-8

Ko, E. M., Estabrooke, I. V., McCarthy, M., and Scammell, T. E. (2003). Wake-related activity of tuberomammillary neurons in rats. *Brain Res.* 992, 220–226. doi: 10.1016/j.brainres.2003.08.044

Krettek, J. E., and Price, J. L. (1978). A description of the amygdaloid complex in the rat and cat with observations on intra-amygdaloid axonal connections. *J. Comp. Neurol.* 178, 255–279. doi: 10.1002/cne.901780205

Kristensen, A. S., Andersen, J., Jørgensen, T. N., Sørensen, L., Eriksen, J., Loland, C. J., et al. (2011). SLC6 neurotransmitter transporters: structure, function, and regulation. *Pharmacol. Rev.* 63, 585–640. doi: 10.1124/pr.108.000869

Lan, Y. J., Tan, S. I., Cheng, S. Y., Ting, W. W., Xue, C., Lin, T. H., et al. (2021). Development of *Escherichia coli* Nissle 1917 derivative by CRISPR/Cas9 and application for gamma-aminobutyric acid (GABA) production in antibiotic-free system. *Biochem. Eng. J.* 168:107952. doi: 10.1016/j.bej.2021.107952

Lebovich, M., and Andrews, L. B. (2022). Surveying the genetic design space for transcription factor-based metabolite biosensors: synthetic gamma-aminobutyric acid and propionate biosensors in *E. coli* Nissle 1917. *Front. Bioeng. Biotechnol.* 10:938056. doi: 10.3389/fbioe.2022.938056

Lebovich, M., and Andrews, L. B. (2023). Genetic circuits for feedback control of gamma-aminobutyric acid biosynthesis in probiotic *Escherichia coli* Nissle 1917. *bioRxiv*. doi: 10.1101/2023.06.09.544351

LeDoux, J. E. (2000). Emotion circuits in the brain. *Annu. Rev. Neurosci.* 23, 155–184. doi: 10.1146/annurev.neuro.23.1.155

Lei, J., Shen, L., Zhang, W., Ma, F., Wang, J., Wei, T., et al. (2023). Comparative chemical characterization of potato powders using (1)H NMR spectroscopy and Chemometrics. *Plant Foods Hum. Nutr.* 78, 590–596. doi: 10.1007/s11130-023-01088-0

Li, J., Zhang, Z., Liu, X., Wang, Y., Mao, F., Mao, J., et al. (2015). Study of GABA in healthy volunteers: pharmacokinetics and pharmacodynamics. *Front. Pharmacol.* 6:260. doi: 10.3389/fphar.2015.00260

Liu, G., Khan, I., Li, Y., Yang, Y., Lu, X., Wang, Y., et al. (2022). Overcoming anxiety disorder by probiotic Lactiplantibacillus plantarum LZU-J-TSL6 through regulating intestinal homeostasis. *Food Secur.* 11:3596. doi: 10.3390/foods11223596

Liu, M. Q., Wang, T., Wang, Q. L., Zhou, J., Wang, B. R., Zhang, B., et al. (2022). Structure-guided discovery of food-derived GABA-T inhibitors as hunters for anti-anxiety compounds. *Food Funct.* 13, 12674–12685. doi: 10.1039/d2fo01315k

Liu, D., Zhang, J., Chen, J., Zhang, C., Yi, H., and Liu, D. (2022). Carrot-based fermentation juice rich in sleep-promoting components improved sleep in mice. *Front. Nutr.* 9:1043055. doi: 10.3389/fnut.2022.1043055

Liwinski, T., Lang, U. E., Brühl, A. B., and Schneider, E. (2023). Exploring the therapeutic potential of gamma-aminobutyric acid in stress and depressive disorders through the gut-brain Axis. *Biomedicines* 11:3128. doi: 10.3390/biomedicines11123128

Luck, B., Horvath, T. D., Engevik, K. A., Ruan, W., Haidacher, S. J., Hoch, K. M., et al. (2021). Neurotransmitter profiles are altered in the gut and brain of mice mono-associated with *Bifidobacterium dentium*. *Biomol. Ther.* 11:1091. doi: 10.3390/biom11081091

Luo, Y., and Balle, T. (2022). GABA(a) receptors as targets for anaesthetics and analgesics and promising candidates to help treat coronavirus infections: a mini-review. *Basic Clin. Pharmacol. Toxicol.* 131, 443–451. doi: 10.1111/bcpt.13798

Lydiard, R. B. (2003). The role of GABA in anxiety disorders. *J. Clin. Psychiatry* 64, 21–27.

Lynch, J. P., Goers, L., and Lesser, C. F. (2022). Emerging strategies for engineering *Escherichia coli* Nissle 1917-based therapeutics. *Trends Pharmacol. Sci.* 43, 772–786. doi: 10.1016/j.tips.2022.02.002

Mattison, K. A., Butler, K. M., Inglis, G. A. S., Dayan, O., Boussidan, H., Bhambhani, V., et al. (2018). SLC6A1 variants identified in epilepsy patients reduce γ-aminobutyric acid transport. *Epilepsia* 59, e135–e141. doi: 10.1111/epi.14531

Mazzoli, R., and Pessione, E. (2016). The neuro-endocrinological role of microbial glutamate and GABA signaling. *Front. Microbiol.* 7:1934. doi: 10.3389/fmicb.2016.01934

Merenstein, D., Pot, B., Leyer, G., Ouwehand, A. C., Preidis, G. A., Elkins, C. A., et al. (2023). Emerging issues in probiotic safety: 2023 perspectives. *Gut Microbes* 15:2185034. doi: 10.1080/19490976.2023.2185034

Morin, C. M., and Benca, R. (2012). Chronic insomnia. *Lancet* 379, 1129–1141. doi: 10.1016/s0140-6736(11)60750-2

Morin, C. M., Drake, C. L., Harvey, A. G., Krystal, A. D., Manber, R., Riemann, D., et al. (2015). Insomnia disorder. *Nat. Rev. Dis. Primers* 1:15026. doi: 10.1038/nrdp.2015.26

Morin, C. M., and Jarrin, D. C. (2022). Epidemiology of insomnia: prevalence, course, risk factors, and public health burden. *Sleep Med. Clin.* 17, 173–191. doi: 10.1016/j.jsmc.2022.03.003

Morris, L. S., McCall, J. G., Charney, D. S., and Murrough, J. W. (2020). The role of the locus coeruleus in the generation of pathological anxiety. *Brain Neurosci. Adv.* 4:2398212820930321. doi: 10.1177/2398212820930321

Mutoh, N., Moriya, M., Xu, C., Kato, K., Arai, S., Iwabuchi, N., et al. (2024). *Bifidobacterium breve* M-16V regulates the autonomic nervous system via the intestinal environment: a double-blind, placebo-controlled study. *Behav. Brain Res.* 460:114820. doi: 10.1016/j.bbr.2023.114820

Nácher, A., Polache, A., Moll-Navarro, M. J., Plá-Delfina, J. M., and Merino, M. (1994). Intestinal absorption pathway of gamma-aminobutyric acid in rat small intestine. *Biopharm. Drug Dispos.* 15, 359–371. doi: 10.1002/bdd.2510150503

Nakamura, H., Takishima, T., Kometani, T., and Yokogoshi, H. (2009). Psychological stress-reducing effect of chocolate enriched with gamma-aminobutyric acid (GABA) in humans: assessment of stress using heart rate variability and salivary chromogranin a. *Int. J. Food Sci. Nutr.* 60, 106–113. doi: 10.1080/09637480802558508

Ngo, D. H., and Vo, T. S. (2019). An updated review on pharmaceutical properties of gamma-aminobutyric acid. *Molecules* 24:2678. doi: 10.3390/molecules24152678

Nutt, D. J. (2005). Overview of diagnosis and drug treatments of anxiety disorders. *CNS Spectr.* 10, 49–56. doi: 10.1017/s1092852900009901

Oketch-Rabah, H. A., Madden, E. F., Roe, A. L., and Betz, J. M. (2021). United States Pharmacopeia (USP) safety review of gamma-aminobutyric acid (GABA). *Nutrients* 13:2742. doi: 10.3390/nu13082742

Okita, Y., Nakamura, H., Kouda, K., Takahashi, I., Takaoka, T., Kimura, M., et al. (2009). Effects of vegetable containing gamma-aminobutyric acid on the cardiac autonomic nervous system in healthy young people. *J. Physiol. Anthropol.* 28, 101–107. doi: 10.2114/jpa2.28.101

Olsen, R. W., and Sieghart, W. (2008). International Union of Pharmacology. LXX. Subtypes of gamma-aminobutyric acid(a) receptors: classification on the basis of subunit composition, pharmacology, and function. Update. *Pharmacol. Rev.* 60, 243–260. doi: 10.1124/pr.108.00505

Palmer, C. A., and Alfano, C. A. (2017). Sleep and emotion regulation: an organizing, integrative review. *Sleep Med. Rev.* 31, 6–16. doi: 10.1016/j.smrv.2015.12.006

Palmer, C. A., and Alfano, C. A. (2020). Anxiety modifies the emotional effects of sleep loss. *Curr. Opin. Psychol.* 34, 100–104. doi: 10.1016/j.copsyc.2019.12.001

Pan, H., Sun, T., Cui, M., Ma, N., Yang, C., Liu, J., et al. (2022). Light-sensitive *Lactococcus lactis* for microbe-gut-brain Axis regulating via Upconversion Optogenetic Micro-Nano system. *ACS Nano* 16, 6049–6063. doi: 10.1021/acsnano.1c11536

Pardridge, W. M. (2005). The blood-brain barrier: bottleneck in brain drug development. *NeuroRx* 2, 3–14. doi: 10.1602/neurorx.2.1.3

Pardridge, W. M. (2007). Blood-brain barrier delivery. *Drug Discov. Today* 12, 54–61. doi: 10.1016/j.drudis.2006.10.013

Pillai, V., Roth, T., Mullins, H. M., and Drake, C. L. (2014). Moderators and mediators of the relationship between stress and insomnia: stressor chronicity, cognitive intrusion, and coping. *Sleep* 37, 1199–1208A. doi: 10.5665/sleep.3838

Pokusaeva, K., Johnson, C., Luk, B., Uribe, G., Fu, Y., Oezguen, N., et al. (2017). GABA-producing *Bifidobacterium dentium* modulates visceral sensitivity in the intestine. *Neurogastroenterol. Motil.* 29:e12904. doi: 10.1111/nmo.12904

Qu, S., Yu, Z., Zhou, Y., Wang, S., Jia, M., Chen, T., et al. (2024). Gut microbiota modulates neurotransmitter and gut-brain signaling. *Microbiol. Res.* 287:127858. doi: 10.1016/j.micres.2024.127858

Ren, X., Zhao, X., Li, J., Liu, Y., Ren, Y., Pruessner, J. C., et al. (2022). The hippocampal-ventral medial prefrontal cortex Neurocircuitry involvement in the Association of Daily Life Stress with Acute Perceived Stress and Cortisol Responses. *Psychosom. Med.* 84, 276–287. doi: 10.1097/psy.0000000000001058

Riedel, A., Benz, F., Deibert, P., Barsch, F., Frase, L., Johann, A. F., et al. (2024). The effect of physical exercise interventions on insomnia: a systematic review and meta-analysis. *Sleep Med. Rev.* 76:101948. doi: 10.1016/j.smrv.2024.101948

Riemann, D., Spiegelhalder, K., Feige, B., Voderholzer, U., Berger, M., Perlis, M., et al. (2010). The hyperarousal model of insomnia: a review of the concept and its evidence. *Sleep Med. Rev.* 14, 19–31. doi: 10.1016/j.smrv.2009.04.002

Roberts, A. G., Peckins, M. K., Gard, A. M., Hein, T. C., Hardi, F. A., Mitchell, C., et al. (2022). Amygdala reactivity during socioemotional processing and cortisol reactivity to a psychosocial stressor. *Psychoneuroendocrinology* 144:105855. doi: 10.1016/j.psyneuen.2022.105855

Robinson, O. J., Pike, A. C., Cornwell, B., and Grillon, C. (2019). The translational neural circuitry of anxiety. *J. Neurol. Neurosurg. Psychiatry* 90, jnnp-2019-321400–jnnp-2019-321360. doi: 10.1136/jnnp-2019-321400

Ruseler-van Embden, J. G., van Lieshout, L. M., Gosselink, M. J., and Marteau, P. (1995). Inability of *Lactobacillus casei* strain GG, L. acidophilus, and *Bifidobacterium bifidum* to degrade intestinal mucus glycoproteins. *Scand. J. Gastroenterol.* 30, 675–680. doi: 10.3109/00365529509096312

Sadeh, A., Keinan, G., and Daon, K. (2004). Effects of stress on sleep: the moderating role of coping style. *Health Psychol.* 23, 542–545. doi: 10.1037/0278-6133.23.5.542

Saper, C. B., Scammell, T. E., and Lu, J. (2005). Hypothalamic regulation of sleep and circadian rhythms. *Nature* 437, 1257–1263. doi: 10.1038/nature04284

Sarasa, S. B., Mahendran, R., Muthusamy, G., Thankappan, B., Selta, D. R. F., and Angayarkanni, J. (2020). A brief review on the non-protein amino acid, gamma-amino butyric acid (GABA): its production and role in microbes. *Curr. Microbiol.* 77, 534–544. doi: 10.1007/s00284-019-01839-w

Shaha, D. P. (2023). Insomnia management: a review and update. *J. Fam. Pract.* 72, S31–s36. doi: 10.12788/jfp.0620

Shutta, K. H., Balasubramanian, R., Huang, T., Jha, S. C., Zeleznik, O. A., Kroenke, C. H., et al. (2021). Plasma metabolomic profiles associated with chronic distress in women. *Psychoneuroendocrinology* 133:105420. doi: 10.1016/j.psyneuen.2021.105420

Shyken, J. M., Babbar, S., Babbar, S., and Forinash, A. (2019). Benzodiazepines in Pregnancy. *Clin. Obstet. Gynecol.* 62, 156–167. doi: 10.1097/grf.0000000000000417

Sigel, E., and Ernst, M. (2018). The benzodiazepine binding sites of GABAA receptors. *Trends Pharmacol. Sci.* 39, 659–671. doi: 10.1016/j.tips.2018.03.006

Soghomonian, J. J., and Martin, D. L. (1998). Two isoforms of glutamate decarboxylase: why? *Trends Pharmacol. Sci.* 19, 500–505. doi: 10.1016/s0165-6147(98)01270-x

Srichomphu, P., Wattanathorn, J., Thukham-Mee, W., and Muchimapura, S. (2022). Anxiety, insomnia, and memory impairment in metabolic syndrome rats are alleviated by the novel functional ingredients from *Anacardium occidentale*. *Antioxidants (Basel)* 11:2203. doi: 10.3390/antiox11112203

Steel, A., Mikkelsen, M., Edden, R. A. E., and Robertson, C. E. (2020). Regional balance between glutamate+glutamine and GABA+ in the resting human brain. *NeuroImage* 220:117112. doi: 10.1016/j.neuroimage.2020.117112

Sternbach, L. H. (1978). The benzodiazepine story. *Prog. Drug Res.* 22, 229–266. doi: 10.1007/978-3-0348-7102-0_5

Strandwitz, P. (2018). Neurotransmitter modulation by the gut microbiota. *Brain Res.* 1693, 128–133. doi: 10.1016/j.brainres.2018.03.015

Strandwitz, P., Kim, K. H., Terekhova, D., Liu, J. K., Sharma, A., Levering, J., et al. (2019). GABA-modulating bacteria of the human gut microbiota. *Nat. Microbiol.* 4, 396–403. doi: 10.1038/s41564-018-0307-3

Streeter, C. C., Whitfield, T. H., Owen, L., Rein, T., Karri, S. K., Yakhkind, A., et al. (2010). Effects of yoga versus walking on mood, anxiety, and brain GABA levels: a randomized controlled MRS study. *J. Altern. Complement. Med.* 16, 1145–1152. doi: 10.1089/acm.2010.0007

Suárez-Pereira, I., Llorca-Torralba, M., Bravo, L., Camarena-Delgado, C., Soriano-Mas, C., and Berrocoso, E. (2022). The role of the locus Coeruleus in pain and associated stress-related disorders. *Biol. Psychiatry* 91, 786–797. doi: 10.1016/j.biopsych.2021.11.023

Takanaga, H., Ohtsuki, S., Hosoya, K., and Terasaki, T. (2001). GAT2/BGT-1 as a system responsible for the transport of gamma-aminobutyric acid at the mouse blood-brain barrier. *J. Cereb. Blood Flow Metab.* 21, 1232–1239. doi: 10.1097/00004647-200110000-00012

Taylor, M., Bhagwagar, Z., Cowen, P. J., and Sharp, T. (2003). GABA and mood disorders. *Psychol. Med.* 33, 387–393. doi: 10.1017/s0033291702006876

Thwaites, D. T., Basterfield, L., McCleave, P. M., Carter, S. M., and Simmons, N. L. (2000). Gamma-aminobutyric acid (GABA) transport across human intestinal epithelial (Caco-2) cell monolayers. *Br. J. Pharmacol.* 129, 457–464. doi: 10.1038/sj.bjp.0703069

Toth, E., and Lajtha, A. (1981). Elevation of cerebral levels of nonessential amino acids *in vivo* by administration of large doses. *Neurochem. Res.* 6, 1309–1317. doi: 10.1007/bf00964352

Van Gelder, N. M., and Elliott, K. A. (1958). Disposition of gamma-aminobutyric acid administered to mammals. *J. Neurochem.* 3, 139–143. doi: 10.1111/j.1471-4159.1958.tb12620.x

Van Someren, E. J. W. (2021). Brain mechanisms of insomnia: new perspectives on causes and consequences. *Physiol. Rev.* 101, 995–1046. doi: 10.1152/physrev.00046.2019

Vann, K., Techaparin, A., and Apiraksakorn, J. (2020). Beans germination as a potential tool for GABA-enriched tofu production. *J. Food Sci. Technol.* 57, 3947–3954. doi: 10.1007/s13197-020-04423-4

Verret, L., Fort, P., Gervasoni, D., Léger, L., and Luppi, P. H. (2006). Localization of the neurons active during paradoxical (REM) sleep and projecting to the locus coeruleus noradrenergic neurons in the rat. *J. Comp. Neurol.* 495, 573–586. doi: 10.1002/cne.20891

Vertes, R. P. (2004). Differential projections of the infralimbic and prelimbic cortex in the rat. *Synapse* 51, 32–58. doi: 10.1002/syn.10279

Vu, V., and Conant-Norville, D. (2021). Anxiety: recognition and treatment options. *Psychiatr. Clin. North Am.* 44, 373–380. doi: 10.1016/j.psc.2021.04.005

Warner, E. N., and Strawn, J. R. (2023). Risk factors for pediatric anxiety disorders. *Child Adolesc. Psychiatr. Clin. N. Am.* 32, 485–510. doi: 10.1016/j.chc.2022.10.001

Watanabe, T., Hayashi, K., Takara, T., Teratani, T., Kitayama, J., and Kawahara, T. (2022). Effect of Oral Administration of Lactiplantibacillus plantarum SNK12 on temporary stress in adults: a randomized, placebo-controlled, double-blind, parallel-group study. *Int. J. Environ. Res. Public Health* 19:8936. doi: 10.3390/ijerph19158936

Wong, M. L., Kling, M. A., Munson, P. J., Listwak, S., Licinio, J., Prolo, P., et al. (2000). Pronounced and sustained central hypernoradrenergic function in major depression with melancholic features: relation to hypercortisolism and corticotropin-releasing hormone. *Proc. Natl. Acad. Sci. USA* 97, 325–330. doi: 10.1073/pnas.97.1.325

Wu, Y., Wang, Y., Hu, A., Shu, X., Huang, W., Liu, J., et al. (2022). *Lactobacillus plantarum*-derived postbiotics prevent Salmonella-induced neurological dysfunctions by modulating gut-brain axis in mice. *Front. Nutr.* 9:946096. doi: 10.3389/fnut.2022.946096

Xiang, T., Liao, J., Cai, Y., Fan, M., Li, C., Zhang, X., et al. (2023). Impairment of GABA inhibition in insomnia disorders: evidence from the peripheral blood system. *Front. Psych.* 14:1134434. doi: 10.3389/fpsyt.2023.1134434

Yamatsu, A., Yamashita, Y., Maru, I., Yang, J., Tatsuzaki, J., and Kim, M. (2015). The improvement of sleep by Oral intake of GABA and Apocynum venetum leaf extract. *J. Nutr. Sci. Vitaminol. (Tokyo)* 61, 182–187. doi: 10.3177/jnsv.61.182

Yamatsu, A., Yamashita, Y., Pandharipande, T., Maru, I., and Kim, M. (2016). Effect of oral γ-aminobutyric acid (GABA) administration on sleep and its absorption in humans. *Food Sci. Biotechnol.* 25, 547–551. doi: 10.1007/s10068-016-0076-9

Yan, L., Zheng, H., Liu, W., Liu, C., Jin, T., Liu, S., et al. (2021). UV-C treatment enhances organic acids and GABA accumulation in tomato fruits during storage. *Food Chem.* 338:128126. doi: 10.1016/j.foodchem.2020.128126

Yoo, S. S., Gujar, N., Hu, P., Jolesz, F. A., and Walker, M. P. (2007). The human emotional brain without sleep--a prefrontal amygdala disconnect. *Curr. Biol.* 17, R877–R878. doi: 10.1016/j.cub.2007.08.007

Yoto, A., Murao, S., Motoki, M., Yokoyama, Y., Horie, N., Takeshima, K., et al. (2012). Oral intake of γ-aminobutyric acid affects mood and activities of central nervous system during stressed condition induced by mental tasks. *Amino Acids* 43, 1331–1337. doi: 10.1007/s00726-011-1206-6

Zafar, S., and Jabeen, I. (2018). Structure, function, and modulation of γ-aminobutyric acid transporter 1 (GAT1) in neurological disorders: a Pharmacoinformatic prospective. *Front. Chem.* 6:397. doi: 10.3389/fchem.2018.00397

Zawistowska-Rojek, A., and Tyski, S. (2018). Are probiotic really safe for humans? *Pol. J. Microbiol.* 67, 251–258. doi: 10.21307/pjm-2018-044

Zeng, Y., Hu, H., He, Y., Deng, Z., Guo, Y., and Zhou, X. (2024). Multi-omics reveal the improvements of nutrient digestion, absorption, and metabolism and intestinal function via GABA supplementation in weanling piglets. *Animals (Basel)* 14:3177. doi: 10.3390/ani14223177

Zhang, J., Lam, S. P., Li, S. X., Ma, R. C., Kong, A. P., Chan, M. H., et al. (2014). A community-based study on the association between insomnia and hypothalamic-pituitary-adrenal axis: sex and pubertal influences. *J. Clin. Endocrinol. Metab.* 99, 2277–2287. doi: 10.1210/jc.2013-3728

Zhang, B., Vogelzang, A., Miyajima, M., Sugiura, Y., Wu, Y., Chamoto, K., et al. (2021). B cell-derived GABA elicits IL-10(+) macrophages to limit anti-tumour immunity. *Nature* 599, 471–476. doi: 10.1038/s41586-021-04082-1

Zhao, W., Li, Y., Ma, W., Ge, Y., and Huang, Y. (2015). A study on quality components and sleep-promoting effects of GABA black tea. *Food Funct.* 6, 3393–3398. doi: 10.1039/c5fo00265f

Zhao, N., Shu, Y., Jian, C., Zhou, Z., Bao, H., Li, X., et al. (2022). Lactobacillus ameliorates SD-induced stress responses and gut Dysbiosis by increasing the absorption of gut-derived GABA in Rhesus monkeys. *Front. Immunol.* 13:915393. doi: 10.3389/fimmu.2022.915393

Zhou, B., Jin, G., Pang, X., Mo, Q., Bao, J., Liu, T., et al. (2022). *Lactobacillus rhamnosus* GG colonization in early life regulates gut-brain axis and relieves anxiety-like behavior in adulthood. *Pharmacol. Res.* 177:106090. doi: 10.1016/j.phrs.2022.106090

Zhou, H., Zhang, S., Zhang, X., Zhou, H., Wen, T., and Wang, J. (2022). Depression-like symptoms due to Dcf1 deficiency are alleviated by intestinal transplantation of Lactobacillus murine and *Lactobacillus reuteri*. *Biochem. Biophys. Res. Commun.* 593, 137–143. doi: 10.1016/j.bbrc.2022.01.026

Zhu, Y., Xie, S. Z., Peng, A. B., Yu, X. D., Li, C. Y., Fu, J. Y., et al. (2024). Distinct circuits from the central lateral amygdala to the ventral part of the bed nucleus of Stria Terminalis regulate different fear memory. *Biol. Psychiatry* 95, 732–744. doi: 10.1016/j.biopsych.2023.08.022

frontiers | Frontiers in Neuroscience

TYPE Review
PUBLISHED 13 June 2025
DOI 10.3389/fnins.2025.1574512

OPEN ACCESS

EDITED BY
Zuleide Maria Ignácio,
Federal University of the Southern Frontier, Brazil

REVIEWED BY
Rajesh Singh Yadav,
Dr. Hari Singh Gour University, India
Xie Zhenchao,
Southern Medical University, China

*CORRESPONDENCE
Yuan Wang
✉ 15168867539@163.com
Yuanyuan Xiang
✉ 15168889383@126.com

RECEIVED 11 February 2025
ACCEPTED 28 May 2025
PUBLISHED 13 June 2025

CITATION
Guo M, Gao H, Wang Y and Xiang Y (2025) Exploring the role of gut microbiota in Parkinson's disease: insights from fecal microbiota transplantation.
Front. Neurosci. 19:1574512.
doi: 10.3389/fnins.2025.1574512

Exploring the role of gut microbiota in Parkinson's disease: insights from fecal microbiota transplantation

Mengqi Guo[1,2], Han Gao[3,4], Yuan Wang[1,2]* and Yuanyuan Xiang[1,2]*

[1]Department of Neurology, Shandong Provincial Hospital, Affiliated to Shandong First Medical University, Jinan, Shandong, China, [2]Department of Neurology, Shandong First Medical University, Jinan, Shandong, China, [3]Department of Ultrasound, Shandong Provincial Hospital, Affiliated to Shandong First Medical University, Jinan, Shandong, China, [4]Department of Ultrasound, Shandong First Medical University, Jinan, Shandong, China

As a common neurodegenerative disease, Parkinson's disease (PD) is typified by α-synuclein (α-syn) aggregation and progressive degeneration of dopaminergic neurons within the substantia nigra. Clinical manifestations encompass motor symptoms and non-motor aspects that severely impair quality of life. Existing treatments mainly address symptoms, with no effective disease-modifying therapies available. The gut microbiota refers to the community of microorganisms that colonize the intestinal tract. The gut microbiota, gut, and brain are all connected via a complicated, mutual communication pathway known as the "gut microbiota-gut-brain axis." Gut microbiota dysbiosis is strongly linked to the onset and course of PD, according to growing data. In individuals with PD, gut dysbiosis correlates with clinical phenotype, disease duration, severity, and progression rates. Mechanistically, gut dysbiosis contributes to PD through enhanced intestinal permeability, increased intestinal inflammation and neuroinflammation, abnormal α-syn aggregation, oxidative stress, and reduced neurotransmitter synthesis. Therefore, focusing on the gut microbiota is regarded as a potentially effective treatment strategy. Fecal microbiota transplantation (FMT) is an emerging approach to modulate gut microbiota, with the goal of recovering microbiota diversity and function by transferring functional intestinal flora from healthy individuals into patients' gastrointestinal tracts. FMT is expected to become a promising therapy of PD and has a broad research and application prospect. Evidence suggests that FMT may restore gut microbiota, ease clinical symptoms, and provide potential neuroprotective benefits. However, the precise therapeutic mechanisms of FMT in PD remain uncertain, necessitating further research to clarify its effectiveness. This review examines alterations in gut microbiota linked to PD, mechanisms through which gut dysbiosis influences the disease, and the latest advancements in FMT research for treating PD, setting the stage for its clinical application.

KEYWORDS

Parkinson's disease, gut microbiota, gut-brain axis, fecal microbiota transplantation, gut dysbiosis

1 Introduction

As a prevalent neurodegenerative illness, Parkinson's disease (PD) is typified by aberrant α-synuclein (α-syn) folding and aggregation, as well as dopaminergic neurons degenerating and losing within the substantia nigra (SN; Lajoie et al., 2021). It has a complicated and multifaceted etiology that includes environmental, genetic, and other variables. Its pathogenesis mainly involves α-syn abnormal aggregation (Tofaris, 2022), neuroinflammation (Marogianni et al., 2020), oxidative stress (Ding et al., 2018), and mitochondrial dysfunction (Malpartida et al., 2021).

According to statistics, in 2015, the number of PD cases worldwide was approximately 6.2 million, and by 2040, that figure is predicted to reach 12.9 million (Dorsey and Bloem, 2018). The incidence of PD is significantly related to age (Qi et al., 2021). The typical course is insidious, slow, and progressive. Clinical manifestations encompass motor aspects like bradykinesia, resting tremor, and rigidity, along with non-motor aspects like gastrointestinal dysfunction and sleep disturbances, which often more significantly affect the quality of life. According to statistical data, from 1999 to 2019, the death rate for PD rose from 5.4 per 100,000 to 8.8 per 100,000 (Rong et al., 2021). The growth rate of PD-related disabilities and deaths is faster than that of any other neurological disease (The Lancet, 2022).

Currently, the treatment of PD is mainly symptomatic, comprising pharmacotherapy and surgical interventions. However, effective disease-modifying therapies are still lacking. Commonly utilized medications in clinical treatment include the dopamine (DA) precursor levodopa, catechol-O-methyltransferase inhibitors, monoamine oxidase B inhibitors, anticholinergic drugs, and DA receptor agonists. Among these, DA replacement therapy remains the first-line intervention for PD, but it only partially improves motor symptoms, without alleviating non-motor symptoms or delaying disease progression.

Furthermore, its efficacy tends to decline after 3–5 years, and prolonged use may produce adverse effects such as the wearing-off phenomenon, the on–off phenomenon, and dyskinesia, resulting in poor patient tolerance (Armstrong and Okun, 2020). Deep brain stimulation, the main surgical treatment for PD, alleviates symptoms by implanting electrodes into specific brain nuclei and delivering microcurrents to stimulate target areas, thereby modifying electrical signals in related neural circuits (Sharma et al., 2020). However, deep brain stimulation controls symptoms without curing the disease. Patients still require long-term medication post-surgery, and the associated medical costs remain high. Currently, no existing therapy has shown significant efficacy in reversing α-syn aggregation, restoring DA neuron degeneration, or delaying disease progression (Suarez-Cedeno et al., 2017).

Recent research has introduced a new perspective: the central nervous system (CNS) may be impacted by the gut microbiota. Gut microbiota is known as the collection of bacteria, viruses, fungi, and other microorganisms inhabiting the intestinal tract, representing the largest and most intricate microflora in the human body. It includes approximately 50 bacterial phyla. Bacteroidetes and Firmicutes comprise more than 90% of the total (Ding et al., 2021).

Under normal circumstances, the relative abundance and diversity of gut microbiota are dynamically balanced, influenced by variables including diet, stress and antibiotics (Sun et al., 2022). Many physiological processes, including nutrient digestion and absorption, energy metabolism, immune function regulation, neural function modulation, and intestinal barrier maintenance, are influenced by the gut microbiota. It is necessary for the immune, endocrine, and nervous systems to grow and mature. Dysbiosis of gut microbiota refers to disturbances in overall microbial composition and the relative abundance of specific flora, disrupting the body's homeostasis. In preclinical studies, gut dysbiosis has been linked to the pathophysiological mechanisms of intestinal diseases (e.g., inflammatory bowel disease, irritable bowel syndrome), mental disorders (e.g., anxiety, depression, autism spectrum disorder), as well as neurological diseases (e.g., multiple sclerosis, Alzheimer's disease [AD], PD, amyotrophic lateral sclerosis; Mou et al., 2022; Sorboni et al., 2022; Kujawa et al., 2023; Solanki et al., 2023; Loh et al., 2024).

Modifications to gut microbiota abundance or diversity and its metabolites have been observed in animal models and clinical cases of PD, highlighting the significance in the onset and progression of the disease. Addressing gut dysbiosis offers PD patients a potential treatment strategy. This article reviews the characteristic changes in gut microbiota among PD patients, the potential mechanisms by which gut dysbiosis may contribute to PD pathogenesis, and recent advancements in fecal microbiota transplantation (FMT) for PD treatment, providing a scientific foundation for future clinical applications.

2 Gut microbiota and the connection with PD

2.1 Gut microbiota-gut-brain axis

Recent research has revealed a sophisticated bidirectional communication pathway connecting the gut microbiota, the gut, and the brain, termed the "gut microbiota-gut-brain axis" (MGBA; Cryan and Dinan, 2012). This axis enables crosstalk between the enteric nervous system (ENS) and the CNS by means of neurological, immunological, endocrine, and metabolic signaling pathways (Matheson and Holsinger, 2023). Dysregulation of this axis has been associated with the pathophysiology of PD.

Braak et al. (2006) first proposed the hypothesis that the origins of PD may be in the gastrointestinal tract. They proposed that a neurotropic pathogen crossing the gastric epithelium could induce α-syn misfolding in the ENS, then propagate to the brain via retrograde axonal transport through a chain of interconnected neurons, driving PD pathology. This hypothesis aligns with clinical observations that gastrointestinal symptoms frequently appear before motor problems in PD patients. According to epidemiological research, patients with inflammatory bowel disease are more likely to acquire PD than people without inflammatory bowel disease (Brudek and van Laar, 2019). Furthermore, vagotomy performed to treat peptic ulcers has been shown to reduce PD risk (Sun et al., 2022). These findings underscore the strong association between PD and the gastrointestinal tract, indirectly supporting the hypothesis of intestinal origin.

Preclinical studies provide additional evidence. Kim et al. (2019) found that α-syn was transferred from the gut, initially appearing in the vagus nerve's dorsal motor nucleus nerve and eventually reaching the SN compacta via sequential diffusion and transmission. Importantly, vagotomy effectively prevented α-syn transfer from the colon to the brain, confirming the role of the vagus nerve. Similarly, Bhattarai et al. (2021) administered rotenone to both germ-free and

conventionally raised mice for 6 weeks. Although tyrosine hydroxylase neurons were lost in each group, only conventionally raised mice exhibited decreased motor strength and coordination, emphasizing the importance of gut microbiota in PD etiology. Collectively, these findings highlight the critical function of this axis in the onset and course of PD.

2.2 Dysbiosis of gut microbiota in PD patients

Numerous case–control investigations have researched the gut microbiota composition of PD patients. While findings vary due to differences in sample size, inclusion and exclusion criteria, experimental design, and individual factors (e.g., age, diet, geography, and genetic background; Li Z. et al., 2022), certain consistent trends have emerged. For example, compared with healthy controls, PD patients exhibit higher abundances of certain genera, including *Bilophila*, *Akkermansia*, Verrucomicrobia, *Lactobacillus*, and *Parabacteroides*, alongside lower abundances of beneficial bacteria, especially Lachnospiraceae, *Roseburia*, *Faecalibacterium*, *Blautia*, and *Prevotella* (Scheperjans et al., 2014; Li et al., 2017; Barichella et al., 2018; Lin et al., 2019; Lubomski et al., 2019; Cirstea et al., 2020; Nishiwaki et al., 2020a; Yu et al., 2023). Furthermore, specific microbial taxa correlate with clinical phenotypes, disease duration, severity, and progression rate. Table 1 summarizes these associations, illustrating the complicated link between microbiota changes and PD pathology.

2.3 Potential mechanisms of gut microbiota dysbiosis in PD pathogenesis

In recent years, research has increasingly supported the hypothesis that gut microbiota dysbiosis acts as a triggering factor for PD (Costa et al., 2022). Dysbiosis and its metabolites are thought to affect the onset and progression via several interconnected mechanisms, including raised intestinal permeability, exacerbated intestinal inflammation and neuroinflammation, aberrant α-syn aggregation, elevated oxidative stress, and reduced neurotransmitter manufacture (Figure 1).

2.3.1 Increase in intestinal permeability

Dysbiosis of gut microbiota can compromise the intestinal epithelial barrier, leading to increased penetration. This "leaky gut" condition permits neuroactive small molecules, including potentially toxic metabolites derived from bacteria and microbial sources, to translocate into systemic circulation, accelerating pathological processes and elevating PD risk. Mucin, a key structural component of gastrointestinal mucosa, is essential for preserving barrier integrity. In PD patients, *Prevotella* deficiency is correlated to impaired mucin production, increased gut permeability, and disease progression (Scheperjans et al., 2014). Fang et al. (2024) demonstrated that chronic rotenone administration significantly reduced colonic mucus thickness and downregulated the expression of tight junction proteins (e.g., Zonula Occludens-1, occludin), confirming the essential role of gut microbiota in maintaining intestinal barrier integrity. Notably, FMT effectively alleviated rotenone-induced intestinal barrier impairment.

TABLE 1 Association between gut microbiota and PD phenotypes.

Ref.	Microbiota changes	Aspects of impact	Correlation
Scheperjans et al. (2014)	Enterobacteriaceae↑	disease severity (postural instability and gait disturbances)	positive
Li et al. (2017)	*Enterococcus*, *Proteus*, *Escherichia*-Shigella↑	disease severity and PD duration	positive
	Blautia, *Faecalibacterium*, *Ruminococcus*↓		negative
Barichella et al. (2018)	Lachnospiraceae↓	disease severity (postural instability, gait disturbances and cognitive impairment)	positive
	Lactobacillaceae↑, Christensenellaceae↑		
Lin et al. (2019)	*Bacteroides*	motor symptom	positive
		in PD patients: with tremor subtype<with non-tremor subtype	---
Aho et al. (2021)	microbial alpha diversity indices	disease severity	positive
Mao et al. (2021)	*Klebsiella*, *Parasutterella*	disease severity and PD duration	positive
	hydrogen-generating *Prevotella*	disease severity	negative
Zheng et al. (2021)	*Lactobacillus gasseri*, Deferribacterales	disease duration	positive
	Escherichia/Shigella, Lachnospiraceae, *Clostridium coccoides*		negative
	Enterobacteriaceae, *Proteus*, *Escherichia*, *Enterococcus*, Lactobacillaceae	disease severity	positive
	Lachnospiraceae, *Blautia*, *Ruminococcus*, *Faecalibacterium*		negative
Cilia et al. (2021)	*Roseburia* (Firmicutes phylum) at baseline↓	disease severity	positive
	Ruminococcaceae and Actinobacteria at baseline↓	faster cognitive impairment	
Nishiwaki et al. (2022)	SCFA-producing genera, *Blautia*, *Fusicatenibacter*, *Faecalibacterium*↓	accelerated disease progression	positive
	mucin-degrading genus *Akkermansia*↑		

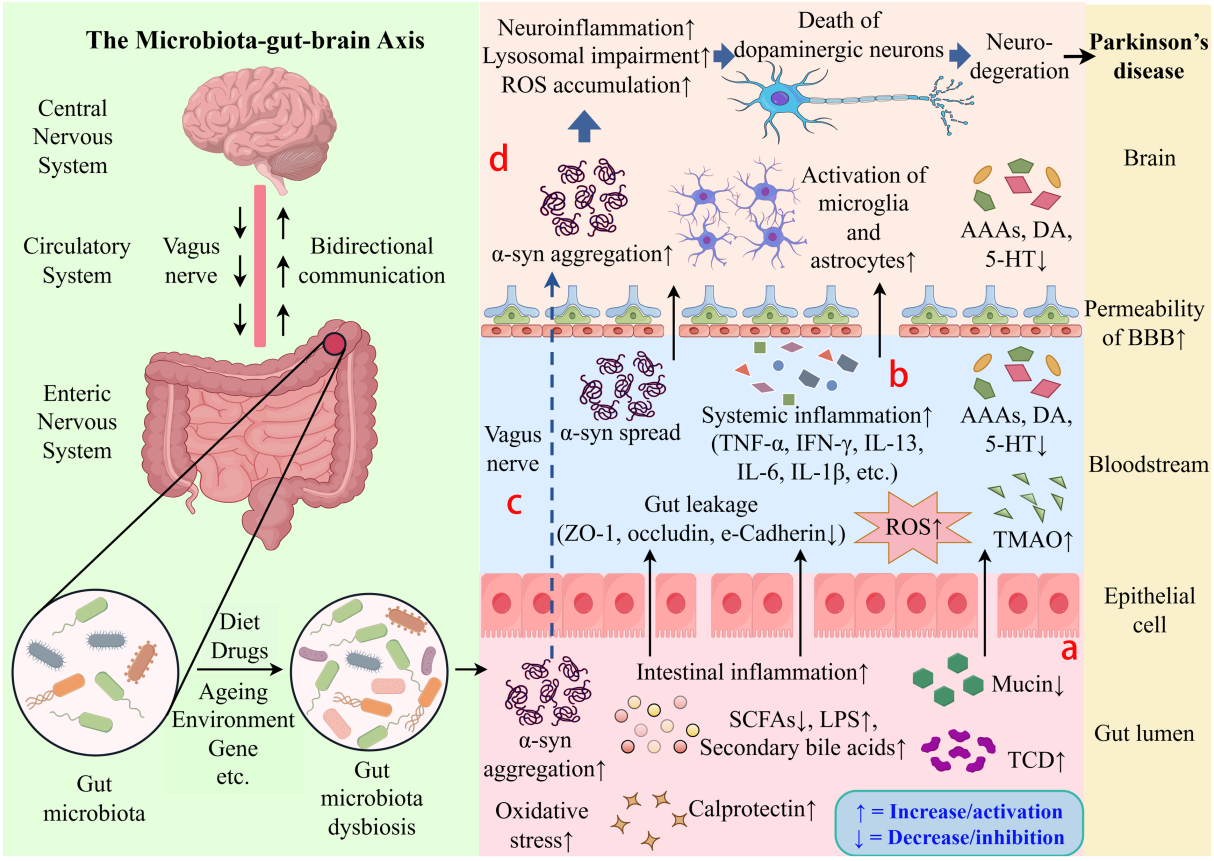


FIGURE 1
Schematic diagram of potential mechanisms by which gut microbiota influences Parkinson's disease. The gut microbiota-gut-brain axis can affect the onset and course of PD. **(a)** Dysbiosis is associated with increased intestinal permeability, dysregulated gut microbiota metabolites, exacerbated oxidative stress and intestinal inflammation. **(b)** Gut leakage can trigger inflammatory cytokines to be released, resulting in systemic inflammation. **(c)** The vagus nerve allows abnormal α-syn aggregation to spread from the ENS to the CNS. **(d)** Pro-inflammatory cytokines can penetrate the blood–brain barrier, cause the activation of microglia and astrocytes, and aggravate neuroinflammation, which results in dopaminergic neuron loss and degeneration. ROS, Reactive Oxygen Species; AAAs, Aromatic Amino Acids; DA, Dopamine; 5-HT, 5-Hydroxytryptamine; α-syn, α-synuclein; BBB, Blood–Brain Barrier; TNF-α, Tumor Necrosis Factor-alpha; IFN-γ, Interferon-gamma; IL-13, Interleukin-13; IL-6, Interleukin-6; IL-1β, Interleukin-1 beta; ZO-1, Zonula Occludens-1; TMAO, Trimethylamine N-Oxide; SCFAs, Short-Chain Fatty Acids; LPS, Lipopolysaccharide; TCD, Total Cumulative Dose.

The study further revealed that gut microbiota dysbiosis promotes excessive hydrogen sulfide production by sulfate-reducing bacteria, which subsequently degrades the mucus layer, disrupts the intestinal epithelial barrier, enhances intestinal permeability, and ultimately contribute to gut leakage (Munteanu et al., 2024). Short-chain fatty acids (SCFAs) contribute to intestinal barrier maintenance. According to studies, PD patients have much less bacteria that produce SCFA, resulting in lower fecal SCFA levels (Bisaglia, 2022). Experimental evidence suggests that administering butyrate to PD animal models can delay disease progression by improving motor function, preserving intestinal barrier integrity, reducing intestinal leakage, and secondary translocation of intestinal contents (Zheng et al., 2021).

2.3.2 Aggravation of intestinal inflammation and neuroinflammation

According to compelling data, chronic intestinal inflammation and neuroinflammation are exacerbated by pro-inflammatory dysbiosis of the gut microbiota, which are thought to potentially contribute to the PD pathophysiology. Lin et al. (2019) reported elevated concentrations of pro-inflammatory cytokines, including Tumor Necrosis Factor-alpha (TNF-α), Interferon-gamma, and Interleukin-13, in the plasma of PD patients. There were positive correlations between the levels of TNF-α and Interferon-gamma and the abundance of *Bacteroides* and Verrucomicrobia, respectively. Additionally, fecal calprotectin, a hallmark of intestinal inflammation, was considerably increased in PD patients (Weis et al., 2019). Keshavarzian et al. (2015) used high-throughput ribosomal RNA sequencing to reveal that PD patients had lower "anti-inflammatory" bacteria, including *Blautia*, *Coprococcus*, and *Roseburia*, alongside more "pro-inflammatory" bacteria like *Faecalibacterium*. Preclinical studies demonstrate that dysbiosis exacerbates neuroinflammation through pathways such as Toll-like receptor 4 (TLR4)/Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), which raises the production of inflammatory markers, such as Glycogen synthase kinase 3 beta, inducible nitric oxide synthase, and Interleukin-1 beta, along with activating microglia and astrocytes in the SN (Varesi et al., 2022).

2.3.3 Abnormal α-syn aggregation

α-syn aggregation, a characteristic of PD pathophysiology, might be impacted by gut dysbiosis. Altered microbiota promotes α-syn misfolding in the ENS and gastrointestinal epithelial cells, promoting pro-inflammatory immune activation and facilitating its spread to the

CNS (Sterling et al., 2022). On the one hand, it has been shown that certain gut bacteria or their secreted metabolites promote the aggregation and dissemination of α-syn. In mice with *leucine-rich repeat kinase 2* gene, Liang et al. (2023) demonstrated that giving them *Escherichia coli* by mouth caused curli-mediated phosphorylation and accumulation of α-syn in the colon, which then spread along the gut-brain axis to the CNS. Additionally, hemolysin A secreted by *Proteus mirabilis* triggers α-syn oligomerization via activation of Mechanistic Target of Rapamycin-dependent autophagy signaling pathways in intestinal cells, ultimately inducing motor deficits and neurodegeneration (Huh et al., 2023). Notably, *Dubosiella* has been implicated in α-syn aggregation via the suppression of branched-chain amino acid catabolism, leading to the peripheral accumulation of valine and isoleucine, which disrupts lysosomal function and hinders α-syn clearance (Wu et al., 2025). On the other hand, Wang et al. (2022) discovered that the probiotic *Lactobacillus plantarum* DP189 could suppress oxidative stress, restore microbial diversity, and decrease α-syn aggregation in the SN of PD mice, thus delaying disease progression. The findings indicate that targeted modulation of gut microbiota could be a possible therapeutic approach to reduce α-syn aggregation in PD pathogenesis.

2.3.4 Increase in oxidative stress

Dysbiosis can worsen oxidative stress by changing microbial metabolism and decreasing antioxidant metabolite production. This promotes neuronal damage and α-syn misfolding in the ENS, which subsequently spreads to the CNS (Bullich et al., 2019; Shandilya et al., 2022). Studies have shown that *Akkermansia* increases intestinal permeability, exposing neurons to oxidative conditions that favor α-syn aggregation (Nishiwaki et al., 2020b). Yu et al. (2023) found that gut dysbiosis aggravated oxidative stress responses and neurobehavioral impairments by downregulating *Nicotinamide mononucleotide adenylyltransferase 2*, a gene involved in NAD^+ synthesis, in PD rat models. Emerging evidence indicates that the modulation of gut microbiota can reduce oxidative stress responses. Zhu et al. (2025) demonstrated that sleep deprivation promotes the synthesis of microbiota-derived adenosine, which elevates the production of reactive oxygen species by upregulating the pro-oxidant enzyme NADPH oxidase 4 and inhibiting the antioxidant factor Nuclear factor erythroid 2-related factor 2, consequently exacerbating oxidative damage to dopaminergic neurons. Probiotic supplements significantly mitigated these effects. Nurrahma et al. (2022) revealed that a high dose of the mangosteen pericarp, abundant in antioxidants, restores gut microbiota balance by diminishing pro-inflammatory bacterial genera (e.g., *Sutterella*, *Rothia*, *Aggregatibacter*), which exhibited a negative correlation with antioxidant gene expression. This improves antioxidant levels and alleviates PD motor deficits. Additionally, Gao et al. (2024) demonstrated that in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced PD mice, administration of ginkgolide C could restore gut microbiota homeostasis, exert antioxidant effects by activating the Protein Kinase B/Nuclear factor erythroid 2-related factor 2/Heme oxygenase-1 pathway in SN4741 neuronal cells, and alleviate pathological damage in mice.

2.3.5 Reduction in production of neurotransmitters

It has been shown that the gut microbiota synthesize numerous neurotransmitters that are present in the human brain, such as DA, serotonin, γ-aminobutyric acid, and noradrenaline. Gut microbiota dysbiosis may disrupt neurotransmitters synthesis, perturbing the CNS homeostasis through gut-brain axis signaling pathways, which may contribute to the pathological progression of PD neurological dysfunction (Strandwitz, 2018; Wang et al., 2021). Research by Gao et al. (2018) revealed that when gut microbiota in experimental animals was changed through antibiotic infusion, there were significant reductions in the concentrations of serotonin, DA, and aromatic amino acids in their blood and hypothalamus compared to a control group infused with normal saline. This discovery emphasizes how vital the gut bacteria is to preserving neurotransmitter and precursor levels. Similarly, van Kessel et al. (2019) investigated the impact of microbial tyrosine decarboxylase in the proximal small intestine—a primary area for Levodopa absorption—in PD patients. They observed an increase in tyrosine decarboxylase activity, which led to premature transformation of L-dopa, significantly reducing its plasma levels and bioavailability. This, in turn, increased therapeutic dose requirements and reduced drug efficacy. According to these findings, gut dysbiosis may directly or indirectly influence the pharmacokinetics, bioavailability, and side effects of medications used to treat PD.

Overall, the findings presented underscore the role that gut microbiota plays in regulating neurotransmitter production and its profound implications for PD pathophysiology as well as the optimization of therapy approaches.

2.4 Metabolites of gut microbiota

The metabolites produced by the gut microbiota play a critical role in regulating neurodegenerative diseases, such as PD, via the MGBA. The pathological mechanisms mediated by four key metabolite classes are outlined.

2.4.1 SCFAs

SCFAs, including acetate, propionate, and butyrate, are microbial metabolites derived from anaerobic fermentation of dietary fibers. Emerging evidence establishes a link between SCFA homeostasis disruption and neurodegenerative pathogenesis. In PD, reduced fecal SCFA levels compromise the structural integrity of the intestinal barrier and the blood–brain barrier (BBB), promote α-syn pathological aggregation, and exacerbate intestinal inflammation and neuroinflammation (Chen et al., 2022; Duan et al., 2023). Preclinical studies demonstrate that SCFA supplementation attenuates dopaminergic neurodegeneration and alleviates motor impairments in PD mice by inhibiting NF-κB/mitogen-activated protein kinase pathway inhibition in the SN and reducing α-syn aggregation (Hou Y. F. et al., 2021; Hou Y. et al., 2021). The neuroprotective effects of SCFAs extend to AD pathophysiology by modulating synaptic plasticity, amyloid-β (Aβ) and tau pathology, and neuroinflammation (Tang et al., 2022). Clinically, mild cognitive impairment patients exhibit a significant reduction of fecal SCFAs that inversely correlates with Aβ burden in cognition-associated brain regions (Gao et al., 2023). Notably, in models of amyotrophic lateral sclerosis, the abundance of butyrate-producing bacteria decreases in $SOD1^{G93A}$ mice, whereas butyrate supplementation enhances gut barrier integrity, reduces $SOD1^{G93A}$ aggregates, decelerates motor neuron degeneration, and prolongs survival of these mice (Loh et al., 2024).

2.4.2 Secondary bile acids

Intestinal microbiota mediate the biotransformation of primary to secondary bile acids. In PD patients, increased levels of deoxycholic acid and lithocholic acid in the cecum are closely related to increased abundance of bile acid-synthesizing microbiota. These secondary bile acids induce pathologic α-syn aggregation and propagation through exerting pro-inflammatory and cytotoxic effects while simultaneously impairing mitochondrial function and autophagy regulation, contributing to neurodegenerative disease pathology (Castro-Caldas et al., 2012; Kiriyama and Nochi, 2023). Notably, taurodeoxycholic acid, a neuroprotective bile acid, demonstrates therapeutic potential across neurodegenerative models. In PD mice, taurodeoxycholic acid administration significantly delays dopaminergic neurodegeneration by inhibiting the c-Jun N-terminal kinase apoptosis pathway, reducing mitochondrial reactive oxygen species, and activating the Protein Kinase B survival pathway (Li et al., 2021). AD rodent models further reveal the capacity of taurodeoxycholic acid to reduce Aβ deposition in the hippocampus and prefrontal cortex and rescue cognitive deficits in spatial, recognition, and contextual memory domains (Lo et al., 2013).

2.4.3 Trimethylamine N-oxide

The gut microbiota mediates enzymatic conversion of dietary choline and carnitine to trimethylamine, which undergoes hepatic oxidation to generate TMAO, a compound implicated in neurodegeneration through various mechanisms (Caradonna et al., 2025). Clinical metabolomic profiling reveals elevated circulating TMAO concentrations in PD patients, though independent of disease progression (Voigt et al., 2022). Mechanistically, TMAO promotes abnormal α-syn conformational changes and pathological aggregation and activates pro-inflammatory signaling pathways, such as NF-κB. Additionally, TMAO penetrates the BBB, exacerbating neuroinflammation and neuronal damage (Caradonna et al., 2024). Lee et al. (2022) demonstrated that TMAO-treated midbrain organoids showed impaired brain-derived neurotrophic factor signaling, loss of dopaminergic neurons, astrocyte activation, and neuromelanin accumulation. Furthermore, TMAO induced the pathological phosphorylation of α-syn and tau proteins, facilitating their aggregation. Vogt et al. (2018) identified a correlation between elevated TMAO levels and AD pathology and markers of neuronal degeneration in the cerebrospinal fluid. Individuals with mild cognitive impairment and AD dementia exhibited higher TMAO levels in the cerebrospinal fluid compared to cognitively normal individuals.

2.4.4 Lipopolysaccharide

LPS, an endotoxin produced by Gram-negative bacteria, plays a multifaceted role in neurodegenerative pathology. Gorecki et al. (2019) found that PD patients had a significantly higher abundance of LPS-producing Gammaproteobacteria in the gut compared to healthy controls, and LPS reduces the expression and disrupts the distribution of intestinal epithelial tight junction markers (e.g., Zonula Occludens-1, e-Cadherin). A clinical study indicated that plasma LPS rose with cognitive decline, and in non-dementia participants, high plasma LPS was independently linked to mild cognitive impairment (Saji et al., 2022). LPS activates TLR4 receptors, triggering downstream Myeloid differentiation primary response protein 88 and TIR-domain-containing adapter-inducing interferon-β pathways. It also induces the release of pro-inflammatory cytokines (e.g., TNF-α, Interleukin-1 beta) from microglia and astrocytes, causes oxidative stress and mitochondrial dysfunction, promotes Aβ deposition, Tau hyperphosphorylation, and α-syn aggregation, and results in neuronal and synaptic damage, thus driving neurodegeneration (Batista et al., 2019; Kesika et al., 2021; Brown et al., 2023).

In summary, the metabolites of gut microbiota regulate organismal homeostasis through complex mechanisms, and their dysregulation may raise the risk of neurodegenerative diseases. Thus, targeting the generation or signaling pathways of these metabolites may provide potential therapeutic strategies for PD and other neurodegenerative conditions.

3 FMT treatment for PD

For PD, the gut microbiota has become a potential treatment focus. Restoring gut microbiota balance to delay or prevent neurodegeneration in PD represents a novel treatment strategy. Interventions that target the gut microbiota include antibiotics, probiotics, prebiotics, dietary modifications, and FMT. Among these, FMT has drawn a lot of interest as a novel and promising approach for treating PD (Varesi et al., 2022).

3.1 Definition and application of FMT

To restore gut microbiota diversity and function, FMT entails transplanting functional intestinal flora from healthy donors' feces into the patients' gastrointestinal tract. This procedure aims to increase beneficial bacteria, reduce harmful bacterial populations, and re-establish gut homeostasis, thereby mitigating disease progression. According to data from ClinicalTrials.gov, more than 400 FMT-related clinical trials have been registered worldwide, underscoring its growing prominence in medical research.

FMT can be administered through two primary methods: capsule transplantation and bacterial liquid transplantation. Bacterial liquid transplantation is further divided into three pathways: (1) the upper gastrointestinal tract route, using nasogastric or nasojejunal tubes or a gastroscope to introduce the transplant; (2) the colonoscope route, involving the insertion of a colonoscope to deliver fecal bacteria to the ileum; and (3) the enema route (König et al., 2016). Donor selection for FMT requires stringent screening criteria, including eight dimensions of assessments. Standardized effectiveness criteria for FMT are currently lacking.

Existing evidence demonstrates the short-term safety of FMT, with most adverse events being mild, self-limiting gastrointestinal symptoms such as abdominal discomfort, diarrhea, constipation, borborygmi, bloating, nausea, and vomiting. Serious adverse events are rare but warrant investigation to improve safety protocols. Notably, the US Food and Drug Administration has approved FMT for the treatment of *Clostridium difficile* infection, achieving cure rates of approximately 90% (Carlucci et al., 2016).

Beyond *Clostridium difficile* infection, FMT holds potential for a variety of diseases linked to gut microbiota dysbiosis, including ulcerative colitis, irritable bowel syndrome, sepsis, depression, type 2 diabetes, autism spectrum disorder, multiple sclerosis, PD, AD, epilepsy, Guillain-Barré syndrome, and amyotrophic lateral sclerosis (Li et al., 2019; Kim et al., 2020b; Kim et al., 2020a; Vendrik et al.,

2020; Wang et al., 2020; Cui et al., 2021; Chen et al., 2023). This broad applicability underscores the promising future of FMT in both intestinal and systemic diseases linked to microbiota dysregulation.

3.2 FMT and PD

FMT has been investigated in preclinical and clinical research for PD. As these studies consistently demonstrate, FMT can effectively restore gut microbiota dysbiosis associated with PD (Table 2).

3.2.1 Preclinical studies

Preclinical evidence reveals several key mechanisms through which FMT improves gastrointestinal function, alleviates motor symptoms, and delays neurodegeneration in PD (Figure 2):

1 **Reduction of Inflammatory Effects and Oxidative Stress**: FMT relieves the neurotoxic effects of microglia and astrocytes, lowers LPS in the colon and SN, and reduces the secretion of pro-inflammatory cytokines while elevating anti-inflammatory factors. Moreover, FMT modulates inflammatory signaling pathways, including TLR4/TANK-binding kinase 1/NF-κB/TNF-α (Sun et al., 2018), TLR4/Phosphatidylinositol 3-kinase/Protein Kinase B/NF-κB (Zhong et al., 2021), and TLR4/Myeloid differentiation primary response protein 88/NF-κB (Zhao et al., 2021). In addition, Xie et al. (2023) confirmed that FMT activates the AMP-activated protein kinase/Superoxide dismutase 2 pathway, mitigating mitochondrial damage and enhancing mitochondrial antioxidative capacity. Studies have indicated that FMT reduced oxidative stress induced by 6-Hydroxydopamine in PD rat models, a known contributor to PD progression (Yu et al., 2023).

2 **Reduction of α-syn Aggregation**: Transplantation of fecal microbiota from PD patients into mice has been shown to promote microglial activation and α-syn aggregation by modulating metabolites such as SCFAs, which exacerbates motor dysfunction (Sampson et al., 2016). In PD mouse models, FMT has been reported to restore gut microbiota diversity, elevate SCFA levels (especially butyrate), and reduce pathological α-syn aggregation in both the ENS and SN, ultimately ameliorating motor dysfunction (Sun et al., 2018; Liang et al., 2023). Ni et al. (2025) demonstrated that FMT may regulate SCFA levels by upregulating SCFA receptors Free Fatty Acid Receptor 2 and Free Fatty Acid Receptor 3, thereby mitigating pathological features. Fang et al. (2024) found that rotenone-induced gut dysbiosis promotes α-syn transcription via activation of the CCAAT/Enhancer-Binding Protein Beta/Asparagine Endopeptidase pathway, while FMT alleviates this pathological damage.

3 **Restoration of BBB Integrity**: FMT has been demonstrated to enhance BBB integrity and mitigate dopaminergic neuronal damage, thereby exerting neuroprotective effects. Studies have revealed that compared to normal controls, germ-free mice and antibiotic-treated mice with gut microbiota depletion exhibit significantly increased BBB permeability. FMT can upregulate the expression of tight junction proteins in the CNS, including Zonula Occludens-1, Zonula Occludens-2, occludin, and claudin-5, thereby restoring BBB integrity and reducing its permeability (Braniste et al., 2014; Sun N. et al., 2021). In PD mouse models, Zhao et al. (2021) found that FMT treatment ameliorated the tight junction structure defects in the SN, alleviated endothelial cell damage, and significantly upregulated the Messenger RNA levels of tight junction proteins.

TABLE 2 The application of FMT for PD: preclinical and clinical research.

Ref.	Models	Fece donors	Fece recipients	Microbiota changes	
				↑	↓
Zhao et al. (2021)	mice	the control group mice	Rotenone-induced PD mice	Proteobacteria, Helicobacteraceae, Lactobacillaceae, Enterobacteriaceae, *Barnesiella*, *Roseburia*, *Butyricicoccus*, *Helicobacter*	Verrucomicrobia, Coriobacteriaceae, *Akkermansia*, *Desulfovibrio*
Sun et al. (2018)	mice	normal control mice/PD mice	MPTP+FMT group, NS + PD-FMT group, NS + FMT group	Firmicutes, Clostridiales	Proteobacteria, Turicibacterales, Enterobacteriales
Xie et al. (2023)	mice	PD patients/ healthy human controls	MPTP+PD FMT group, MPTP+HC FMT group	Verrucomicrobiota, *Akkermansia*	Unclassified Muribaculaceae, *Odoribacter*
Kuai et al. (2021)	human	The China fmtBank (Nanjing, China)	11 PD patients with constipation	*Blautia*, *Prevotella*	Bacteroidetes
Xue et al. (2019)	human	a healthy 20-year-old female	a male PD patient who refused to take drugs because of hallucinations	*Ruminococcus*, *Blautia*, Prevotellaceae, *Faecalibacterium*	*Bacteroides*
DuPont et al. (2023)	human	4 thoroughly screened donors	8 PD patients with constipation	Firmicutes	Proteobacteria
Huang et al. (2019)	human	a 26-year-old male	a 71-year-old male patient presented with intractable constipation	Firmicutes	Proteobacteria, Bacteroidetes

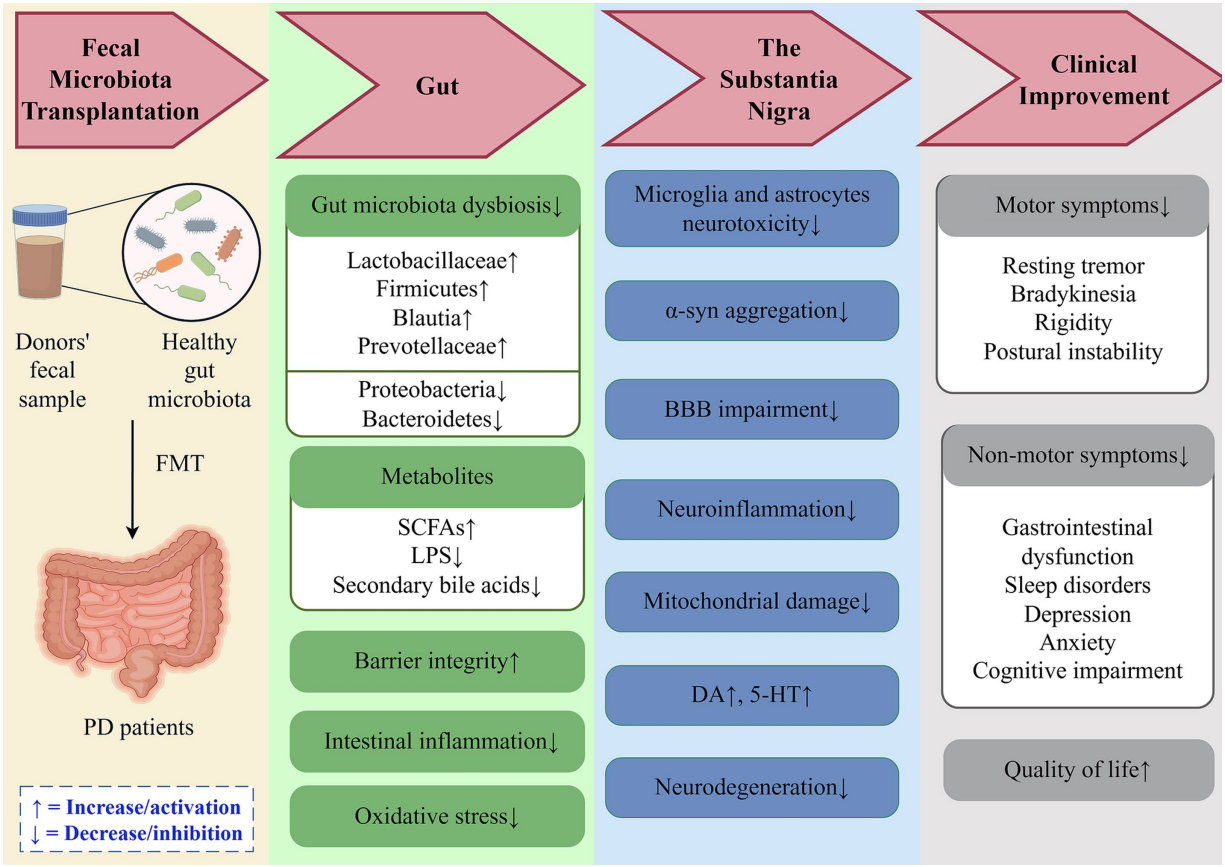


FIGURE 2
Potential mechanisms of fecal microbiota transplantation in PD treatment. FMT restores gut microbial homeostasis in PD patients by correcting dysbiosis, enhancing intestinal barrier integrity, and reducing oxidative stress and inflammatory responses. These effects mitigate neurodegeneration through modulation of the gut-brain axis, ultimately improving clinical manifestations and quality of life. DA, Dopamine; 5-HT, 5-Hydroxytryptamine; α-syn, α-synuclein; BBB, Blood–Brain Barrier; SCFAs, Short-Chain Fatty Acids; LPS, Lipopolysaccharide.

3.2.2 Clinical investigations

Clinical trials involving FMT in PD patients further support these preclinical findings. Fecal samples collected before and after FMT have undergone microbiota sequencing, revealing significant restoration of gut microbiota composition. Symptom assessments using scales such as the Unified Parkinson's Disease Rating Scale, Non-Motor Symptoms Scale, and Parkinson's Disease Questionnaire-39 indicate improvements in motor symptoms, constipation, anxiety, depression, sleep, and cognitive function, all of which improve overall quality of life. Furthermore, adverse events are less common and generally self-limiting (Xue et al., 2019; Kuai et al., 2021; Segal et al., 2021; Cheng et al., 2023; DuPont et al., 2023; Liu et al., 2023).

However, FMT's therapeutic effects appear time-dependent. Research indicates that microbiota composition and related motor and non-motor symptoms, except constipation, may partially revert after a certain period post-transplantation (Huang et al., 2019; Xue et al., 2019). Further investigation is needed to determine long-term efficacy and stability. Additionally, transplantation methods may impact therapeutic outcomes. For instance, Xue et al. (2020) compared colonoscope-administered FMT with nasojejunal tube administration and found that the former yielded superior clinical benefits.

3.2.3 Single-strain microbiota transplantation

Recent studies highlight the promising potential of single-strain microbiota transplantation in PD treatment through modulation of the MGBA. *Lactobacillus plantarum* PS128, a probiotic strain, has been shown to alleviate motor deficits in PD mice through multi-target mechanisms. Specifically, PS128 restores gut microbiota homeostasis, diminishes neuroinflammation via the microRNA-155-5p/Suppressor of Cytokine Signaling 1 pathway, inhibits the neurotoxic activation of microglia and astrocytes, alleviates oxidative stress damage, protects dopaminergic neurons, and ultimately mitigates neurodegeneration (Liao et al., 2020; Lee et al., 2023). Clinical trials further support its therapeutic efficacy, with PS128 supplementation demonstrating significant improvements in motor symptoms and quality of life in PD patients (Lu et al., 2021).

Additionally, other microbial strains have shown promise in the treatment of PD. For example, *Bifidobacterium breve* (CCFM1067, Bif11) and *Lacticaseibacillus rhamnosus* E9 demonstrate neuroprotective effects in PD mouse models by enhancing intestinal

barrier integrity and alleviating pathological progression (Li T. et al., 2022; Aktas et al., 2024; Valvaikar et al., 2024). Oral administration of *Clostridium butyricum* has been shown to restore colonic Glucagon-Like Peptide-1 (GLP-1) and G Protein-Coupled Receptor 41/43 levels, along with cerebral GLP-1 Receptor expression in PD mice, thereby mediating neuroprotection via the GLP-1/GLP-1 Receptor pathway (Sun J. et al., 2021).

These findings collectively emphasize the therapeutic potential of single-strain microbiota transplantation in mitigating PD progression. Future studies should aim to elucidate the molecular mechanisms underlying single-strain interventions and validate the long-term safety and therapeutic efficacy through rigorous clinical trials.

4 Discussion

In summary, the bidirectional regulation and communication through the MGBA provide an innovative framework for investigating the pathological processes underlying PD. There is much evidence now available linking gut microbiota dysbiosis, its metabolites, and PD initiation and progression. While characteristic alterations in the composition have been shown in PD, inconsistencies across studies suggest that a consensus on specific microbial alterations has yet to be reached. Nevertheless, FMT has demonstrated potential in alleviating clinical symptoms and delaying PD progression.

Despite the fact that several research studies have verified the short-term effectiveness and safety of FMT, the field remains in its early stages with limited clinical trials. Most existing research comprises cross-sectional comparisons between PD patients and healthy people, with limited follow-up investigations tracking long-term outcomes. The long-term safety and sustained effectiveness thus require further exploration.

In PD, the gut microbiota represents an emerging potential therapeutic target. However, to fully assess the clinical utility, future research should focus on (1) elucidating the molecular mechanisms underlying gut dysbiosis in PD; (2) conducting rigorous, high-quality clinical trials to validate the efficacy and safety of FMT; and (3) optimizing FMT protocols by determining the optimal transplantation routes, dosing regimens, and administration frequencies. The development of standardized treatment guidelines would facilitate the responsible translation of FMT into clinical practice. Provided that ongoing research continues to demonstrate both safety and efficacy, FMT may potentially emerge as an adjunctive approach in PD management.

Author contributions

MG: Conceptualization, Data curation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing. HG: Writing – original draft. YW: Funding acquisition, Supervision, Writing – review & editing. YX: Funding acquisition, Supervision, Writing – review & editing.

Funding

The author(s) declare that financial support was received for the research and/or publication of this article. This research is supported by the Youth Science Foundation of Shandong First Medical University (No. 202201-068) and Postdoctoral Innovation Project of Shandong Province (SDCX-ZG-202303095).

Acknowledgments

The authors are grateful to all subjects for participation in our study. Figure 1 (export ID: YWSAS475b5), Figure 2 (export ID: YTAPT1d1b1) were created by Figdraw.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare that no Gen AI was used in the creation of this manuscript.

Publisher's note

References

Aho, V. T. E., Houser, M. C., Pereira, P. A. B., Chang, J., Rudi, K., Paulin, L., et al. (2021). Relationships of gut microbiota, short-chain fatty acids, inflammation, and the gut barrier in Parkinson's disease. *Mol. Neurodegener.* 16:6. doi: 10.1186/s13024-021-00427-6

Aktas, B., Aslim, B., and Ozdemir, D. A. (2024). A neurotherapeutic approach with Lacticaseibacillus rhamnosus E9 on gut microbiota and intestinal barrier in MPTP-induced mouse model of Parkinson's disease. *Sci. Rep.* 14:15460. doi: 10.1038/s41598-024-65061-w

Armstrong, M. J., and Okun, M. S. (2020). Diagnosis and treatment of Parkinson disease. *JAMA* 323:548. doi: 10.1001/jama.2019.22360

Barichella, M., Severgnini, M., Cilia, R., Cassani, E., Bolliri, C., Caronni, S., et al. (2018). Unraveling gut microbiota in Parkinson's disease and atypical parkinsonism. *Mov. Disord.* 34, 396–405. doi: 10.1002/mds.27581

Batista, C. R. A., Gomes, G. F., Candelario-Jalil, E., Fiebich, B. L., and de Oliveira, A. C. P. (2019). Lipopolysaccharide-induced Neuroinflammation as a bridge to understand neurodegeneration. *Int. J. Mol. Sci.* 20:2293. doi: 10.3390/ijms20092293

Bhattarai, Y., Si, J., Pu, M., Ross, O. A., McLean, P. J., Till, L., et al. (2021). Role of gut microbiota in regulating gastrointestinal dysfunction and motor symptoms in a mouse model of Parkinson's disease. *Gut Microbes* 13:1866974. doi: 10.1080/19490976.2020.1866974

Bisaglia, M. (2022). Mediterranean diet and Parkinson's disease. *Int. J. Mol. Sci.* 24:42. doi: 10.3390/ijms24010042

Braak, H., de Vos, R. A. I., Bohl, J., and Del Tredici, K. (2006). Gastric α-synuclein immunoreactive inclusions in Meissner's and Auerbach's plexuses in cases staged for

Parkinson's disease-related brain pathology. *Neurosci. Lett.* 396, 67–72. doi: 10.1016/j.neulet.2005.11.012

Braniste, V., Al-Asmakh, M., Kowal, C., Anuar, F., Abbaspour, A., Tóth, M., et al. (2014). The gut microbiota influences blood-brain barrier permeability in mice. *Sci. Transl. Med.* 6:263ra158. doi: 10.1126/scitranslmed.3009759

Brown, G. C., Camacho, M., and Williams-Gray, C. H. (2023). The endotoxin hypothesis of Parkinson's disease. *Mov. Disord.* 38, 1143–1155. doi: 10.1002/mds.29432

Brudek, T., and van Laar, T. (2019). Inflammatory bowel diseases and Parkinson's disease. *J. Parkinsons Dis.* 9, S331–S344. doi: 10.3233/jpd-191729

Bullich, C., Keshavarzian, A., Garssen, J., Kraneveld, A., and Perez-Pardo, P. (2019). Gut vibes in Parkinson's disease: the microbiota-gut-brain Axis. *Movement Disor. Clin. Prac.* 6, 639–651. doi: 10.1002/mdc3.12840

Caradonna, E., Abate, F., Schiano, E., Paparella, F., Ferrara, F., Vanoli, E., et al. (2025). Trimethylamine-N-oxide (TMAO) as a rising-star metabolite: implications for human health. *Meta* 15:220. doi: 10.3390/metabo15040220

Caradonna, E., Nemni, R., Bifone, A., Gandolfo, P., Costantino, L., Giordano, L., et al. (2024). The brain–gut axis, an important player in Alzheimer and Parkinson disease: a narrative review. *J. Clin. Med.* 13:4130. doi: 10.3390/jcm13144130

Carlucci, C., Petrof, E. O., and Allen-Vercoe, E. (2016). Fecal microbiota-based therapeutics for recurrent *Clostridium difficile* infection, ulcerative colitis and obesity. *EBioMedicine* 13, 37–45. doi: 10.1016/j.ebiom.2016.09.029

Castro-Caldas, M., Carvalho, A. N., Rodrigues, E., Henderson, C. J., Wolf, C. R., Rodrigues, C. M. P., et al. (2012). Tauroursodeoxycholic acid prevents MPTP-induced dopaminergic cell death in a mouse model of Parkinson's disease. *Mol. Neurobiol.* 46, 475–486. doi: 10.1007/s12035-012-8295-4

Chen, S.-J., Chen, C.-C., Liao, H.-Y., Lin, Y.-T., Wu, Y.-W., Liou, J.-M., et al. (2022). Association of Fecal and Plasma Levels of short-chain fatty acids with gut microbiota and clinical severity in patients with Parkinson disease. *Neurology* 98, e848–e858. doi: 10.1212/wnl.0000000000013225

Chen, Q., Fan, Y., Zhang, B., Yan, C., Chen, Z., Wang, L., et al. (2023). Specific fungi associated with response to capsulized fecal microbiota transplantation in patients with active ulcerative colitis. *Front. Cell. Infect. Microbiol.* 12:1086885. doi: 10.3389/fcimb.2022.1086885

Cheng, Y., Tan, G., Zhu, Q., Wang, C., Ruan, G., Ying, S., et al. (2023). Efficacy of fecal microbiota transplantation in patients with Parkinson's disease: clinical trial results from a randomized, placebo-controlled design. *Gut Microbes* 15:2284247. doi: 10.1080/19490976.2023.2284247

Cilia, R., Piatti, M., Cereda, E., Bolliri, C., Caronni, S., Ferri, V., et al. (2021). Does gut microbiota influence the course of Parkinson's disease? A 3-year prospective exploratory study in de novo patients. *J. Parkinsons Dis.* 11, 159–170. doi: 10.3233/jpd-202297

Cirstea, M. S., Yu, A. C., Golz, E., Sundvick, K., Kliger, D., Radisavljevic, N., et al. (2020). Microbiota composition and metabolism are associated with gut function in Parkinson's disease. *Mov. Disord.* 35, 1208–1217. doi: 10.1002/mds.28052

Costa, H. N., Esteves, A. R., Empadinhas, N., and Cardoso, S. M. (2022). Parkinson's disease: a multisystem disorder. *Neurosci. Bull.* 39, 113–124. doi: 10.1007/s12264-022-00934-6

Cryan, J. F., and Dinan, T. G. (2012). Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. *Nat. Rev. Neurosci.* 13, 701–712. doi: 10.1038/nrn3346

Cui, J., Lin, Z., Tian, H., Yang, B., Zhao, D., Ye, C., et al. (2021). Long-term follow-up results of fecal microbiota transplantation for irritable bowel syndrome: a single-center, retrospective study. *Front. Med.* 8:710452. doi: 10.3389/fmed.2021.710452

Ding, M., Lang, Y., Shu, H., Shao, J., and Cui, L. (2021). Microbiota–gut–brain Axis and epilepsy: a review on mechanisms and potential therapeutics. *Front. Immunol.* 12:742449. doi: 10.3389/fimmu.2021.742449

Ding, Y., Xin, C., Zhang, C.-W., Lim, K.-L., Zhang, H., Fu, Z., et al. (2018). Natural molecules from Chinese herbs protecting against Parkinson's disease via anti-oxidative stress. *Front. Aging Neurosci.* 10:246. doi: 10.3389/fnagi.2018.00246

Dorsey, E. R., and Bloem, B. R. (2018). The Parkinson pandemic—a call to action. *JAMA Neurol.* 75, 9–10. doi: 10.1001/jamaneurol.2017.3299

Duan, W.-X., Wang, F., Liu, J.-Y., and Liu, C.-F. (2023). Relationship between short-chain fatty acids and Parkinson's disease: a review from pathology to clinic. *Neurosci. Bull.* 40, 500–516. doi: 10.1007/s12264-023-01123-9

DuPont, H. L., Suescun, J., Jiang, Z.-D., Brown, E. L., Essigmann, H. T., Alexander, A. S., et al. (2023). Fecal microbiota transplantation in Parkinson's disease—a randomized repeat-dose, placebo-controlled clinical pilot study. *Front. Neurol.* 14:1104759. doi: 10.3389/fneur.2023.1104759

Fang, X., Liu, S., Muhammad, B., Zheng, M., Ge, X., Xu, Y., et al. (2024). Gut microbiota dysbiosis contributes to α-synuclein-related pathology associated with C/EBPβ/AEP signaling activation in a mouse model of Parkinson's disease. *Neural Regen. Res.* 19, 2081–2088. doi: 10.4103/1673-5374.391191

Gao, X., Fu, S., Wen, J., Yan, A., Yang, S., Zhang, Y., et al. (2024). Orally administered Ginkgolide C alleviates MPTP-induced neurodegeneration by suppressing Neuroinflammation and oxidative stress through microbiota-gut-brain Axis in mice. *J. Agric. Food Chem.* 72, 22115–22131. doi: 10.1021/acs.jafc.4c03783

Gao, C., Li, B., He, Y., Huang, P., Du, J., He, G., et al. (2023). Early changes of fecal short-chain fatty acid levels in patients with mild cognitive impairments. *CNS Neurosci. Ther.* 29, 3657–3666. doi: 10.1111/cns.14252

Gao, K., Pi, Y., Mu, C. L., Peng, Y., Huang, Z., and Zhu, W. Y. (2018). Antibiotics-induced modulation of large intestinal microbiota altered aromatic amino acid profile and expression of neurotransmitters in the hypothalamus of piglets. *J. Neurochem.* 146, 219–234. doi: 10.1111/jnc.14333

Gorecki, A. M., Preskey, L., Bakeberg, M. C., Kenna, J. E., Gildenhuys, C., MacDougall, G., et al. (2019). Altered gut microbiome in Parkinson's disease and the influence of lipopolysaccharide in a human α-Synuclein over-expressing mouse model. *Front. Neurosci.* 13:839. doi: 10.3389/fnins.2019.00839

Hou, Y., Li, X., Liu, C., Zhang, M., Zhang, X., Ge, S., et al. (2021). Neuroprotective effects of short-chain fatty acids in MPTP induced mice model of Parkinson's disease. *Exp. Gerontol.* 150:111376. doi: 10.1016/j.exger.2021.111376

Hou, Y. F., Shan, C., Zhuang, S.-y., Zhuang, Q.-q., Ghosh, A., Zhu, K.-c., et al. (2021). Gut microbiota-derived propionate mediates the neuroprotective effect of osteocalcin in a mouse model of Parkinson's disease. *Microbiome* 9:34. doi: 10.1186/s40168-020-00988-6

Huang, H., Xu, H., Luo, Q., He, J., Li, M., Chen, H., et al. (2019). Fecal microbiota transplantation to treat Parkinson's disease with constipation. *Medicine* 98:e16163. doi: 10.1097/md.0000000000016163

Huh, E., Choi, J. G., Choi, Y., Ju, I. G., Kim, B., Shin, Y.-J., et al. (2023). *P. mirabilis*-derived pore-forming haemolysin, HpmA drives intestinal alpha-synuclein aggregation in a mouse model of neurodegeneration. *EBioMedicine* 98:104887. doi: 10.1016/j.ebiom.2023.104887

Keshavarzian, A., Green, S. J., Engen, P. A., Voigt, R. M., Naqib, A., Forsyth, C. B., et al. (2015). Colonic bacterial composition in Parkinson's disease. *Mov. Disord.* 30, 1351–1360. doi: 10.1002/mds.26307

Kesika, P., Suganthy, N., Sivamaruthi, B. S., and Chaiyasut, C. (2021). Role of gut-brain axis, gut microbial composition, and probiotic intervention in Alzheimer's disease. *Life Sci.* 264:118627. doi: 10.1016/j.lfs.2020.118627

Kim, S. M., DeFazio, J. R., Hyoju, S. K., Sangani, K., Keskey, R., Krezalek, M. A., et al. (2020a). Fecal microbiota transplant rescues mice from human pathogen mediated sepsis by restoring systemic immunity. *Nat. Commun.* 11:2354. doi: 10.1038/s41467-020-15545-w

Kim, S. M., Kim, Y., Choi, H., Kim, W., Park, S., Lee, D., et al. (2020b). Transfer of a healthy microbiota reduces amyloid and tau pathology in an Alzheimer's disease animal model. *Gut* 69, 283–294. doi: 10.1136/gutjnl-2018-317431

Kim, S., Kwon, S.-H., Kam, T.-I., Panicker, N., Karuppagounder, S. S., Lee, S., et al. (2019). Transneuronal propagation of pathologic α-Synuclein from the gut to the brain models Parkinson's disease. *Neuron* 103, 627–641.e7. doi: 10.1016/j.neuron.2019.05.035

Kiriyama, Y., and Nochi, H. (2023). Role of microbiota-modified bile acids in the regulation of intracellular organelles and neurodegenerative diseases. *Gene* 14:825. doi: 10.3390/genes14040825

König, J., Siebenhaar, A., Högenauer, C., Arkkila, P., Nieuwdorp, M., Norén, T., et al. (2016). Consensus report: faecal microbiota transfer – clinical applications and procedures. *Aliment. Pharmacol. Ther.* 45, 222–239. doi: 10.1111/apt.13868

Kuai, X., Yao, X., Xu, L., Zhou, Y., Zhang, L., Liu, Y., et al. (2021). Evaluation of fecal microbiota transplantation in Parkinson's disease patients with constipation. *Microb. Cell Factories* 20:98. doi: 10.1186/s12934-021-01589-0

Kujawa, D., Laczmanski, L., Budrewicz, S., Pokryszko-Dragan, A., and Podbielska, M. (2023). Targeting gut microbiota: new therapeutic opportunities in multiple sclerosis. *Gut Microbes* 15:2274126. doi: 10.1080/19490976.2023.2274126

Lajoie, A. C., Lafontaine, A.-L., and Kaminska, M. (2021). The Spectrum of sleep disorders in Parkinson disease. *Chest* 159, 818–827. doi: 10.1016/j.chest.2020.09.099

Lee, Y. Z., Cheng, S.-H., Chang, M.-Y., Lin, Y.-F., Wu, C.-C., and Tsai, Y.-C. (2023). Neuroprotective effects of *Lactobacillus plantarum* PS128 in a mouse model of Parkinson's disease: the role of gut microbiota and MicroRNAs. *Int. J. Mol. Sci.* 24:6794. doi: 10.3390/ijms24076794

Lee, Y., Kang, J. S., Ham, O.-J., Son, M.-Y., and Lee, M.-O. (2022). Gut metabolite trimethylamine N-oxide induces aging-associated phenotype of midbrain organoids for the induced pluripotent stem cell-based modeling of late-onset disease. *Front. Aging Neurosci.* 14:925227. doi: 10.3389/fnagi.2022.925227

Li, T., Chu, C., Yu, L., Zhai, Q., Wang, S., Zhao, J., et al. (2022). Neuroprotective effects of *Bifidobacterium breve* CCFM1067 in MPTP-induced mouse models of Parkinson's disease. *Nutrients* 14:4678. doi: 10.3390/nu14214678

Li, P., Killinger, B. A., Ensink, E., Beddows, I., Yilmaz, A., Lubben, N., et al. (2021). Gut microbiota Dysbiosis is associated with elevated bile acids in Parkinson's disease. *Meta* 11:29. doi: 10.3390/metabo11010029

Li, Z., Liang, H., Hu, Y., Lu, L., Zheng, C., Fan, Y., et al. (2022). Gut bacterial profiles in Parkinson's disease: a systematic review. *CNS Neurosci. Ther.* 29, 140–157. doi: 10.1111/cns.13990

Li, N., Tian, H., Chen, Q., Yang, B., Ma, C., Lin, Z., et al. (2019). Efficacy analysis of fecal microbiota transplantation in the treatment of 2010 patients with intestinal disorders. *Chin. J. Gastrointest. Surg.* 22, 861–868. doi: 10.3760/cma.j.issn.1671-0274.2019.09.011

Li, W., Wu, X., Hu, X., Wang, T., Liang, S., Duan, Y., et al. (2017). Structural changes of gut microbiota in Parkinson's disease and its correlation with clinical features. *Sci. China Life Sci.* 60, 1223–1233. doi: 10.1007/s11427-016-9001-4

Liang, D., Liu, H., Jin, R., Feng, R., Wang, J., Qin, C., et al. (2023). *Escherichia coli* triggers α-synuclein pathology in the LRRK2 transgenic mouse model of PD. *Gut Microbes* 15:2276296. doi: 10.1080/19490976.2023.2276296

Liao, J.-F., Cheng, Y.-F., You, S.-T., Kuo, W.-C., Huang, C.-W., Chiou, J.-J., et al. (2020). *Lactobacillus plantarum* PS128 alleviates neurodegenerative progression in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced mouse models of Parkinson's disease. *Brain Behav. Immun.* 90, 26–46. doi: 10.1016/j.bbi.2020.07.036

Lin, C.-H., Chen, C.-C., Chiang, H.-L., Liou, J.-M., Chang, C.-M., Lu, T.-P., et al. (2019). Altered gut microbiota and inflammatory cytokine responses in patients with Parkinson's disease. *J. Neuroinflammation* 16:129. doi: 10.1186/s12974-019-1528-y

Liu, J., Chen, Q., Yang, B., Wang, L., Lv, X., Li, N., et al. (2023). Fecal microbiota transplantation for constipation in patients with Parkinson disease: clinical efficacy observation. *Chinese J. Integrated Trad. Western Med. Digestion* 31, 874–878. doi: 10.3969/j.issn.1671-038X.2023.11.10

Lo, A. C., Callaerts-Vegh, Z., Nunes, A. F., Rodrigues, C. M. P., and D'Hooge, R. (2013). Tauroursodeoxycholic acid (TUDCA) supplementation prevents cognitive impairment and amyloid deposition in APP/PS1 mice. *Neurobiol. Dis.* 50, 21–29. doi: 10.1016/j.nbd.2012.09.003

Loh, J. S., Mak, W. Q., Tan, L. K. S., Ng, C. X., Chan, H. H., Yeow, S. H., et al. (2024). Microbiota–gut–brain axis and its therapeutic applications in neurodegenerative diseases. *Signal Transduct. Target. Ther.* 9:37. doi: 10.1038/s41392-024-01743-1

Lu, C.-S., Chang, H.-C., Weng, Y.-H., Chen, C.-C., Kuo, Y.-S., and Tsai, Y.-C. (2021). The add-on effect of *Lactobacillus plantarum* PS128 in patients with Parkinson's disease: a pilot study. *Front. Nutr.* 8:650053. doi: 10.3389/fnut.2021.650053

Lubomski, M., Tan, A. H., Lim, S.-Y., Holmes, A. J., Davis, R. L., and Sue, C. M. (2019). Parkinson's disease and the gastrointestinal microbiome. *J. Neurol.* 267, 2507–2523. doi: 10.1007/s00415-019-09320-1

Malpartida, A. B., Williamson, M., Narendra, D. P., Wade-Martins, R., and Ryan, B. J. (2021). Mitochondrial dysfunction and Mitophagy in Parkinson's disease: from mechanism to therapy. *Trends Biochem. Sci.* 46, 329–343. doi: 10.1016/j.tibs.2020.11.007

Mao, L., Zhang, Y., Tian, J., Sang, M., Zhang, G., Zhou, Y., et al. (2021). Cross-sectional study on the gut microbiome of Parkinson's disease patients in Central China. *Front. Microbiol.* 12:728479. doi: 10.3389/fmicb.2021.728479

Marogianni, C., Sokratous, M., Dardiotis, E., Hadjigeorgiou, G. M., Bogdanos, D., and Xiromerisiou, G. (2020). Neurodegeneration and inflammation—An interesting interplay in Parkinson's disease. *Int. J. Mol. Sci.* 21:8421. doi: 10.3390/ijms21228421

Matheson, J.-A. T., and Holsinger, R. M. D. (2023). The role of fecal microbiota transplantation in the treatment of neurodegenerative diseases: a review. *Int. J. Mol. Sci.* 24:1001. doi: 10.3390/ijms24021001

Mou, Y., Du, Y., Zhou, L., Yue, J., Hu, X., Liu, Y., et al. (2022). Gut microbiota interact with the brain through systemic chronic inflammation: implications on Neuroinflammation, neurodegeneration, and aging. *Front. Immunol.* 13:796288. doi: 10.3389/fimmu.2022.796288

Munteanu, C., Onose, G., Rotariu, M., Postaru, M., Turnea, M., and Galaction, A. I. (2024). Role of microbiota-derived hydrogen sulfide (H_2S) in modulating the gut-brain axis: implications for Alzheimer's and Parkinson's disease pathogenesis. *Biomedicine* 12:2670. doi: 10.3390/biomedicines12122670

Ni, Y., Tong, Q., Xu, M., Gu, J., and Ye, H. (2025). Gut microbiota-induced modulation of the central nervous system function in Parkinson's disease through the gut-brain Axis and short-chain fatty acids. *Mol. Neurobiol.* 62, 2480–2492. doi: 10.1007/s12035-024-04370-7

Nishiwaki, H., Hamaguchi, T., Ito, M., Ishida, T., Maeda, T., Kashihara, K., et al. (2020a). Short-chain fatty acid-producing gut microbiota is decreased in Parkinson's disease but not in rapid-eye-movement sleep behavior disorder. *mSystems* 5, 00797–00720. doi: 10.1128/mSystems.00797-20

Nishiwaki, H., Ito, M., Hamaguchi, T., Maeda, T., Kashihara, K., Tsuboi, Y., et al. (2022). Short chain fatty acids-producing and mucin-degrading intestinal bacteria predict the progression of early Parkinson's disease. *NPJ Parkinsons Dis.* 8:65. doi: 10.1038/s41531-022-00328-5

Nishiwaki, H., Ito, M., Ishida, T., Hamaguchi, T., Maeda, T., Kashihara, K., et al. (2020b). Meta-analysis of gut dysbiosis in Parkinson's disease. *Mov. Disord.* 35, 1626–1635. doi: 10.1002/mds.28119

Nurrahma, B. A., Yeh, T.-H., Hsieh, R.-H., Tsao, S.-P., Chen, C.-W., Lee, Y.-P., et al. (2022). Mangosteen pericarp extract supplementation boosts antioxidant status via rebuilding gut microbiota to attenuate motor deficit in 6-OHDA-induced Parkinson's disease. *Antioxidants* 11:2396. doi: 10.3390/antiox11122396

Qi, S., Yin, P., Wang, L., Qu, M., Kan, G. L., Zhang, H., et al. (2021). Prevalence of Parkinson's disease: a community-based study in China. *Mov. Disord.* 36, 2940–2944. doi: 10.1002/mds.28762

Rong, S., Xu, G., Liu, B., Sun, Y., Snetselaar, L. G., Wallace, R. B., et al. (2021). Trends in mortality from Parkinson disease in the United States, 1999–2019. *Neurology* 97, e1986–e1993. doi: 10.1212/wnl.0000000000012826

Saji, N., Saito, Y., Yamashita, T., Murotani, K., Tsuduki, T., Hisada, T., et al. (2022). Relationship between plasma lipopolysaccharides, gut microbiota, and dementia: a cross-sectional study. *J. Alzheimers Dis.* 86, 1947–1957. doi: 10.3233/jad-215653

Sampson, T. R., Debelius, J. W., Thron, T., Janssen, S., Shastri, G. G., Ilhan, Z. E., et al. (2016). Gut microbiota regulate motor deficits and Neuroinflammation in a model of Parkinson's disease. *Cell* 167, 1469–1480.e12. doi: 10.1016/j.cell.2016.11.018

Scheperjans, F., Aho, V., Pereira, P. A. B., Koskinen, K., Paulin, L., Pekkonen, E., et al. (2014). Gut microbiota are related to Parkinson's disease and clinical phenotype. *Mov. Disord.* 30, 350–358. doi: 10.1002/mds.26069

Segal, A., Zlotnik, Y., Moyal-Atias, K., Abuhasira, R., and Ifergane, G. (2021). Fecal microbiota transplant as a potential treatment for Parkinson's disease – a case series. *Clin. Neurol. Neurosurg.* 207:106791. doi: 10.1016/j.clineuro.2021.106791

Shandilya, S., Kumar, S., Kumar Jha, N., Kumar Kesari, K., and Ruokolainen, J. (2022). Interplay of gut microbiota and oxidative stress: perspective on neurodegeneration and neuroprotection. *J. Adv. Res.* 38, 223–244. doi: 10.1016/j.jare.2021.09.005

Sharma, V. D., Patel, M., and Miocinovic, S. (2020). Surgical treatment of Parkinson's disease: devices and lesion approaches. *Neurotherapeutics* 17, 1525–1538. doi: 10.1007/s13311-020-00939-x

Solanki, R., Karande, A., and Ranganathan, P. (2023). Emerging role of gut microbiota dysbiosis in neuroinflammation and neurodegeneration. *Front. Neurol.* 14:1149618. doi: 10.3389/fneur.2023.1149618

Sorboni, S. G., Moghaddam, H. S., Jafarzadeh-Esfehani, R., and Soleimanpour, S. (2022). A comprehensive review on the role of the gut microbiome in human neurological disorders. *Clin. Microbiol. Rev.* 35:e0033820. doi: 10.1128/cmr.00338-20

Sterling, K. G., Dodd, G. K., Alhamdi, S., Asimenios, P. G., Dagda, R. K., De Meirleir, K. L., et al. (2022). Mucosal immunity and the gut-microbiota-brain-Axis in Neuroimmune disease. *Int. J. Mol. Sci.* 23:13328. doi: 10.3390/ijms232113328

Strandwitz, P. (2018). Neurotransmitter modulation by the gut microbiota. *Brain Res.* 1693, 128–133. doi: 10.1016/j.brainres.2018.03.015

Suarez-Cedeno, G., Suescun, J., and Schiess, M. C. (2017). Earlier intervention with deep brain stimulation for Parkinson's disease. *Parkinson's Dis.* 2017, 1–9. doi: 10.1155/2017/9358153

Sun, N., Hu, H., Wang, F., Li, L., Zhu, W., Shen, Y., et al. (2021). Antibiotic-induced microbiome depletion in adult mice disrupts blood-brain barrier and facilitates brain infiltration of monocytes after bone-marrow transplantation. *Brain Behav. Immun.* 92, 102–114. doi: 10.1016/j.bbi.2020.11.032

Sun, J., Li, H., Jin, Y., Yu, J., Mao, S., Su, K.-P., et al. (2021). Probiotic *Clostridium butyricum* ameliorated motor deficits in a mouse model of Parkinson's disease via gut microbiota-GLP-1 pathway. *Brain Behav. Immun.* 91, 703–715. doi: 10.1016/j.bbi.2020.10.014

Sun, X., Xue, L., Wang, Z., and Xie, A. (2022). Update to the treatment of Parkinson's disease based on the gut-brain Axis mechanism. *Front. Neurosci.* 16:878239. doi: 10.3389/fnins.2022.878239

Sun, M., Zhu, Y., Zhou, Z., Jia, X., Xu, Y., Yang, Q., et al. (2018). Neuroprotective effects of fecal microbiota transplantation on MPTP-induced Parkinson's disease mice: gut microbiota, glial reaction and TLR4/TNF-α signaling pathway. *Brain Behav. Immun.* 70, 48–60. doi: 10.1016/j.bbi.2018.02.005

Tang, H.-d., Chen, S.-d., Liu, X.-l., Xie, R.-y., and Qian, X.-h. (2022). Mechanisms of short-chain fatty acids derived from gut microbiota in Alzheimer's disease. *Aging Dis.* 13, 1252–1266. doi: 10.14336/ad.2021.1215

The Lancet (2022). Parkinson's disease needs an urgent public health response. *Lancet Neurol.* 21:759. doi: 10.1016/s1474-4422(22)00312-x

Tofaris, G. K. (2022). Initiation and progression of α-synuclein pathology in Parkinson's disease. *Cell. Mol. Life Sci.* 79:210. doi: 10.1007/s00018-022-04240-2

Valvaikar, S., Vaidya, B., Sharma, S., Bishnoi, M., Kondepudi, K. K., and Sharma, S. S. (2024). Supplementation of probiotic *Bifidobacterium breve* Bif11 reverses neurobehavioural deficits, inflammatory changes and oxidative stress in Parkinson's disease model. *Neurochem. Int.* 174:105691. doi: 10.1016/j.neuint.2024.105691

van Kessel, S. P., Frye, A. K., El-Gendy, A. O., Castejon, M., Keshavarzian, A., van Dijk, G., et al. (2019). Gut bacterial tyrosine decarboxylases restrict levels of levodopa in the treatment of Parkinson's disease. *Nat. Commun.* 10:310. doi: 10.1038/s41467-019-08294-y

Varesi, A., Campagnoli, L. I. M., Fahmideh, F., Pierella, E., Romeo, M., Ricevuti, G., et al. (2022). The interplay between gut microbiota and Parkinson's disease: implications on diagnosis and treatment. *Int. J. Mol. Sci.* 23:12289. doi: 10.3390/ijms232012289

Vendrik, K. E. W., Ooijevaar, R. E., de Jong, P. R. C., Laman, J. D., van Oosten, B. W., van Hilten, J. J., et al. (2020). Fecal microbiota transplantation in neurological disorders. *Front. Cell. Infect. Microbiol.* 10:98. doi: 10.3389/fcimb.2020.00098

Vogt, N. M., Romano, K. A., Darst, B. F., Engelman, C. D., Johnson, S. C., Carlsson, C. M., et al. (2018). The gut microbiota-derived metabolite trimethylamine N-oxide is elevated in Alzheimer's disease. *Alzheimers Res. Ther.* 10:124. doi: 10.1186/s13195-018-0451-2

Voigt, R. M., Wang, Z., Brown, J. M., Engen, P. A., Naqib, A., Goetz, C. G., et al. (2022). Gut microbial metabolites in Parkinson's disease: association with lifestyle, disease characteristics, and treatment status. *Neurobiol. Dis.* 170:105780. doi: 10.1016/j.nbd.2022.105780

Wang, Y., Peng, L., Song, J., Bai, Y., Sun, Y., and Wang, F. (2020). The effects of fecal microbiota transplantation on chronic unpredictable mild stress induced depression model rats. *Chin. J. Clin. Psychol.* 28, 1083–1093. doi: 10.16128/j.cnki.1005-3611.2020.06.002

Wang, Y., Tong, Q., Ma, S.-R., Zhao, Z.-X., Pan, L.-B., Cong, L., et al. (2021). Oral berberine improves brain dopa/dopamine levels to ameliorate Parkinson's disease by regulating gut microbiota. *Signal Transduct. Target. Ther.* 6:77. doi: 10.1038/s41392-020-00456-5

Wang, L., Zhao, Z., Zhao, L., Zhao, Y., Yang, G., Wang, C., et al. (2022). *Lactobacillus plantarum* DP189 reduces α-SYN aggravation in MPTP-induced Parkinson's disease mice via regulating oxidative damage, inflammation, and gut microbiota disorder. *J. Agric. Food Chem.* 70, 1163–1173. doi: 10.1021/acs.jafc.1c07711

Weis, S., Schwiertz, A., Unger, M. M., Becker, A., Faßbender, K., Ratering, S., et al. (2019). Effect of Parkinson's disease and related medications on the composition of the fecal bacterial microbiota. *NPJ Parkinsons Dis.* 5:28. doi: 10.1038/s41531-019-0100-x

Wu, J., Li, C.-S., Huang, W.-Y., Zhou, S.-Y., Zhao, L.-P., Li, T., et al. (2025). Gut microbiota promote the propagation of pathologic α-syn from gut to brain in a gut-originated mouse model of Parkinson's disease. *Brain Behav. Immun.* 128, 152–169. doi: 10.1016/j.bbi.2025.04.001

Xie, Z., Zhang, M., Luo, Y., Jin, D., Guo, X., Yang, W., et al. (2023). Healthy human fecal microbiota transplantation into mice attenuates MPTP-induced neurotoxicity via AMPK/SOD2 pathway. *Aging Dis.* 14, 2193–2214. doi: 10.14336/ad.2023.0309

Xue, L., Ou, Z., Wang, L., Wei, M., Yang, X., Zheng, J., et al. (2019). A case analyse of Parkinson's disease treated with fecal microbiota transplantation instead of dopaminergic drugs. *J. Clin. Neurol.* 32, 329–332. doi: 10.3969/j.issn.1004-1648.2019.05.006

Xue, L., Yang, X., Tong, Q., Shen, P., Ma, S., Wu, S., et al. (2020). Fecal microbiota transplantation therapy for Parkinson's disease. *Medicine* 99:e22035. doi: 10.1097/md.0000000000022035

Yu, J., Meng, J., Qin, Z., Yu, Y., Liang, Y., Wang, Y., et al. (2023). Dysbiosis of gut microbiota inhibits NMNAT2 to promote neurobehavioral deficits and oxidative stress response in the 6-OHDA-lesioned rat model of Parkinson's disease. *J. Neuroinflammation* 20:117. doi: 10.1186/s12974-023-02782-1

Zhao, Z., Ning, J., Bao, X.-q., Shang, M., Ma, J., Li, G., et al. (2021). Fecal microbiota transplantation protects rotenone-induced Parkinson's disease mice via suppressing inflammation mediated by the lipopolysaccharide-TLR4 signaling pathway through the microbiota-gut-brain axis. *Microbiome* 9:226. doi: 10.1186/s40168-021-01107-9

Zheng, S., Li, H., Xu, R., Miao, W., Dai, M., Ding, S., et al. (2021). Potential roles of gut microbiota and microbial metabolites in Parkinson's disease. *Ageing Res. Rev.* 69:101347. doi: 10.1016/j.arr.2021.101347

Zhong, Z., Chen, W., Gao, H., Che, N., Xu, M., Yang, L., et al. (2021). Fecal microbiota transplantation exerts a protective role in MPTP-induced Parkinson's disease via the TLR4/PI3K/AKT/NF-κB pathway stimulated by α-Synuclein. *Neurochem. Res.* 46, 3050–3058. doi: 10.1007/s11064-021-03411-0

Zhu, W., Hu, Y., Shi, Y., Bao, H., Cheng, X., Jiang, M., et al. (2025). Sleep deprivation accelerates Parkinson's disease via modulating gut microbiota associated microglial activation and oxidative stress. *Microbiol. Res.* 293:128077. doi: 10.1016/j.micres.2025.128077

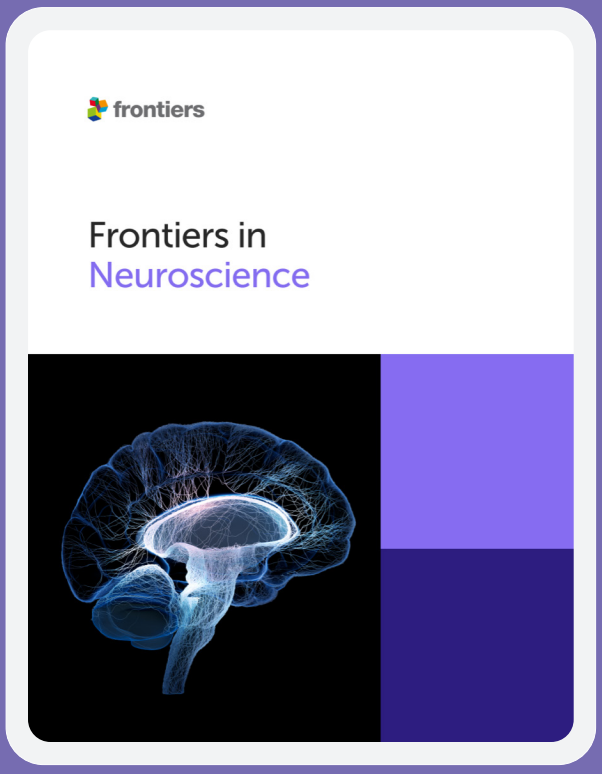
Frontiers in Neuroscience

Provides a holistic understanding of brain function from genes to behavior

Part of the most cited neuroscience journal series which explores the brain - from the new eras of causation and anatomical neurosciences to neuroeconomics and neuroenergetics.

Discover the latest Research Topics

See more →



Frontiers

Avenue du Tribunal-Fédéral 34
1005 Lausanne, Switzerland
frontiersin.org

Contact us

+41 (0)21 510 17 00
frontiersin.org/about/contact