De Remediis
ISSN: 3058-0358
Official Scientific Journal of Faculty of Pharmacy UD

Review

To be or not to be manipulated by our bacteria?

Marina Le Borgne [1,#], Kézia Sossou-Echavidre [2,#], Christine Janssen [3] and Marc Le Borgne [4,*]

[1] Faculté de Médecine Lyon-Est, Université Claude Bernard Lyon 1, Univ Lyon, 69373 Lyon, France
[2] Institut des Sciences Pharmaceutiques et Biologiques, Faculté de Pharmacie, Université Claude Bernard Lyon 1, Univ Lyon, 69373 Lyon, France
[3] Institut de Formation en Masso-Kinésithérapie pour Déficients de la Vue (IFMK DV), F-69373 Lyon, France. 0002-0430-5093 (C. Janssen)
[4] Small Molecules for Biological Targets Team, Centre de recherche en cancérologie de Lyon, Centre Léon Bérard, CNRS 5286, INSERM 1052, Université Claude Bernard Lyon 1, Univ Lyon, 69373 Lyon, France
* Correspondence: marc.le-borgne@univ-lyon1.fr;
# Contributed equally

Abstract

The gut microbiota has become a central research focus since its intricate connection with the brain was identified. Notably, the gut microbiota's influence on mental health opens new prospects for improving psychiatric disorder management. Understanding the bidirectional interactions between the brain, gut, and microbiome is crucial for evaluating the true impact of gut microbiota on mental health and its subsequent implications for psychiatry. Currently, the brain-gut-microbiome axis communicates through five interconnected pathways: the immune system, the vagus nerve, the enteric nervous system, the neuroendocrine system, and the circulatory system. The development of microbiota-based therapeutics signals significant changes in current clinical practices. Furthermore, microbiome-based therapeutics are expected to undergo substantial regulatory transformations in Europe. Thus, the competent European authorities wish to harmonize the regulatory environment concerning intestinal microbiota. From 2027, a new regulation will include intestinal microbiota among substances of human origin to clarify its status, which is currently disparate, being considered either a drug, a therapeutic intervention, or a biological product (tissue and cell). This mini-review aims to explore these aspects to evaluate the potential of gut bacteria to shape mental health interventions.

1. Introduction

An article published in 2013 by Carrie Arnold[1] recounted the story of a teenage daughter, suffering from obsessive-compulsive disorder (OCD) and attention-deficit hyperactivity disorder (ADHD), both classified as psychiatric disorders. Despite undergoing conventional treatments, the patient's condition showed no notable

improvement. A Boston-based psychiatrist then introduced an unconventional approach: targeting her gut health, specifically the balance of her intestinal microbiota. The gut microbiota comprises a complex ecosystem of microorganisms – including non-pathogenic bacteria, viruses, fungi, and parasites – that coexist in symbiosis with the human body. Recognizing the critical role of this microbiota, the psychiatrist prescribed probiotics to restore microbial balance. Remarkably, after a year of treatment, the patient experienced a complete disappearance of her symptoms of mental health disorders, such as intrusive thoughts and repetitive behaviors.

This case is a clear example of the profound connection between gut health and mental well-being. It underscores the need to deepen our understanding of the gut-brain axis, which continues to be a rapidly expanding area of research in neuroscience and microbiology.

We can then ask ourselves the following question: Do our gut bacteria manipulate us? As early as 2009, a study onrats laid the foundations for a link between stress and fecal microbiota. An increase in the production of fecal pellets was observed in maternally separated animals, a significant marker of an altered brain-gut axis[2]. Since then, numerous studies have reported important discoveries concerning the gut microbiota and the importance of its interactions with the brain in psychiatric disorders[3-4]. As **Figure 1** shows, interest in gut microbiota in relation to psychiatric disorders is relatively recent (1 article published in 2008), but is growing rapidly (286 articles published in 2024 alone).

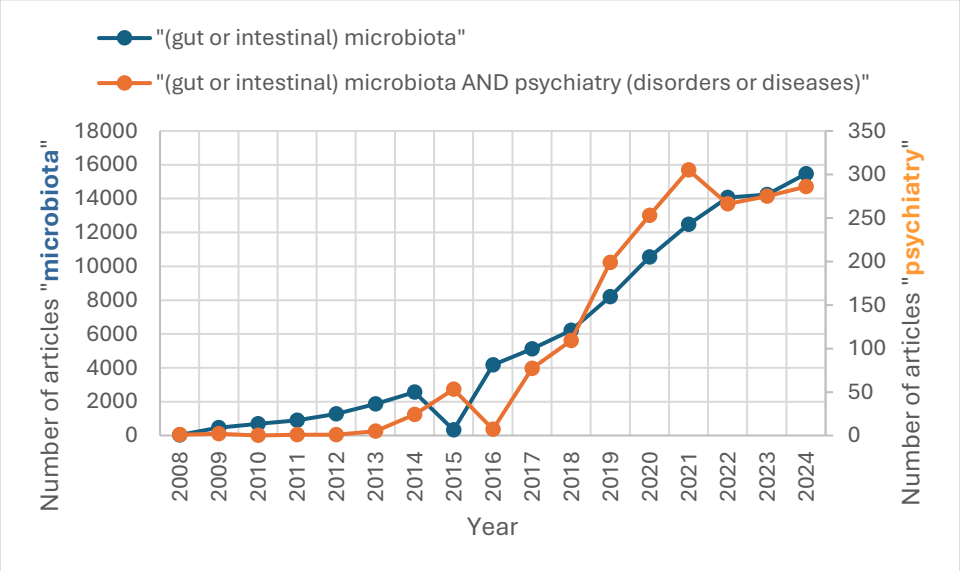

Figure 1: PubMed® queries on gut microbiota AND psychiatry (1 Jan. 2008 to 30 Nov. 2024).

This manuscript will explore the bidirectional communication between the gut microbiota and the brain, examine its influence on psychological and psychiatric conditions, and discuss potential interventions to enhance gut health for improved well-being. The final section will address the regulatory challenges surrounding microbiota-based therapies, emphasizing the necessity for harmonized legal frameworks to guide future developments.

2. How does the gut microbiota communicate with our brain?

To understand the mechanisms of the gut-brain connection, it is essential to first explore some key facts and characteristics of the gut and its microbiota as follows:

- Since birth, we have lived in symbiosis with our gut microbiota – a vast microbial world that contains an estimated 50,000 to 100,000 billion bacteria (50–100 trillion). These microorganisms collectively weigh approximately 1 to 2 kilograms in each individual, making the gut microbiota one of the most significant components of the human body in terms of mass and influence[5];
- The neurotransmitter serotonin is synthesized 90-95% in the gut. A neurotransmitter is a chemical substance that enables communication between our neurons, and in this case, serotonin is essential for regulating not only appetite and pain but also mood. We can thus already glimpse the crucial importance of the gut in modulating our emotional and mental well-being[6].
- The intestine has its own nervous system, known as the enteric nervous system. It contains up to 600 million neurons distributed along its length, enabling it to function independently to a significant extent. This is why it is often called the “second brain”[7].
- Approximately 70-80% of immune cells (e.g., macrophages, natural killer cells, T- and B- lymphocytes) reside in the gut, highlighting the complex interactions between the intestinal microbiota, the epithelial layer, and the local mucosal immune system. Beyond local immune responses, the gut microbiome is increasingly recognized for its impact on systemic immunity[8].

The intestine and its microbiota possess all the necessary components, such as neurotransmitters and neurons, to establish a direct connection with the brain.

Among the significant recent discoveries about the gut microbiota-brain axis, one stands out as particularly important. In 2022, different scientists from the “Institut Pasteur” (a partner research organization of Université Paris Cité), the “Institut National

de la Santé et de la Recherche Médicale" (Inserm), the "Centre National de la Recherche Scientifique" (CNRS) and the Institute of Clinical Molecular Biology (Kiel) discovered in mice that specific neurons in the hypothalamus, located at the center of the brain, are capable of detecting variations in gut microbiota activity[9-10]. Using medical imaging techniques in mice, the researchers obtained the following results:

- In the hypothalamus, certain neurons express Nod2 (Nucleotide Oligomerization Domain 2) receptors, which are capable of detecting muropeptides – cell wall fragments derived from intestinal bacteria. These muropeptides are by-products of the intestinal microbiota. This discovery is groundbreaking because it shows that neurons in the hypothalamus, a brain region crucial for regulating homeostasis, can directly sense molecules from intestinal bacteria. Traditionally, such detection has been considered the domain of immune system cells;
- In the presence of muropeptides, Nod2-expressing neurons exhibit reduced electrical signaling. Consequently, muropeptides suppress the activity of neurons responsible for regulating food intake and body temperature (physiological conditions);
- Conversely, when Nod2 receptors are defective (pathophysiology, genetic disorder), muropeptides can no longer suppress the activity of Nod2-expressing neurons, leading to a loss of brain regulation over food intake and body temperature. As a result, the mice gained weight, and older females exhibited the development of type 2 diabetes.

It is indeed fair to say that our gut microbiota and brain engage in continuous communication, influencing processes such as energy metabolism. According to the article published by the Institut Pasteur[10] and other bibliographical references[11-15], this communication primarily occurs through five interconnected pathways, the immune system (e.g., short-chain fatty acids, secondary bile acids), vague nerve system, enteric nervous system, neuroendocrine system (e.g., serotonin, dopamine) and circulatory system (e.g., metabolites like muropeptides) (**Figure 2**).

This multifaceted interaction underlines the profound influence of the gut microbiota on physical and mental well-being. Given that most human gut bacteria produce "feel-good chemicals" such as serotonin and dopamine, what insights do we have about the connections between gut microbiota and mental health?

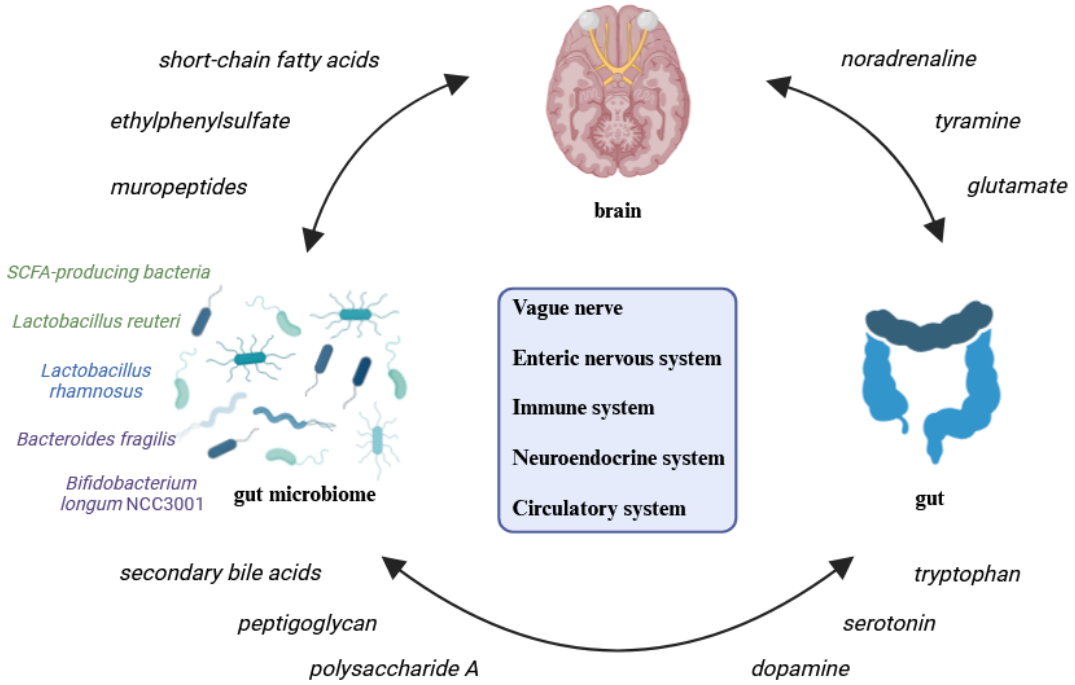

***Figure 2**:* Bidirectional interactions between brain, gut and microbiome (Created with BioRender.com).

3. In other words, can the gut microbiota affect our psychology?

Here are several studies and their findings that aim to address this question. A 2013 study by researchers at the University of Cork (Ireland) demonstrated that bacteria-free or “axenic” mice exhibited impaired social behavior, showing a preference for staying in an empty cage rather than interacting with other mice. However, inoculating their digestive tract to restore the microbiota successfully normalized their behavior[16].

In other studies, also conducted in young axenic mice, colonization with the microbiota of aged mice led to a decline in learning and memory abilities[17]. These findings highlight the significant role of the intestinal microbiota in the development and functioning of the brain.

In 2013, researchers at McMaster University (Canada) investigated the impact of gut bacteria on anxiety in mice. They found that transferring gut bacteria from anxious mice to adventurous mice induced anxiety in the latter, and vice versa[18].

Transferring the fecal microbiota from depressed patients to animals induces anxious and depressive behaviors in the recipients[17]. Other studies have suggested that variations in the gene encoding the Nod2 receptor may also contribute to psychiatric conditions such as anxiety and depression[10].

In 2019, researchers from the Catholic University of Leuven (Belgium) published a study in *Nature Microbiology* revealing that the gut microbiota of patients with depression lacked two specific bacteria, *Coprococcus* and *Dialister* spp. This finding highlights a clear correlation between gut microbiota composition and the mental health status of patients[19]. It is becoming clear that the systematic study of changes in the intestinal microbiota must be integrated into all work undertaken. For example, in the TwinsUK cohort study[20], il a été démontré a link between the abundance of the *Parabacteroides* genus and the diagnosis of mental disorders.

A search of the ClinicalTrials.gov online database of clinical research studies reveals the current interest in studying gut microbiota in relation to psychiatric disorders. Using the combined terms “psychiatric disorders, gut microbiota, recruiting, active, not recruiting studies”, over 130 studies are listed. This covers both adults and children and a wide range of psychiatric disorders (anxiety, depression, ADHD, and even beyond (anorexia nervosa, dementia, autism).

Overall, including both preclinical and clinical studies, understanding gut microbiota-brain interactions not only reveals how gut health impacts mental well-being but also offers insights into new therapeutic avenues for psychiatric disorders.

4. Could a pathology be treated by manipulating the gut microbiota?

Patients can be treated by modifying their intestinal microbiota through fecal transplantation, a technique that replaces their intestinal flora with that of a donor. It is currently used to cure patients suffering from chronic infections caused by *Clostridium difficile* bacteria[21].

In 2013, for the first time, a study carried out by American researchers showed in healthy women that consumption of a probiotic-containing fermented milk product (FMPP) for 4 weeks modified the activity of brain areas - insula, periaqueductal gray, somatosensory regions - involved in emotional control[22]. This study concluded that the activity of brain regions that control the central processing of emotions is affected. This study was financed by Danone, one of the world's leading food companies in dairy and plant-based products, specialized nutrition, and packaged waters. It is clear that the food industry is very interested in this quest for well-being through probiotic-based consumption.

Furthermore, as 70-80% of the immune system is located in the intestine (8), it is important to ensure a diet and lifestyle that will contribute to the health of the gut

microbiota. It is essential to reduce consumption of excess saturated fats (such as those in delicatessen and red meat) and pesticide-contaminated foods, focusing instead on incorporating more prebiotic-rich foods (e.g., chicory roots, Jerusalem artichokes, Dandelion greens) into our diets[23].

A new era is emerging with the development of probiotics, fecal transplants, prebiotics, and even psychobiotics[24] – a specialized category of probiotics designed to influence mental health by targeting the intestinal microbiota. Given the growing potential of microbiome-based therapeutics, it is essential to understand their current and future regulatory framework.

5. Regulatory framework for microbiome-based therapeutics

The heterogeneity of legal statuses worldwide complicates the harmonization of practicesrelated to fecal microbiota transplantation (FMT)[25]. This ambiguity results in a lack of a clear legal definition, creating uncertainties about the qualification of the products[26]. Furthermore, the industrialization of these treatments poses significant challenges in terms of standardizing procedures, characterizing microbial strains, and ensuring quality control.

However, the history of FMT dates back several centuries, with the first documented uses in China in the 4th century[27]. It is only in recent decades that FMT has gained popularity as a medical treatment, mainly due to research on the role of gut microbiota in human health.

Many countries, including the United States, Canada, the United Kingdom, and France, consider FMT as a drug, but there are also disagreements about whether it should be classified as a drug or a biologic drug[28-30]. This classification is justified by the FMT's use to treat diseases and the attribution of its therapeutic effects to the microorganisms it contains[30]. However, the complexity and variability of the intestinal microbiota make it difficult to apply traditional drug regulatory requirements.

The classification of FMT as a medical device is excluded under the European Medical Device Regulation (MDR 2017/745) because it involves the use of living biological material[29-31]. This exclusion also applies to cell therapies, which are defined as products containing cells or tissues that have been substantially manipulated[31].

Some experts advocate for classifying FMT as a transplant product[29-30]. They argue that FMT involves the transfer of living cells and their integration into the recipient's microbiota, similar to an organ transplant.

However, European legislation on tissues and cells (Directive 2004/23/EC) does not apply directly to FMT, as it focuses on human cells and tissues as active components, which is not the case for intestinal microbiota[31]. Nevertheless, countries like Hungary, Italy, and Belgium have decided to apply the Tissue and Cell Directives (**Table 1**)[32-33].

Other countries have adopted a differentiated approach based on certain criteria [29-31]. In Austria, the classification depends on the level of manipulation of the fecal material; an extemporaneously prepared FMT is more likely to be classified as a therapeutic intervention, while an FMT that undergoes multiple procedures before administration may be classified as a drug (**Table 1**). Although the therapeutic intervention does not have a precise regulatory definition, detailed recommendations have been developed by local scientific societies, with the goal of treating the patient through the act of fecal microbiota transplantation. These recommendations cover indications, administration methods, and donor selection criteria to provide a framework for this medical practice[34]. In Denmark, classification depends on the presence of a clinical indication. FMT with a clinical indication is considered as a drug, while FMT for treating dysbiosis in a hospital may be regulated as tissue (**Table 1**)[29]. In Australia, most products intended for FMT are considered biological products. However, if one or more strains of microorganisms naturally present in stools are identified, characterized, and cultivated from standardized isolates, these products can be considered as drugs. Finland, on the other hand, only classifies it as a therapeutic intervention (**Table 1**).

Country	Drug	Therapeutic intervention	Tissue and cell (biological product)
France	✔		
Hungary			✔
Austria	✔	✔	
Belgium			✔
Germany	✔		
Italy			✔
Denmark	✔		✔
Finland		✔	
Sweden	✔		
United Kingdom	✔		
United States of America	✔		
Australia	✔		✔
Canada	✔		

***Table 1**:* Regulatory classification of FMT.

The inclusion of intestinal microbiota in the upcoming regulation of substances of human origin (SoHO) could contribute to the harmonization of FMT regulation in Europe[31]. This regulation aims to establish a unified framework for all substances of human origin, including intestinal microbiota, and to clarify the definitions and requirements applicable to microbiota-derived products. This regulation will come into effect in 2027 for all EU Member States[35]. This new European regulation on substances of human origin, effective from 2027, seeks to strengthen the quality and safety standards for SoHO, including intestinal microbiota, and facilitate their cross-border movement within the EU[35-36]. It broadens the scope of regulation to cover all SoHO, including intestinal microbiota, and enhances oversight by competent authorities.

The regulatory status of intestinal microbiota remains an evolving issue. Harmonizing regulations at both the European and international levels is crucial to ensure safe and effective access to FMT, while also fostering research and development in this promising field. The ongoing evolution of FMT regulation is an area to watch closely, as it will significantly impact the availability and accessibility of this treatment in the future.

6. Conclusion

The answer to the question "Can our gut bacteria manipulate us?" is yes. Bacteria can influence not only our mental state but also processes like energy metabolism and beyond. Nevertheless, research into the gut microbiota requires further investigation to deepen our understanding of bidirectional interactions between brain, gut, and microbiome. Alongside the links between mental health and psychiatry, the gut microbiota is also being studied in the context of other disorders. For example, a June 2024 review synthesized current data around the role of gut microbiota in the context of mild cognitive impairment (MCI)[37]. MCI is often an early stage of certain neurodegenerative conditions like Alzheimer's disease. In the field of oncology, numerous studies have been carried out on the intestinal microbiota, and a study led by Prof. Laurence Zitvogel has demonstrated the importance of two immunogenic bacteria present in the intestinal microbiota in the sensitivity to treatment of metastatic melanoma with ipilimumab[38]. Once again, discoveries linked to the gut microbiota will lead to new therapeutic approaches.

To the question " Can we manipulate our intestinal bacteria?", the answer is also yes: we can introduce new strains to treat disease. Eligo Bioscience, a French company, has succeeded in modifying the DNA of intestinal bacteria in experiments with mice. This innovation opens the way to new targeted gene therapies for chronic diseases. However, it is also becoming urgent for the regulatory environment around microbiome-based therapeutics to be clearly harmonized in Europe and beyond[39]. Alongside genetic manipulation, an individual's diet plays a crucial role in their physical and mental health. Given the significant variability of the intestinal microbiota, it is essential to adopt a tailored approach to diet (e.g., Mediterranean, vegetarian, gluten-free, or ketogenic) to effectively influence it[40]. Numerous studies have also been carried out to measure the effective impact of prebiotics[41] and probiotics[41-42] in the context of dysbiosis of the gut microbiota. As mentioned above, the analysis of ongoing clinical trials will be crucial in the coming years to guide the rational use of microbiome-based therapeutics.

For a happy life, controlling the gut microbiota is essential. To live longer, the gut microbiota must also be preserved and enriched throughout life. A selection of major reviews on this topic is also available for further immersion in this fascinating subject [43-45].

Acknowledgments

Marc Le Borgne would like to thank "Institut Convergence" PLAsCAN (ANR-17-CONV-0002). Marina Le Borgne would like to thank "La Région Auvergne-Rhône-Alpes" for obtaining the 2024 Merit Scholarship.

Data Availability Statement:

Not applicable.

References

1. Arnold C. Gut feelings: the future of psychiatry may be inside your stomach [internet]. The Verge; 2013 [cited 2024 May 8]. Available from: https://www.theverge.com/2013/8/21/4595712/gut-feelings-the-future-of-psychiatry-may-be-inside-your-stomach.
2. O'Mahony SM, Marchesi JR, Scully P, Codling C, Ceolho AM, Quigley EM et al. Early life stress alters behavior, immunity, and microbiota in rats: implications for irritable bowel syndrome and psychiatric illnesses. Biol Psychiatry. 2009 Feb;65(3):263-7. doi: 10.1016/j.biopsych.2008.06.026.

3. Nikolova VL, Smith MRB, Hall LJ, Cleare AJ, Stone JM, Young AH. Perturbations in gut microbiota composition in psychiatric disorders: A review and meta-analysis. JAMA Psychiatry. 2021 Dec;78(12):1343-54. doi: 10.1001/jamapsychiatry.2021.2573.
4. Singh J, Vanlallawmzuali, Singh A, Biswal S, Zomuansangi R, Lalbiaktluangi C et al. Microbiota-brain axis: Exploring the role of gut microbiota in psychiatric disorders - A comprehensive review. Asian J Psychiatr. 2024 Jul;97:104068. doi: 10.1016/j.ajp.2024.104068.
5. Ferranti EP, Dunbar SB, Dunlop AL, Corwin EJ. 20 things you didn't know about the human gut microbiome. J Cardiovasc Nurs. 2014 Nov-Dec;29(6):479-81. doi: 10.1097/JCN.0000000000000166.
6. Chen Y, Xu J, Chen Y. Regulation of neurotransmitters by the gut microbiota and effects on cognition in neurological disorders. Nutrients. 2021 Jun;13(6):2099. doi: 10.3390/nu13062099.
7. Fleming MA 2nd, Ehsan L, Moore SR, Levin DE. The enteric nervous system and its emerging role as a therapeutic target. Gastroenterol Res Pract. 2020 Sep;2020:8024171. doi: 10.1155/2020/8024171.
8. Wiertsema SP, van Bergenhenegouwen J, Garssen J, Knippels LMJ. The interplay between the gut microbiome and the immune system in the context of infectious diseases throughout life and the role of nutrition in optimizing treatment strategies. Nutrients. 2021 Mar;13(3):886. doi: 10.3390/nu13030886.
9. Press release. Decoding a direct dialogue between the gut microbiota and the brain [internet]. Institut Pasteur; 2022 [cited 2024 Nov 12]. Available from: https://www.pasteur.fr/en/press-area/press-documents/decoding-direct-dialog-between-gut-microbiota-and-brain.
10. Gabanyi I, Lepousez G, Wheeler R, Vieites-Prado A, Nissant A, Wagner S et al. Bacterial sensing via neuronal Nod2 regulates appetite and body temperature. Science. 2022 Apr;376(6590):eabj3986. doi: 10.1126/science.abj3986.
11. Kasarello K, Cudnoch-Jedrzejewska A, Czarzasta K. Communication of gut microbiota and brain *via* immune and neuroendocrine signaling. Front Microbiol. 2023 Jan;14:1118529. doi: 10.3389/fmicb.2023.1118529.
12. Carabotti M, Scirocco A, Maselli MA, Severi C. The gut-brain axis: interactions between enteric microbiota, central and enteric nervous systems. Ann Gastroenterol. 2015 Apr-Jun;28(2):203-9.
13. Martin CR, Osadchiy V, Kalani A, Mayer EA. The brain-gut-microbiome axis. Cell Mol Gastroenterol Hepatol. 2018 Apr;6(2):133-48. doi: 10.1016/j.jcmgh.2018.04.003.
14. Cryan JF, O'Riordan KJ, Cowan CSM, Sandhu KV, Bastiaanssen TFS, Boehme M et al. The microbiota-gut-brain axis. Physiol Rev. 2019 Oct;99(4):1877-2013. doi: 10.1152/physrev.00018.2018.
15. Negi S, Das DK, Pahari S, Nadeem S, Agrewala JN. Potential role of gut microbiota in induction and regulation of innate immune memory. Front Immunol. 2019 Oct;10:2441. doi: 10.3389/fimmu.2019.02441.
16. Luczynski P, McVey Neufeld KA, Oriach CS, Clarke G, Dinan TG, Cryan JF. Growing up in a bubble: Using germ-free animals to assess the influence of the gut microbiota on brain and behavior. Int J Neuropsychopharmacol. 2016 Aug;19(8):pyw020. doi: 10.1093/ijnp/pyw020.

17. D'Amato A, Di Cesare Mannelli L, Lucarini E, Man AL, Le Gall G, Branca JJV et al. Faecal microbiota transplant from aged donor mice affects spatial learning and memory via modulating hippocampal synaptic plasticity- and neurotransmission-related proteins in young recipients. Microbiome. 2020 Oct;8(1):140. doi: 10.1186/s40168-020-00914-w.
18. Collins SM, Kassam Z, Bercik P. The adoptive transfer of behavioral phenotype via the intestinal microbiota: experimental evidence and clinical implications. Curr Opin Microbiol. 2013 Jun;16(3):240-5. doi: 10.1016/j.mib.2013.06.004.
19. Valles-Colomer M, Falony G, Darzi Y, Tigchelaar EF, Wang J, Tito RY et al. The neuroactive potential of the human gut microbiota in quality of life and depression. Nat Microbiol. 2019 Apr;4(4):623-32. doi: 10.1038/s41564-018-0337-x.
20. Delanote J, Correa Rojo A, Wells PM, Steves CJ, Ertaylan G. Systematic identification of the role of gut microbiota in mental disorders: a TwinsUK cohort study. Sci Rep. 2024 Feb;14(1):3626. doi: 10.1038/s41598-024-53929-w.
21. Gupta S, Allen-Vercoe E, Petrof EO. Fecal microbiota transplantation: in perspective. Therap Adv Gastroenterol. 2016 Mar;9(2):229-39. doi: 10.1177/1756283X15607414.
22. Tillisch K, Labus J, Kilpatrick L, Jiang Z, Stains J, Ebrat B et al. Consumption of fermented milk product with probiotic modulates brain activity. Gastroenterology. 2013 Jun;144(7):1394-401, 1401.e1-4. doi: 10.1053/j.gastro.2013.02.043.
23. Conlon MA, Bird AR. The impact of diet and lifestyle on gut microbiota and human health. Nutrients. 2014 Dec;7(1):17-44. doi: 10.3390/nu7010017.
24. Del Toro-Barbosa M, Hurtado-Romero A, Garcia-Amezquita LE, García-Cayuela T. Psychobiotics: Mechanisms of action, evaluation methods and effectiveness in applications with food products. Nutrients. 2020 Dec;12(12):3896. doi: 10.3390/nu12123896.
25. Nouguez E. Between medicine and food: inventing microbiota medicine [Internet]. Cogito; 2021 [cited 2024 Nov 25]. Available from: https://www.sciencespo.fr/research/cogito/home/entre-medicament-et-aliment-inventer-la-medecine-du-microbiote/.
26. PRI. Microbiome-based medicinal products [Internet]. Pharmabiotic Research Institute; 2022 [cited 2024 Nov 25]. Available from: https://www.pharmabiotic.org/microbiome-based-medicinal-products/.
27. De Groot PF, Frissen MN, de Clercq NC, Nieuwdorp M. Fecal microbiota transplantation in metabolic syndrome: History, present and future. Gut Microbes. 2017 May;8(3):253-67. doi: 10.1080/19490976.2017.1293224.
28. Thanush D, Venkatesh MP. Fecal microbiota transplantation: history, procedure and regulatory considerations. Presse Med. 2023 Dec;52(4):104204. doi: 10.1016/j.lpm.2023.104204.
29. EU-IN Horizon Scanning Report. Faecal Microbiota Transplantation, EMA/204935/2022 [Internet]. EMA; 2022 [cited 2024 Dec 4]. Available from: https://www.ema.europa.eu/en/committees/working-parties-other-groups/eu-innovation-network-eu.
30. Merrick B, Allen L, Masirah M Zain N, Forbes B, Shawcross DL, Goldenberg SD. Regulation, risk and safety of faecal microbiota transplant. Infect Prev Pract. 2020 Sep;2(3):100069. doi: 10.1016/j.infpip.2020.100069.

31. Desvignes C, Mavoungou L. The EU regulatory framework for microbiota transplantation: An Industry Perspective [Internet]. Voisin Consulting Life Sciences; 2022 [cited 2024 Dec 4]. Available from: https://voisinconsulting.com/resources/blog/eu-regulatory-framework-for-microbiota-transplants/.

32. de Stefano MC, Mazzanti B, Vespasiano F, Lombardini L, Cardillo M. The regulatory approach for faecal microbiota transplantation as treatment for *Clostridioides difficile* infection in Italy. Antibiotics (Basel). 2022 Apr;11(4):480. doi: 10.3390/antibiotics11040480.

33. Nagy GG, Tudlik Z, Gergely L, Kónya J, Orosi P, Rákóczi É, et al. Reconsidering the technical aspects and quality management background of faecal microbiota transplantation due to the novel coronavirus pandemic. Orv Hetil. 2020 Nov;161(44):1858-71. doi: 10.1556/650.2020.32023.

34. Kump PK, Krause R, Allerberger F, Högenauer C. Faecal microbiota transplantation--the Austrian approach. Clin Microbiol Infect. 2014 Nov;20(11):1106-11. doi: 10.1111/1469-0691.12801.

35. News. New European regulation on substances of human origin (SoHO) [Internet]. ANSM; 2024 [cited 2024 Nov 28]. Available from: https://ansm.sante.fr/actualites/nouvelle-reglementation-europeenne-sur-les-substances-dorigine-humaine-soho.

36. Press release. Council adopts new rules on substances of human origin [internet]. Council of the European Union; 2024 [cited 28 nov 2024]. Available from: https://www.consilium.europa.eu/en/press/press-releases/2024/05/27/council-adopts-new-rules-on-substances-of-human-origin/.

37. Gallo A, Martone AM, Liperoti R, Cipriani MC, Ibba F, Camilli S et al. Mild cognitive impairment and microbiota: what is known and future perspectives. Front Med (Lausanne). 2024 Jun;11:1410246. doi: 10.3389/fmed.2024.1410246.

38. Routy B, Le Chatelier E, Derosa L, Duong CPM, Alou MT, Daillère R et al. Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. Science. 2018 Jan;359(6371):91-7. doi: 10.1126/science.aan3706.

39. Brödel AK, Charpenay LH, Galtier M, Fuche FJ, Terrasse R, Poquet C et al. In situ targeted base editing of bacteria in the mouse gut. Nature. 2024 Aug;632(8026):877-84. doi: 10.1038/s41586-024-07681-w.

40. Rinninella E, Tohumcu E, Raoul P, Fiorani M, Cintoni M, Mele MC et al. The role of diet in shaping human gut microbiota. Best Pract Res Clin Gastroenterol. 2023 Feb-Mar;62-63:101828. doi: 10.1016/j.bpg.2023.101828.

41. Yoo S, Jung SC, Kwak K, Kim JS. The role of prebiotics in modulating gut microbiota: Implications for human health. Int J Mol Sci. 2024 Apr;25(9):4834. doi: 10.3390/ijms25094834.

42. Ansari F, Neshat M, Pourjafar H, Jafari SM, Samakkhah SA, Mirzakhani E. The role of probiotics and prebiotics in modulating of the gut-brain axis. Front Nutr. 2023 Jul;10:1173660. doi: 10.3389/fnut.2023.1173660.

43. Thursby E, Juge N. Introduction to the human gut microbiota. Biochem J. 2017 May;474(11):1823-36. doi: 10.1042/BCJ20160510.

44. Hou K, Wu ZX, Chen XY, Wang JQ, Zhang D, Xiao C et al. Microbiota in health and diseases. Signal Transduct Target Ther. 2022 Apr;7(1):135. doi: 10.1038/s41392-022-00974-4.

45. Kiousi DE, Kouroutzidou AZ, Neanidis K, Karavanis E, Matthaios D, Pappa A et al. The Role of the Gut Microbiome in Cancer Immunotherapy: Current Knowledge and Future Directions. Cancers (Basel). 2023 Mar;15(7):2101. doi: 10.3390/cancers15072101.