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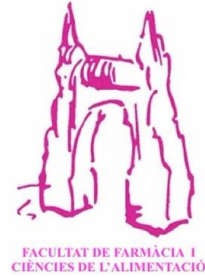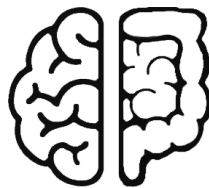
THE MICROBIOTA-GUT-BRAIN AXIS AND ITS ASSOCIATION WITH DEPRESSION

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Abbreviations

A collection of the most relevant abbreviations used in this work is provided below to ease its comprehension:

ANS: autonomic nervous system

APA: American Psychiatric Association

BBB: blood-brain barrier

BDNF: brain-derived neurotrophic factor

CNS: central nervous system

CRH: corticotrophin-releasing hormone

CUMS: chronic unpredictable mild stress

ECC: enterochromaffin cells

ENS: enteric nervous system

FAO: Food and Agriculture Organization

FMT: fecal microbiota transplantation

GALT: gut-associated lymphoid tissue

GB: gut-brain

GF: germ-free

HPA: hypothalamic–pituitary–adrenal

IBS: irritable bowel syndrome

IDO1: indoleamine 23-dioxygenase 1

LPS: lipopolysaccharides

MAO: monoamine oxidase

MDD: major depressive disorder

MGB: microbiota-gut-brain

NCHS: National Center for Health Statistics

PAMPs: pathogen-associated molecular patterns

PRRs: pattern recognition receptors

PVN: paraventricular nucleus

SCFA: short-chain fatty acids

SPF: specific-pathogen-free

SSRI: serotonin-selective reuptake inhibitor

TCA: tricyclic antidepressants

TDO: tryptophan-2,3-dioxygenase

WHO: World Health Organisation

1 Abstract

The gut microbiota is integrated by trillions of microorganisms that symbiotically interact with the host. During the last decade, researchers have focused on determining its role within the gut-brain axis and have suggested a link between their presence and mental disorders such as depression. The present bibliographic review gathers current knowledge on the microbiota-gut-brain axis, its relationship with depression and the use of microbiota as a potential therapeutic strategy. In this context, researchers have mainly used molecular tools to characterise the gut microbiota composition and animal models to understand the effects of a dysbiosis and establish its links with the development of human pathologies. *Firmicutes* and *Bacteroidetes* are the two main phyla present in our intestines. In addition, three enterotypes have been described in humans: *Bacteroides, Prevotella* and *Ruminococcus*. On another note, studies have proved the microbiota-gut-brain connection to be bidirectional, involving neural, endocrine and immunologic pathways. Current research on its contribution in depression is still at its infancy but it has recently been observed a correlation with the *Bacteroides* 2 enterotype in depressed patients and a decrease in *Dialister and Coprococcus* genera. Likewise, current knowledge on alternative therapies for depression based on microbiota interventions is limited but proposes some species from *Bifidobacterium* and *Lactobacillus* genera as well as *Faecalibacterium prausnitzii* as potential probiotic therapies. In conclusion, the microbiota-gut-brain axis is presented as a possible therapeutic target for depression but still requires further investigation.

Resum

La microbiota intestinal està formada per trilions de microorganismes que interaccionen de forma simbiòtica amb l'hoste. Durant l'última dècada, alguns científics han provat de determinar el seu rol dins l'eix intestí-cervell i n'han suggerit la relació amb malalties mentals com ara la depressió. En aquesta revisió bibliogràfica es recull l'actual coneixement sobre l'eix microbiota-intestí-cervell, la seva relació amb la depressió i l'ús de la microbiota com a estratègia terapèutica. En aquest context, s'ha caracteritzat la composició de la microbiota intestinal mitjançant tècniques moleculars. A part, l'ús de models animals ha permès entendre els efectes derivats d'una disbiosis i relacionar-la amb el desenvolupament de malalties. S'ha vist que els principals fílums que colonitzen l'intestí son *Firmicutes* i *Bacteroidetes*. També s'han descrit tres enterotips en humans: *Bacteroides, Prevotella* i *Ruminococcus*. Per altra banda, varis estudis han demostrat la bidireccionalitat de l'eix microbiota-intestí-cervell, que compren rutes neurològiques, endocrines i immunològiques. L'actual investigació sobre la contribució de l'eix en la depressió és encara molt incipient, tot i que recentment s'ha observat una correlació amb l'enterotip *Bacteroides* 2 en pacients deprimits i una reducció dels gèneres *Dialister* i *Coprococcus*. De forma similar, l'actual coneixement sobre teràpies alternatives a l'ús d'antidepressus basades en la microbiota és limitat. Tot i això, algunes espècies dels gèneres *Bifidobacterium* i *Lactobacillus* i l'espècie *Faecalibacterium prausnitzii* s'han proposat com a potencials teràpies probiòtiques. En conclusió, l'eix microbiota-intestí-cervell es presenta com una possible diana terapèutica en la depressió encara que requereix més investigació.

2 Integration of the different fields

One of the main purposes of this project is to offer an insight into the microbiota-gut-brain axis and its connexion to psychiatric diseases such as depression. To do so, an explanation of the different mechanisms that the gut microbiota uses to signal the brain is provided as well as a brief description of the disorder. Hence, this work is mainly framed into the *physiology and pathophysiology* field.

Moreover, the project is related to two additional fields that are represented to a lesser extent: *microbiology* and *pharmacology*. The first one is present at the beginning of the work, when it comes to understanding the characteristics of the human gut microbiota and defining its main functions and taxonomy. The study of the microbiota and its interactions with the host is important to understand the impact that gut microorganisms can have on the brain and on behaviour disorders. Nowadays, there is a general concern about the rise in the rates of depression along with the over-prescription of antidepressant drugs, which has pushed researchers to investigate alternative options to the classic medication. Therefore, at the end of the work, the current drug strategies for depression are discussed from a pharmacological viewpoint and compared to new proposed techniques based on altering the gut microbiota.

3   Introduction

There is a common expression that says the gut is our body's "second brain". Through this term, we allude to the enteric nervous system and indirectly we refer the constant communication that exists between the two distant organs. This connection has been observed since the early 19th century. One of the first to observe a connection between the gut and the brain was an army surgeon who associated mood alterations with changes in gastric secretions (1). From there, studies demonstrated how the brain could have influence over gut inflammation or chronic abdominal pain and how a stress response conditioned changes in the gut, shaping the connections of what we call the gut-brain (GB) axis.

The GB axis is as a communication network that combines neural, immunological and hormonal information circulating from the brain to the gut and vice versa. It intervenes in gastrointestinal processes such as the production of bicarbonate, acid, hormones and mucus as well as in the control of the gut motility. Thus, the main constituents of the axis are the central nervous system (CNS), the sympathetic and parasympathetic limbs of the autonomic nervous system (ANS), the enteric nervous system (ENS) and the endocrine and immune systems.

It was not until the past decade that experts started paying attention to the role that gut microbiota may have inside that complex system, given its strategic location and its bulky genetic contribution within the intestine. Therefore, the term microbiota-gut-brain axis (MGB axis) was coined and spread across the scientific community with the intention of contemplating our gut commensal community as a key participant in this communication system. The increasing attention to the trillions of bacteria, fungi and other microorganisms that inhabit in our gut led to the National Health Institute to start a project called Human Microbiome Project that, similarly to the Human Genome Project, aims to get a deeper understanding on the genetic material that resides in our gut and how it interferes with our health and diseases.

The link between the gut and the brain was initially studied for intestinal and metabolic diseases like irritable bowel syndrome (IBS), colorectal cancer, diabetes and obesity. However, a Canadian study from 2006 analysed a survey from the population of 1996-1997 and observed that IBS patients had three-fold the prevalence of depression than the regular population (2). This set off a growing body of evidence from the last decade suggesting the influence of the microbiota-gut-brain axis in shaping behavioural disorders such as anxiety or depression.

The 20th century is known in history as a period where multiple changes that affected the whole world were set in a short time. Two World Wars shook the entire population and contributed to the rise of nihilistic philosophies in Western societies. Concurrently to this scenario, a rise in the prevalence of depression started. But despite the pessimistic environment that surrounded last century and seemed to justify the increase of depressive disorders, new theories in neurology take the approach of the gut microbiota influence on the brain to find a complementary explanation to the general increase in depression. In 1989 the importance of the microbiota to our health was emphasized through the apparition of the "hygiene hypothesis". This theory

suggests that the lack of contact with microorganisms – accentuated by the 20th century migratory movements from rural areas to cities – has contributed to an increase of chronic inflammatory disorders like depression (3). Later, at the beginning of the new millennium, it was complemented by the "old friends hypothesis" by Rook *et al* (4),which proposes that "our old friends" (i.e. the microorganisms that have lived in our body for thousands of years) have been stimulating our T cells and that a deficient exposure to them may increase immunoregulatory diseases among the population.

Also, the general increase of hygiene during the 20th century as a consequence of the medical progress and collective consciousness of infectious diseases as well as public education in the topic was complemented with an increase in the use of antibiotics and a significant shift on dietary habits in Western cultures. All these changes pointed towards a restructuring of the habitat where the "old friends", that Rook *et al* mentioned in their study (4), live. And a growing body of evidence is pointing towards gut microbiota alterations taking part in mood-associated diseases. Therefore, it is believed that all these factors that contributed to change our gut microbiome may influence the rise in the prevalence of depression.

Recently, the National Center for Health Statistics (NCHS) of the United States detected that in a 15-year span (from 1999 to 2014) the use of antidepressant medications amidst the American population increased by 65% (7.7% - 12.7%) (Figure 1) (5). Yet, not all patients who were prescribed antidepressants were diagnosed with major depressive disorder (MDD) because they are also used in generalised anxiety disorder, bipolar disorder, panic attacks and obsessive-compulsive disorder. In addition, it is estimated that 30-40% of treated patients do not respond to these pharmacologic strategies (6). This has led to a general concern with the over-prescription of antidepressants that carries the consequences of their adverse effects and aggravates the problem of polymedication.

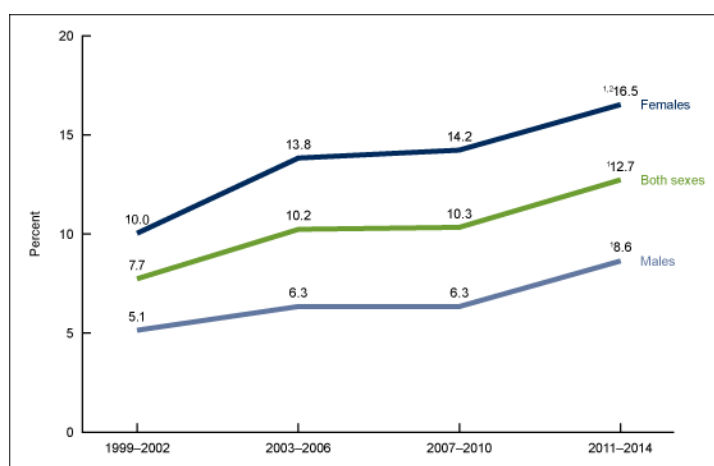**Figure 1.** Illustrative graphic of the antidepressant consumption within the American population from NCHS (5). It depicts the increased tendency of use at the beginning of the millennium.

As a result, it is important to consider new alternatives to the use of classic antidepressants and – as it will be examined in this work – researchers are currently investigating the gut microbiota as a possible therapeutic weapon.

4 Objectives

The principal aim of this project is to deeply examine the relation between the gut microbiota and the brain to later understand its role in mental disorders such as depression, gathering data from initial pioneer studies to the latest discoveries and present the current knowledge of the topic. Therefore, this work plans on answering the following questions to further understand the subject:

1. What do we currently know about the composition of human microbiota? And which tools do researchers use to examine it?
2. Which are the underlying mechanisms of the microbiota-gut-brain axis discovered so far? In addition, is there a connection between gut microbiota and depressive disorders?
3. How do traditional pharmacological therapies used to treat depression and anxiety work? Moreover, what do we know about new approaches such as the use of "psychobiotics"?

5 Materials and methods

This project falls in the category of bibliographical research. First, general physiologic and anatomic concepts have been consulted in essential books from the field. Then, a search using the terms "gut microbiota" "gut brain axis" "depression" "probiotics [AND] depression", "prebiotics [AND] depression", "FMT [AND] depression" and "diet [AND] gut brain axis" has been conducted in databases such as PubMed, Scopus, Nature Reviews, Science Direct, ClinicalTrials.gov and Google Scholar to find reviews on the subject and gather general information. Afterwards, a research on articles depicting the topic during the latest years (2009-2019) has been carried out considering that from the 1600 articles on the subject listed in PubMed, 99% are from the past ten years, so it is considered a recently explored issue.

Besides consulting the previous sources, information from organisations related to the field of inquiry has been used. It is the case of the NIH Human Microbiome Project, the American Psychiatric Association (AMA), the World Health Organisation (WHO) and the Food and Agriculture Organisation (FAO).

6 Results

This section exposes the bibliographical research carried out in this project. It starts by describing the gut microbiota and the techniques used to characterise it and to elucidate its relationship with the host (6.1 section). Later, the mechanisms that intervene in the microbiota-gut-brain connection are dissected (6.2 section), followed by a review of its association with depression (6.3 section). Finally, current pharmacological treatments are compared with proposed approaches based on microbiota modulations (6.4 section).

6.1 The gut microbiota

The human species has been carrying for centuries in its insides a whole ecosystem made of microorganisms. This microscopic community is formed by an estimation of 100 trillion bacteria, 80% of which resides in our gastrointestinal tract receiving the name of gut microbiota. And despite its genetic volume – encoding 150-fold the human genome – it has not been until the last decades when we have started giving it importance and considering the effects of this symbiotic relation (7,8).

The commensal ecosystem inhabiting our gut comprises more than 5000 strains of microorganisms and is mainly composed of bacteria (around 99% of gut microbial genes were found to be bacterial (9)), especially anaerobic. However, fungi, virus, protozoa and archaea are also part of the microbiome but in a lower proportion (10). Within all this diversity two dominant bacterial phyla arise in the adult human gut: *Bacteroidetes* and *Firmicutes*, accounting for 70-75% of the population with a *Firmicutes/Bacteroidetes* ratio of 10.9 in adults (11). They are accompanied in smaller proportions by *Proteobacteria*, *Fusobacteria*, *Actinobacteria* and *Verrucomicrobia* phyla (12).

Since the very beginning of life, there is a determinant contact with gut microbiota and, from there, it evolves dynamically. Due to the aseptic conditions of the uterus, children are microbial-free until birth. New-borns' initial microbiome is influenced by the birthing method: it conditions an exposition to their mother's vaginal and fecal bacteria mainly acquiring *Bifidobacteria* spp., *Prevotella* spp. and *Lactobacillus* spp. (in vaginally delivered babies) or to the maternal skin bacteria, rich in *Staphylococcus* spp. and *Corynebacterium* spp. (in the case of a caesarean section) (13). During the first two years, infants show a rather unstable and variable microbiota composition that can be influenced by the lactation method (natural or artificial). It is the introduction of solid food what adds diversity to their microbiome. So it's around the third year when they start building a more complex microbiome that resembles the adult one where the *Bacteroidetes* and *Firmicutes* genera gain importance (11). During adulthood, the gut microbial composition is characterised by rather stability compared to the extremes of life – infancy and old age – when changes are more dramatic. In addition, the general evolution of our microbiome through the lifespan is marked by our genetic predisposition and external factors such as antibiotic treatment, use of other drugs, infections, diet, stress, etc (8).

This means that despite the certain stability that exists in microbiota's composition during adulthood, there are inter-individual variations of around 90% between healthy individuals (14). In fact, in 2011 a study was conducted by Arumugam *et al* (15) to understand whether that variability allowed the classification of the population in clusters with similar gut microbiota profiles. It analysed the gut microbiota from individuals across the world (Europe, America and Japan) and identified three enterotypes. In other words, the study proposed the existence of three types of gut ecosystems amongst the population that favour the presence of determined bacteria genera. These enterotypes are: *Bacteroides* (*Bacteroidetes)* or enterotype 1, *Prevotella* (*Bacteroidetes*) or enterotype 2, and *Ruminococcus* (*Firmicutes*) or enterotype 3. As it is represented in Figure 2, each enterotype is named after the dominant taxa in the cluster. Other studies just distinguish two clusters as the *Ruminoccocus* enterotype appears more discreet and merged with the *Bacteroides* enterotype (16).
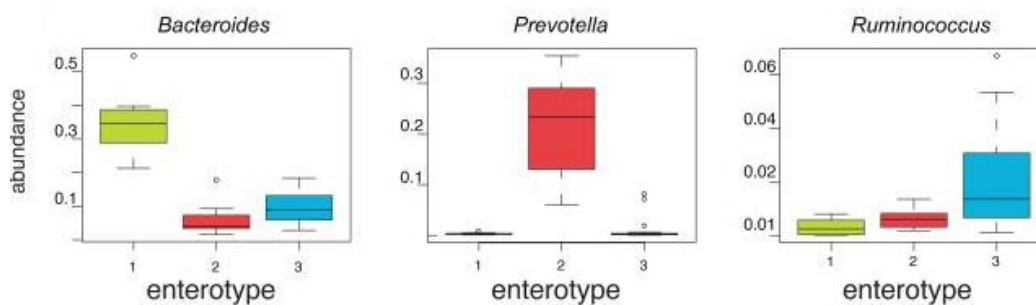

Figure 2. Boxplots obtained from Arumugam *et al* (15). Each graphic represents the abundance of the bacterial genera in each enterotype.

At the same time, it has been suggested that instead of focussing on the microbiota – i.e. the microorganism population – it would be interesting to examine our microbiome – i.e. the collection of microbiota's genetic material – to understand the functional aspects of the commensal community.

Following this line, a study of the Human Microbiome Project shows that there are bacterial metabolic processes that are preserved throughout different taxa (17). This implies that differences in microbiota taxa amongst the population don't necessarily translate into differences in the microbiota functionality, which means that different microbial taxa can perform comparable functions because their bacterial DNA codes for similar processes.

It has been observed that the gut microbiota has an important value for the correct functionality of the body: it participates in digestion (fermenting carbohydrates), it is part of the intestinal barrier, prevents pathogenic occupation, promotes mucus production and the regeneration of cells from gut epithelium, participates in the synthesis of neuroactive substances (like short-chain fatty acids) (18), and – as it will be further discussed in 6.2.1 – it plays a key role in the maturation of the immune system and the endocrine response thanks to their stimulation in the early stages of life.

However, an alteration of the gut microbiota composition creates a state known as dysbiosis. Studies on gut dysbiosis have found it to be involved in multiple diseases as it has been mentioned in section 3, including mental conditions that affect behaviour. As a result, scientists have used analytic tools and animal models to characterise gut

microbiota changes in disorders such as depression and to understand how these variations influence our health. The following section offers a closer look at the current methods used to study the microbiota.

6.1.1 Studying the gut microbiota

Our understanding of the human gut microbiota started with culture-based methods. Nevertheless, these techniques are slow and can't ensure the identification of all present bacteria as some can't grow in laboratory conditions. That is why since molecular biology methods were introduced in the 1990s, our knowledge of microbiota has exponentially grown.

Most of these culture-independent methods rely on DNA extraction from fecal samples and its following amplification of 16S rRNA genes. This is because 16S rRNA genes are enough preserved to assure their amplification but have slight differences that allow taxa identification. These methods are powerful enough to prove the diversity of gut microbiota and identify and quantify bacterial species. Some of the most used techniques are FISH (Fluorescence In Situ Hybridization), DGGE (Denaturing Gradient Gel Electrophoresis), next-generation sequencing of 16S rRNA gene, T-RFLP (Terminal Restriction Fragment Length Polymorphism) and DNA microarrays (19).

In addition, it is important to highlight that most studies on the relationship between the gut-brain axis and the microbiota take as target luminal bacteria from fecal samples, while the microbiota from the biofilm contiguous to the gut mucosa isn't as much considered as its analysis usually requires a more invasive procedure. Yet, future work on these structures will offer help on understanding the magnitude of their interactions with the host (20).

Up until now, techniques that allow a microbiome characterisation have been summarized. However, these methods don't provide us with causational information on the possible influence of microbiota in diseases. This issue can be approached from two sides:

On one hand, it can be explored through metagenomics, which study collective genomes – in this case from the whole bacterial community of the gut. Most studies on the effects of microbiota in health have focused on its taxonomic composition while there is a lack of data about the functional understanding of microbiota genomes. Thus, techniques such as microbiome shotgun sequencing provide information on both aspects and enable correlations between the status of the microbiota and disorders (19).

On another hand, understanding the influence of microbiota in mental diseases requires characterising the underlying mechanisms of the microbiota-brain connection. To do so, it's fundamental to develop a model that allows scientists to further explore the effects of gut microbiota to the host. In this context, the most important animal model in research has been germ-free (GF) mice.

Germ-free mice, as the word suggests, don't have any commensal microbiota. Therefore, the lack of microorganisms reveals the role they play in the development of

body functions. The also-called axenic mice are born through Caesarean sections and raised in sterile isolators with germ-free mothers (21). Despite its major importance in microbiota research, the model is limited when the objective is to see the effects of microbiota alterations. For this purpose, the following strategies are considered:

- One of the alternatives to the GF mice is the treatment with antibiotics – usually non-absorbable antibiotics – which creates a disruption in the microbiota community.

- Specific-pathogen-free (SPF) mice assure the absence of certain pathogens so its derived diseases don't interfere with the experiment.

- Another technique well used to study the microbiota and its interactions with the host is the introduction of external microbiota to a model. It has been applied as an exchange of gut microbiotas from two different rodent models (22) to observe changes in the receiver's behaviour associated to the donor's microbiota as it will be further explained in 6.2 but it also has been used to introduce human gut microbiota from patients suffering an investigated disease to GF and antibiotic-treated mice.

- In addition, probiotics are also used to understand the influence of gut microbiota on the host. For example, probiotics are administered to mice models with intestinal and central-nervous-system disorders to see if they can improve the symptoms. Results from these studies will be discussed in 6.4.2.1.

In order to study the effects of the microbiota in behavioural diseases, researchers have used anxious and depressive-like models to see how changes in their microbiome (germ-free state, microbiome transplants, use of probiotics, antibiotics, etc) modulate the symptoms. To recreate these animal models one of the strategies used for researchers is the maternal separation of the animal during the first weeks of life as it induces an anxiety and depressive-like phenotype in the adult along with changes in monoamine levels, the HPA axis and immune functions (23). Another frequently used model for depression is the chronic unpredictable mild stress (CUMS) model. In this case, rodents are chronically exposed to aleatory micro-stressors (water or food withdrawal, alteration of the light-darkness cycle, etc) (24).

Then, to measure the level of depression and anxiety of the rodents undergoing the experiments, researchers use a series of tests that put the animals under an aversive situation to assess their behaviour. The most used ones are the elevated plus maze, the open-field test, the light/dark test or stress induced hypothermia for evaluating anxious-like behaviours and the forced swimming test, the tail suspension test or the sucrose preference test to measure their depressive-like conduct (25).

Nowadays, despite having the mentioned tools, the translation of the animal model findings to the complexity of the human model is still in its early ages. However, animal models have provided most of the current understanding of the mechanisms that connect the gut microbiota with the brain, which are discussed in the next section.

6.2 Microbiota-gut-brain axis

As it has been introduced in section 3, the GB axis is a network that uses neural, humoral and immune routes to communicate and regulate our physiological functions. Recently, the influence of an additional element to the axis has been noticed, the commensal microscopic community that lives in our guts, which helps to shape the MGB axis.

The main characteristic of the MGB axis is its bidirectionality. On one hand, it is widely accepted that the CNS can induce changes in the intestinal tract (section 3). On the other hand, however, it's less acknowledged the influence that the gut environment may have on the brain. Yet, studies on the matter confirm the bottom-to-top pathway.

It is the case of the research portrayed by Bercik *et al* (22) mentioned in the last section which proved the transfer of psychological phenotypes through microbiota transplants (Figure 3). This study used two mice strains with differences in behaviour and in microbiota profiles. NIH Swiss mice tend to show a more anxious behaviour than BALB/c mice, but when germ-free NIH Swiss mice were colonized with microbiota from BALB/c rodents, they manifested a reduction in their anxious conduct – assessed through their exploratory behaviour – compared to the conventionally-housed NIH Swiss mice. Inversely, germ-free BALB/c mice experienced increased anxious behaviour when receiving the microbiota from the NIH Swiss strain.
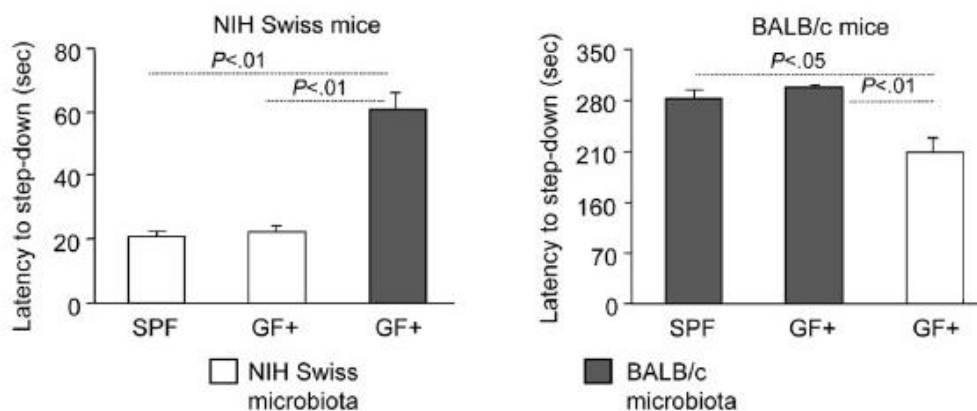
Figure 3. Graphics from Bercik *et al* (22)**.** They asses the behaviour from the different mice groups 3 weeks after microbiota colonisation. On the left, the three NIH Swiss groups are compared: the control (SPF mice) with the non-colonised germ-free (white GF +) and the BALB/c-colonised mice (grey GF +). On the right, the comparison is with the three BALB/c groups. The two graphics evidence the behavioural switch post-colonisation while the non-colonised groups resemble the controls.

This goes to show that alterations in the gut microbiome are likely to influence the communication between the gut and the brain. In this sense, the main question arises: what are the mechanisms used by gut microbiota to influence the CNS and vice versa?

6.2.1 Communication pathways

The strategies that bacteria use to reach the CNS involve direct and indirect circuits as it is illustrated in Figure 4. These include: signalling through neural afferent nerves (autonomic nervous system); triggering hormone secretion in the gut like serotonin released by enterochromaffin cells (endocrine system); stimulating intestinal immune mucosa to produce cytokines capable of influencing the brain (immune system) as well as synthesising products that can interact with the mentioned systems.
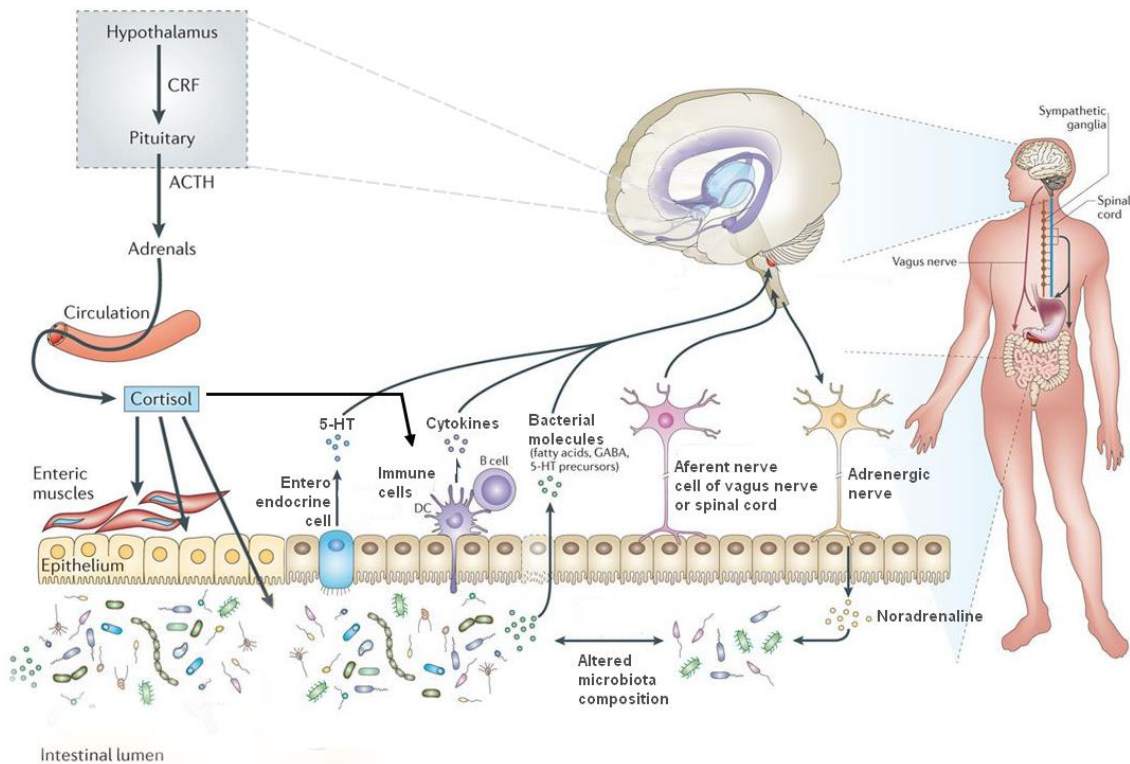

Figure 4. Schematic illustration of the elements that take part in the microbiota-gut-brain axis and their interconnections (modified from Cyan *et al* (26) and Collins *et al* (27)). Sympathetic and parasympathetic nerves innervate the gastrointestinal tract with efferent and afferent fibres. The efferent terminals transduce signals that induce changes in the microbiota environment, while the afferent ones constantly keep the brain informed about the intestinal state. Microbiota-derived metabolites can signal close endocrine cells or travel to the brain via the vagus nerve or the bloodstream. In addition, the permeability of the intestinal barrier eases gut bacterial translocation with a consequent immunologic response and an activation of the HPA axis, which simultaneously controls the inflammatory response.

This section aims to dissect individually the thus far discovered neurobiological pathways and communication systems aforesaid with the intention of helping to shed light upon the influence of microbiota on CNS functions.

6.2.1.1 Autonomic nervous system

One of the main connection pathways between the gut and the brain is the nervous system which, in a bidirectional manner, receives and sends information from and to the gut by the autonomic fibres.

The autonomic nervous system controls body functions through the sympathetic and parasympathetic branches as well as via the enteric nervous system.

On one hand, the sympathetic system decreases gut motility and secretion through the release of neurotransmitters such as noradrenaline. This system plays a major role in the stress response together with the HPA axis (as it will be further discussed in 6.2.1.2). Therefore, in stressful conditions, the sympathetic branch is activated and the signal reaches the intestinal mucosa through the greater splanchnic nerves. Nerve terminals stimulate enterochromaffin cells (ECC) from the epithelium. These cells respond to the signal by releasing norepinephrine into the lumen where the prokaryote community inhabits. Catecholamines and hormones released in stressful situations are known to alter gene expression in some bacteria as well as their conjugative communication system, favouring the presence of some species – especially pathogenic ones – at the expense of others (28). This is one of the multiple mechanisms used by the brain to modulate the gut environment. Moreover, the reduction in gut motility and mucus secretion alters the bacterial habitat and also influences the microbial population.

However, most of the area of the gastrointestinal system is innervated by the parasympathetic limb, especially the vagus nerve. The vagus nerve is also known as the 10th cranial nerve, referring to its origin in the brain. Moreover, its Latin-derived name means "wandering", describing its path from the *medulla oblongata* descending to innervate the larynx, lungs and heart until the abdominal viscera, covering the majority of the organs. Its main actions in the gut are increasing gastrointestinal peristalsis and secretions, counteracting the sympathetic effect and influencing the microbiota and its environment (29).

Vagus ending nerves reach the *lamina propria* from the gut, crossing all intestinal layers except the epithelium barrier. Therefore, the parasympathetic innervation lays close to the whole microbiome that lives inside the intestinal lumen. Moreover, 80% of its fibres are afferents, so there is an important volume of information about the gastrointestinal state being integrated into the brain constantly (30).

On another note, the ENS also has to be taken into account when thinking about the neurologic connection paths between the microbiota and the CNS. It is integrated by around 500 million neurons divided into two types of ganglia: the submucosal (Meissner) and the myenteric (Auerbach) plexuses. The first one is located in the submucosa – as its name indicates – while the second one resides between the circular and longitudinal layers of the muscularis. The ENS can operate independently from the sympathetic and parasympathetic branches, although it is able to exchange information with them (29).

Both vagal and enteric afferent ends don't reach the lumen directly because they don't go across the epithelium but are placed close, in the *lamina propria*. Therefore, lumen-residing microorganisms can get in contact with these sensory terminals when gut permeability increases (in stressful or inflammatory situations) or when epithelial cells from the intestine (such as EEC) receive microbiota inputs and transduce the bacterial signal to the afferent neuronal terminals located within the *lamina propria* via paracrine mediators such as cholecystokinin, histamine, CRH and serotonin. From there, the

signal is transferred via vagus nerve to the brain (31). Below a schematic representation of the three nervous pathways is provided (Figure 5):
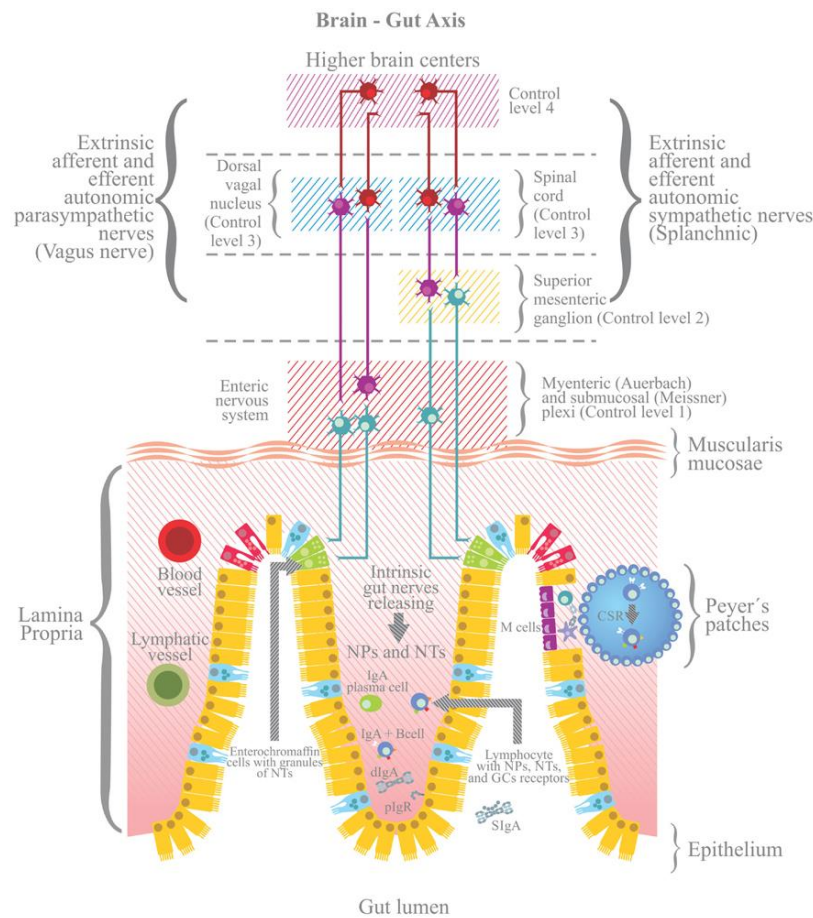

Figure 5. Representation of the sympathetic and parasympathetic pathways from the brain to the gut mucosa, where they convey with the enteric innervations from the myenteric and submucosal plexi. Image obtained from Campos-Rodríguez *et al* (32).

Prove of the ascendant pathway is that GF mice show less excitability of the vagal synaptic ends than mice models with microbiota presence (like SPF) and that the signal from GF mice sensory neurons increases after colonising them with SPF-mice microbiota (33). So as it has been observed, the activity of some microbial species and many probiotics over brain function depends on the activation of vagal afferents (34). However, Bercik *et al* (22) saw that vagotomised animals still had microbiota-derived effects on brain and behaviour, proving the existence of vagus-independent mechanisms of interaction between microbiota and the brain as it will be discussed below.

6.2.1.2 HPA axis

The HPA axis is a complex system of communications subjected to feedback between the paraventricular nucleus (PVN) of the hypothalamus, the pituitary gland and the adrenal cortex. This neuroendocrine system is involved in homeostasis and regulates essential processes like digestion, immune system, emotions, energetic metabolism,

sexual conduct as well as controlling the response to stress. This last point is important in the neurobiology of emotional conditions as anxiety or depression.

Following a stressful state, there's a release of corticotropin-release hormone (CRH) from the PVN area of the hypothalamus. The CRH descends through the hypophyseal stalk to the anterior pituitary gland and stimulates the secretion of corticotropin (ACTH) which enters the bloodstream and targets the cortex of the suprarenal glands to produce glucocorticoid hormones (especially cortisol). One of its major functions is to adapt the organism to the stressful situation by increasing glycaemic levels, inhibiting insulin in order to avoid glucose storage and potentiate its immediate use and, amongst other adaptations, inhibiting the production of IL-2, INF-γ, INF-α and TNF-α in order to prevent inflammation. When cortisol levels are high, the molecule gives a negative feedback to the hypothalamus and pituitary gland by binding to glucocorticoid receptors of the PNV, the hippocampus and the pituitary gland and inhibiting them in a self-regulatory manner (29). However, in a situation of chronic stress, high levels of cortisol are sustained, inducing an alteration in the HPA axis that has been linked to psychopathologies like anxiety and major depressive disorder (MDD).

In this neuroendocrine context, the gut microbiota has a bi-directional relationship. The currently proposed mechanisms for the interaction are the following:

Stress-induced cortisol release can modulate gut motility as well as the secretion of luminal mucus and therefore affect the microbial community residing inside (35). Following this line, cortisol may induce changes in the gene expression profiles of gut microbiota as it has been documented in the oral cavity (36). Moreover, it can also affect the integrity of the gut epithelium by weakening tight junctions from the intestinal barrier and reducing the expression of tight junction protein 2 in the colon (37), leading to a situation known as "leaky gut", that eases the translocation of gut microbial content through the enteric barrier (activating the immune system as it is further explained in section 6.2.1.3).

In practice, experiments performed in animals have illustrated the alteration in the commensal bacteria community in early ages as well as in adult stages when put under stressful environments. For example, in an early study conducted by Bailey *et al* (38), Rhesus monkeys that suffered maternal separation at 6-9 months of age – understood as a stress-triggering procedure – already showed a decrease in *Lactobacillus* spp. and 3 days after the separation. Furthermore, initial levels were normalised after 7 days, confirming the association of the dysbiosis to the stressful situation. In a similar manner, the exposure of adult mice to chronic stress resulted in an alteration of microbiota characterised by a decrease in the *Bacteroides* spp. population and an increase in the *Clostridium* spp. community (39).

Moreover, the communication pathway between gut microbiota and the HPA axis has also been suggested to work in the opposite direction, i.e. from the gut to the brain.

One suggested mechanism of action for the bottom-up pathway is an indirect route derived from a leaky gut state. An increase in the permeability of the enteric barrier allows bacteria to reach the immune mucosal immune system, stimulating the secretion of proinflammatory cytokines that can pass through the blood-brain barrier (BBB) and

activate a neuroendocrine response by the HPA axis in order to control the inflammatory response (40).

In addition, studies have proved that commensal bacteria such as C*ampylobacter jejuni* or *Citrobacter rodentium* can activate stress pathways through vagal activation. Evidence showed that in vagal sensory neurons there was an induction of cFOS – a marker for neuronal activation – after infection with *C.jejuni*, in the absence of immune response (34). These findings were complemented with other studies that noticed cFOS activation in the brain after administrating *C.rodentium* (41), feeding GF mice with probiotics (*Bifidobacterium infantis*) or with a mutated *E.coli* strain (42). These results suggest the implication of not only immune but also neural circuits in the microbiota-derived HPA activation.

Over a decade ago Sudo *et al* (42) observed that GF mice responded with a magnified release of ADCH and corticosterone in comparison with SPF mice (controls) when put under stress conditions (Figure 6). Nevertheless, their anxiety-like behaviour was minimized. Such response was reversed after GF mice received a fecal transplant from control animals or were fed with the probiotic *B. infantis*. In contrast, when associating it with an enteropathogenic strain of *E.coli* the enhanced response to stress was potentiated. In addition, the study noticed that the reversibility of the abnormal response was greater when the colonisation occurred earlier in the life of the animals whereas after 8 weeks it was irreversible. The investigation by Sudo *et al* (42) was replicated in other works, also using probiotics and obtaining positive outcomes (43). Thus, the experiment has been translated to human healthy subjects, who showed a reduced salivary cortisol awakening response after being fed with prebiotics for 3 weeks (44).
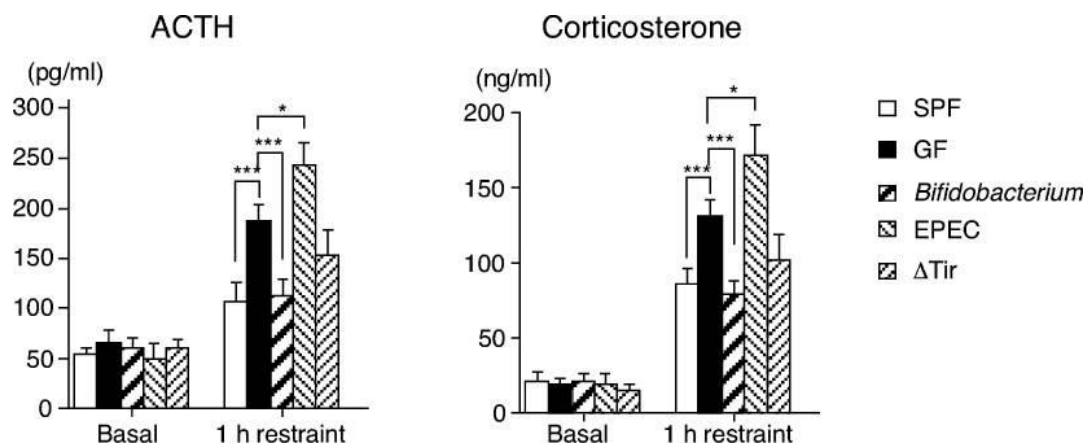
Figure 6. Graphics obtained from the study by Sudo *et al* (42) proving the rise in the stress-triggered HPA response from GF mice, which is reversed with the administration of the probiotic *B. infantis*.

Therefore, such findings indicate that the stress response can be modulated through gut bacteria, which either amplify or reduce the HPA axis setting the start to further studies on the matter. At the same time, they prove that the intestinal microbiota acts as a crucial factor in the maturity and correct function of the HPA axis, highlighting the importance for the colonisation of external microbiota to happen within a concrete time frame in life – early life – to determine the correct development of the neuroendocrine axis.

6.2.1.3 Immune system

Another system that plays a role in the MGB axis is the immune system as it has been mentioned while discussing the HPA axis. Our digestive tract has the largest mucosal surface of the entire body, comparable to a badminton court (45), which hosts the greatest lymphoid tissue of the organism, the gut-associated-lymphoid tissue (GALT). It is in this environment where our microbiota lives and given the magnitude of the epithelium, the contact area between the prokaryote and the body-constitutive eukaryote cells must be taken into account.

In physiological conditions, enterocytes constitute a layer of cells, closely fixed to each other by thigh junctions. If gut microorganisms or its structural molecules such as peptidoglycan monomers or lipopolysaccharides (LPS) get to cross the gut wall and access immune and neuronal cells from ENS, they will trigger an innate response from the immune system of the local mucosa. These pro-inflammatory structures are recognised as pathogen-associated molecular patterns (PAMPs) and thus bind to pattern recognition receptors (PRRs) and toll-like receptors (TLRs) expressed by immune cells unleashing a cytokine-mediated defensive response.

In physiological conditions, a low-grade and constant stimulation of the gut immune system by commensal bacteria determines its maturation, enables its dynamic education and improves its function. A study proved that the immune activity of GF mice was almost non-existent, but it could be generated after receiving microbiota (reviewed in 39). In fact, GF mice have been characterised by the thinner structure of their intestinal wall (with reduced Peyer's patches and *lamina propria*), decreased intestinal levels of CD8+ T cells, CD4+ T cells and Ig A (47) along with limited expression of intestinal TLR (48).

However, this controlled immune response can be disrupted with an excessive or chronic translocation of lumen microbiota or its molecules through the gut wall as a result of the aforementioned "leaky gut" phenomenon eased by chronic stress (explained in 6.2.1.2 section).

The PAMP-PRR contact induces a local release of proinflammatory cytokines (e.g.: IL-6, IL-1, IL-10 and TNFα) which can reach the brain directly or indirectly. The direct humoral response is facilitated by the pathologic existence of permeable regions in the blood-brain barrier. The second case happens when these compounds interact with cytokine receptors expressed by vagal afferents and activate these ascendant neurons (40).

As it has been discussed in the previous section (6.2.1.2), there is a bidirectional influence between the HPA axis and the immune system. In this case, the inflammatory state derived from microbiota stimulation can activate the HPA axis, increasing the cortisol secretion and creating a positive feedback situation for the leaky gut that could exacerbate the condition and worsen microbiota's environment.

6.2.1.4 Neurotransmitters and neuropeptides

Several signalling molecules been identified which may enable a communication between the gut microbiome and the host's ENS and brain. These substances are secreted by gut microbes and include short chain fatty acids (SCFA), bile acid metabolites, neuroactive molecules such as catecholamines (dopamine, noradrenaline), acetylcholine, GABA, serotonin and tryptophan precursors (49). For example, *Lactobacillus* spp. and *Bifidobacterium* spp. produce GABA; *Escherichia* spp., *Streptococcus* spp., *Enterococcus* spp., and *Candida* spp. are associated with the production of serotonin; *Lactobacillus* spp. synthesises acetylcholine; *Bacillus* spp., *Escherichia* spp. and *Saccharomyces* spp. produce noradrenaline; and *Bacillus* spp. produces dopamine (reviewed in 44). In an indirect way, we could also consider the cytokines released by the host's immune system in response to gut microbes.

Therefore, all the above-mentioned compounds can signal to the brain by binding to gut cell receptors or through neural afferents. Not only can microbiota produce some of the neurotransmitters, but they can also alter the endogenous production by modifying its expression or the receptors' expression. In fact, recent studies with GF mice observed a reduction of brain-derived neurotrophic factor (BDNF) in the brain of the animals as well as changes in the expression of NMDA, GABA receptor A and B subunits, serotonin 1A and tryptophan. In some cases, changes in the expression of these receptors were also associated with behaviour alterations (reviewed in 45).

This section examines the currently most relevant molecules produced or modulated by gut microbiota in order to place them within the MGB scheme.

- **Serotonin and tryptophan**

95% of the body's serotonin is synthesized within the gut, mainly by ECC, while the rest resides in the CNS. This neurotransmitter is linked to a vast range of functions, from regulation of intestinal transit and uptake (peripherally) to modulating mood and cognition (in the CNS). This work focuses on the latest as this molecule is one of the most important targets of depression disorder therapies.

Serotonin's precursor is the essential amino acid tryptophan. As it is illustrated in Figure 7, tryptophan can endure a transformation to serotonin that requires a hydroxylation and a subsequent decarboxylation of the amino acid or be metabolised through the kynurenine pathway which is dependent of two rate-limiting enzymes: indoleamine-2,3-dioxygenase 1 (IDO1) and tryptophan-2,3-dioxygenase (TDO). In addition, the activity of these two kynurenine catalysers can be induced by cortisol (TDO) and inflammatory mediators (IDO1). However, 90% of the available tryptophan undergoes the kynurenine pathway (51).
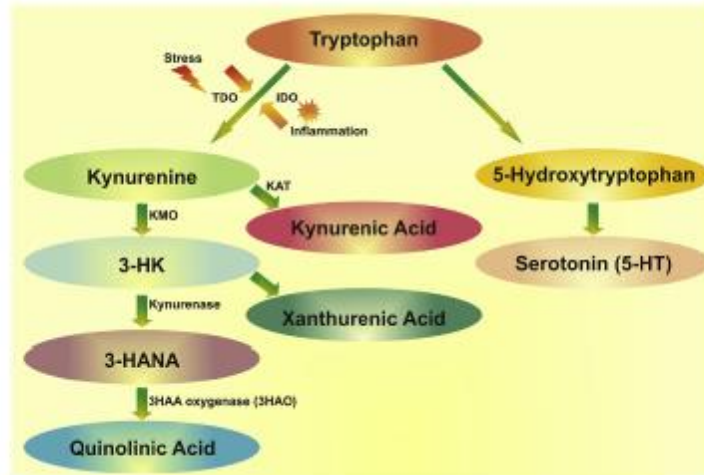
Figure 7. Schematic representation of the tryptophan metabolism pathways obtained from Kennedy *et al* (52).

It is believed that microbiota participates in modifying CNS serotonin levels in a direct and an indirect manner.

First, some bacteria can directly synthesise serotonin like *Escherichia* spp., *Streptococcus* spp., *Enterococcus* spp., and *Candida* spp (reviewed in 44).

Then, it is proposed that they can modulate the levels indirectly using different strategies. On one hand, microbiota can release SCFA which stimulate serotonin production in ECC (53). On the other hand, they may condition the transformation of tryptophan towards the formation of kynurenine or to 5-OH-tryptophan formation. The second metabolite can go through the BBB and thus be used in the CNS for synthesizing serotonin but the first one can't. Consequently, if too much tryptophan undergoes the kynurenine pathway, 5-HT levels in CNS may be lower (52).

Marrying all the aspects together, these studies prove the ability of microbiota to induce changes in the serotoninergic system of the CNS.

▪ **GABA**

GABA is the principal inhibitory neurotransmitter in our body and it derives from glutamate metabolism. An alteration in GABA has been related to the pathogenesis of some diseases like depression and anxiety.

Interestingly, it has been discovered that some bacterial strains modulate GABA levels. For example, some *Lactobacillus* and *Bifidobacterium* genera can metabolize glutamate into GABA (54). Another study suggests that microbiota-synthesised GABA can pass the BBB and enter the CNS (55). It is also proposed that some microbes' signalling through gut vagal afferents alters the expression of GABA receptor in brain areas related to stress such as the hippocampus. It is the case of *Lactobacillus rhamnosus*, whose presence alters GABA receptor population in mice through the vagus nerve and reduces their anxious and depressive behaviours (56). This indicates that gut bacteria can influence the brain by regulating this neurotransmitter.

- **Brain-derived neurotrophic factor (BDNF)**

The brain-derived neurotrophic factor (BDNF) is a protein (neurotrophin) expressed in the CNS. Its main actions rely on neuroplasticity, neural differentiation, and surveillance as well as enabling the synapsis formation.

Animal studies in mice stress models noticed the association of stress and depression with low brain BDNF levels (in the hippocampus) and that antidepressants restored the basal levels (22). Later, experiments noticed a reduction of BDNF mRNA in animals infected with *Trichuris muris* that was restored after the administration of the probiotic *Bifidobacterium longum* (57).

In GF mice, changes in BDNF levels have been documented in comparison with control animals. Yet, when focusing on the hippocampus of GF mice, results are inconsistent: some studies point to an increase and others to a decrease in BDNF hippocampal expression (as it has been reviewed in (21)).

However, the conclusion that can be drawn so far from these investigations is that gut microbiota has an influence over the expression of BDNF in CNS. Defining how these alterations affect the brain and its pathologies still needs clarification.

- **Short-chain fatty acids (SCFA)**

In our diet we ingest variable amounts of fibre. Once the fermentable fibre we eat reaches the gut it can be converted into short-chain fatty acids (SCFA) by colonic microbiota. The three major SCFA molecules are acetic, propionic and butyric acid. These metabolites have a wide amount of properties: they are anti-inflammatory and antitumorigenic, modulate gut permeability and have the ability to activate epithelial cell signalling mechanisms (50).

It has been proposed that these compounds interact with the brain through different pathways. Once they are synthesised in the intestine, they can cross the BBB and reach the hypothalamus, where they modulate GABA and glutamate levels (58).

In addition, it is known that they benefit the intestinal and the blood-brain barrier through modulation of the thigh junctions, thus reducing the "leaky gut" consequences and limiting the access of intestinal metabolites to the brain (59,60).

Another proposed mechanism by which these compounds can transduce their signal to the brain is by binding to G-coupled receptors from enteric cells (61). Enteroendocrine cells and neurons of the submucosal and myenteric ganglia have SCFA receptors and contribute to their signalling (62). Consequently, the gut stimulus can reach the brain.

To sum up, as it has been dissected in this part, gut microbiota uses multiple pathways to access the brain. This leads to scientists questioning if its impact on the CNS could also affect our behaviour. As a result, in the following section, it will be analysed how changes in the MGB axis may influence our mental health, focusing the investigation on depression.

6.3 Depression

According to the American Psychiatric Association, depression is considered a mood disorder in which a feeling of sadness and/or a loss of pleasure in activities that once were considered enjoyable for the person happens during 2 or more weeks and is accompanied with at least 5 of the following symptoms that impair their daily life: weight loss, fatigue, feelings of worthlessness or guilt, insomnia, difficulty of concentration, changes in appetite, recurrent thoughts of death (63).

Depression is the leading mental disease worldwide. Also, it is considered the first cause of disability in the world by the World Health Organisation and it is important to highlight that people with mood disorders have higher rates of mortality due to suicide and a lower life expectancy compared with the average of the population (64). Hence, the upsurge of its prevalence during the last century has increased the interest to find appropriate therapies.

Nevertheless, the cause of such diseases still isn't enough defined. For what is known so far, pathophysiology of major depressive disorder (MDD) essentially is related to the following three aspects: abnormal function of the brain as a result of an imbalance of neurotransmitters and dysfunction on the neural circuitry as well as in neuroplasticity; dysregulation of the HPA axis (patients usually present increased cortisol and CRH levels); and a chronic low-grade inflammatory response involving pro-inflammatory cytokines (65). There have also been links with genetic and environmental factors but in the last decade, some studies have switched the attention to the role that microbiota can play within the disease. Therefore, the next section examines these investigations.

6.3.1 Studies on the influence of the microbiota in depression

One of the first studies to suggest that gut microbiota can be involved in the pathogenesis of depression was conducted by Kelly *et al* (66) in GF rats who received a transplant of fecal microbiota from depressed patients, resulting in a change of behaviour towards an anxious-like mood in the colonised animals along with anhedonia and irregularities in the metabolism of tryptophan. This experiment was carried out five years after Bercik *et al* (22) already hinted the influence of the microbiota in behavioural traits (as it is explained in 6.2).

From the beginning, animal studies have procured most of the data we have nowadays about the gut microbiota-depression relationship, meaning the relationship has mostly been characterised in rodents and it still lacks human data.

A growing body of evidence suggests the influence of gut microbiota on the susceptibility of developing depression in a bidirectional manner that resembles the bidirectionality of the MGB axis itself. This means depression exacerbating gut dysbiosis but also gut alterations impacting brains' health.

As it has been reviewed in 6.2.1 section, a state of dysbiosis is linked to an alteration of the MGB axis pathways including irregularities in inflammation, HPA axis functionality

and an abnormal regulation of the neurotransmitters (66). These three features are also related to the physiopathology of depression and are proposed as possible mechanisms for gut microbiota to influence depression.

One of the mechanisms starts with stress – whether organic or psychologic – increasing the permeability of the gut epithelium (67). In this sense, stress-related disorders such as depression have shown an increase of bacterial translocation through the intestinal wall (68). The increased translocation leads to an immune activation that triggers a cytokine release (as explained in 6.2.1.3 section). The perpetuation of a pro-inflammatory state - with proinflammatory cytokines such as interferon-α – can disrupt the activity of metabolic neurotransmitters and origin symptoms linked to mood changes, fatigue or depression (40). In fact, some antidepressants block these inflammatory cytokines (69). In addition, the work from Berk *et al* (70) suggested that the chronic, low-grade inflammatory state related to depression may be associated with the "leaky gut" state. In support of that hypothesis, a study in human subjects showed higher concentrations of IgA and IgM against LPS of commensal gram-negative gut bacteria in depressed patients than in healthy individuals (71). Also, a study found an increase of TLR-4 expression and detected intestinal bacterial DNA in plasma from depressed patients (72). Therefore, the participation of the microbiota within this scheme gains importance.

The HPA axis is another key element in mood regulation. In depression, it has been reported an alteration in the HPA axis function. The sensitivity of glucocorticoid receptors located in the hippocampus and pituitary gland is impaired and thus the negative feedback is reduced. In this scenario, there is an increase in the production of cortisol (73), which could induce changes in the microbiota composition. Moreover, considering that the microbiota can influence the HPA axis (as it exposed in 6.2.1.2), a dysbiosis may exacerbate the disease.

The third proposed mechanism is an abnormal neurotransmitter signalling. Changes in levels of BDNF, serotonin, noradrenaline, dopamine, plus alterations in GABA and NMDA receptors have been associated with anxiety and depressive-like behaviour in animals (50). These molecules play a role in the MGB axis. In this sense, the aforementioned studies in 6.2.1.4 show experimental evidence of the neurotransmitter changes that follow gut dysbiosis, which strengthens the correlation with mental disorders.

Despite all the research on the field, current evidence of the relationship between gut microbiota and depression is still poor and diffuse. For example, there isn't a firm consensus over the composition of the gut microbiota in depression yet.

A review conducted by Cheung *et al* (74) at the beginning of 2019 gathered the results of all published human studies on gut microbiota in mood disorders and noticed a change in microbial diversity and taxa in comparison to healthy groups. However, the authors of the review stated that present studies have limitations that don't allow a consensus about the most relevant bacterial taxa in depression, starting with the reduced number of the samples, the heterogeneity of the population included in the studies and the presence of depressed patients that were taking antidepressants and thus biasing results.

Interestingly, shortly after, it was published a study by Valles-Colomer *et al* (75) that overcame some of the problems outlined by Cheung *et al* (74). This study carried out a DNA sequencing of feces from 1054 participants in order to establish a link between the present microbiota taxa with the quality of life and depression of participants. Afterwards, results were validated with an independent cohort (n=1063). Researchers found the genera *Coprococcus* and *Dialister* to be reduced in depressed patients even after suppressing from the study the possible bias induced by antidepressant use. In addition, *Faecalibacterium* and *Coprococcus* – two butyrate-producing genera that reduce intestinal inflammation and strengthen the intestinal barrier – were associated with a better life quality (represented in Figure 8. Moreover, It was also noticed the correlation between the quality of life and the bacterial synthesis of a dopamine-derived metabolite (3,4-dihydroxyphenylacetic acid, also referred to as DOPAC). Interestingly, it must be highlighted that it was described for the first time the association of mental health with an enterotype: the *Bacteroides* enterotype 2, which appeared more frequently within the depressed population (as it is illustrated in Figure 8A).

This study has been published recently and still no other investigations have replicated it or further explored the obtained outcomes. However, the results are promising and set a possible direction to explore in future investigations.
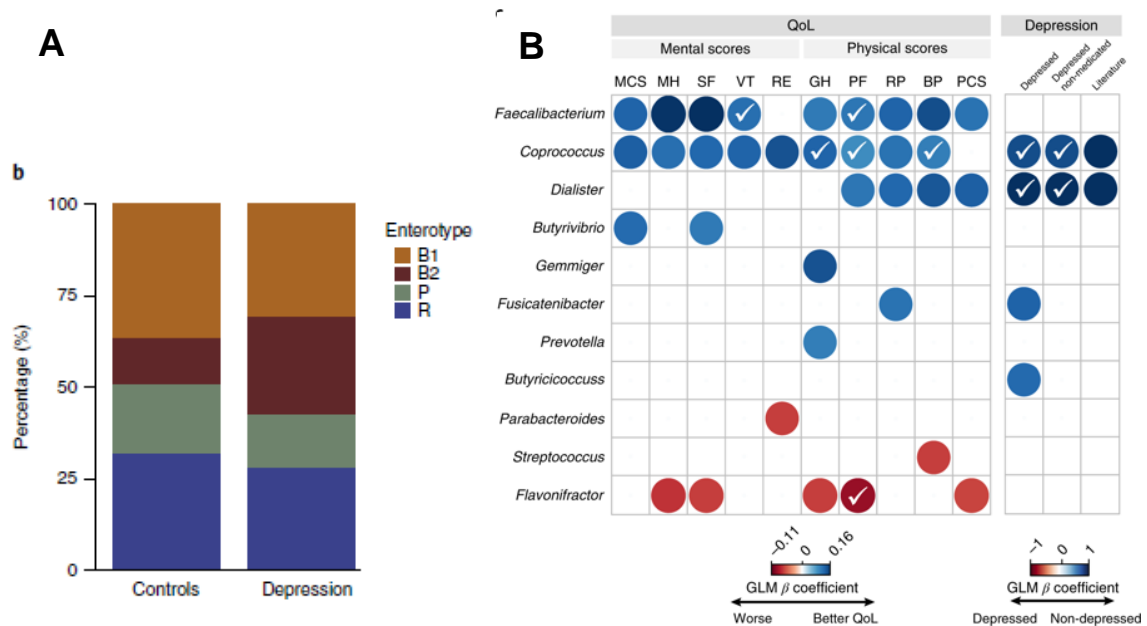

Figure 8. Results obtained from Valles-Colomer *et al* (75). **A**: increment of *Bacteroides* enterotype 2 (B2) in depressed patients opposed to healthy individuals. (B1: *Bacteroides* enterotype 1; B2: *Bacteroides* enterotype 2; P: *Prevotella* enteorype; R: *Ruminococcaceae* enterotype); **B**: It depicts the association between quality of life scores (QoL) and depression with presence of bacterial genera. In blue it is represented the elevated taxa in the non-depressed group whereas in red the increased ones in subjects with a worse QoL.

In most of the existing research examining the microbiota composition in depression, the obtained results lack causal evidence and are just a correlation of observations that happen concomitantly (i.e. changes in microbiota and depression). Not to mention the heterogeneity in the methodology of the different studies or the limited sample sizes that, as Cheung *et al* (74) claimed, doesn't allow a proper meta-analysis of the literature. In addition, the recent publication by Valles-Colomer *et al* (75) cannot prove a cause and effect relationship either. Yet, due to its meticulous experimental design, it

offers the clearest results for now on microbiota taxa alterations in depression, thus, leaving the door open for future studies examining the effects of *Coprococcus* spp. and *Dialister* spp. as probiotics in depressed patients.

6.4   Comparison of therapies

Given the increasing evidence supporting the link of microbiota with depression, a key question should be considered: can treatments that target the MGB axis be used as therapeutic weapons against mood-related disorders? Hence, in the last years, this proposal has been examined and compared with current depression treatments with antidepressants.

Inhere we compare current knowledge of both classic and new proposed therapies based on the MGB axis, considering efficacy and side effects related to both strategies.

6.4.1   Classic strategies

Therapeutic approaches for depression usually use psychotherapy and medications. Also, in resistant cases, brain stimulation therapies have been considered an option. In this section, we digress about the traditional and still currently leading pharmaceutical therapy based in antidepressants.

6.4.1.1 Antidepressants

Nowadays, the common therapeutic approach for depression treatment is based on the monoaminergic hypothesis, which states that depression's origin is found in the imbalance or lack of monoamine neurotransmitters (i.e. noradrenaline, dopamine, serotonin). That theory arose in the 1950s when an anti-tuberculosis drug – iproniazid – was seen to produce euphoric effects on patients. Later it was proved that iproniazid inhibits an enzyme called monoamine oxidase (MAO) which metabolises biogenic amines (like serotonin and catecholamines) by deamination, so it elevates their levels. This lead to the development of the first antidepressant group: the MAO inhibitors (MAOIs). However, MAOIs present dangerous consequences like lethal hypertension and risky interactions with numerous drugs.

Further research facilitated the appearance of tricyclic antidepressants (TCA), which inhibited norepinephrine and serotonin reuptake. These groups were considered the first generation of antidepressants. They are effective thanks to their action increasing serotonin or noradrenaline levels (or both). However, TCA also have antihistaminic and anticholinergic properties and antagonise α1 receptors, unleashing a series of negative side-effects such as dizziness, constipation, drowsiness, dry mouth and weight gain.

From these discoveries and the increasing research on the serotonin's participation in depression, the development of drugs followed a rational design focused on serotonin-

specific reuptake inhibitors (SSRI) like fluoxetine as well as targeting multiple receptors (the case of venlafaxine, bupropion, trazodone, mirtazapine, etc). These new generations of antidepressants got rid of some of the antihistaminic and anticholinergic side-effects thanks to their specific receptor binding.

Besides their effects on the metabolism of monoamines, some antidepressants also modulate another key factor of depression: inflammation. To do so they potentiate IL-10 cytokine (76) and block pro-inflammatory ones like interferon-α (69).

Interestingly, some recent studies point to the antibiotic effect of several antidepressants as one of their possible mechanisms of action, putting as an example isoniazid, the first antidepressant to be discovered, and others as sertraline, fluoxetine, escitalopram or imipramine that have been found to also have antimicrobial effects (77). Hence, proposing a link between their mechanisms and the MGB axis.

Today there are still important limitations in the antidepressant therapy. First, the onset of action of these drugs is delayed for a few weeks and usually comes together with undesirable side-effects. Then, around 30-40% of all treated patients do not respond or show unsatisfactory results to the treatment (6). This could be due to the narrowed monoaminergic approach which doesn't consider the multi-cause idiosyncrasy of the disease.

However, despite the aforementioned weakness of these drugs, the rate of antidepressant consumption is increasing at an alarming rate (5). Therefore, it is suggested that future research should focus on targeting multiple hypotheses of depression for a wider therapeutic approach.

6.4.2 New approaches

New proposed therapies include different methods that alter gut microbiota composition such as the so-called "psychobiotics" (probiotics and prebiotics), fecal microbiota transplantation (FMT) or dietary habits which are discussed below. These are studied as alternatives to classic antidepressant drugs given the current problem with the rise in their prescription.

6.4.2.1 Probiotics

Probiotics are defined by the WHO as "live micro-organisms which, when administered in adequate amounts, confer a health benefit on the host" (78). Initially, they were found to be helpful in the treatment of IBS condition and recently, their possible beneficial effects in depression and behavioural disorders have started to be analysed.

In order to influence the CNS, probiotics use the proposed pathways of connection of the MGB axis (depicted in section 6.2.1). In addition, they improve the gut epithelium permeability (increasing tight junction proteins' expression) and mucus secretion (through regulation of mucin expression). These are two gut defence mechanisms that

prevent microbiota from accessing the *lamina propria*. Hence, its improvement ameliorates the "leaky gut" state (79).

Pioneer studies on the therapeutic benefits of probiotics in mental disorders were conducted in animal models. For example, treatment with specific probiotic strains improved depression- and anxiety-like behaviour in adult mice (56) or reversed the depressive behaviour generated by maternal separation in rats (80). In this last study, separated rats were put in two different groups and treated with an existing antidepressant (citalopram) or the probiotic *B. infantis*. A closer look to their results shows that the probiotic treatment reversed all the negative effects caused by the maternal separation (increased immobility behaviour in the forced swim test, reduced norepinephrine brain levels, increased expression of CRH in amygdala and secretion of proinflammatory cytokines as IL-6). Moreover, when comparing the results between the probiotic and the antidepressant group they found no significant differences in terms of cytokine and corticosterone levels as well as in the behaviour in the swimming test as seen in Figure 9.
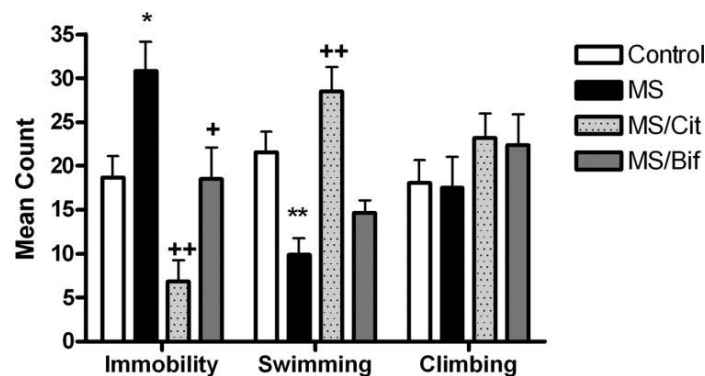

Figure 9. Behaviour of the animals from the study Desbonnet *et al* (80) assessed in the swim test. Non-treated maternal-separated mice (MS) showed an increased anxiety-like behaviour than the groups treated with citalopram (MS/Cit) or *B. infantis* (MS/Bif).

Another publication compared citalopram with another probiotic strain – *Lactobacillus helveticus* – obtaining similar results than in the previous study (81).

However, there are some contradictory results, because when *L. rhamnosus* was tested against another antidepressant (fluoxetine) it showed antidepressant and anxiolytic activity on BALB/c mice but not in Swiss Webster mice (82).

It is also interesting to highlight an interesting study from this year that observed a potential use for *Faecalibacterium prausnitzii* as a psychobiotic. It is worth to mention that this species has the biggest population within the gut and it represents around 5% of the bacterial microbiota. In this study the animal model used was under CUMS conditions and the probiotic administration prevented and treated the CUMS-derived depressive- and anxious-like behaviour with a parallel increase of SCFAs cecum levels, anti-inflammatory cytokines (IL-10) – which has also been observed in antidepressants – and inhibited the corticosterone effects and the release of proinflammatory IL-6 as well as C-reaction protein (83).

As it has been exposed, the relationship with probiotic intake and its impact on animal behaviour has been assessed in multiple preclinical studies. However, clinical research in humans is still limited.

Two double-blind, placebo-controlled clinical trials were conducted on healthy subjects, fed with probiotics (*L. helveticus* and *B. longum* in the first study; probiotic-containing milk in the second) versus placebo, showed that people under probiotic treatment felt less depressed symptoms (84,85). Then, Wang *et al* (86) examined 38 studies on the effects of probiotics to the CNS on humans and animals – 9 of which evaluated depression symptoms –, finding that the most effective species in improving depressive symptoms were *Lactobacillus casei, L. rhamnosus, L. helveticus, Bifidobacterium breve, B. infantis* and *B.longum*. These are results that reaffirm the outcome of previous studies in mice.

Nevertheless, a review from Nadeem *et al* (87) that examined studies of probiotic use in depression up to 2018 found evidence of their effect on anxiety and depression but the volume of samples coming from psychiatric patients was relatively small compared to the healthy group to draw firm conclusions, arguing the need of more participants and longer treatment duration. On a positive note, they concluded that the effects that probiotics produce on patients with depressive disorders are more significant than in healthy populations.

Besides testing the efficacy of probiotics in depressed individuals it is important to assure the security of the therapy. It is worth mentioning that, as opposed to antidepressants, probiotics have insignificant side effects in non-immunocompromised people, usually limited to gases. This is a positive asset for probiotics in a moment when there's a need for finding novel therapeutic strategies for depression and anxiety. Yet, there are reports pointing out that patients with immunosuppression, those who are critically ill or have undergone surgery could have serious side effects such as risky infections associated with probiotic usage (88).

In conclusion, probiotics are interesting candidates for depression treatment – whether as monotherapy or adjuvant therapy with traditional strategies – as they present a safer profile than prevailing drugs. However, further clinical studies in depressed patients are required to better characterise their effects and to investigate the potential role as probiotics of bacterial strains with interesting outcomes in pre-clinical studies (like *Dialister* and *Coproccocus* genera mentioned in 6.3.1).

6.4.2.2 Prebiotics

The Food and Agriculture Organisation (FAO) describes a prebiotic as a "non-viable food component that confers a health benefit on the host associated with modulation of the microbiota" (89). Prebiotics include monosaccharides (like fructose), disaccharides (as lactose), oligosaccharides (fructo- and galacto-oligosaccharides), polyols and FODMAPs (like inulin) (90). Once we ingest these products, they escape intestinal absorption and reach the colon, where they feed the commensal bacteria that live in there – including *Lactobacilli* and *Bifidobacteria*. As they promote the growth and

function of beneficial bacteria, they are proposed as possible treatment strategies for depressive and anxious conditions.

However, the first quantitative analysis of all data relative to prebiotics associated with depression has been carried out this year examining 5 prebiotic trials with depressed patients and found no significant differences with the controls (placebo) (91).

6.4.2.3 Fecal microbiota transplantation

A fecal microbiota transplant is a procedure in which feces from a donor are collected, dissolved in a solution and relocated into the colon of another individual. The use of FMT has had promising results in patients infected with *Clostridium difficile* and has also been applied to other diseases like IBS, Crohn's disease and ulcerative colitis (92).

However, little do we know about the consequences of a microbiota transplant in the context of psychiatric conditions. Studies with microbiota transplants have been carried out in animals to further understand the mechanisms of the MGB axis – like the experiments from Bercik *et al* (22) and Kelly *et al* (66) aforementioned – concluding that with the transference of the microbiome, some phenotypical characteristics of the donor's behaviour can also be transferred to the receiver.

To date, no clinical trials are found using FMT in depressed groups. However, a study conducted by the Psychiatric Hospital of the University of Basel is currently in its recruitment phase to later assess the effects of oral frozen FMT capsules in severely depressed patients (93). Nowadays, evidence is presented as documented cases from individual patients. For example, this year, there was a reported case of a depressed old patient that after not improving with a 6-month treatment of escitalopram, flupentixol and melitracen tablets combined with probiotics and digestive enzymes received a FMT that reverted her initial symptoms six months afterwards (94).

All in all, due to the low evidence level of the studies, it's still too early to draw firm conclusions about its therapeutic potential but it is another option worth to be explored. However, outcomes from the clinical trial lead by the University of Basel will hopefully shine some light on the issue.

6.4.2.4 Diet

Our dietary habits are closely related to the composition of our gut microbiome. For example, it has been studied that the enterotype of people who follow a diet with big proportions of meat and animal fats has greater levels of *Bacteroides* spp. (enterotype 1) whereas individuals more prone to consume fiber in their diets have higher levels of *Prevotella* spp. (enterotype 2) (16). Also, fermentable fibre intake conditions the levels of SCFA as well as the number of bacteria thanks to their prebiotic qualities.

In this sense, studies have observed that diets catalogued as unhealthy and that include refined foods, industrially processed aliments, excessive sugars, saturated fats and/or additives disrupt the microbiota and create an imbalance in the gut immune system with a resulting increase in proinflammatory cytokines and neuroinflammation than can increase the host's susceptibility of developing a depressive phenotype (95). Hence, a Mediterranean diet is likely to protect while a Western diet is related to an increased risk of depression development.

Considering these findings, we conclude that by controlling our intake products we can modify our intestinal microorganisms, but further research is needed to establish if taking specific dietary habits could lead to substantial changes to our mental health.

7   Concluding remarks

The principal conclusions obtained from this work are summarised below:

- The main organisms inhabiting the gut are bacteria, especially from the *Firmicutes* and *Bacteroidetes* phyla. The microbiota is a dynamic entity. Its composition is variable throughout the different life stages and is conditioned by genetic as well as external factors such as birthing method, dietary habits, use of antibiotics, stress or infections. Moreover, 3 enterotypes have been described amongst the population: *Bacteroides* (enterotype 1), *Prevotella* (enterotype 2) and *Ruminococcus* (enterotype 3).

- On one side, molecular techniques allow us to describe gut microbiota composition from fecal samples. Further strategies point to the direction of metagenomics in order to get further insight into the collective functionality of the microbiome rather than the taxonomic aspects. On the other side, germ-free animals are the current major model to study the effects of dysbiosis within the MGB axis.

- The MGB axis is integrated by neural, endocrine and immunologic pathways that connect in a bidirectional manner the brain and the intestinal lumen. The literature suggests that in mood-related disorders there is an alteration of the MGB axis constituents.

- Recent studies show a decrease in *Coprococcus* spp. and *Dialister* spp. concurrently with an increase of the *Bacteroides* 2 enterotype in a depressed population. However, current findings still are correlations and lack of a causal association.

- The most studied alternative for classic antidepressant therapy based on the MGB axis has been the use of probiotics. Their beneficial effects on depression may be related to a decrease in proinflammatory cytokines, in gut epithelium permeability as well as to GABA alterations and HPA modulations. Potential probiotics for depression treatment proposed by different authors include *Bifidobacterium* (*B. infantis, B. breve, B. longum*), *Lactobacillus* (*L. helveticus, L. rhamnosus, L.* case*i)* and *Faecalibacterium prausnitzii*. The effects of probiotics are more significative in depressed patients than in healthy individuals. At the same time, probiotics present a safer profile of side effects as opposed to antidepressants. Hence, its study as an adjuvant therapy for depression may be of interest.

To sum up, research on the effects of the gut microbiota in depression it is still in its infancy and thus requires additional studies before drawing firm conclusions. Finally, positive findings obtained so far justify further research in the field considering the major impact it could have on public health.

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