Impact of gut microbiota on neurological diseases: Diet composition and novel treatments

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Abstract

Gut microbiota has significant effects on the structure and function of the enteric and central nervous system including human behaviour and brain regulation. Herein, we analyze the role of this intestinal ecosystem, the effects of dietary changes and the administration of nutritional supplements, such as probiotics, prebiotics, or fecal transplantation in neuropsychiatric disorders. Numerous factors have been highlighted to influence gut microbiota composition, including genetics, health status, mode of birth delivery and environment. However, diet composition and nutritional status has been repeatedly shown to be one of the most critical modifiable factors of this ecosystem. A comprehensively analysis of the microbiome-intestine-brain axis has been performed, including the impact of intestinal bacteria in alterations in the nervous, immune and endocrine systems and their metabolites. Finally, we discuss the latest literature examining the effects of diet composition, nutritional status and microbiota alterations in several neuropsychiatric disorders, such as autism, anxiety, depression, Alzheimer's disease and anorexia nervosa.

Keywords: Gut microbiota; probiotics; prebiotics; Alzheimer's disease; autism; depression; anorexia nervosa.

Abbreviations

Aβ: Amyloid-β

AD: Alzheimer's disease

AN: Anorexia nervosa

ASD: Autism spectrum disorder

BBB: Blood-brain barrier

BDNF: Brain-derived neurotrophic factor

BFA: Branched fatty acids

CNS: Central nervous system

ENS: Enteric nervous system

FOS: fructooligosaccharides

GABA: γ- Aminobutyric acid

GALT: Gut-associated lymphoid tissue

GF: Germ-free

GOS: Galactooligosaccharides

IL: Interleukin

Lipopolysaccharides

NAC: N-acetyl cysteine

NMDA: N-methyl-D-aspartate

PPA: Propionic acid

SCFA: Short chain fatty acid

SPF: Specific pathogen free

TEA: autism spectrum disorder

TFN-α: Tumor necrosis factor alpha

Introduction

The term microbiota refers to populations of microorganisms present in several ecosystems in the body (for example, the gut microbiota and skin microbiota) (Khanna and Tosh, 2014). Our intestinal microbiota contains one hundred billion microorganisms, including at least 1,000 different bacterial species that comprise more than 3 million genes, 150-fold more than human genome (Blaser, 2017). The number and variety of these bacteria present in our microbiota grow exponentially from the proximal end of the gastrointestinal tract to the distal end, being most of them in the colon. On the other hand, only a third of our intestinal microbiota is common to most people, while the other two thirds are specific to each person. In other words, we could describe it as a personal ID card, unique for each individual. Thus, our future microbiome will depend on multiple variables from the moment that we are born (Leong, et al, 2016).

Neuropsychiatric disorders affect over one billion people all over the world (Chisholm et al, 2016). Thus, the number of people with depression and/or anxiety has risen, and about 10% of all the world population is affected; whereas mental disorders represent 30% of non-deadly disease health care system load in the entire world (Chisholm et al, 2016). Another remarkable neurological disease is autism, whose incidence has multiplied in the last fifteen years among children (Ding et al, 2016). Mental disorders, such as Alzheimer's, must also be considered, and finally we cannot forget about the problem of anorexia nervosa, that is increasingly in teenagers at earlier ages (Jayatilleke et al, 2017).

From the 80's, it was proposed that alterations in the microbial diversity could contribute to chronical inflammatory disorders, including depression (Williamson et al,

2016). Modification of the gut microbiota could be associated to neurological diseases since the first stages of life. For that reason, the use of novel therapies such as probiotics, prebiotics or fecal transplantation is increasing sharply nowadays (Misra & Mohanty, 2017). Probiotics are living organisms with beneficial functions in our body that exert immunomodulatory effects, mostly shown in experimental models but also in human intervention studies (West et al, 2014). Prebiotics are non-digestible ingredients that profit our organism stimulating the development and activity of beneficial bacteria producing short-chain fatty acids (SCFAs), among others. These SCFAs promote gut epithelial integrity and exert immune effects including stimulation of G-protein-coupled receptors, promotion of innate (TLR2) immune responses, and induction of T regulatory cells in the colon (Thorburn et al, 2014). On the other hand, fecal transplantation consists on the introduction of intestinal bacteria from a healthy donor to a patient. It can be hypothesized that antibiotic therapy (among others) could disrupt the normal gut ecology, allowing colonization of undesirable microorganisms. Thus, the aim of this review is to comprehensively analyze the effects of dietary modifications in several neuropsychiatric disorders.

Materials and methods

The data published for this revision were identified by means of search and selection on the following databases: PubMed, Google Scholar, Elvesier and Scielo. Searching was made in two steps. First, a quest was made by using the keywords “microbiota”, “diet”, “intestine”, “gut”, “brain” and “mental disorders”. Secondly, the role of microbiota was assessed separately, in different mental illnesses adding it to a second selection: “autism”, “depression”, “stress”, “Alzheimer’s disease”, “anorexia nervosa”, “inflammation”, “intestinal permeability”, “psychobiotics” and “fecal transplantation”.

Also the bibliography of each selected article was revised for the inclusion of other relevant articles.

Intestinal ecosystem and its evolution

Several investigations suggest that the fetus could be exposed to microorganisms in the uterus, penetrating through the placenta. Thus, a study carried out in 2010, revealed that when the bacterial profile of mothers and newborns was stablished with DNA sequencing, in particular the phylogeny of the rRNA 16s gene, babies born through vaginal via got colonies similar to their mother‘s vaginal microbiome, where *Lactobacillus sp* and *Bacteroides sp* would prevail. These colonies, once installed in the gut, could help the babies with their digestion. On the other hand, babies born via cesarean section would acquire colonies similar to those present in the skin surface, where S*taphylococcus sp* would prevail, being potentially harmful bacteria (Dominguez-Bello et al, 2010). Moreover, three or four days after the birth, the bacterial profile keeps on modifying itself with the microbia in the mother’s milk, appearing an intestinal infantile microbiota similar to the colostrum of the mother (Akkerman et al, 2018; Collado et al, 2016). It is important to remark that a recent study showed that cesarean born children or fed with artificial milk could suffer more risk of deploying inflammatory and immune problems as allergies, asthma or even cancer, and provided new evidences about the effects of the kind of birth and the newborn diet in the formation of the essential microbial community in the childhood (Song et al, 2013). In conclusion, it could be said that the transmission of microorganisms from one generation to another is a fundamental process in life and it depends on factors that are external to us.

Numerous published articles about microbiome comparisons among different cultures help us to understand the impact of several lifestyles and diets in human adaptation and health (Clemente et al, 2015; Gomez et al, 2016; Shankar et al, 2017). In a recent study, intestinal bacteria of two neighbors African communities (Bantu and BaAka) and occidental individuals were compared. The BaAka are hunter-gatherers; whereas the Bantu are agriculturalists. It was observed that microbiome profiles of the three communities follow a gradient that reflects the degree of traditional lifestyle. Thus, the Bantu had a more similar microbiota to that of the industrialized occidental countries; whereas the bacterial diversity of the BaAka was more alike to the one typical of the ancient hunter-gatherer peoples, with a bacterial composition characteristic of the savage primates. The latest consumed high quantities of fiber and tannins which could explain the *Prevotella sp* enrichment, a polysaccharide degrading bacterium that inhabits the bovine rumen. On the other hand, the patterns observed in the gut microbiome of the Bantu may reflect more recent transitions from traditional to modern, agricultural, or western-like lifestyles (Gomez et al, 2016). A former study stated that the Yanomami Indians, a tribe living in the Amazonian rainforest uncontacted with the occidental civilization until 2008, had almost double bacterial diversity compared with citizens of the USA (Clemente et al, 2015). A very recent study remarked the power of the diet to control the composition of the gut microbiota (Shankar et al, 2017). In this study, gut microbiota from Egyptian children following a Mediterranean style diet rich in vegetables was compared to the microbiota of children from the USA, following a diet rich in fat, animal proteins and highly processed carbohydrates. It was observed that the intestinal environment of the Egyptians had abundance in SCFAs that inhibit inflammation and protect against obesity. The guts of the children from the USA had an increase in amino acids associated with lipidic metabolism and in 1-metilhistamina, a

biological marker of allergies. On the other hand, these products of proteic and lipidic degradation appear associated to a higher risk of developing arteriosclerosis and colon cancer (Shankar et al, 2017). All these studies highlight that diet influences on bacterial diversity.

Among all the strains dwelling in our gut, *Bifidobacterium sp. (Filo Actinobacteria) and Lactobacilus sp. (Filo Firmicutes)* are the most beneficial to the host because of their anti-inflammatory, anti-tumoral as well as pathogen excluding properties (Marteau, 2013). Although it would be difficult to trace the frontier between "good" and "bad" bacteria, the general trend says that the key is in diversity and ratio existent among different strains. Many causes can alter the wellbeing of our benefic gut bacteria, such as: administration of antibiotics or non-steroidal anti-inflammatory medicines (NSAIDs), chemical substances present in the environment (until 232 have been found able to cause changes in the microbiota), herbicides, ingredients present in our food (sugar or gluten) or in the water (chlorine). This way, modern lifestyle is the main cause altering the diversity in gut microbiota. This imbalance is called dysbiosis (Principi & Esposito, 2016).

Therapies for the modulation of the microbiome

There are numerous studies analyzing the role of novel therapies used for the modulation of the gut microbiome as a new approach for the cure of psychological disorders (Bercik et al, 2011b; Grimaldi et al, 2016; Jeong et al, 2016; Liu et al, 2015; Mallikarjuna et al, 2016; Marchesi et al, 2015; Numakawa et al, 2014; Saulnier et al, 2013; Wan et al, 2018; Wang et al, 2016; Zamudio-Tiburcio et al, 2017; Zhang et al, 2012).

Probiotics

Probiotics are living organisms with beneficial functions in our body (Figure 1). They help to keep the integrity of the intestinal lining, balance the pH of the body; act as antibiotic; regulate immunity and control inflammation diminishing the levels of liposaccharides (LPS); and boost the brain-derived neutrophic factor (BDNF). BDNF is a protein that promotes the survival of existent neurons and fosters the growth and differentiation of new neurons (neurogenesis), thus playing an essential role in the normal neurological development. When levels of BDNF are low, problems in learning and/or memory arise (Numakawa et al, 2014). On the other hand, probiotic bacteria block the spread and invasion of pathogen bacteria producing anti-microbial substances called bacteriocins. In addition, they facilitate the absorption of food and improve the bioavailability of some nutrients such as A, C and K vitamins and those of B group (Liu et al, 2015).

Although there are not many studies in humans about the role of probiotics in the improvement of the cognitive function, a recent systematic review of 38 studies in animals and humans (25 in animals, 15 in humans and two in both, humans and animals), concluded that probiotics could be effective in improving functions related to psychiatric disease and memory skills (Wang et al, 2016). Among the most important strains with probiotic functions are: *Bifidobacterium sp.* (*B. longum, B. Brevis, B. infantis, B. lactis*), *Lactobacillus sp.* (*L. helveticus, L. rhamnosus, L. plantarum, L. casei, L. acidophilus, L. brevis*) and *Bacteroides fragilis* (Liu et al, 2015). *L. plantarum* controls inflammation and reduces intestinal permeability (Mallikarjuna et al, 2016). *L. brevis* boosts BDNF levels and lowers LPS levels produced by intestinal bacteria (Jeong et al, 2016). In addition, *B. longum* was associated with anxiety reduction and improvement in BDNF production in mice (Bercik et al, 2011b).

Prebiotics

Prebiotics are non-digestible ingredients that profit our organism stimulating the development and activity of beneficial bacteria (Figure 1). They are generally carbohidrates of different size, including: nondigestible oligosaccharides or polysaccharides (inulin), fructooligosaccharides (FOS), galactofructose, galactooligosaccharides (GOS) and xiloligosaccharides (Marchesi et al, 2015). Different studies have shown benefits both in animals and in humans, including: (i) diminish inflammation in intestinal inflammatory disorders, avoiding the presence of inflammatory compounds in the brain (Liu et al, 2015; Saulnier et al, 2013); (ii) improve the intestinal ecosystem and modulate brain function enhancing the composition of the intestinal microbiota, in particular the rate of *Firmicutes/Bacteroidetes* (Liu et al, 2015); and (iii) influence the production of neuro-chemicals. Importantly, a recent study explored the neuro-endocrine and affective aspects of two prebiotics, FOS and Bimuno-GOS (B-GOS), in healthy human beings and concluded that they could have anti-autistic properties (Grimaldi et al, 2016).

Fecal Transplantation

It consists on the introduction of intestinal bacteria from a healthy donor to a patient, by means of the transference of an infusion from a fecal sample through a nasogastric tube, nasoduodenal tube or rectal enema (Marchesi et al, 2015). According to a recent preliminary study including six patients aged 36 to 83 (4 men and 2 women), it was noted an improvement on anxiety as much as 70% after being subject to a transplant of intestinal microbiota (Zamudio-Tiburcio et al, 2017).

Physiopathology of the bowel-brain axis

The mechanisms of signal transmission between the brain and the bowel are complex and not completely elucidated, but they include neural, endocrine, immune and metabolic pathways (Shankar et al, 2017) (Figure 2). The enteric-nervous system, often referred to as the second brain, is the biggest cluster of neurons and neuron protective cells (glia) apart from the central nervous system (de Weerth, 2017). Remarkably, the population of intestinal bacteria affects directly to the stimulation and function of the vagus nerve. Two recent studies confirmed this hypothesis. The first one pointed that the elimination by vagotomy of *Lactobacillus rhamnosus* diminished the risk of neurological diseases such as Parkinson's disease (PD) (Bravo et al, 2011). A later study noted that in patients who had undergone vagotomy at an early age, risk of PD diminished compared to general population (Svensson et al, 2015). It could be hypothesized that PD could be caused by an enteric neurotropic pathogen which enters the brain through the vagus nerve.

On the other hand, enteroendocrine cells found along the intestinal tract represent the biggest producers of hormones and biogen amines (neurotransmitters derived from different amino acids) in the body, remarking the function of histamine, serotonin and catecolamines (dopamine, noradrenalin and adrenalin) (Green & Brown, 2016). These substances are called neurotransmitters or neuromodulators and they are chemical substances created by the body that convey signals from one neuron to the next one (Figure 3). It should be pointed that about 80-90% of the body serotonin is produced in the intestine by intestinal bacteria, thus, more serotonin is produced in the intestine than in the brain. This suggests that depression could be more efficiently modulated by the diet than by antidepressants. The most remarkable intestinal neurotransmitters produced by different intestinal bacteria are: *Lactobacillus sp*. and *Bifidobacterium spp*. produce

aminobutiric acid (GABA); *Escherichia sp, Bacillus sp.* or *Saccaromyces spp.* can synthesize noradrenalin; *Streptococcus sp, Escherichia sp.* and *Enterococcus spp.* can produce serotonin; *Bacillus sp.* produces dopamine and *Lactobacillus sp.* can synthesize acetylcholine. Another remarkable neurotransmitter produced by intestinal bacteria is glutamate, involved in cognition, learning and memory functions (Lyte, 2014).

The brain and the intestine are also connected by the immune system (Figure 3). Inflammatory reaction affects neurological control through the intestine-brain axis, modulating the cooperation between the central nervous system (CNS), the enteric nervous system (ENS) and the gut-associated lymphoid tissue (GALT) (Hale et al, 2015). GALT is the gut's own immune system and accounts for a 70-80% of the whole immune system of the organism (Berer & Krishnamoorthy, 2012). Cytokines are one of the best known indicators of inflammation. They are small proteins released by cells (mainly immune system cells) affecting the behavior of other cells and usually the inflammatory process. Other indicators of inflammation are the C-reactive protein (CRP), interleukins (IL) and tumor necrosis factor alpha (TNF-α). Importantly, a study analyzing the plasma levels of cytokines (IL-6, IL-10 y TNF-α) in women with AD, observed that their levels were significantly higher when compared with control subjects (Baranowska-Bik et al, 2008).

The metabolic system is the fourth and last linkage between the brain and the intestinal microbiota (Figure 3). The microbiota degrades the carbonated chains digested in the intestine (starch, cellulose, hemicellulose, oligosaccharides, sugars and not absorbed alcohols) resulting in the synthesis of short chain fatty acids (SCFAs) and branched fatty acids (BFAs). The most important SCFAs are butyric, acetic and propionic acid (PPA); whereas among the BFAs are isobutyrate, methyl butyrate or isovalerate (Schneider et al, 2017). Depending on the composition of the microbiota, the type of

food and the intestinal transit time, the production of these fatty acids will be different. Thus, higher levels of PPA and BFAs are associated with intestinal harmful bacteria; whereas higher levels of butyric or acetic acids (SCFAs) are associated to healthier microbiota (Marteau, 2013). Furthermore, it has been observed that amines produced by degrading-protein bacteria can overpass the blood-brain barrier (BBB), aggravating the symptoms of autism (MacFabe, 2012).

Social and communicative behaviour and microbiota

Numerous studies have linked the onset of behavioural and learning problems with alterations of the intestinal microbiota (Table 1).

Animal studies

The biggest scientific evidence between the link of behaviour and the microbiome appears in studies on small rodents. Thus, in a study examining whether shifts in bacterial diversity due to dietary manipulation could be correlated with changes in memory and learning in 5-old male mice, it was observed that rodents that received standard rodent chow supplemented with 50% lean ground beef for 3 months improved their learning and memory capacities when compared with standard rodent chow mice (Li et al, 2009).

Moreover, a study comparing germ-free (GF) mice, absent from microbial colonization, and specific-pathogen free (SPF) mice, concluded that GF mice showed less social and more fearful behaviour associated with higher levels of cortisol and lower levels of BDNF (Bercik et al, 2011a). Additionally, colonization of GF mice with microbiota increased exploratory behaviour and higher hippocampal levels of BDNF (Bercik et al, 2011a). A more recent study, confirmed this results, finding a less social behaviour of

GF male rats that preferred and socialized better with an object than with another rodent, when compared with the SPF group (Desbonnet et al, 2014).

Furthermore, numerous studies analyzing the role of treatments such as fecal transplantation, prebiotics and/or probiotics in the improvement of intestinal health and their effects on the brain function have been performed (Buffington et al, 2016; Burokas et al, 2017; Chen et al, 2017; Persico & Napolioni, 2013; Robertson et al, 2017). In a recent study, it was noted that the transplantation of intestinal microbiota from SPF mice to GF mice significantly restored 7 differentially expressed miRNAs and 139 mRNAs affecting gene expression on the prefrontal cortex (Chen et al, 2017). In addition, social avoidance behaviour (autism) was significantly associated with the increase of *Lachnospiraceae, Ruminococcaceae* and *Clostridialis* in adult mice guts. These intestinal bacteria produced *p*-cresol, a neurotoxic substance related to altered behaviour (Persico & Napolioni, 2013).

Recent studies emphasize the role of probiotics in the modulation of social behaviour. A recent investigation found that infant rats whose mothers had ingested a high-fat diet during pregnancy showed an abnormal behaviour. However, the administration of living cultures of *Lactobacillus reuteri* was able to correct deficiencies in their social behaviour, leading to the conclusion that specific bacteria from intestinal microbiota can influence in communicative behaviour (Buffington et al, 2016). Another study carried out with a combination of prebiotics in male mice (FOS, GOS, or a combination of FOS+GOS for 3 weeks), showed that prebiotic treatment exhibited both antidepressant and anxiolytic effects. Moreover, the administration of GOS and the FOS+GOS combination reduced stress-induced corticosterone release (Burokas et al, 2017). Furthermore, prebiotics modified specific gene expression in the hippocampus and hypothalamus, thus enhancing social behaviour of rodents. Besides, in another study, a

supplementation of fatty omega-3 acids were administered to a group of pregnant rodents, observing higher fecal levels of *Bifidobacterium sp.* and *Lactobacillus sp.* in supplemented mice compared with higher levels of *Firmicutes sp. and Bacteroidetes sp.* in mice without supplementation. These differences in gut microbiota were associated with a better social behaviour and enhanced cognition in supplemented pregnant mice, favoring the activation of the hypothalamic-pituitary-adrenal (HPA) axis (Robertson et al, 2017).

Human studies

Albeit only a few, there are important clinical studies displaying how probiotics are able to affect brain functions in human beings. One interesting study in humans was carried out in 2013 with thirty-six women divided into three groups: one group got a mix of yogurt with various probiotics (*Bifidobacterium animalis, Streptococcus thermophiles, Lactobacillus bulgaricus* y *Lactococcus lactis*) along four weeks; the second group got a yogurt without probiotics along four weeks and the third group did not take any of the other two products. Before and after those four weeks, the thirty-six women underwent magnetic resonance imaging to assess brain activity in specific moments (Tillisch et al, 2013). The women who ingested yogurt with probiotics had less activity in a brain area related to emotions and sensorial processing (the midbrain region centered on the periaqueductal gray, the prefrontal cortex, precuneus, basal ganglia, and the parahippocampal gyrus). Inversely, the other two groups showed a higher connectivity in these brain regions (Tillisch et al, 2013). Although further examinations of these pathways in humans are needed, homologous studies in rodent models have been developed, showing that this decrease in activity could be related with a better responsiveness and modulation in pain sensitivity, stress, mood and anxiety. In another study, a double blind controlled study (n=47) using maltodextrin as placebo analyzed

the acute effects of oligofructose-enriched inulin (5 g) over a 4-hour period. On each test day mood and cognitive performance were assessed at baseline (at 8:00) and then following inulin or placebo (at 11:00). Questionnaire results showed that on the day that the inulin was consumed, participants felt happier, had less indigestion and were less hungry than when they consumed the placebo. As for performance and mood tasks, the most consistent effects were on the episodic memory tasks where consumption of inulin was associated with greater accuracy on a recognition memory task, and improved recall performance (immediate and delayed) (Smith et al, 2015). These amazing studies show how changes in our gut microbiome affect the behavior of human brain. In the light of the results exposed here, we could conclude that additional clinical studies are needed to elucidate the role of the microbiota in brain function.

Autism and microbiota

Autism, along with depression and anxiety, are the mental disorders more associated to intestinal microbiota until now. Autism or autism spectrum disorder (ASD) features three characteristics: difficulty for social interaction, verbal and non-verbal communication problems and repetitive behaviours. Despite the mental symptoms of autism, disorders of the gastrointestinal tract are also remarkable, showing food intolerances, constipation and/or diarrhea. The relation between autism, intestinal inflammation, mitochondrial dysfunction and intestinal dysbiosis has boosted the interest about the role of the microbiota in this mental disease (Berding & Donovan, 2016) (Table 2).

Animal studies

Numerous studies in rodents have shown an increase of inflammatory biomarkers in the autistic model in comparison with control groups, highlighting the neurotoxic effects of PPA levels (Foley et al, 2014; Lim et al, 2017). PPA could induce inflammation, activate the immune system, increase oxidative stress, damage proteins, cell membranes and even DNA. A very recent study showed that levels of serotonin in ASD rats were significantly higher in comparison with the control group (Lim et al, 2017). In another study, after PPA administration in pregnant rats, their offspring presented altered social behaviours like anxiety, loss of desire for socialization and obsession for the objects, in contrast with the offspring's control group (Foley et al, 2014), thus showing the neurotoxic role of this metabolite and its association with ASD. On the other hand, a study carried out on ASD mice demonstrated that the administration of the probiotic *Bacteroides infantis* corrected gut permeability, altered microbial composition, and ameliorated defects in communicative, stereotypic, anxiety-like and sensorimotor behaviors (Hsiao et al, 2013).

Remarkably, there are numerous studies in humans about the connection between intestinal dysbiosis and autism, providing us clearer and more convincing evidences.

Human studies

Different studies in humans about intestinal dysbiosis have highlighted an increase of *Phylum Bacteroidetes* and *Proteobacteria*, and a decrease of *Phylum Firmicutes* and *Actinobacteria* in ASD children in comparison with non-autistic children (Ding et al, 2016; Rosenfeld, 2015). These studies have also observed a reduction of *Prevotella sp, Coprococcus sp, Enterococcus sp, Lactobacillus sp, Streptococcus sp, Lactococcus sp, Staphylococcus sp,* and *Bifidobacterium sp,* genera and an increase of *Rominnococcus sp, Sutterella sp, Desulfovibrio sp, Prevotella sp, Pseudomonas sp, Aeromonas sp,*

Enterobacterias and *Clostridium sp* genera in ASD children (Ding et al, 2016; Rosenfeld, 2015). All these studies agree that the genus *Clostridium sp* is augmented in autistic children, causing higher PPA levels and interactions with beneficial bacteria such as *Bifidobacterium sp*. Thus, recent investigations have noted the importance of balancing the microbiome to relieve autism symptoms. As far as we know, a study performed in 2000 showed, for the first time, that alterations in indigenous intestinal bacteria might promote colonization by one or more neurotoxin-producing bacteria, such as *C. botulinum or C. tetani*, contributing, at least in part, to autistic symptomatology. Moreover, treating such alterations with a minimally absorbed oral antibiotic, such as vancomycin, could significantly relief autism symptoms (Sandler et al, 2000).

Furthermore, studies in humans have pointed a relation between intestinal permeability and autism. It has been hypothesized that an increase of intestinal permeability would allow the passage of bacteria, toxins and metabolites and would trigger the immune system activation. This could lead to inflammation and development of intestinal, systemic and brain diseases, such as ASD (Ashwood et al, 2011). In a study performed in 97 ASD children, it was observed that increased levels of proinflammatory cytokines such as IL-1β, IL-6, IL-8 and IL-12 in plasma autistic children were significantly associated to poor social behavior and typical alterations of this disorder when compared with the control group (n=87) (Ashwood et al, 2011). More recently, it was observed a significant dysfunction of the immune system in autistic children and adults, with alterations that include inflammation of brain samples, high levels of proinflammatory cytokines in the cerebrospinal fluid or in the blood and increased autoantibodies in the brain against serotonin receptors, myelin basic protein, heat shock proteins or glial filament proteins, among others (Onore et al, 2012). Interestingly, the

presence of specific anti-fetal brain antibodies in approximately 12% of mothers of children with ASD has been observed, whilst they are absent in mothers of children who are typically developing, thus suggesting a potential inflammatory process occurring in mothers of children with ASD that leads to the production of antibodies directed to the developing brain (Singer et al, 2009). Furthermore, the administration of IgG collected from mothers of children with ASD to pregnant rhesus macaques, induced stereotypic behavior and hyperactivity in the offspring, symptoms that share homology to ASD (Martin et al, 2008).

Another study carried out in 40 ASD children showed that the administration of 1200 mg/day of N-Acetylcysteine (NAC) for 8 weeks induced a significant irritability decrease compared with the placebo group. Moreover, repetitive behaviours and other autistic conducts also diminished (Ghanizadeh & Moghimi-Sarani, 2013). Similarly, a study using a combination of probiotics in 33 ASD children (*Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus delbruecki, Bifidobacterium longum y Bifidobacterium bifidum*) for six months, found changes in the microbiota and consequently an improvement in communication and autistic behaviours (West et al, 2013). Other studies with probiotic therapy have shown similar results (Adams et al, 2011; Russo, 2015). In a study with 49 autistic subjects (39 males; mean age 11.4 years) and 36 controls (29 males; mean age 10.2 years), probiotics substantially lowered the concentration of myeloperoxidase, a marker of inflammation and oxidation, in ASD patients (Russo, 2015). A former study performed in 58 ASD children and 39 healthy typical children of similar ages showed that probiotics substantially diminished fecal levels of PPA in autistic individuals (Adams et al, 2011).

On the other hand, administering GOS to autistic children increased the presence of beneficial bacteria (*Bifidobacterium sp* and *Lactobacillus sp*), improving the features of

autism (Grimaldi et al, 2016). A study performed in 18 autistic children showed that fecal transplantation from children without ASD to children with ASD using a high initial dose followed by daily and lower maintenance doses for 7-8 weeks, caused a reduction of 80% of the typical gastrointestinal symptoms of this disorder, along with a substantial improvement of the autistic behaviour, both of them persisting 8 weeks after treatment (Kang et al, 2017).

All of these observations reveal that probiotics, prebiotics as well as fecal transplantation could be useful options for ASD children treatment.

Anxiety and microbiota

Depression and anxiety are two closely associated mental disorders. Whereas anxiety is characterized by fear, oppression, uneasy thinking and exaggerated preoccupations; depression is related to hopeless feelings (Sloan et al, 2017). Studies carried out in both, animals and humans, revealed that the presence or absence of intestinal microbiota influenced in the development of anxious thinking (Table 3).

Animal studies

Studies in rodents have pointed out that, after exploratory behaviour tests, the anxious conduct appears in the GF group in comparison with SPF group (Bercik et al, 2011a) or EX-GF (commensal fecal microbiota-associated mice) group (Nishino et al, 2013). The latest study showed that the gnotobiotic (EX-GF) mice with normal specific pathogen-free microbiota were less anxious and active than GF mice; thus showing that probiotics diminished anxiety behaviour. In addition, norepinephrine, dopamine, and serotonin turnover rates were higher in the EX-GF mice than in the GF mice in most regions of the brain, suggesting that monoaminergic neurotransmission might increase in the EX-

GF mice comparing the GF mice. All these observations indicated that the presence of intestinal microbiota can lower anxiety. Confirming these observations, the presence of metabolites related with anxiety was analyzed in stress-sensitive F344 rats (Crumeyrolle-Arias et al, 2014), finding that GF rats showed elevated corticotropin releasing factor mRNA expression in the hypothalamus and reduced glucocorticoid receptor mRNA expression in the hippocampus. GF rats also showed lower dopaminergic turnover rate in the frontal cortex, hippocampus and striatum than SPF rats. Moreover, a rise in corticosterone serum levels and an increase of neuro-endocrine responses to stress (cortisol) were found in GF rats, all of them related to anxious behaviour (Crumeyrolle-Arias et al, 2014). All of these observations highlight the importance of the microbiota in the anxious behaviour in animals.

Recently, it was observed that the probiotic *Lactobacillus helveticus* administered to adult SPF Sprague-Dawley rats subjected to 21 days of stress, improved chronic restraint stress-induced behavioral (anxiety and depression) and cognitive dysfunction. Additionally, *L. helveticus* also resulted in lower plasma corticosterone and adrenocorticotropic hormone levels, higher plasma IL-10 levels, restored hippocampal serotonin and norepinephrine levels, and more hippocampal BDNF mRNA expression than in chronic stress rats (Liang et al, 2015). Taken together, these results indicate an anti-depressant effect of *L. helveticus* in rats subjected to chronic restraint stress. Previously, a study was performed to investigate the anxiolytic-like activity of *Lactobacillus helveticus* and *Bifidobacterium longum* in rats, and in healthy human volunteers. In the preclinical study, rats were daily administered with a mixture of the probiotics for 2 weeks, whereas, in the clinical trial, volunteers participated in a double-blind, placebo-controlled, randomized parallel group study with probiotics administration for 30 days. Importantly, daily administration of probiotics significantly

reduced anxiety-like behaviour in rats and alleviated psychological distress in volunteers (Messaoudi et al, 2011).

On the other hand, prebiotics also improved anxious and depressive disorders. A very recent study in rodents evidenced that the administration of GOS and a mix of GOS+FOS improve anxious behaviour in those animals by increasing time in the center of the open field test and a tendency to make more entries into the center in this test. In addition, qPCR results corroborated higher concentration of *Bifidobacterium sp* and *Lactobacillus sp* in the prebiotic administration groups than in stressed animals. Furthermore, FOS and FOS+GOS administration increased serotonin levels in the prefrontal cortex and dihydroxyphenylacetic acid levels in the frontal cortex. Conversely, FOS+GOS administration decreased dihydroxyphenylacetic acid levels in the brainstem. Besides, several changes in SCFA cecum levels were observed. Thus, an increase in acetate and propionate levels and a decrease in corticosterone, isobutyrate and PPA levels were found. All these changes correlated significantly with the positive effects seen on behavior (Burokas et al, 2017).

Human studies

Studies conducted in humans are scarce and focused in corroborating the therapeutic usefulness of probiotics and prebiotics to modulate the microbiome, and therefore, lowering stress, depression and anxiety problems. In a study, 45 healthy adults (aged 18-45) were divided into two groups; in one group, one of two prebiotics, FOS or Bimuno-GOS (B-GOS) was administered, whereas a placebo (maltodextrin) was administered to the second group. After three weeks, participants showed decreased attentional vigilance to negative versus positive information in a dot-probe task only after B-GOS intake compared to placebo intake. Additionally, cortisol levels also

diminished only after B-GOS intake, leading to a reduction of stress and anxiety in this group in comparison with the placebo group (Schmidt et al, 2015). All these results confirm the role of gut microbiota to control stress reactions and therefore, anxiety.

Depression and microbiota

Nowadays, depression cannot be conceived only as a brain disorder and several animal and human studies have confirmed that cytokines and inflammatory processes can trigger depression (Abildgaard et al, 2017; Reichenberg et al, 2001) (Table 4).

Animal studies

Several animal studies have revealed how the modulation of the intestinal microbiota in rodents through diet, fecal transplantation and probiotics had an impact on depression. Thus, a study in rodents showed that the administration of omega-3 polyunsaturated fatty acids (PUFA) was associated with a cytokine levels reduction (Robertson et al, 2017). Thus, omega-3 deficient mice displayed an elevated *Firmicutes/Bacteroidetes* ratio and blunted systemic LPS and concanavalin A (Con A) responsiveness with elevated TNFα ($p=0.018$) and IL-10 ($p=0.007$) levels. In contrast, omega-3 supplemented mice displayed greater fecal *Bifidobacterium sp* and *Lactobacillus sp* abundance. Moreover, omega-3 deficient mice displayed impaired communication, social and depression-related behaviours and omega-3 supplemented rodents displayed enhanced cognition (Robertson et al, 2017). This study, therefore, highlights that diet might modify the gut microbiome and consequently neurobehavioural development. Another study showed that regardless of the type of diet (Occidental *vs* Mediterranean), the administration of eight bacterial strains (*B. bifidum* W23, *B. lactis* W19, W52 and

W58, *L. acidophilus* W37, *L. brevis* W63, *L. casei* W56, *L. salivarius* W24) for ten weeks caused changes in the intestinal ecosystem in all of the rodents and a reduction of TFN-α, IL2 and IL6 levels (Abildgaard et al, 2017). Additionally, probiotics lowered hippocampal transcript levels of factors involved in hypothalamic-pituitary–adrenal axis regulation. These findings in animals have been also corroborated in human studies, showing a positive relation between the ingestion of pre- and probiotics, the improvement of intestinal dysbiosis and mood (Reichenberg et al, 2001; Schmidt et al, 2015; Steenbergen et al, 2015).

Human studies

Several human studies confirm the role of inflammation in depressive disorders. A double-blind, crossover study performed in 20 human healthy volunteers found an increase in the circulating levels of IL6, TNF-α, IL-1 receptor antagonist and cortisol after the injection of *Salmonella abortus* endotoxin, thus correlating in a significant manner with higher levels of anxiety and depressed mood (Reichenberg et al, 2001).

Recent studies have shown differences between the microbiota of healthy and depressive individuals (Aizawa et al, 2016; Zheng et al, 2016). Thus, 58 patients with major depressive disorder (MDD) and 63 matched healthy controls were recruited and their fecal samples were collected. 16S ribosomal RNA gene-sequence based approach was used to compare the gut microbial communities of MDD patients and healthy controls showing that in depressive people, there was an increase of *Firmicutes, Actinobacterias* and *Bacteroides* (Zheng et al, 2016). Strikingly, after the introduction of microbiota from MDD patients in GF rodents, they acquired depressive behaviours that were not present before, providing evidence that microbiota might play a casual role in depression (Zheng et al, 2016). Another study, found that, in MDD patients (43 patients and 57 controls), there was a decrement of beneficial bacteria such as

Bifidobacterium sp and *Lactobacillus sp* using bacterial rRNA-targeted reverse transcription-quantitative polymerase chain reaction (RT-q-PCR) in fecal samples (Aizawa et al, 2016), thus providing new insight into the pathophysiology of MDD and enhancing future research on the use of probiotics in its treatment.

Regarding the use of intestinal microbiota modulators, a recent study was performed in 14,539 men and women (mean age: 37 years) initially free of depression with a median follow-up period of 9.3 years. In this period, 727 incident cases of depression were identified. It was observed that high fat probiotic yogurt induced an improvement in depressive behaviours, but low fat yogurt or the use of prebiotics (fructans and GOS) did not make a difference (Perez-Cornago et al, 2016). In a triple-blind, placebo-controlled, randomized study, 20 healthy participants received a 4-week probiotic food-supplement intervention with a multispecies probiotic containing *Bifidobacterium bifidum*, *Bifidobacterium lactis*, *Lactobacillus acidophilus*, *Lactobacillus brevis*, *Lactobacillus casei*, *Lactobacillus salivarius*, and *Lactococcus lactis*. Participants who received the 4-week multispecies probiotics intervention showed a significantly reduced overall cognitive reactivity to sad mood (Steenbergen et al, 2015), evidencing that the intake of probiotics may help reduce negative thoughts.

Dementia: Alzheimer's disease and microbiota

Alzheimer's disease (AD) is the most common form of dementia. It is a clinicopathological, degenerative, chronical and progressive disease, which exhibits a deterioration of memory, orientation, speech and other functions related to personality and visual and spatial skills. Factors contributing to the pathogenesis of the disease are, mainly, the presence of extracellular amyloid deposits, called neuritic senile plaques, and fibrillary protein deposits inside neurons, known as neurofibrillary bundles,

appearing mainly in the frontal and temporal lobes. The pathogenesis also implies a deficit in multiple neurotransmitters; highlighting the loss of cholinergic markers, choline acetyltransferase and acetylcholinesterase. Furthermore, there is a deficiency of serotonin, noradrenalin, somatostatin and corticotrophin-releasing factors. In addition, the presence of apolipoprotein E4 alleles is also involved in the predisposition to develop the disease (Abate et al, 2017). Therefore, it can be said that whilst the etiology of AD is still somewhat dubious, it is recognized as an interaction between genetic and environmental factors.

Regarding the link between intestinal microbiota and AD, several studies evidenced that increased permeability in the intestinal and BBB, caused by bacterial dysbiosis, can affect the pathogenesis of AD (Table 5). Furthermore, intestinal microbiota can segregate amyloids, LPS and other small inflammatory molecules. These substances, added to intestinal permeability, can favor the occurrence and evolution of this kind of dementia (Jiang et al, 2017).

Animal studies

It has been observed that GF rodents displayed higher BBB and intestinal permeability compared to those of the SPF group, thus predisposing to the development of AD (Braniste et al, 2014). Other studies also performed in rodents have shown that multiples injections of LPS induced an increase in hippocampal amyloid-β (Aβ), the primary cause of AD (Jaeger et al, 2009; Kahn et al, 2012). Along with the inflammation caused by the presence of LPS and the hippocampal Aβ accumulation, serious cognitive deficits also appear in treated rodents (Jaeger et al, 2009; Kahn et al, 2012). Several studies evidenced that, along with LPS, the accumulation of Aβ can also be generated by several bacterial strains, including *Escherichia coli, Bacillus subtilis,*

Salmonella typhimurium, Salmonella enteric, Mycobacterium tuberculosis and *Staphylococcus aureus;* thus increasing the risk of AD (Hill & Lukiw, 2015). Therefore, the use of antibiotic therapy in AD mice significantly reduced Aβ plaques by activating autophagy in a manner that is dependent on the mTOR pathway, and improved spatial memory and learning ability of tested mice (Zhang et al, 2017).

Regarding to diet, it has been observed that after the administration of a high-fat diet to rodents, levels of Aβ increased in the intestine. Moreover, Aβ might be secreted by enterocytes as an apolipoprotein component of chylomicrons, thus evidencing that diets rich in saturated fat could exacerbate cerebral amyloidosis and predispose to AD (Galloway et al, 2009). On the other hand, the ingestion of omega-3 PUFAs are essential to the neuronal and cerebral functions and low levels in the organism are related to neurodegenerative diseases such as AD (Hu et al, 2016; Robertson et al, 2017). Hence, the intake of food like nuts, oily fish and vegetable omega 3-rich oil is recommended, along with antioxidants such as coffee, vitamin C, vitamin E and flavonoids for the improvement of AD (Hu et al, 2016). Furthermore, animal studies showed that the administration of probiotics caused positive effects in the reduction of Aβ plaques and the recess of AD (Jiang et al, 2017).

Human studies

Confirming the results obtained in animal models, LPS has been also associated with AD in human studies. This way, LPS plasma levels were found to be 3-fold higher in AD patients than in healthy controls (Zhang et al, 2009). Interestingly, novel findings about disturbed intestinal barrier function in AD patients have been pointed. In a study with 22 AD patients, fecal concentrations of calprotectin were significantly higher than in controls, thus indicating a disturbed intestinal barrier function associated with AD

(Leblhuber et al, 2015). Calprotectin is a protein released by leukocytes into inflamed tissues. It provides not only bacteriostatic but also cytokine-like effects in the local environment and can be used as inflammatory marker. Recent studies found that calprotectin triggers and promotes the formation and aggregation of Aβ *in vitro* as well as in animals (Kim et al, 2014). On the other hand, the role of neurotransmitters synthetized by intestinal bacteria in the pathogenesis of AD has been also highlighted. Significant reductions in GABA levels have been described in severe cases of AD, which could be underlying the behavioral and psychological symptoms of AD (Solas et al, 2015). Moreover, another study assessed the ability of serotonin to alter brain Aβ levels and plaques in a mouse model of AD and in humans (Cirrito et al, 2011). To test whether serotonin signaling could impact Aβ plaques in humans, brain Aβ load was compared in cognitively normal elderly participants who were exposed to antidepressant drugs for 5 years to participants who were not. Antidepressant-treated participants had significantly less amyloid load as quantified by positron emission tomography (PET). Cumulative time of antidepressant use correlated with less plaque load (Cirrito et al, 2011). These data suggest that serotonin signaling was associated with less Aβ accumulation in cognitively normal individuals.

A recent randomized, double-blind, and controlled clinical trial was conducted among 60 AD patients to assess the effects of probiotic supplementation on cognitive function (Akbari et al, 2016). The patients were randomly divided into two groups (n=30 in each group) treating with either milk (control group) or a mixture of probiotics (probiotic group). The probiotic supplementation contained a mixture of *Lactobacillus acidophilus, Lactobacillus casei, Bifidobacterium bifidum, and Lactobacillus fermentum*. After 12 weeks intervention, compared with the control group, the probiotic treated patients showed a significant improvement in the mini-mental state examination

score, thus demonstrating that probiotic consumption positively affects cognitive function and corroborating animal studies about probiotics and cognitive function.

Anorexia nervosa and microbiota

Anorexia nervosa (AN) is one of the most common chronical diseases in teenagers (Lavender et al, 2015). AN is characterized by insufficient ingest of food and a poor diet, leading to a significantly low body weight and a severe health risk for the individual (Ahlstrom et al, 2017). Investigations about the correlation between eating disorders and the influence of the microbiota are scarce (Table 6). Nevertheless, it has been noted that people with AN presented a "leaky gut", characterized by antigens traversing the intestinal wall, as demonstrated in an animal model of AN (Jésus et al, 2014), and could underlie the low-grade inflammation and increased risk of autoimmune diseases found in AN. This increase of inflammatory substances in the body could affect the mood, cognitive function, depression and anxiety (Herpertz-Dahlmann et al, 2017).

Animal studies

Chronic food restriction during AN may induce gut barrier dysfunction, contributing to disease development and its complications. A recent study characterized intestinal barrier function in mice with activity-based anorexia, an animal model of AN (Jésus et al, 2014). It was observed that the AN group increased colonic permeability in comparison with the control group, thus suggesting that intestinal barrier dysfunction may also occur in AN. Another study pointed intestinal bacteria as one of the main factors associated to severe malnutrition. In this study, fecal transplantation from children suffering kwashiorkor disease, a severe malnutrition syndrome, was performed

on GF mice. Interestingly, a drastic reduction of weight was observed, accompanied by perturbations in amino acid, carbohydrate, and intermediary metabolism that were only transiently ameliorated after the intake of ready-to-use therapeutic food (Smith et al, 2013). This food has become the international standard of treatment for severe acute malnutrition in community-based treatment programs and is composed of peanut paste, sugar, vegetable oil and milk fortified with vitamins and minerals. These observations led to the hypothesis that malnutrition in GF mice had been caused by the microbiota transplantation implicating the gut microbiome as a causal factor in severe malnutrition.

Human studies

A study explored the role of the intestinal microbiota in AN patients analyzing the fecal microbiota and SCFAs in these patients before (n=55) and after weight gain (n=44) in comparison to normal-weight participants (n=55). Profound microbial perturbations were observed in AN patients with lower gut microbial diversity when compared to normal-weight participants. In addition, a significant reduction of *Phylum Bacteroidetes* and a significant increase of *Phylum Firmicutes* were found. Moreover, higher levels of mucin-degraders microorganisms (*Verrucomicrobium sp, Bifidobacterium sp, Anaerotruncus sp*) and members of *Clostridium sp* clusters I, XI and XVIII were identified. Mucin acts as a barrier that protects enterocytes against pathogens and chemical risk factors, contributing to a lower intestinal permeability. Furthermore, reduced levels of the butyrate-producing *Roseburia spp* and elevated SCFA concentrations were also observed. Upon weight gain, microbial richness increased, but SCFA profiles and most of gastrointestinal symptoms did not recover (Mack et al, 2016). These insights provide new clues to modulate the intestinal microbiota in order to improve the outcomes of the standard therapy.

Another study analyzed cortisol levels immediately on awakening in 18 underweight AN women, 15 weight-restored AN women and 26 normal-weight healthy women as a measure of stress and anxiety. Moreover, participants' anxiety levels in the morning of sampling were measured by the State-Trait Anxiety Inventory. It was found that underweight AN patients showed enhanced cortisol level when compared with weight-restored patients and normal-weight healthy women. In addition, BDNF levels diminished in malnourished patients with AN with respect to the control group (Monteleone et al, 2016). All these observations could lead to new therapeutic goals focusing on nutrition and the modulation of the microbiome to improve AN symptoms.

Conclusions and future directions

Albeit we are still in a very early stage regarding the comprehension, evidence suggests that intestinal microbiota plays an important role in the bidirectional interactions occurring between the intestine and the nervous system. This communication happens by means of several pathways, and recent findings point to the vagal nerve, neuroendocrine systems, neurotransmitters of the CNS and inflammatory factors as responsible for this connection. All the studies here analyzed, both in animals and in humans, highlight the potential role of several treatments in the alteration of the composition of the intestinal microbiota, such as probiotics, prebiotics and/or fecal transplantation.

Studies about these pioneer treatments represent a novel approach for the prevention and treatment of diverse psychiatric disorders such as autism, depression, anxiety, AD and AN. Future research in this area may help to elucidate the relation between the microbiota and the CNS and advance in the improvement of cerebral disorders.

Therefore, although the identification of the microbial population in our body will be the beginning of this new discovery, it will be necessary to decipher the meaning of all of this information in terms of health or illness. It will also be necessary to investigate the complex interaction gene-microbiota. But currently, these findings have pointed the diet as a modulator for the microbiota and a potential therapy to cure different neuropsychiatric conditions.

Disclosure of interests

The authors report no conflicts of interest.

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Tables

Table 1. Animal and human studies analyzing the role of microbiota on social and communicative behaviour

Purpose	Patients and Samples	Measurements	Main Findings	References
Analyze how diet causes changes in microbiota and behavior, specifically in learning and memory behavior	Mice	Working memory, reference memory, measure of anxiety-like behavior	Group with supplemented diet obtained significant better results in all the parameters analyzed	Li et al, 2009
Examine whether the intestinal microbiota affects social behavior and brain biochemistry in mice	GF *vs* SPF mice	Step-down and light preference tests. Cortisol and BDNF level measurements	GF mice showed less sociability, higher levels of cortisol and lower levels of BDNF than SPF mice	Bercik et al, 2011
Examine whether the intestinal microbiota affects social behavior	GF *vs* SPF mice	Social assay: Sociability and preference for social novelty	GF mice showed less sociability than SPF mice	Desbonnet et al, 2014
Explore the effect of gut microbiota on hippocampal miRNA and mRNAs expression	GF *vs* SPF mice	Behavioral test and mRNA, miRNA levels	GF showed less social behavior. 7 differentially expressed miRNAs and 139 mRNAs were identified	Chen et al, 2017
Analyze the role of *Lactobacillus reuteri* administration in social behavior of rodents	Mice offspring	Sociability and preference for social novelty	Increased social interaction after probiotic treatment	Buffington et al, 2016
Examine the role of prebiotics in anxiety, depression, cognition, stress response, and social behavior	Male mice	Plasma corticosterone, microbiota composition, cecal SCFAs and hippocampal gene expression.	Prebiotic treatment exhibited antidepressant and anxiolytic effects. Also reduction of corticosterone, propionionate and isobutyrate	Burokas et al, 2017
Milk product consumption supplemented with probiotics affects brain connectivity.	36 women segregated into 3 groups.	Magnetic resonance	Women who consumed probiotics had greater connectivity between brain areas related to cognition.	Tillisch et al, 2013
Analyze the acute effects of oligofructose-enriched inulin (5 g) over a 4-hour period.	47 patients in a double blind controlled study	Questionnaire for memory performance	Consumption of inulin was associated with greater accuracy on a recognition memory task, and improved memory performance	Smith et al, 2015

Abbreviations: SPF: Specific-pathogen free; GF: Germ-free; SCFAs: Short-chain fatty acids; BDNF: Brain-derived neurotrophic factor; miRNA: microRNA; mRNA: messenger RNA.

Table 2. Animal and human studies analyzing the role of microbiota on autism.

Purpose	Patients and Samples	Measurements	Main Findings	References
Analyze the role *Bacteroides infantis* administration in ASD rats	ASD mouse model	Behavioral and physiological parameters	Probiotic administration corrected gut symptoms, communicative, anxiety-like and sensorimotor behaviors	Hsiao et al, 2013
Gut microflora through dietary modification may help to alleviate disorders in autistic patients	Sixty young male western albino rats	Biochemical parameters related to oxidative stress	A balanced diet can protect against PPA neurotoxicity.	El-Ansary et al, 2015
Implication of serotonin in autism.	mouse models of ASD	Serotonin levels	Serotonin is significantly increased in ASD mouse	*Lim et al, 2017*
Implication of PPA in ASD rat model	PPA administration in pregnant rats	Test of their nest seeking response	Offspring presented altered social behaviours like anxiety, loss of desire for socialization and obsession for the objects	Foley et al, 2014
The usefulness of antibiotic (vancomycin) treatment for autism improvement	11 ASD subjects (3-5 age)	Analysis of intestinal ecosystem and behavioral tests	ASD improvement in communication and behavior; absence of anaerobic cocci in feces of children with ASD	Sandler et al, 2000
Analyze the role of proinflammatory cytokines in ASD.	97 ASD patients *vs* 87 controls	Cytokine levels were assessed by multiplex Luminex™ analysis	Increased levels of proinflammatory cytokines were significantly associated with poor social behavior and typical autistic alterations	Ashwood et al, 2011
Efficacy and safety of 1200 mg/day of NAC for treating irritability in ASD	40 Children	Irritability subscale score of Aberrant Behavior Checklist	Group NAC decreased irritability in children and adolescents with ASD	Ghanizadeh et al, 2013
Usefulness of a combination of probiotics for autism improvement	33 children	Autism treatment evaluation checklist (ATEC)	Improvement in communication and autistic behaviours	West et al, 2013
Usefulness of probiotic for the improvement of autism	49 ASD children	ELISA for MPO detection	Decreased plasma MPO	Russo A, 2015
Fecal transplant to improve autism symptoms	18 ASD children	GI Symptom Rating Scale	80% reduction of GI symptoms and a substantial improvement of the autistic behaviour	Kang et al, 2017
Influence of B-GOS on gut microbial ecology and metabolic function	Autistic children	Analysis of intestinal ecosystem	B-GOS increased *Bifidobacterium sp*, *Lactobacillus sp* and improve SCFAs levels.	Grimaldi et al, 2016

Abbreviations: PPA: propionic acid; ASD: Autism spectrum disorder; PPA: Propionic acid; MPO: Myeloperoxidase; GI: Gastrointestinal; B-GOS: Bimuno-Galactooligosaccharides.

Table 3. Animal and human studies analyzing the role of microbiota on anxiety.

Purpose	Patients and Samples	Measurements	Main Findings	References
Compare anxiety behaviors between GF and EX-GF mice	GF *vs* EX-GF mice	Monoamine levels in several regions of the brain. Open-field and marble-burying tests	EX-GF mice were less anxious and active than GF mice	Nishino et al, 2013
Compare anxiety behaviors between GF and SPF rats	GF *vs* SPF	Neurological, behavioral and open-field tests. Biochemical determinations	Absence of the gut microbiota exacerbates the neuroendocrine and behavioral responses to acute stress.	Crumeyrolle-Arias et al, 2014
Administration of probiotics can improve chronic-stress-induced depression	SPF Sprague-Dawley rats	Behavioral tests and biochemical analysis	*L. helveticus* reduced anxiety and depression in rats subjected to chronic restraint stress	Liang et al, 2015
A mixture of probiotics to ameliorate anxiety effects.	Rats and human healthy volunteers.	Anxiety and Depression Scale (HADS) and Perceived Stress Scale (PSS)	Decreased anxiety and alleviated psychological distress after probiotic treatment.	Messaoudi et al, 2011
Examine the role of prebiotics in anxiety, depression, cognition, stress response, and social behavior	Male mice	Plasma corticosterone, microbiota composition, cecal SCFAs and hippocampal gene expression	Prebiotic treatment exhibited antidepressant and anxiolytic effects. Also reduction of corticosterone, propionionate and isobutyrate	Burokas et al, 2017
Corroborate the therapeutic usefulness of prebiotics to decrease stress, depression and anxiety problems	45 healthy adults (aged 18-45)	Dot-probe task and cortisol biochemical analysis	More attention to positive information and cortisol levels decrease only after B-GOS intake.	Schmidt et al, 2015

Abbreviations: GF: Germ-free; SPF: Specific-pathogen free; SCFAs: Short-chain fatty acids; B-GOS: Bimuno-Galactooligosaccharides.

Table 4. Animal and human studies analyzing the role of microbiota on depression.

Purpose	Patients and Samples	Measurements	Main Findings	References
Influence of Omega 3 PUFAs in brain development and function	Pregnant female C57BL/6	Depression, cytokine levels and changes in gut microbiota	Diet might modify the gut microbiome and consequently neurobehavioural development	Robertson et al, 2017
Analyze the effects of probiotics in depression	40 male Sprague-Dawley rats	Behavior tests	Independently of diet, probiotic treatment markedly reduced depressive-like behavior. Probiotics disminished cytokine levels (TFN, IL2 and IL6)	Abildgaard et al, 2017
Confirm the role of inflammation in depressive disorders	20 human healthy volunteers	Biochemical analysis	Increase in the circulating levels of IL6, TNF-α, IL-1 receptor antagonist and cortisol after endotoxin injection	Reichenberg et al, 2001
Difference between the microbiota of a healthy person and MDD. Use these MDD patients microbiota for fecal transplantation in GF mice	MDD patients and matched controls. GF mice	16S rRNA gene	Increased *Firmicutes sp, Actinobacteria sp, and Bacteroidetes sp* in depressed patients. Transplantation of MDD patients' microbiota induces depression like behaviors in GF recipient mice	Zheng et al, 2016
Effect of a mixture of probiotics in depression	20 healthy participants	Leiden index of depression and sensitivity scale	Reduced cognitive reactivity to sad mood after probiotic treatment	Steenbergen et al, 2015
Confirm whether *Bifidobacterium sp* and *Lactobacillus sp* are reduced in MDD patients	43 MDD patients and 57 controls	RT-q-PCR analysis	Lower *Bifidobacterium sp* and/or *Lactobacillus sp* counts in patients with MDD compared to controls	Aizawa et al, 2016
Evaluate association between yogurt and prebiotic consumption and depression risk	14.539 men and women	Questionnaires and diagnosis to evaluate incidence of depression.	Whole-fat yogurt intake was significantly associated with reduced depression risk; however, prebiotic consumption was not	Perez-Cornago et al, 2016

Abbreviations: PUFA: Poly-unsaturated fatty acids; TNF: Tumor necrosis factor; IL: Interleukin; MDD: major depressive disorder; GF: Germ-free; RT-q-PCR: Reverse transcription-quantitative polymerase chain reaction.

Table 5. Animal and human studies analyzing the role of microbiota on Alzheimer's Disease.

Purpose	Patients and Samples	Measurements	Main Findings	References
Effect of LPS in AD	C57BL/6J mice	Cognitive tests. Aβ and cytokines level measurements	Injections of LPS resulted in increased Aβ1-42 in the hippocampus and cognitive deficits in mice	Kahn et al, 2012
Accumulation of Aβ protein in AD	AD Rats	Aβ levels in the brain	Lipopolysaccharide favors increase brain levels of Aβ by blood–brain barrier transport alteration	Jaeger et al, 2009
Compare the efficacy and safety of several antibiotics	40 AD mice	Aβ was detected by ELISA and IHC. Proteins related to autophagy were detected by WB	Antibiotics can alleviate the AD syndrome by activating autophagy	Zhang et al, 2017
Determine if Aβ is expressed in epithelial cells of the small intestine	Mice	*In vivo* immunological approach	Diet enriched in saturated fats doubled the abundance of Aβ, which is secreted by enterocytes	Galloway et al, 2009
Leaky gut, leaky brain and AD	AD patients	Levels of LPS in plasma	Plasma levels of LPS in patients with AD were three times higher than in healthy controls	Zhang et al, 2009
Examined fecal concentrations of calprotectin	22 patients with AD	Levels of fecal calprotectin	Calprotectin indicate a disturbed intestinal barrier function in AD patients. Lower levels of tryptophan, tyrosine and phenylalanine were also found	Leblhuber et al, 2015
Activation of certain neurotransmitter receptors can regulate Aβ metabolism	Mice AD and humans AD	Aβ levels after administration of serotonin	Treatment with citalopram, a SSRI, caused a 50% reduction in amyloid in mice. Antidepressant-treated participants had significantly less amyloid compared to control.	Cirrito et al, 2011
Effect of a mixture of probiotics	60 AD patients	MMSE score and fasting blood samples	Probiotic affects cognitive function and some metabolic statuses in the AD patients.	Akbari et al, 2016

Abbreviations: AD: Alzheimer's disease; LPS: lipopolysaccharide; Aβ: Amyloid beta; SSRI: selective serotonin reuptake inhibitor; ELISA: Enzyme-Linked ImmunoSorbent Assay; IHC: Immunohistochemistry; MMSE: Mini-mental state examination; WB: Western blotting

Table 6. Animal and human studies analyzing the role of microbiota on anorexia nervosa.

Purpose	Patients and Samples	Measurements	Main Findings	References
Restriction during AN may induce gut barrier dysfunction	ABA mice (mice model of AN)	Behavioral test and colonial histology	Increased colonic permeability and histological alterations found in ABA mice	Jésus et al, 2014
Analyze the role of intestinal bacteria in severe malnutrition	Fecal transplantation from children with kwashiorkor disease to GF mice	Biochemical analysis	A drastic reduction of weight was observed, accompanied by perturbations in amino acid, carbohydrate, and intermediary metabolism	Smith et al, 2013
Explore the role of the intestinal microbiota in AN	AN patients before and after recovering weight and control group.	Levels of intestinal bacteria and SCFAs	Profound microbial perturbations in AN patients as compared to controls	Mack et al, 2016
Cortisol and AN	Underweight AN women, weight restored AN women and control group	Saliva samples and Trait Anxiety Inventory test	As compared to control women, underweight AN patients showed significant higher cortisol level.	Monteleone et al, 2016

Abbreviations: AN: Anorexia nervosa; ABA: activity-based anorexia; GF: Germ-free; SCFAs: Short-chain fatty acids.

Figures

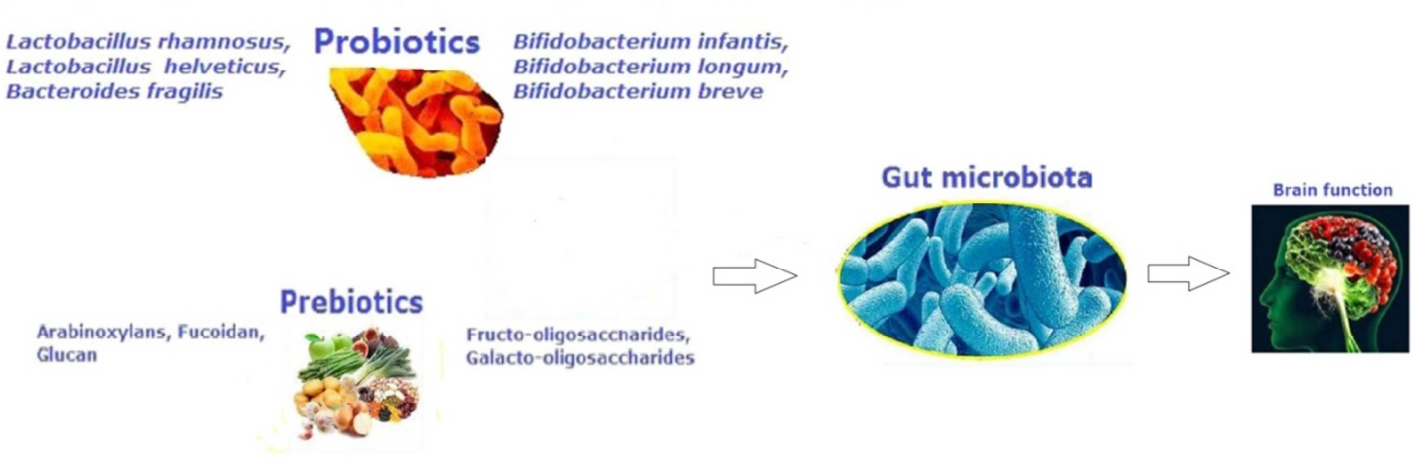

Figure 1. Different pro- and prebiotics therapies and their effect on gut microbiota and brain function.

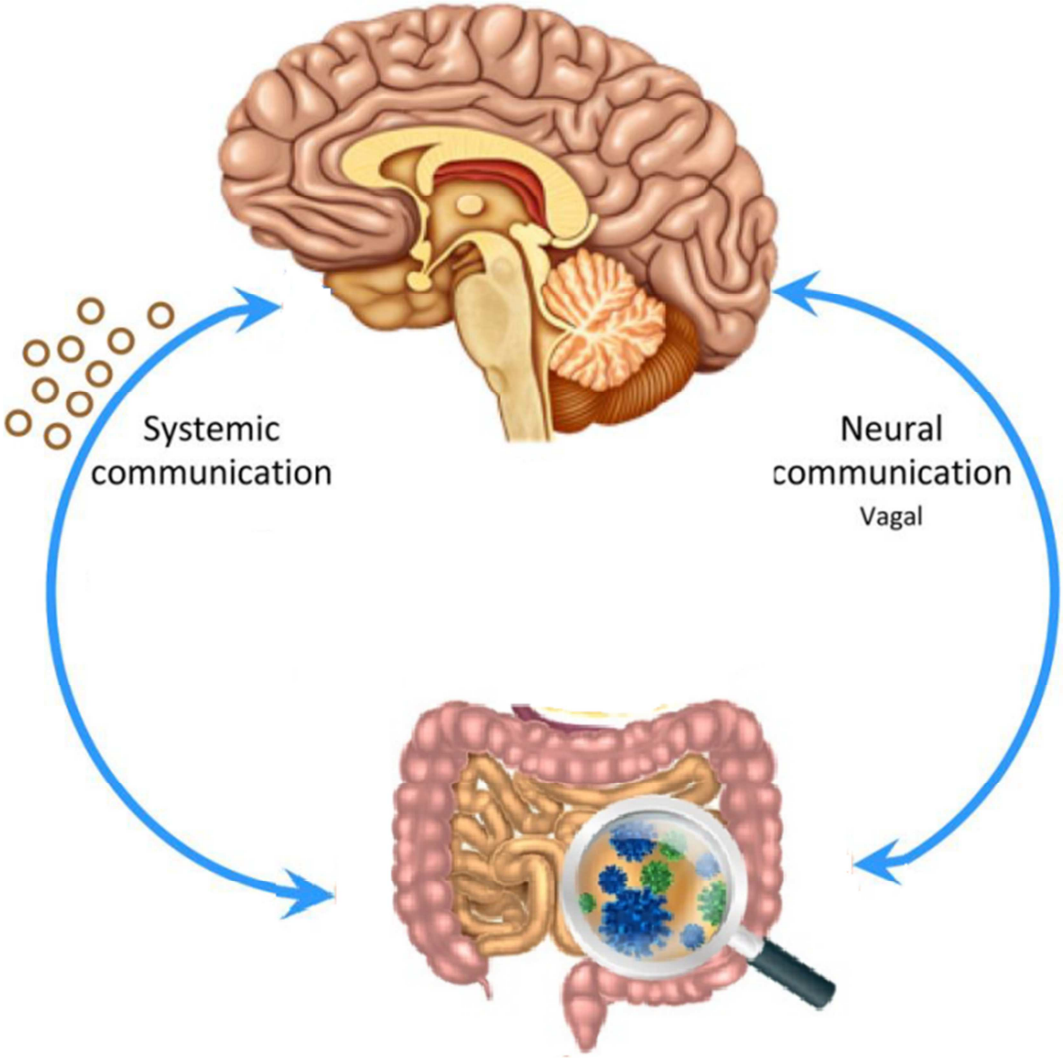

Figure 2. Bidirectional communication between intestine and brain.

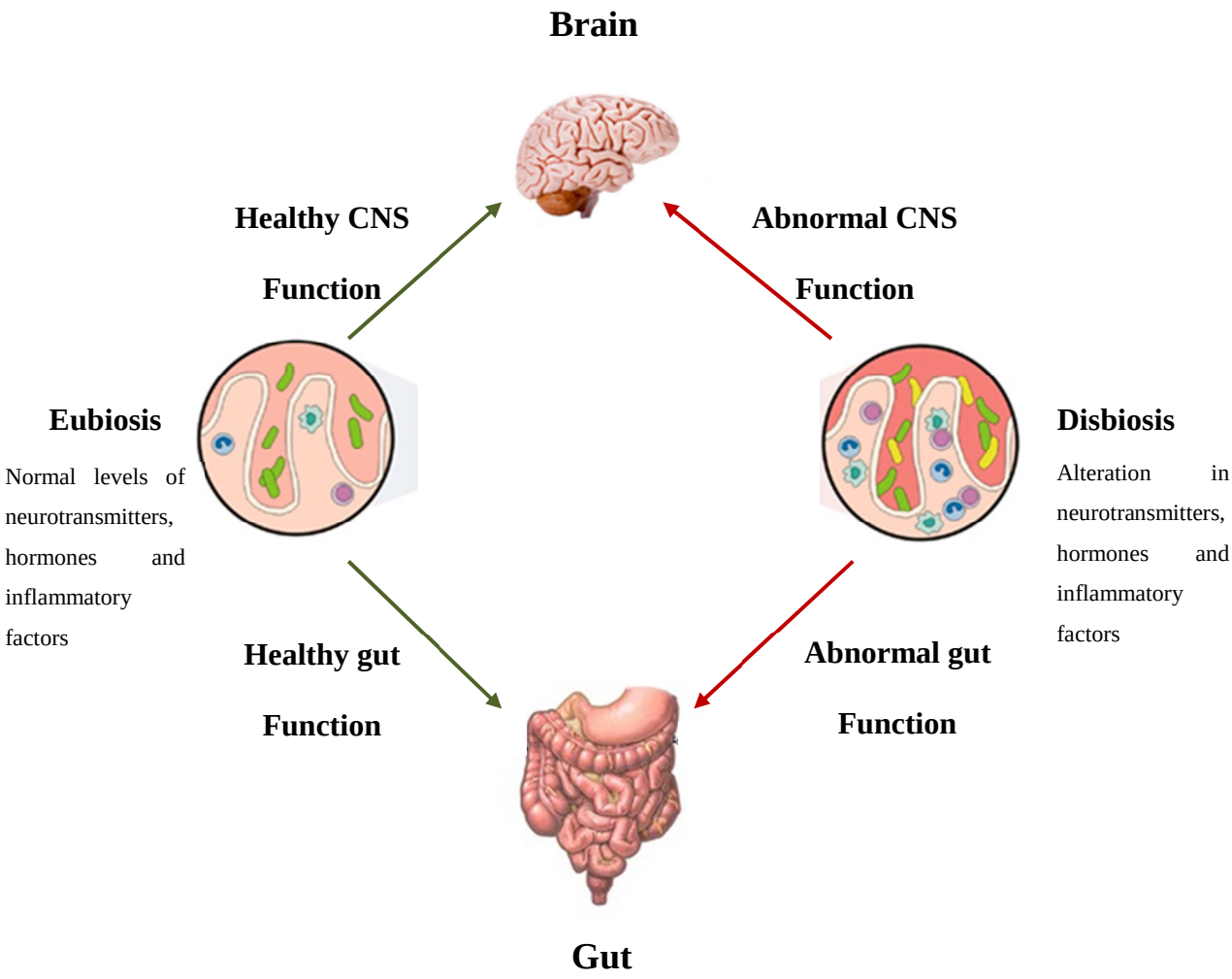

Figure 3. Alteration in neurotransmitters, hormones and inflammatory factors by gut dysbiosis can modify brain and gut functions.