THE GUT-BRAIN AXIS. EFFECT OF PROBIOTICS ON ANXIETY

MICHELE MAGNANO[1], IVANA VALASTRO[1], LUIGI RAMPELLO[2], MARCO VACANTE[1], MICHELE MALAGUARNERA[3], LIBORIO RAMPELLO[2], INNOCENZA ALESSANDRIA[1]
[1]Research Center "The Great Senescence", University of Catania, [2]Department of Neurosciences, University of Catania, Italy, [3]International PhD programme in Neuropharmacology, University of Catania, Italy

[L'asse cervello-intestino. Effetti dei probiotici sull'ansia]

ABSTRACT

The gut microbiota-flora is involved in neural development and functions, both peripherally in the enteric nervous system and centrally in the brain. It can influence the brain activity, the learning and memorizing processes and it could play an important role also in maintaining the health of the host. Gastrointestinal system and brain are not separate entities but rather are closely related. The first one is able to influence the brain by reducing anxiety, modulating the stress levels, the memory and learning process. Perturbations of these systems result in alterations in the stress-response and overall behavior. The gut micro-flora reduces the levels of ACTH compared to control mice; increases $GABA_{B1b}$ mRNA in the brain with increases in cortical regions (cingulate and prelimbic); reduces the expression of GABA mRNA in the hippocampus, amygdala, and *locus coeruleus*, in comparison with control-fed mice; reduces $GABA_{A\alpha2}$ mRNA expression in the prefrontal cortex and amygdala, but increased $GABA_{A\alpha2}$ in the hippocampus. It has also been showed an up-regulation in the expression of brain derived neurotrophic factor (BDNF) mRNA in the dentate gyrus of the hippocampus of these germ-free animals. The alterations of those receptor systems have a great impact in the behavior and control of anxiety. In germ-free mice it has been demonstrated that the absence of a conventional microbiota may result in a reduction in anxiety behavior in the elevated plus maze, a well validated model of anxiolytic action.

Key words: Probiotics, anxiety, bifidobacterium, gut-brain axis.

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Introduction

The term brain–gut axis describes the complex bidirectional communication system that exists between the central nervous system (CNS) and the gastrointestinal tract (GIT)[(1)]. In this context the commensal microbiota play a pivotal role, both on our general wellbeing and on the specific functioning of the brain-gut axis[(2-4)]. Perturbations of these systems result in alterations in the stress-response and overall behavior[(5)].

Recent studies on germ-free (GF) mice have shown that intestinal flora is important for the development of circuits and stress-related behaviors: for example the high co-morbidity between stress-related psychiatric symptoms such as anxiety with gastrointestinal disorders including irritable bowel disorder (IBS) and inflammatory bowel disorder (IBD) are further evidence of the importance of this axis[(1,6,7)]. The GF mice show reduced anxiety-like behavior in the elevated plus maze (EPM) in comparison to specific pathogen-free (SPF) mice, a phenotype that was accompanied by changes in plasticity-related genes in the hippocampus and amygdala, brain areas involved in cognitive processes and memory. This results provide support to the idea that normal healthy gut bacteria may influence the development of the CNS and thereby its function. The intestinal flora influences the brain not only producing metabolites or by altering the metabolism of key molecules such as tryptophan, but also by altering the activity and structure of the landscape tract[(8)].

In fact, the brain is constantly informed on the motility and characteristics of the biofilm, which consists of the microbial communities adhering to the intestinal mucosa. Animal studies have shown that the brain keeps track of the introduction of

invasive non-pathogenic bacteria in the ileocecal tract with an activation of the brainstem (nucleus of the solitary tract and other nuclei), in parallel with an increase in anxiety[9].

The levels of tryptophan in the blood are very low in lactose-intolerant people (dairy products are particularly rich in tryptophan) or disorders of the absorption of fructose. The levels of serotonin are often decreased in depressed patients, which not coincidentally are frequently treated with selective serotonin reuptake inhibitors - SSRI (e.g. fluoxetine, sertraline, citalopram, fluvoxamine, paroxetine). These drugs act on brain synapses preventing the normal physiological uptake and elimination of serotonin (the proportion reabsorbed by the presynaptic terminal is processed by monoamine oxidase, which oxidizes serotonin, then transformed into 5-Hydroxyindolacetic acid excreted in the urine)[10]. It has been showed that not only are bowel disorders related to psychiatric problems associated with stress, but also the bacteria in the digestive system have a direct effect on the brain. To reach this conclusion, the researchers administered to mice a broth containing Lactobacillus rhamnosus JB-1, a bacterium that occurs naturally in the human gut, showing that animals fed in this way showed less signs of stress, anxiety and depression. Mice that had received JB-1, in fact, let loose in a maze, expressed lower levels of corticosterone - a stress hormone - than those which had not been administered. There is increasing, but largely indirect, evidence pointing to an effect of commensal gut microbiota on the central nervous system (CNS)[11].

However, it is unknown whether lactic acid bacteria such as Lactobacillus rhamnosus could have a direct effect on neurotransmitter receptors in the CNS of normal, healthy animals.

GABA is the main CNS inhibitory neurotransmitter and is significantly involved in regulating many physiological and psychological processes. Alterations in central GABA receptor expression are implicated in the pathogenesis of anxiety and depression, which are highly comorbid with functional bowel disorders. Chronic treatment with L. rhamnosus (JB-1) induced region-dependent alterations in $GABA_{B1b}$ mRNA in the brain with increases in cortical regions (cingulate and prelimbic) and concomitant reductions in expression in the hippocampus, amygdala, and locus coeruleus, in comparison with control-fed mice. In addition, L. rhamnosus (JB-1) reduced $GABA_{A\alpha2}$ mRNA expression in the prefrontal cortex and amygdala, but increased $GABA_{A\alpha2}$ in the hippocampus. Importantly, L. rhamnosus (JB-1) reduced stress-induced corticosterone and anxiety- and depression-related behavior. Moreover, the neurochemical and behavioral effects were not found in vagotomized mice, identifying the vagus as a major modulatory constitutive communication pathway between the bacteria exposed to the gut and the brain.

Together, these findings highlight the important role of bacteria in the bidirectional communication of the gut-brain axis and suggest that certain organisms may prove to be useful therapeutic adjuncts in stress-related disorders such as anxiety and depression[12].

Probiotics and anxiety

Recent studies on probiotics administration focused on the role for microbiota in anxiety-like behaviors. Administration of L. helveticus R0052 and B. longum R0175 taken in combination showed anxiolytic-like activity in rats[13].

A study reported that chronic treatment with the probiotic L. rhamnosus (JB-1) over 28 days produced animals with lower levels of stress-induced corticosterone and reduced depressive behaviors in the forced swim test in addition to a less anxious phenotype in the elevated plus-maze (EPM), a well validated model of anxiolytic action.

The L. rhamnosus (JB-1) treated animals also showed alterations of $GABA_{B1b}$ mRNA in the brain with increased expression in cortical regions and decreased expression in the hippocampus, amygdala, and locus coeruleus as well as reduced $GABA_{A\alpha2}$ mRNA expression in the prefrontal cortex and amygdala and increased $GABA_{A\alpha2}$ in the hippocampus. Vagotomized mice did not manifested the neurochemical and behavioral effects of this bacterium, thus implicating the role of the vagus nerve in the direct communication between the bacteria and the brain[14]. A role for the gut microbiota in pain perception has also been proposed[15] and a study demonstrated that specific Lactobacillus strains could induce the expression of μ-opioid and cannabinoid receptors in intestinal epithelial cells and mimic the effects of morphine in promoting analgesia[16].

L. reuteri, a potential probiotic known to modulate the immune system, decreased anxiety as measured on the elevated plus maze as well as reduced the stress-induced increase of corticos-

terone in mice[14]. This probiotic may alter the mRNA expression of both GABAA and GABAB receptors in the central nervous system. Alterations of several kinds of receptors are associated also with anxious and depressive-like behaviors in animal models. Vagotomy in these animals prevented the anxiolytic and antidepressant effects of this bacterium as well as the effects on the central GABA receptors. This suggests that parasympathetic innervation is necessary for L. reuteri to participate in the microbiota-brain interaction. Previous studies have showed the bound between gut microflora and CSN[17,18].

Probiotic agents can modulate antidepressant-like and anti-anxiety behavior: Bifidobacterium infantis had antidepressant properties in the forced swim test, a well-established model in the evaluation of pharmacological antidepressant activity[1].

Studies in female germ-free mice (Neufeld et al, 2011) demonstrated that the absence of a conventional microbiota results in a reduction in anxiety behavior in the elevated plus maze[1]. It has also been showed an upregulation in the expression of brain derived neurotrophic factor (BDNF) mRNA in the dentate gyrus of the hippocampus of these germ-free animals. Those data seem to gainsay a study by Sudo et al.[20], which demonstrated that male germ-free mice have an increased stress response (although no basal changes in hypothalamic pituitary adrenal axis function were noted) coupled with decreased hippocampal and cortical BDNF, and decreased NR1 (hippocampus) and NR2A (hippocampus and cortex)[1]. Gender may play a role in such effects. In fact, neurochemical and endocrine effects of growing up in a germ-free environment are only evident in male animals.

It is clear that the brain-derived neurotrophic factor is crucial for supporting neuronal survival and encouraging the growth and differentiation of new neurons and synapses and is involved in the regulation of multiple aspects of cognitive and emotional behaviors[20]. There is a clear relationship between chronic stress states, major depression and BDNF; the link between anxiety and BDNF appears to be complex with contrasting results on correlation between hippocampal levels and anxiety.

A strategy employing antibiotic-induced dysbiosis of the microbiome (through adiministration of a cocktail consisting of neomycin, bacitracin, and the antifungal agent pimaricin), resulted in mice with less anxiety-like behaviors in both the step down box and the light/dark box tests. Bercik et al. reported altered BDNF levels in the amygdala and hippocampus; discontinuation of the antibiotic cocktail restored the normal behavioral profile of the animals[21-22].

Similarly, perturbation of the microbiota through Citrobacter rodentium increased anxiety-like behavior in mice 7-8 h post infection as measured in the hole board open field apparatus[23] and produced stress-induced memory dysfunction 10 and 30 days post infection.

Interestingly, and somewhat discordant with the behavioral data, an increase in the stress hormone corticosterone was noted in the plasma of the germ-free mice. Moreover, a decrease in the NR2B subunit of the NMDA receptor in the amygdala, but not hippocampus, of germ-free animals was observed thus maybe contributing to the anxiolytic-like effect noted. In addition, a down-regulation of the 5-HT1A auto receptor was also present in the dentate gyrus of the germ-free mice. The administration of broad-spectrum antibiotics, frequently used in both adult and pediatric clinical practices, may reduce the biodiversity of the fecal microbiota and delay the colonization by some probiotic strains, e.g., lactobacilli.

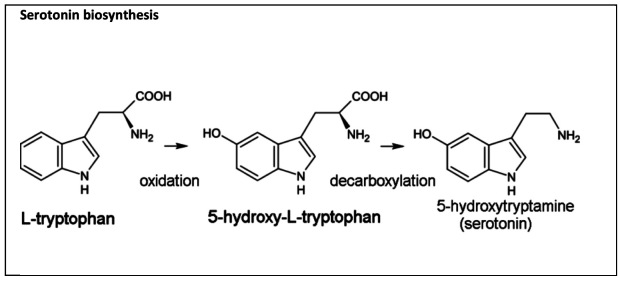

Figure 1: Serotonin biosynthesis is successful when the intestinal microenvironment is balanced. In fact, when intestinal dysbiosis occurs, the prevalence of bifid-acidophilic bacteria (known as lactic acid fermenters), changes the intestinal pH (usually 5.5- 6, or slightly alkaline) and coliforms are no longer able to function properly. The enzyme tryptophan hydroxylase influenced by such changes in pH thus directing the biosynthetic process towards the production of toxic amines.

Conclusions and therapeutic perspectives

The positive role of probiotics has been demonstrated in the treatment of various diseases, for example it has been showed that Bifidobacterium + fructo-oligosaccharides (FOS) decreased ammonia levels in blood and brain and therefore had a role in the treatment of patients with hepatic encephalopathy[24].

Lactobacillus rhamnosus	• increases $GABA_{B1b}$ mRNA in the brain with increases in cortical regions (cingulate and prelimbic); • reduces the expression of GABA mRNA in the hippocampus, amygdala, and locus coeruleus, in comparison with control-fed mice. • reduces $GABA_{A\alpha 2}$ mRNA expression in the prefrontal cortex and amygdala, but increased GABAAα2 in the hippocampus. • reduces stress-induced corticosterone and anxiety- and depression-related behavior.
Bifidobacterium helveticus R0052 and B. longum R0175	• anxiolytic-like activity • prevents learning and memory • increases anxiety-like behavior in mice • produce stress-induced memory dysfunction 10 and 30 days post infection
Citrobacter rodentium	• prevents learning and memory • decreases ammonia levels in blood and brain

Table 1: Effects of probiotic strains on brain activity.

There is an increasing need to understand the molecular, cellular and physiological basis of enteric microbiome-gut-brain communication. The conventional microbiota seems to modify the anxiety levels through alterations in central neurochemistry but the mechanism and the practical implications are still unknown. Limitations in the research conducted so far, are represented by the possibility to translate animal studies to human disease[25]. Moreover, the germfree mice used in the study are different from colonized mice in many aspects. The differences in brain function between germfree mice and colonized mice may be a reflection of maturation of mucosal and systemic changes rather than direct effects on the brain.

In conclusion, a possible role for the microbiota in maintaining the normal brain function offers the intriguing possibility that the therapeutic targeting of the gut microbiome might be a viable strategy in the treatment of CNS disorders[26].

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