Psychobiotics

Alper Evrensel, Barış Önen Ünsalver
and Mehmet Emin Ceylan

Abstract

Psychobiotics are live bacteria that directly and indirectly produce positive effects on neuronal functions by colonizing into the intestinal flora. Preliminary studies, although in limited numbers, have found that these bacteria have anxiolytic and antidepressant activities. No research has yet been published on the antipsychotic efficacy of psychobiotics. However, these preliminary studies have opened up new horizons and raised the idea that a new class is emerging in psychopharmacology. About 70 years have passed since the discovery of chlorpromazine, and while the synaptic transmission is understood in almost all details, there seems to be a paradigm shift in psychopharmacology. In recent years, the perspective has shifted from synapse to intestinal microbiota. In this respect, germ-free and conventional animal experiments and few human studies were examined in a comprehensive manner. In this article, after a brief look at the history of contemporary psychopharmacology, the mechanisms of the gut–brain relationship and the evidence of metabolic, systemic, and neuropsychiatric activities of psychobiotics were discussed in detail. In conclusion, psychobiotics seem to have the potential for treatment of neuropsychiatric

A. Evrensel (✉)
Department of Psychiatry, Uskudar University, NP Brain Hospital, Saray Mah. Ahmet Tevfik İleri Cad. No: 18 PK, 34768 Umraniye, Istanbul, Turkey
e-mail: alperevrensel@gmail.com

B. Ö. Ünsalver
Department of Medical Documentation and Secretariat, Vocational School of Health Services, Uskudar University, Istanbul, Turkey
e-mail: onenunsalver@gmail.com

M. E. Ceylan
Departments of Psychology and Philosophy, Uskudar University, Istanbul, Turkey
e-mail: m.eminceylan@yahoo.com

Y.-K. Kim (ed.), *Frontiers in Psychiatry*, Advances in Experimental Medicine and Biology 1192, https://doi.org/10.1007/978-981-32-9721-0_28

disorders in the future. However, there are many questions and we do not know the answers yet. We anticipate that the answer to these questions will be given in the near future.

Keywords
Probiotics · Psychobiotics · Microbiota · Psychopharmacology · Gut–brain axis · Dysbiosis

Introduction

In modern medicine, there have been four important developments in the field of treatment. These are: Louis Pasteur and Robert Koch's bringing in vaccine to the medicine, the discovery of penicillin, use of oral contraceptives, and psychopharmacology [1]. The term "psychopharmacology" has been first visible in the literature under the title of pharmacologist David Macht's article [2]. Although morphine, codeine, cocaine, bromine, barbiturates, and amphetamines have been discovered until 1920 and have been used in various psychiatric indications, the discovery of chlorpromazine in 1951 is the milestone of psychopharmacology as a modern scientific discipline [3]. The discovery of chlorpromazine not only made a revolutionary difference in the treatment of psychotic patients but also paved the way for an understanding of synaptic transmission and subsequently identified neurotransmitters in the central nervous system [4].

Understanding the synaptic transmission, determining the neurobiological basis of psychiatric disorders and controlling the disease symptoms with drugs is the first paradigm shift in psychopharmacology. With the discovery of chlorpromazine, psychiatry has become one of the other medical disciplines, and psychiatrists have become true physicians rather than being therapists listening only to the problems of their patients [3].

A paradigm shift is being experienced in recent years. In the etiopathogenesis of neuropsychiatric disorders, in addition to synaptic neurotransmission, the role of intestine–microbiota–immune system–brain interactions have begun to be better understood [5]. In recent years, much evidence has been obtained on bidirectional interaction between intestinal microbiota and brain, impaired microbiota composition (dysbiosis), leaky gut, effects of germ-free conditions on neurodevelopment, and neuroinflammation in preclinical and clinical studies [5]. In the light of this evidence, microbiota-based treatments have emerged. The main heading of this treatment is probiotics or, in other words, psychobiotics.

Current Paradigm in Psychopharmacology

Although the discovery of chlorpromazine has been a short while ago, about 70 years, the roots of modern psychopharmacology go back further. Roughly two periods can be defined before the discovery of chlorpromazine. In the first

period (1800s), pharmacological agents were used for the first time in the control of behavioral disorders through the discovery of drugs such as morphine, potassium bromide, chloral hydrate, hyoscine, and paraldehyde. With the introduction of thiamine (vitamin B1), nicotinic acid (niacin, vitamin B3), and penicillin in the first half of the twentieth century, neuropsychiatric disorders related to beriberi disease, psychosis related to pellagra and dementia caused by syphilis have been taken under control, respectively, and the incidence in psychiatric hospitals has decreased significantly. In addition, barbiturates were discovered in this period, and the antimanic property of lithium (1949) was noticed [4]. In order to understand the paradigm shift created by chlorpromazine, it is useful to take a brief look at the period before chlorpromazine.

Before Chlorpromazine

The first fruit of modern psychopharmacology is for sure morphine. Friedrich Wilhelm Adam Sertürner succeeded in decomposing morphine from opium in 1805 and observed that this substance caused sleep in animals, therefore, by being inspired by the ancient Greek god of sleep *Morpheus*, he named it morphine [1]. Morphine has been used for rapid control of aggression and agitation in 1860s [4]. Again, during these years, potassium bromide has been used in the treatment of epilepsy and anxiety [6]. Chloral hydrate is also used for sedation and behavior control, like morphine and potassium bromide [4].

Barbiturates produced in 2500 different derivatives since being synthetized in 1903 have been the leading drug in the treatment of insomnia for nearly 50 years [7]. In the treatment of schizophrenia; electrically induced convulsions (electroconvulsive therapy, ECT) have been used since the mid-1930 s, and camphor or pentetrazol (cardiazol) induced convulsions and insulin coma therapy have been applied throughout the 1940s [4]. From a different angle, these treatment methods are also a reflection of the desperation in psychopharmacology.

Chlorpromazine

Phenothiazine, a precursor of chlorpromazine, was produced in 1883 for use in the blue dye industry [4]. Paul Charpentier et al., working in the French pharmaceutical company Rhone-Poulenc laboratories, succeeded in synthesizing chlorpromazine from the phenothiazine on December 11, 1951, and announced their general anesthetic properties [8]. Surgeon Henri Laborit and his colleagues used it for anesthetic purposes, and published its sedative activity without loss of consciousness in an article named “A new vegetative stabilizer” [9]. The first case treated with chlorpromazine at the psychiatry ward (Jacques Lh, a male, agitated, psychotic manic patient) has been reported at Laborit’s request [10]. One month after this article, on March 22, 1952, Pierre Deniker and Jean Delay started clinical research and published six articles in 6 months [3]. Chlorpromazine has

been recognized and used in psychiatry because of these articles. In November 1952, chlorpromazine was registered in France (inspired by "large in action") as *Largactil* [3].

After Chlorpromazine

Simultaneously with the introduction of chlorpromazine, the mechanisms of synaptic transmission have begun to be understood. At this point, the discovery of spectrophotofluorometer (SPF) is a revolutionary progress [11]. Electrical and chemical activity in the synaptic range has been observed through SPF, and six neurotransmitters (acetylcholine, dopamine, serotonin, gamma-aminobutyric acid, norepinephrine, and substance P) have been identified [3]. In 1963, it has been understood that potent antipsychotics block catecholamine receptors [12]. The information obtained by observing the effect of chlorpromazine on neuronal transmission through SPF pave the way for both the understanding of schizophrenia physiopathology and the discovery of other drugs [13]. Today, around 112 psychotropic drugs (32 antipsychotics, 25 antidepressants, 20 anxiolytics/sedatives/hypnotics, 4 chemical dependency adjuncts, 4 monoamine oxidase inhibitors, 6 mood stabilizers, 8 stimulants, and 17 miscellaneous drugs) are in clinical use (*Last updated: July 10, 2018)* [14].

Paradigm Shift in Psychopharmacology

Over the last half century, enormous knowledge to illuminate the etiopathogenesis of neuropsychiatric disorders and to offer treatment options has been accumulated. The vast majority of this information is associated with neuronal transmission. Dopamine hypothesis in psychotic disorders, serotonin hypothesis in anxiety/depression still remains valid [15]. However, dopamine is not the only neurotransmitter associated with psychosis. Increased evidence in recent years show that serotonin and glutamate are associated with dopamine, the problems in these two neurotransmitter systems (serotonin hyperactivity in 5-HT_2A receptors on glutamatergic neurons in the cerebral cortex and NMDA receptor hypoactivity in GABAergic interneurons in the prefrontal cortex) cause dopamine hyperactivity in D_2 receptors at mesolimbic pathway [16]. Similarly, serotonin plays an important role in peripheral tissues (gastrointestinal system, hematopoiesis, bone metabolism, metabolic homeostasis) and especially in immune system functions [17, 18]. Gardner and Boles, using the term "mitochondrial psychiatry", proposed a new model of serotonergic insufficiency, mitochondrial dysfunction, and inflammation in the pathogenesis of depression and affective spectrum disorders [19, 20]. Han et al. argued that commensal microorganisms living in the intestines communicate with the host mitochondria and thus, it is possible to live long and healthy [21]. Anderson also emphasized the importance of the relationship between

mitochondria and melatonin, inflammation, sirtuin, tryptophan metabolites, DNA repair, and oxidative/nitrosative stress [22].

The focus of psychopharmacology is shifting from synaptic neurotransmission to the peripheral system, intestinal microbiota, mitochondria, immune system, and neuroinflammation. In this period, psychobiotics stand out as the new class of psychotropic drugs [23, 24].

Gut–Brain Communication

Human body is a complex ecosystem where our eukaryotic own cells and prokaryotic commensal microorganisms live together [5]. The term "psychobiotics" has been proposed to describe viable microorganisms colonized in the intestines that have positive effects on neuropsychiatric functions in various ways [24]. Psychobiotics interact with the body and especially the brain through the endocrine, metabolic, and immune system by virtue of the hormones, metabolites, and immune factors they secrete [25]. This bidirectional interaction between the gut and the brain, defined as the "gut–brain axis", is not yet fully elucidated [26]. However, according to the information obtained from a large number of animal and human studies, psychobiotics are effective on brain functions by secreting neuroactive metabolites (serotonin, catecholamine, gamma-aminobutyric acid [GABA], acetylcholine) as well as short-chain fatty acids (SCFAs) [27]. In the presence of dysbiosis, pathogenic live bacteria or bacterial components (endotoxins with lipopolysaccharide and peptidoglycan structure) that are involved in the systemic circulation due to leaky gut can cause a low-grade immune reaction. These components that pass through the blood–brain barrier can induce neuroinflammation by activating microglia [28]. This interaction is discussed below in detail.

Microbiota–Immune System Interaction

In recent years, with the emergence of evidence that the brain has its own lymphatic drainage system, the neuroinflammation hypothesis has become more important [29]. The main function of the immune system is to find and destroy germs. Molecular elements of microorganisms (nucleic acids, cell wall components in lipopolysaccharide, flagella, etc.) activate immune system cells [25]. Microbiota bacteria contact the immune system through pattern recognition receptors (PRRs). The most important member of the PRRs is toll-like receptors (TLRs) [26].

Anti-inflammatory cytokines such as interleukin-10 (IL-10) are produced when PRRs are activated by commensal bacteria [30]. For example, *Bifidobacterium infantis* and *Lactobacillus GG* increase the level of IL-10 in human and reduce the level of pro-inflammatory cytokine and repair impaired blood–brain barrier permeability due to inflammation [31]. In addition, beneficial bacteria block the

pathogenic pro-inflammatory process by activation of TLR-2 and TLR-4 [32]. Pro-inflammatory cytokines induced by pathogens can affect neuronal functioning in several ways. For example, they can change the level of neurotransmitters in the brain [33]. They may also induce prostaglandin synthesis that provokes pro-inflammatory process through another way [34]. Psychobiotics play an important role in reducing low-level neuroinflammation by decreasing the levels of pro-inflammatory cytokines in the systemic circulation.

However, in a trial by Bercik et al. on mice infected with *Trichuris muris* parasites, *Bifidobacterium longum NCC3001* reduced anxiety levels and normalized low hippocampal BDNF levels without any change in cytokine levels [35]. This finding suggests that non-cytokine mechanisms are also present in the probiotic effect.

Microbiota–Enteric Nervous System Interaction

Myenteric neurons are located just below the enterocytes and can be in direct contact with microbiota bacteria as it is close to the lumen [36]. Microbiota may be effective on the electrical activity of the enteric nervous system. For example, Bifidobacterium longum exhibits anxiolytic activity by reducing the action potential of myenteric neurons [37]. In the study of Ma et al., psychobiotic treatment (*Lactobacillus reuteri*) prevented hyperexcitation of dorsal root ganglion neurons in the colon due to noxious stimulation [38]. The excitability levels of myenteric afterhyperpolarization neurons of germ-free mice were found to be lower [39]. In another study, various abnormalities of the enteric nervous system of germ-free mice (increased number of myenteric nitrergic neurons, less neuronal density in ganglions) has been found [40]. By altering ion transport in the colon mucosa and submucosa, microbiota also affects the operation of the myenteric nervous system and peristalsis [41]. Microbiota bacteria play an important and decisive role in the enteric nervous system not only on neurons but also on homeostasis and control of glial cells in lamina propria [42].

In addition, there are neurotransmitters in the intestinal bacteria metabolites. *Bacillus* produces dopamine and noradrenaline, *Bifidobacteria and Lactobacillus* produce GABA, *Escherichia* produces noradrenaline and serotonin, and *Enterococcus* and *Streptococcus* produce serotonin [43]. Although there is no evidence, these neuroactive amines have the potential to affect synaptic activity in the enteric nervous system.

The Role of Nervus Vagus

Nervus vagus is the most important linkage regulating parasympathetic activity between brain and gastrointestinal system and plays a direct role in immune system functions [44]. Stress [45], nutrition [46], and exercise [47] affect vagal activity. Nervus vagus stimulation produces anti-inflammatory [44], analgesic [48],

antiepileptic [49], antidepressant, and anxiolytic [50] effects. On the contrary, there are indications that antidepressant and anxiolytic drugs also affect vagal activity [51].

The effectiveness of psychobiotics has not been observed in many vagotomy applied animal experiments [37, 52]. However, in an experiment on mice, Bercik et al. also found that neurobehavioral effect of *Lactobacillus* continued after vagotomy [53]. Apparently, nervus vagus is one of the pathways responsible for psychobiotic activity in the gut–brain axis.

The Effect of Stress on Leaky Gut

The intestinal epithelial cell (enterocytes) is the largest mucosa in the human body due to its ciliary structure, and its total surface area can reach the size of a tennis court. Tight junction proteins that bind enterocytes make the mucosa intact. The mucous layer secreted by the mucosa is a strong physical barrier between bacteria and the toxic material and the host. Leaky gut may occur for various reasons (stress, glucocorticoids, dysbiosis, and endotoxins). The pathogenic microorganisms and their toxic metabolites in the lipopolysaccharide structure enter into the bloodstream and cause a pro-inflammatory effect. The role of this process in the etiopathogenesis of depression is quite clear [54]. Psychobiotics caused leaky gut-reducing and anti-inflammatory effect in animal experiments [55–57].

The leaky gut effect of glucocorticoids is reversed by psychobiotics and ultimately it results in an anti-inflammatory effect, which is significant in neuropsychiatric disorders accompanied by low-level neuroinflammation. However, it was found that psychobiotics had beneficial effects in cases where leaky gut and associated neuroinflammation are not observed [58, 59].

Bacteria-Induced Active Metabolites and Short-Chain Fatty Acids (SCFAs)

The human genome does not encode enzymes that digest plant-derived polysaccharides. The digestion of these foods in the diet is carried out by enzymes synthesized by microbiota [60]. Digestion of plant-derived polysaccharide fibers results in production of SCFAs (acetate, butyrate, propionate, lactate) [61]. SCFAs are absorbed from the colon, enter into the systemic circulation, and reach the liver and muscles, and then they are involved in the metabolic functions and are perhaps the most important microbiota metabolites [61]. A small amount of SCFAs reaches the central nervous system by crossing the blood–brain barrier [62]. Although the effect of this small amount on synaptic transmission is not yet clear, experimentally it has been shown that high-dose fatty acids have an agonist effect on free fatty acid receptors [63] and change neuromodulation via epigenetic mechanisms [64, 65]. This may have important consequences for neuropsychiatric functions.

Microbiota bacteria metabolites are not limited to SCFAs. Metabolites of microbiota, which are involved in blood circulation, have a very important role in neuroimmune disorders and neuroinflammation [66]. For example, the role of metabolites synthesized from tryptophan (such as serotonin and antioxidant-featured indoxyl sulfate and indole-3-propionic acid) has been shown in germ-free animal experiments [67]. The plasma tryptophan amounts of germ-free mice are higher than that of normal (microbiota-containing) mice, whereas the plasma serotonin levels of normal mice are approximately three times higher [68]. This result may be interpreted as the effect of microbiota bacteria have an effect on the metabolism of enterochromaffin cells secreting serotonin in the intestine.

However, the information obtained so far in this area is unfortunately insufficient and is limited to animal experiments. After it is uncovered in detail from which substrate which metabolites are produced by human microbiota bacteria and which effects they have on all organ systems, its role in maintenance of health and etiopathogenesis of diseases will be clarified.

Psychobiotics

In the scientific literature, Ilya Ilyich Mechnikov (Elie Metchnikoff) is the first person who mentioned the beneficial effects of commensal microorganisms on the host, without using the name psychobiotics [69]. The Nobel Prize in Physiology or Medicine 1908 was awarded to Mechnikov in recognition of his work on immunity [70]. Probably the first research has been published in 1910 on the efficacy of probiotic bacteria in the treatment of depression [71]. After a long period of silence, as summarized in Fig. 1, extensive clinical and preclinical studies have been started to be conducted in recent years on the effectiveness of probiotics [23, 72].

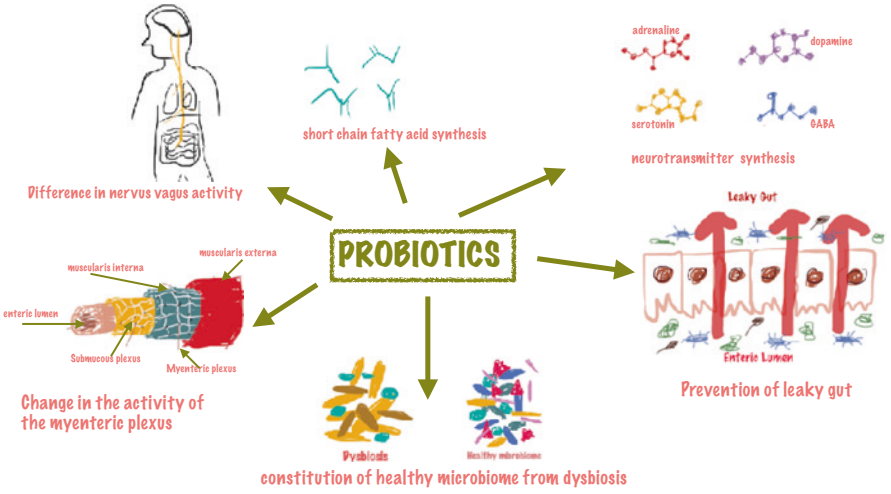

Fig. 1 Overview of the effectiveness of probiotics

Animal Studies

Two of the effective studies conducted in this field have been performed by Desbonnet et al. In the first of these, adult male Sprague-Dawley rats were divided into two groups; 12 for experiment group and 8 for control group. *Bifidobacterium infantis* was administered orally to the experiment group for 14 days. Then, forced swim test and various blood analyses (cytokine, plasma tryptophan, brain monoamine, vasopressin, corticotrophin-releasing factor) were applied to the rats. *Bifidobacterium infantis* showed antidepressant-like activity in serological analyzes, even though there was no change in the test performance of two groups [73]. In their second study, the researchers tested the antidepressant activity of this psychobiotic bacterium by comparing it with citalopram (a selective serotonin reuptake inhibitor antidepressant). At the end of the experiment, no difference has been found between citalopram and *Bifidobacterium infantis* antidepressant activity [74].

Psychobiotics can cause a decrease in anxiety scores as well as in depression scores. In an experiment by Bravo et al., BALB/c mice applied *Lactobacillus rhamnosus JB-1* showed lower scores in forced swim test and elevated plus maze test forced swim test [75]. Additionally, $GABA_{B1b}$ receptor expression levels decreased in amygdala and hippocampus and increased in cingulate and prelimbic areas in the experiment group. These findings can be interpreted as psychobiotics can exhibit anxiolytic activity by modulating inhibitory neurotransmitter (GABA) functions. Janik et al. measured the efficacy of *Lactobacillus rhamnosus JB-1* on brain neurotransmitter levels of BALB/c mice with magnetic resonance spectroscopy (MRS) [76]. GABA, glutamate, and aspartate levels were found to be high in mice fed with psychobiotics. This is the first study showing that probiotics increased the level of central glutamate. Another interesting finding is the difference between the duration of neurotransmitters' elevation and staying high after probiotic administration.

In another study with similar design, the efficacy of *Mycobacterium vaccae* as a psychobiotic bacterium was tested using the Hebb–Williams style complex maze [77]. Test performances of the probiotic applied group were higher than the control group. However, the life span of this effect was limited to 1 week. This finding is significant even if it contradicts some previous studies on the persistent colonization of exogenous probiotics in the intestine [78, 79]. In the study of Liang et al., the efficacy of *Lactobacillus helveticus* and citalopram was compared in various biochemical analyzes and anxiety tests (elevated plus maze and open-field test) [80]. Psychobiotic-fed rats had lower levels of anxiety and higher memory performance, lower hypothalamo-pituitary activity, and higher anti-inflammatory markers than control group. These findings are similar to citalopram activity.

In a study by Moya-Perez et al., the C57BI/6 J male pups under the model of chronic stress induced by maternal separation were divided into two groups [81]. The effects of *Bifidobacterium pseudocatenulatum CECT 7765* were evaluated on the 21st day and on the 41st day of postpartum through various analyzes and tests (corticosterone, neurotransmitters, cytokines, fecal microbiota analysis, elevated

plus maze, open field, acute immobilization). As a result of the experiment, it was found that the bacteria weakened the acute stress response, showed anti-inflammatory effect, decreased the level of anxiety and repaired intestinal dysbiosis.

In a very recent study, the antidepressant-like efficacy of *Lactobacillus rhamnosus JB-1* and fluoxetine was tested on two different mouse strains (BALB/c and Swiss Webster) [82]. The tail suspension test and the corticosterone response to an acute restraint stressor were applied to the laboratory animals. *Lactobacillus rhamnosus JB-1* and fluoxetine showed antidepressant-like behavior in both tests in BALB/c mice (n = 46). However, Swiss Webster mice (n = 36) did not respond to both treatments. In the light of these results, it is seen that the selection of laboratory animal strain in the studies on psychobiotics is of importance on the results.

Human Studies

Although the findings from animal experiments give hope for human studies, they may not always meet expectations. Therefore, it is necessary to increase studies on human and discuss their results. Let's take a look at the small number of human studies in this context.

In an early study on patients with irritable bowel syndrome, 75 participants (48 females, 27 males) received *Lactobacillus salivarius UCC4331, Bifidobacterium infantis 35624* or placebo for 8 weeks. The rates of interleukin 10/interleukin 12 before and after treatment were compared. It was found that this ratio, which was low before treatment and indicated a pro-inflammatory condition, was normalized in the group receiving *Bifidobacterium infantis 35624* [31]. In the following period, many studies showing the probiotic efficacy of the *Lactobacillus* family has been published. One of these, published in 2007, investigated the effect of *Lactobacillus casei Shirota* or a placebo-containing milky drink on mood and cognitive functions after drinking for 3 weeks [83]. No statistically significant difference was found between the two groups as the result of self-report tests (POMS, Wechsler Memory Scale, NART). However, the group with the lowest mood scores was found to be happier after probiotic supplementation. Surprisingly, the probiotic group had lower scores in the tests that measure cognitive functions.

Messaoudi et al. tested the effects of *Lactobacillus helveticus R0052* and *Bifidobacterium longum* on mood and cognition in two randomized controlled trials (RCT) [58, 84]. In experiment group, self-report tests (Hopkins Symptom Checklist-90, Hospital Anxiety and Depression Scale, Coping Checklist) showed a decrease in depression and anxiety scores, but the cognitive function scores did not decrease.

Different *Bifidobacterium* and *Lactobacillus* strains were given to healthy volunteers in another RCT [85]. The participants applied Leiden Index of Depression Sensitivity, The Beck Depression Inventory, The Beck Anxiety Inventory (n = 40) showed a decrease in sad mood levels. Similarly, healthy medical faculty students (n = 47) who had a *Lactobacillus* bacterium (*L. casei strain Shirota*) for 8 weeks before the exam were found to have lower plasma cortisol levels compared to

placebo [86]. Another study that examined the effects of another *Lactobacillus* bacterium (*L. gasseri OLL2809 LG2809*) on student-athletes (n = 44) found decreased symptoms of fatigue and improved mood [87]. These findings can be interpreted as psychobiotics may be useful in reducing performance anxiety.

In the study of Tillish et al., healthy volunteers who were given fermented milk containing psychobiotics (*Bifidobacterium animalis subsp Lactis, Streptococcus thermophiles, Lactobacillus bulgaricus, and Lactococcus lactis subsp Lactis*) were examined under functional magnetic resonance imaging (fMRI). In the experiment group, reduced activity in the emotional and somatosensory centers (insula, somatosensory cortex, and periaqueductal cortex) was found [88]. The findings of this study were duplicated by an RCT on patients with irritable bowel syndrome (n = 44) [89].

However, in a recent study on healthy male volunteers, *Lactobacillus rhamnosus* were found to be similar to placebo in terms of stress and cognitive tests (Cambridge Neuropsychological Test Automated Battery, Socially Evaluated Cold Pressor Test, electroencephalography, cortisol, and cytokine analysis). This finding may be caused by the small sample size (n = 29) [90].

Future Directions and Knowledge Gaps

Although promising evidence has been obtained about psychobiotics, there are still many uncertainties. For example, it cannot be said that psychobiotics change the composition of microbiology permanently and comprehensively. However, there are only short-term studies in this field. There is a need for long-term (months or even years) studies in which fecal samples are examined. Moreover, the composition of the microbiota is dynamic and changes with aging [91]. There are no studies on the effectiveness of psychobiotics in different age groups. Another issue is which dose to be applied in which indication. Psychobiotics may also have a therapeutic range, as in medicines. There may be unresponsiveness due to low dose applications, or there may be side effects caused by high doses. In addition, it is not clear when physiological, metabolic, immunological, and neuropsychiatric effects occur after oral administration of psychobiotics. The number of preliminary studies is quite small. Another problem is; after ending the application of psychobiotics, it is not clear how long the activity lasts. Studies on this subject are limited. This information should be clarified by monitoring clinical and biological parameters for a long time.

The simultaneous increase of the neurotransmitters that interact with each other in the brain (for example, GABA and glutamate) suggests that the total effect can vanish [76]. Local and specific effects shall be emphasized and functional responses of neurotransmitter changes shall be monitored. Evidence of improvement in cognitive functions through psychobiotics has been obtained from preclinical studies [77]. Even if it is not repeated, there is even a study reporting that it causes cognitive impairment [83]. The lack of information in this area needs to be addressed through additional human studies.

Another important problem is the uncertainty about why some bacterial strains have a psychobiotic effect while others do not. Germ-free animal experiments are ideal for separating the neurophysiological effects of a single bacterial strain. However, it should be clarified whether the psychobiotic effect is caused by the synergy of singular or bacterial strains (*quorum sensing*) [92]. Factors that can affect psychobiotic outcome and their impact strength are not known clearly. Some of them may be age, gender, diet, and genotype.

However, there is no study on the interactions of psychobiotics and psychotropic drugs. Most psychotropic drugs have antibiotic activity [93]. For this reason, adding psychobiotics to psychotropics may vanish positive effects. There is a need to clarify which psychotropic has an antibiotic effect on which psychobiotic.

Conclusion

Jacques Lh, the first patient treated with chlorpromazine, received total of 855 mg of medication during his 20-day treatment [10]. Today we know that this dose (40–50 mg/day) is too low for antipsychotic activity [15]. In addition to its role in the synaptic transmission, other activities of chlorpromazine have been discovered. New evidence suggests that it (and many other psychotropics) has antibiotic/antifungal activity [94]. For example, SSRIs kill bacteria through efflux pump inhibition, MAO inhibitors through cell wall synthesis inhibition and tricyclic antidepressants through DNA gyrase inhibition by antiplasmic action (antimicrobial effect) [93]. Here, it is important to remind that the first antidepressant molecule is an antituberculosis drug, iproniazid (an MAO inhibitor) [95]. Perhaps today, distinguished and active psychotropics change the microbiota composition in addition to their monoaminergic and synaptic effects (dysbiosis or restoration) [54].

Thus, about 70 years of the reign of psychotropics has begun to be questioned [96]. The paradigm in psychopharmacology may be changing. The viewpoint seems to shift from synapse to intestinal microbiota and immune system [97]. Although more than 100 years have passed since the discovery of psychotropic activities, the power and role of psychobiotics have just begun to be understood. However, there are many questions to be answered. By answering these questions in the coming years, psychobiotics may be used as a new class of psychotropics.

Acknowledgements We would like to thank Dr. Barış Önen Ünsalver for preparing the image used in this review.

References

1. Köknel O. At the fiftieth anniversary the history of modern psychopharmacology and psychopharmacological researches in Turkey and in the world. Bull Clin Psychopharmacol. 2002;12(4):201–10.
2. Macht DL. Contributions to psychopharmacology. Johns Hopkins Hosp Bull. 1920;31:167–73.

3. Ban TA. Fifty years chlorpromazine: a historical perspective. Neuropsychiatr Dis Treat. 2007;3(4):495–500.
4. Ban TA. Pharmacotherapy of mental illness. A historical analysis. Progress Neuro Psychopharmacol Biol Psychiatry. 2001;25(4):709–727.
5. Evrensel A, Önen Ünsalver B, Ceylan ME. Gut-brain axis and psychiatric disorders. Curr Psychiatry Rev. 2018;14(3):178–186.
6. Ewing JA, Grant WJ. The bromide hazard. South Med J. 1965;58:148–52.
7. Lehmann HE. Before they called it psychopharmacology. Neuropsychopharmacology. 1993;8(4):291–303.
8. Charpentier P, Gailliot P, Jacob R, Gaudechon J, Buisson P. Recherches sur les diméthylaminopropyl-N phénothiazines substituées. Comptes rendus de l'Académie des sciences (Paris). 1952;235:59–60.
9. Laborit H, Huguenard P, Alluaume R. Un noveau stabilisateur vegetatif (le 4560 RP). La Presse Médicale. 1952;60:206–8.
10. Hamon J, Paraire J, Velluz J. Remarques sur l'action du 4560 RP sur l'agitation maniaque. Annales Médico-psychologiques (Paris). 1952;110:331–5.
11. Bowman RL, Caulfield PA, Udenfriend S. Spectrophotometric assay in the visible and ultraviolet. Science. 1955;122(3157):32–3.
12. Carlsson A, Lindqvist M. Effect of chlorpromazine and haloperidol on formation of 3-methoxytyramine and normetanephrine on mouse brain. Acta Pharmacologica et Toxicologica (Copenh). 1963;20:140–4.
13. Bennett MR. Monoaminergic synapses and schizophrenia: 45 years of neuroleptics. J Psychopharmacol. 1998;12(3):289–304.
14. Psychotropic Medications-Consent Drug List Classes of Medications Frequently Used for Psychiatric Indication. Texas Department of State Health Services. https://www.dshs.texas.gov/mhprograms/consentdruglist/.
15. Stahl SM. Stahl's essential psychopharmacology: neuroscientific basis and practical applications. 4th ed. New York: Cambridge University Press; 2013.
16. Stahl SM. Beyond the dopamine hypothesis of schizophrenia to three neural networks of psychosis: dopamine, serotonin, and glutamate. CNS Spectr. 2018;23(3):187–91.
17. Spohn SN, Mawe GM. Non-conventional features of peripheral serotonin signalling - the gut and beyond. Nat Rev Gastroenterol Hepatol. 2017;14(7):412–20.
18. Wu H, Denna TH, Storkersen JN, Gerriets VA. Beyond a neurotransmitter: the role of serotonin in inflammation and immunity. Pharmacol Res. 2018. (in press).
19. Gardner A, Boles RG. Is a "mitochondrial psychiatry" in the future? A Rev Curr Psychiatry Rev. 2005;1(3):255–71.
20. Gardner A, Boles RG. Beyond the serotonin hypothesis: mitochondria, inflammation and neurodegeneration in major depression and affective spectrum disorders. Prog Neuropsychopharmacol Biol Psychiatry. 2011;35(3):730–43.
21. Han B, Sivaramakrishnan P, Lin CJ, Neve IAA, He J, Tay LWR, et al. Microbial genetic composition tunes host longevity. Cell. 2017;169(7):1249–62.
22. Anderson G. Linking the biological underpinnings of depression: Role of mitochondria interactions with melatonin, inflammation, sirtuins, tryptophan catabolites, DNA repair and oxidative and nitrosative stress, with consequences for classification and cognition. Prog Neuropsychopharmacol Biol Psychiatry. 2018;80(Pt C):255–66.
23. Sarkar A, Lehto SM, Harty S, Dinan TG, Cryan JF, Burnet PWJ. Psychobiotics and the manipulation of bacteria-gut-brain signals. Trends Neurosci. 2016;39(11):763–81.
24. Dinan TG, Stanton C, Cryan JF. Psychobiotics: a novel class of psychotropic. Biol Psychiat. 2013;74(10):720–6.
25. Evrensel A, Ceylan ME. Microbiome: the missing link in neuropsychiatric disorders. EMJ Innov. 2017;1(1):83–8.
26. Evrensel A, Ceylan ME. The gut-brain axis: the missing link in depression. Clin Psychopharmacol Neurosci. 2015;13(3):239–44.

27. Evrensel A, Ceylan ME. Fecal microbiota transplantation and its usage in neuropsychiatric disorders. Clin Psychopharmacol Neurosci. 2016;14(3):231–7.
28. Rea K, Dinan TG, Cryan JF. The microbiome: a key regulator of stress and neuroinflammation. Neurobiol Stress. 2016;4:23–33.
29. Louveau A, Smirnov I, Keyes TJ, Eccles JD, Rouhani SJ, Peske JD. Structural and functional features of central nervous system lymphatic vessels. Nature. 2015;523(7560):337–41.
30. Chu H, Mazmanian SK. Innate immune recognition of the microbiota promotes host-microbial symbiosis. Nat Immunol. 2013;14(7):668–75.
31. O'Mahony L, McCarthy J, Kelly P, Hurley G, Luo F, Chen K, et al. Lactobacillus and bifidobacterium in irritable bowel syndrome: symptom responses and relationship to cytokine profiles. Gastroenterology. 2005;128(3):541–51.
32. Zhou W, Lv H, Li MX, Su H, Huang LG, Li J, et al. Protective effects of bifidobacteria on intestines in newborn rats with necrotizing enterocolitis and its regulation on TLR2 and TLR4. Genetics Mol Res. 2015;14(3):11505–14.
33. Miller AH, Haroon E, Raison CL, Felger JC. Cytokine targets in the brain: impact on neurotransmitters and neurocircuits. Depress Anxiety. 2013;30(4):297–306.
34. Felger JC, Lotrich FE. Inflammatory cytokines in depression: neurobiological mechanisms and therapeutic implications. Neuroscience. 2013;246:199–229.
35. Bercik P, Verdu EF, Foster JA, Macri J, Potter M, Huang X, et al. Chronic gastrointestinal inflammation induces anxiety-like behavior and alters central nervous system biochemistry in mice. Gastroenterology. 2010;139(6):2102–12.
36. Foster JA, McVey Neufeld KA. Gut–brain axis: how the microbiome influences anxiety and depression. Trends Neurosci. 2013;36(5):305–12.
37. Bercik P, Park AJ, Sinclair D, Khoshdel A, Lu J, Huang X, et al. The anxiolytic effect of Bifidobacterium longum NCC3001 involves vagal pathways for gut–brain communication. Neurogastroenterol Motil. 2011;23(12):1132–9.
38. Ma X, Mao YK, Wang B, Huizinga JD, Bienenstock J, Kunze W. Lactobacillus reuteri ingestion prevents hyperexcitability of colonic DRG neurons induced by noxious stimuli. Am J Physiol Gastrointest Liver Physiol. 2009; 296(4): G868–G875.
39. McVey Neufeld KA, Mao YK, Bienenstock J, Foster JA, Kunze WA. The microbiome is essential for normal gut intrinsic primary afferent neuron excitability in the mouse. Neurogastroenterol Motil. 2013;25(2):183–8.
40. Collins J, Borojevic R, Verdu EF, Huizinga JD, Ratcliffe EM. Intestinal microbiota influence the early postnatal development of the enteric nervous system. J Neurogastroenterol Motil. 2014;26(1): 98–107.
41. Lomasney KW, Houston A, Shanahan F, Dinan TG, Cryan JF, Hyland NP. Selective influence of host micro-biota on cAMP-mediated ion transport in mouse colon. J Neurogastroenterol Motil. 2014;26(6):887–8.
42. Kabouridis PS, Lasrado R, McCallum S, Chng SH, Snippert HJ, Clevers H, et al. Microbiota controls the homeosta-sis of glial cells in the gut lamina propria. Neuron. 2015;85(2):289–95.
43. Lyte M. Probiotics function mechanistically as delivery vehicles for neuroactive compounds: microbial endocrinology in the design and use of probiotics. BioEssays. 2011;33(8):574–81.
44. Bonaz B, Sinniger V, Pellissier S. The vagus nerve in the neuro-immune axis: Implications in the pathology of the gastrointestinal tract. Front Immunol. 2017;8:1452.
45. Mezzacappa ES, Kelsey RM, Katkin ES, Sloan RP. Vagal rebound and recovery from psychological stress. Psychosom Med. 2001;63:650–7.
46. de Haan JJ, Hadfoune M, Lubbers T, Hodin C, Lenaerts K, Ito A, et al. Lipid-rich enteral nutrition regulates mucosal mast cell activation via the vagal anti-inflammatory reflex. Am J Physiol Gastrointest Liver Physiol. 2013;305(5): G383–G391.
47. Weberruss H, Maucher J, Oberhoffer R, Müller J. Recovery of the cardiac autonomic nervous and vascular system after maximal cardiopulmonary exercise testing in recreational athletes. Eur J Appl Physiol. 2018;118(1):205–11.

48. Kirchner A, Birklein F, Stefan H, Handwerker HO. Left vagus nerve stimulation suppresses experimentally induced pain. Neurology. 2000;55(8):1167–71.
49. Galbarriatu L, Pomposo I, Aurrecoechea J, Marinas A, Agúndez M, Gómez JC, et al. Vagus nerve stimulation therapy for treatment-resistant epilepsy: a 15-year experience at a single institution. Clin Neurol Neurosurg. 2015;137:89–93.
50. Shah AP, Carreno FR, Wu H, Chung YA, Frazer A. Role of TrkB in the anxiolytic-like and antidepressant-like effects of vagal nerve stimulation: comparison with desipramine. Neuroscience. 2016;322:273–86.
51. Abdel Salam OM. Fluoxetine and sertraline stimulate gastric acid secretion via a vagal pathway in anaesthetised rats. Pharmacol Res. 2004;50(3):309–16.
52. Malick M, Gilbert K, Daniel J, Arseneault-Breard J, Tompkins TA, Godbout R, et al. Vagotomy prevents the effect of probiotics on caspase activity in a model of postmyocardial infarction depression. J Neurogastroenterol Motil. 2015;27(5):663–71.
53. Bercik P, Denou E, Collins J, Jackson W, Lu J, Jury J, et al. The intestinal microbiota affect central levels of brain-derived neurotropic factor and behavior in mice. Gastroenterology. 2011;141(2):599–609.
54. Evrensel A, Ceylan ME. Gut-microbiota-brain axis and depression. In: Kim YK, editor. Understanding depression. Singapore: Springer; 2018. p. 197–207.
55. Zareie M, Johnson-Henry K, Jury J, Yang PC, Ngan BY, McKay DM, et al. Probiotics prevent bacterial translocation and improve intestinal barrier function in rats following chronic psychological stress. Gut. 2006;55(11):1553–60.
56. Ait-Belgnaoui A, Durand H, Cartier C, Chaumaz G, Eutamene H, Ferrier L, et al. Prevention of gut leakiness by a probiotic treatment leads to attenuated HPA response to an acute psychological stress in rats. Psychoneuroendocrinology. 2012;37(11):1885–95.
57. Reandon S. Gut-brain link grabs neuroscientists. Nature. 2014;515(7526):175–7.
58. Messaoudi M, Lalonde R, Violle N, Javelot H, Desor D, Nejdi A, et al. Assessment of psychotropic-like properties of a probiotic formulation (Lactobacillus helveticus R0052 and Bifidobacterium longum R0175) in rats and human subjects. Br J Nutr. 2011;105(5):755–64.
59. Schmidt K, Cowen PJ, Harmer CJ, Tzortzis G, Errington S, Burnet PW, et al. Prebiotic intake reduces the waking cortisol response and alters emotional bias in healthy volunteers. Psychopharmacology. 2015;232(10):1793–801.
60. Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, et al. A human gut microbial gene catalogue established by metagenomic sequencing. Nature. 2010;464(7285):59–65.
61. Tan J, McKenzie C, Potamitis M, Thorburn AN, Mackay CR, Macia L. The role of short-chain fatty acids in health and disease. Adv Immunol. 2014;121:91–119.
62. Lei E, Vacy K, Boon WC. Fatty acids and their therapeutic potential in neurological disorders. Neurochem Int. 2016;95:75–84.
63. Sun J, Wang F, Hong G, Pang M, Xu H, Li H, et al. Antidepressant-like effects of sodium butyrate and its possible mechanisms of action in mice exposed to chronic unpredictable mild stress. Neurosci Lett. 2016;618:159–66.
64. Stilling RM, Dinan TG, Cryan JF. Microbial genes, brain & behaviour–epigenetic regulation of the gut–brain axis. Genes Brain Behav. 2014;13(1):69–86.
65. Erny D, Hrabě de Angelis AL, Jaitin D, Wieghofer P, Staszewski O, David E, et al. Host microbiota constantly control maturation and function of microglia in the CNS. Nat Neurosci. 2015;18(7):965–77.
66. Morris G, Berk M, Carvalho A, Caso JR, Sanz Y, Walder K, et al. The role of the microbial metabolites including tryptophan catabolites and short chain fatty acids in the pathophysiology of immune-inflammatory and neuroimmune disease. Mol Neurobiol. 2017;54(6):4432–51.
67. Yano JM, Yu K, Donaldson GP, Shastri GG, Ann P, Ma L, et al. Indigenous bacteria from the gut micro-biota regulate host serotonin biosynthesis. Cell. 2015;161(2):264–76.

68. Wikoff WR, Anfora AT, Liu J, Schultz PG, Lesley SA, Peters EC, et al. Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites. Proc Natl Acad Sci. 2009;106(10):3698–703.
69. Mackowiak PA. Recycling Metchnikoff: probiotics, the intestinal microbiome and the quest for long life. Front Public Health. 2013;1:52.
70. Scott KA, Ida M, Peterson VL, Prenderville JA, Moloney GM, Izumo T, et al. Revisiting Metchnikoff: Age-related alterations in microbiota-gut-brain axis in the mouse. Brain Behav Immun. 2017;65:20–32.
71. Philips JGP. The treatment of melancholia by the lactic acid bacillus. J Ment Sci. 1910;56(234):422–31.
72. Bambury A, Sandhu K, Cryan JF, Dinan TG. Finding the needle in the haystack: systematic identification of psychobiotics. Br J Pharmacol. 2017. (in press).
73. Desbonnet L, Garrett L, Clarke G, Bienenstock J, Dinan TG. The probiotic Bifidobacteria infantis: an assessment of potential antidepressant properties in the rat. J Psychiatr Res. 2008;43(2):164–74.
74. Desbonnet L, Garrett L, Clarke G, Kiely B, Cryan JF, Dinan TG. Effects of the probiotic Bifidobacterium infantis in the maternal separation model of depression. Neuroscience. 2010;170(4):1179–88.
75. Bravo JA, Forsythe P, Chew MV, Escaravage E, Savignac HM, Dinan TG, et al. Ingestion of Lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. Proc Natl Acad Sci. 2011;108(38):16050–5.
76. Janik R, Thomason LAM, Stanisz AM, Forsythe P, Bienenstock J, Stanisz GJ. Magnetic resonance spectroscopy reveals oral Lactobacillus promotion of increases in brain GABA, N-acetyl aspartate and glutamate. NeuroImage. 2016;125:988–95.
77. Matthews DM, Jenks SM. Ingestion of Mycobacterium vaccae decreases anxiety-related behavior and improves learning in mice. Behav Process. 2013;96:27–35.
78. Alander M, Satokari R, Korpela R, Saxelin M, Vilpponen-Salmela T, Mattila-Sandholm T, et al. Persistence of colonization of human colonic mucosa by a probiotic strain, Lactobacillus rhamnosus GG, after oral consumption. Appl Environ Microbiol. 1999;65(1):351–4.
79. Frese SA, Hutton AA, Contreras LN, Shaw CA, Palumbo MC, Casaburi G, et al. Persistence of Supplemented Bifidobacterium longum subsp. infantis EVC001 in Breastfed Infants. mSphere. 2017;2(6): pii: e00501–17.
80. Liang S, Wang T, Hu X, Luo J, Li W, Wu X, et al. Administration of Lactobacillus helveticus NS8 improves behavioral, cognitive, and biochemical aberrations caused by chronic restraint stress. Neuroscience. 2015;310:561–77.
81. Moya-Perez A, Perez-Villalba A, Benitez-Paez A, Campillo I, Sanz Y. Bifidobacterium CECT 7765 modulates early stress-induced immune, neuroendocrine and behavioral alterations in mice. Brain Behav Immun. 2017;65:43–56.
82. McVey Neufeld KA, Kay S, Bienenstock J. Mouse strain affects behavioral and neuroendocrine stress responses following administration of probiotic Lactobacillus rhamnosus JB-1 or traditional antidepressant fluoxetine. Front Neurosci. 2018;12:294.
83. Benton D, Williams C, Brown A. Impact of consuming a milk drink containing a probiotic on mood and cognition. Eur J Clin Nutr. 2007;61(3):355–61.
84. Messaoudi M, Violle N, Bisson JF, Desor D, Javelot H, Rougeot C. Beneficial psychological effects of a probiotic formulation (Lactobacillus helveticus R0052 and Bifi-dobacterium longum R0175) in healthy human volunteers. Gut Microbes. 2011;2(4):256–61.
85. Steenbergen L, Sellaro R, van Hemert S, Bosch JA, Colzato LS. A randomized controlled trial to test the effect of multispecies probiotics on cognitive reactivity to sad mood. Brain Behav Immun. 2015;48:258–64.
86. Kato-Kataoka A, Nishida K, Takada M, Kawai M, Kikuchi-Hayakawa H, Suda K, et al. Fermented milk containing Lacto-bacillus casei strain Shirota preserves the diversity of the gut microbiota and relieves abdominal dysfunction in healthy medical students exposed to academic stress. Appl Environ Microbiol. 2016;82(12):3649–58.

87. Sashihara T, Nagata M, Mori T, Ikegami S, Gotoh M, Okubo K, et al. Effects of Lactobacillus gasseri OLL2809 and α-lactalbumin on university-student athletes: a randomized, double-blind, placebo-controlled clinical trial. Appl Physiol Nutr Metab. 2013;38(12):1228–35.
88. Tillisch K, Labus J, Kilpatrick L, Jiang Z, Stains J, Ebrat B, et al. Consumption of fermented milk product with probiotic modulates brain activity. Gastroenterology. 2013;144(7):1394–401.
89. Pinto-Sanchez MI, Hall GB, Ghajar K, Nardelli A, Bolino C, Lau JT, et al. Probiotic Bifidobacterium longum NCC3001 reduces depression scores and alters brain activity: A pilot study in patients with irritable bowel syndrome. Gastroenterology. 2017;153(2):448–459.e8.
90. Kelly JR, Allen AP, Temko A, Hutch W, Kennedy PJ, Farid N, et al. Lost in translation? The potential psychobiotic Lactobacillus rhamnosus (JB-1) fails to modulate stress or cognitive performance in healthy male subjects. Brain Behav Immun. 2017;61:50–9.
91. Claesson MJ, Cusack S, O'Sullivan O, Greene-Diniz R, de Weerd H, Flannery E, et al. Composition, variability, and tempo-ral stability of the intestinal microbiota of the elderly. Proc Natl Acad Sci. 2011;108(Suppl 1):4586–91.
92. Abisado RG, Benomar S, Klaus JR, Dandekar AA, Chandler JR. Bacterial quorum sensing and microbial community interactions. MBio. 2018;9(3): pii: e02331–17.
93. Macedo D, Filho AJMC, Soares de Sousa CN, Quevedo J, Barichello T, Junior HVN, et al. Antidepressants, antimicrobials or both? Gut microbiota dysbiosis in depression and possible implications of the antimicrobial effects of antidepressant drugs for antidepressant effectiveness. J Affect Disord. 2017;208:22–32.
94. Ishijima SA, Abe S. A novel murine candidiasis model with severe colonization in the stomach induced by n-acetylglucosamine-treatment and its scoring system based on local characteristic stomach symptoms. Med Mycol J. 2015;56(4):E31–9.
95. López-Muñoz F, Alamo C. Monoaminergic neurotransmission: the history of the discovery of antidepressants from 1950s until today. Curr Pharm Des. 2009;15(14):1563–86.
96. Patra S. Psychobiotics: A paradigm shift in psychopharmacology. Indian J Pharmacol. 2016;48(4):469–70.
97. Sato-Kasai M, Kato TA, Ohgidani M, Mizoguchi Y, Sagata N, Inamine S, et al. Aripiprazole inhibits polyI:C-induced microglial activation possibly via TRPM7. Schizophr Res. 2016;178(1–3):35–43.