Role of Gut Microbiota in the Pathophysiology of Stress-Related Disorders: Evidence from Neuroimaging Studies

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Keywords

Brain-gut interaction · Gut microbiota · Neuroimaging · Functional magnetic resonance imaging · Stress

Abstract

Background: The brain and gut communicate bidirectionally via immune, neurological, and endocrine pathways, which is termed the “brain-gut interaction.” Recent studies of gut microbiota as a mediator of this interaction have provided a growing body of scientific evidence that suggests that the gut microbiota influences stress and emotional responses and stress-related disorders. **Summary:** Major advances in analytical methods have led to an increased number of studies that combine gut microbiota and neuroimaging, mainly magnetic resonance imaging, to elucidate the mechanisms. Observational studies have been done to examine brain characteristics related to gut microbiota profiles, and intervention studies have examined brain changes related to probiotic intake. Studies of healthy subjects using negative emotional stimuli have shown that the pattern of emotional response differs depending on the gut microbiota profile and that probiotic intervention can modulate emotional response and be a buffer against the negative effects of stress. In studies on irritable bowel syndrome (IBS), a typi-

cal psychosomatic disorder, IBS-specific gut microbiota were reported to contribute to visceral irritability and pain by affecting the subcortical regions. Studies on psychiatric disorders revealed that a relative abundance of *Bacteroides* that produce γ -aminobutyric acid in feces was associated with a change in brain function specific to depression and that gut microbiota have an influence on abnormalities in the reward system of attention-deficit/hyperactivity disorder.

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Introduction

The brain and the gut communicate bidirectionally via immune, neurological, and endocrine pathways, which is termed the “brain-gut interaction.” Gut microbiota have recently received much attention for the important role they play in this interaction. The human intestine contains more than 1,000 species of gut microbiota, with 10^{11} to 10^{12} bacteria per gram of stool. Next-generation sequencing was introduced in the late 2000s as a method to identify indigenous bacteria, and the use of 16S ribosomal RNA as a marker for molecular biology has enabled detailed analysis.

Research on gut microbiota and their host brain function has also greatly progressed, revealing that gut microbiota influence stress and emotional responses [1]. Furthermore, there is a growing body of scientific evidence suggesting that gut microbiota influence psychosomatic disorders (physical disorders closely related to psychosocial stress that affects the onset or clinical course of the disease (e.g., irritable bowel syndrome [IBS] [2, 3]) and psychiatric disorders (e.g., depression [4] and attention-deficit/hyperactivity disorder [ADHD] [5]).

Mechanisms of the “brain-gut interaction” by gut microbiota have been reported to be pathways mediated by the vagus and spinal afferent nerves, short-chain fatty acids, tryptophan, catecholamines, and other interkingdom signaling-related molecules [1, 6]. In addition to measuring the bioactive substances associated with those pathways, neuroimaging is a useful, noninvasive way to elucidate the mechanisms of the interaction. Major advances in analytical methods for examining the structure, function, and networks of the brain have led to an increasing number of studies that combine gut microbiota and neuroimaging and several neuroimaging studies on the pathophysiology of stress-related disorders.

Methods

We first introduce methods to approach the brain, focusing on magnetic resonance imaging (MRI) and then outline studies of gut microbiota and neuroimaging on stress and emotional responses and stress-related disorders. By reviewing the currently available evidence of the “brain-gut interaction,” we hope to clarify what we have learned by combining microbiota and neuroimaging and propose a perspective for future research.

A literature search was done for articles with terms related to gut microbiota (microbiota OR microbiome OR probiotic) AND others about neuroimaging (neuroimaging OR MRI) AND each topic (emotion, IBS, depression, and ADHD) in PubMed, Web of Science, and Cochrane Library from 2010 to 2019. We then selected as our main studies those that combined gut microbiota and neuroimaging.

Results

Neuroimaging Methods

MRI is widely used as a noninvasive method to examine the brain structure, which can be assessed by voxel-based morphometry (VBM) and diffusion tensor imaging (DTI). VBM is a method to exploratively evaluate the density and volume of gray and white matter in the whole brain, not just specific regions, by dividing the brain into

small voxel units and performing statistical analysis. DTI is a method to visualize the structure of nerve fibers in white matter by quantifying and imaging the 3-dimensional direction and speed of diffusion, which is the micro-movement of water molecules in the tissue.

Functional MRI (fMRI) is a method used to assess brain function that estimates neural activity, mainly by the blood oxygenation level-dependent (BOLD) signal. There are 2 types of measurements: task-based fMRI, which measures neural activity with specific cognitive processing and resting-state fMRI (RS-fMRI), which measures neural activity at rest. Through RS-fMRI studies, a group of regions showing synchrony of BOLD signals was discovered, and functional connectivity (FC), a cross-correlation coefficient between BOLD signals, has been used as an indicator of brain network strength. Previous studies have revealed multiple functional brain networks. One of particular interests is the default mode network (DMN), which is composed of a set of structures including the posterior cingulate cortex and the medial prefrontal cortex (mPFC), which is activated at rest and is thought to be involved in self-referencing, autobiographical memory recall, and prediction of one's future. Among other brain function networks, it is known that the executive control network (ECN), which includes the dorsolateral prefrontal cortex (DLPFC) and the superior parietal cortex, is a network that trades off with the DMN, and the salience network (SN), which includes the dorsal anterior cingulate cortex (dACC) and anterior insular cortex, is involved in detecting external/internal stimuli and switching the DMN and ECN (shown in Fig. 1). Magnetic resonance spectroscopy is a method used to assess brain metabolism. Magnetic resonance spectroscopy can measure various metabolites, including neurotransmitters such as glutamate and γ -aminobutyric acid (GABA), in the region of interest.

Studies on Stress and Emotional Responses

To assess stress response, germ-free (GF) and various artificial microbiota mice were compared in an animal study [7]. The degree of plasma adrenocorticotropin hormone and corticosterone elevation in response to restraint stress was significantly higher in GF mice than in specific pathogen-free (SPF) mice, and monoassociation with *Bifidobacterium infantis* decreased the HPA stress response to SPF. These results indicate that the gut microbiota is related to stress response.

In a recent human intervention study combining neuroimaging, Papalini et al. [8] found that administration of probiotics (Ecologic® Barrier: *Bifidobacterium bifidum*

Fig. 1. **a** DMN is mainly composed of the mPFC, PCC/PCu, hippocampus, and IPL. **b** ECN is mainly composed of the DLPFC, DMPFC, and SPC. **c** Salience network is mainly composed of the dACC, AI, TP, and Amy. DMN, default mode network; mPFC, medial prefrontal cortex; PCC/PCu, posterior cingulate cortex/precuneus; IPL, inferior parietal lobule; ECN, executive control network; DLPFC, dorsolateral prefrontal cortex; DMPFC, dorso-medial prefrontal cortex; SPC, superior parietal cortex; dACC, dorsal anterior cingulate cortex; AI, anterior insular cortex; TP, temporal pole; Amy, amygdala.

W23, *Bifidobacterium lactis* W51, *Bifidobacterium lactis* W52, *Lactobacillus acidophilus* W37, *Lactobacillus brevis* W63, *Lactobacillus casei* W56, *Lactobacillus salivarius* W24, *Lactococcus lactis* W19, and *Lactococcus lactis* W58) acted as a buffer against a stress-induced reduction in working memory performance and that this effect was associated with reduced brain activity in the prefrontal cortex (PFC) during a cognitive control task (probiotics: $n = 28$, placebo: $n = 28$, and age range: 18–41). In general, the PFC becomes more active with increasing difficulty in a

cognitive control task, but the reduced activity of this region under the same condition (when the difficulty level does not change) can be viewed as more efficient.

Furthermore, several neuroimaging studies have examined the association between microbiota and emotion evoked by negative stress stimuli. Tillisch et al. [9] found that consumption of a fermented milk product with probiotics (*Bifidobacterium animalis* ssp. *lactis*, *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, and *Lactococcus lactis* ssp. *lactis*) for 4 weeks was associated with decreased responses in a wide range of brain regions, including the insula, postcentral gyrus (primary somatosensory cortex), periaqueductal gray (PAG), PFC, precuneus, putamen, and parahippocampal gyrus, in response to a task probing attention to negative context (fermented milk product with probiotics: $n = 28$, placebo: $n = 11$, no intervention: $n = 13$, and age range: 18–55). In a resting state, there was a significant decrease in FC between the PAG and regions associated with emotion (insula, cingulate cortex, and amygdala) and somatosensory functioning (postcentral gyrus) and a significant increase in FC between the PAG and regions that regulate emotional and sensory responses (mPFC and DLPFC) (shown in Fig. 2). The PAG is an anatomical and functional interface between the forebrain and the brainstem that receives information to nociceptive and emotional stimuli by internal/external stressors and that integrates and expresses behavioral and autonomic responses [10]. The changes in a PAG-seeded FC showed that probiotic intake modulates emotion and somatosensory functioning, diminishes irritability, and enhances appropriate behavioral and autonomic responses.

In an approach based on the gut microbiota profile [11], a *Prevotella*-rich group had a higher negative affective valence than a *Bacteroides*-rich group when viewing negative valence pictures, which was associated with reduced hippocampal activity (*Prevotella*-rich: $n = 7$, *Bacteroides*-rich: $n = 33$, and age range: 18–55). The hippocampus has close neural circuitry with the amygdala and has been investigated as a target for neurofeedback in emotion regulation [12]. Reduced hippocampal activity has been shown to increase emotional arousal and can be observed in psychiatric disorders such as depression [13] and post-traumatic stress disorder [14]. These findings suggest that the composition of the gut microbiota is a factor in the vulnerability of the individual.

Bagga et al. [15] performed an emotional decision-making (ED) task (presenting 3 neutral/unpleasant pictures and determining the most neutral/unpleasant picture of the 3) and an emotional recognition memory (ER)

Fig. 2. Regions showing changes in functional connectivity with midbrain activity after intervention with probiotics. FC, functional connectivity; PAG, periaqueductal gray; PFC, prefrontal cortex.

Fig. 3. Regions showing changes in brain activation patterns in response to ED and ER tasks after intervention with probiotics. ACC, anterior cingulate cortex; MCC, mid cingulate cortex; PCu, precuneus; ED, emotional decision-making; ER, emotional recognition memory.

task (presenting a neutral/unpleasant pictures and recalling whether or not the subject had seen the pictures during the ED task), before and after 4 weeks of administration of probiotics (Ecologic® 825: *Lactobacillus casei* W56, *Lactobacillus acidophilus* W22, *Lactobacillus paracasei* W20, *Bifidobacterium lactis* W51, *Lactobacillus salivarius* W24, *Lactococcus lactis* W19, *Bifidobacterium lactis* W52, *Lactobacillus plantarum* W62, and *Bifidobacterium bifidum* W23) (probiotics: $n = 15$, placebo: $n = 15$, no intervention: $n = 15$, and age range: 20–40). The number of times a subject in the probiotics group changed the picture most unpleasant to them during the ED task was fewer than the number of changes made by the no intervention and placebo groups, and the accuracy of recalling the unpleasant pictures was increased in the ER task after the intervention. Neuroimaging showed reduced brain activity in the mid-cingulate cortex, precuneus, and parahippocampal gyrus and increased brain activity in the anterior cingulate cortex (ACC) in the ED task and reduced cerebellar activity in the ER task (shown in Fig. 3). In the resting state [16], there was a decreased FC in the DMN (frontal pole, superior frontal gyrus, and paracingulate gyrus) and an increased FC in the SN (cingulate gyrus and precuneus) (shown in Fig. 4). The cingulate cortex consists of the anterior, middle (cingulate motor area), and posterior cingulate cortices, and the ACC links informa-

tion about value input from the orbitofrontal cortex, with emotional information input from the amygdala and behavior output through the mid-cingulate cortex [17]. Further, the ACC, along with the insula, is an important structure of the SN that plays an important role in switching between the DMN and ECN. Changes in the FC in this region may reflect the impact of probiotic administration on ED and efficient attentional control. The results of these 4 studies suggest that the pattern of emotional response to negative stimuli differs depending on the profile of the gut microbiota and that probiotic intervention can modulate emotional response to negative stimuli, buffer against the negative effects of stress, and contribute to appropriate decision-making and attentional control.

Studies on IBS

IBS, a typical psychosomatic disorder, is diagnosed by the Rome IV criteria [18] for recurrent abdominal pain associated with defecation and changes in frequency of defecation or stool form. Psychosocial stress is known to play an important role in the onset and clinical course of IBS symptoms. IBS has been shown to complicate depression and anxiety at a high rate [19].

Labus et al. [2] examined the association between gut microbiota and the brain structure of IBS patients (IBS: $n = 29$, control: $n = 23$, and mean age: 26). In their IBS

Fig. 4. Regions showing changes in functional connectivity within the DMN and SN after intervention with probiotics. SFC, superior frontal cortex; FP, frontal pole; PCu, precuneus; DMN, default mode network; SN, salience network.

group, the relative abundance of Firmicutes-associated Clostridia, which is increased in IBS, was significantly associated with increased volume in the subcortical regions (putamen, caudate nucleus, and nucleus accumbens) and decreased volume in the insula and PFC. In another study [20], they focused on Clostridiales, an order of the class that is related to the regulation of serotonin, and examined its association with brain function and gastrointestinal sensorimotor function (IBS: $n = 65$, control: $n = 21$, and mean age: 33). In their healthy control group, *Lachnospiraceae incertae sedis*, *Clostridium XIVa*, and *Coprococcus*, all classified into the order Clostridiales, were associated with gastrointestinal sensorimotor function via centrality (a degree that assesses the importance of individual nodes in a network, calculated based on the graph theory) of the somatosensory cortex and subcortical areas, while these associations were lost in the IBS group. Focusing on the subcortical regions, the healthy control group showed a negative correlation between *Clostridium XIVa* and the centrality of the putamen and between *Coprococcus* and the caudate nucleus, which was associated with improved gastrointestinal sensorimotor function. In contrast, in the IBS group, *Clostridium XIVa* was positively correlated with the centrality of the putamen, caudate nucleus, and thalamus. DTI studies of IBS showed changes in white matter in the thalamus and basal ganglia

[21], and fMRI studies using rectal stimulation showed that the basal ganglia were involved in IBS-specific changes in pain processing [22]. *Clostridium XIVa* and *Coprococcus* may contribute to the visceral irritability and pain of IBS by affecting these subcortical regions. In a study of IBS patients with depression [3], administration of *Bifidobacterium longum* NCC3001 improved depression and reduced responses to negative emotional stimuli in multiple brain regions, including the amygdala and fronto-limbic regions (probiotics: $n = 22$, placebo: $n = 22$, and age range: 26–58).

Studies on Depression Results

An association between depression and gut microbiota has been hypothesized since the early 1900s [23, 24] and has recently been studied in healthy subjects and patients. In a study of healthy subjects, consumption of dairy products containing *Lactobacillus casei* strain *shirota* was associated with a significant improvement of depressed mood in a group with the highest depression index, compared to placebo [25]. In addition, administration of probiotics (Ecologic® 825) was more closely associated with their depression questionnaire scores than placebo, and it significantly increased positive feelings in terms of hopelessness and risk aversion, blunting vulnerability to depression [15].

Strandwitz et al. [4] found that *Bacteroides* ssp. produces GABA, a major inhibitory neurotransmitter. In fMRI testing of inpatients with a major depressive disorder ($n = 23$), a GABA-related disorder, they found that the relative abundance of *Bacteroides* ssp. in feces was significantly associated with reduced FC between areas of the DMN, including the mPFC and the DLPFC. Additionally, depressed patients are more likely to develop introspective and repetitive thoughts about themselves (negative rumination), and the associated hyperconnectivity of the DMN [26] is supported by a meta-analysis examining RS-fMRI in depression [27]. This study by Strandwitz et al. [4] suggests that the gut microbiota are associated with changes in the brain function of patients with depression.

Studies on ADHD

In an animal study examining gut microbiota and behavioral characteristics, GF mice were found to be more anxious and hyperactive than EX-GF mice whose gut microbiota had been reconstructed with those of normal SPF mice [28]. In addition, mice with gut microbiota transplanted from ADHD patients were more anxious and showed structural alterations of the inner envelope

and hippocampal white matter in DTI [29]. These findings indicate that the gut microbiota affect the behavioral characteristics and brain structure of ADHD patients.

Aarts et al. [5] examined differences in gut microbiota between ADHD patients and healthy controls, using fMRI to investigate their neural relation. In these ADHD patients (ADHD: $n = 19$ and control: $n = 77$), they found increased *Bifidobacterium* genes and enhanced gene encoding enzymes involved in the synthesis of phenylalanine, a precursor of dopamine. The increased function of this gene was significantly associated with a reduced response of the ventral striatum in predicting reward (ADHD: $n = 6$ and control: $n = 22$). The activity of the ventral striatum of ADHD patients was similar for both reward-predictive and non-reward-predictive cues, but when methylphenidate was administered, the activity of the ventral striatum increased only in response to reward-predictive cues, making the 2 cues more easily distinguishable [30]. These findings suggest that the gut microbiota have some influence on the abnormalities seen in the reward system of ADHD.

Limitations

It should be noted that this is not a systematic review and the study is focused on MRI techniques.

Conclusion and Perspective

The combination of microbiota with noninvasive methods for assessing brain function, such as fMRI, has revealed some aspects of the “brain-gut interaction.” Research findings suggest that the gut microbiota are involved in altering the stress and emotional responses of their host, even though the pathophysiological mecha-

nisms are not completely understood. Probiotic interventions may modulate emotional response, contribute to stress relief, and enhance resilience. However, because a few of these intervention studies involved changes in the composition of the gut microbiota, it is unclear what changes were brought about by the administration of the probiotics. Therefore, it is necessary to include biomarkers that reflect the pathways that link the gut microbiota and brain function in new study protocols to identify the precise mechanisms. It has also been suggested that the gut microbiota affect brain activity related to psychosomatic disorders such as IBS and psychiatric disorders such as depression and ADHD. Intervention studies focused on these disorders will be necessary to clarify their pathophysiological mechanisms and to develop probiotic-based therapies.

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Conflict of Interest Statement

We have no conflicts of interest to declare.

Author Contributions

S.I. and K.Y. contributed to the conception of the manuscript, conducted the literature research, and drafted the manuscript. N.S. organized and revised the draft critically. All the authors read and approved the final manuscript.

References

- 1 Sudo N. Role of gut microbiota in brain function and stress-related pathology. *Biosci Microbiota Food Health*. 2019;38(3):75–80.
- 2 Labus JS, Hollister EB, Jacobs J, Kirbach K, Oezguen N, Gupta A, et al. Differences in gut microbial composition correlate with regional brain volumes in irritable bowel syndrome. *Microbiome*. 2017;5(1):49.
- 3 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):448–59.e8.
- 4 Strandwitz P, Kim KH, Terekhova D, Liu JK, Sharma A, Levering J, et al. GABA-modulating bacteria of the human gut microbiota. *Nat Microbiol*. 2019 Mar;4(3):396–403.
- 5 Aarts E, Ederveen THA, Naaijen J, Zwiers MP, Boekhorst J, Timmerman HM, et al. Gut microbiome in ADHD and its relation to neural reward anticipation. *PLoS One*. 2017;12(9):e0183509.
- 6 Sudo N. Biogenic amines: signals between commensal microbiota and gut physiology. *Front Endocrinol*. 2019 Jul 31;10:504.

- 7 Sudo N, Chida Y, Aiba Y, Sonoda J, Oyama N, Yu XN, et al. Postnatal microbial colonization programs the hypothalamic-pituitary-adrenal system for stress response in mice. *J Physiol*. 2004;558(1):263–75.
- 8 Papalini S, Michels F, Kohn N, Wegman J, van Hemert S, Roelofs K, et al. Stress matters: randomized controlled trial on the effect of probiotics on neurocognition. *Neurobiol Stress*. 2019;10:100141.
- 9 Tillisch K, Labus J, Kilpatrick L, Jiang Z, Stains J, Ebrat B, et al. Consumption of fermented milk product with probiotic modulates brain activity. *Gastroenterology*. 2013 Jun;144(7):1394–401.
- 10 Benarroch EE. Periaqueductal gray: an interface for behavioral control. *Neurology*. 2012 Jan;78(3):210–7.
- 11 Tillisch K, Mayer EA, Gupta A, Gill Z, Brazeilles R, Le Nevé B, et al. Brain structure and response to emotional stimuli as related to gut microbial profiles in healthy women. *Psychosom Med*. 2017 Oct;79(8):905–13.
- 12 Zhu Y, Gao H, Tong L, Li Z, Wang L, Zhang C, et al. Emotion regulation of hippocampus using real-time fMRI neurofeedback in healthy human. *Front Hum Neurosci*. 2019 Jul;13:242.
- 13 Lee BT, Seok JH, Lee BC, Cho SW, Yoon BJ, Lee KU, et al. Neural correlates of affective processing in response to sad and angry facial stimuli in patients with major depressive disorder. *Prog Neuropsychopharmacol Biol Psychiatry*. 2008;32(3):778–85.
- 14 Hayes JP, LaBar KS, McCarthy G, Selgrade E, Nasser J, Dolcos F, et al. Reduced hippocampal and amygdala activity predicts memory distortions for trauma reminders in combat-related PTSD. *J Psychiatr Res*. 2011 May;45(5):660–9.
- 15 Bagga D, Reichert JL, Koschutnig K, Aigner CS, Holzer P, Koskinen K, et al. Probiotics drive gut microbiome triggering emotional brain signatures. *Gut Microbes*. 2018;9(6):486–96.
- 16 Bagga D, Aigner CS, Reichert JL, Cecchetto C, Fischmeister FPS, Holzer P, et al. Influence of 4-week multi-strain probiotic administration on resting-state functional connectivity in healthy volunteers. *Eur J Nutr*. 2019;58(5):1821–7.
- 17 Rolls ET. The cingulate cortex and limbic systems for emotion, action, and memory. *Brain Struct Funct*. 2019;224:3001–18.
- 18 Drossman DA, Hasler WL. Rome IV – functional GI disorders: disorders of gut-brain interaction. *Gastroenterology*. 2016;150(6):1257–61.
- 19 Enck P, Aziz Q, Barbara G, Farmer AD, Fukudo S, Mayer EA, et al. Irritable bowel syndrome. *Nat Rev Dis Primers*. 2016 Dec;2(1):16014.
- 20 Labus JS, Osadchiv V, Hsiao EY, Tap J, Derrien M, Gupta A, et al. Evidence for an association of gut microbial Clostridia with brain functional connectivity and gastrointestinal sensorimotor function in patients with irritable bowel syndrome, based on tripartite network analysis. *Microbiome*. 2019;7(1):45.
- 21 Ellingson BM, Mayer E, Harris RJ, Ashe-McNally C, Naliboff BD, Labus JS, et al. Diffusion tensor imaging detects microstructural reorganization in the brain associated with chronic irritable bowel syndrome. *Pain*. 2013 Sep;154(9):1528–41.
- 22 Tillisch K, Mayer EA, Labus JS. Quantitative meta-analysis identifies brain regions activated during rectal distension in irritable bowel syndrome. *Gastroenterology*. 2011 Jan;140(1):91–100.
- 23 Phillips JGP. The treatment of melancholia by the lactic acid Bacillus. *J Ment Sci*. 1910;56(234):422–30.
- 24 Julianelle LA. Implantation of *Bacillus acidophilus* in persons with psychoses. *Arch Neuropsych*. 1923 Jun;9(6):769.
- 25 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.
- 26 Kross E, Davidson M, Weber J, Ochsner K. Coping with emotions past: the neural bases of regulating affect associated with negative autobiographical memories. *Biol Psychiatry*. 2009;65(5):361–6.
- 27 Sundermann B, Olde Lütke Beverborg M, Pfeleiderer B. Toward literature-based feature selection for diagnostic classification: a meta-analysis of resting-state fMRI in depression. *Front Hum Neurosci*. 2014 Sep;8:692.
- 28 Nishino R, Mikami K, Takahashi H, Tomonaga S, Furuse M, Hiramoto T, et al. Commensal microbiota modulate murine behaviors in a strictly contamination-free environment confirmed by culture-based methods. *Neurogastroenterol Motil*. 2013;25(6):521–8.
- 29 Tengeler AC, Dam SA, Wiesmann M, Naaijen J, Van Bodegom M, Belzer C, et al. Gut microbiota from persons with attention-deficit/hyperactivity disorder affects the brain in mice. *Microbiome*. 2020;8(1):44.
- 30 Furukawa E, da Costa RQM, Bado P, Hoefle S, Vigne P, Monteiro M, et al. Methylphenidate modifies reward cue responses in adults with ADHD: an fMRI study. *Neuropharmacology*. 2020;162:107833.