Role of the Microbiome and the Gut-Brain Axis in Alcohol Use Disorder: Potential Implication for Treatment Development

Sophie Leclercq and Philippe de Timary

Contents

Abstract The gut microbiota is constituted by trillions of microorganisms colonizing the human intestine. Studies conducted in patients with alcohol use disorder (AUD) have shown altered microbial composition related to bacteria, viruses, and fungi.

This review describes the communication pathways between the gut and the brain, including the ones related to the bacterial metabolites, the inflammatory cytokines, and the vagus nerve. We described in more detail the gut-derived metabolites that have been shown to be implicated in AUD or that could potentially be involved in the development of AUD due to their immune and/or neuroactive

S. Leclercq
Laboratory of Nutritional Psychiatry, Institute of Neuroscience, Université Catholique de Louvain, Brussels, Belgium

P. de Timary (✉)
Department of Adult Psychiatry, Cliniques Universitaires Saint-Luc, Brussels, Belgium
e-mail: philippe.detimary@saintluc.uclouvain.be

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properties, including tryptophan-derivatives, tyrosine-derivatives, short chain fatty acids.

Finally, we discussed the potential beneficial effects of microbiome-based therapies for AUD such as probiotics, prebiotics, postbiotic, and phage therapy.

Keywords Fecal microbiota transplantation · Gut microbiota · Gut-brain axis · Inflammation · Metabolomics · Prebiotics · Probiotics

Abbreviations

4-EPS	4-Ethylphenylsufate
AhR	Aryl hydrocarbon receptor
AUD	Alcohol use disorder
BBB	Blood brain barrier
BHB	β-hydroxybutyrate
CNS	Central nervous system
CT	Controls
FA	Fractional anisotropy
FMT	Fecal microbiota transplantation
GABA	Gamma-aminobutyric acid
HDAC	Histone deacetylase
IL	Interleukin
ITS	Internal transcribed spacer
LBP	LPS-binding protein
MCT	Monocarboxylate transporter
MRI	Magnetic resonance imaging
NMDA	N-methyl-D-aspartic acid
rDNA	Ribosomal DNA
SCFA	Short chain fatty acids
TLR	Toll-like receptor

1 Introduction

The gut microbiota is a complex ecosystem with huge impact on host's health (Nicholson et al. 2012). The microorganisms living in the gastrointestinal tract, comprising bacteria, viruses, fungi, and archaea, have numerous functionalities such as regulation of the immune system, involvement in many metabolic responses (including absorption of nutrients and minerals, synthesis of vitamins, breakdown of toxic components), and regulation of the nervous system with consequences for brain functions and behavior (Cryan and Dinan 2012). Consequently, unbalance of this ecosystem is usually associated with diseases including inflammatory diseases,

obesity, diabetes, liver diseases, and neurologic or psychiatric disorders (Nicholson et al. 2012; Cryan et al. 2019). The factors that can significantly affect the gut microbiota include dietary habits, lifestyle factors (like stress or sleep patterns), medications used, age, and sex (Zhernakova et al. 2016; Quigley 2017; David et al. 2014).

Alcohol use disorder (AUD) is a chronic relapsing psychiatric disease associated with metabolic, immune, cognitive, mood, and social disturbances. Alcohol abuse leads to several nutritional deficiencies and it is one of the main causes of malnutrition in Western countries (Amadieu et al. 2021). Since nutrition is a major factor shaping the gut microbiota, it is of interest to interrogate the gut microbiota composition of AUD patients. Furthermore, due to the large psychosocial distress of those patients, this clinical population represents an interesting target to investigate the gut-brain relationship.

In this review, we discuss the microbial alterations observed in AUD patients and describe the potential mechanisms by which the microbes could influence brain functions and behavior. We summarize the gut-brain communication pathways that have been described in the scientific literature, which are mainly related to the gut-derived metabolites, to the inflammatory cytokines and to the vagus nerve. While a large amount of data arises from animal experiments, we chose to highlight clinical data when possible. Finally, we discuss the potential benefits of microbiota-based therapies for the improvement of AUD.

2 The Gut Microbiota Composition in AUD Patients

A recent systematic review examined the effect of alcohol consumption on the gut microbiome, including AUD individuals with and without alcoholic liver diseases (Litwinowicz et al. 2020). Seventeen studies were included in this review and only results (related to bacterial changes) confirmed in at least two studies were included. At the phylum level, Bacteroidetes had a lower relative abundance and Proteobacteria (a highly pro-inflammatory phylum) a higher relative abundance in individuals with AUD than in healthy controls. At the family level, the relative abundance of Enterobacteriaceae was increased and Ruminococcaceae reduced. At the genus level, *Ruminococcus*, *Collinsella*, *Prevotella*, *Clostridium*, *Faecalibacterium*, *Paraprevotella*, *Bacteroides*, *Parabacteroides*, *Alistipes*, and *Akkermansia* had lower relative abundances in individuals with AUD. Results regarding *Bifidobacterium* were mixed, with some studies reporting a lower relative abundance in AUD individuals while other reported a higher abundance.

Overall, the results of existing studies are conflicting with little overlap asking for larger and more carefully designed studies. Also, the differences in the methodologies (related to DNA extraction, 16S rDNA sequencing, bioinformatics pipelines) across studies and that are in constant evolution could participate in the heterogeneity of the results. Furthermore, adjustment for confounders including lifestyle factors, nutrition, and medications used is needed. Indeed, drugs commonly used

in AUD patients such as proton pump inhibitors and antidepressants have been shown to largely modify the gut microbiota (Zhernakova et al. 2016; Letchumanan et al. 2021). Also, the status of the liver should be taken into consideration. Specific bacterial changes also characterized subjects presenting with progressive alcoholic liver disease (Maccioni et al. 2020), as well as cirrhotic patients (Addolorato et al. 2020). The team of Bernd Schnabl has carefully investigated the microbial composition of patients with alcoholic hepatitis. They found that the abundance of *Enterococcus faecalis* was 2,700-fold higher in the feces of alcoholic subjects compared to healthy controls, and about 80% of alcoholic hepatitis patients were *E. faecalis* positive in feces (Duan et al. 2019). Interestingly, this bacterium produces an exotoxin, called cytolysin, which is found to be a good predictor of mortality due to liver failure. Indeed, 89% of cytolysin-positive patient with alcoholic hepatitis died within 3 months after hospital admission compared to <4% of cytolysin-negative patients. Also, colonization of germ-free mice with feces from cytolysin-positive patients developed more severe ethanol-induced liver injury, inflammation, steatosis, and fibrosis and have a reduced survival time compared with germ-free mice colonized with feces from cytolysin-negative patients. Those results provide evidence that cytolysin promotes ethanol-induced liver damage (Duan et al. 2019).

In our lab, where we conducted studies in AUD patients that were selected not to present with severe liver disease, we found that only a subset of patients (around 40%) have alterations of the gut microbiota (Leclercq et al. 2014a). The dysbiotic group of alcoholic patients was characterized by decreased Ruminococcaceae and increased Lachnospiraceae families. Among the Ruminococcaceae's family, the genera *Ruminococcus* and *Subdoligranulum* were almost absent in dysbiotic patients, and the level of *Faecalibacterium prausnitzii*, a bacterium with anti-inflammatory properties (Sokol et al. 2008), was 10,000 times lower compared to healthy controls. Interestingly, intestinal dysbiosis of AUD patients was associated with a large increase in intestinal permeability, also called leaky gut (Leclercq et al. 2014a).

While the first decade of gut microbiome research has mainly focused on DNA-based 16S rRNA gene sequencing and shotgun metagenomic sequencing which elucidate the bacterial composition and gene content, more attention is recently given to fungi (amplicon sequencing based on 18S rRNA and ITS) and viruses (Gao et al. 2021a). The human mycobiome diversity is relatively low compared with bacterial communities and is dominated by yeast such as *Candida*, *Saccharomyces,* and *Malassezia*. Dysbiosis of intestinal fungi has been reported in alcoholic patients with an overgrowth of *Candida* (Yang et al. 2017). On the other hand, viruses are key constituents of the gut ecosystem and contribute greatly to its evolution and homeostasis. Viromic sequencing has also been performed in a population of patients with alcoholic hepatitis using metagenomic sequencing. Higher viral diversity was found in fecal samples of alcoholic patients compared to controls (Jiang et al. 2020).

3 The Gut Microbiome-Brain Axis in Patients with Alcohol Use Disorder

The first study investigating the gut-brain axis in AUD patients was published in 2014. The authors showed that the patients characterized by alterations of the gut microbiota had higher scores of depression, anxiety, and alcohol craving compared to AUD patients with no altered microbial composition. In a later study (Leclercq et al. 2020), the authors showed that the leaky gut and the dysbiosis of the microbiota were also associated with altered sociability.

In recent studies (Radjabzadeh et al. 2022; Bosch et al. 2022) assessing the effect of gut microbiota composition on depression scores in more than 2,500 individuals, increased Lachnospiraceae and decreased Ruminococcaceae were consistently associated with depression across ethnicities. *Subdoligranulum,* a butyrate-producing bacteria, was also consistently found to be depleted in individuals with generalized anxiety disorder and depression in several studies (Simpson et al. 2021). Those bacterial changes were also observed in the subset of dysbiotic alcoholic patients presenting with more severe psychological symptoms, suggesting that the gut microbiota might contribute to the development of psychological alterations associated with AUD, including depression, anxiety, and alcohol craving.

In order to demonstrate the causal role of the gut microbiota in the development of the behavioral and neurobiological alterations associated with AUD, we performed a fecal microbiota transplantation experiment (Leclercq et al. 2020). Briefly, mice were first treated with a cocktail of antibiotics to deplete their microbiota. Then, the first group of mice (AUD-recipient) were transplanted with the dysbiotic microbiota of AUD patients, and the second group of mice (CT-recipient) were transferred with the gut microbiota of healthy subjects. Both groups were tested for behavior and different brain areas were collected at the end of the experiment. We found that AUD-recipient mice displayed reduced social behavior, increased depression-like behavior, and increased blood stress marker, corticosterone, compared to CT-recipient mice, while anxiety-like behavior was similar in both groups. Interestingly, mice inoculated with the AUD microbiota exhibited changes in brain functions such as reduced myelination, disturbances of GABA and glutamate neurotransmission in the frontal cortex, and increased inflammatory markers in the striatum. We then investigated the metabolic pathways that could drive the changes in behavior and brain functions. We found that increased ethanol production by the gut microorganisms and reduced β-hydroxybutyrate (BHB) levels, a ketone body produced by the liver known for its multiple neuroprotective, anticonvulsant, and anti-inflammatory properties, could contribute to the brain alterations. Those preclinical findings were confirmed in a cohort of AUD patients where lower blood BHB levels were associated with higher psychological distress, including social impairment, depression, and alcohol craving. To test the implication of BHB in myelination, we conducted an MRI study to extract the fractional anisotropy (FA) maps, a reflect of brain myelination (Pérez-Cervera et al. 2023; De Santis et al. 2019; Spindler et al. 2022), and found that plasma BHB levels were

significantly and positively correlated with FA in two areas of the white matter (Leclercq et al. 2020).

In conclusion, this was the first study showing that the transplantation of a human AUD microbiota to mice recapitulated some of the neurobiological and behavioral alterations associated with addiction, including depression, higher stress level, and social impairments and elucidating the potential mechanisms underlying the gut-brain relationships. Social deficits are also essential features of AUD patients and are related to difficulties in interpreting the emotions of others, sensitivity to social rejection (Maurage et al. 2012), and difficulties in taking into account the perspectives of others (Maurage et al. 2015), which results in high rates of relapses after alcohol withdrawal (Zywiak et al. 2003). Interestingly, while the link between gut microbiome and social behavior has been demonstrated in multiple animal models (Sherwin et al. 2019), our clinical data reported a link between leaky gut, gut dysbiosis, and social impairments in humans, suggesting a role for specific metabolites (Leclercq et al. 2020).

The metabolic pathway is a major communication pathway linking the gut and the brain. Here below, we described a non-exhaustive list of gut-derived metabolites that could potentially contribute to alcohol addiction or to the behavioral alterations associated with AUD, with a special focus on depression and sociability. After that, in the next section, we briefly reviewed the other gut-brain pathways related to inflammation and to the vagus nerve (see Fig. 1).

3.1 Microbial Metabolites Potentially Contributing to Alcohol Use Disorder

Presence or absence of specific microbial taxa has been associated with health or disease outcomes. However, given the redundancies of microbiome pathways, the functions of the microbiota, rather than their mere presence, are more likely to be the major determinants of their impact on human health. Functionality of the microbiome can be evaluated by measuring the metabolites produced by the gut bacteria via metabolomics studies. Metabolomics is an omics approach that makes possible the study of the metabolic changes in the body by measuring small molecules (i.e., metabolites) (Voutilainen and Kärkkäinen 2019). Unlike the genome, the metabolome directly represents the functional changes in cellular metabolism, and therefore provides a view about the current physiological state. In the human blood circulation, metabolites arise from different origins, including the diet, the gut microorganisms, the host, or other exogenous sources. At the crossroads between the gut microbiota and the host, microbial products from the intestine travel through the portal vein and the liver where they are metabolized into bioactive mediators and continue their journey in the systemic blood where they can eventually reach the brain to exert neuroactive effects.

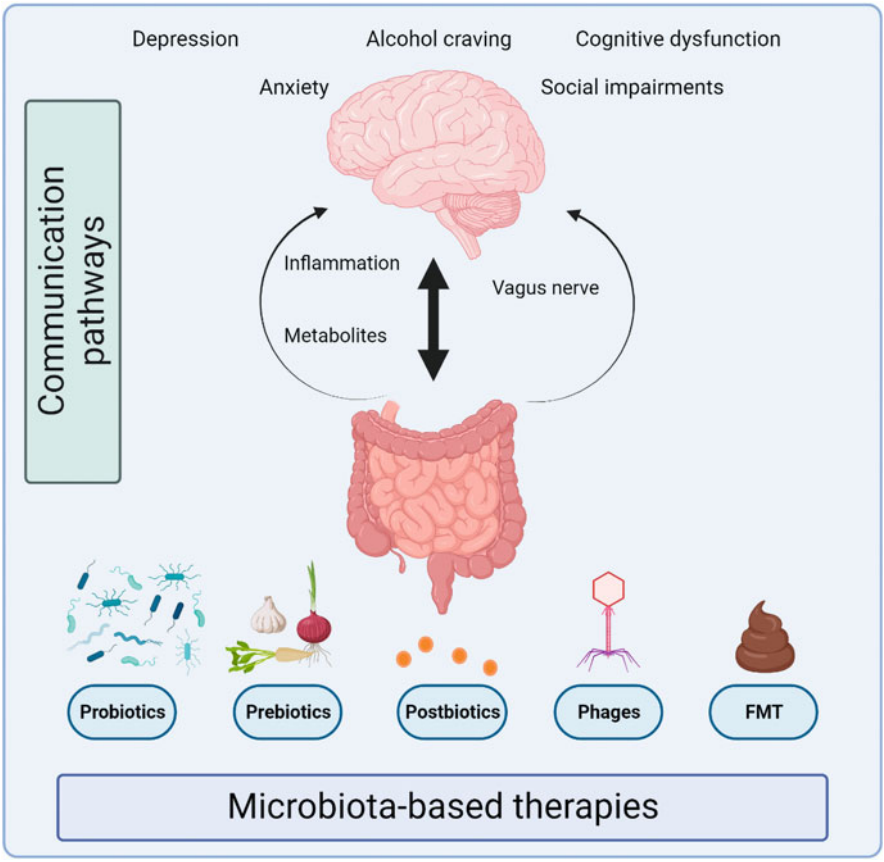

Fig. 1 Gut-brain communication pathways and microbiota-based interventional approaches that could be used in AUD patients to improve emotional, cognitive, and social impairments. FMT: fecal microbiota transplantation. Figure created with BioRender

Here, we reviewed the gut-derived metabolites that have been shown to be implicated in AUD or that could potentially be involved in the development of AUD due to their immune and neuroactive properties.

3.1.1 Neurotransmitters

The gut bacteria are able to synthetize neurotransmitters including dopamine, norepinephrine, glutamate, and GABA. The vast majority (>90%) of serotonin in the human body is produced by enterochromaffin cells of the gut under the influence of bacteria (Reigstad et al. 2015; Yano et al. 2015; Wikoff et al. 2009). Although this may not directly affect brain neurotransmitters levels since neurotransmitters

produced at the gut level may not cross the blood brain barrier (BBB), they likely influence the communication between bacteria and the motility of the gastrointestinal tract through an effect on the enteric nervous system.

3.1.2 Tryptophan Metabolites Including Indole-Derivatives and Kynurenine

Tryptophan is an essential amino acid whose degradation can occur through human or microbial pathways. Tryptophan metabolism is greatly influenced by alcohol consumption and has been widely studied in psychiatry since tryptophan is the precursor of serotonin. In fact, tryptophan can be absorbed in the intestine and enter the host serotoninergic and kynurenine pathways, or it can be converted to indole and its derivatives by gut microorganisms.

Indole-derivatives are ligands for aryl hydrocarbon receptor (AhR) and can accumulate in the brain (Jaglin et al. 2018). AhR signaling pathways have been recently associated with intestinal immune responses and neuronal signaling (Barroso et al. 2021). They are therefore potent mediators of gut-brain communication. Fecal metabolic profiling has confirmed low level of indole-derivatives in AUD patients (Leclercq et al. 2014a; Hendrikx et al. 2018).

Intestinal levels of indole-3-acetic acid are reduced in patients with alcoholic hepatitis and in mice fed ethanol (Hendrikx et al. 2018). Supplementation of ethanol fed mice with indole-3-acetic acid attenuated liver damage and restored the expression of IL-22 and Reg3g implicated in the gut barrier function, through AhR activation (Hendrikx et al. 2018). Also, amelioration of anxiety-like behavior in a humanized mouse model of irritable bowel syndrome was suggested to be mediated by indole-3-acetic acid (Constante et al. 2021).

The role of indoxyl sulfate, another AhR ligand, remains controversial. It has been shown to promote oxidative stress and neuroinflammation in some studies, while in a mouse model of multiple sclerosis, indoxyl sulfate suppressed CNS inflammation (Rothhammer et al. 2016, 2018).

Kynurenine metabolites have gained considerable attention since they are at the interface between intestinal bacteria, host immune response, and brain functions. In situations of stimulated inflammation, the enzyme indoleamine 2,3 dioxygenase is activated and due to this activation, the tryptophan metabolism switches from serotonin to kynurenine pathway. By-products of the kynurenine pathway, such as kynurenic acid (neuroprotective) and quinolinic acid (neurotoxic), modulate glutamatergic neurotransmission, and their blood levels have been associated with alcohol craving and cognitive function in AUD patients (Leclercq et al. 2021). Interestingly, those metabolites were also correlated with specific gut bacteria including *Subdoligranulum*, *Faecalibacterium prausnitzii*, *Akkermansia*, and *Prevotella*, a butyrate-producing bacterium, suggesting that the gut microbiota may influence the circulating levels of neuroactive metabolites. While current pharmacological drugs targeting the NMDA receptors showed only modest efficacy and are accompanied by multiple side effects (Jonas et al. 2014), an indirect

approach targeting glutamatergic neurotransmission through the modulation of the kynurenine pathways might represent an interesting alternative for modulating alcohol-related behavior.

3.1.3 Tyrosine-Derivatives

In a maternal immune activation experiment, a mice model of autism spectrum disorder, Hsiao et al. (Hsiao et al. 2013) showed that serum levels of 4-ethylphenylsufate (4-EPS), a tyrosine-derivative and a metabolite modulated by gut bacteria such as *Bacteroides fragilis*, could contribute to the behavioral alterations related to autism. Later, it was shown that 4-EPS interfered with oligodendrocyte maturation, myelination, and brain activity patterns in the limbic system (Needham et al. 2022). p-Cresol (or 4-methyl phenol) is another tyrosine-derivative metabolite produced by certain bacteria that is able to reduce the amount of myelin produced by brain cells and reduced social behavior (Gacias et al. 2016). The gut-host co-metabolite p-cresol glucuronide has been shown to improve, in vivo, the BBB integrity by acting as a TLR4 antagonist (Stachulski et al. 2022). Interestingly, the fecal levels of p-cresol were found to be modulated by alcohol consumption (Leclercq et al. 2014a).

3.1.4 Short Chain Fatty Acids

Short chain fatty acids (SCFAs) are produced by gut bacteria during the fermentation of nondigestible polysaccharides such as dietary fibers and are considered key mediators of the gut-brain axis due to their immune and neuroactive properties. Acetate (C2), propionate (C3), and butyrate (C4) are the most abundant SCFAs in the human body. Following their production in the colon, they are rapidly absorbed by colonocytes via active transport mediated by monocarboxylate transporters (MCTs), and then, they enter the citric acid cycle in the mitochondria to generate ATP and energy for the cells. Butyrate is produced by specific bacteria, called butyrate-producing bacteria (e.g., *Faecalibacterium prausnitzii*, *Roseburia*, *Subdoligranulum,* etc.) and has a key role in the protection of tight junction proteins of the gut barrier, thereby preventing the translocation of microbial toxins and exerting a protective effect for the liver (Roychowdhury et al. 2019). SCFAs that are not metabolized in the colonocytes are transported in the portal circulation and are used as energy source for the hepatocytes. Consequently, only a minor fraction of colon-derived SCFAs reaches the systemic circulation (Boets et al. 2017).

In vitro and animal studies have shown that SCFAs can cross the BBB and reach the brain likely through the MCTs abundantly expressed in the endothelial cells. In preclinical studies, SCFAs have been involved in numerous brain functions including neuroprotection, neurotransmission, and integrity of the BBB (Dalile et al. 2019; Braniste et al. 2014). In addition, SCFAs regulate gene expression by inhibiting histone deacetylases (HDACs), thereby promoting hyperacetylation of histones,

which have been shown to be protective for the pathophysiology of depression, autism spectrum disorders, and addiction (Volmar and Wahlestedt 2015; You et al. 2014; Kratsman et al. 2016). Studies in mice have shown that systemic administration of sodium butyrate combined with fluoxetine (a selective serotonin reuptake inhibitor antidepressant) significantly reduced depression-like behavior compared to fluoxetine alone, while histone hyperacetylation was observed in the hippocampus and frontal cortex (Schroeder et al. 2007). Also, intraperitoneal injection of sodium butyrate for 10 days attenuated the social deficits in a mouse model of autism (Kratsman et al. 2016).

However, in humans, the brain uptake of SCFAs appears to be minimal and it is currently unclear whether butyrate at physiological concentrations induces HDAC inhibition. This implies that the effects of SCFAs may rather result from peripheral signaling instead of direct uptake to the brain as seen in animal models. The effects of SCFAs on the brain could be driven by their effects on systemic inflammation which can modulate neuroinflammation (Hoogland et al. 2015). Preclinical evidence suggests that the gut microbiota might affect systemic inflammation and central neuroimmune function, with SCFAs being candidate mediators of these effects (Dalile et al. 2019). In humans, a meta-analysis (McLoughlin et al. 2017) supports the systemic anti-inflammatory effects of prebiotic and synbiotic supplementation, which are known to promote SCFAs production. However, whether those gut-targeted interventions result in decrease neuroinflammation is currently unknown but PET imaging studies could fill this gap of knowledge.

3.2 Inflammatory Pathways Contributing to Alcohol Use Disorder

AUD patients are characterized by a chronic systemic low-grade inflammation as witnessed by elevated plasma levels of TNFα, IL-1β, IL-6, IL-8, IL-10, and hsCRP (Leclercq et al. 2012, 2014b). Ethanol is likely not sufficient to induce a peripheral inflammatory response in AUD patients, as elevated circulating cytokines are also found in detoxified patients. This suggests that other stimuli might challenge the immune system, such as the gut which is a source of pro-inflammatory agents.

By yet incompletely understood mechanisms, chronic alcohol abuse increases intestinal permeability, leading to the translocation of bacterial components such as lipopolysaccharides and peptidoglycans derived from the cell wall of Gram-negative and Gram-positive bacteria, respectively, candidalysin, cytolysin, β-glucan from the gut lumen to the systemic circulation (Gao et al. 2021b). These gut-derived bacterial products are recognized by Toll-like receptors of the immune cells circulating in the blood or residing in target organs, which consequently synthesize and release pro-inflammatory cytokines. Circulating cytokines are considered important mediators of the gut–brain communication, as they can reach the central nervous system

and induce neuroinflammation that is associated with change in mood, cognition, and drinking behavior (Leclercq et al. 2017).

3.3 The Vagal Communication

The vagus nerve is composed of up to 80% of afferent fibers that are not in direct contact with the gut microbiota or luminal content but can be stimulated by neurotransmitters and gut microbial metabolites including serotonin (Ye et al. 2021), SCFAs (Muller et al. 2020), and indoles (Jaglin et al. 2018) through diffusion across the intestinal barrier.

A hallmark study conducted by Bravo et al (Bravo et al. 2011) showed that oral administration of *Lactobacillus rhamnosus* JB1 to mice reduced depression-like behavior, anxiety-like behavior, and stress level and modified glutamate, GABA, and N-acetyl aspartate in several brain areas (Janik et al. 2016). Noteworthily, the behavioral effects of the bacterium were not observed in vagotomized animals, indicating that the vagus nerve is the main route of communication linking the bacterial strain to the brain.

In the context of AUD, Ezquer et al. (Ezquer et al. 2021) showed that, in rats selectively bred for their high ethanol intake, innate gut microbiota profile (rich in Proteobacteria, for instance) influences voluntary alcohol consumption and that, interestingly, subdiaphragmatic vagotomy led to an overall 80% reduction in voluntary ethanol intake compared with sham-operated animals.

4 Promises of Microbiome-Based Therapies for Alcohol Use Disorder

Strategies targeting the gut microbiota, including probiotics, prebiotics, and fecal microbiota transplantations (FMT) have been evaluated in AUD patients. Other strategies, including postbiotics and phage therapy, have never been tested in AUD patients but preclinical results highlight their potential beneficial effect for alcohol addiction (Fig. 1).

4.1 Probiotics

Probiotics are defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" (Hill et al. 2014).

Kirpish et al. (Kirpich et al. 2008) have shown that short-term oral supplementation with *Bifidobacterium bifidum* and *Lactobacillus plantarum 8PA3* was

associated with greater improvement in alcohol-induced liver injury than standard therapy alone.

In an open-label study (Stadlbauer et al. 2008), patients with alcoholic cirrhosis received *Lactobacillus casei Shirota* for 4 weeks. Probiotics restored neutrophil phagocytic capacity, possibly by changing IL10 secretion and TLR4 expression.

However, currently, no study has evaluated the effects of probiotics on the behavioral dimensions of AUD.

4.2 Prebiotics

A prebiotic is defined as "a substrate that is selectively utilized by host microorganisms conferring a health benefit" (Gibson et al. 2017). Prebiotics act as substrates for bacteria in the colon which in turn ferment them into SCFAs.

We have performed the first randomized, double-blind, placebo-controlled study to test the effect of a prebiotic fiber supplementation on gut microbiota, immune markers, liver function, and behavior of AUD patients (Amadieu et al. 2022a; Amadieu et al. 2022b). The prebiotic inulin, a polysaccharide that is indigestible by the gut human enzymes but accessible to fermentation by the gut microorganisms, was given during a 3-week detoxification program at the hospital. Biological measurements and psychological assessments were performed before and after the intervention. Inulin induced changes in the gut microbiota composition such as an increase in *Bifidobacterium* and decrease in *Bacteroides* abundance. All patients showed an improvement in depression, anxiety, and alcohol craving scores during alcohol withdrawal regardless of the intervention group. Interestingly, only patients treated with inulin significantly improved the sociability score and had an increased serum level of brain-derived neurotrophic factor (Amadieu et al. 2022a). We then tried to elucidate the mechanisms by which the prebiotic could modulate social behavior. We did not find any improvement of systemic inflammatory markers and liver function suggesting that, in this study, inflammatory cytokines did not contribute to social behavior (Amadieu et al. 2022b). Consequently, we are currently performing blood metabolomics analysis in order to identify which gut-derived metabolites with potential neuroactive properties could contribute to the improvement of sociability score upon inulin exposure.

4.3 Fecal Microbiota Transplantation

FMT from healthy donors to patients has been used to change the gut microbiota of the recipient and represents an untargeted therapeutic approach.

The first FMT experiment in AUD patients was performed in 2017 in steroid-ineligible patients with severe alcohol hepatitis and showed improved liver disease and survival at 1 year (Philips et al. 2017). Then, in a randomized clinical trial of

FMT for AUD-related cirrhosis patients, Bajaj et al. (Bajaj et al. 2020) showed that patients receiving FMT had improved cognition and psychosocial quality of life compared to the placebo group. Serum inflammation (IL-6) and LPS-binding protein (LBP) were reduced while butyrate and fecal Ruminococcaceae were increased compared to baseline in the FMT group but not in the placebo group. A recent systematic review including 4 human studies showed that FMT overall led to an improvement in neurocognitive function in patients with hepatic encephalopathy (Madsen et al. 2021).

One major concern regarding FMT is the risk of infection, since AUD patients can have increased intestinal permeability and immune dysfunction. Also, selection of safe donors for FMT is quite complex, particularly given the potential transfer of multidrug-resistant bacteria. Since the definition of a "healthy" gut microbiota has not been determined yet, stool donors should be screened for several criteria. For instance, obesity (body mass index >30 kg/m^2), active cigarette smoking, gastrointestinal, autoimmune, atopic, allergic, metabolic, neurologic, and psychiatric conditions, as well as abnormal serologic laboratory tests are exclusion criteria. Recent data from a stool bank estimate the donor eligibility rate at 3%, highlighting that healthy donors are not easy to find (Kassam et al. 2019).

4.4 Postbiotics

While microbially generated metabolites have been related to the development of metabolic, immune, and psychosocial disturbances related to AUD, they can also serve as therapeutic opportunities. The microbial metabolites were previously included in the broad-spectrum definition of postbiotics, but this has been changed by a panel of experts in 2021. The current definition of postbiotic is "preparation of inanimate microorganisms and/or their components that confers a health benefit on the host" (Salminen et al. 2021). This includes deliberately inactivated microbial cells with or without metabolites or cell components that contribute to demonstrate health benefits.

In the context of addiction, depletion of the gut microbiota by antibiotics showed enhanced sensitivity to cocaine reward in mice while administration of SCFAs normalized the reward response (Kiraly et al. 2016). Only one randomized, placebo-controlled trial has been performed to investigate the neuroactive effect of SCFAs supplementation. Authors demonstrated that 1-week colonic SCFA-mixture delivery significantly attenuates the cortisol response to psychosocial stress in healthy men (Dalile et al. 2020).

4.5 Phage Therapy

Alcoholic hepatitis is the most severe form of alcohol-related liver disease, with 75% of patients dying within 90 days of diagnosis (Maddrey et al. 1978; Thursz et al. 2015). Therapy with corticosteroids has limited efficiency (Thursz et al. 2015) and liver transplantation is not available for all patients. Therefore, new approaches that target the gut microbiota could be of importance for those patients. Phage-based treatment is a potential interesting tool to edit very precisely the gut microbiota. Previous work has demonstrated the key role of cytolytic *E. faecalis* in ethanol-induced liver damage and mortality in patients with alcoholic hepatitis (Duan et al. 2019). Therefore, eradication of those specific bacterial strains that produce cytolysin might result in better outcome compared to current treatments of alcoholic hepatitis. Phages or bacteriophages are viruses infecting bacteria in a highly-strain specific manner. In a preclinical study, mice were colonized with feces from cytolysin-positive patients and then administered with a bacteriophage cocktail containing phages against cytolytic *E. faecalis*. The treatment with phages reduced the fecal amount of *Enterococcus* without affecting the overall composition of the gut microbiota and interestingly, it abolished ethanol-induced liver injury (Duan et al. 2019). A clinical trial is now required to validate the human relevance of those findings.

5 Conclusion

The fields of microbiology and neuroscience in modern medicine have largely developed in recent years, but following distinct trajectories. However, accumulating evidence demonstrated that the gut microbiota can greatly influence all aspects of physiology including brain functions and behavior (Cryan and Dinan 2012). It should be emphasized that the majority of the gut microbiota-brain axis studies have been performed in rodent models. While modelling complex human psychiatric disorders such as alcohol addiction in animals is challenging, it should be noted that important discrepancies between rodent and human microbiota do exist (Nguyen et al. 2015). This review has therefore put the focus on human studies, whenever possible.

Characterization of the human alcoholic gut microbiota composition is challenging. Results of existing studies are conflicting likely due to many confounding factors that influence the microbial composition, including lifestyle factors, diet, and drugs used. Therefore, combining metagenomics and metabolomics approaches in well-defined AUD population could be a better way to uncover the intricate communication within the gut-brain axis. Some bacterial metabolites have been proposed in this review to contribute to the physiopathology of AUD. The role of other microbiota-derived metabolites with neuroactive properties in other pathological contexts has been reviewed elsewhere (Ahmed et al. 2022).

We believe that modulating certain type of neuroactive metabolites, by acting on nutrition and gut microbiota, represents an interesting strategy to manage neuropsychiatric diseases such as alcohol addiction. It appears probable that, in the future, a detailed analysis of the patient's microbiome and metabolome will be performed, in relation to discrete behavioral impairments, to determine which personalized microbiota-centered therapies might be the most useful to restore microbe-host homeostasis (Bajaj et al. 2022).

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