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Review

2023 ESBRA Nordmann Award Lecture: Involvement of the gut microbiome-brain axis in alcohol use disorder

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Abstract

The human intestine is colonized by a variety of microorganisms that influence the immune system, the metabolic response, and the nervous system, with consequences for brain function and behavior. Unbalance in this microbial ecosystem has been shown to be associated with psychiatric disorders, and altered gut microbiome composition related to bacteria, viruses, and fungi has been well established in patients with alcohol use disorder. This review describes the gut microbiome–brain communication pathways, including the ones related to the vagus nerve, the inflammatory cytokines, and the gut-derived metabolites. Finally, the potential benefits of microbiota-based therapies for the management of alcohol use disorder, such as probiotics, prebiotics, and fecal microbiota transplantation, are also discussed.

Keywords: gut microbiota; gut–brain axis; nutrition; metabolomics

Introduction

The gastro-intestinal tract hosts a complex and dynamic ecosystem comprising bacteria, viruses, yeasts, fungi, and archaea, collectively called as microbiota, which has a major impact on host's health (Nicholson et al. 2012). Those microorganisms living in the gut have numerous functionalities including regulation of the immune system, involvement in many metabolic responses (such as absorption of nutrients and minerals, synthesis of vitamins), 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). Among the factors that are known to influence the gut microbiota, such as age, sex, lifestyle factors (like stress and sleep patterns), and medications used, the dietary habits have likely the greatest influence on microbial composition (David et al. 2014; Zhernakova et al. 2016; Quigley 2017). Alcohol use disorder (AUD), in its severe form, affects the nutritional status and can lead to malnutrition (Stickel et al. 2003; Ross et al. 2012). Knowing the impact of the gut microbiota on brain functions and behavior, it is of interest to interrogate the gut microbiota composition of AUD patients and to investigate the link between nutrition, gut microbiota, and the psychological symptoms developed by AUD patients, such as depression, anxiety, alcohol craving, and social impairments.

In this "ESBRA Nordmann Award"-related narrative review, we highlighted the work done by our own research group and by other scientists focusing on the impact of alcohol on the human gut microbiome, on the involvement of the gut–brain axis in the development of AUD, and we discussed the potential benefits of microbiota-based therapies for the improvement of AUD.

Alcohol abuse as a modulator of the gut microbiota

A systematic review, published in 2020, aimed at listing the changes in the composition of the microbiome in AUD patients, using 16S ribosomal RNA gene sequencing (Litwinowicz et al. 2020). Seventeen studies were retrieved in this review, which included AUD individuals with and without alcoholic liver diseases. At the phylum level, Bacteroidetes had a lower relative abundance and Proteobacteria (a highly pro-inflammatory phylum) had 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 was reduced. At the genus level, *Ruminococcus*, *Collinsella*, *Prevotella*, *Clostridium*, *Faecalibacterium*, *Paraprevotella*, *Bacteroides*, *Parabacteroides*, *Alistipes*, and *Akkermansia* (also found in Addolorato et al. 2020) 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 others reported a higher abundance.

Overall, the microbial changes observed in the existing studies are conflicting with little overlap, asking for larger and more carefully designed study. Indeed, many confounding factors should be considered when analyzing the gut microbiota of AUD patients. Among them, nutritional habits and medications should be listed. In a recent study analyzing the nutritional intakes of AUD patients (Amadieu et al. 2021), we found that their diet included low fiber intakes compared to the diet of healthy controls. Furthermore, while

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ethanol brought >40% of the total energy intake, a significant reduction of carbohydrates, lipids, and proteins was observed in AUD patients. Those nutritional deficiencies and overall unbalanced diet could contribute to the alterations of gut microbiota. In addition, 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 characterized subjects presenting with progressive alcoholic liver disease (Maccioni et al. 2020), as well as cirrhotic patients (Addolorato et al. 2020). In addition, the type of alcoholic beverages (beer *vs* wine *vs* spirits) and the drinking status (actively drinking *vs* short- or long-term abstinence) might also influence the microbial composition. Finally, the differences in the methodologies (related to DNA extraction, 16S ribosomal DNA sequencing, bioinformatics pipelines) across studies and that are in constant evolution could participate to the heterogeneity of the results.

While a large majority of the studies has mainly focused on DNA-based 16S rRNA gene sequencing and shotgun metagenomic sequencing that elucidate the bacterial composition and gene content, more attention is recently given to viruses and fungi (amplicon sequencing based on 18S rRNA and internal transcribed spacer) (Gao et al. 2021a). Viruses are key constituents of the gut ecosystem and contribute greatly of 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 AUD patients compared to controls (Jiang et al. 2020). However, the human mycobiome diversity is relatively low compared with bacterial populations and is dominated by yeast such as *Candida*, *Saccharomyces,* and *Malassezia*. Dysbiosis of intestinal fungi has been reported in AUD patients with an overgrowth of *Candida* (Yang et al. 2017; Lang et al. 2020).

Obviously, there is no consensus on which bacteria, viruses, and fungi are effectively modulated by chronic alcohol abuse. Furthermore, to identify potential bacteria involved in AUD development, the reference to a healthy control group without a history of alcohol abuse is needed but the microbial features of a "healthy microbiota" are still a matter of debate (Bäckhed et al. 2012). However, several studies including those from our laboratory found that only a subset of AUD patients is characterized by altered gut microbiota composition (Mutlu et al. 2012; Leclercq et al. 2014b; de Timary et al. 2015; Maccioni et al. 2020). In a study evaluating both the intestinal permeability and the microbial composition, we found that ∼40% of AUD patients presented with higher intestinal permeability (leaky gut) and dysbiosis compared to healthy controls, while 60% of AUD patients had gut permeability and microbial composition similar to that of controls (Leclercq et al. 2014b).

The gut microbiome–brain axis in alcohol use disorder

Intestinal barrier function is crucial for maintaining homeostasis. Many preclinical and clinical studies have shown that alcohol-induced dysregulation of the gut barrier lead to the translocation of bacterial endotoxins, such as lipopolysaccharides (LPS) or peptidoglycan, from the gut lumen to the systemic circulation (Adachi et al. 1995; Mathurin et al. 2000; Rao et al. 2004; Yan et al. 2011; Leclercq et al. 2014a; Donnadieu-Rigole et al. 2018). Those endotoxins are then recognized by immune cells, through Toll-like receptors, which synthesize and release inflammatory cytokines (Akira et al. 2006). This has huge consequences for the development of alcoholic liver damage (Wheeler 2003). Cytokines, released in the periphery, are also known to reach the brain and induce psychological impairments including depression (Dantzer et al. 2008).

Interestingly, AUD patients who presented with increased intestinal permeability and dysbiosis also presented with a more severe form of alcohol addiction. Indeed, the dysbiotic patients had higher score of depression, anxiety, and alcohol craving compared to AUD patients with a microbial composition and intestinal permeability similar to that of healthy controls, particularly at the end of a 3-week detoxification program (Leclercq et al. 2014b). This psychological distress, associated with leaky gut and dysbiosis, could make the patients more susceptible to relapse. In a later study, we found that altered gut microbiota composition was also strongly associated with altered sociability in AUD patients (Leclercq et al. 2020a). Social impairments are 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 also results in high rates of relapses after alcohol withdrawal (Zywiak et al. 2003).

In order to demonstrate the causal role of the gut microbiota in the development of the psychological alterations associated with AUD, we conducted a preclinical experiment of fecal microbiota transplantation (FMT) (Leclercq et al. 2020a). Briefly, a first group of mice was transplanted with the dysbiotic microbiota of AUD patients, and a second group of mice was transplanted with the gut microbiota of healthy subjects. Both groups of mice were subsequently tested for behavior. We found that mice transplanted with the AUD microbiota displayed increased depression-like behavior, reduced social behavior, and increased stress level (as reflected by increased serum corticosterone level), compared to control mice transplanted with the microbiota of healthy subjects. At the end of the behavioral testing, different brain areas were collected to analyze brain functions. Mice inoculated with the AUD microbiota exhibited several changes including reduced myelination, disturbances of gamma-aminobutyric acid (GABA) and glutamate neurotransmission in the frontal cortex, and increased inflammatory response (inflammatory cytokines and markers of activated microglia) in the striatum. Interestingly, the role of striatal immune reactions for reward processes and alcohol-seeking behavior is supported by rodents studies, where voluntary ethanol consumption was induced by a targeted neuro-immune response (Chen et al. 2011; Blednov et al. 2012; Valenta and Gonzales 2016).

We then investigated the metabolic pathways that could drive the changes in brain functions and behavior induced by the FMT. 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 AUD patients: lower blood BHB levels were associated with higher psychological

distress, including social impairment, depression, and alcohol craving (Leclercq et al. 2020a).

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. This experiment demonstrated that "something in the gut" can changes the host metabolism, with consequences for brain functions and behavior. Whether "something" is a bacterium, a virus, a fungus or a metabolite produced gut microorganisms remained (a big challenge) to be determined.

Mechanisms underlying gut–brain interactions

Accumulating data now indicate that the gut microbiota communicates with the central nervous system, through neural, immune, and metabolic pathways, and thereby influences brain functions and behavior. In line with that, germ-free mice exhibit behavioral and neurobiological disturbances such as reduced anxiety (Neufeld et al. 2011), reduced social behavior (Desbonnet et al. 2014), hyperactivity of the hypothalamus–pituitary–adrenal axis (Sudo et al. 2004), leaky blood brain barrier (Braniste et al. 2014), axon hypermyelination (Hoban et al. 2016), etc. Those gut–brain communication pathways are briefly described here below.

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), short-chain fatty acids (SCFAs) (Muller et al. 2020), and indoles (Jaglin et al. 2018) through diffusion across the intestinal barrier.

The implication of the vagus nerve in the neuroactive properties of a bacterium was first demonstrated by Bravo et al. (2011). 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. Interestingly, the behavioral effect of this bacterium was not observed in vagotomized animals, indicating that the vagus nerve is the main route of communication linking the bacterial strain to the brain.

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

The immune activation

AUD patients present with chronic low-grade systemic inflammation, as witnessed by elevated plasma levels of tumor necrosis factor alpha, interleukin (IL)-1β, IL-6, IL-8, IL-10, and high sensitivity C-reactive protein (Leclercq et al. 2012, 2014a), even in the absence of actual bacterial or viral infection. *In vivo*, ethanol is likely not sufficient to induce the peripheral inflammatory response observed in AUD patients, as elevated plasma proinflammatory cytokines are still found after a period of sobriety (Leclercq et al. 2012). 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 LPS 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). In our study, circulating cytokines were significantly correlated with the psychological symptoms associated with AUD, including depression, anxiety, and alcohol craving (Leclercq et al. 2012).

The metabolic pathways

While it is almost impossible to identify microbial taxa responsible for the development of AUD in human pathology, it might be more relevant to interrogate the metabolome—which represents the large catalog of metabolites produced or to some extent influenced by the gut microbiota. Untargeted metabolomics analysis of stools, urine, or blood, using mass spectrometry or nuclear magnetic resonance spectroscopy approaches, is useful to gain pathophysiological insight into the disease process by identifying potential key metabolites involved in AUD. Indeed, the circulating metabolome reflects the crosstalk between nutrition, microbiome, and host metabolism (Ahmed et al. 2022), with diet and microbiome being the strongest determinants of the human blood metabolome (Bar et al. 2020; Chen et al. 2022). In 2019, a review summarized the results obtained from 23 studies that have used a metabolomics approach for measuring changes in metabolite profiles in relation to alcohol use (Voutilainen and Kärkkäinen 2019). Changes in lipids have been highlighted as the most consistent changes across studies. In addition to altered levels of SCFAs and long chain fatty acids, changes in bile acids, dimethyl sulfide, dimethyl trisulfide, indole compounds, phenol, cytolysin – an exotoxin secreted by *Enterococcus feacalis*, fungal β-glucan, and candidalysin – a toxin secreted by *Candida albicans* have been observed in animal models and AUD patients (Xie et al. 2013; Leclercq et al. 2014b; Couch et al. 2015; Wang et al. 2018). In a recent study (Leclercq et al. 2024), not yet published, we have conducted a non-targeted metabolomics analysis in AUD patients, at the beginning and end of alcohol withdrawal, and we have correlated the blood metabolites with the psychological symptoms in order to identify circulating metabolites with potential neuroactive properties. We found that some bacterial metabolites, including hippuric acid and pyrocatechol sulfate, and dietary metabolites (like caffeine metabolites) were strongly correlated with depression, anxiety, and alcohol craving.

Finally, the metabolites arising from the tryptophan–kynurenine pathway have gained considerable attention since they are at the interface between intestinal bacteria, host-immune response, and brain functions. The essential amino

acid tryptophan is the precursor of serotonin, which plays an important in mood regulation. In mammals, >90% of tryptophan is degraded through the kynurenine pathway, which generates a range of metabolites that regulate the immune response and neurotransmission (Vécsei et al. 2013). Increased concentration of the neurotoxic metabolite quinolinic acid and decreased levels of the neuroprotector metabolite kynurenic acid modulate glutamatergic neurotransmission, which were observed in AUD patients (Leclercq et al. 2021).

Overall, those results suggest that innovative approaches targeting the gut microbiome, or the associated metabolic pathways, through for instance nutritional interventions might be promising in the management of AUD.

Microbiota-based therapies for the improvement of AUD

Since nutrition is a major factor impacting the gut microbiota (David et al. 2014), it seems interesting to modulate the microbial composition through nutritional approaches. Several attempts have been done in AUD patients, using probiotics and prebiotics (see Probiotics and Prebiotics).

Probiotics

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

Most probiotics contain *Lactobacillus* and *Bifidobacterium* genera, which are saccharolytic bacteria that can ferment carbohydrates and produce SCFAs including acetate, propionate, and butyrate. These SCFAs are known to inhibit the growth of pathogens, reinforce the gut barrier function, and exert anti-inflammatory actions (Koh et al. 2016). Several randomized clinical studies have been performed to evaluate the impact of probiotic supplementation on alcoholic liver disease (see review, Leclercq et al. 2020b), with a small but significant effect on the reduction of liver enzymes and some inflammatory parameters. Currently, no study has evaluated the effects of probiotics on the psychological aspects of AUD.

Prebiotics

A prebiotic is defined as "a substrate that is selectively utilized by host microorganisms conferring a health benefit" (Gibson et al. 2017). Dietary fibers including fructooligosaccharides, inulin, galactooligosaccharides, which are not digested in the upper part of intestinal tract, reach the colon and are selectively fermented by bacteria into SCFAs (Fu et al. 2022). In a recent study (Amadieu et al. 2021), we conducted detailed dietary anamneses in AUD patients and observed that, not surprisingly, 90% of the patients had a dietary fiber intake below the recommendation. Interestingly, low dietary fiber intake was associated with higher anxiety and lower sociability score, suggesting that adequate intake of dietary fiber may be beneficial for the recovery of psychological and social impairment. This hypothesis was tested in a randomized, double-blind, placebo-controlled study in which AUD patients were supplemented with a prebiotic (inulin) during a 3-week detoxication program (Amadieu et al. 2022a). 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* genera abundance. All patients showed an improvement in depression, anxiety, and alcohol craving scores during alcohol withdrawal regardless of the intervention group (inulin *vs* placebo). 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). However, in this study, we did not find improvement of inflammatory marker and liver function suggesting that those parameters do not play a role in social behavior (Amadieu et al. 2022b).

Fecal microbiota transplantation

Another area of interest has been the role of FMT in AUD treatment (Wolstenholme et al. 2024). The first FMT experiment in AUD patients was performed in 2017 in eight steroid-ineligible patients with severe alcohol hepatitis and showed improved liver disease and survival at 1 year (Philips et al. 2017). Another open-label study comparing FMT in 13 alcohol-associated hepatitis patients with standard care in 20 patients reported a significant increase in 90-day survival in favor of the FMT group (Sharma et al. 2022). In an extended study including more patients ($n = 60$ in each group), Pande et al. (2023) showed a significant improvement in 90-day survival in the FMT group compared to the prednisolone group. Importantly, there were fewer deaths related to infections in the FMT group, indicating the safety of the procedure.

In a randomized clinical trial of FMT for AUD-related cirrhosis patients, 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 family were increased compared to baseline in the FMT group but not in the placebo group.

Finally, FMT conducted in 35 severe alcohol-associated hepatitis patients showed increase 3-year survival (66 *vs* 39%), reduction of alcohol relapse (29 *vs* 54%), and reduction of time to relapse compared to patients receiving standard of care. Gut microbiota analyses indicated increased relative abundance of *Bifidobacterium* and reduction of *Acinetobacter* genera in the FMT group (Philips et al. 2022).

Conclusion

Accumulating evidence demonstrated that the gut microbiota can greatly influence all aspects of physiology, including brain functions and behavior (Cryan and Dinan 2012). Characterization of the human AUD 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 a well-defined AUD population could be a better way to uncover the intricate communication within the gut–brain axis. Identification of circulating metabolites with immune and/or neuroactive properties could bring new insights in the physiopathology of AUD and potential treatments. Indeed, the modulation of specific metabolites, by acting on nutrition and on the gut microbiome, represents an interesting strategy to manage AUD. It appears probable that,

in the future, a detailed analysis of the patient's microbiome and metabolome will be performed in order to determine which personalized microbiota-centered therapies might be the most useful to restore microbe–host homeostasis (Bajaj et al. 2022). In addition, we should keep in mind that the gut and the brain are engaged in a bidirectional relationship, where both influence each other. Specific behaviors such as stress and social isolation are known to influence the microbial composition. The combination of microbiota-based therapies with behavioral therapies aiming at reducing stress and increasing social support should be considered in the management of AUD.

List of abbreviations

AUD: alcohol use disorder; BHB: β-hydroxybutyrate; FMT: fecal microbiota transplantation; GABA: gamma-aminobutyric acid; hsCRP: high sensitivity C-reactive protein; IL-: interleukin; ITS: internal transcribed spacer; LPS: lipopolysaccharide; SCFAs: short-chain fatty acids; TNFα: tumor necrosis factor alpha; 16S rDNA: 16S ribosomal DNA (a component of the 30S small subunit of prokaryotic ribosomes); 18S rDNA: 18S ribosomal DNA (part of the 40S small subunit of eukaryotic ribosomes, is used for broader eukaryotic organism identification, including fungi).

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Data availability

None.

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