Chapter 10
Bacterial Neuroactive Compounds Produced by Psychobiotics

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Abstract We recently coined the phrase 'psychobiotics' to describe an emerging class of probiotics of relevance to psychiatry [Dinan et al., Biol Psychiatry 2013;74 (10):720–726]. Such "mind-altering" probiotics may act via their ability to produce various biologically active compounds, such as peptides and mediators normally associated with mammalian neurotransmission. Several molecules with neuroactive functions such as gamma-aminobutyric acid (GABA), serotonin, catecholamines and acetylcholine have been reported to be microbially-derived, many of which have been isolated from bacteria within the human gut. Secreted neurotransmitters from bacteria in the intestinal lumen may induce epithelial cells to release molecules that in turn modulate neural signalling within the enteric nervous system and consequently signal brain function and behaviour of the host. Consequently, neurochemical containing/producing probiotic bacteria may be viewed as delivery vehicles for neuroactive compounds and as such, probiotic bacteria may possibly have the potential as a therapeutic strategy in the prevention and/or treatment of certain neurological and neurophysiological conditions.

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Abbreviations

5-HT	5-Hydroxytryptamine
AA	Arachidonic acid
ASD	Autism spectrum disorders
CLA	Conjugated linoleic acid
CNS	Central nervous system
DHA	Docosahexaenoic acid
GABA	Gamma-aminobutyric acid
GAD	Glutamate decarboxylase
GF	Germ-free
GIT	Gastrointestinal tract
IPA	Indole-3-propionic acid
LAB	Lactic acid bacteria
LC-PUFA	Long-chain fatty acid
PPAR γ	Peroxisome proliferator-activated receptor gamma
SCFA	Short chain fatty acid
TNF	Tumor necrosis factor

Introduction

We recently coined the phrase 'psychobiotics' to describe an emerging class of probiotics of relevance to psychiatry [1]. Such "mind-altering" probiotics may act via their ability to produce various biologically active compounds, such as peptides and mediators normally associated with mammalian neurotransmission. Several molecules with neuroactive functions such as gamma-aminobutyric acid (GABA), serotonin, catecholamines and acetylcholine have been reported to be microbially-derived, many of which have been isolated from bacteria within the human gut. Secreted neurotransmitters from bacteria in the intestinal lumen may induce epithelial cells to release molecules that in turn modulate neural signalling within the enteric nervous system and consequently signal brain function and behaviour of the host. Consequently, neurochemical containing/producing probiotic bacteria may be viewed as delivery vehicles for neuroactive compounds and as such, probiotic bacteria may possibly have the potential as a therapeutic strategy in the prevention and/or treatment of certain neurological and neurophysiological conditions.

In recent years, interdisciplinary investigation has revealed strong evidence of the existence of a bidirectional signalling between the intestine and the brain, the so called "brain-gut axis". This communication system integrates neural, hormonal and immunological signalling between the gut and the brain and is critical to maintain homeostasis [2]. More recently, however, this axis concept was expanded to the "microbiota-gut-brain axis", when it became clear that not only the intestinal tract itself but also its 100 trillion microbial inhabitants can affect the functioning of the central nervous system (CNS) and consequently mood and behaviour [3, 4]. The

brain communicates with the enteric microbiota directly by releasing signalling molecules into the gut lumen, and indirectly by altering gastric motility, secretion and intestinal permeability [5]. Equally, the enteric microbiota can communicate with the host via epithelial cells, receptor-mediated signalling, and stimulation of cells of the lamina propria [6]. Changes in the composition of the gut microbiota may lead to deterioration in gastrointestinal, neuroendocrine, or immune pathways and relationships, which in turn could lead to alterations in brain-gut interactions and consequently result in disease [7].

Recently, the microbial endocrinology-based theory was introduced which claimed that probiotics (i.e. live microorganisms that, when ingested in adequate amounts, exerts a health benefit on the host [8]) function as pharmacological agents and hence act as drug delivery vehicles due to their ability to synthesize neuroactive compounds [9]. As such, probiotics may affect the brain in a direct manner by producing neurotransmitters and neuromodulators and may therefore have the potential to act as a novel treatment for neuropsychiatric diseases. The delivery of neurochemicals by probiotics may either be in the amount already contained in the bacterium at time of ingestion or what is actively produced by the bacterium once inside the gastrointestinal tract (GIT).

It is well recognized that some bacteria within the human GIT have the capacity to produce many neurotransmitters and neuromodulators. For example, *Lactobacillus* spp. and *Bifidobacterium* spp. have been reported to produce GABA; *Escherichia* spp., and *Bacillus* spp. have been reported to produce norepinephrine; *Streptococcus* spp., *Escherichia* spp. and *Enterococcus* spp. have been reported to produce serotonin; *Bacillus* spp. have been reported to produce dopamine, and *Lactobacillus* spp. have been reported to produce acetylcholine and histamine [10–14]. It is possible that the secreted neurotransmitters from bacteria in the intestinal lumen may induce epithelial cells to release molecules that in turn modulate neural signalling within the enteric nervous system, or act directly on primary afferent axons [15]. Other bacterially-produced metabolites with proven neuroactive functions include short chain fatty acids (SCFAs) and long chain fatty acids such as conjugated linoleic acid (CLA). Table 10.1 provides a list of a range of neuroactive chemicals isolated from bacteria within the human gut (it should be noted that this is representative of neuroactives isolated, but is not a complete and comprehensive list). The production of these metabolites and the aforementioned neuroactives by bacteria naturally inhabiting the human gut will be discussed in this chapter.

Bacterial Metabolites

The microbiome has a capability to produce a spectrum of neuroactive compounds, and although we are still in an early stage of exploring its capacity, there is an expanding volume of evidence supporting the role of our intestinal inhabitants as being factories for neurochemicals. Studies comparing germ-free (GF) animals (lacking gut microbiota) with conventional animals (with a normal gut microbiota)

Table 10.1 Representative list of neurochemicals isolated from bacteria within the human gut

Genus	Neurochemical	References
Lactobacillus, Bifidobacterium	GABA	[10]
Streptococcus, Escherichia, Enterococcus, Lactococcus, Lactobacillus	Serotonin	[17, 49]
Escherichia, Bacillus	Norepinephrine	[14, 49]
Escherichia, Bacillus, Lactococcus, Lactobacillus, Streptococcus	Dopamine	[14, 17, 49]
Lactobacillus, Bacillus	Acetylcholine	[12, 13, 59, 61]
Lactobacillus, Lactococcus, Streptococcus, Enterococcus	Histamine	[11, 66, 67]

have demonstrated that the commensal microbiota influence monoamine levels in specific brain regions of the host brain [3, 16]. Neurochemicals that have been isolated from gut bacteria include GABA, noradrenaline, serotonin, dopamine and acetylcholine [10, 17, 18], which may directly affect the brain. The use of probiotic bacteria that can deliver neurochemicals has further been suggested as a novel treatment for neuropsychiatric diseases [9]. Other bacterial metabolites with neuroactive functions include SCFAs such as propionate and long chain fatty acids such as CLA [19–22].

It is not yet clear as to why certain bacteria harbour the genes responsible for the production of neuroactive molecules. It has been proposed that late horizontal gene transfer can explain the existence of genes encoding many of the enzymes involved in the synthetic and metabolic pathways of catecholamines, acetylcholine, and GABA from bacteria. This concept is concordant with increasing evidence that signalling molecules of quorum-sensing systems, used by bacteria to communicate and coordinate their actions [23] can also bind to mammalian receptors and directly influence the host [24, 25]. Neurotransmitters that are produced by the host can furthermore influence the function of members of the microbiota. As an example, the QseC sensor kinase, present in *Escherichia coli* O157:H7 is a bacterial receptor for host-derived epinephrine/norepinephrine which triggers the transcription of virulence genes in bacteria, a response which can be blocked by adrenergic antagonists [26].

Gamma-Aminobutyric Acid

Gamma-aminobutyric acid (GABA) is a major inhibitory neurotransmitter in the brain regulating many physiological and psychological processes, and dysfunctions in GABA signalling have been linked to anxiety and depression [27]. We have recently demonstrated that human intestinally derived strains of lactobacilli and bifidobacteria produce GABA from monosodium glutamate (MSG) in culture [10] and it has been suggested that microbially produced GABA may have an effect on the brain-gut axis [28]. The production of GABA by commensal bacteria occurs via

the same biosynthetic pathway as in neuronal tissue involving conversion of glutamate by the action of the enzyme glutamate decarboxylase (GAD) and vitamin co-factor pyridoxal phosphate [29]. The GABA-producing capability held by some bacterial strains is thought to protect the organism from the acidic environment encountered in the stomach, since its synthesis involves proton exchange for the uptake of glutamate [30]. Many studies have reported the presence of a *gad* gene in lactic acid bacteria (LAB) [29, 31, 32] and given the heightened interest in the physiological effects associated with GABA, many GABA-enriched fermented food products, using dairy starter cultures with GABA-producing capabilities, have been developed in the past 10 years [31, 33, 34]. The levels of GABA that can be achieved in vitro by probiotic organisms are quite large. For example, in the production of fermented foodstuffs, such as Japanese funa-sushi and Chinese traditional paocai, which uses lactobacilli as starter cultures, GABA levels in the millimolar range have been detected in the final products [35, 36].

The prevalence of GABA-producing lactobacilli and bifidobacteria in the human GIT is not as widespread as it is among food-derived LAB. We screened 91 strains of human-derived lactobacilli and bifidobacteria for their ability to produce GABA from MSG, and found that five strains had the ability [10], with *Lactobacillus brevis* and *Bifidobacterium dentium* being the most efficient GABA producers. *L. brevis* DPC6108 was the most efficient of the strains tested, and it retained the capability to produce GABA in the presence of other gut-derived bacteria (in faecal fermentations). A recent study also demonstrated that GABA production in black soybean milk by *L. brevis* FPA3709 and its administration to rats resulted in an antidepressant effect similar to that of fluoxetine (a common antidepressant drug) but without the side effects of lost appetite and decreased weight [37]. Interestingly, neuronal cells have been shown to respond to nanomolar concentrations of GABA [38]. At the level of gene expression, ingestion of the *Lactobacillus* strain, *Lactobacillus rhamnosus* (JB-1), altered the mRNA expression of both $GABA_A$ and $GABA_B$ receptors. These receptors are implicated in anxiety and depression, and are widely expressed in key brain regions responsible for maintaining normal fear and mood responses [39].

A number of further potential health benefits of GABA have been described, including induction of hypotension, diuretic effects, and tranquilizer effects [40, 41]. Furthermore, GABA has a receptor-mediated role in a number of immunological (i.e. down-regulation of cytokine released by proinflammatory cells release) and intestinal neurophysiological (i.e. secretion of neuropeptides by intrinsic and extrinsic intestinal nerve fibers) processes [38, 42, 43]. Given the broad health benefits associated with GABA, the use of a GABA-secreting bacterium, acting on dietary glutamate, could have potential in both neuropsychiatric diseases and in inflammatory conditions such as inflammatory bowel disease (IBD), however, in vivo studies are required to ascertain whether the host would benefit from microbially- produced GABA.

Serotonin and 5-HT Precursors

Serotonin (5-hydroxytryptamine, 5-HT) is a metabolite of the essential amino acid tryptophan and plays an important role in the regulation of a number of bodily functions, including mood. Today, the vast majority of antidepressant drugs lead to increases in the levels of serotonin in the brain. Serotonin is ubiquitously distributed in nature and has been found in some plants (fruits and nuts) and in both vertebrates and invertebrates animals [44]. Some studies also indicate that bacteria can synthesize serotonin and/or induce its production by the host. Wikoff et al. [45] utilized a metabolomics-based approach to study the metabolic products of the microbiome in mice which may impact health, and unexpectedly found that serotonin plasma levels were nearly threefold higher in conventional mice compared with GF mice, whereas plasma concentrations of tryptophan was 40 % lower in conventional animals than in their GF counterparts. The authors postulated that the increased plasma serotonin levels observed could indirectly result from an as yet undefined host microbe interaction [45]. Furthermore, increased serotonin turnover and altered levels of related metabolites in the striatum [3] and hippocampus [46] of GF mice have been reported. The levels of serotonin in the cortex and hippocampus were also significantly reduced in GF mice [4], suggesting a role for the microbiota in maintaining serotonin levels. Rats that were given *Bifidobacterium infantis* for 14 days had increased concentrations of the serotonin precursor tryptophan in plasma, suggesting that commensal bacteria have the ability to influence tryptophan metabolism [47]. This effect on tryptophan metabolism may be mediated by the impact of the microbiota on the expression of indoleamine-2,3-dioxygenase, a key enzyme in the physiologically dominant kynurenine pathway of tryptophan degradation [48]. Moreover, Özogul [17] investigated the influences of LAB on biogenic amine formation by foodborne pathogens using single and mix cultures. All the LAB species used in their study, including *Lactococcus lactis* subsp. *cremoris* (MG 1363), *L. lactis* subsp. *lactis* (IL1403), *Lactobacillus plantarum* (FI8595) and *Streptococcus thermophilus* (NCFB2392) produced serotonin to some extent [17]. Shishov et al. [49] also demonstrated that *E. coli* K-12 was capable of producing serotonin at nanomolar concentrations in culture.

Catecholamines

Catecholamines such as dopamine and norepinephrine (also known as noradrenaline) are the major neurotransmitters that mediate a variety of the CNS functions, such as motor control, cognition, memory processing, emotion and endocrine regulation [50]. Dysfunctions in catecholamine neurotransmission are implicated in some neurological and neuropsychiatrical disorders, including Parkinson's disease [51], Alzheimer's disease [52] and major depressive disorders [53].

Both norepinephrine and dopamine were identified in bacteria in a study by Tsavkelova et al. [14]. Dopamine, in concentrations from 0.45 to 2.13 mmol/L was found in the biomass of *Bacillus cereus*, *B. mycoides*, *B. subtilis*, *Proteus vulgaris*, *Serratia marcescens*, *S. aureus*, and *E. coli*, and norepinephrine was detected (0.21–1.87 mmol/L) in *B. mycoides*, *B. subtilis*, *P. vulgaris*, and *S. marcescens*. Moreover, it was demonstrated that bacteria, particularly *B. subtilis* may release norepinephrine and dopamine out of the cell and perhaps in this way might participate in intercellular microbe–microbe and microbe–host communications [14]. Shishov et al. [49] used the *E. coli* K-12 strain to investigate the production of catecholamines in vitro and demonstrated that this *E. coli* strain can produce dopamine and norepinephrine and also their precursor, DOPA, in culture. The culture fluid of *E. coli* contained micromolar concentrations of DOPA and nanomolar concentrations of dopamine and norepinephrine [49]. Moreover, Özogul [17] demonstrated the production of dopamine by some LAB species in culture, namely *L. lactis* subsp. *cremoris* (MG 1363), *L. lactis* subsp. *lactis* (IL1403), *L. plantarum* (FI8595) and *S. thermophilus* (NCFB2392). A recent study by Asano et al. [54] demonstrated that bacteria which constitute the normal microbiome in mice are capable of the in vivo production of large quantities of norepinephrine. Furthermore, adoptive transfer of the microbiome of mice that could produce norepinephrine in vivo to GF mice resulted in the in vivo elaboration of norepinephrine within the murine GIT [54]. Interestingly, in many cases, the content of catecholamines found in bacteria is higher than in human blood, for example concentrations of norepinephrine in human blood are found to be 0.04 mmol/L [55].

Acetylcholine

Acetylcholine is a well-known neurotransmitter in the central and peripheral nervous systems that plays a critical role in cognitive function, particularly in memory and learning. It is synthesized by choline acetyltransferase in the CNS and by both choline acetyltransferase and carnitine acetyltransferase in the peripheral system [56, 57]. Acetylcholine has also been identified in non-neuronal tissues, including gastrointestinal, respiratory and urogenital epithelial cells [58]. In addition, acetylcholine has been found to be a component of bacteria and its production was discovered in a strain of *L. plantarum* [13, 59, 60]. Cell free enzyme(s) participating in acetylcholine synthesis were also found in *L. plantarum* [59]. Horiuchi et al. [61] tested three different bacterial strains for acetylcholine content and synthesis, *E. coli* JCM 5491, *Staphylococcus aureus* JCM 2151 and *Bacillus subtilis* PCI 219. Among these, a substantial amount of acetylcholine was detected in *B. subtilis* (55.7 pmol/10^{10} colony forming units), while much smaller amounts were found in *E. coli* (2.22 pmol) and *S. aureus* (0.39 pmol). Although acetylcholine synthesis was detected in all bacterial samples, the levels were low and the authors suggested that an acyltransferase other than choline

acetyltransferase and carnitine acetyltransferase was responsible for acetylcholine synthesis in these bacteria [61].

Histamine

Histamine acts as a modulatory neurotransmitter in the mammalian brain and has an important role in the maintenance of wakefulness, while dysfunction in the histaminergic system has been linked to narcolepsy [62]. Moreover, behavioural studies suggest that the histaminergic system in the brain has important roles in cognitive function [63]. Levels of histamine are decreased in the hippocampus, temporal cortex and hypothalamus of patients with Alzheimer's disease, suggesting that histaminergic neurons undergo degeneration and contribute to cognitive decline in this disorder [64].

Histamine is produced via histidine decarboxylase (HDC) of L-histidine, an essential amino acid for humans that is present in many dietary foods [65]. Some fermentative bacteria, including strains of *Lactobacillus*, *Lactococcus*, *Streptococcus*, *Pediococcus* and *Enterococcus* have been reported to possess the HDC-gene and to produce histamine at different levels [66, 67]. However, the production of histamine by certain bacterial strains has caused alarm as a health risk in food and as a marker of food spoilage. Ingestion of food containing high concentrations of histamine has been linked with headaches, vomiting and hypertension [68]. Nonetheless, histamine production by the probiotic strain *Lactobacillus reuteri* (ATCC PTA 6475) was recently reported, resulting in a suppression of human proinflammatory tumor necrosis factor (TNF) production [11]. Moreover, histidine supplementation increased the expression of HDC-genes and the production of histamine by *L. reuteri*. In addition to the role of histamine in immunomodulation observed in the study by Thomas et al. [11], luminal production of histamine by *L. reuteri* 6475 may influence signalling in the enteric nervous system. However, in vivo studies are needed to gain a better understanding of the role of bacterially-produced histamine in the gut and that such production does not induce negative side-effects.

Indole-3-Propionic Acid

Indole-3-propionic acid (IPA) is a deamination product of tryptophan and is found in plasma and cerebrospinal fluid [69, 70]. IPA has been shown to be a powerful antioxidant [71] and has been considered as a possible treatment for Alzheimer's disease [72] due to its ability to protect neurons and neuroblastoma cells against oxidative damage and death [73, 74].

Wikoff et al. [45] demonstrated that the production of IPA was completely dependent on the presence of gut microbiota and could also be established by

colonizing germ-free mice with the bacterium *Clostridium sporogenes*. An earlier study by Jellet et al. [75] also demonstrated that IPA was present in the spent bacterial media of *C. sporogenes* and that addition of the precursor tryptophan to the media greatly enhanced the formation of IPA. In addition, IPA was shown to be produced in vitro when human large intestinal contents were incubated with tryptophan and indolelactate [76].

Short-Chain Fatty Acids

Short chain fatty acids (SCFA) are the major products of the bacterial fermentation of carbohydrates and proteins in the GIT [19, 77]. The main compounds are acetic, propionic and n-butyric acids, occurring roughly in molar ratios of 60:20:20 in the colon [78]. Through their absorption and metabolism, the host is able to salvage energy from foodstuffs, particularly resistant starch and fibers that are not digested in the upper part of the GIT. The main site for SCFA production and absorption is the proximal large intestine, where the fermentation of undigested food by colonic bacteria occurs at high rates. Bacteria that produce SCFA include, but are not limited to, *Bacteroides*, *Bifidobacterium*, *Propionibacterium*, *Eubacterium*, *Lactobacillus*, *Clostridium*, Roseburia and Prevotella [19]. SCFA have a multiplicity of effects in the body, and affect epithelial cell transport and metabolism, epithelial cell growth and differentiation, and hepatic control of lipid and carbohydrates, while providing energy sources for muscles and kidneys, as well as the heart and brain [79]. Epithelial cells in the distal colon derive 60–70 % of their energy requirements from bacterial fermentation products [80]. SCFA also act as signalling molecules. Propionate, acetate, and to a lesser extent butyrate are ligands for at least two G protein-coupled receptors (GPCRs), Gpr41 and Gpr43, which are broadly expressed in the distal small intestine, colon and adipocytes [81, 82]. SCFA interaction with Gpr43 can profoundly affect inflammatory responses. For example, mice treated with oral acetate showed a substantial decrease in inflammation. This protection was mediated by acetate binding to Gpr43, because acetate had no effect in Gpr43-deficient mice. Furthermore, it was shown that Gpr43 exhibited enhanced expression in neutrophils and eosinophils, suggesting that SCFA-Gpr43 signalling is one of the molecular pathways whereby commensal bacteria regulate immune and inflammatory responses [83]. Gpr43 is also induced during adipocyte differentiation and exhibits increased levels during high-fat feeding in rodents, suggesting that Gpr43 may also affect adipocyte function [84]. Hong et al. [85] demonstrated that acetate and propionate act on lipid accumulation and inhibition of lipolysis mainly through Gpr43 [85]. Gpr41 has been shown to be implicated in microbiota-dependent regulation of host adiposity and leptin production [86].

SCFA can cross the blood-brain barrier and enter the CNS [87] and are taken up by glia and, to a lesser extent, by neurons, where they are thought to comprise a major energy source in cellular metabolism, particularly during early brain

development [88–90]. They also play a role in cell signalling [91] and neurotransmitter synthesis and release [92]. Moreover, SCFA have been shown to increase the synthesis of dopamine and its related catecholamines through induction of tyrosine hydroxylase, a key enzyme in the synthesis of catecholamines [93]. Propionate, in particular has been shown to alter dopamine, serotonin, and glutamate systems in a manner similar to that observed in autism spectrum disorders (ASD) [94, 95]. Furthermore, intraventricular infusion of propionate in rats was shown to impair social behaviour and cause brain abnormalities, similar to those detected in human autism [96–98] and furthermore to alter brain phospholipid composition [99]. Some clinical studies have also found that a subset of ASD patients have high levels of *Clostridial* and *Bacteriodetes* species in the gut [100, 101], species which are efficient propionate-producers [19]. This highlights that although propionate is beneficial at appropriate levels, such as lowering lipogenesis, serum cholesterol levels and improving insulin sensitivity [102], excessive propionate may have negative effects on health and behaviour.

Butyrate is known to exhibit many important physiological functions in eukaryotic cells [19]. One of the most recognised cellular mechanisms for the action of butyrate is its effects on histone acetylation [103], where the inhibition of histone deacetylase facilitates hyperacetylation of histone proteins to occur, thus facilitating the access of DNA repair enzymes. Interestingly, sodium butyrate has been demonstrated to elicit an antidepressant effect in the murine brain [104]. When injected systemically, sodium butyrate induced a short-lasting, transient acetylation of histones in frontal cortex and hippocampus, in conjunction with dynamic changes in expression of brain-derived neurotrophic factor (BDNF), thereby resulting in an antidepressant-like behavioral response [104].

Long-Chain Fatty Acids

Long-chain fatty acids (LC-PUFAs) play numerous roles in the brain, including structural (forming the physico-chemical properties in the lipid bilayer of cellular membranes) and signalling functions. Moreover, they influence neurogenesis and neurotransmission within the nervous tissue. Arachidonic acid (AA, C20:4n-6) and docosahexaenoic acid (DHA, C22:6n-3) are highly concentrated in the brain and are vital fatty acids for neurological development [105, 106]. Furthermore, there is a growing body of evidence for their role in mental health across the lifespan [107]. Being the major structural components of brain cells [108], AA and DHA influence cell membrane physical properties, enzyme activity, regulation of ion channels and neuroreceptors and their signalling (neurotransmission) [109, 110].

Recently, we have reported that administration of a *Bifidobacterium breve* strain, *B. breve* NCIMB702258 to mice had a significant impact on the fatty acid composition of brain [111]. Mice that received this strain for 8 weeks exhibited significantly higher concentrations of AA and DHA in the brain when compared to unsupplemented mice. Interestingly, this effect was bacterial strain-dependent, as

it was not induced by the *B. breve* strain DPC6330. The mechanism by which the *B. breve* strain alters the fatty acid composition is currently unknown. Possible explanations include modulations of fat-absorption processes in the small intestine and/or desaturase activities involved in the metabolism of fatty acids to the longer-chain unsaturated derivatives caused either directly by the strain administered or by alterations in the gut microbiota. Interestingly, it was previously postulated that different members of the gut microbiota promote fatty acid absorption via distinct mechanisms [112]. Semova et al. [112] used the zebrafish model to investigate how microbiota and diet interact to regulate lipid absorption in the gut epithelium. By comparing GF zebrafish with conventional zebrafish, the authors demonstrated that the microbiota stimulate fatty acid uptake and lipid droplet formation in both the intestinal epithelium and liver [112]. Previous studies have also demonstrated that manipulation of the gut microbiota by probiotics resulted in altered fat composition in the host [113–115]. Although the adult microbiome is not known to be particularly enriched in genes involved in fatty acid metabolism [116], there are indications that interactions between fatty acids and components of the gut microbiota occur which could affect the biological roles of both. However, a deeper knowledge of such interactions and what consequences they have for the host are warranted.

Conjugated Linoleic Acid

Conjugated linoleic acid (CLA) comprises a mixture of positional and geometric isomers of linoleic acid (*cis*-9, *cis*-12 C18:2n-6) characterised by the presence of conjugated double bonds with *cis* or *trans* configurations. CLA is a natural component of ruminant milk and tissue fat as a result of the action of the ruminal microbiota on dietary linoleic acid [117]. A range of health-promoting activities have been attributed to the consumption of CLA, most notably anticarcinogenic, immune-modulatory, anti-obesity and anti-atherosclerotic activities [118–121]. In contrast, in certain conditions, some CLA isomers can also exert potentially negative effects such as liver steatosis and insulin resistance [122, 123]. Regarding the action of CLA on the CNS, it is known that CLA crosses the blood-brain barrier and is incorporated and metabolized in the brain [124]. Dietary CLA has been shown to reduce cerebral prostaglandin E2 in the peripheral and CNS [125], and to exert antiangiogenic actions in the brain [126]. Furthermore, Hunt et al. [127] showed that CLA protects cortical neurons from excitotoxicity at concentrations likely achieved by consumption of CLA as a dietary supplement.

Evidence of the role of the gut microbiota in the endogenous production of CLA was first reported by Chin et al. [128] who observed that increasing the amount of linoleic acid in the diet increased the tissue content of CLA in conventional rats but not in GF rats. Since then, commensal lactobacilli and bifidobacteria from the human GIT, most notably *B. breve* strains and *L. plantarum* strains, have been shown to produce CLA, predominantly the *cis*-9, *trans*-11 (c9, t11) isomer from free linoleic acid [21–23, 129]. These bacteria convert linoleic acid to CLA in a

similar manner to ruminant bacteria via the action of the enzyme linoleic acid isomerase [130], which also has been sequenced in some *Lactobacillus* and *Bifidobacterium* strains [131, 132]. We have reported that the CLA-producing bacterium, *B. breve* NCIMB702258 converts linoleic acid to CLA in the murine gut, resulting in significantly elevated c9, t11 CLA in the liver [133]. Moreover, Bassaganya-Riera et al. [134] demonstrated that administration of the probiotic mixture VSL#3 to mice with colitis resulted in high colonic concentrations of c9, t11 CLA and that this locally produced CLA improved colitis by activating peroxisome proliferator-activated receptor gamma (PPAR γ) in macrophages. Two studies have also reported the in vivo production of the CLA isomer, *trans*-10, *cis*-12 (t10, c12) CLA, by using two strains of human origin, *L. rhamnosus* PL60 and *L. plantarum* PL62. Administration of these two strains to mice resulted in increased t10, c12 CLA concentrations in sera with subsequent reductions in adipose tissue and body weight [135, 136]. Druart et al. [137] further demonstrated that prebiotic supplementation increased CLA content in caecal tissue, by increasing substrate availability and by modulating gut microbiota composition.

Although dietary CLA has been reported to affect the CNS, the consequences of bacterially-produced CLA on nervous system function are yet to be discovered.

Conclusion

Although we are still at the very early stages of understanding the complex communication systems between gut bacteria and the brain, we know that certain bacteria within the human gut have the ability to produce molecules with neuroactive functions which could affect the brain in a direct manner. However, only cultivable bacteria have been tested for their capacity to produce neuroactive compounds in vitro and only a limited number of bacterial strains have been tested up to now. Moreover, in complex microbial ecosystems such as in the human gut, interactions and competition exist between bacteria, which are not studied upon simple culture conditions in vitro. This highlights the need for in vivo studies to elucidate the role of metabolite-producing bacteria and what effect such bacteria, and their components, have on nervous system function and behaviour. Such future studies may also facilitate our understanding of the consequences of neuroactive compound production by the microbiota and how probiotic bacteria can influence the CNS, and could furthermore identify the potential for neurochemical containing/producing probiotic bacteria as a therapeutic strategy in the treatment of certain neurological and neurophysiological conditions. Given that molecular tools have now been developed for many intestinal organisms, the possibility exists now to overproduce neuroactive compounds and/or to regulate their production in response to gut metabolites such as bile.

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