

INTERPRETING THE BIOCHEMICAL LANGUAGE OF THE GUT-BRAIN AXIS

JONATHAN R. SWANN

School of Human Development and Health, Faculty of Medicine, University of
Southampton, UK, and Department of Metabolism, Digestion and Reproduction,
Faculty of Medicine, Imperial College, London, UK

SUMMARY

A complex, bidirectional communication network exists between the gut microbiota and the brain. This interaction involves multiple pathways, with microbial metabolites playing a central role. These compounds can act locally on the enteric nervous system or reach the central nervous system to influence brain function. Across the lifespan, microbiota-derived metabolites contribute to neurodevelopment and are implicated in neuropsychiatric and neurodegenerative disorders. Major metabolite classes involved in gut-brain communication include short-chain fatty acids, bile acids, choline-derived compounds, and those related immune responses to microbial signals. Metabolomics, a systems biology approach that captures the full range of metabolites in biological samples, provides a powerful framework for studying this biochemical dialogue, encompassing both host- and microbiota-derived molecules.

INTRODUCTION

Over the past twenty years, major advances have transformed our understanding of the bidirectional communication between the gut and the brain. Central to this dialogue are the trillions of microorganisms inhabiting the gastrointestinal tract, including bacteria, viruses, fungi, and protozoa, collectively termed the gut microbiota. This ecosystem influences brain function and behaviour and has been increasingly implicated in neurodevelopmental, psychiatric, and neurodegenerative conditions. Communication occurs through immunological, neural, and biochemical pathways; here, we focus on the biochemical mediators.

The gut microbiota has co-evolved with humans and plays a fundamental role in host physiology. Its collective genome, the metagenome, contains an estimated three million genes, vastly exceeding the 24,000 genes of the human genome (*Qin et al.*, 2010). This expanded biochemical repertoire enables metabolic reactions absent from the host, broadening the diversity of molecules that be processed and the spectrum of compounds to which the host is exposed. As a result, humans can be considered holobionts, whose overall fitness is shaped by the integrated activities arising from both human and microbial genomes.

METABOLOMICS AND THE MICROPHENOME

Metabolomics, the comprehensive profiling of small molecules within biolog-

ical systems, has been instrumental in characterizing host-microbe metabolic

interactions. Metabolomic profiles reflect endogenous host pathways as well as exogenous influences from diet, environment and the microbial metabolism. Because the metagenome contains 100-150 times more genes than the human genome, microbial biotransformation substantially expands host metabolic flexibility.

Key classes of microbiota-derived or microbiota-modulated metabolites include:

- Short chain fatty acids
- Bile acids
- Amino acids and their derivatives
- Choline-related metabolites
- Immunomodulatory and neuroactive compounds

Collectively, these microbial-associated molecules can be referred to as the microphenome and several have been highlighted to participate in gut-brain communication.

Short chain fatty acids

Short chain fatty acids (SCFA) are produced through microbial fermentation of dietary carbohydrates and, to a lesser extent, proteins. The key SCFAs are acetate, propionate, and butyrate with smaller amounts of valerate, isobutyrate, isovalerate, and caproate also present. These molecules support gut barrier integrity, modulate immune responses and can serve as an energy source for the host. Following absorption from the gut, SCFAs enter the systemic circulation and reach the brain. While faecal SCFAs can be measured by ^1H nuclear magnetic resonance (NMR) spectroscopy (acetate, ~30-80 mmol/kg wet stool; propionate, ~10-30 mmol/kg; butyrate ~10-30 mmol/kg) the lower concentrations found in plasma samples (acetate, 20-200 mM; propionate, 1-15 mM; butyrate 1-10 mM) requires the enhanced sensitivity of mass spectrometry platforms typically hyphenated with gas chromatography (GCMS) or liquid chromatography (LCMS).

Relevant to the gut-brain axis, *in vivo* studies have demonstrated that acetate can alter appetite and feeding behaviour at both the gut level, stimulating the release of appetite hormones (e.g. GLP-1, PYY) from enteroendocrine cells, and in the brain by influencing central appetite circuits (Frost et al., 2014). Analysis of the Flemish Gut Flora Project including over 1,000 participants, discovered positive associations between butyrate-producing taxa (e.g. *Faecalibacterium*, *Coprococcus*) and quality of life, with higher abundance correlated with lower depressive symptoms (Valles-Colomer et al., 2019). Moreover, these fermentation products have been shown to maintain blood-brain-barrier (BBB) integrity, with propionate and butyrate providing protection against lipopolysaccharide (LPS)-induced elevations in its permeability (Hoyle et al., 2018). In addition, butyrate is an epigenetic regulator, found to act as a histone deacetylase (HDAC) inhibitor capable of supporting neuronal health (Park and Sohrabji, 2016; Patnala et al., 2017).

Bile acids

Bile acids exemplify trans-genomic metabolism, arising from coordinated host-microbial processing. The first step is catalysed by host enzymes in the liver synthesising primary bile acids from cholesterol. These primary bile acids include cholic acid, chenodeoxycholic acid, and muricholic acid. These bile acids are subsequently conjugated primarily with glycine and taurine, to increase their solubility, before entering the bile and stored in the gall bladder. Following the consumption of a meal, the gall bladder contracts to release these conjugated primary bile acids into the small intestine where they facilitate lipid digestion and absorption. Approximately 95% of these bile acids are

absorbed from the small intestine and return to the liver. Bile acids that escape this absorption, reach the colon and serve as substrate for the microbiota. First, microbial bile salt hydrolase (BSH) enzymes deconjugate the bile acids releasing the unconjugated primary bile acids, which can then be further modified through dehydroxylation and epimerization reactions to produce secondary bile acids such as deoxycholic acid, lithocholic acid, ursodeoxycholic acid and hyodeoxycholic acid. These bile acids are either reabsorbed and returned to the liver or excreted in the faeces. Upon reaching the liver, secondary bile acids are re-conjugated and released into the bile. As a result, these trans-genomic molecules continually shuttle between the host liver (genome) and the metagenome.

In addition to their key role in digestion, bile acids also act as important signalling molecules capable of regulating host metabolism and immune function. This class of metabolites are typically measured by targeted LC-MS and GC-MS-based metabolomics assays, and bile acids have been detected in the brain, alongside their receptors and transporters. They interact with receptors such as farnesoid-X-receptor (FXR), Takeda G-protein coupled receptor-5 (TGR5), and vitamin D receptor (VDR), and can influence brain processes. Different bile acids have different signalling potencies, and we have previously demonstrated the strong influence of the metagenome on the diversity of bile acids contained within the pool (Swann et al., 2011; Martin et al., 2018). Using a targeted LCMS approach to quantify bile acids in plasma collected from healthy participants every two hours over a 36-hour period, we have demonstrated that bile acids follow a daily rhythm (Bello et al., 2024). This human study identified that the environment exerted a stronger

influence on these rhythms than the host circadian system. Here, conjugated bile acids absorbed in the small intestine began to rise in the plasma before lunchtime peaking shortly after an evening meal. In contrast, the unconjugated forms arising from bacterial BSH activity were absorbed in the colon and exhibited similar patterns but delayed by 4-6 hours. This daily fluctuation in circulating bile acids is hypothesized to prepare the metabolic readiness of the host in anticipation for nutrient intake. Desynchronization between dietary intake and bile acid signalling could have implications for obesity and diabetes. Interestingly, these outcomes are significantly higher in shift workers than non-shift workers (Gan et al., 2015). Huang and colleagues demonstrated the importance of bile acid-FXR signalling in the gut-brain axis, showing that FXR deficiency in mice altered neurotransmission, anxiety-like behaviour and memory (Huang et al., 2015). Intriguingly, a meta-analysis of longitudinal studies (7 studies; 28,431 participants) found that shift workers had a 28-30% higher risk of adverse mental health outcomes overall compared to non-shift workers (Torquati et al., 2019).

Amino acids and their related compounds

Many gut bacteria, including *Bacteroides*, *Prevotella*, *Clostridium*, *Lactobacillus* and *Bifidobacterium*, possess complete biosynthetic pathways for all 20 amino acids. Microbial metabolism of amino acids also generates neuroactive compounds such as serotonin, γ -aminobutyric acid (GABA), dopamine, tyramine, tryptamine, and polyamines. Microbial-derived neurotransmitters act primarily in the gut environment rather than directly on the brain. For example, the most immediate target is the enteric nervous system, where they can regulate gut motility,

visceral pain sensitivity, and secretion. Bacterial neurotransmitters can also signal through the vagus nerve where they can activate vagal sensory endings, alter firing patterns and influence brain-stem nuclei. This is considered a major route for microbiome-brain communication. Microbial synthesis of neurotransmitters can indirectly impact on central neurotransmission through alterations in precursor bioavailability. This can be seen in germ-free animals where the microbial synthesis of serotonin is absent. As a result, there is an increased availability of its precursor, tryptophan in the gut, leading to higher circulating concentrations of this amino acids resulting in higher amounts of serotonin in the germ-free brain compared to conventional animals (Clarke et al., 2013).

In the work presented in Zuffa *et al.*, we identified early alterations in the gut-brain axis in infants at elevated likelihood of developing autism spectrum disorder (ASD), before overt behavioural symptoms emerge (Zuffa et al., 2023). A potential mechanistic role was highlighted for GABA, a key neuroactive metabolite linking microbial ecology with neurodevelopment. Applying an NMR spectroscopy-based metabolomic approach infants at elevated likelihood of ASD were found to have persistently lower faecal GABA from as early as 5 months of age. In contrast, infants at low-likelihood of ASD showed higher early-life GABA that naturally declined with maturation. This is noteworthy because GABA is the principal inhibitory neurotransmitter in the mature brain, but during infancy it also acts as an excitatory developmental signal that regulates neuronal maturation, synapse formation, and circuit refinement. Hence, these findings suggest that altered microbial regulation of GABA during critical windows of development could influence early brain

development. It was demonstrated in both humans and *in vitro* experiments that *Bifidobacterium* species produced GABA while *Clostridium* species consumed this metabolite and the ratio of *Bifidobacterium*: *Clostridium* species was related to faecal GABA abundance. Variation in dietary preferences is a major confounder in many studies exploring the relationship between the gut microbiota and ASD. In this study, these biochemical differences were observed at 5 months of age despite similarities in dietary intake (*e.g.* all infants breast-fed). Hence, this work identified GABA as a candidate microbial-neurodevelopmental mediator in ASD risk.

In addition to neurotransmitter synthesis from amino acids, bacterial species in the gut can also produce a range of other compounds including *p*-cresol (4-cresol) from tyrosine, phenylacetate from phenylalanine, and indoles from tryptophan. Following the absorption of *p*-cresol from the gut, this toxic metabolite is sulphated in the liver to produce *p*-cresyl-sulphate (PCS), which is subsequently excreted in the urine. Similarly, phenylacetate produced in the gut, via phenylpyruvate, is absorbed, conjugated with glutamine and excreted as phenylacetylglutamine (PAG). Through the application of ¹H NMR spectroscopy-based metabolomics to two large scale human studies in Taiwan and the US, both PCS and PAG were observed to increase in the urine with age in those between ~50-90 years old (Swann et al., 2013). These findings indicate that the gut microbial production of *p*-cresol and phenylacetate, and hence gut exposure and systemic circulation, is increased with age. Changes in PAG and phenylacetate metabolism have been observed in a range of neurodevelopmental and neuropsychiatric disorders including ASD, schizophrenia, major depressive disorder, Parkinson's disease, and Alzheimer's disease

(Bertarini et al., 2025; Lee et al., 2025).

Such changes can impact on gut health. Transepithelial electrical resistance (TEER) analysis using Caco-2 cells to study the permeability of colonic cells demonstrated that increasing concentrations of *p*-cresol increase the permeability of the cells (Al Hinai et al., 2019). These findings suggest that alterations in gut microbial metabolism seen with age may increase gut permeability with potential implications for bacterial translocation, leading to both systemic and neuroinflammation. One consequence of this inflammation is disruption to the tryptophan-kynurenine pathway. Following detection of inflammatory (e.g. LPS) and immune signalling pathways (e.g. interferon- γ , TNF- α), the enzyme indoleamine 2,3-dioxygenase (IDO) is induced. This is a major enzyme that acts as an immunometabolic brake, catalyzing the breakdown of tryptophan to kynurenine. This depletes tryptophan, suppressing immune cell activity, while kynurenine and its metabolites actively promote immune tolerance by activating the aryl hydrocarbon receptor (AhR) to promote regulatory T cells and suppress Th1 and Th17 responses. This is beneficial for shifting the immune system to a tolerogenic state dampening inflammation, but it has implications for mood. The induction of IDO and increase in the kynurenine:tryptophan (K:T) ratio, leads to ‘tryptophan steal’, reducing the availability of this amino acid for serotonin synthesis impacting on mood. IDO induction has been demonstrated in major depressive disorder (Myint et al., 2007). Interestingly, quinolinic acid, a downstream metabolite of kynurenine is neurotoxic and has been associated with cognitive impairment and brain fog (Dehhaghi et al., 2022). Hence, bacterial or LPS-induced activation of IDO highlights a mechanism where the microbiota can modulate brain pro-

cesses and function by altering the immune-metabolic systems.

Choline-related metabolites

Another class of compounds modulated by the microbiota that impact on the brain are those related to choline. The diet is a major source of choline, including foods such as eggs, red meat and dairy. As it passes through the gut, microbes can convert choline to trimethylamine (TMA), with further demethylation able to occur producing dimethylamine (DMA) and methylamine (MA). TMA absorbed from the gut is further processed in the host liver by flavin monooxygenase 3 (FMO3) to produce trimethylamine-*N*-oxide (TMAO). This microbial-mammalian co-metabolite can be measured in the urine and blood or it is released back into the gut where the microbiota can reduce TMAO back to TMA, often referred to a microbial retroversion. It should be noted that TMAO can also be derived from the diet being particularly high in marine fish.

Microbial choline degradation in the gut modifies the bioavailability of this important compound. As it is the precursor for acetylcholine, such activity can impact on neurotransmission. Choline also serves as a methyl donor via betaine in the methionine cycle, generating *S*-adenosylmethionine (SAM), the universal methyl donor for DNA methylation and histone methylation. This is fundamentally important for brain development, regulating how neural genes are turned on and off across embryonic development, neuronal differentiation, synapse formation and long-term plasticity. The availability of SAM, and therefore choline, is essential for neurodevelopment. The influence of gut microbial choline degradation on DNA methylation and brain function was highlighted in a mouse study by Romano and colleagues (Romano et al., 2017). These authors showed that colo-

nizing germ-free animals with an *E. coli* strain possessing the *cutC* gene, providing the metabolic machinery to break down choline to TMA, resulted in dietary choline being degraded, leading to hypomethylation and increased anxiety-like behaviour in their offspring. These effects were not observed when colonizing mice with an *E. coli* strain lacking the *cutC* gene. This highlights the potential for the microbiota to influence choline availability with downstream epigenetic implications that can impact brain development and function.

Interestingly, TMA has been observed to be damaging to the BBB, while TMAO can exert a protective effect at physiological concentrations (Hoyle et al., 2021). Here, TMA

disrupted tight junction organisation in the BBB and induced cellular stress responses weakening endothelial barrier integrity. In contrast, TMAO reduced BBB permeability and improved cortical actin organisation. Mouse experiments found that TMAO could protect cognition during chronic low-grade inflammatory stress induced by LPS, reducing inflammatory activation of astrocytes and microglia. Consistent with these findings, we have also shown that circulating TMAO at 12 months of age was positively associated with early neurodevelopment measured in infants 6 months later, particularly language acquisition (Wimborne et al., 2025).

CONCLUSIONS

Microbial metabolites are central mediators of gut-brain communication, influencing neurodevelopment, behaviour, and disease risk. Metabolomics has been pivotal in identifying these molecules and mapping their functional roles. Integrating metabolomics with metagenomics, transcriptomics, proteomics and immune profiling will deepen

our understanding of host-microbe interactions and support the development of targeted interventions. Because the microbiome is modifiable through diet, lifestyle, and therapeutics, it represents a promising avenue for strategies aimed at optimising brain health across the lifespan.

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