

Old Herborn University Seminar Monograph

37. MICROBIOME AND NEUROLOGICAL DISORDERS

EDITORS:

PETER J. HEIDT
TILLMANN RUSCH
VOLKER RUSCH



Old Herborn University

Old Herborn University Seminar

Monograph 37

ISBN 3-923022-49-2

ISSN 1431-6579

COPYRIGHT © 2026 BY THE OLD HERBORN UNIVERSITY FOUNDATION.

THIS PUBLICATION OR PARTS OF IT MAY BE REPRODUCED OR TRANSMITTED IN ANY FORM UNDER THE RESTRICTION THAT THE SOURCE SHOULD BE MENTIONED.

THIS PUBLICATION CAN BE FOUND ON, AND DOWN-LOADED FROM:

<http://www.old-herborn-university.de/>

EDITORS:

Prof. Dr. Peter J. Heidt
Willem-Alexander Children's Hospital
Leiden University Medical Center
Albinusdreef 2
2333 ZA - Leiden
The Netherlands

Dr. med. Tillmann Rusch, M.Sc.
University Hospital Gießen and Marburg
Department of Hematology, Oncology and Immunology
Baldingerstraße 1
35043 Marburg
Germany

Dr. rer. nat. Volker Rusch
Old Herborn University Foundation
Postfach 1765
D-35727 Herborn-Dill
Germany



Old Herborn University

Old Herborn University Foundation
Postfach 1765
D-35727 Herborn-Dill
Germany

Contents

Participating authors	V
I. THE MICROBIOTA-GUT-BRAIN AXIS IN NEURODEVELOPMENT: EMERGING ROLES OF PEPTIDOGLYCAN SIGNALLING (<i>Rochellys Diaz Heijtz</i>)	1
Summary	1
Introduction	2
Microbial signals and the brain	4
Peptidoglycan: an underappreciated signal in microbiota-brain communication	5
Peptidoglycan signalling at the maternal-foetal interface	7
Translating microbiota-brain findings to humans	9
Targeting the microbiota to promote brain health	10
Conclusions and perspectives	12
Acknowledgements	14
Literature	14
II. THE GUT MICROBIOTA-HISTIDINE-UROCANATE AXIS AND COGNITION: MECHANISTIC CONVERGENCE ACROSS PRECLINICAL AND CLINICAL EVIDENCE (<i>Antonio Suárez</i>)	19
Summary	19
Introduction	19
Materials and methods	21
Results	22
Discussion	29
Conclusion	30
Literature	31
III. INTERPRETING THE BIOCHEMICAL LANGUAGE OF THE GUT-BRAIN AXIS (<i>Jonathan R. Swann</i>)	35
Summary	35
Introduction	35
Metabolomics and the microphenome	35
Conclusions	40
Literature	40

Contents (continued)

IV.	FAECAL MICROBIOTA TRANSPLANTATION FOR TREATMENT OF PARKINSON'S DISEASE (<i>Filip Scheperjans</i>)	43
	Introduction	43
	Parkinson's disease as a multisystem disorder with a long prodromal phase	43
	Body-first and brain-first Parkinson's disease	44
	Gastrointestinal epidemiology and microbiota-associated risk signals	44
	Gut microbiota signatures and links to symptoms and progression	45
	Potential mechanisms linking the gut microbiota to Parkinson's disease biology	46
	From mechanism to intervention: preclinical and early clinical modulation studies	46
	Faecal microbiota transplantation as a therapeutic concept	48
	Clinical faecal microbiota transplantation trials in Parkinson's disease ..	48
	Interpretation and future directions	49
	Conclusions	49
	Literature	49
V.	THE GUT-BRAIN AXIS IN MULTIPLE SCLEROSIS: UNRAVELING THE ROLE OF GUT MICROBIOTA (<i>Ashutosh Mangalam</i>)	53
	Introduction	53
	Gut dysbiosis in people with multiple sclerosis	53
	From bedside to bench: <i>Prevotella histicola</i> as a bacterial drug (BRUG) ..	54
	Gut bacteria, phyto-oestrogens, and the mechanism of microbiome-mediated immunomodulation	55
	Translational significance: diet switching as a therapeutic strategy	56
	Identification of a gut microbiota-based biomarker for multiple sclerosis discovery in the EAE model: the <i>Bifidobacterium:Akkermansia</i> ratio ..	57
	From disease marker to predictive biomarker: a causal role for MS-enriched bacteria	58
	Conclusions: from dysbiosis to clinical translation	59
	Disclosure.....	60
	Acknowledgements	61
	Literature	61

V.	THE ROLE OF THE MICROBIOTA-GUT-BRAIN AXIS IN PSYCHIATRY AND BEYOND (<i>Valery Taylor</i>)	63
	Introduction.....	63
	Influence of anti-depressant medications on the gut microbiota of patients with depression	63
	Clinical trials using faecal microbial transplantation	65
	Literature	67

Participating Authors

Assoc. Prof. Rochellys Diaz Heijtz., Ph.D.

Department of Neuroscience
Karolinska Institutet
S-171 77 Stockholm, Sweden
(E-mail: Rochellys.heijtz@ki.se).

Prof. Antonio Suarez, Ph.D.

Department of Biochemistry and Molecular Biology II
Faculty of Pharmacy
University of Granada
18071 Granada
Spain
(E-mail: asuarez@ugr.es)

Prof. Jonathan R. Swann, Ph.D.

School of Human Development and Health
Faculty of Medicine
University of Southampton
Southampton, SO17 1BJ
United Kingdom
(E-mail: J.Swann@soton.ac.uk)

Assoc. Prof. Filip Scheperjans, M.D., Ph.D.

Department of Neurology
Helsinki University Hospital
University of Helsinki
Haartmaninkatu 4
00290 Helsinki
Finland
(E-mail: Filip.Scheperjans@hus.fi)

Assoc. Prof. Ashutosh Mangalam, Ph.D.

Department of Pathology
Carver College of Medicine
University of Iowa
451 Newton Road
Iowa City, IA 52242,
USA
(E-mail: ashutosh-mangalam@uiowa.edu)

Prof. Valerie Taylor, M.D., Ph.D.

Department of Psychiatry
University of Calgary
3280 Hospital Dr. NW
Calgary, AB T2N 4Z6
Canada
(E-mail: valerie.taylor@ucalgary.ca)

THE MICROBIOTA-GUT-BRAIN AXIS IN NEURODEVELOPMENT: EMERGING ROLES OF PEPTIDOGLYCAN SIGNALLING

ROCHELLYS DIAZ HEIJTZ

Department of Neuroscience, Karolinska Institutet, S-171 77, Stockholm, Sweden

SUMMARY

The gut microbiota has emerged as an important regulator of brain development, function and behaviour. Studies using germ-free, gnotobiotic and other experimental models have demonstrated that microbial colonization influences multiple processes involved in brain development, including blood-brain barrier formation and integrity, microglial maturation, and myelination. A major challenge is now to identify the molecular mechanisms underlying these effects, translate findings from experimental models to humans, and develop strategies to harness the gut microbiota to promote brain health. Microbiota-brain communication involves multiple neuronal, metabolic, immune and endocrine pathways. While considerable attention has focused on microbial metabolites, accumulating evidence indicates that structural bacterial components may also function as physiological signals. In particular, peptidoglycan (PGN) fragments released by commensal bacteria can cross biological barriers and be recognized by host PGN-sensing pathways. Experimental studies demonstrate that microbiota-derived PGN can reach the brain and that PGN sensing influences brain development and behaviour, with sex-dependent effects. More recent findings extend this concept to prenatal life, showing that maternal exposure to the PGN fragment muramyl dipeptide (MDP) can alter the foetal environment and PGN sensing and transport pathways in the foetal brain, with persistent sex-specific effects on offspring behaviour in the absence of overt foetal neuroinflammation. Translating these mechanisms to humans will require integration of experimental models with human stem cell-based model systems and prospective longitudinal studies. Early-life differences in microbial composition and metabolic function have been detected in infants at elevated likelihood of developing autism spectrum disorder (ASD) before measurable behavioural differences emerge, supporting a potential contribution of the gut microbiota to the neurobiology of ASD. At the same time, experimental and emerging clinical studies with *Limosilactobacillus reuteri* demonstrate the potential, as well as the complexity, of microbiota-based interventions to modulate neural circuits underlying social behaviour. Their effects depend on microbial strain, developmental timing and host characteristics, including biological sex. Together, these findings support a shift from largely descriptive microbiota studies towards understanding the precise molecular mechanisms mediating gut-brain interactions. Such mechanistic knowledge will be essential for translating microbiota-gut-brain research into targeted strategies to promote brain health in humans.

INTRODUCTION

The microbiota-gut-brain axis has emerged as an important framework for understanding how indigenous microorganisms residing in the gastrointestinal tract interact with the brain. The gut microbiota is a complex and dynamic ecosystem comprising bacteria, archaea, fungi and viruses that contributes to host physiology through a broad repertoire of metabolic, immune, endocrine and neuronal signals. Over the past two decades, evidence from germ-free (GF), antibiotic-exposed, gnotobiotic and other animal models has demonstrated that the gut microbiota can influence brain development, function and behaviour, with sex-specific effects. These studies have implicated the microbiota in processes ranging from blood-brain barrier (BBB) formation and integrity and microglial maturation to stress responses, myelination, synaptic development and complex behaviours (*Sudo et al., 2004; Diaz Heijtz et al., 2011; Braniste et al., 2014; Erny et al., 2015; Hoban et al., 2016*). Together with a growing, although still largely associative, body of evidence from human studies, these findings support the possibility that microbiota-host interactions contribute to brain development and function across the lifespan and that disruption of these interactions may be relevant to neurodevelopmental, psychiatric, neurological and neurodegenerative disorders (*Cryan et al., 2020*).

Gut microbiota and brain interactions may be particularly important during early life, although the nature of microbial influence differs fundamentally between the prenatal and postnatal periods. Current evidence does not support the presence of a resident foetal gut microbiota during healthy, uncomplicated pregnancy (*Kennedy et al., 2023*). Before birth, the maternal microbiota is therefore more likely to influence the

developing foetus indirectly through maternal and microbiota-derived signals than through direct microbial colonization. These signals may include microbial metabolites, immune mediators and structural microbial products that enter the maternal circulation and, in some cases, reach the placenta and foetal compartment (*Pessa-Morikawa et al., 2022; Wang et al., 2025*). Maternal infection, inflammation, stress, antibiotic exposure, diet and other environmental or pregnancy-related factors may alter the maternal microbiota and the nature or magnitude of these signals, thereby modifying the foetal environment. Following birth, exposure to maternal and environmental microorganisms initiates establishment of the infant gut microbiota (*Stewart et al., 2018; Sinha et al., 2026*), which subsequently undergoes substantial ecological and functional maturation during the first years of life.

During prenatal and early postnatal life, the brain undergoes extensive cellular and structural changes, including neurogenesis, neuronal migration, axonal and dendritic growth, synaptogenesis, synaptic pruning, myelination and maturation of glial populations (*Semple et al., 2013; Silbereis et al., 2016*). Maternal microbiota-derived signals may therefore influence the developing brain even before establishment of the infant gut microbiota and, after birth, microbial signals increasingly originate from the infant's own colonizing microbial community. Compelling evidence that postnatal microbial colonization contributes to these processes has come from GF and gnotobiotic models. GF mice exhibit alterations in behaviour and stress responsiveness together with changes in synaptic pathways, BBB integrity, myelination and microglial maturation (*Cryan, 2025*).

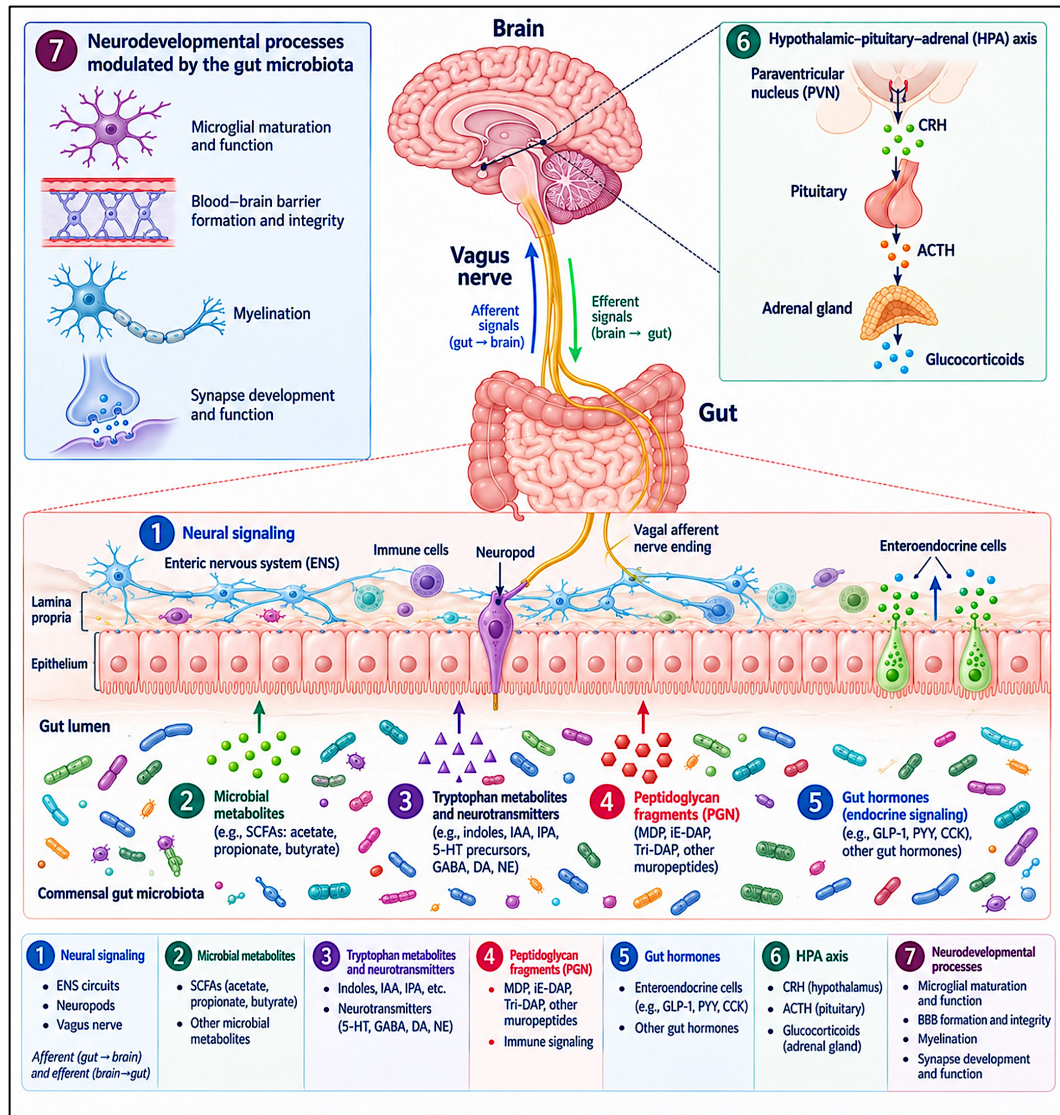


Figure 1: Biological signalling pathways and processes involved in microbiota–gut–brain communication.

The gut microbiota communicates with the developing brain through multiple, interconnected pathways. These include neural signalling through the enteric nervous system (ENS), neuropods, and bidirectional vagal pathways (1); microbial metabolites, including short-chain fatty acids (SCFAs) (2); tryptophan metabolites and neurotransmitter-related signalling (3); peptidoglycan (PGN) fragments and associated immune signalling (4); gut hormones released by enteroendocrine cells (5); and modulation of the hypothalamic–pituitary–adrenal (HPA) axis (6). Together, these signals can influence neurodevelopmental processes, including microglial maturation and function, blood–brain barrier formation and integrity, myelination, and synapse development and function (7). The relative contribution of individual pathways varies across developmental stages and physiological contexts, and substantial bidirectional and functional interactions occur among them. SCFAs, short-chain fatty acids; IAA, indole-3-acetic acid; IPA, indole-3-propionic acid; 5-HT, serotonin; GABA, γ -amino-butyric acid; DA, dopamine; NE, norepinephrine; MDP, muramyl dipeptide; iE-DAP, γ -D-glutamyl-meso-diaminopimelic acid (DAP); Tri-DAP, L-Ala- γ -D-Glu-meso-DAP; GLP-1, glucagon-like peptide-1; PYY, peptide YY; CCK, cholecystikinin; CRH, corticotropin-releasing hormone; ACTH, adrenocorticotrophic hormone; BBB, blood–brain barrier.

Controlled colonization studies have further begun to identify contributions of specific early-life microorganisms, including evidence that human infant-associated *Bifidobacterium* species can influence synaptic development and pruning in the cerebellum (Luck et al., 2020). Importantly, microbiota-dependent effects on the brain can differ between males and females and depend on the developmental period during which microorganisms or their signals are absent, introduced or restored.

Despite substantial progress, three major challenges remain central to the field: defining the mechanisms through which the gut microbiota influences brain development, function and behaviour; translating observations from experimental animal models to human

biology; and determining whether microbiota-based interventions can ultimately be harnessed to promote brain health. These three questions provide the framework for this monograph. Addressing them requires moving beyond associations between microbial community composition and brain-related phenotypes toward identifying the molecular signals responsible for communication with the host. Which microbial molecules are sensed? Can they cross intestinal, placental, blood-brain or other biological barriers? How are they transported through the body? Which receptors, cell populations and brain circuits respond to them? And how do developmental timing, biological sex, host genetics and physiological state shape these responses?

MICROBIAL SIGNALS AND THE BRAIN

Microbiota-brain communication occurs through multiple interacting pathways, including the vagus nerve and enteric nervous system, enteroendocrine signalling, immune mediators, microbial metabolites such as short-chain fatty acids (SCFAs) and tryptophan metabolites, and structural microbial components (Figure 1). One of the most direct routes is the vagus nerve, which conveys sensory information from the gastrointestinal tract to the brain. Experimental studies have shown that the behavioural effects of several probiotic strains depend on intact vagal signalling. For example, the effects of *Lactobacillus rhamnosus* JB1 on stress-related behaviour are abolished by vagotomy (Bravo et al., 2011), while vagal signalling is also required for *Limosilactobacillus reuteri* to restore social deficits in several mouse models relevant to autism spectrum disorder (ASD) (Sgritta et al., 2019). Communication between the intestinal epithelium

and vagal afferents may also involve specialized enteroendocrine cells with axon-like basal processes, commonly referred to as neuropod cells. These cells can form synaptic connections with vagal sensory neurons, providing a rapid route through which luminal signals may be communicated to the brain (Kaelberer et al., 2018). Experimental activation of gut-innervating vagal sensory neurons has also been linked to reward-related behaviour and dopaminergic signalling (Han et al., 2018), illustrating how intestinal signals can influence specific brain circuits.

Other microbiota-derived signals reach distant host tissues through the circulation. SCFAs, particularly acetate, propionate and butyrate, are generated predominantly through microbial fermentation of dietary substrates in the colon and can influence host cells through several mechanisms, including G protein-coupled receptors and the regulation of gene transcription. Studies in

GF animals have provided compelling evidence that SCFAs contribute to brain homeostasis. Erny and colleagues showed that the absence of a microbiota results in immature and functionally altered microglia and that treatment with SCFAs can partially restore these abnormalities (Erny et al., 2015). SCFAs have also been implicated in BBB integrity and in the regulation of microglial function in neurodevelopmental, psychiatric and neurodegenerative animal models. The molecular repertoire generated through microbiota-host interactions extends considerably beyond SCFAs. Commensal microorganisms can produce or modify neurotransmitters and numerous metabolites that enter the circulation, and microbial or microbiota-host co-metabolites have been detected in the brain from early postnatal life (Swann et al., 2020). Although the functional significance of many of these molecules for brain development remains poorly understood, their presence demonstrates that the developing brain is exposed to a chemically diverse repertoire of signals whose abundance depends, at least in part, on the gut microbiota.

Microbial signals may influence brain development even before establishment of the infant gut microbiota. Although current evidence does not support colonization of the foetus by a resident microbiota during healthy pregnancy, functional molecular signals generated by the maternal gut microbiota can enter the maternal circulation and reach the foetal compartment. Experimental studies have demonstrated that maternal microbiota-derived metabolites can influence foetal devel-

opment, including the programming of peripheral tissues (Kimura et al., 2020) and thalamocortical axon development in the foetal brain (Vuong et al., 2020). These findings establish an important conceptual principle: microbial colonization of the developing organism is not required for microbial signals to influence brain development. Before birth, the maternal microbiota can provide molecular information to the developing foetus; after birth, microbial signals increasingly originate from the infant's own colonizing microbial community. The nature and magnitude of these signals are likely to vary with the maternal and postnatal environment. Infection, inflammation, stress, diet, antibiotic exposure and other factors can alter microbial composition, microbial metabolism, intestinal barrier function or combinations of these processes and may consequently modify host exposure to microbiota-derived molecules. Their biological effects are therefore likely to depend on the identity and concentration of the signal, developmental timing, biological sex, host genetics and physiological state.

While much attention has focused on microbial metabolites, bacteria also continuously release structural components during growth, division, remodelling and lysis. These molecules can be recognized by host pattern-recognition systems and provide another potential source of molecular information about the microbial environment. Among them, PGN represents a particularly intriguing and comparatively underappreciated signal in microbiota-brain communication.

PEPTIDOGLYCAN: AN UNDERAPPRECIATED SIGNAL IN MICROBIOTA-BRAIN COMMUNICATION

PGN is an essential structural component of the bacterial cell wall and is

absent from mammalian cells. It consists of glycan chains composed of

alternating N-acetylglucosamine and N-acetylmuramic acid residues cross-linked by short peptide chains (*Wheeler et al., 2014*). Despite its structural role, PGN is highly dynamic. During bacterial growth, division, remodelling and turnover, fragments of the cell wall are continuously released into the surrounding environment. Different bacterial groups generate structurally distinct PGN motifs. Meso-diaminopimelic acid (DAP)-containing PGN is characteristic of Gram-negative bacteria and some Gram-positive species, whereas lysine-type PGN is found in many Gram-positive bacteria. Muramyl dipeptide (MDP) represents a minimal bioactive motif shared by PGN from both Gram-positive and Gram-negative bacteria.

The mammalian host possesses specialized pattern-recognition systems capable of detecting these structures. Two major families are the intracellular nucleotide-binding oligomerization domain receptors NOD1 and NOD2 and the peptidoglycan recognition proteins PGLYRP1-4 (*Royet et al., 2011; Mukherjee et al., 2019*). NOD1 preferentially detects DAP-containing PGN motifs, whereas NOD2 recognizes MDP. Historically, these pathways have been studied primarily in the context of bacterial infection and innate immune defence. However, the enormous and continuous source of PGN provided by the commensal microbiota raises a different possibility: PGN sensing may also participate in physiological host-microbe communication. An important demonstration of this principle came from Clarke and colleagues, who showed that PGN originating from the indigenous intestinal microbiota can translocate across the intestinal barrier and reach systemic tissues in healthy mice. Microbiota-derived PGN was sufficient to prime neutrophil function through NOD1 signalling (*Clarke et al., 2010*). More recent demonstrations of

systemic dissemination of microbiota-derived PGN have reinforced the concept of a circulating repertoire of PGN fragments generated by commensal bacteria (*Wheeler et al., 2023*).

Our work extended this concept to the developing brain. Arentsen and colleagues demonstrated that PGN fragments derived from the indigenous gut microbiota reach the developing mouse brain (*Arentsen et al., 2017*). Brain PGN levels increased in parallel with postnatal microbial colonization, suggesting that exposure of the brain to bacterial cell-wall components is part of normal postnatal development rather than being restricted to infection or pathological disruption of the BBB. Importantly, molecules required for PGN sensing are expressed in the developing brain. Members of the PGLYRP and NOD receptor families show developmentally regulated expression, as does the peptide transporter PepT1. Their expression varies according to brain region, developmental stage and sex, suggesting that the biological effects of PGN signalling are likely to be strongly context dependent. The functional importance of this pathway was demonstrated using mice lacking Pglyrp2. Juvenile Pglyrp2-deficient mice displayed alterations in the expression of genes involved in brain development and synaptic function, including *c-Met* and *Bdnf*, together with changes in social behaviour. Male, but not female, Pglyrp2-deficient mice exhibited a pronounced hypersocial phenotype, and additional sex-dependent behavioural alterations emerged later in life (*Arentsen et al., 2017, 2018*). Other studies have subsequently strengthened the connection between PGN sensing and brain function. NOD1-dependent signalling in intestinal epithelial cells has been implicated in stress-related behavioural responses, while NOD2 has been identified in hypothalamic neurons, where bacterial

PGN sensing contributes to the regulation of appetite and body temperature (Pusceddu et al., 2019; Gabanyi et al., 2022).

The biological activity of microbiota-derived PGN may depend not only on bacterial abundance or total PGN production but also on how the bacterial cell wall is remodelled. As a proof of principle, bacterial PGN hydrolases, including DL-endopeptidases, can alter the repertoire of soluble muropeptides available for host sensing and thereby modify NOD-dependent signalling (Gao et al., 2022). This emerging concept suggests that the functional PGN

output of a microbial community cannot necessarily be inferred from its taxonomic composition alone. Together, these findings suggest that PGN-sensing pathways should not be regarded exclusively as components of antimicrobial defence. Instead, they may represent an evolutionarily conserved molecular interface through which the host, including the developing brain, obtains information about its microbial environment. The finding that PGN reaches the brain during postnatal development raises a further question: could PGN signalling begin even earlier, before birth?

PEPTIDOGLYCAN SIGNALLING AT THE MATERNAL-FOETAL INTERFACE

During pregnancy, the maternal intestinal microbiota provides a continuous source of microbial molecules, including bacterial structural components, while the placenta and other maternal-foetal barriers regulate their access to the developing foetus. Changes in maternal intestinal permeability, microbial composition, bacterial growth or cell-wall turnover could therefore alter the amount and composition of PGN fragments entering the maternal circulation and reaching the foetal compartment. To investigate whether maternal PGN exposure could influence the foetal environment, we used MDP as a defined bioactive PGN motif. Pregnant mice were exposed to MDP through drinking water during late gestation, from gestational day 16 to 19. Using a cellular reporter assay based on HEK-Blue cells expressing mouse NOD2, we detected increased NOD2-activating activity in amniotic fluid 24 hours after maternal MDP exposure, with a significant increase observed in samples from male foetuses (Martinez Sanchez et al., 2025). This observation provides evi-

dence that changes in maternal exposure to a defined PGN fragment can influence the foetal environment and alter the molecular milieu surrounding the developing foetus.

We next examined whether the foetal brain showed molecular responses to this altered PGN environment. We focused on candidate transport systems for small bacterial peptides. Members of the SLC15 family, including SLC15A1/PepT1, SLC15A2/PepT2, SLC15A3/PHT2 and SLC15A4/PHT1, together with members of the SLC46 family, have been implicated in the transport of small PGN fragments in peripheral tissues (Bastos et al., 2021). In the foetal brain, maternal MDP exposure produced sex-dependent changes in the mRNA expression of genes associated with PGN transport and sensing. Male foetuses showed increased expression of *Slc15a1*, *Slc15a2* and *Slc46a2*, whereas *Nod2* expression was increased in females (Martinez Sanchez et al., 2025). One particularly important observation was the absence of significant changes in the mRNA expression

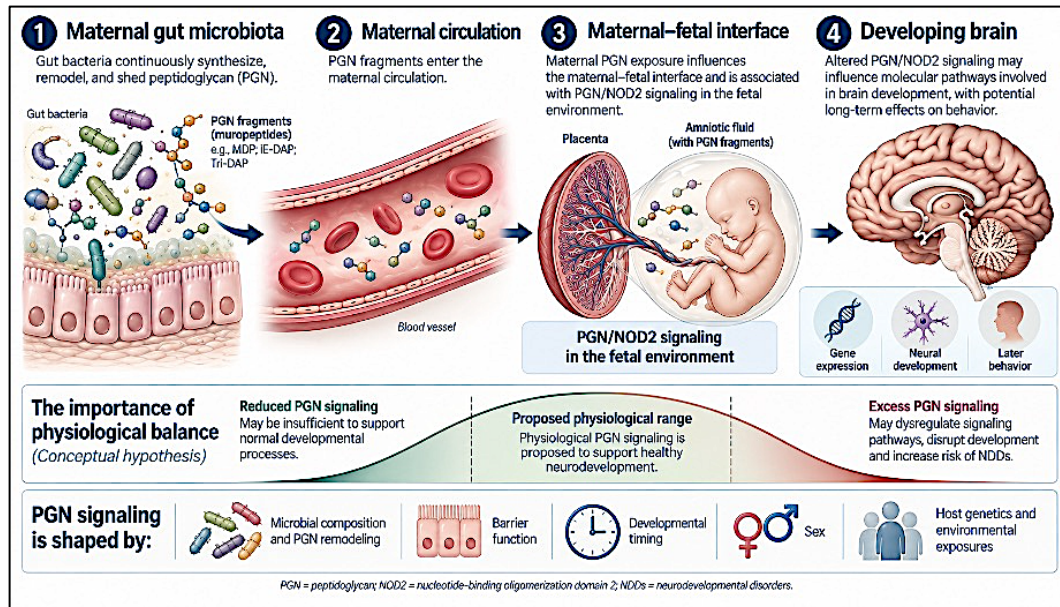


Figure 2. Maternal microbiota-derived peptidoglycan signalling during neurodevelopment.

The maternal gut microbiota continuously generates peptidoglycan (PGN) fragments, or muropeptides, through bacterial growth and cell-wall remodelling. These fragments can enter the maternal circulation and potentially influence the maternal-foetal interface and foetal environment, where PGN-sensing pathways, including NOD2 signalling, may contribute to developmental processes. Experimental studies indicate that perturbation of PGN/NOD2 signalling during gestation can alter molecular pathways involved in brain development and produce long-term effects on behaviour. The lower panel illustrates a conceptual model in which PGN signalling operates within a physiological range that supports normal neurodevelopment, whereas reduced or excessive signalling may disrupt developmental processes and increase susceptibility to neurodevelopmental disorders. The magnitude and consequences of PGN signalling are likely shaped by microbial composition and PGN remodelling, barrier function, developmental timing, sex, host genetics, and environmental exposures. MDP, muramyl dipeptide; iE-DAP, γ -D-glutamyl-meso-diaminopimelic acid; Tri-DAP, L-Ala- γ -D-Glu-meso-DAP; NOD2, nucleotide-binding oligomerization domain-containing protein 2; NDDs, neurodevelopmental disorders.

of inflammatory and microglia-related genes in the foetal brain. This distinction is conceptually important. Maternal immune activation models commonly rely on strong inflammatory stimuli and are generally interpreted through cytokine-mediated effects on foetal brain development. The MDP findings suggest an additional possibility: bacterial molecules may modify developmental programs through changes in PGN-associated sensing and transport pathways without necessarily provoking classical neuroinflammation. However, given that these observations were based on gene expression, further stud-

ies will be required to establish the underlying cellular mechanisms. The consequences of prenatal PGN exposure were not restricted to foetal molecular responses. Juvenile offspring displayed distinct sex-specific behavioural phenotypes. Prenatally exposed males showed reduced direct social interaction, whereas females displayed reduced novelty-induced locomotion and impaired social recognition. These behavioural alterations were accompanied by changes in the expression of synaptic and microglia-related genes in the prefrontal cortex (Martinez Sanchez et al., 2025).

Taken together with our earlier work showing that microbiota-derived PGN reaches the postnatal brain, these observations suggest a broader developmental framework (Figure 2). PGN signalling may operate across multiple developmental windows, beginning with maternal microbial signals during foetal life and continuing as the infant acquires its own intestinal microbiota after birth. The identity and concentration of PGN fragments, developmental stage, expression of sensing and transport pathways, brain region, biological sex, host genetics and physiological context may

collectively determine the biological response.

This raises an important question for future research. Rather than asking whether PGN is simply “beneficial” or “harmful,” should we instead consider the existence of an optimal developmental range of PGN signalling? Both insufficient and excessive exposure could potentially disturb developmental processes. Such a model would also help explain why environmental factors that alter microbial composition, bacterial turnover or barrier permeability may have different consequences depending on when they occur.

TRANSLATING MICROBIOTA-BRAIN FINDINGS TO HUMANS

Experimental animal models have been essential for establishing causal relationships between the gut microbiota and brain development. GF animals, antibiotic exposure, controlled microbial colonization and administration of defined microbial molecules make it possible to manipulate individual components of the microbiota-brain axis and examine their effects on brain development and behaviour. Translation to humans, however, remains challenging. Human microbial communities are highly heterogeneous and are shaped by host genetics, diet, medications, lifestyle, geography and numerous environmental exposures. Moreover, many experimental manipulations routinely used in animal models cannot be applied during human pregnancy or infancy. A translational approach therefore requires integration of complementary experimental systems. Human neural stem cell-based models and brain organoids may help determine whether microbial metabolites or structural bacterial components act directly on human brain cells and identify candidate receptors, transporters and molecular pathways

involved. Prospective human cohort studies can then determine whether related microbial features or molecular signals are detectable during relevant developmental windows and whether they precede differences in developmental outcomes. Metagenomic and metabolomic approaches provide an additional opportunity to move beyond taxonomic descriptions of the microbiota toward characterizing its functional capacity and molecular output.

Our longitudinal study of infants at elevated likelihood of developing ASD provides one example of the importance of studying the microbiota before behavioural differences become apparent. Infants with and without a family history of ASD were followed across the first 36 months of life (Zuffa et al., 2023). At 5 months, infants at elevated likelihood harboured lower abundances of *Bifidobacterium* and higher abundances of *Clostridium* and *Klebsiella* species compared with low-likelihood infants. Faecal γ -aminobutyric acid (GABA) was also lower in the elevated-likelihood group. Integrated microbiome-metabolome analysis revealed pos-

itive associations between GABA and *Bifidobacterium* species and negative associations with *Clostridium* species. *In vitro* experiments further demonstrated that bifidobacteria and clostridia differentially influence GABA availability, with *Clostridium* species consuming GABA. Importantly, these microbial and metabolic differences were already present at 5 months, when behavioural measures did not differ significantly between the groups. Differences in developmental and ASD-related measures became evident later, at 36 months. Although these observational findings cannot establish causality, they demonstrate the value of longitudinal studies beginning before measurable behavioural differences emerge and raise the possibility that early microbial functional differences may contribute to later atypical neurodevelopmental trajectories (Zuffa et al., 2023).

The same emphasis on microbial function rather than taxonomy may ultimately be important for PGN. Recent work provides proof of principle that bacterial PGN-remodelling capacity can determine the production of host-active

muropeptides (Gao et al., 2022). A recent study in preterm infants further linked early antibiotic exposure with altered microbial PGN-processing capacity and host responses (Shen et al., 2026). These findings concern intestinal and immune physiology rather than brain development and should therefore not be extrapolated directly to neurodevelopment. Nevertheless, they demonstrate that environmental perturbations in early life can modify the functional PGN output of the human microbiota.

The strongest translational evidence will ultimately emerge from convergence across experimental systems: mechanistic studies in animals, validation of candidate pathways in human cell-based models and longitudinal human studies demonstrating that related microbial functions or molecular signals are relevant during human development. For PGN, important questions include which muropeptides circulate during pregnancy and infancy, how their abundance and molecular composition change with development, and whether variation in these signals is associated with different trajectories of brain development.

TARGETING THE MICROBIOTA TO PROMOTE BRAIN HEALTH

Understanding the molecular mechanisms linking microbes to the brain is also essential for developing rational microbiota-based interventions. Rather than seeking to establish a universally “healthy microbiome,” a more realistic translational goal may be to identify microbial functions, molecules, and host signalling pathways that can be targeted to modulate specific brain circuits and functions in a predictable manner. Studies with *Limosilactobacillus reuteri* illustrate both the potential and the complexity of microbiota-based interventions to modulate neural circuits under-

lying social behaviour. Buffington and colleagues showed that maternal high-fat diet altered the offspring gut microbiota and was associated with impaired social behaviour (Buffington et al., 2016). These behavioural changes were accompanied by reduced numbers of oxytocin-immunoreactive neurons in the hypothalamus and impaired synaptic potentiation in the ventral tegmental area (VTA) in response to social interaction. Microbial transfer experiments supported a causal role for the altered gut microbiota in the social deficits. Remarkably, reconstitution

with *L. reuteri* restored hypothalamic oxytocin levels, social interaction-induced synaptic plasticity in the VTA and social behaviour (Buffington et al., 2016). Subsequent work demonstrated that the effects of *L. reuteri* are not restricted to a single environmental model. Sgritta and colleagues showed that *L. reuteri* treatment selectively rescued social deficits across genetic, environmental and idiopathic mouse models relevant to ASD (Sgritta et al., 2019). Importantly, the behavioural effects were not mediated by restoration of the overall gut microbiota composition. Instead, *L. reuteri* acted in a vagus nerve-dependent manner and restored social interaction-induced synaptic plasticity in the VTA, while its effects were absent in oxytocin receptor-deficient mice. Together, the Buffington and Sgritta studies established a mechanistic connection between a defined commensal bacterium, vagal signalling, oxytocin-dependent modulation of dopaminergic reward circuitry and social behaviour.

More recent work has reinforced this pathway in another environmental context. In offspring exposed to prenatal stress, *L. reuteri* supplementation during critical developmental windows restored oxytocin levels in the paraventricular nucleus, improved dopamine reward pathway function and ameliorated social deficits. These effects were abolished by either oxytocin receptor antagonism or subdiaphragmatic vagotomy, further supporting the importance of vagal afferent signalling and oxytocin-dopamine circuitry in *L. reuteri*-mediated modulation of social behaviour (Zhang et al., 2026). Importantly, early clinical studies suggest that some of these preclinical observations may translate to humans. In a pilot double-blind, randomized, placebo-controlled trial in children with ASD, treatment with a combination of

L. reuteri strains ATCC-PTA-6475 and DSM-17938 improved social functioning across several measures, although it did not alter overall autism severity, restricted and repetitive behaviours, microbiome composition or immune profiles (Mazzone et al., 2024). Preclinical comparison of the strains further indicated strain specificity: ATCC-PTA-6475, but not the parental strain of DSM-17938, rescued social deficits in an ASD mouse model. These findings illustrate an important principle for microbiota-based therapeutics: closely related bacterial strains cannot be assumed to exert equivalent effects on brain function and behaviour.

Developmental timing and biological sex add further layers of complexity. We recently investigated the long-term consequences of *L. reuteri* supplementation during a defined perinatal window, from gestational day 6 to postnatal day 7, in BALB/c mice (Sieglar Lathrop et al., 2025). Perinatal supplementation produced persistent improvements in sociability and social recognition in adult males, together with changes in gut microbiota composition and increased *Il10* gene expression in the striatum and colon. Expression of the oxytocin receptor (*Oxtr*) was increased in both the prefrontal cortex and striatum in males and females. However, females showed a distinct behavioural profile, including impaired social recognition, demonstrating that the same early-life microbial intervention can produce divergent long-term outcomes according to sex. Interestingly, expression of the PGN-associated transporters *Slc46a2* and *Slc46a3* was also altered in a sex-dependent manner, raising the possibility that microbial interventions may influence not only vagal and neuroendocrine pathways but also host responsiveness to structural bacterial signals. Collectively, these observations highlight several variables likely to

determine the success of early-life microbial interventions. Timing matters because prenatal and postnatal microbial signals encounter fundamentally different developmental environments. Host factors, including sex, genetics and immune status, can modify responsiveness. Microbial characteristics, particularly strain identity, dose and mode of administration, determine the functions delivered to the host. Finally, environmental context, including maternal diet, stress, inflammation and antibiotic exposure, may enhance, modify or inter-

fere with an intervention.

These considerations argue against a one-size-fits-all approach to microbiota-based therapeutics. Instead, future strategies will require increasing precision in defining when, how and for whom microbial interventions are likely to be effective. Ultimately, identifying the molecular functions and signals generated by individual microbial strains may allow interventions to be selected on the basis of biological mechanism rather than microbial taxonomy alone.

CONCLUSIONS AND PERSPECTIVES

Research on the microbiota-gut-brain axis has progressed from demonstrating that microorganisms influence brain development, function and behaviour toward identifying the molecular signals responsible for these effects. This transition from association toward mechanism is essential if microbiome research is to explain how microbial environments shape brain development and ultimately determine whether these pathways can be modified to promote brain health. PGN illustrates this conceptual shift particularly well. Once viewed primarily as a pathogen-associated molecule that activates innate immunity during infection, PGN is increasingly recognized as a component of normal host-microbiota communication. The intestinal microbiota continuously generates a diverse repertoire of PGN fragments, collectively forming a peptidoglycome shaped by bacterial growth, turnover and cell-wall remodeling. A proportion of these fragments can cross biological barriers and reach distant host tissues. PGN-sensing and candidate transport pathways are present in the developing brain, and experimental manipulation of PGN exposure or sensing can alter molecular programs

involved in brain development and behaviour.

Our recent findings extend this framework into prenatal life. Maternal exposure to the NOD2 ligand MDP alters NOD2-activating activity within the amniotic fluid and alters the expression of genes associated with PGN sensing and candidate transport pathways in the foetal brain in a sex-dependent manner. These molecular changes occur without detectable changes in the expression of microglial or inflammatory markers in the foetal brain, while transient prenatal PGN overexposure is followed by persistent, sex-specific behavioural and molecular alterations in juvenile offspring. These findings raise the possibility that PGN can function not simply as an inflammatory stimulus but as a developmental microbial signal whose biological consequences depend on molecular structure, concentration, developmental timing, tissue context and host characteristics.

An important next step will be to understand how the microbial PGN signal itself is generated. PGN is continuously remodelled by bacterial enzymes that determine the molecular repertoire of soluble fragments released into the

intestinal environment. Recent evidence that bacterial DL-endopeptidase activity can regulate the generation of host-active mucopeptides provides proof of principle that microbial PGN remodelling can influence host signalling. The functional PGN output of the microbiota may therefore depend not simply on which bacteria are present, but on the enzymatic activities that determine which PGN fragments become available for host sensing. This concept may be particularly important during early development. The infant microbiota undergoes profound ecological and functional maturation during the same period in which brain circuits, glial populations, the immune system and physiological barriers undergo extensive development and maturation. It is therefore conceivable that maturation of the microbiota is accompanied by maturation of its PGN-remodelling capacity, generating developmentally distinct peptidoglycomes. Antibiotics, infection, diet and other environmental perturbations could modify this process by changing not only microbial community structure but also the quantity and molecular composition of PGN fragments presented to the host.

Many fundamental questions remain. Which PGN fragments circulate under physiological conditions during pregnancy and infancy? How does their molecular composition change during development? How do individual mucopeptides cross intestinal, placental and brain barriers? Which transport systems regulate their tissue and cellular distribution? Which brain and peripheral cell populations respond to particular PGN structures? And how do developmental timing, biological sex, host genetics and environmental exposures modify these responses? Extending this framework to humans represents a major challenge. Interindividual variation in microbial PGN biosynthesis and remodelling,

intestinal permeability, systemic clearance, transport and innate immune sensing could generate substantially different PGN exposures even among individuals with apparently similar microbiota compositions. Longitudinal studies integrating metagenomic analysis of PGN-related microbial functions with characterization of circulating mucopeptides will be required to determine whether distinct PGN signatures accompany typical developmental trajectories and whether altered PGN signalling is associated with atypical brain development.

The same mechanistic precision will be required for microbial interventions. The *L. reuteri* studies illustrate both the promise and complexity of this approach. Developmental timing, biological sex, host genetics, microbial strain and environmental context can all influence the response. Identifying the molecular signals generated by specific microorganisms may therefore allow the field to move beyond interventions defined principally by microbial taxonomy toward approaches based on defined microbial functions.

The developing brain exists in continuous molecular dialogue with the microbial world. Deciphering this dialogue will require understanding not only which microorganisms are present, but also which molecules they generate, how those molecules are modified, where they travel and how they are sensed by the developing host. PGN provides a compelling model for this emerging view of microbiota-brain communication. Defining the developmental peptidoglycome, and the microbial and host mechanisms that shape it, may ultimately reveal new principles governing brain development and identify molecular pathways through which early-life microbial environments influence vulnerability or resilience to neurodevelopmental and neurological disorders.

ACKNOWLEDGMENTS

Work from the laboratory of R.D.H. cited in this review has been supported by grants from the Swedish Research Council, The Swedish Brain Foundation, the Olle Engkvist Byggmästare Foundation, and the Frimurare Barnhus Foundation.

LITERATURE

- Arentsen, T., Qian, Y., Gkotsis, S., Femenia, T., Wang, T., Udekwu, K., Forssberg, H., and Diaz Heijtz, R.: The bacterial peptidoglycan-sensing molecule Pglyrp2 modulates brain development and behavior. *Mol. Psychiatry* 22: 257-266 (2017).
- Arentsen, T., Khalid, R., Qian, Y., and Diaz Heijtz, R.: Sex-dependent alterations in motor and anxiety-like behavior of aged bacterial peptidoglycan sensing molecule 2 knockout mice. *Brain Behav. Immun.* 67: 345-354 (2018).
- Bastos, P.A.D., Wheeler, R., and Boneca, I.G.: Uptake, recognition and responses to peptidoglycan in the mammalian host. *FEMS Microbiol. Rev.* 45: fuaa044 (2021).
- Braniste, V., Al-Asmakh, M., Kowal, C., Anuar, F., Abbaspour, A., Tóth, M., Korecka, A., Bakocevic, N., Ng, L.G., Kundu, P., Gulyás, B., Halldin, C., Hultenby, K., Nilsson, H., Hebert, H., Volpe, B.T., Diamond, B., and Pettersson, S.: The gut microbiota influences blood-brain barrier permeability in mice. *Sci. Transl. Med.* 6: 263ra158 (2014).
- Bravo, J.A., Forsythe, P., Chew, M.V., Escaravage, E., Savignac, H.M., Dinan, T.G., Bienenstock, J., and Cryan, J.F.: Ingestion of *Lactobacillus* strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. *Proc. Natl. Acad. Sci. USA* 108: 16050-16055 (2011).
- Buffington, S.A., Di Prisco, G.V., Auchtung, T.A., Ajami, N.J., Petrosino, J.F., and Costa-Mattioli, M.: Microbial Reconstitution Reverses Maternal Diet-Induced Social and Synaptic Deficits in Offspring. *Cell* 165: 1762-1775 (2016).
- Clarke, T.B., Davis, K.M., Lysenko, E.S., Zhou, A.Y., Yu, Y., and Weiser, J.N.: Recognition of peptidoglycan from the microbiota by Nod1 enhances systemic innate immunity. *Nat. Med.* 16: 228-231 (2010).
- Cryan, J.F., O'Riordan, K.J., Sandhu, K., Peterson, V., and Dinan, T.G.: The gut microbiome in neurological disorders. *Lancet Neurol.* 19: 179-194 (2020).
- Cryan, J.F.: Microbiome and Brain Development: A Tale of Two Systems. *Ann. Nutr. Metab.* 81(Suppl 1): 34-46 (2025).
- Diaz Heijtz, R., Wang, S., Anuar, F., Qian, Y., Björkholm, B., Samuelsson, A., Hibberd, M.L., Forssberg, H., and Pettersson, S.: Normal gut microbiota modulates brain development and behavior. *Proc. Natl. Acad. Sci. USA* 108: 3047-3052 (2011).
- Erny, D., Hrabě de Angelis, A.L., Jaitin, D., Wieghofer, P., Staszewski, O., David, E., Keren-Shaul, H., Mhlahkoi, T., Jakobshagen, K., Buch, T., Schwierzeck, V., Utermöhlen, O., Chun, E., Garrett, W.S., McCoy, K.D., Diefenbach, A., Staeheli, P., Stecher, B., Amit, I., and Prinz, M.: Host microbiota constantly control maturation and function of microglia in the CNS. *Nat. Neurosci.* 18: 965-977 (2015).
- Gabanyi, I., Lepousez, G., Wheeler, R., Vieites-Prado, A., Nissant, A., Chevalier, G., Wagner, S., Moigneu, C., Dulauroy, S., Hicham, S., Polomack, B., Verny, F., Rosenstiel, P., Renier, N., Boneca, I.G., Eberl, G., and Lledo, P.M.: Bacterial sensing via neuronal Nod2 regulates appetite and body temperature. *Science* 376: eabj3986 (2022).
- Gao, J., Zhao, X., Hu, S., Huang, Z., Hu, M., Jin, S., Lu, B., Sun, K., Wang, Z., Fu, J., Weersma, R.K., He, X., and Zhou, H.: Gut microbial DL-endopeptidase alleviates Crohn's disease via the NOD2 pathway. *Cell*

- Host Microbe 30: 1435-1449 (2022).
- Han, W., Tellez, L.A., Perkins, M.H., Perez, I.O., Qu, T., Ferreira, J., Ferreira, T.L., Quinn, D., Liu, Z.W., Gao, X.B., Kaelberer, M.M., Bohórquez, D.V., Shammah-Lagnado, S.J., de Lartigue, G., de Araujo, I.E.: A Neural Circuit for Gut-Induced Reward. *Cell* 175: 665-678 (2018).
- Hoban, A.E., Stilling, R.M., Ryan, F.J., Shanahan, F., Dinan, T.G., Claesson, M.J., Clarke, G., and Cryan, J.F.: Regulation of prefrontal cortex myelination by the microbiota. *Transl. Psychiatry* 6: e774 (2016).
- Kaelberer, M.M., Buchanan, K.L., Klein, M.E., Barth, B.B., Montoya, M.M., Shen, X., and Bohórquez, D.V. A gut-brain neural circuit for nutrient sensory transduction. *Science* 361(6408): eaat5236 (2018).
- Kennedy, K.M., de Goffau, M.C., Perez-Muñoz, M.E., Arrieta, M.C., Bäckhed, F., Bork, P., Braun, T., Bushman, F.D., Dore, J., de Vos, W.M., Earl, A.M., Eisen, J.A., Elovitz, M.A., Ganai-Vonarburg, S.C., Gänzle, M.G., Garrett, W.S., Hall, L.J., Hornef, M.W., Huttenhower, C., Konnikova, L., Lebeer, S., Macpherson, A.J., Massey, R.C., McHardy, A.C., Koren, O., Lawley, T.D., Ley, R.E., O'Mahony, L., O'Toole, P.W., Pamer, E.G., Parkhill, J., Raes, J., Rattei, T., Salonen, A., Segal, E., Segata, N., Shanahan, F., Sloboda, D.M., Smith, G.C.S., Sokol, H., Spector, T.D., Surette, M.G., Tannock, G.W., Walker, A.W., Yassour, M., and Walter, J.: Questioning the fetal microbiome illustrates pitfalls of low-biomass microbial studies. *Nature* 613: 639-649 (2023).
- Kimura, I., Miyamoto, J., Ohue-Kitano, R., Watanabe, K., Yamada, T., Onuki, M., Aoki, R., Isobe, Y., Kashiara, D., Inoue, D., Inaba, A., Takamura, Y., Taira, S., Kumaki, S., Watanabe, M., Ito, M., Nakagawa, F., Irie, J., Kakuta, H., Shinohara, M., Iwatsuki, K., Tsujimoto, G., Ohno, H., Arita, M., Itoh, H., and Hase, K.: Maternal gut microbiota in pregnancy influences offspring metabolic phenotype in mice. *Science* 367: eaaw8429 (2020).
- Luck, B., Engevik, M.A., Ganesh, B.P., Lackey, E.P., Lin, T., Balderas, M., Major, A., Runge, J., Luna, R.A., Sillitoe, R.V., and Versalovic, J.: Bifidobacteria shape host neural circuits during postnatal development by promoting synapse formation and microglial function. *Sci. Rep.* 10: 7737 (2020).
- Martínez Sánchez, I., Spielbauer, J., and Diaz Heijtz, R.: Maternal peptidoglycan overexposure during late pregnancy alters neurodevelopment and behavior in juvenile offspring. *Brain Behav. Immun.* 127: 96-102 (2025).
- Mazzone, L., Dooling, S.W., Volpe, E., Uljarević, M., Waters, J.L., Sabatini, A., Arturi, L., Abate, R., Riccioni, A., Siracusano, M., Pereira, M., Engstrand, L., Cristofori, F., Adduce, D., Francavilla, R., Costa-Mattioli, M., and Hardan, A.Y.: Precision microbial intervention improves social behavior but not autism severity: A pilot double-blind randomized placebo-controlled trial. *Cell Host Microbe* 32: 106-116 (2024).
- Mukherjee, T., Hovingh, E.S., Foerster, E.G., Abdel-Nour, M., Philpott, D.J., and Girardin, S.E.: NOD1 and NOD2 in inflammation, immunity and disease. *Arch. Biochem. Biophys.* 670: 69-81 (2019).
- Pessa-Morikawa, T., Husso, A., Kärkkäinen, O., Koistinen, V., Hanhineva, K., Iivanainen, A., and Niku, M.: Maternal microbiota-derived metabolic profile in fetal murine intestine, brain and placenta. *BMC Microbiol.* 22: 46 (2022).
- Pusceddu, M.M., Barboza, M., Keogh, C.E., Schneider, M., Stokes, P., Sladek, J.A., Kim, H.J.D., Torres-Fuentes, C., Goldfild, L.R., Gillis, S.E., Brust-Mascher, I., Rabasa, G., Wong, K.A., Lebrilla, C., Byndloss, M.X., Maisonneuve, C., Bäuml, A.J., Philpott, D.J., Ferrero, R.L., Barrett, K.E., Reardon, C., and Gareau, M.G.: Nod-like receptors are critical for gut-brain axis signalling in mice. *J. Physiol.* 597: 5777-5797 (2019).
- Royet, J., Gupta, D., and Dziarski, R.: Peptidoglycan recognition proteins: modulators of the microbiome and inflammation. *Nat. Rev. Immunol.* 11: 837-

- 851 (2011).
- Semple, B.D., Blomgren, K., Gimlin, K., Ferriero, D.M., and Noble-Haeusslein, L.J.: Brain development in rodents and humans: Identifying benchmarks of maturation and vulnerability to injury across species. *Prog. Neurobiol.* 106-107: 1-16 (2013).
- Sgritta, M., Dooling, S.W., Buffington, S.A., Momin, E.N., Francis, M.B., Britton, R.A., and Costa-Mattioli, M.: Mechanisms Underlying Microbial-Mediated Changes in Social Behavior in Mouse Models of Autism Spectrum Disorder. *Neuron* 101: 246-259 (2019).
- Shen, W., Wang, H., Wang, J., Yuan, Y., Luo, Y., Chen, X., Li, J., Liu, Y., Yin, Y., Wang, M., Lin, L., Zhou, L., Li, J., Xie, R., Dai, Y., Wu, F., Huang, Z., Zhou, Y., Xia, F., Wu, F., Cao, P., Gao, J., He, X., Clemente, J.C., Chen, H., Yang, J., Huang, W., Zhou, H., and He, Y.: Gut microbiota immaturity with DL-endopeptidase deficiency links antibiotic use to preterm late-onset sepsis. *Cell Host Microbe* 34: 672-691 (2026).
- Siegler Lathrop, T., Martínez Sanchez, I., Chronakis, I.S., and Diaz Heijtz, R.: Sex-Specific Long-Term Effects of Perinatal *Limosilactobacillus reuteri* on Social Cognition, Gene Expression, and Gut Microbiota. *J. Neurochem.* 169: e70199 (2025).
- Silbereis, J.C., Pochareddy, S., Zhu, Y., Li, M., and Sestan, N.: The Cellular and Molecular Landscapes of the Developing Human Central Nervous System. *Neuron* 89: 248-268 (2016).
- Sinha, T., Brushett, S., Fernández-Pato, A., Garmaeva, S., Andreu-Sánchez, S., Spreckels, J.E., Mallon, C.A., Kuzub, N., Gois, M.B., Wu, J., Kruk, M., Jankipersadsing, S.A., Dekens, J.A.M., Gacesa, R., Vila, A.V., Bang, C., Perenboom, C., Franke, A., Tytgat, H.L.P., Mottaz, S.C., Peters, L., de Jonge, A., Verkade, H.J., Swertz, M.A., Wijmenga, C., Kuipers, F., Scherjon, S., Sikkema, J., Sprikkelman, A.B., de Kroon, M.L.A., Prins, J.R., Gordijn, S.J., Koppelman, G.H., Reijneveld, S.A.; Lifelines NEXT cohort study; Fu, J., Yassour, M., Kurilshikov, A., and Zhernakova, A.: Maternal influences on infant gut microbiome and health. *Nature*. Epub ahead of print, doi: 10.1038/s41586-026-10922-9 (2026).
- Stewart, C.J., Ajami, N.J., O'Brien, J.L., Hutchinson, D.S., Smith, D.P., Wong, M.C., Ross, M.C., Lloyd, R.E., Doddapaneni, H., Metcalf, G.A., Muzny, D., Gibbs, R.A., Vatanen, T., Huttenhower, C., Xavier, R.J., Rewers, M., Hagopian, W., Toppari, J., Ziegler, A.G., She, J.X., Akolkar, B., Lernmark, A., Hyoty, H., Vehik, K., Krischer, J.P., and Petrosino, J.F.: Temporal development of the gut microbiome in early childhood from the TEDDY study. *Nature* 562: 583-588 (2018).
- Sudo, N., Chida, Y., Aiba, Y., Sonoda, J., Oyama, N., Yu, X.N., Kubo, C., and Koga, Y.: Postnatal microbial colonization programs the hypothalamic-pituitary-adrenal system for stress response in mice. *J. Physiol.* 558: 263-275 (2004).
- Swann, J.R., Spitzer, S.O., and Diaz Heijtz, R.: Developmental Signatures of Microbiota-Derived Metabolites in the Mouse Brain. *Metabolites* 10:172 (2020).
- Vuong, H.E., Pronovost, G.N., Williams, D.W., Coley, E.J.L., Siegler, E.L., Qiu, A., Kazantsev, M., Wilson, C.J., Rendon, T., and Hsiao, E.Y.: The maternal microbiome modulates fetal neurodevelopment in mice. *Nature* 586: 281-286 (2020).
- Wang, W., Gu, W., Schweitzer, R., Koren, O., Khatib, S., Tseng, G., and Konnikova, L.: In utero human intestine contains maternally derived bacterial metabolites. *Microbiome* 13: 116 (2025).
- Wheeler, R., Chevalier, G., Eberl, G., and Gomperts Boneca, I.: The biology of bacterial peptidoglycans and their impact on host immunity and physiology. *Cell Microbiol.* 16: 1014-1023 (2014).
- Wheeler, R., Bastos, P.A.D., Disson, O., Rifflet, A., Gabanyi, I., Spielbauer, J., Bérard, M., Lecuit, M., and Boneca, I.G.: Microbiota-induced active translocation of peptidoglycan across the intestinal barrier dictates its within-host dissemination. *Proc.*

- Natl. Acad. Sci. USA 120: e2209936120 (2023).
- Zhang, F., Xu, W., Zeng, R., Chen, J., and Huang, J.: *Limosilactobacillus reuteri* normalizes gut microbiota dysfunction and social deficits of rat offspring associated with prenatal exposure to stress. Gut Microbes 18: 2649440 (2026).
- Zuffa, S., Schimmel, P., Gonzalez-Santana, A., Belzer, C., Knol, J., Bölte, S., Falck-Ytter, T., Forssberg, H., Swann, J., and Diaz Heijtz, R.: Early-life differences in the gut microbiota composition and functionality of infants at elevated likelihood of developing autism spectrum disorder. Transl. Psychiatry 13: 257 (2023).

THE GUT MICROBIOTA–HISTIDINE–UROCANATE AXIS AND COGNITION: MECHANISTIC CONVERGENCE ACROSS PRECLINICAL AND CLINICAL EVIDENCE

ANTONIO SUÁREZ

Institute of Nutrition and Food Technology "José Mataix Verdú", Centre for Biomedical Research, University of Granada; Institute of Biosanitary Research of Granada, Health Sciences Technological Park, Granada; and Department of Biochemistry and Molecular Biology II. Faculty of Pharmacy. University of Granada. Granada, Spain

SUMMARY

Histidine and its non-histaminergic downstream metabolite trans-urocanic acid (trans-urocanate, UCA) have been implicated in a broad spectrum of neurological phenotypes, ranging from hippocampus-dependent learning and memory in rodents to fatigue, mood regulation, and neurodevelopmental outcomes in humans. The studies describe four distinct exposure categories: (i) dietary or supplemental histidine or UCA, (ii) histidinaemia resulting from histidine ammonia-lyase (HAL; histidase) deficiency, (iii) exogenous administration of UCA, and (iv) endogenous UCA derived from host or gut microbial histidine catabolism. Recent evidence demonstrates that infant gut microbiota composition, microbial histidine ammonia-lyase (HutH) activity, and host histidine-UCA metabolite profiles are associated with cognitive performance, thereby repositioning microbially derived UCA - rather than histidine or histamine - as a potential mechanistic contributor to the microbiota-gut-brain axis. In parallel, experimental studies indicate that systemic UCA crosses the blood-brain barrier (BBB), accumulates in brain regions involved in memory processing, and modulates synaptic plasticity through urocanase (UROC1)-dependent conversion to glutamate, as well as via non-glutamatergic mechanisms including GABA-A receptor modulation, oxidative stress pathways, and cGAS-STING-associated cellular senescence. This review synthesizes preclinical and clinical evidence along the histidine ammonia-lyase → UCA → BBB transport → hippocampus → memory axis and critically evaluates the strength of evidence supporting causal roles for histidine, UCA, or both in cognitive function. Histaminergic and histidine decarboxylase-dependent pathways are intentionally excluded, as they represent a distinct branch of histidine metabolism previously reviewed elsewhere.

INTRODUCTION

L-histidine is an essential amino acid whose neurobiology has traditionally been dominated by its role as the precursor of histamine via histidine decarboxylase (HDC) (*Holeček, 2020a*). However, histidine is also catabolized

through a quantitatively major alternative pathway initiated by HAL (EC 4.3.1.3), which deaminates histidine to UCA. UCA is subsequently metabolized by UROC1 to generate downstream intermediates including gluta-

mate and formiminoglutamate, linking histidine metabolism to central carbon and folate-dependent one-carbon pathways. Loss-of-function mutations in HAL cause histidinaemia, one of the most frequently identified inborn errors of amino acid metabolism in newborn screening programs (*Widhalm and Virmani, 1994; Rojas et al., 2013*). Whether histidinaemia is associated with clinically meaningful neurological impairment has remained unresolved for decades (*Widhalm and Virmani, 1994; Coulombe et al., 1983; Rosenmann et al., 1983*), and this uncertainty has shaped longstanding debate regarding the physiological relevance of the HAL-UCA pathway in human cognition.

Historically, interest in UCA as a biologically active metabolite was largely confined to dermatology and photoimmunology. This view changed following experimental evidence that moderate UV exposure increases systemic UCA levels, enabling its passage across BBB and its participation in a previously unrecognized neuronal metabolic route leading to glutamate synthesis. In rodents, UV-induced enhancement of rotarod performance and object recognition memory is reproduced by systemic administration of UCA and abolished by inhibition or knockdown of neuronal UROC1, indicating a mechanistic dependence on UCA metabolism within the brain (*Zhu et al., 2018*). Consistently, subsequent pharmacological studies demonstrated that systemic UCA enhances acquisition, consolidation, and reconsolidation of object recognition and object location memory, as well as spatial working memory in behavioural paradigms such as the Y-maze (*Yu et al., 2021; Yu et al., 2022; Wang et al., 2023*).

More recently, the mechanistic framework has been extended by micro-

biome-focused evidence. Cerdó and colleagues integrated infant cohort data, metaproteomics of gut microbiota, targeted metabolomics, and faecal microbiota transplantation (FMT) into germ-free mice to demonstrate that gut microbial histidine ammonia-lyase (HutH) activity and host histidine-UCA metabolite profiles are associated with cognitive performance, and that memory-related phenotypes are transferable via microbiota (*Cerdó et al., 2023*). This study provides the first *in vivo* human-linked evidence implicating bacterial histidine catabolism in cognitive outcomes and reframes earlier observations by positioning microbially derived UCA as a potential mechanistic intermediary linking gut microbial function to brain physiology.

Against this background, the present review has three objectives. First, it synthesizes evidence for neurological effects of histidine and UCA across human and animal studies, while explicitly distinguishing among physiological dietary intake, pharmacological supplementation, histidinaemia, exogenous UCA administration, and endogenous microbially derived UCA-exposures that are frequently conflated in the literature. Second, it evaluates a mechanistic model in which HAL-derived or microbiota-derived UCA crosses BBB, accumulates in brain regions involved in memory processing, and modulates synaptic plasticity via both UROC1-dependent conversion to glutamate and non-glutamatergic mechanisms, including GABA-A receptor modulation, oxidative stress regulation, and cGAS-STING-mediated cellular senescence pathways. Third, it critically assesses the current evidence for causal roles of histidine, UCA, or both in cognitive function and identifies key experimental gaps required to resolve remaining uncertainty.

MATERIALS AND METHODS

Review design and data collection

This narrative systematic review was conducted in accordance with PRISMA 2020 guidelines, adapted to a heterogeneous evidence base spanning inborn errors of metabolism, pharmacological rodent studies, human dietary intervention trials, and microbiome-omics investigations. Literature identification, screening, and data extraction were performed by a single reviewer. A second independent reviewer assessed a random subset of included records to verify eligibility decisions and extraction accuracy. Discrepancies were resolved through consensus. Given the integrative and interpretive nature of the synthesis, no quantitative meta-analysis was pre-specified. Where relevant prior meta-analyses existed (e.g., histidine-containing dipeptides and cognition), their pooled effect estimates were incorporated into the qualitative synthesis (Bell et al., 2024).

Literature search strategy and data sources

Systematic searches were conducted using a federated academic search platform indexing PubMed/MEDLINE, Semantic Scholar, and OpenAlex databases. These searches were supplemented by targeted retrieval of the key reference study by Cerdó and colleagues and its accompanying editorial (Cerdó et al., 2023; Rabot, 2023).

Search strategies were designed to capture six semantically distinct domains, each queried independently and in combination:

1. Mechanistic studies of UCA in brain function, memory, and hippocampal physiology.
2. HAL biology, including genetic knockouts and enzymatic deficiency.
3. Histidinaemia and related inborn

errors of histidine metabolism with neurological outcomes.

4. Dietary or supplemental histidine interventions assessing cognition, fatigue, or mood.
5. Microbiome-brain axis studies linking histidine metabolism or UCA to neural outcomes.
6. BBB transport and CNS accessibility of UCA.

Free-text search terms combined MeSH-equivalent descriptors and metabolite synonyms, including “urocanic acid,” “urocanate,” “histidine ammonia-lyase,” “histidase,” “histidinaemia,” “urocanase,” and “UROC1.” Grey literature and supplementary records were identified through manual screening of reference lists from the study by Cerdó and colleagues (Cerdó et al. (2023) and two comprehensive reviews of histidine biology (Holeček, 2020a; Thalacker-Mercer and Gheller, 2020). The search included all records available up to July 2026. Bibliographic metadata and abstracts were retrieved for screening. Full texts were obtained for all shortlisted studies and were used for data extraction when studies contributed mechanistic, quantitative, or clinically relevant findings.

Eligibility criteria

Inclusion criteria

Studies were included if they were peer-reviewed original research articles or systematic reviews/meta-analyses and reported at least one neurological, cognitive, behavioural, electrophysiological, or neurodevelopmental outcome. Eligible studies involved one or more of the following exposures: Histidine administration or dietary manipulation; histidinaemia (human cohorts or animal models); trans- or cis-urocanic acid administration; manipulation of HAL, HutH, or UROC1; or microbiota-

derived histidine catabolism. Human studies of any age and all rodent strains were eligible. Germ-free, gnotobiotic, and FMT studies were prioritised for causal inference.

Exclusion criteria

Studies were excluded if their primary focus was histaminergic signalling, including histamine receptors (H1-H4) or HDC, unless required to distinguish histaminergic from non-histaminergic histidine effects. Studies on histidine-containing dipeptides (e.g., carnosine, anserine) were excluded unless supported by formal meta-analytic synthesis of cognitive outcomes (Bell et al., 2024). Purely photochemical or photoimmunological studies of urocanic acid without neurological endpoints were excluded. Case reports lacking neuropsychological or neurological outcome measures were also excluded.

Data extraction and synthesis

For each included study, the following variables were extracted: study population or experimental model, exposure type (compound, dose, route, duration), comparator condition, primary neurological outcome, reported effect sizes

with precision metrics where available, and mechanistic readouts (biochemical, electrophysiological, or histological). Given the substantial heterogeneity in dosing regimens (spanning three orders of magnitude), administration routes (oral, intraperitoneal, intravenous, and intracerebroventricular), and exposure durations, all interventions were standardised into compound-dose-route-duration frameworks to enable structured comparison across studies. Neurological outcomes were categorised *a priori* into learning, memory acquisition, memory consolidation, reconsolidation, long-term memory, spatial and non-spatial memory, hippocampal plasticity, locomotor activity, anxiety-related behaviour, mood and fatigue measures, and neurodevelopmental outcomes. Findings were synthesised narratively along the HAL or HutH→UCA→BBB transport→hippocampus→memory axis. Where inconsistencies between studies were identified, these were evaluated in relation to differences in exposure classification (histidine versus UCA versus histidinaemia), dosing, route of administration, and outcome measurement paradigms.

RESULTS

Characteristics of included studies

The synthesis included studies grouped into six partially overlapping domains:

- (i) pharmacological studies administering UCA in rodents and assessing recognition and spatial memory outcomes (Zhu et al., 2018; Yu et al., 2021; Yu et al., 2022; Wang et al., 2023);
- (ii) a mechanistic study of UCA in D-galactose-induced cognitive impairment identifying ZCCHC3 as a molecular target and implicating the cGAS–STING pathway (Hu et al., 2026);
- (iii) electrophysiological studies demonstrating UCA activity at ionotropic receptors, particularly GABA-A receptors (Uusi-Oukari et al., 2000);
- (iv) histidinaemia case reports, retrospective analyses, prospective screening cohorts, and dietary intervention follow-ups spanning several decades (Rosenmann et al., 1983; Coulombe et al., 1983; Ishikawa, 1987; Widhalm and Virmani, 1994; Suárez et al., 2017);

- (v) rodent models of experimentally induced histidinaemia via HAL inhibition or dietary histidine loading (*Dutra-Filho et al., 1989; Tansini et al., 2004; Rojas et al., 2012; 2013*); and
- (vi) human interventional and observational studies of histidine supplementation assessing cognition, fatigue, mood, or safety outcomes (*Sasahara et al., 2015; Loy et al., 2019; Holeček, 2020a; Thalacker-Mercer and Gheller, 2020; Bell et al., 2024; Shimomasuda et al., 2026*).

These datasets are complemented by microbiome-brain axis studies, particularly the work of Cerdó and colleagues (*Cerdó et al., 2023; Rabot, 2023*). Additional supporting evidence comes from studies associating microbial histidine metabolism with systemic metabolic phenotypes such as non-alcoholic fatty liver disease and glycaemic regulation (*Quesada-Vázquez et al., 2023; Warmbrunn et al., 2024*).

Human studies were generally small and heterogeneous. Histidinaemia investigations ranged from isolated case reports (*Suárez et al., 2017*) to the Austrian newborn screening cohort reported by *Widhalm and Virmani (1994)*. They detected 124 children with histidinaemia in the Austrian Neonates Screening Program, receiving long-term follow-up. Fifty nine patients were treated with a histidine-restricted diet (35 mg/kg). The follow-up included determining biochemical parameters, physical examination and psychological assessment of 58 treated and 43 untreated patients which were followed longitudinally to school age. Adult supplementation trials included a crossover study involving 20 participants assessing repeated histidine administration (*Sasahara et al., 2015*) and a single-dose randomized trial in a comparable sample (*Shimomasuda et al., 2026*). A meta-analysis of histidine-

containing dipeptides integrated ten randomized controlled trials from an initial screening of 2,653 records (*Bell et al., 2024*).

Rodent studies were predominantly single-laboratory pharmacological experiments with group sizes of 8-20 animals. Behavioural paradigms included novel object recognition (NOR), object location recognition (OLR), Y-maze, radial arm maze, Morris water maze, elevated plus maze, and open-field testing. Interventions ranged from acute intraperitoneal UCA administration (10-30 mg/kg) (*Wang et al., 2023*), to chronic dietary histidine manipulation, to genetic or pharmacological modulation of UROC1 activity (*Zhu et al., 2018*). Across studies, outcome measures converged primarily on hippocampus-dependent learning and memory processes.

Neurological outcomes in animal models

UCA enhances hippocampus-dependent memory

Across independent studies, UCA consistently enhances hippocampus-dependent learning and memory. The most robust and reproducible finding is that systemic administration of UCA facilitates both recognition and spatial memory.

Zhu and colleagues established the primary mechanistic framework by demonstrating that moderate UV exposure increases circulating UCA levels, that UCA crosses BBB, and that neuronal UROC1-dependent metabolism of UCA to glutamate in hippocampus and motor cortex contributes to improved rotarod performance and enhanced object recognition memory (*Zhu et al., 2018*). These effects were reproduced by intravenous UCA administration and abolished by UROC1 inhibition or neuronal knockdown, supporting a causal

role for UCA metabolism in cognitive enhancement.

Subsequent pharmacological studies confirmed and extended these findings. Intraperitoneal administration of UCA facilitated acquisition of NOR and OLR memory at 10 mg/kg, while a higher dose (30 mg/kg) selectively enhanced long-term memory in NOR, indicating a dose-dependent dissociation between short- and long-term memory processes (Wang et al., 2023). In addition, UCA modulated both memory consolidation and reconsolidation when administered within defined post-training or post-reactivation windows, but not when delivered outside the consolidation period, indicating temporal specificity of action (Yu et al., 2021). UCA also improved spatial working memory in the Y-maze and spatial recognition performance in OLR paradigms (Yu et al., 2022). Importantly, these cognitive effects occurred without significant alterations in locomotor activity or exploratory behaviour, indicating that they are not attributable to nonspecific changes in arousal or motor function (Wang et al., 2023; Yu et al., 2021). Network-based analyses further implicated MAPK, NF- κ B, insulin signalling, and serotonergic synapse pathways as potential downstream mediators of UCA-induced cognitive effects (Yu et al., 2022).

UCA acts through multiple molecular mechanisms

Beyond its role as a metabolic intermediate in the UCA-to-glutamate pathway, UCA engages additional molecular targets. Uusi-Oukari and colleagues demonstrated that UCA acts as a stereoselective modulator of GABA-A receptors in *Xenopus* oocytes expressing recombinant $\alpha 1\beta 1\gamma 2S$ subunits, with efficacy enhanced under acidic conditions, whereas cis-UCA lacked activity (Uusi-Oukari et al., 2000). Although the

physiological relevance of this mechanism under physiological brain pH remains uncertain, the findings indicate that UCA can directly modulate inhibitory neurotransmission. Hu and colleagues further showed that UCA ameliorates cognitive impairment in D-galactose-induced aging models by binding ZCCHC3 and suppressing cGAS–STING signalling, resulting in reduced astrocytic senescence, apoptosis, and inflammatory cytokine release. Genetic or pharmacological activation of the pathway abolished the cognitive benefits of UCA, supporting a causal mechanistic role (Hu et al., 2026). These findings suggest that UCA may influence cognition through both metabolic conversion to glutamate and direct regulation of inflammatory and senescence-associated pathways. Collectively, these mechanisms are not mutually exclusive and may represent parallel or context-dependent pathways downstream of systemic UCA availability.

Histidine effects in rodents: mixed and pathway-dependent

Systemic histidine administration produces heterogeneous behavioural outcomes that often reflect downstream histaminergic signalling rather than direct histidine action. Song and colleagues reported that histidine administration improved cognitive performance in a chronic cerebral hypoperfusion model, accompanied by increased neurogenesis, improved microvascular perfusion, and upregulation of the tight junction protein zonula occludens-1, suggesting partial restoration of BBB integrity (Song et al., 2018). In contrast, several studies show that histidine-induced improvements in pharmacologically impaired cognition (e.g., scopolamine-, 5-HT_{1A} agonist-, or N-methyl-D-aspartate (NMDA) antagonist-induced deficits) are abolished by HDC inhibition or H₁ receptor antagonism,

indicating that these effects are mediated primarily through histamine signalling rather than histidine per se (Miya-zaki et al., 1995a; 1995b; Isayama et al., 2001). Dietary histidine depletion in rodents reduces brain histamine levels and produces paradoxical behavioural effects, including enhanced performance in some spatial learning tasks alongside increased susceptibility to NMDA antagonist-induced deficits (Nishiga et al., 2002). These findings underscore the necessity of distinguishing histidine metabolism from histaminergic neurotransmission when interpreting behavioural outcomes. Although outside the primary scope of this review, these data are relevant for interpretation because behavioural effects attributed to histidine frequently depend on whether histidine is acting as a precursor for histamine, a substrate for alternative catabolic pathways, or a metabolic perturbation of amino acid homeostasis.

Rodent histidinemia models and systemic effects

Experimental histidinaemia, induced by combined histidine administration and histidine ammonia-lyase inhibition, reduces locomotor activity but does not consistently produce robust cognitive impairment in adult rodents (Dutra-Filho et al., 1989). Developmental exposure, however, yields more pronounced effects. Maternal histidine loading during gestation and lactation induces oxidative stress in offspring hippocampus and cortex, with partial normalization during postnatal maturation (Rojas et al., 2012). It also disrupts energy metabolism, including alterations in pyruvate kinase and creatine kinase activity and dysregulation of adenylate kinase systems (Rojas et al., 2013). *In vitro* studies indicate that histidine induces oxidative stress only at supraphysiological concentrations,

whereas imidazolelactic acid exhibits dose-dependent antioxidant properties (Tansini et al., 2004). Collectively, these findings suggest that the neurological consequences of histidinaemia are modest in adulthood but may depend on developmental timing, metabolic context, and redox balance.

Consensus and divergence across studies

Across multiple independent rodent studies, UCA emerges as a consistent positive modulator of hippocampus-dependent learning and memory across acquisition, consolidation, and reconsolidation phases (Zhu et al., 2018; Yu et al., 2021; Yu et al., 2022; Wang et al., 2023). However, two main sources of heterogeneity are evident. First, a dose-dependent effect. Lower doses of UCA (10 mg/kg) enhance both short- and long-term memory, whereas higher doses (30 mg/kg) preferentially affect long-term memory, suggesting non-linear dose-response dynamics (Wang et al., 2023). Second, cognitive effects vary depending on behavioural paradigm, timing of administration, and whether outcomes reflect acquisition, consolidation, or reconsolidation processes. Overall, the convergence of behavioural, pharmacological, and molecular evidence supports a robust cognitive effect of UCA in rodents, while also indicating complex dose- and context-dependent modulation.

Neurological outcomes in humans

Histidinaemia exhibits a substantially milder and more heterogeneous neurological phenotype than early case reports suggested

In a retrospective analysis of 43 probands and 26 siblings, Rosenmann and colleagues reported that 79% of probands and approximately 50% of siblings exhibited a “disadaptive” central nervous system phenotype, including

intellectual disability, speech impairment, seizures, behavioural abnormalities, and learning difficulties, with a modal IQ of ~70. Importantly, no correlation was observed between blood histidine concentration and severity of neurological presentation (Rosenmann et al., 1983). The same authors estimated that clinically reported cases with neurological involvement represent approximately 1% of all individuals with histidinaemia, indicating substantial ascertainment bias in the historical case literature. Prospective cohort data contrast sharply with these early reports. In the study by Coulombe and colleagues, 21 individuals identified through newborn screening (including probands and siblings) demonstrated normal cognitive development, including full-scale, verbal, and performance IQ, as well as intact visual-motor integration and school achievement. Outlier values were not associated with the degree of histidinaemia, leading the authors to conclude that histidinaemia identified through population screening is largely a non-disadaptive condition (Coulombe et al., 1983). Longitudinal data from the Austrian newborn screening cohort (Widhalm and Virmani, 1994; n = 124) further support this interpretation. Although dietary histidine restriction reduced plasma histidine concentrations, treated children (aged 4 and 6 years) exhibited significantly lower IQ scores than untreated peers ($p < 0.05$), and clinical symptoms - when present - were similarly distributed between groups. The authors concluded that dietary histidine restriction does not confer cognitive benefit in histidinaemia (Widhalm and Virmani, 1994). Additional reports from Japanese longitudinal cohorts describe a subset of individuals with histidinaemia exhibiting autistic traits, language impairment, reduced visual-motor performance, and abnormal electroencephalographic findings

(Ishikawa, 1987). Case reports of atypical histidinaemia continue to describe motor, cognitive, and academic impairments (Suárez et al., 2017). However, these studies are strongly influenced by selection bias and are not representative of population-level outcomes.

Collectively, the human literature supports four converging conclusions:

- (i) histidinaemia is biochemically common but clinically mild in most individuals
- (ii) a minority of individuals exhibit neurological symptoms
- (iii) symptom severity does not correlate with circulating histidine levels
- (iv) dietary histidine restriction does not improve and may worsen cognitive outcomes (Widhalm and Virmani, 1994; Rosenmann et al., 1983; Coulombe et al., 1983).

This pattern is difficult to reconcile with a model in which histidine itself is directly neurotoxic. It is more consistent with a model in which flux through HAL and downstream metabolites - particularly UCA - may be more relevant to neurological outcomes than absolute histidine concentration.

Dietary and supplemental histidine effects in humans

Controlled trials indicate that histidine supplementation at physiological to moderate pharmacological doses influences fatigue, mood, and select cognitive domains. In a placebo-controlled, double-blind crossover trial involving 20 adults with elevated fatigue and sleep disruption scores, daily histidine supplementation over two weeks significantly reduced Profile of Mood States fatigue T-scores, improved working memory reaction times, and increased subjective ratings of attentiveness and clear thinking (all $p < 0.05$) (Sasahara et al., 2015). A subsequent randomized controlled trial using a single-dose design reported

no significant effects in the overall cohort. However, prespecified subgroup analyses showed that individuals with high baseline fatigue experienced reductions in fatigue-inertia, confusion-bewilderment, and depression-dejection, alongside increased vigor-activity ($p = 0.022$ - 0.049) (Shimomasuda et al., 2026). A systematic review and meta-analysis of ten randomized controlled trials evaluating histidine-containing dipeptides (primarily carnosine) reported improvements in delayed recall on the Wechsler Memory Scale (weighted mean difference 1.5; 95% CI 0.6-2.5; $p < 0.01$), while showing no significant effects on global cognitive measures such as Alzheimer's Disease Assessment Scale-Cognitive Subscale, Mini-Mental State Examination, or immediate recall indices (Bell et al., 2024). Cross-sectional evidence further indicates that serum histidine levels are lower in women with multiple sclerosis who report higher fatigue compared with those reporting lower fatigue (64.57 vs. 70.48 nmol/mL; $p = 0.048$; Hedges' $g = 0.75$), independent of depressive symptoms (Loy et al., 2019).

Safety, dose dependence, and adverse effects

Histidine exhibits a narrow and non-linear dose-response profile. Dietary intakes of approximately 4.0-4.5 g/day have been associated with improvements in metabolic parameters, including reductions in body mass index, adiposity, glycaemic markers, inflammatory cytokines, and oxidative stress indicators (Thalacker-Mercer and Gheller, 2020). In contrast, intakes exceeding ~24 g/day have been associated with adverse outcomes, including reduced serum zinc levels and cognitive impairment, although a formal tolerable upper intake level has not been established due to limited data between 4.5 and 24 g/day (Thalacker-Mercer and

Gheller, 2020). High-dose intravenous histidine administration, such as that used in cardioplegic solutions (raising plasma concentrations from ~70 μ M to ~21,000 μ M), induces marked metabolic perturbations, including increased blood and urinary ammonia, elevated urea production, depletion of hepatic and muscular ATP, and reductions in circulating branched-chain amino acids, accompanied by increases in glutamine and alanine at 24 hours (Holeček and Vodeníčarovová, 2019). Consistent with these findings, Holeček and colleagues caution against histidine supplementation in hepatic dysfunction due to the combined burden of ammonia accumulation, glutamine elevation, and branched-chain amino acid depletion (Holeček, 2020a; 2020b).

Integrated interpretation across exposure classes

When considered as a unified dataset, the four principal human exposure classes—dietary intake, supplementation, histidinemia, and high-dose pharmacological exposure—exhibit a coherent but non-linear relationship with neurological outcomes. Physiological dietary histidine intake supports normal cognitive function and is associated with reduced fatigue in clinical populations such as multiple sclerosis. Moderate supplementation (approximately 2–4 g/day) improves fatigue, mood, and select working memory outcomes. In contrast, histidinemia - despite markedly elevated circulating histidine - is generally neurologically benign in unbiased populations. At very high intake levels (>24 g/day or acute pharmacological exposure), histidine is associated with metabolic dysregulation and cognitive impairment. This pattern is best described as an inverted-U shaped dose-response relationship. Importantly, the dissociation between circulating histidine levels and neurological phenotype suggests

that histidine itself is unlikely to be the proximal effector. Instead, neurological effects may be more closely related to flux through downstream metabolic pathways, particularly histidine ammonia-lyase-mediated production of UCA.

Biochemical and mechanistic pathways

The integrated mechanistic model supported by the reviewed evidence is: Histidine → histidine ammonia-lyase (host and microbial) → UCA → BBB transport → accumulation in hippocampus and perirhinal cortex → UROC1-dependent conversion to glutamate and receptor-mediated signalling → modulation of synaptic plasticity → effects on recognition and spatial memory.

Multiple convergent lines of evidence support this pathway. First, HAL is expressed in host tissues, including liver and skin, and in gut microbial taxa. Microbial HutH activity correlates with infant cognitive performance and systemic histidine metabolite profiles (Cerdó et al., 2023). Second, systemic UCA - derived from ultraviolet-induced skin metabolism, exogenous administration, or microbial histidine catabolism - crosses BBB and accumulates in memory-related brain regions, including hippocampus and prefrontal cortex (Zhu et al., 2018; Yu et al., 2022). Third, neuronal UCA serves as a substrate for UROC1-dependent conversion to glutamate, which is incorporated into synaptic vesicles and released at glutamatergic synapses within hippocampal and cortical circuits (Zhu et al., 2018). Fourth, UCA directly modulates GABA-A receptor function in a stereoselective and pH-dependent manner, indicating additional non-metabolic neuromodulatory activity (Uusi-Oukari et al., 2000). Fifth, UCA suppresses cGAS-STING-mediated astrocytic senescence via interaction with ZCCHC3, suggesting a role in neuroinflammatory regulation and brain aging

(Hu et al., 2026). Sixth, network pharmacology analyses converge on MAPK, NF-κB, insulin signalling, serotonergic synapses, and amino acid metabolic pathways as downstream mediators of UCA-associated cognitive effects (Yu et al., 2022).

Causal and adjacent mechanisms

Microbiome-brain axis studies further support a role for microbial histidine metabolism in host physiology. The study by Cerdó and colleagues demonstrated that microbial histidine ammonia-lyase (HutH) activity correlates with infant cognitive performance, and that behavioural phenotypes are transferable via faecal microbiota transplantation into germ-free mice (Cerdó et al., 2023). Cognitive performance was associated with lower faecal histidine and altered UCA-to-histidine and UCA-to-glutamate ratios in brain tissue, consistent with altered flux through the histidine-UCA pathway. Adjacent human studies show that oral histidine reshaped gut microbiota and mucosal-associated invariant T cell populations in type 2 diabetes (Warmbrunn et al., 2024), and that microbial histidine catabolism modified non-alcoholic fatty liver disease phenotypes (Quesada-Vázquez et al., 2023), indicating that microbe-mediated histidine turnover had systemic effects beyond the CNS. Besides, histidine is a physiologically important zinc chelator; supplementation above ~24 g/d lowered serum zinc and, plausibly through this route, impaired cognition, supporting the U-shaped safety curve (Thalacker-Mercer and Gheller, 2020). Histidine has also both antioxidant (physiological concentrations) and pro-oxidant (>2.5 mM *in vitro*) properties, and maternal histidine loading disrupted offspring cortical and hippocampal redox balance and phosphoryl-transfer capacity (Tansini et al., 2004; Rojas et al., 2012; 2013). Finally, UCA

increased glutamatergic tone via UROC1-dependent glutamate synthesis (Zhu et al., 2018) while also modulating GABA-A receptors (Uusi-Oukari et al., 2000), positioning it as a candidate regulator of excitation-inhibition balance in hippocampal circuits.

Overall, the current evidence sup-

ports UCA - not histidine - as the proximal effector of the histidine-brain axis, with host HAL and microbial HutH activity determining the size of the pool that reaches the brain, and neuronal UROC1 determining how much of that pool is channelled into glutamate.

DISCUSSION

Cross-species integration

Evidence across species supports a partially conserved histidine-UCA axis, although translational fidelity is asymmetric between rodent models and humans. In rodents, systemic UCA crosses BBB, is metabolized to glutamate via neuronal UROC1, and enhances hippocampus- and perirhinal cortex-dependent memory performance. In humans, key enzymatic components of this pathway, including HAL and UROC1, are expressed in liver and peripheral tissues, and loss-of-function mutations in UROC1 cause urocanic aciduria, confirming pathway conservation at the biochemical level (Cerdó et al., 2023). Moreover, the association between microbial HutH activity, systemic histidine metabolism, and infant cognitive outcomes reported by Cerdó and colleagues provides *in vivo* human evidence linking this metabolic axis to neurodevelopment (Cerdó et al., 2023).

However, translational extrapolation from rodents to humans remains limited at the level of behavioural outcomes. Rodent NOR and OLR tasks depend on hippocampal and perirhinal cortex circuits and are considered partial analogues of human declarative recognition memory, but they do not fully recapitulate its complexity. Consequently, extrapolating acute intraperitoneal UCA effects in mice (e.g., 10 mg/kg improving 24-hour memory performance) to human cognitive enhancement is not

currently justified. Furthermore, no controlled studies have evaluated systemic UCA administration in humans, precluding direct assessment of dose-response relationships, including the inverted-U pattern observed in rodents.

Species-specific differences further constrain quantitative translation. These include differences in HAL expression, microbial ecosystem composition, and UROC1 activity between rodents and humans. In particular, laboratory mouse microbiomes and histidine metabolic fluxes differ substantially from those of free-living humans, limiting direct scaling of microbial UCA production. Rodent histidine turnover is also generally more rapid, potentially amplifying metabolic signals that may be attenuated in humans.

Behavioural interpretation is additionally complicated by state-dependent effects. Although locomotor confounding has been largely excluded in UCA studies, state-dependent memory phenomena - well documented for histidine-mediated histaminergic signaling - remain a potential confounder. Histidine can induce H1 receptor-dependent state-dependent alterations in memory performance, yet no studies have systematically evaluated whether UCA exhibits similar state dependence (Gianlorenço et al., 2011).

Histidine versus urocanate

Although histidine and UCA produce

partially overlapping behavioural phenotypes, their mechanistic bases are distinct. Systemic administration of UCA consistently enhances hippocampus-dependent learning across acquisition, consolidation, and reconsolidation phases in rodents (*Zhu et al., 2018; Yu et al., 2021; Yu et al., 2022; Wang et al., 2023*). In contrast, histidine exerts cognitive and behavioural effects primarily through conversion to histamine rather than via histidine itself. Pharmacological dissection consistently supports a histaminergic mechanism for histidine-induced behavioural effects. The amelioration of scopolamine-, MK-801-, and 5-HT1A agonist-induced cognitive deficits by histidine is abolished by inhibition of HDC or blockade of H1 receptors, indicating dependence on histamine signalling (*Miyazaki et al., 1995a; 1995b; Isayama et al., 2001*). Similarly, histidine-induced enhancement of prefrontal cortex-dependent working memory is mediated by histaminergic activation of basal forebrain-prefrontal circuits rather than direct histidine action (*Nakamura et al., 2022*). In humans, improvements in fatigue and working memory following dietary histidine supplementation are consistent with a histaminergic contribution, although a potential contribution of downstream UCA has not been directly assessed.

Two observations specifically implicate UCA rather than histidine as the relevant cognitive intermediate. First, in histidinaemia, dietary histidine restriction reduces substrate availability for HAL and therefore decreases downstream UCA production. Despite this, dietary restriction does not improve - and in some cases worsens - cognitive outcomes (*Widhalm and Virmani, 1994*). This finding is inconsistent with histidine toxicity as the primary driver of neurological phenotype and is more compatible with a deficiency of a downstream metabolite within the HAL pathway. Second, microbiome-linked evidence from Cerdó and colleagues demonstrates that higher cognitive performance in infants is associated with reduced faecal histidine and altered UCA-related ratios in brain tissue, consistent with increased metabolic flux through microbial histidine catabolism and more efficient downstream processing of UCA (*Cerdó et al., 2023*). Together with rodent data showing that UCA is converted to glutamate at glutamatergic terminals to support memory enhancement (*Zhu et al., 2018*), these findings support a model in which UCA occupies a central intermediate position, with either its metabolic conversion to glutamate or its direct neuromodulatory actions mediating cognitive effects.

CONCLUSION

Collectively, the available evidence supports a moderate-strength causal role for UCA - rather than histidine itself - as a proximal effector within the histidine-brain axis, particularly in rodent models of hippocampus-dependent recognition and spatial memory.

This conclusion is supported by four convergent lines of evidence:

(i) multiple independent rodent studies demonstrating that systemic

UCA enhances acquisition, consolidation, and reconsolidation of memory (*Zhu et al., 2018; Wang et al., 2023; Yu et al., 2021; 2022*).

(ii) a validated neuronal mechanism in which UCA is metabolized to glutamate via UROC1, with genetic loss-of-function confirmation (*Zhu et al., 2018*).

(iii) mechanistically distinct anti-senescence effects mediated

- through ZCCHC3 and suppression of cGAS-STING signalling, supported by pharmacological and genetic validation (Hu et al., 2026).
- (iv) the first *in vivo* human evidence linking microbial histidine catabolism, systemic metabolite profiles, and cognitive outcomes, with microbiota-dependent transmissibility in germ-free mice (Cerdó et al., 2023).

In contrast, histidine itself appears to exert indirect and context-dependent effects. At physiological to moderate supplemental doses, histidine modulates fatigue, mood, and select cognitive measures in humans, but mechanistic studies consistently attribute behavioural effects in rodents to downstream histamine signalling rather than histidine *per se* (Miyazaki et al., 1995a; 1995b; Isayama et al., 2001; Sasahara et al., 2015; Bell et al., 2024; Shimomatsuda et al., 2026). In population-level analyses, histidinaemia is largely non-disadaptive when ascertained without bias (Coulombe et al., 1983; Widhalm

and Virmani, 1994), and dietary histidine restriction fails to improve - and may worsen - cognitive outcomes. This pattern is more consistent with reduced flux through the HAL–UCA pathway than with histidine toxicity.

Several key uncertainties remain. It is not known whether the cognitive effects of UCA observed in rodents translate quantitatively to humans. It also remains unclear whether orally administered UCA achieves sufficient brain concentrations to engage neuronal UROC1-dependent pathways. In addition, the causal contribution of microbial UCA to the cognitive phenotype observed by Cerdó and colleagues (Cerdó et al., 2023) remains to be experimentally isolated from broader microbial metabolic shifts. Finally, the biological basis of neurological impairment in the small subset of individuals with histidinaemia-associated CNS phenotypes remains unresolved and may reflect distinct downstream metabolic or developmental modifiers.

LITERATURE

- Bell, S., Hariharan, R., Laud, P., Jenkinson, C., and Corfe, B.M.: Histidine-containing dipeptide supplementation improves delayed recall: a systematic review and meta-analysis. *Nutr. Rev.* 82: 906-918 (2024).
- Cerdó, T., Ruiz-Rodríguez, A., Acuña, I., Torres-Espínola, F.J., Menchén-Márquez, S., Gámiz, F., Gallo, M., Jehmlich, N., Haange, S.B., von Bergen, M., Campoy, C., and Suárez, A.: Infant gut microbiota contributes to cognitive performance in mice. *Cell Host Microbe* 31: 1974-1988 (2023).
- Coulombe, J., Kammerer, B., Levy, H.L., Rosenmann, A., Scriver, C.R., Clow, C., Shih, V.E., and MacCready, R.A.: Histidinaemia. Part III: impact; a prospective study. *J. Inher. Metab. Dis.* 6: 117-120 (1983).
- Dutra-Filho, C.S., Wannmacher, C.M., Pires, R.F., Clovel, M.A., Ribeiro, M.F., Wajner, M., and Sarkis, J.J.: Reduced locomotor activity of rats made histidinemic by injection of histidine. *J. Nutr.* 119: 1223-1228 (1989).
- Gianlorenço, A.C.L., Canto-de-Souza, A., and Mattioli, R.: L-histidine induces state-dependent memory deficit via H1 receptor. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 35: 136-141 (2011).
- Holeček, M. and Vodeníčarovová, M.: Effects of histidine load on ammonia, amino acids, and adenine nucleotides in rats. *Amino Acids* 51: 1029-1038 (2019).
- Holeček, M.: Histidine in health and disease: metabolism, physiological importance, and use as a supplement. *Nutrients* 12: 848 (2020a).

- Holeček, M.: Influence of histidine administration on ammonia and amino acid metabolism: a review. *Physiol. Res.* 69: 99-111 (2020b).
- Hu, S., Kou, Z., Chen, B., Wang, J., Zhou, X., Zhang, Y., and Liu, L.: Urocanic acid alleviates cognitive impairment by targeting ZCCHC3 and suppressing the cGAS-STING-mediated senescence. *Neurochem. Res.* 51: 411-423 (2026).
- Isayama, S., Sugimoto, Y., Nishiga, M., and Kamei, C.: Effects of histidine on working memory deficits induced by 8-OH-DPAT. *Jpn. J. Pharmacol.* 86: 451-456 (2001).
- Ishikawa, M.: Developmental disorders in histidinemia-follow-up study of language development. *Acta Paediatr. Jpn.* 29: 153-161 (1987).
- Loy, B.D., Fling, B., Sage, K., Horak, F., and Cameron, M.H.: Serum histidine is lower in fatigued women with multiple sclerosis. *Fatigue* 7: 109-120 (2019).
- Miyazaki, S., Imaizumi, M., and Onodera, K.: Ameliorating effects of histidine on scopolamine-induced learning deficits using an elevated plus-maze in mice. *Life Sci.* 57: 2137-2144 (1995a).
- Miyazaki, S., Imaizumi, M., and Onodera, K.: Ameliorating effects of histidine on learning deficits in an elevated plus-maze test in mice: involvement of cholinergic systems. *Methods Find. Exp. Clin. Pharmacol.* 17: 567-572 (1995b).
- Nakamura, T., Naganuma, F., Kudomi, U., Yoshikawa, T., Miura, Y., Eisawa, S., Miura, T., and Yanai, K.: Oral histidine improves working memory via histaminergic activation. *Biochem. Biophys. Res. Commun.* 606: 90-96 (2022).
- Nishiga, M., Sugimoto, Y., Taga, C., and Kamei, C.: Effects of MK-801 on radial maze performance in histidine-deficient rats. *Life Sci.* 71: 2339-2348 (2002).
- Quesada-Vázquez, S., Castells-Nobau, A., Latorre, J., Oliveras-Cañellas, N., de la Vega-Correa, L., Biarnés, C., Martínez-Gili, L., Elosua-Bayod, M., Tobajas, Y., Cifuentes, M., Sabater, M., Puig, J., Gich, I., Fernández-Real, J.M., Mayneris-Perxachs, J., Portero-Otin, M., Pamplona, R., Jové, M., Sol, J., Ortega, F., Ricart, W., Burokas, A., Maldonado, R., and Martín-García, E.: Therapeutic implications of histidine catabolism by the gut microbiota in NAFLD with obesity. *Cell Rep. Med.* 4: 101341 (2023).
- Rabot, S.: tHIS way to cognitive development. *Cell Host Microbe* 31: 1937-1939 (2023).
- Rojas, D.B., Gemelli, T., Andrade, R., Sgaravatti, A.M., Garcia, J.C., Vannucchi, H., Wyse, A.T., and Dutra-Filho, C.S.: Administration of histidine to female rats induces changes in oxidative status in cortex and hippocampus of the offspring. *Neurochem. Res.* 37: 1167-1174 (2012).
- Rojas, D.B., Andrade, R., Gemelli, T., Sgaravatti, A.M., Garcia, J.C., Vannucchi, H., Wyse, A.T., and Dutra-Filho, C.S.: Effect of histidine administration to female rats during pregnancy and lactation on enzyme activity of the phosphoryltransfer network in cerebral cortex and hippocampus of the offspring. *Metab Brain Dis.* 28: 63-72 (2013).
- Rosenmann, A., Scriver, C.R., Clow, C., and Levy, H.L.: Histidinaemia. Part II: impact; a retrospective study. *J. Inherit. Metab. Dis.* 6: 113-116 (1983).
- Sasahara, I., Fujimura, N., Nozawa, Y., Furuhashi, S., and Tanaka, M.: The effect of histidine on mental fatigue and cognitive performance in subjects with high fatigue and sleep disruption scores. *Physiol. Behav.* 147: 238-244 (2015).
- Shimomasuda, M., Fujiyoshi, H., Kajiyama, Y., Oku, H., Takahashi, M., and Sato, T.: Effects of a single dose of L-histidine on mental fatigue and vigor: a randomized controlled trial. *Sci. Rep.* 16: 3104 (2026).
- Song, J., Yang, L., Nan, D., Gao, Z., Wang, X., Wu, J., Wang, Z., Zhao, Y., and Zhang, J.: Histidine alleviates impairments induced by chronic cerebral hypoperfusion in mice. *Front. Physiol.* 9: 662 (2018).
- Suárez, R., Benítez, Y.R., Bringa, M.D., and Rodríguez, D.F.: Histidinemia atípica y desarrollo cognitivo: evaluación neuropsicológica longitudinal. *Rev. Neurol.* 64: 351-356 (2017).
- Tansini, C., Durigon, K., Testa, C., Sgaravatti,

- A., de Assis, A.M., Dutra-Filho, C.S., Wannmacher, C.M., Wyse, A.T., and Wajner, M.: Effects of histidine and imidazolelactic acid on oxidative stress parameters in cerebral cortex of young rats. *Int. J. Dev. Neurosci.* 22: 25-32 (2004).
- Thalacker-Mercer, A. and Gheller, M.E.: Benefits and adverse effects of histidine supplementation. *J. Nutr.* 150 (Suppl. 1): 2517S-2526S (2020).
- Uusi-Oukari, M., Soini, S., Heikkilä, J., Honkaniemi, J., and Korpi, E.R.: Stereospecific modulation of GABA-A receptor function by urocanic acid isomers. *Eur. J. Pharmacol.* 400: 11-20 (2000).
- Wang, L., Tan, Y., Wang, H., He, Z., and Yu, X.D.: Urocanic acid facilitates acquisition of object recognition memory in mice. *Physiol. Behav.* 266: 114201 (2023).
- Warmbrunn, M.V., Attaye, I., Aron-Wisniewsky, J., de Clercq, N.C., Frissen, M.N., Levin, E., Levels, J.H.M., de Vos, W.M., Nieuwdorp, M., and Groen, A.K.: Oral histidine affects gut microbiota and MAIT cells, improving glycemic control in type 2 diabetes. *Gut Microbes* 16: 2370616 (2024).
- Widhalm, K. and Virmani, S.: Long-term follow-up of 58 patients with histidinemia treated with a histidine-restricted diet: no effect of therapy. *Pediatrics* 94: 861-866 (1994).
- Yu, X.D., Mo, Y.X., He, Z., Wang, L., Tan, Y., Li, X., Yao, L., Wang, N., and Xiong, W.: Urocanic acid enhances memory consolidation and reconsolidation in a novel object recognition task. *Biochem. Biophys. Res. Commun.* 574 98-104 (2021).
- Yu, X.D., Li, A., Li, X., Mo, Y.X., He, Z., Wang, L., and Tan, Y.: Trans-urocanic acid facilitates spatial memory: implications for Alzheimer's disease. *Physiol. Behav.* 248: 113827 (2022).
- Zhu, H., Wang, N., Yao, L., Chen, Q., Zhang, R., Qian, J., Hou, Y., Guo, W., Fan, S., Liu, S., Li, Q., Du, F., Xie, X., Guo, C., Xu, F., Zhang, J., Yu, Y., Hansson, T., Xu, S., Zhou, J., Cao, P., Zhong, H., Xu, X., and Xiong, W.: Moderate UV exposure enhances learning and memory by promoting a novel glutamate biosynthetic pathway in the brain. *Cell* 173: 1716-1727 (2018).

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 (Hoyles 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.

LITERATURE

- Al Hinai, E.A., Kullamethee, P., Rowland, I.R., Swann, J., Walton, G.E., and Commane, D.M.: Modelling the role of microbial p-cresol in colorectal genotoxicity. *Gut Microbes* 10: 398-411 (2019).
- Bello, A.T., Sarafian, M.H., Wimborne, E.A., Middleton, B., Revell, V.L., Raynaud, F.I., Chowdhury, N.R., van der Veen, D.R., Skene, D.J., and Swann, J.R.: Exposing 24-hour cycles in bile acids of male humans. *Nat. Commun.* 15: 10014 (2024).
- Bertarini, L., Imbeni, F., Brighenti, V., Martusciello, I., Pellati, F., and Alboni, S.: Para-Cresol and the Brain: Emerging Role in Neurodevelopmental and Neurodegenerative Disorders and Therapeutic Perspectives. *ACS Pharmacol. Transl. Sci.* 8: 3432-3452 (2025).
- Clarke, G., Grenham, S., Scully, P., Fitzgerald, P., Moloney, R.D., Shanahan, F., Dinan, T.G., and Cryan, J.F.: The microbiome-gut-brain axis during early life regulates the hippocampal serotonergic system in a sex-dependent manner. *Mol. Psychiatry* 18: 666-673 (2013).

- Dehghani, M., Panahi, H.K.S., Kavyani, B., Heng, B., Tan, V., Braidy, N., and Guillemin, G.J.: The Role of Kynurenine Pathway and NAD⁺ Metabolism in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome. *Aging Dis.* 13: 698-711 (2022).
- Frost, G., Sleeth, M.L., Sahuri-Arisoylu, M., Lizarbe, B., Cerdan, S., Brody, L., Anastasovska, J., Ghourab, S., Hankir, M., Zhang, S., Carling, D., Swann, J.R., Gibson, G., Viardot, A., Morrison, D., Thomas, E.L., and Bell, J.D.: The short-chain fatty acid acetate reduces appetite via a central homeostatic mechanism. *Nat. Commun.* 5: 3611 (2014).
- Gan, Y., Yang, C., Tong, X., Sun, H., Cong, Y., Yin, X., Li, L., Cao, S., Dong, X., Gong, Y., Shi, O., Deng, J., Bi, H., and Lu, Z.: Shift work and diabetes mellitus: a meta-analysis of observational studies. *Occup. Environ. Med.* 72: 72-78 (2015).
- Hoyles, L., Snelling, T., Umlai, U.K., Nicholson, J.K., Carding, S.R., Glen, R.C., and McArthur, S.: Microbiome-host systems interactions: protective effects of propionate upon the blood-brain barrier. *Microbiome* 6: 55 (2018).
- Hoyles, L., Pontifex, M.G., Rodriguez-Ramiro, I., Anis-Alavi, M.A., Jelane, K.S., Snelling, T., Solito, E., Fonseca, S., Carvalho, A.L., Carding, S.R., Müller, M., Glen, R.C., Vauzour, D., and McArthur, S.: Regulation of blood-brain barrier integrity by microbiome-associated methylamines and cognition by trimethylamine N-oxide. *Microbiome* 9: 235 (2021).
- Huang, F., Wang, T., Lan, Y., Yang, L., Pan, W., Zhu, Y., Lv, B., Wei, Y., Shi, H., Wu, H., Zhang, B., Wang, J., Duan, X., Hu, Z., and Wu, X.: Deletion of mouse FXR gene disturbs multiple neurotransmitter systems and alters neurobehavior. *Front. Behav. Neurosci.* 9: 70 (2015).
- Lee, S., Kim, J., Baek, J.W., Jung, K.Y., Lee, Y., Koh, A., and Kim, H.J.: Enrichment of gut-derived metabolites in a Parkinson's disease subtype with REM sleep behavior disorder. *NPJ Parkinsons Dis.* 11: 189 (2025).
- Martin, G., Kolida, S., Marchesi, J.R., Want, E., Sidaway, J.E., and Swann, J.R.: In Vitro Modeling of Bile Acid Processing by the Human Fecal Microbiota. *Front. Microbiol.* 9: 1153 (2018).
- Myint, A.M., Kim, Y.K., Verkerk, R., Scharpé, S., Steinbusch, H., and Leonard, B.: Kynurenine pathway in major depression: Evidence of impaired neuroprotection. *J. Affect. Disord.* 98: 143-151 (2007).
- Patnala, R., Arumugam, T. V., Gupta, N., and Dheen, S.T.: HDAC Inhibitor Sodium Butyrate-Mediated Epigenetic Regulation Enhances Neuroprotective Function of Microglia During Ischemic Stroke. *Mol. Neurobiol.* 54: 6391-6411 (2017).
- Park, M.J. and Sohrabji, F.: The histone deacetylase inhibitor, sodium butyrate, exhibits neuroprotective effects for ischemic stroke in middle-aged female rats. *J. Neuroinflammation* 13: 300 (2016).
- Qin, J., Li, R., Raes, J., Arumugam, M., Burgdorf, K.S., Manichanh, C., Nielsen, T., Pons, N., Levenez, F., Yamada, T., Mende, D.R., Li, J., Xu, J., Li, S., Li, D., Cao, J., Wang, B., Liang, H., Zheng, H., Xie, Y., Tap, J., Lepage, P., Bertalan, M., Batto, J.M., Hansen, T., Le Paslier, D., Linneberg, A., Nielsen, H.B., Pelletier, E., Renault, P., Sicheritz-Ponten, T., Turner, K., Zhu, H., Yu, C., Li, S., Jian, M., Zhou, Y., Li, Y., Zhang, X., Li, S., Qin, N., Yang, H., Wang, J., Brunak, S., Doré, J., Guarner, F., Kristiansen, K., Pedersen, O., Parkhill, J., Weissenbach, J.; MetaHIT Consortium; Bork, P., Ehrlich, S.D., and Wang, J.: A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* 464: 59-65 (2010).
- Romano, K.A., Martinez-del Campo, A., Kasa-hara, K., Chittim, C.L., Vivas, E.I., Amador-Noguez, D., Balskus, E.P., and Rey, F.E.: Metabolic, Epigenetic, and Transgenerational Effects of Gut Bacterial Choline Consumption. *Cell Host Microbe* 22: 279-290.e7 (2017).
- Swann, J.R., Want, E.J., Geier, F.M., Spagou, K., Wilson, I.D., Sidaway, J.E., Nicholson, J.K., and Holmes, E.: Systemic gut microbial

- modulation of bile acid metabolism in host tissue compartments. *Proc. Natl. Acad. Sci. USA* 108 Suppl. 1: 4523-4530 (2011).
- Swann, J.R., Spagou, K., Lewis, M., Nicholson, J.K., Glei, D.A., Seeman, T.E., Coe, C.L., Goldman, N., Ryff, C.D., Weinstein, M., and Holmes, E: Microbial-mammalian cometabolites dominate the age-associated urinary metabolic phenotype in Taiwanese and American populations. *J. Proteome Res.* 12: 3166-3180 (2013).
- Torquati, L., Mielke, G.I., Brown, W.J., Burton, N.W., and Kolbe-Alexander, T.L.: Shift Work and Poor Mental Health: A Meta-Analysis of Longitudinal Studies. *Am. J. Public Health* 109: e13-e20 (2019).
- Valles-Colomer, M., Falony, G., Darzi, Y., Tigchelaar, E.F., Wang, J., Tito, R.Y., Schiweck, C., Kurilshikov, A., Joossens, M., Wijmenga, C., Claes, S., Van Oudenhove, L., Zhernakova, A., Vieira-Silva, S., and Raes, J.: The neuroactive potential of the human gut microbiota in quality of life and depression. *Nat. Microbiol.* 4: 623-632 (2019).
- Wimborne, E.A., Hampel, D., Allen, L., van der Veen, D.R., Panic, G., Panchal, M., Plattsmills, J.A., Mduma, E., Blacy, L., Jatsoh, S., Mdoe, P., Scharf, R., DeBoer, M.D., and Swann, J.R.: Birth season shapes the infant metabolome and development in Tanzania: a secondary explorative analysis of the early life interventions for childhood growth and development in Tanzania (ELICIT) trial. *Nat. Commun.* 16: 11469 (2025).
- Zuffa, S., Schimmel, P., Gonzalez-Santana, A., Belzer, C., Knol, J., Bölte, S., Falck-Ytter, T., Forssberg, H., Swann, J., and Diaz Heijtz, R.: Early-life differences in the gut microbiota composition and functionality of infants at elevated likelihood of developing autism spectrum disorder. *Transl. Psychiatry* 13: 257 (2023).

FAECAL MICROBIOTA TRANSPLANTATION FOR TREATMENT OF PARKINSON'S DISEASE

FILIP SCHEPERJANS

Department of Neurology, Helsinki University Hospital,
and NeuroBiome Ltd., Helsinki, Finland

INTRODUCTION

There is a rapidly growing field of microbiota-based interventions in Parkinson's disease (PD). Although PD is usually defined by bradykinesia, rigidity and tremor, many patients develop gastrointestinal symptoms years before the motor syndrome becomes evident. This temporal sequence, together with accumulating epidemiological, microbiological and experimental evidence, has raised the possibility that the gut is not merely affected secondarily in PD, but may participate in disease initiation and progression in at least a subgroup of patients (*Kalia and Lang, 2015; Schapira et al., 2017; Horsager et al., 2020*).

PD remains one of the most challenging chronic neurodegenerative

disorders in clinical neurology. It is the second most common neurodegenerative disease, leads to progressive disability and frequently cognitive decline, and still lacks a disease-modifying therapy (*Kalia and Lang, 2015*). The highly relevant question is whether the intestinal microbiota can be used not only to better understand the biology of PD, but if it also can be used as a therapeutic target. This review moves stepwise from the prodromal phase of PD and gut-based epidemiology to microbiota signatures, plausible biological mechanisms, preclinical intervention studies and finally to human clinical trials, with particular emphasis on faecal microbiota transplantation (FMT).

PARKINSON'S DISEASE AS A MULTISYSTEM DISORDER WITH A LONG PRODROMAL PHASE

One of the core messages is that PD should be viewed as a multisystem disease rather than as an isolated degeneration of nigrostriatal dopaminergic neurons. Many non-motor features, including constipation, rapid eye movement sleep behaviour disorder (RBD), hyposmia, depression, fatigue, pain and autonomic dysfunction, may occur years before the diagnosis is made (*Kalia and Lang, 2015; Schapira et al., 2017*). This long prodromal interval is clinically important because it indicates that the pathological process is already

active when the classical motor syndrome first emerges.

Figure 1 illustrates how constipation can precede diagnosis by decades, while other non-motor symptoms gradually accumulate before motor impairment and later complications become prominent. In practical terms, this means that the gastrointestinal tract is involved early in the disease course and deserves attention not only as a source of symptoms, but also as a potential window into pathogenesis.

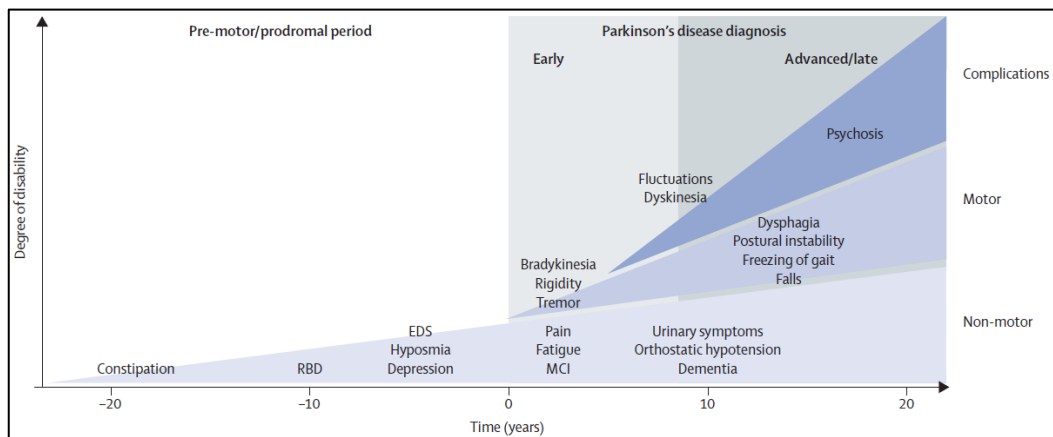


Figure 1: The prodromal phase of PD can extend for years and is dominated by non-motor symptoms such as constipation, RBD, hyposmia and depression before the classical motor syndrome becomes established. (Figure from *Kalia and Lang*, 2015).

BODY-FIRST AND BRAIN-FIRST PARKINSON'S DISEASE

An increasingly influential concept claims that PD may consist of at least two major biological routes of disease spread. In the body-first subtype, pathology may initially arise in the enteric or peripheral autonomic nervous system and ascend to the brainstem and higher structures. In the brain-first subtype, the earliest pathology is thought to begin within the central nervous system and only later involves the peripheral autonomic system and gut (Figure 2) (*Horsager et al.*, 2020). This framework helps to explain why some patients have a striking prodromal burden of gastrointestinal and autonomic symptoms, whereas others present more directly with central motor features.

For microbiota-oriented research, this distinction is crucial. If a subgroup of patients truly has a body-first form of synucleinopathy, then gut microbial changes may be more than an epiphenomenon. They could be mechanistically linked to early disease biology and therefore represent rational targets for intervention. The presentation emphasized that imaging and clinical work from the Borghammer group has provided a coherent conceptual basis for this subdivision, and that this framework is especially relevant when interpreting microbiome findings and treatment responses (*Horsager et al.*, 2020).

GASTROINTESTINAL EPIDEMIOLOGY AND MICROBIOTA-ASSOCIATED RISK SIGNALS

There are several epidemiological observations that support a gut-brain connection in PD. Constipation is one of the strongest and best replicated prodromal gastrointestinal features of PD, with

meta-analytic data suggesting a clearly increased later risk of diagnosis. Similar associations have been reported for irritable bowel syndrome (IBS) and inflammatory bowel disease. The key point is

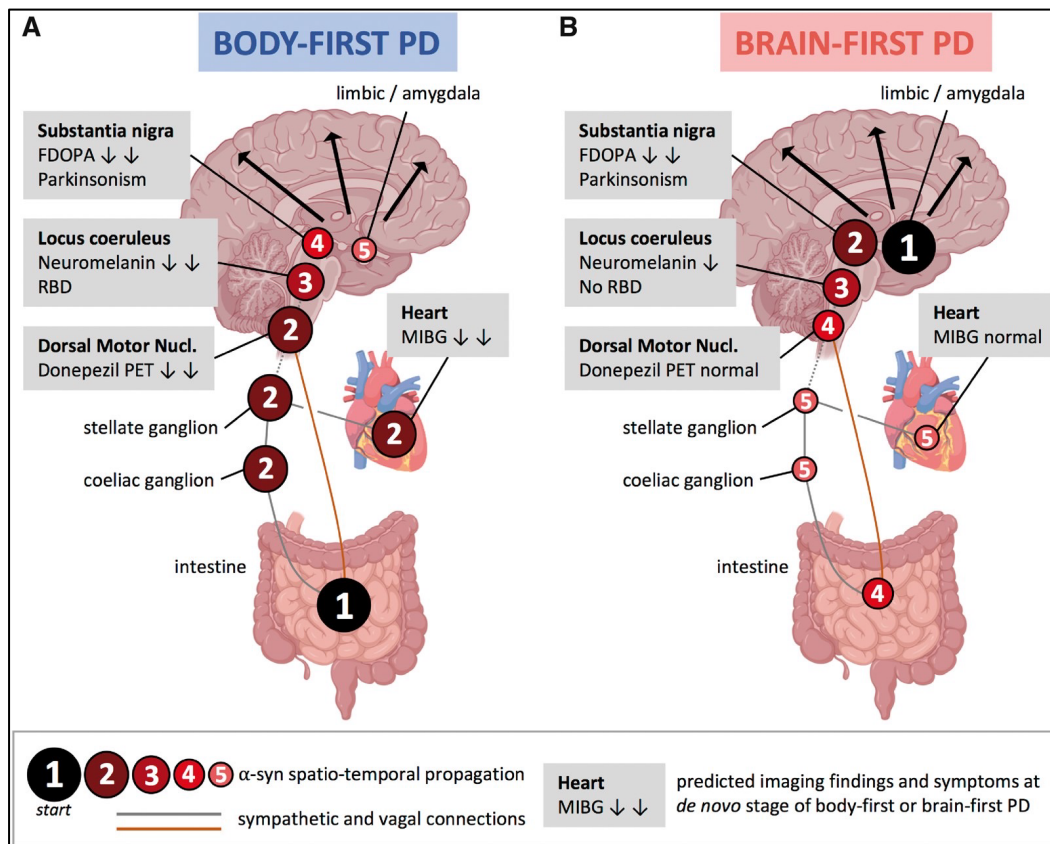


Figure 2: Schematic distinction between body-first and brain-first PD. In the body-first subtype, pathology is hypothesized to start in the enteric/autonomic nervous system and ascend centrally; in the brain-first subtype, the earliest pathology is thought to arise within the brain. (Figure from Horsager et al., 2020).

that these disorders are not direct causes of PD, but that they consistently point toward early gastrointestinal involvement and toward inflammatory or microbial factors that may modify susceptibility (Lai et al., 2014; Adams-Carr et al., 2016; Zhu et al., 2019).

Of especially interest is antibiotic exposure. In a large Finnish nationwide case-control study, exposure to certain oral antibiotics was associated with an

elevated later risk of PD, with the timing of exposure fitting the idea of a long pre-diagnostic interval. Such findings are important because antibiotics are among the strongest routine modifiers of the intestinal microbiome. Although antibiotic use itself cannot be equated with causation, these data again support the idea that perturbation of gut microbial ecology may be linked to PD risk over the long term (Mertsalmi et al., 2020).

GUT MICROBIOTA SIGNATURES AND LINKS TO SYMPTOMS AND PROGRESSION

Extensive literature describes alterations in the gut microbiota of patients with PD. Alpha-diversity measures such

as richness, evenness and Shannon index are not consistently changed, and loss of overall diversity is not a

consistent feature of PD. What appears more reproducible are specific compositional shifts. Across studies, differences in taxa related to short-chain fatty acid (SCFA) production, *Prevotella*, *Akkermansia*, *Lactobacillus*, *Bifidobacterium* and several other genera have repeatedly been reported (Plassais et al., 2021; Romano et al., 2021).

Important are longitudinal as well as symptom-linked findings. In the work from Aho and colleagues (Aho et al., 2019), microbiota differences remained relatively stable over time, and certain

microbial patterns appeared to relate to disease progression. Other studies linked reduced butyrate-producing bacteria or lower faecal butyrate to depressive symptoms, while additional datasets associated specific microbial profiles with constipation, IBS symptoms, cognitive measures and motor subdomains. Taken together, these observations suggest that the microbiome may help explain part of the well-known clinical heterogeneity of PD (Aho et al., 2019; Xie et al., 2022).

POTENTIAL MECHANISMS LINKING THE GUT MICROBIOTA TO PARKINSON'S DISEASE BIOLOGY

The mechanistic section of this review integrates microbiology, immunology and alpha-synuclein biology into a coherent pathophysiological model. Dysbiosis may reduce the production of beneficial SCFAs, alter bile acid metabolism and weaken the intestinal barrier. This can increase local immune activation, facilitate exposure to bacterial products such as lipopolysaccharide and affect enteroendocrine signalling, enteric glial function and vagal pathways. In parallel, bacterial amyloids and inflammatory mediators may influence alpha-synuclein expression and aggregation, while systemic immune activation may further amplify neuroinflammation (Tan et al., 2022).

The review by Tan and colleagues (Tan et al., 2022) summarized these interacting pathways elegantly: altered fibre fermentation and metabolite production, barrier dysfunction, innate immune activation, cytokine signalling,

blood-borne mediators and neural propagation are all plausible contributors (Figure 3). The important point is that the microbiota does not need to 'cause' PD in a simplistic one-microbe-one-disease manner. Rather, it may shape the intestinal and immune environment in a way that promotes or protects against pathogenic processes relevant to PD.

This mechanistic plausibility is strengthened by experimental data. Germ-free or microbiota-depleted alpha-synuclein-overexpressing mice show reduced motor deficits, less microglial activation and improved gut motility compared with conventionally colonized animals (Sampson et al., 2016). Such results do not automatically translate into treatment effects in humans, but they provide strong proof of principle that microbial manipulation can modify PD-relevant phenotypes *in vivo*.

FROM MECHANISM TO INTERVENTION: PRECLINICAL AND EARLY CLINICAL MODULATION STUDIES

Several preclinical studies suggest that transferring a healthier microbial community or administering microbiota-associated products can attenuate

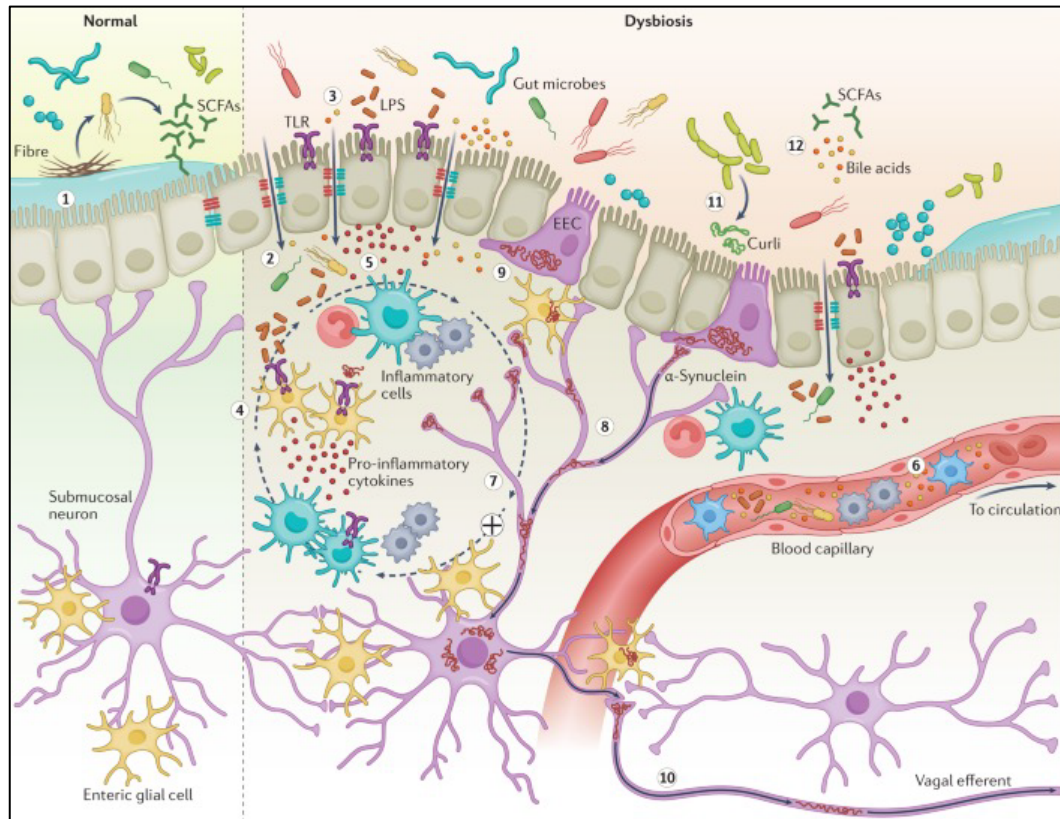


Figure 3: Proposed mechanisms by which gut dysbiosis may contribute to PD pathophysiology, including altered microbial metabolites, impaired epithelial barrier function, innate and adaptive immune activation, inflammatory signalling, effects on enteroendocrine and enteric nervous system neurons, and possible propagation of alpha-synuclein-related pathology through neural and systemic routes. (Figure from *Tan et al., 2022*).

PD-like pathology in toxin-based animal models. Reported mechanisms include reduced microglial activation, suppression of TLR4-related inflammatory signalling, improved intestinal function and restoration of microbial metabolites such as butyrate. These studies remain model-dependent and should be interpreted cautiously, but together they suggest that PD-related mechanisms are not fixed and may be manipulable through the microbiota (*Sun et al., 2018*).

The human intervention literature remains less mature but is growing steadily. Probiotic trials and the RESISTA-PD resistant-starch study

suggest that gastrointestinal outcomes are presently the most consistent target, with improvements reported for constipation-related measures and, in some studies, quality of life. By contrast, benefits on global motor scores have been smaller or less reproducible. This imbalance is not surprising. Gut-targeted therapies are most likely to show their earliest clinical effects on bowel symptoms, whereas robust effects on established central motor manifestations may require either longer treatment periods, more targeted patient selection or disease stages in which gut-driven biology is still especially relevant (*Jin et al., 2024; Becker et al., 2024*).

FAECAL MICROBIOTA TRANSPLANTATION AS A THERAPEUTIC CONCEPT

FMT represents the most direct available strategy for altering the intestinal microbial ecosystem. Instead of supplying one or a few strains, it transfers an entire microbial community from a carefully screened donor to the recipient. In established gastroenterological practice, FMT is best known as a highly effective treatment for recurrent *Clostridioides difficile* infection. For PD, however, it remains an experimental intervention. The translational challenge is fundamentally different: the goal is not to eradicate a single pathogen, but to remodel a complex host-

microbiota interaction that may contribute to symptoms and possibly to progression.

This distinction has several consequences. In PD, the relevant donor profile is uncertain, the optimal route of administration is not established, the durability of engraftment is unknown, and clinically meaningful endpoints are broader than simple microbiological change. FMT in PD should therefore be interpreted as a platform for biological discovery as much as a treatment in itself.

CLINICAL FAECAL MICROBIOTA TRANSPLANTATION TRIALS IN PARKINSON'S DISEASE

Two randomized trials reported on the use of FMT in PD patients. The first was the Belgian GUT-PARFECT trial, a double-blind phase 2 study in early-stage PD that compared one-time nasojejunal application of healthy donor stool with autologous transplantation. The trial showed that FMT is feasible in PD and that adverse effects were mainly mild transient gastrointestinal symptoms such as abdominal cramps and nausea. The donor-FMT group showed encouraging small improvements in motor symptoms as compared to the control group (Bruggeman et al., 2024).

The second dataset was the Finnish randomized placebo-controlled double-blind trial led by Scheperjans and colleagues (Scheperjans et al., 2024). In this study, patients with PD were screened for dysbiosis, randomized to one-time intracaecal infusion of healthy donor stool or placebo carrier solution, and eventually underwent bowel preparation and colonoscopy. The design is noteworthy because it treated the micro-

biota phenotype as a clinically relevant variable and because it allowed careful follow-up of both symptoms and microbial dynamics. A proportion of patients shifted from a dysbiotic to a non-dysbiotic state shortly after donor FMT, yet this effect diminished over time. In contrast, the placebo group showed a slower but more sustained improvement in dysbiosis status. On the clinical side, signals were seen in bowel-related outcomes and in selected motor subdomains, while the overall pattern suggested that efficacy may depend on the dynamics of dysbiosis recovery rather than on the label of donor versus placebo alone (Scheperjans et al., 2024). This leads to one of the most original conclusions. Because all participants underwent bowel cleansing before the intervention, bowel cleansing itself may have contributed substantially to the observed benefit. In other words, the trial may have revealed not only information about donor microbiota transfer, but also an effect of resetting the intesti-

nal environment. This possibility is clinically attractive because bowel cleansing is simpler, cheaper and easier to standardize than FMT. These hypo-

theses now require direct testing in specifically designed trials (*Scheperjans et al., 2024*).

INTERPRETATION AND FUTURE DIRECTIONS

Gastrointestinal and microbiota disturbances are real, clinically relevant and mechanistically plausible features of PD, especially in patients with a body-first biology or marked bowel dysfunction. Microbiota modulation therefore deserves serious clinical investigation, but future studies must define which patients to treat, which biological endpoints to monitor and which interventions are most rational at different disease stages (*Horsager et al., 2020; Tan et al., 2022; Scheperjans et al., 2024*).

In this perspective, microbiota-based phenotyping may become as important

as the intervention itself. If only a subset of patients has microbiota-driven symptom generation or progression, then broad unselected trials will underestimate true treatment effects. Repeated microbiological sampling, metabolite measurements and deeper analysis of donor-recipient dynamics may help identify responders and reveal new live biotherapeutic or metabolite-based treatment candidates that are easier to standardize than whole-stool transplantation (*Aho et al., 2019; Scheperjans et al., 2024*).

CONCLUSIONS

The evidence reviewed supports several linked conclusions on why the gut deserves serious attention in PD:

- Gastrointestinal symptoms and microbiota disturbances are integral features of PD and may precede motor diagnosis by many years.
- The body-first versus brain-first framework offers an important biological context for interpreting microbiome findings.
- Gut microbial composition is linked not only to case-control status but also to symptom profiles and disease

progression.

- Experimental work shows that microbiota manipulation can influence PD-relevant biology, supporting therapeutic exploration.
- First-generation clinical trials of FMT and other microbiota-modifying approaches have produced encouraging but complex results, suggesting that treatment effects may depend on route of administration, patient selection, dysbiosis recovery and possibly bowel cleansing itself.

LITERATURE

Adams-Carr, K.L., Bestwick, J.P., Shribman, S., Lees, A., Schrag, A., and Noyce, A.J.: Constipation preceding Parkinson's disease: a systematic review and meta-analysis. *J. Neurol. Neurosurg. Psychiatry* 87: 710-716

(2016).

Aho, V.T.E., Pereira, P.A.B., Voutilainen, S., Paulin, L., Pekkonen, E., Auvinen, P., and Scheperjans, F: Gut microbiota in Parkinson's disease: temporal stability and

- relations to disease progression. *EBioMedicine* 44: 691-707 (2019).
- Becker, A., Schmartz, G.P., Groeger, L., Grammes, N., Galata, V., Philippeit, H., Weiland, J., Ludwig, N., Meese, E., Tierling, S., Walter, J., Schwirtz, A., Spiegel, J., Wagenpfeil, G., Faßbender, K., Keller, A., and Unger, M.M.: Effects of resistant starch on symptoms, fecal markers, and gut microbiota in Parkinson's disease - the RESISTA-PD trial. *Genomics Proteomics Bioinformatics* 22: 274-287 (2024).
- Bruggeman, A., Vandendriessche, C., Hamerlinck, H., De Looze, D., Tate, D.J., Vuylsteke, M., De Commer, L., Devolder, L., Raes, J., Verhasselt, B., Laukens, D., Vandenbroucke, R.E., and Santens, P.: Safety and efficacy of faecal microbiota transplantation in patients with mild to moderate Parkinson's disease (GUT-PARFECT): a double-blind, placebo-controlled, randomised, phase 2 trial. *EClinicalMedicine* 71: 102563 (2024).
- Horsager, J., Andersen, K.B., Knudsen, K., Skjærbæk, C., Fedorova, T.D., Okkels, N., Schaeffer, E., Bonkat, S.K., Geday, J., Otto, M., Sommerauer, M., Danielsen, E.H., Bech, E., Kraft, J., Munk, O.L., Hansen, S.D., Pavese, N., Göder, R., Brooks, D.J., Berg, D., and Borghammer, P.: Brain-first versus body-first Parkinson's disease: a multimodal imaging case-control study. *Brain* 143: 3077-3088 (2020).
- Jin, X., Dong, W., Chang, K., Yan, Y., and Liu, X.: Efficacy of probiotic supplements on Parkinson's disease: a systematic review and meta-analysis. *Complement. Ther. Med.* 82: 103045 (2024).
- Kalia, L.V. and Lang, A.E.: Parkinson's disease. *Lancet* 386: 896-912 (2015).
- Lai, S.W., Liao, K.F., Lin, C.L., and Sung, F.C.: Irritable bowel syndrome correlates with increased risk of Parkinson's disease in Taiwan. *Eur. J. Epidemiol.* 29: 57-62 (2014).
- Mertsalmi, T.H., Pekkonen, E., and Scheperjans, F.: Antibiotic exposure and risk of Parkinson's disease in Finland: a nationwide case-control study. *Mov. Disord.* 35: 431-442 (2020).
- Plassais, J., Gbikpi-Benissan, G., Figarol, M., Scheperjans, F., Gorochov, G., Derkinderen, P., and Cervino, A.C.L.: Gut microbiome alpha-diversity is not a marker of Parkinson's disease and multiple sclerosis. *Brain Commun.* 3: fcab113 (2021).
- Romano, S., Savva, G.M., Bedarf, J.R., Charles, I.G., Hildebrand, F., and Narbad, A.: Meta-analysis of the Parkinson's disease gut microbiome suggests alterations linked to intestinal inflammation. *NPJ Parkinsons Dis.* 7: 27 (2021).
- Sampson, T.R., Debelius, J.W., Thron, T., Janssen, S., Shastri, G.G., Ilhan, Z.E., Challis, C., Schretter, C.E., Rocha, S., Gradinaru, V., Chesselet, M.F., Keshavarzian, A., Shannon, K.M., Krajmalnik-Brown, R., Wittung-Stafshede, P., Knight, R., and Mazmanian, S.K.: Gut microbiota regulate motor deficits and neuroinflammation in a model of Parkinson's disease. *Cell* 167: 1469-1480.e12 (2016).
- Schapira, A.H.V., Chaudhuri, K.R., and Jenner, P.: Non-motor features of Parkinson disease. *Nat. Rev. Neurosci.* 18: 435-450 (2017).
- Scheperjans, F., Levo, R., Bosch, B., Lääperi, M., Pereira, P.A.B., Smolander, O.P., Aho, V.T.E., Vetkas, N., Toivio, L., Kainulainen, V., Fedorova, T.D., Lahtinen, P., Ortiz, R., Kaasinen, V., Satokari, R., and Arkkila, P.: Fecal Microbiota Transplantation for Treatment of Parkinson Disease: A Randomized Clinical Trial. *JAMA Neurol.* 81: 925-938 (2024).
- Sun, M.F., Zhu, Y.L., Zhou, Z.L., Jia, X.B., Xu, Y.D., Yang, Q., Cui, C., and Shen, Y.Q.: Neuroprotective effects of fecal microbiota transplantation on MPTP-induced Parkinson's disease mice: gut microbiota, glial reaction and TLR4/TNF-alpha signaling pathway. *Brain Behav. Immun.* 70: 48-60 (2018).
- Tan, A.H., Lim, S.Y., and Lang, A.E.: The microbiome-gut-brain axis in Parkinson disease — from basic research to the clinic. *Nat. Rev. Neurol.* 18: 476-495 (2022).
- Xie, A., Ensink, E., Li, P., Gordevičius, J., Marshall, L.L., George, S., Pospisilik, J.A., Aho, V.T.E., Houser, M.C., Pereira, P.A.B., Rudi,

K., Paulin, L., Tansey, M.G., Auvinen, P., Brundin, P., Brundin, L., Labrie, V., and Scheperjans, F.: Bacterial Butyrate in Parkinson's Disease Is Linked to Epigenetic Changes and Depressive Symptoms. *Mov. Disord.* 37: 1644-1653 (2022).

Zhu, F., Li, C., Gong, J., Zhu, W., Gu, L., and Li, N.: The risk of Parkinson's disease in inflammatory bowel disease: a systematic review and meta-analysis. *Dig. Liver Dis.* 51: 38-42 (2019).

THE GUT-BRAIN AXIS IN MULTIPLE SCLEROSIS: UNRAVELING THE ROLE OF GUT MICROBIOTA

ASHUTOSH MANGALAM

Department of Pathology, Carver College of Medicine, University of Iowa, and
VA Iowa City Healthcare System, Iowa City, USA

INTRODUCTION

The gut microbiome is increasingly recognized as a key environmental factor in the pathobiology of multiple sclerosis (MS), with special emphasis on phyto-oestrogen-metabolizing gut bacteria, beneficial symbionts, and disease-promoting pathobionts.

MS is an autoimmune disease of the central nervous system (CNS) in which both genetic and environmental factors contribute to disease pathogenesis. Importantly, while genetic factors, primarily HLA class II alleles, account for

only 2-27% of disease risk, environmental factors contribute as much as 73-98%. Among these environmental determinants, the gut microbiome has emerged as one of the strongest factors linked to MS susceptibility, resistance, and progression. The gut microbiota can contribute to disease susceptibility and progression but can equally confer protection against disease development or worsening, making the microbiome a uniquely tractable target for both diagnostics and therapy.

GUT DYSBIOSIS IN PEOPLE WITH MULTIPLE SCLEROSIS

There are two independent MS microbiome cohorts published: the first is the “Mayo Clinic Rochester cohort” (Chen et al., 2016); the second is the “University of Iowa cohort” (Yadav et al., 2022). In both cohorts, beta-diversity ordination analyses demonstrated a statistically distinct gut microbial composition in MS subjects compared to healthy controls (HC). Beyond this overall compositional shift, targeted analysis of specific microbiota revealed a consistent pattern of depletion of particular bacterial genera in MS patients, most notably *Prevotella*, *Parabacteroides*, and *Adlercreutzia*. To place these findings in a broader context, MS microbiome studies from research groups across the globe were compared. The results of this comparison are shown in Table 1. Strikingly, the majority of these independent

studies reported depletion of one or more of these same three bacterial genera, underscoring the robustness and reproducibility of the dysbiosis signal across different populations, sequencing platforms, and study designs.

Certain bacteria, specifically *Prevotella* and *Sutterella*, were found to be enriched in MS patients receiving disease-modifying therapies (DMT). These observations suggest that effective MS treatment may partially restore the gut microbiota toward a healthier compositional state and further underscores the intimate relationship between gut microbial ecology and MS disease activity.

In summary, these human microbiome data from multiple cohorts worldwide provide compelling evidence that MS is associated with gut dysbiosis

Table 1: Alteration of *Prevotella*, *Parabacteroides*, and *Adlercreutzia*, reported in patients with MS across independent international studies

Study (Reference)	Bacteria depleted in MS vs. HC	Bacteria enriched after DMT
Chen et al., 2016	<i>Prevotella</i> , <i>Parabacteroides</i> , <i>Adlercreutzia</i>	-
Jangi et al., 2016	<i>Prevotella</i> , <i>Parabacteroides</i>	<i>Prevotella</i> , <i>Sutterella</i>
Miyake et al., 2015	<i>Prevotella</i> , <i>Parabacteroides</i>	-
Castillo-Alvarez et al., 2018	<i>Prevotella</i>	-
Cekanaviciute et al., 2017	<i>Prevotella</i> , <i>Parabacteroides</i>	-
Berer et al., 2017	<i>Adlercreutzia</i>	-
Cosorich et al., 2017	<i>Prevotella</i> (mucosal biopsies)	-

HC = healthy controls; DMT = disease-modifying therapy; “-” not reported.

characterized specifically by consistent depletion of *Prevotella*, *Parabacteroides*, and *Adlercreutzia* genera, and that restoration of some of these bacteria may accompany clinical benefit from

DMT. These findings laid the conceptual foundation for all subsequent mechanistic and translational work described here.

FROM BEDSIDE TO BENCH: *PREVOTELLA HISTICOLA* AS A BACTERIAL DRUG (BRUG)

Based on the fact that *Prevotella* species are depleted in stool of MS patients, a series of preclinical experiments were carried out to test the therapeutic hypothesis: if a bacterium consistently lacking in MS subjects is provided back to the host, can it ameliorate disease?

Using the experimental autoimmune encephalomyelitis (EAE) mouse model, the most widely employed preclinical model of MS, it was demonstrated that oral administration of *Prevotella histicola*, a human commensal bacterium, significantly suppressed EAE severity. The mechanism involved induction of immunoregulatory cells and restoration of both the intestinal epithelial barrier (gut barrier) and the blood-brain barrier (Mangalam et al., 2017). This dual barrier-protective effect is particularly relevant to MS pathophysiology, in which breakdown of both barriers is thought to

be an early step in CNS immune infiltration.

It was also examined whether *P. histicola* could synergize with approved MS drugs. Therefore, *Prevotella histicola* was tested in combination with Copaxone (glatiramer acetate) (Shahi et al., 2019) and with interferon-beta (Betaseron) (Shahi et al., 2020), two standard-of-care DMTs. While the combinations did not demonstrate synergy beyond either agent alone, a critical finding emerged: *P. histicola* as a single agent was as effective in suppressing EAE as Copaxone alone or Betaseron alone.

The term **BRUG** (beneficial bacteria as **drug**) was used to describe the concept of deploying human commensal bacteria as precision drug-like therapies. This term was recognized by the magazine *WIRED*, where it was featured as a

"Jargon Watch" entry in their November 2017 issue, reflecting broader interest in the therapeutic potential of commensal microorganisms.

In summary, these bedside-to-bench studies established that providing a human commensal bacterium that is deficient in MS subjects can ameliorate

disease in an animal model of MS, performing comparably to approved DMTs. This work provided the first direct proof-of-concept for the BRUG concept and supports the potential of *Prevotella histicola* and related organisms as microbiome-based therapeutics for MS.

GUT BACTERIA, PHYTO-OESTROGENS, AND THE MECHANISM OF MICROBIOME-MEDIATED IMMUNOMODULATION

Nutrient-based evolution and the microbiome

The fundamental question is: through what mechanism does the gut microbiota modulate MS? This question is framed within the context of nutrient-based evolution, arguing that the human microbiome exists largely because of its role in nutrient absorption and energy harvesting from food, a relationship refined over millions of years of co-evolution (Cady et al., 2020). In utero, the foetus harbours little or no microbiota. After birth, a simple microbiome is established, adapted to a milk-based diet. As infants transition from milk to solid foods, there is an enormous expansion of the gut microbiota, coinciding with physical elongation of the intestinal tract and the emergence of complex microbial communities capable of fermenting plant-based, indigestible polysaccharides to extract additional nutrients and energy. This evolutionary perspective places diet as the primary driver of microbiome composition, and the microbiome as the primary mediator of diet's immunological effects.

Three MS-depleted bacteria and their shared role in phyto-oestrogen metabolism

There is a striking shared characteristic of the three bacterial genera consistently depleted in MS: *Adlercreutzia*, *Parabacteroides*, and *Prevotella* are all

involved in the intestinal metabolism of phyto-oestrogens, plant-derived compounds that include isoflavones (found in soy products) and lignans (found in seeds, whole grains, and legumes). Humans lack the enzymatic machinery to metabolize these compounds directly; intestinal bacteria convert them into bioactive metabolites, principally S-equol and enterolactone, which share structural similarities with endogenous oestrogens.

This connection is biologically significant because oestrogens are known to be protective in MS (Gold and Voskuhl, 2009). Women with MS experience fewer relapses during pregnancy, a high-oestrogen state, and there are ongoing clinical trials of oestriol therapy in MS subjects. If MS patients are depleted of the bacteria required to generate oestrogen-like metabolites from dietary phyto-oestrogens, they may be functionally deficient in this protective pathway regardless of what they eat. To test this hypothesis rigorously there are three specific questions with regard to the EAE model:

- Do phyto-oestrogens have the ability to modulate disease in an animal model of MS?
- Are gut bacteria required for phyto-oestrogen-mediated disease protection?
- What is the mechanism of phyto-oestrogen-mediated immunomodulation?

Isoflavone diet protects against EAE across multiple mouse models

To address the first question, mice were randomized to either a phyto-oestrogen-free (PF) diet or an isoflavone-rich diet for six weeks prior to EAE induction. Mice on the PF diet developed a severe form of EAE, whereas those on the isoflavone diet showed significantly milder disease, a protective effect that was reproducible across three independent mouse strains: the C57BL/6 mice (standard chronic EAE model), the HLA-DR2 transgenic humanized model, and SJL mice (relapsing-remitting model). These data established unequivocally that dietary phyto-oestrogens have disease-protective properties in EAE (Jensen et al., 2021).

Gut bacteria are required for isoflavone-mediated disease protection

The isoflavone diet reshapes the gut microbiome in a pattern strikingly parallel to the difference between MS patients and healthy controls. Isoflavone-fed mice showed enrichment of *Parabacteroides* and *Adlercreutzia*, precisely the genera depleted in MS subjects, mirroring the microbiome of healthy controls (Jensen et al., 2021). Notably, *Prevotella* was not enriched, which was attributed to the fact that mice from Jackson Laboratory do not harbour *Prevotella* at baseline; the diet therefore could not expand a genus that was already absent. Conversely, mice on the phyto-oestrogen-free diet showed

enrichment of *Akkermansia muciniphila*, a species that had been reported by others to be elevated in MS subjects.

To formally demonstrate that gut bacteria mediate the protective effect, intestinal microbiota were depleted using an antibiotic cocktail. Antibiotic treatment abolished the disease-protective effect of the isoflavone diet. Mice on isoflavone diet but depleted of gut bacteria developed disease as severe as those on the PF diet. Crucially, when antibiotic-treated mice on the isoflavone diet were reconstituted by oral gavage with a combination of the phyto-oestrogen-metabolizing bacteria *Parabacteroides* and *Adlercreutzia*, disease protection was restored. By contrast, reconstitution of antibiotic-treated mice on the phyto-oestrogen-free diet with the same bacteria conferred no protection, establishing that both the appropriate diet and the bacteria are required simultaneously (Jensen et al., 2021).

In summary, the phyto-oestrogen experiments demonstrated that dietary isoflavones can protect against inflammatory disease in EAE, and that this protection is dependent on the presence of specific phyto-oestrogen-metabolizing gut bacteria. Since MS subjects are systematically depleted of exactly these bacteria, the data support a model in which MS-associated dysbiosis creates a functional phyto-oestrogen deficiency that contributes to the disease's pro-inflammatory milieu.

TRANSLATIONAL SIGNIFICANCE: DIET SWITCHING AS A THERAPEUTIC STRATEGY

To test the translational relevance of these findings, a clinically important question should be answered: if the gut microbiome of MS subjects resembles that of mice on a phyto-oestrogen-free diet, and the microbiome of healthy con-

trols resembles that of mice on an isoflavone-rich diet, then switching a patient from an inflammatory dietary pattern to an isoflavone-rich one should ameliorate disease, provided this can first be demonstrated in the animal model.

In a diet-switch EAE experiment, mice initially maintained on a phyto-oestrogen-free diet were switched to an isoflavone diet and four weeks later EAE was induced (Ghimire et al., 2022). In contrast, a parallel group was switched in the opposite direction (from isoflavone to phyto-oestrogen-free). Mice switched from the phyto-oestrogen-free to the isoflavone diet developed significantly milder EAE compared to those switched in the opposite direction. Microbiome analysis of these diet-switch animals revealed that the isoflavone diet caused expansion of

Bifidobacterium species, while the phyto-oestrogen-free diet promoted *Akkermansia*, a pattern that would become the foundation of the biomarker work described in the next section.

These data provide compelling evidence for the translational significance of phyto-oestrogen-rich diet and phyto-oestrogen-metabolizing bacteria as potential therapeutic strategies for MS patients, by demonstrating in an animal model that dietary correction, even after disease onset, can meaningfully reduce disease severity.

IDENTIFICATION OF A GUT MICROBIOTA-BASED BIOMARKER FOR MULTIPLE SCLEROSIS DISCOVERY IN THE EAE MODEL: THE *BIFIDOBACTERIUM*:*AKKERMANSIA* RATIO

The next question to be answered was whether a gut microbiota-based biomarker could be identified that is translatable to human MS. Using the EAE mouse model, three experimental groups were used: PBS-immunized controls, adjuvant-only controls (CFA + pertussis toxin), and CNS-antigen plus adjuvant-immunized mice (MOG₃₅₋₅₅ + CFA + pertussis toxin), of which only the last group develops EAE disease. Faecal and caecal microbiome samples were collected at baseline, day 7 (disease onset), and day 14 (peak disease).

Analysis revealed a clear and progressive trend: the ratio of *Bifidobacterium adolescentis* (BA) to *Akkermansia muciniphila* (AM), hereafter the BA:AM ratio, was higher in both control groups (PBS and adjuvant only) but significantly lower and declining in the EAE group in both the faecal and caecal compartments. Reanalysis of the diet-switch microbiome data from the phyto-oestrogen study corroborated this: the BA:AM ratio was higher in isoflavone-diet mice (the protective condition) compared to phyto-oestrogen-free-diet mice (the disease-promoting condition),

further strengthening the association of this ratio with inflammatory disease state (Ghimire et al., 2025).

Validation in human MS cohorts

Next, the BA:AM ratio in human subjects was validated. In the University of Iowa cohort, shotgun metagenomic sequencing was performed in 45 RRMS patients and 51 age- and sex-matched healthy controls. The BA:AM ratio, was significantly lower in MS patients compared to healthy controls ($p = 0.041$) (Ghimire et al., 2025).

This finding was then validated in the International Multiple Sclerosis Microbiome Study (iMSMS), the largest MS microbiome dataset currently available, comprising 576 MS subjects and 1,152 genetically unrelated household controls. The BA:AM ratio was again significantly lower in MS subjects ($p = 1 \times 10^{-5}$), confirming the finding across a completely independent cohort, different geography, and higher statistical power (Ghimire et al., 2025).

Importantly, the BA:AM ratio was not only a case-control discriminator but also correlated with disease severity: in

the iMSMS cohort, the ratio showed a significant negative correlation with EDSS score ($R = -0.13$, $p = 0.0041$) and with disease duration ($R = -0.14$, $p = 0.0023$), and a trend toward correlation with the MS Severity Score ($R = -0.075$, $p = 0.093$). Patients with greater disability and longer disease duration had lower BA:AM ratios, consistent with the hypothesis that dysbiosis tracks with, and may actively contribute to, disease progression rather than being a passive bystander (Ghimire et al., 2025).

Preliminary unpublished data further show that Parkinson's disease (PD)

subjects also exhibit a lower BA:AM ratio compared to healthy controls ($p = 0.0083$), raising the intriguing possibility that this ratio may represent a broader biomarker of neuroinflammatory and neurodegenerative disease (Ghimire et al., unpublished).

Taken together, these human microbiome studies from two independent MS cohorts, spanning hundreds of subjects, strongly suggest that the BA:AM ratio represents a robust, quantitative, stool-based biomarker of MS disease and severity.

FROM DISEASE MARKER TO PREDICTIVE BIOMARKER: A CAUSAL ROLE FOR MS-ENRICHED BACTERIA

Is the reduced BA:AM ratio merely a marker of established disease, or does it have predictive and causative significance? To identify the specific bacteria driving the microbiome differences between MS patients and healthy controls, topic modelling was applied (Fitzjerrrells et al., 2024) and co-occurrence network analysis to the shotgun metagenomic dataset. This analysis revealed that *Blautia* species were the dominant drivers of the MS-associated microbial community, whereas *Prevotella copri* drove the healthy control-associated community (Ghimire et al., 2025).

To test causality, a pseudo-germ-free mouse model was used: intestinal microbiota were depleted by antibiotic treatment, and mice were then colonized by oral gavage with one of three bacteria, (i) the HC-driving *Prevotella copri*, (ii) the MS-driving *Blautia wexlerae*, or (iii) a control human commensal (*Phocaeicola vulgatus*). All groups then received a common human faecal microbiota transplant to allow background community development, and microbiome composition was tracked at days 9, 13, 17, 22, and 27.

The results were striking as by day 27, mice initially colonized with the MS-driving *Blautia wexlerae* showed a significantly lower BA:AM ratio compared to those colonized with HC-driving *Prevotella copri* ($p = 2.2 \times 10^{-5}$). This reduction was temporally progressive, emerging as the microbial communities matured and the initial colonizer exerted its influence on community structure. Furthermore, the *Blautia*-colonized group went on to develop significantly more severe EAE after disease induction compared to the *Prevotella copri*-colonized group, and the magnitude of the BA:AM ratio reduction correlated directly with cumulative EAE disease scores ($R = -0.42$, $p = 0.025$).

Mechanistic investigation revealed that *Blautia wexlerae*-colonized mice also showed significantly elevated faecal lipocalin-2 and calprotectin, validated markers of gut inflammation, compared to both the *Prevotella copri*- and *Phocaeicola vulgatus*-colonized groups (Ghimire et al., 2025).

These studies provide compelling evidence that the enrichment of MS-

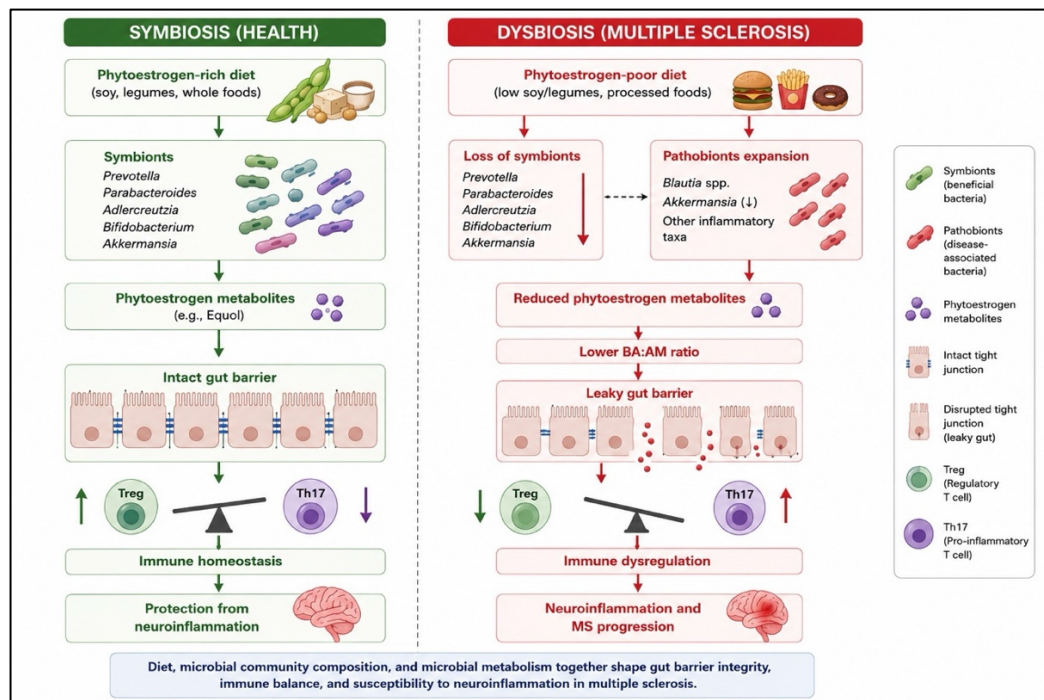


Figure 1: Diet-microbiome-immune interactions in health and disease (MS). Proposed model illustrating how a phyto-oestrogen-rich diet and beneficial gut bacteria promote production of immunoregulatory metabolites, maintain gut barrier integrity, and support immune homeostasis. In MS, depletion of phyto-oestrogen-metabolizing symbionts and enrichment of inflammatory pathobionts are associated with a lower BA:AM ratio, gut barrier dysfunction, immune dysregulation, and increased neuroinflammation.

associated bacteria such as *Blautia* species actively drives gut inflammation and promotes a lower BA:AM ratio, which in turn predicts greater disease severity in EAE. (Ghimire et al., 2025). Critically, this colonization experiment

establishes a causative rather than merely correlative role for gut microbiota in MS pathobiology, a mechanistic proof that moves the field beyond association studies toward actionable therapeutic and preventive targets.

CONCLUSIONS: FROM DYSBIOSIS TO CLINICAL TRANSLATION

With respect to gut dysbiosis in MS, this work and that of others have established that:

- (i) people with MS harbour a distinct gut microbiome characterized by depletion of multiple bacteria including phyto-oestrogen-metabolizing bacteria (*Prevotella*, *Parabacteroides*, *Adlercreutzia*);
- (ii) this dysbiosis has been mechanistically validated in animal models to

be functionally important, not merely associative;

- (iii) maximum disease protection requires both the correct diet and the appropriate bacteria acting in concert, neither alone is sufficient; and
- (iv) MS-associated dysbiosis is enriched for bacteria such as *Blautia* that promote gut inflammation and a lower *Bifidobacterium*:*Akkermansia* ratio.

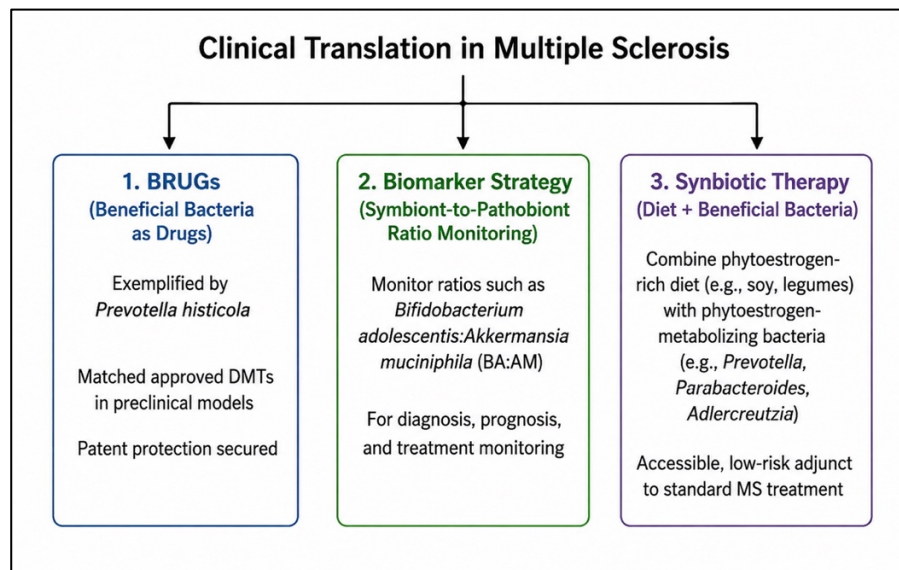


Figure 2. Microbiome-based strategies for clinical translation in MS.

Gut dysbiosis in MS provides opportunities for therapeutic and diagnostic development, including BRUGs (beneficial bacteria as drugs), symbiont-to-pathobiont ratios such as BA:AM as diagnostic/prognostic biomarkers, and synbiotic approaches combining phyto-oestrogen-rich diet with phyto-oestrogen-metabolizing bacteria.

With respect to biomarkers, the *Bifidobacterium adolescentis*:*Akkermansia muciniphila* (BA:AM) ratio has emerged from both animal and human studies as a promising diagnostic and prognostic gut microbiome biomarker for MS, validated in over 600 MS patients across two independent international cohorts and correlated with disability and disease duration (Figure 1).

With respect to clinical translation, the data collectively support three therapeutic strategies:

(i) BRUGs, beneficial bacteria as drugs, exemplified by *Prevotella histicola* which matched approved DMTs in preclinical models and for which patent protection has been secured;

(ii) symbiont-to-pathobiont ratio monitoring as a diagnostic and prognostic tool; and

(iii) synbiotic therapy, combining phyto-oestrogen-rich diet with phyto-oestrogen-metabolizing bacteria, as a potentially accessible and low-risk adjunct to standard MS treatment (Figure 2).

Future directions will focus on understanding the microbial functional pathways modulated during dysbiosis, including LPS biosynthesis, phyto-oestrogen metabolism, and short-chain fatty acid production, using multi-omic approaches, and on advancing the most promising candidates toward clinical trials in people with MS.

DISCLOSURE

The author declares the following patent interests relevant to the content of this presentation: (1) *Prevotella histicola* for treatment of autoimmune conditions

(Patent #US8617536B2, 2015); and (2) phyto-oestrogen combined with gut bacteria as treatment of disease (Patent #US20240091279, 2022)

ACKNOWLEDGMENTS

Dr. Mangalam first thanks all human subjects for providing biospecimens for the clinical studies. He gratefully also acknowledges the contributions of the Mangalamanians, the trainees and staff who conducted this research, including Samantha Jensen, PhD; Shailesh Shahi, PhD; Sudeep Ghimire, PhD; Nicole Cady, PhD; Stephanie Peterson, PhD; Apurva Patel, PhD; Katie Hereford, PhD; Rachel Shrode, PhD; Peter Lehman; Ti-Ara Turner; Aracely Miron-Ocampo; and undergraduate researchers Gavin Clark, Sushil Desai, Lian Alt, and Francesca Cannon. The Microbiome Core (Leeann Aguilar) provided essential sequencing support. Clinical collaboration was provided by the UIHC MS Clinic team: Dr. Tracey Cho, MD; Dr. Christina Gill, MD; and

Dr. Deema Fattal, MD; with biostatistical support from Dr. Kai Wang and clinical trial coordination from Heena Olalde and Maegan Tyrrell. External collaborators include Dr. Shailendra Giri (Henry Ford Health System), Dr. Amanda Ramer-Tait (University of Nebraska), Dr. Christina Camell (University of Minnesota), and Dr. Pavan Bhargava (Johns Hopkins University). Funding was provided by the U.S. Department of Veterans Affairs (BX006091, CX002212), the National Multiple Sclerosis Society (RG 5138A1), the NIH/NIAID (R01AI137075), Carver Trust Medical Research Initiative Grants, and a generous philanthropic gift from Margaret Heppelmann and Michael Wacek.

LITERATURE

- Berer, K., Gerdes, L.A., Cekanaviciute, E., Jia, X., Xiao, L., Xia, Z., Liu, C., Klotz, L., Stauffer, U., Baranzini, S.E., Kämpfel, T., Hohlfeld, R., Krishnamoorthy, G., and Wekerle, H.: Gut microbiota from multiple sclerosis patients enables spontaneous autoimmune encephalomyelitis in mice. *Proc. Natl. Acad. Sci. USA.* 114 (40): 10719-10724 (2017).
- Cady, N., Peterson, S.R., Freedman, S.N., and Mangalam, A.K.: Beyond Metabolism: The Complex Interplay Between Dietary Phytoestrogens, Gut Bacteria, and Cells of Nervous and Immune Systems. *Front. Neurol.* 11: 150 (2020).
- Castillo-Álvarez, F., Pérez-Matute, P., Oteo, J.A., and Marzo-Sola, M.E.: The influence of interferon β -1b on gut microbiota composition in patients with multiple sclerosis. *Neurologia (Engl. Ed.)* 36 (7): 495-503 (2021).
- Cekanaviciute, E., Yoo, B.B., Runia, T.F., Debelius, J.W., Singh, S., Nelson, C.A., Kanner, R., Bencosme, Y., Lee, Y.K., Hauser, S.L., Crabtree-Hartman, E., Sand, I.K., Gacias, M., Zhu, Y., Casaccia, P., Cree, B.A.C., Knight, R., Mazmanian, S.K., and Baranzini, S.E.: Gut bacteria from multiple sclerosis patients modulate human T cells and exacerbate symptoms in mouse models. *Proc. Natl. Acad. Sci. USA* 114 (40): 10713-10718 (2017).
- Chen, J., Chia, N., Kalari, K.R., Yao, J.Z., Novotna, M., Paz Soldan, M.M., Luckey, D.H., Marietta, E.V., Jeraldo, P.R., Chen, X., Weinshenker, B.G., Rodriguez, M., Kantarci, O.H., Nelson, H., Murray, J.A., and Mangalam, A.K.: Multiple sclerosis patients have a distinct gut microbiota compared to healthy controls. *Sci. Rep.* 6: 28484 (2016).
- Cosorich, I., Dalla-Costa, G., Sorini, C., Ferrarese, R., Messina, M.J., Dolpady, J., Radice, E., Mariani, A., Testoni, P.A., Canducci, F., Comi, G., Martinelli, V., and Falcone, M.:

- High frequency of intestinal T_H17 cells correlates with microbiota alterations and disease activity in multiple sclerosis. *Sci. Adv.* 3 (7): e1700492 (2017).
- Fitzjerrells, R.L., Ollberding, N.J., and Mangalam, A.K.: Looking at the full picture, using topic modeling to observe microbiome communities associated with disease. *Gut Microbes Rep.* 1 (1): 1-11 (2024).
- Ghimire, S., Cady, N.M., Lehman, P., Peterson, S.R., Shahi, S.K., Rashid, F., Giri, S., and Mangalam, A.K.: Dietary Isoflavones Alter Gut Microbiota and Lipopolysaccharide Biosynthesis to Reduce Inflammation. *Gut Microbes.* 14 (1): 2127446 (2022).
- Ghimire, S., Lehman, P.C., Aguilar Meza, L.S., Shahi, S.K., Hoang, J., Olalde, H., Paullus, M., Cherwin, C., Wang, K., Gill, C., Cho, T., and Mangalam, A.K.: Specific microbial ratio in the gut microbiome is associated with multiple sclerosis. *Proc. Natl. Acad. Sci. USA* 122 (10): e2413953122 (2025).
- Gold, S.M. and Voskuhl, R.R.: Estrogen treatment in multiple sclerosis. *J. Neurol. Sci.* 286 (1-2): 99-103 (2009).
- Jangi, S., Gandhi, R., Cox, L.M., Li, N., von Glehn, F., Yan, R., Patel, B., Mazzola, M.A., Liu, S., Glanz, B.L., Cook, S., Tankou, S., Stuart, F., Melo, K., Nejad, P., Smith, K., Topçuoğlu, B.D., Holden, J., Kivisäkk, P., Chitnis, T., De Jager, P.L., Quintana, F.J., Gerber, G.K., Bry, L., and Weiner, H.L.: Alterations of the human gut microbiome in multiple sclerosis. *Nat. Commun.* 7: 12015 (2016).
- Jensen, S.N., Cady, N.M., Shahi, S.K., Peterson, S.R., Gupta, A., Gibson-Corley, K.N., and Mangalam, A.K.: Isoflavone diet ameliorates experimental autoimmune encephalomyelitis through modulation of gut bacteria depleted in patients with multiple sclerosis. *Sci. Adv.* 7 (28): eabd4595 (2021).
- Mangalam, A., Shahi, S.K., Luckey, D., Karau, M., Marietta, E., Luo, N., Choung, R.S., Ju, J., Sompallae, R., Gibson-Corley, K., Patel, R., Rodriguez, M., David, C., Taneja, V., and Murray, J.: Human Gut-Derived Commensal Bacteria Suppress CNS Inflammatory and Demyelinating Disease. *Cell Rep.* 20 (6): 1269-1277 (2017).
- Miyake, S., Kim, S., Suda, W., Oshima, K., Nakamura, M., Matsuoka, T., Chihara, N., Tomita, A., Sato, W., Kim, S.W., Morita, H., Hattori, M., and Yamamura, T.: Dysbiosis in the Gut Microbiota of Patients with Multiple Sclerosis, with a Striking Depletion of Species Belonging to Clostridia XIVa and IV Clusters. *PLoS One* 10 (9): e0137429 (2015).
- Shahi, S.K., Freedman, S.N., Murra, A.C., Zarei, K., Sompallae, R., Gibson-Corley, K.N., Karandikar, N.J., Murray, J.A., and Mangalam, A.K.: *Prevotella histicola*, A Human Gut Commensal, Is as Potent as COPAXONE® in an Animal Model of Multiple Sclerosis. *Front. Immunol.* 10: 462 (2019).
- Shahi, S.K., Jensen, S.N., Murra, A.C., Tang, N., Guo, H., Gibson-Corley, K.N., Zhang, J., Karandikar, N.J., Murray, J.A., and Mangalam, A.K.: Human Commensal *Prevotella histicola* Ameliorates Disease as Effectively as Interferon-Beta in the Experimental Autoimmune Encephalomyelitis. *Front. Immunol.* 11: 578648 (2020).
- Yadav, M., Ali, S., Shrode, R.L., Shahi, S.K., Jensen, S.N., Hoang, J., Cassidy, S., Olalde, H., Guseva, N., Paullus, M., Cherwin, C., Wang, K., Cho, T., Kamholz, J., and Mangalam, A.K.: Multiple sclerosis patients have an altered gut mycobiome and increased fungal to bacterial richness. *PLoS One* 17 (4): e0264556 (2022).

THE ROLE OF THE MICROBIOTA-GUT-BRAIN AXIS IN PSYCHIATRY AND BEYOND

VALERIE TAYLOR

Department of Psychiatry, University of Calgary,
3280 Hospital Dr. NW, Calgary, AB T2N 4Z6, Canada

INTRODUCTION

The idea that bacteria in the human gut could play a role in mental health is not revolutionary, and the prehistory of this area provides important context for present-day research. The concept of auto-intoxication, a state of being poisoned by toxic substances produced within the body, was widely accepted in the 1800's and ultimately led to methods for dealing with 'cases requiring change of the intestinal flora', such as those used to treat illnesses that would be characterized as mirroring today's mental illnesses by people such as Dr. John Kellogg at the Battle Creek Sanitarium (*Kellogg*, 1919). The microbiota-gut-brain (MGB) axis is a bidirectional pathway between the brain and microbiota in the gastrointestinal system (*McVey Neufeld et al.*, 2024). It is comprised of humoral, neural, and cellular pathways that ultimately connect the gut microbiota with the central nervous system

(CNS). That the gut microbiota can impact the symptoms of mental illness through a variety of purported mechanisms has been extensively researched and work has been done to evaluate this connection as a potential therapeutic target via faecal microbiota transplantation (FMT) or probiotics (*Wallace et al.*, 2020; *Settani et al.*, 2021; *Chung et al.*, 2022; *Queiroz et al.*, 2022; *Nikolova et al.*, 2023). This has resulted in the intriguing possibility of the MGB axis being a possible treatment target. The probiotic literature for mental illness is mixed (*Hofmeister et al.*, 2021), however, in part because probiotics are not designed to change the gut microbiome (*Wallace et al.*, 2017). FMT may offer a better biotherapeutic option as evidence suggest it may result in more long-term effects on the MGB axis (*Wallace et al.*, 2017; *Weingarden et al.*, 2017; *Chinna Meyyappan et al.*, 2020).

INFLUENCE OF ANTI-DEPRESSANT MEDICATIONS ON THE GUT MICROBIOTA OF PATIENTS WITH DEPRESSION

This led us to explore translational clinical trials using FMT but prior to this, we had done research to show that anti-depressant medications alter the gut microbiota of patients with depression, indicating support for studies taking this further. As published earlier (*Bharwani et al.*, 2020), we recruited participants were between 18 and 60 years of age with Major Depressive Disorder (MDD)

as confirmed by the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID) (*American Psychiatric Association*, 1994) and moderate depression as indicated by a Montgomery-Asberg Depression Rating Scale (MADRS; *Montgomery and Asberg*, 1979) score of >19. Exclusion criteria included psychiatric medication use (other than sedatives for sleep and benzodiazepines) at

minimum 1 week prior to visit 1, medical conditions or current use of medications or natural health products known to impact the microbiota, and current substance abuse. After the screening visit, participants were given a 3-day food diary that was returned prior to the baseline visit and repeated at 3- and 6-month assessments. Anthropomorphic measurements (height, weight, and waist circumference) were also completed, and the MADRS administered, at the same time points. Participants then initiated therapy with Citalopram or Escitalopram. Specimens were stored at 80°C. Bacterial community profiling using 16S rRNA gene sequencing was carried out using a modified bar-coded Illumina sequencing method (Chung et al, 2022). Using data rarefied in QIIME at the lowest sequencing depth, phylogenetic diversity was analysed. For beta diversity, Jackknife resampling was used to generate Bray-Curtis distances. To identify differentially abundant operational taxonomic units (OTUs), OTUs not observed in 75% of samples were eliminated, and the data set was analysed using the DESeq2 software package and the Benjamini-Hochberg procedure.

In total, fifteen participants (mean age = 36.9; SD = 12.9; 12 women) were studied. The average baseline MADRS score was 22.53 (SD = 6.63). At 6 months, 11 participants were classified as remitters (MADRS < 12) and 4 as non-remitters (MADRS > 13). Citalopram was taken by 3 participants in the remitter group and 1 participant in the non-remitter group; the remaining 11 took Escitalopram. Remitters exhibited greater diversity at baseline than non-remitters (Mann-Whitney U test = 5, $P = 0.026$). Bray-Curtis distances revealed no differences in variability (Mann-Whitney U test = 138, $P = 0.517$) and a lack of community clustering based upon eventual treatment response

(PERMANOVA; $F = 0.720$, $P = 0.824$). At baseline, 22 OTUs were differentially abundant between the remitter and non-remitter groups.

Comparisons at 3- and 6-months following treatment showed no change in diversity in remitter or non-remitters. Differences in diversity between groups were no longer significant at 3 months but were again significant at 6 months ($F = 4.773$, $P = 0.048$, post hoc $P < 0.05$). Analysis of the composition in remitters showed no clustering by time point (PERMANOVA; $F = 0.262$, $P = *1$) and no changes in within-subject distances (Friedman statistic = 0.546, $P = 0.844$). Similarly, non-remitters showed no clustering of the community across treatment (PERMANOVA; $F = 0.433$, $P = 0.951$) or changes in within-subject distances (Friedman statistic = 3.50, $P = 0.273$).

Next, we examined whether specific OTUs were altered after treatment. In remitters, a single OTU was altered (a *Clostridiales*, elevated at 6 months). No OTUs were altered in non-remitters. At 3 months, 35 OTUs were significantly different between remitters and non-remitters but 16 were no longer different by the 6-month time point. At 6 months, 42 OTUs were different between groups: 23 were unique while the remaining 19 were also differentially abundant at 3 months. There was no impact of dietary composition as analysed by Nutritionist Pro diet analysis software or antidepressant exposure.

Our findings indicated that antidepressant treatment affects the microbiota of MDD patients at the OTU level, based on response to treatment. Assessment at 3 and 6 months following treatment revealed no changes to the diversity or profile of the community relative to baseline, which is consistent with previous work demonstrating the lack of effects of antidepressant medications on bacterial taxa (Queiroz et al., 2022).

It was therefore interesting that notable differences in the microbial community were observed in analyses based on treatment response. Despite similar baseline depression severity scores, non-remitters exhibited lower diversity prior to treatment initiation. Additionally, 22 microbial groups were differentially abundant between response groups. Our data provide compelling preliminary evidence that baseline differences in the microbiota may be correlated with antidepressant medication efficacy.

To further examine the impact of antidepressant medications, we compared changes in the microbiota in remitters and non-remitters across 6 months of treatment. There was no change in diversity or overall profile in either response group, paralleling our findings of limited community-wide changes. However, baseline differences between remitters and non-remitters

were still evident after 6 months of treatment: Remitters continued to exhibit greater diversity. These observations further suggest a microbial community signature distinguishing treatment response groups.

Our data indicate that antidepressant medications may alter the gut microbiota of patients with MDD and outline the disparate nature of their effects in remitters versus non-remitters. Limitations include the small sample size and the lack of a placebo controls. Larger studies are needed to investigate whether the gut microbiome may serve as a potential biomarker to aid in guiding treatment. But this work supported the concept of a microbiota phenotype associate with treatment response in depression (*Bharwani et al., 2020*), and added to the growing support for the gut microbiome as a target for the treatment of mental illness. This led us in to trials.

CLINICAL TRIALS USING FAECAL MICROBIOTA TRANSPLANTATION

A growing body of literature has found that individuals with Bipolar Disorder (BD) have alterations in the gut microbiome composition or function and that these alterations are associated with illness severity in BD (*Coello et al., 2019; Painold et al., 2019*). We decided to investigate this with a phase 2/3, double-blind randomized control trial (RCT). In this study, all participants with BD on, but failing to respond to, first-line treatment were given a medical grade bowel preparation and then randomized to receive either (1) allogenic FMT from healthy donors or (2) autologous FMT with their own faeces by colonoscopy. FMT was manufactured into a liquid filtrate and administered via colonoscopy by a trained gastroenterologist. Participants were subsequently

followed for 24 weeks for assessment of primary and secondary study outcomes.

In terms of data, we recruited adults 18 to 65 years of age, who had a Mini-International Neuropsychiatric Interview (*Sheenan et al., 1998*) confirmed diagnosis of BD (type I or II) on a stable first-line treatment (*Yatham et al., 2018*) or ≥ 8 weeks prior to study entry, who were currently experiencing a depressive episode. A participant met inclusion criteria if their current episode was characterized by a MADRS (*Montgomery and Asberg, 1979*) score at screening and baseline of ≥ 12 . Exclusion criteria included a Young Mania Rating Scale (*Young et al., 1978*) score of ≥ 12 at screening; Diagnostic and Statistical Manual of Mental Disorders IV (DSM-IV) criteria (*American Psychiatric*

Association, 1994) for substance abuse within the past 6 months or lifetime dependency; active suicidal ideation, an active eating disorder; schizophrenia or schizoaffective disorder; chronic gastrointestinal diseases; conditions causing immunosuppression; a significant bleeding disorder; a history of incomplete colonoscopy; pregnancy, regular use of non-steroidal anti-inflammatory drugs, antibiotics, prebiotics or probiotics for medical purposes, or iron supplements within 3 months prior to study entry.

Participants were randomly allocated (1:1) to groups receiving either allogenic or autologous FMT and once enrolled, FMT administration was delivered via colonoscopy after a standard bowel cleanse consisting of 4 L of an over-the-counter polyethylene glycol laxative solution. The primary outcome of this study is to determine the effectiveness, safety, and tolerability of FMT as an adjunctive treatment in adults with BD depression, as well as the viability of a larger trial. Feasibility was measured by participant recruitment, retention and completion rates, adherence to the intended study protocol, participant acceptability, and robustness of study methodology. Safety was measured by the assessment of severe adverse events while Tolerability was monitored by the Toronto Side Effect Scale (TSES) (Vanderkooy et al., 2002), with each symptom rated for both frequency and severity on a five-point scale (1 = Never/No trouble; 5 = Everyday/Extreme trouble), as well as weight changes. Adverse events were also assessed via a symptom diary reviewed at each visit. Effectiveness, the primary clinical outcome, was measured by change in MADRS score from baseline to the end of follow-up.

At the end of the study, MADRS scores significantly improved from baseline to the last visit in both treat-

ment arms. There was no significant difference between allogenic FMT (16.74-point improvement) and autologous FMT (15.4-point improvement) regarding clinical efficacy ($t = -0.47$, p -value = .64, 95% confidence interval [CI] = -7.3 - 4.6). Microbiota analysis showed that allogenic FMT lead to a bacterial profile similar to the healthy donor and increased bacterial diversity at the 6-month mark, whereas those receiving autologous FMT did not. The intervention was well tolerated with no significant adverse events. Recruitment, randomization, and retention metrics support feasibility of a larger trial.

There were clear limitations associated with this FMT methodology, related to the many unknowns still existing in this field, including the use of a single dose FMT, as there is still debate regarding the best frequency of FMT to produce appropriate results. Colonoscopy (Cammarota et al., 2017) was chosen as the method of intervention as it is reasonable to believe that better FMT distribution increases the chance of donor microbiota engraftment, which has been shown to be associated with treatment outcome (Wilson et al., 2019) and current evidence indicates this is better achieved with colonoscopy (Skjevling et al., 2023). A recent study of FMT by enema for depression, however, indicates other methods of administration may be adequate as well (Green et al., 2023). No antibiotic was used as evidence indicated that it could impact engraftment (Zhao et al., 2020; Singh et al., 2022), but again, the data is equivocal and thus further work needs to be done (Pocari et al., 2023). The use of bowel preparation may also have an impact on outcomes (McGuinness et al., 2024) but evidence from studies showing efficacy after the 6 months indicates that the effect may be transient (Brugeman et al., 2024). We also notice trends toward increased diversity in the

allogenic group, indicating something other than the bowel preparation was having an effect.

This study was designed as a feasibility study to inform outcomes of future research, as such studies are essential to inform key next steps. It adds to the pos-

itive findings of two case reports reporting the efficacy of FMT in refractory BD (*Hinton, 2020*) and a probiotic RCT showing improvements in mania (*Parker et al., 2022*) building on the evidence base supporting microbial manipulation being a viable therapeutic target

LITERATURE

- American Psychiatric Association: DSM-IV: Diagnostic and Statistical Manual of Mental Disorders (Fourth Edition). American Psychiatric Publishing, Inc., Washington, DC (1994).
- Bharwani, A., Bala, A., Surette, M., Bienenstock, J., Vigod, S.N., and Taylor, V.H.: Gut Microbiome Patterns Associated With Treatment Response in Patients With Major Depressive Disorder. *Can. J. Psychiatry* 65: 278-280 (2020).
- Bruggeman, A., Vandendriessche, C., Hamerlinck, H., De Looze, D., Tate, D.J., Vuylsteke, M., De Commer, L., Devolder, L., Raes, J., Verhasselt, B., Laukens, D., Vandenbroucke, R.E., and Santens, P.: Safety and efficacy of faecal microbiota transplantation in patients with mild to moderate Parkinson's disease (GUT-PARFECT): a double-blind, placebo-controlled, randomised, phase 2 trial. *EclinicalMedicine* 71: 102563 (2024).
- Cammarota, G., Ianiro, G., Tilg, H., Rajilić-Stojanović, M., Kump, P., Satokari, R., Sokol, H., Arkkila, P., Pintus, C., Hart, A., Segal, J., Aloï, M., Masucci, L., Molinaro, A., Scaldaferri, F., Gasbarrini, G., Lopez-Sanroman, A., Link, A., de Groot, P., de Vos, W.M., Högenauer, C., Malfertheiner, P., Mattila, E., Milosavljević, T., Nieuwdorp, M., Sanguinetti, M., Simren, M., and Gasbarrini, A.: European FMT Working Group. European consensus conference on faecal microbiota transplantation in clinical practice. *Gut* 66: 569-580 (2017).
- Chinna Meyyappan, A., Forth, E., Wallace, C.J.K., and Milev, R.: Effect of fecal microbiota transplant on symptoms of psychiatric disorders: a systematic review. *BMC Psychiatry* 20: 299 (2020).
- Chung, S.Y., Kostev, K., and Tanislav, C.: Dysbiosis: A Potential Precursor to the Development of a Depressive Disorder. *Healthcare (Basel)* 10: 1503 (2022).
- Coello, K., Hansen, T.H., Sørensen, N., Munkholm, K., Kessing, L.V., Pedersen, O., and Vinberg, M.: Gut microbiota composition in patients with newly diagnosed bipolar disorder and their unaffected first-degree relatives. *Brain Behav. Immun.* 75: 112-118 (2019).
- Green, J.E., Berk, M., Mohebbi, M., Loughman, A., McGuinness, A.J., Castle, D., Chatterton, M.L., Perez, J., Strandwitz, P., Athan, E., Hair, C., Nierenberg, A.A., Cryan, J.F., and Jacka, F.: Feasibility, Acceptability, and Safety of Faecal Microbiota Transplantation in the Treatment of Major Depressive Disorder: A Pilot Randomized Controlled Trial. *Can. J. Psychiatry* 68: 315-326 (2023).
- Hinton, R.: A case report looking at the effects of faecal microbiota transplantation in a patient with bipolar disorder. *Aust. N. Z. J. Psychiatry* 54: 649-650 (2020).
- Hofmeister, M., Clement, F., Patten, S., Li, J., Dowsett, L.E., Farkas, B., Mastikhina, L., Egunsola, O., Diaz, R., Cooke, N.C.A., and Taylor, V.H.: The effect of interventions targeting gut microbiota on depressive symptoms: a systematic review and meta-analysis. *CMAJ Open* 9: E1195-E1204 (2021).
- Kellogg, J.H.: Autointoxication or Intestinal Toxemia. Battle Creek : The Modern Medicine Publishing Co. (1919).
- McGuinness, A.J., O'Hely, M., Stupart, D., Waters, D., Dawson, S.L., Hair, C., Berk, M.,

- Mohebbi, M., Loughman, A., Guest, G., and Jacka, F.N.: Depressive Symptoms and Gut Microbiota after Bowel Preparation and Colonoscopy: A Pre-Post Intervention Study. *Microorganisms* 12: 1960 (2024).
- McVey Neufeld, S.F., Ahn, M., Kunze, W.A., and McVey Neufeld, K.A.: Adolescence, the Microbiota-Gut-Brain Axis, and the Emergence of Psychiatric Disorders. *Biol. Psychiatry* 95: 310-318 (2024).
- Montgomery, S.A. and Asberg M.: A new depression scale designed to be sensitive to change. *Br. J. Psychiatry* 134: 2-9 (1979).
- Nikolova, V.L., Cleare, A.J., Young, A.H., and Stone, J.M.: Acceptability, Tolerability, and Estimates of Putative Treatment Effects of Probiotics as Adjunctive Treatment in Patients With Depression: A Randomized Clinical Trial. *JAMA Psychiatry* 80: 842-847 (2023).
- Painold, A., Mörk, S., Kashofer, K., Halwachs, B., Dalkner, N., Bengesser, S., Birner, A., Fellendorf, F., Platzer, M., Queissner, R., Schütze, G., Schwarz, M.J., Moll, N., Holzer, P., Holl, A.K., Kapfhammer, H.P., Gorkiewicz, G., and Reininghaus, E.Z.: A step ahead: Exploring the gut microbiota in inpatients with bipolar disorder during a depressive episode. *Bipolar Disord.* 21: 40-49 (2019).
- Parker, G., Spoelma, M.J., and Rhodes, N.: Faecal microbiota transplantation for bipolar disorder: A detailed case study. *Bipolar Disord.* 24: 559-563 (2022).
- Porcari, S., Benech, N., Valles-Colomer, M., Segata, N., Gasbarrini, A., Cammarota, G., Sokol, H., and Ianiro, G.: Key determinants of success in fecal microbiota transplantation: From microbiome to clinic. *Cell Host Microbe* 31: 712-733 (2023).
- Queiroz, S.A.L., Ton, A.M.M., Pereira, T.M.C., Campagnaro, B.P., Martinelli, L., Picos, A., Campos-Toimil, M., and Vasquez, E.C.: The Gut Microbiota-Brain Axis: A New Frontier on Neuropsychiatric Disorders. *Front. Psychiatry* 13: 872594 (2022).
- Settanni, C.R., Ianiro, G., Bibbò, S., Cammarota, G., and Gasbarrini, A.: Gut microbiota alteration and modulation in psychiatric disorders: Current evidence on fecal microbiota transplantation. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 109: 110258 (2021).
- Sheehan, D.V., Lecrubier, Y., Sheehan, K.H., Amorim, P., Janavs, J., Weiller, E., Hergueta, T., Baker, R., and Dunbar, G.C.: The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *J. Clin. Psychiatry* 59, Suppl. 20: 22-33 (1998).
- Singh, P., Alm, E.J., Kelley, J.M., Cheng, V., Smith, M., Kassam, Z., Nee, J., Iturrino, J., and Lembo, A.: Effect of antibiotic pretreatment on bacterial engraftment after Fecal Microbiota Transplant (FMT) in IBS-D. *Gut Microbes* 14: 2020067 (2022).
- Skjevling, L.K., Hanssen, H.M., Valle, P.C., Goll, R., Juul, F.E., Arlov, Ø., and Johnsen, P.H.: Colonic distribution of FMT by different enema procedures compared to colonoscopy - proof of concept study using contrast fluid. *BMC Gastroenterol.* 23: 363 (2023).
- Vanderkooy, J.D., Kennedy, S.H., and Bagby, R.M.: Antidepressant side effects in depression patients treated in a naturalistic setting: a study of bupropion, moclobemide, paroxetine, sertraline, and venlafaxine. *Can. J. Psychiatry* 47: 174-80 (2002).
- Wallace, C.J.K. and Milev, R.: The effects of probiotics on depressive symptoms in humans: a systematic review. *Ann. Gen. Psychiatry* 16: 14 (2017).
- Wallace, C.J.K., Foster, J.A., Soares, C.N., and Milev, R.V.: The Effects of Probiotics on Symptoms of Depression: Protocol for a Double-Blind Randomized Placebo-Controlled Trial. *Neuropsychobiology* 79: 108-116 (2020).
- Weingarden, A.R., and Vaughn, B.P.: Intestinal microbiota, fecal microbiota transplantation, and inflammatory bowel disease. *Gut Microbes* 8: 238-252 (2017).
- Wilson, B.C., Vatanen, T., Cutfield, W.S., and O'Sullivan, J.M.: The Super-Donor Phenomenon in Fecal Microbiota Transplantation. *Front. Cell. Infect. Microbiol.* 9: 2 (2019).

- Yatham, L.N., Kennedy, S.H., Parikh, S.V., Schaffer, A., Bond, D.J., Frey, B.N., Sharma, V., Goldstein, B.I., Rej, S., Beaulieu, S., Alda, M., MacQueen, G., Milev, R.V., Ravindran, A., O'Donovan, C., McIntosh, D., Lam, R.W., Vazquez, G., Kapczinski, F., McIntyre, R.S., Kozicky, J., Kanba, S., Lafer, B., Suppes, T., Calabrese, J.R., Vieta, E., Malhi, G., Post, R.M., and Berk, M.: Canadian Network for Mood and Anxiety Treatments (CANMAT) and International Society for Bipolar Disorders (ISBD) 2018 guidelines for the management of patients with bipolar disorder. *Bipolar Disord.* 20: 97-170 (2018).
- Young, R.C., Biggs, J.T., Ziegler, V.E., and Meyer, D.A.: A rating scale for mania: reliability, validity and sensitivity. *Br. J. Psychiatry* 133: 429-435 (1978).
- Zhao, H.L., Chen, S.Z., Xu, H.M., Zhou, Y.L., He, J., Huang, H.L., Xu, J., and Nie, Y.Q.: Efficacy and safety of fecal microbiota transplantation for treating patients with ulcerative colitis: A systematic review and meta-analysis. *J. Dig. Dis.* 21: 534-548 (2020).

