BRAIN
REVIEW ARTICLE

From bugs to brain: unravelling the GABA signalling networks in the brain–gut–microbiome axis

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Convergent data across species paint a compelling picture of the critical role of the gut and its resident microbiota in several brain functions and disorders. The chemicals mediating communication along these sophisticated highways of the brain–gut–microbiome (BGM) axis include both microbiota metabolites and classical neurotransmitters. Amongst the latter, GABA is fundamental to brain function, mediating most neuronal inhibition. Until recently, GABA's role and specific molecular targets in the periphery within the BGM axis had received limited attention. Yet, GABA is produced by neuronal and non-neuronal elements of the BGM, and recently, GABA-modulating bacteria have been identified as key players in GABAergic gut systems, indicating that GABA-mediated signalling is likely to transcend physiological boundaries and species.

We review the available evidence to better understand how GABA facilitates the integration of molecularly and functionally disparate systems to bring about overall homeostasis and how GABA perturbations within the BGM axis can give rise to multi-system medical disorders, thereby magnifying the disease burden and the challenges for patient care.

Analysis of transcriptomic databases revealed significant overlaps between $GABA_AR$ subunits expressed in the human brain and gut. However, in the gut, there are notable expression profiles for a select number of subunits that have received limited attention to date but could be functionally relevant for BGM axis homeostasis.

GABAergic signalling, via different receptor subtypes, directly regulates BGM homeostasis by modulating the excitability of neurons within brain centres responsible for gastrointestinal (GI) function in a sex-dependent manner, potentially revealing mechanisms underlying the greater prevalence of GI disturbances in females. Apart from such top-down regulation of the BGM axis, a diverse group of cell types, including enteric neurons, glia, enteroendocrine cells, immune cells and bacteria, integrate peripheral GABA signals to influence brain functions and potentially contribute to brain disorders.

We propose several priorities for this field, including the exploitation of available technologies to functionally dissect components of these GABA pathways within the BGM, with a focus on GI and brain-behaviour-disease. Furthermore, *in silico* ligand-receptor docking analyses using relevant bacterial metabolomic datasets, coupled with advances in knowledge of $GABA_AR$ 3D structures, could uncover new ligands with novel therapeutic potential. Finally, targeted design of dietary interventions is imperative to advancing their therapeutic potential to support GABA homeostasis across the BGM axis.

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Introduction: the long and winding road between gut, bacteria, the immune system and the brain

Just as greater use of common languages and technology has advanced communication across diverse communities, extraordinary evidence is emerging about how our individual organ systems have long employed common chemical messengers for effective internal communication across biological borders. A striking example is the appreciation of the roles of certain neurotransmitters beyond neural communication. Central to this pursuit has been the advancement in understanding the profound functional interconnectedness between the gut, its resident microbes, the local gut and systemic immune systems, and the brain, commonly termed the brain–gut–microbiome (BGM) axis.[1]

An array of chemicals is emerging as mediators of this BGM interconnectedness. These chemicals include neurotransmitters, bacterial metabolites and immune mediators, which traditionally were thought to be produced predominantly by neurons, bacteria and immune cells, respectively. However, some chemical messengers and targets are common to all such systems, notably GABA and its receptors. Here, we review the evidence for GABA as an important common messenger that integrates the collective functions of these diverse systems and how these pathways may provide novel therapeutic targets for brain disorders, specifically in psychiatry.

GABA in the brain and beyond—more than a conventional inhibitory transmitter across the BGM axis

GABA signalling within and between neural circuits of the BGM axis

A detailed discussion of the complex organization of the BGM axis is beyond the scope of this review and is extensively described elsewhere.[1-3] Here, we briefly outline the main elements of the BGM axis to provide the molecular, functional and theoretical context for a discussion of the role of GABA in the subsequent sections.

The term BGM axis has come to describe, in the animal kingdom, a bidirectional communication system between the brain and the gastrointestinal (GI) system, the latter often referred to as the gut. This complex highway utilizes specific neuronal pathways, most notably the vagus nerve,[4] together with endocrine and immune signals and pathways, and a range of chemical messengers, many of which are of microbial origin. These include classical neurotransmitters such as serotonin (5-HT) and GABA, short-chain fatty acids (SCFAs), bile acids and their metabolites,[5-7] all of which act together with GI resident microbiota to provide optimal homeostatic regulation of the host's functions and physiology. Focusing on GABA, we begin by examining the neuronal roles of this molecule both in the brain and in GI functions and reviewing how GABA participates in reciprocal communication between the two ends of the BGM axis through tailored neuronal pathways.

GABA regulation of neuronal excitability in the central and enteric nervous systems

The quintessential notion of GABA's role in mammalian neural function is to provide the principal mechanism for neuronal inhibition. The canon of GABA-mediated neuronal inhibition in the CNS is founded on evidence, primarily in the brain, showing the exquisite organization of diverse sources of GABA, including both neuronal elements, notably interneurons,[8] and glial cells.[9,10] Furthermore, the release of GABA is spatially and temporally coordinated across distinct sub-cellular domains of target neurons and glia[11] in alignment with different GABA receptor subtypes in a cell-type- and brain-region-specific manner.[12-14] The functional consequences are a myriad of pre- and postsynaptic inhibitory signatures. This signalling diversity, coupled with the burgeoning evidence of non-synaptic, persistent forms of tonic inhibition (see Box 1), serves to orchestrate neuronal activity within and across brain regions.[15] However, the role of GABA extends beyond the brain since GABA appears to be a modulator of a variety of GI functions. Molecular analyses provide direct evidence for the expression of GABA, as well as its two main receptor types, the ionotropic $GABA_A$ receptor ($GABA_AR$) and the G-protein coupled metabotropic $GABA_B$ receptor ($GABA_BR$) within the enteric nervous system (ENS), also known as the second brain.[16] However, functional roles for GABA in mediating fast synaptic transmission in the gut are largely indirect and based on the application of exogenous GABA or a combination of receptor ligands. Yet, recent evidence indicates that neurally-released GABA strongly modulates slow inhibitory potentials in the mouse ileum via ρ-containing $GABA_ARs$ (previously known as $GABA_CRs$), which exhibit a distinct pharmacology, e.g. they are insensitive to bicuculline block (see also the 'Gastrointestinal GABA regulation of brain function' section).[17,18]

Collectively, studies of GABA's physiological actions suggest a myriad of functions, including the modulation of motility, secretions and immune function. Providing unequivocal evidence for the contribution of endogenous GABA and/or other putative endogenous GABA agonists (e.g. taurine) to native GI function is a key priority for future research. Salient aspects of GABA–receptor signalling in the brain are summarized in Box 1. An important

Box 1 Molecular, cellular and biophysical properties of $GABA_AR$ and $GABA_BR$ signalling in the brain

- GABA fulfils a canonical inhibitory role via two main distinct modalities: (i) the rapid, locally and temporally precise mode of inhibitory transmission mediated by the vesicular release of sub- to high-mM GABA concentrations acting mainly on synaptic GABA type A receptors ($GABA_ARs$); and (ii) a sustained, temporally diffused mode of tonic inhibition or a combination of both.[19] The latter is mediated by both extrasynaptic $GABA_ARs$[13,20,21] and GABA type B receptors ($GABA_BRs$),[22] located beyond the synaptic domain, and activated by low micromolar GABA concentrations. Because of their conspicuous localization on presynaptic nerve terminals, the role of $GABA_BRs$ in neuronal inhibition was initially viewed through the lens of the inhibition of transmitter release across neuronal networks. However, their participation in post-synaptic transmission, originally described in the hippocampus,[23] and tonic inhibition has received growing attention.[15]
- $GABA_ARs$ and $GABA_BRs$ exhibit distinct inhibitory mechanisms as members of ligand-gated and G-protein coupled receptor families, respectively. $GABA_ARs$ are ligand-gated channels where GABA binding results in the rapid (on a sub-ms to ms time scale) opening of an associated channel, selectively conducting mainly negatively charged Cl^- ions. In both the brain and spinal cord, Cl^- ions mostly, but not always, flow into neurons because of an electrochemical gradient that drives Cl^- influx, resulting in a membrane hyperpolarization that makes neurons less excitable.[13] Importantly, this gradient is imposed by both the neuronal resting membrane potential and the relative expression of the two transport systems regulating the extrusion (KKC2) and influx (NKCC) of Cl^-.[24] Apart from being responsible for a hyperpolarizing response, GABA additionally produces an associated so-called shunting inhibitory action by decreasing the neuronal membrane resistance, as per Ohm's law (V = IR), making neurons less likely to reach the membrane potential threshold for action potential generation in response to excitatory signals, e.g. glutamate, the main excitatory transmitter in the CNS.[25]
- $GABA_BRs$ are G-protein receptors, coupled to either voltage-gated Ca^{2+} or K^+ channels, and their activation results in a delayed net efflux of positively charged ions due to closure and opening of the G-protein linked Ca^{2+} and K^+ channels. The net effect is hyperpolarizing but on a significantly longer time scale than $GABA_ARs$, i.e. hundreds of ms.[26]
- Even within the brain, there are exceptions to this predominant inhibitory role for GABA. For example, during development, $GABA_AR$ signalling in the brain, via chloride-permeable $GABA_ARs$, is depolarizing and excitatory,[27-29] largely due to the delayed postnatal expression of the chloride-extruder KCC2, compared with NKCC,[30] in comparison to adult levels, when the former dominates the latter. Similarly, GABA can be hyperpolarizing and inhibitory or depolarizing and potentially excitatory on individual cells, depending on the GABA release sites across its surface and the associated electrochemical Cl^- gradients. For example, GABAergic interneuron targeting of somatic and dendritic regions of cortical pyramidal neurons is hyperpolarizing but GABAergic axo-axonic cells, which selectively synapse on the pyramidal cell axon initial segments,[31] have excitatory effects[32] due to a reverse axo-somato-dendritic chloride gradient.[33] Nevertheless, depolarizing actions may still be inhibitory because of a prevailing shunting effect.[34,35]

difference to GABA's predominantly hyperpolarizing and inhibitory effect in the adult brain is that in the gut and peripheral organs, it is generally, but not always (Box 1 and the 'Gastrointestinal GABA regulation of brain function' section), considered to be excitatory.[34] This excitatory action through $GABA_ARs$, is driven by an electrochemical gradient that leads to chloride (Cl^-) efflux rather than influx, resulting in membrane depolarization.

A fundamental feature of $GABA_AR$-mediated inhibition, underpinning its widespread and complex influence in both brain and GI function, is the considerable receptor heterogeneity that supports a rich pharmacology. This diversity is provided by the molecular and structural organization of $GABA_ARs$, which are pentameric complexes derived from 19 different subunits (6α, 3β, 3γ, δ, ϵ, ϑ, π, 3ρ)[36] [Fig. 1A(i–iii)]. In principle, this heterogeneity allows for a potentially staggering number of receptor subtypes. However, the rules governing the specific subunit assembly *in vivo*, with initial estimates proposing 20–30 major subtypes, are yet to be fully understood.[37,38] More recent studies strongly suggest an even greater degree of heterogeneity.[39,40] The most commonly occurring receptor subtypes, at least in the brain, contain two α and two β subunits together with a γ, δ or ϵ subunit [Fig. 1A(ii)]. It should be noted that $GABA_ARs$ are members of the Cys-loop family of membrane-spanning neurotransmitter-gated ion channels, which include most notably nicotinic acetylcholine, 5-HT3 and glycine receptors, and that there is a degree of cross-activity between some of the antagonists acting at these different receptors.

This $GABA_AR$ diversity gives rise to an extensive pharmacology,[36,38,42,43] with numerous therapeutically useful agents that enhance receptor function allosterically. These agents act via sites distinct from that of GABA itself, functioning as positive allosteric modulators (PAMs). Such ligands include benzodiazepines (BZs), a variety of general intravenous anaesthetics, including etomidate and propofol, and a class of brain-made steroids called neurosteroids (NSs)—notably the progesterone metabolite, 5α-pregnan-3α-ol-20-one, known as allopregnanolone (ALLO), and tetrahydrodeoxycorticosterone (THDOC), which are devoid of classical hormonal actions[44] [Fig. 1A(iv) and Box 3].

The receptor subunit composition impacts function, localization and pharmacology. For example, receptors incorporating the γ2 subunit together with either the α1,2,3 or 5 subunits are particularly sensitive to BZ ligands and localize, albeit not exclusively, to synaptic locations to mediate fast synaptic inhibition.[13] Receptors without the γ2 subunit are not responsive to BZs,[38] whereas receptors incorporating the δ subunit are insensitive to classical BZ ligands but exquisitely sensitive to the agonist gaboxadol and exclusively localized to extrasynaptic neuronal domains.[20,36,45] In each instance, the receptor subtypes display unique properties ideally suited to the form of inhibition they mediate. For example,

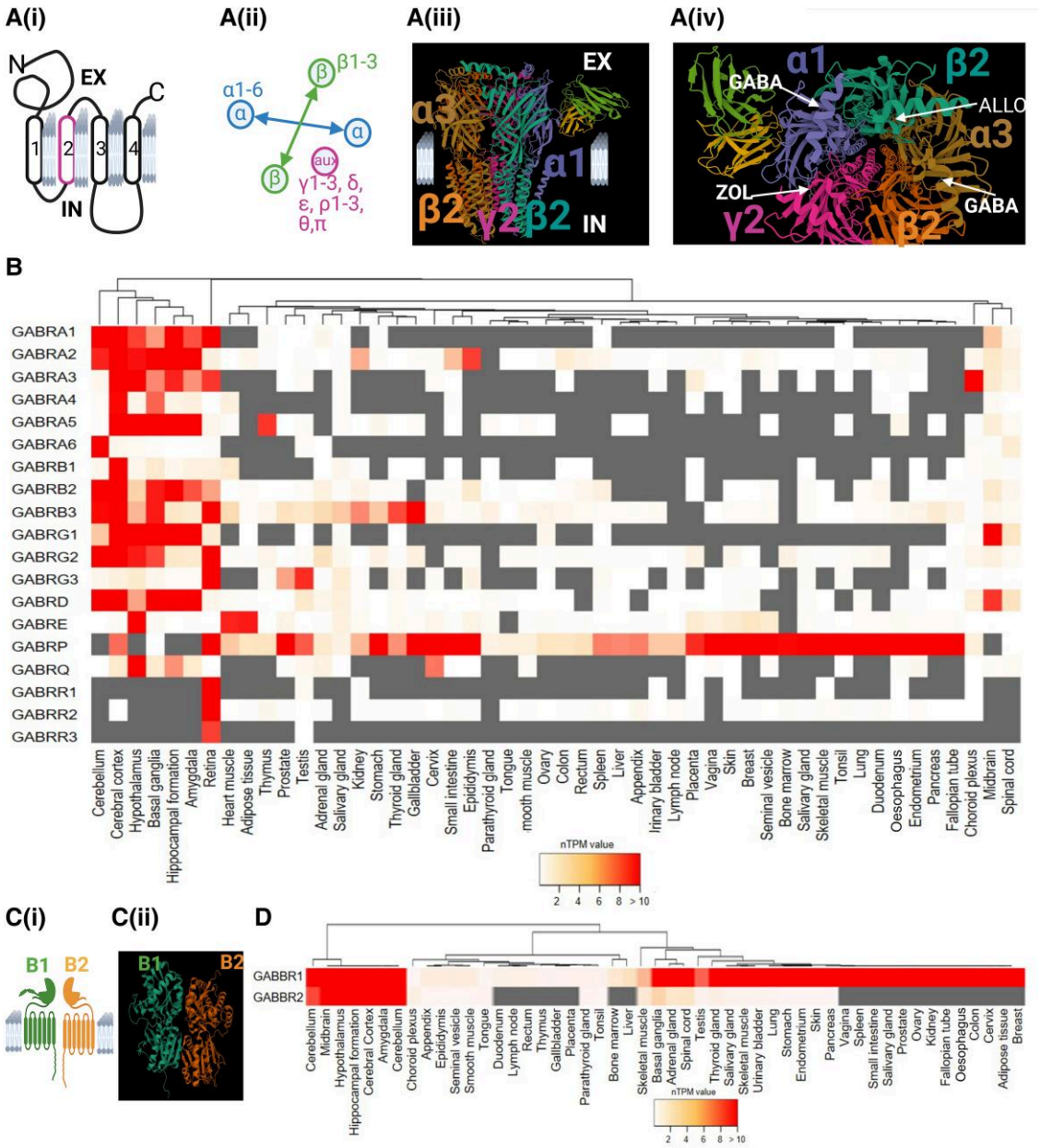

Figure 1 Molecular, pharmacological and expression diversity of mammalian GABA receptors throughout the body. [**A(i)**] The basic structure of an individual GABA type A receptor ($GABA_AR$) subunit (viewed from the side and flattened), when anchored in the plasma membrane. These proteins consist of four transmembrane domains (TM 1–4), two intracellular loops and one extracellular loop, an extracellular amino terminus (N) and carboxyl (C) termini. TM 2 is distinguished by putatively forming the lining of the ion channel. EX = extracellular; IN = intracellular. [**A(ii)**] The molecular diversity of individual $GABA_AR$ subunits, as well as their general stoichiometry, assembling the pentameric $GABA_AR$ complex, which contains two α- and two β-subunits and a single auxiliary (AUX) subunit. [**A(iii)**] Model of the 3D crystal structure of an assembled $GABA_AR$, viewed from the side. The image was generated by the Protein Data Bank in Europe, 8g5h, and is based on data originally published by Sun *et al.*[40] [**A(iv)**] provides a view from the top of the $GABA_AR$ mentioned in **iii**, illustrating (arrows) the diversity of receptor sites at which ligands have been shown to bind. These include separate sites for the endogenous ligand and neurotransmitter GABA, the exogenous, positive allosteric modulator zolpidem (ZOL) and the endogenous neurosteroid allopregnanolone (ALLO). The data indicate that the GABA binding site is located at the interface of the α- and β-subunits, zolpidem binds to the extracellular domain interface of the α1- and 2-subunits, and the binding pockets for ALLO are located at the interface between TM 1 and 4 of the α-subunit and TM 3 of the adjacent β-subunit.[40] **B** illustrates the relative mRNA expression levels of 19 different human $GABA_AR$ subunits across various tissues in the human body, based on consensus datasets from the Protein Atlas database (https://www.proteinatlas.org/). The heat map was generated using Heatmapper (http://www.heatmapper.ca/). Expression levels are represented by colour intensity: grey indicates no expression, red indicates high expression levels and white indicates low expression levels. The values are presented in normalized transcripts per million (nTPM). [**C(i)**] The basic structure of an individual GABA type B receptor ($GABA_BR$). The receptor is composed of two subunits, GABAB1 and GABAB2, and belongs to the superfamily of metabotropic G-protein coupled receptors. [**C(ii)**] Model of the 3D crystal structure of the extracellular regions of an assembled $GABA_BR$, viewed from the side. The image was generated by the Protein Data Bank in Europe, 4mr7, and is based on data originally published by Geng *et al.*[41] (**D**) The relative mRNA expression levels of the two $GABA_BR$ subunits across various tissues in the human body, based on consensus datasets from the Protein Atlas database, created in a similar manner to **C**. The figure was prepared using BioRender (www.biorender.com).

δ-subunit containing $GABA_A$Rs (δ-$GABA_A$Rs) have a high affinity for GABA and exhibit limited desensitization, both congruent for mediating sustained tonic inhibition. Indeed, some δ-$GABA_A$Rs spontaneously open in the absence of GABA.[46,47] We refer to comprehensive reviews for an in-depth discussion.[20,48]

Although a relative consensus has been reached in terms of our understanding of the assembly,[37-40] function and pharmacology of $GABA_A$Rs in the brain,[20,48] a similar knowledge base is a priority for other organ systems. This development is important because data indicate extensive and diverse $GABA_A$R subunit expression throughout most organs, at least at the transcriptomic level in humans (Fig. 1B). This diversity is also evident in other animals.[49-52]

The molecular configuration of metabotropic $GABA_B$Rs is much simpler since only two different subunits are required for their function, $GABA_{B1}$ and $GABA_{B2}$. The GABA binding is on the $GABA_{B1}$ subunit, while the $GABA_{B2}$ subunit induces intracellular signalling[53] (Fig. 1C). As with $GABA_A$Rs, $GABA_B$Rs are widespread in the periphery (Fig. 1D), including the ENS,[54-56] where they not only modulate presynaptic transmitter release[57] but may contribute to both synaptic and tonic inhibition, the latter via extrasynaptically located receptors, in a manner similar to that in the brain.[58]

Organization of brain GABAergic pathways that regulate gut functions

The complex anatomical and physiological organization of brain GABAergic signalling in highly organized laminated regions such as the cortex has been widely described.[12,59-63] However, we know comparatively less about the anatomically more diffuse cell groupings of subcortical brain regions that mediate gut function. Of particular importance is the full molecular, morphological and physiological characterization of individual GABAergic cell types. Furthermore, it is imperative to identify any distinctive GABA pharmacological and physiological signatures and their collective contribution to brain and gut functions. Specifically, GABA-mediated top-down CNS regulation of GI function is important in the context of BGM signalling in health and disease, as alterations to GI parameters commonly occur in a range of brain disorders, most notably those associated with maladaptive responses to psychosocial stress (see the 'Gastrointestinal GABA regulation of brain function' section). Here, we focus on three key nuclei, the medullary dorsal motor nucleus of the vagus (DMV), the pontine locus coeruleus (LC) nucleus and Barrington's nucleus (BN), since they modulate motor pathways to the stomach/small intestinal (DMV) and large intestinal (LC and BN) segments of the GI tract (GIT), respectively.

The primary CNS motor output to the upper GIT is mediated by spontaneously firing DMV neurons,[64,65] the majority of which are cholinergic.[66,67] GABAergic regulation of DMV neuronal excitability significantly modulates the function of the GIT and other viscera (Fig. 2A). This DMV GABAergic tone arises from inputs of the neighbouring nucleus tractus solitarii (NTS)[68] and from a subpopulation of putative GABAergic local circuit interneurons.[69,70] Immunolocalization analyses indicate that human DMV neurons express at least α1–3, β2–3 and γ2 $GABA_A$R subunits.[71] This is corroborated by pharmacological analyses of $GABA_A$R-mediated synaptic and tonic currents in rodent DMV neurons using the PAM zolpidem,[72,73] which exhibits preferable affinity and efficacy at α1- versus α2/3-, and none for α5-, subunit-containing $GABA_A$Rs[74,75] [Fig. 2A(i)] and the δ-preferring $GABA_A$R agonist gaboxadol,[36] respectively.[73] Indeed, a recent single-cell RNA sequencing (scRNA-seq) transcriptomic analysis revealed two populations of DMV cholinergic cell types co-expressing either cholecystokinin or prodynorphin and projecting selectively to either ENS cholinergic or nitrergic neurons of the stomach[76] [Fig. 2A(ii)]. Our analysis of their deposited data for $GABA_A$R subunit mRNA expression levels revealed significant differences between these two populations of DMV neurons [Fig. 2A(iii)]. Irrespectively, the net effect of activating DMV $GABA_A$Rs is decreased GI function. This is evidenced by the *in vivo* application of the $GABA_A$R antagonist bicuculline to the DMV, which increases gastric motor function, intragastric pressure, gastric motility and gastric secretion.[77,78]

Intriguingly, this $GABA_A$R-mediated inhibition of DMV neurons is sexually dimorphic. Indeed, the application of the $GABA_A$R antagonist bicuculline to DMV neurons induced a more significant increase in the firing rate of DMV neurons, as well as gastric tone and motility, in female rats that were in the high-oestrogen/low-progesterone stage of their cycle, compared to those in the low-oestrogen/high-progesterone stage and male rats.[89] This difference in GABAergic tone could contribute to the documented sex differences in native GI functions[90] and GI disorders.[91] In contrast to the inhibitory effect of DMV $GABA_A$R activation on GI function, the activation of DMV $GABA_B$Rs enhances its output.[92] $GABA_B$Rs are often located at axon terminals, such as those of GABAergic interneurons, where they might reduce their output, suggesting that pre- and postsynaptic GABAergic tone on DMV neurons dynamically contributes to GI homeostasis.

The direct influence of DMV neurons on GI function arises, in part, because most of these neurons project to ENS neurons in the myenteric plexus (see the 'Gut neuronal GABAergic pathways' section; Fig. 2) as well as to the pacemaker interstitial cells of Cajal (ICC) in the upper GI tract.[93-95] BN is another pontine centre[96] important for regulating visceral organ function. Its preeminent role is in the neural modulation of micturition,[97] but it also regulates the voiding behaviour of the gut.[98] Whilst the DMV primarily modulates upper GI functions related to the oesophagus, stomach and small intestine, the BN-LC circuitry has been shown to regulate distal colonic functions and those of other pelvic viscera. Indeed, distension of the urinary bladder, distal colon, rectum or stomach induces significant activation of the principal noradrenergic neurons of the LC[99,100] (Fig. 2B). This regulation appears to be bidirectional since the activation of a key input to the LC, namely BN, increases colonic intraluminal pressure.[98] Convergent data indicate a trans-neuronal circuit[101] linking LC and BN activities[102] to the regulation of pelvic organ voiding behaviours.[103] Similar to the DMV, both $GABA_A$Rs and $GABA_B$Rs are expressed within the LC[71,85,104] and regulate the spontaneous firing rate of the principal neurons.[105-107] The sources of GABA input are diverse and include putative inhibitory neurons in the LC pericoeruleur dendritic region[84] and the LC nuclear core.[81-83] However, contrary to the DMV, where both centres contain α1/2/3-containing $GABA_A$R subtypes, within the LC, these subunits are expressed selectively on either principal cells or local circuit interneurons. Indeed, LC noradrenergic neurons express only α2/3-containing $GABA_A$R subtypes and not the α1 subtype.[85,86] The deletion of the α3 subunit increases LC noradrenergic neuron excitability.[86] In contrast, local GABAergic interneurons, which mediate the inhibitory regulation of LC noradrenergic neurons, express the α1 subunit. Activation of these receptors is likely to decrease the activity of such local inhibitory inputs to LC noradrenergic neurons. This increases noradrenergic tone, mirroring other brain regions with similar $GABA_A$R expression patterns, such as the ventral tegmental area.[108] Importantly, pontine GABAergic somatostatin-expressing neurons,

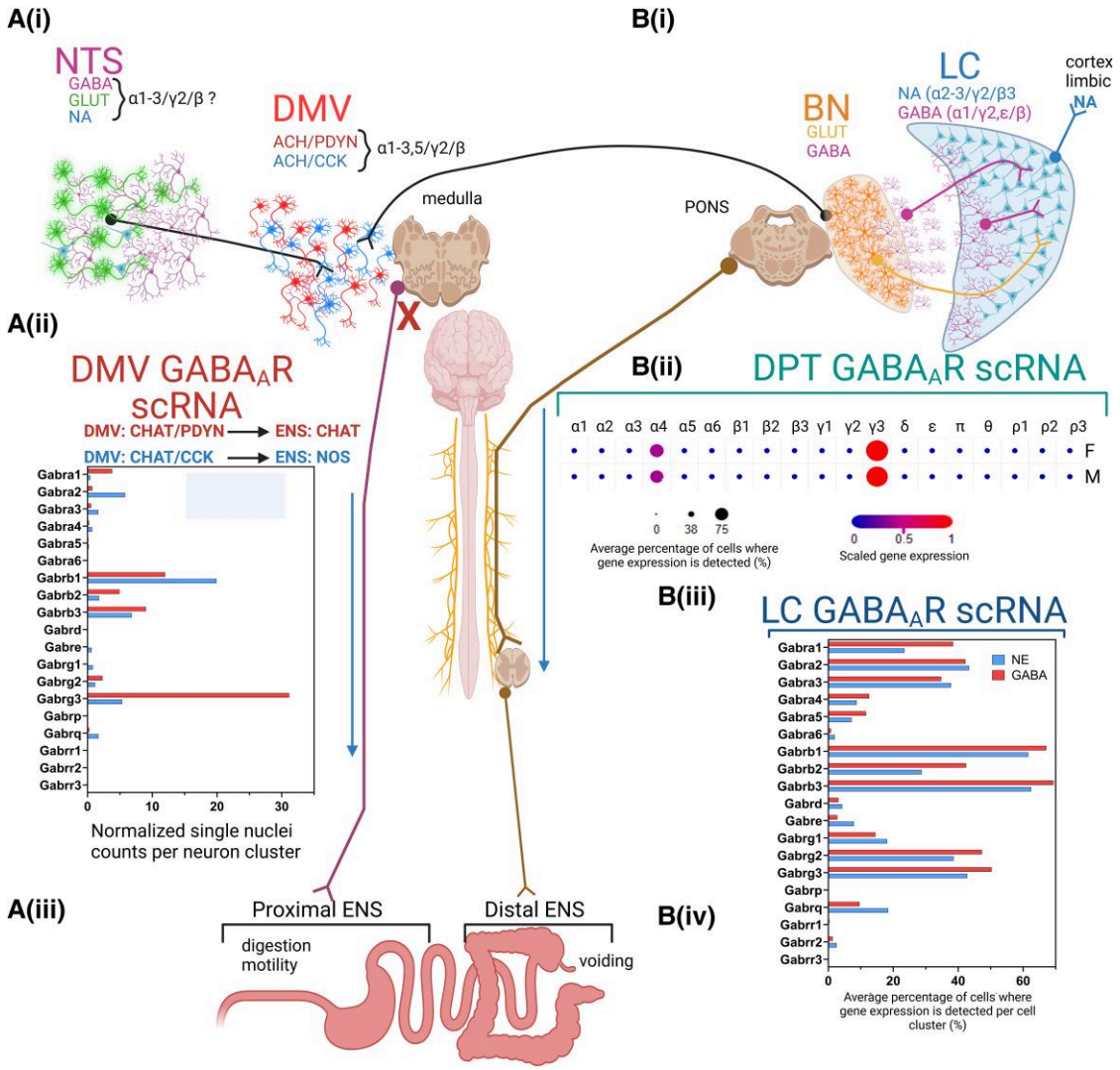

Figure 2 Roles of GABAergic signalling in brain-to-gut neuronal pathways. (**A**) Key brain centres located within the medulla that are involved in providing motor output to the gastrointestinal tract (GIT). [**A(i)**] Organization between neurochemically diverse neurons of the nucleus of the solitary tract (NTS) and one of its major projection targets, the dorsal motor nucleus of the vagus (DMV). GABAergic inputs from the NTS onto DMV neurons are mediated by a range of GABA type A receptor ($GABA_AR$) subtypes.[71-73] Whilst the majority of DMV neurons express acetylcholine (ACh), they can also be divided into two subpopulations based on their co-expression of either cholecystokinin (CCK) or prodynorphin (PDYN).[76] DMV axons project via the 10th cranial nerve (X) to make direct synaptic connections with their enteric nervous system targets in the myenteric plexus. [**A(ii)**] The two distinct types of DMV cholinergic neurons, CCK (blue) and PDYN (red), contact different enteric neuronal types (Chat+ and NOS1+) to induce gastric contraction and relaxation, respectively. A single-nucleus RNA sequence (snRNA-seq) analysis performed on data retrieved from a report by Tao *et al.*,[76] revealed the differential expression of $GABA_AR$ subunits between the two types of DMV cholinergic neurons, CCK and PDYN, in mouse models. **A(iii)** shows that this NTS–DMV pathway, as well as the associated $GABA_AR$ subtypes, preferentially regulates the proximal regions of the GIT. (**B**) Key brain centres located within the pons that are involved in providing motor output to the distal GIT (B4). [**B(i)**] Barrington's nucleus (BN) interacts with the locus coeruleus (LC) in regulating voiding behaviour of the gut viscera through their modulation of the distal colon. The principal noradrenergic (NA) neurons of the LC receive excitatory input from BN neurons.[79] They also receive inhibitory inputs from GABAergic interneurons located near BN[80] as well as within the LC.[81-84] Whilst LC NA neurons have been shown to express α2/3-β-γ-$GABA_AR$ subunits, non-NA, GABAergic LC neurons express α1-β-γ-$GABA_AR$ subunits.[71,85,86] [**B(ii)**] Single-nucleus RNA sequencing (RNA-seq) data reported by Nardone *et al.*[87] were inspected in the Broad Institute Single Cell portal (https://singlecell.broadinstitute.org/single_cell), reference 'pons_exc_neurons_snrnaseq' to compare the gene expression of $GABA_AR$ subunits between female (F) and male (M) mice in the dorsal pontine tegmentum (DPT) region. The sizes of the dots in the dot plot indicate the percentage of cells in which gene expression was detected, and the colour scale indicates the magnitude of gene expression (scaled gene expression defines the expression maximum value as 1 and the minimum as 0). The DPT includes the pre-LC, LC, BRN and mesencephalic trigeminal nucleus (MTN) centres. [**B(iii)**] An analysis of mouse LC single-cell RNA-seq data reported by Luskin *et al.*[88] shows the average percentage of $GABA_AR$ subunit gene expression per cell cluster. Noradrenergic (NE) neurons are shown in blue and GABA neurons in red. [**B(iv)**] The selective innervation of distal colon by this pathway and its involvement primarily in voiding behaviour. The figure was prepared using BioRender (www.biorender.com).

putatively linked to LC-BRN circuits that regulate visceral organs, provide GABAergic modulation of LC neurons[80] [Fig. 2B(i)]. Our detailed analysis of data from recent scRNA-seq studies of dorsal pons[87] and LC cells[88] is shown in Fig. 2(ii and iii) and offers a more comprehensive picture of $GABA_AR$ subunit expression. In summary, $GABA_AR$ ligands, depending on their pharmacological profiles, potentially either increase or decrease BN-LC neuron activity and thus influence downstream targets, such as pelvic viscera,

in comparison with DMV neurons, which are invariably inhibited. Collectively, the impact of these central GABA pathways in both the DMV and BN-LC extends to vast swathes of GI homeostatic mechanisms.

Organization of gut neuronal GABAergic pathways and how they modulate brain function

Whilst the DMV provides top-down brain modulation of GI function, its activity is strongly mediated by GABAergic neurons from the NTS, which in turn are strongly modulated by GI afferents via the vago-vagal reflex.[109] These afferent signals represent integrated information from a constellation of different cell types within the ENS of the stomach, duodenum and upper jejunum. These signals are conveyed by sensory axons arising from the inferior ganglion neurons of the vagus nerve, the nodose ganglia. Nodose ganglionic centrally projecting axons innervate the NTS[110] [Fig. 3A(i)]. Similarly, sensory information about the physiological status of the distal colon is conveyed via sensory axons of lumbosacral dorsal root ganglion neurons (LSDRG). Centrally projecting axons from LSDRG innervate sacral spinal projecting neurons to activate brain centres, including BN and the LC [Fig. 3A(ii)].

A number of GI neuronal and non-neuronal cell types have been documented to express different $GABA_AR$ subunits, including intrinsic primary afferents, enteric glia, enterocytes, tuft cells and enteroendocrine cells (EECs) [Fig. 3A(iii)]. Several EEC subtypes have been identified based on location and the peptides and hormones they secrete, and they are particularly important in integrating GI, endocrine and brain signalling.[131] An important subtype of EECs, the enterochromaffin cells (ECs), utilize 5-HT to signal, but there is a sub-population that is immunoreactive for GABA.[120] Furthermore, 5-HT-containing ECs also express $GABA_ARs$,[121,122] and their activation has been shown to induce 5-HT release.[132,133] Here, 5-HT is thought to act in a paracrine manner to stimulate nodose sensory neurons.[134] $GABA_ARs$ are also likely to be present on other EEC subtypes, where they may modulate the release of a variety of hormones and peptides, e.g. cholecystokinin.[135]

GABA also plays an important role in the intrinsic nervous system of the gut (ENS). The ENS regulates all facets of GI function, including motility, secretions and immune response[136] using a plethora of functionally and neurochemically diverse neurons distributed throughout the two neural networks of the myenteric and submucosal plexuses.[136] Remarkably, ENS neuronal activation has also been shown to modulate the gut microbiome, metagenome, transcriptome, metabolome and proteome directly.[137] A range of corroborative methods indicate the expression of GABA-containing neurons in the mammalian ENS, including humans.[138,139] Most of the GABA-containing neurons have been detected in the myenteric plexus and are estimated to comprise 5%–8% of total ENS neurons.[140] However, the extent and diversity of GAB_ARs throughout the intestine provide a more informative appraisal of the likely importance of GABAergic signalling to overall GI function. Functionally and pharmacologically distinct $GABA_AR$ subtypes are expressed on neurochemically and functionally diverse ENS neurons[50,115,141] [Fig. 3B(i)], and their activation has contrasting effects on GI parameters including contractility[51] [Fig. 3B(ii)] and immune status[127,128] [Fig. 3B(iii)].

GABA signalling within the ENS has also been shown to include enteric glia [Fig. 3A(iii)], which have established roles in the BGM axis in health and disease.[142,143] Enteric glia produce GABA[116] and thus can directly modulate GI functions such as motility.[117] The result is an ENS-GABA-mediated modulation of a range of GI functions, including receptor subtype-specific relaxation and enhancement of intestinal contractility, secretions, mucosal function and blood flow.[144] Our analysis of previously published ENS scRNA-seq data[129] revealed expression for a range of $GABA_AR$ subunits within these cell types (Fig. 3C). The consequences of GABA signalling at the local GI level can extend to the brain via tailored anatomical connections as illustrated in Fig. 3 or via diffuse systemic messengers (discussed in the 'Gastrointestinal GABA regulation of brain function' section).

BGM GABA signalling beyond neurons and glia: bacterial GABA in brain health and disease

A traditional neuroscience tenet is that the neurotransmitters that modulate neural function are of neural origin. This view broadly holds true for central neural pathways within the blood–brain barrier (BBB), where the communities of cell types are largely homogeneous—that is, only neurons and glia. However, in peripheral pathways, neurons co-exist with a range of non-neuronal cells, glia and tissues, without the parcellation that comes with the BBB. This renders PNS neurons susceptible to modulation by neurotransmitters produced by such non-neuronal elements. Given the influence of PNS pathways on brain function discussed earlier, such diverse peripheral neurotransmitters will impinge on brain function, albeit indirectly. There is no greater demonstration of this constellation of non-neuronal producers of neurotransmitters than the GI tract. In this organ, non-neuronal neurotransmitter sources include immune cells[145] (see the 'Gastrointestinal GABA regulation of brain function' section) and endocrine cells. Moreover, it has recently become apparent that resident gut microbes, most notably bacteria, can produce and utilize a variety of chemical messengers, including neurotransmitters, to mediate homeostasis within the microbiome, the gut and putatively beyond, i.e. the brain. This is important because a disrupted equilibrium in the gut microbiome, known as dysbiosis, is implicated in various medical conditions, including various brain disorders.[146,147] We summarize the growing evidence that resident gut microbiota, specifically bacteria, are a significant source of GABA and how this may contribute to health and disease.

Microbial GABA synthesis: a focus on bacteria

A bacterial source of GABA in the mammalian GI was initially inferred from the finding of reduced GABA content in both the stool and blood of germ-free mice.[148] This finding was corroborated by (i) the observation that faecal GABA levels are decreased by antibiotic use[149] and (ii) genetic analysis of the bacterial community in mammalian gut isolates.[150] While most evidence discussed here is derived from studies on colon-resident bacteria, emerging evidence suggests an important role for the less-investigated, although documented, bacteria that reside in the small intestine,[151] some of which (e.g. *Lactobascilli*) produce GABA.[152]

Microbes synthesize GABA via several routes, but the glutamic acid decarboxylase (GAD) and (to a minor extent) putrescine pathways predominate (Fig. 4A). GABA biosynthesis was first reported in strains from the genera *Lactobacillus, Lactococcus*, *Streptococcus* and *Bifidobacterium*,[153] with additional genera and species subsequently added.[154,155] Using GAD gene expression as a proxy for the ability to synthesize GABA, a variety of bacterial genera/species from the human gut microbiome have been identified, with the

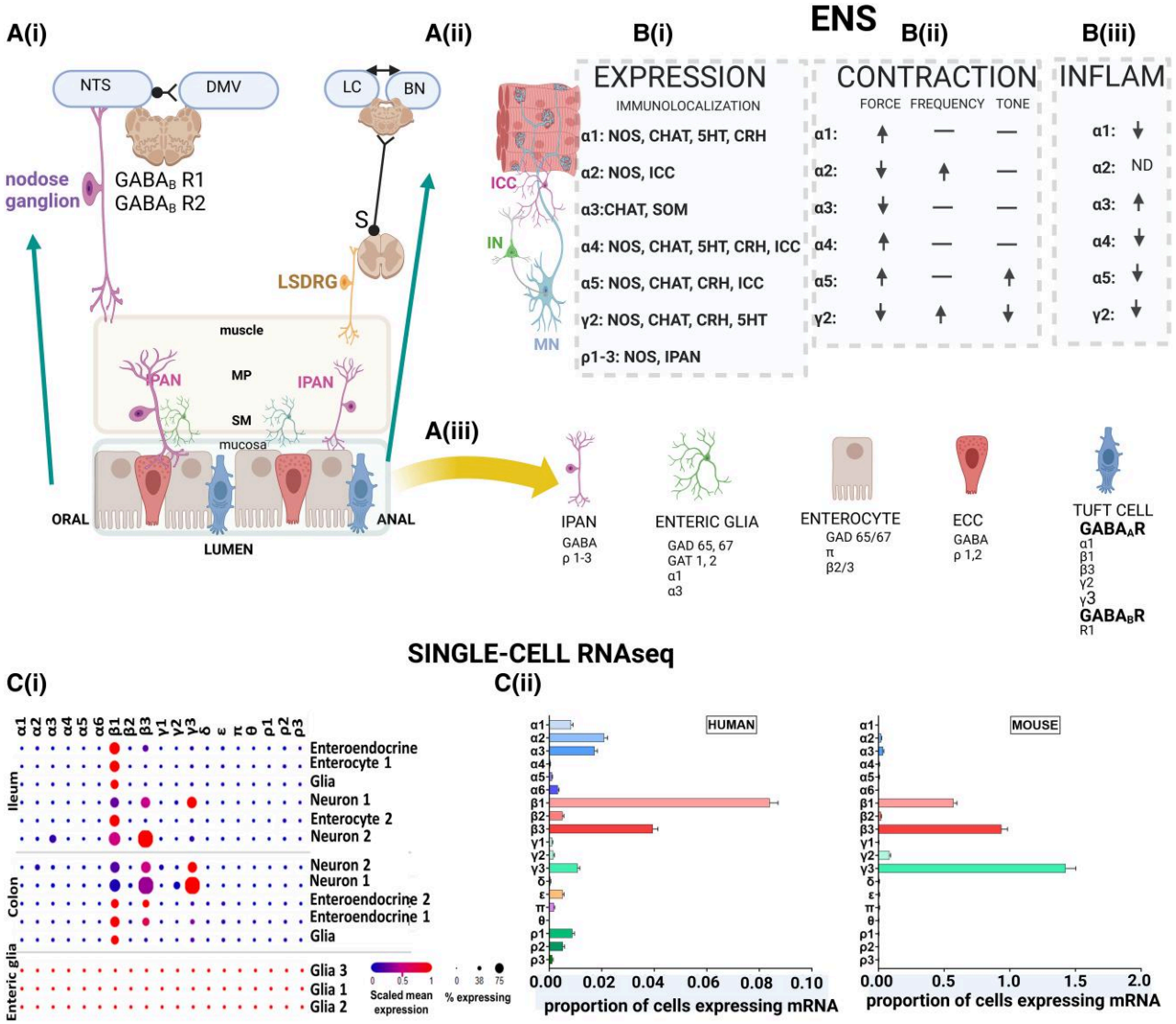

Figure 3 The roles of GABAergic signalling in neuronal and non-neuronal gut-to-brain pathways. (**A**) Schematic summary of the circuitry relaying gastrointestinal (GI) signals to the brain. [**A(i)**] In proximal (ORAL) regions, nodose ganglion (NG) sensory axons innervate the wall of the GI tract (GIT), with their centrally projecting axons innervating the nucleus tractus solitarii (NTS). The NTS in turn projects to neurons within the dorsal nucleus of the vagus (DMV). The NG is immunopositive for GABA type B receptors (GABA$_B$Rs) but not type A receptors (GABA$_A$Rs).[111] While NG cells are devoid of synaptic inputs, GABA may act here as diffusible transmitter released non-synaptically, for example, from local satellite glial cells.[112,113] [**A(ii)**] In distal (ANAL) regions of the GIT, sensory axons of lumbosacral dorsal root ganglion (LSDRG) neurons make a variety of contacts throughout the GIT, including the muscle, enteric nervous system (ENS) and submucosa.[114] LSDRG centrally projecting axons innervate sacral (S) spinal neurons that project to Barrington's nucleus (BN), which in turn activates neurons in the locus coeruleus (LC). [**A(iii)**] An overview of different cell types located in the mucosa and the associated GABAergic molecular machinery they have been demonstrated to express, including IPANS,[115] enteric glia,[116-118] enterocytes,[119] enteroendocrine cells (EEC)[120-122] and tuft cells.[123-126] MP = myenteric plexus; SP = submucosal plexus The green arrows indicate the direction of efferent information flow from the gut to the brain. **B** summarizes the expression and function of different GABA$_A$R subtypes within the mouse ENS. ICC = interstitial cells of Cajal; IN = interneuron; MN = motor neuron. [**B(i)**] The neurochemical identity of ENS neurons that have been shown immunohistochemically to express specific GABA$_A$R subunits.[50,51,115,127] [**B(ii)**] The effects of the pharmacological activation of different GABA$_A$R subtypes on various parameters underlying spontaneous contractions of the mouse colon.[51] [**B(iii)**] The effects of the pharmacological activation of different GABA$_A$R subtypes on intrinsic colonic inflammation.[128] N.D. = not determined. (**C**) An analysis of previously published single-cell RNA sequencing datasets of ENS GABA$_A$R subunit mRNA expression. [**C(i)**] An analysis of data published by Drokhlyansky *et al.*[129] The sizes of the dots in the dot plot indicate the percentage of cells in which gene expression was detected, and the colour scale indicates the magnitude of gene expression (scaled gene expression defines the expression maximum value as 1 and the minimum as 0). [**C(ii)**] An analysis of data published by Wright *et al.*[130] depicting the proportion of cells expressing subunits. The figure was prepared using BioRender (www.biorender.com).

genus *Bacteroides* the most abundantly represented[150,154] (Fig. 4B). Conversely, some strains that require GABA for growth have also been isolated from the human microbiome.[156] Further indirect support for a physiological role for bacterial GABA came from a recent in-depth screening of intestinal *Bacteroides*,[157] which demonstrated a high prevalence of GABA producers in human gut isolates. Crucially, 16/17 strains tested produced GABA at concentrations ranging from 0.09 to 60.84 mM, comparable with levels observed in high GABA-producing *Lactobacillus* and *Bifidobacterium* strains.[153] These levels fall within a physiological bioactive concentration

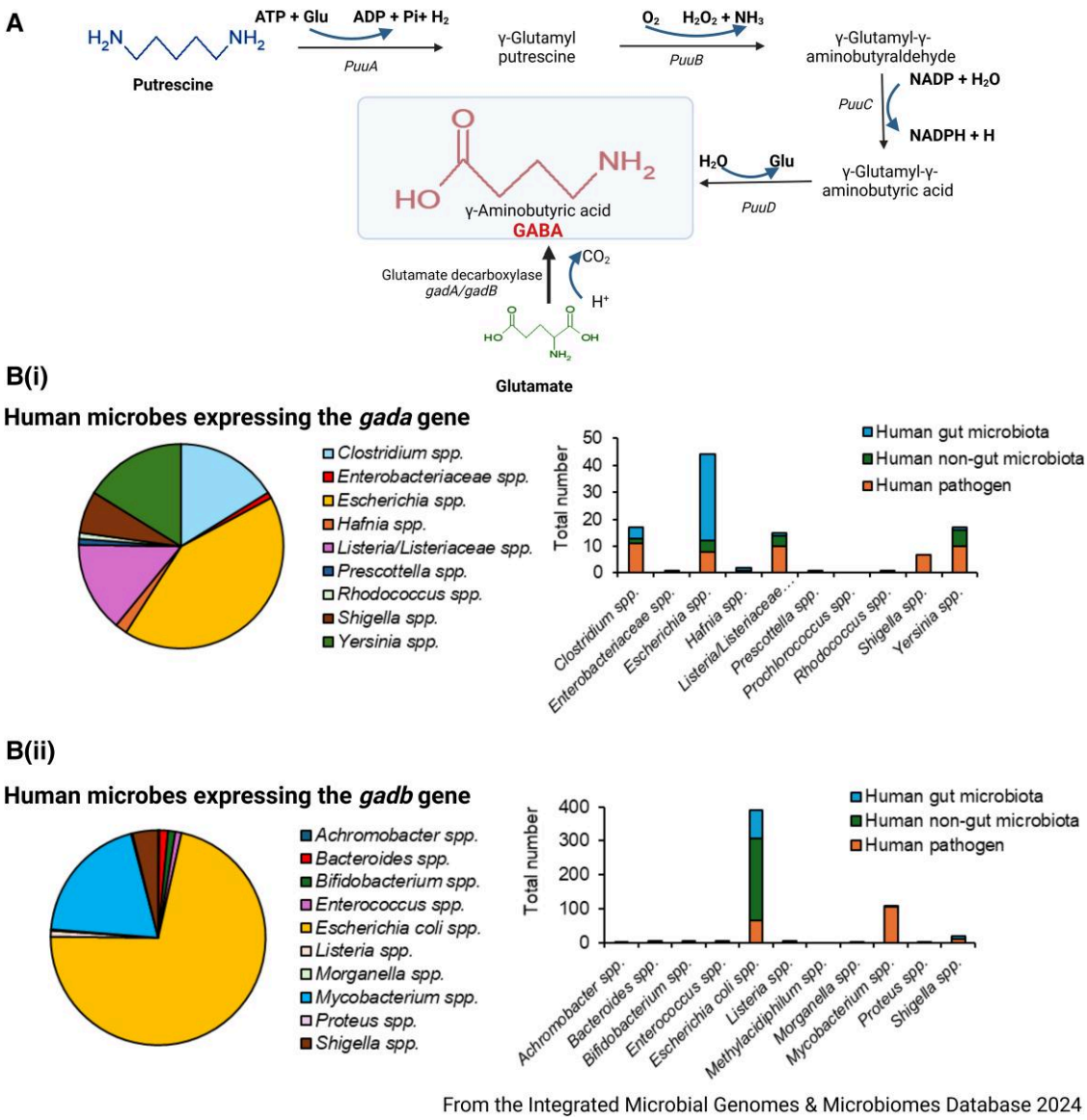

Figure 4 Bacterial GABA synthesis. (**A**) Pathways utilized by bacteria to synthesize GABA. The main synthetic route occurs via glutamic acid decarboxylase (GAD), encoded by *gadA/B* genes, while the putrescine pathways represent a minor route used by certain species such as *Escherichia coli*. These pathways are absent from *Lactobacillus* and *Bifidobacterium* strains, which instead utilize the GAD pathway.[158] In addition to the *gadA/B* genes, the bacterial GAD operon also includes a *gadC* gene, which encodes an antiporter, allowing GABA to be exported from the cell, as reviewed by Sarasa *et al.*,[155] Diez-Gutierrez *et al.*[158] and Yogeswara *et al.*[159] Note that the measured concentrations (sub to low mM) of GABA produced by human gut bacteria fall within a physiological bioactive range.[157] (**B**) An overview of human microbes expressing (**i**) *gadA* and (**ii**) *gadB* gene. Human microbes expressing either gene were identified from the Integrated Microbial Genomes & Microbiomes database (accessed on 30 June 2024). These microbes were categorized into gut microbiota, non-gut microbiota and pathogens. Pie charts illustrate the distribution, including types and relative contributions of all human microbial species from the database. Histograms depict the distribution of gut microbiota, non-gut microbiota and pathogens. The total number and percentages of human microbial species obtained from the database are described in the Supplementary material. The figure was prepared using BioRender (www.biorender.com).

range (Box 1). Dysbiosis of GABA-producing bacteria is increasingly associated with medical disorders, notably GI and psychiatric conditions, suggesting an important role for microbial GABA in the optimal functioning of the entire BGM axis.

A role for bacterially derived GABA in brain disorders? A focus on psychopathology

The importance of GABAergic signalling within the CNS for homeostasis and some brain disorders is well documented.[43,160] However, emerging evidence associates perturbations of bacterially-derived GABA from BGM dysregulation as a putative contributory factor to certain brain disorders, although the evidence is currently correlative rather than unequivocally causative. However, it is noteworthy that alterations in bacterially-derived GABA and/or the expression of GAD-system genes have been reported for a number of psychiatric and neurological disorders, including autism,[161,162] depression,[156,163,164] schizophrenia,[165] alcohol use disorder[166,167] and Alzheimer's disease.[168,169] Next, we focus specifically on the emerging evidence regarding mood disorders.

Bacteria from the genus *Bacteroides* are some of the main GABA producers implicated in altered mood. However, available evidence paints a complex and, in places, contradictory picture of how bacterial abundance is linked to health and disease. Several

reports suggest that deficits in *Bacteroides* numbers may negatively impact mood. In a recent functional MRI study, the abundance of *Bacteroides* was inversely correlated with both connectivity within the default mode network and the clinical depressive profile of a population with major depressive disorder (MDD). This finding is important because the default mode network, a group of brain regions showing coherent activity, is involved in negative rumination and self-referential thinking and shows elevated connectivity in the depressive state, which is normalized in response to antidepressant treatment.[170] Further corroborating evidence is provided by the reduced abundance of *Bacteroides* in faecal samples of MDD subjects.[171-173]. Human faecal metagenomic analyses indicate a potential role of altered microbial GABA production in depression.[164] Conversely, in healthy subjects, *Bacteroides* levels associate positively with grey matter volume in cortical regions, coupled with diminished anxiety/distress in healthy women exposed to images with emotional content.[174] Finally, the abundance of GABA-producing bacteria (*Bacteroides* and *Parabacteroides*) appears inversely related to fear behaviour in red fowls,[175] which could indicate that the BGM mechanisms linking emotional regulation with particular gut bacteria are conserved across species.

Although the *Bacteroides* genus is most consistently associated with brain connectivity studies,[176] the association between their abundance and psychopathology is inconsistent across publications, with some studies documenting an increase rather than a decrease in depressive clinical cohorts.[177] These results, coupled with findings from previous studies of MDD clinical populations, which uncovered additional significant changes in other microbiota genera and species (e.g. decreased *Faecalibacterium*[178] and increased *Prevotella* and *Klebisiella*[179,180]), strongly suggest that, while playing an important role, the *Bacteroides* genus alone cannot fully account for the depressive phenotype in human MDD. Instead, a balance amongst different microbiota species is likely a crucial driver of health or pathology. This complexity suggests that a more granular analysis at the species or strain level is necessary, as supported by a recent study that individually phenotyped mice colonized with one of the four most represented individual *Bacteroides* species identified in the microbiome of a 40-strong medicine-free MDD patient versus control cohort. This analysis revealed that only colonization with *Bacteroides fragilis*, *B. uniformis* or to a lesser extent *B. caccae* but not *B. ovatus* (see later) recapitulated both the behavioural and molecular indices of the stress-induced 'depressive' phenotype observed following transplantation with the whole microbiome from the MDD cohort.[181] Similar mechanistic approaches using mixed human-animal models are a major priority for establishing definitive causal relationships between gut bacteria and human mental states.[182]

The immunomodulatory profile of bacterial species may be particularly relevant in health and disease. A case-control study in school-aged children with depression revealed that a significant decrease in the *Bacteroides* genus correlated positively with a variety of inflammatory markers. Conversely, a negative association was evident for anti-inflammatory indicators.[183] This is consistent with growing evidence indicating that gut-associated systemic inflammation is a driver of mood disorders, e.g. depression.[184-189] Furthermore, as microbiota can generate a diverse signalling system, including but not limited to GABA, it is likely that the type and abundance of additional microbial products, e.g. SCFAs such as butyrate or acetate or other neurotransmitters, e.g. 5-HT[190,191] (see the 'Gastrointestinal GABA interaction with SCFA and 5-HT' section), participate in the observed clinical phenotypes.

In terms of the role of microbial GABA, specifically GABA derived from *Bacteroides*, in regulating mood, certain but not all *Bacteroides* species may contribute to the functional GABAergic deficits in the CNS that have been implicated consistently in clinical studies of depressive illness.[192-194] Of specific interest is a recent investigation revealing that colonization by *B. ovatus* influences the abundance of intestinal GABA and SCFA but not dopamine or noradrenaline.[195] Furthermore, *B. ovatus* is the most effective stimulator of IgA production[196] (Fig. 5), which is essential to gut homeostasis.[197] This finding is significant in view of the study discussed earlier,[181] where only mice transplanted with *B. ovatus* but not *B. fragilis, B. uniformis* or *B. caccae* appeared resistant to the stress-induced depressive phenotype and showed a selective increase in hippocampal BDNF, a putative biomarker of antidepressant action.[181] Finally, many of these findings are also relevant to substance use disorders since a disrupted microbiome, inflammation and psychiatric disturbances such as anxiety and depression have been implicated in the development and maintenance of chronic drug consumption,[198,199] notably alcohol use disorder.[166,189,200-204]

Mechanisms of gastrointestinal-derived GABA regulation of brain function

The mechanisms by which brain-derived GABA contributes to brain function in health and disease are widely reported.[43,225,226] In contrast, precisely how peripheral GABA systems modulate brain function via their influence on the GI system is less well understood. Since GABA is unlikely to cross the BBB,[227-229] peripherally produced GABA almost certainly influences brain function indirectly. Here, we focus on three key mechanisms: (i) the activation of $GABA_A$Rs in the ENS; (ii) $GABA_A$R activation in the immune system; and (iii) GABA stimulation of exosome-mediated signalling (Fig. 5). Additionally, GI-derived GABA may influence brain function as a modulator of gut microbiota by acting as a nutrient, fine-tuning microbial communities or targeting bacterial $GABA_A$Rs (Fig. 5 and Box 2).

Gastrointestinal GABA modulates brain function by activating GABA receptors on ENS neurons

$GABA_A$R subtypes expressed on neurochemically diverse ENS neurons modulate various GI functions, including motility, local immune function and barrier integrity.[51,128] These observations are important because, as discussed later, alterations to these GI parameters commonly occur in a range of brain disorders, especially those associated with maladaptation to psychosocial stress. For example, exposing rodents to stress that induces anxiogenic- and depressive-like behaviour increased colonic contractility, induced GI inflammation and impaired barrier function.[128] This overlap of GI and brain symptoms is commonly present in patients with stress-associated conditions such as dysmotility and inflammation in irritable bowel syndrome (IBS)[244] as well as mental illnesses such as anxiety.[245] Importantly, some $GABA_A$R PAMs ameliorate both GI and brain symptoms, further evidencing the interconnectedness of GABA pathways throughout the BGM axis in health and disease. For example, the widely used anxiolytic alprazolam, a known PAM of $GABA_A$Rs, induces a robust relaxation of GI tone in animals.[51] This may represent the amelioration of dysmotility, a core somatic component of anxiety. Moreover, a novel role has emerged for α3-$GABA_A$Rs, since in a rodent model of early-life stress (ELS), α3-$GABA_A$Rs appear necessary and sufficient for the ELS-evoked

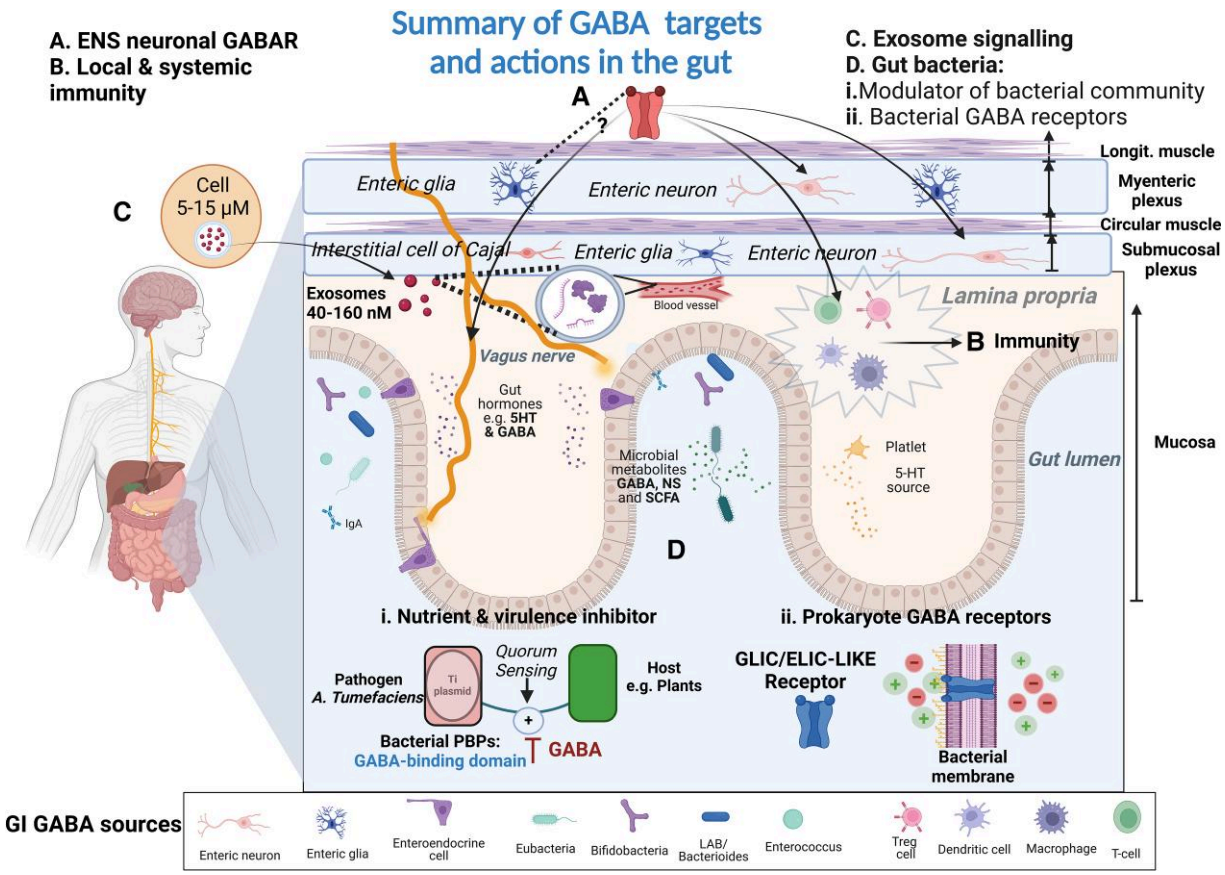

Figure 5 Versatile sources, actions and targets of GABA in the gastrointestinal tract. GABA and putatively neurosteroids (NSs)[205] exhibit pleiotropic actions and targets in the gastrointestinal (GI) tract. (**A**) GABA targets neuronal and potentially glial GABA receptors in the submucosal and myenteric plexuses of the enteric nervous system and modulates vagal transmission.[110,206-209] GABA can act on local and systemic immune cells[145,210] (**B**), which express both GABA type A and B receptors ($GABA_A$Rs and $GABA_B$Rs).[211] As the gut-associated lymphoid system accounts for ~70% of the body's immune cells,[212] the GI GABA system is ideally placed to influence immunity. Although the specific mechanisms are yet to be fully elucidated, gut GABA can also induce blood-detected exosome signalling associated with brain plasticity, e.g. in the hippocampus[213-215] (**C**). Finally, GABA can act on bacterial communities to modulate their relative abundance [**D(i)**], either by acting as an essential nutrient[156] or by affecting the expression or transfer of crucial genes, e.g. as a virulence inhibitor of invading plasmids from pathogenic species such as *Agrobacterium tumefaciens*.[216,217] GABA specifically promotes the degradation of quorum sensing signals that control plasmid transfer.[218] Interestingly, unique GABA binding motifs have been identified in the periplasmic bacterial protein (PBP) Atu4243 of *A. tumefaciens*, which functions as a selective GABA-sensor and transporter to satisfy nutritional requirements.[217] These opposite roles of GABA suggest that GABA participates in and fine-tunes the complex relationship between hosts and biotrophic pathogens. Albeit speculative at present, GABA may also target bacterial receptors [**D(ii)**], akin to the identified GLIC (Gleobacter ligand-gated ion channel)[219] and ELIC (Erwinia ligand-gated ion channel)[220,221] prokaryotic GABA receptors of the *Cyanobacteria* phylum, closely related to some recently identified human gut bacteria.[222] In indirect support, recent evidence has revealed that GABA modifies the bioelectrical properties of a known commensal bacterial strain *Lactobacillus reuteri* to impact their growth,[223] congruent with the known bacterial use of change in membrane potential to convey and process key information.[224] The figure was prepared using BioRender (www.biorender.com).

inflammatory and anxious phenotype, which is characterized by disruption of gut barrier function along with a concomitant significant increase in a range of inflammatory markers.[128]

Here, we surmise that the following $GABA_AR$ subtypes are relevant in terms of modulating connected BGM axis functions. Convergent genetic and pharmacological studies strongly implicate α2/3-$GABA_AR$ subtypes in anxiolysis produced by benzodiazepines[246] and neurosteroids.[247] It is notable from the expression data in Fig. 1B that these α-subunits are abundantly expressed in both brain and gut. As mentioned earlier, α3-$GABA_A$Rs are implicated in stress-induced GI inflammation.[128] Given the prevalence of GI symptoms and systemic inflammation in comorbid mental illness such as anxiety and depression (see the 'GABA's role in immunity' section), assessing the efficacy of α2/3-$GABA_A$R-selective ligands that do or do not have brain penetrance in such patient populations would improve our understanding of which $GABA_AR$ subtypes within specific segments of the BGM axis contribute to such complex medical conditions. Based on human expression data (Fig. 1B), ϵ-$GABA_AR$ subtypes could also be integral to BGM axis regulation. The highest brain expression of ϵ-$GABA_AR$ is in the hypothalamus, with considerable expression across several peripheral organs (Fig. 1B). Given the preeminent role of the hypothalamus in coordinating body homeostasis, the expression of the same $GABA_AR$ subtypes throughout these brain-body pathways is likely to enhance coordination across functionally diverse organ systems. Thus, the development of appropriate ϵ-$GABA_AR$ research tools (verifiably specific antibodies; selective ligands; gene-deleted mice) could be instrumental in advancing science and medicine across diverse disciplines. Interestingly, ρ-subunit-containing $GABA_A$Rs, originally described in the retina[248] and previously called $GABA_C$Rs, but apparently expressed more widely (Fig. 1B), including in the ENS,[115] have been implicated in modulating rodent intestinal

Box 2 GABA actions on gut bacteria

Modulation of gut bacteria

- GABA may affect brain function by modulating key bacterial communities either as a nutrient or a fine tuner/modulator. The former, i.e. as a source of carbon and nitrogen, is well documented in plants,[230-233] where GABA was first identified in potato tubers over 70 years ago[234] before being recognized as the main inhibitory neurotransmitter in the vertebrate brain. Therefore, it is no surprise that GABA is a vital nutrient within the human microbiome.[156] This raises the prospect that GABA-dependent microbes may be beneficial for human health and GABA may be an intermediate inter-kingdom messenger able to confer directly and/or indirectly, a range of health advantages for animals and humans.
- GABA may additionally modulate the presence and abundance of specific microbial communities via more tailored strategies as described for plant-microbial interactions. For example, there is ample evidence that plants utilize GABA to overcome biotic stress brought about by pathogenic microbes.[235-237] The GABA-mediated defence approach by plants embraces a variety of mechanisms including support for key energetic pathways.[237] In addition, GABA can reduce the virulence of some pathogens such as *Agrobacterium tumefaciens* and *Pseudomonas syringae* by interfering with the production of molecules by quorum sensing, a complex signalling system utilized by bacteria to control several processes including survival and colonization of specific ecosystems.[218,238] The protective role of GABA against pathogenic colonization by inhibiting specific quorum signalling molecules appears important to preventing the conjugation and amplification of the Ti plasmid, used by *A. tumefaciens* to transfer DNA and transform the plants it invades.[239] Intriguingly, *A. tumefaciens* possesses a selective GABA-binding protein where highly conserved amino acid residues have been proven critical for the inactivation of the quorum signal implicated in Ti plasmid actions.[216,217] Thus, a specific and primordial mechanism exists to enable GABA protective actions against microbial pathogens, suggesting a similar approach may also be operative in mammalian gastrointestinal systems.

GABA as an agonist of bacterial GABA receptors

- GABA may also regulate the microbial ecosystem by modifying their bioelectrical properties, which are dynamic and can be utilized to serve a variety of functions and communication strategies.[224] In support, two recent pioneering studies employing population measurements of bacterial membrane potential with fluorescent voltage-sensitive dyes revealed that bacterial membrane depolarization is associated with bacterial growth. Furthermore, sub-micromolar concentrations of GABA can reduce the degree of depolarization of known microbiome bacterial strains *Lactobacillus reuteri*[223] and *Enterococcus foecalis*.[240] The impact of GABA on bacterial growth remains to be established since the ultimate effect of depolarization appears dependent on the activated or inhibited state of the bacteria.[241] Potential molecular targets of this GABA effect include bacterial homologues of pentameric ligand-gated channels, including $GABA_A$Rs, previously identified, e.g. Erwinia ligand-gated ion channels (ELICs)[220,221] and Gleobacter ligand-gated ion channels (GLICs)[219]from *Erwinia chrysanthemi* and *Gloeobacter violaceus* of the *Cyanobacteria* phylum, respectively These prokaryotic receptor homologues are sensitive to GABA (i.e. ELIC)[220,221,242] and/or GABA-active ligands including neurosteroids.[243] Interestingly, bacteria closely related to *Cyanobacteria* have been identified in both the human and animal gastrointestinal tracts,[222] with tantalizing implications for human health.

contractility.[17,249] Specifically, receptors with a ρ-type pharmacology were implicated in the contractility of the mouse duodenum[249] and increased cholecystokinin release in a neuroendocrine tumour intestinal cell line (STC-1),[135] which shares many properties with native intestinal enteroendocrine cells.[250] Importantly, ρ-$GABA_A$Rs are distinct amongst other subtypes. This is due to their relatively high affinity for GABA, insensitivity to the classical $GABA_A$R antagonist bicuculline and lack of desensitization.[18] These features make them ideally tailored to mediating sustained GABA inhibition via a shunting action (Box 1). It is important to determine precisely how ρ-$GABA_A$Rs differ from other subtypes in terms of regulating GI function, e.g. by inducing ENS neuronal inhibition, despite the reported local GABA depolarizing actions.[251]

Gastrointestinal GABA modulates local and systemic immunity by activating immune-cell GABA receptors

A compelling body of evidence implicates immune system dysregulation in psychopathology.[189] However, there are many crucial unanswered questions about whether associated systemic inflammation drives brain pathology or primary alterations to brain function result in changes to immune function in the rest of the body. This has stimulated the assessment of neurochemical involvement, most notably GABA, in immune processes.[145,210] What has emerged is convincing evidence that the GABA molecular apparatus is expressed in both arms of the immune system—in the innate, e.g. mononuclear phagocytes, and adaptive response factors, e.g. T and B cells. The components of the molecular apparatus include molecules underpinning GABA metabolism, GABA transport and secretion, both $GABA_A$ and $GABA_B$Rs, GABA signal regulation by cation chloride co-transporters (CCCs) and effector Ca^{2+} signalling via voltage-dependent channels.[145,210] Furthermore, functional studies confirm that GABA signalling via both A and B receptor subtypes regulates inflammatory and anti-inflammatory responses in both arms of the immune system.[211,252] These findings suggest that the GI GABA system is ideally placed to influence immune function since the gut-associated lymphoid system accounts for ~70% of the body's immune cells[212] (Fig. 5). In support, the activation of specific $GABA_A$R subtypes induces or suppresses colonic inflammation in addition to impacting barrier permeability.[128] Thus, there is the potential for such GABA-mediated production of local GI immune mediators to distribute more widely throughout the body, allowing systemic inflammation to impair BBB integrity. This would result in a variety of molecules obtaining access to the brain, such as inflammatory

cytokines, which negatively impact brain function, thereby contributing to psychological symptoms.

Key questions remain in terms of which specific $GABA_AR$ subtypes drive changes in immune function, as well as their cellular location, with evidence indicating that specific subtypes are important for brain and systemic immune pathways.[211] There is also heterogeneity in the expression of $GABA_AR$ subtypes across distinct immune cells,[145] a feature shared with both the CNS[37,253,254] and ENS.[50,51] However, a full mechanistic appreciation of the role of $GABA_{A/B}Rs$ in modulating local and systemic immunity will require novel knowledge of the specific receptor subtypes and associated roles within specific immune cell populations, along with a better understanding of the specific biological context driving the expression of the identified receptor subtypes. Moreover, given that immune cells can additionally synthesize GABA, e.g. in phagocytes, via cytosolic GAD67[255] or T cells,[256] via a vesicle-independent process, elucidation of the specific GABA secretory pathway(s) will aid the understanding of GABA's role in the regulation of immune tone and whether GABA may function as an autocrine or paracrine messenger.[256,257] Intriguingly, studies have revealed that sub-micromolar concentrations of GABA affect specific functions, e.g. cytokine release.[255] These observations raise the prospect that these effects are mediated by receptor subtypes associated with extra-synaptic function in neuronal populations, which are also activated by low GABA concentrations and show limited desensitization[21,45,258] (see the 'GABA signalling within the BGM axis' section). This suggestion is supported by the abundant expression in immune cells of subunits typically associated with neuronal extrasynaptic subtypes, e.g. α4, α5, α6 and δ, and ρ1/2 receptor isoforms, also known to exhibit high affinity for GABA and limited desensitization.[145,248,255,256,259,260] Nevertheless, the additional expression of receptor subunits that are typically linked with synaptic activation in neuronal networks e.g. α1 and/or α2/3-γ2-containing subtypes coupled with expression of proteins, e.g. GABARAP,[256] long known to support synapse stability,[261] suggests that GABA concentration gradients may also be required for modulation of specific immune functions.

Gastrointestinal GABA modulates brain function via exosome-mediated signalling

Exosomes are a subtype of extracellular vesicles (~40–160 nm in diameter) with a lipid-enveloping structure. They are released extracellularly by different cell types and function like carriages, shuttling basic cell biomolecules, including DNA, mRNA, miRNA, lipids and proteins (Fig. 5), to mediate short- as well as long-distance communications[262-264] in both physiological and pathological processes.[213] A role for GABA-induced exosomes from intestinal tissue has recently emerged. Exosomes isolated from the blood of mice orally administered with GABA but not those derived from vehicle-treated mice produced neuronal activity in SH-SY5A cell lines via a unique complement of genes associated with neuronal function.[214,215] Moreover, their presence in the blood of GABA-treated mice was associated with hippocampal changes in the expression profile of genes governing neuronal function.[215] The exact mechanisms of exosome-mediated gut-to-brain GABA-evoked communication are yet to be uncovered. GABA could either target the brain directly via exosomes or indirectly by activating GABARs in the intestinal tissue to modulate exosome content. Future studies will provide clarity and uncover potential new mechanisms that may be exploited to therapeutic advantage.

Gastrointestinal GABA modulation of brain function: other microbiota metabolites and neurotransmitters

Evidence suggests that peripheral GABA has the potential to influence the production of, or interact with, other important mediators of the BGM axis with neuroactive properties and, thus, has brain-modulating potential. Here, we focus on two such classes of BGM axis messengers, namely SCFA and 5-HT, because of their relevance to mental health.

GABA and short-chain fatty acids

SCFAs are the main metabolites produced in the colon by bacterial fermentation of dietary fibres and resistant starch.[265] Apart from being a necessary waste product for resident bacteria to balance redox equivalent production in the anaerobic environment of the gut,[266] compelling evidence points to microbiome-derived SCFAs playing a beneficial role in brain function and behaviour.[267] Thus, mechanisms that stimulate SCFA production could, in theory, positively influence brain processes. Whilst the evidence is still in its infancy, GABA has been shown to increase caecal SCFA content in mice,[268] suggesting a potential therapeutic target. One particular SCFA, acetate, not only crosses the BBB[269,270] but also participates in neuronal GABA synthesis (approximately 30%).[271] Furthermore. studies in rodents demonstrate that direct acetate supplementation improves depressive-like behaviours.[272] Additionally, acetate and GABA are implicated in reducing depressive- and anxious-like behaviours in mice supplemented with the probiotic *B. licheniformis*, with a positive correlation reported between the increased levels of acetic acid and GABA.[273] Collectively, these findings support further investigations regarding the underlying signalling mechanisms.

GABA and 5-HT

Another well-documented chemical messenger and regulator of the BGM axis is 5-HT.[274] Significant evidence implicates dysregulation of the 5-HT system (including metabolism of its key precursor, tryptophan) at both ends of the BGM axis in psychopathology.[1,274-277] While an extensive discussion of the roles and targets of the 5-HT signalling system in the working of the BGM axis[276] is beyond the scope of this review, we focus on its potential interaction with GABA in the context of BGM signalling, specifically in the homeostatic regulation of emotional states.

Different lines of evidence support directly or indirectly a GABA-5-HT interaction not only in the brain[278] but also potentially in the periphery and gut. Dysregulation of both GABAergic and serotonergic systems has been documented in clinical cohorts[279-284] and animal models of psychopathology.[193,285-287] Intriguingly, emerging evidence also implicates, directly or indirectly, both GABA (see the 'Future research priorities' section) and 5-HT[288-291] in the beneficial effects of certain dietary interventions. For example, *Bifidobacteria*-based probiotic interventions, which are amongst the most widely used and effective for the prevention and treatment of psychological disturbances,[292-294] have been reported to involve modulation of both the serotonergic[295] and GABAergic[296,297] systems. In further indirect support, most utilized pharmacological treatments for anxiety and depressive disturbances target GABAergic (e.g. benzodiazepines) and/or serotonergic transmission (i.e. selective serotonin reuptake inhibitors). Collectively, these observations suggest a possible synergy between these neurotransmitters to optimize emotional regulation.

This proposal is consistent with the role of 5-HT as a neuromodulator, and that dysregulation of the 5-HT system in depression and anxiety represents a biological risk factor rather than being the primary cause of psychopathology.[298] Similarly, CNS functional GABAergic deficits, while apparent in many clinical cohorts, do not appear sufficient to be the sole cause of depression.[43,299]

How could GABAergic and serotonergic systems interact in the context of BGM signalling? In the brain, various mechanisms have been proposed to underpin physiologically and pathologically important interactions between GABA and 5-HT,[299] including modulation of transmitter release. For example, GABA release can be influenced by the activation of 5-HT targets, i.e. increased by excitatory 5-HT3 receptors (5HT3R) expressed in GABAergic interneurons, particularly in the amygdala[300] and hippocampus.[301] Conversely, 5-HT release is modulated by GABAergic transmission, e.g. in the dorsal raphe via $GABA_A$[302] and $GABA_B$ receptors,[303,304] which mediate a decrease and increase, respectively. Although functional evidence is currently lacking, similar mechanisms may also occur in the gut where 5HT3Rs are abundantly expressed, for example, on inhibitory motor neurons in the ENS,[305] which comprise the majority of GABAergic neurons.[306]

When considering the regulation of 5-HT levels both in the brain and the GIT, it is important to emphasize that approximately 95% of 5-HT in the body is produced in the gut, and of this 80% is produced by ECs in the GI epithelium (Fig. 5), whereas ENS neurons are a minor, albeit important, source.[277,307,308] Interestingly, both $GABA_A$Rs and $GABA_B$Rs are expressed in ECs,[132] providing a means for GABA-mediated regulation of 5-HT levels at its main source.

Gut microbiota also modulate 5-HT levels directly or indirectly.[274,288,309] Conversely, 5-HT can modulate bacterial colonization in the gut.[310] For example, the synthesis of 5-HT is modulated by microbiota products such as SCFA, which can upregulate the expression of the key enzyme involved in 5-HT synthesis in ECs, namely tryptophan hydroxylase 1 (*TPH1*).[311] As discussed earlier, GABA may regulate SCFAs levels[268,312] and so may also indirectly modulate 5-HT levels. Intriguingly, *Clostridium perfringens*, an anaerobic gram-positive spore-forming bacillus associated with dangerous gastrointestinal infections and long known to modulate the production of gut 5-HT also displays high amounts of GAD, the key GABA synthesizing enzyme.[313] Neurosteroids (Box 3) may also be implicated in the regulation of gut 5-HT levels, probably via $GABA_A$Rs. Thus, withdrawal from chronic inhibition of neurosteroid synthesis with finasteride, a selective inhibitor of a key neurosteroid synthetic enzyme, 5α-reductase, produces profound changes in rat colon neurosteroid levels, while concomitantly increasing 5-HT levels and altering local $GABA_A$R expression. Importantly, both changes are reversed by treatment with ALLO.[314]

Immunomodulation is a key element of BGM signalling[337] and another potential converging platform for 5-HT and GABAergic interactions. As noted earlier, evidence implicates inflammation and immune dysregulation in psychopathology.[185,187,189,338] Notably, both are key features of irritable bowel syndrome.[339] This condition is also known to be co-morbid with both anxiety and depressive disorders[340-342] and accompanied by dysregulated GABAergic[343] and serotonergic signalling.[270,344,345] Some elements of the alterations in GABAergic and 5-HT systems may be mediated via the immune system. Thus, the role of 5-HT as a key regulator of the local and systemic immune responses (e.g. via 5-HT-enriched platelets; Fig. 5) is well established.[346] However, some immune dysregulation may also implicate and/or modulate GABA. For example, GABA may modulate 5-HT receptor expression in immune cells both locally and systemically, thus affecting their function and vice versa. When exploring potential mechanisms underpinning GABA–5-HT interaction, the depolarizing actions of GABA in the periphery compared to its traditional hyperpolarizing role in the majority of CNS neurons are likely important. Indeed, depolarizing action by GABA could be followed by the activation of specific voltage-gated channels, for example, Ca^{2+}-mediated conductances. This would, in turn, trigger various signalling cascades, possibly leading to changes in the genetic expression of receptors and other cellular components. If and how GABA and 5-HT interact to regulate immunity locally in the gut and systemically remains to be established and could offer a new understanding of some molecular underpinnings of BGM homeostasis and dysfunction.

Finally, the vagal afferent system (see the 'GABA signalling within the BGM axis' section and Fig. 5) offers another pathway whereby GABAergic and serotonergic signalling may interact.[4] From an anatomical perspective, vagal innervation of the small intestine is well documented,[347] while recent reports provide further compelling evidence[348,349] to support earlier studies on vagal innervation and regulation of the colon where, common to the entire GIT, intraganglionic lamellar endings and related structures in the myenteric ganglia mediate the interaction between ENS and vagal afferents.[347,350,351] Both GABA and 5-HT receptors are present on vagal terminals[110,206,207,352] where $5HT_3$Rs are abundantly expressed[353] and implicated in gut-derived GABA-[208] and 5-HT-mediated[354] effects. Moreover, vagotomy prevents both GABAergic[209] and serotonergic[355] effects on behavioural and molecular end points of gut-targeted interventions. Conversely, stimulation of the vagus nerve, which can reduce anxiety and depressive-like behaviour in rodents[356,357] and is a US Food and Drug Administration-approved treatment for resistant depression (since 2005),[358] increases levels of 5-HT in anatomically relevant brain centres, i.e. dorsal raphe.[359,360] The vagally mediated behavioural effects implicating GABA and 5-HT may also involve modulation of the immune system.[361] In summary, a GABA–5-HT interaction is well documented in the brain and highly likely to occur in the GI tract, supporting the bidirectional conversation between the two ends of the gut-brain axis.

Key priorities for future research

Identifying direct evidence for neuronally-released GABA regulation of gastrointestinal function

As discussed in Box 1, within the CNS, there is compelling evidence for the variety mechanisms by which the release of endogenous GABA alters the excitability of target neurons, including fast phasic or persistent tonic currents. In stark contrast, such direct evidence for GABA-mediated postsynaptic potentials in the ENS is lacking.[362] This is despite the array of studies, using exogenously applied GABA or GABA receptor ligands, providing compelling evidence for the roles of GABA receptors in a variety of GI functions, most notably smooth muscle contractility. There is evidence for GABA expression in excitatory motor neurons, descending interneurons and inhibitory motor neurons[306] and of neurally released GABA to modulate slow inhibitory potential in the mouse ileum via ρ-containing $GABA_A$Rs.[17] Yet, clear evidence of GABA acting directly on GI muscle is lacking. However, $GABA_A$Rs have been detected by immunohistochemistry in non-neuronal ICCs,[51] which lie at the interface between the ENS and intestinal smooth muscle. Given that the activity of ICC is believed to underlie spontaneous intestinal contractions,[363] ICC $GABA_A$Rs may represent a key molecular link through which GABA modulates GI muscle contractility.

Box 3 A role for neurosteroids in the regulation of the brain-gut-microbiota axis?

- A class of endogenously occurring steroids synthesized in the CNS, neurosteroids, are documented potent efficacious positive allosteric modulators (PAMs) of GABA type A receptor ($GABA_AR$) function at physiologically and pathologically relevant levels.[44,315-319] Most research investigating the $GABA_AR$-active properties of neurosteroids has focused on CNS excitability. However, converging evidence indicates that local production of $GABA_AR$-active neurosteroids within the gastrointestinal tract is also relevant to CNS function and behaviour.
- Such local production may be regulated dynamically by the resident microbiota. Specifically, an abundant expression of the steroidogenic machinery that regulates levels of neurosteroids and their precursors is apparent in the adult rat colon, suggesting local synthesis.[320] Importantly, enzymes (e.g. 3α-hydroxysteroid-oxido reductase) critical to the synthesis of the $GABA_AR$-active neurosteroid allopregnanolone (ALLO) are more densely expressed in the colon than in brain steroidogenic tissue, e.g. the cerebral cortex.[320] In agreement, the levels of ALLO are markedly higher in the colon than the cortex/plasma.[320] Indirect support for microbiota influencing neurosteroid levels comes from studies of germ-free mice, where both the brain and peripheral abundance of $GABA_AR$-active steroids are altered, albeit differentially in the brain compared to the periphery.[321] Neurosteroids may also originate from gut microbiota, as a significant population of the human gastrointestinal flora express the necessary enzymes to convert progesterone and testosterone.[322,323] Moreover, certain human gut bacteria, namely *Gordonibacter pamelaeae* and *Eggerthella lenta*, convert abundant, micromolar, biliary corticoids into $GABA_AR$-active neurosteroids, particularly during pregnancy.[205] The microbiota contribution to neurosteroid modulation of neuronal homeostasis is further emphasized by the inhibition of neurosteroid synthesis by finasteride. This inhibitor of the 5α-reductase enzyme is associated in male rats with a depressive-like phenotype, neuroinflammation and concomitantly an alteration of the gut microbiota composition.[324] Importantly, in rodents, ALLO provides protection against gut inflammation induced by withdrawal from chronic finasteride treatment. Thus, ALLO decreases the raised expression levels of some inflammatory cytokines, namely IL-1β and TNFα and reverses the concomitant mRNA changes in β2-, β3- (decrease) and δ- (increase) $GABA_AR$ subunits,[314] thus implicating $GABA_ARs$ in ALLO's protective actions. These findings may be clinically relevant to post-finasteride syndrome, a condition affecting vulnerable male patients treated with finasteride for benign prostatic hyperplasia or alopecia, who develop mood and cognitive impairments amongst other side effects which persist even after finasteride discontinuation.[320,325-328] In this patient population, the documented abnormal plasma and CSF neurosteroid levels[329,330] and gut dysbiosis[331] may result in peripheral and brain neuroinflammation. Similarly, the protective effect of neurosteroids against CNS neuroinflammation that features both in psychopathology[189] and neurodegenerative conditions[332] may arise from their peripheral effects in the gut, reducing systemic inflammation via the brain–gut–microbiome (BGM) axis.
- Overall, these findings suggest a putative role for neurosteroids in regulating the function of the enteric nervous system (ENS), microbiota composition and BGM axis, impacting the modulation of brain function.
- A role for neurosteroids is also emerging in the regulation of neuroendocrine pathways of the BGM, implicated in brain homeostasis. A variety of putative targets and mechanisms are likely to mediate neurosteroid actions within the gastrointestinal tract. In common with GABA, $GABA_AR$-acting neurosteroids modulate the function of $GABA_ARs$ located in the gastrointestinal tract, thus including those expressed both in the ENS and immune cells. Growing evidence also supports an important role for the peroxisome proliferator-activated receptor (PPAR)-α receptor as a key molecular link between neurosteroids, gut bacteria, inflammation and physiologically relevant behavioural and molecular phenotypes. This nuclear receptor is ubiquitously expressed throughout the gastrointestinal tract, including epithelial and immune cells, and its absence associates with significant dysbiosis and an inflammatory phenotype.[333,334] Importantly, PPARα activation both stimulates neuro-steroidogenesis, e.g. of ALLO, and anti-inflammatory responses, while also preventing both stress-induced depressive phenotypes in animal models and the concomitant brain decrease in $GABA_AR$-acting neurosteroids.[334,335] These findings are significant for GABAergic signalling, as evidence of a causal relationship between neurosteroid metabolism and expression of $GABA_AR$ subtypes already exists in animals.[336]

Future targeted investigations should explore this possibility. A major confound derives from the co-expression of GABA with other neurotransmitters known to modulate smooth muscle contractility, such as acetylcholine. The exploitation of transgenic models allowing for the selective activation of subsets of GABAergic cell types whilst recording physiological responses with neighbouring cells could be instructive in terms of defining connected cell circuits.

Characterizing individual segments of the BGM with relevance to gut physiology and brain diseases

Compelling evidence links the integration of different cell groups and circuits of the brain-gut axis in mediating homeostasis and their involvement in medical disorders. Going forward, it will be important to disentangle the functional contributions of individual elements and how these change in disease. This progression is imperative if we are to address associated medical conditions by targeting and modifying the underlying mechanisms, rather than providing purely symptomatic relief. Whilst technical challenges have in the past prevented the selective manipulation of individual cell types or cell circuits during different behavioural or physiological states, the latest technologies allow for such direct interrogation of brain-body mechanisms. For example, using a combination of cutting-edge genetic, physiological and behavioural technologies, the cardiogenic control of affective behavioural states has recently been elegantly demonstrated in mice.[364] This study showed that the activation of heart rate can induce anxiogenic-like behavioural states, depending on the context of the environment, thereby illustrating the profound influence of peripheral physiological states in driving brain function and behaviour. It would be

intriguing to determine whether certain GI physiological states have such profound impacts on brain function and behaviour or whether the influence is predominantly unidirectional in a top-down manner. Recent reports demonstrate the feasibility of assessing the GI molecular and functional consequences of selectively activating specific ENS neurons.[137] It would be instructive to determine how such specific GI manipulations impact brain function and behaviour related to psychopathology. Furthermore, $GABA_AR$ subunit-specific genetically modified mice (gene-deleted or rendered insensitive to specific ligands) have advanced our understanding of their contributions to native brain function, behaviour and disorders, as well as the pharmacological profiles of GABAergic ligands.[43,365-368] However, the next generation of animal models should target the manipulation of individual subunit expression discretely in selected peripheral organs to gain a more granular understanding of their roles throughout the body and to realize the therapeutic potential of targeting these peripheral receptors in associated inflammatory conditions of other organs as in inflammatory bowel disease.

Allied to this, and given the mutual interactions between the brain and gut, it will be important to unequivocally identify the primary site/s of action of GABA-based compounds in mediating their therapeutic efficacy. For example, are the effects of anxiolytics solely driven by changes in brain function, or do they work in combination with changes in peripheral organs? A further key question is which $GABA_AR$ subtypes to target. Given the plethora of subunits expressed throughout the body (Fig. 1B), it is essential to identify the contribution of specific receptor subtypes to specific GI functions if we are to minimize unwanted effects. This is exemplified by *in vitro* studies demonstrating that compounds with different $GABA_AR$ subtype profiles exert contrasting effects on basic GI functions, such as the force and frequency of colonic contractions,[51] as well as immune functions (Fig. 3B).[127,128]

Targeting the gut and microbiome: psychoactive GABA modulators and GABA-based dietary interventions

Identifying endogenous allosteric modulators of GABA receptors: relevance to psychopathology

Whilst GABA is understandably responsible for the majority of GABAR tone, there are some important exceptions. For example, certain endogenous neurosteroids act as potent efficacious enhancers of $GABA_ARs$ at a specific site,[369,370] thereby influencing neuronal activity and consequently behaviour (see the 'GABA signalling within the BGM axis' section and Boxes 1 and 3).[44,315,316] Indeed, perturbations of neurosteroid levels are implicated in a variety of neurological and psychiatric disorders such as certain types of epilepsy and postpartum and major depression.[36,371-375]

Although neurosteroid research has primarily focused on their role in the CNS, high levels of these powerful $GABA_AR$ PAMs are found in the GI tract, and their peripheral abundance influences brain function (Box 3). Therefore, if such significant endogenous PAMs of $GABA_ARs$ are found in the periphery, it begs the question of whether other such chemicals exist. These could be bacterial metabolites, including neurosteroids (Box 3) that then impart the microbiome's functional influence on the brain. The discovery of such ligand-receptor interactions has traditionally relied on serendipity. However, the combination of public databases hosting bacterial metabolomic arrays, alongside structural and biophysical models of individual $GABA_ARs$, using high-throughput *in silico* ligand-receptor docking techniques provide unique opportunities for the targeted identification of novel, endogenous compounds potentially able to influence $GABA_AR$ function. The potential outcome could be to identify a variety of novel potentially druggable compounds.

GABA-based dietary interventions to therapeutically target microbial GABA pathways in the gut microbiome

There is increasing evidence identifying bacterial metabolites, including but not limited to GABA, that affect $GABA_ARs$ and impart beneficial effects (see the 'Gastrointestinal GABA interaction with SCFA and 5-HT' section). A potential strategy to naturally exploit such signalling pathways could be to stimulate their production indirectly with agents that influence the microbiome. Such an approach could be in the form of dietary interventions based on the use of prebiotics, probiotics or a combination of both (synbiotics).[376] Such preparations have become increasingly popular worldwide because of their putative positive effect on human health via actions beneficial to GI homeostasis.[377] Amongst the biotic-type of dietary interventions, a considerable pre-clinical (in animal models) and clinical literature over the past 20 years has developed around those based on GABA, which is offered either in fermented food/beverages or via supplements containing GABA-producing bacteria (see Braga *et al.*[154] for a recent excellent review). Next, we will briefly discuss GABA-based probiotic and prebiotic approaches.

Probiotic interventions

Amongst the GABA-producing microorganisms utilized in dietary interventions, those most used in the food and drink industry belong to the genera *Lactobacillus* and *Bifidobacterium*. Several beneficial health claims for such GABA-based dietary products (both food and beverages) have been reported, including improvements in psychiatric conditions such as anxiety,[166,209,378,379] depression,[380-383] schizophrenia[384] and cognition,[385] neurological disorders such as epilepsy[386] and pain,[150] and cardiovascular disorders such as hypertension[387,388] and cancer.[389] Here, we focus on the evidence concerning the putative mental health benefits of GABA-based dietary interventions and discuss what advances are necessary to realize their translational potential.

Studies in animal models offer generally compelling evidence for the beneficial profile of GABA-based diets in many of the disorders mentioned. Recent systematic reviews of the clinical literature also offer a promising outlook. Specifically, reviews of randomized control trials (RCTs) for the treatment of anxiety and/or depressive disorders have concluded that probiotics—especially those known to produce, albeit not exclusively, GABA; that is, the *Lactobacilli* and *Bifidobacteria* strains—may be effective in improving psychological symptoms associated with depression[390,391] and possibly anxiety.[294] Moreover, overall, psychological symptom reduction is more consistently evident in clinical rather than healthy cohorts.[392-394]

Yet, while these studies are promising, a consensus is still to be reached in the clinical arena for probiotic dietary interventions in psychopathology.[294] Confounding factors include the limited scale and heterogeneity of the clinical studies, which have complicated the interpretation of the data. Clearly, these limitations need to be addressed, together with Mendelian randomization,[395,396] to draw firm conclusions on the causal relationship between the intervention and the measured clinical outcome.

Prebiotic interventions

GABA appears also to play a role in the health-promoting actions of certain prebiotics, taken alone or in combination with probiotic

strains such as *L. plantarum*.[397,398] The prebiotics most utilized belong to the polysaccharide group and include fructo-oligosaccharides (FOS), inulin, abundant in fruits and vegetables,[399] galacto-oligosaccharides (GOS) and human milk oligosaccharides (HMOs), which have recently received significant attention,[400] within the context of dietary approaches for ameliorating IBS.[401-403]

The beneficial effects of the prebiotics on gut health in humans appear to be mediated by enhancing the abundance of specific microbiota strains and, thus, the levels of their metabolic products. These chemicals include but are not limited to GABA and SCFAs (see the 'Gastrointestinal GABA interaction with SCFA and 5-HT' section). Specifically, these prebiotics enrich classical probiotics of the genera *Lactobacillus* and *Bifidobacterium*. Many prebiotic-supported species also reside in the human gut and produce SCFAs and GABA amongst their main metabolic products.[153,296] While the genera *Lactobacillus* and *Bifidobacterium* are some of the most widely characterized and used GABA-producers in the food industry, individual prebiotics or combinations of prebiotics may also promote the abundance of other human gut GABA-producing probiotic strains. These include the *Bacteroides* and *Eubacterium* genera.[156] Indeed, inulin-degrading species are widely distributed across *Bacteroides* and *Eubacterium*,[404] and these strains may play an indirect role in a healthy gut (see later). Moreover, recently, in an *in vitro* gut model, treatment of humans with certain HMOs alone or in various combination blends was shown to increase GABA in faeces in children and, especially, in adults.[405] Notably, HMOs boosted GABA production, likely mediated by an increase in various microbial species and especially *Bifidobacterium adolescentis*, consistent with previous studies.[153,296] On the other hand, in the same study, a marked correlation was also evident between GABA and *Bacteroides* species when fructans were used as prebiotics, thus indicating a prebiotic-selective effect on the gut microbiota profile.[406] This finding suggests specific prebiotic combinations may achieve optimal health benefits at least partially by increasing GABA synthesis via specific microbial species. Furthermore, evidence from a recent report[407] using an *in vitro* human model demonstrated a significant physiological increase in GABA production with a combined 2′-fucosyllactose and oligofructose supplementation.

Another class of prebiotics of considerable interest includes non-carbohydrate molecules or polyphenols. They are abundant in plants and linked with a variety of health benefits including mental health.[408,409] Such ligands could also be utilized in the development of targeted GABA-based dietary interventions directly by supporting GABA-producing species or indirectly by supporting their enzymatic capabilities, e.g. bacterial vitamin B6 for GABA production.[406,410-413] Emerging evidence indicates that GABA-consuming bacteria in the human gut[156,190] may indirectly promote other bacteria, indicating mutualistic interactions amongst gut bacteria that are essential for a healthy and adaptable gut ecosystem.[402,407,414-416] Thus, the impact of GABA in the context of a healthy BGM axis could be far-reaching and on present evidence, probably both directly and indirectly mediated.

In summary, whilst there is evidence supporting GABA-based dietary interventions to modulate brain function, larger and better-designed RCTs for both probiotic- and prebiotic-type interventions are needed to strengthen these observations. Specifically, there is a lack of studies with rigorous controls for: concomitant pharmacological treatment; the pre-existence of dysbiosis; and both the duration and role of diet before, during and after the intervention. Furthermore, more homogenous methods of analysis are required to improve consensus amongst different studies. Such advances, coupled with a more targeted identification of beneficial prebiotic and/or probiotic interventions, e.g. based on newly acquired knowledge on the beneficial profile of specific species and their key nutritional requirements, will open a new chapter in the design of effective and advantageous brain modulating dietary interventions.

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Supplementary material

Supplementary material is available at *Brain* online.

References

1. Cryan JF, O'Riordan KJ, Cowan CSM, et al. The microbiota-gut-brain axis. *Physiol Rev*. 2019;99:1877-2013.
2. Morais LH, Schreiber HL 4th, Mazmanian SK. The gut microbiota-brain axis in behaviour and brain disorders. *Nat Rev Microbiol*. 2021;19:241-255.
3. Lai Y, Dhingra R, Zhang Z, Ball LM, Zylka MJ, Lu K. Toward elucidating the human gut microbiota-brain axis: Molecules, biochemistry, and implications for health and diseases. *Biochemistry*. 2022;61:2806-2821.
4. Bonaz B, Bazin T, Pellissier S. The vagus nerve at the interface of the microbiota-gut-brain axis. *Front Neurosci*. 2018;12:49.
5. Schroeder BO, Bäckhed F. Signals from the gut microbiota to distant organs in physiology and disease. *Nat Med*. 2016;22:1079-1089.
6. Krautkramer KA, Fan J, Bäckhed F. Gut microbial metabolites as multi-kingdom intermediates. *Nat Rev Microbiol*. 2021;19:77-94.
7. Miri S, Yeo J, Abubaker S, Hammami R. Neuromicrobiology, an emerging neurometabolic facet of the gut microbiome? *Front Microbiol*. 2023;14:1098412.
8. Bowery N, Enna SJ, Olsen RW. Six decades of GABA. *Biochem Pharmacol*. 2004;68:1477-1478.
9. Andersen JV, Schousboe A. Milestone review: Metabolic dynamics of glutamate and GABA mediated neurotransmission—The essential roles of astrocytes. *J Neurochem*. 2023;166:109-137.
10. Kilb W, Kirischuk S. GABA release from astrocytes in health and disease. *Int J Mol Sci*. 2022;23:15859.
11. Klausberger T, Somogyi P. Neuronal diversity and temporal dynamics: The unity of hippocampal circuit operations. *Science*. 2008;321:53-57.

12. Fritschy J-M, Panzanelli P. GABAA receptors and plasticity of inhibitory neurotransmission in the central nervous system. *Eur J Neurosci*. 2014;39:1845-1865.
13. Farrant M, Nusser Z. Variations on an inhibitory theme: Phasic and tonic activation of GABA(A) receptors. *Nat Rev Neurosci*. 2005;6:215-229.
14. Bassetti D. Keeping the balance: $GABA_B$ receptors in the developing brain and beyond. *Brain Sci*. 2022;12:419.
15. Villalobos N. Disinhibition is an essential network motif coordinated by GABA levels and GABA B receptors. *Int J Mol Sci*. 2024;25:1340.
16. Fung C, Vanden Berghe P. Functional circuits and signal processing in the enteric nervous system. *Cell Mol Life Sci*. 2020; 77:4505-4522.
17. Koussoulas K, Swaminathan M, Fung C, Bornstein JC, Foong JPP. Neurally released GABA acts via GABA(C) receptors to modulate Ca(2+) transients evoked by trains of synaptic inputs, but not responses evoked by single stimuli, in myenteric neurons of mouse ileum. *Front Physiol*. 2018;9:97.
18. Naffaa MM, Hung S, Chebib M, Johnston GAR, Hanrahan JR. GABA-ρ receptors: Distinctive functions and molecular pharmacology. *Br J Pharmacol*. 2017;174:1881-1894.
19. Herd MB, Brown AR, Lambert JJ, Belelli D. Extrasynaptic GABA(A) receptors couple presynaptic activity to postsynaptic inhibition in the somatosensory thalamus. *J Neurosci*. 2013;33: 14850-14868.
20. Koh W, Kwak H, Cheong E, Lee CJ. GABA tone regulation and its cognitive functions in the brain. *Nat Rev Neurosci*. 2023;24: 523-539.
21. Walker MC, Semyanov A. Regulation of excitability by extrasynaptic GABA(A) receptors. *Results Probl Cell Differ*. 2008;44:29-48.
22. Khatri SN, Wu WC, Yang Y, Pugh JR. Mechanisms of $GABA_B$ receptor enhancement of extrasynaptic $GABA_A$ receptor currents in cerebellar granule cells. *Sci Rep*. 2019;9:16683.
23. Newberry NR, Nicoll RA. Direct hyperpolarizing action of baclofen on hippocampal pyramidal cells. *Nature*. 1984;308: 450-452.
24. Schulte JT, Wierenga CJ, Bruining H. Chloride transporters and GABA polarity in developmental, neurological and psychiatric conditions. *Neurosci Biobehav Rev*. 2018;90:260-271.
25. Mody I, De Koninck Y, Otis TS, Soltesz I. Bridging the cleft at GABA synapses in the brain. *Trends Neurosci*. 1994;17:517-525.
26. Shaye H, Stauch B, Gati C, Cherezov V. Molecular mechanisms of metabotropic $GABA_B$ receptor function. *Sci Adv*. 2021;7: eabg3362.
27. Cherubini E, Rovira C, Gaiarsa JL, Corradetti R, Ben Ari Y. GABA mediated excitation in immature rat CA3 hippocampal neurons. *Int J Dev Neurosci*. 1990;8:481-490.
28. Mueller AL, Chesnut RM, Schwartzkroin PA. Actions of GABA in developing rabbit hippocampus: An in vitro study. *Neurosci Lett*. 1983;39:193-198.
29. Ben-Ari Y, Cherubini E, Corradetti R, Gaiarsa JL. Giant synaptic potentials in immature rat CA3 hippocampal neurones. *J Physiol*. 1989;416:303-325.
30. Rivera C, Voipio J, Payne JA, et al. The K+/Cl- co-transporter KCC2 renders GABA hyperpolarizing during neuronal maturation. *Nature*. 1999;397:251-255.
31. Somogyi P. A specific 'axo-axonal' interneuron in the visual cortex of the rat. *Brain Res*. 1977;136:345-350.
32. Szabadics J, Varga C, Molnár G, Oláh S, Barzó P, Tamás G. Excitatory effect of GABAergic axo-axonic cells in cortical microcircuits. *Science*. 2006;311:233-235.
33. Khirug S, Yamada J, Afzalov R, Voipio J, Khiroug L, Kaila K. GABAergic depolarization of the axon initial segment in cortical principal neurons is caused by the Na-K-2Cl cotransporter NKCC1. *J Neurosci*. 2008;28:4635-4639.
34. Kim HR, Martina M. Bidirectional regulation of $GABA_A$ reversal potential in the adult brain: Physiological and pathological implications. *Life (Basel)*. 2024;14:143.
35. Staley KJ, Mody I. Shunting of excitatory input to dentate gyrus granule cells by a depolarizing GABAA receptor-mediated postsynaptic conductance. *J Neurophysiol*. 1992;68:197-212.
36. Belelli D, Hales TG, Lambert JJ, et al. GABAA receptors in GtoPdb v.2021.3. *IUPHAR BPS Guide Pharm CITE*. 2021;2021: 10.2218/gtopdb/F72/2021.3.
37. McKernan RM, Whiting PJ. Which GABAA-receptor subtypes really occur in the brain? *Trends Neurosci*. 1996;19:139-143.
38. Olsen RW, Sieghart W. International Union of Pharmacology. LXX. Subtypes of γ-aminobutyric acid(A) receptors: Classification on the basis of subunit composition, pharmacology, and function. Update. *Pharmacol Rev*. 2008;60:243-260.
39. Sente A, Desai R, Naydenova K, et al. Differential assembly diversifies $GABA_A$ receptor structures and signalling. *Nature*. 2022;604:190-194.
40. Sun C, Zhu H, Clark S, Gouaux E. Cryo-EM structures reveal native $GABA_A$ receptor assemblies and pharmacology. *Nature*. 2023;622:195-201.
41. Geng Y, Bush M, Mosyak L, Wang F, Fan QR. Structural mechanism of ligand activation in human GABA(B) receptor. *Nature*. 2013;504:254-259.
42. Castellano D, Shepard RD, Lu W. Looking for novelty in an "old" receptor: Recent advances toward our understanding of $GABA_A$Rs and their implications in receptor pharmacology. *Front Neurosci*. 2020;14:616298.
43. Thompson SM. Modulators of GABA(A) receptor-mediated inhibition in the treatment of neuropsychiatric disorders: Past, present, and future. *Neuropsychopharmacology*. 2024;49: 83-95.
44. Belelli D, Lambert JJ. Neurosteroids: Endogenous regulators of the GABA(A) receptor. *Nat Rev Neurosci*. 2005;6:565-575.
45. Belelli D, Harrison NL, Maguire J, Macdonald RL, Walker MC, Cope DW. Extrasynaptic GABAA receptors: Form, pharmacology, and function. *J Neurosci*. 2009;29:12757-12763.
46. Sexton CA, Penzinger R, Mortensen M, Bright DP, Smart TG. Structural determinants and regulation of spontaneous activity in GABA. *Nat Commun*. 2021;12:5457.
47. Wlodarczyk AI, Sylantyev S, Herd MB, et al. GABA-independent GABAA receptor openings maintain tonic currents. *J Neurosci*. 2013;33:3905-3914.
48. Arslan A. Extrasynaptic δ-subunit containing $GABA_A$ receptors. *J Integr Neurosci*. 2021;20:173-184.
49. Zeiter DK, Li X, Broussard DL. Identification of the GABAA receptor alpha-subunit mRNA in rat intestine. *Brain Res Mol Brain Res*. 1996;39:241-244.
50. Poulter MO, Singhal R, Brown LA, Krantis A. GABA(A) receptor subunit messenger RNA expression in the enteric nervous system of the rat: Implications for functional diversity of enteric GABA(A) receptors. *Neuroscience*. 1999;93:1159-1165.
51. Seifi M, Brown JF, Mills J, et al. Molecular and functional diversity of GABA-A receptors in the enteric nervous system of the mouse colon. *J Neurosci*. 2014;34:10361-10378.
52. Everington EA, Gibbard AG, Swinny JD, Seifi M. Molecular characterization of GABA-A receptor subunit diversity within major peripheral organs and their plasticity in response to early life psychosocial stress. *Front Mol Neurosci*. 2018;11:18.
53. Fritzius T, Stawarski M, Isogai S, Bettler B. Structural basis of $GABA_B$ receptor regulation and signaling. *Curr Top Behav Neurosci*. 2022;52:19-37.

54. Nakajima K, Tooyama I, Kuriyama K, Kimura H. Immunohistochemical demonstration of GABAB receptors in the rat gastrointestinal tract. *Neurochem Res*. 1996;21:211-215.
55. Kataoka Y, Niwa M, Yamashita K, Taniyama K. GABA receptor function in the parasympathetic ganglia. *Jpn J Physiol*. 1994; 44(Suppl 2):S125-S129.
56. Hyland NP, Cryan JF. A gut feeling about GABA: Focus on GABA(B) receptors. *Front Pharmacol*. 2010;1:124.
57. Blackshaw LA. Receptors and transmission in the brain-gut axis: Potential for novel therapies. IV. GABA(B) receptors in the brain-gastroesophageal axis. *Am J Physiol Gastrointest Liver Physiol*. 2001;281:G311-G315.
58. Boyes J, Bolam JP. Localization of GABA receptors in the basal ganglia. *Prog Brain Res*. 2007;160:229-243.
59. Berggaard N, Seifi M, van der Want JJL, Swinny JD. Spatiotemporal distribution of GABAA receptor subunits within layer II of mouse medial entorhinal cortex: Implications for grid cell excitability. *Front Neuroanat*. 2018;12:46.
60. Klausberger T, Roberts JD, Somogyi P. Cell type- and input-specific differences in the number and subtypes of synaptic GABA(A) receptors in the hippocampus. *J Neurosci*. 2002;22: 2513-2521.
61. Nusser Z, Sieghart W, Benke D, Fritschy JM, Somogyi P. Differential synaptic localization of two major gamma-aminobutyric acid type A receptor alpha subunits on hippocampal pyramidal cells. *Proc Natl Acad Sci U S A*. 1996;93: 11939-11944.
62. Panzanelli P, Gunn BG, Schlatter MC, et al. Distinct mechanisms regulate GABAA receptor and gephyrin clustering at perisomatic and axo-axonic synapses on CA1 pyramidal cells. *J Physiol*. 2011;589(Pt 20):4959-4980.
63. Prenosil GA, Schneider Gasser EM, Rudolph U, Keist R, Fritschy JM, Vogt KE. Specific subtypes of GABAA receptors mediate phasic and tonic forms of inhibition in hippocampal pyramidal neurons. *J Neurophysiol*. 2006;96:846-857.
64. Travagli RA, Hermann GE, Browning KN, Rogers RC. Brainstem circuits regulating gastric function. *Annu Rev Physiol*. 2006;68: 279-305.
65. Fox EA, Powley TL. Morphology of identified preganglionic neurons in the dorsal motor nucleus of the vagus. *J Comp Neurol*. 1992;322:79-98.
66. O'Leary KT, Loughlin SE, Chen Y, Leslie FM. Nicotinic acetylcholine receptor subunit mRNA expression in adult and developing rat medullary catecholamine neurons. *J Comp Neurol*. 2008;510:655-672.
67. Cheng SB, Lu GQ. Progress in the study of the dorsal motor nucleus of the vagus nerve. *Sheng Li Ke Xue Jin Zhan*. 1996; 27:13-18.
68. Xu H, Boychuk JA, Boychuk CR, Uteshev VV, Smith BN. Nicotine enhances inhibition of mouse vagal motor neurons by modulating excitability of premotor GABAergic neurons in the nucleus tractus solitarii. *J Neurophysiol*. 2015;113:1165-1174.
69. Gao H, Glatzer NR, Williams KW, Derbenev AV, Liu D, Smith BN. Morphological and electrophysiological features of motor neurons and putative interneurons in the dorsal vagal complex of rats and mice. *Brain Res*. 2009;1291:40-52.
70. McMenamin CA, Travagli RA, Browning KN. Inhibitory neurotransmission regulates vagal efferent activity and gastric motility. *Exp Biol Med (Maywood)*. 2016;241:1343-1350.
71. Waldvogel HJ, Baer K, Eady E, et al. Differential localization of γ-aminobutyric acid type A and glycine receptor subunits and gephyrin in the human pons, medulla oblongata and uppermost cervical segment of the spinal cord: An immunohistochemical study. *J Comp Neurol*. 2010;518:305-328.
72. Gao H, Smith BN. Zolpidem modulation of phasic and tonic GABA currents in the rat dorsal motor nucleus of the vagus. *Neuropharmacology*. 2010;58:1220-1227.
73. Gao H, Smith BN. Tonic GABAA receptor-mediated inhibition in the rat dorsal motor nucleus of the vagus. *J Neurophysiol*. 2010;103:904-914.
74. Crestani F, Martin JR, Mohler H, Rudolph U. Mechanism of action of the hypnotic zolpidem in vivo. *Br J Pharmacol*. 2000;131: 1251-1254.
75. Pritchett DB, Seeburg PH. γ-Aminobutyric $acid_A$ receptor α_5-subunit creates novel type II benzodiazepine receptor pharmacology. *J Neurochem*. 1990;54:1802-1804.
76. Tao J, Campbell JN, Tsai LT, Wu C, Liberles SD, Lowell BB. Highly selective brain-to-gut communication via genetically defined vagus neurons. *Neuron*. 2021;109: 2106-2115.e4.
77. Sivarao DV, Krowicki ZK, Hornby PJ. Role of GABAA receptors in rat hindbrain nuclei controlling gastric motor function. *Neurogastroenterol Motil*. 1998;10:305-313.
78. Washabau RJ, Fudge M, Price WJ, Barone FC. GABA receptors in the dorsal motor nucleus of the vagus influence feline lower esophageal sphincter and gastric function. *Brain Res Bull*. 1995;38:587-594.
79. Page ME, Akaoka H, Aston-Jones G, Valentino RJ. Bladder distention activates noradrenergic locus coeruleus neurons by an excitatory amino acid mechanism. *Neuroscience*. 1992;51: 555-563.
80. DuBar SG, Cosio D, Korthas H, et al. Somatostatin neurons in the mouse pontine nucleus activate $GABA_A$ receptor mediated synaptic currents in locus Coeruleus neurons. *Front Synaptic Neurosci*. 2021;13:754786.
81. Kuo C-C, Hsieh J-C, Tsai H-C, et al. Inhibitory interneurons regulate phasic activity of noradrenergic neurons in the mouse locus coeruleus and functional implications. *J Physiol*. 2020;598: 4003-4029.
82. Breton-Provencher V, Sur M. Active control of arousal by a locus coeruleus GABAergic circuit. *Nat Neurosci*. 2019;22: 218-228.
83. Jin X, Li S, Bondy B, et al. Identification of a group of GABAergic neurons in the dorsomedial area of the locus coeruleus. *PLoS One*. 2016;11:e0146470.
84. Aston-Jones G, Zhu Y, Card JP. Numerous GABAergic afferents to locus ceruleus in the pericerulear dendritic zone: Possible interneuronal pool. *J Neurosci*. 2004;24:2313-2321.
85. Corteen NL, Cole TM, Sarna A, Sieghart W, Swinny JD. Localization of GABA-A receptor alpha subunits on neurochemically distinct cell types in the rat locus coeruleus. *Eur J Neurosci*. 2011;34:250-262.
86. Kelly L, Seifi M, Ma R, et al. Identification of intraneuronal amyloid beta oligomers in locus coeruleus neurons of Alzheimer's patients and their potential impact on inhibitory neurotransmitter receptors and neuronal excitability. *Neuropathol Appl Neurobiol*. 2021;47:488-505.
87. Nardone S, De Luca R, Zito A, et al. A spatially-resolved transcriptional atlas of the murine dorsal pons at single-cell resolution. *Nat Commun*. 2024;15:1966.
88. Luskin AT, Li L, Fu X, et al. 2023. A diverse network of pericoerulear neurons control arousal states. *bioRxiv*. [Preprint] https://doi.org/10.1101/2022.06.30.498327
89. Jiang Y, Babic T, Travagli RA. Sex differences in GABAergic neurotransmission to rat DMV neurons. *Am J Physiol Gastrointest Liver Physiol*. 2019;317:G476-G483.
90. Datz FL, Christian PE, Moore J. Gender-related differences in gastric emptying. *J Nucl Med*. 1987;28:1204-1207.

91. Narayanan SP, Anderson B, Bharucha AE. Sex- and gender-related differences in common functional gastroenterologic disorders. *Mayo Clin Proc*. 2021;96:1071-1089.
92. Bellusci L, Kim E, Garcia DuBar S, Gillis RA, Vicini S, Sahibzada N. Brainstem activation of GABA. *Front Neurosci*. 2022;16: 961042.
93. Berthoud HR, Patterson LM, Zheng H. Vagal-enteric interface: Vagal activation-induced expression of c-Fos and p-CREB in neurons of the upper gastrointestinal tract and pancreas. *Anat Rec*. 2001;262:29-40.
94. Beckett EAH, McGeough CA, Sanders KM, Ward SM. Pacing of interstitial cells of Cajal in the murine gastric antrum: Neurally mediated and direct stimulation. *J Physiol*. 2003; 553(Pt 2):545-559.
95. Zheng H, Berthoud HR. Functional vagal input to gastric myenteric plexus as assessed by vagal stimulation-induced Fos expression. *Am J Physiol Gastrointest Liver Physiol*. 2000;279: G73-G81.
96. Verstegen AMJ, Vanderhorst V, Gray PA, Zeidel ML, Geerling JC. Barrington's nucleus: Neuroanatomic landscape of the mouse "pontine micturition center". *J Comp Neurol*. 2017;525: 2287-2309.
97. Barrington FJ. Affections of micturition resulting from lesions of the nervous system. *Proc R Soc Med*. 1927;20:722-727.
98. Pavcovich LA, Yang M, Miselis RR, Valentino RJ. Novel role for the pontine micturition center, Barrington's nucleus: Evidence for coordination of colonic and forebrain activity. *Brain Res*. 1998;784:355-361.
99. Lechner SM, Curtis AL, Brons R, Valentino RJ. Locus coeruleus activation by colon distention: Role of corticotropin-releasing factor and excitatory amino acids. *Brain Res*. 1997;756:114-124.
100. Elam M, Thorén P, Svensson TH. Locus coeruleus neurons and sympathetic nerves: Activation by visceral afferents. *Brain Res*. 1986;375:117-125.
101. Valentino RJ, Kosboth M, Colflesh M, Miselis RR. Transneuronal labeling from the rat distal colon: Anatomic evidence for regulation of distal colon function by a pontine corticotropin-releasing factor system. *J Comp Neurol*. 2000; 417:399-414.
102. Rouzade-Dominguez ML, Curtis AL, Valentino RJ. Role of Barrington's nucleus in the activation of rat locus coeruleus neurons by colonic distension. *Brain Res*. 2001;917:206-218.
103. Valentino RJ, Guyenet P, Hou XH, Herman M. Central network dynamics regulating visceral and humoral functions. *J Neurosci*. 2017;37:10848-10854.
104. Wang HY, Kuo ZC, Fu YS, Chen RF, Min MY, Yang HW. GABAB receptor-mediated tonic inhibition regulates the spontaneous firing of locus coeruleus neurons in developing rats and in citalopram-treated rats. *J Physiol*. 2015;593:161-180.
105. Wu RN, Kuo CC, Min MY, Chen RF, Yang HW. Extracellular signal-regulated kinases mediate an autoregulation of GABA. *Sci Rep*. 2020;10:7869.
106. Kawahara Y, Kawahara H, Westerink BH. Tonic regulation of the activity of noradrenergic neurons in the locus coeruleus of the conscious rat studied by dual-probe microdialysis. *Brain Res*. 1999;823:42-48.
107. Shefner SA, Osmanović SS. $GABA_A$ and $GABA_B$ receptors and the ionic mechanisms mediating their effects on locus coeruleus neurons. *Prog Brain Res*. 1991;88:187-195.
108. Tan KR, Brown M, Labouebe G, et al. Neural bases for addictive properties of benzodiazepines. *Nature*. 2010;463:769-774.
109. Powley TL. Brain-gut communication: Vagovagal reflexes interconnect the two "brains". *Am J Physiol Gastrointest Liver Physiol*. 2021;321:G576-G587.
110. Kupari J, Haring M, Agirre E, Castelo-Branco G, Ernfors P. An atlas of vagal sensory neurons and their molecular specialization. *Cell Rep*. 2019;27:2508-2523.e4.
111. Rohof WO, Aronica E, Beaumont H, Troost D, Boeckxstaens GE. Localization of mGluR5, $GABA_B$, $GABA_A$, and cannabinoid receptors on the vago-vagal reflex pathway responsible for transient lower esophageal sphincter relaxation in humans: An immunohistochemical study. *Neurogastroenterol Motil*. 2012; 24:383-e173.
112. Shoji Y, Yamaguchi-Yamada M, Yamamoto Y. Glutamate- and GABA-mediated neuron-satellite cell interaction in nodose ganglia as revealed by intracellular calcium imaging. *Histochem Cell Biol*. 2010;134:13-22.
113. Yokoyama T, Fukuzumi S, Hayashi H, Nakamuta N, Yamamoto Y. GABA-mediated modulation of ATP-induced intracellular calcium responses in nodose ganglion neurons of the rat. *Neurosci Lett*. 2015;584:168-172.
114. Spencer NJ, Kyloh MA, Travis L, Dodds KN. Identification of spinal afferent nerve endings in the colonic mucosa and submucosa that communicate directly with the spinal cord: The gut-brain axis. *J Comp Neurol*. 2020;528:1742-1753.
115. Fletcher EL, Clark MJ, Senior P, Furness JB. Gene expression and localization of GABA(C) receptors in neurons of the rat gastrointestinal tract. *Neuroscience*. 2001;107:181-189.
116. Fletcher EL, Clark MJ, Furness JB. Neuronal and glial localization of GABA transporter immunoreactivity in the myenteric plexus. *Cell Tissue Res*. 2002;308:339-346.
117. Fried DE, Watson RE, Robson SC, Gulbransen BD. Ammonia modifies enteric neuromuscular transmission through glial γ-aminobutyric acid signaling. *Am J Physiol Gastrointest Liver Physiol*. 2017;313:G570-G580.
118. Deng Z, Li D, Yan X, et al. Activation of GABA receptor attenuates intestinal inflammation by modulating enteric glial cells function through inhibiting NF-κB pathway. *Life Sci*. 2023;329: 121984.
119. Ma X, Sun Q, Sun X, et al. Activation of $GABA_A$ receptors in colon epithelium exacerbates acute colitis. *Front Immunol*. 2018;9: 987.
120. Oomori Y, Iuchi H, Ishikawa K, Satoh Y, Ono K. Gamma-aminobutyric acid immunoreactivity in the enterochromaffin cells of the rat stomach. *Acta Anat (Basel)*. 1992;145:127-131.
121. Modlin IM, Kidd M, Pfragner R, Eick GN, Champaneria MC. The functional characterization of normal and neoplastic human enterochromaffin cells. *J Clin Endocrinol Metab*. 2006;91: 2340-2348.
122. Schäfermeyer A, Gratzl M, Rad R, Dossumbekova A, Sachs G, Prinz C. Isolation and receptor profiling of ileal enterochromaffin cells. *Acta Physiol Scand*. 2004;182:53-62.
123. Bezençon C, Fürholz A, Raymond F, et al. Murine intestinal cells expressing Trpm5 are mostly brush cells and express markers of neuronal and inflammatory cells. *J Comp Neurol*. 2008;509:514-525.
124. Arora P, Andersen D, Moll JM, et al. Small intestinal tuft cell activity associates with energy metabolism in diet-induced obesity. *Front Immunol*. 2021;12:629391.
125. Haber AL, Biton M, Rogel N, et al. A single-cell survey of the small intestinal epithelium. *Nature*. 2017;551:333-339.
126. Hendel SK, Kellermann L, Hausmann A, Bindslev N, Jensen KB, Nielsen OH. Tuft cells and their role in intestinal diseases. *Front Immunol*. 2022;13:822867.
127. Seifi M, Swinny JD. Developmental and age-dependent plasticity of GABA(A) receptors in the mouse colon: Implications in colonic motility and inflammation. *Auton Neurosci*. 2019;221: 102579.

128. Seifi M, Rodaway S, Rudolph U, Swinny JD. GABAA receptor subtypes regulate stress-induced colon inflammation in mice. *Gastroenterology*. 2018;155:852-864.e3 .
129. Drokhlyansky E, Smillie CS, Van Wittenberghe N, et al. The human and mouse enteric nervous system at single-cell resolution. *Cell*. 2020;182:1606-1622.e23.
130. Wright CM, Schneider S, Smith-Edwards KM, et al. scRNA-Seq reveals new enteric nervous system roles for GDNF, NRTN, and TBX3. *Cell Mol Gastroenterol Hepatol*. 2021;11:1548-1592.e1.
131. Gribble FM, Reimann F. Enteroendocrine cells: Chemosensors in the intestinal epithelium. *Annu Rev Physiol*. 2016;78:277-299.
132. Racke K, Schworer H. Regulation of serotonin release from the intestinal mucosa. *Pharmacol Res*. 1991;23:13-25.
133. Schwörer H, Racké K, Kilbinger H. GABA receptors are involved in the modulation of the release of 5-hydroxytryptamine from the vascularly perfused small intestine of the guinea-pig. *Eur J Pharmacol*. 1989;165:29-37.
134. Galligan JJ. 5-HT secretion by enterochromaffin cells is a very touching story. *J Physiol*. 2017;595:3.
135. Jansen A, Hoepfner M, Herzig KH, Riecken EO, Scherubl H. GABA(C) receptors in neuroendocrine gut cells: A new GABA-binding site in the gut. *Pflugers Arch*. 2000;441:294-300.
136. Sharkey KA, Mawe GM. The enteric nervous system. *Physiol Rev*. 2023;103:1487-1564.
137. Griffiths JA, Yoo BB, Thuy-Boun P, et al. Peripheral neuronal activation shapes the microbiome and alters gut physiology. *Cell Rep*. 2024;43:113953.
138. Jessen KR, Mirsky R, Dennison ME, Burnstock G. GABA may be a neurotransmitter in the vertebrate peripheral nervous system. *Nature*. 1979;281:71-74.
139. Miki Y, Taniyama K, Tanaka C, Tobe T. GABA, glutamic acid decarboxylase, and GABA transaminase levels in the myenteric plexus in the intestine of humans and other mammals. *J Neurochem*. 1983;40:861-865.
140. Taniyama K, Miki Y, Tanaka C. Presence of γ-aminobutyric acid and glutamic acid decarboxylase in Auerbach's plexus of cat colon. *Neurosci Lett*. 1982;29:53-56.
141. Krantis A, Shabnavard L, Nichols K, de Blas AL, Staines W. Localization of GABAA receptor immunoreactivity in NO synthase positive myenteric neurones. *J Auton Nerv Syst*. 1995;53:157-165.
142. D'Antongiovanni V, Pellegrini C, Antonioli L, et al. Enteric glia and brain astroglia: Complex communication in health and disease along the gut-brain axis. *Neuroscientist*. 2024;30:493-510.
143. Seguella L, Gulbransen BD. Enteric glial biology, intercellular signalling and roles in gastrointestinal disease. *Nat Rev Gastroenterol Hepatol*. 2021;18:571-587.
144. Krantis A. GABA in the mammalian enteric nervous system. *News Physiol Sci*. 2000;15:284-290.
145. Bhandage AK, Barragan A. GABAergic signaling by cells of the immune system: More the rule than the exception. *Cell Mol Life Sci*. 2021;78:5667-5679.
146. DeGruttola AK, Low D, Mizoguchi A, Mizoguchi E. Current understanding of dysbiosis in disease in human and animal models. *Inflamm Bowel Dis*. 2016;22:1137-1150.
147. Zhang Y-J, Li S, Gan R-Y, Zhou T, Xu D-P, Li H-B. Impacts of gut bacteria on human health and diseases. *Int J Mol Sci*. 2015;16:7493-7519.
148. Matsumoto M, Ooga T, Kibe R, Aiba Y, Koga Y, Benno Y. Colonic absorption of low-molecular-weight metabolites influenced by the intestinal microbiome: A pilot study. *PLoS One*. 2017;12:e0169207.
149. Fujisaka S, Avila-Pacheco J, Soto M, et al. Diet, genetics, and the gut microbiome drive dynamic changes in plasma metabolites. *Cell Rep*. 2018;22:3072-3086.
150. Pokusaeva K, Johnson C, Luk B, et al. GABA-producing *Bifidobacterium dentium* modulates visceral sensitivity in the intestine. *Neurogastroenterol Motil*. 2017;29:e12904.
151. Sekirov I, Russell SL, Antunes LC, Finlay BB. Gut microbiota in health and disease. *Physiol Rev*. 2010;90:859-904.
152. Ruigrok RAAA, Weersma RK, Vich Vila A. The emerging role of the small intestinal microbiota in human health and disease. *Gut Microbes*. 2023;15:2201155.
153. Barrett E, Ross RP, O'Toole PW, Fitzgerald GF, Stanton C. γ-Aminobutyric acid production by culturable bacteria from the human intestine. *J Appl Microbiol*. 2012;113:411-417.
154. Braga JD, Thongngam M, Kumrungsee T. Gamma-aminobutyric acid as a potential postbiotic mediator in the gut-brain axis. *NPJ Sci Food*. 2024;8:16.
155. Sarasa SB, Mahendran R, Muthusamy G, Thankappan B, Selta DRF, Angayarkanni J. A brief review on the non-protein amino acid, gamma-amino butyric acid (GABA): Its production and role in microbes. *Curr Microbiol*. 2020;77:534-544.
156. Strandwitz P, Kim KH, Terekhova D, et al. GABA-modulating bacteria of the human gut microbiota. *Nat Microbiol*. 2019;4:396-403.
157. Otaru N, Ye K, Mujezinovic D, et al. GABA production by human intestinal *Bacteroides* spp.: Prevalence, regulation, and role in acid stress tolerance. *Front Microbiol*. 2021;12:656895.
158. Diez-Gutierrez L, San Vicente L, Barron LJR, Villaran MD, Chavarri M. γ-Aminobutyric acid and probiotics: Multiple health benefits and their future in the global functional food and nutraceuticals market. *J Funct Foods*. 2020;64:103669.
159. Yogeswara IBA, Maneerat S, Haltrich D. Glutamate decarboxylase from lactic acid bacteria-a key enzyme in GABA synthesis. *Microorganisms*. 2020;8:1923.
160. Mohler H. GABA(A) receptor diversity and pharmacology. *Cell Tissue Res*. 2006;326:505-516.
161. Kang D-W, Adams JB, Coleman DM, et al. Long-term benefit of Microbiota Transfer Therapy on autism symptoms and gut microbiota. *Sci Rep*. 2019;9:5821.
162. Averina OV, Kovtun AS, Polyakova SI, Savilova AM, Rebrikov DV, Danilenko VN. The bacterial neurometabolic signature of the gut microbiota of young children with autism spectrum disorders. *J Med Microbiol*. 2020;69:558-571.
163. Zheng P, Zeng B, Zhou C, et al. Gut microbiome remodeling induces depressive-like behaviors through a pathway mediated by the host's metabolism. *Mol Psychiatry*. 2016;21:786-796.
164. Valles-Colomer M, Falony G, Darzi Y, et al. The neuroactive potential of the human gut microbiota in quality of life and depression. *Nat Microbiol*. 2019;4:623-632.
165. Wang Z, Yuan X, Zhu Z, et al. Multiomics analyses reveal microbiome-gut-brain crosstalk centered on aberrant gamma-aminobutyric acid and tryptophan metabolism in drug-naive patients with first-episode schizophrenia. *Schizophr Bull*. 2024;50:187-198.
166. Leclercq S, de Timary P, Delzenne NM, Starkel P. The link between inflammation, bugs, the intestine and the brain in alcohol dependence. *Transl Psychiatry*. 2017;7:e1048.
167. Chen G, Shi F, Yin W, et al. Gut microbiota dysbiosis: The potential mechanisms by which alcohol disrupts gut and brain functions. *Front Microbiol*. 2022;13:916765.

168. Megur A, Baltriukiene D, Bukelskiene V, Burokas A. The microbiota-gut-brain axis and Alzheimer's disease: Neuroinflammation is to blame? *Nutrients*. 2020;13:37.
169. Kesika P, Suganthy N, Sivamaruthi BS, Chaiyasut C. Role of gut-brain axis, gut microbial composition, and probiotic intervention in Alzheimer's disease. *Life Sci*. 2021;264:118627.
170. Liston C, Chen AC, Zebley BD, et al. Default mode network mechanisms of transcranial magnetic stimulation in depression. *Biol Psychiatry*. 2014;76:517-526.
171. Rhee SJ, Kim H, Lee Y, et al. The association between serum microbial DNA composition and symptoms of depression and anxiety in mood disorders. *Sci Rep*. 2021;11:13987.
172. Rong H, Xie X-H, Zhao J, et al. Similarly in depression, nuances of gut microbiota: Evidences from a shotgun metagenomics sequencing study on major depressive disorder versus bipolar disorder with current major depressive episode patients. *J Psychiatr Res*. 2019;113:90-99.
173. Jiang H, Ling Z, Zhang Y, et al. Altered fecal microbiota composition in patients with major depressive disorder. *Brain Behav Immun*. 2015;48:186-194.
174. Tillisch K, Mayer EA, Gupta A, et al. Brain structure and response to emotional stimuli as related to gut microbial profiles in healthy women. *Psychosom Med*. 2017;79:905-913.
175. Puetz LC, Delmont TO, Aizpurua O, et al. Gut microbiota linked with reduced fear of humans in red junglefowl has implications for early domestication. *Adv Genet (Hoboken)*. 2021;2:2100018.
176. Mulder D, Aarts E, Arias Vasquez A, Bloemendaal M. A systematic review exploring the association between the human gut microbiota and brain connectivity in health and disease. *Mol Psychiatry*. 2023;28:5037-5061.
177. Yang J, Zheng P, Li Y, et al. Landscapes of bacterial and metabolic signatures and their interaction in major depressive disorders. *Sci Adv*. 2020;6:eaba8555.
178. Sanada K, Nakajima S, Kurokawa S, et al. Gut microbiota and major depressive disorder: A systematic review and meta-analysis. *J Affect Disord*. 2020;266:1-13.
179. Lin P, Ding B, Feng C, et al. Prevotella and Klebsiella proportions in fecal microbial communities are potential characteristic parameters for patients with major depressive disorder. *J Affect Disord*. 2017;207:300-304.
180. Liu L, Wang H, Zhang H, et al. Toward a deeper understanding of gut microbiome in depression: The promise of clinical applicability. *Adv Sci (Weinh)*. 2022;9:e2203707.
181. Zhang Y, Fan Q, Hou Y, et al. Bacteroides species differentially modulate depression-like behavior via gut-brain metabolic signaling. *Brain Behav Immun*. 2022;102:11-22.
182. Cryan JF, Mazmanian SK. Microbiota-brain axis: Context and causality. *Science*. 2022;376:938-939.
183. Ling Z, Cheng Y, Chen F, et al. Changes in fecal microbiota composition and the cytokine expression profile in school-aged children with depression: A case-control study. *Front Immunol*. 2022;13:964910.
184. Dowlati Y, Herrmann N, Swardfager W, et al. A meta-analysis of cytokines in major depression. *Biol Psychiatry*. 2010;67:446-457.
185. Caso JR, MacDowell KS, Gonzalez-Pinto A, et al. Gut microbiota, innate immune pathways, and inflammatory control mechanisms in patients with major depressive disorder. *Transl Psychiatry*. 2021;11:645.
186. Felger JC, Lotrich FE. Inflammatory cytokines in depression: Neurobiological mechanisms and therapeutic implications. *Neuroscience*. 2013;246:199-229.
187. Haroon E, Raison CL, Miller AH. Psychoneuroimmunology meets neuropsychopharmacology: Translational implications of the impact of inflammation on behavior. *Neuropsychopharmacology*. 2012;37:137-162.
188. Lee C-H, Giuliani F. The role of inflammation in depression and fatigue. *Front Immunol*. 2019;10:1696.
189. Goldsmith DR, Bekhbat M, Mehta ND, Felger JC. Inflammation-related functional and structural dysconnectivity as a pathway to psychopathology. *Biol Psychiatry*. 2023;93:405-418.
190. Dicks LMT. Gut bacteria and neurotransmitters. *Microorganisms*. 2022;10:1838.
191. Eicher TP, Mohajeri MH. Overlapping mechanisms of action of brain-active bacteria and bacterial metabolites in the pathogenesis of common brain diseases. *Nutrients*. 2022;14:2661.
192. Levinson AJ, Fitzgerald PB, Favalli G, Blumberger DM, Daigle M, Daskalakis ZJ. Evidence of cortical inhibitory deficits in major depressive disorder. *Biol Psychiatry*. 2010;67:458-464.
193. Luscher B, Fuchs T. GABAergic control of depression-related brain states. *Adv Pharmacol*. 2015;73:97-144.
194. Luscher B, Mohler H. Brexanolone, a neurosteroid antidepressant, vindicates the GABAergic deficit hypothesis of depression and may foster resilience. *F1000Res*. 2019;8:F1000 Faculty Rev-751.
195. Horvath TD, Ihekweazu FD, Haidacher SJ, et al. *Bacteroides ovatus* colonization influences the abundance of intestinal short chain fatty acids and neurotransmitters. i*Science*. 2022;25:104158.
196. Yang C, Mogno I, Contijoch EJ, et al. Fecal IgA levels are determined by strain-level differences in *Bacteroides ovatus* and are modifiable by gut microbiota manipulation. *Cell Host Microbe*. 2020;27:467-475.e6.
197. Sutherland DB, Suzuki K, Fagarasan S. Fostering of advanced mutualism with gut microbiota by immunoglobulin A. *Immunol Rev*. 2016;270:20-31.
198. Angoa-Perez M, Kuhn DM. Evidence for modulation of substance use disorders by the gut microbiome: Hidden in plain sight. *Pharmacol Rev*. 2021;73:571-596.
199. Chivero ET, Sil S, Kumar M, Buch S. Substance use, microbiome and psychiatric disorders. *Pharmacol Biochem Behav*. 2022;219:173432.
200. Meroni M, Longo M, Dongiovanni P. Alcohol or gut microbiota: Who is the guilty? *Int J Mol Sci*. 2019;20:4568.
201. Lang S, Schnabl B. Microbiota and fatty liver disease-the known, the unknown, and the future. *Cell Host Microbe*. 2020;28:233-244.
202. Anker JJ, Kushner MG. Co-occurring alcohol use disorder and anxiety: Bridging psychiatric, psychological, and neurobiological perspectives. *Alcohol Res*. 2019;40:arcr.v40.1.03.
203. Kronsten VT, Tranah TH, Pariante C, Shawcross DL. Gut-derived systemic inflammation as a driver of depression in chronic liver disease. *J Hepatol*. 2022;76:665-680.
204. Leclercq S, de Timary P. Role of the microbiome and the gut-brain axis in alcohol use disorder: Potential implication for treatment development. *Curr Top Behav Neurosci*. Published online 25 June 2024. doi: 10.1007/7854_2024_478.
205. McCurry MD, D'Agostino GD, Walsh JT, et al. Gut bacteria convert glucocorticoids into progestins in the presence of hydrogen gas. *Cell*. 2024;187:2952-2968.e13.
206. Ashworth-Preece M, Krstew E, Jarrott B, Lawrence AJ. Functional GABAA receptors on rat vagal afferent neurones. *Br J Pharmacol*. 1997;120:469-475.
207. Smid SD, Young RL, Cooper NJ, Blackshaw LA. GABA(B)R expressed on vagal afferent neurones inhibit gastric mechanosensitivity in ferret proximal stomach. *Am J Physiol Gastrointest Liver Physiol*. 2001;281:G1494-G1501.

208. Nakamura U, Nohmi T, Sagane R, et al. Dietary gamma-aminobutyric acid (GABA) induces satiation by enhancing the postprandial activation of vagal afferent nerves. *Nutrients*. 2022;14:2492.
209. Bravo JA, Forsythe P, Chew MV, et al. Ingestion of lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. *Proc Natl Acad Sci U S A*. 2011;108:16050-16055.
210. Tian J, Kaufman DL. The GABA and GABA-receptor system in inflammation, anti-tumor immune responses, and COVID-19. *Biomedicines*. 2023;11:254.
211. Auteri M, Zizzo MG, Serio R. GABA and GABA receptors in the gastrointestinal tract: From motility to inflammation. *Pharmacol Res*. 2015;93:11-21.
212. Vighi G, Marcucci F, Sensi L, Di Cara G, Frati F. Allergy and the gastrointestinal system. *Clin Exp Immunol*. 2008;153(Suppl 1): 3-6.
213. Cicero A L, Stahl PD, Raposo G. Extracellular vesicles shuffling intercellular messages: For good or for bad. *Curr Opin Cell Biol*. 2015;35:69-77.
214. Inotsuka R, Uchimura K, Yamatsu A, Kim M, Katakura Y. γ-Aminobutyric acid (GABA) activates neuronal cells by inducing the secretion of exosomes from intestinal cells. *Food Funct*. 2020;11:9285-9290.
215. Inotsuka R, Udono M, Yamatsu A, Kim M, Katakura Y. Exosome-mediated activation of neuronal cells triggered by γ-aminobutyric acid (GABA). *Nutrients*. 2021;13:2544.
216. Planamente S, Vigouroux A, Mondy S, Nicaise M, Faure D, Morera S. A conserved mechanism of GABA binding and antagonism is revealed by structure-function analysis of the periplasmic binding protein Atu2422 in *Agrobacterium tumefaciens*. *J Biol Chem*. 2010;285:30294-30303.
217. Planamente S, Mondy S, Hommais F, Vigouroux A, Morera S, Faure D. Structural basis for selective GABA binding in bacterial pathogens. *Mol Microbiol*. 2012;86:1085-1099.
218. Chevrot R, Rosen R, Haudecoeur E, et al. GABA controls the level of quorum-sensing signal in *Agrobacterium tumefaciens*. *Proc Natl Acad Sci U S A*. 2006;103:7460-7464.
219. Bocquet N, de Carvalho LP, Cartaud J, et al. A prokaryotic proton-gated ion channel from the nicotinic acetylcholine receptor family. *Nature*. 2007;445:116-119.
220. Zimmermann I, Dutzler R. Ligand activation of the prokaryotic pentameric ligand-gated ion channel ELIC. *PLoS Biol*. 2011;9: e1001101.
221. Spurny R, Ramerstorfer J, Price K, et al. Pentameric ligand-gated ion channel ELIC is activated by GABA and modulated by benzodiazepines. *Proc Natl Acad Sci U S A*. 2012;109: E3028-E3034.
222. Hu CL, Rzymski P. Non-Photosynthetic melainabacteria (Cyanobacteria) in human gut: Characteristics and association with health. *Life-Basel*. 2022;12:476.
223. Muñoz-Rodríguez D, Bourqqia-Ramzi M, García-Esteban MT, , et al. Bioelectrical state of bacteria is linked to growth dynamics and response to neurotransmitters: Perspectives for the investigation of the microbiota-brain axis. *Int J Mol Sci*. 2023; 24:13394.
224. Benarroch JM, Asally M. The microbiologist's guide to membrane potential dynamics. *Trends Microbiol*. 2020;28:304-314.
225. Richardson RJ, Petrou S, Bryson A. Established and emerging $GABA_A$ receptor pharmacotherapy for epilepsy. *Front Pharmacol*. 2024;15:1341472.
226. Witkin JM, Shafique H, Cerne R, et al. Mechanistic and therapeutic relationships of traumatic brain injury and γ-aminobutyric acid (GABA). *Pharmacol Ther*. 2024;256:108609.
227. Boonstra E, de Kleijn R, Colzato LS, Alkemade A, Forstmann BU, Nieuwenhuis S. Neurotransmitters as food supplements: The effects of GABA on brain and behavior. *Front Psychol*. 2015;6: 1520.
228. Hepsomali P, Groeger JA, Nishihira J, Scholey A. Effects of oral gamma-aminobutyric acid (GABA) administration on stress and sleep in humans: A systematic review. *Front Neurosci*. 2020;14:923.
229. Louveau A, Smirnov I, Keyes TJ, et al. Structural and functional features of central nervous system lymphatic vessels. *Nature*. 2015;523:337-341.
230. Shelp BJ, Bown AW, McLean MD. Metabolism and functions of gamma-aminobutyric acid. *Trends Plant Sci*. 1999;4:446-452.
231. Fait A, Nesi AN, Angelovici R, et al. Targeted enhancement of glutamate-to-gamma-aminobutyrate conversion in Arabidopsis seeds affects carbon-nitrogen balance and storage reserves in a development-dependent manner. *Plant Physiol*. 2011;157:1026-1042.
232. Batushansky A, Kirma M, Grillich N, et al. Combined transcriptomics and metabolomics of *Arabidopsis thaliana* seedlings exposed to exogenous GABA suggest its role in plants is predominantly metabolic. *Mol Plant*. 2014;7:1065-1068.
233. Fromm H. GABA signaling in plants: Targeting the missing pieces of the puzzle. *J Exp Bot*. 2020;71:6238-6245.
234. Steward F, Thompson J, Dent C. γ-Aminobutyric acid, a constituent of the potato tuber. *Science*. 1949;110:439-440.
235. Wang GP, Kong J, Cui DD, et al. Resistance against *Ralstonia solanacearum* in tomato depends on the methionine cycle and the γ-aminobutyric acid metabolic pathway. *Plant J*. 2019;97: 1032-1047.
236. Tarkowski LP, Signorelli S, Hofte M. γ-Aminobutyric acid and related amino acids in plant immune responses: Emerging mechanisms of action. *Plant Cell Environ*. 2020;43:1103-1116.
237. Shelp BJ, Aghdam MS, Flaherty EJ. γ-Aminobutyrate (GABA) regulated plant defense: Mechanisms and opportunities. *Plants (Basel)*. 2021;10:1939.
238. Dagorn A, Hillion M, Chapalain A, et al. Gamma-aminobutyric acid acts as a specific virulence regulator in *Pseudomonas aeruginosa*. *Microbiology-Sgm*. 2013;159(Pt 2):339-351.
239. Li L, Dou N, Zhang H, Wu CX. The versatile GABA in plants. *Plant Signal Behav*. 2021;16:1862565.
240. Bourqqia-Ramzi M, Mansilla-Guardiola J, Muñoz-Rodriguez D, et al. From the microbiome to the electrome: Implications for the microbiota-gut-brain axis. *Int J Mol Sci*. 2024; 25:6233.
241. Stratford JP, Edwards CLA, Ghanshyam MJ, et al. Electrically induced bacterial membrane-potential dynamics correspond to cellular proliferation capacity. *Proc Natl Acad Sci U S A*. 2019; 116:9552-9557.
242. Thompson AJ, Alqazzaz M, Ulens C, Lummis SCR. The pharmacological profile of ELIC, a prokaryotic GABA-gated receptor. *Neuropharmacology*. 2012;63:761-767.
243. Ghosh B, Tsao T-W, Czajkowski C. A chimeric prokaryotic-eukaryotic pentameric ligand gated ion channel reveals interactions between the extracellular and transmembrane domains shape neurosteroid modulation. *Neuropharmacology*. 2017;125:343-352.
244. Lacy BE, Patel NK. Rome criteria and a diagnostic approach to irritable bowel syndrome. *J Clin Med*. 2017;6:99.
245. Midenfjord I, Polster A, Sjövall H, Törnblom H, Simrén M. Anxiety and depression in irritable bowel syndrome: Exploring the interaction with other symptoms and pathophysiology using multivariate analyses. *Neurogastroenterol Motil*. 2019;31:e13619.

246. Atack JR. GABAA receptor subtype-selective modulators. I. α2/α3-selective agonists as non-sedating anxiolytics. *Curr Top Med Chem*. 2011;11:1176-1202.
247. Durkin EJ, Muessig L, Herlt T, et al. 2018. Brain neurosteroids are natural anxiolytics targeting α2 subunit γ-aminobutyric acid type-A receptors. *bioRxiv*. [Preprint] https://doi.org/10.1101/462457
248. Enz R, Cutting GR. Molecular composition of GABAC receptors. *Vision Res*. 1998;38:1431-1441.
249. Zizzo MG, Mulè F, Serio R. Functional evidence for GABA as modulator of the contractility of the longitudinal muscle in mouse duodenum: Role of GABA(A) and GABA(C) receptors. *Neuropharmacology*. 2007;52:1685-1690.
250. McCarthy T, Green BD, Calderwood D, et al. STC-1 cells. In: Verhoeckx K, Cotter P, López-Expósito I, Kleiveland C, Lea T, Mackie A, eds. *The impact of food bioactives on health: In vitro and ex vivo models*. Springer International Publishing; 2015:211-220.
251. Zarei Eskikand P, Koussoulas K, Gwynne RM, Bornstein JC. Computational simulations and Ca2+ imaging reveal that slow synaptic depolarizations (slow EPSPs) inhibit fast EPSP evoked action potentials for most of their time course in enteric neurons. *PLoS Comput Biol*. 2022;18:e1009717.
252. Vezzani A, Viviani B. Neuromodulatory properties of inflammatory cytokines and their impact on neuronal excitability. *Neuropharmacology*. 2015;96(Pt A):70-82.
253. Wisden W, Laurie DJ, Monyer H, Seeburg PH. The distribution of 13 GABAA receptor subunit mRNAs in the rat brain. I. Telencephalon, diencephalon, mesencephalon. *J Neurosci*. 1992;12:1040-1062.
254. Olsen RW, Sieghart W. GABA A receptors: Subtypes provide diversity of function and pharmacology. *Neuropharmacology*. 2009;56:141-148.
255. Bhandage AK, Olivera GC, Kanatani S, et al. A motogenic GABAergic system of mononuclear phagocytes facilitates dissemination of coccidian parasites. *Elife*. 2020;9:e60528.
256. Kang S, Liu L, Wang T, et al. GAB functions as a bioenergetic and signalling gatekeeper to control T cell inflammation. *Nat Metab*. 2022;4:1322-1335.
257. Zhang B, Vogelzang A, Miyajima M, et al. B cell-derived GABA elicits IL-10(+) macrophages to limit anti-tumour immunity. *Nature*. 2021;599:471-476.
258. Storustovu SI, Ebert B. Pharmacological characterization of agonists at delta-containing GABAA receptors: Functional selectivity for extrasynaptic receptors is dependent on the absence of γ2. *J Pharmacol Exp Ther*. 2006;316:1351-1359.
259. Bhandage AK, Hellgren C, Jin Z, Olafsson EB, Sundstrom-Poromaa I, Birnir B. Expression of GABA receptors subunits in peripheral blood mononuclear cells is gender dependent, altered in pregnancy and modified by mental health. *Acta Physiol (Oxf)*. 2015;213:575-585.
260. Kim JK, Kim YS, Lee H-M, et al. GABAergic signaling linked to autophagy enhances host protection against intracellular bacterial infections. *Nat Commun*. 2018;9:4184.
261. Chen L, Wang H, Vicini S, Olsen RW. The gamma-aminobutyric acid type A ($GABA_A$) receptor-associated protein (GABARAP) promotes $GABA_A$ receptor clustering and modulates the channel kinetics. *Proc Natl Acad Sci U S A*. 2000;97:11557-11562.
262. Zhou W, Zhao L, Mao Z, Wang Z, Zhang Z, Li M. Bidirectional communication between the brain and other organs: The role of extracellular vesicles. *Cell Mol Neurobiol*. 2023;43:2675-2696.
263. Figueira I, Bastos P, Gonzalez-Sarrias A, Espin JC, Costa-Silva B, Nunes Dos Santos C. Can exosomes transfer the preconditioning effects triggered by (poly)phenol compounds between cells? *Food Funct*. 2023;14:15-31.
264. Wang S, Luo J, Wang H, et al. Extracellular vesicles: A crucial player in the intestinal microenvironment and beyond. *Int J Mol Sci*. 2024;25:3478.
265. Morrison DJ, Preston T. Formation of short chain fatty acids by the gut microbiota and their impact on human metabolism. *Gut Microbes*. 2016;7:189-200.
266. van Hoek MJA, Merks RMH. Redox balance is key to explaining full vs. partial switching to low-yield metabolism. *BMC Syst Biol*. 2012;6:22.
267. Stilling RM, van de Wouw M, Clarke G, Stanton C, Dinan TG, Cryan JF. The neuropharmacology of butyrate: The bread and butter of the microbiota-gut-brain axis? *Neurochem Int*. 2016;99:110-132.
268. Xie M, Chen H-H, Nie S-P, Yin J-Y, Xie M-Y. Gamma-aminobutyric acid increases the production of short-chain fatty acids and decreases pH values in mouse colon. *Molecules*. 2017;22:653.
269. Frost G, Sleeth ML, Sahuri-Arisoylu M, et al. The short-chain fatty acid acetate reduces appetite via a central homeostatic mechanism. *Nat Commun*. 2014;5:3611.
270. Shufflebotham J, Hood S, Hendry J, et al. Acute tryptophan depletion alters gastrointestinal and anxiety symptoms in irritable bowel syndrome. *Am J Gastroenterol*. 2006;101:2582-2587.
271. Rowlands BD, Klugmann M, Rae CD. Acetate metabolism does not reflect astrocytic activity, contributes directly to GABA synthesis, and is increased by silent information regulator 1 activation. *J Neurochem*. 2017;140:903-918.
272. Huang W, Hu W, Cai L, et al. Acetate supplementation produces antidepressant-like effect via enhanced histone acetylation. *J Affect Disord*. 2021;281:51-60.
273. Feng S, Meng C, Liu Y, et al. *Bacillus licheniformis* prevents and reduces anxiety-like and depression-like behaviours. *Appl Microbiol Biotechnol*. 2023;107:4355-4368.
274. O'Mahony SM, Clarke G, Borre YE, Dinan TG, Cryan JF. Serotonin, tryptophan metabolism and the brain-gut-microbiome axis. *Behav Brain Res*. 2015;277:32-48.
275. Margolis KG, Cryan JF, Mayer EA. The microbiota-gut-brain axis: From motility to mood. *Gastroenterology*. 2021;160:1486-1501.
276. Shine JM, O'Callaghan C, Walpola IC, et al. Understanding the effects of serotonin in the brain through its role in the gastrointestinal tract. *Brain*. 2022;145:2967-2981.
277. Najjar SA, Hung LY, Margolis KG. Serotonergic control of gastrointestinal development, motility, and inflammation. *Compr Physiol*. 2023;13:4851-4868.
278. Nutt DJ, Cowen PJ. Diazepam alters brain 5-HT function in man: Implications for the acute and chronic effects of benzodiazepines. *Psychol Med*. 1987;17:601-607.
279. Heninger GR, Charney DS, Sternberg DE. Serotonergic function in depression. Prolactin response to intravenous tryptophan in depressed patients and healthy subjects. *Arch Gen Psychiatry*. 1984;41:398-402.
280. Heninger GR. Indoleamines: The role of serotonin in clinical disorders. In: Bloom FE, Kupfer DJ, eds. *Psychopharmacology: The fourth generation of progress*. Raven Press Ltd.; 1995:471-482.
281. Ghosal S, Hare B, Duman RS. Prefrontal cortex GABAergic deficits and circuit dysfunction in the pathophysiology and treatment of chronic stress and depression. *Curr Opin Behav Sci*. 2017;14:1-8.
282. Hu Y-T, Tan Z-L, Hirjak D, Northoff G. Brain-wide changes in excitation-inhibition balance of major depressive disorder:

A systematic review of topographic patterns of GABA- and glutamatergic alterations. *Mol Psychiatry*. 2023;28:3257-3266.
283. Sanacora G, Gueorguieva R, Epperson CN, et al. Subtype-specific alterations of γ-aminobutyric acid and glutamate in patients with major depression. *Arch Gen Psychiatry*. 2004;61:705-713.
284. Erritzoe D, Godlewska BR, Rizzo G, et al. Brain serotonin release is reduced in patients with depression: A [^{11}C]Cimbi-36 positron emission tomography study with a d-amphetamine challenge. *Biol Psychiatry*. 2023;93:1089-1098.
285. Israelyan N, Del Colle A, Li Z, et al. Effects of serotonin and slow-release 5-hydroxytryptophan on gastrointestinal motility in a mouse model of depression. *Gastroenterology*. 2019; 157:507-521.e4.
286. Luscher B, Shen Q, Sahir N. The GABAergic deficit hypothesis of major depressive disorder. *Mol Psychiatry*. 2011;16:383-406.
287. Zhang Y, Fan K, Liu Y, Liu G, Yang X, Ma J. Cathepsin C aggravates neuroinflammation involved in disturbances of behaviour and neurochemistry in acute and chronic stress-induced murine model of depression. *Neurochem Res*. 2018;43:89-100.
288. Legan TB, Lavoie B, Mawe GM. Direct and indirect mechanisms by which the gut microbiota influence host serotonin systems. *Neurogastroenterol Motil*. 2022;34:e14346.
289. Li B, Li M, Luo Y, Li R, Li W, Liu Z. Engineered 5-HT producing gut probiotic improves gastrointestinal motility and behavior disorder. *Front Cell Infect Microbiol*. 2022;12:1013952.
290. Lindseth G, Helland B, Caspers J. The effects of dietary tryptophan on affective disorders. *Arch Psychiatr Nurs*. 2015;29: 102-107.
291. Shaw K, Turner J, Del Mar C. Tryptophan and 5-hydroxytryptophan for depression. *Cochrane Database Syst Rev*. 2002;1: CD003198.
292. Ansari F, Neshat M, Pourjafar H, Jafari SM, Samakkhah SA, Mirzakhani E. The role of probiotics and prebiotics in modulating of the gut-brain axis. *Front Nutr*. 2023;10:1173660.
293. Lalonde R, Strazielle C. Probiotic effects on anxiety-like behavior in animal models. *Rev Neurosci*. 2022;33:691-701.
294. Vitellio P, Chira A, De Angelis M, Dumitrascu DL, Portincasa P. Probiotics in psychosocial stress and anxiety. A systematic review. *J Gastrointestin Liver Dis*. 2020;29:77-83.
295. Li J, Wang J, Wang M, et al. *Bifidobacterium*: A probiotic for the prevention and treatment of depression. *Front Microbiol*. 2023; 14:1174800.
296. Duranti S, Ruiz L, Lugli GA, et al. Bifidobacterium adolescentis as a key member of the human gut microbiota in the production of GABA. *Sci Rep*. 2020;10:14112.
297. Rehman A, Di Benedetto G, Bird JK, et al. Development of a workflow for the selection, identification and optimization of lactic acid bacteria with high γ-aminobutyric acid production. *Sci Rep*. 2023;13:13663.
298. Jans LA, Riedel WJ, Markus CR, Blokland A. Serotonergic vulnerability and depression: Assumptions, experimental evidence and implications. *Mol Psychiatry*. 2007;12:522-543.
299. Pehrson AL, Sanchez C. Altered γ-aminobutyric acid neurotransmission in major depressive disorder: A critical review of the supporting evidence and the influence of serotonergic antidepressants. *Drug Des Devel Ther*. 2015;9:603-624.
300. Koyama S, Matsumoto N, Kubo C, Akaike N. Presynaptic 5-HT3 receptor-mediated modulation of synaptic GABA release in the mechanically dissociated rat amygdala neurons. *J Physiol*. 2000;529(Pt 2):373-383.
301. Choi I-S, Cho J-H, Kim J-T, et al. Serotoninergic modulation of GABAergic synaptic transmission in developing rat CA3 pyramidal neurons. *J Neurochem*. 2007;103:2342-2353.
302. Tao R, Auerbach SB. Regulation of serotonin release by GABA and excitatory amino acids. *J Psychopharmacol*. 2000;14:100-113.
303. Abellán MT, Adell A, Honrubia MA, Mengod G, Artigas F. GABAB-RI receptors in serotonergic neurons: Effects of baclofen on 5-HT output in rat brain. *Neuroreport*. 2000;11:941-945.
304. Varga V, Szekely AD, Csillag A, Sharp T, Hajos M. Evidence for a role of GABA interneurones in the cortical modulation of midbrain 5-hydroxytryptamine neurones. *Neuroscience*. 2001;106: 783-792.
305. Dickson EJ, Heredia DJ, Smith TK. Critical role of 5-HT1A, 5–HT3, and 5-HT7 receptor subtypes in the initiation, generation, and propagation of the murine colonic migrating motor complex. *Am J Physiol Gastrointest Liver Physiol*. 2010;299:G144-G157.
306. Nestor-Kalinoski A, Smith-Edwards KM, Meerschaert K, et al. Unique neural circuit connectivity of mouse proximal, middle, and distal colon defines regional colonic motor patterns. *Cell Mol Gastroenterol Hepatol*. 2022;13:309-337.e3.
307. Gershon MD. 5-Hydroxytryptamine (serotonin) in the gastrointestinal tract. *Curr Opin Endocrinol Diabetes Obes*. 2013;20: 14-21.
308. Humenick A, Chen BN, Wattchow DA, et al. Characterization of putative interneurons in the myenteric plexus of human colon. *Neurogastroenterol Motil*. 2021;33:e13964.
309. Yano JM, Yu K, Donaldson GP, et al. Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. *Cell*. 2015;161:264-276.
310. Fung TC, Vuong HE, Luna CDG, et al. Intestinal serotonin and fluoxetine exposure modulate bacterial colonization in the gut. *Nat Microbiol*. 2019;4:2064-2073.
311. Reigstad CS, Salmonson CE, Rainey JF 3rd, et al. Gut microbes promote colonic serotonin production through an effect of short-chain fatty acids on enterochromaffin cells. *FASEB J*. 2015;29:1395-1403.
312. Jeong S-B, Kim YB, Lee J-W, et al. Role of dietary gamma-aminobutyric acid in broiler chickens raised under high stocking density. *Animal Nutrition*. 2020;6:293-304.
313. Cozzani I, Barsacchi R, Dibenedetto G, Saracchi L, Falcone G. Regulation of breakdown and synthesis of L-glutamate decarboxylase in *Clostridium perfringens*. *J Bacteriol*. 1975;123: 1115-1123.
314. Diviccaro S, Giatti S, Cioffi L, et al. Paroxetine effects in adult male rat colon: Focus on gut steroidogenesis and microbiota. *Psychoneuroendocrinology*. 2022;143:105828.
315. Belelli D, Phillips GD, Atack JR, Lambert JJ. Relating neurosteroid modulation of inhibitory neurotransmission to behaviour. *J Neuroendocrinol*. 2022;34:e13045.
316. Maguire JL, Mennerick S. Neurosteroids: Mechanistic considerations and clinical prospects. *Neuropsychopharmacology*. 2024;49:73-82.
317. Almeida FB, Pinna G, Barros HMT. The role of HPA axis and allopregnanolone on the neurobiology of major depressive disorders and PTSD. *Int J Mol Sci*. 2021;22:5495.
318. Cutler AJ, Mattingly GW, Maletic V. Understanding the mechanism of action and clinical effects of neuroactive steroids and GABAergic compounds in major depressive disorder. *Transl Psychiatry*. 2023;13:228.
319. Gunduz-Bruce H, Takahashi K, Huang M-Y. Development of neuroactive steroids for the treatment of postpartum depression. *J Neuroendocrinol*. 2022;34:e13019.
320. Diviccaro S, Giatti S, Borgo F, et al. Steroidogenic machinery in the adult rat colon. *J Steroid Biochem Mol Biol*. 2020;203:105732.
321. Diviccaro S, Caputi V, Cioffi L, et al. Exploring the impact of the microbiome on neuroactive steroid levels in germ-free animals. *Int J Mol Sci*. 2021;22:12251.

322. Kisiela M, Skarka A, Ebert B, Maser E. Hydroxysteroid dehydrogenases (HSDs) in bacteria: A bioinformatic perspective. *J Steroid Biochem Mol Biol*. 2012;129:31-46.
323. Sato Y, Atarashi K, Plichta DR, et al. Novel bile acid biosynthetic pathways are enriched in the microbiome of centenarians. *Nature*. 2021;599:458-464.
324. Diviccaro S, Giatti S, Borgo F, et al. Treatment of male rats with finasteride, an inhibitor of 5alpha-reductase enzyme, induces long-lasting effects on depressive-like behavior, hippocampal neurogenesis, neuroinflammation and gut microbiota composition. *Psychoneuroendocrinology*. 2019;99:206-215.
325. Rahimi-Ardabili B, Pourandarjani R, Habibollahi P, Mualeki A. Finasteride induced depression: A prospective study. *BMC Clin Pharmacol*. 2006;6:7.
326. Irwig MS. Depressive symptoms and suicidal thoughts among former users of finasteride with persistent sexual side effects. *J Clin Psychiatry*. 2012;73:1220-1223.
327. Traish AM, Melcangi RC, Bortolato M, Garcia-Segura LM, Zitzmann M. Adverse effects of 5α-reductase inhibitors: What do we know, don't know, and need to know? *Rev Endocr Metab Disord*. 2015;16:177-198.
328. Ganzer CA, Jacobs AR. Emotional consequences of finasteride: Fool's gold. *Am J Mens Health*. 2018;12:90-95.
329. Melcangi RC, Caruso D, Abbiati F, et al. Neuroactive steroid levels are modified in cerebrospinal fluid and plasma of post-finasteride patients showing persistent sexual side effects and anxious/depressive symptomatology. *J Sex Med*. 2013;10:2598-2603.
330. Melcangi RC, Santi D, Spezzano R, et al. Neuroactive steroid levels and psychiatric and andrological features in post-finasteride patients. *J Steroid Biochem Mol Biol*. 2017;171:229-235.
331. Borgo F, Macandog AD, Diviccaro S, et al. Alterations of gut microbiota composition in post-finasteride patients: A pilot study. *J Endocrinol Invest*. 2021;44:1263-1273.
332. Yilmaz C, Karali K, Fodelianaki G, et al. Neurosteroids as regulators of neuroinflammation. *Front Neuroendocrinol*. 2019;55:100788.
333. Grabacka M, Plonka PM, Pierzchalska M. The PPAR alpha regulation of the gut physiology in regard to interaction with microbiota, intestinal immunity, metabolism, and permeability. *Int J Mol Sci*. 2022;23:14156.
334. Pinna G. Role of PPAR-allopregnanolone signaling in behavioral and inflammatory gut-brain axis communications. *Biol Psychiatry*. 2023;94:609-618.
335. Locci A, Pinna G. Stimulation of peroxisome proliferator-activated receptor-α by N-palmitoylethanolamine engages allopregnanolone biosynthesis to modulate emotional behavior. *Biol Psychiatry*. 2019;85:1036-1045.
336. Isles AR, Davies W, Burrmann D, Burgoyne PS, Wilkinson LS. Effects on fear reactivity in XO mice are due to haploinsufficiency of a non-PAR X gene: Implications for emotional function in Turner's syndrome. *Hum Mol Genet*. 2004;13:1849-1855.
337. Rutsch A, Kantsjo JB, Ronchi F. The gut-brain axis: How microbiota and host inflammasome influence brain physiology and pathology. *Front Immunol*. 2020;11:604179.
338. Foster JA, Baker GB, Dursun SM. The relationship between the gut microbiome-immune system-brain axis and major depressive disorder. *Front Neurol*. 2021;12:721126.
339. Craig CF, Filippone RT, Stavely R, Bornstein JC, Apostolopoulos V, Nurgali K. Neuroinflammation as an etiological trigger for depression comorbid with inflammatory bowel disease. *J Neuroinflammation*. 2022;19:4.
340. Fadgyas-Stanculete M, Buga A-M, Popa-Wagner A, Dumitrascu DL. The relationship between irritable bowel syndrome and psychiatric disorders: From molecular changes to clinical manifestations. *J Mol Psychiatry*. 2014;2:4.
341. Mayer EA, Ryu HJ, Bhatt RR. The neurobiology of irritable bowel syndrome. *Mol Psychiatry*. 2023;28:1451-1465.
342. Stasi C, Bellini M, Bassotti G, Blandizzi C, Milani S. Serotonin receptors and their role in the pathophysiology and therapy of irritable bowel syndrome. *Tech Coloproctol*. 2014;18:613-621.
343. Aggarwal S, Ahuja V, Paul J. Dysregulation of GABAergic signalling contributes in the pathogenesis of diarrhea-predominant irritable bowel syndrome. *J Neurogastroenterol Motil*. 2018;24:422-430.
344. Fu R, Chen M, Chen Y, Mao G, Liu S. Expression and clinical significance of 5-HT and 5-HT(3)R in the intestinal mucosa of patient with diarrhea-type irritable bowel syndrome. *Exp Ther Med*. 2019;17:3077-3082.
345. Singh SV, Ganguly R, Jaiswal K, Yadav AK, Kumar R, Pandey AK. Molecular signalling during cross talk between gut brain axis regulation and progression of irritable bowel syndrome: A comprehensive review. *World J Clin Cases*. 2023;11:4458-4476.
346. Shajib MS, Khan WI. The role of serotonin and its receptors in activation of immune responses and inflammation. *Acta Physiol (Oxf)*. 2015;213:561-574.
347. Berthoud HR, Neuhuber WL. Functional and chemical anatomy of the afferent vagal system. *Auton Neurosci*. 2000;85:1-17.
348. Osman S, Tashtush A, Reed DE, Lomax AE. Analysis of the spinal and vagal afferent innervation of the mouse colon using neuronal retrograde tracers. *Cell Tissue Res*. 2023;392:659-670.
349. Spencer NJ, Kyloh MA, Travis L, Hibberd TJ. Identification of vagal afferent nerve endings in the mouse colon and their spatial relationship with enterochromaffin cells. *Cell Tissue Res*. 2024;396:313-327.
350. Wang FB, Powley TL. Topographic inventories of vagal afferents in gastrointestinal muscle. *J Comp Neurol*. 2000;421:302-324.
351. Wang FB, Powley TL. Vagal innervation of intestines: Afferent pathways mapped with new en bloc horseradish peroxidase adaptation. *Cell Tissue Res*. 2007;329:221-230.
352. Wallis DI, Stansfeld CE, Nash HL. Depolarizing responses recorded from nodose ganglion cells of the rabbit evoked by 5-hydroxytryptamine and other substances. *Neuropharmacology*. 1982;21:31-40.
353. Andrews PL, Davis CJ, Bingham S, Davidson HI, Hawthorn J, Maskell L. The abdominal visceral innervation and the emetic reflex: Pathways, pharmacology, and plasticity. *Can J Physiol Pharmacol*. 1990;68:325-345.
354. Browning KN. Role of central vagal 5-HT3 receptors in gastrointestinal physiology and pathophysiology. *Front Neurosci*. 2015;9:413.
355. McVey Neufeld KA, Bienenstock J, Bharwani A, et al. Oral selective serotonin reuptake inhibitors activate vagus nerve dependent gut-brain signalling. *Sci Rep*. 2019;9:14290.
356. Cunningham JT, Mifflin SW, Gould GG, Frazer A. Induction of c-Fos and DeltaFosB immunoreactivity in rat brain by vagal nerve stimulation. *Neuropsychopharmacology*. 2008;33:1884-1895.
357. Krahl SE, Senanayake SS, Pekary AE, Sattin A. Vagus nerve stimulation (VNS) is effective in a rat model of antidepressant action. *J Psychiatr Res*. 2004;38:237-240.
358. Austelle CW, O'Leary GH, Thompson S, et al. A comprehensive review of vagus nerve stimulation for depression. *Neuromodulation*. 2022;25:309-315.
359. Breit S, Kupferberg A, Rogler G, Hasler G. Vagus nerve as modulator of the brain-gut axis in psychiatric and inflammatory disorders. *Front Psychiatry*. 2018;9:44.

360. Dorr AE, Debonnel G. Effect of vagus nerve stimulation on serotonergic and noradrenergic transmission. *J Pharmacol Exp Ther*. 2006;318:890-898.
361. Bonaz B, Sinniger V, Pellissier S. Anti-inflammatory properties of the vagus nerve: Potential therapeutic implications of vagus nerve stimulation. *J Physiol*. 2016;594:5781-5790.
362. Nurgali K, Stebbing MJ, Furness JB. Correlation of electrophysiological and morphological characteristics of enteric neurons in the mouse colon. *J Comp Neurol*. 2004;468:112-124.
363. Sanders KM, Ward SM. Interstitial cells of Cajal: A new perspective on smooth muscle function. *J Physiol*. 2006;576(Pt 3): 721-726.
364. Hsueh B, Chen R, Jo Y, et al. Cardiogenic control of affective behavioural state. *Nature*. 2023;615:292-299.
365. Rudolph U, Mohler H. GABA-based therapeutic approaches: GABAA receptor subtype functions. *Curr Opin Pharmacol*. 2006; 6:18-23.
366. Maramai S, Benchekroun M, Ward SE, Atack JR. Subtype selective γ-aminobutyric acid type A receptor (GABA. *J Med Chem*. 2020;63:3425-3446.
367. Antkowiak B, Rudolph U. New insights in the systemic and molecular underpinnings of general anesthetic actions mediated by γ-aminobutyric acid A receptors. *Curr Opin Anaesthesiol*. 2016;29:447-453.
368. Stephens DN, King SL, Lambert JJ, Belelli D, Duka T. $GABA_A$ receptor subtype involvement in addictive behaviour. *Genes Brain Behav*. 2017;16:149-184.
369. Laverty D, Thomas P, Field M, et al. Crystal structures of a GABA(A)-receptor chimera reveal new endogenous neurosteroid-binding sites. *Nat Struct Mol Biol*. 2017;24:977-985.
370. Mortensen M, Bright DP, Fagotti J, Dorovykh V, Cerna B, Smart TG. Forty years searching for neurosteroid binding sites on $GABA_A$ receptors. *Neuroscience*. doi: 10.1016/j.neuroscience. 2024.06.002. Online ahead of print.
371. Sharma R, Bansal P, Saini L, Sharma N, Dhingra R. Zuranolone, a neuroactive drug, used in the treatment of postpartum depression by modulation of $GABA_A$ receptors. *Pharmacol Biochem Behav*. 2024;238:173734.
372. Patterson R, Balan I, Morrow AL, Meltzer-Brody S. Novel neurosteroid therapeutics for post-partum depression: Perspectives on clinical trials, program development, active research, and future directions. *Neuropsychopharmacology*. 2024;49:67-72.
373. Khan P, Saini S, Hussain S, Majid H, Gupta S, Agarwal N. A systematic review and meta-analysis on efficacy and safety of ganaxolone in epilepsy. *Expert Opin Pharmacother*. 2024;25: 621-632.
374. Lamb YN. Ganaxolone: First approval. *Drugs*. 2022;82:933-940.
375. Lévesque M, Biagini G, Avoli M. Neurosteroids and focal epileptic disorders. *Int J Mol Sci*. 2020;21:9391.
376. Salminen S, Collado MC, Endo A, et al. The International Scientific Association of Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of postbiotics. *Nat Rev Gastroenterol Hepatol*. 2022;19:551.
377. Kazemi A, Soltani S, Ghorabi S, et al. Effect of probiotic and synbiotic supplementation on inflammatory markers in health and disease status: A systematic review and meta-analysis of clinical trials. *Clinical Nutrition*. 2020;39:789-819.
378. Bharwani A, Mian MF, Surette MG, Bienenstock J, Forsythe P. Oral treatment with Lactobacillus rhamnosus attenuates behavioural deficits and immune changes in chronic social stress. *BMC Med*. 2017;15:7.
379. Bagga D, Reichert JL, Koschutnig K, et al. Probiotics drive gut microbiome triggering emotional brain signatures. *Gut Microbes*. 2018;9:486-496.
380. Messaoudi M, Violle N, Bisson J-F, Desor D, Javelot H, Rougeot C. Beneficial psychological effects of a probiotic formulation (Lactobacillus helveticus R0052 and Bifidobacterium longum R0175) in healthy human volunteers. *Gut Microbes*. 2011;2: 256-261.
381. Firth J, Marx W, Dash S, et al. The effects of dietary improvement on symptoms of depression and anxiety: A meta-analysis of randomized controlled trials. *Psychosom Med*. 2019;81:265-280.
382. Bayes J, Schloss J, Sibbritt D. The effect of a Mediterranean diet on the symptoms of depression in young males (the "AMMEND: A Mediterranean diet in MEN with depression" study): A randomized controlled trial. *Am J Clin Nutr*. 2022; 116:572-580.
383. Jacka FN, O'Neil A, Opie R, et al. A randomised controlled trial of dietary improvement for adults with major depression (the 'SMILES' trial). *BMC Med*. 2017;15:23.
384. Munawar N, Ahmad A, Anwar MA, Muhammad K. Modulation of gut microbial diversity through non-pharmaceutical approaches to treat schizophrenia. *Int J Mol Sci*. 2022;23:2625.
385. Barros-Santos T, Silva KSO, Libarino-Santos M, et al. Effects of chronic treatment with new strains of *Lactobacillus plantarum* on cognitive, anxiety- and depressive-like behaviors in male mice. *PLoS One*. 2020;15:e0234037.
386. Bagheri S, Heydari A, Alinaghipour A, Salami M. Effect of probiotic supplementation on seizure activity and cognitive performance in PTZ-induced chemical kindling. *Epilepsy Behav*. 2019;95:43-50.
387. Inoue K, Shirai T, Ochiai H, et al. Blood-pressure-lowering effect of a novel fermented milk containing gamma-aminobutyric acid (GABA) in mild hypertensives. *Eur J Clin Nutr*. 2003;57:490-495.
388. Abd El-Fattah A, Sakr S, El-Dieb S, Elkashef H. Developing functional yogurt rich in bioactive peptides and gamma-aminobutyric acid related to cardiovascular health. *LWT-Food Sci Technol*. 2018;98:390-397.
389. Gori S, Inno A, Belluomini L, et al. Gut microbiota and cancer: How gut microbiota modulates activity, efficacy and toxicity of antitumoral therapy. *Crit Rev Oncol Hematol*. 2019;143:139-147.
390. Ribera C, Sánchez-Ortí JV, Clarke G, Marx W, Mörkl S, Balanzá-Martínez V. Probiotic, prebiotic, synbiotic and fermented food supplementation in psychiatric disorders: A systematic review of clinical trials. *Neurosci Biobehav Rev*. 2024;158: 105561.
391. Binda S, Tremblay A, Iqbal UH, et al. Psychobiotics and the microbiota-gut-brain axis: Where do we go from here? *Microorganisms*. 2024;12:634.
392. Reis DJ, Ilardi SS, Punt SEW. The anxiolytic effect of probiotics: A systematic review and meta-analysis of the clinical and preclinical literature. *PLoS One*. 2018;13:e0199041.
393. Chao R-Y, Cheng C-H, Wu S-N, Chen P-C. Defective trafficking of Kv2.1 channels in MPTP-induced nigrostriatal degeneration. *J Neurochem*. 2018;144:483-497.
394. Chao LM, Liu C, Sutthawongwadee S, et al. Effects of probiotics on depressive or anxiety variables in healthy participants under stress conditions or with a depressive or anxiety diagnosis: A meta-analysis of randomized controlled trials. *Front Neurol*. 2020;11:421.
395. Lv B-M, Quan Y, Zhang H-Y. Causal inference in microbiome medicine: Principles and applications. *Trends Microbiol*. 2021; 29:736-746.
396. Chaudhari SN, McCurry MD, Devlin AS. Chains of evidence from correlations to causal molecules in microbiome-linked diseases. *Nat Chem Biol*. 2021;17:1046-1056.

397. Lee HS, Kim MA, Valentino RJ, Waterhouse BD. Glutamatergic afferent projections to the dorsal raphe nucleus of the rat. *Brain Res*. 2003;963:57-71.
398. Lee M-H, Kim J, Kim G-H, Kim M-S, Yoon S-S. Effects of *Lactiplantibacillus plantarum* FBT215 and prebiotics on the gut microbiota structure of mice. *Food Sci Biotechnol*. 2023;32: 481-488.
399. Roberfroid MB. Introducing inulin-type fructans. *Br J Nutr*. 2005;93:S13-S25.
400. Suligoj T, Vigsnaes LK, Van den Abbeele P, et al. Effects of human milk oligosaccharides on the adult gut microbiota and barrier function. *Nutrients*. 2020;12:2808.
401. Iribarren C, Magnusson MK, Vigsnaes LK, et al. The effects of human milk oligosaccharides on gut microbiota, metabolite profiles and host mucosal response in patients with irritable bowel syndrome. *Nutrients*. 2021;13:3836.
402. Morales PS, Wijeyesekera A, Robertson MD, Jackson PPJ, Gibson GR. The potential role of human milk oligosaccharides in irritable bowel syndrome. *Microorganisms*. 2022;10:2338.
403. Pham VT, Steinert RE, Duysburgh C, Ghyselinck J, Marzorati M, Dekker PJT. In vitro effect of enzymes and human milk oligosaccharides on FODMAP digestion and fecal microbiota composition. *Nutrients*. 2023;15:1637.
404. Briggs JA, Grondin JM, Brumer H. Communal living: Glycan utilization by the human gut microbiota. *Environ Microbiol*. 2021;23:15-35.
405. Bajic D, Wiens F, Wintergerst E, Deyaert S, Baudot A, Van den Abbeele P. HMOs exert marked bifidogenic effects on children's gut microbiota ex vivo, due to age-related *Bifidobacterium* species composition. *Nutrients*. 2023;15:1701.
406. Fan ST, Zhang ZH, Zhao YS, et al. Recent advances in targeted manipulation of the gut microbiome by prebiotics: From taxonomic composition to metabolic function. *Curr Opin Food Sci*. 2023;49:100959.
407. Jackson PPJ, Wijeyesekera A, Rastall RA. Oligofructose alone and in combination with 2′fucosyllactose induces physiologically relevant changes in gamma-aminobutyric acid and organic acid production compared to sole 2′fucosyllactose supplementation: An in vitro study. *FEMS Microbiol Ecol*. 2023; 99:fiad100.
408. Pferschy-Wenzig EM, Pausan MR, Ardjomand-Woelkart K, et al. Medicinal plants and their impact on the gut microbiome in mental health: A systematic review. *Nutrients*. 2022;14:2111.
409. Wan MLY, Co VA, El-Nezami H. Dietary polyphenol impact on gut health and microbiota. *Crit Rev Food Sci Nutr*. 2021;61: 690-711.
410. Mittenhuber G. Phylogenetic analyses and comparative genomics of vitamin B6 (pyridoxine) and pyridoxal phosphate biosynthesis pathways. *J Mol Microbiol Biotechnol*. 2001;3:1-20.
411. Scott KP, Grimaldi R, Cunningham M, et al. Developments in understanding and applying prebiotics in research and practice-an ISAPP conference paper. *J Appl Microbiol*. 2020; 128:934-949.
412. Wan Z, Zheng J, Zhu Z, et al. Intermediate role of gut microbiota in vitamin B nutrition and its influences on human health. *Front Nutr*. 2022;9:1031502.
413. Rodriguez-Daza MC, Pulido-Mateos EC, Lupien-Meilleur J, Guyonnet D, Desjardins Y, Roy D. Polyphenol-mediated gut microbiota modulation: Toward prebiotics and further. *Front Nutr*. 2021;8:689456.
414. Jackson PPJ WA, Rastall RA. Determining the metabolic fate of human milk oligosaccharides: It may just be more complex than you think? *Gut Microbiome*. 2022;3:e39.
415. Smith NW, Shorten PR, Altermann E, Roy NC, McNabb WC. The classification and evolution of bacterial cross-feeding. *Front Ecol Evol*. 2019;7:153.
416. Culp EJ, Goodman AL. Cross-feeding in the gut microbiome: Ecology and mechanisms. *Cell Host Microbe*. 2023;31:485-499.