Bile acids, bioactive signalling molecules in interoceptive gut-to-brain communication.

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Abstract

Aside from facilitating solubilisation and absorption of dietary lipids and lipid-soluble vitamins, amphipathic bile acids (BAs) also act as bioactive signalling molecules. A plethora of conjugated or un-conjugated primary and bacterially-modified secondary BA moieties have been identified, with significant divergence between species. These molecules are excreted into the external environment of the intestinal lumen, yet nuclear and membrane receptors that are sensitive to BAs are expressed internally in the liver, intestinal and neural tissues, amongst others. The diversity of BAs and receptors underpins the multitude of distinct bioactive functions attributed to BAs, but also hampers elucidation of the physiological mechanisms underpinning these actions. In this topical review, we have considered the potential of BAs as cross-barrier signalling molecules that contribute to interoceptive pathways informing the central nervous system of environmental changes in the gut lumen. Activation of BAs on FGF_{19}-secreting enterocytes, enteroendocrine cells coupled to sensory nerves or intestinal immune cells would facilitate indirect signalling, whereas direct activation of BA receptors in the brain are likely to occur primarily under pathophysiological conditions when concentrations of BAs are elevated.

Context

The continuous flow of information between the brain and the gut is significant in the context of maintaining physiological homeostasis (Mayer, 2011). Neural, immune, endocrine and metabolic pathways have all been implicated in this communication axis. Moreover, accumulating evidence, providing microbes with a role in modulating brain function and host behaviour (Stilling *et al.*, 2015), has resulted in renaming of this bidirectional circuit as the microbiota-gut-brain axis (Bienenstock *et al.*, 2015; Chakrabarti *et al.*, 2022). Microbes interact with the host immune system and are also capable of stimulating sensory enteroendocrine cells (El Aidy *et al.*, 2015; O'Malley, 2016). However, they also have an innate capacity to produce neuromodulatory factors, including central neurotransmitters and short-chain fatty acids (Lyte, 2014; Fung *et al.*, 2021). Increasing attention is also being focussed on the dynamic, symbiotic relationship that exists between bacteria with bile salt hydrolase (BSH) activity and luminal bile acids (BAs).

Most physiologists have familiarity with the digestive function of BAs, where their amphipathic structure enables solubilisation and absorption of dietary lipids and fat-soluble vitamins in the proximal intestine. However, given that numerous cell types, both in the gut and in other peripheral and central organs, express BA receptors (Gadaleta *et al.*, 2011a; Ward *et al.*, 2013; Deutschmann *et al.*, 2018; Jonas *et al.*, 2019), BAs are also classified as bioactive signalling molecules. The primary human BAs, cholic acid (CA) and chenodeoxycholic acid (CDCA) are synthesised and conjugated by liver hepatocytes. Conjugation makes BAs more soluble in the aqueous environment of the intestinal lumen and minimises potentially damaging interactions between BAs and apical facing plasma membranes. The majority of BAs are transported from the distal ileum back to the liver via the portal vein as part of the entero-hepatic cycle. However, a small proportion escape reuptake and pass into the colon, the gut region where BSH-comprising bacteria are most abundant. Interactions between BSH-bacteria and primary BAs result in deconjugation reactions, which in turn, facilitates further

microbially-mediated transformations. BSH activity is a ubiquitous trait in gut-residing bacteria that may have evolved through host-driven selection (Jones *et al.*, 2008). Dynamic interactions between microbes and BAs results in a great diversity of microbially-modified secondary BA species (Guzior & Quinn, 2021), including deoxycholic acid (DCA) and lithocholic acid (LCA) in a variety of conjugated and unconjugated forms (figure 1). There are many receptors which bind BAs, with variable binding affinities and response potencies, resulting in a multitude of distinct bioactive functions. However, we will focus on the two BA receptors that exclusively bind BAs, nuclear farnesoid X receptors (FXR) and membrane-expressed G protein-coupled BA receptor 1 (GPBAR-1), also named Takeda G-protein-coupled receptor 5 (TGR5). In the context of this rapidly evolving research field, we have examined BAs as potential signalling molecules participating in interoceptive signalling from the intestines to the central nervous system (CNS).

Bile acid synthesis and secretion

BAs are a class of metabolites that interact with microbiota and have significant importance both in gut and whole-body homeostasis (Chavez-Talavera *et al.*, 2017). Location-specific changes in concentrations and diversity of BAs are influenced by diet, host metabolites and microbial interactions (Chiang & Ferrell, 2020). Derived from cholesterol, BAs are the major component of hepatic bile and act as lipid emulsifiers in the small intestine. Two inter-regulated pathways, the classical pathway in the endoplasmic reticulum, regulated by cytochrome CYP7A1 and the alternative pathway in the mitochondria regulated through cytochromes CYP27A1 and CYP8B1, produce CA and CDCA, together and separately, respectively. Regulation of all three enzymes is mediated through microbial actions on the liver (Sayin *et al.*, 2013) and the flux through each pathway can be altered by cholesterol cell localisation and overload (Ren *et al.*, 2004) and by endoplasmic reticulum stress (Henkel *et al.*, 2017). Therefore, all cells and tissues carrying mitochondria have the potential to produce CDCA.

BAs classically function at a critical miceller concentration to spread or emulsify fats for digestion and for cellular uptake from the gut lumen. The critical miceller concentration is different for individual BA moieties. This is important when considering microbial modifications of BAs and in the context of the sometimes-convergent nature of BA representation called signatures, associated with different disease states. To reduce their pKa and optimise the critical miceller concentration, BAs are conjugated to an amino acid, usually taurine or glycine, which represent approximately 25% and 75%, respectively, of conjugates in humans. Variations in the ratios of glyco- and tauro-conjugated BAs are influenced by host enzymes and also by diet. Glycine conjugation is favoured in adult humans, pigs and cows, where vegan or herbivore diets are more prevalent (Vessey, 1978), whereas in animals with an omnivore or carnivore diet, BAs are primarily conjugated to taurine. In newborns, taurine-conjugated BAs predominate and indeed, liver taurine levels are higher in infants. Interestingly, in the first week of life CA is the main BA with representation of CA : CDCA as being 2.5 : 1, this normalises in the first month of life to 1.2 : 1 (Murphy & Signer, 1974). Conjugated BAs in areas of low pH are toxic to some bacteria and may influence gut residency and colonisation resistance (Ducarmon *et al.*, 2019). Other modifications, such as glucuronidation or sulphonation, target BAs for excretion (Takikawa *et al.*, 1985), although all of these BA modifications can be reversed by gut bacteria.

Functionally, BAs act as signalling molecules by binding to and activating BA receptors, of which there are many. However, only two bind BAs exclusively. Activation of nuclear FXR is key to regulating *de novo* BA synthesis (Laffitte *et al.*, 2000). Indeed, mice lacking FXR exhibit BA dyshomeostasis (Degirolamo *et al.*, 2015), in addition to defects in lipid metabolism (Hanniman *et al.*, 2005), changes in centrally-regulated behaviours (Huang *et al.*, 2015) and immune response (Gadaleta *et al.*, 2011b). Membrane-expressed GPBAR-1 or TGR5 is a G-protein-coupled BA receptor,

which induces cyclic adenosine monophosphate (cAMP) synthesis leading to activation of the protein kinase-A pathway and gene transcription (Kawamata *et al.*, 2003). LCA is the most potent natural agonist of TGR5 (Kawamata *et al.*, 2003), and has been shown to influence glucose metabolism, neuronal function, immune system control and liver regeneration (Guo *et al.*, 2016).

There is, however, significant variability in receptor binding affinity and response potency, depending on BA species and conjugation state, resulting in a plethora of biological outcomes. It is important to note that significant species diversity exists between rodents and humans. In mice, the primary BAs are CA and α-and β muricholic acid (MCA), which is derived from CDCA through the actions of 6β-hydroxylase. Ursodeoxycholic acid (UDCA), a primary BA in mice but a 'tertiary' bile acid in humans, is a weak TGR5 agonist (Carino *et al.*, 2019) and neutral toward FXR. 6β-hydroxylation alters the physicochemical properties of BAs, such that these molecules are more hydrophilic but less potent detergents. The signalling properties of murine BAs is also significantly altered as CDCA, the most potent endogenous FXR agonist, is converted into MCA, which is actually an FXR antagonist (Guo & Chiang, 2020). Additionally, murine BAs are almost exclusively conjugated to taurine (Dawson & Karpen, 2015) and differences in rodent microbial profiles results in different secondary bile acids (figure 1). These factors make it difficult to translate observations from rodent studies to human conditions. Indeed, FXR agonists, which exhibited promise in experimental models of chronic liver disease (Ali *et al.*, 2015), have not been as successful in humans. For example, the efficacy of FXR agonists for the treatment of non-alcoholic steatohepatitis was limited by does-related side effects (Fiorucci *et al.*, 2020).

Interactions between bile acids and intestinal microbes

The human body is host to its own unique, co-evolved microbial ecosystems where microbes (bacteria, virus and fungi) reside. Bacteria have evolved to occupy specific spatial and temporal intestinal niches. They can form complex, sometimes symbiotic interactions with the host, as well as syntrophic interactions with other microbes. These interactions result in microbial-produced factors and metabolites that may act as signalling molecules. The functional diversity of the intestinal tract confers it with the capacity to both impact and indicate health and disease status (Long *et al.*, 2017). Modern meta-omics approaches have made characterisation and connection possible. Meta-genomics identify microbes and their genetic potential, meta-transcriptomics examine potential functionalities, meta-proteomics identify expressed functionality and meta-metabolomics asses the metabolites and actual functional outcomes. The major phyla (>90%) represented in the mature gut are *Firmicutes* and *Bacteroidetes* (Hugon et al., 2015). Diet, immunity and genetics add further to the complexity of microbial intestinal colonisation, enrichment and its associated metabolism (Scepanovic *et al.*, 2019). Indeed, debate continues regarding the features of a healthy microbiota and its associated healthy metabolic capacity (Moya & Ferrer, 2016).

In the absence of a gut microbial community, BA moieties would have remained a conservative set of molecules. Bacterial enzymatic modifications in the gut lumen are responsible for the huge diversity of BAs detected regionally in the liver, the gut, the systemic circulation (Staley *et al.*, 2017) and even the brain (Zheng *et al.*, 2016), breast milk (Forsyth *et al.*, 1983) and ovarian follicles (Yang *et al.*, 2021). Often, secondary bile acids are more potent agonists for BA receptors (Ridlon *et al.*, 2016). The gatekeepers, microbial BSHs, are a ubiquitous feature of almost all phyla represented in the gut environment (Jones *et al.*, 2008). BSHs belong to the N-terminal nucleophile (Ntn) hydrolase superfamily of proteins. Discovered in 1995, these enzymes hydrolyse the amide bond conjugated to the BA steroid nucleus and differ in their substrate specificity (Brannigan *et al.*, 1995). Individual species of bacteria can carry none, one or indeed, multiple copies of BSHs (Fang *et al.*, 2009; Prete *et*

al., 2020), that may or not be active. These enzymes selectively remove the amine from liver-conjugated BAs, rendering them susceptible to further modification by microbes (Joyce *et al.*, 2014; Song *et al.*, 2019).

BSH activity may confer a protective advantage for bacteria species survival and colonisation, so that BSH activity is among selection criteria for probiotics (Vizoso Pinto *et al.*, 2006; Jones *et al.*, 2008). Additionally, in liberating amino acids, glycine and taurine, bacteria may be able to use these amino acids as an energy source. Therefore, another role for conjugated BAs could be to carry these amino acids to the intestines for further use, or alternatively, to act as a sink for nitrogen elimination in the faeces. BAs that escape ileal uptake and dihydroxylation can undergo microbial re-amidation, oxidation esterification, deglucuronidation and desulphatation (Ridlon *et al.*, 2006; Ridlon & Hylemon, 2012; Quinn *et al.*, 2020). BAs that have undergone oxidation, epimerization and dehydroxylation are usually recycled to the liver, repaired and re-secreted into bile, whereas the majority of BAs excreted in the faeces are products of 7α-dehydroxylation (Hirano *et al.*, 1981).

Direct bile acid signalling in the central nervous system.

Only a small proportion of BAs escape the enterohepatic circuit to gain entry to the systemic circulation allowing them to function as steroid hormones. However, with the exception of UDCA and tauro-UDCA, which can cross the blood brain barrier at physiological concentrations, most BAs are restricted to the peripheral circulation (Parry *et al.*, 2010). Nonetheless, BA receptors including TGR5 (Keitel *et al.*, 2010) and FXR (Huang *et al.*, 2016) have been detected in neurons, astrocytes and microglia in the brain. Indeed, endogenous neurosteroids also bind to TGR5, resulting in increases intracellular cAMP and calcium central neurons and glial cells (Keitel *et al.*, 2010). Neurosteroids have been implicated in the pathological consequences of hepatic encephalopathy in the CNS. In a mouse model of hepatic encephalopathy, activation of TGR5 is protective against

neurological decline evoked, by suppressing neuroinflammation (McMillin *et al.*, 2015b). The brain also represents a potential site of BA synthesis. Indeed, twenty BAs and oxysterols were detected in rat brain regions (Zheng *et al.*, 2016). Altered CYP8B1 and CYP7A1 expression were also detected in human brain tissue (Cali *et al.*, 1991; Ogundare *et al.*, 2010). Additionally, when serum BAs are at supra-physiological concentrations, as in the case in cholestasis, a leaky blood brain barrier facilitates passive movement of BA into the CNS. In an animal model of cholestasis, specific BAs were taken up into hypothalamic neurons, which express BA transporters (McMillin *et al.*, 2015a), resulting in suppression of corticotropin-releasing factor (CRF) synthesis and secretion (Quinn et al., 2014). Others have reported that BAs have an indirect inhibitory effect on the hypothalamic-pituitary-adrenal (HPA) stress axis, either through their actions on glucocorticoid receptors (McMillin *et al.*, 2015a) or by suppression of hepatic glucocorticoid clearance (McNeilly et al., 2010).

CRF is secreted as part of an adaptive response to a perceived environmental threat and thereby activates HPA axis activity. Chronic activation of the HPA axis is associated with altered bowel morphology, function and visceral pain sensitivity (O'Malley *et al.*, 2010; Parker *et al.*, 2019) and is frequently co-morbid in individuals with the functional bowel disorder, irritable bowel syndrome (IBS) (Spiller, 2004). Stress and the subsequent activation of the HPA axis also impacts on the luminal environment of the gut, as noted by direct changes in luminal BA profiles (Silvennoinen *et al.*, 2015) and indirect effects through modification of the microbiome (Madison & Kiecolt-Glaser, 2019). Although more research is needed to elucidate the precise consequences of BA-induced suppression of the HPA axis activity on gut luminal contents, an impaired stress response is associated with an inadequate host defence against pathogens and an imbalance in intestinal microbiota would, in turn, modify the BA pool. Thus, under certain pathophysiological conditions, BAs may influence central regulation of gut function (Ni Dhonnabhain *et al.*, 2021).

Sensitivity of intestinal sensory nerves to bile acids

Intrinsic to a bidirectional axis are signalling conduits that inform the CNS about the luminal environment of the intestine. Direct and indirect neural, immune and endocrine pathways facilitate this function (Mayer, 2011; Öhman *et al.*, 2015). The versatility of BAs as signalling molecules is underpinned by widespread expression of both nuclear and membrane receptors throughout the organism, in addition to direct and indirect modes of signalling. BA profiles vary with diet, transit through the intestine and changes in the microbiome. Although not comparable to any human condition, germ-free mice, have proved extremely useful in investigating the capacity of luminal microbial factors to modify host physiology. Germ-free mice exhibit elevated levels of BAs and increased activation of membrane-expressed TGR5 receptors (Selwyn *et al.*, 2015). In the absence of luminal microbes, intrinsic primary afferent neurons, proposed to be a neural starting point for the relay of information regarding the gut lumen to the brain, are less excitable (McVey Neufeld *et al.*, 2015). TGR5 receptors have been detected on intrinsic primary afferent neurons (Alemi *et al.*, 2013), indicating a possible neurally-regulated signalling pathway. Moreover, activation of TGR5 receptors have been implicated in gut-to-brain satiety-related signalling via the vagus nerve (Wu *et al.*, 2020). The vagus nerve appears to have a central role in mediating microbiome-CNS communication, as in mouse studies, vagotomy prevented behavioural changes brought about by modifying the gut microbiome (Bercik *et al.*, 2011; Bravo *et al.*, 2011). Moreover, we and others have recorded changes in the excitability of rodent vagal afferents in the jejunum (Perez-Burgos *et al.*, 2013; Perez-Burgos *et al.*, 2015) and colon (Buckley & O'Malley, 2018; Buckley *et al.*, 2019; Buckley *et al.*, 2020) following mucosal exposure to specific bacterial products. As vagal afferent sensory endings are sensitive to oleanolic acid, a TGR5-specific agonist (Wu *et al.*, 2020), they are appropriately equipped to facilitate neural transmission of information about BAs in the luminal environment, however, further research is needed in this area.

Endocrine-mediated gut-brain signalling evoked by bile acids.

Embedded within the intestinal epithelium are enteroendocrine cells, specialised chemosensors with luminal and basolateral sides. When activated, these polarised sensory cells release endocrine factors basolaterally (Raybould, 2010) and represent an indirect mode of interoceptive signalling. Expressing a multitude of receptors, luminal contents including nutrients, microbial products and BAs all stimulate these sensory cells, resulting in release of endocrine or neuromodulatory molecules. BAs stimulate cholecystokinin-mediated gallbladder contraction, induction of lipase activity and pancreatic enzyme and bicarbonate release. In the colon, but not the small intestine, TGR5 is expressed on serotonin-secreting enterochromaffin cells (Lund et al., 2018), which release serotonin when stimulated by bile salts. Interestingly, neoplastic enterochromaffin cells exhibit enhanced sensitivity to bile salt ligands (Kidd *et al.*, 2008). Although serotonin cannot cross the blood brain barrier, enterochromaffin cells are coupled to sensory nerves (Bellono *et al.*, 2017), revealing a clear neural pathway to facilitate signalling between the gut lumen and the CNS.

Glucagon-like peptide-1 (GLP-1) and peptide YY (PYY)-secreting L-cells are another subset of enteric biosensors. Electrically excitable L-cells express an abundance of receptors including receptors for microbially-produced factors such as short-chain fatty acids (Tolhurst et al., 2012), GABA (Gameiro et al., 2005), serotonin (Lund et al., 2018), as well as FXR and TGR5 both in rodents (Katsuma et al., 2005; Christiansen et al., 2019) and humans (Trabelsi et al., 2015; Calderon et al., 2020). In isolated human enterocytes, almost three quarters of GLP-1-expressing enteroendocrine cells were found to express TGR5, whereas only 16% of GLP-1 negative cells expressed the BA receptor, indicating that GLP-1-secreting L-cells are the predominant cell type activated by TGR5-stimulating BAs (Calderon et al., 2020). Stimulation of L-cells by potent TGR5 agonists, particularly LCA, increased intracellular cAMP and calcium resulting in secretion of GLP-1 (Parker et al., 2012). Moreover, elevated circulating concentrations of GLP-1 following inhibition of ileal BA transporters was explained by elevated luminal levels of BAs interacting with colonic L-cells (Rudling et al., 2015). It is noteworthy

that TGR5 is expressed on the basolateral membrane of L-cells (Brighton et al., 2015; Christiansen et al., 2019; Calderon et al., 2020), indicating that BAs must first traverse the epithelial barrier to stimulate GLP-1 release from L-cells. GLP-1 receptors are expressed in several regions of the central nervous system (Baggio & Drucker, 2014) and this hormone may be able to cross the blood brain barrier by simple diffusion (Kastin *et al.*, 2002). However, the enzyme, dipeptidyl peptidase-4 rapidly degrades circulating GLP-1, therefore high concentrations of this hormone are primarily detected in the intestinal lamina propria. An alternative signalling pathway would be through neural signals generated in vagal afferents, which express GLP-1 receptors (Nakagawa *et al.*, 2004; Ronveaux *et al.*, 2014). Similar to the coupling of enterochromaffin cells with peripheral afferents (Bellono *et al.*, 2017), L-cells also form a neuroepithelial circuit, directly synapsing with sensory afferents (Bohorquez et al., 2015), which signal to the CNS (Buckley *et al.*, 2020). In this way, L-cells could act as sensory transducers to facilitate indirect transmission of cross-barrier sensory signals from luminal BAs to the CNS.

In contrast to the stimulatory action of conjugated BAs on the TGR5-GLP-1 signalling pathway, primary BAs, which have a higher affinity for nuclear FXR, cause the release of FGF_{19}, which decreases expression of GLP-1 (Calderon et al., 2020). Furthermore, crosstalk between these receptors contribute to the regulation of glucose homeostasis (Kim & Fang, 2018) and functional antagonism has also been reported between these two BA receptors in the context of autophagy relating to fed and fasting states (Carino *et al.*, 2021). These studies exemplify the complexity of BA signalling resulting in diverse biological outcomes. Activation of FXR in the terminal ileum induces secretion of FGF_{19}, which is transported via the portal vein to the liver where is stimulates hepatic fibroblast growth factor receptor (FGR) 4. FGR4 regulates *de novo* synthesis and mobilisation of hepatic BAs (De Magalhaes Filho *et al.*, 2017). However, FGF_{19} may also cross the blood brain barrier (Hsuchou *et al.*, 2013) and bind to FGRs in select brain regions including the hypothalamus, where it may modulate feeding behaviour (Mertens *et al.*, 2017), but could also impact upon gut function

through activation of the HPA axis. This indirect signalling cascade, involving enteroendocrine cells and neural afferents, elucidates a potential pathway by which intestinal BA receptors could modify central neurocircuitry (figure 2).

Bile acids stimulate immune signalling molecules.

BAs exhibit antimicrobial properties as evidenced in the small intestine, where high bile concentrations prevent small intestinal bacterial overgrowth (Dawson & Karpen, 2015). Mechanistically, this may be mediated through direct cytotoxicity (Staley *et al.*, 2017), or by stimulating innate immune mechanisms (D'Aldebert et al., 2009). One such mechanism employed to protect the intestinal epithelium from cytotoxic BAs is the stimulation of BA diarrhoea by high colonic levels of BAs (Hegyi et al., 2018). Moreover, monocytes, macrophages, dendritic and natural killer cells all express TGR5 and FXR receptors (Maruyama et al., 2002; Vavassori et al., 2009; Cipriani et al., 2011). Generally, it appears that BA moieties and their conjugates are important for fine-tuning the immune response to the diversity of antigens to which the gut is exposed (Sun *et al.*, 2021), with a bias in favour of gut tolerance (Fiorucci et al., 2018). Indeed, TGR5-induced activation of monocytes and macrophages inhibits phagocytic activity and secretion of pro-inflammatory cytokines (Pols et al., 2011; Haselow et al., 2013; Perino et al., 2014). Cytokine and chemokine release from monocytes and dendritic cells is also suppressed following FXR activation and overall, it is protective against the effects of intestinal inflammation (Gadaleta *et al.*, 2011b). Changes in BA pools impacts on intestinal immune function (Fiorucci *et al.*, 2021), both directly through their variable affinity for different BA receptors on host immune cells and indirectly, by modifying luminal microbial profiles. Previously largely ignored, formation of BA minor species or intermediates (Doden & Ridlon, 2021), as a consequence of mature secondary BA formation (DCA from CA, LCA and UDCA from CDCA), is important, as many are now being assigned roles as mediators of both innate and adaptive immunity (Meng *et al.*, 2018; Song *et al.*, 2019; Campbell *et al.*, 2020). Given the established interactions between luminal factors and immune cells residing in the gut, the

circulation and the CNS (Fung, 2020), BAs could also employ immune-mediated gut-to-brain signalling pathways (Buckley *et al.*, 2014; O'Malley, 2016) in interoceptive communication with the CNS (figure 2).

Bile acid-mediated signalling under pathophysiological conditions

Diversity and richness of the gut microbiome is an indicator of good gut health (Human Microbiome Project, 2012), and, this in turn, appears to maintain a healthy diversity of BAs across the spectrum. However, in disease states, where gut microbial dysbiosis occurs, the expected consequence is alterations to the size and the diversity of BA pool (Joyce *et al.*, 2014; Contijoch *et al.*, 2019). Elevated activity of BSH has been associated with inflammation (Parasar *et al.*, 2019) and BSHs are central to the drive towards infection (Mullish *et al.*, 2019), which could, in turn, influence immune-mediated signalling to the CNS. A role for microbes and BA signatures in intestinal and microbial health is clearly defined in the incidence of recurrent *Clostridia difficile* infections, where microbial diversity is reduced through recurrent antibiotic use. This resulted in altered BA signatures characterised by loss of secondary BAs with a concomitant gain in primary BAs (Brown *et al.*, 2018). Inflammatory bowel diseases (Crohn's disease and Ulcerative Colitis) are also linked to alterations in BA signatures. Faecal microbiota from individuals with inflammatory bowel disease is less diverse and more unstable (Pascal *et al.,* 2017) and associations to inflamed and non-inflamed regions point to specific microbial drivers of inflammation and epigenetic regulation (Ryan *et al.*, 2020). Although signatures differed, a common emerging theme in these patients was increased CA and a selective decrease in circulating secondary BAs (Duboc *et al.*, 2013; Weng *et al.*, 2019; Sinha *et al.*, 2020).

It is generally accepted that manifestation of symptoms characteristic of the functional bowel disorder, IBS, are due to dysfunction gut-brain communication (Enck *et al.*, 2016). The gut

microbiome is altered in IBS patients (Rajilic-Stojanovic *et al.*, 2011). As deconjugated bile acids can drive microbial phylum level shifts (Islam *et al.*, 2011), changes in bacterial profiles mediated by bile acids may be a contributary factor in IBS. Indeed, chronic watery diarrhoea is a symptom of luminal accumulation of bile acids due to malabsorption (Conley *et al.*, 1976; Coyne *et al.*, 1976) and a subset (~25%) of individuals with IBS-D exhibit elevated fecal BAs resulting in accelerated colonic transit, which is linked with diarrhea and visceral pain sensitivity (Slattery *et al.*, 2015). Bile acids may also influence motility in this subtype (Peleman *et al.*, 2017). Interestingly, treatment with colestipol, which binds BAs and prevents ileal reabsorption, improved IBS symptoms (Bajor *et al.*, 2015) and the therapeutic potential of BAs in functional bowel disorders is currently being explored (Rao *et al.*, 2010).

Treatment with BAs may also have benefits in age-related cognitive decline. In mouse models of Alzheimer's disease, a neurodegenerative condition characterised by progressive cognitive impairment, dietary supplementation with TUDCA positively altered amyloid plaque deposition and neuronal injury (Nunes *et al.*, 2012) and in Parkinson's disease, defective mitochondrial function in was in part restored on administration of UDCA (Mortiboys *et al.*, 2013). Indeed, BA profiles change with age. In mouse studies, BA concentrations increased, particularly conjugated BAs and secondary BAs (Fu *et al.*, 2012), whereas in humans the levels of BA-committed precursor 7alpha-hydroxy-4-cholesten-3-one, was inversely correlated with aging (Bertolotti *et al.*, 2007). Interestingly, Sato and colleagues reported that the minor BA intermediates associated with immune cell differentiation were prevalent in centenarians (Sato *et al.*, 2021). BA activities were related to enhanced pathogen resistance, and the authors speculated on their roles in toxin clearance, better bone health and immune functions in maintaining health in aging. Thus, host cell senescence in ageing goes hand-in-hand with microbial bacterial cell senescence leading to changes in BA profiles and BA-mediated signalling.

Concluding remarks.

Gaining an understanding of the physiological mechanisms by which microbes, residing in the external environment of the intestinal lumen, influence interoceptive signalling from the gut to the brain, has received substantial research interest in recent times. While it is generally accepted that a dynamic and interactive relationship exists between microbiota and BAs, the potential contribution of BAs as independent bioactive signalling molecules in gut-brain communication has been somewhat overlooked.

As discussed, BAs may signal to the CNS using both direct and indirect mechanisms (figure 2). Once in the systemic circulation BAs may operate as endocrine factors and cross the blood brain barrier to directly bind to BA receptors expressed on neural cells in the brain. However, this appears to occur primarily under pathophysiological conditions when concentrations of BAs are elevated. Under physiological conditions, it is more likely that indirect pathways represent the predominant signalling conduits for BAs. This may be through activation of BA receptor-expressing enterocytes or enteroendocrine cells. Hormone-secreting biosensors release neuromodulatory factors such as serotonin and GLP-1 that have the capacity to stimulate vagal afferent fibres. An alternative signalling route is through activation of FXR-expressing enterocytes, which secrete $FGF_{19,}$ a neuromodulatory factor that can cross the blood brain barrier. Further research is needed to elucidate these possible signalling routes. Finally, we described the predominantly anti-inflammatory effects of BAs on the immune system and how changes to the BA pool could influence immune-mediated gut-to-brain signalling. Even when focussed on a single signalling molecule such as BAs, the simplicity of the term 'gut-brain axis' belies the complexity of direct and indirect signalling pathways, which may be activated by a multitude of BA species which have variable affinities for several BAs receptors. It has yet to be determined if the actions of nuclear or membrane receptor binding BAs have contrasting or complimentary effects in terms of interoceptive signalling. It is also

not clear whether regional differences in BA profiles in the proximal and distal intestine relay distinct information to the CNS. Moreover, pathophysiological changes differentially modify these signalling pathways adding to the complexity. Much remains to be elucidated regarding the physiological mechanisms of these intriguing bioactive signalling molecules.

Additional Information Section

Competing Interests

No competing interests declared.

Author Contributions

SJ and DO'M prepared this review together. Both authors approved the final version of this manuscript. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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References

Alemi F, Poole DP, Chiu J, Schoonjans K, Cattaruzza F, Grider JR, Bunnett NW & Corvera CU. (2013). The receptor TGR5 mediates the prokinetic actions of intestinal bile acids and is required for normal defecation in mice. *Gastroenterology* **144,** 145-154.

Ali AH, Carey EJ & Lindor KD. (2015). Recent advances in the development of farnesoid X receptor agonists. *Ann Transl Med* **3,** 5.

Baggio LL & Drucker DJ. (2014). Glucagon-like peptide-1 receptors in the brain: controlling food intake and body weight. *J Clin Invest* **124,** 4223-4226.

Bajor A, Tornblom H, Rudling M, Ung KA & Simren M. (2015). Increased colonic bile acid exposure: a relevant factor for symptoms and treatment in IBS. *Gut* **64,** 84-92.

Bellono NW, Bayrer JR, Leitch DB, Castro J, Zhang C, O'Donnell TA, Brierley SM, Ingraham HA & Julius D. (2017). Enterochromaffin Cells Are Gut Chemosensors that Couple to Sensory Neural Pathways. *Cell* **170,** 185-198 e116.

Bercik P, Park AJ, Sinclair D, Khoshdel A, Lu J, Huang X, Deng Y, Blennerhassett PA, Fahnestock M, Moine D, Berger B, Huizinga JD, Kunze W, McLean PG, Bergonzelli GE, Collins SM & Verdu EF. (2011). The anxiolytic effect of Bifidobacterium longum NCC3001 involves vagal pathways for gut-brain communication. *Neurogastroenterol Motil* **23,** 1132-1139.

Bertolotti M, Gabbi C, Anzivino C, Crestani M, Mitro N, Del Puppo M, Godio C, De Fabiani E, Macchioni D, Carulli L, Rossi A, Ricchi M, Loria P & Carulli N. (2007). Age-related changes in bile acid synthesis and hepatic nuclear receptor expression. *Eur J Clin Invest* **37,** 501-508.

Bienenstock J, Kunze W & Forsythe P. (2015). Microbiota and the gut-brain axis. *Nutr Rev* **73 Suppl 1,** 28-31.

Bohorquez DV, Shahid RA, Erdmann A, Kreger AM, Wang Y, Calakos N, Wang F & Liddle RA. (2015). Neuroepithelial circuit formed by innervation of sensory enteroendocrine cells. *J Clin Invest* **125,** 782-786.

Brannigan JA, Dodson G, Duggleby HJ, Moody PC, Smith JL, Tomchick DR & Murzin AG. (1995). A protein catalytic framework with an N-terminal nucleophile is capable of self-activation. *Nature* **378,** 416-419.

Bravo JA, Forsythe P, Chew MV, Escaravage E, Savignac HM, Dinan TG, Bienenstock J & Cryan JF. (2011). 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* **108,** 16050-16055.

Brighton CA, Rievaj J, Kuhre RE, Glass LL, Schoonjans K, Holst JJ, Gribble FM & Reimann F. (2015). Bile Acids Trigger GLP-1 Release Predominantly by Accessing Basolaterally Located G Protein-Coupled Bile Acid Receptors. *Endocrinology* **156,** 3961-3970.

Brown JR, Flemer B, Joyce SA, Zulquernain A, Sheehan D, Shanahan F & O'Toole PW. (2018). Changes in microbiota composition, bile and fatty acid metabolism, in successful faecal microbiota transplantation for Clostridioides difficile infection. *BMC Gastroenterol* **18,** 131.

Buckley MM, O'Brien R, Brosnan E, Ross RP, Stanton C, Buckley JM & O'Malley D. (2020). Glucagon-Like Peptide-1 Secreting L-Cells Coupled to Sensory Nerves Translate Microbial Signals to the Host Rat Nervous System. *Front Cell Neurosci* **14,** 95.

Buckley MM, O'Brien R, Buckley JM & O'Malley D. (2019). GHSR-1 agonist sensitizes rat colonic intrinsic and extrinsic neurons to exendin-4: A role in the manifestation of postprandial gastrointestinal symptoms in irritable bowel syndrome? *Neurogastroenterol Motil***,** e13684.

Buckley MM, O'Mahony SM & O'Malley D. (2014). Convergence of neuro-endocrine-immune pathways in the pathophysiology of irritable bowel syndrome. *World J Gastroenterol* **20,** 8846-8858.

Buckley MM & O'Malley D. (2018). Development of an ex Vivo Method for Multi-unit Recording of Microbiota-Colonic-Neural Signaling in Real Time. *Front Neurosci* **12,** 112.

Calderon G, McRae A, Rievaj J, Davis J, Zandvakili I, Linker-Nord S, Burton D, Roberts G, Reimann F, Gedulin B, Vella A, LaRusso NF, Camilleri M, Gribble FM & Acosta A. (2020). Ileo-colonic delivery of conjugated bile acids improves glucose homeostasis via colonic GLP-1-producing enteroendocrine cells in human obesity and diabetes. *EBioMedicine* **55,** 102759.

Cali JJ, Hsieh CL, Francke U & Russell DW. (1991). Mutations in the bile acid biosynthetic enzyme sterol 27-hydroxylase underlie cerebrotendinous xanthomatosis. *J Biol Chem* **266,** 7779-7783.

Campbell C, McKenney PT, Konstantinovsky D, Isaeva OI, Schizas M, Verter J, Mai C, Jin WB, Guo CJ, Violante S, Ramos RJ, Cross JR, Kadaveru K, Hambor J & Rudensky AY. (2020). Bacterial metabolism of bile acids promotes generation of peripheral regulatory T cells. *Nature* **581,** 475-479.

Carino A, Biagioli M, Marchiano S, Fiorucci C, Zampella A, Monti MC, Scarpelli P, Ricci P, Distrutti E & Fiorucci S. (2019). Ursodeoxycholic acid is a GPBAR1 agonist and resets liver/intestinal FXR signaling in a model of diet-induced dysbiosis and NASH. *Biochim Biophys Acta Mol Cell Biol Lipids* **1864,** 1422-1437.

Carino A, Marchiano S, Biagioli M, Scarpelli P, Bordoni M, Di Giorgio C, Roselli R, Fiorucci C, Monti MC, Distrutti E, Zampella A & Fiorucci S. (2021). The bile acid activated receptors GPBAR1 and FXR exert antagonistic effects on autophagy. *FASEB J* **35,** e21271.

Chakrabarti A, Geurts L, Hoyles L, Iozzo P, Kraneveld AD, La Fata G, Miani M, Patterson E, Pot B, Shortt C & Vauzour D. (2022). The microbiota-gut-brain axis: pathways to better brain health. Perspectives on what we know, what we need to investigate and how to put knowledge into practice. *Cell Mol Life Sci* **79,** 80.

Chavez-Talavera O, Tailleux A, Lefebvre P & Staels B. (2017). Bile Acid Control of Metabolism and Inflammation in Obesity, Type 2 Diabetes, Dyslipidemia, and Nonalcoholic Fatty Liver Disease. *Gastroenterology* **152,** 1679-1694 e1673.

Chiang JYL & Ferrell JM. (2020). Bile acid receptors FXR and TGR5 signaling in fatty liver diseases and therapy. *Am J Physiol Gastrointest Liver Physiol* **318,** G554-G573.

Christiansen CB, Trammell SAJ, Wewer Albrechtsen NJ, Schoonjans K, Albrechtsen R, Gillum MP, Kuhre RE & Holst JJ. (2019). Bile acids drive colonic secretion of glucagon-like-peptide 1 and peptide-YY in rodents. *Am J Physiol Gastrointest Liver Physiol* **316,** G574-G584.

Cipriani S, Mencarelli A, Chini MG, Distrutti E, Renga B, Bifulco G, Baldelli F, Donini A & Fiorucci S. (2011). The bile acid receptor GPBAR-1 (TGR5) modulates integrity of intestinal barrier and immune response to experimental colitis. *PLoS One* **6,** e25637.

Conley DR, Coyne MJ, Bonorris GG, Chung A & Schoenfield LJ. (1976). Bile acid stimulation of colonic adenylate cyclase and secretion in the rabbit. *Am J Dig Dis* **21,** 453-458.

Contijoch EJ, Britton GJ, Yang C, Mogno I, Li Z, Ng R, Llewellyn SR, Hira S, Johnson C, Rabinowitz KM, Barkan R, Dotan I, Hirten RP, Fu SC, Luo Y, Yang N, Luong T, Labrias PR, Lira S, Peter I,

Grinspan A, Clemente JC, Kosoy R, Kim-Schulze S, Qin X, Castillo A, Hurley A, Atreja A, Rogers J, Fasihuddin F, Saliaj M, Nolan A, Reyes-Mercedes P, Rodriguez C, Aly S, Santa-Cruz K, Peters L, Suarez-Farinas M, Huang R, Hao K, Zhu J, Zhang B, Losic B, Irizar H, Song WM, Di Narzo A, Wang W, Cohen BL, DiMaio C, Greenwald D, Itzkowitz S, Lucas A, Marion J, Maser E, Ungaro R, Naymagon S, Novak J, Shah B, Ullman T, Rubin P, George J, Legnani P, Telesco SE, Friedman JR, Brodmerkel C, Plevy S, Cho JH, Colombel JF, Schadt EE, Argmann C, Dubinsky M, Kasarskis A, Sands B & Faith JJ. (2019). Gut microbiota density influences host physiology and is shaped by host and microbial factors. *Elife* **8**.

Coyne MJ, Bonorris GG, Chung A, Conley DR, Croke J & Schoenfield LJ. (1976). Inhibition by propranolol of bile acid stimulation of rabbit colonic adenylate cyclase in vitro. *Gastroenterology* **71,** 68-71.

D'Aldebert E, Biyeyeme Bi Mve MJ, Mergey M, Wendum D, Firrincieli D, Coilly A, Fouassier L, Corpechot C, Poupon R, Housset C & Chignard N. (2009). Bile salts control the antimicrobial peptide cathelicidin through nuclear receptors in the human biliary epithelium. *Gastroenterology* **136,** 1435-1443.

Dawson PA & Karpen SJ. (2015). Intestinal transport and metabolism of bile acids. *J Lipid Res* **56,** 1085-1099.

De Magalhaes Filho CD, Downes M & Evans RM. (2017). Farnesoid X Receptor an Emerging Target to Combat Obesity. *Dig Dis* **35,** 185-190.

Degirolamo C, Modica S, Vacca M, Di Tullio G, Morgano A, D'Orazio A, Kannisto K, Parini P & Moschetta A. (2015). Prevention of spontaneous hepatocarcinogenesis in farnesoid X receptor-null mice by intestinal-specific farnesoid X receptor reactivation. *Hepatology (Baltimore, Md)* **61,** 161-170.

Deutschmann K, Reich M, Klindt C, Droge C, Spomer L, Haussinger D & Keitel V. (2018). Bile acid receptors in the biliary tree: TGR5 in physiology and disease. *Biochim Biophys Acta Mol Basis Dis* **1864,** 1319-1325.

Doden HL & Ridlon JM. (2021). Microbial Hydroxysteroid Dehydrogenases: From Alpha to Omega. *Microorganisms* **9**.

Duboc H, Rajca S, Rainteau D, Benarous D, Maubert MA, Quervain E, Thomas G, Barbu V, Humbert L, Despras G, Bridonneau C, Dumetz F, Grill JP, Masliah J, Beaugerie L, Cosnes J, Chazouilleres O, Poupon R, Wolf C, Mallet JM, Langella P, Trugnan G, Sokol H & Seksik P. (2013).

Connecting dysbiosis, bile-acid dysmetabolism and gut inflammation in inflammatory bowel diseases. *Gut* **62,** 531-539.

Ducarmon QR, Zwittink RD, Hornung BVH, van Schaik W, Young VB & Kuijper EJ. (2019). Gut Microbiota and Colonization Resistance against Bacterial Enteric Infection. *Microbiol Mol Biol Rev* **83**.

El Aidy S, Dinan TG & Cryan JF. (2015). Gut Microbiota: The Conductor in the Orchestra of Immune-Neuroendocrine Communication. *Clin Ther* **37,** 954-967.

Enck P, Aziz Q, Barbara G, Farmer AD, Fukudo S, Mayer EA, Niesler B, Quigley EM, Rajilic-Stojanovic M, Schemann M, Schwille-Kiuntke J, Simren M, Zipfel S & Spiller RC. (2016). Irritable bowel syndrome. *Nat Rev Dis Primers* **2,** 16014.

Fang F, Li Y, Bumann M, Raftis EJ, Casey PG, Cooney JC, Walsh MA & O'Toole PW. (2009). Allelic variation of bile salt hydrolase genes in Lactobacillus salivarius does not determine bile resistance levels. *J Bacteriol* **191,** 5743-5757.

Fiorucci S, Biagioli M, Sepe V, Zampella A & Distrutti E. (2020). Bile acid modulators for the treatment of nonalcoholic steatohepatitis (NASH). *Expert Opin Investig Drugs* **29,** 623-632.

Fiorucci S, Biagioli M, Zampella A & Distrutti E. (2018). Bile Acids Activated Receptors Regulate Innate Immunity. *Front Immunol* **9,** 1853.

Fiorucci S, Carino A, Baldoni M, Santucci L, Costanzi E, Graziosi L, Distrutti E & Biagioli M. (2021). Bile Acid Signaling in Inflammatory Bowel Diseases. *Dig Dis Sci* **66,** 674-693.

Forsyth JS, Ross PE & Bouchier IA. (1983). Bile salts in breast milk. *Eur J Pediatr* **140,** 126-127.

Fu ZD, Csanaky IL & Klaassen CD. (2012). Gender-divergent profile of bile acid homeostasis during aging of mice. *PLoS One* **7,** e32551.

Fung C, Cools B, Malagola S, Martens T, Tack J, Kazwiny Y & Vanden Berghe P. (2021). Luminal short-chain fatty acids and 5-HT acutely activate myenteric neurons in the mouse proximal colon. *Neurogastroenterol Motil* **33,** e14186.

Fung TC. (2020). The microbiota-immune axis as a central mediator of gut-brain communication. *Neurobiol Dis* **136,** 104714.

Gadaleta RM, Oldenburg B, Willemsen EC, Spit M, Murzilli S, Salvatore L, Klomp LW, Siersema PD, van Erpecum KJ & van Mil SW. (2011a). Activation of bile salt nuclear receptor FXR is repressed by pro-inflammatory cytokines activating NF-kappaB signaling in the intestine. *Biochim Biophys Acta* **1812,** 851-858.

Gadaleta RM, van Erpecum KJ, Oldenburg B, Willemsen EC, Renooij W, Murzilli S, Klomp LW, Siersema PD, Schipper ME, Danese S, Penna G, Laverny G, Adorini L, Moschetta A & van Mil SW. (2011b). Farnesoid X receptor activation inhibits inflammation and preserves the intestinal barrier in inflammatory bowel disease. *Gut* **60,** 463-472.

Gameiro A, Reimann F, Habib AM, O'Malley D, Williams L, Simpson AK & Gribble FM. (2005). The neurotransmitters glycine and GABA stimulate glucagon-like peptide-1 release from the GLUTag cell line. *J Physiol* **569,** 761-772.

Guo C, Chen WD & Wang YD. (2016). TGR5, Not Only a Metabolic Regulator. *Front Physiol* **7,** 646.

Guo GL & Chiang JYL. (2020). Is CYP2C70 the key to new mouse models to understand bile acids in humans? *J Lipid Res* **61,** 269-271.

Guzior DV & Quinn RA. (2021). Review: microbial transformations of human bile acids. *Microbiome* **9,** 140.

Hanniman EA, Lambert G, McCarthy TC & Sinal CJ. (2005). Loss of functional farnesoid X receptor increases atherosclerotic lesions in apolipoprotein E-deficient mice. *J Lipid Res* **46,** 2595-2604.

Haselow K, Bode JG, Wammers M, Ehlting C, Keitel V, Kleinebrecht L, Schupp AK, Haussinger D & Graf D. (2013). Bile acids PKA-dependently induce a switch of the IL-10/IL-12 ratio and reduce proinflammatory capability of human macrophages. *J Leukoc Biol* **94,** 1253-1264.

Hegyi P, Maleth J, Walters JR, Hofmann AF & Keely SJ. (2018). Guts and Gall: Bile Acids in Regulation of Intestinal Epithelial Function in Health and Disease. *Physiol Rev* **98,** 1983-2023.

Henkel AS, LeCuyer B, Olivares S & Green RM. (2017). Endoplasmic Reticulum Stress Regulates Hepatic Bile Acid Metabolism in Mice. *Cell Mol Gastroenterol Hepatol* **3,** 261-271.

Hirano S, Nakama R, Tamaki M, Masuda N & Oda H. (1981). Isolation and characterization of thirteen intestinal microorganisms capable of 7 alpha-dehydroxylating bile acids. *Appl Environ Microbiol* **41,** 737-745.

Hsuchou H, Pan W & Kastin AJ. (2013). Fibroblast growth factor 19 entry into brain. *Fluids Barriers CNS* **10,** 32.

Huang C, Wang J, Hu W, Wang C, Lu X, Tong L, Wu F & Zhang W. (2016). Identification of functional farnesoid X receptors in brain neurons. *FEBS Lett* **590,** 3233-3242.

Huang F, Wang T, Lan Y, Yang L, Pan W, Zhu Y, Lv B, Wei Y, Shi H, Wu H, Zhang B, Wang J, Duan X, Hu Z & Wu X. (2015). Deletion of mouse FXR gene disturbs multiple neurotransmitter systems and alters neurobehavior. *Front Behav Neurosci* **9,** 70.

Hugon P, Dufour JC, Colson P, Fournier PE, Sallah K & Raoult D. (2015). A comprehensive repertoire of prokaryotic species identified in human beings. *Lancet Infect Dis* **15,** 1211-1219.

Human Microbiome Project C. (2012). Structure, function and diversity of the healthy human microbiome. *Nature* **486,** 207-214.

Islam KB, Fukiya S, Hagio M, Fujii N, Ishizuka S, Ooka T, Ogura Y, Hayashi T & Yokota A. (2011). Bile acid is a host factor that regulates the composition of the cecal microbiota in rats. *Gastroenterology* **141,** 1773-1781.

Jonas MI, Kurylowicz A, Bartoszewicz Z, Lisik W, Jonas M, Kozniewski K & Puzianowska-Kuznicka M. (2019). Vitamin D Receptor Gene Expression in Adipose Tissue of Obese Individuals is Regulated by miRNA and Correlates with the Pro-Inflammatory Cytokine Level. *Int J Mol Sci* **20**.

Jones BV, Begley M, Hill C, Gahan CG & Marchesi JR. (2008). Functional and comparative metagenomic analysis of bile salt hydrolase activity in the human gut microbiome. *Proc Natl Acad Sci U S A* **105,** 13580-13585.

Joyce SA, MacSharry J, Casey PG, Kinsella M, Murphy EF, Shanahan F, Hill C & Gahan CG. (2014). Regulation of host weight gain and lipid metabolism by bacterial bile acid modification in the gut. *Proc Natl Acad Sci U S A* **111,** 7421-7426.

Kastin AJ, Akerstrom V & Pan W. (2002). Interactions of glucagon-like peptide-1 (GLP-1) with the blood-brain barrier. *J Mol Neurosci* **18,** 7-14.

Katsuma S, Hirasawa A & Tsujimoto G. (2005). Bile acids promote glucagon-like peptide-1 secretion through TGR5 in a murine enteroendocrine cell line STC-1. *Biochem Biophys Res Commun* **329,** 386-390.

Kawamata Y, Fujii R, Hosoya M, Harada M, Yoshida H, Miwa M, Fukusumi S, Habata Y, Itoh T, Shintani Y, Hinuma S, Fujisawa Y & Fujino M. (2003). A G protein-coupled receptor responsive to bile acids. *The Journal of biological chemistry* **278,** 9435-9440.

Keitel V, Gorg B, Bidmon HJ, Zemtsova I, Spomer L, Zilles K & Haussinger D. (2010). The bile acid receptor TGR5 (Gpbar-1) acts as a neurosteroid receptor in brain. *Glia* **58,** 1794-1805.

Kidd M, Modlin IM, Gustafsson BI, Drozdov I, Hauso O & Pfragner R. (2008). Luminal regulation of normal and neoplastic human EC cell serotonin release is mediated by bile salts, amines, tastants, and olfactants. *Am J Physiol Gastrointest Liver Physiol* **295,** G260-272.

Kim H & Fang S. (2018). Crosstalk between FXR and TGR5 controls glucagon-like peptide 1 secretion to maintain glycemic homeostasis. *Lab Anim Res* **34,** 140-146.

Laffitte B, Kast H, Nguyen C, Zavacki A, Moore D & Edwards P. (2000). Identification of the DNA binding specificity and potential target genes for the farnesoid X-activated receptor. *The Journal of biological chemistry* **275,** 10638-10647.

Long SL, Gahan CGM & Joyce SA. (2017). Interactions between gut bacteria and bile in health and disease. *Mol Aspects Med* **56,** 54-65.

Lund ML, Egerod KL, Engelstoft MS, Dmytriyeva O, Theodorsson E, Patel BA & Schwartz TW. (2018). Enterochromaffin 5-HT cells - A major target for GLP-1 and gut microbial metabolites. *Mol Metab* **11,** 70-83.

Lyte M. (2014). Microbial endocrinology: Host-microbiota neuroendocrine interactions influencing brain and behavior. *Gut Microbes* **5,** 381-389.

Madison A & Kiecolt-Glaser JK. (2019). Stress, depression, diet, and the gut microbiota: human-bacteria interactions at the core of psychoneuroimmunology and nutrition. *Curr Opin Behav Sci* **28,** 105-110.

Maruyama T, Miyamoto Y, Nakamura T, Tamai Y, Okada H, Sugiyama E, Nakamura T, Itadani H & Tanaka K. (2002). Identification of membrane-type receptor for bile acids (M-BAR). *Biochem Biophys Res Commun* **298,** 714-719.

Mayer EA. (2011). Gut feelings: the emerging biology of gut-brain communication. *Nat Rev Neurosci* **12,** 453-466.

McMillin M, Frampton G, Quinn M, Divan A, Grant S, Patel N, Newell-Rogers K & DeMorrow S. (2015a). Suppression of the HPA Axis During Cholestasis Can Be Attributed to Hypothalamic Bile Acid Signaling. *Mol Endocrinol* **29,** 1720-1730.

McMillin M, Frampton G, Tobin R, Dusio G, Smith J, Shin H, Newell-Rogers K, Grant S & DeMorrow S. (2015b). TGR5 signaling reduces neuroinflammation during hepatic encephalopathy. *J Neurochem* **135,** 565-576.

McNeilly AD, Macfarlane DP, O'Flaherty E, Livingstone DE, Mitic T, McConnell KM, McKenzie SM, Davies E, Reynolds RM, Thiesson HC, Skott O, Walker BR & Andrew R. (2010). Bile acids modulate glucocorticoid metabolism and the hypothalamic-pituitary-adrenal axis in obstructive jaundice. *J Hepatol* **52,** 705-711.

McVey Neufeld KA, Perez-Burgos A, Mao YK, Bienenstock J & Kunze WA. (2015). The gut microbiome restores intrinsic and extrinsic nerve function in germ-free mice accompanied by changes in calbindin. *Neurogastroenterol Motil* **27,** 627-636.

Meng F, Kennedy L, Hargrove L, Demieville J, Jones H, Madeka T, Karstens A, Chappell K, Alpini G, Sybenga A, Invernizzi P, Bernuzzi F, DeMorrow S & Francis H. (2018). Ursodeoxycholate inhibits mast cell activation and reverses biliary injury and fibrosis in Mdr2(-/-) mice and human primary sclerosing cholangitis. *Lab Invest* **98,** 1465-1477.

Mertens KL, Kalsbeek A, Soeters MR & Eggink HM. (2017). Bile Acid Signaling Pathways from the Enterohepatic Circulation to the Central Nervous System. *Front Neurosci* **11,** 617.

Mortiboys H, Aasly J & Bandmann O. (2013). Ursocholanic acid rescues mitochondrial function in common forms of familial Parkinson's disease. *Brain* **136,** 3038-3050.

Moya A & Ferrer M. (2016). Functional Redundancy-Induced Stability of Gut Microbiota Subjected to Disturbance. *Trends Microbiol* **24,** 402-413.

Mullish BH, McDonald JAK, Pechlivanis A, Allegretti JR, Kao D, Barker GF, Kapila D, Petrof EO, Joyce SA, Gahan CGM, Glegola-Madejska I, Williams HRT, Holmes E, Clarke TB, Thursz MR & Marchesi JR. (2019). Microbial bile salt hydrolases mediate the efficacy of faecal microbiota transplant in the treatment of recurrent Clostridioides difficile infection. *Gut* **68,** 1791-1800.

Murphy GM & Signer E. (1974). Bile acid metabolism in infants and children. *Gut* **15,** 151-163.

Nakagawa A, Satake H, Nakabayashi H, Nishizawa M, Furuya K, Nakano S, Kigoshi T, Nakayama K & Uchida K. (2004). Receptor gene expression of glucagon-like peptide-1, but not glucose-dependent insulinotropic polypeptide, in rat nodose ganglion cells. *Auton Neurosci* **110,** 36-43.

Ni Dhonnabhain R, Xiao Q & O'Malley D. (2021). Aberrant Gut-To-Brain Signaling in Irritable Bowel Syndrome - The Role of Bile Acids. *Front Endocrinol (Lausanne)* **12,** 745190.

Nunes AF, Amaral JD, Lo AC, Fonseca MB, Viana RJ, Callaerts-Vegh Z, D'Hooge R & Rodrigues CM. (2012). TUDCA, a bile acid, attenuates amyloid precursor protein processing and amyloid-beta deposition in APP/PS1 mice. *Mol Neurobiol* **45,** 440-454.

O'Malley D. (2016). Neuroimmune Cross Talk in the Gut. Neuroendocrine and neuroimmune pathways contribute to the pathophysiology of irritable bowel syndrome. *Am J Physiol Gastrointest Liver Physiol* **311,** G934-G941.

O'Malley D, Julio-Pieper M, Gibney SM, Dinan TG & Cryan JF. (2010). Distinct alterations in colonic morphology and physiology in two rat models of enhanced stress-induced anxiety and depression-like behaviour. *Stress* **13,** 114-122.

Ogundare M, Theofilopoulos S, Lockhart A, Hall LJ, Arenas E, Sjovall J, Brenton AG, Wang Y & Griffiths WJ. (2010). Cerebrospinal fluid steroidomics: are bioactive bile acids present in brain? *J Biol Chem* **285,** 4666-4679.

Öhman L, Törnblom H & Simrén M. (2015). Crosstalk at the mucosal border: importance of the gut microenvironment in IBS. *Nat Rev Gastroenterol Hepatol* **12,** 36-49.

Parasar B, Zhou H, Xiao X, Shi Q, Brito IL & Chang PV. (2019). Chemoproteomic Profiling of Gut Microbiota-Associated Bile Salt Hydrolase Activity. *ACS Cent Sci* **5,** 867-873.

Parker CH, Naliboff BD, Shih W, Presson AP, Videlock EJ, Mayer EA & Chang L. (2019). Negative Events During Adulthood Are Associated With Symptom Severity and Altered Stress Response in Patients With Irritable Bowel Syndrome. *Clin Gastroenterol Hepatol* **17,** 2245-2252.

Parker HE, Wallis K, le Roux CW, Wong KY, Reimann F & Gribble FM. (2012). Molecular mechanisms underlying bile acid-stimulated glucagon-like peptide-1 secretion. *Br J Pharmacol* **165,** 414-423.

Parry GJ, Rodrigues CM, Aranha MM, Hilbert SJ, Davey C, Kelkar P, Low WC & Steer CJ. (2010). Safety, tolerability, and cerebrospinal fluid penetration of ursodeoxycholic Acid in patients with amyotrophic lateral sclerosis. *Clin Neuropharmacol* **33,** 17-21.

Peleman C, Camilleri M, Busciglio I, Burton D, Donato L & Zinsmeister AR. (2017). Colonic Transit and Bile Acid Synthesis or Excretion in Patients With Irritable Bowel Syndrome-Diarrhea Without Bile Acid Malabsorption. *Clin Gastroenterol Hepatol* **15,** 720-727 e721.

Perez-Burgos A, Wang B, Mao YK, Mistry B, McVey Neufeld KA, Bienenstock J & Kunze W. (2013). Psychoactive bacteria Lactobacillus rhamnosus (JB-1) elicits rapid frequency facilitation in vagal afferents. *Am J Physiol Gastrointest Liver Physiol* **304,** G211-220.

Perez-Burgos A, Wang L, McVey Neufeld KA, Mao YK, Ahmadzai M, Janssen LJ, Stanisz AM, Bienenstock J & Kunze WA. (2015). The TRPV1 channel in rodents is a major target for antinociceptive effect of the probiotic Lactobacillus reuteri DSM 17938. *The Journal of physiology* **593,** 3943-3957.

Perino A, Pols TW, Nomura M, Stein S, Pellicciari R & Schoonjans K. (2014). TGR5 reduces macrophage migration through mTOR-induced C/EBPbeta differential translation. *J Clin Invest* **124,** 5424-5436.

Pols TW, Noriega LG, Nomura M, Auwerx J & Schoonjans K. (2011). The bile acid membrane receptor TGR5 as an emerging target in metabolism and inflammation. *J Hepatol* **54,** 1263-1272.

Prete R, Long SL, Gallardo AL, Gahan CG, Corsetti A & Joyce SA. (2020). Beneficial bile acid metabolism from Lactobacillus plantarum of food origin. *Sci Rep* **10,** 1165.

Quinn M, McMillin M, Galindo C, Frampton G, Pae HY & DeMorrow S. (2014). Bile acids permeabilize the blood brain barrier after bile duct ligation in rats via Rac1-dependent mechanisms. *Dig Liver Dis* **46,** 527-534.

Quinn RA, Melnik AV, Vrbanac A, Fu T, Patras KA, Christy MP, Bodai Z, Belda-Ferre P, Tripathi A, Chung LK, Downes M, Welch RD, Quinn M, Humphrey G, Panitchpakdi M, Weldon KC, Aksenov A, da Silva R, Avila-Pacheco J, Clish C, Bae S, Mallick H, Franzosa EA, Lloyd-Price J, Bussell R, Thron T, Nelson AT, Wang M, Leszczynski E, Vargas F, Gauglitz JM, Meehan MJ, Gentry E, Arthur TD, Komor AC, Poulsen O, Boland BS, Chang JT, Sandborn WJ, Lim M, Garg N, Lumeng JC, Xavier RJ, Kazmierczak BI, Jain R, Egan M, Rhee KE, Ferguson D, Raffatellu M, Vlamakis H, Haddad GG, Siegel D, Huttenhower C, Mazmanian SK, Evans RM, Nizet V, Knight R & Dorrestein PC. (2020). Global chemical effects of the microbiome include new bile-acid conjugations. *Nature* **579,** 123-129.

Rajilic-Stojanovic M, Biagi E, Heilig HG, Kajander K, Kekkonen RA, Tims S & de Vos WM. (2011). Global and deep molecular analysis of microbiota signatures in fecal samples from patients with irritable bowel syndrome. *Gastroenterology* **141,** 1792-1801.

Rao AS, Wong BS, Camilleri M, Odunsi-Shiyanbade ST, McKinzie S, Ryks M, Burton D, Carlson P, Lamsam J, Singh R & Zinsmeister AR. (2010). Chenodeoxycholate in females with irritable bowel syndrome-constipation: a pharmacodynamic and pharmacogenetic analysis. *Gastroenterology* **139,** 1549-1558, 1558 e1541.

Raybould HE. (2010). Gut chemosensing: interactions between gut endocrine cells and visceral afferents. *Auton Neurosci* **153,** 41-46.

Ren S, Hylemon PB, Marques D, Gurley E, Bodhan P, Hall E, Redford K, Gil G & Pandak WM. (2004). Overexpression of cholesterol transporter StAR increases in vivo rates of bile acid synthesis in the rat and mouse. *Hepatology* **40,** 910-917.

Ridlon JM, Harris SC, Bhowmik S, Kang DJ & Hylemon PB. (2016). Consequences of bile salt biotransformations by intestinal bacteria. *Gut Microbes* **7,** 22-39.

Ridlon JM & Hylemon PB. (2012). Identification and characterization of two bile acid coenzyme A transferases from Clostridium scindens, a bile acid 7alpha-dehydroxylating intestinal bacterium. *J Lipid Res* **53,** 66-76.

Ridlon JM, Kang D-J & Hylemon PB. (2006). Bile salt biotransformations by human intestinal bacteria. *Journal of Lipid Research* **47,** 241-259.

Ronveaux CC, de Lartigue G & Raybould HE. (2014). Ability of GLP-1 to decrease food intake is dependent on nutritional status. *Physiol Behav* **135,** 222-229.

Rudling M, Camilleri M, Graffner H, Holst JJ & Rikner L. (2015). Specific inhibition of bile acid transport alters plasma lipids and GLP-1. *BMC Cardiovasc Disord* **15,** 75.

Ryan FJ, Ahern AM, Fitzgerald RS, Laserna-Mendieta EJ, Power EM, Clooney AG, O'Donoghue KW, McMurdie PJ, Iwai S, Crits-Christoph A, Sheehan D, Moran C, Flemer B, Zomer AL, Fanning A, O'Callaghan J, Walton J, Temko A, Stack W, Jackson L, Joyce SA, Melgar S, DeSantis TZ, Bell JT, Shanahan F & Claesson MJ. (2020). Colonic microbiota is associated with inflammation and host epigenomic alterations in inflammatory bowel disease. *Nat Commun* **11,** 1512.

Sato Y, Atarashi K, Plichta DR, Arai Y, Sasajima S, Kearney SM, Suda W, Takeshita K, Sasaki T, Okamoto S, Skelly AN, Okamura Y, Vlamakis H, Li Y, Tanoue T, Takei H, Nittono H, Narushima S, Irie J, Itoh H, Moriya K, Sugiura Y, Suematsu M, Moritoki N, Shibata S, Littman DR, Fischbach MA, Uwamino Y, Inoue T, Honda A, Hattori M, Murai T, Xavier RJ, Hirose N & Honda K. (2021). Novel bile acid biosynthetic pathways are enriched in the microbiome of centenarians. *Nature* **599,** 458-464.

Sayin SI, Wahlstrom A, Felin J, Jantti S, Marschall HU, Bamberg K, Angelin B, Hyotylainen T, Oresic M & Backhed F. (2013). Gut microbiota regulates bile acid metabolism by reducing the levels of tauro-beta-muricholic acid, a naturally occurring FXR antagonist. *Cell Metab* **17,** 225-235.

Scepanovic P, Hodel F, Mondot S, Partula V, Byrd A, Hammer C, Alanio C, Bergstedt J, Patin E, Touvier M, Lantz O, Albert ML, Duffy D, Quintana-Murci L, Fellay J & Milieu Interieur C. (2019). A comprehensive assessment of demographic, environmental, and host genetic associations with gut microbiome diversity in healthy individuals. *Microbiome* **7,** 130.

Selwyn FP, Csanaky IL, Zhang Y & Klaassen CD. (2015). Importance of Large Intestine in Regulating Bile Acids and Glucagon-Like Peptide-1 in Germ-Free Mice. *Drug Metab Dispos* **43,** 1544-1556.

Silvennoinen R, Quesada H, Kareinen I, Julve J, Kaipiainen L, Gylling H, Blanco-Vaca F, Escola-Gil JC, Kovanen PT & Lee-Rueckert M. (2015). Chronic intermittent psychological stress promotes macrophage reverse cholesterol transport by impairing bile acid absorption in mice. *Physiol Rep* **3**.

Sinha SR, Haileselassie Y, Nguyen LP, Tropini C, Wang M, Becker LS, Sim D, Jarr K, Spear ET, Singh G, Namkoong H, Bittinger K, Fischbach MA, Sonnenburg JL & Habtezion A. (2020). Dysbiosis-Induced Secondary Bile Acid Deficiency Promotes Intestinal Inflammation. *Cell Host Microbe* **27,** 659-670 e655.

Slattery SA, Niaz O, Aziz Q, Ford AC & Farmer AD. (2015). Systematic review with meta-analysis: the prevalence of bile acid malabsorption in the irritable bowel syndrome with diarrhoea. *Aliment Pharmacol Ther* **42,** 3-11.

Song Z, Cai Y, Lao X, Wang X, Lin X, Cui Y, Kalavagunta PK, Liao J, Jin L, Shang J & Li J. (2019). Taxonomic profiling and populational patterns of bacterial bile salt hydrolase (BSH) genes based on worldwide human gut microbiome. *Microbiome* **7,** 9.

Spiller RC. (2004). Irritable bowel syndrome. *Br Med Bull* **72,** 15-29.

Staley C, Weingarden AR, Khoruts A & Sadowsky MJ. (2017). Interaction of gut microbiota with bile acid metabolism and its influence on disease states. *Appl Microbiol Biotechnol* **101,** 47-64.

Stilling RM, Dinan TG & Cryan JF. (2015). The brain's Geppetto-microbes as puppeteers of neural function and behaviour? *J Neurovirol*.

Sun R, Xu C, Feng B, Gao X & Liu Z. (2021). Critical roles of bile acids in regulating intestinal mucosal immune responses. *Therap Adv Gastroenterol* **14,** 17562848211018098.

Takikawa H, Beppu T & Seyama Y. (1985). Profiles of bile acids and their glucuronide and sulphate conjugates in the serum, urine and bile from patients undergoing bile drainage. *Gut* **26,** 38-42.

Tolhurst G, Heffron H, Lam YS, Parker HE, Habib AM, Diakogiannaki E, Cameron J, Grosse J, Reimann F & Gribble FM. (2012). Short-chain fatty acids stimulate glucagon-like peptide-1 secretion via the G-protein-coupled receptor FFAR2. *Diabetes* **61,** 364-371.

Trabelsi MS, Daoudi M, Prawitt J, Ducastel S, Touche V, Sayin SI, Perino A, Brighton CA, Sebti Y, Kluza J, Briand O, Dehondt H, Vallez E, Dorchies E, Baud G, Spinelli V, Hennuyer N, Caron S, Bantubungi K, Caiazzo R, Reimann F, Marchetti P, Lefebvre P, Backhed F, Gribble FM, Schoonjans K, Pattou F, Tailleux A, Staels B & Lestavel S. (2015). Farnesoid X receptor inhibits glucagon-like peptide-1 production by enteroendocrine L cells. *Nat Commun* **6,** 7629.

Vavassori P, Mencarelli A, Renga B, Distrutti E & Fiorucci S. (2009). The bile acid receptor FXR is a modulator of intestinal innate immunity. *J Immunol* **183,** 6251-6261.

Vessey DA. (1978). The biochemical basis for the conjugation of bile acids with either glycine or taurine. *Biochemical Journal* **174,** 621-626.

Vizoso Pinto MG, Franz CMAP, Schillinger U & Holzapfel WH. (2006). Lactobacillus spp. with in vitro probiotic properties from human faeces and traditional fermented products. *International Journal of Food Microbiology* **109,** 205-214.

Ward JB, Mroz MS & Keely SJ. (2013). The bile acid receptor, TGR5, regulates basal and cholinergic-induced secretory responses in rat colon. *Neurogastroenterol Motil* **25,** 708-711.

Weng YJ, Gan HY, Li X, Huang Y, Li ZC, Deng HM, Chen SZ, Zhou Y, Wang LS, Han YP, Tan YF, Song YJ, Du ZM, Liu YY, Wang Y, Qin N, Bai Y, Yang RF, Bi YJ & Zhi FC. (2019). Correlation of diet, microbiota and metabolite networks in inflammatory bowel disease. *J Dig Dis* **20,** 447-459.

Wu X, Li JY, Lee A, Lu YX, Zhou SY & Owyang C. (2020). Satiety induced by bile acids is mediated via vagal afferent pathways. *JCI Insight* **5**.

Yang X, Wu R, Qi D, Fu L, Song T, Wang Y, Bian Y & Shi Y. (2021). Profile of Bile Acid Metabolomics in the Follicular Fluid of PCOS Patients. *Metabolites* **11**.

Zheng X, Chen T, Zhao A, Wang X, Xie G, Huang F, Liu J, Zhao Q, Wang S, Wang C, Zhou M, Panee J, He Z & Jia W. (2016). The Brain Metabolome of Male Rats across the Lifespan. *Sci Rep* **6,** 24125.

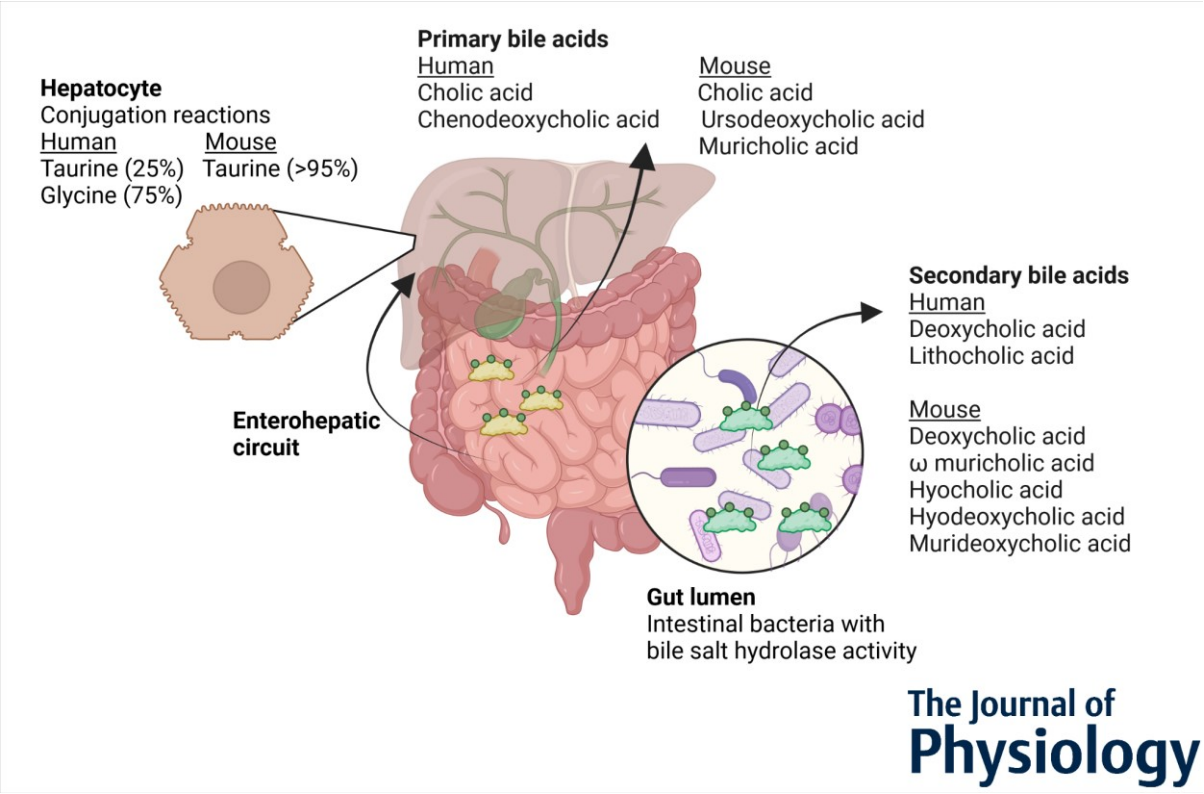

Figure 1: Species divergence in bile acid profiles.

The figure illustrates the critical role that luminal bacteria play in generating a diverse array of bile acids. It also highlights species dfferences between mice and humans in the range and conjugation status of bile acids.

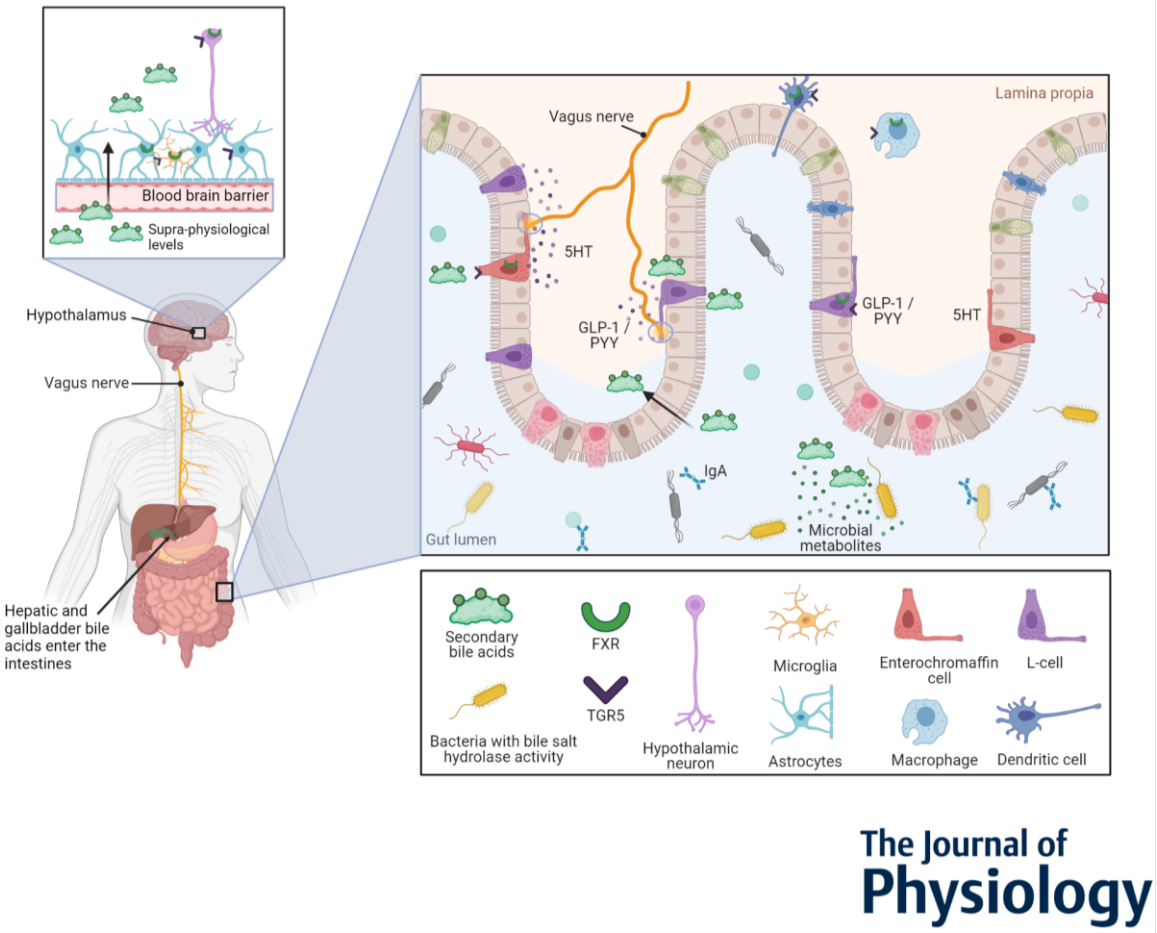

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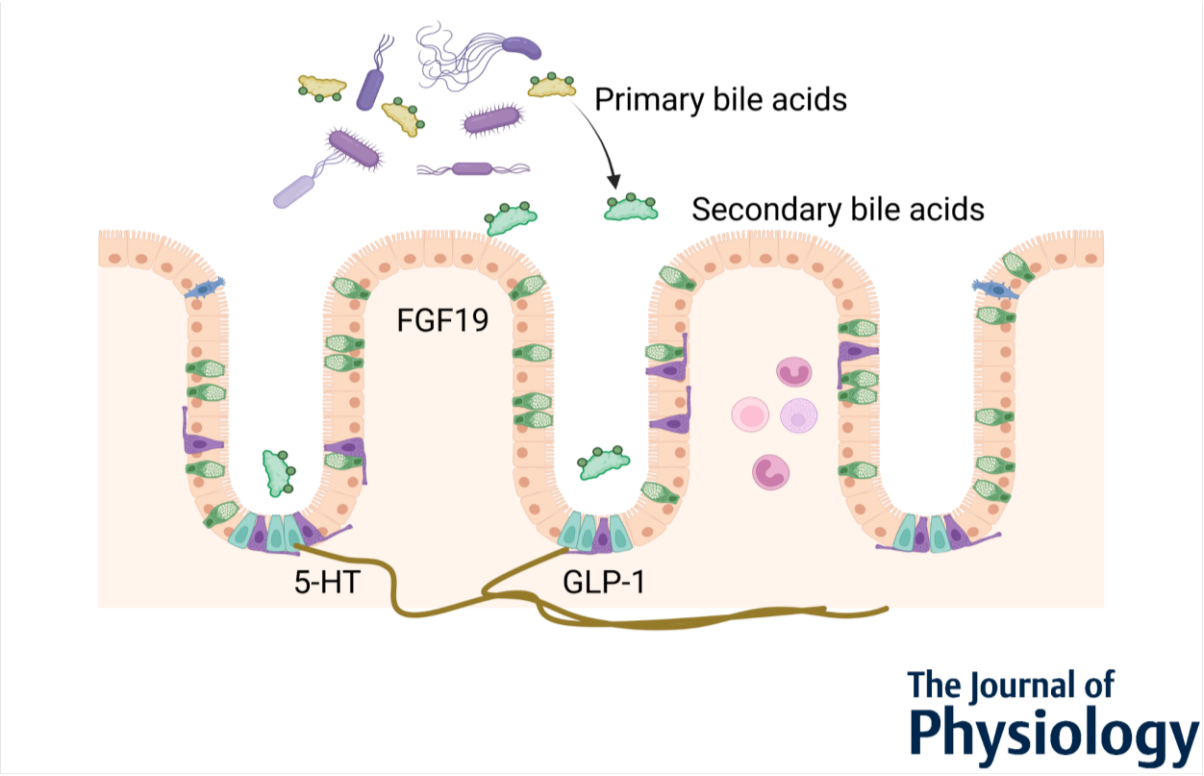
Figure 2: Bile acids as bioactive molecules in the gut-brain signalling axis.

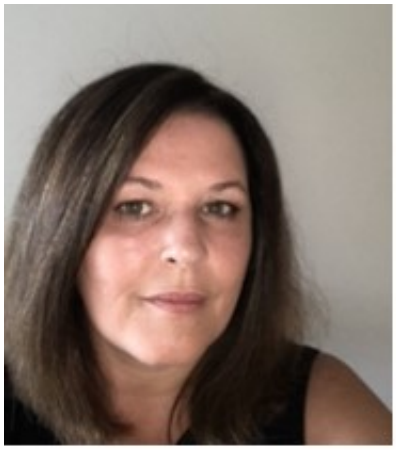
The illustration depicts interactions between colonic microbes with bile salt hydrolase activity and luminal bile acids (BAs). These BAs may subsequently bind to BA receptors, which are expressed on enteroendocrine, immune and neural cells. When circulating BA levels are elevated (under pathophysiological conditions), BAs may cross the blood brain barrier and bind to TGR5 and FXR, which are expressed on neural cells, astrocytes and microglia. The figure is adapted from a "Gut-brain axis" template, by BioRender.com (2021). Retrieved from https://app.biorender.com/biorender-templates



Abstract figure

The figure illustrates the microbial modification of hepatic primary bile acids into secondary bile acids. In addition to facilitating lipid digestion and absorption, bile acids act as bioactive signalling molecules by binding to bile acid receptors expressed on enterocytes, neural afferent-coupled enteroendocrine cells and immune cells.

Susan Joyce



Dr Susan Joyce is a lecturer and a principal investigator interested in metabolism and in metabolites produced by microbes that influence host responses, among them bile acids and fatty acids. She applies microbial and cellular molecular approaches combined with mass spectrometry applications and clinical investigations to her scientific questions. Her group enjoys research funding from Science foundation Ireland, Department of Food and Marine, EU and Government of Irish Research Council.

Dervla O'Malley

Dr Dervla O'Malley is a lecturer and principal investigator with a research focus on neuro-endocrine regulation of gut function and the physiological mechanisms underlying gastrointestinal dysfunction. Using imaging and electrophysiological techniques, her research team aims to elucidate and understand how luminal factors, including bile acids, signal across the gut barrier to the peripheral and central nervous systems.