Short chain fatty acids: The microbiome's route to the brain?

Dalile, B., Van Oudenhove, L., Vervliet, B., Verbeke, K.

Abstract

Short chain fatty acids (SCFA), the main metabolites produced by bacterial fermentation of dietary fibre in the colon, are speculated to play a key role in microbiota-gut-brain crosstalk. However, the pathways through which they may influence psychological functioning, including affective and cognitive processes and their neural basis, have not been fully elucidated. Furthermore, research directly exploring the role of SCFA as potential mediators of the impact of microbiota-targeted interventions on affective and cognitive functioning is sparse, especially in humans. The purpose of this review is to (a) summarise the existing knowledge on SCFA and their potential to mediate microbiota-gut-brain interactions directly or indirectly, (b) review the impact of microbiota-targeted interventions on psychological functioning and its neural basis, and the putative mediating role of SCFA signaling herein, (c) discuss the literature that examines the relationship between SCFA and psychobiological processes, and (d) outline future directions to facilitate direct investigation of the impact of SCFA on psychological functioning.

Keywords:

Short chain fatty acids, gut-brain axis, mood, emotion, cognition, microbiota, fibre

Competing interests

The authors have no competing interests to declare.

Contents

1. Introduction

The gut-brain axis refers to the bidirectional signaling mechanisms between the gastrointestinal (GI) tract and the central nervous system (CNS)[1]. Through complex neuro-humoral pathways, signals from the brain can alter the gut's sensorimotor and secretory functions, and conversely, visceral afferent signals originating in the GI tract can modulate brain function. The gut microbiota is the ecological community of commensal, symbiotic and pathogenic micro-organisms present in the gut and is critically involved in gut-brain communication[2]. Imbalances in the gut's microbial composition are present in GI and metabolic disorders[3] as well as in mental disorders including eating disorders[4], autism spectrum disorders (ASD)[5], and mood and anxiety disorders[6]. Microbiota-gut-brain (MGB) communication can theoretically occur through multiple systems comprising the gut-brain axis, including the autonomic and enteric nervous system (ENS), neuroendocrine systems, and the immune system. Nevertheless, the specific mechanisms of this communication and its putative effects on brain development, behaviour, cognition, and mood in humans are largely unknown.

To explore the influence of the gut microbiome on psychological functioning, the microbiome can be modified by means of prebiotic, probiotic, and dietary interventions. Experimental studies adopting this approach have demonstrated modulation of stress reactivity, affective and cognitive processes, and behaviour in animals and, to a lesser extent, humans[7]. Although the biological mediators driving these effects remain largely unknown, short-chain fatty acids (SCFA), microbial metabolites that constitute the major products of bacterial fermentation of dietary fibre in the colon, are often considered key candidate mediators. SCFA may be directly or indirectly involved in communication along the MGB axis due to their neuroactive properties and effects on other gut-brain signaling pathways including the immune and endocrine systems[8,9]. However, research directly exploring the role of SCFA as potential mediators of the impact of microbiota-targeted interventions on affective and cognitive functioning is sparse, especially in humans. In this non-exhaustive but comprehensive review we (a) summarise the existing knowledge on SCFA and their potential to mediate MGB interactions, (b) review the impact of microbiota-targeted interventions on psychological functioning and its neural basis, and the putative mediating

role of SCFA signaling herein, (c) discuss the literature that examines the relationship between SCFA and psychobiological processes, and (d) outline future directions to facilitate investigation of the impact of SCFA on psychological functioning.

2. Metabolism of SCFA

SCFA are saturated fatty acids with a chain length ranging from one to six carbon atoms and are the main products of fermentation of dietary fibre in the large intestine[10]. Approximately 500 to 600 mmol of SCFA are produced per day depending on the dietary fibre content of the diet[11]. Acetic acid (C2), propionic acid (C3) and butyric acid (C4) are the most abundant SCFA as well as the most abundant anions in the large intestine. They are responsible for the drop in pH when progressing from the terminal ileum to the proximal colon. These SCFA are present in the colon in an approximate molar ratio of 60:20:20, respectively[12], although the amount and relative proportion of each SCFA depends on the substrate, the microbiota composition, and gut transit time[13] (Table 1). Following their production in the colon, SCFA are rapidly absorbed by the colonocytes, mainly via active transport mediated by members of the family of monocarboxylate transporters (MCT) (FIG. 1). MCT-1 transports SCFA in an H^+-dependent electroneutral manner whereas SCFA anion transport by the electrogenic sodium-dependent monocarboxylate transporter 1 (SMCT-1) is coupled to Na^+-transport. A comprehensive list of all known SCFA transporters is shown in Table 2. Absorption of undissociated SCFA by passive diffusion is probably quantitatively less important, as well as exchange for HCO_3^- via downregulated-in-adenoma (DRA) or solute carrier family 26, member 3 (SLC26A3)[14]. After absorption in the cells, SCFA enter the citric acid cycle in the mitochondria to generate ATP and thus energy for the cells. SCFA that escape metabolism in the colonocytes are transported into the portal circulation where concentrations of SCFA have been reported to be 260, 30 and 30 µM for acetate, propionate and butyrate, respectively[15]. In the liver, all three SCFA are used as energy substrates for the cells. In addition, acetate is a substrate for cholesterol and fatty acid synthesis[16-18]. Propionate is a known precursor for the synthesis of glucose in the liver, at least in ruminants[19], but gluconeogenesis from propionate in the human liver is quantitatively less important[16]. Consequently, only a minor fraction of the SCFA produced in the

colon reaches the systemic circulation and peripheral tissues. Plasma concentrations of acetate, propionate and butyrate have been reported in a range of 25-250 µM, 1.4-13.4 µM and 0.5-14.2 µM, respectively[12]. Other sources of plasma acetate include endogenous production from fatty acid oxidation and amino acid metabolism[20,21], ketogenesis in hepatocytes[21], or oxidation of ethanol by microsomal cytochroom P450 enzymes[22]. Bovine milk fats provide a source of butyrate as 5-10 % of the triacylglycerides mixture in bovine milk contains butyrate which is released by gastric lipase[23].

3. SCFA in gut-brain signaling

Besides exerting local effects in the colon, SCFA affect gene expression by inhibiting histone deacetylases (HDAC), and act as endogenous ligands for orphan G-protein coupled receptors (GPRs). Additionally, SCFA affect inflammation and hormonal regulation, and interact with vagal afferents. In what follows, we outline the interactions of SCFA with specific cellular systems and gut-brain signaling pathways, arguing for a potential key role of SCFA in MGB communication (FIG. 2).

3.1. Impact on cellular systems

3.1.1. SCFA receptors

The best studied SCFA receptors are GPR43 and GPR41, later renamed as free fatty acid receptor (FFAR) 2 and FFAR3, respectively. Those receptors are activated by SCFA anions such as formate, acetate, propionate, butyrate and pentanoate, although with differing specificity for carbon chain length. FFAR2 is expressed mainly in enteroendocrine L cells, vasculature and immune cells, including lymphocytes, neutrophils, and monocytes[24-26]. FFAR3 is expressed in the colon, kidneys, sympathetic nervous system, and blood vessels. Finally, GPR109a, a receptor initially identified as a receptor for niacin present on adipocytes, immune cells and colonocytes, is activated by β-D-hydroxy butyrate as well as butyrate[27,28]. Other receptors activated by SCFA are listed in Table 3.

Evidence exists for presence of functional SCFA receptors in the CNS and peripheral nervous system (PNS). FFAR3 is highly expressed in rat brain tissue[29], and in sympathetic ganglia, specifically the superior cervical ganglion, in adult mice[30]. The expression of FFAR3 appears

important in controlling sympathetic nerve activity, as reduced activity was observed in *Ffar3*$^{-/-}$ mice[30]. Furthermore, intraperitoneal administration of propionate (1 g/kg) resulted in increased heart rate in wild-type and *Ffar2*$^{-/-}$ mice, but not in *Ffar3*$^{-/-}$ mice. Nøhr et al.[31] confirmed expression of FFAR3 in the superior cervical ganglion and revealed its expression in the sympathetic ganglia of the thoracic and lumbar sympathetic trunk, as well as in both autonomic and sensory ganglia such as the vagal ganglion, spinal dorsal root ganglion, and trigeminal ganglion. Contrary to evidence in rats[29], however, FFAR3 was not found in the brain or spinal cord in mice[31]. Through gut-brain neurocircuitry involving FFAR3, propionate improved glucose control and insulin sensitivity in mice[32]. Specifically, FFAR3 was expressed in the portal vein wall and propionate-induced intestinal gluconeogenesis depended on FFAR3 signaling. Importantly, propionate influenced CNS regions implicated in signaling from the portal area, whereby propionate feeding induced c-Fos expression in all areas of the dorsal vagal complex, C1 segment of the spinal cord, and the parabrachial nucleus. Hypothalamic areas, namely the paraventricular nucleus, the lateral hypothalamus, and the arcuate nucleus, which receive input from the parabrachial nucleus, also exhibited c-Fos activation in response to propionate feeding[32]. Together, these findings suggest that through binding of GPRs, SCFA might affect the central and peripheral nervous systems, which is a prerequisite for their putative effects on psychological processes.

3.1.2. Histone deacetylase inhibition

Gene expression is regulated by modulating the coiling of the DNA around histones mostly via acetylation of the histones. Acetylated histones are less compact and result in more transcriptionally active chromatin. Conversely, removal of the acetyl groups by HDAC leads to condensed and transcriptionally silenced chromatin. Studies showed that intracellular butyrate and propionate[33], as well as acetate[34] inhibit the activity of HDAC, promoting hyperacetylation of histones. Importantly, HDACs are involved in brain development and a range of psychopathologies[35]. Furthermore, preclinical studies suggest that HDAC inhibitors act as cognitive enhancers in fear, anxiety and trauma-related processes and may be used in conjunction with psychotherapy to promote long-term positive treatment outcomes and relapse prevention[36].

Evidence demonstrating SCFA-mediated HDAC inhibition and its impact on the brain mostly comes from animal research with sodium butyrate. Chronic (28 days) and acute systemic administration of butyrate combined with fluoxetine (a selective serotonin reuptake inhibitor antidepressant) significantly decreased behavioural despair[37], which correlated with changes in brain-derived neurotrophic factor (BDNF) transcript levels, suggesting that upregulation of BDNF expression may be important to the observed effect. Notably, histone hyperacetylation following systemic injection of a single dose of sodium butyrate (1.2 g/kg) was observed in the hippocampus and frontal cortex and explained the superior antidepressant effects of the combined treatment over fluoxetine alone. Chronic inhibition of HDAC by sodium butyrate (daily for four weeks, 1.2 g/kg^{-1}) also improved learning and memory in wild-type mice and mice with brain atrophy[38]. Furthermore, systemic (1.2 g/kg) and intrahippocampal (55 mmol/L) injection of sodium butyrate in mice induced enhanced and persistent extinction of fear[39]. For a more extensive summary of studies on the impact of butyrate administration on brain physiology and function, the reader is directed to the review by Stilling et al.[8].

The dose of butyrate may be critical in determining the effects on behavioural and psychophysiological processes. Intraperitoneal injection of sodium butyrate (100 mg/kg, 10 days) attenuated social deficits in a mouse model for ASD, with no side effects on locomotor and anxiety-related behaviours. The dose normally used to induce HDAC inhibition (1.2 g/kg), on the other hand, did not affect the examined social behaviours[40]. The high dose of butyrate induced global changes in histone acetylation whereas the low dose selectively modified the expression of genes involved in excitatory and inhibitory pathways in the prefrontal cortex. In another study, the dose of 1.2 g/kg of sodium butyrate acted as pharmacological stressor, increasing plasma levels of stress markers corticosterone and adrenocorticotropic hormone (ACTH), as well as glucose[41] whereas a low dose (200 mg/kg) only slightly increased ACTH. As argued by Stilling et al.[8], butyrate is usually administered at supraphysiological concentrations. This entails that, at physiological concentrations (a) butyrate influences the brain through a different mechanism than HDAC inhibition, (b) butyrate still influences the brain through HDAC inhibition, or c) butyrate may not influence the brain. The last possibility is questionable as a physiological oral dose of butyrate impacted brain metabolism

and hippocampal neurogenesis in pigs[42]. Some studies also showed that diet modifies histone acetylation[43] and that SCFA are at least partially responsible for this modification[44]. Together, these findings suggest that SCFA do play a role in diet-induced chromatin changes, but whether these changes also occur at the level of the brain, or reach the brain indirectly (e.g., via expression of genes associated with immune function), remains to be demonstrated.

Other histone modifications including crotonylation, butyrylation, and hydroxybutyrylation have been identified in recent years, but their functional significance remains unclear. Histone crotonylation correlates with gene expression and promotes histone acetylation, but may even play a more direct role in promoting transcription compared to histone acetylation[45]. Moreover, crotonyl-coenzyme A, an intermediate in fermentation of butyrate, increased histone crotonylation and subsequently transcription *in vivo* and *in vitro*[45]. Fellow and colleagues[46] found that histone crotonylation is abundant in both the intestinal epithelium and the brain. Notably, gut microbiota and SCFA were important to histone crotonylation, with butyrate specifically promoting histone crotonylation in intestinal cell and organoid culture[46]. Since SCFA can enter the brain, they may induce CNS histone crotonylation, thereby influencing brain functions[46].

Although studies in mice solely used butyrate, propionate and acetate are also capable of inhibiting HDAC, albeit to a lesser extent[34,47]. In a rat hepatoma cell line, propionate administration resulted in buildup of acetylated forms of histones[47]. Furthermore, using purified calf thymus histone deacetylases, propionate inhibited HDAC *in vitro* to similar extents as butyrate[47]. Acetate increases acetylated histones not only by inhibition of deacetylation but also by stimulating acetylation. Acetate supplementation using a single dose of glyceryl triacetate (6 g/kg) in rats increased acetylation in brain histones, possibly via inhibition of HDAC 2, since increased brain histone acetylases-states temporally coincided with decreases in HDAC 2 protein levels[34]. In contrast, long-term acetate supplementation did not affect total brain HDAC, and exhibited variable effects on class I and II HDAC, but increased histone acetylation by increasing brain histone acetyl transferase (HAT)[34], suggesting that HDAC inhibition may become desensitised in the long-term and other transcription processes may be at play.

In sum, SCFA may influence brain function via interaction with FFARs and/or inhibition of HDAC. Studies exploring SCFA interactions with these cellular systems are, however, lacking in humans. Exploring the dose-response effect of individual SCFA on HDAC inhibition and posttranslational modifications is needed. Specifically, determining whether a given SCFA dose produces global, potentially unfavourable, HDAC inhibition versus more specific HDAC inhibition that modulates a psychological response in the desired direction is critical.

3.2. Impact on gut-brain pathways

3.2.1. Immune pathways

Immune responses and inflammation may be involved in the pathogenesis of psychiatric disorders (FIG. 3)[48,49]. CNS-cytokine interactions influence neural processes, thereby affecting the function of neurocircuits that regulate mood, motor activity, and motivation[50]. Microglia dysregulation was reported in a range of psychiatric disorders including major depression, schizophrenia, ASD, and obsessive-compulsive disorder[51]. Effects of SCFA on mucosal immunity are well documented[52], yet SCFA may also affect the peripheral immune system to modulate brain function. Systemic inflammation may be reduced indirectly by improving the intestinal barrier and preventing translocation of bacteria and bacterial products, or by direct interaction with immune cells, which may in turn reduce neuroinflammation at the level of the brain.

Early studies found that physiological concentrations of butyrate[53,54], but also acetate and propionate[54], enhance intestinal barrier function as indexed by increased transepithelial electrical resistance (TEER) in a Caco-2 cell monolayer model. Butyrate appears to enhance intestinal barrier function by regulating the expression of tight junction proteins, and this effect is mediated by the activation of AMP-activated protein kinase (AMPK)[55]. Another effect of butyrate is downregulation of claudin-2 expression (a cation-selective pore), which may improve barrier function as claudin-2 upregulation partially explains barrier function disturbances[56]. In dextran sulfate sodium-induced colitis in rats, *ex vivo* treatment with butyrate restored TEER[57]. Recently, SCFA mixtures representing compositions produced by fermentation of different dietary fibres improved barrier function and protected against disrupting agents such as LPS and TNF-α in a Caco-2 cell model[58].

By maintaining intestinal barrier integrity, SCFA decrease bacterial translocation[59] into the systemic circulation, which may in turn reduce systemic inflammation.

In addition, SCFA directly regulate a host of immune cells and immune modulators to maintain homeostasis[52]. SCFAs regulate the differentiation, recruitment and activation of immune cells including neutrophils, dendritic cells (DCs), macrophages and monocytes, and T-lymphocytes. Specifically, SCFA modulate the recruitment of neutrophils, effector function, and survival in the affected tissue[60]. SCFA can also modify production of inflammatory cytokines from neutrophils, such as tumor necrosis factor alpha (TNF-α), and regulate growth and function of monocytes, macrophages, and DCs, altering their abilities to capture antigens and produce cytokines, such as interleukin (IL) 10 and IL-12[52,61]. Finally, SCFA are able to modulate adaptive immune responses by direct or indirect modulation of T-lymphocyte differentiation and proliferation, through effects on DCs, promoting the production of IL-10 to downregulate inflammatory response, or via the generation of regulatory T cells (Tregs) and T-helper 1 and 17 cells[62,63]. Multiple mechanisms are involved in the effects of SCFA on immune cells, including their interaction with FFARs present on immune cells[64-68] as well as inhibition of HDAC[61,69].

Since SCFA can reach the bloodstream, they have the potential to modulate immune cell function in the systemic circulation and potentially influence brain and neuronal function. Oral administration of butyrate and propionate promoted peripheral Treg generation[70]. A study by Möhle et al.[71] suggested that Ly6C^{hi} monocytes may be important for hippocampal neurogenesis. Specifically, hippocampal neurogenesis and memory retention were decreased following antibiotic treatment in mice and restored upon reconstitution of a normal microbiota combined with probiotics or physical exercise. These mice had higher numbers of Ly6C^{hi} monocytes in the brain than antibiotic-treated mice. Depletion of these monocytes decreased neurogenesis in the brain whereas adoptive transfer of Ly6C^{hi} monocytes rescued neurogenesis in antibiotic-treated mice, demonstrating that Ly6C^{hi} monocytes are important messengers signaling from the periphery to restore brain homeostasis. Further, SCFA modulate human, but not mouse, monocyte inflammatory responses via activation of FFAR2 and FFAR3, resulting in increased p38 phosphorylation and decreased proinflammatory cytokine expression[72,73]. Thus, these cells can likely be regulated via

microbially-derived SCFA, and subsequently reach the bloodstream and the brain, influencing neural structure and function, and in turn higher-order brain functions.

Human studies on modulation of systemic inflammation by SCFA are scarce and yielded inconsistent results. In a recent systematic review[74], only two of five studies administering SCFA showed significant decreases in serum inflammatory markers. TNF-α levels significantly decreased in hyperinsulinaemic female subjects after intravenous or rectal administration of acetate[75]. In the second study, fasting levels of IL-1β significantly decreased following colonic infusion of a SCFA mixture (40 mmol/200mL)[76]. The studies that failed to demonstrate an effect of SCFA on systemic inflammation administered SCFA for periods between 3 and 20 days[77-79], suggesting that effects of SCFA on pro-inflammatory cytokines may only be observable after acute administration. However, it remains difficult to compare these studies given the small sample sizes (<16 participants), and the heterogeneity of the study populations. Comparatively more studies have investigated the effects of prebiotic and synbiotic (mixtures of prebiotics and probiotics) supplementation on systemic inflammation[74]. Inflammatory markers were decreased in 48% of the prebiotic and 53% of the synbiotic studies, with no effect on inflammation in the remaining studies, and in very few studies even an increase in inflammation. Even though the meta-analysis did not take into account changes in SCFA levels reported in some of the reviewed studies[80], SCFA were suggested to drive the effects on systemic inflammation[74].

Systemic inflammation is highly important in brain immunity, and can modulate neuroinflammation[81,82]. Butyrate decreased lipopolysaccharide (LPS)-induced inflammation in rat primary microglia, hippocampal slice cultures, and co-cultures of rat cerebellar granule neurons, astrocytes and microglial cells[83]. However, in murine N9 transformed microglia cells, butyrate had a pro-inflammatory effect. Recently, the microbiota was found to influence homeostasis, maturation, and function of microglia in the CNS[84]. Specifically, germ-free mice exhibited compromised innate immune responses due to microglia-related defects. When challenged with LPS and lymphocytic choriomeningitis virus, their microglia innate immune response was severely reduced compared to that of specific pathogen-free mice. Furthermore, microbiota depletion via antibiotic administration in specific pathogen-free mice severely compromised microglia homeostasis, resembling that in

germ-free mice[84]. Notably, 4-week oral administration of a SCFA mix in the drinking water of germ-free mice resulted in restored microglial cell morphology and reversed microglial immaturity. In further support of a crucial role of SCFA, *Ffar2−/−* mice continued to exhibit malformed microglia in terms of major alterations of dendrite length, number of segments, branching points, terminal points and increased cell volumes[84].

Prebiotic treatment also altered neuroimmune responses potentially via SCFA. Mice treated with β-galacto-oligosaccharides (BGOS) showed reduced anxiety following LPS-induced inflammation compared to control mice[85]. Furthermore, LPS-induced increases in IL-1β and 5-HT2ARs in the frontal cortex were lower in the BGOS group compared to the control group. These attenuated neuroimmune responses were attributed to prebiotic fermentation into SCFA. For another study[86], see section 4. Modulation of SCFA production.

Taken together, the gut microbiota may impact systemic inflammation and central neuroimmune function, with SCFA being candidate mediators of these effects. SCFA strengthen gut barrier integrity and interact with a host of immune cells, influencing systemic inflammation, and affecting the structural and functional integrity and microglia-related activation involved in neuroinflammation. Prebiotic interventions that show decreased systemic inflammation could benefit from concurrent measurement of plasma SCFA to confirm a potential mediational effect of SCFA on pro-inflammatory and anti-inflammatory markers. Results from mice studies look promising but translation to and replication in humans is lacking and may be challenging.

3.2.2. Endocrine pathways

SCFA may also exert their effects on the gut-brain axis by modulating secretion of gut hormones (FIG. 3). Activation of G-protein coupled receptors by SCFA in the colon stimulates the release of glucagon-like peptide 1 (GLP-1) and peptide YY (PYY) from enteroendocrine L-cells[87-89] which interfere with brain circuits involved in appetite and food intake regulation, either through the systemic circulation[75] or through vagal afferents[90].

GLP-1 is best known as an incretin hormone that enhances glucose-dependent insulin secretion. However, it is also secreted in the nucleus of the solitary tract of the brainstem[91]. GLP-1 can influence brain function via humoral and neural pathways[92]. GLP-1 receptors are widespread

across the body, including the pancreas, intestines, heart, and lungs, as well as the CNS and PNS[93]. GLP-1 may have various effects on brain function. Administration of GLP-1 or a GLP-1 receptor agonist influenced responses to food pictures in reward-related brain regions in obese participants[94], had anxiogenic and antidepressant effects in rats[95], and increased ACTH and cortisol in animals and humans[96]. In mice, GLP-1 improved learning and memory[97,98], neuroprotection and neuroplasticity in the hippocampus[99,100], reduced beta-amyloid (Aβ) plaques, and microglia activation in animal models of Alzheimer's disease (AD)[99].

PYY is another anorexigenic neuropeptide that inhibits gastric motility and reduces appetite. In the human brain, PYY is expressed in the frontal, temporal, and occipital lobes, thalamus, hypothalamus, hippocampus, pons, medulla oblongata and cerebellum, with the highest concentrations found in the hypothalamus and the pituitary[101]. The most common form of circulating PYY is PYY_{3-36}, which preferentially binds to the Y2 neuropeptide Y receptor[102]. Animal studies suggest that the mechanisms by which PYY influences appetite and brain function involve either crossing the blood-brain barrier (BBB)[103] or activation of vagal afferent pathways to the brainstem[104]. PYY influences affective state, but evidence regarding the direction of its effects is contradictory. Knockout of PYY enhanced both depressive- and anxiety-like behaviour in mice[105], whereas in another study, depressive-like but not anxiety-like behaviour was enhanced[106]. Knockout of Y2 receptors enhanced ability to cope with stress and reduced anxiety in mice[107], while stimulating them increased anxiety-like and depressive behaviour[108].

To our knowledge, the extent to which SCFA-induced changes in GLP-1 and PYY mediate brain function is not well investigated. One study found that increased colonic propionate by administration of inulin-propionate ester influenced brain anticipatory reward responses in the caudate and nucleus accumbens during an fMRI food picture evaluation task in non-obese men[109]. In parallel, decreases in subjective appeal of high energy food picture and reduced energy intake during an *ad libitum* meal was also observed[109]. However, no changes in PYY and GLP-1 were observed. Using the same propionate-delivery method, however, acute supplementation increased plasma PYY and GLP-1 levels, but long-term supplementation did not[89], suggesting that gut hormones may be differently involved in the SCFA-brain effects in the long- versus short-term. The

studies cited regarding the direct effect of PYY and GLP-1 on the brain also render it difficult to judge whether injection of GLP-1 and PYY induces similar psychological changes as when produced by the enteroendocrine L-cells. In addition, evidence is conflicting as to whether (SCFA-prompted) GLP-1 and PYY production attenuates or enhances stress, anxiety, and depressive-like behaviour.

Other hormones that influence brain function and are affected by SCFA include leptin, ghrelin, and insulin. However, they have been studied less extensively than PYY and GLP-1. Leptin is an anorexigenic hormone mainly known for its regulatory role of energy balance by activating hypothalamic receptors. All SCFA appear to regulate leptin production but the directionality and mechanisms are not clear[110]. Acetate and propionate increased leptin expression in adipocytes[26,111-113]. Whereas one study[114] found that butyrate had no effect on leptin secretion, another found that fasting leptin levels were significantly reduced following chronic supplementation of all SCFA including butyrate[115]. Furthermore, while some findings implicated the involvement of FFAR2 in SCFA-induced leptin regulation[111,113,114], others suggest that FFAR3 is involved[26,116], yet others found no effect of SCFA nor expression of FFARs in relation to leptin[117]. The BBB and the vagus nerve have been implicated in the effect of leptin on the brain, although transport- and receptor-related mechanisms are not clear[118-122]. Leptin signaling also influences non-hypothalamic areas such as the cortex and the hippocampus, thereby modulating a range of brain functions including reward, motivation, cognition, as well as brain structure, neuronal and synaptic function, and plasticity[123]. Disruption in leptin signaling has been associated with AD, depression, bipolar disorder, and schizophrenia[124].

Ghrelin, the main orexigenic hormone, is produced by ghrelin cells which are mainly present in the stomach and duodenum, and functions as a neuropeptide in the CNS. Ghrelin influences the brain through the vagus nerve[125] or by crossing the blood brain barrier[126] and acts on the hypothalamus to increase hunger and prepare the body for food intake[127]. Plasma ghrelin concentrations decreased after injection of SCFA in wethers[128], independent of circulating glucose and insulin concentrations. Furthermore, ingestion of inulin increased serum SCFA and reduced ghrelin in lean and obese participants[129,130]. The mechanisms that mediate this inverse relationship

between SCFA and ghrelin are not clear. However, ghrelin modulates a number of brain functions. Specifically, intravenous administration of ghrelin activated brain regions (including the amygdala and the orbitofrontal cortex) implicated in reward and incentive value of food cues[131], suggesting modulation of hedonic, as opposed to solely homeostatic, responses to food. In mice, ghrelin modulated neuronal and synaptic function in the hippocampus, which were paralleled by enhanced learning and memory[132,133]. Furthermore, ghrelin modulated stress, depression, and anxiety via the Hypothalamo-Pituitary-Adrenal (HPA) axis, the serotonergic system, and the sympathetic nervous system[134].

Insulin is a hormone produced by the pancreas that allows the body to maintain blood sugar levels, preventing hyperglycemia and hypoglycemia[135]. Earlier work found that infusion of propionate and butyrate, but not acetate, in sheep increased plasma insulin levels 4- and 14- fold, respectively, without affecting plasma glucose. These effects were also found in cows, but not in non-ruminant species (rats, rabbit, or pigs)[136,137]. In humans, supplementation of resistant starch (30g/d for 4 weeks) increased both SCFA and systemic and muscle insulin sensitivity despite lower insulin levels[138]. Insulin is also a CNS regulatory peptide, and peripheral insulin is taken up by the brain but the mechanisms remains a matter of debate[139]. Insulin may influence the brain from the periphery, following intravenous administration, or centrally, following intranasal and intracereboventricular routes to the CNS[139] or intracranial or intrathecal transplantation of pancreatic islets[140]. A large body of literature suggests that insulin availability and/or alterations in insulin receptor sensitivity/availability is relevant to brain function. This has been shown in relation to cognitive function and mood in AD[141-144], bipolar disorder[145] but not in major depressive disorder[146]. However, a neural circuit involving the ventral striatum, insula, and anterior mid-cingulate cortex linked higher insulin resistance with depressed mood in healthy humans[147]. Intranasal administration of insulin to a healthy population improved learning and memory, as well as responses to psychosocial stress[148,149].

More research is needed to discern whether there is a direct effect of SCFA on leptin, ghrelin, and insulin and the mechanisms underpinning these effects. Surprisingly, chronic intragastric acetate infusion doubled acetate turnover in the brain under high-fat diet in comparison

to chow diet in mice and increased insulin and ghrelin secretion, caloric intake, and weight gain. These effects were prevented by vagotomy, suggesting that the effect of chronic acetate is mediated by parasympathetic activation[150]. Whether SCFA-prompted release of these hormones would have positive or negative effects on the brain and its psychobiological functions is difficult to predict. Nonetheless, the studies cited above suggests that (a) SCFA increase the production of some hormones in the GI tract and (b) these hormones influence mood and cognition. Therefore, investigating whether these GI hormones may be a mechanism through which SCFA may impact on psychological functioning would be a fruitful endeavor.

3.2.3. Vagal pathways

The vagus nerve contains 80% afferent and 20% efferent fibres and innervates almost all the digestive tract. Vagal afferents are not in direct contact with the gut luminal microbiota but can indirectly sense luminal signals, through diffusion of bacterial compounds or metabolites[151], such as serotonin and gut hormones released from the enteroendocrine cells[152,153]. Electric stimulation of vagal afferents modulates neurotransmitter levels in the brain[154] and is a last-resort for treatment of epilepsy and depression[155]. Bacteria can also stimulate vagal afferents, modulating brain neurotransmitter gene expression and related stress-, anxiety-, and depressive-like behaviours[156]. SCFA are able to directly activate vagal afferents. Luminal perfusion of sodium butyrate (10 mM) in the jejunum of anaesthetised male rats evoked vagal afferent nerve discharge that was abolished following subdiaphragmatic vagotomy[157]. Importantly, butyrate likely acted directly on vagal afferent terminals, at least independent of CCK-A receptors present on vagal afferents. More recently, intraperitoneal injection of acetate, propionate, and butyrate (separately, each at 6 mmol/kg) suppressed food intake in mice in the order of butyrate>propionate>>acetate. This effect was attenuated following desensitisation of vagal afferents by systemic capsaicin treatment and via hepatic branch vagotomy[158]. Finally, butyrate directly interacted with single neurons isolated from the nodose ganglion and increased $Ca^{2}+$[158]. Since FFAR3 were expressed in nodose ganglion neurons[31], the effect of SCFA on appetite suppression via the vagus nerve may be further mediated

by the presence of FFAR3[151]. It would be interesting to investigate whether the vagus nerve mediates SCFA effects on other higher-order brain functions.

3.2.3. Other direct humoral pathways

An intact BBB is critical to brain development and preservation of CNS homeostasis, as it ensures a controlled passage of molecules and nutrients from the circulation to the brain (FIG. 3). SCFA crossed the BBB in a cell-culture model[159] possibly due to the abundant expressions of MCTs[160,161]. Brain uptake of SCFA in rats is in the order of butyrate>propionate>>acetate[162]. In human brain tissues, concentrations of 17.0 pmol/mg of brain tissue for butyrate and 18.8 pmol/mg for propionate have been reported[163]. Using positron emission tomography (PET) imaging, it was shown that approximately 3% of intravenously infused ^{11}C-acetate was immediately taken up in rat brain, whereas approximately 2% was taken up 20 minutes following colonic infusion[164]. However, brain uptake of ^{11}C-labelled butyrate in primates was limited to only 0,006% of injected dose/ml[165]. In human PET studies, no measurable brain uptake of ^{11}C-acetate was detected[166,167] up to 76 min after injection[167]. In sum, brain uptake of SCFA appears to be minimal.

Compared to normal mice, germ-free mice have increased BBB permeability and butyrate treatmented decreases BBB permeability to a level similar to that of pathogen-free mice[168]. Propionate also protected BBB integrity, as exposure of human cerebromicrovascular endothelial cell line (hCMEC/D3) to 1 µM propionate for 24h lead to the inhibition of a number of pathways associated with non-specific inflammatory responses to microbial inflections *in vitro*[169]. For example, exposure of hCMEC/D3 monolayers for 12h to physiological concentration of propionate (1µM) and butyrate (1µM), but not acetate (65µM) attenuated the permeablising effect of LPS derived from *Escherichia coli*. Propionate also protected the BBB from oxidative stress via NFE2L2 signalling and protected against LPS-induced disruptions in tight junction proteins occludin, claudin-5 and zona occludens-1. Given the low brain and systemic SCFA concentrations[16], it is unlikely that colonic SCFA affect brain function through uptake in the brain, but the limited evidence in mice does not completely rule out this possibility.

There is some evidence that SCFA that cross into the CNS have neuroactive properties. In mice, acetate taken up through the BBB altered the level of the neurotransmitters glutamate, glutamine, and gamma-aminobutyric acid (GABA) in the hypothalamus, and increased anorexigenic neuropeptide expression[164]. Propionic acid induced the expression of tryptophan 5-hydoxylase 1, the enzyme involved in synthesis of serotonin in PC12 cells, a pheochromocytoma cell line. Furthermore, propionate and butyrate also induced tyrosine hydroxylase gene transcription[170]. Tyrosine hydroxylase facilitates the conversion of L-tyrosine to L-3,4-dihydroxyphenylalanine (L-DOPA), which is the rate-limiting step in the biosynthesis of dopamine, noradrenaline and adrenaline[171].

The mechanisms by which dietary fibre and butyrate affect the brain may involve neurotrophic factors, such as nerve growth factor (NGF), BDNF, and glial cell line-derived neurotrophic factor (GDNF). These are small proteins that regulate the growth, survival, and differentiation of neurons and synapses in the CNS and PNS[172], playing important roles in learning, memory, and a range of brain disorders. Fructo-oligosaccharides (FOS) and BGOS feeding in rats elevated hippocampal BDNF and N-methyl-d-aspartate receptors[173]. These effects may be mediated by butyrate as butyrate injection enhanced levels of neurotrophic factors alongside positive effects on mood-related behaviours[174,175]. Effects of butyrate on neurotrophic factors also benefit learning and memory. Upregulation of hippocampal BDNF, following exercise and intraperitoneal administration of butyrate, was necessary to transform a weak learning event to long-term memory in sedentary mice[176]. Similarly, butyrate prevented memory impairment and reversed levels of hippocampal BDNF and GDNF in a mouse model of pneumococcal meningitis[177]. Finally, effects of butyrate on neurotrophic factors also support neurogenesis and neuroprotection[178,179].

Serotonin biosynthesis constitutes another humoral gut-brain communication pathway that may be impacted by SCFA. Serotonin (5-hydroxytryptamine; 5-HT) is derived from tryptophan and functions as a neurotransmitter in the CNS and in the periphery. More than 90% of the body's 5-HT is synthesised in the enterochromaffin cells in the GI tract where it regulates diverse GI functions[180]. The remainder is synthesised in the CNS in the raphe nuclei located in the brainstem, the ascending projections from which are involved in regulation of mood, appetite, memory, learning, and sleep[181].

Intraluminal administration of physiological concentrations of SCFA into the proximal colon stimulated the release of 5-HT from enterochromaffin cells (ECs)[182]. Reigstad et al.[183] found that acetate and butyrate promoted tryptophan hydroxylase 1 (the rate limiting enzyme for mucosal 5-HT synthesis) transcription in a human-derived EC cell model, suggesting that SCFA are crucially involved in enteric 5-HT production and homeostasis. Similarly, Yano et al.[184] found that specific microbial metabolites, including the SCFA butyrate and propionate, play a critical role in promoting host 5-HT biosynthesis, regulating both colon and serum levels of 5-HT. Taken together, these findings suggest that SCFA may modulate the peripheral levels of serotonin, which may in turn regulate brain function by influencing the immune system[185], fetal brain development[186,187], or signalling to the brain via 5-HT receptors on vagal afferent fibres[188].

In sum, SCFA may directly influence the brain by crossing the BBB, reinforcing BBB integrity, modulating neurotransmission, influencing levels of neurotrophic factors, and promoting serotonin biosynthesis. Due to the invasive nature of these studies, research into the direct humoral effects of SCFA on brain function is limited to *in vitro* and animal studies. Importantly, it remains to be established whether alterations in microbiota-derived SCFAs exert similar effects on the BBB, neurotransmission, and neurotrophins, and whether microbiota-derived SCFAs reach physiologically relevant concentrations in the CNS in humans.

4. Modulation of SCFA production

SCFA production can be modulated by manipulating the intestinal microbiota via the ingestion of live beneficial bacteria, known as probiotics[189, 190]. An alternative strategy is the intake of prebiotics which are defined as "a substrate that is selectively utilised by host microorganisms conferring a health benefit"[191]. Prebiotics act as a substrate for beneficial bacteria in the colon, which in turn ferment them into SCFA. Finally, habitual diet significantly influences gut health and its microbial composition. As an example, increased consumption of plant foodstuffs, as in a Mediterranean diet, increases the availability of fermentable substrates for advantageous bacteria, thereby modulating SCFA formation. Ample evidence exists that interventions with prebiotics[12,192-195] and probiotics[196,197] or adherence to the Mediterranean diet[198-200] increases large intestinal SCFA

production. On the other hand, the number of studies, in animals and humans, demonstrating positive effects of prebiotics, probiotics and adherence to a Mediterranean diet on psychological functioning is increasing. Endpoints include depressive-like and anxiety-like behaviour, stress-related behaviour, and cognitive functioning. An overview of these studies is provided in Supplementary Information (Supplementary Tables 1 and 2). Unfortunately, less than a handful of studies on psychological impact of these dietary interventions quantified SCFA. In mice, administration of *Clostridium Butyricum* increased butyrate levels in faeces and in the brain in a model of vascular dementia, showed significant attenuation of cognitive impairment and histopathological changes in mice hippocampus, and activated BDNF-PI3K/Akt Pathway-related proteins[201]. A 3-week supplementation of FOS and GOS attenuated stress-induced corticosterone release, reduced pro-inflammatory cytokine levels, modified gene expression in hippocampus and hypothalamus, increased cecal acetate and proprionate, and reduced cecal isobutyrate concentration. Importantly, changes in cecal SCFA levels correlated with reduced depression- and anxiety-like behaviours[86].

5. SCFA, psychological functioning, and neuropsychiatric disorders

Despite the knowledge of multiple pathways that support a potential key role of SCFA in MGB axis signaling, studies linking SCFA directly with one or more aspects of psychological functioning are sparse, heterogeneous in terms of design and study population, and yield conflicting results. Notwithstanding these limitations, SCFA have been implicated in a range of neuropsychiatric disorders. First, there is controversy over the role of SCFA in Parkinson's disease (PD). PD patients have lower abundance of SCFA-producing bacteria compared to healthy controls[202-205], and exhibit lower faecal SCFA concentrations than age-matched healthy controls[206], suggesting that those patients could benefit from SCFA supplementation. Butyrate administration in animal models of Parkinson's disease improved motor impairment and dopamine deficiency[207-209]. However, in a similar mouse model, orally-administered SCFA mixtures promoted neuroinflammation and motor deficits in comparison to germ-free or antibiotic-treated mice[210], suggesting that under genetic predisposition, SCFA may exacerbate symptoms associated with PD.

Further, key neuropathological processes underlying AD may also be modulated by SCFA. Butyrate administration recovered memory function and increased expression of genes implicated in associative learning in a mouse model of AD via HDAC inhibition[211]. Valeric, butyric, and propionic acid interfered with protein-protein interactions among Aβ peptides, thereby disrupting Aβ assembly into neurotoxic oligomers; specifically, SCFA attenuated conversion of monomeric Aβ1-40 and Aβ1-42 into Aβ fibrils *in vitro*[212].

The role of SCFA in ASD is also controversial. Children with ASD have been reported with both lower[213] and higher[214] faecal SCFA levels than controls. However, altered faecal SCFA levels in children with ASD may have various causes such as variability in fibre intake, changes in microbiota composition, or variability in gut transit time[213]. Animal studies have shown higher levels of butyrate in the cecum of offspring exposed to valproic acid (murine model of ASD)[215] but another study demonstrated that treatment with butyrate attenuated social behaviour deficits via modulation of GABA signaling and modification of inhibitory and/or excitatory gene transcription in the frontal cortex of a mouse model of ASD[40]. Finally, the extensive work of MacFabe and colleagues[216] suggested that treating rodents with SCFA, predominantly propionate, via various methods of administration (intracerebroventricular, intraperitoneal, subcutaneous, and oral) induces behavioural and brain alterations consistent with those exhibited in patients with ASD[216].

With respect to affective symptomatology, faecal SCFA concentrations were lower in depressed patients compared to controls in one study[217] but not in another[218]. Moreover, faecal microbiota transplantation from depressed patients to microbiota-depleted rats transmitted the anxiety-like behaviour, yet resulted in higher faecal SCFA concentrations than in rats receiving donor feces from healthy controls[218]. In another study, faecal butyrate unexpectedly correlated with reported emotional problems in children[219]. In animal models of mania, sodium butyrate reversed behavioural hyperactivity and restored hypoactivity of mitochondrial respiratory-chain complexes in the prefrontal cortex, hippocampus, striatum, and amygdala[220], as well as reversed depressive-like and manic-like behaviours in rats[221].

In relation to addiction-related behaviour, administration of SCFA normalised reward responses to cocaine in mice with antibiotic-induced depletion of gut microbiota[222].

In addition to the putative direct influences outlined above, the role of SCFA in neuropsychiatric disorders may also be indirect, such that instead of alleviating (or exacerbating) symptoms *per se*, they may alleviate (or exacerbate) symptoms of comorbid physical or psychological symptoms, thereby indirectly reducing (or enhancing) severity of the primary neuropsychiatric illness. For instance, enriching SCFA in patients with schizophrenia via Mediterranean diet-based interventions has been suggested to improve immune and cardiovascular outcomes associated with increased mortality in these patients[223].

A couple of functional brain imaging studies have been conducted looking at the effects of SCFA in relation to food reward, cognitive function and brain functional connectivity. See section 3.2.2. Endocrine pathways for one propionate-fMRI study on food reward[109]. In another study, high fat diet supplemented with 5% of butyrate for two months restored metabolic adaptions, impairments in spatial memory, systolic blood pressure and cerebral blood flow, functional connectivity, and neuroinflammation (decreased number of activated microglia in hippocampus and thalamus, but no changes in TNF-α, IL-6, or IL-1β) in mice. Finally, butyrate-related changes in microbiota correlated with changes in neuroinflammation[224].

5.1. Limitations and future directions

The above findings suggest that SCFA may indeed play a role in a range of neurological and neuropsychiatric conditions, as well as psychological functioning in general. However, it is premature to conclude whether their effect is favourable or unfavourable. A major commonality across these studies is the quantification of faecal SCFA in both observational and experimental studies. Faecal SCFA, however, provide information on the resultant non-absorbed SCFA, but do not reflect *in situ* production rates, absorption, and interaction with other biologically relevant molecules or cell types[195]. This hampers our ability to understand the influence of SCFA on (psycho)biological functions. This gap in our understanding stems, presumably, from (a) the inability to sample the intestinal content, (b) the extensive extraction and metabolism of SCFA in the splanchnic area (small intestine, liver and colon) resulting in low plasma SCFA concentration, and (c) analytical challenges in quantifying low plasma SCFA levels[225]. Knowledge of the systemic availability – that is, the fraction of the administered dose of unchanged compound that reaches the

systemic circulation – of SCFA is critical to understanding their full biological relevance, and to decipher the extent to which systemic SCFA mediate gut-brain communication through the MGB axis. Recently, our group[16] quantified the systemic availability and metabolism of colonically-produced SCFA in healthy subjects using stable isotope technology. Known amounts of either ^{13}C-labelled acetate, propionate, or butyrate were directly administered to the colon using colon delivery capsules after which the concentrations of ^{13}C-labelled SCFA were quantified in plasma. The use of stable isotopes allowed for selective and sensitive quantification of the SCFA originating from the colon. On average 36% of the administered acetate, 9% of the administered propionate, and 2% of the administered butyrate were recovered in the systemic circulation.

Nevertheless, quantification of circulating SCFA only will not solve discrepancies between animal and human literature, nor within-species discrepancies, specifically on the role of SCFA in neuropsychiatric disorders. Animal models of psychiatric disorders in MGB axis research must grapple with two major criteria, (a) adequately model the human microbiome and GI tract and (b) recapitulate the symptom or phenotype of interest. With respect to (a), it should be emphasised that the overwhelming majority of cited research in this article is based on rodent models. Important discrepancies between human and rodent (especially mouse) microbiota may render translation to humans highly challenging[226]. First, the dominant bacterial genera and their relative abundance in mouse and man are clearly different. Second, the distinct GI tract anatomy between mouse and human (e.g., large relative size of cecum) suggests that it may not adequately mirror metabolic interactions, such as microbiota composition across the GI tract, fibre fermentation, and metabolism and absorption of SCFA, in the human GI tract[226]. Better models for animal and human GI tract and gut microbiota are the pig[227] and the chimpanzee[228], yet practical and ethical considerations restrict their use in MGB axis studies.

With respect to (b), it is well established that modeling complex human psychiatric disorders in animals is extremely challenging, and some argue it cannot mirror the multifaceted nature of any given psychiatric disorder[229]. Inherent limitations lie in using Diagnostic and Statistical Manual of Mental Disorders (DSM) criteria to construct animal models, as in humans, multiple symptom combinations can result in the same diagnosis of depressive disorder. In addition, the boundaries

between multiple psychiatric disorders, as well as between normality and disorder are quite arbitrary in humans[229]. Moreover, phenomenology of certain symptoms such as sadness and guilt in animals are impossible to validate. Finally, a pathophysiology-based animal model of a neuropsychiatric disorder usually consists of a single mutation that may associate with multiple disorders, as was the case with the DISC1 mutation that resulted in major depressive disorder, bipolar disorder, and schizophrenia in one family[230]. While animal models are invaluable for studying highly specified molecular, cellular, genetic, or neural abnormalities in a given neuropsychiatric disorder, no model may be capable of sufficiently recapitulating etiological factors, developmental processes, and temporal dynamics that rendered a human a psychiatric patient. Currently, it is advised to regard animal models of psychiatric disorders as endophenotype/symptom-based models and consider them a "biological system representing a *distinct* pathological process, but not a *nosological entity*"[231]. That is, to refrain from modelling all interdependent dimensions of a disorder and instead restrict the model to a single symptom, provided that its various dimensions/endophenotypes are adequately distinct and separable[231,232].

Taken together, animal studies are instrumental in advancing our understanding of the neurobiological underpinnings of psychiatric disorders and have unquestionably permitted the exploration of molecular mechanisms that are impossible, or at best unethical, to reveal in humans. Future animal studies on the role of SCFA in MGB axis should therefore draw on models that adequately mirror the GI tract and gut microbiome and focus on a single endophenotype of a given psychiatric disorder. Difficulty in satisfying these conditions may, for instance, partially account for failing to translate the effect of *Lactobacillus rhamnosus* (JB-1) on emotional behaviour from animals[156] to humans[233]. True significance of the findings in animal models is their translatability in humans, thus advancing methodologies for studying the role of SCFA in the MGB axis in humans is needed. As reviewed, SCFA can be directly administered to humans orally, intravenously, colonically (via an enema), or by using an esterified fibre to facilitate colonic SCFA delivery[89,234]. While no single method may be perfect, findings from such studies would complement animal studies to increase our understanding of the different pathways by which SCFA may influence brain and behaviour. Finally, in humans, multiple factors, such as dietary fibre intake, microbiota

composition, and gut transit time, can contribute to SCFA production, and throughout the GI tract, multiple "stations" can modify absorption rate and incorporation to relevant biological processes. Variability of these mechanisms is larger in humans with GI and neuropsychiatric disorders compared to healthy populations. Thus, drawing solid conclusions on the role of SCFA in MGB axis may be an unsurmountable challenge if knowledge on their role in healthy humans is not systematically researched prior to that in patient populations. A set of recommendations to better address the role of SCFA in the MGB axis is summarised in (Box 1).

6. Conclusion

This paper synthesised a broad literature, supporting a role of SCFA as mediators of MGB interactions. By virtue of their putative effects on brain function via various gut-brain signaling pathways, they may act as a mediator of the effects of probiotics, prebiotics, and dietary interventions on a range of psychological functions. The link between SCFA and psychological functioning is slowly solidifying in animal research, but there is a dearth of human research, and little convergence in human and animal studies. Interventions using direct SCFA administration in humans should therefore be carried out. Prior to making claims on a mechanistic role of SCFA in the MGB axis, information on dose-effect relationships with respect to various psychological functions and the individual contributions of the major SCFA to observe such changes are needed.

7. References

1 Mayer, E. A. Gut feelings: the emerging biology of gut-brain communication. *Nat Rev Neurosci* **12**, 453-466, doi:10.1038/nrn3071 (2011).

2 Cryan, J. F. & Dinan, T. G. Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. *Nat Rev Neurosci* **13**, 701-712, doi:10.1038/nrn3346 (2012).

3 De Palma, G., Collins, S. M., Bercik, P. & Verdu, E. F. The microbiota-gut-brain axis in gastrointestinal disorders: stressed bugs, stressed brain or both? *The Journal of physiology* **592**, 2989-2997, doi:10.1113/jphysiol.2014.273995 (2014).

4 Kleiman, S. C. *et al.* The Intestinal Microbiota in Acute Anorexia Nervosa and During Renourishment: Relationship to Depression, Anxiety, and Eating Disorder Psychopathology. *Psychosom Med* **77**, 969-981, doi:10.1097/PSY.0000000000000247 (2015).

5 Kang, D. W. *et al.* Reduced incidence of Prevotella and other fermenters in intestinal microflora of autistic children. *PLoS One* **8**, e68322, doi:10.1371/journal.pone.0068322 (2013).

6 Jiang, H. *et al.* Altered fecal microbiota composition in patients with major depressive disorder. *Brain Behav Immun* **48**, 186-194, doi:10.1016/j.bbi.2015.03.016 (2015).

7 Liu, X., Cao, S. & Zhang, X. Modulation of gut microbiota–brain axis by probiotics, prebiotics, and diet. *Journal of agricultural and food chemistry* **63**, 7885-7895 (2015).

8 Stilling, R. M. *et al.* The neuropharmacology of butyrate: The bread and butter of the microbiota-gut-brain axis? *Neurochemistry International* **99**, 110-132, doi:https://doi.org/10.1016/j.neuint.2016.06.011 (2016).

9 Clarke, G. *et al.* Minireview: Gut Microbiota: The Neglected Endocrine Organ. *Molecular Endocrinology* **28**, 1221-1238, doi:10.1210/me.2014-1108 (2014).

10 Miller, T. L. & Wolin, M. J. Pathways of acetate, propionate, and butyrate formation by the human fecal microbial flora. *Appl Environ Microbiol* **62**, 1589-1592 (1996).

11 den Besten, G. *et al.* Gut-derived short-chain fatty acids are vividly assimilated into host carbohydrates and lipids. *Am J Physiol Gastrointest Liver Physiol* **305**, G900-910, doi:10.1152/ajpgi.00265.2013 (2013).

12 Cummings, J. H., Pomare, E. W., Branch, W. J., Naylor, C. P. & Macfarlane, G. T. Short chain fatty acids in human large intestine, portal, hepatic and venous blood. *Gut* **28**, 1221-1227 (1987).

13 Macfarlane, S. & Macfarlane, G. T. Regulation of short-chain fatty acid production. *Proc Nutr Soc* **62**, 67-72, doi:10.1079/PNS2002207 (2003).

14 Stumpff, F. A look at the smelly side of physiology: transport of short chain fatty acids. *Pflugers Archiv : European journal of physiology* **470**, 571-598, doi:10.1007/s00424-017-2105-9 (2018).

15 Bloemen, J. G. *et al.* Short chain fatty acids exchange across the gut and liver in humans measured at surgery. *Clin Nutr* **28**, 657-661, doi:10.1016/j.clnu.2009.05.011 (2009).

16 Boets, E. *et al.* Systemic availability and metabolism of colonic-derived short-chain fatty acids in healthy subjects: a stable isotope study. *The Journal of physiology* **595**, 541-555, doi:10.1113/JP272613 (2017).

17 Hellman, L., Rosenfeld, R. S. & Gallagher, T. F. Cholesterol synthesis from C14-acetate in man. *J Clin Invest* **33**, 142-149, doi:10.1172/JCI102881 (1954).

18 Hellerstein, M. K. *et al.* Measurement of de novo hepatic lipogenesis in humans using stable isotopes. *J Clin Invest* **87**, 1841-1852, doi:10.1172/JCI115206 (1991).

19 Wiltrout, D. W. & Satter, L. D. Contribution of propionate to glucose synthesis in the lactating and nonlactating cow. *J Dairy Sci* **55**, 307-317, doi:10.3168/jds.S0022-0302(72)85487-0 (1972).

20 Layden, B. T., Angueira, A. R., Brodsky, M., Durai, V. & Lowe, W. L., Jr. Short chain fatty acids and their receptors: new metabolic targets. *Transl Res* **161**, 131-140, doi:10.1016/j.trsl.2012.10.007 (2013).

21 Yamashita, H., Kaneyuki, T. & Tagawa, K. Production of acetate in the liver and its utilization in peripheral tissues. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids* **1532**, 79-87, doi:https://doi.org/10.1016/S1388-1981(01)00117-2 (2001).

22 Bell-Parikh, L. C. & Guengerich, F. P. Kinetics of cytochrome P450 2E1-catalyzed oxidation of ethanol to acetic acid via acetaldehyde. *The Journal of biological chemistry* **274**, 23833-23840 (1999).

23 Bugaut, M. Occurrence, absorption and metabolism of short chain fatty acids in the digestive tract of mammals. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry* **86**, 439-472, doi:https://doi.org/10.1016/0305-0491(87)90433-0 (1987).

24 Karaki, S.-i. *et al.* Short-chain fatty acid receptor, GPR43, is expressed by enteroendocrine cells and mucosal mast cells in rat intestine. *Cell and tissue research* **324**, 353-360 (2006).

25 Tazoe, H. *et al.* Expression of short-chain fatty acid receptor GPR41 in the human colon. *Biomedical Research* **30**, 149-156 (2009).

26 Xiong, Y. *et al.* Short-chain fatty acids stimulate leptin production in adipocytes through the G protein-coupled receptor GPR41. *Proceedings of the National Academy of Sciences of the United States of America* **101**, 1045-1050 (2004).

27 Thangaraju, M. *et al.* GPR109A is a G-protein-coupled receptor for the bacterial fermentation product butyrate and functions as a tumor suppressor in colon. *Cancer research* **69**, 2826-2832, doi:10.1158/0008-5472.CAN-08-4466 (2009).

28 Ahmed, K., Tunaru, S. & Offermanns, S. GPR109A, GPR109B and GPR81, a family of hydroxy-carboxylic acid receptors. *Trends Pharmacol Sci* **30**, 557-562, doi:10.1016/j.tips.2009.09.001 (2009).

29 Bonini, J. A., Anderson, S. M. & Steiner, D. F. Molecular cloning and tissue expression of a novel orphan G protein-coupled receptor from rat lung. *Biochem Biophys Res Commun* **234**, 190-193, doi:10.1006/bbrc.1997.6591 (1997).

30 Kimura, I. *et al.* Short-chain fatty acids and ketones directly regulate sympathetic nervous system via G protein-coupled receptor 41 (GPR41). *Proc Natl Acad Sci U S A* **108**, 8030-8035, doi:10.1073/pnas.1016088108 (2011).

31 Nohr, M. K. *et al.* Expression of the short chain fatty acid receptor GPR41/FFAR3 in autonomic and somatic sensory ganglia. *Neuroscience* **290**, 126-137, doi:10.1016/j.neuroscience.2015.01.040 (2015).

32 De Vadder, F. *et al.* Microbiota-generated metabolites promote metabolic benefits via gut-brain neural circuits. *Cell* **156**, 84-96, doi:10.1016/j.cell.2013.12.016 (2014).

33 Waldecker, M., Kautenburger, T., Daumann, H., Busch, C. & Schrenk, D. Inhibition of histone-deacetylase activity by short-chain fatty acids and some polyphenol metabolites formed in the colon. *J Nutr Biochem* **19**, 587-593, doi:10.1016/j.jnutbio.2007.08.002 (2008).

34 Soliman, M. L. & Rosenberger, T. A. Acetate supplementation increases brain histone acetylation and inhibits histone deacetylase activity and expression. *Molecular and cellular biochemistry* **352**, 173-180, doi:10.1007/s11010-011-0751-3 (2011).

35 Volmar, C.-H. & Wahlestedt, C. Histone deacetylases (HDACs) and brain function. *Neuroepigenetics* **1**, 20-27, doi:https://doi.org/10.1016/j.nepig.2014.10.002 (2015).

36 Whittle, N. & Singewald, N. HDAC inhibitors as cognitive enhancers in fear, anxiety and trauma therapy: where do we stand? *Biochemical Society transactions* **42**, 569-581, doi:10.1042/bst20130233 (2014).

37 Schroeder, F. A., Lin, C. L., Crusio, W. E. & Akbarian, S. Antidepressant-like effects of the histone deacetylase inhibitor, sodium butyrate, in the mouse. *Biological psychiatry* **62**, 55-64, doi:10.1016/j.biopsych.2006.06.036 (2007).

38 Fischer, A., Sananbenesi, F., Wang, X., Dobbin, M. & Tsai, L. H. Recovery of learning and memory is associated with chromatin remodelling. *Nature* **447**, 178-182, doi:10.1038/nature05772 (2007).

39 Stafford, J. M., Raybuck, J. D., Ryabinin, A. E. & Lattal, K. M. Increasing histone acetylation in the hippocampus-infralimbic network enhances fear extinction. *Biological psychiatry* **72**, 25-33, doi:10.1016/j.biopsych.2011.12.012 (2012).

40 Kratsman, N., Getselter, D. & Elliott, E. Sodium butyrate attenuates social behavior deficits and modifies the transcription of inhibitory/excitatory genes in the frontal cortex of an autism model. *Neuropharmacology* **102**, 136-145, doi:10.1016/j.neuropharm.2015.11.003 (2016).

41 Gagliano, H., Delgado-Morales, R., Sanz-Garcia, A. & Armario, A. High doses of the histone deacetylase inhibitor sodium butyrate trigger a stress-like response. *Neuropharmacology* **79**, 75-82, doi:10.1016/j.neuropharm.2013.10.031 (2014).

42 Val-Laillet, D. *et al.* Oral sodium butyrate impacts brain metabolism and hippocampal neurogenesis, with limited effects on gut anatomy and function in pigs. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* **32**, 2160-2171, doi:10.1096/fj.201700547RR (2018).

43 Carrer, A. *et al.* Impact of a High-fat Diet on Tissue Acyl-CoA and Histone Acetylation Levels. *The Journal of biological chemistry* **292**, 3312-3322, doi:10.1074/jbc.M116.750620 (2017).

44 Krautkramer, K. A. *et al.* Diet-Microbiota Interactions Mediate Global Epigenetic Programming in Multiple Host Tissues. *Molecular cell* **64**, 982-992, doi:10.1016/j.molcel.2016.10.025 (2016).

45 Sabari, B. R. *et al.* Intracellular crotonyl-CoA stimulates transcription through p300-catalyzed histone crotonylation. *Molecular cell* **58**, 203-215, doi:10.1016/j.molcel.2015.02.029 (2015).

46 Fellows, R. *et al.* Microbiota derived short chain fatty acids promote histone crotonylation in the colon through histone deacetylases. *Nat Commun* **9**, 105, doi:10.1038/s41467-017-02651-5 (2018).

47 Cousens, L. S., Gallwitz, D. & Alberts, B. M. Different accessibilities in chromatin to histone acetylase. *Journal of Biological Chemistry* **254**, 1716-1723 (1979).

48 Miller, A. H. & Raison, C. L. The role of inflammation in depression: from evolutionary imperative to modern treatment target. *Nat Rev Immunol* **16**, 22-34, doi:10.1038/nri.2015.5 (2016).

49 Segerstrom, S. C. & Miller, G. E. Psychological stress and the human immune system: a meta-analytic study of 30 years of inquiry. *Psychological bulletin* **130**, 601 (2004).

50 Capuron, L. & Miller, A. H. Immune system to brain signaling: neuropsychopharmacological implications. *Pharmacology & therapeutics* **130**, 226-238 (2011).

51 Frick, L. R., Williams, K. & Pittenger, C. Microglial dysregulation in psychiatric disease. *Clinical and Developmental Immunology* **2013** (2013).

52 Correa-Oliveira, R., Fachi, J. L., Vieira, A., Sato, F. T. & Vinolo, M. A. Regulation of immune cell function by short-chain fatty acids. *Clin Transl Immunology* **5**, e73, doi:10.1038/cti.2016.17 (2016).

53 Peng, L., He, Z., Chen, W., Holzman, I. R. & Lin, J. Effects of butyrate on intestinal barrier function in a Caco-2 cell monolayer model of intestinal barrier. *Pediatric research* **61**, 37-41, doi:10.1203/01.pdr.0000250014.92242.f3 (2007).

54 Mariadason, J. M., Barkla, D. H. & Gibson, P. R. Effect of short-chain fatty acids on paracellular permeability in Caco-2 intestinal epithelium model. *American Journal of Physiology-Gastrointestinal and Liver Physiology* **272**, G705-G712 (1997).

55 Peng, L., Li, Z. R., Green, R. S., Holzman, I. R. & Lin, J. Butyrate enhances the intestinal barrier by facilitating tight junction assembly via activation of AMP-activated protein kinase in Caco-2 cell monolayers. *J Nutr* **139**, 1619-1625, doi:10.3945/jn.109.104638 (2009).

56 Daly, K. & Shirazi-Beechey, P. S. P. Microarray Analysis of Butyrate Regulated Genes in Colonic Epithelial Cells. *DNA and Cell Biology* **25**, 49-62, doi:10.1089/dna.2006.25.49 (2006).

57 Venkatraman, A., Ramakrishna, B. S., Pulimood, A. B., Patra, S. & Murthy, S. Increased permeability in dextran sulphate colitis in rats: time course of development and effect of butyrate. *Scandinavian journal of gastroenterology* **35**, 1053-1059 (2000).

58 Chen, T. *et al.* Dietary fibre-based SCFA mixtures promote both protection and repair of intestinal epithelial barrier function in a Caco-2 cell model. *Food Funct* **8**, 1166-1173, doi:10.1039/c6fo01532h (2017).
59 Lewis, K. *et al.* Enhanced translocation of bacteria across metabolically stressed epithelia is reduced by butyrate. *Inflammatory bowel diseases* **16**, 1138-1148, doi:10.1002/ibd.21177 (2010).
60 Rodrigues, H. G., Takeo Sato, F., Curi, R. & Vinolo, M. A. R. Fatty acids as modulators of neutrophil recruitment, function and survival. *European journal of pharmacology* **785**, 50-58, doi:https://doi.org/10.1016/j.ejphar.2015.03.098 (2016).
61 Chang, P. V., Hao, L., Offermanns, S. & Medzhitov, R. The microbial metabolite butyrate regulates intestinal macrophage function via histone deacetylase inhibition. *Proceedings of the National Academy of Sciences of the United States of America* **111**, 2247-2252, doi:10.1073/pnas.1322269111 (2014).
62 Kim, C. H., Park, J. & Kim, M. Gut Microbiota-Derived Short-Chain Fatty Acids, T Cells, and Inflammation. *Immune Network* **14**, 277-288, doi:10.4110/in.2014.14.6.277 (2014).
63 Furusawa, Y. *et al.* Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. *Nature* **504**, 446, doi:10.1038/nature12721

https://www.nature.com/articles/nature12721#supplementary-information (2013).
64 Singh, N. *et al.* Activation of Gpr109a, receptor for niacin and the commensal metabolite butyrate, suppresses colonic inflammation and carcinogenesis. *Immunity* **40**, 128-139, doi:10.1016/j.immuni.2013.12.007 (2014).
65 Masui, R. *et al.* G protein-coupled receptor 43 moderates gut inflammation through cytokine regulation from mononuclear cells. *Inflammatory bowel diseases* **19**, 2848-2856, doi:10.1097/01.MIB.0000435444.14860.ea (2013).
66 Nilsson, N. E., Kotarsky, K., Owman, C. & Olde, B. Identification of a free fatty acid receptor, FFA2R, expressed on leukocytes and activated by short-chain fatty acids. *Biochem Biophys Res Commun* **303**, 1047-1052 (2003).
67 Kim, M. H., Kang, S. G., Park, J. H., Yanagisawa, M. & Kim, C. H. Short-chain fatty acids activate GPR41 and GPR43 on intestinal epithelial cells to promote inflammatory responses in mice. *Gastroenterology* **145**, 396-406.e391-310, doi:10.1053/j.gastro.2013.04.056 (2013).
68 Smith, P. M. *et al.* The Microbial Metabolites, Short-Chain Fatty Acids, Regulate Colonic T_{reg} Cell Homeostasis. *Science* (2013).
69 Park, J. *et al.* Short-chain fatty acids induce both effector and regulatory T cells by suppression of histone deacetylases and regulation of the mTOR-S6K pathway. *Mucosal immunology* **8**, 80-93, doi:10.1038/mi.2014.44 (2015).
70 Arpaia, N. *et al.* Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. *Nature* **504**, 451-455, doi:10.1038/nature12726 (2013).
71 Möhle, L. *et al.* Ly6Chi Monocytes Provide a Link between Antibiotic-Induced Changes in Gut Microbiota and Adult Hippocampal Neurogenesis. *Cell Reports* **15**, 1945-1956, doi:https://doi.org/10.1016/j.celrep.2016.04.074 (2016).
72 Ang, Z. *et al.* Human and mouse monocytes display distinct signalling and cytokine profiles upon stimulation with FFAR2/FFAR3 short-chain fatty acid receptor agonists. *Scientific Reports* **6**, 34145, doi:10.1038/srep34145

https://www.nature.com/articles/srep34145#supplementary-information (2016).
73 Cox, M. A. *et al.* Short-chain fatty acids act as antiinflammatory mediators by regulating prostaglandin E(2) and cytokines. *World Journal of Gastroenterology : WJG* **15**, 5549-5557, doi:10.3748/wjg.15.5549 (2009).
74 McLoughlin, R. F., Berthon, B. S., Jensen, M. E., Baines, K. J. & Wood, L. G. Short-chain fatty acids, prebiotics, synbiotics, and systemic inflammation: a systematic review and meta-analysis. *Am J Clin Nutr*, doi:10.3945/ajcn.117.156265 (2017).

75 Freeland, K. R. & Wolever, T. M. Acute effects of intravenous and rectal acetate on glucagon-like peptide-1, peptide YY, ghrelin, adiponectin and tumour necrosis factor-alpha. *Br J Nutr* **103**, 460-466, doi:10.1017/S0007114509991863 (2010).

76 Canfora, E. E. *et al.* Colonic infusions of short-chain fatty acid mixtures promote energy metabolism in overweight/obese men: a randomized crossover trial. *Sci Rep* **7**, 2360, doi:10.1038/s41598-017-02546-x (2017).

77 Hamer, H. M. *et al.* Butyrate modulates oxidative stress in the colonic mucosa of healthy humans. *Clin Nutr* **28**, 88-93, doi:10.1016/j.clnu.2008.11.002 (2009).

78 Hamer, H. M. *et al.* Effect of butyrate enemas on inflammation and antioxidant status in the colonic mucosa of patients with ulcerative colitis in remission. *Clin Nutr* **29**, 738-744, doi:10.1016/j.clnu.2010.04.002 (2010).

79 van der Beek, C. M. *et al.* Distal, not proximal, colonic acetate infusions promote fat oxidation and improve metabolic markers in overweight/obese men. *Clinical science (London, England : 1979)* **130**, 2073-2082, doi:10.1042/cs20160263 (2016).

80 Lecerf, J. M. *et al.* Xylo-oligosaccharide (XOS) in combination with inulin modulates both the intestinal environment and immune status in healthy subjects, while XOS alone only shows prebiotic properties. *Br J Nutr* **108**, 1847-1858, doi:10.1017/s0007114511007252 (2012).

81 Varatharaj, A. & Galea, I. The blood-brain barrier in systemic inflammation. *Brain, Behavior, and Immunity* **60**, 1-12, doi:https://doi.org/10.1016/j.bbi.2016.03.010 (2017).

82 Hoogland, I. C. M., Houbolt, C., van Westerloo, D. J., van Gool, W. A. & van de Beek, D. Systemic inflammation and microglial activation: systematic review of animal experiments. *Journal of Neuroinflammation* **12**, 114, doi:10.1186/s12974-015-0332-6 (2015).

83 Huuskonen, J., Suuronen, T., Nuutinen, T., Kyrylenko, S. & Salminen, A. Regulation of microglial inflammatory response by sodium butyrate and short-chain fatty acids. *Br J Pharmacol* **141**, 874-880, doi:10.1038/sj.bjp.0705682 (2004).

84 Erny, D. *et al.* Host microbiota constantly control maturation and function of microglia in the CNS. *Nat Neurosci* **18**, 965-977, doi:10.1038/nn.4030 (2015).

85 Savignac, H. M. *et al.* Prebiotic administration normalizes lipopolysaccharide (LPS)-induced anxiety and cortical 5-HT2A receptor and IL1-β levels in male mice. *Brain, Behavior, and Immunity* **52**, 120-131, doi:https://doi.org/10.1016/j.bbi.2015.10.007 (2016).

86 Burokas, A. *et al.* Targeting the Microbiota-Gut-Brain Axis: Prebiotics Have Anxiolytic and Antidepressant-like Effects and Reverse the Impact of Chronic Stress in Mice. *Biological psychiatry* **82**, 472-487, doi:10.1016/j.biopsych.2016.12.031 (2017).

87 Tolhurst, G. *et al.* Short-chain fatty acids stimulate glucagon-like peptide-1 secretion via the G-protein-coupled receptor FFAR2. *Diabetes* **61**, 364-371, doi:10.2337/db11-1019 (2012).

88 Psichas, A. *et al.* The short chain fatty acid propionate stimulates GLP-1 and PYY secretion via free fatty acid receptor 2 in rodents. *Int J Obes (Lond)* **39**, 424-429, doi:10.1038/ijo.2014.153 (2015).

89 Chambers, E. S. *et al.* Effects of targeted delivery of propionate to the human colon on appetite regulation, body weight maintenance and adiposity in overweight adults. *Gut* **64**, 1744-1754, doi:10.1136/gutjnl-2014-307913 (2015).

90 Sam, A. H., Troke, R. C., Tan, T. M. & Bewick, G. A. The role of the gut/brain axis in modulating food intake. *Neuropharmacology* **63**, 46-56, doi:10.1016/j.neuropharm.2011.10.008 (2012).

91 Trapp, S. & Richards, J. E. The gut hormone glucagon-like peptide-1 produced in brain: is this physiologically relevant? *Current Opinion in Pharmacology* **13**, 964-969, doi:10.1016/j.coph.2013.09.006 (2013).

92 Katsurada, K. & Yada, T. Neural effects of gut- and brain-derived glucagon-like peptide-1 and its receptor agonist. *Journal of diabetes investigation* **7 Suppl 1**, 64-69, doi:10.1111/jdi.12464 (2016).

93 Alvarez, E. *et al.* The expression of GLP-1 receptor mRNA and protein allows the effect of GLP-1 on glucose metabolism in the human hypothalamus and brainstem. *Journal of neurochemistry* **92**, 798-806, doi:10.1111/j.1471-4159.2004.02914.x (2005).

94 van Bloemendaal, L. *et al.* GLP-1 Receptor Activation Modulates Appetite- and Reward-Related Brain Areas in Humans. *Diabetes* **63**, 4186 (2014).

95 Anderberg, R. H. *et al.* GLP-1 is both anxiogenic and antidepressant; divergent effects of acute and chronic GLP-1 on emotionality. *Psychoneuroendocrinology* **65**, 54-66, doi:https://doi.org/10.1016/j.psyneuen.2015.11.021 (2016).

96 Gil-Lozano, M. *et al.* GLP-1(7-36)-amide and Exendin-4 stimulate the HPA axis in rodents and humans. *Endocrinology* **151**, 2629-2640, doi:10.1210/en.2009-0915 (2010).

97 During, M. J. *et al.* Glucagon-like peptide-1 receptor is involved in learning and neuroprotection. *Nature medicine* **9**, 1173-1179, doi:10.1038/nm919 (2003).

98 Isacson, R. *et al.* The glucagon-like peptide 1 receptor agonist exendin-4 improves reference memory performance and decreases immobility in the forced swim test. *European journal of pharmacology* **650**, 249-255, doi:10.1016/j.ejphar.2010.10.008 (2011).

99 McClean, P. L., Parthsarathy, V., Faivre, E. & Holscher, C. The diabetes drug liraglutide prevents degenerative processes in a mouse model of Alzheimer's disease. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **31**, 6587-6594, doi:10.1523/jneurosci.0529-11.2011 (2011).

100 Porter, D. W., Irwin, N., Flatt, P. R., Hölscher, C. & Gault, V. A. Prolonged GIP receptor activation improves cognitive function, hippocampal synaptic plasticity and glucose homeostasis in high-fat fed mice. *European journal of pharmacology* **650**, 688-693 (2011).

101 Morimoto, R. *et al.* Expression of peptide YY in human brain and pituitary tissues. *Nutrition* **24**, 878-884, doi:10.1016/j.nut.2008.06.011 (2008).

102 Murphy, K. G. & Bloom, S. R. Gut hormones and the regulation of energy homeostasis. *Nature* **444**, 854-859, doi:10.1038/nature05484 (2006).

103 Nonaka, N., Shioda, S., Niehoff, M. L. & Banks, W. A. Characterization of blood-brain barrier permeability to PYY3-36 in the mouse. *The Journal of pharmacology and experimental therapeutics* **306**, 948-953, doi:10.1124/jpet.103.051821 (2003).

104 Koda, S. *et al.* The role of the vagal nerve in peripheral PYY3-36-induced feeding reduction in rats. *Endocrinology* **146**, 2369-2375, doi:10.1210/en.2004-1266 (2005).

105 Painsipp, E., Herzog, H. & Holzer, P. The gut-mood axis: a novel role of the gut hormone peptide YY on emotional-affective behaviour in mice. *BMC Pharmacology* **9**, A13-A13, doi:10.1186/1471-2210-9-S2-A13 (2009).

106 Painsipp, E., Herzog, H., Sperk, G. & Holzer, P. Sex-dependent control of murine emotional-affective behaviour in health and colitis by peptide YY and neuropeptide Y. *Br J Pharmacol* **163**, 1302-1314, doi:10.1111/j.1476-5381.2011.01326.x (2011).

107 Tschenett, A. *et al.* Reduced anxiety and improved stress coping ability in mice lacking NPY-Y2 receptors. *The European journal of neuroscience* **18**, 143-148 (2003).

108 Heilig, M. The NPY system in stress, anxiety and depression. *Neuropeptides* **38**, 213-224, doi:10.1016/j.npep.2004.05.002 (2004).

109 Byrne, C. S. *et al.* Increased colonic propionate reduces anticipatory reward responses in the human striatum to high-energy foods. *The American Journal of Clinical Nutrition* **104**, 5-14, doi:10.3945/ajcn.115.126706 (2016).

110 Byrne, C. S., Chambers, E. S., Morrison, D. J. & Frost, G. The role of short chain fatty acids in appetite regulation and energy homeostasis. *International Journal of Obesity (2005)* **39**, 1331-1338, doi:10.1038/ijo.2015.84 (2015).

111 Hong, Y. H. *et al.* Acetate and propionate short chain fatty acids stimulate adipogenesis via GPCR43. *Endocrinology* **146**, 5092-5099, doi:10.1210/en.2005-0545 (2005).

112 Al-Lahham, S. H. *et al.* Regulation of adipokine production in human adipose tissue by propionic acid. *European journal of clinical investigation* **40**, 401-407, doi:10.1111/j.1365-2362.2010.02278.x (2010).

113 Ivan, J. *et al.* The Short-Chain Fatty Acid Propionate Inhibits Adipogenic Differentiation of Human Chorion-Derived Mesenchymal Stem Cells Through the Free Fatty Acid Receptor 2. *Stem cells and development* **26**, 1724-1733, doi:10.1089/scd.2017.0035 (2017).

114 Zaibi, M. S. *et al.* Roles of GPR41 and GPR43 in leptin secretory responses of murine adipocytes to short chain fatty acids. *FEBS letters* **584**, 2381-2386, doi:10.1016/j.febslet.2010.04.027 (2010).

115 Lin, H. V. *et al.* Butyrate and Propionate Protect against Diet-Induced Obesity and Regulate Gut Hormones via Free Fatty Acid Receptor 3-Independent Mechanisms. *PLoS ONE* **7**, e35240, doi:10.1371/journal.pone.0035240 (2012).

116 Samuel, B. S. *et al.* Effects of the gut microbiota on host adiposity are modulated by the short-chain fatty-acid binding G protein-coupled receptor, Gpr41. *Proc Natl Acad Sci U S A* **105**, 16767-16772, doi:10.1073/pnas.0808567105 (2008).

117 Frost, G. *et al.* Effect of short chain fatty acids on the expression of free fatty acid receptor 2 (Ffar2), Ffar3 and early-stage adipogenesis. *Nutrition & diabetes* **4**, e128, doi:10.1038/nutd.2014.25 (2014).

118 Banks, W. A. Leptin transport across the blood-brain barrier: implications for the cause and treatment of obesity. *Curr Pharm Des* **7**, 125-133 (2001).

119 Kastin, A. J. & Pan, W. Dynamic regulation of leptin entry into brain by the blood–brain barrier. *Regulatory Peptides* **92**, 37-43, doi:https://doi.org/10.1016/S0167-0115(00)00147-6 (2000).

120 Banks, W. A., Niehoff, M. L., Martin, D. & Farrell, C. L. Leptin transport across the blood-brain barrier of the Koletsky rat is not mediated by a product of the leptin receptor gene. *Brain research* **950**, 130-136 (2002).

121 Sachot, C., Rummel, C., Bristow, A. F. & Luheshi, G. N. The role of the vagus nerve in mediating the long-term anorectic effects of leptin. *Journal of neuroendocrinology* **19**, 250-261, doi:10.1111/j.1365-2826.2006.01528.x (2007).

122 de Lartigue, G., Ronveaux, C. C. & Raybould, H. E. Deletion of leptin signaling in vagal afferent neurons results in hyperphagia and obesity. *Molecular Metabolism* **3**, 595-607, doi:10.1016/j.molmet.2014.06.003 (2014).

123 Morrison, C. D. Leptin signaling in brain: A link between nutrition and cognition? *Biochimica et biophysica acta* **1792**, 401-408, doi:10.1016/j.bbadis.2008.12.004 (2009).

124 Farr, O. M., Tsoukas, M. A. & Mantzoros, C. S. Leptin and the brain: influences on brain development, cognitive functioning and psychiatric disorders. *Metabolism: clinical and experimental* **64**, 114-130, doi:10.1016/j.metabol.2014.07.004 (2015).

125 Date, Y. Ghrelin and the vagus nerve. *Methods in enzymology* **514**, 261-269, doi:10.1016/b978-0-12-381272-8.00016-7 (2012).

126 Cabral, A., De Francesco, P. N. & Perello, M. Brain Circuits Mediating the Orexigenic Action of Peripheral Ghrelin: Narrow Gates for a Vast Kingdom. *Frontiers in Endocrinology* **6**, doi:10.3389/fendo.2015.00044 (2015).

127 Wren, A. *et al.* Ghrelin enhances appetite and increases food intake in humans. (2001).

128 Fukumori, R. *et al.* Plasma ghrelin concentration is decreased by short chain fatty acids in wethers. *Domestic Animal Endocrinology* **41**, 50-55, doi:https://doi.org/10.1016/j.domaniend.2011.04.001 (2011).

129 Tarini, J. & Wolever, T. M. The fermentable fibre inulin increases postprandial serum short-chain fatty acids and reduces free-fatty acids and ghrelin in healthy subjects. *Applied physiology, nutrition, and metabolism = Physiologie appliquee, nutrition et metabolisme* **35**, 9-16, doi:10.1139/h09-119 (2010).

130 Rahat-Rozenbloom, S., Fernandes, J., Cheng, J. & Wolever, T. M. S. Acute increases in serum colonic short-chain fatty acids elicited by inulin do not increase GLP-1 or PYY responses but may reduce ghrelin in lean and overweight humans. *European Journal Of Clinical Nutrition* **71**, 953, doi:10.1038/ejcn.2016.249

https://www.nature.com/articles/ejcn2016249#supplementary-information (2016).
131 Malik, S., McGlone, F., Bedrossian, D. & Dagher, A. Ghrelin Modulates Brain Activity in Areas that Control Appetitive Behavior. *Cell Metabolism* **7**, 400-409, doi:https://doi.org/10.1016/j.cmet.2008.03.007 (2008).
132 Diano, S. *et al.* Ghrelin controls hippocampal spine synapse density and memory performance. *Nat Neurosci* **9**, 381-388, doi:10.1038/nn1656 (2006).
133 Li, E. *et al.* Ghrelin directly stimulates adult hippocampal neurogenesis: implications for learning and memory. *Endocrine journal* **60**, 781-789 (2013).
134 Bali, A. & Jaggi, A. S. An Integrative Review on Role and Mechanisms of Ghrelin in Stress, Anxiety and Depression. *Current drug targets* **17**, 495-507 (2016).
135 Wilcox, G. Insulin and Insulin Resistance. *Clinical Biochemist Reviews* **26**, 19-39 (2005).
136 Horino, M., Machlin, L. J., Hertelendy, F. & Kipnis, D. M. Effect of short-chain fatty acids on plasma insulin in ruminant and nonruminant species. *Endocrinology* **83**, 118-128, doi:10.1210/endo-83-1-118 (1968).
137 Trenkle, A. Effects of Short-chain Fatty Acids, Feeding, Fasting and Type of Diet on Plasma Insulin Levels in Sheep. *The Journal of Nutrition* **100**, 1323-1330, doi:10.1093/jn/100.11.1323 (1970).
138 Robertson, M. D., Bickerton, A. S., Dennis, A. L., Vidal, H. & Frayn, K. N. Insulin-sensitizing effects of dietary resistant starch and effects on skeletal muscle and adipose tissue metabolism. *Am J Clin Nutr* **82**, 559-567, doi:10.1093/ajcn.82.3.559 (2005).
139 Gray, S. M., Meijer, R. I. & Barrett, E. J. Insulin Regulates Brain Function, but How Does It Get There? *Diabetes* **63**, 3992-3997, doi:10.2337/db14-0340 (2014).
140 Daniel, L., Pnina, V. & Konstantin, B. Anti-diabetic and neuroprotective effects of pancreatic islet transplantation into the central nervous system. *Diabetes/Metabolism Research and Reviews* **32**, 11-20, doi:doi:10.1002/dmrr.2644 (2016).
141 Craft, S., Baker, L. D., Montine, T. J. & et al. Intranasal insulin therapy for alzheimer disease and amnestic mild cognitive impairment: A pilot clinical trial. *Archives of Neurology* **69**, 29-38, doi:10.1001/archneurol.2011.233 (2012).
142 Reger, M. A. *et al.* Intranasal insulin improves cognition and modulates β-amyloid in early AD. *Neurology* **70**, 440-448, doi:10.1212/01.wnl.0000265401.62434.36 (2008).
143 Stanley, M., Macauley, S. L. & Holtzman, D. M. Changes in insulin and insulin signaling in Alzheimer's disease: cause or consequence? *The Journal of experimental medicine* **213**, 1375-1385, doi:10.1084/jem.20160493 (2016).
144 Swaminathan, S. K. *et al.* Insulin differentially affects the distribution kinetics of amyloid beta 40 and 42 in plasma and brain. *Journal of Cerebral Blood Flow & Metabolism* **38**, 904-918, doi:10.1177/0271678X17709709 (2017).
145 McIntyre, R. S. *et al.* A randomized, double-blind, controlled trial evaluating the effect of intranasal insulin on neurocognitive function in euthymic patients with bipolar disorder. *Bipolar disorders* **14**, 697-706, doi:10.1111/bdi.12006 (2012).
146 Cha, D. S. *et al.* A randomized, double-blind, placebo-controlled, crossover trial evaluating the effect of intranasal insulin on cognition and mood in individuals with treatment-resistant major depressive disorder. *Journal of Affective Disorders* **210**, 57-65, doi:https://doi.org/10.1016/j.jad.2016.12.006 (2017).
147 Ryan, J. P., Sheu, L. K., Critchley, H. D. & Gianaros, P. J. A Neural Circuitry Linking Insulin Resistance to Depressed Mood. *Psychosomatic Medicine* **74**, 476-482, doi:10.1097/PSY.0b013e31824d0865 (2012).
148 Benedict, C. *et al.* Intranasal insulin improves memory in humans. *Psychoneuroendocrinology* **29**, 1326-1334, doi:https://doi.org/10.1016/j.psyneuen.2004.04.003 (2004).
149 Bohringer, A., Schwabe, L., Richter, S. & Schachinger, H. Intranasal insulin attenuates the hypothalamic–pituitary–adrenal axis response to psychosocial stress. *Psychoneuroendocrinology* **33**, 1394-1400, doi:https://doi.org/10.1016/j.psyneuen.2008.08.002 (2008).

150 Perry, R. J. *et al.* Acetate mediates a microbiome–brain–β-cell axis to promote metabolic syndrome. *Nature* **534**, 213, doi:10.1038/nature18309

https://www.nature.com/articles/nature18309#supplementary-information (2016).

151 Bonaz, B., Bazin, T. & Pellissier, S. The Vagus Nerve at the Interface of the Microbiota-Gut-Brain Axis. *Frontiers in Neuroscience* **12**, 49, doi:10.3389/fnins.2018.00049 (2018).

152 Li, Y., Hao, Y., Zhu, J. & Owyang, C. Serotonin released from intestinal enterochromaffin cells mediates luminal non-cholecystokinin-stimulated pancreatic secretion in rats. *Gastroenterology* **118**, 1197-1207 (2000).

153 Strader, A. D. & Woods, S. C. Gastrointestinal hormones and food intake. *Gastroenterology* **128**, 175-191 (2005).

154 Ressler, K. J. & Mayberg, H. S. Targeting abnormal neural circuits in mood and anxiety disorders: from the laboratory to the clinic. *Nat Neurosci* **10**, 1116-1124, doi:10.1038/nn1944 (2007).

155 Bonaz, B., Picq, C., Sinniger, V., Mayol, J. F. & Clarencon, D. Vagus nerve stimulation: from epilepsy to the cholinergic anti-inflammatory pathway. *Neurogastroenterol Motil* **25**, 208-221, doi:10.1111/nmo.12076 (2013).

156 Bravo, J. A. *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* **108**, 16050-16055, doi:10.1073/pnas.1102999108 (2011).

157 Lal, S., Kirkup, A. J., Brunsden, A. M., Thompson, D. G. & Grundy, D. Vagal afferent responses to fatty acids of different chain length in the rat. *Am J Physiol Gastrointest Liver Physiol* **281**, G907-915, doi:10.1152/ajpgi.2001.281.4.G907 (2001).

158 Goswami, C., Iwasaki, Y. & Yada, T. Short-chain fatty acids suppress food intake by activating vagal afferent neurons. *The Journal of Nutritional Biochemistry* **57**, 130-135, doi:https://doi.org/10.1016/j.jnutbio.2018.03.009 (2018).

159 Mitchell, R. W., On, N. H., Del Bigio, M. R., Miller, D. W. & Hatch, G. M. Fatty acid transport protein expression in human brain and potential role in fatty acid transport across human brain microvessel endothelial cells. *Journal of neurochemistry* **117**, 735-746, doi:10.1111/j.1471-4159.2011.07245.x (2011).

160 Vijay, N. & Morris, M. E. Role of monocarboxylate transporters in drug delivery to the brain. *Curr Pharm Des* **20**, 1487-1498 (2014).

161 Kekuda, R., Manoharan, P., Baseler, W. & Sundaram, U. Monocarboxylate 4 mediated butyrate transport in a rat intestinal epithelial cell line. *Dig Dis Sci* **58**, 660-667, doi:10.1007/s10620-012-2407-x (2013).

162 Oldendorf, W. Carrier-mediated blood-brain barrier transport of short-chain monocarboxylic organic acids. *American Journal of Physiology-Legacy Content* **224**, 1450-1453, doi:10.1152/ajplegacy.1973.224.6.1450 (1973).

163 Bachmann, C., Colombo, J.-P. & Berüter, J. Short chain fatty acids in plasma and brain: Quantitative determination by gas chromatography. *Clinica Chimica Acta* **92**, 153-159, doi:https://doi.org/10.1016/0009-8981(79)90109-8 (1979).

164 Frost, G. *et al.* The short-chain fatty acid acetate reduces appetite via a central homeostatic mechanism. *Nat Commun* **5**, 3611, doi:10.1038/ncomms4611 (2014).

165 Kim, S. W. *et al.* Whole-body pharmacokinetics of HDAC inhibitor drugs, butyric acid, valproic acid and 4-phenylbutyric acid measured with carbon-11 labeled analogs by PET. *Nucl Med Biol* **40**, 912-918, doi:10.1016/j.nucmedbio.2013.06.007 (2013).

166 Song, W. S., Nielson, B. R., Banks, K. P. & Bradley, Y. C. Normal organ standard uptake values in carbon-11 acetate PET imaging. *Nuclear medicine communications* **30**, 462-465, doi:10.1097/MNM.0b013e32832aa7ce (2009).

167 Seltzer, M. A. *et al.* Radiation dose estimates in humans for (11)C-acetate whole-body PET. *Journal of nuclear medicine : official publication, Society of Nuclear Medicine* **45**, 1233-1236 (2004).

168 Braniste, V. *et al.* The gut microbiota influences blood-brain barrier permeability in mice. *Sci Transl Med* **6**, 263ra158, doi:10.1126/scitranslmed.3009759 (2014).

169 Hoyles, L. *et al.* Microbiome–host systems interactions: protective effects of propionate upon the blood–brain barrier. *Microbiome* **6**, 55, doi:10.1186/s40168-018-0439-y (2018).

170 Nankova, B. B., Agarwal, R., MacFabe, D. F. & La Gamma, E. F. Enteric bacterial metabolites propionic and butyric acid modulate gene expression, including CREB-dependent catecholaminergic neurotransmission, in PC12 cells--possible relevance to autism spectrum disorders. *PLoS One* **9**, e103740, doi:10.1371/journal.pone.0103740 (2014).

171 Nagatsu, T. Tyrosine hydroxylase: human isoforms, structure and regulation in physiology and pathology. *Essays Biochem* **30**, 15-35 (1995).

172 Morís, G. & Vega, J. A. [Neurotrophic factors: basis for their clinical application]. *Neurologia* **18**, 18-28 (2003).

173 Savignac, H. M. *et al.* Prebiotic feeding elevates central brain derived neurotrophic factor, N-methyl-D-aspartate receptor subunits and D-serine. *Neurochem Int* **63**, 756-764, doi:10.1016/j.neuint.2013.10.006 (2013).

174 Varela, R. B. *et al.* Sodium butyrate and mood stabilizers block ouabain-induced hyperlocomotion and increase BDNF, NGF and GDNF levels in brain of Wistar rats. *Journal of psychiatric research* **61**, 114-121, doi:10.1016/j.jpsychires.2014.11.003 (2015).

175 Sun, J. *et al.* Antidepressant-like effects of sodium butyrate and its possible mechanisms of action in mice exposed to chronic unpredictable mild stress. *Neurosci Lett* **618**, 159-166, doi:10.1016/j.neulet.2016.03.003 (2016).

176 Intlekofer, K. A. *et al.* Exercise and sodium butyrate transform a subthreshold learning event into long-term memory via a brain-derived neurotrophic factor-dependent mechanism. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* **38**, 2027-2034, doi:10.1038/npp.2013.104 (2013).

177 Barichello, T. *et al.* Sodium Butyrate Prevents Memory Impairment by Re-establishing BDNF and GDNF Expression in Experimental Pneumococcal Meningitis. *Molecular neurobiology* **52**, 734-740, doi:10.1007/s12035-014-8914-3 (2015).

178 Kim, H. J., Leeds, P. & Chuang, D. M. The HDAC inhibitor, sodium butyrate, stimulates neurogenesis in the ischemic brain. *Journal of neurochemistry* **110**, 1226-1240, doi:10.1111/j.1471-4159.2009.06212.x (2009).

179 Wu, X. *et al.* Histone deacetylase inhibitors up-regulate astrocyte GDNF and BDNF gene transcription and protect dopaminergic neurons. *The international journal of neuropsychopharmacology / official scientific journal of the Collegium Internationale Neuropsychopharmacologicum (CINP)* **11**, 1123-1134, doi:10.1017/S1461145708009024 (2008).

180 Gershon, M. D. & Tack, J. The serotonin signaling system: from basic understanding to drug development for functional GI disorders. *Gastroenterology* **132**, 397-414, doi:10.1053/j.gastro.2006.11.002 (2007).

181 Lucki, I. The spectrum of behaviors influenced by serotonin. *Biological psychiatry* **44**, 151-162 (1998).

182 Fukumoto, S. *et al.* Short-chain fatty acids stimulate colonic transit via intraluminal 5-HT release in rats. *American journal of physiology. Regulatory, integrative and comparative physiology* **284**, R1269-1276, doi:10.1152/ajpregu.00442.2002 (2003).

183 Reigstad, C. S. *et al.* Gut microbes promote colonic serotonin production through an effect of short-chain fatty acids on enterochromaffin cells. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* **29**, 1395-1403, doi:10.1096/fj.14-259598 (2015).

184 Yano, J. M. *et al.* Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. *Cell* **161**, 264-276, doi:10.1016/j.cell.2015.02.047 (2015).

185 Stasi, C., Bellini, M., Bassotti, G., Blandizzi, C. & Milani, S. Serotonin receptors and their role in the pathophysiology and therapy of irritable bowel syndrome. *Techniques in Coloproctology* **18**, 613-621, doi:10.1007/s10151-013-1106-8 (2014).

186 Bonnin, A. & Levitt, P. Fetal, Maternal and Placental Sources of Serotonin and New Implications for Developmental Programming of the Brain. *Neuroscience* **197**, 1-7, doi:10.1016/j.neuroscience.2011.10.005 (2011).

187 Côté, F. *et al.* Maternal serotonin is crucial for murine embryonic development. *Proceedings of the National Academy of Sciences* **104**, 329 (2007).

188 Browning, K. N. Role of central vagal 5-HT(3) receptors in gastrointestinal physiology and pathophysiology. *Frontiers in Neuroscience* **9**, 413, doi:10.3389/fnins.2015.00413 (2015).

189 Sanders, M. E. Probiotics: definition, sources, selection, and uses. *Clin Infect Dis* **46 Suppl 2**, S58-61; discussion S144-151, doi:10.1086/523341 (2008).

190 LeBlanc, J. G. *et al.* Beneficial effects on host energy metabolism of short-chain fatty acids and vitamins produced by commensal and probiotic bacteria. *Microb Cell Fact* **16**, 79, doi:10.1186/s12934-017-0691-z (2017).

191 Gibson, G. R. *et al.* Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nat Rev Gastroenterol Hepatol* **14**, 491-502, doi:10.1038/nrgastro.2017.75 (2017).

192 Macfarlane, G. T. & Macfarlane, S. Fermentation in the human large intestine: its physiologic consequences and the potential contribution of prebiotics. *J Clin Gastroenterol* **45 Suppl**, S120-127, doi:10.1097/MCG.0b013e31822fecfe (2011).

193 Koh, A., De Vadder, F., Kovatcheva-Datchary, P. & Backhed, F. From Dietary Fiber to Host Physiology: Short-Chain Fatty Acids as Key Bacterial Metabolites. *Cell* **165**, 1332-1345, doi:10.1016/j.cell.2016.05.041 (2016).

194 Roberfroid, M. *et al.* Prebiotic effects: metabolic and health benefits. *Br J Nutr* **104 Suppl 2**, S1-63, doi:10.1017/s0007114510003363 (2010).

195 Verbeke, K. A. *et al.* Towards microbial fermentation metabolites as markers for health benefits of prebiotics. *Nutrition research reviews* **28**, 42-66, doi:10.1017/s0954422415000037 (2015).

196 Derrien, M. & van Hylckama Vlieg, J. E. Fate, activity, and impact of ingested bacteria within the human gut microbiota. *Trends in microbiology* **23**, 354-366, doi:10.1016/j.tim.2015.03.002 (2015).

197 Sakata, T., Kojima, T., Fujieda, M., Takahashi, M. & Michibata, T. Influences of probiotic bacteria on organic acid production by pig caecal bacteria in vitro. *Proc Nutr Soc* **62**, 73-80, doi:10.1079/pns2002211 (2003).

198 David, L. A. *et al.* Diet rapidly and reproducibly alters the human gut microbiome. *Nature* **505**, 559-563, doi:10.1038/nature12820 (2014).

199 De Filippis, F. *et al.* High-level adherence to a Mediterranean diet beneficially impacts the gut microbiota and associated metabolome. *Gut* **65**, 1812-1821, doi:10.1136/gutjnl-2015-309957 (2016).

200 Gutierrez-Diaz, I., Fernandez-Navarro, T., Sanchez, B., Margolles, A. & Gonzalez, S. Mediterranean diet and faecal microbiota: a transversal study. *Food Funct* **7**, 2347-2356, doi:10.1039/c6fo00105j (2016).

201 Liu, J. *et al.* Neuroprotective effects of Clostridium butyricum against vascular dementia in mice via metabolic butyrate. *BioMed research international* **2015** (2015).

202 Hopfner, F. *et al.* Gut microbiota in Parkinson disease in a northern German cohort. *Brain research* **1667**, 41-45 (2017).

203 Li, W. *et al.* Structural changes of gut microbiota in Parkinson's disease and its correlation with clinical features. *Science China Life Sciences* **60**, 1223-1233 (2017).

204 Bedarf, J. R. *et al.* Functional implications of microbial and viral gut metagenome changes in early stage L-DOPA-naïve Parkinson's disease patients. *Genome medicine* **9**, 39 (2017).

205 Keshavarzian, A. *et al.* Colonic bacterial composition in Parkinson's disease. *Movement Disorders* **30**, 1351-1360 (2015).
206 Unger, M. M. *et al.* Short chain fatty acids and gut microbiota differ between patients with Parkinson's disease and age-matched controls. *Parkinsonism & related disorders* **32**, 66-72, doi:10.1016/j.parkreldis.2016.08.019 (2016).
207 Paiva, I. *et al.* Sodium butyrate rescues dopaminergic cells from alpha-synuclein-induced transcriptional deregulation and DNA damage. *Human molecular genetics* **26**, 2231-2246 (2017).
208 Laurent, R. S., O'Brien, L. M. & Ahmad, S. T. Sodium butyrate improves locomotor impairment and early mortality in a rotenone-induced Drosophila model of Parkinson's disease. *Neuroscience* **246**, 382-390 (2013).
209 Sharma, S., Taliyan, R. & Singh, S. Beneficial effects of sodium butyrate in 6-OHDA induced neurotoxicity and behavioral abnormalities: Modulation of histone deacetylase activity. *Behavioural brain research* **291**, 306-314 (2015).
210 Sampson, T. R. *et al.* Gut Microbiota Regulate Motor Deficits and Neuroinflammation in a Model of Parkinson's Disease. *Cell* **167**, 1469-1480.e1412, doi:10.1016/j.cell.2016.11.018 (2016).
211 Govindarajan, N., Agis-Balboa, R. C., Walter, J., Sananbenesi, F. & Fischer, A. Sodium butyrate improves memory function in an Alzheimer's disease mouse model when administered at an advanced stage of disease progression. *Journal of Alzheimer's disease : JAD* **26**, 187-197, doi:10.3233/jad-2011-110080 (2011).
212 Ho, L. *et al.* Protective Roles of Intestinal Microbiota derived Short Chain Fatty Acids in Alzheimer's Disease-type Beta-Amyloid Neuropathological Mechanisms. *Expert review of neurotherapeutics* **18**, 83-90, doi:10.1080/14737175.2018.1400909 (2018).
213 Adams, J. B., Johansen, L. J., Powell, L. D., Quig, D. & Rubin, R. A. Gastrointestinal flora and gastrointestinal status in children with autism – comparisons to typical children and correlation with autism severity. *BMC Gastroenterology* **11**, 22, doi:10.1186/1471-230x-11-22 (2011).
214 Wang, L. *et al.* Elevated fecal short chain fatty acid and ammonia concentrations in children with autism spectrum disorder. *Digestive diseases and sciences* **57**, 2096-2102 (2012).
215 de Theije, C. G. *et al.* Altered gut microbiota and activity in a murine model of autism spectrum disorders. *Brain Behav Immun* **37**, 197-206, doi:10.1016/j.bbi.2013.12.005 (2014).
216 MacFabe, D. F. Enteric short-chain fatty acids: microbial messengers of metabolism, mitochondria, and mind: implications in autism spectrum disorders. *Microbial Ecology in Health and Disease* **26**, 28177, doi:10.3402/mehd.v26.28177 (2015).
217 Szczesniak, O., Hestad, K. A., Hanssen, J. F. & Rudi, K. Isovaleric acid in stool correlates with human depression. *Nutr Neurosci* **19**, 279-283, doi:10.1179/1476830515Y.0000000007 (2016).
218 Kelly, J. R. *et al.* Transferring the blues: Depression-associated gut microbiota induces neurobehavioural changes in the rat. *Journal of psychiatric research* **82**, 109-118, doi:10.1016/j.jpsychires.2016.07.019 (2016).
219 Michels, N., Van de Wiele, T. & De Henauw, S. Chronic Psychosocial Stress and Gut Health in Children: Associations With Calprotectin and Fecal Short-Chain Fatty Acids. *Psychosom Med* **79**, 927-935, doi:10.1097/PSY.0000000000000413 (2017).
220 Moretti, M. *et al.* Behavioral and neurochemical effects of sodium butyrate in an animal model of mania. *Behavioural pharmacology* **22**, 766-772 (2011).
221 Resende, W. R. *et al.* Effects of sodium butyrate in animal models of mania and depression: implications as a new mood stabilizer. *Behavioural pharmacology* **24**, 569-579 (2013).
222 Kiraly, D. D. *et al.* Alterations of the Host Microbiome Affect Behavioral Responses to Cocaine. *Sci Rep* **6**, 35455, doi:10.1038/srep35455 (2016).
223 Joseph, J., Depp, C., Shih, P.-a. B., Cadenhead, K. S. & Schmid-Schönbein, G. Modified Mediterranean Diet for Enrichment of Short Chain Fatty Acids: Potential Adjunctive

Therapeutic to Target Immune and Metabolic Dysfunction in Schizophrenia? *Frontiers in Neuroscience* **11**, 155, doi:10.3389/fnins.2017.00155 (2017).
224 Arnoldussen, I. A. C. *et al.* Butyrate restores HFD-induced adaptations in brain function and metabolism in mid-adult obese mice. *International Journal Of Obesity* **41**, 935, doi:10.1038/ijo.2017.52

https://www.nature.com/articles/ijo201752#supplementary-information (2017).
225 Powers, L. *et al.* Assay of the concentration and stable isotope enrichment of short-chain fatty acids by gas chromatography/mass spectrometry. *Journal of Mass Spectrometry* **30**, 747-754, doi:doi:10.1002/jms.1190300514 (1995).
226 Nguyen, T. L. A., Vieira-Silva, S., Liston, A. & Raes, J. How informative is the mouse for human gut microbiota research? *Disease Models & Mechanisms* **8**, 1-16, doi:10.1242/dmm.017400 (2015).
227 Lamendella, R., Domingo, J. W., Ghosh, S., Martinson, J. & Oerther, D. B. Comparative fecal metagenomics unveils unique functional capacity of the swine gut. *BMC microbiology* **11**, 103, doi:10.1186/1471-2180-11-103 (2011).
228 Moeller, A. H. *et al.* Chimpanzees and Humans Harbor Compositionally Similar Gut Enterotypes. *Nature communications* **3**, 1179-1179, doi:10.1038/ncomms2159 (2012).
229 Nestler, E. J. & Hyman, S. E. Animal Models of Neuropsychiatric Disorders. *Nature neuroscience* **13**, 1161-1169, doi:10.1038/nn.2647 (2010).
230 Blackwood, D. H. R. *et al.* Schizophrenia and Affective Disorders—Cosegregation with a Translocation at Chromosome 1q42 That Directly Disrupts Brain-Expressed Genes: Clinical and P300 Findings in a Family. *The American Journal of Human Genetics* **69**, 428-433, doi:https://doi.org/10.1086/321969 (2001).
231 Anderzhanova, E., Kirmeier, T. & Wotjak, C. T. Animal models in psychiatric research: The RDoC system as a new framework for endophenotype-oriented translational neuroscience. *Neurobiology of Stress* **7**, 47-56, doi:https://doi.org/10.1016/j.ynstr.2017.03.003 (2017).
232 Salgado, J. V. & Sandner, G. A critical overview of animal models of psychiatric disorders: challenges and perspectives. *Revista Brasileira de Psiquiatria* **35**, S77-S81 (2013).
233 Kelly, J. R. *et al.* Lost in translation? The potential psychobiotic Lactobacillus rhamnosus (JB-1) fails to modulate stress or cognitive performance in healthy male subjects. *Brain, Behavior, and Immunity* **61**, 50-59, doi:https://doi.org/10.1016/j.bbi.2016.11.018 (2017).
234 Annison, G., Illman, R. J. & Topping, D. L. Acetylated, propionylated or butyrylated starches raise large bowel short-chain fatty acids preferentially when fed to rats. *J Nutr* **133**, 3523-3528, doi:10.1093/jn/133.11.3523 (2003).

8. Key-points

- SCFA are speculated to play a mediational role in the microbiota-gut-brain axis crosstalk.
- SCFA may influence psychological functioning via interaction with G-protein coupled receptors, histone deacetylase inhibition, and exert their effects on the brain via direct humoral effects, indirect hormonal and immune pathways, and neural routes.
- Dietary intervention studies indirectly implicate a mediational role of SCFA on cognition and emotion.
- Animal studies provide direct evidence of the effects of SCFA on neuropsychiatric disorders and psychological functioning, whereas human studies are sparse, suffer from methodological limitations, and offer inconsistent conclusions.
- SCFA should be quantified in the systemic circulation in dietary intervention studies where the effects on psychological functioning and psychopathology are an outcome of interest.
- SCFA could be used as intervention substances to target microbiota-gut-brain interactions in humans.

Box 1.

Future directions in the study of SCFA as a mediator of microbiota-gut-brain interactions

1. Quantification of systemic concentrations of SCFA in probiotic, prebiotic, and dietary-intervention studies and use of mediation analysis to determine the extent to which SCFA mediate the effects of the intervention on the psychological outcome of interest.
2. Utilize SCFA as intervention substances and directly test their effects on psychological functions in humans.
3. Quantification of systemic concentrations of SCFA as opposed to faecal SCFA.
4. Examine whether changes in other relevant microbiota-gut-brain interaction pathways, including the immune and endocrine systems, are driven by changes in SCFA production.

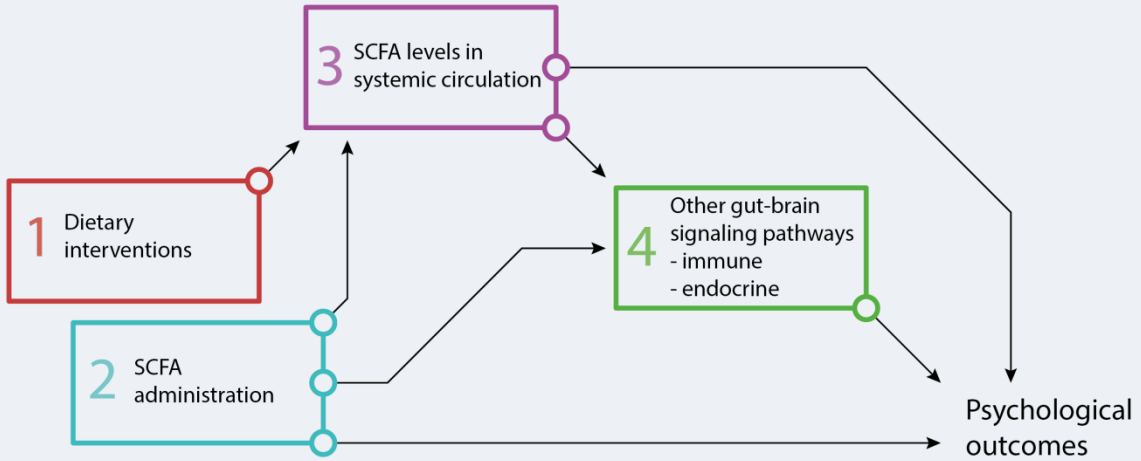

FIG 1. Metabolism of SCFA: From dietary fibre to systemic circulation.

Fermentation of dietary fibre by commensal bacteria in the colon leads to the production of SCFA. SCFA are rapidly absorbed by colonic cells via monocarboxylate transporters, and produce energy for the cells. SCFA that are not absorbed by the colonic cells travel via the basolateral membrane into the portal circulation, whereby all SCFA provide energy for the cells. In the liver SCFA are incorporated in glucose, cholesterol, and fatty acids. Only small amount of the colonically-produced SCFA thus reach systemic circulation (part of the figure was adapted from den Besten et al. (2013)).

FIG 2. Potential pathways through which SCFA may modulate brain function. Dietary fibre, prebiotics, and probiotics contribute to increases in SCFA via proliferation of beneficial bacteria or fermentation of complex carbohydrates. SCFA may influence gut-brain communication and, hence, brain function by interacting with FFARs on colonocytes, acting as histone deacetylase inhibitors, influencing gut permeability and systemic inflammation, and inducing the release of gut hormones. If SCFA successfully crossed the BBB, they may exert direct effects on the brain, including influencing neurotransmission and neuroinflammation. SCFA: short-chain fatty acids, FFARs: free fatty acid receptors, DC: dendritic cell, GLP-1: glucagon-like peptide-1, PYY: peptide YY, SNS: sympathetic nervous system, HDAC: histone deacetylase, NTFs: neurotrophic factors, MCTs: monocarboxylate transporters, BBB: blood-brain barrier.

FIG 3. Gut-brain signaling pathways.

The immune system, endocrine system, vagus nerve, and the humoral system mediate interactions between the gut (and potentially SCFA) and the brain. PYY: Peptide YY, GLP-1: glucagon-like peptide-1, BBB: blood-brain barrier, BDNF: brain-derived neurotrophic factor, NGF: nerve growth factor, GDNF: Glial cell-derived neurotrophic factor; TEER: transepithelial electrical resistance.

9. Acknowledgements

The financial support for the PhD of BD from an unrestricted grant from Nestlé is highly appreciated.

10. Author information

Affiliations

Translational Research Center in Gastrointestinal Disorders (TARGID), Department of Chronic Diseases, Metabolism, and Ageing, Faculty of Medicine, KU Leuven

Boushra Dalile (M.Sc.), PhD student
Prof. Dr. Lukas van Oudenhove, and
Prof. Dr. Pharm. Kristin Verbeke

Laboratory of Biological Psychology, KU Leuven

Prof. Dr. Bram Vervliet

Contributions

BD performed the literature review and wrote the manuscript. LVO, BV, and KV revised the intellectual content of the manuscript critically.

Corresponding author

Prof. Dr. Pharm. Kristin Verbeke

O&N I Herestraat 49 - box 701
3000 Leuven, Belgium
TEL. +32 16 33 01 50
kristin.verbeke@kuleuven.be | www.targid.eu

Glossary

Term	Definition
Microbiota-gut-brain axis	The bidirectional communication occurring between the central nervous system and the gastrointestinal tract, including the enteric nervous system and the gut microbiota.
Short-chain fatty acids	Mainly including acetate, propionate, and butyrate, the major end products of bacterial fermentation of dietary fibre in the colon.
Probiotic	Live microorganisms that, when administered in adequate amounts, confer a health benefit on the host.
Prebiotic	A substrate that is selectively utilized by host microorganisms conferring a health benefit
Dietary fibre	A fraction of edible parts of plants, including polysaccharides, oligosaccharides, and lignins, that resists digestion in the small intestinal and is partially or fully fermented in the large intestine.
Transepithelial electrical resistance (TEER)	An index of the integrity and permeability of a barrier by quantification of electrical resistance across a cellular monolayer
Hypothalamo-Pituitary-Adrenal (HPA) axis	The neuroendocrine system comprising a set of interactions between the hypothalamus, pituitary gland, and the adrenal glands, which is highly involved in the regulation of the organism's stress response, as well as other bodily processes.
Fear extinction	The decline in fear response to a previously conditioned stimulus following multiple non-reinforced presentations of the conditioned stimulus

1