

**UCC Library and UCC researchers have made this item openly available.  
Please [let us know](#) how this has helped you. Thanks!**

<b>Title</b>	Bacterial modulation of visceral sensation: mediators and mechanisms
<b>Author(s)</b>	Lomax, Alan E.; Pradhananga, Sabindra; Sessenwein, Jessica L.; O'Malley, Dervla
<b>Publication date</b>	2019-07-10
<b>Original citation</b>	Lomax, A. E., Pradhananga, S., Sessenwein, J. L. and O'Malley, D. (2019) 'Bacterial modulation of visceral sensation: mediators and mechanisms', American Journal of Physiology-Gastrointestinal and Liver Physiology, In Press, doi: 10.1152/ajpgi.00052.2019
<b>Type of publication</b>	Article (peer-reviewed)
<b>Link to publisher's version</b>	<a href="https://www.physiology.org/doi/abs/10.1152/ajpgi.00052.2019">https://www.physiology.org/doi/abs/10.1152/ajpgi.00052.2019</a> <a href="http://dx.doi.org/10.1152/ajpgi.00052.2019">http://dx.doi.org/10.1152/ajpgi.00052.2019</a> Access to the full text of the published version may require a subscription.
<b>Rights</b>	© 2019, The American Physiological Society. All rights reserved.
<b>Embargo information</b>	Access to this article is restricted until 12 months after publication by request of the publisher.
<b>Embargo lift date</b>	2020-07-10
<b>Item downloaded from</b>	<a href="http://hdl.handle.net/10468/8423">http://hdl.handle.net/10468/8423</a>

Downloaded on 2021-11-27T07:23:40Z

1 **Bacterial modulation of visceral sensation: mediators and mechanisms**

2

3

4 **Alan E Lomax\*<sup>1</sup>, Sabindra Pradhananga<sup>1</sup>, Jessica L Sessenwein<sup>1</sup>, Dervla O'Malley<sup>2,3</sup>**

5

6 *<sup>1</sup>Gastrointestinal Diseases Research Unit, Queen's University, Kingston, ON, Canada*

7 *<sup>2</sup>APC Microbiome Ireland, University College Cork, Ireland*

8 *<sup>3</sup>Department of Physiology, University College Cork, Ireland*

9

10 *\*Author for Correspondence:*

11 *Alan Lomax*

12 *GIDRU Wing,*

13 *Kingston General Hospital,*

14 *Kingston ON K7L 2V7*

15 *Canada*

16 **Email: lomaxa@queensu.ca**

17

18

19

20

21

22

23

24

25 **Abstract**

26 The potential role of the intestinal microbiota in modulating visceral pain has received increasing  
27 attention during recent years. This has led to the identification of signaling pathways that have  
28 been implicated in communication between gut bacteria and peripheral pain pathways. In  
29 addition to the well-characterised impact of the microbiota on the immune system, which in turn  
30 affects nociceptor excitability, bacteria can modulate visceral afferent pathways by effects on  
31 enterocytes, enteroendocrine cells and the neurons themselves. Proteases produced by bacteria,  
32 or by host cells in response to bacteria, can increase or decrease the excitability of nociceptive  
33 dorsal root ganglion (DRG) neurons depending on the receptor activated. Short chain fatty acids  
34 generated by colonic bacteria are involved in gut-brain communication, and intracolonic short  
35 chain fatty acids have pro-nociceptive effects in rodents but may be anti-nociceptive in humans.  
36 Gut bacteria modulate the synthesis and release of enteroendocrine cell mediators including  
37 serotonin and glucagon-like peptide-1, which activate extrinsic afferent neurons. Deciphering the  
38 complex interactions between visceral afferent neurons and the gut microbiota may lead to the  
39 development of improved probiotic therapies for visceral pain.

40

## 41 **Introduction**

42           Visceral pain is a common and debilitating symptom of many digestive diseases,  
43 including inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS) (17). Visceral  
44 pain is often resistant to conventional analgesics and can sometimes be exacerbated by opioid  
45 drugs (45, 55). In light of this, new therapeutics to relieve visceral pain are urgently needed.  
46 Progress towards this goal will be accelerated by a more complete understanding of the  
47 peripheral signaling molecules that modulate nociception in the gut.

48           The perception of pain is accomplished by neural pathways that connect the gut to the  
49 brain via the spinal cord. The first neurons in this chain have cell bodies in dorsal root ganglia  
50 (DRG), project sensory axons into the gut and form excitatory synapses in the dorsal horn of the  
51 spinal cord. A subpopulation of these neurons, called nociceptors, detects noxious stimuli and  
52 activates pain circuits in the brain. Host-derived mediators from biopsies of IBS and IBD  
53 patients induce hyperexcitability in nociceptive DRG neurons, leading to an exaggerated  
54 response to stimuli such as distension or a bowel movement (16, 26, 60). This change in  
55 nociceptor sensitivity is a major driver of visceral pain. Superimposed upon these peripheral  
56 changes are changes in central nervous system (CNS) circuits that amplify synaptic inputs from  
57 the periphery (17, 20). Thus, visceral pain results from a combination of peripheral sensitisation  
58 and central plasticity. Combating these pro-nociceptive influences are host-derived analgesic  
59 substances including endogenous opioids and cannabinoids (22, 124). This balance between pro-  
60 nociceptive and anti-nociceptive influences on DRG neuron excitability dictates the transmission  
61 of pain stimuli to the CNS and the perception of pain. Recent investigations have identified the  
62 gut microbiota as an additional factor in pain modulation, capable of either worsening or  
63 ameliorating pain (8, 88). Microbial modulation of visceral pain may have translational

64 relevance given the changes in microbiota composition associated with IBD and IBS. Although  
65 intestinal fungi may also play important roles in modulating visceral pain (21), in this review, we  
66 discuss the potential mediators of bacterial modulation of peripheral visceral pain pathways.

### 67 **A potential role for gut bacteria in visceral pain signalling**

68         The mutualistic relationship that has evolved between bacteria and eukaryotes includes  
69 the ability of commensal bacteria in the gut to influence behavior and pain (24, 40, 88, 96, 122).  
70 Although probiotics have been marketed for the treatment of visceral pain for over a decade,  
71 there is a lack of mechanistic insight into which bacteria, bacterial metabolites, or signaling  
72 pathways are most important. To date, much of the evidence in support of a role for the  
73 microbiota in regulating pain is derived from *in vivo* studies demonstrating that germ-free mice,  
74 or mice treated with antibiotics that alter the microbiota early in life, have heightened pain  
75 sensitivity (39-41, 74, 88, 90, 98). However, changes to pain sensitivity in germ-free mice may  
76 not be due solely to direct microbial-neuronal interaction, as germ-free mice exhibit a number of  
77 potentially confounding developmental changes to the immune system. Similarly, a study of  
78 visceral pain sensitivity in mice treated with a cocktail of antibiotics reported an increase in  
79 visceral pain accompanied by an increase in colonic myeloperoxidase activity, which is  
80 indicative of immune system activation (126). This suggested a role for inflammatory changes in  
81 nociceptive effects of modulating the microbiota. Although there is potential for bacterial  
82 products to directly activate nociceptive neurons, the evidence until recently, largely supported a  
83 role for epithelial and immune cells in mediating many of the effects of the gut microbiota on  
84 pain pathways *in vivo* (Table 1) (5, 80, 84, 131).

### 85 **Bacteria as a source of host modulatory factors**

86           There is a growing appreciation that the gut microbiota can be considered an endocrine  
87 organ, having the capability to directly or indirectly regulate different gastrointestinal and stress  
88 hormones, which may modify host physiological function (33). Intriguingly, the transfer of  
89 faecal matter from IBS patients is sufficient to evoke visceral hypersensitivity in gnotobiotic rats.  
90 This is not due to changes in mucosal permeability or immune activation, raising the possibility  
91 that bacterial metabolites in IBS patient stool directly modify gut-brain signalling (35). DRG  
92 neurons are capable of “sensing” the presence of microbes. They express functional microbial  
93 pattern recognition molecules, including toll like receptors and nucleotide-binding  
94 oligomerization domains 1 and 2 (91), whose activation can modulate neuronal excitability.  
95 Furthermore, the pathogenic bacterium *Staphylococcus aureus* directly excites DRG neurons  
96 through a toxin that forms cation-permeable pores in DRG neuronal membranes and through  
97 secretion of N-formylated peptides (32). In contrast to the pro-nociceptive effects of this skin  
98 pathogen however, the commensal gut microbes studied to date have inhibitory effects on DRG  
99 neuron excitability (88, 93, 109). Given the potential importance of the microbiota as a  
100 modulator of visceral pain, identification of the specific species involved and mediators  
101 responsible will be particularly important. Gut microbes produce a plethora of neuro-active  
102 compounds such as proteases (116), short chain fatty acids (SCFA) (99) and also classical  
103 neurotransmitters such as  $\gamma$ -amino butyric acid (GABA), dopamine and norepinephrine (94). We  
104 will consider the available evidence in support of a role for specific bacterial mediators in terms  
105 of their capability to directly access and act upon nerve circuits to modulate their function (39,  
106 88, 137). We will also discuss microbe-mediated modulation of visceral pain pathways by using  
107 immune cells and enterocytes as cellular transducers (Figure 1).

#### 108 **Direct signalling by bacterial metabolites**

109 ***Proteases***

110 Extracellular proteases, in particular serine and cysteine proteases, are important modulators of  
111 visceral pain (127). Proteases are released from many eukaryotic cell types, including mast cells,  
112 neutrophils and enterocytes (97, 104). Recent *in vivo* and *in vitro* work has identified the gut  
113 microbiota as an important source of proteases (116) capable of affecting peripheral pain  
114 pathways (8, 81, 109). Pain regulation by proteases most often occurs through the activation of  
115 protease activated receptors (PARs). PARs are a family of four G-protein coupled receptors that  
116 lack conventional ligand binding sites and are instead activated via protease-mediated hydrolysis  
117 of amino acid residues. Upon protease cleavage, a tethered ligand within the receptor is revealed  
118 that activates intracellular signaling pathways (97). The net effect of receptor signaling depends  
119 not just on the PAR subtype involved but the specific amino acids hydrolysed (97). A consistent  
120 finding from numerous laboratories is that PAR-2 activation causes sustained hyperexcitability  
121 of DRG neurons (6, 34, 51, 136). Indeed, activation of nociceptor PAR-2 by mast cell tryptase  
122 and enterocyte derived trypsin-3 (85, 104) has been implicated in visceral pain (12, 63).  
123 However, nociceptive neurons also express PAR-1 and PAR-4. Activation of PAR-1 and PAR-4  
124 reduces DRG neuron excitability and is anti-nociceptive (10, 11, 66, 104). PAR-2 activation *in*  
125 *vivo* by cysteine proteases in fecal supernatants from IBS patients enhanced the visceromotor  
126 response to colorectal distension in rats, an *in vivo* assay of visceral pain. In contrast, activation  
127 of PAR-4 by commensal microbes has an analgesic effect *in vivo* and *in vitro* (81, 109). The  
128 opposing effects of PAR-2, PAR-1 and -4 suggest that the balance between PAR-2, and PAR-1 -  
129 4 activation could be a critical determinant of nociception.

130           While it seems clear that activation of PARs by proteases derived from the microbiota  
131 can modulate pain, an important unresolved issue is whether these proteases exert this influence

132 via actions on mucosal cells, immune cells or directly on DRG nerve terminals. The intestinal  
133 barrier is comprised of a mucus-coated epithelial monolayer whose integrity is maintained by  
134 tight junction proteins, which regulate the paracellular movement of luminal molecules. Beneath  
135 the epithelial layer, intrinsic and extrinsic neurons relay neural information both within the GI  
136 tract but also between the gut and the CNS. However, evidence that this communication system  
137 extends beyond the epithelial barrier to the microbially-dominated environment of the gut lumen,  
138 has resulted in it being referred to as the microbiota-gut-brain axis (19, 47, 76). It appears that at  
139 least in some circumstances, the impact of PAR activation on visceral pain is due to modulation  
140 of epithelial barrier function. Using a model of IBS in rodents, Miquel and colleagues found that  
141 proteases derived from *Faecalibacterium prausnitzii* inhibited the increase in visceral pain that  
142 results from neonatal maternal separation. In this case, the decrease in visceral pain was ascribed  
143 to PAR-4 mediated reversal of the increase in mucosal permeability in this model of visceral  
144 pain (81). Faecal supernatants from patients with chronic ulcerative colitis led to a decrease in  
145 visceromotor response to colorectal distention due to activation of PAR-4 (8). In a separate  
146 study, serine proteases from *Faecalibacterium prausnitzii* acted directly on nerve terminals to  
147 inhibit colonic sensory nerve spike discharge and reduced the excitability of colon-projecting  
148 DRG neurons via PAR-4 activation (109). Furthermore, these proteases reversed DRG neuronal  
149 hyperexcitability caused by the dextran sulphate sodium model of colitis in mice (109).

150       Opposite findings have been reported for microbial activation of PAR-2. Luminal  
151 administration of faecal supernatants from patients with diarrhea-predominant IBS increased  
152 visceral pain sensitivity and impaired mucosal barrier function *in vivo* via PAR-2 activation (49).  
153 Consistent with the ability of luminal proteases to have pronociceptive effects, luminal  
154 administration of the PAR-2 activating serine protease, cathepsin S, was sufficient to increase



155 visceromotor responses in mice in a PAR-2-dependent manner (27). Similarly, activation of  
156 PAR-2 by host derived proteases causes a sustained increase in the excitability of mouse DRG  
157 neurons (67). Thus, although there is abundant evidence that activation of neuronal PAR-2 has  
158 pro-nociceptive effects, it remains unclear whether neuronal PAR-2, in addition to mucosal  
159 PAR-2, participates in the pro-nociceptive effects of bacterial proteases. Cell-specific receptor  
160 knockout strategies will be important tools in identifying which PAR-expressing cells are most  
161 important to visceral pain modulation *in vivo*.

162 In addition to microbial-derived proteases, the microbiota is a rich source of protease  
163 inhibitors (54) including siropins, which has been shown to mitigate the effect of host-derived  
164 proteases implicated in IBD pathogenesis (82). A recent study using a rodent model of post-  
165 inflammatory hypersensitivity provided valuable evidence that synthetic protease inhibitors can  
166 mitigate the pro-nociceptive effects of proteases in this model (28). It therefore appears that the  
167 balance between the activity of proteases and protease inhibitors can influence visceral  
168 perception and may be an important target for novel therapeutics (128).

### 169 ***Short chain fatty acids***

170 Short chain fatty acids (SCFAs) are produced by the fermentation of dietary polysaccharides that  
171 are metabolized by the anaerobic bacteria found in the cecum and colon. Formate, acetate,  
172 butyrate, and propionate are the major byproducts of this fermentation process (83). Earlier  
173 reports have identified *Fecalibacterium prausnitzii*, *Eubacterium rectale*, *Eubacterium hallii* and  
174 *Roseburia faecis* as bacteria capable of producing butyrate. Likewise, acetate and pyruvate are  
175 produced by enteric bacteria such as *Blautia hydrogenotrophica*; propionate, on the other hand,  
176 can be produced by *Bacteroidetes* and *Firmicutes* (72).

177 A well-established effect of butyrate is inhibition of bowel inflammation and  
178 enhancement of mucosal repair, which would have an indirect effect on inflammatory visceral  
179 pain (103). SCFAs also modulate the enteric nervous system (113) and have been posited as an  
180 important mediator of microbiota-gut-brain communication (88). Microbial dysbiosis, due to the  
181 administration of antibiotics or due to modulation of diet, led to a decrease in SCFA and an  
182 increase in visceral sensitivity (38, 90, 100, 112). This suggests an association between SCFA  
183 and visceral pain modulation but does not directly establish a causal relationship. Contrary to  
184 these studies, when SCFAs were administered to control rats and rats with TNBS-induced colitis,  
185 visceral hypersensitivity was not improved by any of the SCFAs (acetate, propionate and  
186 butyrate) used (121). In fact, butyrate administration decreased the noxious pressure threshold in  
187 rats, indicating a pronociceptive effect; this phenomenon was more pronounced in control rats  
188 than in TNBS- treated rats. This observation is supported by a report that rectal administration of  
189 sodium butyrate induced colonic hypersensitivity in rats (133). This pronociceptive effect was  
190 associated with neuronal activation of extracellular signal related kinase (ERK)1/2 and an  
191 enhancement of DRG neuronal excitability. However, a study of healthy human volunteers  
192 concluded that butyrate treatment induced a dose-dependent reduction of visceral sensitivity  
193 (125). In summary, despite evidence implicating SCFAs in mediating gut-brain communication  
194 in general, there are conflicting findings regarding the role of SCFAs in modulating visceral  
195 pain.

### 196 *Microbial neurotransmitters and neurotrophic factors*

197 Microbial depletion and recolonization studies have linked microbial modification of neuroactive  
198 compounds in the gut-brain communication axis to diseases of the peripheral and central nervous  
199 system (119). Germ-free studies illustrate the crucial role of microbes in the development of

200 brain function and expression of central neurochemicals (15, 23) however, antibiotic treatment in  
201 mature animals can avoid the confounding developmental effects of early-life microbial  
202 alterations. Hoban and colleagues reported modification of central monoamines, serotonin and  
203 brain derived neurotrophic factor (BDNF) following sustained antibiotic administration to adult  
204 rats. These changes were accompanied by altered behaviors and diminished visceral pain  
205 sensitivity to colorectal distension (58). Interestingly, antibiotic-related alterations in  
206 neurotransmitters can be long-lasting and have different functional outcomes when administered  
207 early in life. A gender-specific increase in visceral sensitivity, which was linked to decreases in  
208 spinal cord expression of transient receptor potential (TRP)V1,  $\alpha$ 2A adrenergic receptors and  
209 cholecystinin B receptors, was noted in male rats treated with vancomycin from postnatal days  
210 4-13 (90).

211 In addition to modification of host neurotransmitters, microbes also exhibit the capacity  
212 to secrete functional neurotransmitters and neurotrophins. GABA, the major inhibitory  
213 neurotransmitter, is synthesized by several *Lactobacilli* and *Bifidobacteria* (14, 129). As GABA  
214 receptor agonists can suppress visceral pain responses to colorectal distension (56) and  
215 inflammation-induced pain signals (73), this may contribute to nociceptive signaling from the  
216 gut (62). Dopamine and norepinephrine, which have reported anti-nociceptive effects of visceral  
217 pain sensitivity (37, 92), are also produced by several gut bacterial species, including *Bacilli* and  
218 *Escherichia* (94, 129). BDNF, an important neurotrophic regulator of synaptic plasticity and  
219 neurogenesis, is purported to be a hallmark of altered microbiota-gut-brain axis signaling, given  
220 that its expression is altered in germ-free mice (87, 120) and in antibiotic- (58) and prebiotic-  
221 treated mice (107). Moreover, BDNF is expressed on TRPV1-expressing nociceptive DRG  
222 neurons (132) and neutralizing BDNF blocked visceral hypersensitivity in inflammatory colonic

223 hypersensitivity (42). In IBS patients, increased expression of nerve growth factor (NGF)  
224 correlated with visceral pain sensitivity (134), which may be due to sensitization of pro-  
225 nociceptive receptors on primary afferent neurons. Indeed, NGF increases TRPV1 expression in  
226 DRGs (110). In the context of microbial modification of host molecules, an *in vitro* study  
227 demonstrated that *Lactobacillus rhamnosus* induces anti-inflammatory effects in human  
228 epithelial cells which is mediated by NGF (75). Although intriguing, evidence that gut bacteria  
229 have the capacity to secrete neurotransmitters and neurotrophins, does not explain how  
230 neuromodulatory molecules in the external environment of the gut lumen can modify gut-to-  
231 brain nociceptive signalling. As afferent nerves do not reach through the epithelium into the gut  
232 lumen, further mechanistic studies are needed to determine how bacterially-derived  
233 neuromodulatory factors can cross the gut barrier to influence gut-brain signalling.

234

### 235 **Indirect signaling**

#### 236 *Serotonin secretion from Enterochromaffin cells*

237 Serotonin has long been recognised as a critical regulator of gut function, inflammation and pain  
238 (50, 77). Accordingly, the release of serotonin from enterochromaffin (EC) cells and its sites of  
239 action are important therapeutic targets for visceral pain. Two recent independent reports  
240 delineated the ability of microbes to modulate serotonin synthesis by EC cells. One study  
241 reported an increase in serotonin production in mice colonised with human fecal microbiota,  
242 compared to germ-free mice (99). This was associated with an increase in expression of  
243 tryptophan hydroxylase 1 (TPH1), the rate limiting enzyme for serotonin synthesis in EC cells.  
244 Consistent with the ability of microbial metabolites to increase TPH1 expression, the SCFAs,  
245 sodium acetate and sodium butyrate, increased TPH1 expression in a human-derived EC cell

246 line. The second study identified spore-forming bacteria as important modulators of serotonin  
247 production by EC cells, and revealed that this effect occurred in the colon but not the small  
248 intestine (135). Furthermore, EC cell serotonin modulation by microbiota was also observed in  
249 RAG1 knockout mice which lack T and B cells, suggesting a direct action on EC cells rather  
250 than an indirect effect via immunomodulation. SCFAs were also implicated as modulators of EC  
251 cell function, which may be an important mechanism of pain modulation by microbiota. Other  
252 bacterial metabolites, such as bile acids and p-aminobenzoate, have also been implicated in  
253 regulating serotonin production. From these findings it appears that several bacterial signaling  
254 pathways depend on the release of serotonin from EC cell as a means of modulating gut function,  
255 inflammation and visceral pain. In addition to microbial modulation of serotonin release, Kwon  
256 and colleagues have recently (69) demonstrated that host-derived serotonin has direct and  
257 species-specific effects on the growth of commensal microbes *in vivo* and *in vitro*. Furthermore,  
258 the secretion of the anti-microbial peptide  $\alpha$ -defensin from the HT-29 epithelial cell line was  
259 inhibited by serotonin (69). These findings illustrate the complex and bidirectional nature of the  
260 interactions between gut microbes and enterochromaffin cells.

### 261 ***GLP-1 secretion from L-cells***

262 Similar to EC cells, GLP-1-secreting L-cells may act as chemosensory sentinels, conveying  
263 information about the luminal environment to the host. L-cells are polarised, electrically  
264 excitable enteroendocrine cells (31), which sense the arrival of nutrients, such as glucose and  
265 amino acids, in the small intestine. Despite the reduced probability of nutrients being present, the  
266 abundance of GLP-1-secreting L-cells increases towards the distal end of the GI tract (117).  
267 Consistent with the contents of the colonic lumen, L-cells in this region express receptors for  
268 SCFAs and bile acids (101, 123). Moreover, dietary supplementation with SCFAs (123), the

269 introduction of specific commensal strains (9, 118) or antibiotic treatment (61) increased GLP-1  
270 levels. Somewhat counter-intuitively, one study determined that serum GLP-1 was also elevated  
271 in germ-free mice (108), although other researchers found that germ-free mice exhibited a strong  
272 state of GLP-1 resistance, with impaired GLP-1 evoked gut-brain signalling and enteric nervous  
273 system function (52). A clinical trial in IBS patients found that administration of a GLP-1  
274 mimetic reduced acute abdominal pain in patients (57). GLP-1 can act as a classical endocrine  
275 hormone, however GLP-1 also has direct neurostimulatory actions on vagal afferent neurons  
276 (78). Furthermore, there is evidence of direct, physical contact between a pseudopod-like  
277 elongation of L-cells and afferent nerve fibres (18), providing for a potential neural signalling  
278 pathway in the modification of GI function. Thus, L-cells are appropriately positioned to  
279 facilitate cross-barrier signalling from the gut lumen to the host peripheral nervous system and  
280 on to the CNS, and should be investigated as a potential modulator of visceral pain.

### 281 *Histamine release from mast cells*

282 Histamine, which is mainly secreted by mast cells, promotes allergic inflammation but also  
283 appears to play a role in visceral nociception. Indeed, histamine-containing secretions from IBS  
284 patient mucosal mast cells have been shown to excite rat nociceptive visceral afferent nerves,  
285 and are thus likely to participate in relaying visceral pain signals (13). Of the four histamine  
286 receptor subtypes, H1R and H2R are most prevalent in the gut. Similar to the opposing actions of  
287 PAR subtypes described earlier, activation of H1R promotes pro-inflammatory pathways (30),  
288 whereas H2R suppresses inflammation (111). In patients with IBD, reduced expression of H2R  
289 may underlie decreased suppression of TLR-induced cytokine secretion in this patient population  
290 (111). H1R antagonists decreased abdominal pain in IBS patients (68) and in a rat model of  
291 visceral hypersensitivity (115). Moreover, IBS patient biopsies display increased expression of

292 H1R (106). Histamine may also be secreted by bacterial species such as *Lactobacillus reuteri*  
293 6475, a commonly-used probiotic (114), which can reduce intestinal inflammation (48) and may  
294 also have an impact of visceral pain sensitivity.

### 295 **Vagal afferent pathways**

296 Vagal afferent neurons may also participate in the sensory arm of gut-brain nociceptive  
297 signaling. Although electrical stimulation of abdominal vagal afferents does not induce pain *per*  
298 *se*, nociceptive signaling may be modulated by vagal activity (7). Vagal nerve activation may in  
299 fact, induce an inhibitory modulation of chemically or mechanically-provoked insults (29, 53), as  
300 noted in a rat model of visceral pain where vagal nerve stimulation had an anti-nociceptive effect  
301 (138). Vagal afferent terminals are located within enteric ganglia, and in the smooth muscle and  
302 mucosal layers, where they are well-positioned to sense chemo-nociceptive signals (70, 95, 130).  
303 Given the essential role of the vagus nerve in mediating microbe-gut-brain communication (15,  
304 23), future work should address whether modulation of vagal afferent pathways by bacteria  
305 impacts visceral pain.

### 306 **Conclusions**

307 There is abundant evidence that the microbiota is capable of modifying visceral pain *in vivo*.  
308 However, clinical trials of probiotics as therapies for visceral pain have yielded equivocal results.  
309 This may reflect patient heterogeneity, patient compliance, or the variety of probiotic  
310 formulations used, which is in turn reflects a relative paucity of mechanistic work identifying the  
311 most important microbial species and mediators to target for clinical benefit. A number of issues  
312 remain unresolved in bridging the gaps between our present state of knowledge and successful  
313 manipulation of the gut microbiota to alleviate pain. For example, the detection of high  
314 threshold noxious stimuli in rodents is accomplished by visceral afferent neurons with terminals

315 that lie along serosal and mesenteric blood vessels (25). Furthermore, based on a limited number  
316 of recordings from visceral afferent neurons from human bowel, the majority of afferent  
317 terminals that have been characterized to date have been located in the muscle and vasculature.  
318 Thus, it appears that luminal mediators from the microbiota may have traverse the epithelial  
319 barrier and enter the circulation to access and modulate gut nociceptive terminals. Future studies  
320 of full-thickness resected bowel preparations from patients may provide insight into how the  
321 luminal microbiota accesses these terminals. Another potential caveat when translating findings  
322 from rodents to patients is that signaling mechanisms that are inhibitory in rodents may be  
323 excitatory in patients, and vice versa. A recent  $Ca^{2+}$  imaging study of PAR activation in human  
324 DRG neurons reported that PAR-1 activation in human neurons is excitatory (43), whereas PAR-  
325 1 is inhibitory in rodents (10). By increasing mechanistic insights into the interplay between  
326 the microbiota and peripheral pain pathways, particularly using patient microbiota and human  
327 DRG neurons (59), improved therapies that harness the analgesic properties of the microbiota  
328 may soon be on the horizon.

329

330



331

332 **References**

- 333 1. **Agostini S, Gubern M, Tondereau V, Salvador-Cartier C, Bezirard V, Leveque M,**  
334 **Keranen H, Theodorou V, Bourdu-Naturel S, Goupil-Feuillerat N, Legrain-Raspaud S, and**  
335 **Eutamene H.** A marketed fermented dairy product containing *Bifidobacterium lactis* CNCM I-  
336 2494 suppresses gut hypersensitivity and colonic barrier disruption induced by acute stress in  
337 rats. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal*  
338 *Motility Society* 24: 376-e172, 2012.
- 339 2. **Ait-Belgnaoui A, Eutamene H, Houdeau E, Bueno L, Fioramonti J, and Theodorou V.**  
340 *Lactobacillus farciminis* treatment attenuates stress-induced overexpression of Fos protein in  
341 spinal and supraspinal sites after colorectal distension in rats. *Neurogastroenterology and*  
342 *motility : the official journal of the European Gastrointestinal Motility Society* 21: 567-573,  
343 e518-569, 2009.
- 344 3. **Ait-Belgnaoui A, Han W, Lamine F, Eutamene H, Fioramonti J, Bueno L, and Theodorou**  
345 **V.** *Lactobacillus farciminis* treatment suppresses stress induced visceral hypersensitivity: a  
346 possible action through interaction with epithelial cell cytoskeleton contraction. *Gut* 55: 1090-  
347 1094, 2006.
- 348 4. **Ait-Belgnaoui A, Payard I, Rolland C, Harkat C, Braniste V, Theodorou V, and Tompkins**  
349 **TA.** *Bifidobacterium longum* and *Lactobacillus helveticus* Synergistically Suppress Stress-related  
350 Visceral Hypersensitivity Through Hypothalamic-Pituitary-Adrenal Axis Modulation. *J*  
351 *Neurogastroenterol Motil* 24: 138-146, 2018.
- 352 5. **Al-Nedawi K, Mian MF, Hossain N, Karimi K, Mao Y, Forsythe P, Min KK, Stanisz AM,**  
353 **Kunze WA, and Bienenstock J.** Gut commensal microvesicles reproduce parent bacterial signals  
354 to host immune and enteric nervous systems. *FASEB J*, 2014.
- 355 6. **Amadesi S, Cottrell GS, Divino L, Chapman K, Grady EF, Bautista F, Karanjia R, Barajas-**  
356 **Lopez C, Vanner S, Vergnolle N, and Bunnett NW.** Protease-activated receptor 2 sensitizes  
357 TRPV1 by protein kinase C epsilon- and A-dependent mechanisms in rats and mice. *The Journal*  
358 *of physiology* 575: 555-571, 2006.
- 359 7. **Andrews PL and Sanger GJ.** Abdominal vagal afferent neurones: an important target for  
360 the treatment of gastrointestinal dysfunction. *Current opinion in pharmacology* 2: 650-656,  
361 2002.
- 362 8. **Annahazi A, Gecse K, Dabek M, Ait-Belgnaoui A, Rosztoczy A, Roka R, Molnar T,**  
363 **Theodorou V, Wittmann T, Bueno L, and Eutamene H.** Fecal proteases from diarrheic-IBS and  
364 ulcerative colitis patients exert opposite effect on visceral sensitivity in mice. *Pain* 144: 209-217,  
365 2009.
- 366 9. **Aoki R, Kamikado K, Suda W, Takii H, Mikami Y, Suganuma N, Hattori M, and Koga Y.** A  
367 proliferative probiotic *Bifidobacterium* strain in the gut ameliorates progression of metabolic  
368 disorders via microbiota modulation and acetate elevation. *Sci Rep* 7: 43522, 2017.
- 369 10. **Asfaha S, Brussee V, Chapman K, Zochodne DW, and Vergnolle N.** Proteinase-activated  
370 receptor-1 agonists attenuate nociception in response to noxious stimuli. *British journal of*  
371 *pharmacology* 135: 1101-1106, 2002.

- 372 11. **Auge C, Balz-Hara D, Steinhoff M, Vergnolle N, and Cenac N.** Protease-activated  
373 receptor-4 (PAR 4): a role as inhibitor of visceral pain and hypersensitivity.  
374 *Neurogastroenterology and motility : the official journal of the European Gastrointestinal*  
375 *Motility Society* 21: 1189-e1107, 2009.
- 376 12. **Barbara G, Stanghellini V, De Giorgio R, Cremon C, Cottrell GS, Santini D, Pasquinelli G,**  
377 **Morselli-Labate AM, Grady EF, Bunnett NW, Collins SM, and Corinaldesi R.** Activated mast  
378 cells in proximity to colonic nerves correlate with abdominal pain in irritable bowel syndrome.  
379 *Gastroenterology* 126: 693-702, 2004.
- 380 13. **Barbara G, Wang B, Stanghellini V, de Giorgio R, Cremon C, Di Nardo G, Trevisani M,**  
381 **Campi B, Geppetti P, Tonini M, Bunnett NW, Grundy D, and Corinaldesi R.** Mast cell-  
382 dependent excitation of visceral-nociceptive sensory neurons in irritable bowel syndrome.  
383 *Gastroenterology* 132: 26-37, 2007.
- 384 14. **Barrett E, Ross RP, O'Toole PW, Fitzgerald GF, and Stanton C.** gamma-Aminobutyric  
385 acid production by culturable bacteria from the human intestine. *J Appl Microbiol* 113: 411-417,  
386 2012.
- 387 15. **Bercik P, Park AJ, Sinclair D, Khoshdel A, Lu J, Huang X, Deng Y, Blennerhassett PA,**  
388 **Fahnestock M, Moine D, Berger B, Huizinga JD, Kunze W, McLean PG, Bergonzelli GE, Collins**  
389 **SM, and Verdu EF.** The anxiolytic effect of *Bifidobacterium longum* NCC3001 involves vagal  
390 pathways for gut-brain communication. *Neurogastroenterology and motility : the official*  
391 *journal of the European Gastrointestinal Motility Society* 23: 1132-1139, 2011.
- 392 16. **Beyak MJ and Vanner S.** Inflammation-induced hyperexcitability of nociceptive  
393 gastrointestinal DRG neurones: the role of voltage-gated ion channels. *NeurogastroenterolMotil*  
394 17: 175-186, 2005.
- 395 17. **Bielefeldt K, Davis B, and Binion DG.** Pain and inflammatory bowel disease.  
396 *Inflammatory bowel diseases* 15: 778-788, 2009.
- 397 18. **Bohorquez DV, Shahid RA, Erdmann A, Kreger AM, Wang Y, Calakos N, Wang F, and**  
398 **Liddle RA.** Neuroepithelial circuit formed by innervation of sensory enteroendocrine cells. *J Clin*  
399 *Invest* 125: 782-786, 2015.
- 400 19. **Bonaz B, Bazin T, and Pellissier S.** The Vagus Nerve at the Interface of the Microbiota-  
401 Gut-Brain Axis. *Front Neurosci* 12: 49, 2018.
- 402 20. **Bonaz BL and Bernstein CN.** Brain-gut interactions in inflammatory bowel disease.  
403 *Gastroenterology* 144: 36-49, 2013.
- 404 21. **Botschuijver S, Roeselers G, Levin E, Jonkers DM, Welting O, Heinsbroek SEM, de**  
405 **Weerd HH, Boekhout T, Fornai M, Masclee AA, Schuren FHJ, de Jonge WJ, Seppen J, and van**  
406 **den Wijngaard RM.** Intestinal Fungal Dysbiosis Is Associated With Visceral Hypersensitivity in  
407 Patients With Irritable Bowel Syndrome and Rats. *Gastroenterology* 153: 1026-1039, 2017.
- 408 22. **Boue J, Basso L, Cenac N, Blanpied C, Rolli-Derkinderen M, Neunlist M, Vergnolle N,**  
409 **and Dietrich G.** Endogenous regulation of visceral pain via production of opioids by colitogenic  
410 CD4(+) T cells in mice. *Gastroenterology* 146: 166-175, 2014.
- 411 23. **Bravo JA, Forsythe P, Chew MV, Escaravage E, Savignac HM, Dinan TG, Bienenstock J,**  
412 **and Cryan JF.** Ingestion of *Lactobacillus* strain regulates emotional behavior and central GABA  
413 receptor expression in a mouse via the vagus nerve. *Proc Natl Acad Sci U S A* 108: 16050-16055,  
414 2011.

- 415 24. **Bravo JA, Julio-Pieper M, Forsythe P, Kunze W, Dinan TG, Bienenstock J, and Cryan JF.**  
416 Communication between gastrointestinal bacteria and the nervous system. *Current opinion in*  
417 *pharmacology* 12: 667-672, 2012.
- 418 25. **Brierley SM, Hibberd TJ, and Spencer NJ.** Spinal Afferent Innervation of the Colon and  
419 Rectum. *Front Cell Neurosci* 12: 467, 2018.
- 420 26. **Brierley SM and Linden DR.** Neuroplasticity and dysfunction after gastrointestinal  
421 inflammation. *Nature reviews Gastroenterology & hepatology* 11: 611-627, 2014.
- 422 27. **Cattaruzza F, Lyo V, Jones E, Pham D, Hawkins J, Kirkwood K, Valdez-Morales E,**  
423 **Ibeakanma C, Vanner SJ, Bogyo M, and Bunnett NW.** Cathepsin S is activated during colitis and  
424 causes visceral hyperalgesia by a PAR2-dependent mechanism in mice. *Gastroenterology* 141:  
425 1864-1874 e1861-1863, 2011.
- 426 28. **Ceuleers H, Hanning N, Heirbaut J, Van Remoortel S, Joossens J, Van Der Veken P,**  
427 **Francque SM, De Bruyn M, Lambeir AM, De Man JG, Timmermans JP, Augustyns K, De**  
428 **Meester I, and De Winter BY.** Newly developed serine protease inhibitors decrease visceral  
429 hypersensitivity in a post-inflammatory rat model for irritable bowel syndrome. *British journal*  
430 *of pharmacology* 175: 3516-3533, 2018.
- 431 29. **Chen SL, Wu XY, Cao ZJ, Fan J, Wang M, Owyang C, and Li Y.** Subdiaphragmatic vagal  
432 afferent nerves modulate visceral pain. *American journal of physiology* 294: G1441-1449, 2008.
- 433 30. **Chen X, Egly C, Riley AM, Li W, Tewson P, Hughes TE, Quinn AM, and Obukhov AG.**  
434 PKC-dependent Phosphorylation of the H1 Histamine Receptor Modulates TRPC6 Activity. *Cells*  
435 3: 247-257, 2014.
- 436 31. **Chimerel C, Emery E, Summers DK, Keyser U, Gribble FM, and Reimann F.** Bacterial  
437 metabolite indole modulates incretin secretion from intestinal enteroendocrine L cells. *Cell Rep*  
438 9: 1202-1208, 2014.
- 439 32. **Chiu IM, Heesters BA, Ghasemlou N, Von Hehn CA, Zhao F, Tran J, Wainger B,**  
440 **Strominger A, Muralidharan S, Horswill AR, Bubeck Wardenburg J, Hwang SW, Carroll MC,**  
441 **and Woolf CJ.** Bacteria activate sensory neurons that modulate pain and inflammation. *Nature*  
442 501: 52-57, 2013.
- 443 33. **Clarke G, Stilling RM, Kennedy PJ, Stanton C, Cryan JF, and Dinan TG.** Minireview: Gut  
444 microbiota: the neglected endocrine organ. *Mol Endocrinol* 28: 1221-1238, 2014.
- 445 34. **Coelho AM, Vergnolle N, Guiard B, Fioramonti J, and Bueno L.** Proteinases and  
446 proteinase-activated receptor 2: a possible role to promote visceral hyperalgesia in rats.  
447 *Gastroenterology* 122: 1035-1047, 2002.
- 448 35. **Crouzet L, Gaultier E, Del'Homme C, Cartier C, Delmas E, Dapoigny M, Fioramonti J,**  
449 **and Bernalier-Donadille A.** The hypersensitivity to colonic distension of IBS patients can be  
450 transferred to rats through their fecal microbiota. *Neurogastroenterol Motil* 25: e272-282,  
451 2013.
- 452 36. **Dai C, Guandalini S, Zhao DH, and Jiang M.** Antinociceptive effect of VSL#3 on visceral  
453 hypersensitivity in a rat model of irritable bowel syndrome: a possible action through nitric  
454 oxide pathway and enhance barrier function. *Mol Cell Biochem* 362: 43-53, 2012.
- 455 37. **Danzebrink RM and Gebhart GF.** Antinociceptive effects of intrathecal adrenoceptor  
456 agonists in a rat model of visceral nociception. *J Pharmacol Exp Ther* 253: 698-705, 1990.
- 457 38. **De Filippo C, Cavalieri D, Di Paola M, Ramazzotti M, Poullet JB, Massart S, Collini S,**  
458 **Pieraccini G, and Lionetti P.** Impact of diet in shaping gut microbiota revealed by a comparative

459 study in children from Europe and rural Africa. *Proc Natl Acad Sci U S A* 107: 14691-14696,  
460 2010.

461 39. **De Palma G, Blennerhassett P, Lu J, Deng Y, Park AJ, Green W, Denou E, Silva MA,**  
462 **Santacruz A, Sanz Y, Surette MG, Verdu EF, Collins SM, and Bercik P.** Microbiota and host  
463 determinants of behavioural phenotype in maternally separated mice. *Nature communications*  
464 6: 7735, 2015.

465 40. **De Palma G, Collins SM, and Bercik P.** The microbiota-gut-brain axis in functional  
466 gastrointestinal disorders. *Gut microbes* 5: 419-429, 2014.

467 41. **De Palma G, Lynch MD, Lu J, Dang VT, Deng Y, Jury J, Umeh G, Miranda PM, Pigrau**  
468 **Pastor M, Sidani S, Pinto-Sanchez MI, Philip V, McLean PG, Hagelsieb MG, Surette MG,**  
469 **Bergonzelli GE, Verdu EF, Britz-McKibbin P, Neufeld JD, Collins SM, and Bercik P.**  
470 Transplantation of fecal microbiota from patients with irritable bowel syndrome alters gut  
471 function and behavior in recipient mice. *Science translational medicine* 9, 2017.

472 42. **Delafroy L, Gelot A, Ardid D, Eschalier A, Bertrand C, Doherty AM, and Diop L.**  
473 Interactive involvement of brain derived neurotrophic factor, nerve growth factor, and  
474 calcitonin gene related peptide in colonic hypersensitivity in the rat. *Gut* 55: 940-945, 2006.

475 43. **Desormeaux C, Bautzova T, Garcia-Caraballo S, Rolland C, Barbaro MR, Brierley SM,**  
476 **Barbara G, Vergnolle N, and Cenac N.** Protease-activated receptor 1 is implicated in irritable  
477 bowel syndrome mediators-induced signaling to thoracic human sensory neurons. *Pain* 159:  
478 1257-1267, 2018.

479 44. **Distrutti E, Cipriani S, Mencarelli A, Renga B, and Fiorucci S.** Probiotics VSL#3 protect  
480 against development of visceral pain in murine model of irritable bowel syndrome. *PLoS one* 8:  
481 e63893, 2013.

482 45. **Drossman DA, Morris CB, Edwards H, Wrennall CE, Weinland SR, Aderoju AO, Kulkarni-**  
483 **Kelapure RR, Hu YJ, Dalton C, Bouma MH, Zimmerman J, Rooker C, Leserman J, and**  
484 **Bangdiwala SI.** Diagnosis, characterization, and 3-month outcome after detoxification of 39  
485 patients with narcotic bowel syndrome. *The American journal of gastroenterology* 107: 1426-  
486 1440, 2012.

487 46. **Eutamene H, Lamine F, Chabo C, Theodorou V, Rochat F, Bergonzelli GE, Cortesy-**  
488 **Theulaz I, Fioramonti J, and Bueno L.** Synergy between *Lactobacillus paracasei* and its bacterial  
489 products to counteract stress-induced gut permeability and sensitivity increase in rats. *The*  
490 *Journal of nutrition* 137: 1901-1907, 2007.

491 47. **Forsythe P, Bienenstock J, and Kunze WA.** Vagal pathways for microbiome-brain-gut  
492 axis communication. *Advances in experimental medicine and biology* 817: 115-133, 2014.

493 48. **Ganesh BP, Hall A, Ayyaswamy S, Nelson JW, Fultz R, Major A, Haag A, Esparza M,**  
494 **Lugo M, Venable S, Whary M, Fox JG, and Versalovic J.** Diacylglycerol kinase synthesized by  
495 commensal *Lactobacillus reuteri* diminishes protein kinase C phosphorylation and histamine-  
496 mediated signaling in the mammalian intestinal epithelium. *Mucosal Immunol* 11: 380-393,  
497 2018.

498 49. **Gecse K, Roka R, Ferrier L, Leveque M, Eutamene H, Cartier C, Ait-Belgnaoui A,**  
499 **Rosztoczy A, Izbeki F, Fioramonti J, Wittmann T, and Bueno L.** Increased faecal serine protease  
500 activity in diarrhoeic IBS patients: a colonic luminal factor impairing colonic permeability and  
501 sensitivity. *Gut* 57: 591-599, 2008.

- 502 50. **Gershon MD.** Serotonin is a sword and a shield of the bowel: serotonin plays offense  
503 and defense. *Transactions of the American Clinical and Climatological Association* 123: 268-280;  
504 discussion 280, 2012.
- 505 51. **Grant AD, Cottrell GS, Amadesi S, Trevisani M, Nicoletti P, Materazzi S, Altier C, Cenac**  
506 **N, Zamponi GW, Bautista-Cruz F, Lopez CB, Joseph EK, Levine JD, Liedtke W, Vanner S,**  
507 **Vergnolle N, Geppetti P, and Bunnett NW.** Protease-activated receptor 2 sensitizes the  
508 transient receptor potential vanilloid 4 ion channel to cause mechanical hyperalgesia in mice.  
509 *The Journal of physiology* 578: 715-733, 2007.
- 510 52. **Grasset E, Puel A, Charpentier J, Collet X, Christensen JE, Terce F, and Burcelin R.** A  
511 Specific Gut Microbiota Dysbiosis of Type 2 Diabetic Mice Induces GLP-1 Resistance through an  
512 Enteric NO-Dependent and Gut-Brain Axis Mechanism. *Cell metabolism* 25: 1075-1090 e1075,  
513 2017.
- 514 53. **Gschossmann JM, Mayer EA, Miller JC, and Raybould HE.** Subdiaphragmatic vagal  
515 afferent innervation in activation of an opioidergic antinociceptive system in response to  
516 colorectal distension in rats. *Neurogastroenterol Motil* 14: 403-408, 2002.
- 517 54. **Guo CJ, Chang FY, Wyche TP, Backus KM, Acker TM, Funabashi M, Taketani M, Donia**  
518 **MS, Nayfach S, Pollard KS, Craik CS, Cravatt BF, Clardy J, Voigt CA, and Fischbach MA.**  
519 Discovery of Reactive Microbiota-Derived Metabolites that Inhibit Host Proteases. *Cell* 168:  
520 517-526 e518, 2017.
- 521 55. **Hanson KA, Loftus EV, Jr., Harmsen WS, Diehl NN, Zinsmeister AR, and Sandborn WJ.**  
522 Clinical features and outcome of patients with inflammatory bowel disease who use narcotics: a  
523 case-control study. *Inflammatory bowel diseases* 15: 772-777, 2009.
- 524 56. **Hara K, Saito Y, Kirihara Y, Yamada Y, Sakura S, and Kosaka Y.** The interaction of  
525 antinociceptive effects of morphine and GABA receptor agonists within the rat spinal cord.  
526 *Anesth Analg* 89: 422-427, 1999.
- 527 57. **Hellstrom PM, Hein J, Bytzer P, Bjornsson E, Kristensen J, and Schambye H.** Clinical  
528 trial: the glucagon-like peptide-1 analogue ROSE-010 for management of acute pain in patients  
529 with irritable bowel syndrome: a randomized, placebo-controlled, double-blind study. *Aliment*  
530 *Pharmacol Ther* 29: 198-206, 2009.
- 531 58. **Hoban AE, Moloney RD, Golubeva AV, McVey Neufeld KA, O'Sullivan O, Patterson E,**  
532 **Stanton C, Dinan TG, Clarke G, and Cryan JF.** Behavioural and neurochemical consequences of  
533 chronic gut microbiota depletion during adulthood in the rat. *Neuroscience* 339: 463-477, 2016.
- 534 59. **Hockley JRF, Smith ESJ, and Bulmer DC.** Human visceral nociception: findings from  
535 translational studies in human tissue. *Am J Physiol Gastrointest Liver Physiol* 315: G464-G472,  
536 2018.
- 537 60. **Hughes P, Brierly S, and Blackshaw LA.** Post inflammatory modification of colonic  
538 afferent mechanosensitivity. *ClinExpPharmacolPhysiol*, 2009.
- 539 61. **Hwang I, Park YJ, Kim YR, Kim YN, Ka S, Lee HY, Seong JK, Seok YJ, and Kim JB.**  
540 Alteration of gut microbiota by vancomycin and bacitracin improves insulin resistance via  
541 glucagon-like peptide 1 in diet-induced obesity. *FASEB J* 29: 2397-2411, 2015.
- 542 62. **Hyland NP and Cryan JF.** A Gut Feeling about GABA: Focus on GABA(B) Receptors.  
543 *Frontiers in pharmacology* 1: 124, 2010.
- 544 63. **Ibeakanma C, Ochoa-Cortes F, Miranda-Morales M, McDonald T, Spreadbury I, Cenac**  
545 **N, Cattaruzza F, Hurlbut D, Vanner S, Bunnett N, Vergnolle N, and Vanner S.** Brain-gut

- 546 interactions increase peripheral nociceptive signaling in mice with postinfectious irritable bowel  
547 syndrome. *Gastroenterology* 141: 2098-2108 e2095, 2011.
- 548 64. **Johnson AC, Greenwood-Van Meerveld B, and McRorie J.** Effects of *Bifidobacterium*  
549 *infantis* 35624 on post-inflammatory visceral hypersensitivity in the rat. *Digestive diseases and*  
550 *sciences* 56: 3179-3186, 2011.
- 551 65. **Kannampalli P, Pochiraju S, Chichlowski M, Berg BM, Rudolph C, Bruckert M, Miranda**  
552 **A, and Sengupta JN.** Probiotic *Lactobacillus rhamnosus* GG (LGG) and prebiotic prevent  
553 neonatal inflammation-induced visceral hypersensitivity in adult rats. *Neurogastroenterology*  
554 *and motility : the official journal of the European Gastrointestinal Motility Society* 26: 1694-  
555 1704, 2014.
- 556 66. **Karanja R, Spreadbury I, Bautista-Cruz F, Tsang ME, and Vanner S.** Activation of  
557 protease-activated receptor-4 inhibits the intrinsic excitability of colonic dorsal root ganglia  
558 neurons. *Neurogastroenterology and motility : the official journal of the European*  
559 *Gastrointestinal Motility Society* 21: 1218-1221, 2009.
- 560 67. **Kayssi A, Amadesi S, Bautista F, Bunnnett NW, and Vanner S.** Mechanisms of protease-  
561 activated receptor 2-evoked hyperexcitability of nociceptive neurons innervating the mouse  
562 colon. *J Physiol* 580: 977-991, 2007.
- 563 68. **Klooker TK, Braak B, Koopman KE, Welting O, Wouters MM, van der Heide S,**  
564 **Schemann M, Bischoff SC, van den Wijngaard RM, and Boeckstaens GE.** The mast cell  
565 stabiliser ketotifen decreases visceral hypersensitivity and improves intestinal symptoms in  
566 patients with irritable bowel syndrome. *Gut* 59: 1213-1221, 2010.
- 567 69. **Kwon YH, Wang H, Denou E, Ghia JE, Rossi L, Fontes ME, Bernier SP, Shajib MS,**  
568 **Banskota S, Collins SM, Surette MG, and Khan WI.** Modulation of Gut Microbiota Composition  
569 by Serotonin Signaling Influences Intestinal Immune Response and Susceptibility to Colitis.  
570 *Cellular and molecular gastroenterology and hepatology* 7: 709-728, 2019.
- 571 70. **Lamb K, Kang YM, Gebhart GF, and Bielefeldt K.** Gastric inflammation triggers  
572 hypersensitivity to acid in awake rats. *Gastroenterology* 125: 1410-1418, 2003.
- 573 71. **Li YJ, Dai C, and Jiang M.** Mechanisms of Probiotic VSL#3 in a Rat Model of Visceral  
574 Hypersensitivity Involves the Mast Cell-PAR2-TRPV1 Pathway. *Digestive diseases and sciences*  
575 64: 1182-1192, 2019.
- 576 72. **Louis P, Hold GL, and Flint HJ.** The gut microbiota, bacterial metabolites and colorectal  
577 cancer. *Nat Rev Microbiol* 12: 661-672, 2014.
- 578 73. **Lu Y and Westlund KN.** Effects of baclofen on colon inflammation-induced Fos, CGRP  
579 and SP expression in spinal cord and brainstem. *Brain Res* 889: 118-130, 2001.
- 580 74. **Luczynski P, Tramullas M, Viola M, Shanahan F, Clarke G, O'Mahony S, Dinan TG, and**  
581 **Cryan JF.** Microbiota regulates visceral pain in the mouse. *eLife* 6, 2017.
- 582 75. **Ma D, Forsythe P, and Bienenstock J.** Live *Lactobacillus rhamnosus* [corrected] is  
583 essential for the inhibitory effect on tumor necrosis factor alpha-induced interleukin-8  
584 expression. *Infect Immun* 72: 5308-5314, 2004.
- 585 76. **Martin CR, Osadchiy V, Kalani A, and Mayer EA.** The Brain-Gut-Microbiome Axis. *Cell*  
586 *Mol Gastroenterol Hepatol* 6: 133-148, 2018.
- 587 77. **Mawe GM and Hoffman JM.** Serotonin signalling in the gut--functions, dysfunctions and  
588 therapeutic targets. *Nature reviews Gastroenterology & hepatology* 10: 473-486, 2013.

- 589 78. **McKee DP and Quigley EM.** Intestinal motility in irritable bowel syndrome: is IBS a  
590 motility disorder? Part 1. Definition of IBS and colonic motility. *Digestive diseases and sciences*  
591 38: 1761-1772, 1993.
- 592 79. **McKernan DP, Fitzgerald P, Dinan TG, and Cryan JF.** The probiotic Bifidobacterium  
593 infantis 35624 displays visceral antinociceptive effects in the rat. *Neurogastroenterology and*  
594 *motility : the official journal of the European Gastrointestinal Motility Society* 22: 1029-1035,  
595 e1268, 2010.
- 596 80. **McVey Neufeld KA, Mao YK, Bienenstock J, Foster JA, and Kunze WA.** The microbiome  
597 is essential for normal gut intrinsic primary afferent neuron excitability in the mouse.  
598 *Neurogastroenterology and motility : the official journal of the European Gastrointestinal*  
599 *Motility Society* 25: 183-e188, 2013.
- 600 81. **Miquel S, Martin R, Lashermes A, Gillet M, Meleine M, Gelot A, Eschalier A, Ardid D,**  
601 **Bermudez-Humaran LG, Sokol H, Thomas M, Theodorou V, Langella P, and Carvalho FA.** Anti-  
602 nociceptive effect of Faecalibacterium prausnitzii in non-inflammatory IBS-like models. *Sci Rep*  
603 6: 19399, 2016.
- 604 82. **Mkaouer H, Akermi N, Mariaule V, Boudebouze S, Gaci N, Szukala F, Pons N, Marquez**  
605 **J, Gargouri A, Maguin E, and Rhimi M.** Siropins, novel serine protease inhibitors from gut  
606 microbiota acting on human proteases involved in inflammatory bowel diseases. *Microb Cell*  
607 *Fact* 15: 201, 2016.
- 608 83. **Morrison DJ and Preston T.** Formation of short chain fatty acids by the gut microbiota  
609 and their impact on human metabolism. *Gut Microbes* 7: 189-200, 2016.
- 610 84. **Muller PA, Koscsó B, Rajani GM, Stevanovic K, Berres ML, Hashimoto D, Mortha A,**  
611 **Leboeuf M, Li XM, Mucida D, Stanley ER, Dahan S, Margolis KG, Gershon MD, Merad M, and**  
612 **Bogunovic M.** Crosstalk between muscularis macrophages and enteric neurons regulates  
613 gastrointestinal motility. *Cell* 158: 300-313, 2014.
- 614 85. **Nasser Y, Boeckxstaens GE, Wouters MM, Schemann M, and Vanner S.** Using human  
615 intestinal biopsies to study the pathogenesis of irritable bowel syndrome.  
616 *Neurogastroenterology and motility : the official journal of the European Gastrointestinal*  
617 *Motility Society* 26: 455-469, 2014.
- 618 86. **Nebot-Vivinus M, Harkat C, Bziouche H, Cartier C, Plichon-Dainese R, Moussa L,**  
619 **Eutamene H, Pishvaie D, Holowacz S, Seyrig C, Piche T, and Theodorou V.** Multispecies  
620 probiotic protects gut barrier function in experimental models. *World J Gastroenterol* 20: 6832-  
621 6843, 2014.
- 622 87. **Neufeld KM, Kang N, Bienenstock J, and Foster JA.** Reduced anxiety-like behavior and  
623 central neurochemical change in germ-free mice. *Neurogastroenterol Motil* 23: 255-264, e119,  
624 2011.
- 625 88. **O' Mahony SM, Dinan TG, and Cryan JF.** The gut microbiota as a key regulator of  
626 visceral pain. *Pain* 158 Suppl 1: S19-S28, 2017.
- 627 89. **O'Mahony L, McCarthy J, Kelly P, Hurley G, Luo F, Chen K, O'Sullivan GC, Kiely B,**  
628 **Collins JK, Shanahan F, and Quigley EM.** Lactobacillus and bifidobacterium in irritable bowel  
629 syndrome: symptom responses and relationship to cytokine profiles. *Gastroenterology* 128:  
630 541-551, 2005.
- 631 90. **O'Mahony SM, Felice VD, Nally K, Savignac HM, Claesson MJ, Scully P, Woznicki J,**  
632 **Hyland NP, Shanahan F, Quigley EM, Marchesi JR, O'Toole PW, Dinan TG, and Cryan JF.**

633 Disturbance of the gut microbiota in early-life selectively affects visceral pain in adulthood  
634 without impacting cognitive or anxiety-related behaviors in male rats. *Neuroscience* 277: 885-  
635 901, 2014.

636 91. **Ochoa-Cortes F, Ramos-Lomas T, Miranda-Morales M, Spreadbury I, Ibeakanma C,**  
637 **Barajas-Lopez C, and Vanner S.** Bacterial cell products signal to mouse colonic nociceptive  
638 dorsal root ganglia neurons. *AmJ Physiol GastrointestLiver Physiol* 299: G723-G732, 2010.

639 92. **Okumura T, Nozu T, Kumei S, Takakusaki K, Miyagishi S, and Ohhira M.** Involvement of  
640 the dopaminergic system in the central orexin-induced antinociceptive action against colonic  
641 distension in conscious rats. *Neuroscience letters* 605: 34-38, 2015.

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

646 94. **Pokusaeva K, Johnson C, Luk B, Uribe G, Fu Y, Oezguen N, Matsunami RK, Lugo M,**  
647 **Major A, Mori-Akiyama Y, Hollister EB, Dann SM, Shi XZ, Engler DA, Savidge T, and Versalovic**  
648 **J.** GABA-producing *Bifidobacterium dentium* modulates visceral sensitivity in the intestine.  
649 *Neurogastroenterology and motility : the official journal of the European Gastrointestinal*  
650 *Motility Society* 29, 2017.

651 95. **Powley TL, Spaulding RA, and Haglof SA.** Vagal afferent innervation of the proximal  
652 gastrointestinal tract mucosa: chemoreceptor and mechanoreceptor architecture. *J Comp*  
653 *Neurol* 519: 644-660, 2011.

654 96. **Pusceddu MM and Gareau MG.** Visceral pain: gut microbiota, a new hope? *J Biomed Sci*  
655 25: 73, 2018.

656 97. **Ramachandran R, Altier C, Oikonomopoulou K, and Hollenberg MD.** Proteinases, Their  
657 Extracellular Targets, and Inflammatory Signaling. *Pharmacological reviews* 68: 1110-1142,  
658 2016.

659 98. **Rea K, O'Mahony SM, Dinan TG, and Cryan JF.** The Role of the Gastrointestinal  
660 Microbiota in Visceral Pain. *Handbook of experimental pharmacology* 239: 269-287, 2017.

661 99. **Reigstad CS, Salmonson CE, Rainey JF, 3rd, Szurszewski JH, Linden DR, Sonnenburg JL,**  
662 **Farrugia G, and Kashyap PC.** Gut microbes promote colonic serotonin production through an  
663 effect of short-chain fatty acids on enterochromaffin cells. *FASEB J* 29: 1395-1403, 2015.

664 100. **Reijnders D, Goossens GH, Hermes GD, Neis EP, van der Beek CM, Most J, Holst JJ,**  
665 **Lenaerts K, Kootte RS, Nieuwdorp M, Groen AK, Olde Damink SW, Boekschoten MV, Smidt H,**  
666 **Zoetendal EG, Dejong CH, and Blaak EE.** Effects of Gut Microbiota Manipulation by Antibiotics  
667 on Host Metabolism in Obese Humans: A Randomized Double-Blind Placebo-Controlled Trial.  
668 *Cell Metab* 24: 63-74, 2016.

669 101. **Reimann F, Habib AM, Tolhurst G, Parker HE, Rogers GJ, and Gribble FM.** Glucose  
670 sensing in L cells: a primary cell study. *Cell metabolism* 8: 532-539, 2008.

671 102. **Ringel-Kulka T, Goldsmith JR, Carroll IM, Barros SP, Palsson O, Jobin C, and Ringel Y.**  
672 *Lactobacillus acidophilus* NCFM affects colonic mucosal opioid receptor expression in patients  
673 with functional abdominal pain - a randomised clinical study. *Alimentary pharmacology &*  
674 *therapeutics* 40: 200-207, 2014.

675 103. **Roediger WE.** Role of anaerobic bacteria in the metabolic welfare of the colonic mucosa  
676 in man. *Gut* 21: 793-798, 1980.



- 677 104. **Rolland-Fourcade C, Denadai-Souza A, Cirillo C, Lopez C, Jaramillo JO, Desormeaux C,**  
678 **Cenac N, Motta JP, Larauche M, Tache Y, Berghe PV, Neunlist M, Coron E, Kirzin S, Portier G,**  
679 **Bonnet D, Alric L, Vanner S, Deraison C, and Vergnolle N.** Epithelial expression and function of  
680 trypsin-3 in irritable bowel syndrome. *Gut* 66: 1767-1778, 2017.
- 681 105. **Rousseaux C, Thuru X, Gelot A, Barnich N, Neut C, Dubuquoy L, Dubuquoy C, Merour E,**  
682 **Geboes K, Chamailard M, Ouwehand A, Leyer G, Carcano D, Colombel JF, Ardid D, and**  
683 **Desreumaux P.** Lactobacillus acidophilus modulates intestinal pain and induces opioid and  
684 cannabinoid receptors. *Nature medicine* 13: 35-37, 2007.
- 685 106. **Sander LE, Lorentz A, Sellge G, Coeffier M, Neipp M, Veres T, Frieling T, Meier PN,**  
686 **Manns MP, and Bischoff SC.** Selective expression of histamine receptors H1R, H2R, and H4R,  
687 but not H3R, in the human intestinal tract. *Gut* 55: 498-504, 2006.
- 688 107. **Savignac HM, Corona G, Mills H, Chen L, Spencer JP, Tzortzis G, and Burnet PW.**  
689 Prebiotic feeding elevates central brain derived neurotrophic factor, N-methyl-D-aspartate  
690 receptor subunits and D-serine. *Neurochem Int* 63: 756-764, 2013.
- 691 108. **Selwyn FP, Csanaky IL, Zhang Y, and Klaassen CD.** Importance of Large Intestine in  
692 Regulating Bile Acids and Glucagon-Like Peptide-1 in Germ-Free Mice. *Drug Metab Dispos* 43:  
693 1544-1556, 2015.
- 694 109. **Sessenwein JL, Baker CC, Pradhananga S, Maitland ME, Petrof EO, Allen-Vercoe E,**  
695 **Noordhof C, Reed DE, Vanner SJ, and Lomax AE.** Protease-Mediated Suppression of DRG  
696 Neuron Excitability by Commensal Bacteria. *The Journal of neuroscience : the official journal of*  
697 *the Society for Neuroscience* 37: 11758-11768, 2017.
- 698 110. **Shen S, Al-Thumairy HW, Hashmi F, and Qiao LY.** Regulation of transient receptor  
699 potential cation channel subfamily V1 protein synthesis by the phosphoinositide 3-kinase/Akt  
700 pathway in colonic hypersensitivity. *Experimental neurology* 295: 104-115, 2017.
- 701 111. **Smolinska S, Groeger D, Perez NR, Schiavi E, Ferstl R, Frei R, Konieczna P, Akdis CA,**  
702 **Jutel M, and O'Mahony L.** Histamine Receptor 2 is Required to Suppress Innate Immune  
703 Responses to Bacterial Ligands in Patients with Inflammatory Bowel Disease. *Inflammatory*  
704 *bowel diseases* 22: 1575-1586, 2016.
- 705 112. **Song Z, Xie W, Chen S, Strong JA, Print MS, Wang JI, Shareef AF, Ulrich-Lai YM, and**  
706 **Zhang JM.** High-fat diet increases pain behaviors in rats with or without obesity. *Sci Rep* 7:  
707 10350, 2017.
- 708 113. **Soret R, Chevalier J, De Coppet P, Poupeau G, Derkinderen P, Segain JP, and Neunlist**  
709 **M.** Short-chain fatty acids regulate the enteric neurons and control gastrointestinal motility in  
710 rats. *Gastroenterology* 138: 1772-1782, 2010.
- 711 114. **Spinler JK, Sontakke A, Hollister EB, Venable SF, Oh PL, Balderas MA, Saulnier DM,**  
712 **Mistretta TA, Devaraj S, Walter J, Versalovic J, and Highlander SK.** From prediction to function  
713 using evolutionary genomics: human-specific ecotypes of Lactobacillus reuteri have diverse  
714 probiotic functions. *Genome Biol Evol* 6: 1772-1789, 2014.
- 715 115. **Stanisor OI, van Diest SA, Yu Z, Welting O, Bekkali N, Shi J, de Jonge WJ, Boeckxstaens**  
716 **GE, and van den Wijngaard RM.** Stress-induced visceral hypersensitivity in maternally  
717 separated rats can be reversed by peripherally restricted histamine-1-receptor antagonists. *PLoS*  
718 *one* 8: e66884, 2013.
- 719 116. **Steck N, Mueller K, Schemann M, and Haller D.** Bacterial proteases in IBD and IBS. *Gut*  
720 61: 1610-1618, 2012.

721 117. **Steinert RE, Feinle-Bisset C, Asarian L, Horowitz M, Beglinger C, and Geary N.** Ghrelin,  
722 CCK, GLP-1, and PYY(3-36): Secretory Controls and Physiological Roles in Eating and Glycemia in  
723 Health, Obesity, and After RYGB. *Physiological reviews* 97: 411-463, 2017.

724 118. **Stenman LK, Waget A, Garret C, Briand F, Burcelin R, Sulpice T, and Lahtinen S.**  
725 Probiotic B420 and prebiotic polydextrose improve efficacy of antidiabetic drugs in mice.  
726 *Diabetol Metab Syndr* 7: 75, 2015.

727 119. **Strandwitz P.** Neurotransmitter modulation by the gut microbiota. *Brain research* 1693:  
728 128-133, 2018.

729 120. **Sudo N, Chida Y, Aiba Y, Sonoda J, Oyama N, Yu XN, Kubo C, and Koga Y.** Postnatal  
730 microbial colonization programs the hypothalamic-pituitary-adrenal system for stress response  
731 in mice. *The Journal of physiology* 558: 263-275, 2004.

732 121. **Tarrerias AL, Millecamps M, Alloui A, Beaughard C, Kemeny JL, Bourdu S, Bommelaer**  
733 **G, Eschaliier A, Dapoigny M, and Ardid D.** Short-chain fatty acid enemas fail to decrease colonic  
734 hypersensitivity and inflammation in TNBS-induced colonic inflammation in rats. *Pain* 100: 91-  
735 97, 2002.

736 122. **Theodorou V, Ait Belgnaoui A, Agostini S, and Eutamene H.** Effect of commensals and  
737 probiotics on visceral sensitivity and pain in irritable bowel syndrome. *Gut microbes* 5: 430-436,  
738 2014.

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

742 124. **Valdez-Morales E, Guerrero-Alba R, Ochoa-Cortes F, Benson J, Spreadbury I, Hurlbut D,**  
743 **Miranda-Morales M, Lomax AE, and Vanner S.** Release of endogenous opioids during a chronic  
744 IBD model suppresses the excitability of colonic DRG neurons. *Neurogastroenterology and*  
745 *motility : the official journal of the European Gastrointestinal Motility Society* 25: 39-46 e34,  
746 2013.

747 125. **Vanhoutvin SA, Troost FJ, Kilkens TO, Lindsey PJ, Hamer HM, Jonkers DM, Venema K,**  
748 **and Brummer RJ.** The effects of butyrate enemas on visceral perception in healthy volunteers.  
749 *Neurogastroenterol Motil* 21: 952-e976, 2009.

750 126. **Verdu EF, Bercik P, Verma-Gandhu M, Huang XX, Blennerhassett P, Jackson W, Mao Y,**  
751 **Wang L, Rochat F, and Collins SM.** Specific probiotic therapy attenuates antibiotic induced  
752 visceral hypersensitivity in mice. *Gut* 55: 182-190, 2006.

753 127. **Vergnolle N.** Protease-activated receptors as drug targets in inflammation and pain.  
754 *Pharmacology & therapeutics* 123: 292-309, 2009.

755 128. **Vergnolle N.** Protease inhibition as new therapeutic strategy for GI diseases. *Gut* 65:  
756 1215-1224, 2016.

757 129. **Wall R, Cryan JF, Ross RP, Fitzgerald GF, Dinan TG, and Stanton C.** Bacterial neuroactive  
758 compounds produced by psychobiotics. *Adv Exp Med Biol* 817: 221-239, 2014.

759 130. **Wang FB and Powley TL.** Topographic inventories of vagal afferents in gastrointestinal  
760 muscle. *J Comp Neurol* 421: 302-324, 2000.

761 131. **Wu RY, Pasyk M, Wang B, Forsythe P, Bienenstock J, Mao YK, Sharma P, Stanis AM,**  
762 **and Kunze WA.** Spatiotemporal maps reveal regional differences in the effects on gut motility  
763 for *Lactobacillus reuteri* and *rhamnosus* strains. *Neurogastroenterology and motility : the*  
764 *official journal of the European Gastrointestinal Motility Society* 25: e205-214, 2013.

- 765 132. **Xia CM, Gulick MA, Yu SJ, Grider JR, Murthy KS, Kuemmerle JF, Akbarali HI, and Qiao**  
766 **LY.** Up-regulation of brain-derived neurotrophic factor in primary afferent pathway regulates  
767 colon-to-bladder cross-sensitization in rat. *Journal of neuroinflammation* 9: 30, 2012.
- 768 133. **Xu D, Wu X, Grabauskas G, and Owyang C.** Butyrate-induced colonic hypersensitivity is  
769 mediated by mitogen-activated protein kinase activation in rat dorsal root ganglia. *Gut* 62:  
770 1466-1474, 2013.
- 771 134. **Xu XJ, Zhang YL, Liu L, Pan L, and Yao SK.** Increased expression of nerve growth factor  
772 correlates with visceral hypersensitivity and impaired gut barrier function in diarrhoea-  
773 predominant irritable bowel syndrome: a preliminary explorative study. *Alimentary*  
774 *pharmacology & therapeutics* 45: 100-114, 2017.
- 775 135. **Yano JM, Yu K, Donaldson GP, Shastri GG, Ann P, Ma L, Nagler CR, Ismagilov RF,**  
776 **Mazmanian SK, and Hsiao EY.** Indigenous bacteria from the gut microbiota regulate host  
777 serotonin biosynthesis. *Cell* 161: 264-276, 2015.
- 778 136. **Zhao P, Lieu T, Barlow N, Metcalf M, Veldhuis NA, Jensen DD, Kocan M, Sostegni S,**  
779 **Haerteis S, Baraznenok V, Henderson I, Lindstrom E, Guerrero-Alba R, Valdez-Morales EE,**  
780 **Liedtke W, McIntyre P, Vanner SJ, Korbmacher C, and Bunnett NW.** Cathepsin S causes  
781 inflammatory pain via biased agonism of PAR2 and TRPV4. *The Journal of biological chemistry*  
782 289: 27215-27234, 2014.
- 783 137. **Zhou SY, Gilliland M, 3rd, Wu X, Leelasinjaroen P, Zhang G, Zhou H, Ye B, Lu Y, and**  
784 **Owyang C.** FODMAP diet modulates visceral nociception by lipopolysaccharide-mediated  
785 intestinal inflammation and barrier dysfunction. *J Clin Invest* 128: 267-280, 2018.
- 786 138. **Zurowski D, Nowak L, Wordliczek J, Dobrogowski J, and Thor PJ.** Effects of vagus nerve  
787 stimulation in visceral pain model. *Folia Med Cracov* 52: 57-69, 2012.

788

789

790

791 **Table 1: *In vivo* studies of the effects of probiotics on visceral pain.**

Probiotic strain	Reference	Main finding	Proposed mechanism
<i>Lactobacillus rhamnosus</i> and/or prebiotics polydextrose/galactooligosaccharide	(65)	Neonatal zymosan-treated rats treated with probiotic did not exhibit visceral hyperalgesia in response to CRD in adulthood	Altered CNS neurotransmitters
<i>Lactobacillus reuteri</i>	(93)	Inhibited the bradycardia induced by painful gastric distension in rats	TRPV1 modulation
<i>Lactobacillus paracasei</i>	(126)	Normalized visceral sensitivity to CRD in antibiotic treated mice in mice	Immunomodulation
	(46)	Prevented the maternal deprivation increased visceral sensitivity in response to CRD in rats	Epithelial barrier regulation
<i>Lactobacillus acidophilus</i>	(105)	Normalized visceral pain responses to CRD in mice and rats	Altered epithelial expression of opioid and cannabinoid receptors
	(102)	Reduced bloating symptoms in patients with functional bowel diseases experiencing abdominal pain in females	Modulated $\mu$ -opioid receptor expression and activity
<i>Lactobacillus farciminis</i>	(3)	Reversed visceral hypersensitivity induced by partial restraint stress (PRS) in rats	Epithelial barrier regulation
	(2)	Inhibited Fos protein expression at spinal and supraspinal levels as a marker of visceral pain in response to CRD in rats after PRS	None specified
<i>Bifidobacterium infantis</i>	(64)	Reversed post-inflammatory (TNBS) visceral hypersensitivity in rats	Immunomodulation
<i>Bifidobacterium lactis</i>	(1)	Inhibited PRS-induced visceral hypersensitivity in rats	Epithelial barrier regulation
<i>Bifidobacterium longum</i> and <i>Lactobacillus helveticus</i>	(4)	Reduced chronic stress-induced visceral hypersensitivity in mice	Regulation of hypothalamic-pituitary-adrenal axis

<i>Bifidobacterium infantis</i> , <i>Lactobacillus salivarius</i> , <i>Bifidobacterium breve</i>	(79)	Reduced CRD-induced visceral pain behaviours in rats	None specified
<i>Bifidobacterium infantis</i> or <i>Lactobacillus salivarius</i>	(89)	<i>Bifidobacterium infantis</i> decreased visceral pain more than <i>Lactobacillus salivarius</i> or placebo in IBS patients	Immunomodulation
<i>Lactibiane Tolerance</i> ®: <i>Lactobacillus acidophilus</i> , <i>Lactobacillus plantarum</i> , <i>Lactobacillus salivarius</i> <i>Bifidobacterium lactis</i>	(86)	Reversed visceral hypersensitivity induced by water-avoidance stress or IBS fecal supernatant administration in mice	Epithelial barrier regulation
VSL#3 <i>Bifidobacterium</i> ( <i>B. longum</i> , <i>B. infantis</i> and <i>B. breve</i> ); <i>Lactobacillus</i> ( <i>L. acidophilus</i> , <i>L. casei</i> , <i>L. delbrueckii</i> ssp. <i>bulgaricus</i> and <i>L. plantarum</i> ); and <i>Streptococcus salivarius</i> ssp. <i>Thermophilus</i>	(44)	Early life administration of VSL#3 reduced visceral pain perception in a model of IBS in rats	Altered colonic expression of genes influencing pain and inflammation
	(36)	Decreases acetic-acid-induced visceral hypersensitivity in rats	Epithelial barrier regulation
	(71)	Decreases acetic-acid-induced visceral hypersensitivity in rats	Immunomodulation
<i>Faecalibacterium prausnitzii</i>	(81)	Decreased colonic hypersensitivity induced by either NMS in mice or partial restraint stress in rats	Epithelial barrier regulation

792

793 **Figure 1: Microbial modulation of visceral afferent pathways**

794 The figure illustrates potential mechanisms by which microbes in the gut lumen could modify  
795 afferent signaling from the gut to the central nervous system. The microbiota can affect the  
796 sensitivity of peripheral pain pathways by direct effects on the peripheral terminals of DRG  
797 neurons or indirectly by changing mediator release from enteroendocrine cells, immune cells or  
798 enterocytes. NTS: nucleus tractus solitarius, DRG: dorsal root ganglion, ENS: enteric nervous  
799 system, ECC: enterochromaffin cell, TLRs: Toll-like receptors.

800

Figure 1

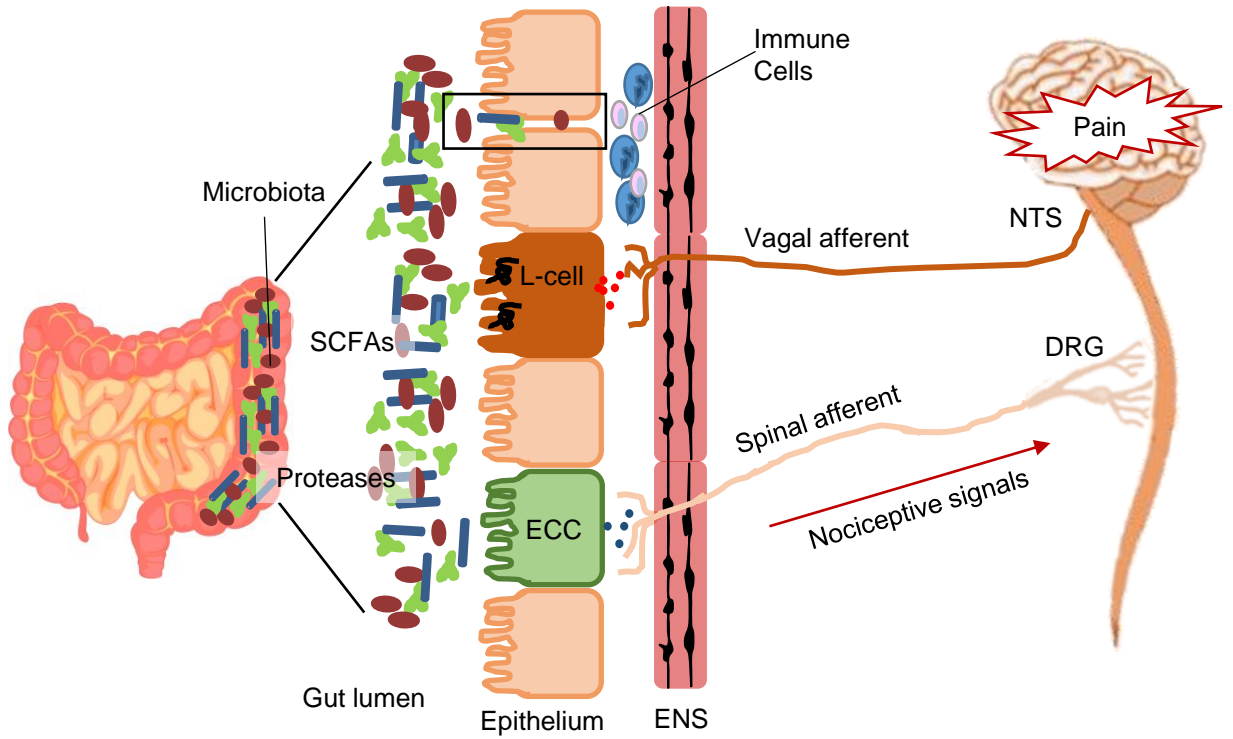


Figure 1

