# The Gastrointestinal Microbiota; A key regulator in Visceral Pain

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## Gastrointestinal Microbiota

The human gut harbours a complex and dynamic microbial ecosystem. We each harbour a unique microbiota signature of bacteria, archaea, yeasts, single-celled eukaryotes, as well as helminth parasites and viruses, including bacteriophage [1]. Numbering approximately 100 trillion, these microorganisms have an estimated collective mass of 1–2 kg [2], approximately the same weight as the human brain. Genes within the human gut microbiota significantly outnumber host human genes and are capable of producing a plethora of centrally-acting compounds, influencing virtually all aspects of human physiology and biology [3].

The microbiome-gut-brain axis is a complex bi-directional system including the central nervous system (CNS), the neuroendocrine and neuroimmune systems, the autonomic nervous system, the enteric nervous system (ENS), and, of course, the gut microbiome. Through endocrine, immune and neuropeptide/neurotransmitter systems, the microbiota can relay information about health status of the gut. This in turn can profoundly impact on neuronal signalling in the brain and thus impact on emotional systems and behavioural response [3,4]. This may be true for visceral pain, as the facilitation or inhibition of pain processing is mediated at a central level, while ascending visceral afferents are mediated through the spinal cord, as a consequence of primary visceral nociceptor activation.

## Visceral Pain

Pain is a multimodal experience combining a discriminative sensory component with a complex graded emotional response. This physiological survival facility is inherent to all sentient organisms to protect against potential or existing tissue damage. While the anatomical pathways and signalling mechanisms involved in somatic/musculoskeletal pain (skin and deep tissue) are relatively well defined, the mechanisms underlying visceral pain (internal organs) and its treatment are proving a difficult target for therapeutic intervention. Visceral pain is believed to affect up to 40% of the population at some stage in their lifetime and is commonly associated with an aching or throbbing sensation with varying degrees of discomfort, and often difficult to localise to a precise anatomical region.

Functional gastrointestinal disorders (FGID's) including functional dyspepsia, irritable bowel syndrome (IBS) and infant colic represent the more common forms of visceral pain. IBS alone affects an estimated 10-15% of the population in developed countries with an estimated economic burden to healthcare systems in the billions (Canavan et al. 2014). Other commonly

reported malaises include myocardial infarction (heart attack), dysmenhorrea, appendicitis, bladder pain and pelvic pain.

The perception of gastrointestinal pain and discomfort involves complex mechanisms. These include peripheral sensitization of sensory nerves and, at a central level, regulation of thalamic and corticolimbic signalling pathways. Of interest, there is substantial overlap in the brain areas underlying visceral pain and those that are involved in the processing of psychological stress, a key predisposing factor for visceral hypersensitivity.

**Visceral Pain Pathways**

After an event such as visceral injury, stress or infection; the nociceptive information coding for visceral pain, is propagated from the site of origin to the spinal cord, and then through ascending spinal pathways to the brain. Nociceptors in the viscera respond to mechanical stimulation such as distension or pressure, tissue damage and chemical stimulation as a consequence of inflammation, infection or ischaemia. These receptors are localised on bare nerve endings containing transient receptor potential (TRP) channels that detect tissue damage or injury. Chemicals that activate TRP channels include globulin, protein kinases, arachidonic acid, prostaglandins, histamine, nerve growth factor, substance P, calcitonin gene-related peptide, serotonin, acetylcholine, ATP and changes in pH [5].

At the dorsal horn of the spinal cord, biochemically active agents including substance P, glutamate, aspartate, vasoactive intestinal peptide (VIP), cholecystokinin (CCK), somatostatin, calcitonin gene-related peptide (CGRP) and galanin are released from the nerve terminals of the visceral primary afferents to propagate the nociceptive signal to second order neurons. Under normal physiological conditions, these neurons are under 'gated' control. However, once a certain threshold of stimulation is surpassed, these neurons are no longer suppressed and the nociceptive information coding for general location and intensity projects to supraspinal sites. The two major ascending pain pathways in mammals are the spinothalamic and the spinoparabrachial tracts, which encode the sensory-discriminatory and affective aspects of pain respectively [6]. Once the nociceptive information has been processed, the descending pathways (from brain to spinal cord) can exert an inhibitory or facilitatory effect on the sensation of pain. Interestingly, the supraspinal and peripheral anatomical regions involved in facilitation and inhibition of nociception often overlap. Sensitization of receptors in these anatomical loci as a consequence of repeated or prolonged activation can often lead to chronic, repeated and often unpredictable bouts of visceral pain. Thus by targeting key bioactive chemicals or receptor systems on these sensory afferent neurons, the sensation of visceral pain could be significantly ameliorated.

**The Gastrointestinal Microbiota and Visceral Pain**

At the source, the sensitisation of primary afferent nociceptors may lead to visceral hypersensitivity in FGIDs. A number of different receptor types are involved in the process of peripheral sensitization including the TRPV family, proteinase activated receptors,

cholecystokinin receptors, serotonin receptors, cannabinoid receptors, as well as an array of ion channels including ATP-gated ion channels, voltage-gated sodium and calcium channels, and acid-sensing ion channels [7]. The gastrointestinal microbiota can activate these receptors directly or indirectly through immune responses at the mucosal surface during infection, inflammation and autoimmunity, formyl peptides and protease release, polyunsatturated fatty acid (PUFA) release, SCFA (short chain fatty acid) production, neurotransmitter production and hormone secretion [8]. Gastrointestinal microbiota can also stimulate the release of the body's natural pain-suppressing biomolecules including opioids from innate neutrophils and monocytes, endocannabinoids from colonic tissue, as well as other pain modulators [9] including monoamines. Microbial metabolites can also influence epigenetic mechanisms, by altering substrate concentrations or by direct inhitibiton of enzymatic machinery in epigenetic pathways [10]. However, the extent to which these mechanisms either individually or collectively has in the aetiology of FGIDs remains unaddressed.

From a physiological perspective relevant to IBS visceral sensitivity; stress can influence gut motility, alter gastroinestinal secretions and exacerbate intestinal permeability – all of which can have a negative impact on gastroinestinal microbiota number and diversity. While most individuals are routinely exposed to intermittent stress, some are more susceptible and this can lead to stress-related disorders and related comorbidities. It may well be that this maladaptive stress response is mediated by a lack or overexpression of specific gastrointestinal microbiota.

Interestingly, adverse early-life events are linked with a maladaptive stress response and might increase the vulnerability of individuals to visceral sensitivity and other stress-related disorders later in life. While it is difficult to conclusively attribute early life stress and associated changes in gastrointestinal microbiota with the presentation of visceral sensitivity in later life in humans, the use of animals to establish this link has been very informative.

## Empirical Evidence for Gastrointestinal Microbiota Involvement in Visceral Pain Response

Animals raised in a sterile environment (germ-free mice) have an exaggerated stress response and reduced perception of pain following different inflammatory stimuli. In naïve animals, antibiotic-mediated depletion of gastrointestinal microbiota decreased visceral pain-related response in mice and rats; and early-life exposure to antibiotics predisposed animals to visceral sensitivity in adulthood [11]. Recently, faecal matter from IBS patients characterised by hypersensitivity to colorectal distension was transplanted to germ-free rats, and the response to colorectal distension was enhanced in these animals [12]. Accumulating empirical evidence supports a role for positively influencing gastrointestinal microbiota with probiotic treatment in the treatment of visceral sensitivity and other ailments – provided vagus nerve integrity was maintained (Bravo et al., 2011).

In contrast to the provocative preclinical evidence for a role for gut microbiota in visceral pain, clinical studies remain inconclusive with a large 'non-responder' population in many probiotic trials. Anecdotal evidence suggests that antibiotics may be useful in treating bacterial infections

that may be contributing to the discomfort and pain associated with FGID and inflammation, however further research is required to confirm these assertions. It is clear that longitudinal, placebo-controlled, double-blind studies with whole-system analysis [13,14] and complimentary brain imaging are necessary to integrate central, peripheral and behavioural alterations before, during and after treatment of visceral pain.

**Summary**

A growing body of preclinical and clinical evidence supports a relationship between the complexity and diversity of the microorganisms that inhabit our gut (human gastrointestinal microbiota) and health status. Under normal homeostatic conditions this microbial population helps maintain intestinal peristalsis, mucosal integrity, pH balance, immune priming and protection against invading pathogens. Furthermore, these microbes can influence centrally regulated emotional behaviour through mechanisms including microbially-derived bioactive molecules (amino acid metabolites, short chain fatty acids, neuropeptides and neurotransmitters), mucosal immune and enteroendocrine cell activation, as well as vagal nerve stimulation.

Changes to the microbial environment, as a consequence of illness, stress or injury can lead to a broad spectrum of physiological and behavioural effects locally including a decrease in gut barrier integrity, altered gut motility, inflammatory mediator release, as well as nociceptive and distension receptor sensitisation. Centrally mediated events including hypothalamic-pituitary-adrenal (HPA) axis, neuroinflammatory events and neurotransmitter systems are concomitantly altered. Thus, both central and peripheral pathways associated with pain manifestation and perception are altered as a consequence of the microbiota-gut-brain axis imbalance.

The dogmatic approach of antibiotic treatment in the latter century for the treatment of many diseases and conditions, has undergone a radical change. We are 90% microbe, and pragmatism suggests that we manipulate this ecosystem for the treatment of various ailments, stress dysfunction and affective disorders, including the alleviation of visceral sensitivity.

**References**


1 Human Microbiome Jumpstart Reference Strains, C. *et al.* A catalog of reference genomes from the human microbiome. *Science* **328**, 994-999, doi:10.1126/science.1183605 (2010).

2 Frank, D. N. & Pace, N. R. Gastrointestinal microbiology enters the metagenomics era. *Current opinion in gastroenterology* **24**, 4-10, doi:10.1097/MOG.0b013e3282f2b0e8 (2008).

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

4 Mayer, E. A., Knight, R., Mazmanian, S. K., Cryan, J. F. & Tillisch, K. Gut microbes and the brain: paradigm shift in neuroscience. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **34**, 15490-15496, doi:10.1523/JNEUROSCI.3299-14.2014 (2014).

5 Cortright, D. N. & Szallasi, A. TRP channels and pain. *Current pharmaceutical design* **15**, 1736-1749 (2009).

6 Millan, M. J. The induction of pain: an integrative review. *Progress in neurobiology* **57**, 1-164 (1999).

7 Akbar, A., Walters, J. R. & Ghosh, S. Review article: visceral hypersensitivity in irritable bowel syndrome: molecular mechanisms and therapeutic agents. *Alimentary pharmacology & therapeutics* **30**, 423-435, doi:10.1111/j.1365-2036.2009.04056.x (2009).

8 Rea, K., O'Mahony, S. M., Dinan, T. G. & Cryan, J. F. The Role of the Gastrointestinal Microbiota in Visceral Pain. *Handbook of experimental pharmacology*, doi:10.1007/164_2016_115 (2016).

9 Moloney, R. D. *et al.* Stress and the Microbiota-Gut-Brain Axis in Visceral Pain: Relevance to Irritable Bowel Syndrome. *CNS neuroscience & therapeutics* **22**, 102-117, doi:10.1111/cns.12490 (2016).

10 Stilling, R. M., Dinan, T. G. & Cryan, J. F. Microbial genes, brain & behaviour - epigenetic regulation of the gut-brain axis. *Genes, brain, and behavior* **13**, 69-86, doi:10.1111/gbb.12109 (2014).

11 O'Mahony, S. M. *et al.* Disturbance of the gut microbiota in early-life selectively affects visceral pain in adulthood without impacting cognitive or anxiety-related behaviors in male rats. *Neuroscience* **277**, 885-901, doi:10.1016/j.neuroscience.2014.07.054 (2014).

12 Crouzet, L. *et al.* The hypersensitivity to colonic distension of IBS patients can be transferred to rats through their fecal microbiota. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society* **25**, e272-282, doi:10.1111/nmo.12103 (2013).

13 Mayer, E. A., Tillisch, K. & Gupta, A. Gut/brain axis and the microbiota. *The Journal of clinical investigation* **125**, 926-938, doi:10.1172/JCI76304 (2015).

14 Mayer, E. A., Gupta, A., Kilpatrick, L. A. & Hong, J. Y. Imaging brain mechanisms in chronic visceral pain. *Pain* **156 Suppl 1**, S50-63, doi:10.1097/j.pain.0000000000000106 (2015).