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Title	High and Mighty? Cannabinoids and the microbiome in pain
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Publication date	2021-02-20
Original Citation	Rea, K., O'Mahony, S. M. and Cryan, J. F. (2021) 'High and Mighty? Cannabinoids and the microbiome in pain', Neurobiology Of Pain, 9, 100061 (7pp). doi: 10.1016/j.ynpai.2021.100061
Type of publication	Article (peer-reviewed)
Link to publisher's version	10.1016/j.ynpai.2021.100061
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Download date	2026-11-06 12:37:05
Item downloaded from	https://hdl.handle.net/10468/13025

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Neurobiology of Pain 9 (2021) 100061

Contents lists available at ScienceDirect

Neurobiology of Pain

journal homepage: www.sciencedirect.com/journal/neurobiology-of-pain

Review

High and Mighty? Cannabinoids and the microbiome in pain

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ARTICLE INFO

Keywords:
Endocannabinoids
Microbiota
Inflammation
Pain
Gut

ABSTRACT

Within the human gut, we each harbour a unique ecosystem represented by trillions of microbes that contribute to our health and wellbeing. These gut microbiota form part of a complex network termed the microbiota-gut-brain axis along with the enteric nervous system, sympathetic and parasympathetic divisions of the autonomic nervous system, and neuroendocrine and neuroimmune components of the central nervous system. Through endocrine, immune and neuropeptide/neurotransmitter systems, the microbiota can relay information about health status of the gut. This in turn can profoundly impact neuronal signalling not only in the periphery, but also in the brain itself and thus impact on emotional systems and behavioural responses. This may be true for pain, as the top-down facilitation or inhibition of pain processing occurs at a central level, while ascending afferent nociceptive information from the viscera and systemic areas travel through the periphery and spinal cord to the brain. The endogenous cannabinoid receptors are ubiquitously expressed throughout the gut, periphery and in brain regions associated with pain responding, and represent targets for endogenous and exogenous manipulation. In this review, we will focus on the potential role of the endogenous cannabinoids in modulating microbiota-driven changes in peripheral and central pain processing. We also focus on the overlap in mechanisms whereby commensal gut microbiota and endocannabinoid ligands can regulate inflammation and further aim to exploit our understanding of their role in microbiota-gut-brain axis communication in pain processing.

1. Introduction

1.1. The endocannabinoid system

The endogenous cannabinoid system is a neuroactive lipid signalling system comprised of two G-protein coupled receptors CB_1 (Devane et al., 1988) and CB_2 (Matsuda et al., 1990), endogenous ligands that bind to and activate these receptors, and the enzymes that either synthesise or degrade these compounds. The two best characterised endogenous ligands are anandamide (N-arachidonoylethanolamide; AEA) (Devane et al., 1992) and 2-arachidonoylglycerol (2-AG) (Sugiura et al., 1995), which are synthesised by N-acylphosphatidylethanolamine-specific Phospholipase D (NAPE-PLD) (Di Marzo et al., 1996) and diacylglycerol lipase (Stella et al., 1997) respectively. These compounds are atypical as 'neurotransmitters' as they are synthesised on demand in the post-synaptic neuron, and travel retrogradely to the presynaptic neuron to mediate their inhibitory effects prior to being catabolised by fatty acid amide hydrolase (FAAH), N-acylethanolamine acid amidase or monoacylglycerol lipase (MAG-Lipase) and α/β hydrolase 6 and 12 respectively (Cravatt et al., 1996; Dinh et al., 2002).

The CB_1 receptor can be found in the brain and in the periphery including the gut where it is enriched in both the enteric nervous system and in non-neuronal cells in the intestinal mucosa, including enteroendocrine cells, immune cells, and enterocytes (Izzo & Sharkey, 2010) to influence gut motility and permeability, gastric juice secretion, and neurotransmitter and hormone synthesis and release. Indeed it has been speculated that the effects of the endocannabinoidome on gut barrier function are dependent on inflammatory status of the gut, and lipid availability from the diet (Cani et al., 2016). Low to moderate levels of CB_2 receptors are found in the brain, with these receptors being far more prevalent on immune cells in peripheral tissue, including the gut (Howlett & Abood, 2017). The endocannabinoid system is implicated in inflammation and pain processing at both the gut and central nervous system (CNS) levels (Russo et al., 2018). In this context, endocannabinoids and biochemically related mediators, by being important modulators of immune crosstalk, are strongly implicated in inflammation-based pathological states, including neuropathic, inflammatory and arthritic pain (Starowicz & Finn, 2017).

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https://doi.org/10.1016/j.ynpai.2021.100061
Received 12 October 2020; Received in revised form 21 December 2020; Accepted 28 January 2021
Available online 16 February 2021
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Outside the CB_1 and CB_2 receptors, endocannabinoids also express activity at other G-protein coupled receptors including GPR18, GPR55 and GPR19, as well as peroxisome proliferator-activated receptors (PPARs) and several transient receptor potential cation channels, subfamily V (TRPV1) (Di Marzo, 2018). Other compounds that resemble the prototypical endocannabinoids that can engage the same synthesising and catabolising enzymes, or by inactivating their degradation include N-acylethanolamines (NAEs), N-palmitoylethanolamine (PEA), N-oleylethanolamine (OEA), N-stearoylethanolamine (SEA) and N-linoylethanolamine (LEA), 2-palmitoylglycerol (2PG) and 2-oleylglycerol (2-OG), and are commonly referred to as 'entourage compounds' of the endocannabinoid system (Di Marzo, 2018). However, it is important to point out that these compounds can also exhibit biological activity at other receptors distinct from the cannabinoid receptors. Thus care must be taken when interpreting data from cannabinoid research, as many of the exogenous cannabinoid agents (e.g. MAG lipase inhibitors or FAAH inhibitors) influence multiple compounds with the ability to alter cannabinoid, and other, signalling pathways.

Herein we discuss the potential role of endocannabinoids in microbiota-mediated influence of pain response.

1.2. The gastrointestinal microbiota

The human gastrointestinal tract hosts a number of microorganisms including bacteria, archaea, yeasts, single-celled eukaryotes, helminth and viruses collectively referred to as the microbiota (Eckburg et al., 2005; Gaci et al., 2014; Lankelma et al., 2015; Scarpellini et al., 2015; Williamson et al., 2016). The bacterial gut microbiota is largely defined by two dominant phylotypes, Bacteroidetes and Firmicutes, with Proteobacteria, Actinobacteria, Fusobacteria, and Verrucomicrobia phyla present in relatively low abundance (Qin et al., 2010; Lankelma et al., 2015). At a basic level, the gut microbiota interact with the host in a mutualistic relationship that begins at birth. In humans and other mammals, colonisation of the infant gut essentially begins at birth, when delivery through the birth canal exposes the infant to the mother's vaginal microbiota, thereby initiating a critical maternal influence over the offspring's lifelong microbial signature (Donnet-Hughes et al., 2010; Collado et al., 2012; Bäckhed et al., 2015). Other factors associated with 'seeding' of the newborn gut include those from the environment, diet (breastfeeding or bottle-feeding) as well as early introduction of antibiotics or other medication (Borre et al., 2014).

The gastrointestinal tract provides an energy rich, anaerobic environment in which the microbes can thrive, while the microbes contribute to host metabolism, protection and immune development and maintenance. The bi-directional communication along the gut-brain axis is thought to involve a number of complex pathways including activating host immune or inflammatory mediators, releasing or stimulating the release of host neurotransmitters or neuropeptides from the epithelium, activation of host receptors from microbial by-products including short-chain fatty acids (SCFAs), or peptidoglycans, and by activation of the enteric nervous system and vagus nerve (Cryan et al., 2019).

Despite the seclusion of the gut microbes to the gastrointestinal tract, they are capable of interacting with the host across the single-celled, mucous-coated epithelial layer. As well as restricting microbes to the lumen of the gut, the gut epithelium serves in the regulation of nutrient intake and secretes a protective mucous layer. It also acts as a physical barrier against pathogens, and houses the dense population of immunogenic cells that line the gut (Farhadi et al., 2003). This epithelial monolayer is also interspersed with complex tight-junction proteins to allow the diffusion of fluids and solvents between adjacent epithelial cells and can regulate gut barrier permeability (Frazier et al., 2011).

Under homeostatic conditions there exists a healthy, resting inflammatory tone in the gut where the microbiota stimulate cytokines and chemokines release, which in turn regulates local levels of bacteria and other microbes in the gut. As well as influencing local immune responses at the epithelium, microbiota can synthesise and release neurotransmitters and SCFAs, as well as influence the release of neuropeptides and hormones from enteroendocrine cells of the intestines (Cani et al., 2013).

These gut peptides such as ghrelin, gastrin, orexin, galanin, cholecystokinin, leptin, neuropeptide Y and neuroactive lipids like cannabinoids are thought to influence peripheral neural communication from the gut and can also act centrally to influence behaviour (Cryan et al., 2019). Current hypotheses suggest that these circulating cytokines, chemokines, endocrine messengers and microbial by-products can infiltrate the blood and lymphatic systems, or influence neural messages carried by the vagal and spinal afferent neurons to impact on centrally-mediated events, including emotive responding such as pain.

1.3. Pain response

Pain is a multimodal experience combining a discriminative sensory component with a complex graded emotional response. While for most individuals pain is transient, both acute and chronic pain can be debilitating, and can influence one's state of mind, which in turn can amplify the psychological intensity of the pain (Zhang et al., 2019). In general, pain types can be categorised as nociceptive pain, inflammatory pain or neuropathic pain. Nociceptive pain is the classical response to a painful stimulus such as a pin-prick, where superficial pain receptors sense the stimulus and transmit the signal to our brains. Inflammatory pain results from activation and sensitisation of nociceptors by inflammatory biochemicals, such as some mediators produced at the site of injured tissue, which include serotonin (5-HT), kinins, histamine, nerve growth factors (NGF), adenosine triphosphate (ATP), prostaglandins, glutamate, leukotrienes, nitric oxide (NO), norepinephrine, protons, vasoactive peptides, bioactive lipids, eicosanoids, pro-inflammatory cytokines, and acute-phase proteins (Bueno & Fioramonti, 1999). Under homeostatic conditions, an acute injury such as painful information coding for heat (inflammation) or pain, is propagated from the site of origin to vagal afferents or through ascending spinal pathways to the brain. The brain can localise where this pain originates and issues an appropriate response to the painful stimulus. Inflammatory pain is normally transient and usually dissipates once the tissue repairs. However, persistent inflammatory pain can occur and can manifest as a clinical condition if left untreated.

Pain receptors in our visceral organs, and in skin and tissue respond to mechanical stimulation such as distension or pressure, tissue damage and chemical (due to inflammation, infection or ischaemia) stimulation. However, following prolonged or chronic activation, neurons involved in transmitting nociceptive information can also become sensitised or dysfunctional leading to the conductance of a painful signal to what should be an innocuous stimulus (neuropathic pain). Dysfunction along pain pathways, including in the anatomical loci at the site of injury, or the neural communication from the site of injury along the spinal cord to the brain, as well as supraspinal regions involved in descending pain facilitation and inhibition may lead to chronic, repeated and often unpredictable bouts of pain, and may be the root from which neuropathic pain stems (examples including neuropathic pain, fibromyalgia, migraine and headache) (Woolf et al., 1998).

The gastrointestinal tract is lined with receptors that can mediate the pain response including the transient receptor potential channels, of the vanilloid subtype (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 (Akbar et al., 2009). Pain receptors locally in the gut can be directly or indirectly activated by gastrointestinal microbes (Holzer et al., 2017), by releasing or promoting the release of formyl peptides and proteases, pH changes, polyunsaturated fatty acid (PUFA) release, SCFA production, neurotransmitter production, and hormone secretion (Rea et al., 2017). Incoming visceral information from the gut via the sympathetic and

parasympathetic branches of the autonomic nervous system (Fig. 1) are further processed in the brain, and are subjected to feedback loops to initiate the appropriate behavioural and physiological (modulate gut motility and permeability, epithelial fluid maintenance, luminal osmolarity, secretion of bile, carbohydrate levels, mechanical distortion of the mucosa, bicarbonate and mucus production as well as the mucosal immune response and intestinal-fluid control) response (Mayer & Tillisch, 2011).

2. Evidence for a role of gastrointestinal microbiota in pain response

There is limited clinical and preclinical evidence directly supporting a role for gut microbiota in the pathophysiology of the pain state. Irritable bowel syndrome (IBS) in human observational and intervention studies, and visceral pain studies in animal models vastly outnumber other researched pain modalities with respect to the involvement of the gut microbiota (Rea et al., 2017). However, one cannot overlook the involvement of the microbiota in symptoms that are comorbid with chronic pain especially affective disorders.

Useful preclinical tools in elucidating the role of the microbiome in pain-related behaviours have included the use of germ-free mice, antibiotic administration, faecal matter transplantation, prebiotic and probiotic administration. Germ-free animals were shown to have increased sensitivity to pain in the colorectal distension model (Luczynski et al., 2017) (animal model of visceral hypersensitivity), increased pain responding to inflammatory mediator injection into the paw (Amaral et al., 2008) but lower pain response in chemotherapy-induced neuropathic pain (Shen et al., 2017). Microbiota depletion with antibiotics decreased visceral pain response in mice (Aguilera et al., 2015) and rats (Hoban et al., 2016), with administration of antibiotics in early life predisposing animals to visceral hypersensitivity in later life (O'Mahony et al., 2014).

A reduction in chemotherapy-induced neuropathic pain (Shen et al., 2017) was also observed in antibiotic treated animals. The transfer of faecal matter from human subjects with IBS into germ-free rats was reported to exacerbate their pain response to colorectal distension (Crouzet et al., 2013). Furthermore, a recent preclinical neuropathic pain study, using sciatic nerve ligation to sensitise the nerve to previously innocuous pressure, demonstrated that faecal matter transplant

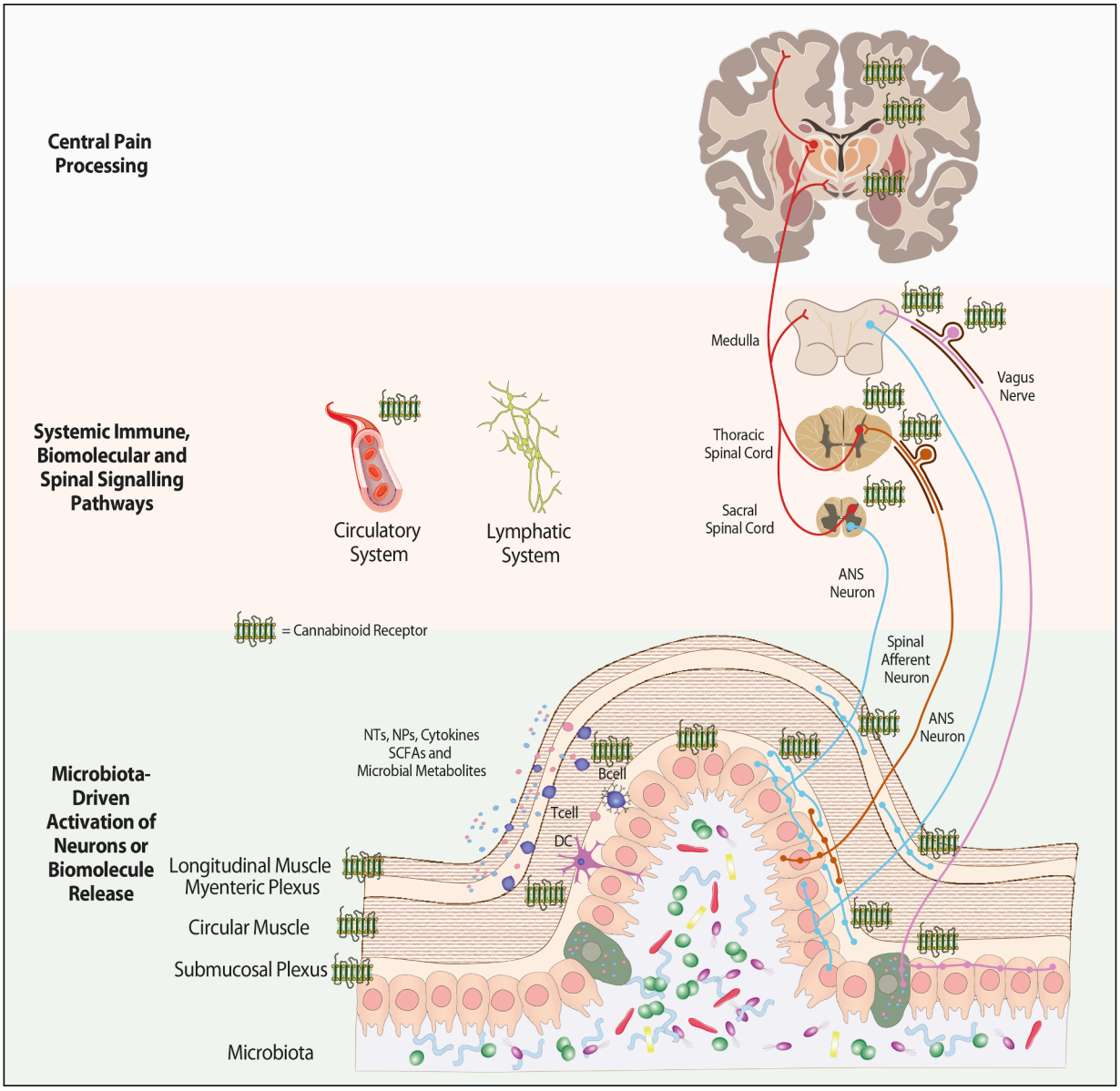


Fig. 1. Modes of communication between the gut microbiota and central pain processing pathways, identifying endocannabinoid receptor location and involvement.

from pain-sensitive animals to antibiotic-treated mice conferred a painful phenotype (Yang et al., 2019) reinforcing an involvement of microbes in pain response.

From a clinical perspective, randomised, double-blind, placebo-controlled studies investigating the role of the microbiome in pain research are lacking. The data on gut microbiota in functional gastrointestinal disorders are too limited to draw conclusions yet (Halkjær et al., 2017). But recently, the potential role of the human microbiome in visceral, inflammatory, and neuropathic pain states, including fibromyalgia, migraine, cancer and chemotherapy-associated pain has been highlighted (Rea et al., 2019).

3. Endocannabinoids and the microbiome

While there is separate evidence supporting a role for microbiome modulation and endocannabinoids in pain management, there are limited studies that have tried to directly link changes in microbiome-endocannabinoidome in pain response and the mechanisms involved. However, studies looking at the role of endocannabinoids in microbiota-mediated changes in physiology suggest that the mechanism largely involves a microbiome-driven change in gut permeability (associated with an obesogenic phenotype) that facilitates an increase in systemic inflammatory tone regulated by endocannabinoids. In human observational studies, dietary intakes of specific fatty acids were associated with 2-AG and omega-3 fatty acid-derived endocannabinoids, irrespective of the body fat distribution. These changes in endocannabinoids were associated with changes in *Peptostreptococcaceae,* V*eillonellaceae* and *Akkermansiaceae.* A 2-day Mediterranean diet intervention in this study increased circulating levels of endocannabinoids in agreement with fatty acid intake, however no microbiota sequencing was performed on this short intervention (Castonguay-Paradis et al., 2020). Increased gut permeability leads to the translocation of gram-negative bacterial components (e.g. lipopolysaccharides (LPS)) that trigger an immune response and the production of endocannabinoids (Maccarrone et al., 2001; Liu et al., 2003; Zhu et al., 2011). This increase in endocannabinoid tone can further exacerbate gut permeability, particularly in obesity, leading to a viscous cycle with further increase in LPS translocation and an increased inflammatory tone (Muccioli et al., 2010). Indeed, knockout of the gene encoding myeloid differentiation primary response protein MyD88, which is involved in the signalling of most Toll-like receptors following activation by translocating bacterial components, attenuated inflammation and gut permeability alterations in an animal model of obesity. Furthermore, this change resulted in an altered microbiota composition and increased levels of 2-AG and 2-OG, but decreased levels of anandamide (Everard et al., 2014). This has led to the hypothesis that the endocannabinoids can act as 'gate-openers' and 'gate-keepers' in gut permeability (Silvestri and Di Marzo, 2013), as well as influencing some of the central effects associated with microbiota perturbation (Russo et al., 2018).

A recent study determined that gene expression of cannabinoid receptors and enzymes and lipid mediators in the juvenile and adult ileum, duodenum and colon are altered in germ-free animals. Interestingly, FMT from donor age-matched conventional animals partially or completely reversed changes in gene expression and lipid mediator levels after only 1 week treatment (Manca et al., 2020). Despite the fact that this study does not look at pain readouts, these findings lend credence to the hypothesis that intestinal microorganisms exploit endocannabinoid signalling to exert some of their physiological functions.

Further evidence that intestinal microbes can influence the endocannabinoid system is provided in another recent study. It was determined that the yeast C*andida albicans* can manipulate the endocannabinoid system resulting in changes in neuroendocrine levels resulting in changes in anxiety-like behaviour (Markey et al., 2020). Further, increasing AEA levels using the well-characterized FAAH inhibitor URB597 was sufficient to reverse both the neuroendocrine and behavioural phenotypes in these *C. albicans*-colonized mice, suggesting an interaction between the microbiome and endocannabinoid system in these effects.

In obese mice, CB_1 receptor antagonism with Rimonabant (SR141716A) attenuated the increased gut permeability, altered tight junction protein expression, and inflammatory tone associated with obesity, and resulted in an increased relative abundance of *Akkermansia muciniphila* (Mehrpouya-Bahrami et al., 2017). Similarly, chronic Δ^9-tetrahydrocannabinol (THC), a neuroactive component of cannabis, attenuated diet-induced obesity and altered the microbiota with an increased Firmicutes:Bacteroidetes ratio (Cluny et al., 2015). In a separate study, the administration of *Akkermansia muciniphila* in high-fat diet (HFD) mice increased intestinal levels of 2-AG, 2-OG, and 2-PG, as well as reversing HFD-related changes in gut permeability (Everard et al., 2013). Further work from this group using a mouse model of inducible intestinal epithelial cell (IEC)-specific deletion of NAPE-PLD determined an altered gastrointestinal microbiota and metabolic function in these animals, that was partly reversible with *Akkermansia* administration (Everard et al., 2019).

Recently, genetic manipulation of the bacterium *Escherichia coli* to produce NAPE-PLD was able to attenuate diet-induced obesity (Dosoky et al., 2019). Taken together, it is evident that the exogenous administration of cannabinoid compounds can influence microbiota readouts (in an altered microbiome metabolic state) and vice versa.

In a 6-week feeding study in female mice, α-linoleic acid (to increase polyunsaturated fatty acids) decreased liver 2-AG, OEA and AEA, while increasing PEA, while there were no effects on endocannabinoid levels in epididymal adipose tissue. Supplementation of these ALA-treated groups with *B. breve* strains NCIMB 702,258 and DPC6330 further exacerbated changes in the liver, but induced a pronounced increase in epididymal adipose tissue levels of AEA and DHEA. These findings were postulated to be linked to microbiota driven changes in inflammatory response through toll-like receptor activation (Patterson et al., 2017) further supporting the hypothesis that endocannabinoids may play a role in gut microbe-driven changes in inflammatory tone.

Using the FAAH inhibitor PF3845, and thus promoting levels of anandamide (and other lipids) there was a reduction in immune readouts and pain response in an animal model of 2,4,6-trinitrobenzene sulfonic acid (TNBS)- but not Dextran sulfate sodium (DSS)-induced colitis (Sałaga et al., 2014). Similarly, increasing 2-AG levels using MAG-lipase inhibitor prevented TNBS-colitis and prevented further systemic and central inflammation (Alhouayek et al., 2011). This may be through the classic cannabinoid receptors or other fatty acid ethanolamines that have demonstrated powerful anti-inflammatory effects *in vitro* (Alhamoruni et al., 2010; Muccioli et al., 2010; Geurts et al., 2013). In an animal model of multiple sclerosis, the autoimmune encephalitis model, there is an increase in systemic and central inflammatory tone, as well as changes in microbiota complexity and diversity, including increased levels of *Akkermansia muciniphila*. Administration of a combination of cannabidiol and THC significantly reduced levels of *Akkermansia* and attenuated the systemic and central inflammatory response (Al-Ghezi et al., 2019). These studies strengthen the link between the microbiome and the endocannabinoid system, potentially through immune modulation to exert beneficial effects.

However, while some of these studies show reduced local inflammatory tone, these changes have not been linked with pain response (Table 1). In an animal model of visceral hypersensitivity, the colorectal distension model, *Lactobacillus acidophilus* administration was shown to reduce abdominal pain through cannabinoid and opioid receptors – as their effects were blocked with selective antagonists (Rousseaux et al., 2007). However, a thorough evaluation of where these two physiological systems intersect was not further explored. In an animal model of neuropathic pain, using deprivation of vitamin D, an altered microbiome with a reduction in *Verrucomicrobia* and *Bacteroidetes*, an increase in tactile allodynia (a painful response to a normally non-noxious stimulus) and changes in endocannabinoid levels at the level of the spinal cord,

Table 1
Studies examining possible connection between gut microbiota and cannabinoid signalling.

Animal	Effect	Intervention	Author/Year
Germ-free male mice C57BL/6NTac	Gene expression of endocannabinoidome receptors and enzymes and lipid mediators in juvenile and adult ileum, duodenum and colon were altered in germ-free animals	FMT from donor age-matched conventional animals partially or completely reversed changes in gene expression and lipid mediator levels after 1 week treatment	Manca et al., 2020
5–6 w old Female C57BL/6 Mice	The fungus *Candida albicans* increased serum corticosterone levels, altered lipid metabolism and increased anxiety-like behaviour	1 mg/kg URB597 i.p. attenuated these changes in neuroendocrine, metabolic and behavioural outputs	Markey et al., 2020
Adult male C57BL/6 Mice	Diet-induced obesity (DIO) model increased gut permeability, increased anandamide levels and CB1R mRNA in adipose tissue, altered glucose and SCFA metabolism, altered tight junction protein expression, and increased inflammatory tone in blood, gut and fat tissues	10 mg/kg daily oral gavage of CB1R antagonist SR141716A for 4 weeks attenuated DIO-mediated changes in physiology and metabolism	Mehrpouya-Bahrami et al., 2017
Adult male C57BL/6N mice	DIO increased weight gain, had no effect on gut transit time, and altered gut microbiota	2 mg/kg THC i.p. for 3 weeks and 4 mg/kg for 1 additional week attenuated DIO-mediated weight gain and increased Firmicutes: Bacteroidetes ratio with an increase in *Akkermansia muciniphila* abundance	Cluny et al., 2015
Genetic ob/ob, HFD, type 2 diabetic and naïve male C57BL/6 Mice	All groups had increased weight gain and adipose tissue weight, increased serum LPS levels and *Akkermansia muciniphila* was significant lower (>100 fold decrease) in all groups	The prebiotic oligofructose (10 g/100 g of diet for ob/ob study or 0.3 g per day HFD study) attenuated weight gain, fat mass accumulation, increase in serum LPS levels and decreases in *Akkermansia muciniphila*	Everard et al., 2013
10w old adult male C57BL/6 Mice	HFD increased weight gain and adipose tissue weight, increased serum LPS levels and *Akkermansia muciniphila* was significantly reduced	2 X 10^8 cfu/0.2 mL daily *Akkermansia muciniphila* (ATTC BAA-835) oral gavage or heat-killed control for 4 weeks attenuated serum LPS levels, weight gain, fat mass accumulation, and restored gut barrier function and increased intestinal endocannabinoids in DIO mice	Everard et al., 2013
Male adult mice with Intestinal epithelial cell-specific deletion of NAPE-PLD	NAPE-PLD deletion decreased levels of AEA, OEA, PEA, 2-AG; exacerbated obesity, reduced energy expenditure and altered gut microbiota	2 X 10^8 cfu/0.2 mL daily *Akkermansia muciniphila* (ATTC BAA-835) oral gavage reduced body weight and fat mass in HFD NAPE-PLD deleted mice, without further altering endocannabinoid levels	Everard et al., 2019
Adult male C57BL/6 Mice	DIO increased weight gain	Administration of genetically manipulated *Escherichia coli* to produce NAPE-PLD attenuated increases in weight gain	Dosoky et al., 2019
Adult female C57BL/6 Mice	Autoimmune Encephalopathy model induced an inflammatory phenotype and altered microbiota	10 mg/kg each of THC + CBD daily i.p. administration from day 10 reduced levels of Verrucomicrobia, specifically *Akkermansia muciniphila* and attenuated the systemic and central inflammatory response associated with EAE model	Al-Ghezi et al., 2019
Male adult Balb C mice and Male adult Sprague-Dawley rats	Colorectal distension model increased pain response as visceral hypersensitivity	*L. acidophilus* NCFM (1 X 10^9 CFU/mL) daily attenuated the pain response in mice and rats. 3 mg/kg i.p. administration of the CB2-selective antagonist AM-630 reversed this effect.	Rousseaux et al., 2007
Adult male C57BL/6 Mice	Vitamin D deficiency exacerbated allodynia associated with spinal nerve sensitisation, reduced Verrucomicrobia and Bacteroidetes and reduced spinal CB1R expression	10 mg/kg i.p. PEA administration attenuated pain response in spinal nerve injury, and attenuated changes in microbiota including *Akkermansia muciniphila*	Guida et al., 2020
Adult female Balb C mice	6 week α-linoleic acid decreased liver triacylglycerides, increased tissue fatty acid composition and decreased liver endocannabinoid levels	*B. breve* strains NCIMB 702,258 and DPC6330 exacerbate the changes in liver metabolites, but increase endocannabinoid tone in adipose tissue only	Patterson et al., 2017
Adult male and female human participants	Dietary intakes of specific fatty acids were associated with 2-AG and omega-3-fatty acid-derived endocannabinoids, irrespective of the body fat distribution, and changes in *Peptostreptococcaceae, Veillonellaceae*, and *Akkermansiaceae*	2-day Mediterranean diet intervention increased circulating levels of NAEs and 2-MAGs in agreement with changes in FA intake	Castonguay-Paradis et al., 2020

duodenum and colon were observed. PEA administration attenuated both the pain behaviour and spinal biochemical changes in vitamin D deficient mice, whilst increasing the levels of *Akkermansia*, *Eubacterium* and *Enterobacteriaceae*, as compared with vehicle-treated mice (Guida et al., 2020). However, whether changes in the microbiome were causal or as a consequence of the sensitivity to noxious stimuli remain

unresolved.

In a similar vein, recent reviews using the available meta-analyses have drawn association and noted correlations between the endocannabinoid system and the microbiota-gut-brain axis in a range of disorders that can be comorbid with certain aspects of pain including obesity and metabolic disorders, multiple sclerosis and autoimmune disorders, arthritis and inflammatory disorders, neurodegenerative disorders, IBS, IBD and gastrointestinal disorders (Cani et al., 2016; Mestre et al., 2018; Russo et al., 2018).

4. Conclusion and future research directions

Both the endocannabinoid system and the gastrointestinal microbiota can individually contribute to the manifestation of pain and our endogenous response in pain management. Research into the complex interactions of these systems in the pain response is in its infancy, with very limited research on local events at the level of the gut, and no studies looking at supraspinal effects. Furthermore, there is no comprehensive study looking at the impact of chronic cannabinoid compounds on the microbiome *per se,* in the presence or absence of pain. Given the overlapping roles of the endocannabinoid system and the microbiome on many critical biological systems including metabolism, immune response, satiety, gut function and their ability to modulate mood and behaviour – future research directions could look to the modulation of endogenous pain control systems including the endocannabinoid system via the microbiome. The immunomodulatory properties of probiotics and prebiotics that have been reported in clinical and preclinical studies suggest that they could be used as adjunct therapies in the treatment of certain pain modalities. Knowing that a high-fat diet contributes to an altered microbiome and pro-inflammatory profile, a more holistic approach promoting exercise and diets including healthy amounts of polyphenols, polyunsaturated fatty acids, fibre, fermented foods etc. that promote and support the growth of beneficial bacteria could also help in the alleviation of inflammatory-related pain, possibly through the endocannabinoid system. In conclusion, the microbiome represents an accessible and adaptable target, to activate receptors (including cannabinoid receptors) in the endogenous pain control pathways. Despite the limited literature dedicated to these interactions to date, further work in this exciting research field is anticipated (Fig. 1).

Declaration of Competing Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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