Regulation of pain perception by microbiota in Parkinson’s Disease

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Non-standard abbreviations:
AHR: aryl-hydrocarbon receptor; BBB: blood-brain barrier; BDNF: brain-derived neurotrophic factor; CCl: chronic constriction injury; CCLn: C–C motif chemokine n; CCRn: C–C chemokine receptor n; COX2: cyclooxygenase 2; CXCLn: C–X–C motif chemokine n; CXCRn: C–X–C motif chemokine receptor n; Cx43: connexin 43; CX3CR1: fractalkine receptor; CIPN: chemotherapy induced peripheral neuropathy; CNS: central nervous system; DAP12: DNAX-activation protein 12; DRG: dorsal root ganglia; ERBB2: receptor tyrosine-protein kinase erbB2; ERK: extracellular signal-regulated kinases; FGF2: fibroblast growth factor 2; GF: germ
free; GFAP: glial fibrillary acidic protein; HDAC: histone deacetylase; IASP: international association for the study of pain; IBS: irritable bowel syndrome; IL-n: interleukin n; IFN-I: type I interferon; IFNγ: interferon γ; IRF: interferon regulatory factors; IRFn: interferon regulatory factor n; JAK: janus kinases; JNK: c-Jun N-terminal kinases; KCC2: K-Cl cotransporter 2; LPS: lipopolysaccharide; MAPK: mitogen-activated protein kinases; MPTP: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NFPR: N-formyl peptide receptors; NF-κB: nuclear factor κB; NLRP3: NOD-, LRR- and pyrin domain-containing protein 3; PGN: prostaglandin n; PLC: phospholipase C; PTGIS: prostaglandin I2 synthase; PUFA: polyunsaturated fatty acids; P2Xn: purinoceptor Xn; P2Yn: purinoceptor Yn; SCFA: short-chain fatty acids; SPF: specific pathogen free; S100β: S-100 calcium-binding protein β; TGFn: transforming growth factor n; Thy1-SNCA: transgenic mice overexpressing human α-synuclein under the Thy1-promoter; TLRn: toll-like receptor n; TLRs: toll-like receptors; TNFα: tumor necrosis factor α; TNFR: TNFα receptor; TREM2: triggering receptor expressed on myeloid cells 2; TRKB: tyrosine receptor kinase B; TRPA1: transient receptor potential cation channel A1; TRPVn: transient receptor potential cation channel Vn; WNT3A: WNT family member 3A; 5-HTn receptor: serotonin receptor n.
Abstract

Pain perception involves current stimulation in peripheral nociceptive nerves and the subsequent stimulation of postsynaptic excitatory neurons in the spinal cord. Importantly, in chronic pain, the neural activity of both peripheral nociceptors and postsynaptic neurons in the central nervous system is influenced by several inflammatory mediators produced by the immune system. Growing evidence has indicated that the commensal microbiota plays an active role in regulating pain perception by either acting directly on nociceptors or indirectly through the modulation of the inflammatory activity on immune cells. This symbiotic relationship is mediated by soluble bacterial mediators or intrinsic structural components of bacteria that act on eukaryotic cells, including neurons, microglia, astrocytes, macrophages, T-cells, enterochromaffin cells, and enteric glial cells. The molecular mechanisms involve bacterial molecules that act directly on neurons affecting their excitability, or indirectly on non-neuronal cells inducing changes in the production of pro-inflammatory or anti-inflammatory mediators. Importantly, Parkinson’s disease, a neurodegenerative and inflammatory disorder that affect mainly the dopaminergic neurons implicated in the control of voluntary movements, involves not only a motor decline but also non-motor symptomatology, including chronic pain. Of note, several recent studies have shown that Parkinson’s disease involves a dysbiosis in the composition of the gut microbiota. In this review, first, we summarize, integrate and classify the molecular mechanisms implicated in the microbiota-mediated regulation of chronic pain. Secondly, we analyze the changes on the commensal microbiota associated to Parkinson’s disease and propose how these changes affect the development of chronic pain in this pathology.
Significance Statement

The microbiota regulates chronic pain through the action of bacterial signals into two main locations: the peripheral nociceptors and the postsynaptic excitatory neurons in the spinal cord. The dysbiosis associated to Parkinson’s disease reveals increased representation of commensals that potentially exacerbate chronic pain and reduced levels of bacteria with beneficial effects on pain. This review encourages further research to better understand the signals involved in bacteria-bacteria and bacteria-host communication to get the clues for the development of probiotics with therapeutic potential.
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I. Introduction

Parkinson disease (PD) is a neurodegenerative disorder that affects the control of voluntary movements, although it can also affect the somatosensory system. Indeed, chronic pain is a common symptom in Parkinson's, affecting up to 90% of people with this condition (Buhmann et al., 2017; Mylius et al., 2021).

Pain can be classified according to its pathophysiology as nociceptive, neuropathic, or nociplastic (Clauw et al., 2019; Orr et al., 2017), and their respective definitions can be found in the international association for the study of pain (IASP) resources. Pain can also be classified according to anatomical location as somatic or visceral. And, according to duration, pain can be acute, which is a rapid response against injury or trauma and is usually limited to the duration of the underlying cause (Orr et al., 2017). On the other side, acute pain may experience a transition to chronic pain, which persist beyond the standard time of healing without a clear biological purpose (Clauw et al., 2019). Pain in PD has been described as being nociceptive (inflamed muscles or joints), neuropathic (secondary to neurodegeneration of central and peripheral somatosensory system) or nociplastic (on-going nociceptive pain with a sensitization component) (Buhmann et al., 2020). The mechanisms of pain in PD are various as the disease affects many of the pain processing structures as well as affecting nociceptor excitability (Reichling and Levine, 2011).

Several lines of evidence have shown that the microbiota might regulate pain (Amaral et al., 2008; Ding et al., 2021; Pusceddu and Gareau, 2018; SM et al.,...
The microbiota is defined as a community of microorganisms living in a specific environment, such as mucosal tissues and skin (Marchesi and Ravel, 2015). Although the contribution of intestinal microbiota has classically been considered the most dominant (Shreiner et al., 2015), the microbiota is distributed throughout different niches, including the oral cavity, genital and pulmonary mucosa, among others, from which oral microbiota is the second largest community in humans (Deo and Deshmukh, 2019). It has been established that these anatomical sites harbor specific microbial communities. In line with this, in a steady state, these microbial communities coexist in a mutualistic (Willing et al., 2011) and tolerogenic (Zheng et al., 2020) symbiotic relationship with the host. Under homeostatic conditions, the microbiota reaches a “Eubiotic” state, characterized by microbial communities with high taxonomic diversity, elevated microbial gene richness, and stable core microbiota (Fan and Pedersen, 2021). This “healthy” microbiota impacts physiological functions such as metabolism, epithelial barrier function, hematopoiesis, protection against pathogens, nutrient extraction, and biosynthesis or degradation of bioactive molecules such as vitamins, amino acids, neurotransmitters and, lipids (Campos-Acuna et al., 2019; D’Argenio and Salvatore, 2015; Tremaroli and Backhed, 2012).

While the eubiotic state of microbial communities (Ileba et al., 2016) is linked to the maintenance of host homeostasis, dysbiosis involves significant changes in the composition of resident commensal communities relative to eubiosis (Ileba et al., 2016; Petersen and Round, 2014). These disturbances of microbial communities impact several physiological processes and are closely associated with pathological processes from which neurodegenerative diseases are included, such
as Alzheimer’s disease, Parkinson’s disease (PD), Huntington disease, multiple sclerosis, and amyotrophic lateral sclerosis (Marizzoni et al., 2017; Singh et al., 2022; Wang et al., 2022; Zhang et al., 2022). For a more in-depth discussion about the state of the art of microbiota about its dynamics, distribution, and host interactions during healthy and pathological states, reviews have been addressed (Hou et al., 2022; Zheng et al., 2020).

PD involves an autoimmune response mediated by the peripheral immune system (Gonzalez and Pacheco, 2014). Since the immune response might modulate pain perception (Calvo et al., 2012), and the function of immune cells may be affected by the microbiota (Campos-Acuna et al., 2019), peripheral immune cells seem to be critical mediators connecting the effects of microbiota in pain associated to PD. The autoimmune response associated with PD involves the activation of both the adaptive (the specific arm of the immune system) and the innate (non-specific arm of the immune system) immunity (Harms et al., 2021). The adaptive immunity activated in PD involves mainly T-cells specific to antigens derived from the α-synuclein (Pacheco, 2021), a protein associated with transporting presynaptic vesicles, which undergoes oxidation and aggregation in PD (Lim and Zhang, 2013). This peripheral T-cell response is directed to neo-epitopes derived from phosphorylated and nitrated forms of the α-synuclein and involves CD4⁺ and CD8⁺ T-cells (Sulzer et al., 2017). Autoreactive CD8⁺ T-cells associated with PD display cytotoxic activity (cytotoxic T lymphocytes, CTL) that might directly kill cells (neurons) presenting autoantigens on major histocompatibility complex (MHC) class I (Pacheco, 2021). Autoreactive CD4⁺ T-cells involved in PD display the
inflammatory phenotypes T-helper 1 (Th1) and Th17, which upon recognition of autoantigens presented on class II MHC by myeloid cells (i.e. macrophages/monocytes or microglia), produce inflammatory cytokines (i.e., IFN\(\gamma\), IL-17, TNF\(\alpha\)) exacerbating the inflammatory and neurotoxic potential of myeloid cells (Gonzalez et al., 2015). According to the involvement of a T-cell mediated chronic inflammation in PD, many experimental therapies sought to induce regulatory T-cells (Treg), which are CD4\(^+\) T-cells with suppressive activity over the function of Th1, Th17, and CTL (Pacheco, 2021).

Some studies have shown the presence of antibodies specific to \(\alpha\)-synuclein-derived antigens in PD (Scott, 2022), suggesting a role of B cells in the pathogenesis of this disorder. However, recent studies have provided evidence that the lack of B cells does not affect the disease development in animal models (George et al., 2021; Karikari et al., 2022), thereby suggesting a minor role of these adaptive immune cells in PD pathogenesis. On the other hand, peripheral innate immune response associated with PD involves monocytes/macrophages, which upon stimulation by pathological forms of \(\alpha\)-synuclein (i.e., oxidized and aggregated), acquire inflammatory function (Harms et al., 2021) and also present \(\alpha\)-synuclein-derived antigens associated to class II MHC on their cell surface (Gonzalez et al., 2014). Of note, the local activation of myeloid cells (monocyte/macrophages or microglia) might recruit T-cells, which in turn provide feedback on these myeloid cells potentiating the production of inflammatory mediators. Moreover, the local activation of T-cells promotes further recruitment and tissue infiltration of peripheral monocytes/macrophages. Thus, the adaptive
and innate immune responses work together, promoting chronic inflammation in PD (Gonzalez et al., 2014; Gonzalez and Pacheco, 2014).

Several studies have addressed the impact of gut microbiota in developing motor impairment and neurodegeneration in PD. However, whether and how the commensal gut microbiota could affect the development of pain associated with this disorder remains poorly understood. Furthermore, it is unclear if the microbiota from other anatomical regions different from the gut mucosa may regulate the development of pain in PD. In this review, we collect and integrate the evidence pointing to a potential link between microbiome composition from the gut and other niches with the regulation of pain sensitivity in the context of PD. The main goals of this review are: i. To summarize, integrate and classify the molecular mechanisms implicated in the microbiota-mediated regulation of chronic pain, including the analysis of the bacterial-derived mediators and the host's cell types participating in these processes; ii. To analyze the changes in the commensal microbiota associated with PD and propose how these changes affect the development of chronic pain in this pathology.

II. Evidence of regulation of chronic pain by microbiota

Pain is defined by the International Association for the Study of Pain (IASP) as an unpleasant sensory and emotional experience associated with actual or potential tissue damage (Raja et al., 2020). Chronic pain persists over time, usually defined as over three months. Chronic pain is highly prevalent, affecting around 30% of
adults. (Cohen et al., 2021; Duran et al., 2023). It has devastating consequences on the individual who suffers it and society. Chronic pain is one of the leading causes of disability worldwide (Disease et al., 2017) and has an enormous economic cost. Chronic pain affects the person who suffers it multidimensionally, with complex interactions between biological, psychological, and social factors. Pain can be categorized depending on the duration as acute (less than three months; usually present while there is tissue damage, and disappears with tissue healing, it is regarded as a protective mechanism) or chronic (longer than three months; persists after the tissue has healed, it is regarded as maladaptive). Pain is also classified depending on the primary mechanism driving it as nociceptive (from tissue injury), neuropathic (from nerve injury), or nociplastic (from a sensitized nervous system). However, in practice, there is considerable overlap in the different types of pain within and between patients, making us think about it as a continuum rather than categories. Chronic pain can also be categorized depending on the region of the body it affects: somatic (muscles, bones, or soft tissues) or visceral (internal organs and blood vessels). Somatic and visceral nociceptive signals travel via different pathways to reach the spinal cord; therefore, they are considered different pain types.

II.1. Pain types in which a role of the microbiota has been described

II.1.1. Neuropathic pain. Recent studies have found that gut microbiota could play a significant role in the development of neuropathic pain in preclinical models of chemotherapy induced peripheral neuropathy (CIPN). For instance, the depletion
of microbiota induced by the treatment of mice with an antibiotic cocktail or by breeding mice in germ-free (GF) conditions ameliorates hyperalgesia, allodynia, and cytokines production by dorsal root ganglia (DRG) neurons in a model of CIPN (Ma et al., 2022; Shen et al., 2017). Moreover, the transplant of fecal microbiota obtained from specific pathogen free (SPF; animals living in conventional environmental conditions associated with most animal facilities, where the absence of some specific pathogens is periodically tested) into GF animals recapitulated pain-like behaviors in models of CIPN using oxaliplatin or paclitaxel (Ramakrishna et al., 2019; Shen et al., 2017). However, it seems that not all commensal bacteria are deleterious, as a probiotic formulation (SLAB51) was able to protect from mechanical or cold hypersensitivity in a CIPN model induced by paclitaxel (Cuozzo et al., 2021). Nevertheless, although the promising effects of microbiota’s modulation on CIPN, orally administered probiotics *L. reuteri* LR06 or *Bifidobacterium* BL5b in other models of neuropathic pain such as that induced by chronic constriction injury (CCI) in rats have failed to alleviate pain (Huang et al., 2019). The discrepancy between these studies might be due to the use of different host species (mouse versus rats), different models of neuropathic pain (CIPN versus CCI), and different probiotics formulations.

II.1.2. Visceral pain. The gut microbiota has been shown to modulate pain in gastrointestinal disorders such as irritable bowel syndrome (IBS). Using GF animals, it has been demonstrated that commensals are required to develop pain sensitivity. Indeed, the fecal transplant from IBS patients into GF mice reproduced some features of IBS in these mice, including hypersensitivity to colorectal
distension (Pusceddu and Gareau, 2018). Similar results have been recently reported in a rat model of colitis induced by the intrarectal administration of 2,4-dinitrobenzenesulfonic acid (DNBS). After antibiotics-mediated microbiota depletion, the fecal transplantation from DNBS-treated donors in recipient rats induced a long-lasting visceral hypersensitivity. Furthermore, the authors demonstrated that fecal transplantation from healthy donors ameliorates visceral hypersensitivity in DNBS-treated animals (Lucarini et al., 2022). In addition, using a model of visceral pain induced by colorectal distension in mice, antibiotic treatment generates an imbalance of the microbiota composition that was associated with visceral hypersensitivity, which was prevented with probiotics (Verdu et al., 2006). Similar conclusions were obtained using the model of visceral pain induced by colorectal distension in GF mice. In this regard, GF mice display visceral hypersensitivity accompanied by up-regulation of Toll-like receptors (TLRs) and inflammatory cytokines in the spinal cord. All these effects were abolished when GF mice were postnatally colonized with microbiota obtained from SPF mice (Luczynski et al., 2017). Furthermore, the colonization of GF mice with microbiota from SPF mice rescued the excitability of enteric sensory neurons (McVey Neufeld et al., 2013), suggesting that commensal microbiota is necessary for the normal excitability of these neurons. Altogether, these studies suggest that gut microbiota might exacerbate or reduce visceral sensitivity depending on the paradigm of visceral pain. However, it is unclear what specific species of bacteria are increasing or decreasing visceral sensitivity.
According to the critical role of microbiota in regulating visceral pain in animal models, clinical investigation has shown positive applications in humans. In a randomized clinical trial in patients with IBS, two-week treatment with the antibiotic rifaximin reduced abdominal pain (Pimentel et al., 2011). In addition, a meta-analysis showed that the administration of *Lactobacillus rhamnosus* GG moderately decreased the intensity of abdominal pain-related functional gastrointestinal disorders in children (Horvath et al., 2011). Similarly, administering a probiotic mixture composed of *Bifidobacterium infantis* M-63, breve M-16V, and longum BB536 ameliorated abdominal pain in children with IBS (Giannetti et al., 2017). Altogether, these preclinical and clinical studies support the involvement of the gut microbiota in regulating visceral pain associated with GI disorders, which encourages us to keep working in this promising field with translational significance.

II.1.3. Inflammatory (nociceptive) pain. Compared to SPF, GF mice display a reduced sensitivity to several inflammatory mediators injected in the paw, including carrageenan, lipopolysaccharide (LPS), chemokine CXCL1, TNFα, and IL-1β. This reduced hypernociception in GF animals was accompanied by increased IL-10 levels, and anti-IL-10 antibodies abrogated the effect (Amaral et al., 2008). In the same direction, LPS and bacterial lysates from *Escherichia coli* NLM28 directly increase excitability of mouse colonic nociceptive neurons (Ochoa-Cortes et al., 2010). According to the critical role of microbiota in the induction of inflammatory pain in animal models, a more recent study tested the analgesic potential of the
probiotics *Lactobacillus reuteri* LR06 or *Bifidobacterium* BL5b in a model of inflammatory pain induced by complete Freund’s adjuvant (CFA) and CCI in rats. The authors did not detect pain amelioration in mechanical or thermal hyperalgesia (Huang et al., 2019). Further research is required to adequately determine the function of precise components from the microbiota in the modulation of inflammatory pain.

II.2. Mechanisms involved in microbiota-mediated pain regulation

Depending on the involvement of neural circuits of the central nervous system (CNS) or peripheral nociceptors, the mechanisms associated with the microbiota-mediated regulation of pain can be classified as peripheral or central mechanisms.

II.2.1. Peripheral mechanisms with direct action on nociceptors

Some bacterial metabolites or structural components of bacteria could directly affect the excitability of nociceptive neurons while other might indirectly modulate pain signaling through the action on non-neuronal cells (Ochoa-Cortes et al., 2010) (Figure 1). The evidence indicates that some bacterial components might regulate the neuronal excitability. This regulation is conducted through intrinsic structural components (i.e. LPS or flagellin), the production of enzymes that affect the levels of bile acids, and the secretion of soluble factors able to act directly on neurons, including γ-aminobutyric acid (GABA), short-chain fatty acids (SCFA), polyunsaturated fatty acids (PUFA), and bacterial peptides (N-formylated peptides...
and α-hemolysin) (Banerjee et al., 2016; Campos-Acuna et al., 2019; Ji et al., 2016).

- **Structural components (LPS, flagellin):** It has been reported that the LPS, a surface structural component of gram-negative bacteria, can promote the excitability of nociceptive sensory neurons, promoting hyperalgesia and pain by a direct action on transient receptor potential cation channel A1 (TRPA1). Despite TLR4 being the canonical receptor described for many LPS-mediated effects, the TRPA1-evoked hyperalgesia induced by LPS is TLR4-independent (Meseguer et al., 2014) (Figure 1, top panel). Furthermore, by TLR4-dependent mechanisms, LPS could sensitize trigeminal sensory neurons via transient receptor potential cation channel V1 (TRPV1) modulation (Diogenes et al., 2011) (Figure 1, top panel). Another structural component of some commensals is flagellin, which is part of the flagella and might induce pro-inflammatory effects in the host through the stimulation of TLR5 (Xiao et al., 2015). Of note, it has been demonstrated that TLR5 is present on peripheral sensory neurons (Xu et al., 2015). It is important to consider that the TLR5-mediated recognition of flagellin by intestinal immune cells triggers the production of flagellin-specific IgA by mucosal B cells, which induce the down-regulation of flagellar motility genes in several bacteria of the gut microbiota, including members of the phyla *Firmicutes* and *Proteobacteria*. This TLR5-mediated mechanism significantly contributes to maintaining the integrity of the epithelial layer of the gut mucosa, as TLR5-deficiency results in the loss of flagellin-specific
antibodies and high levels of flagellin expressed in the gut microbiota, which promotes increased intestinal permeability (Cullender et al., 2013). Thus, the evidence suggests that TLR5 stimulation on intestinal immune cells in healthy conditions promotes gut barrier integrity and homeostasis; nevertheless, when intestinal permeability is increased, TLR5-signaling in nociceptors favors hypersensitivity.

- **SCFA:** Acetate, propionate, and butyrate, which are metabolites derived from the fermentation of fibers by some bacteria of the commensal microbiota (Campos-Acuna et al., 2019), can also directly affect the excitability of nociceptive neurons. These metabolites might directly stimulate specific surface G protein-couple receptors on the host, including GPR41, GPR43, and GPR109a. Some SCFA receptors are expressed on primary nociceptive neurons, by which these metabolites might affect neuronal excitability and pain perception (Jameson et al., 2020; Lagomarsino et al., 2021; Nohr et al., 2015) (Figure 1, top panel). Of note, certain SCFA do not only act through the stimulation of surface receptors but also by the inhibition of the histone deacetylase (HDAC) in the cytosol or nucleus (Lin et al., 2015), thereby changing the epigenetic modifications of chromatin and altering gene expression (Campos-Acuna et al., 2019) (Figure 1, top panel). The SCFA receptor GPR41 has been found expressed in postganglionic sympathetic and sensory neurons in both the autonomic and somatic peripheral nervous system (Nohr et al., 2015), which makes possible a direct interaction between the commensal microbiota and the peripheral nervous system. Interestingly, using a rat CCI model it was
shown that butyrate attenuates neuropathic pain (Kukkar et al., 2014). This effect was probably due to the butyrate-mediated HDAC inhibition, as other HDAC inhibitors such as suberanilohydroxamic acid, MS-275, and trichostatin A have also been shown to attenuate hypersensitivity in animal models of neuropathic pain (Danaher et al., 2018; Zhao and Wu, 2018). Another study shows that genetic deficiency of ffar2 (the gene encoding GPR43) abrogated the acetate-mediated hyperexcitability in a mouse model of gout (Vieira et al., 2015). In relation with this, the main gut commensals producing acetate are Prevotella spp., Ruminococcus spp., Bifidobacterium spp., Bacteroides spp., Clostridium spp., Streptococcus spp., A. muciniphila, and B. hydrogenotrophica (Nogal et al., 2021). It is unclear if butyrate-mediated effects on pain perception through HDAC or GPR43 are exerted directly on sensory neurons or indirectly on non-neuronal cells.

- **GABA**: The GABA<sub>B1</sub> receptor is expressed on peripheral nociceptive terminals (Hanack et al., 2015), and GABA can evoke inhibitory currents, thus reducing the TRPV1-mediated excitatory effect on the nociceptive transmission (Du et al., 2017) (Figure 1, top panel). Of note, GABA can be produced by some commensal bacteria, including Lactobacillus, Escherichia coli, and Bifidobacterium (Duranti et al., 2020; Pacheco, 2019; Pokusaeva et al., 2017). Interestingly, oral administration of Bifidobacterium dentium reduced sensitization of colonic afferents in a mouse model of visceral hypersensitivity, an effect that was dependent on the bacteria-derived GABA (Pokusaeva et al., 2017).
• **Bacterial peptides and lipids:** The bacterial products N-formylated peptides and the pore-forming toxin α-hemolysin might directly activate nociceptive sensory neurons in mice respectively through the stimulation of N-formyl peptide receptors (NFPR) and by direct membrane depolarization through pore-assembly leading to ionic influx, eliciting hyperalgesia (Chiu et al., 2013) (**Figure 1**, top panel). Other mediators secreted by bacteria that can modulate nociceptors are the PUFA. In this regard, it has been described that 5,6-epoxyeicosatrienoic acid directly stimulates TRPV4 in primary afferent sensory neurons in mice, eliciting visceral pain (Cenac et al., 2015) (**Figure 1**, top panel).

• **Deconjugation of bile acids:** Another mechanism by which gut microbiota regulates hypersensitivity is through bile acids deconjugation, which is mediated by the bile salt hydrolase activity expressed by some colonic commensal bacteria (Song et al., 2020). Importantly, some deconjugated bile acids might increase the TRPA1 sensitivity on primary sensory neurons through the stimulation of the G protein-coupled bile acids receptor 1 (GPBAR1) (Lieu et al., 2014) (**Figure 1**, top panel). For instance, it has been shown that chronic morphine administration induces a microbiota dysbiosis involving a reduction in bile-deconjugating strains with significant decrease on the levels of primary and secondary bile acids in the gut (Banerjee et al., 2016). This may not mean a net reduction on pain sensitivity, as chronic morphine administration also induces hyperalgesia (Corder et al., 2017) and modify neuronal μ opioid receptors function.
Thus, the peripheral mechanisms that involve direct interaction of microbiota with nociceptors include bacterial signals that increase (deconjugated bile acids, LPS, flagellin, bacterial peptides, PUFAs) and reduce (SCFA, GABA) nociceptive sensitivity. Since many bacteria from commensal microbiota might produce many of these mediators, the net effect of dysbiosis on pain sensitivity might be very complex to predict. Further research into how the bacterium-bacterium interactions affect the production of these different bacterial regulators of pain is necessary to increase the understanding of how a dysbiotic change might affect pain perception.

II.2.2. Peripheral mechanisms through activation of non-neuronal cells.

The main cell sources participating as a bridge between microbiota and the modulation of pain are macrophages, T-cells, enterochromaffin cells and enteric glial cells.

-Macrophages: macrophages can be activated through TLRs and polarized to a proinflammatory state with the production of the proinflammatory cytokines (i.e. tumor necrosis factor $\alpha$ (TNF$\alpha$) and interleukin-1$\beta$ (IL-1$\beta$)) and prostaglandins (i.e. PGE2), which in turn stimulate their respective receptors on nociceptive neurons (Barker et al., 2022; Das et al., 2016; Jang et al., 2020). Signaling pathways activated in afferent neurons induced by these inflammatory mediators include the
increase in intracellular Ca\textsuperscript{2+} levels ([Ca\textsuperscript{2+}]\text{in}) and stimulation of the cyclic AMP (cAMP) production, and phospholipase C (PLC), activation of many protein kinases, including the protein kinase A (PKA), PKC, mitogen-activated protein kinases (MAPKs), Janus kinases (JAK), phosphatidylinositol-3 kinase (PI3K) and Src, and ultimately, the activation of transcription factors, including the nuclear factor-κB (NF-κB), and signal transducer and activator of transcription (STAT), which may in turn functionally upregulate TRPA1, TRPV1, Ca\texttextsubscript{v}3.2 T-type Ca\textsuperscript{2+} channels and voltage-gated sodium channels NaV1.7, NaV1.8 and NaV1.9 (Chen et al., 2020; Domoto et al., 2021) (Figure 1, bottom panel). On the other side, macrophages with an anti-inflammatory/profibrotic phenotype produce IL-10 and transforming growth factor β (TGFβ), which attenuate the signaling pathways triggered by inflammation, dampening pain sensitivity (Chen et al., 2020). This reduction on pain sensitivity is apparently mediated by modulation of voltage gated sodium channels in the case of IL-10 (Shen et al., 2013). Interestingly, some components of the commensal microbiota might regulate the acquisition of the functional phenotype of macrophages (Deng et al., 2016; Li et al., 2019), thereby indicating that microbiota might modulate pain sensitivity via macrophages' functional polarization. For instance, Bacteroides fragilis (the ZY-312 strain) facilitates the polarization of macrophages to a proinflammatory phenotype (Deng et al., 2016). Conversely, another study reported that changes in the gut microbiota composition might induce the TLR4-dependent polarization of macrophages into an anti-inflammatory/profibrotic phenotype (Li et al., 2019). In addition, in a model of CCI of the sciatic nerve, an agonist of the serotonin receptor 5-HT\textsubscript{2B} prevented
mechanical allodynia and reduced cold allodynia (Urtikova et al., 2012) which was associated with the 5-HT$_{2B}$ receptor immunoreactivity in neurons and macrophages of the lumbar DRG. Accordingly, some reports provide evidence indicating that serotonin might regulate the functional phenotype of macrophages through the 5-HT$_{2B}$ receptor (de Las Casas-Engel and Corbi, 2014; Nieto et al., 2020). Moreover, macrophages express surface receptors for SCFA, and through their stimulation or by the inhibition of HDAC, SCFA have been shown to down-regulate the production of TNF$\alpha$, IL-1$\beta$, IL-10, TGF$\beta$ and PGE2 (Campos-Acuna et al., 2019; Chang et al., 2014; Vinolo et al., 2011). See a summary of the role of macrophages as a mediator of indirect peripheral mechanisms by which microbiota modulates pain in Figure 1 (bottom panel).

-T-cells: T-cells might potently regulate the phenotype of macrophages (Gonzalez and Pacheco, 2014), thereby affecting nociceptive pain. Interferon $\gamma$ (IFN$\gamma$), TNF$\alpha$, and GM-CSF produced by Th1 and Th17 cells induce the acquisition of a proinflammatory phenotype in macrophages, while IL-10, TGF$\beta$, and IL-4 secreted by Treg and Th2 cells promote an anti-inflammatory/profibrotic phenotype in macrophages (Gonzalez et al., 2015). Interestingly, despite their inflammatory function, it has been shown that Th1 and Th17 lymphocytes might produce opioids and reduce inflammation-associated visceral hypersensitivity. For instance, using two different animal models of inflammatory colitis, Boué and colleagues found that these inflammatory T-cell subsets promote colonic tissue damage, but simultaneously they reduced the abdominal pain (Boue et al., 2014). Thus, the final
balance of Th1/Th17 consequences on pain depends on the integrated inflammatory effects induced through macrophages and the analgesic potential mediated by T-cells derived opioids. Although this analgesic effect of inflammatory T-cells might be relevant beyond the mouse models of colitis, the role of microbiota in the production of T-cells-derived opioids is pending to be explored. Microbiota may affect the production of dopamine, GABA, serotonin, glutamate, SCFA, and bile acids, all able to act on their receptors expressed on T-cells, dendritic cells or epithelial cells and finally affecting the inflammatory/anti-inflammatory behavior of T-cells (Pacheco, 2019; Song et al., 2020). For instance, it has been described that commensal microbiota induces the generation of extrathymic Treg in the colonic mucosa, which are characterized by the expression of the transcription factor RORγt (Kim et al., 2016b; Ohnmacht et al., 2015). A systematic analysis using gnotobiotic mice (mice harboring known microbiota, which are generated by colonizing GF mice with known bacteria) provided evidence that this effect was induced by a mix of 17 Clostridia species, which triggered the activation of STAT3 during T-cell differentiation. Of note, these intestinal RORγt+ Treg are potent inhibitors of Th2-mediated responses, including dietary allergies (Ohnmacht et al., 2015). SCFA produced by commensals upon homeostatic conditions, have been described to play a critical role in the generation of intestinal Treg and the consequent oral tolerance. Mechanistic analyses have indicated that this tolerogenic effect of SCFA is mediated through the inhibition of HDAC activity in dendritic cells (Arpaia et al., 2013) and by the stimulation of the surface receptors GPR109a and GPR43 on dendritic cells and epithelial cells respectively (Tan et al.,
Moreover, the direct stimulation of GPR43 on colonic Treg favor their expansion and suppressive activity (Smith et al., 2013). Interestingly, whereas SCFA (derived from commensal microbiota) favor immune tolerance, middle- and long- chain fatty acids (derived from diet) promote inflammatory T-cell responses, involving Th1 and Th17 phenotypes (Haghikia et al., 2015).

Other important microbial-dependent regulators of T-cell mediated immunity are bile acids. Through the deconjugation and oxidation of bile acids, some bacteria of the gut microbiota metabolize conjugated primary bile acids (synthesized within hepatocytes) to yield deconjugated primary and secondary bile acids, which stimulate bile acid receptors on T-cells promoting the generation of RORγt+ Treg in the colonic mucosa (Song et al., 2020). In addition to the role of SCFA and bile acids, many other mediators whose concentrations are strongly affected by gut microbiota exert regulation on the T-cell response (Figure 1, bottom panel), including dopamine, GABA, serotonin, and glutamate (for these effects see the reviews (Franco et al., 2007; Pacheco et al., 2010) and the book chapter (Pacheco et al., 2012)).

- Enterochromaffin cells: SCFA produced by the gut microbiota might also act on enterochromaffin cells modulating the secretion of serotonin (Reigstad et al., 2015), which has been described to directly act on serotoninergic receptors expressed on nociceptive neurons, modulating their excitability. For instance, serotonin induced an exacerbated TRPV1-mediated calcium influx in nociceptive trigeminal ganglia (Loyd et al., 2011) apparently mediated by the 5-HT2B receptor.
(Su et al., 2016) (Figure 1, bottom panel). In this regard, it has been shown how 5-HT\textsubscript{2B} receptor antagonists exert antinociceptive effects in different animal models of pain, including models of visceral pain and migraine (O'Mahony et al., 2010; Ohashi-Doi et al., 2010; Schaerlinger et al., 2003). The connection between enterochromaffin cells, microbiota-derived metabolites and visceral hypersensitivity has been recently addressed (Bayrer et al., 2023). In this regard, it has been shown that serotonin derived from enterochromaffin cells was sufficient to elicit both, acute and persistent visceral hypersensitivity to gut distension in response to isovalerate, a SCFA associated with gastrointestinal inflammation and pain in IBD (Zhang et al., 2019a). Of note, serotonin has been also described to exert an important immunomodulatory role for many years (Franco et al., 2007; Pacheco et al., 2012), which opens the possibility that serotonin would be involved in communication between microbiota, immune cells, and nociceptors.

-**Enteric glial cells:** These cells share morphological, phenotypical, and functional features with astrocytes, specifically those related with enteric neurons providing trophic and protective support (Gulbransen and Sharkey, 2012) expressing as well glial fibrillary acidic protein (GFAP) (Jessen and Mirsky, 1983) and S-100 calcium-binding protein \( \beta \) (S100\( \beta \)) (Ferri et al., 1982). Besides, enteric glial cells have been shown to affect both the excitability of nociceptive neurons and to regulate the function of local immune cells. These tasks are conducted by the release of soluble mediators such as adenosine triphosphate (ATP), GABA, IL-6, TNF\( \alpha \), and IL-1\( \beta \) and the removal of neuromodulators through enzymatic degradation by
ectoenzymes, such as NTPDase2 (Morales-Soto and Gulbransen, 2019). Several studies suggest that microbiota produces some proteases and other mediators that can act on enteric glial cells affecting their function, colonization, development, renewal and consequently modulating abdominal pain (Ceuleers et al., 2016; Garrido et al., 2002; Kabouridis et al., 2015; Moloney et al., 2016). A recent study provided causal evidence indicating how enteric glia participates in visceral pain perception using a model of inflammatory colitis induced by DNBS in rats. When enteric glia activity was targeted by fluorocitrate, visceral hyperalgesia was abrogated, which was explained by the reduced expression of TRPV1 in the colonic myenteric plexuses, dorsal root ganglion, and periaqueductal grey area (Lucarini et al., 2021). Nevertheless, the mechanisms by which the microbiota-enteric glia interactions are related with pain modulation are still incipient. See a summary of the role of enteric glial cells as a mediator of indirect peripheral mechanisms by which microbiota modulates pain in the Figure 1 (bottom panel).

These indirect mechanisms of peripheral modulation of pain perception by the microbiota involve a complex network of bacterial mediators with a set of intermediate cells, including macrophages, T-cells, enterochromaffin cells, and enteric glial cells, which in turn might exacerbate or attenuate nociceptive excitability. Thereby, these mechanisms add another layer of complexity to direct mechanisms of peripheral regulation of pain by microbiota, making the prediction of how dysbiosis might affect nociceptive excitability even more complex. In this regard, further studies addressing the precise communication between different bacteria of the microbiota with the intermediate cells (macrophages, T-cells,
enterochromaffin cells, and enteric glial cells) are necessary to improve our understanding of the cross-talk between microbiota and peripheral nociceptors excitability and pain perception.

II.2.3. Central mechanisms

Over the past three decades, research has revealed that chronic pain occurs not only as the result of direct neuronal communication but requires cross-talk between neurons, glia, and immune cells (Grace et al., 2021). Here we do a brief review on the role of the immune system on increased excitability of dorsal horn neurons to understand how microbiota could have a role. This summary is not intended as a comprehensive review on the topic (some excellent reviews are available elsewhere as (Inoue and Tsuda, 2018)).

Microglia, which are tissue-resident macrophages of the CNS, have been shown to be necessary and sufficient to activate the molecular and cellular alterations that underlie chronic pain. Microglia change from their surveillance mode to a reactive state by stimulation through molecules secreted by damaged nociceptors in the dorsal horn of the spinal cord. Approximately 40 of these molecules have now been identified (Inoue and Tsuda, 2018). These activated microglia produce and release a variety of bioactive diffusible factors that can influence superficial dorsal horn neuronal function via direct or indirect actions. Some of the well-known pathways after nerve injury are the following:

- **Brain-derived neurotrophic factor (BDNF):** Nerve injury induces secretion of ATP by dorsal horn nociceptors which in turn induces an upregulation and
stimulation of the P2X purinoceptor 4 (P2X4) on microglia, triggering neuroinflammation (Tsuda et al., 2009; Ulmann et al., 2008). These microglia release BDNF which in turn downregulates K-Cl cotransporter 2 (KCC2) in dorsal horn neurons expressing the BDNF receptor, the tyrosine receptor kinase B (TRKB). This causes an increase in intracellular Cl\(^-\) and leads to a depolarizing shift in the anion reversal potential (Coull et al., 2005). Under these conditions, GABA or glycine are released because of innocuous stimulation induces neuronal depolarization.

**- IL-1β:** IL-1β secretion by microglia is induced by: i) Stimulation of TLR2 and/or TLR4, which trigger the activation of NF-κB (Heneka et al., 2014; Kim et al., 2007; Tanga et al., 2005); ii) Stimulating the triggering receptor expressed on myeloid cells 2 (TREM2), which is coupled to DNAX-activation protein 12 (DAP12) (Kobayashi et al., 2016), inducing the consequent activation of the transcription factors interferon regulatory factor 8 (IRF8), and IRF1 (Masuda et al., 2015; Masuda et al., 2012); iii) Stimulating the fractalkine receptor (CX3CR1) via activation of p38 MAPK (Clark et al., 2015), among others. IL-1β is post-transcriptionally activated by the NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3) inflammasome, which generates the mature form of IL-1β (Heneka et al., 2014). Released IL-1β acts on dorsal horn neurons to enhance glutamate excitatory synaptic transmission and decrease GABA-mediated and glycine-mediated synaptic inhibition (Kawasaki et al., 2008) (**Figure 2**).

**-TNF:** TNF\(\alpha\) is induced in microglia after nerve injury via p38 MAPK (Kanda et al., 2017), which is activated by signals from purinoceptors P2X7 and P2Y12,
fractalkine receptor CX3CR1, receptor tyrosine-protein kinase erbB2 (ERBB2) and TLR2 (Inoue, 2022). TNFα receptors (TNFRs) are present in dorsal horn neurons, in the presynaptic terminals of primary afferents, in microglia, in astrocytes and in endothelial cells. In dorsal horn neurons, TNFα increases glutamate-mediated responses. At the presynaptic terminals of primary afferents, TNFα modulates glutamate release (Park et al., 2011). TNFα acts via microglial TNFRs to induce the microglial BDNF (Liu et al., 2017b). In astrocytes, TNFα induces the release of chemokines, which increase excitatory synaptic transmission via their receptors (Chen et al., 2014; Gao et al., 2009b) (Figure 2). In endothelial cells, TNFR activation upregulates cyclooxygenase 2 (COX2) and prostaglandin I2 synthase (PTGIS), which in turn leads to prostaglandin I2 (PGI2) production (Kanda et al., 2017). PGI2 acts on PGI2 receptors (IP receptors) expressed on presynapses and postsynapses and enhances glutamatergic excitatory synaptic transmission (Kanda et al., 2017) (Figure 2).

Astrocytes are also crucial actors in central mechanisms of chronic pain (Ji et al., 2019). Upon nociceptor damage microglia secrete TNFα and IL-1β that activate astrocytes (Inoue and Tsuda, 2018). Dorsal horn neurons release C–X–C chemokine ligand 13 (CXCL13) in chronic pain that can also activate astrocytes through the stimulation of the C–X–C motif chemokine receptor 5 (CXCR5) (Jiang et al., 2016). TLR4 are present in astrocytes and contribute to activation of them too (Tanga et al., 2005). Astrocytes change in morphology, upregulate GFAP and connexin 43 hemichannels (Cx43) (Guo et al., 2007), and activate extracellular signal-regulated kinase (ERK) and c-Jun N-terminal kinase (JNK) signaling (Jin et
al., 2003; Tsuda et al., 2004; Zhuang et al., 2005; Zhuang et al., 2006). These results in the increased release of pro-inflammatory chemokines (CXCL1 and C–C motif chemokine 2 (CCL2)) (Chen et al., 2014; Gao et al., 2009a), of glutamate and ATP (Ji et al., 2019) (Figure 2), of growth factors including WNT family member 3A (WNT3A) (Zhang et al., 2013), and of fibroblast growth factor 2 (FGF2) (Ji et al., 2006). These mediators can alter neural excitability through modulation of excitatory and inhibitory synaptic transmission.

The influence of the adaptive immune response in the CNS, although has a critical role in some neurodegenerative diseases (Gonzalez and Pacheco, 2014), has proven controversial in chronic pain, specifically in neuropathic pain. In diseases where the blood-brain barrier (BBB) is damaged, T-cells are important players in development of pain, but this is not the case in peripheral nerve damage, where the BBB remains intact. Some initial reports suggested that T-cells infiltrate the spinal cord after peripheral nerve injury (Costigan et al., 2009; Leger et al., 2011), however it was in very low numbers. The evidence now shows that T-cells may have a role in hyperalgesia by infiltrating peripheral damaged nerves but not the CNS (Laumet et al., 2019; Moalem et al., 2004).

Altogether, the activation of these signaling pathways in microglia and astrocytes lead to neuroinflammation, central sensitization and to the long-term potentiation of pain circuits.

II.3.1. Microbiota-microglia interaction. It has been demonstrated that commensal microbiota is required for the proper maturation and functional
response to infections of the CNS. Mechanistic analyses indicated that these effects were mediated by the secretion of SCFA and their effects on GPR43 (Erny et al., 2015). Of note, in the context of PD (see also section III), it has been demonstrated that the absence of commensal microbiota, induced by treatment with antibiotics or by GF conditions, attenuates the microglial activation and the consequent neuroinflammation and motor impairment in preclinical models (Sampson et al., 2016). Furthermore, the reconstitution of GF animals with microbiota from PD patients induced microglial activation and the development of motor impairment in a more severe intensity than GF animals reconstituted with microbiota isolated from healthy subjects (Sampson et al., 2016). Interestingly, the comparison of gut microbiota and microbial metabolic pathways obtained from PD patients and healthy subjects revealed the presence of higher levels and altered proportions of SCFA associated with PD. Accordingly, the treatment with SCFA rescued the microglial activation, neuroinflammation and motor impairment manifestation in GF mice (Sampson et al., 2016). A later study identified *Proteus mirabilis* as bacteria particularly enriched in the gut microbiota of three different animal models of PD, including the mouse models induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), MPTP plus probenecid, and 6-hydroxydopamine. Furthermore, the oral administration of *Proteus mirabilis* exacerbated microglial activation, neurodegeneration and motor impairment in preclinical models of PD (Choi et al., 2018) (see also section III.4).

The influence of microbiota on the function of microglia has not only been observed in the context of PD, but also in other pathological contexts. For instance, it has been recently reported that due to the increased permeability of the intestinal...
barrier associated with aging, the gut microbiota induces an accumulation of N6-carboxymethyllysine in the microglia of the aging brain in mouse and human. This metabolite led to impaired mitochondrial function, with a consequent increase in oxidative stress and decreased ATP reservoirs in the microglia of aging brains (Mossad et al., 2022). Moreover, using GF animals in a mouse model of Alzheimer's disease it was recently demonstrated that the absence of microbiota leads to pronounced alterations in the transcriptomic profile of microglia, which was accompanied by a substantial reduction in amyloid-β deposition. This effect was emulated by the supplementation of GF animals with SCFA (Colombo et al., 2021), indicating again an important role of these bacterial metabolites in the communication with microglia and the development of neurodegenerative diseases.

Another interesting example illustrates how in some cases, the microbiota-microglia communication involves the participation of the vagus nerve. In this regard, it has been shown that high-fat diet and high-sugar diet induce changes in the gut microbiota composition, which triggers a reorganization of the gut-brain vagal communication that leads to microglial activation and enhanced body fat accumulation (Sen et al., 2017; Vaughn et al., 2017). It is also important to consider that microglia may work as a bridge for the action of microbiota over astrocytes, which might subsequently induce regulation of neuronal excitability affecting central mechanisms of pain (see more detail in section II.3.3).
Integrating these examples and other studies addressing the interaction between microbiota and microglia, four different mechanisms have been proposed to mediate this communication: i) The modulation of neural signals in the enteric nervous system by microbiota-derived metabolites, which might alter vagal afferent currents affecting microglial activation (Sen et al., 2017; Vaughn et al., 2017); ii) Direct action of bacterial-derived metabolites that cross the BBB, such as the SCFA, by stimulating their corresponding receptors on the microglia (Colombo et al., 2021; Wenzel et al., 2020); iii) Endocrine regulation exerted by bacterial metabolites on peripheral glands (i.e., pancreas or thyroid), which can have an impact on microglial activation (Knezevic et al., 2020; Thomas and Jobin, 2020); and iv) Modulation of the functional behavior of peripheral immune cells in the gut mucosa by microbiota-derived metabolites, which might infiltrate the brain parenchyma and exert regulation on the microglial function (Abdel-Haq et al., 2019; Campos-Acuna et al., 2019). The role of microglia in central mechanisms of microbiota-mediated regulation of pain is illustrated in Figure 2.

Of note, although most studies analyzing the role of brain-macrophages in microbiota-mediated regulation of pain through central mechanisms have been focused on microglia (parenchymal CNS resident macrophages), it is likely that non-parenchymal CNS resident macrophages play a similar role, including those located in choroid plexus, meninges and perivascular spaces (Li and Barres, 2018). Indeed, recent evidence has shown that functional features of non-parenchymal CNS resident macrophages might be influenced by the microbiota (Hasavci and Blank, 2022; Sankowski et al., 2021). Furthermore, it has recently been shown a fundamental role of non-parenchymal CNS resident macrophages in...
neuroinflammation associated with PD (Schonhoff et al., 2023). In addition, in those processes involving infiltration of peripheral immune cells into the CNS, such as the case of pathogen-induced (De Vlaminck et al., 2022) or nerve-injury-induced (Kiguchi et al., 2018) neuroinflammation, it is possible that macrophages from peripheral origin might also display a functional performance similar to microglia in central mechanisms of microbiota-mediated regulation of pain perception.

II.3.2. Microbiota and adaptive immune system interaction. Another way by which microbiota might affect neuroinflammation is through the regulation of T-cell function (Campos-Acuna et al., 2019). In this regard, depending on the functional phenotype, T-cells exert a potent regulation on the polarization of microglia when infiltrate the CNS (Gonzalez et al., 2015; Gonzalez and Pacheco, 2014). Whereas Th1 and Th17 cells induce the acquisition of a pro-inflammatory phenotype in microglia, Treg and Th2 cells favor the differentiation of microglia with an anti-inflammatory functional phenotype (Gonzalez et al., 2014) (Figure 2). However, the participation of T-cells in the regulation of pain in the CNS is restricted to those pathological conditions that involve the BBB disruption, such as the case of PD or multiple sclerosis (Gonzalez et al., 2015; Gonzalez and Pacheco, 2014). For instance, by targeting the T-cell response, the treatment with rapamycin reduced the threshold of neuropathic pain in a model of multiple sclerosis (Lisi et al., 2012). In many models of chronic pain both, inflammatory, and neuropathic, CD4+ T-cells have been found to be able to modulate pain. They infiltrate the DRG and the nerve in neuropathic models, or the site of inflammation in inflammatory models. T-cells can both suppress and promote chronic pain as they can secrete pro- and
anti-inflammatory cytokines, endogenous opioids, and proteases that regulate nociception either via a direct effect on sensory neurons or indirectly though the modulation of neuroinflammation (for more information see review in (Laumet et al., 2019)). In a model of neuropathic pain, antibiotics could attenuate pain like behaviours apparently via increase in anti-inflammatory regulatory T-cells, as depletion of these T-cells reversed the protection offered by gut microbiota changes (Ding et al., 2021). However, it is unclear where these T-cells were located, as the authors report them in the spinal cord. Nevertheless, most articles now suggest no infiltration of T-cells after peripheral nerve injury.

II.3.3. Microbiota interactions with astrocytes. The activity of astrocytes is getting more importance regarding the microbiota-gut-brain axis during last years (Zhao et al., 2021a). For instance, it was shown that NLRP3 knockout mice, which display significant differences in the gut microbiota composition compared with wild-type littermates, were resistant to depressive-like behavior induced by chronic unpredictable stress. Moreover, the fecal microbiota transplantation from NLRP3 knockout mice could ameliorate depressive-like behavior in wild-type mice exposed to chronic unpredictable stress. Mechanistic analyzes revealed that depressive-like behavior induced by chronic unpredictable stress involved astrocyte dysfunction in the hippocampus of wild-type mice, and the fecal microbiota transplantation from NLRP3 knockout mice ameliorated this astrocyte dysfunction (Zhang et al., 2019b). Microbiota may regulate astrocyte activity by the production of metabolites derived from the tryptophan such as indole, indoxyl-3-sulfate, indole-3-propionic acid, and indole-3-aldehyde, which are generated by the tryptophanase activity. Once
produced, they might reach the brain and act on the ligand-activated transcription factor aryl-hydrocarbon receptor (AHR) on astrocytes, a receptor whose expression is stimulated by type I interferon (IFN-I) signaling (Rothhammer et al., 2016). Besides, by acting on microglia, the microbiota can also indirectly modulate the pathogenic activity of astrocytes. For instance, it has been described that microbiota composition might affect the microglial production of transforming growth factor α (TGFα) and VEGF-B, which respectively regulate negatively and positively the pathogenic activity of astrocytes (Rothhammer et al., 2018) (for other effects of microglia-mediated effects of microbiota in central mechanisms of pain see section II.3.1). A summary of the central mechanisms by which microbiota might modulate pain is illustrated in the Figure 2.

Thus, central mechanisms of pain regulation by microbiota involve indirect effects of microbiota on microglia, astrocytes and T-cells, which subsequently exert actions on neurons of the DRG affecting their excitability and pain perception. Thereby, similar to the peripheral mechanisms of pain regulation by microbiota, the central mechanisms of pain regulation involve two layers of complexity as several bacterial mediators might affect the functional response of a set of intermediate cells (i.e. T-cells, astrocytes and microglia), which in turn respond producing several mediators that might potentially affect neuronal excitability. Moreover, it is important to consider that there is also a complex cross-talk between the different types of intermediate cells, affecting the functional response of each other. Considering all these levels of complexity, it is difficult to predict the final effect of
dysbiosis on the central pathways of pain. Further research in understanding the
precise communication between intermediate cells (T-cells, astrocytes, and
microglia), the cross-talk involved in bacteria-bacteria, and between bacteria and
intermediate cells is required to predict how precise changes in the microbiota will
affect pain perception.

III. Microbiota-gut-brain axis in Parkinson’s disease
Growing evidence suggests that PD begins with early symptoms in the gut, such
as inflammation and impaired motility, and subsequently the disease spreads to
the brain (Campos-Acuna et al., 2019). This early symptomatology is strongly
associated with dysbiosis involving enhanced intestinal epithelial barrier
permeability (Chassard et al., 2012; Stolfi et al., 2022). In consequence, this higher
permeability initiates a local inflammatory response, increasing the oxidative stress
in macrophages of the gut mucosa and promoting the aggregation of α-synuclein in
enteric neurons (Forsyth et al., 2011; Glass et al., 2010). Afterward, these
pathological α-synuclein would migrate through the vagus nerve into the brain stem
to initiate the brain pathology and subsequent neurodegeneration of dopaminergic
neurons of the substantia nigra (Campos-Acuna et al., 2019). On the other hand,
dysbiosis present in PD patients (Huang et al., 2021) might be accompanied by
changes in the levels of several soluble mediators and metabolites such as
dopamine, glutamate, GABA, serotonin, SCFA, all of which may stimulate their
receptors expressed on enteric neurons and immune cells affecting later the brain
pathology (Campos-Acuna et al., 2019; Pacheco, 2019).
III.1. Involvement of constipation

Constipation represents prodromal symptomatology associated with dysbiosis affecting 50 – 80% of PD patients (Chen et al., 2015; Verbaan et al., 2007). In 20% of the cases, this symptomatology appears from 10 up to 20 years before the clinical characterization (Chen et al., 2015; Poirier et al., 2016; Savica et al., 2009). Thereby, constipation is considered a risk factor (Abbott et al., 2001; Yao et al., 2023) and biomarker (Grillo et al., 2022) of PD related to gut dysbiosis (Chassard et al., 2012). Nonetheless, how gut dysbiosis and constipation might be functionally associated with other early symptomatology in PD remains unexplored.

Primary chronic constipation involves symptoms of predominantly difficult, infrequent, or incomplete defecation that may be accompanied by abdominal pain and bloating (Ford et al., 2014a; Ford et al., 2014b). In addition, it has been shown that PD patients present a loss of catecholaminergic neurons and other types of enteric neurons within the colon (Lebouvier et al., 2010), which would explain the slower peristalsis. Concerning this, it has been shown that dopamine might inhibit indomethacin-induced intestinal hypermotility through DRD2 signaling (Miyazawa et al., 2003). Moreover, it has been shown that infection-induced inflammation might promote the loss of enteric neurons and reduced intestinal motility in mice, which was limited by adrenergic signaling in tissue resident macrophages. Of note, this reduction in enteric neurons and intestinal motility was correlated with dysbiosis, and these changes were abrogated by the transfer of fecal microbiota obtained from non-infected SPF animals (Matheis et al., 2020). Altogether, these studies suggest that intestinal dysmotility associated with PD is a consequence of...
microbial dysbiosis, which might be mediated by mechanisms dependent on the loss of enteric neurons.

### III.2. Involvement of the vagus nerve

Different lines of evidence support the idea that α-synuclein pathology spreads from the intestine to the CNS through the vagus nerve in PD. For instance, some studies have shown that complete but not partial vagotomy may represent a protective factor for the development of PD (Liu et al., 2017a; Svensson et al., 2015). Furthermore, the delivery of preformed fibrils of α-synuclein in the duodenal and pyloric muscularis layer led to the spreading of pathogenic α-synuclein to the CNS, accompanied by motor and non-motor symptoms. The spreading of pathogenic α-synuclein to the CNS, the consequent neurodegeneration, and associated symptomatology were abrogated by truncal vagotomy (Kim et al., 2019). Similar results were observed upon the gastric delivery of preformed fibrils of α-synuclein in mice (Uemura et al., 2018). Moreover, it has been recently shown that the stereotaxic delivery of PD intestine or vagus nerve lysates into the striatum reproduced synuclein pathology, microglial activation, astrogliosis, and dopaminergic neurodegeneration in rats (Yang et al., 2023). Altogether, these studies support the hypothesis that early synuclein pathology observed in the intestine spreads later to the CNS through the vagus nerve in PD patients and animal models.

### III.3. Involvement of the vermiform appendix
Since its anatomical location is close to cells of the nervous system, the immune system, and local microbiota, the involvement of the vermiform appendix in PD has gained attention during the last few years (Cosentino et al., 2019). In this regard, the appendix not only constitutes a site enriched in lymphoid tissue and cells of the immune system (Kooij et al., 2016) but also is considered a “microbial reservoir” as it harbors bacterial biofilms that are essential constituents of the gut microbiota (Girard-Madoux et al., 2018). Notably, it has been reported that lysates from healthy appendix might induce rapid cleavage and oligomerization of full-length recombinant α-synuclein (Killinger et al., 2018), thus suggesting a potential role of this tissue in the initiation of synuclein pathology. In this way, the appendix could generate new α-synuclein epitopes, triggering an autoimmune response to α-synuclein-derived antigens (Sulzer et al., 2017) and potentially affecting the development or progression of PD. However, the current evidence obtained through the analysis of different cohorts of PD patients is controversial, as some studies suggest that appendectomy would have a protective role in PD development and progression (Killinger et al., 2018; Kim and Bandres-Ciga, 2019; Liu et al., 2020a; Mendes et al., 2015), while other works conclude that appendectomy constitutes a risk factor for PD (Sheriff et al., 2019; Svensson et al., 2016). Moreover, another group of studies concludes that appendectomy history is not related to PD risk (Dahbour et al., 2021; Yilmaz et al., 2017). As far as we know, appendectomy has yet to be addressed in preclinical PD models. Another unresolved issue is to explore the appendix’s contribution during acute inflammatory conditions such as appendicitis or diverticulitis (Cosentino et al.,
2019). Nonetheless, even controversial, the appendix constitutes a crossroad that deserves further exploration in PD pathogenesis.

III.4. Involvement of the gut microbiota dysbiosis

Several studies conducted in patients and animal models have consistently shown that PD involves a dysbiosis of the gut microbiota, with some bacteria displaying higher representation (Table 1), and another group of bacteria that are decreased (Table 2), which might be implicated in the pathogenesis of the disease in many ways. For instance, the enhanced representation of some bacteria involved in SCFA metabolism, such as Enterococcaceae (Barichella et al., 2019; Li et al., 2017), Papillibacter cinnamivorans and Butyricoccus (Devriese et al., 2017; Vanden Abbeele et al., 2013), may contribute to change the homeostatic ratio of acetate:propionate:butyrate in the intestinal mucosa. These changes can lead to detrimental effects, such as increased permeability of the epithelial gut barrier, inducing an inflammatory profile in lymphocytes and innate immune cells, and/or promoting pain hypersensitivity through stimulation of surface SCFA receptors and inhibition of HDAC (see sections II.2.1). In addition, the increased proportion of some bacteria that produce LPS, including Enterococcaceae (Barichella et al., 2019; Li et al., 2017), Escherichia coli, and Gammaproteobacteria (Gorecki et al., 2019; He et al., 2013; Kim et al., 2016a; Rapsinski et al., 2015), might trigger an inflammatory profile in innate immune cells of the gut mucosa by stimulating TLR, favoring oxidative stress and synuclein pathology. Similar to these pathogenic bacteria in human PD, it has been shown a higher proportion of Proteus mirabilis in a number of PD mouse models, whose oral administration into healthy mice can
trigger α-synuclein aggregation in the intestine and the brain, neuroinflammation, and neurodegeneration (Choi et al., 2018) (see also section II.3.1). Mechanistic analysis revealed that LPS produced by this bacteria induced down-regulation of tight junctions expression in gut epithelial cells, thereby disrupting the integrity of the epithelial intestinal barrier, with the subsequent TLR-mediated inflammatory response by mucosal immune cells and the generation of α-synuclein aggregation (Choi et al., 2018). Changes in the representation of bacteria with glucuronidase activity, including *Escherichia coli* (Beraud and Maguire-Zeiss, 2012; Shishov et al., 2009; Tsavkelova et al., 2000) and *Clostridium clusters IV* (*Clostridium leptum*) and *Clostridium cluster XIVa* (*Clostridium coccoide* (Asano et al., 2012), may affect the levels of free catecholamines in the intestinal mucosa, thereby impacting the inflammatory behavior of mucosal immune cells and promoting intestinal dysmotility and constipation (Fruhwald et al., 2002; Miyazawa et al., 2003) (see also section IV.1.3). Furthermore, reducing bacteria with beneficial effects may also contribute to PD pathogenesis. In this regard, some bacteria that contribute to the production of mucin are decreased in PD dysbiosis, such as the case of *Prevo*tella and *Ruminococcus* and *Lachnospiraceae* (Blautia, Roseburia) (Aho et al., 2019; Cakmak, 2015; Cosma-Grigorov et al., 2020; Hill-Burns et al., 2017; Li et al., 2017; Lubomski et al., 2022b), which consequently result in impaired gut barrier integrity and increased permeability. Moreover, the decreased representation of some bacteria producing butyrate and propionate, such as *Dorea longicatena* (Berni Canani et al., 2016; Louis and Flint, 2017) and *Prevo*tella *copri* (Bullich et al., 2019; DeCastro et al., 2005), might lead to an altered proportion of these
SCFA, thus triggering detrimental effects in the gut permeability and the inflammatory profile of mucosal immune cells. For instance, a recent study using a mouse model of CNS autoimmunity provided evidence of how the reduction of propionate in the gut mucosa results in decreased barrier function and in a switch on the profile of mucosal T-cells from anti-inflammatory to pro-inflammatory and in the escape of autoreactive T-cells from the intestinal mucosa to reach the CNS (Prado et al., 2023).

Interestingly, a recent study found alterations in genera and species that might contribute to constipation in PD, including genera Anaerostipes, Fusicatenibacter, Lachnospiraceae, Blautia, Ruminococcaceae, Coprococcus, Faecalibacterium, Dorea and the species Anaerostipes hadrus, Fusicatenibacter saccharivorans, Coprococcus comes and Dorea longicatena (Fu et al., 2022). Interestingly, they reported a reduction of Coprococcus, Blautia, Ruminococcaceae, Faecalibacterium, and Prevotella, which may be involved in pain-related mechanisms (Table 4).

Similar to the rest of the intestine, dysbiosis has been found in the appendix of PD patients (Li et al., 2021). This dysbiosis involves the enrichment of the families Peptostreptococcaceae (Clostridium cluster XI), Lachnospiraceae (Clostridium cluster XIVa), and Burkholderiaceae, genus Burkholderia. On the other hand, they found a reduction in Methanobacteriaceae, genus Methanobrevibacter, and genera Odoribacter, Clostridium, unclassified Sutterellaceae, and Escherichia (Li et al., 2021). In addition to PD patients, the authors also analyzed the cecal dysbiosis in a transgenic mouse model of PD involving the overexpression of human α-synuclein (harboring the A30P mutation) challenged with dextran sodium sulfate (DSS) to
induce gut inflammation. In the absence of DSS, these mice displayed increased *Lachnospiraceae*, *Christensenellaceae*, *Ruminococcaceae*, *Clostridiales*, and *Burkholderiaceae* and decreased *Enterobacteriaceae* and *Bacteroidales* at family level in comparison with wild-type mice. The exposure of transgenic mice to DSS intensified the overrepresentation of Clostridiales, *Ruminococcaceae* and also induced an increase in the genus Coriobacteriales and Desulfovibrionales (Li et al., 2021). Consistently, higher *Desulfovibrio* levels have also been found in other PD models (Zhao et al., 2021b). Interestingly, this microbiota alteration potentially contributes to pain mechanisms in PD (Table 3).

IV. Microbiota in pain and Parkinson’s disease

Pain is a frequent manifestation of PD in 68-85% of patients (Allen et al., 2016; Beiske et al., 2009). Pain is twice as frequent in PD patients than in individuals without PD, even after adjustment for osteoarticular comorbidities (Negre-Pages et al., 2008). Pain in PD is more frequent in females, although the causes for this are unclear (Gao et al., 2022). Pain can appear anytime during the disease and can be present before diagnosis. The most common type of pain is musculoskeletal (nociceptive), which comes from muscle cramps, dystonia, and joints due to rigidity. PD patients also suffer from peripheral neuropathic pain, which may be radicular (discopathies are common as a result of dystonia (Hollern et al., 2021)) or coming from the dying back of the small nerve fibers in the skin (small fiber neuropathy (Jeziorska et al., 2019)). Central neuropathic pain is common (up to
27% of the patients) and is usually described as bizarre painful sensations such as painful burning, stabbing, aching, itching, or tingling sensations anywhere in the body. It has been recently shown that altered parabrachial nucleus nociceptive processing may underlie this type of pain (Pautrat et al., 2023). Gastrointestinal dysfunctions in PD are well-recognized problems often associated with visceral pain. This type of pain may involve either the central or the enteric nervous systems (Kim and Sung, 2015), and probably gut dysbiosis can have a direct effect. The possible roles of the microbiota in pain in PD are discussed below.

IV.1. Gut microbiota dysbiosis potentially involved in pain in Parkinson’s disease

IV.1.1. The involvement of LPS. Sampson and colleagues demonstrated the causal influence of gut microbiota in the development of parkinsonism in a transgenic mouse model involving the overexpression of human α-synuclein under the control of the Thy1-promoter (Thy1-SNCA mice) (Sampson et al., 2016). In a later study, metagenomic sequencing of the prokaryotic 16S ribosomal RNA gene was conducted with stool samples from PD patients and from Thy-SNCA mice showing a significant dysbiosis. The changes included a relative higher abundance of mucin-degrading Verrucomicrobiae and LPS-producing Gammaproteobacteria in PD patients, whereas in Thy1-SNCA mice only involved a significant reduction of Verrucomicrobiae (Gorecki et al., 2019). Moreover, LPS administration to Thy1-SNCA mice induced an earlier motor impairment compared to those non-treated Thy1-SNCA mice (Gorecki et al., 2019). Of note, LPS produced by gram-negative
bacteria is a recognized trigger of inflammatory pain that binds to monocyte TLR4 leading the activation of MyD88-induced NF-κB/IRF/MAPK pathways (Levy et al., 2009) or that can stimulate currents in DRG neurons through the gating of TRPA1 (Meseguer et al., 2014; Ochoa-Cortes et al., 2010) (Figure 3).

IV.1.2. The involvement of SCFA. Other study revealed a reduction in the bacterial phylum Bacteroidetes and the bacterial family Prevotellaceae, (Petrov et al., 2017; Unger et al., 2016) with a concomitant decrease in SCFA production in PD patients (Unger et al., 2016). Genera Dorea, Bacteroides, Prevotella, and Faecalibacterium were also reduced in samples from PD patients (Keshavarzian et al., 2015; Petrov et al., 2017; Shen et al., 2021). Similarly, other SCFA-producer bacteria were also found diminished in patients, including Butyricicoccus, Fusicatenibacter, Lachnospiraceae ND3007 group, and Erysipelotrichaceae UCG-003 (Lubomski et al., 2022a). Importantly, the butyrate-producing bacteria (with anti-inflammatory activity) from the genera Blautia, Coprococcus, and Roseburia were also reduced in PD patients (Keshavarzian et al., 2015). Of note, through their inhibitory effect over HDAC, SCFA have been shown to attenuate pain and to reduce the levels of TNFα in a peripheral nerve injury model of neuropathic pain (Kukkar et al., 2014). Is important to consider that butyrate might also act on the AHR expressed in glial cells triggering anti-inflammatory effects (Marinelli et al., 2019) (see also section IV.1.5). Thereby, altogether these findings suggest that, through the general reduction of SCFA levels, the dysbiosis associated with PD might promote the development of chronic pain (Figure 3).
**IV.1.3. Dopamine regulation.** Another potential modulator of pain in PD is dopamine. Many studies suggest that dopamine levels are altered in the intestine of PD patients. In this regard, the colonic mucosa is a major source of dopamine, reaching levels from $10^{-7}$ to $10^{-4}$ M in healthy conditions (Channer et al., 2023). This high dopamine content is mainly due to the metabolic activity of some gut microbiota bacteria. Most dopamine reaches the gut lumen as glucuronide conjugate that is biologically inactive. Nevertheless, a group of *Clostridium* species in the gut mucosa express $\beta$-glucuronidase activity, which catalyzes the production of free dopamine in the colonic mucosa (Asano et al., 2012). Accordingly, the levels of luminal dopamine in the gut are strongly decreased (more than 20-fold decreased in the cecum) in GF conditions. Moreover, the transference of 46 *Clostridium* species obtained from SPF into GF animals rescued the levels of luminal dopamine (Asano et al., 2012), thus indicating a major role of these bacteria in the production of intestinal dopamine. Of note, a study analyzing changes in bacterial metagenome in the gut of PD patients and healthy controls found a significant reduction of $\beta$-glucuronidase associated to PD dysbiosis (Bedarf et al., 2017) (see also section IV.1.8). In addition, *in vitro* experiments have shown that some bacteria from the gut microbiota can produce dopamine, including *Bacillus cereus*, *Bacillus mycoides*, *Bacillus subtilis*, *Proteus vulgaris*, *Serratia marcescens*, *Staphylococcus aureus* (Tsavkelova et al., 2000), *Escherichia coli* (Shishov et al., 2009; Tsavkelova et al., 2000), *Hafnia alvei*, *Klebsiella pneumoniae*, and *Morganella morganii* (Özoğul, 2004). Conversely, through its molybdenum-
dependent dehydroxylase activity, *Eggerthella lenta* might metabolize dopamine to yield m-tyramine (Maini Rekdal et al., 2019). Importantly, *Eggerthella lenta* has been found significantly increased in a monkey PD model (Yan et al., 2021). Another study has shown the protective effects of *Bacillus subtilis* in a model of PD in C. elegans (Goya et al., 2020). All this evidence strongly suggests that gut dopamine would be decreased in PD. Of note, inflammatory bowel diseases, which have been positively correlated with PD risk (Kang et al., 2023; Lin et al., 2016; Zhu et al., 2022), also involve a significant reduction in the levels of intestinal dopamine (Magro et al., 2002).

Importantly, alterations in dopamine levels might affect the inflammatory behavior of immune cells, including T-cells and myeloid cells (Pacheco, 2019; Pacheco et al., 2014), all of which might modulate pain through peripheral and central mechanisms (Figures 1 and 2). High dopamine levels observed in the gut mucosa upon homeostatic conditions stimulate low-affinity dopamine receptors, including DRD1 and DRD2, which have been associated with anti-inflammatory effects in immune cells. For instance, DRD1-signaling on macrophages attenuates the activation of the inflammasome NLRP3, thus dampening neuroinflammation in a mouse PD model (Yan et al., 2015) and also reducing systemic inflammation in animal models of sepsis (Torres-Rosas et al., 2014; Yan et al., 2015). Moreover, DRD2-signaling in CD4+ T-cells has been shown to reduce the differentiation into inflammatory phenotypes Th1 and Th17 and promote the immunosuppressive phenotype Treg, consequently decreasing the extent of neuroinflammation and neurodegeneration in a mouse PD model (Liu et al., 2021). On the other hand, the reduced levels of intestinal dopamine associated with gut inflammation might result
in the selective stimulation of high-affinity dopamine receptors, including DRD3 and DRD5, which have been associated with proinflammatory consequences in several studies (Pacheco, 2017; Prado et al., 2013). In this regard, DRD3-stimulation in CD4\(^+\) T-cells promotes Th1 differentiation and favors Th17 expansion (Contreras et al., 2016; Franz et al., 2015; Gonzalez et al., 2013) both *in vitro* and *in vivo*. In addition, DRD3-signaling in Treg limits their suppressive activity and impairs their intestinal tropism (Ugalde et al., 2021). DRD3 stimulation also potentiates the IL-2 production in CD8\(^+\) T-cells, increasing their effector and inflammatory function (Chovar-Vera et al., 2022). Consequently, DRD3-signaling on T-cells strengthens gut inflammation in animal models of inflammatory colitis (Contreras et al., 2016; Ugalde et al., 2021) and favors neuroinflammation in PD mouse models (Chen et al., 2013; Elgueta et al., 2017; Elgueta et al., 2019; Gonzalez et al., 2013). DRD5-signaling in T-cells and myeloid cells has also been consistently shown as a pro-inflammatory signal. For instance, DRD5-stimulation in T-cells has been shown to promote Th17 responses in the context of CNS autoimmunity (Osorio-Barrios et al., 2018). In addition, DRD5 (together with the chemokine receptor CCR9) favors the recruitment of effector T-cells into the intestinal mucosa, which constitutes a critical step for gut inflammation (Osorio-Barrios et al., 2021). Moreover, it has been described that DRD5-signaling in dendritic cells induces the polarization of T-cell responses toward Th1 and Th17, thereby exacerbating CNS autoimmunity (Prado et al., 2012; Prado et al., 2018). Thus, summarizing the role of dopamine in pain associated with PD, the potential reduction of dopamine levels in the gut mucosa might change the type of dopamine receptors stimulated from DRD1 and DRD2 toward DRD3 and DRD5. This change in the stimulated dopamine receptors
would promote Th1 and Th17 responses while dampening Treg function, thereby favoring hypersensitivity through peripheral (Figure 1) and central (Figure 2) pain mechanisms. It is important to note that although many studies have shown pro-inflammatory consequences upon stimulation of immune cells with low dopamine levels (≤ 200 nM) and anti-inflammatory effects when stimulating immune cells with high dopamine concentrations (≥ 1 µM), there are some exceptions to these “rules”. For instance, it has been shown that treating human macrophages with high dopamine levels (1 µM) triggers the activation of NF-κB and the consequent production of IL-1β (Nolan et al., 2020). Of note, depending on the presence and abundance of Enterococcus faecalis in the intestinal microbiota of patients, the administration of L-DOPA might contribute to an increase in the gut levels of dopamine due to the Pyridoxal phosphate-dependent tyrosine decarboxylase activity of this bacterium (Maini Rekdal et al., 2019).

IV.1.4. Alterations in serotonin levels. The evidence has shown how fecal transfer from healthy control mice into MPTP-treated mice (a murine model of PD) was sufficient to reduce the motor impairment and neurodegeneration and also to decrease the expression/production of the TLR4-TNFα axis in the colon and the striatum. Interestingly, all these effects induced by MPTP were accompanied by reduced levels of striatal dopamine and serotonin (Sun et al., 2018), which might exert several immunomodulatory effects (Franco et al., 2007; Pacheco et al., 2012). The analysis of the dysbiosis involved in these mice revealed the reduction of the phylum Firmicutes and the order Clostridiales and augmented levels of the
phylum Proteobacteria and the orders Enterobacteriales and Turicibacterales upon MPTP treatment (Sun et al., 2018). Interestingly, different bacteria within the phylum Proteobacteria, present in human gut in normal conditions or in dysbiosis produce serotonin (Koopman et al., 2021). Moreover, among Clostridiales, it has been demonstrated how *Clostridium ramosum* is able to regulate the availability of gut serotonin through the stimulation of enterochromaffin cells in a mouse model of high fat diet (Mandic et al., 2019). Serotonin acts on peripheral neurons or in the CNS (see Figures 1 and 2), and depending on the cell types expressing serotonin receptors, and the subtype of serotonin receptors, this neurotransmitter might inhibit pain, favor pain, or even maintain the nociceptive response (Cortes-Altamirano et al., 2018). Thus, although there is no direct evidence indicating that PD involves serotonin-mediated regulation of pain, these previous findings together suggest this possibility (Figure 3).

**IV.1.5. Involvement of tryptophan-derived AHR ligands from bacteria.** These metabolites have been described to be produced by *Lactobacillus* spp. (Zelante et al., 2013) and *Clostridium sporogenes* (Dodd et al., 2017). They act on microglia through the aryl hydrocarbon receptor (AHR) inducing the secretion of TGFα and vascular endothelial growth factor-B (VEGF-B). These molecules can increase or inhibit astrocyte inflammatory response respectively (Rothhammer et al., 2018). Of note, butyrate has been proven to be an AHR ligand as well (Marinelli et al., 2019) (see also section IV.1.2). Since different AHR ligands might trigger pro- or anti-inflammatory effects and due to the broad range of *Lactobacillus* genus it is difficult
to predict the final effect of significant changes of *Lactobacillus* spp in the AHR-mediated modulation of pain in PD patients.

**IV.1.6. Involvement of GABAergic signaling from gut microbiota.** Interestingly, the administration of probiotics such as *Lactobacillus rhamnosus* (JB-1) to healthy mice promotes the expression of GABA receptors in specific areas of the brain (Bravo et al., 2011). Besides, other strains of *Lactobacillus* synthesize GABA that potentially inhibits downstream responses caused by nociceptor activation (Cui et al., 2020). Furthermore, *Bifidobacterium spp* and *Bifidobacterium dentium*, which are GABA-producer bacteria, exert a modulatory sensory activity in a rat model of visceral hypersensitivity induced by fecal retention (Pokusaeva et al., 2017). Importantly, the beneficial effects were abrogated when using a mutant version of *Bifidobacterium dentium* encoding a glutamate decarboxylase (the enzyme involved in the synthesis of GABA) without enzymatic activity, confirming that this modulation of nociceptor excitability was conducted through bacterial GABA (Pokusaeva et al., 2017). Apparently, these effects of GABA would be mediated through the GABA$_A$ receptor in DRG neurons (Du et al., 2017) (Figure 3).

**IV.1.7. Changes in the Firmicutes-to-Bacteroidetes ratio.** Changes in Firmicutes-to-Bacteroidetes ratio have been correlated with relative abundance of putative pro-inflammatory bacteria *Rikenellaceae* and *Allobacculum* and decreased relative abundance of putative anti-inflammatory bacteria *Bifidobacterium*. This ratio is indeed decreased in a mouse model of PD induced by rotenone, a pattern
compatible with a putative pro-inflammatory dysbiotic microbial community (Perez-Pardo et al., 2018) (Figure 3).

**IV.1.8. Other microbial metabolites involved in the dysbiosis associated to PD.**

Dysbiosis in PD is associated with reduced hydrogen-producing bacteria (Hasegawa et al., 2015) and downregulation of β-glucuronate degradation genes (Bedarf et al., 2017). Importantly, reduced microbiota-mediated β-glucuronidation would impact free dopamine generation in the intestine (Asano et al., 2012). Of note, this reduction in the levels of dopamine might induce a pro-inflammatory behavior in immune cells, thus favoring chronic pain (see section IV.1.3). On the contrary, genes involved in the synthesis of LPS and type III secretion system were enhanced in commensal bacteria from PD patients (Keshavarzian et al., 2015). For instance, the genus *Desulfovibrio* was correlated with the increase of the severity of PD due to its ability to produce hydrogen sulfide and LPS (Murros et al., 2021) (Figure 3).

These studies provide some causal evidence of how the composition of the gut microbiota could influence on the development of pain associated to PD (Figure 3). Nonetheless, a direct connection between this triad of microbiota-neuroinflammation-pain pathways is still incipient and needs to be further explored. Commensal bacteria that are up-regulated and down-regulated in PD that
potentially are involved in pain modulation are summarized in Tables 3 and 4 respectively.

IV.2. Probiotics used for pain in Parkinson’s disease and others

Due to the high impact of gut microbiota dysbiosis in the pathophysiology of PD and the beneficial effects exerted by fecal transplants from healthy individuals in PD animal models, some authors have formulated probiotics as therapeutic approaches to treat PD. Probiotics are defined as live microorganisms that, when administered in adequate amounts, confer healthy benefits to the host (Hill et al., 2014). Whereas some probiotic formulations have shown beneficial effects at the level of motor impairment, neurodegeneration, and neuroinflammation in animal models (Castelli et al., 2020; Fang et al., 2019; Hsieh et al., 2020; Srivastav et al., 2019), the study of probiotics in PD patients has been limited to improve symptomatology so far. In this regard, probiotics for treating PD patients have been focused on improving constipation, bloating, and abdominal pain (Barichella et al., 2016; Cassani et al., 2011; Georgescu et al., 2016; Tan et al., 2021). Interestingly, these probiotics still had beneficial effects on PD symptomatology when co-administered with dopaminergic agonists (Sun et al., 2022), which opens the possibility of using probiotics as adjuvant therapy to complement classical PD medication. Nevertheless, the mechanisms underlying these beneficial effects still need to be better understood, and further research is required to improve the understanding of the biology behind them.

Concerning the application of probiotics for the treatment of visceral hypersensitivity, formulations including *Bifidobacterium* or *Lactobacillus* have
shown promising results (Figure 3). For instance, oral delivery of two active pharmaceutical ingredients (Lcr Lenio® and Lcr Restituo®) derived from the probiotic bacterial strain *Lactobacillus rhamnosus* Lcr35® was associated with antinociceptive activity in two different rats IBS models (Darbaky et al., 2017). Similar effects have been observed with the probiotic *Lactobacillus plantarum* PS128 (PS128) (Liu et al., 2020c). In another animal model of IBS, a mixture of probiotics containing *Lactobacillaceae* (*L. acidophilus, L. plantarum, L. casei*, and *L. bulgaricus*), *Bifidobacteriaceae* (*B. breve, B. longum*, and *B. infantis*), and *Streptococcus thermophilus* (Figure 3) commercially called VSL#3 presented beneficial effects in terms of pain behavior and reversion of the expression of inflammatory genes in the colon (Distrutti et al., 2013). Another recent study evaluated the impact of microbiota depletion in 25-day-old male mice and found higher brain levels of oxidative stress and visceral hypersensitivity, among other behavioral issues (Arslanova et al., 2021). Interestingly, the oral administration of *L. rhamnosus* B-8238 and *L. plantarum* 8PA3 prevented all these negative changes, including the alleviation of visceral pain (Arslanova et al., 2021). Another study provided evidence that *Lactobacillus reuteri* (DSM 17938), and its conditioned medium, were shown to reduce nociception evoked in a rodent model of visceral pain induced by gut distension or capsaicin in TRPV1-dependent manner (Perez-Burgos et al., 2015). These examples illustrate the important impact of microbiota on pain hypersensitivity and behavioral issues and encourage the use of probiotics to treat visceral pain in PD patients.

IV.3. Other anatomical regions of dysbiosis potentially involved in pain in PD
Although most studies describing the impact of commensal bacteria in pain have been focused on the gut microbiota, it is important to consider that dysbiosis in other anatomical locations might impact pain. In this regard, oral microbiota might affect the composition of commensals in the lower gastrointestinal tract. Thus, the current evidence indicates that the potential translocation of oral bacteria to other niches of the gastrointestinal tract might trigger gut dysbiosis and intestinal inflammation (Elmaghrawy et al., 2020). Indeed, due to the easy collection of samples, oral microbiome has been suggested as a promising biomarker of intestinal health and IBD (Elmaghrawy et al., 2020). Beyond the gastrointestinal tract, the perturbation of the oral microbiome has been recently associated with several systemic disorders, including diabetes mellitus, cardiovascular disease, and bacterial pneumonia (Li et al., 2000). Nevertheless, the current knowledge on how oral microbiome regulates pain is still incipient but promising. For instance, LPS derived from the odontogenic pathobiont Porphyromonas gingivalis can elicit dose-dependent calcium influx and inward currents, sensitizes TRPV1 to capsaicin, and stimulates the release of calcitonin gene-related peptide in trigeminal ganglia (Diogenes et al., 2011). Moreover, the tryptophan-derived metabolite indole produced by oral (i.e. Porphyromonas gingivalis and Fusobacterium nucleatum) and intestinal (i.e. Escherichia coli) microorganisms may stimulate TRPA1 in DRG neurons and the consequent nocifensive behaviors in mice (Chung et al., 2022). These studies in animal models illustrate how the oral microbiome might regulate pain perception by targeting a range of receptors in sensory neurons. Concerning patients with chronic pain conditions, a study conducted with a cohort of IBS patients concluded that pain severity was strongly
correlated to the abundance of some bacterial taxa, suggesting a potential causal relationship (Fourie et al., 2016). Another study carried out with burning mouth syndrome patients found similar results, concluding that a higher abundance of *Streptococcus, Granulicatella, Bergeyella,* and *Rothia* genus in the oral microbiome was associated with this chronic pain condition (Lee et al., 2022). Nonetheless, further research is necessary to decipher the underlying mechanisms involved in the role of these oral bacteria in chronic pain. Importantly, oral dysbiosis has been reported in PD patients (Fleury et al., 2021; Pereira et al., 2017), even at the early stages of the disease development (Mihaila et al., 2019). Moreover, oral dysbiosis in PD has been associated with gut dysbiosis (Jo et al., 2022), with functional alterations including the down-regulation of genes involved in the biosynthesis of glutamate and arginine and the upregulation of antimicrobial resistance markers (Jo et al., 2022), and with a gingival inflammatory environment (Fleury et al., 2021; Radaic and Kapila, 2021). Thereby, the current evidence indicates that the oral microbiome, also called “oralome”, represents a relevant regulator of pain sensitivity, which still needs extensive mechanistic exploration. In addition to the oral microbiome, axillary microbiota dysbiosis has recently been correlated with cognitive impairment in PD patients. Of note, the profile of axillary microbiota dysbiosis allowed to differentiate between healthy controls, mild cognitive impairment PD patients, and PD patients with dementia, which were, in turn, correlated with the depletion of the *Anaerococcus, Peptoniphilus,* and W5053 (Arikan et al., 2022). Interestingly, early in the disease, PD patients present α-synuclein oligomers deposits in the skin and a reduction of intraepidermal nerve
fibers (many of which are nociceptors) (Vacchi et al., 2021). Thereby, these studies encourage the exploration of the role of skin microbiota in the development of pain mediated by small fiber neuropathy in PD.

V. Concluding remarks and perspectives

The commensal microbiota regulates chronic pain through the action of bacterial signals into two main locations: the peripheral nociceptors (peripheral mechanisms) and the postsynaptic excitatory neurons in the spinal cord (central mechanisms). Both kinds of mechanisms involve the participation of structural bacterial components (i.e. LPS, flagellin) as well as soluble mediators generated by commensals, including SCFA, bile acids, PUFA, neurotransmitters, and AHR ligands. These mediators ultimately affect the sensitivity or activity of TRPV1/TRPA1 channels in nociceptive neurons or NMDA/AMPA receptors in postsynaptic excitatory neurons in the spinal cord, thus modulating pain perception. This modulation of pain perception is triggered by the action of bacterial components directly on neurons or indirectly by acting on intermediate actors, such as T-cells, macrophages, enterochromaffin cells, enteric glial cells, microglia, and astrocytes. These mechanisms of pain regulation by microbiota involve many layers of complexity as several bacterial mediators affect the functional response of a set of intermediate actors, which in turn respond by producing some molecules that potentially affect neuronal excitability. Thereby, to understand the outcome of dysbiosis in pain perception, further research should be focused on understanding i. How does the cross-talk between different bacteria in
the microbiota consortium influence the final production of bacterial mediators; ii. How the cross-talk between the different types of intermediate cells affects the functional response of each other and the final production of molecular regulators of nociceptors or postsynaptic excitatory neurons in the spinal cord. This research will allow us to predict how precise changes in the microbiota will affect pain perception.

Interestingly, the detailed analysis of the dysbiosis associated to PD reveals several changes that potentially favor the development of chronic pain. These changes include the increased representation of intestinal bacteria that regulates the levels of SCFA, GABA, serotonin and the stimulation of TLRs by flagellin and LPS. Moreover, the dysbiosis associated to PD also includes a reduced representation of beneficial intestinal bacteria that synthesizes SCFA, GABA and AHR-derived ligands. Many studies addressing the involvement of microbiota-mediated regulation of pain provide associative evidence, but not causal evidence. Consequently, further research is required to determine unequivocally the role and the relevance of bacterial mediators in the central and peripheral mechanisms of pain. Also, there are probably some more bacterial components, still non-identified and with relevant roles in the regulation of pain. Thereby, additional unbiased metabolomic, proteomic, lipidomic and, glycomic analyses would help the discovery of new bacterial regulators of pain.

Despite the controversy, some studies analyzing PD patients and healthy subjects have proposed that appendectomy might be relevant in triggering/protecting PD development. However, obtaining clear conclusions about this issue in humans may be very complex, as many heterogeneous factors might darken the results,
including the age of appendectomy, the age of PD diagnosis, PD stage, eating and exercise habits, and genetic factors. Thereby, further research using preclinical models of PD should evaluate the impact of appendectomy on the pathology’s development and progression. In addition, a few studies have suggested an important role of oral and skin microbiota in developing pain and cognitive impairment PD. These studies encourage further exploring the role of skin and oral microbiota in PD.

Interestingly, it has recently been proposed the existence of a "Brain microbiome" (Link, 2021). Although it is still considered controversial due to the potential contamination of CNS samples analyzed with microbial artifacts, various reports support the involvement of microbial colonization of the CNS parenchyma, especially in pathological processes. However, it needs to be clarified if this colonization takes place in a steady state. This should be evaluated with adequate controls to reduce artifacts due to contamination (Lusk, 2014; Mangul et al., 2018; Salter et al., 2014). Either in a steady state or in association with pathological conditions, the action of local microorganisms in the CNS parenchyma should exert profound effects on direct or indirect stimulation of pain pathways.

Most research studying the causal impact of microbiota in pain (or other biological processes) has been conducted comparing the effects observed on SPF animals with those observed on animals treated with broad-spectrum antibiotics cocktail or maintained in GF conditions. However, these experimental approaches deplete entirely or nearly completely microbiota, which is different from the case of an actual dysbiosis where still exists a bacterial consortium. Since antibiotics or GF conditions do not recapitulate the potential pathogenic communications given in the
bacterial consortium of a dysbiosis, these animal models may not be the most appropriate approaches to study the pathogenic consequences and mechanisms involved in a dysbiosis. In this regard, using gnotobiotic mice harboring precise bacteria mixtures may better represent the biological mechanisms involved in dysbiosis.

Further research should clarify the specific bacteria species or combination of bacteria species as well as the proper relative proportions of them able to rescue a healthy consortium of intestinal bacteria producing a mixture of bacterial products that attenuate chronic pain and rescue the normal nociception. This knowledge would allow the design of probiotics and prebiotics with therapeutic potential not only for PD, but also for the treatment of chronic pain in diverse scenarios. Furthermore, a detailed understanding of the behavior of the consortium of intestinal bacteria, the mediators involved in the intercommunication, and ultimately, the signals produced for the communication with the host would provide the clues to manipulate many inflammatory processes in the host beyond chronic pain.
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DATA AVAILABILITY

The authors declare that all the data supporting the findings of this study are contained within the paper.

AUTHORSHIP CONTRIBUTIONS

Participated in research design: Manjarres Z., Calvo M., and Pacheco R.

Performed data analysis: Manjarres Z., Calvo M., and Pacheco R.

Wrote or contributed to the writing of the manuscript: Manjarres Z., Calvo M., and Pacheco R.
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appendix impacts the risk of developing Parkinson’s disease. *Science translational medicine* **10**.


synuclein oligomers and small nerve fiber pathology in skin are potential biomarkers of Parkinson's disease. *NPJ Parkinson's disease* 7:119.


FOOTNOTES

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*No author has an actual or perceived conflict of interest with the contents of this article.

¶ IASP link resource:

https://www.iasp-pain.org/resources/terminology/?navItemNumber=576#Pain
Table 1. Bacteria increased in the gut microbiota in Parkinson’s disease

<table>
<thead>
<tr>
<th>Model approach</th>
<th>Phylum</th>
<th>Class</th>
<th>Family</th>
<th>Genera/specie</th>
<th>Reference</th>
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<tbody>
<tr>
<td>PD patients</td>
<td>Verrucomicrobiaceae</td>
<td>Akkermansiaceae</td>
<td>Akkermansia spp.</td>
<td>Akkermansia muciniphila</td>
<td>(Keshavarzian et al., 2015; Khedr et al., 2021; Nishiwaki et al., 2020)</td>
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<td>PD patients</td>
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<td>Desulfovibrio</td>
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<td>(Li et al., 2023)</td>
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<td>Gammaproteobacteria</td>
<td>Ruminococcaceae</td>
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<tr>
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<td>Lactobacillus spp.; Lactobacillus mucosae</td>
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<td>Clostridium cluster</td>
<td></td>
<td>(Qian et al., 2018)</td>
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<tr>
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<td>Clostridium cluster</td>
<td></td>
<td>(Qian et al., 2018)</td>
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<td>(Keshavarzian et al., 2015; Li et al., 2023; Petrov et al., 2017; Qian et al., 2018; Yang et al., 2017)</td>
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<td>Bifidobacteriaceae</td>
<td>Bifidobacterium</td>
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<td>Escherichia coli</td>
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<td>(Li et al., 2023)</td>
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<tr>
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<td>Proteobacteria</td>
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<td>-</td>
<td></td>
<td>(Sun et al., 2018)</td>
</tr>
<tr>
<td>MPTP mice and Thy1-SNCA mice</td>
<td>Enterobacteriaceae</td>
<td>Proteus mirabilis</td>
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<td>(Choi et al., 2018)</td>
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<td>(Zhao et al., 2021b)</td>
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Table 2. Bacteria decreased in the gut microbiota in Parkinson’s disease

<table>
<thead>
<tr>
<th>Model approach</th>
<th>Phylum</th>
<th>Family</th>
<th>Genera/specie</th>
<th>Reference</th>
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<td>-</td>
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<td>-</td>
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<td>Prevotella spp; Prevotella copri</td>
<td>(Li et al., 2023; Lin et al., 2019; Petrov et al., 2017)</td>
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<td>Ruminococcaceae</td>
<td>Ruminococcus spp. Ruminococcus callidus; Faecalibacterium sp. Faecalibacterium prausnitzii</td>
<td>(Hill-Burns et al., 2017; Keshavarzian et al., 2015; Li et al., 2023; Petrov et al., 2017; Unger et al., 2016)</td>
</tr>
<tr>
<td>PD patients</td>
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<td>Butyricoccus sp.</td>
<td></td>
<td>(Scheperjans et al., 2015)</td>
</tr>
<tr>
<td>PD patients</td>
<td>-</td>
<td>Clostridium spp; Clostridium cocoides</td>
<td></td>
<td>(Bedarf et al., 2017; Hasegawa et al., 2015)</td>
</tr>
<tr>
<td>PD patients</td>
<td>-</td>
<td>Lachnospiraceae</td>
<td>Blautia spp; Blautia glucerasea; Fusicatenibacter; Coprococcus spp; Coprococcus eutactus; Roseburia spp; Dorea sp.; Dorea longicatena</td>
<td>(Hill-Burns et al., 2017; Keshavarzian et al., 2015; Li et al., 2023; Petrov et al., 2017; Scheperjans et al., 2015)</td>
</tr>
<tr>
<td>PD patients</td>
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<td>Eubacteriaceae</td>
<td>Eubacterium biforme</td>
<td>(Bedarf et al., 2017; Keshavarzian et al., 2015)</td>
</tr>
<tr>
<td>PD patients</td>
<td>Bacteroidetes</td>
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<td>-</td>
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<td>MPTP mouse model</td>
<td>Costridiales</td>
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<td>Clostridium ramosum</td>
<td>(Sun et al., 2018)</td>
</tr>
<tr>
<td>Rotenone mouse model</td>
<td>Actinobacteria</td>
<td>Bifidobacteriaceae</td>
<td>Bifidobacterium spp.</td>
<td>(Perez-Pardo et al., 2018)</td>
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<tr>
<td>Thy1-SNCA mice</td>
<td>Verrucomicrobia</td>
<td>-</td>
<td></td>
<td>(Gorecki et al., 2019)</td>
</tr>
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</table>
Table 3. Bacteria increased in the gut microbiota in Parkinson’s disease associated to mechanisms of pain

<table>
<thead>
<tr>
<th>Bacteria increased in PD</th>
<th>Mediator(s) involved</th>
<th>Specific mechanism</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterococcus sp</td>
<td>Superoxide Hydrogen peroxide Serotonin</td>
<td>Some <em>Enterococcus</em> species damages colonic epithelial cell DNA by producing extracellular superoxide and hydrogen peroxide. -Serotonin production</td>
<td>(Barichella et al., 2019; Dinan et al., 2015; Huycke et al., 2002; Li et al., 2017)</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>LPS (Lipid A endotoxin) Neurotoxins Serotonin</td>
<td>-Enhanced α-synuclein pathology through LPS production. -Endotoxins and neurotoxins releasing. -Inflammatory TLR-induced response -Serotonin production</td>
<td>(Beraud and Maguire-Zeiss, 2012; Gorecki et al., 2019; He et al., 2013; Kim et al., 2016a; Rapsinski et al., 2015; Shishov et al., 2009; Tsavkelova et al., 2000)</td>
</tr>
<tr>
<td>Akkermansia muciniphila</td>
<td>Mucin-degrading enzymes</td>
<td>Degradation of mucus layer in absence of dietary fibers might favor enteric pathogen infection risk and increased barrier permeability.</td>
<td>(Desai et al., 2016; Liu et al., 2020b)</td>
</tr>
<tr>
<td>Ralstonia</td>
<td>Pro-inflammatory cytokines</td>
<td>Proinflammatory dysbiosis in PD gut mucosa</td>
<td>(Engen et al., 2017; Keshavarzian et al., 2015; Munoz-Pinto et al., 2021)</td>
</tr>
<tr>
<td>Gammaproteobacteria:</td>
<td>LPS</td>
<td>-Enhanced α-synuclein pathology through LPS production -Associated with intestinal inflammation -LPS production</td>
<td>(Gorecki et al., 2019; He et al., 2013; Kim et al., 2016a; Shin et al., 2015)</td>
</tr>
<tr>
<td>Desulfovibrio</td>
<td>LPS (Lipid A endotoxin) Hydrogen sulfide (H₂S)</td>
<td>-Enhanced α-synuclein pathology through LPS and H₂S production -Neurotoxicity associated to H₂S at high –concentrations -H₂S can promote α-synuclein oligomerization and aggregation.</td>
<td>(Haouzi et al., 2020; Murros et al., 2021)</td>
</tr>
<tr>
<td>Proteus sp</td>
<td>Pro-inflammatory cytokines</td>
<td>Associated with inflammation, α-synuclein aggregation and pathology</td>
<td>(Choi et al., 2018)</td>
</tr>
</tbody>
</table>

*This table shows bacteria that are increased in the gut microbiota of PD patients and their association with mechanisms described to regulate pain.*
Table 4. Bacteria decreased in the gut microbiota in Parkinson’s disease associated to mechanisms of pain

<table>
<thead>
<tr>
<th>Bacteria decreased in PD</th>
<th>Mediator involved</th>
<th>Specific mechanism</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Prevotella</em> spp.</td>
<td>SCFA (Acetate)</td>
<td>Beneficial bacteria</td>
<td>(Forsyth et al., 2011; Liu et al., 2020b)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Increased permeability and decreased gut barrier integrity with systemic exposure of bacterial soluble toxins and expression of α-synuclein in colon.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Disruption of mucin maintenance through lower anti-inflammatory SCFA production</td>
<td></td>
</tr>
<tr>
<td><em>Bacteroides spp</em>,</td>
<td>Hydrogen water</td>
<td>These bacteria mediate protective effects through hydrogen water production in a rat model of PD</td>
<td></td>
</tr>
<tr>
<td><em>Bacteroides fragilis</em></td>
<td>Acetate GABA</td>
<td></td>
<td>(Bullich et al., 2019; Fu et al., 2009; Marashly and Bohlega, 2017; Strandwitz et al., 2019)</td>
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<tr>
<td></td>
<td></td>
<td>Reduction in: GABA neurotransmitter synthesis, the neuroprotective molecule riboflavin and anti-inflammatory SCFA such as acetate</td>
<td></td>
</tr>
<tr>
<td><em>Blautia spp</em>, <em>Coprococcus spp</em>, <em>Roseburia spp</em>, <em>Faecalibacterium sp.</em></td>
<td>SCFA (Butyrate, Acetate)</td>
<td>Butyrate is a SCFA with anti-inflammatory properties and related to differentiation of T-regulatory cells subsets.</td>
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<tr>
<td></td>
<td></td>
<td>Butyrate can induce anti-inflammatory cytokines gene expression by inhibiting histone deacetylase.</td>
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<tr>
<td><em>Bifidobacterium</em></td>
<td>Anti-inflammatory cytokines</td>
<td>Promote protective cytokines such as TGF-β and IL-10, improving intestinal permeability and</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Hang et al., 2022; Krumbeck et al., 2018; O’Neill et al., 2017)</td>
<td></td>
</tr>
<tr>
<td><em>Eubacterium</em></td>
<td>Anti-inflammatory SCFA</td>
<td>Reduction in anti-inflammatory SCFA</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>(Mukherjee et al., 2020)</td>
<td></td>
</tr>
</tbody>
</table>

Pharmrev Fast Forward. Published on 20 October 2023 as DOI 10.1124/pharmrev.122.000674 This article has not been copyedited and formatted. The final version may differ from this version.
Reduction in: GABA neurotransmitter synthesis, the neuroprotective molecule riboflavin and anti-inflammatory SCFA such as acetate

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>SCFA (Butyrate, Acetate)</th>
<th>Description</th>
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<tr>
<td>Blautia spp; Coprococcus spp; Roseburia spp; Faecalibacterium sp.</td>
<td>SCFA (Butyrate, Acetate)</td>
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<td>Eubacterium</td>
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<td>Reduction in anti-inflammatory SCFA</td>
<td>Mukherjee, 2020</td>
</tr>
</tbody>
</table>

*This table shows bacteria that are decreased in the gut microbiota of PD patients and their association with mechanisms described to regulate pain.*
FIGURE LEGENDS

Figure 1. Peripheral mechanisms involved in microbiota-mediated regulation of pain. Top panel shows direct mechanisms: 1. LPS (a bacterial structural component) might exacerbate pain by stimulating sensory neurons through TLR4-dependent or -independent ways increasing TRPA1 sensitivity. 2-4. Bacterial products, such as N-formylated peptides (NFP), the pore-forming toxin α-haemolysin and PUFAs can directly activate nociceptive terminals respectively by stimulating the NFPR, by assembling pore channels, or by direct stimulation of TRPV4. 5. SCFA might attenuate pain sensitivity by two ways, i) By stimulating surface receptors (SCFAR) expressed on primary nociceptive neurons or ii) By directly inhibition of HDAC. 6. Bacteria-derived GABA stimulates GABA receptors on nociceptive neurons, dampening pain. 7. Some bacteria expressing bile salt hydrolase activity can deconjugate bile acids which in turn stimulate cognate surface receptors on sensory neurons, increasing TRPA1 sensitivity and pain. Bottom panel shows indirect mechanisms. 1. By targeting TLR-4, SCFAR, 5-HT2B and HDAC in macrophages, bacterial products might regulate the acquisition of pro-inflammatory or anti-inflammatory functional profiles, increasing or reducing pain. CR: Cytokine receptors; EP: PGE receptors. 2. By the generation of dopamine, GABA, serotonin, glutamate, SCFA, and bile acids microbiota regulates T-cell function and differentiation into Th1, Th2, Th17 or Treg, thus affecting cytokine production and macrophages phenotype, and ultimately regulating pain. 3. SCFA might induce 5-HT release by enterochromaffin cells, which subsequently
enhances the calcium influx by TRPV1 on primary nociceptive neurons. 4. Some bacterial proteases and mediators act on enteric glia cells inducing the release of ATP, GABA, cytokines and ectoenzymes that modulate local immune cells, catalyze the degradation of neuromodulators, and upregulate TRPV1 expression promoting pain. Figure created with BioRender.com.

**Figure 2. Central mechanisms involved in microbiota-mediated regulation of pain.** The microbiota might exert regulation on central pain pathways by acting on relevant actors of neuroinflammation including neurons, glia, and immune cells. 1. Microbiota might induce inflammatory microglial activation through SCFAR simulation, by inducing changes on vagal afferent currents, and eventually by TLR stimulation. Of note, upon nerve injury, pre-synaptic neuron release ATP, which might also induce microglial activation through P2X7 and P2Y12 purinoceptors stimulation. 2. Activated microglia produces IL-1β, IL-6, IL-18, TNFα and PGE2. These mediators act on post-synaptic excitatory neurons enhancing glutamatergic transmission and reducing the function of inhibitory receptors GABAR and GlyR. 3. TNFα produced by activated microglia acts on microglial TNFR exacerbating their inflammatory activity. In addition, TNFα stimulates the production of PGI2 by endothelial cells, which subsequently favors excitatory transmission. Moreover, TNFα acts directly on pre- and post-synaptic terminals favoring glutamatergic transmission. 4. TNFα and IL-1β can also act on astrocytes triggering the release of CCL1, CCL2, glutamate (Glu) and ATP, all of which stimulate excitatory transmission in post-synaptic neurons. 5. Under certain pathological circumstances
the BBB is disrupted and T-cells infiltrate the brain. Whereas Th1/Th17 favor the proinflammatory function on microglia, Th2/Treg promote an anti-inflammatory/profibrotic phenotype. Of note, inflammatory T-cells might also produce TNFα, promoting further pain. Microbiota can produce AHR ligands, which can reach the brain and act directly on astrocytes inducing anti-inflammatory effects. However, AHR ligands also act on microglial inducing the production of TGFα and VEGF-B, which promote respectively anti- and pro-inflammatory effects on astrocytes. Microbial metabolites can also act on pericytes and perivascular fibroblast-like cells, inducing the secretion of CCL2, which subsequently act on postsynaptic excitatory neurons enhancing their excitability. The bottom-left insert indicates the area of spinal cord where these processes occur. Figure created with BioRender.com.

**Figure 3. Potential bacteria involved in the regulation of pain manifestation in PD.** PD-associated dysbiosis involves increased representation of some bacteria that might promote pain (indicated with ↑) or reduction of bacteria that might dampen pain (indicated with ↓). Thus, these changes would involve increased levels of mediators that exacerbate pain (i.e. LPS, ROS), and reduced production of molecules that attenuate pain (i.e. GABA, SCFA). All these changes might contribute to inflammation and/or direct nociceptor stimulation in dorsal root ganglia afferents in the gut mucosa (red lines). Moreover, some bacteria that cause direct damage to the gut epithelium by H₂S or enhance the gut barrier permeability are also increased in PD dysbiosis (red dotted line), favoring the loss of a tolerogenic
environment in the gut mucosa, promoting inflammation and affecting systemic pathways. Conversely, probiotics based on *Bifidobacterium, Lactobacillus, Streptococcus thermophilus* (blue box) have been proven to modulate inflammation through IL-10 and other anti-inflammatory mediators and directly abrogate pain manifestation (blue lines). For blue and red lines, arrow heads indicate promoting a process, while flat heads indicate inhibition of a process. Figure created with Biorender.com.