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The microbiota-immune-neural axis in chronic pain: a mechanism-centered narrative review

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Abstract

Chronic pain is increasingly recognized as a complex neuroimmune disorder rather than a purely nociceptive phenomenon. Emerging evidence suggests that microbial ecosystems, particularly the gut microbiota, act as upstream modulators of immune and neural signaling pathways that shape pain perception and chronification. This narrative review synthesizes current mechanistic and translational evidence supporting the concept of a microbiome-immune-neural axis in chronic pain conditions.We examine how dysbiosis and epithelial barrier dysfunction promote systemic translocation of microbial-derived molecules, including lipopolysaccharide and other pathogen-associated molecular patterns, leading to activation of innate immune pathways (e.g., Toll-like receptor 4, inflammasomes) and sustained cytokine release. These immune signals sensitize peripheral nociceptors, prime spinal microglia, and alter descending inhibitory circuits, thereby facilitating peripheral and central sensitization. Attention is given to nociplastic pain syndromes, neuropathic pain, musculoskeletal pain, visceral pain, and primary headache disorders, where low-grade inflammation and immune dysregulation intersect with microbial alterations. We further discuss microbial metabolites, short-chain fatty acids, tryptophan-kynurenine derivatives, secondary bile acids, and neurotransmitter-modulating metabolites, as bidirectional regulators of neuroimmune homeostasis. Finally, we evaluate translational implications, including dietary and probiotic interventions, fecal microbiota transplantation, and immune- and microglia-targeted strategies. While causality remains incompletely established and methodological heterogeneity limits definitive conclusions, converging data support a model in which microbial-immune signaling functions as a mechanistic amplifier of pain persistence. Positioning chronic pain within a microbial-immunological framework may redefine therapeutic targets and open precision-medicine pathways aimed at reducing pain by restoring neuroimmune and microbial homeostasis.

Key words: chronic pain; gastrointestinal microbiome; neuroimmune communication; short-chain fatty acids; dysbiosis.

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Introduction

Chronic pain exerts an enormous personal and societal burden and affects more than 30% of people worldwide in many epidemiological estimates, reflecting its high prevalence across musculoskeletal, neuropathic, visceral, and nociplastic phenotypes.[1,2] While canonical models emphasize peripheral nociceptor activation, nerve injury, and maladaptive central plasticity, there is increasing recognition that chronic pain is also shaped by systems-level biology, including immune dysregulation and host-microbe interactions.[3] In parallel with advances in metagenomics and metabolomics, the gut microbiota has been proposed as a candidate upstream modulator of immune tone and neuroimmune signaling relevant to pain initiation, amplification, and chronification, although the strength of this evidence varies considerably across pain phenotypes.[4]

A systematic review and meta-analysis of chronic pain cohorts supports the presence of gut dysbiosis, including reduced alpha-diversity and non-specific but recurrent taxonomic shifts (e.g., lower relative abundance of SCFA-associated genera such as *Faecalibacterium* and *Roseburia* and increased *Eggerthella* spp.), while also highlighting methodological heterogeneity and the need for causal validation.[5] Importantly, recent translational evidence strengthens the causality argument: fecal microbiota transplantation from individuals with fibromyalgia induced pain behaviors and immune/metabolic alterations in germ-free mice, whereas microbiota replacement attenuated these phenotypes, positioning the microbiome as a modifiable contributor to pain.[6]

Mechanistically, microbial modulation of pain is increasingly conceptualized through an integrated microbiota-immune-brain axis, in which innate and adaptive immune signaling constitutes a major communication channel between the gut ecosystem and the

nervous system.[7] Dysbiosis may promote epithelial barrier dysfunction and facilitate systemic exposure to microbial-associated molecular patterns such as lipopolysaccharide, engaging pattern-recognition receptors (notably TLR4) and amplifying pro-inflammatory cytokine signaling that sensitizes peripheral afferents and supports central neuroinflammatory priming.[8] Related TLR4-dependent pathways have also been synthesized in the context of visceral pain and hypersensitivity states, reinforcing the immunological plausibility of microbial pattern sensing as a pain amplifier.[9]

Conversely, microbiota-derived metabolites can exert immunoregulatory and barrier-protective effects. A recent authoritative synthesis details how short-chain fatty acids (butyrate, propionate, acetate) regulate epithelial integrity and mucosal/systemic immunity via GPCR signaling and histone deacetylase mechanisms, including effects on phagocytes and regulatory/effector T-cell balance, processes with direct relevance to inflammatory pain amplification and resolution.[10] Neuroimmune interfaces further extend into the CNS: foundational experimental work demonstrates that the microbiota is required for normal microglial maturation and function, supporting a mechanistic bridge from peripheral microbial ecology to central immune set-points that shape nociceptive processing.[11] Contemporary pain-focused syntheses further integrate these pathways across peripheral sensitization, central sensitization, and metabolite-receptor interactions (e.g., TLRs, GPCRs, TRP channels), while critically appraising microbiota-targeted interventions.[12] Recent domain reviews also consolidate emerging immune-mechanistic links between dysbiosis and neuropathic pain phenotypes,[13] and provide broader overviews positioning the gut microbiota as a modulator and therapeutic target in chronic pain.[14]

The present narrative review is mechanism-centered and focuses on chronic pain. It examines: i) microbiota profiles across major chronic pain phenotypes; ii) innate and adaptive immune mechanisms linking microbial perturbations to peripheral and central sensitization; iii) metabolite-mediated neuroimmune signaling; and iv) translational evidence for microbiota-targeted strategies, with emphasis on causal inference, reproducibility, and mechanistic specificity. Acute pain, cancer-related pain, and computational/AI-based microbiome profiling fall outside the scope of this synthesis and are not covered here.

Methods

This narrative review was designed and conducted in accordance with the Scale for the Assessment of Narrative Review Articles (SANRA) framework, which provides a structured tool to enhance transparency, methodological rigor, and interpretative balance in narrative syntheses.[15]

Justification and aim

The review was undertaken to synthesize emerging mechanistic and translational evidence linking microbial ecosystems, immune signaling pathways and pain modulation. Given the heterogeneity of study designs (pre-clinical mechanistic studies, translational models, observational clinical studies, Mendelian randomization analyses, and prior systematic, scoping, and narrative reviews), a narrative methodology was considered most appropriate to integrate immunological, microbiological, and neurobiological domains into a coherent conceptual framework. Consistent with the conventions of narrative synthesis, an inclusive rather than restrictive approach to study selection was adopted, prioritizing biological coherence and contributory value to the conceptual model over uniformity of design or endpoint type.

Literature search strategy

A structured, non-systematic but transparent literature search was performed in PubMed/MEDLINE, Scopus, and Web of Science from January 2010 to the present, with emphasis on studies published from 2018 onward to capture recent mechanistic advances. Search terms included combinations of controlled vocabulary (MeSH) and free-text keywords: *microbiota*, *microbiome*, *dysbiosis*, *immune signaling*, *TLR4*, *inflammasome*, *microglia*, *short-chain fatty acids*, *gut–brain axis*, *nociception*, *neuropathic pain*, *fibromyalgia*, and *chronic pain*. Boolean operators ("AND", "OR") were used to refine specificity. Reference lists of key articles were hand-searched to identify additional relevant studies.

Study selection and appraisal

Reflecting the cross-disciplinary and still-emerging nature of the field, a deliberately inclusive selection strategy was adopted. Preference was given, where available, to: i) PubMed-indexed primary mechanistic and translational studies, particularly those incorporating causal-inference designs (e.g., germ-free and pseudo-germ-free models, fecal microbiota transplantation, antibiotic-mediated microbiota depletion, Mendelian randomization, or pharmacological pathway modulation); ii) high-impact peer-reviewed reviews (e.g., *Nature Reviews*, *Neuron*, *Pain*, *Brain, Behavior, and Immunity*); and iii) clinical studies with defined pain phenotypes. Beyond these primary sources, the review also incorporates observational case–control and cross-sectional human studies that are largely associative, systematic reviews and meta-analyses (including those analyzing systemic immune or fecal biomarkers without primary microbiome sequencing), scoping and narrative reviews, and a limited number of pharmacological or neurophysiological studies that probe gut–brain–immune signaling without direct microbiome profiling. Such sources were retained when they contributed convergent mechanistic plausibility, addressed phenotypes for which primary microbiome data remain sparse, or provided integrative synthesis of pathways central to the conceptual framework. Sources were excluded only when they lacked methodological clarity, were not peer reviewed, or did not contribute interpretively to the mechanistic narrative. Inevitably, this approach yields a heterogeneous evidence base; this heterogeneity is acknowledged as an intrinsic feature of narrative synthesis in this field rather than a methodological lapse, and is revisited explicitly in the *Limitations* paragraph.

Data synthesis

Evidence was synthesized thematically according to predefined domains: i) microbiota alterations across pain phenotypes; ii) innate and adaptive immune mechanisms linking microbiota and pain; iii) microbial metabolites and neuroimmune signaling; and iv) translational and therapeutic implications. Emphasis was placed on mechanistic plausibility, biological coherence, and convergence of evidence across heterogeneous study designs rather than on quantitative aggregation or strict uniformity of methodology. In line with SANRA principles, the review explicitly addresses scientific justification, structured literature search description, transparent referencing, critical appraisal of evidence strength, balanced interpretation, and delineation of limitations to minimize narrative bias and enhance scholarly rigor.

Microbiota alterations across pain phenotypes

Accumulating evidence indicates that distinct pain phenotypes are associated with reproducible, though not entirely uniform, alterations in gut microbial composition and function.[16] While methodological heterogeneity remains substantial (differences in sequencing platforms, bioinformatic pipelines, dietary control, and phenotypic stratification), convergent patterns suggest that dysbiosis may represent a shared biological substrate across nociceptive, neuropathic and nociplastic pain states.[17] Crucially, the strength and nature of the evidence linking microbial alterations to pain differ markedly across phenotypes and the present section should be read in that light. For interpretive clarity, three levels of evidence are distinguished. Level 1 (translationally supported): phenotypes for which dedicated human microbiome studies are complemented by mechanistic translational experiments providing directional evidence (e.g., germ-free recipients of human fecal microbiota, antibiotic depletion, FMT trials in patients). Level 2 (emerging mechanistic plausibility): phenotypes with either substantive pre-clinical mechanistic evidence but limited human microbiome data, or substantive associative human evidence with limited direct mechanistic anchoring. Level 3 (preliminary): phenotypes for which microbiome-specific evidence rests largely on small observational human studies, indirect biomarker work, or extrapolation from broader neuroimmune frameworks rather than from condition-specific microbiome experiments. This stratification is intended to qualify, not to dismiss, the evidence in less mature domains; it is also acknowledged that the boundaries between levels are not absolute and reflect the current state of a rapidly evolving literature. Level assignments throughout this section should additionally be read against the substantial confounding and reverse-causation concerns specific to chronic pain populations, including diet, obesity, polypharmacy (notably opioids, antidepressants, PPIs, and NSAIDs), sleep disturbance, physical inactivity, mood comorbidities and gastrointestinal comorbidities such as IBS which materially constrain causal interpretation of human microbiome-pain associations. Each phenotype subsection below closes with an explicit level assignment.

Chronic widespread pain and fibromyalgia

Among nociplastic disorders, fibromyalgia (FM) has received the most systematic microbiome investigation. A landmark study by Minerbi *et al*.[18] demonstrated that women with FM exhibit significant alterations in gut microbial composition compared with healthy controls, including differences in taxa correlated with pain intensity and symptom severity. More recently, a causal dimension was introduced by Cai *et al*.,[6] who showed that fecal microbiota transplantation (FMT) from individuals with FM into germ-free mice induced hyperalgesia and metabolic alterations, whereas microbiota replacement attenuated these phenotypes. These data support the hypothesis that microbial communities may contribute directly to pain amplification rather than merely reflecting disease status. Systematic synthesis further supports dysbiosis in chronic pain populations. A recent meta-analysis reported reduced alpha diversity and recurrent shifts in short-chain fatty acid (SCFA)-producing genera such as *Faecalibacterium* and *Roseburia*, alongside enrichment of pro-inflammatory taxa in chronic pain cohorts.[5]

Among the phenotypes considered here, fibromyalgia therefore represents the most translationally supported example (Level 1): the available evidence combines well-controlled human case-control studies, including the Minerbi cohort[18] with explicit dietary and antibiotic stratification,[18] with directional evidence from human-to-germ-free mouse FMT experiments[6]. Even so, human findings remain susceptible to residual confounding (sex, comorbid mood and sleep disorders, medication exposure) and replication across independent cohorts is still limited.

Neuropathic pain

pre-clinical models of neuropathic pain (NP) demonstrate microbiota-dependent modulation of pain behaviors. Germ-free or antibiotic-treated rodents show altered mechanical allodynia and immune signaling following nerve injury, implicating microbial regulation of macrophage and microglial activation.[13] Foundational mechanistic research further establishes that the microbiota shapes microglial maturation and function, providing a neuroimmune bridge relevant to central sensitization.[11] Human data in NP remain associative but suggest reduced microbial diversity and immune-linked compositional shifts. Reviews synthesizing these findings emphasize TLR4-mediated inflammatory pathways and microbial metabolite signaling as candidate mechanisms.[8]

The evidence base in neuropathic pain is best characterized as level 2: pre-clinical mechanistic data from germ-free, antibiotic-depletion and reciprocal FMT paradigms are relatively strong and a Mendelian randomization analysis provides preliminary genetic-instrument support for directionality in selected neuropathic subtypes,[19] but adequately powered, mechanistically anchored human microbiome studies in clearly defined neuropathic pain cohorts remain sparse.

Visceral pain and irritable bowel syndrome

Visceral pain conditions such as irritable bowel syndrome (IBS) display robust microbiota alterations. Meta-analytic evidence demonstrates compositional differences and functional metabolic changes in IBS patients compared with controls.[20] TLR4-associated inflammatory signaling has been implicated in IBS-related visceral hypersensitivity, reinforcing microbial-immune mechanistic plausibility.[9]

IBS-related visceral pain occupies a Level 2 position with phenotype-specific characteristics: human evidence is comparatively abundant but predominantly associative, with much of the synthesized data pertaining to systemic immune and intestinal-permeability biomarkers rather than to direct microbiome-pain causal experiments,[20] and TLR4-pathway inferences derive substantially from scoping rather than primary mechanistic syntheses.[9] Reverse causation, whereby visceral hypersensitivity, altered transit, or repeated dietary modification shape microbial composition rather than the converse, is a particularly relevant consideration in this phenotype.

Musculoskeletal and low back pain

Although fewer studies exist, emerging evidence suggests that gut dysbiosis may be linked to chronic low back pain (CLBP). A recent publication reported altered microbial diversity in individuals with CLBP compared with controls, with correlations between microbial composition and pain severity.[21] Mechanistic extrapolation implicates systemic low-grade inflammation and microbial metabolite imbalance as potential modulators of nociceptive sensitivity. Evidence in CLBP remains preliminary (Level 3): the available human studies are cross-sectional and observational,[21,22] sample sizes are modest and metagenomic or metabolomic anchoring is restricted to subsets and no causal-inference experiments specific to this phenotype have yet been reported. Mechanistic links to systemic low-grade inflammation are at present extrapolated from broader neuroimmune frameworks rather than demonstrated in CLBP-specific microbiome interventions.

Orofacial and headache disorders

Migraine and temporomandibular disorders have also been linked to microbiota perturbations.[23] Reviews highlight alterations in gut microbial composition alongside immune activation and altered tryptophan metabolism in migraineurs. These findings align with broader gut-brain axis models integrating microbial metabolites, cytokine signaling, and trigemino-vascular sensitization.[24] Headache and orofacial pain disorders likewise represent a level 3 evidence base. A pediatric migraine study combining clinical microbiome profiling, a capsaicin rat model and an exploratory probiotic pilot provides one of the more integrated phenotype-specific datasets currently available,[25] and a recent case-control study has highlighted oral rather than gut dysbiosis as a candidate signal in migraine.[26] However, several frequently cited supporting studies probe gut-brain-trigeminal modulation pharmacologically without direct microbiome profiling,[24] and dedicated microbiome-specific evidence in temporomandibular disorders is at present minimal. Conclusions in this domain should accordingly be regarded as hypothesis-generating.

Metabolic-immune integration across phenotypes

Across pain conditions, recurrent themes include reduced SCFA-producing taxa and increased inflammatory signatures. SCFAs are recognized regulators of epithelial integrity and immune homeostasis.[10] Reduced SCFA availability may predispose to epithelial barrier dysfunction and systemic immune priming, amplifying nociceptive signaling pathways. Taken together, the literature reviewed here is more heterogeneous than uniform. The strongest case for a contributory role of microbial alterations is currently restricted to a small set of translationally anchored phenotypes, most notably fibromyalgia, where directional evidence from human-to-germ-free FMT and an open-label patient trial complements substantive observational data (Level 1). Neuropathic pain and IBS-related visceral pain occupy an intermediate position (Level 2), with strong pre-clinical or substantive associative human evidence respectively, but with persistent gaps in phenotype-specific causal experimentation. CLBP, headache disorders and temporomandibular disorders rest on a comparatively preliminary base (Level 3) and at present rely on small observational studies, indirect biomarker work, or extrapolation from broader neuroimmune frameworks. Recurrent themes, reduced SCFA-producing taxa, low-grade inflammatory signatures, and barrier dysfunction, therefore constitute a unifying conceptual scaffold whose weight in any given chronic pain condition must be calibrated to the maturity and nature of the underlying evidence rather than assumed to be uniform across phenotypes.

Collectively, the analyzed literature supports a cross-phenotypic pattern in which dysbiosis, characterized by diminished microbial diversity, depletion of beneficial metabolite-producing taxa, and enrichment of pro-inflammatory organisms, intersects with immune dysregulation and neuroimmune sensitization. Causality has been demonstrated only in a small number of translational contexts (notably FM FMT models in mice). The available human evidence remains predominantly associative and is vulnerable to confounding by diet, medication, comorbidity and reverse causation. With these caveats, the cross-phenotypic convergence of pre-clinical and observational findings is consistent with, but does not establish, a contributory role of microbiota alterations in pain vulnerability and persistence.[27] All the key studies are summarized in Table 1.

It should be emphasized that the SCFA-depletion / inflammatory-priming theme is supported far more uniformly in pre-clinical and translational systems than across the human pain phenotypes discussed above and that its weight in human disease likely varies considerably between, for example, fibromyalgia and CLBP or temporomandibular disorders. Cross-phenotype metabolic-immune themes should therefore be regarded as a unifying conceptual scaffold rather than as evidence of a single, uniformly validated pathway operating across all chronic pain conditions.

Innate and adaptive immune mechanisms linking microbiota and pain

The microbiota-immunity interface is mechanistically the most extensively characterized component of the proposed axis, although the strength of this characterization is uneven: rodent germ-free, antibiotic-depletion, and FMT paradigms provide convergent directional evidence,[28, 29] whereas adequately powered human studies linking specific microbial alterations to defined immune phenotypes in chronic pain populations are comparatively few.[30] The sections that follow distinguish, for each pathway, what has been demonstrated mechanistically (predominantly in pre-clinical systems), what has been established correlatively in humans and what remains plausible but unproven. This distinction is essential because a recurrent feature of the field is that the strength of mechanistic plausibility in animal models is commonly mistaken for the strength of evidence in patients.

Innate immune signaling

Innate immunity constitutes the first line of host-microbial interaction and is critically dependent on pattern-recognition receptors (PRRs), including Toll-like receptors (TLRs) and nucleotide-binding oligomerization domain-like receptors (NLRs).[31] Among these, TLR4 has been particularly implicated in pain modulation.[32] TLR4 recognizes lipopolysaccharide (LPS), a component of Gram-negative bacterial cell walls, and activates downstream MyD88-dependent signaling cascades culminating in NF-κB-mediated transcription of pro-inflammatory cytokines such as IL-1β, TNF-α, and IL-6. Synthetic reviews of TLR4 signaling in neuropathic and inflammatory pain have summarized this evidence,[33-35] although direct human pain-cohort data linking TLR4 expression to specific phenotypes remain limited.

Experimental blockade of TLR4 attenuates mechanical allodynia in rodent nerve injury models, supporting causal involvement.[36] Importantly, TLR4 is expressed not only on peripheral immune cells but also on microglia and astrocytes, linking microbial pattern recognition to central neuroinflammatory amplification. The NLRP3 inflammasome further represents a critical innate immune platform integrating microbial and danger-associated signals.[37] Activation of NLRP3 leads to caspase-1-mediated maturation of IL-1β and IL-18, cytokines strongly implicated in pain hypersensitivity. Inflammasome activation has been documented in neuropathic and inflammatory pain models.[38] Systematic syntheses confirm NLRP3 involvement in persistent pain states.[39]

Microglial activation constitutes a pivotal mechanism bridging innate immunity and central sensitization. Foundational work demonstrated that peripheral nerve injury induces microglial proliferation and activation in the dorsal horn, contributing to neuropathic pain.[40] Microbiota-dependent regulation of microglial maturation and function has been established, providing a direct mechanistic link between microbial ecosystems and CNS immune tone.[11]

Table 1. Key studies examined in this review.

Domain	Author (year)	Design	Cohort/ phenotype	Microbiome profiling endpoints	Main findings (clinical and mechanistic link)	Level	Confounders/ limitations
Chronic widespread pain/fibromyalgia	Minerbi *et al.* (2019)[18]	Human observational case-control 3 control groups defined (relatives/ household/ unrelated)	77 FM vs 79 controls (n=156; women 30–60)	Stool; 16S (V5-V6) + WGS; diversity, DA taxa;	Overall diversity similar, but higher-resolution analyses show altered composition in FM; taxa correlate with symptom severity. Serum SCFAs: butyrate ↑, propionate ↓. Microbiome-only classifier achieved ROC-AUC ~87.8%	1	Diet/ABX controlled; observational; female, mostly Caucasian
	Weber *et al.* (2022)[61]	Single-center case-control	25 FMS vs 26 age/sex-matched controls; FMS diagnosed by ACR 2016; QST (DFNS)	Stool; 16S rRNA (V4); α/β diversity, CCA, DESeq2	Peripheral + central sensitization on QST and higher depression/anxiety/stress in FMS, but no significant differences in fecal microbiome composition or α/β diversity	1	Small sample; ~20% dropout; some recent antibiotics/meds (NSAIDs, antidepressants, PPIs) may affect microbiome; V4-only sequencing; lifestyle residual confounding
	Fang *et al.* (2024)[62]	Open-label randomized, nonplacebo-controlled (FMT + standard care vs standard care)	45 completed: FMT n=22 vs control n=23	Stool microbiome assessed in a subset (15 FMT patients pre/post); α/β diversity + compositional shift post-FMT	Pain (NRS) significantly lower at 2-12 months; WPI/ SS/ HADS/ PSQI improved; 5-HT & GABA ↑, glutamate ↓ at 6 months; higher "effective rate" (90.9% vs 56.5%)	1	Open-label/no placebo; modest sample size; mechanistic inference limited; microbiome analyses only in a subset
	Cai *et al.* (2025)[6]	Translational: human→ germ-free mouse FMT (FM vs HC) + healthy microbiota "replacement"; plus open-label pilot FMT in women with FM	Donors: women with primary FM, screened negative for anxiety/ depression/ IBS; recipients: germ-free female mice; pilot: women with FM	Stool 16S + WGS; multi-omics/mechanisms: untargeted metabolomics, PBMC scRNA-seq, spinal microglia phenotyping; pain assays	FM microbiota induces multimodal pain hypersensitivity and immune/ metabolomic changes; microglia contribute (partial attenuation with PLX5622); bile-acid pathway implicated (UDCA/ursodiol partially analgesic); healthy microbiota replacement reverses pain; pilot FMT associated with reduced pain & improved QoL	1	Female-only (mice + human); human arm open-label/non-controlled (no blinding/control); donor selection may limit generalizability
Neuropathic pain	Allani *et al.*(2026)[63]	Mechanistic study. Rat CCI neuropathic pain; pseudo-germ-free (antibiotics) + wild-type replication; hFMT vs dFMT; behavior + DRG/spinal molecular assays	Adult male SD rats (200–250 g), n=8/group; 16S subset n=3	Fecal 16S rRNA (V3-V4); alpha/beta diversity, LEfSe; DRG/spinal RT-PCR/WB (barrier/immune/ion channels)	CCI dysbiosis (*Proteobacteria/Fusobacteriota ↑; Actinobacteriota ↓);* hFMT reduces hyperalgesia but not mechanical allodynia; dFMT induces hypersensitivity in healthy PGF rats; hFMT restores claudin-5, TGF-β/IL-10 and downregulates TRPM8, Nav1.7/1.8, TRPA1; dFMT increases IBA1, TNF-α, IL-1β	2	pre-clinical study; male-only; microbiome sequencing small subset; effect on allodynia limited; dFMT effects less robust in conventional rats

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Table 1. Continued from previous page.

Domain	Author (year)	Design	Cohort/ phenotype	Microbiome profiling endpoints	Main findings (clinical and mechanistic link)	Level	Confounders/ limitations
	Ramakrishna (2019)[64]	Mechanistic study Mouse strain comparison (B6 sensitive vs 129 resistant) + reciprocal FMT + antibiotic depletion; spinal/brainstem immune profiling	WT + GF/FMT mice (male+ female); paclitaxel 4× i.p. (cum. 16 mg/kg); pain assays (mechanical/ heat/cold)	Fecal 16S rRNA (V4–V5); beta diversity/OTUs ; OUT-pain/ microglia correlations; flow cytometry microglia in spinal cord/ brainstem	Microbiota drives CIPN: B6 microbiota → pain sensitivity; 129 microbiota → resistance; antibiotics prevent CIPN. Pain phenotype tracks with spinal microglial expansion; paclitaxel associated with ↓ *Akkermansia muciniphila* (barrier/ inflammation hypothesis)	2	pre-clinical (mouse-only); strain/batch microbiome variability; limited human translatability; some mechanistic inference (microglia association/ threshold), not definitive in humans
	Pan *et al.* (2024)[19]	Bidirectional two-sample MR + mediation (immune cells/inflammatory factors)	Exposures: 412 gut microbiota traits, 731 immune-cell traits, 91 inflammatory factors; Outcomes: drug-induced neuropathy, PHN, sciatica, trigeminal neuralgia, unspecified neuralgia	GWAS-based taxa + microbial pathway abundances; IVW primary + sensitivity (MR-Egger/weighted median/mode, MR-PRESSO, leave-one-out); mediation proportion	30 causal links (26 taxa → 5 NP subtypes) + 35 pathway abundances linked to NP; no reverse causality (NP → gut microbiota). Two immune mediators identified: HLA-DR+ + monocyte % leukocyte mediates *Proteobacteria* → sciatica (~36%); CD11c on CD62L+ myeloid DC mediates assimilatory sulfate reduction → trigeminal neuralgia (~28%)	2	European-ancestry bias; relaxed IV threshold for microbiota (P<1e−5) → weak-instrument risk; multiple-testing/false positives possible; no individual-level confounders (diet/ABX/meds) for subgrouping; hypothesis-generating
Visceral pain and irritable bowel syndrome	Wan (2024)[9]	Scoping review	40 included studies (clinical TLR4 expression + mechanistic + TLR4-targeted therapies)	Not primary microbiome sequencing; synthesizes TLR4 expression/status, TLR4–MyD88–NF-κB and related pathways; links to dysbiosis, barrier dysfunction, visceral sensitization; summarizes interventions targeting TLR4	Aberrant TLR4 associates with IBS symptoms (incl. pain/diarrhea); implicated in inflammation, barrier damage, visceral hypersensitivity, dysbiosis; multiple therapies (e.g., acupuncture/ herbs/ probiotics/ hormones) may improve IBS by inhibiting TLR4 pathways	2-3	Heterogeneous IBS subtypes/samples; small-study evidence with inconsistent TLR4 findings across some clinical studies; scoping-review synthesis (limited causal inference)
	Burns *et al.*(2024)[20]	Systematic review + random-effects meta-analysis	115 studies; 14,169 IBS; 7,263 healthy controls; 4,190 organic GI disease	Serum + fecal markers	IBS shows ↑ serum TNF-α/ IL-6/ IFN-γ, ↑ fecal calprotectin, ↓ fecal valerate → supports low-grade immune activation/ permeability link	2	High heterogeneity; publication-bias signals in some analyses; subtype reporting often limited

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Table 1. Continued from previous page.

Domain	Author (year)	Design	Cohort/ phenotype	Microbiome profiling endpoints	Main findings (clinical and mechanistic link)	Level	Confounders/ limitations
	Weng *et al.* (2023)[65]	Human case-control + rat NCI model; folic acid (FA) intervention; spinal patch-clamp	IBS (Rome IV) n=27 vs healthy n=27; stool 16S subset n=11/group; fecal H2S subset n=6/group; serum FA n=10/group. Rats: NCI n=12 vs CON n=12; fecal H2S n=6/group; FA-treated microbiome n=3/group	Fecal 16S rDNA; α-diversity; *Clostridiales* differential abundance; fecal H2S; pain endpoints: GSRS abdominal pain score (humans) + CRD threshold (rats); SG neuron sEPSCs (patch-clamp)	IBS: ↑α-diversity + ↑*Clostridiales*; ↑fecal H2S correlates with visceral pain score. NCI rats: ↑*Clostridiales* + ↑H2S; H2S negatively correlates with CRD pain threshold. IBS: ↓serum FA; FA in NCI rats ↓*Clostridiales*/H2S and ↑CRD threshold; FA ↓SG sEPSC frequency	2	Cross-sectional human association + modest subsample sizes for 16S/H2S; FA-microbiome sequencing underpowered (n=3/group); electrophysiology assessed spontaneous EPSCs only
Musculoskeletal and low back pain	Tieppo *et al.*(2024) [21]	Human observational case-control	40 chronic LBP (≥2 months) *vs* 33 asymptomatic controls; age 20-50; BMI ≤30 (n=73)	Stool 16S rRNA (V3-V4); alpha/beta diversity; differential species	LBP group showed higher species richness; *Clostridioides* difficile more abundant; 52 species differed between groups → suggests a gut microbiota "signature" in chronic LBP	3	Excluded recent antibiotics (3 months) and major comorbidities/ continuous anti-inflammatories/ antidepressants; no imaging; 16S only (no shotgun/ functional profiling); observational association (no causality)
	Li *et al.* (2025)[22]	Cross-sectional, MRI-subphenotyped case-control	Chronic LBP n=107 (LBP+FR n=54, LBP n=53) + HC n=31	Fecal 16S on 138 samples; ↓α-diversity/β-separation metrics. shotgun metagenomics n=36; serum untargeted metabolomics (discovery: 40/40; HC 30) + targeted BCAA quant; RF prediction AUC 0.95 (LBP vs HC) and 0.94 (FR)	LBP (±FR) vs HC: ↓α-diversity, distinct β-diversity; enriched families (e.g., *Streptococcaceae/ Prevotellaceae*) & depleted *Ruminococcaceae/ Lachnospiraceae*; FR-discriminators: ↑R. *gnavus, ↓Roseburia hominis, ↓Lachnospiraceae* bacterium 8 1 57FAA; FR: ↓serum BCAAs; "valine/leucine/isoleucine degradation" most significant pathway; Mechanistic: BCAA stimulation → ↑BM-MSC adipogenesis markers (PPARγ, FABP4); ; SIRT4 promotes adipogenesis via BCKDHA axis	3	Groups matched for sex/age/BMI/ lipids; clinical differences modest (VAS/ODI below MCID thresholds); two-stage design with small metagenomics subset; cross-sectional → causality not established; geographical variation acknowledged

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Table 1. Continued from previous page.

Domain	Author (year)	Design	Cohort/ phenotype	Microbiome profiling endpoints	Main findings (clinical and mechanistic link)	Level	Confounders/ limitations
Orofacial and headache disorders	Suthivanich (2025)[24]	Rat CSD (KCl) model + ex vivo TG patch-clamp; 4-week oral probiotic gavage	Adult male Wistar rats, 6 groups, n=8/group	No microbiome sequencing; neurophysiology endpoints: CSD frequency/ amplitude (DC-ECoG) + TG neuron excitability	Strain-specific effects: B. longum ↓ CSD frequency; *L. plantarum* (± *B. longum*) ↓ CSD amplitude; probiotics reduce TG hyperexcitability (↓ spikes, hyperpolarized RMP, ↑ threshold–RMP gap), supporting gut-brain-trigeminal modulation of central/peripheral sensitization	3	Acute 1-h CSD paradigm; male-only; no microbiome/metabolomics (mechanism inferential); key mediators (e.g., CGRP/ TRPV1/ c-Fos) not measured
	Fan *et al.* (2026)[25]	Prospective pediatric migraine vs healthy controls + capsaicin migraine rat model + exploratory randomized double-blind probiotic pilot	126 migraine (6-19y) + 50 controls; GI disorder 59/126; stool n=119, blood n=107; pilot n=23 (17 *B. longum*, 6 control)	Fecal 16S (V3-V4); plasma CGRP/cytokines; fecal calprotectin; rat trigeminal activation (c-Fos in TCC, CGRP in TG)	Migraine vs controls: distinct microbiota with ↓*B. longum* and ↑*Bacteroides*; GI comorbidity: abdominal pain ↑ and PedMIDAS ↑ plus calprotectin ↑ and *S. gallolyticus* enrichment; *F. prausnitzii* linked to milder symptoms (no-GI group). *B. longum* reduces trigeminal activation in rats and improves headache days/pain in pilot	2-3	Excluded recent antibiotics (2 weeks) and probiotics (1 month); single-center; pilot small; male-only rat experiments; human microbiome findings are associative
	Cho *et al.* (2025)[66]	Cross-sectional case–control	Adults 19-65 meeting ICHD-3 criteria. saliva, stool, blood collected	16S rRNA profiling of saliva + stool; alpha (taxa count), beta, differential abundance; PICRUSt/ KEGG functional inference; correlation + clustering; ML classifiers (random forest)	Oral dysbiosis > gut dysbiosis; 13 oral genera altered vs HC after adjustment incl. diet. Oral signatures linked to carbohydrate pathways, depleted taxa to nitrogen/ SCFA-related pathways; classifier AUC 0.83–0.88	3	Adjusted for age/sex/BMI + lifestyle/diet; comorbid anxiety/ depression/ FM considered as covariates. Cross-sectional (no causality); 16S limits species-level resolution; ML internally validated only.

Adaptive immune contributions

Beyond innate pathways, adaptive immunity plays an increasingly recognized role in chronic pain. T-cell subsets modulate both peripheral inflammation and spinal cord neuroimmune interactions. Th1 and Th17 polarization promotes pro-inflammatory cytokine production, while regulatory T cells (Tregs) exert counter-regulatory effects. Experimental depletion of T cells attenuates neuropathic pain in murine models, demonstrating adaptive immune contribution.[41] Conversely, Treg expansion has been shown to reduce pain hypersensitivity and neuroinflammation.[42] Microbiota profoundly influences T-cell differentiation. Short-chain fatty acids (SCFAs) derived from microbial fermentation promote Treg differentiation via epigenetic mechanisms.[43] A comprehensive review further details how SCFAs regulate immune homeostasis and inflammatory responses.[10] Reduced SCFA-producing taxa in dysbiosis may therefore impair Treg-mediated anti-inflammatory buffering, predisposing to persistent inflammatory pain. Additionally, gut microbiota regulates systemic IL-17 production, linking microbial composition to Th17-mediated inflammation.[44] IL-17 signaling has been implicated in neuropathic and inflammatory pain states.[45]

Collectively, the literature is consistent with a working model in which microbiota-derived signals can influence innate and adaptive immune landscapes. TLR4 engagement, inflammasome activation, microglial priming, and altered T-cell polarization have each been

linked to peripheral and central sensitization in pre-clinical models, although the extent to which these pathways operate in concert in human chronic pain remains to be established. Conversely, microbial metabolites such as SCFAs promote immunoregulatory pathways that may mitigate pain amplification. These findings provide mechanistic plausibility for microbiota-targeted immunomodulatory strategies in chronic pain disorders. These pathways are summarized schematically in Figure 1; the schematic is conceptual, and its constituent steps differ in the degree to which they are supported by human as opposed to pre-clinical evidence.

Microbial metabolites and neuroimmune signaling

The functional output of the microbiota, its metabolome, provides a more mechanistically tractable point of entry to the axis than taxonomic composition alone, because individual metabolite classes engage defined host receptors with defined downstream effects. This tractability, however, has not yet been matched by direct evidence in chronic pain patients: most of the data summarized below derive from in vitro systems, germ-free or gnotobiotic rodents and observational human cohorts in which metabolite measurements are decoupled from direct microbiome profiling. The metabolite classes considered here, short-chain fatty acids, tryptophan-pathway derivatives, secondary bile acids, lipopolysaccharide and neurotransmitter precursors, are therefore presented as biologically coherent candidate pathways rather than as established human pain mechanisms and the section closes by identifying which links currently rest on the strongest and which on the weakest, empirical foundations.

Short-chain fatty acids

Short-chain fatty acids (SCFAs), principally acetate, propionate, and butyrate, are generated through microbial fermentation of dietary fibers. SCFAs exert pleiotropic immunoregulatory effects through G-protein-coupled receptors (FFAR2/GPR43, FFAR3/GPR41, GPR109A) and through inhibition of class I and II histone deacetylases (HDACs). A review details how SCFAs regulate epithelial barrier function, macrophage polarization, dendritic cell maturation, and T-cell differentiation, including expansion of regulatory T cells.[10] Earlier seminal work demonstrated that butyrate promotes colonic Treg differentiation *via* epigenetic modification of the Foxp3 locus.[43]

From a neuroimmune perspective, SCFAs influence microglial maturation and functional state. Germ-free mice display immature microglia, and recolonization or SCFA supplementation restores microglial homeostasis, establishing a causal link between microbial metabolites and CNS immune tone.[11] Given the established role of microglial activation in neuropathic pain and central sensitization,[46]

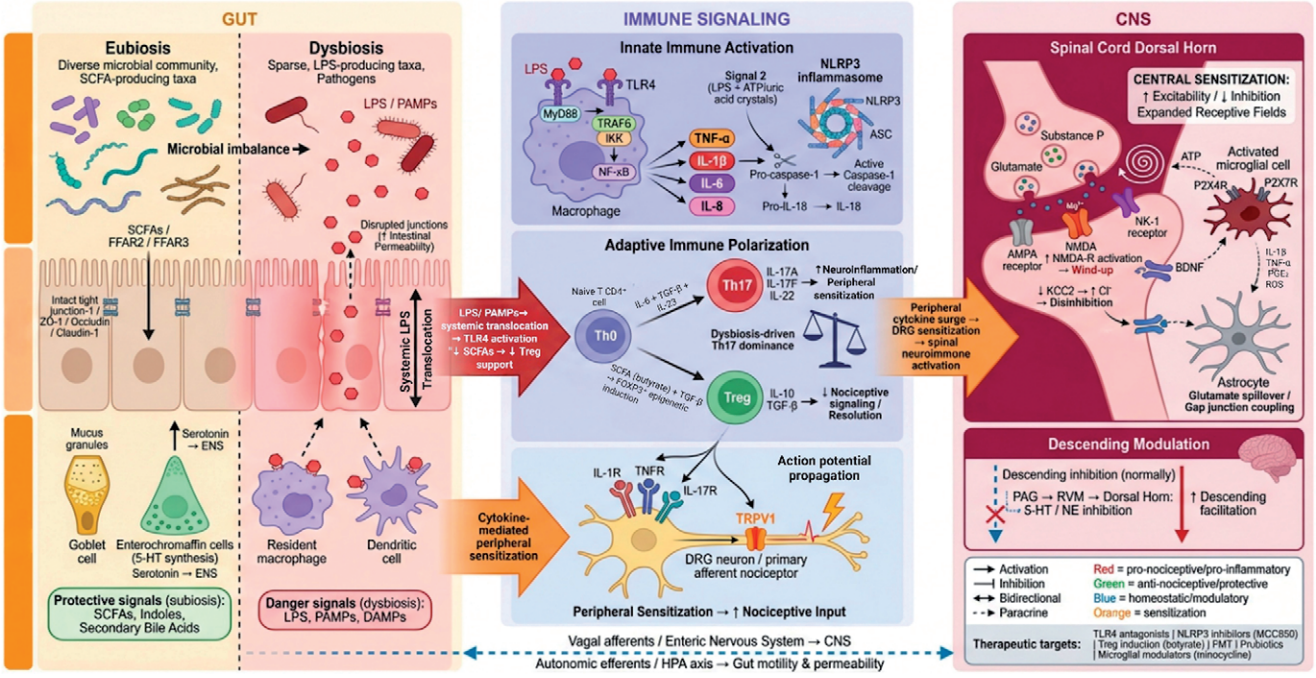

Figure 1. Proposed microbiota-immune-neural interactions implicated in chronic pain. Schematic integration of pathways supported, to varying degrees, by pre-clinical, translational and human evidence. Left, dysbiosis and reduced SCFA-producing taxa have been associated with altered epithelial barrier integrity and translocation of microbial-derived molecules including LPS and other PAMPs. Centre, peripherally, LPS engagement of TLR4 and assembly of the NLRP3 inflammasome can promote pro-inflammatory cytokine release (TNF-α, IL-1β, IL-6, IL-18); reduced SCFA availability has been linked to impaired Treg differentiation and a relative Th17 bias; these signals may sensitize primary afferents and dorsal root ganglion neurons, in part via TRP-channel modulation. Right, in the spinal dorsal horn, microglial activation and altered descending modulation (PAG-RVM) have been implicated in central sensitization. The bottom bidirectional arrow indicates gut-brain crosstalk via vagal and HPA-axis pathways. The sequence depicted is conceptual and should not be read as a uniformly established linear cascade; the relative weight of each step varies across pain phenotypes, and most human evidence remains associative.

SCFA-mediated modulation of microglial function represents a mechanistically coherent pathway linking microbial metabolism to pain amplification.

Tryptophan metabolism and aryl hydrocarbon receptor signaling

Microbiota regulate host tryptophan metabolism, generating indole derivatives that activate the aryl hydrocarbon receptor (AhR), a transcription factor involved in mucosal immunity and barrier integrity. A detailed review of microbial tryptophan metabolites and immune regulation is provided by Wang *et al*.[47] AhR signaling influences IL-22 production and epithelial defense mechanisms, thereby modulating inflammatory tone.[47]

Importantly, alterations in the kynurenine pathway, another branch of tryptophan metabolism, have been implicated in pain and neuroinflammation. Kynurenine metabolites influence NMDA receptor activity and excitotoxic signaling, mechanisms directly relevant to central sensitization.[48] Thus, microbiota-dependent shifts in tryptophan metabolism may modulate both immune and glutamatergic pathways central to pain chronification.

Secondary bile acids and immune modulation

Gut microbes convert primary bile acids into secondary bile acids, which engage host receptors such as farnesoid X receptor (FXR) and Takeda G-protein-coupled receptor 5 (TGR5).[49] These signaling pathways regulate inflammatory responses and epithelial integrity. A synthesis of bile acid-immune interactions is provided by Tong *et al*.[50] Bile acid signaling influences macrophage polarization and cytokine production, processes implicated in inflammatory and neuropathic pain states.

Lipopolysaccharide and neuroimmune activation

Lipopolysaccharide (LPS), a structural component of Gram-negative bacteria, functions as a potent activator of TLR4-dependent innate immune signaling. LPS administration induces hyperalgesia and neuroinflammation in experimental models, effects mediated *via* microglial activation and cytokine release.[51] A recent review summarizes TLR4-pathway evidence in pain amplification,[52] noting that most causal evidence derives from rodent models. Increased intestinal permeability during dysbiosis can facilitate systemic LPS exposure, contributing to low-grade systemic inflammation and central immune priming. The mechanistic framework linking barrier dysfunction, microbial metabolites, and neuroinflammation has been articulated principally by Cryan *et al.,*[53] although translation of this framework to specific chronic pain phenotypes remains incompletely tested.

Neurotransmitter-modulating metabolites

Microbiota also synthesize or modulate neurotransmitter precursors, including γ-aminobutyric acid (GABA), serotonin, and dopamine. Microbial influence on host neurotransmission and behavior has been reviewed elsewhere;[54] its specific contribution to descending pain modulation in humans is less well established. Serotonin biosynthesis in enterochromaffin cells is modulated by spore-forming bacteria, influencing gut-brain communication Given serotonin's established role in descending pain modulation, microbial regulation of its biosynthesis provides an additional neuroimmune interface.

Microbial metabolites regulate immune polarization, barrier integrity, microglial maturation, and neurotransmitter systems through defined molecular pathways. SCFAs promote Treg differentiation and anti-inflammatory signaling; tryptophan metabolites influence AhR and glutamatergic pathways; bile acids regulate macrophage activation; and LPS engages TLR4-dependent inflammatory cascades (Figure 2). These mechanisms outline a biologically coherent, but largely pre-clinical, framework in which the microbiota-derived metabolome may contribute to neuroimmune signaling relevant to pain. Direct human evidence linking specific metabolite profiles to pain phenotypes remains limited.

Translational and therapeutic implications

Translational implications follow from the mechanistic framework outlined above, but their current evidentiary status is highly uneven across intervention class and across pain phenotype. Among the strategies considered below, FMT in IBS-related visceral pain has the strongest controlled-trial evidence; FMT in fibromyalgia has accumulated mechanistic translational support and one open-label clinical trial; probiotic and dietary interventions have generated reproducible but modest signals largely confined to functional gastrointestinal phenotypes; and immune-targeted approaches (TLR4 and NLRP3 modulation) have been tested predominantly in pre-clinical models, with no current chronic-pain trials. Each subsection below states explicitly the current ceiling of evidence rather than treating the strategies as broadly equivalent.[14]

Probiotics, prebiotics, and dietary modulation

Dietary fiber-driven modulation of the gut microbiota represents the most accessible translational avenue. SCFAs generated through microbial fermentation of complex carbohydrates, reinforce epithelial barrier integrity and promote regulatory T-cell (Treg) differentiation, thereby attenuating inflammatory signaling.[43] A further synthesis details the immunoregulatory properties of SCFAs and their systemic effects on inflammatory tone.[10] Given that chronic pain states frequently display low-grade inflammatory signatures, dietary strategies aimed at restoring SCFAs production may offer adjunctive benefit.

Clinical trials investigating probiotic supplementation in IBS, a visceral pain condition with well-characterized microbiota alterations, demonstrate modest but reproducible reductions in abdominal pain and global symptom burden.[55] While heterogeneity in strains and dosing limits generalization, these findings provide proof-of-concept that microbiota-directed interventions can influence pain endpoints.

Fecal microbiota transplantation

More direct microbiota manipulation is achieved *via* fecal microbiota transplantation (FMT). Although primarily established for recurrent *Clostridioides difficile* infection,[56] FMT has demonstrated symptomatic benefit in IBS, including improvements in abdominal pain.[57] Mechanistically, restoration of microbial diversity and metabolic output may recalibrate mucosal immune activation and visceral hypersensitivity.

Translational causality has been strengthened by experimental work showing that microbiota from individuals with fibromyalgia induce hyperalgesia in germ-free mice, supporting a microbial contribution to pain biology.[6] Although therapeutic FMT in nociplastic pain remains investigational, such findings underscore the need for rigorously controlled interventional trials.

Immune-targeted microbiota modulation

Given that microbial influences on pain are largely mediated through immune signaling, therapeutic strategies targeting shared immune pathways may have indirect microbiota relevance. Toll-like receptor 4 (TLR4) antagonism attenuates neuropathic pain behaviors in pre-clinical models.[36] Reviews synthesize the role of TLR4 and innate immune activation in chronic pain.[58] Although systemic TLR4 blockade carries safety concerns, selective modulation of microbiota-derived inflammatory triggers represents a more physiologically nuanced approach.

Similarly, targeting the NLRP3 inflammasome has shown analgesic effects in experimental models.[59] Because inflammasome activation can be driven by microbial signals, microbiota-oriented strategies that reduce inflammasome priming may represent a complementary intervention.

Neuroimmune modulation and microglial targets

Microbiota-dependent regulation of microglial maturation[11] and the well-established role of microglial activation in central sensitization[46] together motivate the conceptual proposal that microbiota-directed interventions might secondarily restore microglial homeostasis. At present, however, this proposal rests on inference from two separate literatures rather than on direct evidence: no human chronic pain study has demonstrated that a microbiota-targeted intervention modifies microglial state, and the existing pre-clinical evidence (e.g., the partial analgesic effect of microglial depletion with PLX5622 in the Cai *et al*.[6] fibromyalgia translational model) is suggestive but not specific to therapeutic microbiota manipulation. Microglial restoration is therefore best regarded as an aspirational rather than an established therapeutic mechanism in this context.

Precision medicine and biomarker development

Future translational strategies require biomarker integration. Multi-omics approaches combining metagenomics, metabolomics, cytokine profiling, and neuroimaging could identify microbiota-linked inflammatory endotypes within heterogeneous pain populations. Conceptual frameworks integrating microbiota and neuroimmune pathways have been advanced in prior syntheses,[33, 53] and extended in subsequent work,[60] from which the schematic in Figure 3 is adapted. A detailed treatment of multi-omics integration and computational profiling lies beyond the mechanistic scope of this review.

Despite promising mechanistic plausibility, clinical relevant analgesic evidence remains hypothetical and preliminary. Variability in probiotic strains, dosing, dietary adherence, and host genetics complicates translation. Moreover, causality in humans requires rigorously designed, adequately powered randomized controlled trials with predefined immune, microbial and analgesic endpoints.

In summary, microbiota-targeted interventions, ranging from

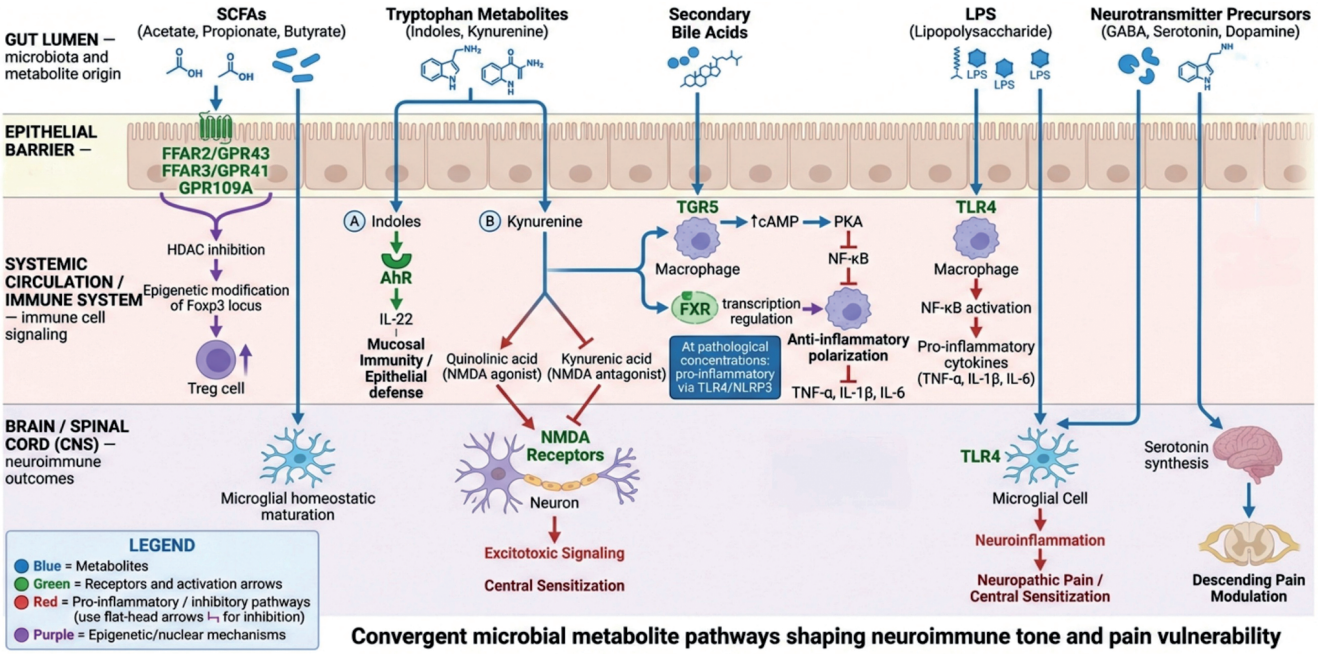

Figure 2. Candidate microbial metabolite pathways relevant to neuroimmune signaling. Receptor and effector targets through which microbiota-derived metabolites have been reported to influence immune polarization, epithelial integrity, and central nervous system signaling. 1) SCFAs (acetate, propionate, butyrate) signal via FFAR2/3 and GPR109A and, in the case of butyrate, inhibit HDACs; these mechanisms have been linked, primarily in pre-clinical models, to Treg differentiation and microglial homeostasis. 2) Tryptophan metabolism yields AhR ligands (indoles) and the kynurenine-pathway metabolites quinolinic acid (NMDA agonist) and kynurenic acid (NMDA antagonist); the relative balance is altered in some dysbiotic states. 3) Secondary bile acids engage TGR5 and FXR, with predominantly anti-inflammatory effects, although high concentrations have also been reported to engage TLR4/NLRP3. 4) LPS activates TLR4-NF-κB signaling in peripheral and central immune cells. 5) Microbiota-modulated biosynthesis of neurotransmitter precursors (e.g., serotonin) may influence descending pain modulation. Pathway weights and clinical relevance differ across pain phenotypes; the schematic is intended to illustrate plausible mechanisms rather than to represent an integrated, causally validated network in humans.

dietary modulation to FMT and immune pathway refinement, could represent biologically plausible adjunctive strategies in chronic pain management. Their successful translation will depend on mechanistic stratification, standardized microbial analytics, and integration of immune biomarkers to ensure precision and reproducibility.

Limitations

This narrative review is subject to several methodological and evidentiary limitations inherent to non-systematic syntheses, of which the most consequential and the most underappreciated in the broader microbiome-pain literature relate to confounding and to the directionality of the proposed associations.

Selection and synthesis bias. Although conducted in accordance with SANRA principles, the literature search was not designed as a formal systematic review or meta-analysis and selection bias cannot be entirely excluded. The evidence base summarized here is intentionally heterogeneous in design and endpoint type, ranging from translational germ-free and FMT experiments and Mendelian randomization analyses to associative observational studies, meta-analyses of systemic immune biomarkers, scoping and narrative reviews, and pharmacological or neurophysiological studies in which microbial composition was not directly profiled. While this breadth was necessary to integrate mechanistic, translational and clinical perspectives across pain phenotypes for which primary microbiome data are unevenly available, it limits the strength of any individual causal claim and reduces comparability across studies.

Translational asymmetry. Much of the mechanistic foundation linking microbiota, immune signaling, and pain derives from preclinical models, germ-free animals, antibiotic manipulation and fecal microbiota transplantation experiments. While these provide strong directional inference, extrapolation to clinically heterogeneous human chronic pain phenotypes must be approached cautiously.

Confounding in chronic pain microbiome research. The interpretation of human microbiome studies in chronic pain populations is uniquely vulnerable to confounding and these confounders are not peripheral statistical issues but central fears to causal inference. Several act simultaneously and in the same direction, generating apparent microbiota-pain associations that may be partially or entirely attributable to shared upstream determinants. Diet is among the most powerful modifiers of gut microbial composition and is systematically altered in chronic pain populations as a consequence of pain itself, comorbid mood symptoms, gastrointestinal symptoms, or therapeutic recommendations; few cited studies adequately control for habitual dietary intake. Obesity and metabolic status independently shape both microbial composition and systemic inflammatory tone and are over-represented in several chronic pain phenotypes (notably musculoskeletal and low back pain). Pharmacological exposures are pervasive: antibiotics produce direct and durable compositional shifts; proton pump inhibitors alter gastric acidity and are associated with overgrowth of oral-type taxa; antidepressants (par-

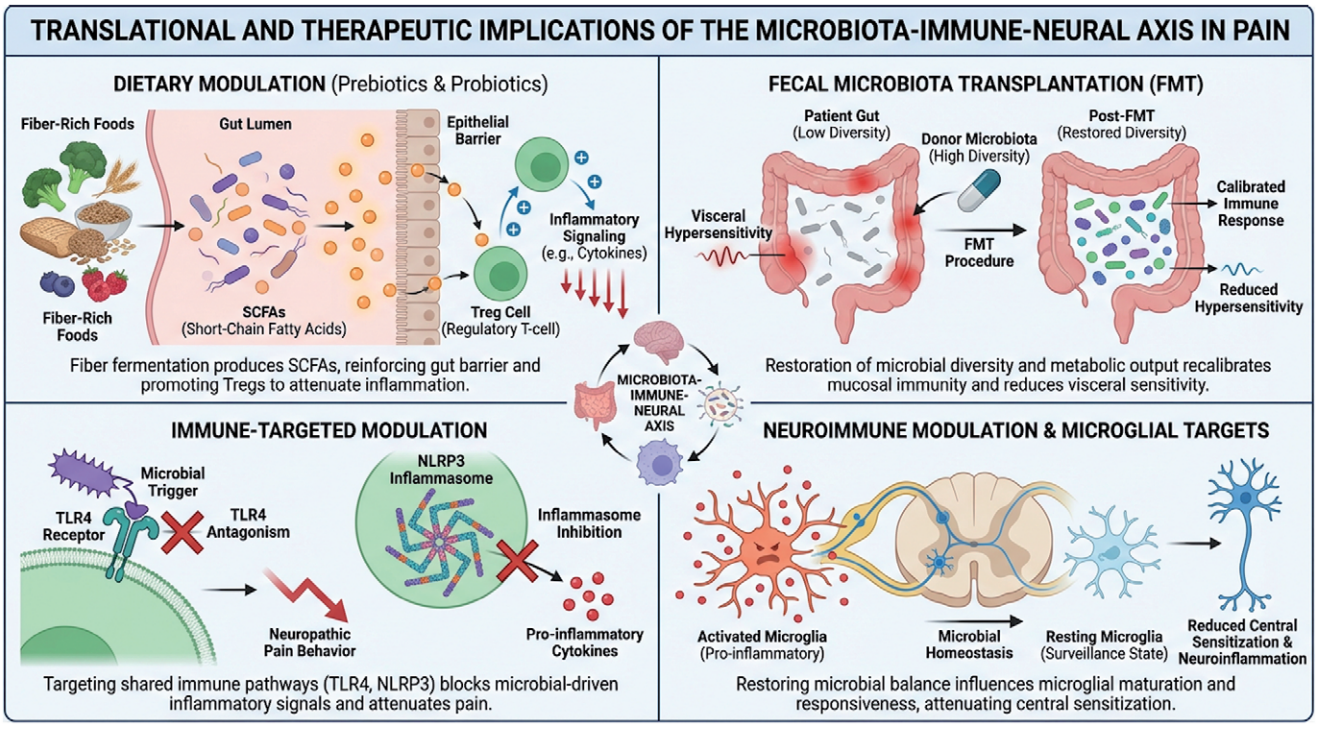

Figure 3. Candidate microbiota-targeted strategies in chronic pain. Schematic of intervention categories proposed in the literature: dietary modulation and prebiotic/probiotic approaches aimed at supporting SCFA production and barrier function; fecal microbiota transplantation, with the strongest current evidence in IBS-related visceral pain; and immune- or microglia-directed strategies (e.g., TLR4 or NLRP3 modulation) tested predominantly in pre-clinical models. Clinical evidence remains preliminary, heterogeneous in methodology, and largely confined to selected pain phenotypes; the figure should be read as a translational map of investigational approaches rather than as established therapeutic options.

ticularly SSRIs and tricyclics, both used as analgesic adjuvants) have intrinsic antimicrobial activity and modify motility; opioids alter transit, mucus secretion and barrier function and are independently associated with dysbiosis; and NSAIDs and other analgesics modify epithelial integrity and microbial communities. Sleep disturbance and physical inactivity, both highly prevalent in chronic pain, independently perturb microbial diurnal rhythms and beta-diversity. Anxiety and depression, ubiquitous comorbidities, are themselves associated with microbial alterations, complicating efforts to disentangle pain-specific from mood-related signals. Finally, gastrointestinal comorbidities, particularly IBS, which co-occurs at high frequency with fibromyalgia, migraine, and chronic pelvic pain, produce microbial signatures that may be erroneously attributed to the pain phenotype under study. Most published human studies, including several included in the present synthesis, address only a subset of these factors and the cumulative effect on the literature is to inflate apparent phenotype-specific microbial signals.

Reverse causation. Closely related to, but distinct from, classical confounding is the question of directionality. Several of the mechanisms proposed in this review can plausibly operate in the reverse direction that is, chronic pain and the physiological, behavioral and pharmacological consequences of pain may themselves drive microbial alterations rather than the converse. Sustained nociceptive and stress signaling alters autonomic tone, intestinal motility, mucosal immunity and HPA-axis output, all of which shape the gut microbial niche; reduced physical activity, dietary restriction, sleep fragmentation and chronic exposure to opioids, antidepressants, NSAIDs and PPIs reinforce these effects. In phenotypes such as IBS-related visceral pain, where altered transit and visceral hypersensitivity directly modify the luminal environment, reverse causation is a particularly strong consideration. With the partial exception of human-to-germ-free FMT experiments and Mendelian randomization analyses, the directionality of the microbiome-pain association in human chronic pain populations cannot at present be resolved with available evidence. The interpretive framework adopted throughout this review should accordingly be read as bidirectional: microbial alterations are best understood as plausible contributors to, and concurrent consequences of, the chronic pain state, with the relative weight of each direction varying across phenotypes and stages of disease.

Methodological and field-maturity limitations. In addition, microbial composition does not equate to functional metabolic output and paired metagenomic-metabolomic data remain comparatively limited; sequencing platforms, bioinformatic pipelines and taxonomic resolution differ substantially across studies; and the rapidly evolving nature of microbiome science may render specific interpretations provisional. High-quality longitudinal cohorts with rigorous control for the confounders enumerated above, mechanistically stratified randomized interventions and study designs explicitly able to address directionality (e.g., pre/post-onset cohorts, properly instrumented Mendelian randomization, and FMT trials with mechanistic readouts) are required to advance causal inference and therapeutic precision.

Conclusions

Available evidence is consistent with a model in which microbial ecosystems can influence pain phenotypes through interactions with innate and adaptive immune pathways relevant to peripheral and central sensitization, although the strength of this influence in humans remains to be defined. Alterations in microbial composition and metabolic output can promote epithelial barrier dysfunction and facilitate systemic dissemination of microbial-derived molecules, including lipopolysaccharide and other pathogen-associated molecular patterns. These signals are proposed to activate pattern-recognition receptors, amplify cytokine cascades, and prime glial cells within the spinal cord and supraspinal structures, potentially lowering nociceptive thresholds and sustaining neuroinflammatory signaling. Integrative approaches that combine high-resolution microbiota profiling with immunophenotyping, cytokine signatures, neuroimaging, and electrophysiological biomarkers may enable mechanistically informed stratification of pain phenotypes. Such multimodal characterization holds substantial promise for redefining chronic pain as a neuroimmune-microbial disorder and for guiding precision-based therapeutic strategies aimed at restoring immune and microbial homeostasis.

References

1. Cohen SP, Vase L, Hooten WM. Chronic pain: an update on burden, best practices, and new advances. Lancet 2021;397:2082-97.
2. Camilloni A, Nati G, Maggiolini P, et al. Chronic non-cancer pain in primary care: an Italian cross-sectional study. Signa Vitae 2021;17:54-62.
3. Rosario D, Boren J, Uhlen M, et al. Systems biology approaches to understand the host–microbiome interactions in neurodegenerative diseases. Front Neurosci 2020;14:716.
4. Pak R, Cho M, Pride K, et al. The gut microbiota and chronic pain. Curr Pain Headache Rep 2024;28:259-69.
5. Goudman L, Demuyser T, Pilitsis JG, et al. Gut dysbiosis in patients with chronic pain: a systematic review and meta-analysis. Front Immunol 2024;15:1342833.
6. Cai W, Haddad M, Haddad R, et al. The gut microbiota promotes pain in fibromyalgia. Neuron 2025;113:2161-75.e13.
7. O'Riordan KJ, Moloney GM, Keane L, et al. The gut microbiota-immune-brain axis: Therapeutic implications. Cell Rep Med 2025;6:101982.
8. Guo R, Chen L-H, Xing C, et al. Pain regulation by gut microbiota: molecular mechanisms and therapeutic potential. Br J Anaesth 2019;123:637-54.
9. Wan X, Wang L, Wang Z, et al. Toll-like receptor 4 plays a vital role in irritable bowel syndrome: a scoping review. Front Immunol 2024;15:1490653.
10. Mann ER, Lam YK, Uhlig HH. Short-chain fatty acids: linking diet, the microbiome and immunity. Nat Rev Immunol 2024;24:577-95.
11. Erny D, Hrabě De Angelis AL, Jaitin D, et al. Host microbiota constantly control maturation and function of microglia in the CNS. Nat Neurosci 2015;18:965-77.
12. Zhao M, Zhang L, Liu Z. Gut microbiota-mediated pain sensitization: mechanisms and therapeutic implications. Front Pain Res (Lausanne) 2025:6:1626515.
13. Gong Z, Zou J, Fang Y, et al. The role of gut microbiota in neuropathic pain: insights into immune mechanisms. J Headache Pain 2025;27:28.
14. Lou L, Zhou L, Wang Y. Gut microbiota: a modulator and therapeutic target for chronic pain. Mol Neurobiol 2025;62:5875-90.
15. Baethge C, Goldbeck-Wood S, Mertens S. SANRA—a scale for the quality assessment of narrative review articles. Res Integr Peer Rev 2019;4:5.

16. Gonzalez E, Sahar T, Haddad M, et al. Altered gut microbiome composition and function in individuals with complex regional pain syndrome. Anesthesiology 2025;143:142-55.
17. Corriero A, Giglio M, Soloperto R, et al. Microbial symphony: exploring the role of the gut in osteoarthritis-related pain. A narrative review. Pain Ther 2024;13:409-33.
18. Minerbi A, Gonzalez E, Brereton NJB, et al. Altered microbiome composition in individuals with fibromyalgia. Pain 2019;160:2589-602.
19. Pan H, Liu C-x, Zhu H-j, et al. Immune cells mediate the effects of gut microbiota on neuropathic pain: a Mendelian randomization study. J Headache Pain 2024;25:196.
20. Burns G, Roberts F, Wark JA, et al. Serological and faecal markers of the irritable bowel syndrome: a systematic review and meta-analysis. EBioMedicine 2026;126:106198.
21. Tieppo AM, Tieppo JS, Rivetti LA. Analysis of intestinal bacterial microbiota in individuals with and without chronic low back pain. Curr Issues Mol Biol 2024;46:7339-52.
22. Li W, Tu J, Zheng J, et al. Gut microbiome and metabolome changes in chronic low back pain patients with vertebral bone marrow lesions. JOR Spine 2025;8:e70042.
23. Rosa A, Gargari M, Martelli M. Gut-brain-jaw axis: the emerging role of gut microbiota in temporomandibular disorders and orofacial pain - A narrative review. J Oral Rehabil 2026;53:939-44.
24. Suthivanich P, Vongseenin S, Auvichayapat V, et al. Probiotic modulation of central and peripheral sensitization in a migraine model via the gut–brain–trigeminal axis. Sci Rep 2025;15:41103.
25. Fan P-C, Chua H-H, Lin C-R, et al. Targeting the microbiome in pediatric migraine: gastrointestinal manifestations and the therapeutic role of Bifidobacterium longum. Gut Microbes 2026;18:2606487.
26. Cho S, Jung Y, Oh H-S, et al. Oral and gut dysbiosis in migraine. Neurol Neuroimmunol Neuroinflamm 2025;12:e200437.
27. Pratt ML, Plumb AN, Manjrekar A, et al. Microbiome contributions to pain: a review of the pre-clinical literature. Pain 2025;166:262-81.
28. Manske S. The microbiome's role in chronic pain and inflammation. Integr Med (Encinitas) 2024;23:10-5.
29. Li M, She K, Zhu P, et al. Chronic pain and comorbid emotional disorders: neural circuitry and neuroimmunity pathways. Int J Mol Sci 2025;26:436.
30. Böckels L, Alexa D, Antal DC, et al. The Microbiome–neurodegeneration interface: mechanisms, evidence, and future directions. Cells 2026;15:135.
31. Majeed M, Sajid R, Sehar A, et al. Genetic variation in innate immune genes shapes gut microbiota composition and host-microbe interactions: a review. Kashf J Multidiscip Res 2026;3:24-43.
32. Mandlem VKK, Rivera A, Khan Z, et al. TLR4 induced TRPM2 mediated neuropathic pain. Front Pharmacol 2024;15:1472771.
33. Grace PM, Hutchinson MR, Maier SF, et al. Pathological pain and the neuroimmune interface. Nat Rev Immunol 2014;14:217-31.
34. Fiore NT, Debs SR, Hayes JP, et al. Pain-resolving immune mechanisms in neuropathic pain. Nat Rev Neurol 2023;19:199-220.
35. Babu N, Gadepalli A, Akhilesh, et al. TLR-4: a promising target for chemotherapy-induced peripheral neuropathy. Mol Biol Rep 2024;51:1099.
36. Tong S-H, Zhou J, Ye F, et al. Activation of TLR4/CCL2 in Intact neurons drives radicular injury-induced global nerve trunk hypersensitivity in radiculopathy pre-clinical models. J Pain Res 2025;18:3903-18.
37. Alam MI, Farhana F, Sakai E. Emerging role of the NLRP3 inflammasome in the onset of oral diseases and its potential as a therapeutic target. Int J Mol Sci 2026;27:1098.
38. Chen R, Yin C, Fang J, et al. The NLRP3 inflammasome: an emerging therapeutic target for chronic pain. J Neuroinflamm 2021;18:84.
39. Chen C, Smith MT. The NLRP3 inflammasome: role in the pathobiology of chronic pain. Inflammopharmacology 2023;31:1589-603.
40. Pottorf TS, Rotterman TM, McCallum WM, et al. The role of microglia in neuroinflammation of the spinal cord after peripheral nerve injury. Cells 2022;11:2083.
41. Austin PJ, Kim CF, Perera CJ, et al. Regulatory T cells attenuate neuropathic pain following peripheral nerve injury and experimental autoimmune neuritis. Pain 2012;153:1916-31.
42. Zhang C, Li Y, Yu Y, et al. Impact of inflammation and Treg cell regulation on neuropathic pain in spinal cord injury: mechanisms and therapeutic prospects. Front Immunol 2024;15:1334828.
43. Furusawa Y, Obata Y, Fukuda S, et al. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. Nature 2013;504:446-50.
44. Ivanov II, Atarashi K, Manel N, et al. Induction of intestinal Th17 cells by segmented filamentous bacteria. Cell 2009;139:485-98.
45. Kim CF, Moalem-Taylor G. Interleukin-17 Contributes to neuroinflammation and neuropathic pain following peripheral nerve injury in mice. J Pain 2011;12:370-83.
46. Malcangio M, Sideris-Lampretsas G. How microglia contribute to the induction and maintenance of neuropathic pain. Nat Rev Neurosci 2025;26:263-75.
47. Wang Y, Li J, Lian Z, et al. Tryptophan metabolites at the service of neuroimmune sensing of microbes. Trends Mol Med 2026;32:547-63.
48. Schwarcz R, Bruno JP, Muchowski PJ, et al. Kynurenines in the mammalian brain: when physiology meets pathology. Nat Rev Neurosci 2012;13:465-77.
49. Tyagi A, Kumar V. The gut microbiota-bile acid axis: a crucial regulator of immune function and metabolic health. World J Microbiol Biotechno. 2025;41:215.
50. Tong Y, Lou X. Interplay between bile acids, gut microbiota, and the tumor immune microenvironment: mechanistic insights and therapeutic strategies. Front Immunol 2025;16:1638352.
51. Svitko S, Ermakova E, Gilizhdinova K, et al. LPS-induced neuroinflammation increases serotonin-evoked activity of trigeminal afferents and aggravates mechanical allodynia and photophobic behavior in rat migraine model. Int J Mol Sci 2025;26:11983.
52. Zhou L, Liang L, He Q, et al. Advances in the study of microglia in neuropathic pain. Mod Health Sci 2025;8:85-90.
53. Cryan JF, O'Riordan KJ, Cowan CSM, et al. The microbiota-gut-brain axis. Physiol Rev 2019;99:1877-2013.
54. Gurow K, Joshi DC, Gwasikoti J, et al. Gut microbial control of neurotransmitters and their relation to neurological disorders: a comprehensive review. Horm Metab Res 2025;57:315-25.

55. Ford AC, Quigley EMM, Lacy BE, et al. Efficacy of prebiotics, probiotics, and synbiotics in irritable bowel syndrome and chronic idiopathic constipation: systematic review and meta-analysis. Am J Gastroenterol 2014;109:1547-61.
56. Van Nood E, Vrieze A, Nieuwdorp M, et al. Duodenal infusion of donor feces for recurrent Clostridium difficile. N Engl J Med 2013;368:407-15.
57. El-Salhy M, Hatlebakk JG, Gilja OH, et al. Efficacy of faecal microbiota transplantation for patients with irritable bowel syndrome in a randomised, double-blind, placebo-controlled study. Gut 2020;69:859-67.
58. Paolini L, Sigaux J, Boissier MC, et al. Immune control of pain Joint Bone Spine 2026;93:105999.
59. Su R, Wang Z, Zhang G, et al. The analgesic effect of NLRP3 inflammasome in the relief of inflammatory pain by electroacupuncture: a systematic review and meta-analysis of animal studies. J Pain Res 2025;18:4413-30.
60. Wheeler MA, Quintana FJ. The neuroimmune connectome in health and disease. Nature 2025;638:333-42.
61. Weber T, Tatzl E, Kashofer K, et al. Fibromyalgia-associated hyperalgesia is related to psychopathological alterations but not to gut microbiome changes. PloS One 2022;17:e0274026.

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