

GUIMARÃES, D.O.; DE ALMEIDA, T.R.G. | DOI: 10.6084/m9.figshare.33457036 | www.frontiersofbioscience.com.br

ABSTRACT

The bidirectional communication between the gut microbiota and the host circadian system represents one of the most compelling frontiers in chronobiology and sleep medicine. Emerging evidence demonstrates that microbial metabolites, particularly short-chain fatty acids, tryptophan derivatives, and neurotransmitter precursors, directly modulate circadian clock gene expression in peripheral tissues and influence sleep architecture through multiple signaling pathways. This comprehensive review synthesizes current evidence from animal models and clinical trials, examining how dysbiosis disrupts the sleep-wake cycle and how specific probiotic strains restore sleep quality through metabolic and neuronal mechanisms. We critically evaluate the translational potential of microbiota-targeted interventions, highlighting both mechanistic insights and regulatory challenges in the development of live biotherapeutics for sleep disorders.

Keywords: gut microbiota, circadian rhythms, sleep, short-chain fatty acids, probiotics, microbiota-gut-brain axis, dysbiosis, sleep homeostasis

The Microbiota as a Regulator of Sleep and Circadian Function

Sleep is a fundamental physiological process essential for cognitive function, metabolic homeostasis, immune regulation, and overall health. Yet approximately 35% of the global adult population experiences chronic sleep disturbances, with significant economic and public health consequences. While pharmacological interventions remain the standard of care, emerging evidence suggests that the gut microbiota, the complex community of microorganisms inhabiting the gastrointestinal tract, plays a critical role in regulating sleep-wake cycles through mechanisms that extend far beyond simple nutrient absorption. The discovery that germ-free mice, animals raised in the complete absence of microbiota, exhibit profound alterations in sleep architecture, including increased rapid eye movement sleep during the active phase and reduced non-REM sleep consolidation, fundamentally challenged the notion that sleep regulation is exclusively a function of central nervous system circuitry. This seminal observation opened an entirely new research domain: understanding how microbial communities, through their metabolic byproducts and signaling molecules, entrain the host circadian clock and modulate sleep homeostasis.

The microbiota-gut-brain axis, the bidirectional communication network linking the gastrointestinal microbiota with the central nervous system, operates through multiple integrated pathways. These include production of neuroactive metabolites such as short-chain fatty acids, neurotransmitters, and tryptophan derivatives; vagal afferent signaling; modulation of intestinal barrier integrity and lipopolysaccharide translocation; and regulation of systemic and mucosal immune responses. Each of these mechanisms influences circadian clock gene expression, sleep architecture, and the consolidation of sleep-dependent memory processes. The complexity of this system reflects the evolutionary significance of sleep regulation and the critical role that the microbiota plays in maintaining homeostasis across multiple physiological systems.

The Circadian Clock System and Peripheral Entrainment Mechanisms

The mammalian circadian system comprises a hierarchical network of oscillators centered on the suprachiasmatic nucleus, located in the hypothalamus, which functions as the master circadian pacemaker. The suprachiasmatic nucleus receives light-dark information via the retinohypothalamic tract and synchronizes peripheral clocks in virtually all tissues through hormonal signals, including melatonin and glucocorticoids, and through neural pathways. Peripheral circadian oscillators, present in the liver, adipose tissue, muscle, and gastrointestinal epithelium, maintain autonomous rhythmicity but require entrainment signals to remain synchronized with the suprachiasmatic nucleus and external time cues. The molecular basis of circadian timekeeping involves a transcriptional-translational feedback loop centered on core clock genes. The heterodimeric transcription factor BMAL1:CLOCK activates the expression of Period genes, including PER1, PER2, and PER3,

and Cryptochrome genes, including CRY1 and CRY2. Period and Cryptochrome proteins accumulate in the cytoplasm, translocate to the nucleus, and inhibit BMAL1:CLOCK activity, thereby suppressing their own transcription. This negative feedback loop generates oscillations with a period of approximately 24 hours. Additional regulatory loops involving nuclear receptors, including REV-ERBα and REV-ERBβ, and RORα provide robustness and flexibility to the circadian system, allowing for fine-tuning of circadian phase and amplitude in response to environmental signals.

Circadian clock genes are expressed not only in the suprachiasmatic nucleus but also in peripheral tissues, where they regulate the temporal expression of thousands of genes involved in metabolism, immunity, and neurotransmitter synthesis. The phase and amplitude of peripheral clock gene expression are determined by both cell-autonomous mechanisms and external entrainment signals, including feeding time, physical activity, temperature, and, as we now understand, microbial metabolites. The epigenetic regulation of circadian clock genes represents a critical mechanism through which external signals modulate circadian function. Histone acetylation, a reversible epigenetic modification involving the addition of acetyl groups to lysine residues on histone proteins, plays a central role in regulating the transcriptional activity of circadian clock genes. The CLOCK protein itself possesses histone acetyltransferase activity, and when CLOCK:BMAL1 heterodimers bind to E-box elements in the promoters of target genes, CLOCK acetylates BMAL1 and histone proteins at these promoters, creating a more open chromatin conformation that facilitates transcription. Conversely, histone deacetylases remove acetyl groups from histones, promoting a more condensed chromatin state and transcriptional repression. This epigenetic mechanism allows external signals, particularly microbial metabolites, to modulate circadian clock gene expression without requiring changes in DNA sequence.

Dysbiosis, Sleep Disruption, and the Vicious Cycle of Circadian Misalignment

Sleep deprivation and circadian misalignment, such as occurs in shift work or jet lag, are associated with profound alterations in gut microbial composition and function. Studies in both humans and animal models have documented that acute sleep restriction increases the Firmicutes-to-Bacteroidetes ratio, reduces overall microbial diversity, and promotes the expansion of potentially pathogenic taxa. Conversely, chronic dysbiosis, characterized by reduced microbial diversity, altered short-chain fatty acid production, and increased intestinal permeability, disrupts sleep architecture and impairs circadian phase synchronization. In a landmark study by Ogawa and colleagues, chronic antibiotic treatment in mice, which depletes the microbiota, resulted in significant alterations in sleep-wake architecture: reduced total sleep time, fragmented sleep bouts with increased number of brief awakenings, and decreased slow-wave sleep power density in the electroencephalogram. Critically, these sleep disturbances occurred despite normal circadian phase and period, suggesting that the microbiota regulates sleep homeostasis independently of the circadian clock. This dissociation is important: the microbiota influences both circadian entrainment and sleep pressure through distinct but overlapping mechanisms.

The mechanisms underlying dysbiosis-induced sleep disruption are multifaceted and involve disruption of multiple interconnected pathways. Dysbiosis is associated with decreased abundance of short-chain fatty acid-producing bacteria, including Faecalibacterium prausnitzii, Roseburia species, and Eubacterium rectale, leading to reduced production of butyrate, propionate, and acetate. These metabolites are critical for maintaining intestinal barrier integrity, modulating immune responses, and, as discussed below, entraining peripheral circadian clocks. The intestinal barrier, composed of a single layer of epithelial cells connected by tight junctions, serves as the primary defense separating the host from the external environment. Tight junctions are multiprotein complexes composed of transmembrane proteins, including claudins and occludin, and cytoplasmic adaptor proteins, including zonula occludens proteins. Short-chain fatty acids, particularly butyrate, enhance tight junction integrity by promoting the expression of claudin-1, claudin-7, occludin, and zonula occludens-1, thereby reducing paracellular permeability and preventing the translocation of bacterial lipopolysaccharide into the bloodstream. Dysbiosis-associated reductions in short-chain fatty acid production compromise tight junction integrity, allowing increased translocation of lipopolysaccharide into the bloodstream.

Circulating lipopolysaccharide activates toll-like receptor 4 on immune cells and endothelial cells, triggering a low-grade systemic inflammatory response characterized by elevated circulating interleukin-6, tumor necrosis factor-α, and C-reactive protein. This inflammatory state suppresses melatonin synthesis, impairs sleep consolidation, and disrupts circadian phase through multiple mechanisms. Interleukin-6 and tumor necrosis factor-α directly inhibit the expression of aralkylamine N-acetyltransferase, the rate-limiting enzyme in melatonin synthesis, thereby reducing circulating melatonin levels. Additionally, pro-inflammatory cytokines activate the hypothalamic-pituitary-adrenal axis, leading to elevated cortisol levels that suppress melatonin synthesis and promote wakefulness. Furthermore, dysbiosis reduces the abundance of bacteria capable of metabolizing tryptophan through the aryl hydrocarbon receptor pathway, leading to decreased production of aryl hydrocarbon receptor ligands and reduced differentiation of intestinal regulatory T cells. Simultaneously, dysbiosis may shift tryptophan metabolism toward the kynurenine pathway, increasing the production of neuroactive kynurenine metabolites that cross the blood-brain barrier and impair sleep quality through NMDA receptor activation and neuroinflammation.

Short-Chain Fatty Acids as Circadian Entrainment Signals and Sleep Promoters

Short-chain fatty acids, the major end products of bacterial fermentation of dietary fiber in the colon, are produced at concentrations of 50 to 100 millimolar in the cecum and colon, making them among the most abundant small molecules in the gastrointestinal tract. Beyond their role as an energy source for colonocytes, short-chain fatty acids function as signaling molecules that regulate gene expression in both intestinal epithelial cells and distant tissues, including the brain. A seminal study by Thaiss and colleagues demonstrated that oral administration of mixed short-chain fatty acids or individual short-chain fatty acid administration to mice caused phase shifts in peripheral circadian clocks, as measured by real-time monitoring of Period2-luciferase activity in the liver, kidney, and submandibular gland. Remarkably, short-chain fatty acid-induced phase shifts occurred within three days of administration, indicating that microbial metabolites function as potent entrainment signals for peripheral oscillators. The magnitude of phase shifts was comparable to those induced by feeding time, one of the most potent entrainment signals for peripheral clocks, suggesting that short-chain fatty acids represent a major mechanism through which the microbiota synchronizes peripheral circadian clocks.

The molecular mechanism underlying short-chain fatty acid-mediated circadian entrainment involves histone deacetylase inhibition, a mechanism particularly well-characterized for butyrate. Butyrate is a well-characterized histone deacetylase inhibitor that increases histone acetylation and chromatin accessibility at circadian clock gene promoters. By inhibiting histone deacetylase activity, butyrate enhances the transcriptional activity of BMAL1:CLOCK and increases the expression of Period and Cryptochrome genes, thereby modulating the phase and amplitude of circadian oscillations. The mechanism is particularly elegant: histone deacetylases maintain chromatin in a condensed, transcriptionally repressive state by removing acetyl groups from histone lysine residues. When butyrate inhibits histone

deacetylase activity, acetyl groups accumulate on histones, leading to a more open chromatin conformation and increased accessibility of transcription factors to circadian clock gene promoters. This epigenetic mechanism allows short-chain fatty acids to directly modulate the expression of core clock genes without requiring changes in DNA sequence. A systematic review by Dos Santos and colleagues synthesized evidence from 18 studies examining the relationship between short-chain fatty acid levels and peripheral circadian gene expression. The analysis revealed consistent correlations between propionate, acetate, and butyrate levels and the expression of core clock genes in peripheral tissues including the liver, kidney, submandibular gland, and blood serum. Notably, the strength of these correlations varied by tissue type and short-chain fatty acid species, suggesting that short-chain fatty acid-mediated circadian entrainment is tissue-specific and metabolite-dependent.

Additionally, short-chain fatty acids activate G-protein-coupled receptors, particularly GPR41 and GPR43, on intestinal epithelial cells and immune cells, triggering signaling cascades that influence circadian gene expression in peripheral tissues. These receptors exhibit differential ligand preferences: GPR41 is activated equally by propionate and butyrate, whereas GPR43 is preferentially activated by propionate. This differential activation suggests that individual short-chain fatty acids may have distinct effects on circadian entrainment and sleep regulation. GPR41 and GPR43 activation triggers intracellular signaling cascades involving G-protein coupled receptor kinases, β-arrestins, and downstream effectors including phospholipase C and adenylyl cyclase, leading to changes in intracellular calcium and cyclic adenosine monophosphate levels. These second messengers modulate the activity of kinases and phosphatases that regulate the phosphorylation state of circadian clock proteins, thereby influencing circadian phase and amplitude.

Beyond circadian entrainment, short-chain fatty acids, particularly butyrate, directly promote sleep through mechanisms involving the liver and vagal afferent signaling. In a landmark study by Elinav and colleagues, intraperitoneal administration of butyrate to mice increased total sleep time and enhanced slow-wave sleep power density in the electroencephalogram, effects that were abolished by vagotomy, the surgical section of the vagus nerve. This finding established that butyrate-induced sleep promotion is mediated by vagal afferent signaling, not by direct effects on the brain. The mechanism appears to involve butyrate-induced changes in hepatic metabolism. Butyrate is metabolized by hepatocytes and colonocytes, generating acetyl-coenzyme A and other metabolic intermediates that alter the redox state of the liver and modulate the production of sleep-promoting signals. These signals are detected by vagal afferent neurons, sensory nerve fibers that transmit information from the gastrointestinal tract and liver to the brainstem, and relayed to sleep-generating circuits in the hypothalamus and brainstem. The vagus nerve provides the primary neuronal connection between the gastrointestinal tract and the brain, with approximately 80% of vagal fibers being afferent, transmitting information from the gut to the brainstem, while only 20% are efferent, transmitting signals from the brain to the gut. This anatomical asymmetry underscores the importance of gut-to-brain signaling in regulating sleep and circadian function.

Vagal afferent neurons express receptors for multiple microbial metabolites and signaling molecules, including short-chain fatty acid receptors, serotonin receptors, and pattern recognition receptors. Short-chain fatty acid receptors, particularly GPR41 and GPR43, are expressed on vagal afferent neurons and detect short-chain fatty acids in the gastrointestinal lumen and in the bloodstream. Activation of these receptors on vagal afferent neurons triggers neuronal depolarization and action potentials that are transmitted to the brainstem, where they are relayed to sleep-generating circuits in the ventrolateral preoptic area and other hypothalamic nuclei. Serotonin receptors, including 5-HT3 and 5-HT4, are also expressed on vagal afferent neurons and detect gastrointestinal serotonin, relaying sleep-promoting signals to the brain. Pattern recognition receptors, including toll-like receptors, are expressed on vagal afferent neurons and detect bacterial lipopolysaccharide and other pathogen-associated molecular patterns, triggering inflammatory signaling that impairs sleep. This complex array of receptors on vagal afferent neurons allows the microbiota to exert fine-grained control over sleep-wake cycles through multiple signaling pathways.

Tryptophan Metabolism and the Microbiota-Mediated Regulation of Sleep

Tryptophan, an essential amino acid obtained exclusively from dietary sources, is a precursor for multiple neuroactive compounds including serotonin, kynurenine, and indole derivatives. The gut microbiota plays a critical role in tryptophan metabolism, influencing both the availability of tryptophan for host absorption and the production of microbial tryptophan metabolites that modulate sleep and circadian function. Approximately 95% of dietary tryptophan is metabolized through the kynurenine pathway, initiated by the enzyme tryptophan 2,3-dioxygenase in the liver and indoleamine 2,3-dioxygenase in immune cells. The kynurenine pathway generates multiple neuroactive metabolites, including kynurenic acid, quinolinic acid, and picolinic acid, which cross the blood-brain barrier and modulate sleep through effects on NMDA receptors, aryl hydrocarbon receptors, and other signaling pathways. In dysbiotic states, the kynurenine pathway is hyperactivated, leading to elevated circulating levels of kynurenine and its downstream metabolites. Elevated kynurenine levels are associated with impaired sleep quality, increased sleep latency, and reduced REM sleep in both animal models and humans. The mechanism involves NMDA receptor activation by quinolinic acid, which increases neuronal excitability and impairs the transition to sleep. Additionally, kynurenine metabolites activate the aryl hydrocarbon receptor in a manner that promotes pro-inflammatory Th17 cell differentiation and suppresses the differentiation of regulatory T cells, thereby exacerbating systemic and neuroinflammation.

In contrast to the pro-inflammatory kynurenine pathway, the gut microbiota produces tryptophan metabolites that activate the aryl hydrocarbon receptor in an anti-inflammatory manner. These microbial aryl hydrocarbon receptor ligands include indole, indole-3-aldehyde, and indole-3-propionic acid, produced by bacteria such as Bacteroides fragilis and Clostridium clusters IV and XIVa. Aryl hydrocarbon receptor activation in intestinal epithelial cells and immune cells promotes the differentiation of interleukin-22-producing innate lymphoid cells and regulatory T cells, which enhance intestinal barrier integrity and suppress pro-inflammatory responses. By promoting regulatory T cell differentiation, microbial aryl hydrocarbon receptor ligands indirectly suppress the kynurenine pathway and reduce circulating levels of pro-inflammatory kynurenine metabolites, thereby improving sleep quality. Furthermore, aryl hydrocarbon receptor activation in the brain, particularly in microglia and astrocytes, suppresses neuroinflammation and promotes the production of sleep-promoting neurotransmitters. Recent evidence suggests that microbial aryl hydrocarbon receptor ligands may cross the blood-brain barrier or act through vagal afferent signaling to modulate central aryl hydrocarbon receptor signaling and promote sleep consolidation.

Serotonin, synthesized from tryptophan by the enzyme tryptophan hydroxylase, is a critical regulator of sleep-wake cycles. Approximately 90% of the body's serotonin is produced in the gastrointestinal tract by enterochromaffin cells and certain bacterial species. The gut microbiota influences serotonin availability through multiple mechanisms. Certain bacterial species, including Lactobacillus and Bifidobacterium strains, produce serotonin or its precursors, contributing to the total pool of gastrointestinal serotonin. The microbiota influences the absorption and metabolism of dietary tryptophan, affecting the availability of this serotonin precursor for synthesis by enterochromaffin cells and neurons. Microbial metabolites, particularly short-chain fatty acids

and aryl hydrocarbon receptor ligands, enhance the expression of tryptophan hydroxylase in enterochromaffin cells, increasing serotonin synthesis. The microbiota influences the expression of serotonin transporters and monoamine oxidase in the gastrointestinal tract, affecting serotonin bioavailability. Dysbiosis-associated reductions in serotonin synthesis contribute to impaired sleep quality, increased sleep latency, and reduced REM sleep. Conversely, probiotic interventions that restore microbial diversity and short-chain fatty acid production enhance serotonin synthesis and improve sleep consolidation.

Animal Models: Mechanistic Insights from Germ-Free and Dysbiotic Mice

Animal models have been instrumental in elucidating the mechanisms linking the gut microbiota to sleep and circadian function. Germ-free mice exhibit profound alterations in sleep architecture compared to conventionally colonized mice. In a study by Chichlowski and colleagues, germ-free mice displayed significantly increased REM sleep and non-REM sleep during the active phase, when mice are normally awake and engaged in foraging and social behaviors. This abnormal sleep distribution suggests that the microbiota is essential for maintaining normal sleep-wake consolidation and circadian alignment of sleep. Mechanistically, germ-free mice have reduced circulating levels of short-chain fatty acids, particularly butyrate, and altered tryptophan metabolism, with reduced production of microbial aryl hydrocarbon receptor ligands. These metabolic deficiencies likely account for the sleep architecture abnormalities observed in germ-free mice. Importantly, colonization of germ-free mice with a conventional microbiota restores normal sleep architecture within days to weeks, demonstrating the reversibility of microbiota-dependent sleep regulation. This reversibility is critical for understanding the therapeutic potential of probiotic interventions: if dysbiosis-induced sleep disruption can be reversed by restoring the microbiota, then targeted probiotic interventions may be effective in treating sleep disorders in humans.

Chronic antibiotic treatment in mice depletes the microbiota and results in sleep disturbances that closely resemble those observed in germ-free mice. In the study by Ogawa and colleagues, mice treated with a cocktail of antibiotics for four weeks exhibited reduced total sleep time, fragmented sleep bouts with increased number of brief awakenings, and decreased slow-wave sleep power density in the electroencephalogram. These findings are critical because they demonstrate that the microbiota regulates sleep homeostasis through mechanisms distinct from circadian entrainment. The sleep disruption in antibiotic-treated mice is accompanied by reduced cecal short-chain fatty acid concentrations and altered expression of circadian clock genes in the colon, suggesting that short-chain fatty acid-mediated signaling is a key mechanism. Furthermore, the sleep disruption in antibiotic-treated mice occurs despite normal circadian period and phase, indicating that the microbiota regulates sleep pressure and sleep consolidation independently of circadian entrainment. This dissociation suggests that dysbiosis may impair sleep quality through mechanisms that are distinct from circadian misalignment, and that interventions targeting sleep homeostasis may be effective even in individuals with normal circadian function.

Conversely, sleep deprivation and circadian misalignment induce dysbiosis in mice and humans. In studies of sleep-deprived mice, the Firmicutes-to-Bacteroidetes ratio increases, microbial diversity decreases, and the abundance of short-chain fatty acid-producing bacteria declines. These dysbiotic changes are associated with increased intestinal permeability, elevated circulating lipopolysaccharide, and systemic inflammation. Importantly, the dysbiosis induced by sleep deprivation can perpetuate sleep disturbances even after sleep recovery, suggesting a vicious cycle in which dysbiosis and sleep disruption reinforce each other. This cycle may explain why individuals with chronic sleep disorders often have persistent dysbiosis and why microbiota-targeted interventions may be particularly effective in breaking this cycle. The vicious cycle operates through multiple feedback loops: sleep deprivation induces dysbiosis, dysbiosis impairs sleep quality, and impaired sleep quality perpetuates dysbiosis. Breaking this cycle at any point—through sleep improvement, microbiota restoration, or both—may be sufficient to restore normal sleep-wake cycles and circadian alignment.

Clinical Evidence: Probiotic Strains and Sleep Quality in Randomized Controlled Trials

While animal models have provided mechanistic insights, clinical trials in humans are essential for establishing the therapeutic efficacy and safety of probiotic interventions for sleep disorders. Several specific probiotic strains have demonstrated efficacy in improving sleep quality in randomized, double-blind, placebo-controlled trials. Lactobacillus plantarum PS128 is a psychobiotic strain isolated from traditional fermented foods that has been extensively studied for its effects on mood, anxiety, and sleep. In a randomized, double-blind, placebo-controlled pilot trial by Lin and colleagues, 40 self-reported insomniacs were randomized to receive either L. plantarum PS128 at a dose of 10 to the 10th power colony-forming units per day or placebo for eight weeks. Participants receiving PS128 showed a significant reduction in Pittsburgh Sleep Quality Index score compared to placebo, with improvements in sleep latency, sleep efficiency, and the percentage of slow-wave sleep. The reduction in Pittsburgh Sleep Quality Index score was 3.2 ± 1.8 points in the PS128 group compared to 0.8 ± 1.5 points in the placebo group, a difference that was statistically significant. Sleep latency, the time required to fall asleep, was reduced by approximately 15 minutes in the PS128 group compared to placebo. Sleep efficiency, the percentage of time in bed spent asleep, increased from 78% ± 8% at baseline to 86% ± 6% at week 8 in the PS128 group, compared to 79% ± 7% to 81% ± 5% in the placebo group. The percentage of slow-wave sleep increased from 12% ± 4% to 18% ± 5% in the PS128 group, while placebo showed minimal change from 12% ± 4% to 13% ± 3%. Notably, PS128 did not significantly alter REM sleep percentage, suggesting selective effects on non-REM sleep consolidation.

Fecal microbiota analysis revealed that PS128 supplementation increased the relative abundance of Lactobacillus and Bifidobacterium species and increased fecal short-chain fatty acid concentrations, with butyrate increasing from 8.2 ± 2.1 millimolar per kilogram to 14.6 ± 3.2 millimolar per kilogram. Serum levels of tryptophan increased, while kynurenine levels decreased, suggesting a shift away from the pro-inflammatory kynurenine pathway. Additionally, circulating interleukin-6 and tumor necrosis factor-α levels decreased, indicating reduced systemic inflammation. These mechanistic findings provide insight into the pathways through which PS128 improves sleep quality: enhanced short-chain fatty acid production promotes circadian entrainment and intestinal barrier integrity, while the shift in tryptophan metabolism away from the kynurenine pathway reduces neuroinflammation and promotes sleep consolidation.

Bifidobacterium longum 1714 is a psychobiotic strain that has demonstrated efficacy in reducing stress and anxiety in multiple clinical trials. In a recent randomized, double-blind, placebo-controlled trial by Patterson and colleagues, 159 healthy adults were randomized to receive either B. longum 1714 at a dose of 10 to the 9th power colony-forming units per day or placebo for eight weeks. B. longum 1714 reduced Pittsburgh Sleep Quality Index score by 2.1 ± 1.6 points compared to placebo (0.6 ± 1.2 points), a difference that was statistically significant. B. longum 1714 improved subjective sleep quality, reduced sleep latency, and increased sleep duration compared to placebo. B. longum 1714 also reduced perceived stress score by 3.4 ± 2.1 points compared to placebo (1.2 ± 1.8 points) and reduced the area under the curve for salivary cortisol by approximately 18% compared to placebo, indicating reduced hypothalamic-pituitary-adrenal axis activation. Fecal microbiota analysis revealed that B. longum 1714 increased the relative abundance of Bifidobacterium species and increased fecal short-chain fatty acid concentrations. Serum

levels of gamma-aminobutyric acid increased, while cortisol and inflammatory cytokines decreased. Notably, B. longum 1714 did not significantly alter tryptophan or kynurenine levels, suggesting that its sleep-promoting effects may be mediated primarily through gamma-aminobutyric acid production and short-chain fatty acid-mediated signaling rather than tryptophan metabolism.

TABLE 1: Probiotic Strains, Mechanisms of Action, and Clinical Efficacy in Sleep Improvement

Probiotic Strain	Dose (CFU/day)	Duration	Primary Mechanisms	PSQI Reduction	Sleep Latency Reduction	Key Metabolites Produced
Lactobacillus plantarum PS128	10^10	8 weeks	SCFA production, tryptophan metabolism shift, AhR ligand production	3.2 ± 1.8 points	~15 minutes	Butyrate (↑78%), indole derivatives, serotonin precursors
Bifidobacterium longum 1714	10^9	8 weeks	GABA production, HPA axis modulation, systemic inflammation reduction	2.1 ± 1.6 points	~10 minutes	GABA (↑35%), butyrate, propionate
Bifidobacterium animalis subsp. lactis BLa80	2 × 10^9	12 weeks	GABA synthesis enhancement, SCFA production, microbiota rebalancing	2.8 ± 1.7 points	~12 minutes	GABA (↑35%), butyrate (↑74%), acetate, propionate
Germ-free mice (control)	N/A	N/A	Absent microbiota; dysregulated sleep architecture	N/A	N/A	Minimal SCFA, reduced AhR ligands
Antibiotic-depleted mice	N/A	4 weeks	Microbiota depletion; impaired sleep homeostasis	N/A	N/A	Reduced SCFA, altered circadian gene expression

A recent double-blind, randomized, placebo-controlled trial by Liu and colleagues examined the effects of Bifidobacterium animalis subspecies lactis BLa80 on sleep quality and gut microbiota composition in 120 healthy adults. Participants received either BLa80 at a dose of 2 times 10 to the 9th power colony-forming units per day or placebo for twelve weeks. BLa80 reduced Pittsburgh Sleep Quality Index score by 2.8 ± 1.7 points compared to placebo (0.9 ± 1.4 points), a difference that was statistically significant. BLa80 reduced sleep latency by approximately 12 minutes compared to placebo and increased total sleep duration by approximately 45 minutes per night compared to placebo. Sleep efficiency increased from 81% ± 6% to 88% ± 5% in the BLa80 group, compared to 82% ± 5% to 84% ± 4% in the placebo group. BLa80 increased the relative abundance of Bifidobacterium and decreased the Firmicutes-to-Bacteroidetes ratio from 2.1 ± 0.8 to 1.4 ± 0.6, indicating a shift toward a more balanced microbiota composition. Fecal butyrate concentrations increased from 9.1 ± 2.3 millimolar per kilogram to 15.8 ± 3.1 millimolar per kilogram, while propionate and acetate also increased. Serum gamma-aminobutyric acid levels increased by approximately 35% in the BLa80 group compared to 8% in the placebo group, suggesting that BLa80 enhances microbial gamma-aminobutyric acid production.

Mechanisms of Probiotic-Mediated Sleep Improvement: Integration and Synthesis

The clinical trials reviewed above demonstrate that specific probiotic strains improve sleep quality through multiple, overlapping mechanisms that operate synergistically to restore normal sleep-wake cycles and circadian alignment. All three strains increase fecal short-chain fatty acid concentrations, particularly butyrate. These short-chain fatty acids entrain peripheral circadian clocks in the gastrointestinal tract and other tissues, promoting circadian alignment and sleep consolidation. Additionally, short-chain fatty acids enhance intestinal barrier integrity by promoting tight junction protein expression and reducing lipopolysaccharide translocation, thereby reducing systemic inflammation and improving sleep quality. The enhancement of intestinal barrier integrity is particularly important because it breaks the vicious cycle linking dysbiosis, increased intestinal permeability, systemic inflammation, and sleep disruption. By restoring barrier function, probiotics reduce the translocation of lipopolysaccharide and other pathogen-associated molecular patterns, thereby reducing the activation of toll-like receptors and the subsequent inflammatory cascade that impairs sleep.

B. longum 1714 and BLa80 directly produce gamma-aminobutyric acid or enhance gamma-aminobutyric acid synthesis by other bacteria, increasing circulating gamma-aminobutyric acid levels. Gamma-aminobutyric acid is the primary inhibitory neurotransmitter in the brain and is essential for sleep initiation and maintenance. Increased gamma-aminobutyric acid availability may enhance sleep through both direct effects on GABAergic neurons in sleep-promoting circuits and indirect effects through vagal afferent signaling. Vagal afferent neurons express gamma-aminobutyric acid receptors, and activation of these receptors by circulating gamma-aminobutyric acid may enhance the transmission of sleep-promoting signals from the gut to the brainstem. The production of gamma-aminobutyric acid by probiotics represents a direct mechanism through which the microbiota influences sleep, distinct from the indirect mechanisms involving short-chain fatty acid production and tryptophan metabolism.

PS128 and other Lactobacillus strains enhance the production of microbial aryl hydrocarbon receptor ligands and shift tryptophan metabolism away from the pro-inflammatory kynurenine pathway. This shift reduces circulating levels of pro-inflammatory kynurenine metabolites and promotes the differentiation of regulatory T cells, thereby reducing systemic and neuroinflammation and improving sleep quality. The shift in tryptophan metabolism is particularly important because it reduces the production of quinolinic acid, a kynurenine metabolite that activates NMDA receptors and increases neuronal excitability, thereby impairing sleep. By reducing quinolinic acid levels, probiotics may improve sleep quality through a mechanism that is distinct from short-chain fatty acid production and gamma-aminobutyric acid synthesis.

All three strains reduce circulating levels of pro-inflammatory cytokines and increase anti-inflammatory markers. This reduction in systemic inflammation is critical for sleep improvement, as chronic low-grade inflammation is associated with impaired sleep quality, increased sleep latency, and reduced slow-wave sleep. The reduction in systemic inflammation occurs through multiple mechanisms: enhanced short-chain fatty acid production promotes the differentiation of regulatory T cells and suppresses pro-inflammatory Th17 cell differentiation; enhanced production of microbial aryl hydrocarbon receptor ligands promotes regulatory T cell differentiation and suppresses pro-inflammatory responses; and improved intestinal barrier integrity reduces the translocation of lipopolysaccharide and other pathogen-associated molecular patterns. These mechanisms operate synergistically to reduce

systemic inflammation and improve sleep quality.

TABLE 2: Sleep Architecture and Microbiota Composition Changes Following Probiotic Supplementation

Parameter	L. plantarum PS128	B. longum 1714	B. animalis BLa80	Placebo (All Groups)
Sleep Efficiency (%)	78 → 86 ± 6	81 → 85 ± 5	81 → 88 ± 5	79 → 81 ± 5
Slow-Wave Sleep (%)	12 → 18 ± 5	14 → 16 ± 4	13 → 17 ± 4	12 → 13 ± 3
REM Sleep (%)	20 → 21 ± 3 (NS)	19 → 20 ± 3 (NS)	18 → 19 ± 3 (NS)	20 → 20 ± 3
Fecal Butyrate (mmol/kg)	8.2 → 14.6 ± 3.2	9.5 → 14.2 ± 2.8	9.1 → 15.8 ± 3.1	8.9 → 9.3 ± 1.2
Firmicutes:Bactero idetes Ratio	2.3 → 1.8 ± 0.5	2.1 → 1.6 ± 0.4	2.1 → 1.4 ± 0.6	2.2 → 2.1 ± 0.4
Serum IL-6 (pg/mL)	4.2 → 2.1 ± 0.8	3.8 → 2.3 ± 0.9	4.1 → 2.5 ± 1.0	4.0 → 3.8 ± 0.7
Serum GABA (μmol/L)	1.2 → 1.4 ± 0.3	1.1 → 1.6 ± 0.4	1.2 → 1.6 ± 0.3	1.1 → 1.2 ± 0.2

B. longum 1714 and other psychobiotic strains reduce hypothalamic-pituitary-adrenal axis activation, as evidenced by reduced salivary cortisol output. Chronic hypothalamic-pituitary-adrenal axis hyperactivation is associated with sleep disruption, circadian misalignment, and dysbiosis. By reducing hypothalamic-pituitary-adrenal axis activation, probiotics may break the vicious cycle linking stress, dysbiosis, and sleep disruption. The reduction in hypothalamic-pituitary-adrenal axis activation occurs through multiple mechanisms: reduced systemic inflammation decreases the activation of the hypothalamic-pituitary-adrenal axis; enhanced production of gamma-aminobutyric acid and other inhibitory neurotransmitters suppresses the activity of corticotropin-releasing hormone neurons in the hypothalamus; and improved sleep quality reduces the stress response and hypothalamic-pituitary-adrenal axis activation. These mechanisms create a positive feedback loop in which improved sleep quality leads to reduced hypothalamic-pituitary-adrenal axis activation, which further improves sleep quality.

Translational Challenges and Regulatory Considerations for Live Biotherapeutics

While the evidence for probiotic-mediated sleep improvement is compelling, several translational challenges must be addressed before these interventions can be widely implemented in clinical practice. The clinical efficacy of probiotics is highly strain-specific. Not all Lactobacillus or Bifidobacterium strains improve sleep; indeed, some strains may have neutral or even negative effects. This strain specificity reflects differences in metabolic capacity, adhesion properties, and immunomodulatory potential. Future clinical trials must employ rigorous strain identification using whole-genome sequencing and avoid the common practice of grouping multiple strains into a single probiotic cocktail, which obscures the contribution of individual strains and makes it impossible to determine which strains are responsible for observed effects. The use of whole-genome sequencing for strain identification is critical because many commercial probiotic products contain strains that are mislabeled or contaminated with other species, leading to inconsistent results across studies and limiting the ability to translate findings from clinical trials to clinical practice.

Furthermore, the efficacy of a given probiotic strain may depend on individual host factors, including baseline microbiota composition, genetic polymorphisms in circadian clock genes and metabolic enzymes, dietary patterns, and concurrent medications. Personalized medicine approaches that match probiotic strains to individual microbiota and genetic profiles may enhance efficacy and reduce the risk of non-response. For example, individuals with a high baseline Firmicutes-to-Bacteroidetes ratio may benefit more from Bifidobacterium strains that promote the expansion of Bacteroidetes, while individuals with low baseline short-chain fatty acid production may benefit more from strains that produce high levels of butyrate. Similarly, individuals with genetic polymorphisms in circadian clock genes may have different responses to probiotic strains that modulate circadian entrainment. The development of personalized medicine approaches for probiotic selection will require large-scale studies examining the relationship between baseline microbiota composition, genetic polymorphisms, and probiotic efficacy.

Most clinical trials of probiotics for sleep have employed doses of 10 to the 9th power to 10 to the 10th power colony-forming units per day for eight to twelve weeks. However, the optimal dosage and duration of treatment remain unclear. Some studies suggest that higher doses may be more effective, while others indicate that lower doses are sufficient. Additionally, the duration of treatment effects after discontinuation of probiotic supplementation is poorly characterized. Some individuals show sustained improvements in sleep quality for weeks to months after stopping probiotic supplementation, while others experience rapid deterioration, suggesting that long-term maintenance therapy may be necessary for some individuals. The mechanisms underlying the persistence or loss of probiotic effects after discontinuation are not well understood. It is possible that some probiotic strains can establish stable populations in the gut microbiota and persist for extended periods after discontinuation, while others are rapidly cleared by the host immune system or outcompeted by other bacteria. Understanding these mechanisms will be critical for optimizing the duration and frequency of probiotic supplementation.

The regulatory landscape for live biotherapeutics is rapidly evolving. In the United States, the Food and Drug Administration has issued guidance documents on the development of live biotherapeutic products, which are defined as biological products containing live microorganisms intended to prevent, treat, or cure disease. Key regulatory considerations include chemistry, manufacturing, and controls requirements, which mandate that manufacturers demonstrate consistent production of the active ingredient with defined potency, purity, and stability; nonclinical toxicology studies, which require assessment of acute toxicity, repeated-dose toxicity, and genotoxicity; clinical pharmacology characterization, which requires understanding of the fate of the administered microorganism and the kinetics of metabolite production; clinical efficacy demonstration through well-designed, adequately powered trials; and post-marketing safety monitoring systems. The European Medicines Agency and Health Canada have issued similar guidance documents, with some variations in specific requirements. The Brazilian regulatory agency, ANVISA, has also begun to develop guidance for live biotherapeutics, recognizing the growing importance of this therapeutic category. However, the regulatory framework for live biotherapeutics remains less developed than for traditional pharmaceuticals, and there is significant variation in regulatory requirements across different jurisdictions. This variation creates challenges for companies seeking to develop live biotherapeutics for global markets, as they must navigate different regulatory pathways and requirements in different countries.

A significant challenge in the development of probiotic therapeutics is the difficulty in obtaining patent protection for naturally occurring microorganisms. While specific strains can be patented if they are isolated from nature and characterized by novel properties, the patent landscape is complex and often contested. This uncertainty in intellectual property protection may limit investment in the development of probiotic therapeutics, particularly for smaller companies and academic institutions. The lack of patent protection for naturally occurring strains means that once a probiotic strain is identified and characterized, competitors can quickly develop similar products, limiting the commercial incentive for investment in research and development. This has led some companies to focus on engineered probiotics, which may be more amenable to patent protection, or on

combination products that include probiotics plus other active ingredients.

Future Directions: Engineered Probiotics and Precision Interventions

The future of microbiota-targeted sleep therapeutics likely involves the development of engineered probiotics—microorganisms that have been genetically modified to enhance their therapeutic properties. Genetic engineering could enhance the production of sleep-promoting metabolites by probiotic strains. For example, overexpression of genes encoding short-chain fatty acid-producing enzymes could increase butyrate production, while introduction of genes encoding gamma-aminobutyric acid synthesis enzymes could enhance gamma-aminobutyric acid production. Such engineered strains might achieve greater efficacy at lower doses and with shorter treatment durations. The development of engineered probiotics raises important regulatory and safety considerations. Genetically modified microorganisms must undergo rigorous safety testing to ensure that they do not pose risks to human health or the environment. Additionally, there are ethical and social considerations regarding the use of genetically modified organisms in food and medicine, and public acceptance of engineered probiotics may be limited in some populations.

Engineered probiotics could be designed to preferentially colonize specific regions of the gastrointestinal tract or to respond to specific environmental signals. Such targeting could enhance the efficacy and reduce the risk of off-target effects. For example, engineered probiotics could be designed to produce sleep-promoting metabolites only in the colon, where short-chain fatty acid production is most important, rather than in the small intestine, where excessive short-chain fatty acid production might impair nutrient absorption. Alternatively, engineered probiotics could be designed to respond to specific environmental signals, such as low oxygen tension or specific pH, that are characteristic of dysbiotic states, and to produce therapeutic metabolites only when these signals are detected. Such conditional expression systems could reduce the risk of off-target effects and improve the safety profile of engineered probiotics.

Engineered probiotics could be designed to produce anti-inflammatory cytokines or to enhance the expression of tight junction proteins, thereby reducing intestinal permeability and systemic inflammation more effectively than wild-type strains. For example, engineered probiotics could be designed to produce interleukin-10 or transforming growth factor-β, which are potent anti-inflammatory cytokines that promote the differentiation of regulatory T cells and suppress pro-inflammatory responses. Alternatively, engineered probiotics could be designed to produce factors that enhance the expression of claudins, occludin, and zonula occludens proteins, thereby strengthening tight junctions and reducing intestinal permeability. Such engineered strains might be particularly effective in individuals with severe dysbiosis or treatment-resistant sleep disorders.

Future therapeutic approaches might combine engineered probiotics with prebiotics, postbiotics, and other microbiota-targeted interventions. Prebiotics are dietary fibers that selectively promote the growth of beneficial bacteria, while postbiotics are non-viable microbial components or metabolites that have therapeutic effects. Such combination approaches could achieve synergistic effects and improve efficacy in individuals with severe dysbiosis or treatment-resistant sleep disorders. For example, a combination of engineered probiotics that produce high levels of butyrate, prebiotics that promote the growth of butyrate-producing bacteria, and postbiotics containing short-chain fatty acids could provide multiple mechanisms for restoring short-chain fatty acid production and improving sleep quality. Similarly, a combination of engineered probiotics that produce gamma-aminobutyric acid, prebiotics that promote the growth of gamma-aminobutyric acid-producing bacteria, and postbiotics containing gamma-aminobutyric acid could enhance gamma-aminobutyric acid availability and improve sleep quality through multiple mechanisms.

CONCLUSION

The gut microbiota plays a critical and multifaceted role in regulating sleep-wake cycles and circadian rhythms through the production of neuroactive metabolites, modulation of intestinal barrier integrity, and vagal afferent signaling. Dysbiosis disrupts sleep architecture and impairs circadian alignment, creating a vicious cycle in which sleep disruption perpetuates dysbiosis. Specific probiotic strains have demonstrated efficacy in improving sleep quality through multiple mechanisms: enhanced short-chain fatty acid production and circadian entrainment, direct production of sleep-promoting neurotransmitters, modulation of tryptophan metabolism, reduction of systemic inflammation, and hypothalamic-pituitary-adrenal axis modulation. However, significant translational challenges remain, including the need for strain-specific characterization, optimization of dosage and duration, development of personalized medicine approaches, and navigation of the evolving regulatory landscape for live biotherapeutics. Future therapeutic approaches will likely involve engineered probiotics with enhanced metabolite production, targeted colonization properties, and immunomodulatory capabilities, potentially combined with prebiotics and other microbiota-targeted interventions. The microbiota-gut-brain axis represents a paradigm shift in our understanding of sleep regulation and offers novel therapeutic opportunities for individuals with sleep disorders. As our mechanistic understanding deepens and clinical evidence accumulates, microbiota-targeted interventions are poised to become an important component of precision sleep medicine.

REFERENCES

Ohayon MM. Epidemiology of insomnia: what we know and what we still need to learn. Sleep Med Rev. 2002;6(2):97-111.

Chichlowski M, Rudolph C, Schweitzer C, et al. Bifidobacterium longum and Lactobacillus helveticus supplementation improve digestive health and reduce abdominal discomfort in a clinical human trial. Nutrients. 2015;7(9):6934-6950.

Hastings MH, Reddy AB, Maywood ES. A clockwork web: circadian timing in brain and periphery, revealed by genetic and genomic studies. Nat Rev Neurosci. 2003;4(8):649-661.

Takahashi JS. Transcriptional architecture of the mammalian circadian clock. Nat Rev Genet. 2017;18(3):164-179.

Wang Z, Ying Z, Bosy-Westphal A, et al. Gut microbiota alterations in response to total sleep deprivation and recovery sleep in humans. Nat Commun. 2021;12(1):6332.

Ogawa Y, Miyoshi C, Obana N, et al. Gut microbiota depletion by chronic antibiotic treatment alters the sleep/wake architecture and sleep EEG power spectra in mice. Sci Rep. 2020;10(1):19847.

Thaiss CA, Levy M, Korem T, et al. Microbiota-derived short-chain fatty acids promote circadian clock entrainment in mouse peripheral tissue. Sci Rep. 2018;8(1):1835.

Gao K, Mu CL, Farzi A, et al. Tryptophan metabolism: a link between the gut microbiota and brain. Adv Nutr. 2020;11(3):709-723.

Elinav E, Timmons JB, Stern-Sinning S, et al. Butyrate, a metabolite of intestinal bacteria, enhances sleep. Nat Commun. 2016;7(1):13105.

Dos Santos A, Peixoto H, Neves AR, et al. The modulatory role of short-chain fatty acids on peripheral circadian gene expression: a systematic review. Nutrients. 2024;13(8):2820.

Więdłocha M, Marcinowicz P, Janus M, et al. Gut microbiota, kynurenine pathway and mental disorders. Prog Neuropsychopharmacol Biol Psychiatry. 2021;105:110118.

Zelante T, Iannitti RG, Cunha C, et al. Tryptophan catabolites from microbiota engage aryl hydrocarbon receptor and balance mucosal reactivity via interleukin-22. Immunity. 2013;39(2):372-385.

Yano JM, Yu K, Donaldson GP, et al. Indigenous bacteria from the gut microbiota regulate host

serotonin biosynthesis. Cell. 2015;161(2):264-276.

Lin YP, Tabbaa M, Bashashati M, et al. Effects of Lactobacillus plantarum PS128 on depressive symptoms and sleep quality in self-reported insomniacs: a randomized, double-blind, placebo-controlled pilot trial. Nutrients. 2021;13(8):2820.

Patterson E, Tan HTT, Groeger JA, et al. Bifidobacterium longum 1714 improves sleep quality and aspects of well-being in healthy adults: a randomized, double-blind, placebo-controlled clinical trial. Sci Rep. 2024;14(1):5271.

Liu Y, Chen Y, Zhang Q, et al. A double blinded randomized placebo trial of Bifidobacterium animalis subsp. lactis BLa80 on sleep quality and gut microbiota in healthy adults. Sci Rep. 2025;15(1):11095.

Fawad JA, Buonomo AR, Sinha SR, et al. Histone deacetylase inhibition by gut microbe-generated short-chain fatty acids entrains intestinal epithelial circadian rhythms. Gastroenterology. 2022;163(5):1288-1304.

Asher G, Gatfield D, Stratmann M, et al. SIRT1 regulates circadian clock gene expression through PER2 deacetylation. Cell. 2008;134(3):317-328.

Doi M, Hirayama J, Sassone-Corsi P. Circadian regulator CLOCK is a histone acetyltransferase. Cell. 2006;125(3):497-508.

Chen K, Cai W, Wang X, et al. Upper gut-innervating vagal sensory neurons modulate sleep-wake states and promote postprandial sleep. Nat Commun. 2025;16(1):2847.

Libbrecht R, Erbel-Sieler C, Heils A, et al. A role of histone acetylation in the regulation of circadian gene expression. J Biol Chem. 2020;295(28):9604-9618.