

Host Circadian Rhythm, Microbial Patterns, and Sleep Architecture

Poorvi Kurundkar

Oakridge International School, Gachibowli, Nanakramguda Road, Cyberabad, Hyderabad, Telangana, 500 008, India;
kurundkar.poorvi@gmail.com

ABSTRACT: The human gut houses roughly 38 trillion microbes and contains about 1.3 times more microbial cells than the entire human body, making it an epicenter of revolutionary science. A growing body of research has demonstrated that intestinal microbiota not only modulates the human body's immune and neural systems, but also forms a close relation with circadian rhythm, which is predominantly regulated by the suprachiasmatic nucleus (the Central biological clock system) and peripheral clock systems. This paper aims to discuss the effects of feeding patterns, dietary interventions, and supplementation on sleep quality, thereby creating a bidirectional pathway governed by circadian rhythm. This provides a more effective way for shaping sleep structure and quality, including paradoxical sleep.

KEYWORDS: Biology, Chronobiology, Intestinal Clock Genes, Feeding Patterns, Sleep Disorders.

■ Introduction

Studying sleep disorders has become a physiological and psychological priority given their prevalence in today's population. Difficulty sleeping or insomnia symptoms are among the most common medical complaints affecting nearly a third of the adult population.¹ Short sleep has been linked with various adverse effects, such as mortality rate, diabetes mellitus, hypertension, cardiovascular disease, and obesity.² A Latin American Survey outlined that strategies patients used to cope with sleep problems included specific behaviors such as taking a warm bath, reading or watching TV (44 %), taking herbal beverages (17 %), or taking sleeping pills (10%).³

Although such practices have temporary symptomatic improvements due to increasing parasympathetic activity and reducing sleep onset latency, emerging evidence acknowledges that gut microbiota, particularly the interaction between circadian processes and microbial diversity, significantly affect sleep quality. This occurs through a bidirectional path, where circadian rhythmicity and sleep architecture function in symbiosis. Studies exploring the proliferation dynamics of gut microbiota are therefore essential not only for the regulation of sleep quality but also because sleep may modulate microbial balance itself.

Historically, mammalian physiology has adapted to periods in which mammals either forage and feed or sleep and fast.⁴ The adaptation of physiology to these oscillations is controlled by a timekeeping system, which primarily encompasses the synchronization between light-dark signals from the eye with peripheral systems, including muscle, adipose tissue, liver, and intestine. It is also influenced by the gut-brain axis through the involvement of the neurotransmitters serotonin, melatonin, and the immune system. The key control for synchronizing external light cues with internal rhythm is the suprachiasmatic nucleus (SCN), sometimes referred to as the central circadian pacemaker, which is located in the hypothalamus and is responsible for this coordination of tissue-specific rhythms according

to the light input it receives from the outside world.⁵ The SCN utilizes a 24-hour pacemaking rhythm that is maintained by the cycling of a transcriptional-translational loop of CLOCK genes (Circadian Locomotor Output Cycles Kaput).

The clock gene mechanism is also present in cells throughout the brain and the body,⁶ with genes such as *Per1*, *Per2*, and *Bmal1* that regulate their own transcription by a negative feedback loop, generating cyclical inhibition of transcription factors. These act as timing signals in the gut, allowing certain microbial species to align their growth and metabolic patterns with the host's sleep-wake cycle.⁷ In essence, the circadian rhythm in our bodies is directly associated with the light-dark cycle, which coordinates with metabolic rhythms that are primarily controlled by host signaling pathways and microbial activity in the gut.

Explorations into how sleep deprivation disrupts the immune system have blossomed into the discovery that it also promotes bacterial translocation and gut dysbiosis.⁸ Today, it is well-established that a healthy gut microbiota is important for maintaining a healthy host and promoting optimal physiological function.⁹ This will, in turn, aid in shaping the cyclical pattern of restorative sleep throughout a sleep period, which is known as sleep architecture. Sleep itself can be broken down into REM (rapid eye movement) and NREM (non-rapid eye movement) stages, which cyclically alternate throughout the night in a predictable structure known as sleep architecture, transitioning from lighter stages of NREM to deep slow-wave sleep before entering REM sleep.¹⁰ The induction of many sleep disorders begins with the disorganization of this healthy structure and alternation of these stages.¹¹

Many doctors have begun prescribing targeted probiotics for gut health as a way to manage certain sleep disorders. For example, according to a 2017 study, *Lactobacillus gasseri* CP2305 supplementation has improved the PSQI (Pittsburgh Sleep Quality Index) score by targeting stress-related insomnia.¹²

This growing trend fortifies connections between gut microbiota and sleep architecture, which this review will explore by evaluating patterns of feeding and dietary supplements with their effect on circadian rhythm.

More broadly, this review examines the ways in which gut microbiota influence circadian rhythm and sleep through interactions with intestinal clock genes and feeding patterns, thus depicting the importance of microbial synchronization for effective regulation of sleep.

■ Discussion

For more than half a century now, researchers have investigated the paradox that the brain's pacemaking center, despite occupying so little space, exerts influence over nearly every physiological process tied to timekeeping. Each cell in the SCN contains an autonomous molecular clock, and the SCN is composed of multiple of these single-cell oscillators, which can govern behavioral and physiological rhythmicity and be reset by environmental light. The key is that the heterogeneous network formed by the cellular clocks within the SCN must synchronize to maintain timekeeping activity.¹³

Host Pacemaking Mechanisms:

The discovery that the SCN contains tissue necessary for the generation of circadian rhythmicity was made through autoradiographic tracing methods in 1972.¹⁴ The study found that specialized retinal ganglion cells (ipRGCs) in the eye contain melanopsin, a light-sensitive pigment that sends signals via the nerve fibers from the retina, which terminate at a specific region in the Hypothalamus- the SCN.

In the SCN, transcription factors are used to operate in a cyclical manner involving core clock genes (such as *CLOCK*, *BMAL1*, *PER*, and *CRY*) that regulate their own expression in a negative feedback loop.

The pioneering research that investigated the nature of this underlying feedback loop was first published in 1971 and used *Drosophila Melanogaster* (fruit fly) as the primary subject.¹⁵ The method consisted of isolating the three distinct mutations that disrupted the flies' normal ~24-hour circadian rhythms: an arrhythmic mutant, a mutant with a shortened period of approximately 19 hours, and one with a lengthened period of approximately 28 hours. When these X-linked mutations were mapped in male offspring, all three different clock mutants always tracked to the same functional gene. This was termed the period "PER" gene, but it was still widely unknown how it worked until the late 1980s, when scientists discovered that PER protein levels cycle in ~24 h cycles and PER mRNA levels also cycle,¹⁶ though delayed, suggesting a negative feedback mechanism.¹⁷

It turned out that *CLOCK* (CLK) forms a complex with *CYCLE* (CYC) and activates transcription of *PER*. Subsequently, *PER*, along with another protein, *CRY*, enters the nucleus and represses this transcription factor (CLK-CYC complex).¹⁸ The mammalian functional equivalents of CLK and CYC are *CLOCK* and *BMAL1*, which form a heterodimer that binds to E-box elements in DNA, activating transcription of *PER* and *CRY* genes. This activation typically

occurs in the morning, and repression (negative peak) occurs at night.⁸

Additionally, it is important to note that this is a two-loop feedback system, with core genes being *PER/CRY*, which control *CLOCK* expression; and secondary genes being *REV-ERB/RORα*, wherein *REV-ERBα* represses *BMAL1* expression, and *RORα* activates *BMAL1* expression.⁸

CLOCK and BMAL1 regulation in the SCN:

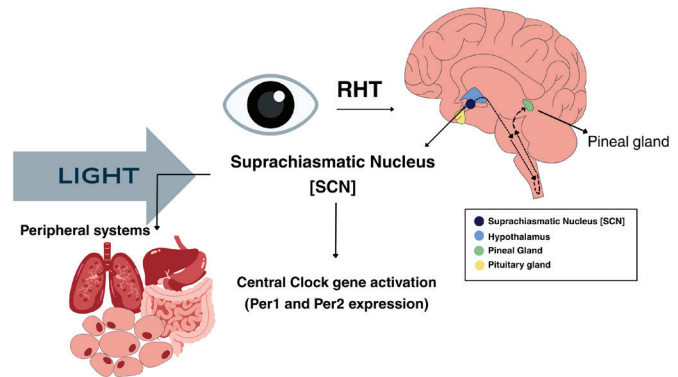


Figure 1: Light input from the retina reaches the SCN via the RHT, where it entrains central clock gene expression (*Per1*, *Per2*) and synchronizes circadian rhythm in peripheral bodily systems. Reproduced from: Matenchuk *et al.* 2020; 53:101340. doi:10.1016/j.smrv.2020.101340- Figure 2. Figure created with canva.com

In order for the SCN to coordinate these peripheral molecular clocks via the RHT, external light cues must cause a rapid increase in *PER1* and *PER2* transcription in SCN neurons (see Figure 1), which causes a circadian clock reset each day.¹⁹

A 2002 study outlined that this aforementioned process requires neither rods nor cones, which were the only known retinal photoreceptors from previous publications. Instead, the study suggested the importance of specialized retinal ganglion cells that are intrinsically photosensitive to depolarization in response to light.²⁰ Thus, the retinal ganglion cells (ipRGCs) act as primary photoreceptors and fire action potentials via the RHT to the SCN through glutamate intersynaptic signaling upon light input to the retina. Within the SCN, glutamate binds to ionotropic glutamate receptors: AMPA Receptors (AMPA) and NMDA Receptors (NMDARs). The by-product of this is a Ca^{2+} influx, which activates intracellular signaling cascades (such as CaMK and PKA), and promotes *PER1* and *PER2* transcription. Transcription of the *PER* gene will then repress the *CLOCK: BMAL1* complex (which ties to the same cycle mentioned above), hence delaying *PER1* and *PER2* expression.

This process highlights the importance of timely light signals when it comes to circadian rhythm. Furthermore, since the *PER* gene expression oscillates across an approximate 24-hour period, light-induced changes or irregular light cues can alter the biological rhythm. This alteration is most apparent in conditions like jet lag, night-shift work, or chronic sleep disorders.²¹ Such disruptions not only impair sleep and alertness but also extend to metabolic health and gut misalignment. For example, a 2018 study conducted among nursing staff revealed that the prevalence of metabolic syndrome (MetS: a cluster of

conditions that increase the risk of heart disease, stroke, and type II diabetes) in workers exposed to night shifts for up to 10 years was 37.5% higher when compared to diurnal workers.²²

This establishes a clear relation between night work and metabolic syndrome as well as inflammatory dysfunction, which can be caused by gut dysbiosis (increased gut permeability), allowing bacterial components to translocate out of the gut and into the body, initiating low-grade systemic inflammation.²³

CLOCK and BMAL1 regulation in the gut:

We have discussed how the SCN is a master regulator of circadian rhythm and acts to synchronize light exposure with peripheral clocks in the rest of the body, like the gut. Growing evidence pertaining to diurnal rhythmicity has revealed a strong bidirectional connection with the SCN and the microbiome situated in the gut, mediated through feeding-fasting cycles and microbial activity.

CLOCK and BMAL1 are expressed in intestinal epithelial cells (IECs), which was first discovered in 2007, when the effects of timed feeding and vagotomy were measured in mice.²⁴ The vagotomy, which consists of the removal of the vagus nerve, thus removing the connection between the gastrointestinal tract and SCN, showed that clock genes were expressed rhythmically throughout the gastrointestinal tract. Timed feeding shifted clock gene expression at the RNA and protein level but did not shift clock gene expression in the central clock. Thus, it is deduced that daytime feeding in nocturnal rodents is a strong synchronizer of gastrointestinal clock genes, but this synchronization occurs independently of the central clock. Furthermore, gastric clock gene expression is not mediated through the vagal nerve. The experiment proved the involvement of separate peripheral gastrointestinal clock genes, but their definite functions were not yet determined. However, subsequent research has revealed that this gastrointestinal clock was closely linked to feeding patterns, which act as a dominant external time cue, or zeitgeber, for the gut.²⁵

Clock genes can affect a variety of functions in the gut. For example, BMAL1 and CLOCK increase expression of tight junction proteins, bolstering epithelial barrier function and allowing diurnal changes in intestinal permeability.²⁶ Expression of the nutrient transporters PEPT1 and SGLT1 is also controlled by clock gene oscillations. Furthermore, immune and inflammatory gene signatures are regulated by central transcription factors such as NF- κ B, thus regulating inflammation according to feeding cycles.²⁷

An example of this can be seen in a Circadian Rhythm Disruption (CRD) mouse model, along with induced down-regulation of BMAL1, which was found to exhibit more severe colitis, accompanied by decreased tight junction protein levels and increased apoptosis of IECs compared with wild-type mice. This further suggests the tight link between clock genes like BMAL1, intestinal barrier function, and the expression of immune-related genes (apoptotic IEC's).²⁸

Dietary Regimens and their influence on microbial rhythms:

Rhythmic food intake not only synchronizes the intestinal clock but also drives microbial oscillations, which illustrates the interdependence of host behavior and microbiota function within the diurnal framework.²⁹ A deeper dive into barrier integrity and permeability in the context of feeding patterns can facilitate a more complete understanding of this framework.

This rhythmic opening and tightening of the barrier may be adaptive: During the day, increased feeding promotes a controlled increase in permeability, allowing nutrient exchange and strengthening of immune barriers such as inflammatory cytokines. However, at night, tightening prevents inflammation and bacterial translocation.

Figure 2 captures information obtained from Scanning Electron Micrographs (SEMs) in mice,³⁰ which are nocturnal and have a dark phase (ZT12–ZT18) that corresponds to their active feeding period, whereas the light phase (ZT0–ZT6) is their resting or fasting period. Keeping this in mind, we can infer that during the feeding phase, the surfaces show dense bacterial adherence and microvilli crowding, as depicted in Figure 2. Along with this, the mucosal surface is coated with filamentous or rod-like microbial structures.

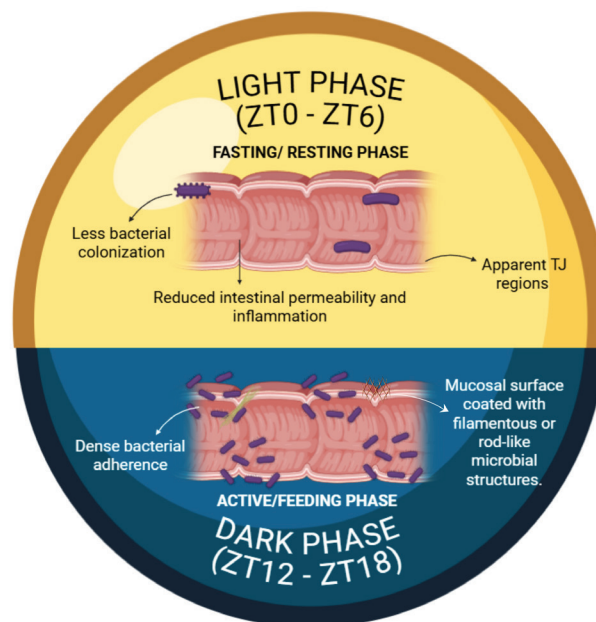


Figure 2: Model showing circadian variation in colonic epithelial adherence of mice. Bacterial attachment is markedly higher during the dark phase (ZT12–ZT18) compared to the light phase (ZT0–ZT6). Reproduced from Thaïss *et al.*, 2016 (*Cell*, 167(6), 1495–1510.e12)- Figure 2. Figure created with with BioRender.com

This indicates increased epithelial adherence and correlates with high nutrient availability due to feeding activity. During the fasting phase, the epithelial surface topography becomes visible and tight junctions are more apparent, appearing smoother and cleaner, with less bacterial colonization. This visually indicates less epithelial adherence, likely due to reduced nutrient availability.

Even if total bacterial numbers don't fluctuate dramatically, the genes expressed by microbes show evident oscillation. For example, carbohydrate metabolism enzymes (like glycosidases,

polysaccharide-degrading enzymes) peak during feeding times when nutrient availability is highest. On the other hand, genes for flagellar motility (to move towards nutrient-rich areas) and host mucin degradation (enzymes like sialidases, fucosidases) peak during fasting times, when bacteria may need to move further for nutrients or consider other carbohydrate sources like mucin.²⁹ Another change is seen in the oscillation of SCFA (Short Chain Fatty Acid) production, which forms diurnal metabolites. These microbial metabolites then feed back into host circadian pathways by modulating histone acetylation, and thus regulating PER/CRY/CLOCK/BMAL1 expression in gut epithelial cells.³⁰

This proves how different food-intake patterns (feeding and fasting cycles) can affect bacterial populations in the gut. This rhythmic switching is exactly what intermittent fasting (IF) and time-restricted feeding (TRF) are based on. IF schemes have mainly two types, which have been applied to rodents, namely, daily fasting (DF) (e.g., 16:8, 20:4) and Alternate-Day Fasting (ADF). These methods restrict the timing of eating, not necessarily the content of the diet. IF triggers evolutionarily conserved cellular responses such as improved glucose regulation, stress resistance, and reduced inflammation by mimicking periods of scarcity (as in evolutionary history). A diet program of fasting every other day increased Firmicutes in male mice, while increasing *Lactobacillus* and *Bacteroides* in females. IF treatment in diabetic mice enriched beneficial bacteria like *Lactobacillus* and reduced harmful ones such as *Akkermansia*.³¹

On the other hand, High-Fat Diets (HFD) can increase the prevalence of metabolic diseases such as obesity, diabetes, non-alcoholic fatty liver disease, hypertension, and hyperuricemia. It is characterized by a significant intake of fats, typically comprising 60% or more of total daily calories, often with low carbohydrate intake. HFD-fed mice were observed to have a decrease in *Bacteroidetes* and an increase in Firmicutes (which break down complex carbohydrates into SCFAs), thus increasing the absorption of calories from food, contributing to obesity via adipose tissue inflammation.³² Furthermore, an HFD has also been linked to disruptions in circadian rhythm, where mice on a regular chow diet maintained a normal free-running circadian rhythm (~23.6 hours), but consumption of an HFD lengthened their circadian period within just one week.³³ Interestingly enough, differences in mean body weight did not occur until the third week of HF feeding. Thus, the increase in period length was independent of body weight and overall activity levels.

While intermittent fasting affects the dynamics of when and how our gut microbes work by creating feeding windows, another daily behavior plays an important role in shaping the gut microbiome: sleep. Changes in sleep duration or quality can alter the balance of microbial species over the course of a day.

Dietary supplements and their impact on sleep and disorders:

Tononi and Cirelli's synaptic homeostasis hypothesis – "Sleep is the price the brain pays for plasticity" – suggests that disturbances in the brain's restorative processes can disrupt diurnal metabolic systems. Sleep is widely said to be directed

by 2 processes: Sleep/wake homeostasis (regulating sleep needs based on the duration of wakefulness) and the circadian clock. The SCN acts as the primary circadian clock in this case, since it distinguishes between light-dark cues and also dictates hormonal fluctuations. For example, norepinephrine is present at higher levels in the morning, while melatonin and serotonin peak during the phase of darkness.³⁴ These hormonal timings help construct sleep's internal structure, which consists of alternating cycles of NREM and REM cycles. NREM is typically longer (75-80% of total sleep) and is categorized by parasympathetic activation, such as low blood pressure, low heart rate, and decreased temperature. REM, on the other hand, consists of shorter periods (20-25%), with a decrease in muscle tone, which is the continuous passive muscle contraction that provides resistance to passive stretch during a resting state.¹⁰ REM also causes greater sympathetic activation, such as increased heart rate, breathing, blood pressure, and temperature. REM sleep has more structured dreams, while NREM sleep has more unstructured "bizarre" dreams.³⁵

Today, with a significant rise in irregular circadian timings, insomnia is affecting 11-16% of the global population.³⁶ It is characterized by decreased slow wave sleep due to reduced REM latency and increased REM density, implying that though the time taken to enter REM sleep is less, the number of rapid eye movements per minute is above average. This increased REM density is observed in eating disorders, narcolepsy, presenile dementia, and other neuropsychiatric diseases due to reduced or absent REM inhibition.³⁷ This imbalance of REM sleep stages has recently been linked to the impairment of intestinal permeability, causing a "leaky gut," through which bacteria and their end products have been found to translocate to the mesenteric lymph nodes, liver, and the brain. In Poroyko *et al.*,³⁸ mice were exposed to sleep fragmentation (SF) for 4 weeks, which caused increased food intake and reversible changes in gut microbiota composition, such as an increase in Lachnospiraceae and Ruminococcaceae and a decrease in Lactobacillaceae families. Excess Lachnospiraceae is known for impairment of glucose metabolism,³⁹ leading to inflammation, obesity, and promoting the onset of Type 1 diabetes,⁴⁰ depicting how sleep disruption can have vast effects on the gut.

However, the gut-brain axis can also impact sleep because a powerful innate sleep-inducing mechanism occurs via gut microbiota. Microbial activity, driven by feeding and fasting cycles, produces neuroactive metabolites such as Short-Chain Fatty Acids (SCFAs) or Gamma-aminobutyric acid (GABA). GABA acts as an inhibitory neurotransmitter that slows down neural signaling by acting as an anti-fight-or-flight chemical messenger.⁴¹ Naturally produced from glutamate, GABA facilitates cross-barrier communication between the intestinal clock genes and central sleep-regulating centers, thereby affecting sleep onset latency and circadian regulation.⁴² When this symbiotic relationship is disrupted through circadian misalignment or dietary regimens, it often manifests as sleep disorders such as insomnia or sleep fragmentation.⁴³ Thus, GABA can directly impact sleep quality as well as the GI tract. This connection has been evident in studies that revealed diminished levels of GABA in IBS-D patients as compared to controls.⁴⁴ GABA

also reduced the expression of proinflammatory cytokines, thus linking GI problems with disruptions in GABA signaling. Consequently, targeted dietary supplementation with probiotics or amino acids may serve as a strategy for restoring the structural integrity of sleep and mitigating disorders.

This means that exogenous supplementation may serve as a strategy to mend microbial diurnal oscillations that were broken due to jet lag, shift work, or sleep fragmentation. These interventions generally target the gut-brain axis through two distinct pathways: regulating the SCN and peripheral microbiome modification.

Primarily targeting the SCN, one of the most extensively researched and prescribed dietary supplements impacting sleep is Melatonin (MLT). MLT is a hormone naturally synthesized by the pineal gland, which acts via MT1 & MT2 receptors in the SCN. When it becomes dark, the SCN sends signals to the pineal gland to make melatonin, a hormone that promotes relaxation and reduced alertness by decreasing body temperature, thus aiding in sleep onset.⁴⁵ Patients with chronic insomnia were found to have significantly lower nighttime melatonin levels, with peak melatonin concentrations averaging 82.5 pg/mL, compared to 116.8 pg/mL in healthy sleepers.⁴⁶ MLT supplements are often utilized to manage sleep-related issues and have been purported to regulate sleep patterns and alleviate sleep disorders such as insomnia. More specifically, they're often prescribed to shift workers who have irregular sleep-wake schedules due to working at night, or patients with intense jet lag.⁴⁷

Clinical trials have shown mixed results in humans, with a general decrease in sleep latency, but no consistent effect on sleep quality. Melatonin is known to be soporific, since it signals biological night to the SCN by telling it when to sleep; it is not restorative since it doesn't signal how much to sleep.

In animals, however, though melatonin is species & time-dependent, it can control architecture, such as reducing REM sleep after MLT during the dark period in nocturnal animals.⁴⁵ For example, Wang *et al.* outline that supplementation with melatonin ameliorates disturbances in the circadian rhythm homeostasis of the gut microbiota due to sleep restriction, by rescuing families like Muribaculaceae and Lachnospiraceae, which are sensitive to SF.⁴⁸ This suggests that supplementation can restore circadian rhythm as well as microbial diversity, and is likely why exogenous melatonin is commonly administered orally in cases of sleep disorders.⁴⁹

Other than supplementation with melatonin itself, dietary strategies to enhance endogenous melatonin synthesis, particularly through tryptophan-rich foods, have been examined for their effects on sleep. According to a meta-analysis,⁵⁰ Tryptophan (Trp) supplementation has been found to shorten wake after sleep onset (~81.03 min/g).

Trp typically becomes Serotonin (see Figure 3), which converts to melatonin once triggered by darkness.⁵¹

Trp, being an essential amino acid, breaks down to form its metabolite, Tryptamine, which has been linked to the induction of melatonin synthesis in the past. In Lee *et al.*,⁵¹ a study measuring melatonin synthesis in 10-week-old wild-type male C57BL/6 mice, Tryptamine was found to increase

sleep duration to 31.6 ± 5.6 min compared to 18.6 ± 3.4 min in the control group. However, sleep onset latency and blood melatonin concentrations showed no significant increase for orally administered Tryptamine trials in the mice. This aligns with the aforementioned meta-analysis, which concluded that though Trp can increase sleep duration, Trp supplementation did not affect other sleep components like sleep efficiency or REM/NREM ratios. On the other hand, ITE, which is formed from the methylation of another metabolite of Trp (Indolokine A5), increased the expression of key genes for melatonin biosynthesis (Tph1a, Aadc, Aanat1, Aanat2, and Hiomt) in zebrafish embryos.⁵¹

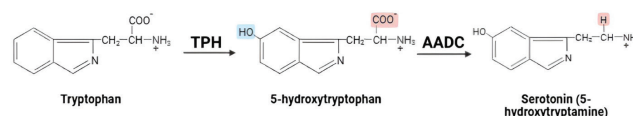


Figure 3: Biosynthetic pathway from L L-tryptophan to serotonin, which is subsequently converted to melatonin. ITE showed an incr ease in expression levels of key enzymes in this pathway. Reproduced from Lee *et al.*, ACS Omega, 2024, 9, 43875 43875–43883. Copyright 2024.

Emerging evidence suggests that other amino acids, such as phenylalanine, may exert even more pronounced effects on sleep. This is supported by Lee *et al.*, where phenylalanine's metabolite, phenethylamine, not only showed one of the longest sleep durations at 36.2 ± 3.4 min, but it also increased blood melatonin concentrations. The study thus concluded by restating phenethylamine's and ITE's potent role as a potential therapeutic agent for sleep disorders due to their role in melatonin synthesis. Despite these promising findings, it is important to remember that though melatonin in humans can decrease sleep latency and affect wake after sleep onset, there are much milder impacts on overall sleep efficiency and REM/NREM architecture.⁴⁵

While melatonin and amino acid precursors primarily target central sleep architecture and neurotransmitter synthesis from the top down, an alternative strategy involves modulating the system from the bottom up via the gastrointestinal tract. This involves the employment of the gut-brain axis, specifically probiotics, for sleep regulation. Probiotics are living nutraceutical microorganisms, such as bacteria or yeast, that aid in digestion and supplement gastrointestinal flora.⁵² Of the probiotics associated with sleep regulation, the *Lactobacillus* species have been of particular interest because they can produce neuroactive compounds that dictate sleep-wake rhythmicity.

Lactobacilli are characterized by their gram-positive rods and are the most common genus in the lactic acid bacteria (LAB) category.⁵³ LAB species can decarboxylate glutamate to produce γ -aminobutyric acid (GABA).

Though this production of GABA primarily occurs in the brain, the Enteric Nervous System (which governs the GI tract) is lined with GABA receptors, indicating GABA secreted by gut bacteria can be sensed locally by cells of the intestinal epithelial tract.⁵⁴ This is what connects *Lactobacilli* with sleep quality, as seen in Yu *et al.*, where milk was fermented with *Lactobacillus brevis* DL1-11, a strain with high GABA-producing capacity, and administered in different concentrations

to mice (the highest concentration was termed high-dose GABA fermented milk (HGFM, 33.33 mg/kg.bw)).⁵⁵ The results indicated decreases in anxiety behavior after oral HGFM administration. Moreover, mice in the HGFM group exhibited a significantly prolonged sleep time and a shortened sleep latency. These results demonstrate a beneficial effect of *Lactobacillus brevis* on sleep. Additionally, significant increases in the relative abundances of *Ruminococcus*, *Adlercreutzia*, and *Allobaculum*, and the levels of certain SCFAs such as butyric acid were observed in the HGFM group.

This suggests that GABA-fermented milk and oral consumption of *Lactobacillus* strains with high GAD activity may improve sleep and regulate gut microbiota as well as SCFAs levels. The same is supported by various studies, such as one measuring sleep deprivation (SD) in Rhesus Monkeys, which speculated that SD-induced gut dysbiosis might be an outcome of reduced gut-derived GABA absorption.⁵⁶ The study thus stated that GABA-producing *Lactobacillus* supplementation might be beneficial for the treatment of SD-induced intestinal dysfunction.

Alongside *Lactobacilli*, *Bifidobacteria*, and similar psychobiotics can also influence sleep efficiency via stress modulation. They are gram-positive, nonmotile, and often branched anaerobic bacteria that are administered as dietary supplements for their probiotic properties.

A study measuring sleep efficiency (SE) found that higher SE, specifically by decreased Wake after sleep onset (WASO), is associated with higher levels of two *Bifidobacterium* species, *breve* and *saguini*.⁵⁷

Targeting the gut microbiome as a therapeutic strategy for psychological disorders has shown promising outcomes in recent years.⁵⁸ This is why *bifidobacteria* are present in probiotics as well as psychobiotics. Species such as *Bifidobacterium longum* 1714 have been shown to influence stress responses and modulate neural responses during social stress, thereby impacting sleep quality.⁵⁹ In a study measuring eighty-nine adults with impaired sleep quality, *B. longum* 1714 significantly improved the PSQI component of sleep quality and daytime dysfunction due to sleepiness after 4 weeks and social functioning and energy/vitality after 8 weeks.⁶⁰ *B. longum* 1714 may modulate the HPA axis, and the SCFAs produced by the strain could influence melatonin synthesis, which emphasizes the multidirectional ability of certain probiotics to modulate neural activity, stress, mood, sleep and microbial diversity.

Overall, the evidence suggests that no single intervention fully restores circadian rhythm homeostasis, sleep architecture, and microbial rhythmicity simultaneously. Current evidence suggests that melatonin-based therapies possess the strongest translational capacity for circadian realignment, since melatonin supplementation remains the most clinically established intervention discussed. Unlike peripheral microbiome interventions, melatonin acts centrally, making it the most effective in disorders characterized primarily by circadian misalignment, such as jet lag, delayed sleep phase syndrome, or shift-work.⁶¹ However, though the ability of exogenous melatonin to reduce sleep onset latency is consistently supported across human trials, recent studies have emerged suggesting that melatonin

primarily functions as a chronobiotic rather than a restorative hypnotic agent.⁴⁵ This means that it can't efficiently improve total sleep efficiency, change NREM/REM ratios, or alter underlying sleep architecture in healthy adults compared to traditional hypnotic drugs such as benzodiazepines.

At the same time, the overall evidence supporting amino acid precursor therapies remains comparatively weak. Although certain metabolites increased sleep duration and melatonin concentrations in animal studies, many failed to significantly improve sleep onset latency, sleep efficiency, or REM/NREM architecture. Additionally, much of the current evidence derives from murine or zebrafish models, whose neuroendocrine regulation differs substantially from that of humans.⁶² The mechanistic complexity of tryptophan metabolism also presents limitations, since tryptophan is diverted into multiple competing pathways, such as the kynurenine pathway.⁶³ Around 95% of tryptophan is processed via the kynurenine pathway, while only a small fraction (1%–2%) goes toward serotonin and melatonin synthesis,⁶⁴ which reduces the predictability of therapeutic outcomes.

Among microbiome-targeted therapies, psychobiotics such as *Bifidobacterium longum* 1714 may currently possess the greatest long-term promise because, unlike melatonin, which only affects the circadian rhythm, psychobiotics are able to affect the HPA axis, inflammation signaling, mood regulation, and neurotransmitters produced through SCFAs. This is particularly relevant because chronic insomnia in humans is frequently associated with hyperarousal, stress dysregulation, and anxiety rather than circadian misalignment alone. The observed improvements in PSQI scores, daytime dysfunction, energy levels, and social functioning following *B. longum* 1714 supplementation provides stronger human translational evidence than many other probiotic interventions. However, although psychobiotics may represent the future of sleep therapeutics, they are not yet as clinically standardized. Hence, further research should focus on optimizing such psychobiotic-based interventions through the identification of standardized dosages, treatment durations, and emphasis on specific probiotic strains. Greater emphasis will also likely be placed on determining how inter-individual gut microbiota variations influence therapeutic responsiveness,⁶⁵ which could allow psychobiotic treatments to become more personalized and clinically reliable for the management of sleep disorders.

■ Conclusion

This paper primarily explored the interconnectedness between three mechanisms in the human body: gut microbiota, CLOCK gene expression, and sleep architecture, as seen in Figure 4.

Specifically, it discussed how gut-driven processes can affect sleep architecture. While central regulation of REM and NREM sleep is primarily governed by the brainstem and hypothalamic networks, peripheral signals originating from the gut can modulate sleep onset latency and quality. Microbiota-driven changes in GABAergic signaling, serotonergic–melatonergic pathways, and immune mediators are examples of mechanisms

by which IECs' clocks can shape sleep. This is further strengthened by research on dietary supplements, with compounds like exogenous Melatonin, Tryptophan, and Phenylalanine, which can induce sleep-like symptoms. The review highlights the importance of understanding how probiotic and psychobiotic supplementation, such as *Lactobacillus* and *Bifidobacterium* strains, can improve GABA secretion, thus supporting the idea that targeting the gut microbiota may offer a more holistic approach to circadian as well as sleep regulation compared to existing hormonal supplementation.

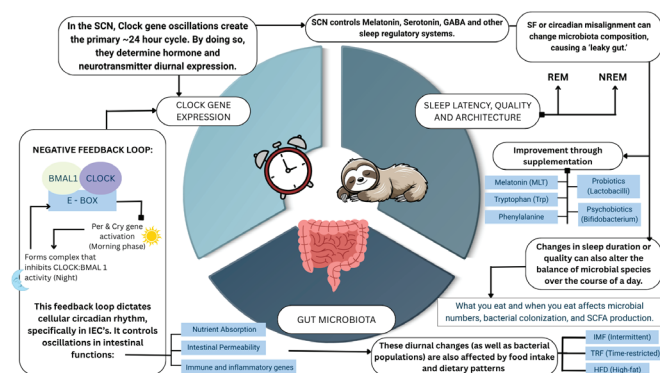


Figure 4: Summary of relationship between gut microbiota, sleep quality and clock gene expression.

Overall, this research highlights how gut microbiota and intestinal CLOCK genes interact with central neural pathways to influence sleep timing and architecture. These findings imply that optimization of sleep patterns requires targeting nutritional and microbiome-related factors rather than only focusing on central nervous system regulation. This perspective could enable the development of personalized chrononutrition strategies that focus on meal timing, dietary composition, and probiotic supplementation with an individual's endogenous circadian phase. This can be increasingly beneficial for overall human health because of the growing trends of sleep deprivation, shift work, and jet lag due to vast urbanization and globalization. Future research in this field should prioritize this kind of systems-level perspective, linking gut microbiota to sleep disorder therapies through well-controlled human studies and longitudinal monitoring studies, which have the potential to create interventions that can practically integrate chronobiological systems and the microbiome.

Acknowledgments

I sincerely thank Indigo Research as well as my mentor, Dr. Brydie R Huckestein (PhD. Microbiology and Immunology, University of Pittsburgh School of Medicine), for her invaluable guidance and support throughout the process of creating this review.

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■ Authors

Poorvi Kurundkar, currently studying in the International Baccalaureate (IB) program at Oakridge International School, is deeply fascinated by how the brain orchestrates complex physiological systems. As a result, she aspires to pursue a career in neuroscience-based research.