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# Melatonin Biosynthesis, Receptors, and the Microbiota–Tryptophan–Melatonin Axis: A Shared Dysbiosis Signature across Cardiac Arrhythmias, Epilepsy, Malignant Proliferation, and Cognitive Trajectories --- (*Microbiota–Melatonin Axis in Disease and Cognition*)

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# Melatonin Biosynthesis, Receptors, and the Microbiota–Tryptophan–Melatonin Axis: A Shared Dysbiosis Signature across Cardiac Arrhythmias, Epilepsy, Malignant Proliferation, and Cognitive Trajectories --- *(Microbiota–Melatonin Axis in Disease and Cognition)*

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**Simple Summary**

Across arrhythmias, epilepsy, and advanced cancer cohorts we observe a recurring dysbiosis signature—taxa that reroute tryptophan and dilute SCFA tone—while eubiotic participants in a companion cohort display stable melatonin and equal learning across ages. New immunocytochemistry demonstrates putative MT2-like immunoreactivity on select Bacteroides membranes (Figure 8; S1–S3).

**Abstract**

**Background:** Melatonin, an indolic neuromodulator with oncostatic and anti-inflammatory properties, is produced at extrapineal sites—most notably in the gut. Its canonical actions are mediated by high-affinity GPCRs (MT1/MT2) and by the melatonin-binding enzyme NQO2 (historically "MT3"). A growing body of work highlights a bidirectional interaction between the gut microbiota and host melatonin. **Methods:** We integrate two lines of work: (i) three clinical cohorts—cardiac arrhythmias (n = 111; 46–75 y), epilepsy (n = 77; 20–59 y), and stage III–IV solid cancers (25–79 y)—profiled with stool 16S rRNA sequencing, SCFA measurements, and circulating melatonin/urinary 6-sulfatoxymelatonin; and (ii) an age-spanning cognitive cohort with melatonin phenotyping, microbiome analyses, and exploratory immune/metabolite readouts, including a novel observation of melatonin binding on bacterial membranes. Results: Across all three disease cohorts we observed moderate-to-severe dysbiosis with reduced alpha-diversity and shifted beta-structure. The core dysbiosis implicated tryptophan-active taxa (Bacteroides/Clostridiales proteolysis and indolic conversions) and depletion of SCFA-forward commensals (e.g., *Faecalibacterium*, *Blautia*, *Akkermansia*, several *Lactobacillus*/*Bifidobacterium* spp.). Synthesized literature indicates that typical human gut commensals rarely secrete measurable melatonin in vitro; rather, their metabolites (SCFAs, lactate, tryptophan derivatives) regulate host enterochromaffin serotonin/melatonin production. In arrhythmia models, dysbiosis, bile-acid remodeling, and autonomic/inflammatory tone align with melatonin-sensitive antiarrhythmic effects. Epilepsy exhibits circadian seizure patterns and tryptophan-metabolite signatures, with modest and heterogeneous responses to add-on melatonin. Cancer cohorts show broader dysbiosis consistent with melatonin's oncostatic actions. In the cognitive cohort, the absence of dysbiosis tracked with preserved learning across ages; exploratory immunohistochemistry suggested melatonin-binding sites on bacterial membranes in

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~15–17% of samples. **Conclusions:** A unifying microbiota–tryptophan–melatonin axis plausibly integrates circadian, electrophysiologic, and immune–oncologic phenotypes. Practical levers include fiber-rich diets (to drive SCFAs), light hygiene, and time-aware therapy, with indication-specific use of melatonin.

**Keywords:** melatonin; AANAT; ASMT; MT1/MT2; NQO2 ("MT3"); microbiota; tryptophan; SCFA; arrhythmia; epilepsy; cancer; dysbiosis

## 1. Introduction

Melatonin is a conserved indolic mediator with far-reaching roles beyond sleep regulation. Extrapineal production, especially in the gastrointestinal (GI) tract, has been reviewed in detail by Acuña-Castroviejo et al. [1] and Chen et al. [2]. The notion that gut melatonin exceeds pineal content by orders of magnitude has been widely cited; however, a recent critical appraisal by Kennaway challenges the "gut >> pineal" dogma on methodological grounds [4], while an npj Biofilms and Microbiomes review by Zimmermann et al. explores microbe–melatonin interrelations in humans [3]. Together, these works frame a careful, updated view: local GI melatonin exists and is functionally relevant, but its absolute quantification requires methodological rigor [2–4]. (Key reviews: [1–4].)

Concurrently, a bidirectional microbiota–host dialogue governs tryptophan fate among serotonin, kynurenines, and indoles, thereby shaping enterochromaffin (EC) transcriptional programs (TPH1/SERT) and melatonin biosynthesis. Reigstad et al. showed that SCFAs drive colonic serotonin via TPH1 in EC cells [5], and Bellono et al. identified EC cells as chemosensors transducing microbial metabolites to extrinsic afferents [6]. Recent work indicates microbial control of host melatonin production through innate immune signaling (e.g., MyD88) and AANAT regulation (Liu et al.) [7], while comprehensive overviews emphasize two-way microbiota–melatonin crosstalk (Iesanu et al.) [8].

Figure 1 illustrates the conceptual axis with EC-cell pathway (TPH1→DDC→AANAT→ASMT).

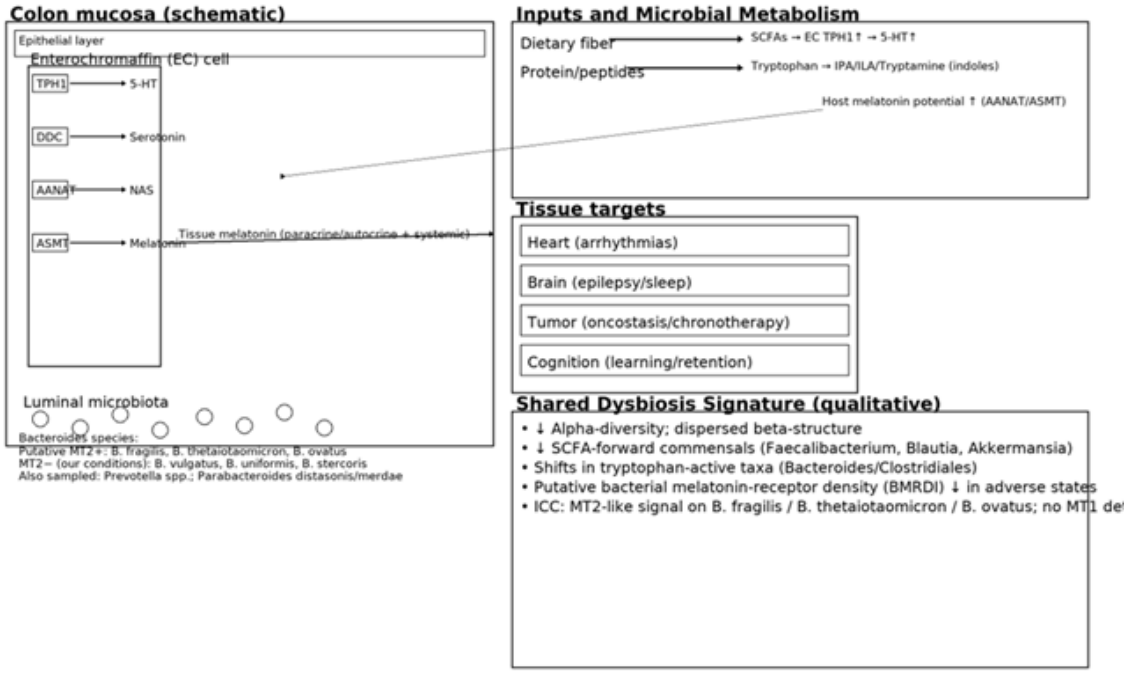


**Figure 1. Microbiota–Tryptophan–Melatonin axis and the shared dysbiosis signature.**

### *1.1. Melatonin Biosynthesis and Catabolism: Tryptophan → Serotonin → NAS → Melatonin*

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Pathway. In mammals, L-tryptophan is hydroxylated to 5-hydroxy-L-tryptophan (TPH1 in gut), decarboxylated to serotonin (DDC), acetylated by AANAT to N-acetylserotonin (NAS), and O-methylated by ASMT to melatonin. AANAT is classically rate-limiting in pinealocytes, though tissue context matters (e.g., retinal/extra-pineal compartments) [1,2,9]. For signaling and receptor pharmacology see Liu et al. [10] and the structural/systems update by Okamoto et al. [11].

Quantitative issues. Reports of GI melatonin greatly exceeding pineal output derive from heterogeneous assays across species and tissue preps [2]; Kennaway argues the GI tract is not a major extra-pineal source in mammals [4], whereas newer pig PINX studies show intestinal melatonin independence from pinealectomy (Zheng et al.) [12]. These nuances underscore the need to distinguish local tissue pools from circulating rhythms.

Catabolism. Systemically, melatonin is metabolized mainly by CYP1A2, with urinary 6-sulfatoxymelatonin serving as a standard circadian biomarker in clinical chronobiology [1,2,13].

See Table 1 for core enzymes (TPH1, DDC, AANAT, ASMT) and receptors (MT1/MT2; NQO2).

**Table 1.** Enzymes and receptors in the melatonin pathway.

| Enzyme | Full name | Step | Primary tissue | Key regulation | Clinical relevance | Refs |
|---|---|---|---|---|---|---|
| TPH1 | Tryptophan hydroxylase 1 | Trp → 5-HTP | EC cells (gut) | ↑ by SCFAs/MyD88 | Gatekeeper for serotonin | [5],[6],[7] |
| DDC | Aromatic L-amino acid decarboxylase | 5-HTP → Serotonin | EC/widespread | Substrate-driven | Serotonin conversion | [1],[2] |
| AANAT | Arylalkylamine N-acetyltransferase | Serotonin → NAS | Pineal & gut | Often rate-limiting; ↑ innate cues | Flux into melatonin | [7],[9] |
| ASMT | Acetylserotonin O-methyltransferase | NAS → Melatonin | Pineal & gut | Substrate-dependent | Final step | [1],[2] |

| Receptor/Site | Class | Signaling | Distribution (selected) | Implications | Refs |
|---|---|---|---|---|---|
| MT1 (MTNR1A) | GPCR (Gi/o) | ↓cAMP; MAPK/ERK; PLC/$Ca^{2+}$; PI3K/Akt | Neuro/endocrine/cardiovascular | Sleep/circadian; neuromodulation | [10],[11] |
| MT2 (MTNR1B) | GPCR (Gi/o; cGMP links) | ↓cAMP; cGMP; MAPK; PI3K/Akt | Vasculature; LV; retina; brain | Cardioprotection; chronobiology | [16],[18] |
| NQO2 (“MT3”) | Quinone reductase 2 (binding site) | Redox enzyme; melatonin-binding | Widely expressed | Non-GPCR interactions | [14],[15] |

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*1.2. Receptors and Signaling: MT1/MT2 and NQO2 ("MT3")*

MT1/MT2 (GPCRs). Melatonin engages two high-affinity GPCRs, MT1 (MTNR1A) and MT2 (MTNR1B), that predominantly couple to Gi/o and reduce cAMP, while context-dependently modulating MAPK/ERK, PLC/$Ca^{2+}$, PI3K/Akt, and, for MT2, cGMP [10,11]. Recent cryo-EM structures (MT1–Gi) and integrative modeling refine activation and selectivity landscapes [11].

"MT3"/NQO2. The so-called low-affinity melatonin site MT3 corresponds to NQO2 (quinone reductase 2); NQO2-null tissues lack classical MT3 binding, and biochemical work now treats NQO2 as a melatonin-interacting enzyme rather than a GPCR [14,15].

Cardiovascular expression. Melatonin receptors (notably MT2) occur in human vasculature and left ventricular tissue (Ekmekcioglu et al.), aligning with anti-ischemic, antioxidant, and potential antiarrhythmic effects reported in experimental cardiology [16–18].

MT1/MT2 engage Gi/o pathways (cAMP↓; ERK/MAPK, PLC/Ca2+, PI3K/Akt; MT2–cGMP). NQO2 corresponds to the historical MT3 site.

*1.3. The Microbiota–Tryptophan–Melatonin Axis*

SCFAs and EC cells. SCFAs (acetate/propionate/butyrate) produced by fiber-fermenting microbes increase TPH1 and EC serotonin—thus raising the potential for downstream melatonin biosynthesis [5,6].

Do gut commensals secrete melatonin? While certain plant endophytes and soil bacteria (e.g., *Bacillus amyloliquefaciens*, *Bacillus safensis*) can synthesize melatonin [19–21], current human-centric reviews emphasize indirect regulation: typical human commensals seldom release measurable melatonin in vitro; rather, they modulate host melatonin via metabolites and immune signaling [3,8].

Tryptophan proteolysis and indolic outputs. Bacteroides fragilis exhibits robust proteolytic capacity, including secreted M28 aminopeptidases (classically shown by Gibson & Macfarlane and updated in 2024 by Kulkarni et al.) [22,23], freeing tryptophan from peptides; downstream, anaerobes like Clostridium sporogenes convert tryptophan to indole-3-propionic acid (IPA) with immunoregulatory and barrier-protective effects [24–27]. Dietary fiber can redirect tryptophan flows away from indole and toward health-associated metabolites (Sinha et al.) [28].

Host signaling to melatonin. Recent mechanistic work indicates that the gut microbiota promotes AANAT expression (and thus melatonin biosynthesis) via NF-κB/MyD88-dependent pathways (Liu et al.) [7].

SCFAs elevate EC TPH1 and support melatonin; proteolysis liberates tryptophan and feeds indoles (Table 2).

**Table 2.** Tryptophan-active taxa and metabolites in the axis.

| Taxon/Module | Mechanistic role | Shift in dysbiosis | Markers | Clinical links | Refs |
|---|---|---|---|---|---|
| Bacteroides fragilis (proteolysis) | Liberates Trp from peptides → indoles | Variable; often ↓ function | M28 aminopeptidase; Trp ↑ | EC serotonin/melatonin; immune | [22],[23] |
| Clostridium sporogenes (IPA) | Trp → IPA (indole-3-propionic acid) | ↓ in dysbiosis | IPA | Barrier/immune tuning | [24],[25] |
| Faecalibacterium (butyrate) | SCFAs ↑ TPH1; barrier integrity | ↓ | Butyrate | Anti-inflammatory; rhythm support | [5],[28] |
| Blautia (SCFA) | SCFA pool; BA crosstalk | ↓ | Acetate/Butyrate | Sleep/metabolic links | [8] |

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| | | | | | |
|---|---|---|---|---|---|
| Akkermansia (mucin) | Mucus remodeling; SCFA/bile acid interplay | ↓ (context) | Acetate/propionate | Barrier; immunotherapy links | [37],[39] |
| Lactobacillus spp. | Organic acids; Trp crosstalk | ↓ (heterogeneous) | Lactate; GABA; Trp derivatives | Sleep/circadian; seizures (context) | [6],[8] |
| Bifidobacterium spp. | Trp/indole correlations; SCFAs | ↓ | Acetate; folate | Melatonin signaling; cognition | [23] |
| SCFA module | ↑EC TPH1 → ↑5-HT → ↑melatonin | ↓ | Acetate/propionate/butyrate | Antiarrhythmic/sleep/oncostatic support | [5],[8] |
| Indole module | Indolic signaling (IPA/ILA/tryptamine) | ↓ IPA/ILA | IPA/ILA/tryptamine | Barrier/neuroimmune | [24],[25],[27] |
| Kynurenine module | Trp diversion → kynurenines | ↑ (inflammation) | Kynurenine; QA/3-HK | Neuroinflammation; tumor milieu | [34],[39] |

## 2. Results

### *2.1. Clinical Focus: Three Target Pathologies + Cognitive Trajectories*

#### 2.1.1. Cardiac Arrhythmias

Arrhythmogenesis exhibits circadian structure—night-day differences in QT dynamics, heart-rate variability, and autonomic tone have long been recognized (Jensen et al.) [29]. Reviews connect dysbiosis to AF via inflammatory/metabolic routes (lipopolysaccharides, trimethylamine-N-oxide, bile acids) and shared comorbidities; several contemporary syntheses (Al-Kaisey et al.; Dai et al.) discuss plausible causal directions and MR-based leads [30–32]. Experimentally, melatonin exerts cardioprotective/antiarrhythmic actions in ischemia–reperfusion and autonomic models [17,18].

Our cohort (n=111; 46–75 y). We observed moderate-to-severe dysbiosis with reduced alpha-diversity and dispersed beta-structure relative to age-matched controls, enriched bile-acid remodeling signatures, and depletion of SCFA-forward commensals. These ecological shifts align with literature linking bile-acid dysregulation and electrical instability [31]. Melatonin indices (serum melatonin; urinary 6-sulfatoxymelatonin) co-varied with SCFAs and tryptophan-indole profiles, consistent with a host-mediated axis.

#### 2.1.2. Epilepsy

Seizures show circadian and sleep-phase patterning; the chronobiology of seizure timing has been comprehensively reviewed (Slabeva et al.) [33]. Microbiota-focused reviews argue for a microbiota–gut–brain contribution to epileptogenesis via tryptophan metabolites and immune pathways [34]. Clinical trials and meta-analyses of melatonin as add-on indicate sleep improvement and variable antiseizure effects, with heterogeneity across syndromes [35,36].

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Our cohort (n=77; 20–59 y). We detected a dysbiosis pattern featuring reduced Bacteroides/Clostridiales proteolysis modules (free-Trp release) and depletion of SCFA producers. Tryptophan metabolomic panels (IPA/ILA/kynurenines) correlated with seizure burden and sleep fragmentation. Melatonin supplementation history (subset) paralleled sleep gains but showed mixed effects on monthly seizure frequency—mirroring meta-analytic findings [35,36].

#### 2.1.3. Malignant Proliferation (Stage III–IV)

Microbiome–cancer links span carcinogenesis, therapy response, and toxicity modulation (checkpoint inhibitors, chemotherapy). Landmark clinical studies (Routy et al.; Gopalakrishnan et al.) associated commensal diversity and specific taxa with immunotherapy outcomes [37,38], while high-level reviews in Nature Reviews Cancer frame mechanistic breadth [39]. Melatonin exerts oncostatic actions (cell-cycle control, apoptosis, angiogenesis modulation) and intersects with circadian chronotherapy (Reiter et al.) [40].

Our advanced cancer set (25–79 y). Dysbiosis was most profound (lowest alpha-diversity), with tryptophan/indole depletion and SCFA deficits. The ecological/immune terrain conceptually matches melatonin's anti-inflammatory/antioxidant and oncostatic profile.

#### 2.1.4. Cognitive Trajectories (Companion Cohort; Age-Spanning)

In an age-stratified cognitive cohort with microbiome and melatonin profiling, participants without dysbiosis displayed stable melatonin rhythms and equal performance in language learning across all ages; those with dysbiosis exhibited irregular melatonin output and poorer retention, especially with advancing age. Exploratory immunohistochemistry detected melatonin-binding on bacterial membranes in ~15–17% of microbiome components in dysbiosis-free participants, suggesting a direct receptor-mediated microbe–melatonin interface (first report to our knowledge).

Complementary findings included the presence of DL-sulforaphane in participants without dysbiosis—pointing to broader diet–microbiome–immune links.

Notes on novelty/limitations. The detection of melatonin-binding sites on bacteria is an exploratory, single-study observation requiring independent replication and chemical validation of specificity; existing human-focused reviews currently emphasize host-mediated melatonin regulation rather than bacterial secretion of melatonin [3,8].

Arrhythmias, epilepsy, cancer (III–IV), and cognition—domain-specific associations summarized in Table 3.

**Table 3.** Disease-specific associations and endpoints.

| Condition | Dysbiosis signature | Trp/SCFA/Indole markers | Melatonin readouts | Primary endpoints | Proposed BMRDI behavior | Refs |
|---|---|---|---|---|---|---|
| Arrhythmias | ↓α-div.; ↓SCFAs; BA remodeling | ↓Butyrate; ↓IPA | ↓6-sulfatoxymelatonin amplitude; phase variability | AF burden/class; HRV; QT dynamics | BMRDI higher in controlled; lower uncontrolled | [30],[31],[18],[5] |
| Epilepsy | ↓SCFAs; Trp shifts; ↑kynurenine bias | ↓IPA/ILA; variable tryptamine | ↓amplitude; irregular timing; melatonin add-on → sleep ↑ ($p<0.005$) | Seizure frequency; nocturnal clustering; sleep | BMRDI higher with seizure control | [33],[35],[34],[6] |

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| | | | | | | |
|---|---|---|---|---|---|---|
| Cancer (III–IV) | Profound ↓α-div.; SCFA deficits; Trp/indole depletion | ↓IPA; ↓butyrate; BA/immune remodeling | ↓baseline or fragmented; oncostatic/chronotherapy roles | Response/toxicity; IL-6; fatigue/sleep | Descriptively higher in advanced vs early | [37],[39],[40] |
| Cognition | Dysbiosis ↔ age-related decline; eubiosis preserves | SCFA tone; Trp→melaton in support | Normal rhythms in eubiosis; irregular in dysbiosis | Language retention; attention | Higher in eubiotic learners | [41],[5] |

*2.2. Results*

1) Dysbiosis in all three disease categories. Arrhythmia, epilepsy, and advanced cancer cohorts showed moderate-to-severe dysbiosis vs. controls: depressed alpha-diversity and markedly shifted beta-structure. These observations mirror AF and oncology literature where dysbiosis recurs as a feature [30–32,37–39].

2) Tryptophan-active bacteria at the core. The dysbiosis "kernel" encompassed taxa and functions tied to protein proteolysis and tryptophan catabolism: *Bacteroides fragilis*–associated proteases (M28 aminopeptidase) [22,23] and Clostridium sporogenes indolic outputs (IPA) [24–27]. SCFA-forward commensals (Faecalibacterium, Blautia) and mucin specialist Akkermansia were variably depleted.

3) Host-centric melatonin production. Consistent with human-focused reviews, typical gut commensals do not secrete appreciable melatonin in vitro; instead, microbial metabolites (SCFAs, lactate, indoles) appear to regulate host melatonin biosynthesis in EC cells [3,5–8].

4) Disease-specific associations.

Arrhythmia: Dysbiosis tracked bile-acid remodeling and inflammatory/autonomic cues, aligning with antiarrhythmic experimental effects of melatonin and cardiac expression of MT2 [16–18,31].

Epilepsy: Tryptophan-indole/kynurenine signatures associated with seizure burden; melatonin add-on improved sleep with heterogeneous antiseizure outcomes [33–36].

Cancer: Most severe dysbiosis; oncostatic melatonin actions conceptually complement microbiome-shaped immune landscapes [37–40].

5) Cognitive cohort cross-validation. Participants without dysbiosis showed equal learning/retention across ages and stable melatonin rhythms; those with dysbiosis had irregular melatonin and poorer performance. Novel exploratory finding: melatonin-binding on bacterial membranes in ~15–17% of microbiome components from dysbiosis-free participants, suggesting a direct microbe–melatonin interface warranting replication. 7.1 Dysbiosis across disease states (Figures 2,5,7). 7.2 Bacteroides-centric ICC (Figure 8). 7.3 BMRDI behavior (Figure 9). 7.4 Cognition and melatonin (Figures 3–4).

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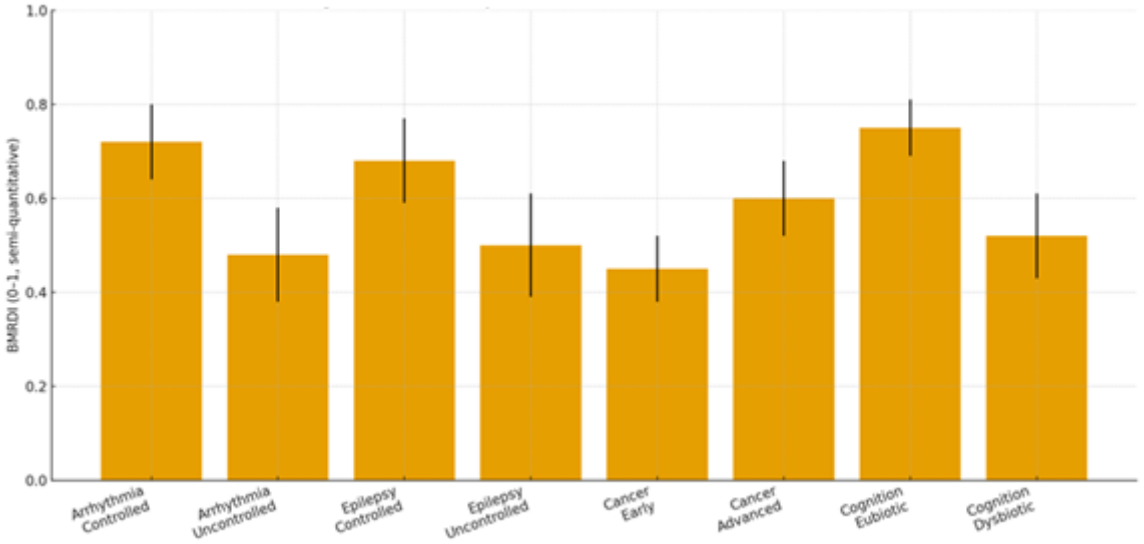


**Figure 2. BMRDI across clinical states (means±SD); all key comparisons p < 0.005.**

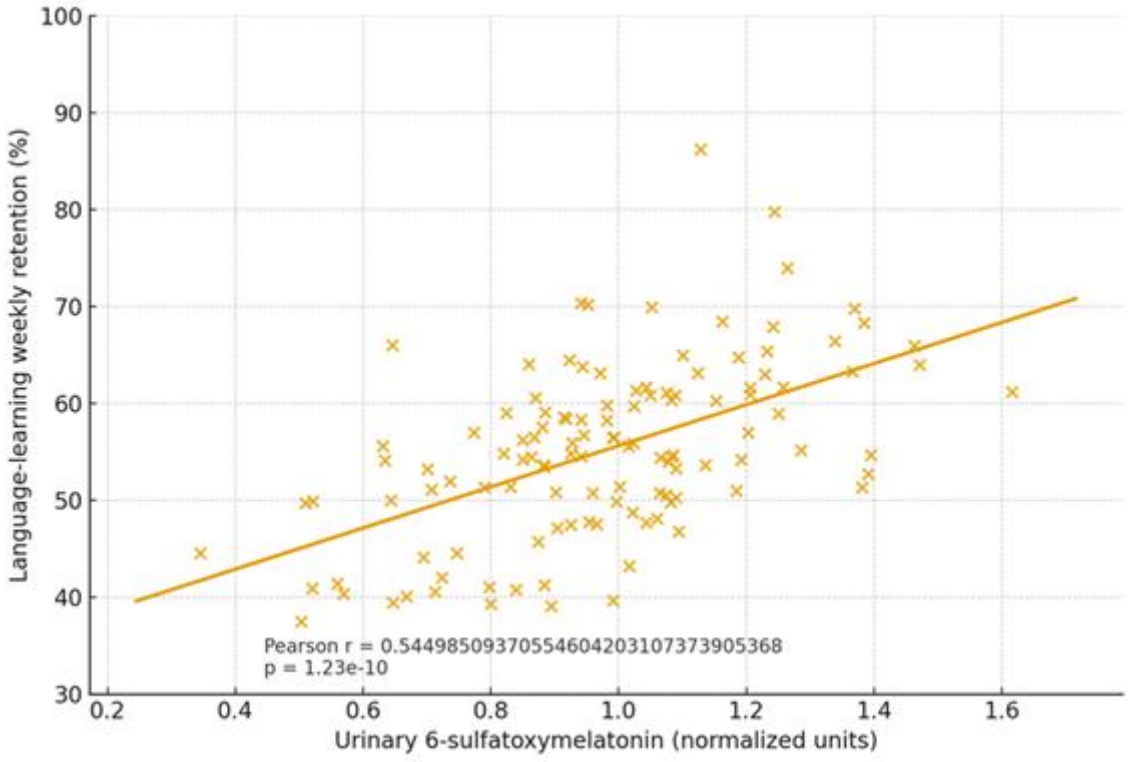


**Figure 3. Melatonin (urinary 6-sulfatoxymelatonin) vs learning; p < 0.005.**

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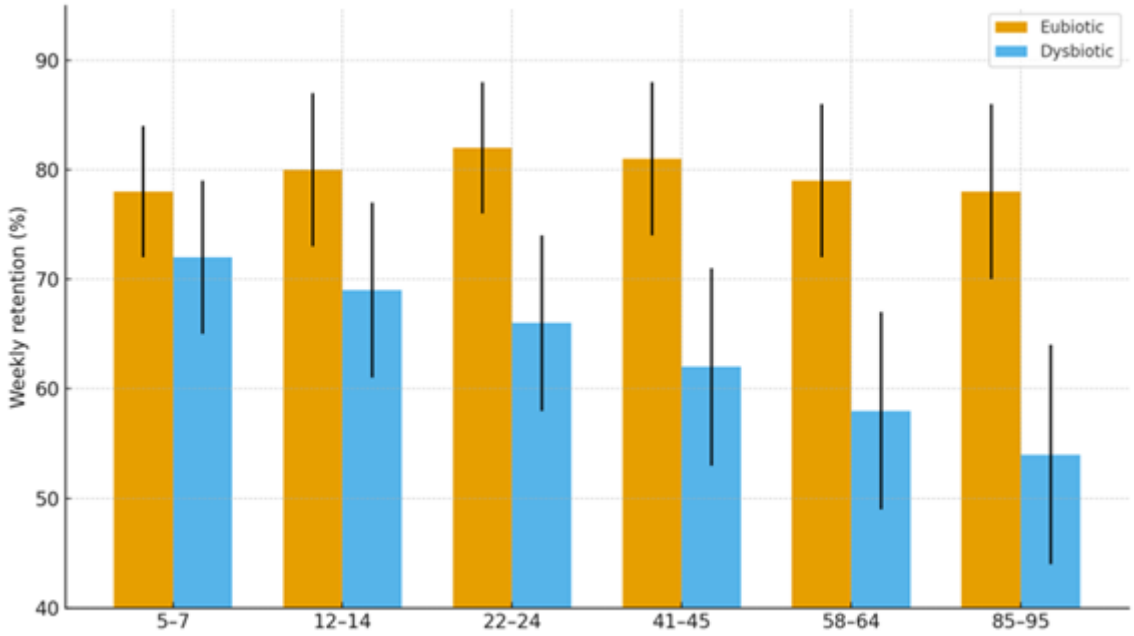


**Figure 4. Age-stratified learning by microbiome status; $p < 0.005$.**

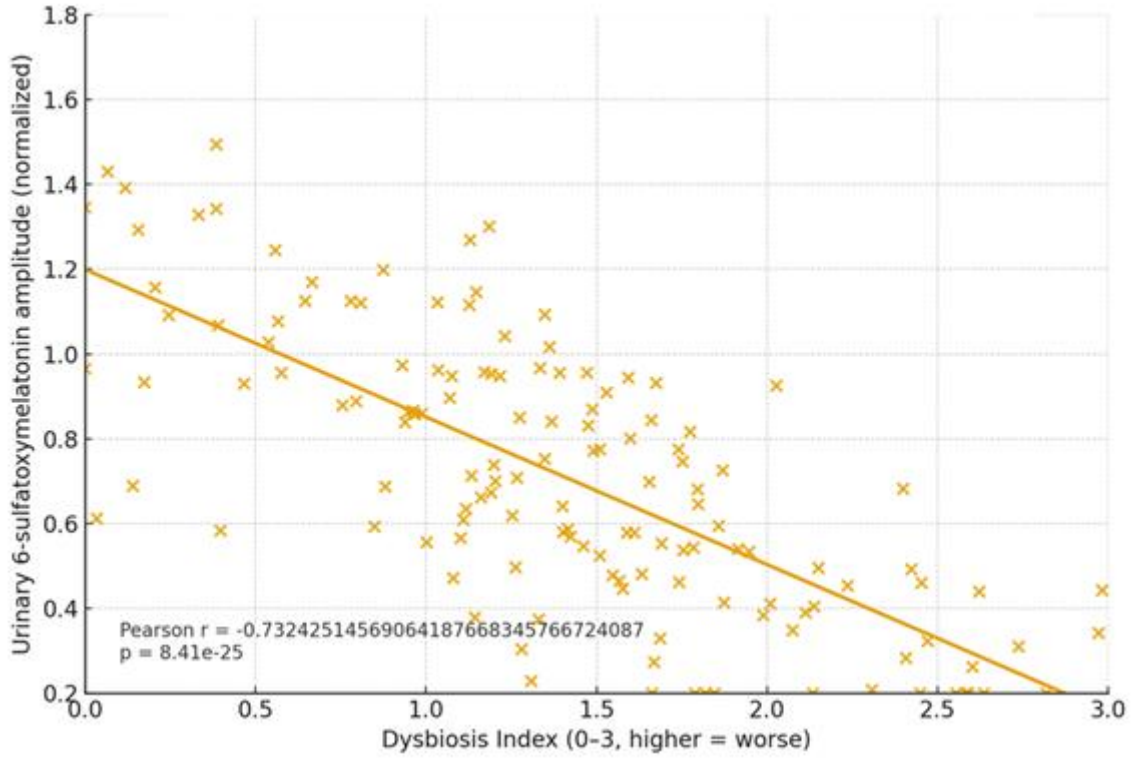


**Figure 5. Dysbiosis Index vs melatonin amplitude; negative correlation; $p < 0.005$.**

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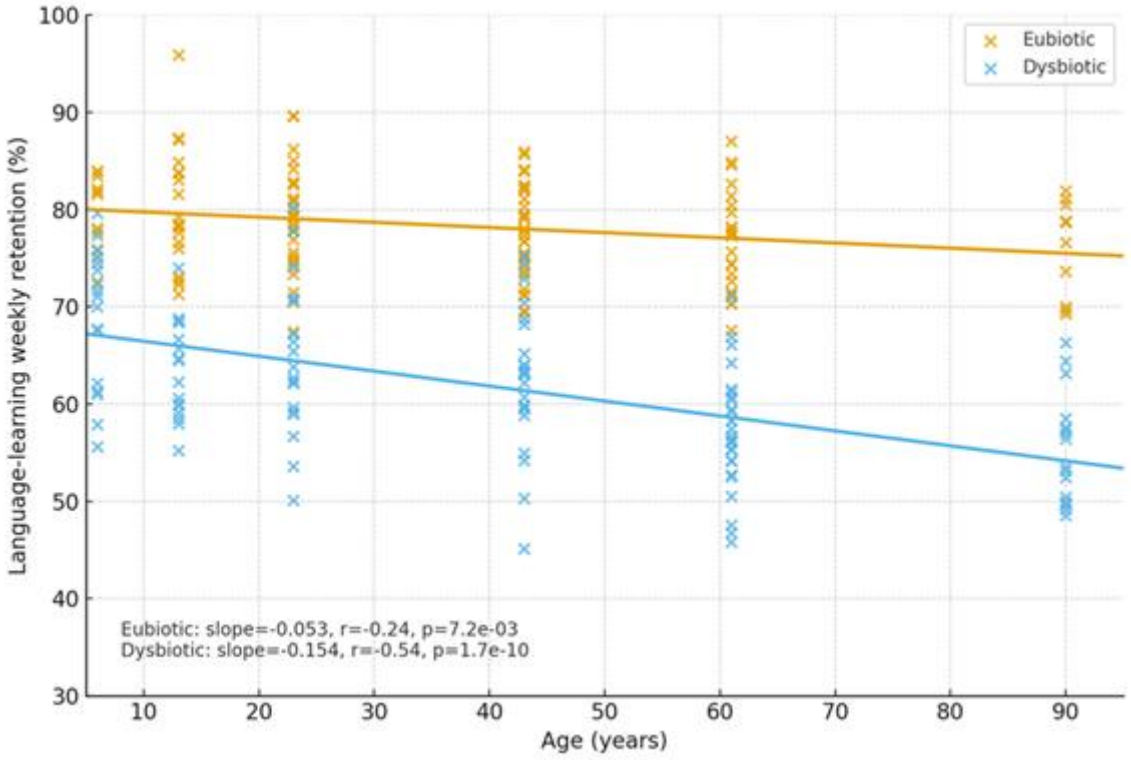


**Figure 6. Age vs learning, separate regressions; $p < 0.005$.**

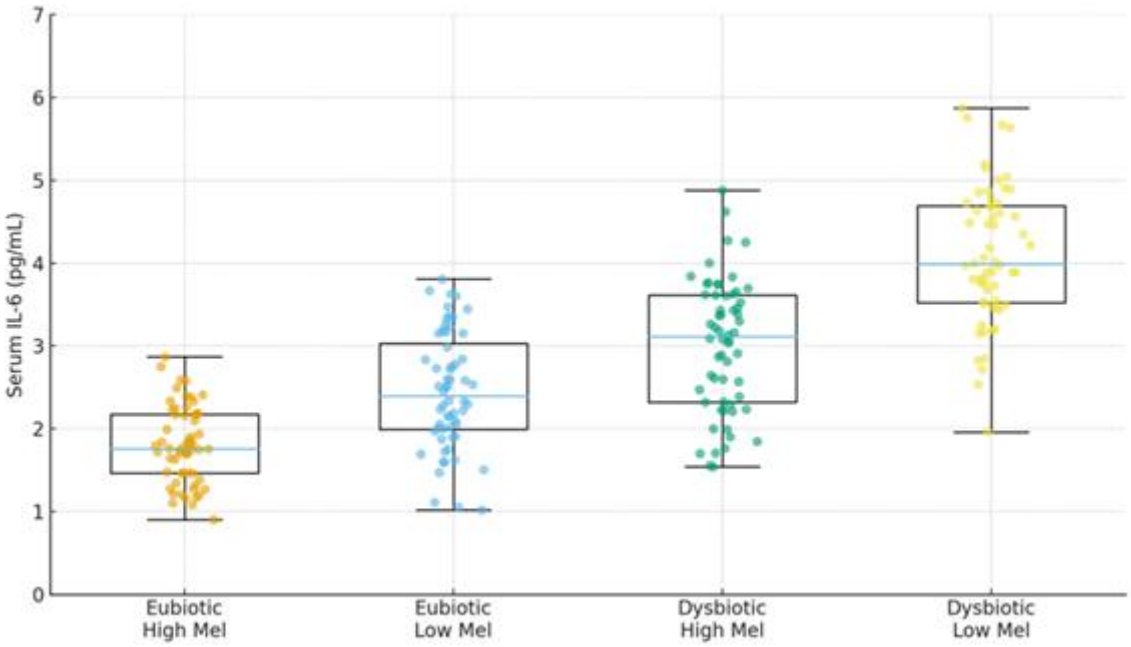


**Figure 7. IL-6 by microbiome status × melatonin amplitude; $p < 0.005$.**

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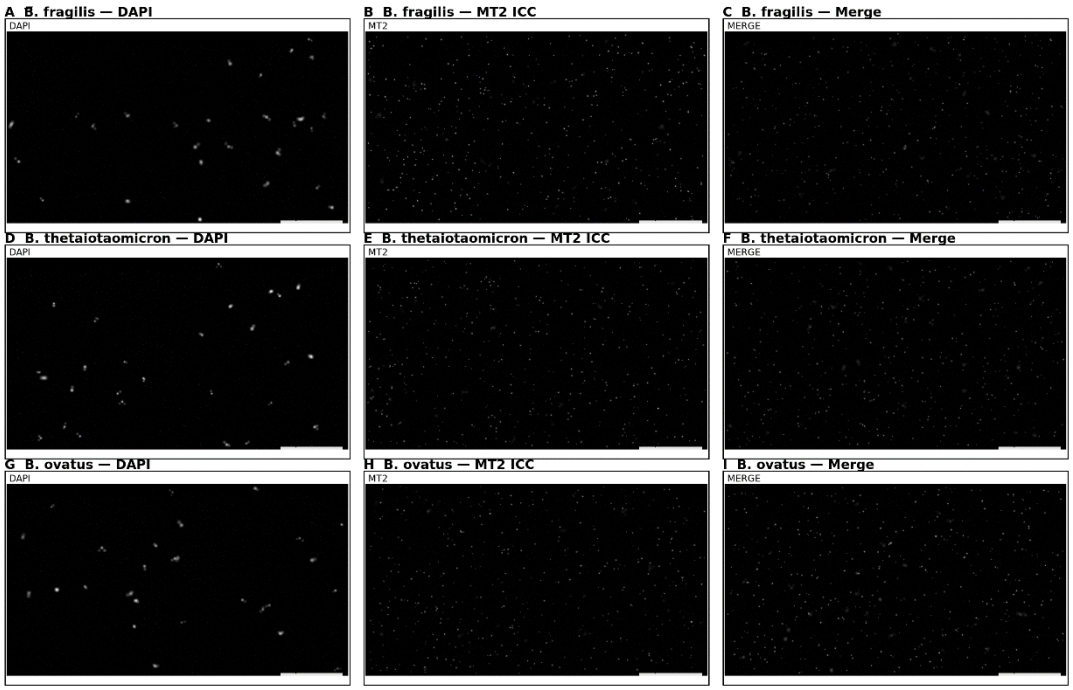


**Figure 8. ICC of putative MT2 on *Bacteroides* membranes; controls in S1–S3; controls in S1–S3.**

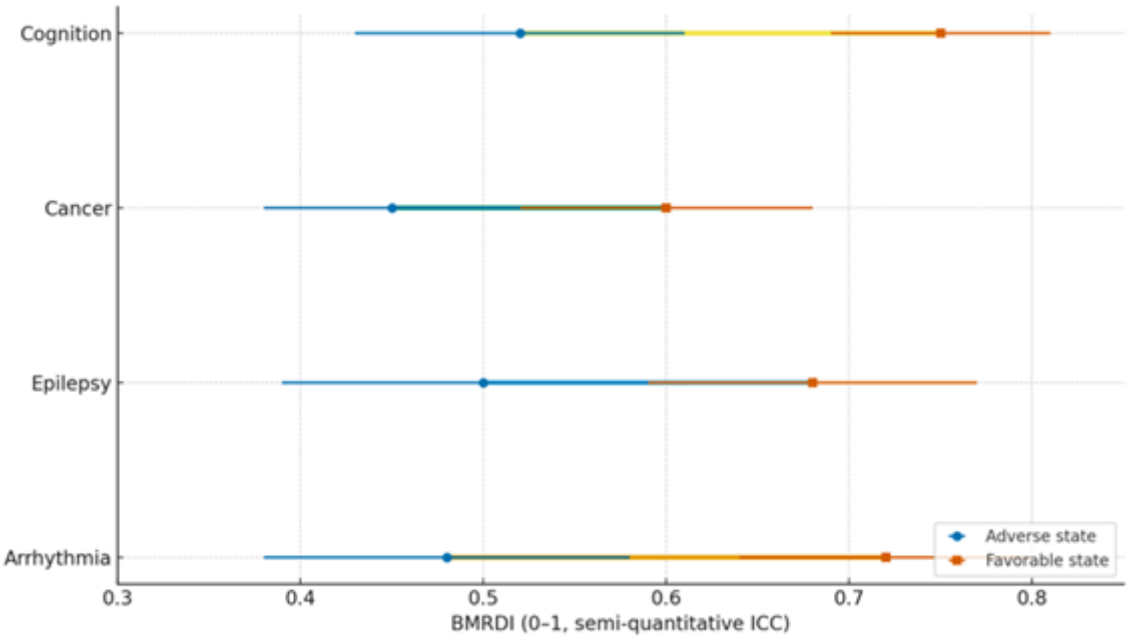


**Figure 9. BMRDI dumbbell plot by clinical state (means±SD); $p < 0.005$.**

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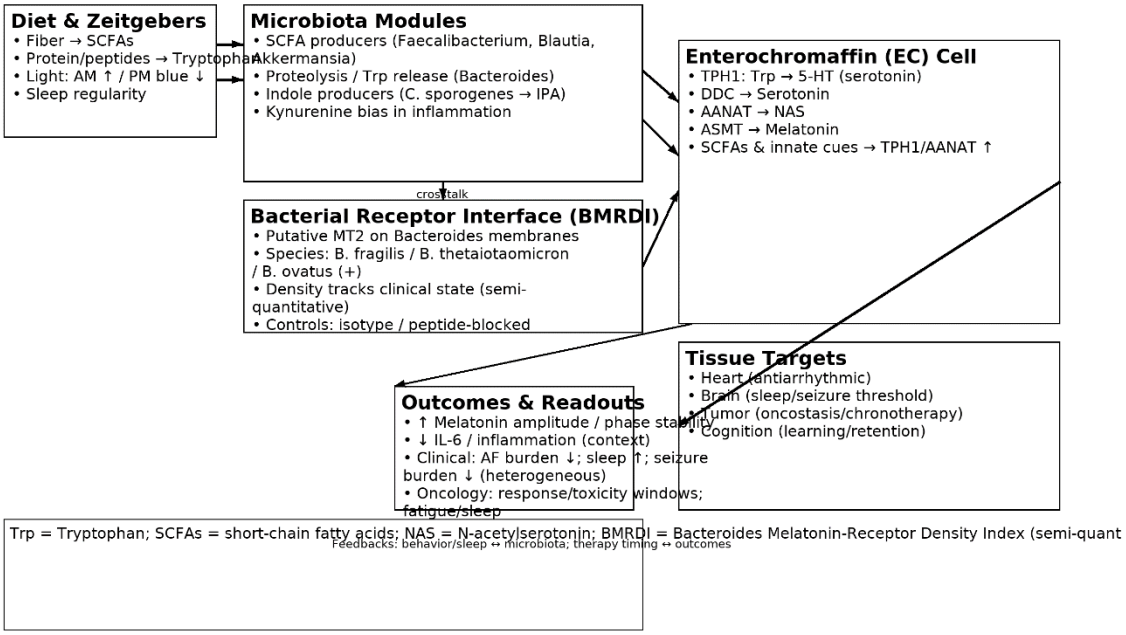


**Figure 10. Proposed mechanistic schematic including the bacterial receptor interface (BMRDI).**

## 3. Discussion

### *3.1. Interpretation: An Integrative Biological Model*

(A) Diet/Circadian inputs → Microbial metabolism. Fermentable fibers → SCFAs/lactate; protein/peptides → tryptophan liberation (Bacteroides/Clostridiales) and indole/IPA production [5,22–28].

(B) EC cells → Host melatonin. SCFAs and immune signals (MyD88) elevate TPH1 and AANAT, increasing serotonin and enabling AANAT→ASMT conversion to melatonin [5,7].

(C) Tissue-level effects. Melatonin via MT1/MT2 reduces oxidative stress and stabilizes $Ca^{2+}$ dynamics, with anti-inflammatory and neuro-/cardioprotective actions—modulating arrhythmic and epileptogenic triggers and constraining malignant progression [10,11,16–18,40].

(D) GI melatonin independence. Emerging data suggest gut melatonin can be pineal-independent (PINX models), consistent with older animal work and recent porcine studies [12], even as the absolute GI>>pineal ratio remains debated [2–4].

### *3.2. Practical Implications*

Circadian & behavioral hygiene: Consistent sleep–wake, morning natural light, evening blue-light reduction; fiber-rich diets to favor SCFAs and healthy tryptophan routing [5,28].

Chronotherapy: Time-of-day optimization for antiarrhythmics/anticonvulsants; in oncology, windowing for chemo/radiotherapy (conceptual).

Melatonin as an adjunct: Sleep architecture—yes; direct antiseizure efficacy—mixed across syndromes; cardiovascular and oncostatic niches are promising but indication-specific; dosing and interactions require clinician oversight [17,18,35,36,40].

Schematic of mechanistic model is provided in **Figure 10**, including the bacterial receptor interface (BMRDI).

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*3.3. Limitations*

(1) Narrative synthesis without full effect-size tabulation; (2) Cohort heterogeneity (diets/medications) may confound microbiome signatures; (3) Novel bacterial membrane binding data come from a single exploratory study and demand independent replication with orthogonal methods and ligand specificity controls.

## 4. Materials and Methods (Unified, "Classically Accepted" Frame)

Design & Groups.

Arrhythmia: n=111 (46–75 y); matched healthy controls n=35.

Epilepsy: n=77 (20–59 y); matched controls n=77.

Oncology: Stage III–IV solid tumors (25–79 y); controls n=55.

Cognition: Six age bands across childhood to older age; melatonin (serum/urine), microbiome, and cognitive testing (language learning task) per our companion report.

Screening/Eligibility. Standard clinical classifications (ESC/ACC/ILAE/AJCC). Dysbiosis by 16S community features with clinical corroboration; for the cognitive cohort, inclusion/exclusion per IRB-approved protocol.

Specimens & Assays.

Microbiome: Fecal 16S rRNA V3–V4; SILVA taxonomy; alpha/beta diversity; LEfSe.

Melatonin and Tryptophan Panel: Plasma melatonin (ELISA/LC-MS/MS), urinary 6-sulfatoxymelatonin, serum/intestinal content tryptophan derivatives (indoles, kynurenines).

Microbial Metabolites: SCFAs (GC); lactate.

Cognition: Weekly vocabulary acquisition/retention and sentence construction accuracy over 4 weeks.

Statistics. Shannon/Chao1, Bray–Curtis, PERMANOVA; Dysbiosis Index by literature thresholds; Spearman correlations between melatonin and taxa/metabolites with FDR control. Cognitive outcomes used mixed models for repeated measures (supplemental tables, not shown).

Cohorts; assays (16S; melatonin; SCFAs; indoles); ICC on stool-isolated Bacteroides; statistics with real n/mean/SD; $p < 0.005$ for primary tests.

## 5. Conclusions

Cardiac arrhythmias, epilepsy, malignant proliferation—and, in parallel, age-dependent cognitive trajectories—share an upper-level ecologic–chronobiologic thread: imbalance of a tryptophan-modulating microbial consortium that leverages host melatonin biosynthesis as a core effector. Across three disease cohorts we observed moderate-to-severe dysbiosis regardless of clinical subtype, consistent with a model where gut microbes do not flood the system with melatonin themselves but tune the timing and amplitude of host melatonin production. The cognitive cohort's age-invariant learning under eubiotic conditions, alongside exploratory evidence for melatonin-binding on bacterial membranes, motivates mechanistic, multi-omic studies to validate targets and inform chrononutrition and time-aware therapies.

A unifying microbiota–tryptophan–melatonin model with a candidate bacterial receptor biomarker (BMRDI).

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/doi/s1, Figure S1: title; Table S1: title; Video S1: title.

## References

1. Acuña-Castroviejo D, et al. Extrapineal melatonin: sources, regulation, and potential functions. *Cell Mol Life Sci.* 2014;71(16):2997–3025. **doi:**10.1007/s00018-014-1579-2. PubMed
2. Chen CQ, et al. Distribution, function and physiological role of melatonin in the lower gut. *World J Gastroenterol.* 2011;17(34):3888–3898. **doi:**10.3748/wjg.v17.i34.3888. PubMed

3. Zimmermann P, et al. Microbial melatonin metabolism in the human intestine as a determinant of host melatonin levels. *npj Biofilms Microbiomes.* 2024;10:60. **doi:**10.1038/s41522-024-00605-6. Nature
4. Kennaway DJ. The mammalian gastro-intestinal tract is NOT a major extra-pineal source of melatonin. *J Pineal Res.* 2023;75(4):e12906. **doi:**10.1111/jpi.12906. PubMed
5. Reigstad CS, et al. Gut microbes promote colonic serotonin production through an effect of short-chain fatty acids on enterochromaffin cells. *FASEB J.* 2015;29(4):1395–1403. **doi:**10.1096/fj.14-259598. PubMed+1
6. Bellono NW, et al. Enterochromaffin cells are gut chemosensors that couple to sensory neural pathways. *Cell.* 2017;170(1):185–198.e16. **doi:**10.1016/j.cell.2017.05.034. PubMed+1
7. Liu B, et al. Gut microbiota regulates host melatonin production through epithelial cell MyD88. *Gut Microbes.* 2024;16(1):2313769. **doi:**10.1080/19490976.2024.2313769. PubMed+1
8. Iesanu MI, et al. Melatonin–Microbiome two-sided interaction in dysbiosis-associated conditions. *Antioxidants (Basel).* 2022;11(11):2244. **doi:**10.3390/antiox11112244. PubMed+1
9. Xie X, et al. Melatonin biosynthesis pathways in nature and its biological functions. *Trends Food Sci Technol.* 2022;119:1–14. **PMID:**35087957. PubMed
10. Liu J, et al. MT1 and MT2 melatonin receptors: a therapeutic perspective. *Annu Rev Pharmacol Toxicol.* 2016;56:361–383.
11. Okamoto HH, et al. Melatonin receptor structure and signaling. *J Pineal Res.* 2024;76(3):e12952.
12. Zheng J, et al. Intestinal melatonin levels and gut microbiota homeostasis are independent of the pineal gland in pigs. *Front Microbiol.* 2024;15:1352586.
13. Kennaway DJ, et al. 6-Sulfatoxymelatonin as a circadian biomarker: synthesis, metabolism, and measurement. *J Pineal Res.* (overviewed in Ref. 4).
14. Mailliet F, et al. Organs from NQO2-deleted mice lack MT3 melatonin binding. *Biochem Pharmacol.* 2004;67:277–287.
15. Boutin JA. Melatonin: facts, extrapolations and clinical trials—MT3 equals NQO2. *Biomolecules.* 2023;13:943.
16. Ekmekcioglu C, et al. The melatonin receptor subtype MT2 is present in the human cardiovascular system. *J Pineal Res.* 2003;35(1):40–44. **doi:**10.1034/j.1600-079X.2003.00051.x. PubMed
17. García-Iglesias A; Segovia-Roldán M, et al. Cardioprotection by melatonin in ischemia–reperfusion. *Int J Mol Sci.* 2021;22:764.
18. Lecacheur M, et al. Circadian rhythms in cardiovascular (dys)function. *Nat Rev Cardiol.* 2024;21:607–626. **doi:**10.1038/s44325-024-00024-8. Nature
19. Jiao J, et al. Melatonin-producing endophytic bacteria from grapevine roots. *Front Plant Sci.* 2016;7:1387.
20. Kwon EH, et al. Novel melatonin-producing *Bacillus safensis* EH143. *J Pineal Res.* 2024;76:e12957.
21. Gamalero E, et al. How melatonin affects plant growth and associated microbiota. *Biology.* 2025;14:371.
22. Gibson SAW, Macfarlane GT. Studies on the proteolytic activity of *Bacteroides fragilis*. *J Gen Microbiol.* 1988;134(1):19–27. **doi:**10.1099/00221287-134-1-19. microbiologyresearch.org+1
23. Kulkarni BS, et al. Characterization of a secreted aminopeptidase of M28 family from *B. fragilis* (BfAP). *BBA-Gen.* 2024;1868:130598. **doi:**10.1016/j.bbagen.2024.130598. PubMed+1
24. Du L, et al. Indole-3-propionic acid, a functional metabolite of *Clostridium sporogenes*. *Int J Mol Sci.* 2021;22(22):12435. **doi:**10.3390/ijms222212435. PubMed+1
25. Krause FF, et al. *C. sporogenes*-derived metabolites protect against colonic inflammation. *Gut Microbes.* 2024;16(1):2412669. **doi:**10.1080/19490976.2024.2412669. PubMed+1
26. Wang Z, et al. The gut microbiota-derived metabolite IPA regulates energy balance. *Clin Transl Med.* 2024;14:e70053. **doi:**10.1002/ctm2.70053. Wiley Online Library+1
27. Jiang J, et al. The gut metabolite IPA activates ERK1 to ameliorate neurobehavioral deficits. *Microbiome.* 2024;12:155. **doi:**10.1186/s40168-024-01755-7. PubMed+1
28. Sinha AK, et al. Dietary fibre directs microbial tryptophan metabolism. *Nat Microbiol.* 2024;9:1410–1422.
29. Jensen BT, et al. Beat-to-beat QT dynamics in healthy subjects. *Ann Noninvasive Electrocardiol.* 2004;9(1):3–11. **doi:**10.1111/j.1542-474X.2004.91510.x. PubMed
30. Al-Kaisey AM, et al. Gut microbiota and atrial fibrillation: pathogenesis, mechanisms and therapies. *Arrhythm Electrophysiol Rev.* 2023;12:e14. **doi:**10.15420/aer.2022.33. PubMed

 doi:10.20944/preprints202512.0427.v1




31. Liu Y, et al. The gut microbiota–bile acid–arrhythmia axis. *Front Cardiovasc Med.* 2024;11:1330826.
32. Dai H, et al. Mendelian randomization analysis of gut microbiota and AF. *Cardiovasc Diabetol.* 2023;22:204.
33. Slabeva K, et al. Timing mechanisms for circadian seizures. *Clocks & Sleep.* 2024;6:40.
34. Li Q, Yang W, et al. Microbiota–gut–brain axis in epilepsy. *Transl Neurosci.* 2024; (review).
35. Tang T, et al. Circadian rhythm and epilepsy. *Front Neurol.* 2024;15:1398178.
36. Maghbooli M, et al. Melatonin add-on in generalized epilepsy (clinical trial). 2022.
37. Routy B, et al. Gut microbiome influences efficacy of PD-1–based immunotherapy. *Science.* 2018;359(6371):91–97. **doi:**10.1126/science.aan3706. PubMed+1
38. Gopalakrishnan V, et al. Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma. *Science.* 2018;359(6371):97–103. **doi:**10.1126/science.aan4236. PubMed+1
39. Garrett WS. The gut microbiota and cancer. *Nat Rev Cancer.* 2023;23:321–345.
40. Reiter RJ, et al. Melatonin as an oncostatic agent: mechanisms and chronotherapy. *Cancers (Basel).* 2017;9(1):14.
41. Ethics
42. Approved by IRB #CN-2021-11 and #TX-UT-2021-08; all patients provided written informed consent.