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Review

The Chrono-Immunological Axis in Viral Encephalitis: Circadian Clocks, Melatonin, and Dynamic Microglial States

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Abstract

Viral encephalitis is a severe neurological condition in which direct viral injury and dysregulated neuroimmune responses jointly determine disease progression and long-term outcomes. Microglia are central to this process, supporting antiviral defense and tissue repair while also contributing to excessive inflammation and neuronal damage. Growing evidence indicates that microglial functions and antiviral immunity are temporally regulated by molecular circadian clocks. Melatonin may connect these systems through its chronobiotic, immunomodulatory, antioxidant, and mitochondrial effects. This comprehensive review examines whether melatonin and circadian clock pathways modulate microglial states and functions during viral encephalitis. We integrate evidence from neurotropic viral infections, experimental encephalitis models, and mechanistic studies of clock–immune interactions, with emphasis on CLOCK–BMAL1, PER–CRY, REV-ERB–ROR, NF-κB, type I interferon, and NLRP3 signaling. Current findings suggest that the effects of melatonin are context-dependent and may vary according to viral species, dose, circadian phase, cellular target, and disease stage. Although preclinical observations support the individual components of a potential chrono-immunological framework, evidence directly integrating these components remains limited. Future studies should incorporate time-of-day-controlled designs, dynamic microglial phenotyping, and stage-specific melatonin administration. Understanding this axis may enable more precise chronotherapeutic strategies that preserve antiviral immunity while limiting neuroinflammatory injury.

Keywords: viral encephalitis; melatonin; circadian clocks; microglia; neuroinflammation; antiviral immunity; molecular clock; chronotherapy

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1. Introduction

Viral encephalitis is a severe inflammatory disorder of the central nervous system (CNS) that may cause seizures, cognitive impairment, persistent neurological disability, or death. Although inflammation is essential for restricting viral replication and dissemination, an excessive or prolonged response can disrupt the blood–brain barrier, impair synaptic function, and intensify neuronal damage [1]. Understanding the mechanisms that regulate the balance between antiviral protection and neuroinflammatory injury is therefore critical for developing safer therapeutic strategies.

Microglia, the resident immune cells of the CNS, are central to this balance. Following viral invasion, they detect pathogen- and damage-associated signals and initiate interferon, cytokine, chemokine, phagocytic, and immune-cell recruitment responses that support viral clearance and tissue repair. Persistent activation, however, may promote oxidative stress, mitochondrial dysfunction, synaptic loss, and secondary neuronal injury [2]. Rather than fitting binary pro- and anti-inflammatory categories, microglia display dynamic functional states shaped by viral species, brain region, cellular environment, and disease stage [2,3].

Circadian clocks may provide an important temporal layer of regulation for these neuroimmune responses. Molecular clock components, including CLOCK, BMAL1, PER, CRY, REV-ERB, and ROR proteins, interact with pathways governing interferon signaling, cytokine production, inflammasome activation, oxidative stress, and cellular metabolism. Microglia possess functional cellular clocks and exhibit time-dependent variations in inflammatory responsiveness, phagocytosis, and metabolic activity [4]. Thus, the timing of viral exposure or immune activation may influence neuroinflammation, while infection-associated sleep disruption may disturb circadian organization.

Melatonin may link circadian regulation to microglial function through MT1/MT2 signaling and its effects on inflammatory pathways, antioxidant defenses, and mitochondrial homeostasis [5]. These properties suggest that melatonin may influence microglial responses during viral encephalitis. Its effects, however, are likely to depend on the infecting virus, dose, treatment timing, cellular target, and disease stage. Suppressing inflammation during early infection could weaken antiviral defense, whereas limiting persistent inflammation during later stages may protect neural tissue.

Research on viral encephalitis, microglial biology, circadian immunity, and melatonin has largely progressed in parallel, with limited integration of these fields. This review integrates evidence on melatonin, molecular circadian clocks, and microglial states in neurotropic viral models, distinguishes direct from indirect evidence, and proposes a chrono-immunological framework with testable research priorities.

2. Literature Search Strategy

This review used a structured narrative approach and targeted literature searches to assess the directness and translational maturity of evidence linking circadian timing, melatonin signaling, and microglial responses in viral encephalitis.

PubMed/MEDLINE, Scopus, and Web of Science were searched through July 2026, supplemented by reference-list screening. Searches combined terms for viral encephalitis and neurotropic viruses with microglia, circadian clocks, melatonin, neuroinflammation, antiviral immunity, chronotherapy, and related molecular pathways.

Peer-reviewed English-language studies and reviews were considered, with priority given to primary experimental and clinical evidence. Evidence was classified as direct viral encephalitis, partially direct neurotropic-virus, or indirect mechanistic evidence and interpreted according to viral species, experimental model, disease stage, circadian timing, melatonin exposure, and outcome. Because of substantial heterogeneity, findings were synthesized narratively rather than through meta-analysis.

Throughout this review, clock or Zeitgeber time denotes externally defined time, whereas circadian time or phase refers to endogenous biological timing; diurnal variation alone is not considered evidence of circadian rhythmicity.

3. Neuroimmune Landscape of Viral Encephalitis

3.1. Viral Entry into the CNS

Neurotropic viruses can reach the CNS through hematogenous or neuronal routes [6]. Hematogenous spread may involve cerebral endothelial infection, blood–brain barrier dis-

ruption, choroid plexus passage, or infected immune cells and predominates in flaviviruses such as Japanese encephalitis and West Nile viruses [6,7]. By contrast, herpes simplex and rabies viruses may enter through peripheral nerves and retrograde axonal transport [7].

After entry, cellular tropism differs among viruses. Neurons are major targets for several encephalitic viruses, whereas astrocytes, microglia, oligodendrocytes, and endothelial cells may also become infected or respond to nearby infection. The affected cell type and brain region influence clinical manifestations, viral persistence, and the pattern of neurological injury. Viral replication within neurons may cause direct cellular dysfunction or death, while infection of glial or vascular cells can amplify inflammation and weaken the blood–brain barrier [6,7].

3.2. Innate Antiviral Responses

Viral nucleic acids and cellular damage activate Toll-like receptors, RIG-I-like receptors, and cGAS–STING signaling, inducing type I interferons, cytokines, and chemokines that restrict viral replication and alert surrounding cells [8].

Type I interferons induce interferon-stimulated genes that restrict multiple stages of viral replication and spread. Chemokines recruit natural killer cells, monocytes, and virus-specific lymphocytes from the circulation. Astrocytes and microglia contribute to these responses by producing inflammatory mediators and coordinating communication among neural, vascular, and immune cells. Endothelial activation also facilitates immune-cell entry into infected tissue [9].

As infection progresses, cytotoxic and helper T cells, together with neutralizing antibodies, support viral control. Their effectiveness depends on viral immune evasion, interferon kinetics, host age and immune status, and prior exposure or vaccination [7,8].

3.3. From Viral Control to Neuroinflammatory Injury

Antiviral inflammation becomes harmful when its intensity or duration exceeds what is required for viral control. Sustained cytokine and chemokine production increases blood–brain barrier permeability and promotes continued immune-cell infiltration. Activated immune and glial cells may release reactive oxygen and nitrogen species, excitotoxic mediators, proteolytic enzymes, and additional inflammatory signals that damage nearby uninfected cells [10].

The same pathways may therefore produce different effects across disease stages. Early interferon and inflammatory responses can restrict viral spread, whereas prolonged activation after viral control may contribute to neuronal dysfunction and tissue injury. Persistent IL-1β, IL-6, tumor necrosis factor, and poorly controlled immune cytotoxicity can disrupt synaptic signaling, mitochondrial function, and neuronal survival [10,11].

Neurological injury may continue even after viral replication has declined [11]. Persistent glial activation, impaired blood–brain barrier integrity, altered synaptic organization, and incomplete resolution of inflammation may contribute to cognitive impairment, seizures, sleep disturbances, and other long-term complications [12]. The outcome of viral encephalitis therefore depends on coordinated viral clearance, immune regulation, and neural repair, processes in which microglia transition among antiviral, inflammatory, phagocytic, and reparative functional states [10,11].

4. Microglial States and Functional Programs

4.1. Transition from Homeostasis to Antiviral Defense

Homeostatic microglia survey the brain parenchyma, remove cellular debris, regulate synaptic organization, and communicate with neural cells, whereas viral invasion rapidly reprograms their morphology, metabolism, transcriptional activity, and secretory profile.

Transcriptomic analyses of neurotropic viral infection have demonstrated a reduction in homeostatic signatures accompanied by enrichment of cytokines, antigen-presentation, and leukocyte-regulatory programs, supporting a transition from surveillance to context-dependent antiviral states [13].

Early antiviral programs include pattern-recognition and type I interferon signaling, interferon-stimulated genes, antigen presentation, and chemokine-mediated immune-cell recruitment. Microglia may also phagocytose viral particles, infected cells, and damaged neural material. Experimental evidence indicates that these responses are shaped by cell-intrinsic viral-sensing pathways, including mitochondrial antiviral-signaling protein signaling, although their magnitude and protective value vary with the infectious context [14].

The response also depends on whether microglia undergo productive or abortive infection or are activated indirectly by signals released from infected neurons, astrocytes, endothelial cells, or infiltrating immune cells. Direct infection may alter microglial viability and antiviral capacity, whereas indirect or bystander activation can generate a strong inflammatory response even when productive viral replication within microglia is absent. Susceptibility to infection and the resulting functional phenotype may also differ according to viral species and the experimental microglial model employed [15].

4.2. Inflammatory and Neurotoxic Programs

During active infection, microglia produce cytokines, chemokines, reactive oxygen and nitrogen species, and other antimicrobial mediators. NF-κB drives inflammatory gene expression, whereas NLRP3 activation promotes caspase-1-dependent IL-1β maturation. This involvement has been demonstrated in selected viral systems, including human microglia exposed to SARS-CoV-2 or its spike protein, but cannot be generalized to all viral encephalitides [11,16].

Persistent production of IL-1β, IL-6, and tumor necrosis factor can disrupt neuronal signaling, promote oxidative and mitochondrial stress, and weaken the blood–brain barrier. Reactive oxygen and nitrogen species may additionally damage cellular proteins, lipids, nucleic acids, and mitochondria. Evidence from Japanese encephalitis virus models supports the involvement of these interconnected inflammatory pathways, while a mouse Zika virus model demonstrated sustained microglial activation associated with synaptic loss and neuronal dysfunction after brain viral RNA had returned to baseline levels [17,18].

Microglial inflammation is therefore not inherently harmful. Its biological effect depends on its intensity, duration, anatomical location, cellular environment, and relationship to the stage of infection. A response that supports viral restriction during early replication may become damaging if it persists after viral control, whereas premature suppression could compromise antiviral defense.

4.3. Resolution and Tissue Repair

Resolution is an active process rather than a simple return to the homeostatic state. As infection is controlled, microglia may acquire or expand immunoregulatory, efferocytic, debris-clearing, and tissue-supportive functions. These include removal of apoptotic cells and damaged myelin, restoration of extracellular homeostasis, regulation of inflammatory signaling, and production of factors that support neuronal survival and repair. The contribution of these activities nevertheless varies according to the viral model, brain region, and participation of infiltrating monocyte-derived macrophages [10].

Successful resolution requires preservation of sufficient antiviral activity to prevent viral persistence or reactivation while limiting secondary tissue injury. In a neurotropic coronavirus model, microglial depletion during viral clearance impaired myelin-debris

removal and remyelination without altering viral-clearance kinetics, indicating that reparative microglial functions can be temporally and mechanistically distinguishable from antiviral control [19].

Neurological recovery may therefore depend on the timing and coordination of antiviral, phagocytic, and reparative microglial programs.

4.4. Microglial States Beyond the M1/M2 Model

The M1/M2 framework classifies M1-like microglia as pro-inflammatory and M2-like microglia as immunoregulatory or reparative. Although convenient, this binary terminology does not capture the diversity, plasticity, or context dependence of microglial states in vivo; current recommendations therefore favor state descriptions grounded in context and measured function [3].

Microglia may simultaneously express antiviral/interferon-responsive, inflammatory, phagocytic, metabolic, reparative, or disease-associated programs that vary with brain region, infection stage, viral species, and local environment. These descriptors do not denote fixed subtypes or uniformly beneficial or harmful functions; for example, phagocytosis may support debris clearance yet also contribute to pathological synaptic loss [20].

Microglial states should therefore be defined using convergent molecular and functional evidence. Single-cell and spatial transcriptomic findings should be integrated with protein, metabolic, phagocytic, viral-control, anatomical, and neural-outcome measures [21].

Accordingly, this review uses functional state descriptors supported by the cited evidence and interprets them according to viral species, infection stage, brain region, circadian phase, cellular environment, and treatment timing.

5. Circadian Regulation of Antiviral Immunity

Antiviral immunity is temporally organized rather than constant across the day. Circadian clocks coordinate immune activity with daily changes in behavior, metabolism, neuroendocrine signaling, and environmental exposure. This organization can influence viral replication and host responses, but evidence from peripheral infections should not automatically be interpreted as evidence for the same mechanisms within the infected CNS.

5.1. Molecular Organization of the Circadian Clock

The suprachiasmatic nucleus (SCN), entrained predominantly by the light–dark cycle, coordinates distributed clocks through neural and humoral outputs. Body temperature, sleep–wake activity, and feeding cycles provide additional timing cues, while peripheral oscillators, including cell-autonomous microglial clocks, regulate local functions [4,22].

At the cellular level, the CLOCK–BMAL1 heterodimer activates the transcription of Period (PER1–3) and Cryptochrome (CRY1/2) genes. As PER and CRY proteins accumulate and enter the nucleus, they inhibit CLOCK–BMAL1-dependent transcription, completing the principal transcriptional–translational feedback loop. In an auxiliary loop, REV-ERB proteins repress and ROR proteins activate BMAL1 expression, contributing to the phase, amplitude, and robustness of molecular rhythmicity [23].

Together with post-transcriptional, post-translational, and metabolic regulation, these interacting loops generate circadian variation in gene expression and contribute to the temporal organization of energy metabolism, redox homeostasis, and inflammatory signaling. Their functional consequences remain cell- and context-dependent; an intact molecular clock should therefore not automatically be equated with an anti-inflammatory state [24].

5.2. Circadian Control of Antiviral Responses

Pathogen recognition, cytokine production, antigen processing, leukocyte trafficking, and T-cell responses show circadian variation, allowing exposure time to influence viral burden and disease severity in experimental models. Time-of-day differences require repeated phase sampling, constant conditions, or clock manipulation to establish endogenous control. In a vesicular stomatitis virus model, β2-adrenergic signaling entrained clock-gene oscillation in CD8+ T cells and shaped their differentiation after infection [25–27].

Clock proteins also interact with inflammatory and antiviral pathways. BMAL1 and REV-ERB family members can modulate cytokine output, type I interferon-linked responses, and noncanonical inflammasome activation. In myeloid-specific BMAL1-deficient mice, BMAL1 loss enhanced noncanonical inflammasome-mediated pyroptosis through a REV-ERBα–C/EBPβ–SAA1 pathway, whereas canonical responses were not similarly increased [28]. Whether this pathway operates in microglia during viral encephalitis remains unknown.

The circadian phase associated with a favorable outcome is not consistent across infections. Circadian effects may operate through viral entry or replication, host resistance, inflammatory tolerance, or adaptive immune differentiation, and these endpoints need not peak together. Their direction and magnitude depend on the virus, infected tissue, exposure route, host condition, and disease stage; evidence from non-neural infections therefore cannot be transferred directly to viral encephalitis [29].

5.3. Circadian Regulation of Microglial Function

Microglia express core clock genes rhythmically and show time-of-day variation in selected functions, although the evidence differs across brain regions and experimental paradigms. In adult mice, isolated microglia displayed rhythmic clock-gene expression, while microglia-specific BMAL1 knockdown abolished a time-dependent CD68/Iba1-based phagocytic pattern under high-fat-diet challenge and increased phagocytic responses during metabolic stress or memory-related neuronal remodeling. These findings support temporal organization of microglial phagocytosis but do not establish a universal cell-autonomous mechanism [30].

Clock components can causally modify microglial inflammatory and metabolic programs, but the direction of the effect is context-dependent. In a spinal cord injury model using global BMAL1-deficient mice and in lipopolysaccharide-stimulated BV2 cells, reduced BMAL1 activity enhanced NF-κB/MMP9 signaling, NLRP3-associated pyroptosis, and inflammatory mediator release. In contrast, microglia-specific REV-ERBα deletion increased inflammatory signaling, disrupted lipid metabolism, promoted lipid-droplet accumulation, and impaired tau phagocytosis, with the principal in vivo effects observed in male mice. These findings argue against treating BMAL1 as universally protective or REV-ERBα as a simple anti-inflammatory switch [31,32].

These findings make it plausible that microglial clocks modify antiviral recognition, inflammation, and repair during encephalitis. However, causal evidence derives mainly from nonviral models; direct tests of microglia-specific clock manipulation on viral burden and neurological outcome are still needed, with attention to rodent phase, sex, brain region, and disease model [33].

5.4. Bidirectional Disruption During Viral Encephalitis

Bidirectional disruption is biologically plausible, although not yet demonstrated within a single viral encephalitis model. In mice, systemic lipopolysaccharide-induced inflammation perturbed clock-gene oscillations across the hypothalamus, hippocampus, and

liver and altered the diurnal dynamics of hippocampal microglia, supporting inflammation-to-clock signaling [34].

Clinical interpretation must separate disease effects from the treatment environment. Sleep–wake and circadian disturbances may persist after tick-borne encephalitis, while pain, noise, artificial lighting, nursing interventions, sedation, and mechanical ventilation can disrupt sleep during intensive care. These factors may amplify disease-related dysregulation but also confound circadian assessment [35,36].

Accordingly, experimental studies should record infection, treatment, and sampling time; light–dark conditions; biological phase; and relevant feeding and sleep schedules. Clinical studies should document habitual sleep timing, light exposure, intensive care procedures, and medications affecting sleep or melatonin. These measures enable direct testing of melatonin as a temporal regulator of microglial antiviral responses.

6. Melatonin as a Chrono-Immunological Mediator

Melatonin combines an endocrine signal of biological night with receptor-mediated and pharmacological effects on immune, redox, and metabolic pathways. These properties make it a plausible link between circadian disruption and altered microglial function during viral encephalitis. However, evidence demonstrating an integrated melatonin–clock–microglia mechanism in this disease remains predominantly preclinical.

6.1. Production and Signaling

In mammals, the pineal gland is the principal source of circulating melatonin under physiological conditions. Melatonin synthesis and release increase during the biological night under the control of the suprachiasmatic nucleus and are acutely suppressed by nocturnal light exposure. The resulting endocrine rhythm conveys information about internal night to the brain and peripheral tissues and contributes to the temporal organization of sleep, metabolism, neuroendocrine activity, and immune function [37].

Melatonin biosynthetic capacity has also been reported in extrapineal immune and neural compartments, where locally generated melatonin may act through autocrine or paracrine mechanisms without substantially contributing to the circulating nighttime rhythm. Its temporal profile is likely tissue-specific. Although mitochondrial synthesis has been demonstrated in selected systems, its in vivo relevance across tissues and in human microglia remains uncertain [38,39].

The best-characterized physiological effects of melatonin are mediated by the high-affinity MT1 and MT2 G protein-coupled receptors. These receptors signal predominantly through Gi/o proteins but can engage additional pathways depending on receptor expression, cellular context, and ligand concentration. Because melatonin can cross biological membranes, receptor-independent redox and mitochondrial effects have also been reported, particularly at pharmacological concentrations. These actions should be distinguished from physiological MT1/MT2-mediated signaling, and antioxidant effects should not automatically be attributed to direct radical scavenging without mechanistic evidence [40,41].

6.2. Modulation of Antiviral Immunity

Melatonin may influence viral infection through host-directed and intracellular antiviral pathways. In Japanese encephalitis virus-infected SH-SY5Y cells, pharmacological melatonin administered after viral entry reduced infectious progeny, apoptosis, and inflammatory signaling but did not directly inactivate extracellular virions; proposed viral-protein interactions remain based on docking. In dengue-infected cells, SIRT1 blockade partly reversed antiviral effects and altered interferon-related gene expression, supporting a

host-directed component [42,43]. These studies demonstrate activity in selected flavivirus models without establishing broad-spectrum direct antiviral action.

Melatonin's immunological effects are context-dependent. In Japanese encephalitis virus-infected neuronal cells, it reduced viral RNA, inflammatory signaling, and neurotoxicity through calcineurin- and autophagy-related mechanisms. Conversely, in an encephalomyocarditis virus model, melatonin suppressed macrophage IFN-β and inducible nitric oxide synthase responses and partly exacerbated brain infection. Treatment timing also altered responses in poly(I)-stimulated intestinal organoids [44–46]. Thus, immune attenuation may protect tissue or compromise antiviral defense depending on the model and disease stage, and later-phase benefit remains a testable hypothesis.

Virus, host, dose, formulation, and treatment schedule may alter melatonin's effects. In recent Zika and dengue virus type 4 mouse neuroinfection studies, pharmacological melatonin reduced brain viral loads and improved survival only in the dengue model, while inflammatory and microglial markers changed in virus-specific directions. These findings support melatonin as a context-dependent experimental adjunct but do not define clinical efficacy, optimal dosing, administration phase, or patient selection [47].

6.3. Effects on Microglial Function

Melatonin has modified several inflammatory programs in preclinical microglial systems, although the observed mechanisms vary among models. In lipopolysaccharide-challenged organotypic hippocampal cultures and HMC3 cells, melatonin opposed NF-κB- and caspase-1-associated responses and enhanced selected neurotrophic features, with evidence for the involvement of MT1 and SIRT1. In corticosterone-exposed microglial cells and chronically stressed rats, melatonin reduced cathepsin B/NLRP3 signaling and microglial pyroptosis. Neither system models viral infection, however, and these findings do not establish that the same pathways govern virus-exposed microglia during encephalitis [48,49].

Mitochondrial protection provides another plausible mechanism, although the microglia-specific evidence is less direct than is sometimes implied. In neuronal cells exposed to oxygen–glucose deprivation and reoxygenation, melatonin preserved mitochondrial structure and respiratory-chain activity, reduced oxidative injury and cytosolic mitochondrial-DNA release, and attenuated cGAS–STING-associated inflammatory signaling. These findings connect mitochondrial integrity with innate immune regulation, but an ischemic neuronal model cannot establish the same mechanism in virus-exposed microglia. Moreover, many experimental antioxidant effects have been obtained using micromolar-to-millimolar melatonin concentrations, and the contribution of direct radical scavenging at clinically achievable exposures remains uncertain [50,51].

Evidence that melatonin promotes complete inflammatory resolution or tissue repair is even less definitive. In non-viral experimental models, melatonin decreased selected pro-inflammatory markers and increased markers commonly associated with anti-inflammatory or trophic activity. Such changes do not demonstrate a binary transition between fixed M1 and M2 phenotypes, nor do they establish improved viral clearance, efferocytosis, debris removal, synaptic preservation, or tissue repair. Melatonin should instead be interpreted as potentially reconfiguring inflammatory, trophic, and metabolic microglial programs. Effects on phagocytosis, inflammatory resolution, and repair must be evaluated directly rather than inferred from a limited marker panel [52,53].

6.4. Restoration of Temporal and Immune Homeostasis

The proposed homeostatic effects of melatonin should be separated into chronobiotic and pharmacological components. Appropriately timed exogenous melatonin can alter

circadian phase, but the direction and magnitude of this effect depend on biological time rather than clock time alone. Administration near habitual bedtime may facilitate sleep without necessarily producing meaningful circadian resetting. Consequently, increased melatonin exposure or improved sleep cannot by itself demonstrate restoration of central or microglial rhythmicity [54].

Melatonin could affect microglia directly through receptor-dependent or metabolic pathways and indirectly through circadian synchronization. Demonstrating the latter requires repeated measures of circadian phase and amplitude together with viral burden, microglial states, inflammatory resolution, and neurological outcomes; reduced cytokine concentrations alone are insufficient.

Endogenous secretion and pharmacological administration are not equivalent. Dose, formulation, route, timing, and bioavailability determine exogenous exposure. Equal 5 mg doses of immediate- and sustained-release melatonin produced different peak concentrations, times to peak, and elimination half-lives, while clinical sleep studies also show dose- and timing-dependent effects. Optimal regimens for viral encephalitis remain unknown [55,56].

7. Evidence Across Viral Encephalitis Models

Evidence linking circadian clocks, melatonin signaling, and microglial function in viral encephalitis remains heterogeneous. Few studies examine all components within the same system; most address microglial responses, clock-dependent antiviral immunity, or melatonin-sensitive inflammatory and metabolic pathways separately.

For the purposes of this review, the evidence is interpreted at three levels. Direct evidence comprises confirmed viral encephalitis models in which circadian phase, clock manipulation, or melatonin administration is evaluated together with microglial, virological, and neurological outcomes. Partial evidence includes encephalitis studies assessing microglial responses alongside either clock-related or melatonin-related endpoints. Indirect evidence derives from systemic infection, non-neural viral models, or sterile neuroinflammation and is used only to support mechanistic plausibility.

Across viral groups, the involvement of interferon signaling, inflammasome activation, oxidative and mitochondrial stress, blood–brain barrier dysfunction, and inflammatory resolution varies with viral species, tropism, disease stage, brain region, and experimental model. The following sections distinguish established observations from proposed connections and consider whether reported benefits reflect viral control, host-directed neuroprotection, or both.

7.1. Arboviral Encephalitis

Neuroinvasive flaviviruses and alphaviruses differ in tropism, replication, immune evasion, neuroinvasion, and clinical severity; findings from one virus therefore cannot define a uniform arboviral mechanism. Nevertheless, these infections commonly involve type I interferon signaling, glial and leukocyte responses, redox and metabolic stress, blood–brain barrier dysfunction, and incomplete inflammatory resolution [57,58].

7.1.1. Flaviviral Models

Japanese encephalitis virus, West Nile virus, and tick-borne encephalitis virus are established causes of human neuroinvasive disease, whereas much Zika virus evidence derives from congenital, neonatal, or immunodeficient models. Japanese encephalitis virus-infected mouse brains show microglial activation and peripheral immune-cell recruitment, while human microglial cultures support strain-dependent tick-borne encephalitis virus infection and inflammatory responses [59,60].

Microglial functions vary with disease stage. In West Nile virus infection, microglia may support early viral containment but later contribute to synaptic elimination and neurological dysfunction. Recovery is also associated with distinct programs, including an LGALS3BP-associated state; microglial activation alone therefore does not identify an antiviral, inflammatory, phagocytic, or reparative function [61,62].

Pharmacological melatonin has reduced viral replication, apoptosis, inflammatory signaling, or neurological injury in selected Japanese encephalitis, Zika, and dengue models. These findings provide partial evidence because endogenous melatonin rhythms, circadian phase, and microglia-specific clock function were not evaluated together [42,44,47].

Translation is limited by differences in viral strain, host, disease model, melatonin dose, route, and treatment schedule. Moreover, reduced inflammation may result from lower viral burden rather than direct immunomodulation; host-directed neuroprotection would require neurological improvement beyond that explained by reduced viral replication.

7.1.2. Alphaviral Models

Encephalitic alphaviruses can cause acute CNS inflammation involving neural, glial, endothelial, and infiltrating immune cells. Early responses may restrict viral dissemination, whereas persistent glial and vascular dysfunction may extend neurological injury beyond the acute phase.

In Venezuelan equine encephalitis virus-infected macaques, viral RNA, activated microglia, and inflammatory mediators persisted after the acute phase, accompanied by electroencephalographic changes and an altered derived circadian index. These findings link infection, neuroinflammation, and temporal disruption but do not demonstrate cell-autonomous clock dysfunction and may be confounded by fever, activity, feeding, and sickness behaviour [63].

A longitudinal study using the attenuated TC-83 strain identified persistent neurobehavioural abnormalities, hippocampal neuronal loss, glial activation, and cell-specific transcriptional changes after acute infection [64]. These findings indicate long-term cellular remodelling but may not reproduce wild-type neuropathogenesis, and persistent glial activation does not necessarily indicate continued infectious-virus production.

Direct evidence for melatonin treatment in alphaviral encephalitis remains sparse. Neurological manifestations associated with chikungunya virus may offer mechanistic information, but systemic inflammation and peripheral disease frequently predominate. Such findings should therefore be classified as indirect evidence rather than treated as equivalent to confirmed primary encephalitis.

7.1.3. Chrono-Immunological Interpretation

Across arboviral models, the strongest evidence concerns the stage-dependent contribution of microglial and recruited myeloid responses to viral control and secondary neurological injury. Melatonin-sensitive antiviral, inflammatory, redox, and mitochondrial pathways provide partial support for the proposed framework, whereas evidence for circadian regulation remains comparatively limited.

The optimal timing of immunomodulation remains unresolved: suppressing early microglial responses could impair antiviral protection, whereas post-acute intervention may limit persistent inflammation. Studies should therefore measure viral, microglial, circadian, and neurological outcomes together and distinguish prophylactic from therapeutic administration.

Overall, arboviral models provide partial support for the proposed framework, but the complete chrono-immunological axis and its causal relationships remain unproven.

7.2. Herpesvirus Encephalitis

Herpesvirus encephalitis includes neurologically distinct infections caused by herpes simplex virus types 1 and 2 and varicella-zoster virus, although most mechanistic evidence relevant to microglial and circadian regulation derives from herpes simplex virus type 1. Findings vary with viral strain, host characteristics, inoculation route, and route of CNS entry; ocular, intranasal, intracerebral, and peripheral infection models should therefore not be considered biologically equivalent [65–67].

7.2.1. Microglial Responses and Disease Stage

Microglia recognize herpes simplex virus type 1 through DNA-sensing and pattern-recognition pathways that promote type I interferon and antiviral responses. Their depletion before or during early CNS invasion increases viral burden and disease severity while reducing interferon and chemokine responses, demonstrating that early microglial activation contributes to antiviral protection [68].

Temporally resolved single-cell analysis has identified early innate immune programs followed by changes in microglial states and peripheral immune-cell recruitment. Single-time-point measurements may therefore obscure transitions among antiviral, inflammatory, phagocytic, and recovery-associated programs [69].

Infection through olfactory and trigeminal pathways produces regionally distinct viral and microglial responses. Whole-brain measurements and markers shared by microglia and infiltrating macrophages may therefore obscure regional and cell-specific contributions [70].

Neurological deficits may persist despite antiviral suppression of active replication because of neuronal injury, persistent glial activation, synaptic disruption, autoimmune complications, or incomplete inflammatory resolution. Microglial activation is therefore a plausible contributor to long-term sequelae but not a sufficient explanation for post-encephalitic disability.

7.2.2. Circadian Clock–Herpesvirus Interactions

Findings outside the CNS indicate that herpesvirus replication and antiviral responses may vary with circadian state. Clock disruption can alter viral replication, interferon-related defense, metabolism, and inflammatory signaling, although the direction and magnitude of these effects depend on the virus, cell type, and experimental design [71].

In neuronal cells, BMAL1 and CLOCK were reported to regulate herpes simplex virus type 1 entry through NECTIN-1. This finding links clock machinery to neural susceptibility but does not establish that microglial clocks regulate encephalitis severity or recovery in vivo [72].

Because CLOCK also functions as a transcriptional regulator and histone acetyltransferase, its involvement in viral transcription does not alone demonstrate circadian regulation. Most herpes simplex encephalitis studies lack repeated phase sampling or microglial clock manipulation, while fever, reduced activity, altered feeding, sleep disruption, and light exposure may confound behavioural rhythms.

7.2.3. Melatonin and Translational Interpretation

Pharmacological melatonin has reduced herpes simplex virus type 1 replication in vitro, but the use of non-neural systems and relatively high concentrations limits its relevance to human encephalitis. These findings do not establish CNS exposure, microglial modulation, circadian restoration, or efficacy as an adjunct to antiviral therapy [73].

Melatonin could influence herpes simplex encephalitis through antioxidant, mitochondrial, immunomodulatory, and chronobiotic mechanisms, particularly during inflammatory

amplification or recovery. Improved sleep–wake organization might provide supportive benefit but would not demonstrate direct regulation of microglial immunity.

Treatment timing remains critical because suppressing early interferon or microglial responses could impair viral control, whereas intervention after replication is controlled may limit secondary inflammation. Melatonin should therefore be considered only as a potential adjunct, not an alternative, to established antiviral therapy.

Overall, herpes simplex encephalitis provides strong evidence for the stage-dependent role of microglia and partial evidence for interactions between herpesviruses and molecular clock pathways. It does not yet demonstrate that circadian timing and melatonin jointly regulate microglial responses, viral clearance, and neurological recovery.

7.3. Other Neurotropic Viral Infections

Enteroviruses, rabies virus, and coronaviruses differ in neuroinvasion, tropism, immune evasion, and mechanisms of neurological injury and should not be treated as a uniform category. Their neuroimmune involvement is well supported, but direct evidence connecting microglial responses with circadian phase or melatonin remains limited.

7.3.1. Enteroviral Encephalitis

Enterovirus A71 is a well-characterized cause of CNS disease involving the brainstem and spinal cord. Its pathogenesis includes neuronal infection, perivascular inflammation, neuronophagia, microglial nodules, and immune responses that may support viral control but contribute to injury when excessive [74].

In a human blood–brain barrier model, Enterovirus A71 crossed in association with infected immune cells, supporting a potential Trojan-horse route that may expose microglia to infected cells and infiltrating leukocytes [75].

Astrocytic activation of the complement C5a–C5aR1 axis can increase CXCL1 signaling and aggravate Enterovirus A71 neuropathogenesis. Changes attributed broadly to glial activation should therefore not be assigned to microglia without cell-resolved evidence [76].

Findings from Enterovirus A71 cannot be generalized to enteroviruses with different tropism, anatomical distribution, or clinical presentation. In particular, acute flaccid myelitis and predominantly spinal motor-neuron disease are not biologically equivalent to encephalitis.

Enteroviral models support the neuroimmune component of the proposed axis, but direct studies integrating circadian timing or melatonin with viral burden, microglial function, and neurological outcomes remain lacking.

7.3.2. Rabies Virus Encephalitis

Rabies virus reaches the CNS primarily through retrograde peripheral nerve transport and preferentially infects neurons. Severe disease may occur without proportional neuronal destruction, indicating contributions from altered signaling, immune evasion, autonomic disturbance, and network dysfunction [77].

In BV-2 microglial cells, rabies virus activated TLR7–MyD88–NF-κB-associated inflammatory pathways. This finding demonstrates microglial responsiveness but does not establish whether the response is protective or pathological in naturally acquired encephalitis [78].

Wild-type viruses, attenuated or vaccine strains, and modified vectors produce different patterns of neuroinvasion, immune activation, and disease severity, and even individual protein substitutions may alter neurovirulence. Findings from attenuated strains or tracing vectors therefore cannot directly represent natural rabies encephalitis [79].

A 2026 study using synchronized cells, cerebral organoids, and mice demonstrated time-dependent susceptibility to neurotropic viruses and rabies virus-induced disruption

of host clock components. This directly links infection timing and clock integrity to rabies-related outcomes but does not address microglia-specific clocks, endogenous melatonin, or the complete chrono-immunological axis [80].

Evidence for melatonin in rabies encephalitis remains insufficient; any proposed benefit represents a mechanistic hypothesis rather than a demonstrated virus-specific treatment effect.

7.3.3. Coronavirus-Associated Neurological Disease

Direct coronavirus encephalitis, para- or post-infectious immune-mediated encephalitis, and encephalopathy caused by systemic inflammation, hypoxemia, vascular injury, metabolic disturbance, or critical illness are biologically distinct. Neurological symptoms or inflammatory markers alone do not establish direct CNS infection [81].

Mouse-adapted SARS-CoV-2 may produce neuroinflammatory and behavioural changes without productive brain infection. Such models inform systemic-to-CNS signaling but should not be classified as direct viral encephalitis models [82].

Neurotropic murine coronaviruses provide direct CNS infection models involving acute encephalitis, persistence, demyelination, or long-term neurological abnormalities. Microglia may support acute antiviral recruitment and disease restriction but contribute to later inflammatory injury. However, these viruses differ from human SARS-CoV-2 in receptor usage, tropism, and neuropathology [83].

Melatonin studies in systemic coronavirus infection provide indirect support through reported effects on inflammation, oxidative stress, mitochondrial function, endothelial stability, and sleep. They do not establish effects on microglial clocks, CNS viral clearance, or encephalitic injury [84].

During critical illness, loss of environmental time cues, sedation, mechanical ventilation, irregular nutrition, and sleep fragmentation independently disrupt circadian organization. Associations among severe coronavirus disease, altered melatonin rhythms, and neuroinflammation therefore cannot be attributed solely to direct brain infection.

Melatonin-based combinations have been proposed for systemic COVID-19, but these hypothesis-driven studies did not evaluate confirmed encephalitis, CNS viral clearance, microglial clock function, or neurological efficacy [85–87].

7.4. Major Gaps and Boundaries of the Available Evidence

Evidence across viral encephalitis models supports a hierarchy of relationships rather than a fully demonstrated causal pathway. The strongest evidence concerns microglial participation in viral sensing, immune-cell recruitment, debris clearance, and tissue repair. However, excessive or unresolved responses may also promote inflammation, oxidative stress, synaptic dysfunction, blood–brain barrier injury, and neuronal damage.

Evidence that circadian clocks and melatonin regulate pathways relevant to antiviral immunity and neuroinflammation is less complete. Although time-dependent susceptibility has been demonstrated in selected neurotropic viral models, no study has integrated biological phase, endogenous melatonin, microglia-specific clock activity, viral replication, and neurological outcomes within the same encephalitis model.

Time-of-day differences or isolated clock-gene measurements do not establish endogenous rhythmicity; repeated phase sampling, environmental controls, and, where feasible, clock manipulation are required [88,89].

Melatonin studies differ substantially in dose, formulation, route, and administration time, and physiological nighttime signaling is not equivalent to pharmacological exposure [54–56]. Moreover, studies that measure inflammatory or oxidative markers without infectious viral burden cannot distinguish direct antiviral activity from host-directed

neuroprotection. Viral RNA alone is also insufficient evidence of continued infectious-virus production.

Cellular attribution is limited because whole-brain measurements and conventional markers may not distinguish microglia from infiltrating macrophages, while small M1/M2 marker panels oversimplify dynamic functional states [90]. Comparisons are also affected by viral strain, inoculation route, brain region, disease stage, host characteristics, and the definition of encephalitis. Translation from nocturnal rodents requires particular caution because rodent strains differ in melatonin production and experimental clock time does not directly correspond to human biological phase [91].

Clinical evidence is the weakest component of the proposed axis. Viral heterogeneity, uncertain infection onset, fever, seizures, sedation, artificial lighting, mechanical ventilation, altered nutrition, and sleep disruption complicate circadian assessment. Current data cannot establish whether circadian status predicts progression or whether melatonin improves viral clearance or neurological outcomes; no evidence-based dose, timing, therapeutic window, or patient-selection strategy can yet be defined.

The chrono-immunological axis should therefore be regarded as a hypothesis-generating framework rather than an established therapeutic pathway. It proposes that the effects of melatonin signaling and microglial clocks depend on biological phase, viral species, brain region, host condition, and disease stage. Section 8 integrates these partially connected evidence streams into a testable mechanistic model.

Table 1 summarizes this evidence imbalance: microglial involvement is well supported, whereas integrated evidence connecting infection timing, cellular clocks, and melatonin remains limited.

Table 1. Comparative evidence relevant to the proposed chrono-immunological axis across viral encephalitis models.

Viral Group and Examples	Main Neuroimmune and Microglial Features	Relevance to the Circadian–Melatonin Axis	Main Evidence Gap	Reference(s)
Herpesviruses: HSV-1 (principal mechanistic evidence), HSV-2, and VZV	In HSV-1 models, early microglial cGAS–type I interferon and chemokine responses restrict CNS spread; subsequent responses are spatially and temporally heterogeneous and involve recruited monocytes and lymphocytes. HSV-2 and VZV have distinct disease phenotypes.	Rhythmic BMAL1/CLOCK regulation of neuronal NECTIN-1 has been linked to HSV-1 entry, but not to microglial clock function or encephalitis outcomes. Melatonin evidence is confined to a pharmacological, non-neural HSV-1 cell-culture study.	No study jointly evaluates circadian phase, microglia-specific clock function, and timed melatonin administration in established herpes simplex encephalitis while measuring infectious viral burden and neurological outcomes.	[65]
Flaviviruses: JEV, WNV, ZIKV, and TBEV	Virus- and stage-dependent interferon and cytokine responses involve microglia, astrocytes, endothelial cells, and recruited myeloid populations. Inflammasome activity, blood–brain barrier dysfunction, oxidative injury, and neuronal or synaptic damage may occur.	Pharmacological melatonin has reduced viral, inflammatory, or neurological endpoints in selected JEV and ZIKV models. However, endogenous circadian phase and microglia-specific clock function were not assessed.	No integrated study simultaneously measures circadian phase, cellular clock function, melatonin signaling, infectious viral burden, cell-resolved microglial responses, and neurological recovery. Many ZIKV findings derive from neonatal or immunodeficient models.	[42]
Alphaviruses: VEEV, EEEV, WEEV, and CHIKV-associated neurological disease	Acute neuronal infection can be accompanied by microglial and astrocytic activation, leukocyte recruitment, vascular dysfunction, and oxidative injury. VEEV models indicate that viral RNA, gliosis, and neurological abnormalities may persist after the acute phase.	Direct circadian evidence has not been established in alphaviral encephalitis, and virus-specific evidence for melatonin remains indirect and limited.	Direct studies of timed melatonin administration and microglia-specific clock pathways are lacking. Findings obtained with attenuated strains should not be generalized to wild-type alphaviral encephalitis.	[58]

Table 1. *Cont.*

Viral Group and Examples	Main Neuroimmune and Microglial Features	Relevance to the Circadian–Melatonin Axis	Main Evidence Gap	Reference(s)
Enteroviruses: EV-A71 and other neurotropic enteroviruses	EV-A71-associated CNS disease involves neuronal injury and multicellular neuroinflammation. Infected leukocyte passage across an experimental blood–brain barrier and astrocytic C5a–C5aR1/CXCL1 signaling indicate that inflammatory changes should not be attributed exclusively to microglia.	Innate immune, inflammatory, and metabolic pathways provide mechanistic plausibility, but a circadian–melatonin mechanism has not been demonstrated in enteroviral encephalitis.	No study integrates circadian phase or clock manipulation, melatonin treatment, cell-resolved microglial outcomes, infectious viral burden, and neurological recovery. Findings from EV-A71 should not be generalized to all enteroviruses, and acute flaccid myelitis should not be equated with encephalitis.	[74]
Rabies virus	Predominantly neuronal retrograde spread and network dysfunction occur alongside strain-, region-, and stage-dependent neuroimmune responses. Rabies virus can activate TLR7–MyD88–NF-κB-associated inflammatory signaling in BV-2 microglial cells.	Direct circadian evidence indicates that biological time and host clock integrity can influence susceptibility to rabies virus, while infection can disrupt molecular clock components. The role of melatonin remains hypothetical.	Existing circadian experiments did not examine microglia-specific clocks, endogenous melatonin, melatonin treatment, or naturally acquired human rabies. Results obtained with fixed, attenuated, vaccine, or tracing strains should not be equated with wild-type clinical rabies.	[77]
Coronavirus-associated neurological disease	Systemic cytokine signaling, endothelial and blood–brain barrier dysfunction, hypoxia, and microglial reactivity may occur without demonstrable brain viral replication. Neurotropic murine coronaviruses provide more direct CNS infection models with stage-dependent microglial functions.	Melatonin and circadian disruption have been studied mainly in systemic coronavirus infection, whereas neurotropic murine coronavirus models primarily support the microglial component of the proposed axis.	Direct viral encephalitis must be distinguished from immune-mediated encephalitis and systemic or critical-illness encephalopathy. SARS-CoV-2 findings cannot be conflated with neurotropic murine coronavirus models, and no integrated CNS circadian–melatonin–microglia study exists.	[81]

8. The Integrated Chrono-Immunological Axis

The proposed chrono-immunological axis integrates the stage-sensitive relationships among circadian timing, melatonin signaling, and microglial responses. These components have largely been studied separately, but the framework proposes that microglial responses may depend on both the viral stimulus and the biological phase of infection, immune activation, or treatment [71,84].

The proposed framework is illustrated in Figure 1.

8.1. Circadian Setting of the Microglial Response

Cell-autonomous clocks may generate time-dependent differences in microglial surveillance, metabolism, inflammation, and phagocytosis [92]. Microglia-specific BMAL1 loss can alter diurnal morphology, inflammatory features, tissue homeostasis, and behaviour, although this evidence derives from the retinal niche rather than viral encephalitis [93].

Circadian phase may influence susceptibility before microglial activation. Neurotropic-virus experiments have demonstrated rhythmic viral-entry receptor expression and phase-dependent susceptibility in cellular, organoid, and animal models, without establishing whether neurological outcomes are mediated by microglial clocks [80].

The model does not predict a universally protective phase; outcomes may reflect interactions among exposure timing, viral kinetics, tissue-specific clocks, and phase-dependent antiviral immunity and may differ by virus, brain region, and disease stage.

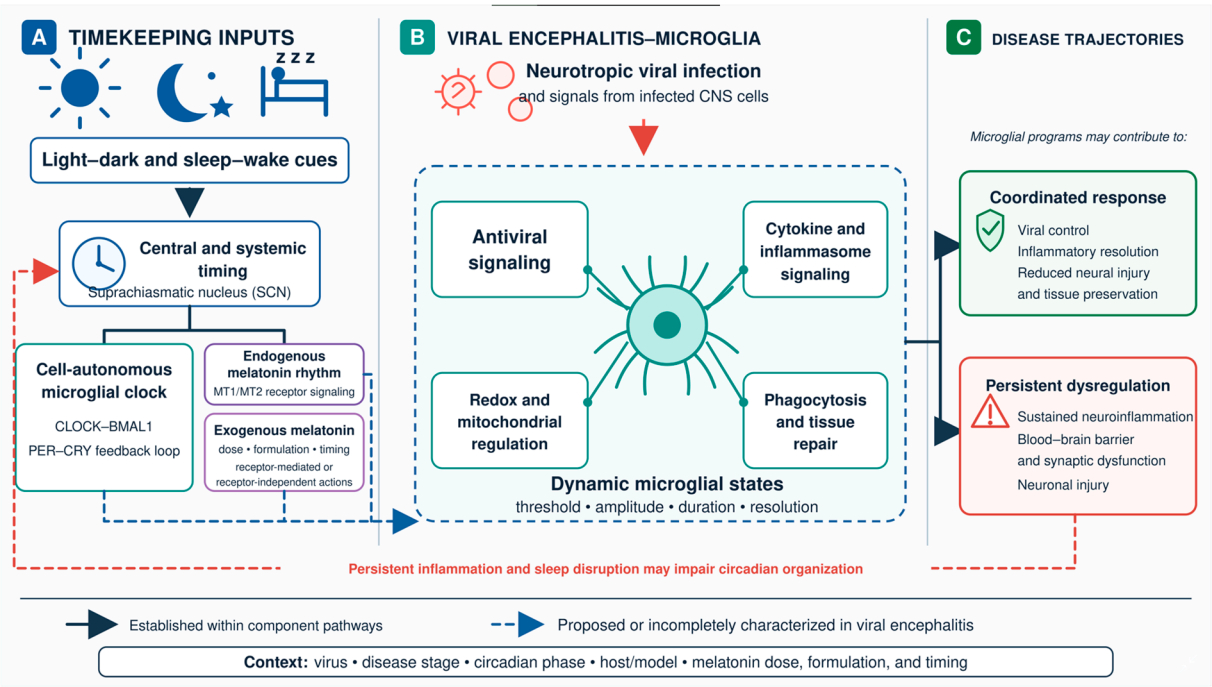


Figure 1. Proposed chrono-immunological framework in viral encephalitis. (**A**) Environmental timing cues, central and systemic timing, cell-autonomous microglial clocks, and endogenous and exogenous melatonin. (**B**) Dynamic microglial programs involving antiviral signaling, cytokine and inflammasome signaling, redox and mitochondrial regulation, phagocytosis, and tissue repair. (**C**) Potential disease trajectories, ranging from coordinated viral control and recovery to persistent neuroinflammation and neural injury. Solid arrows indicate relationships supported within individual pathways, whereas dashed arrows indicate proposed or incompletely characterized links. Effects may vary with viral species, disease stage, biological phase, host model, and melatonin exposure.

8.2. Stage-Dependent Microglial Regulation

During early neuroinvasion, microglial pattern-recognition, interferon, and chemokine responses may restrict viral spread and recruit additional immune cells. Microglial depletion in West Nile virus and herpes simplex virus type 1 models increases viral burden or disease severity, supporting an early protective role [61,68].

Persistent viral or damage signals may sustain inflammatory microglial states through NF-κB and inflammasome activity, mitochondrial stress, reactive oxygen species, and interactions with infiltrating cells [16,17,20]. Because resident and recruited immune populations change during infection, single-time-point measurements may be misleading [69,83].

During resolution, microglia support debris clearance, synaptic remodeling, metabolic recovery, and tissue repair [19,20]. Persistent reactivity may represent residual antiviral surveillance or incomplete repair rather than neurotoxicity alone; longitudinal functional measurements are therefore required to distinguish these states [62,64].

8.3. The Dual Role of Melatonin

Endogenous melatonin acts primarily as a nocturnal timing signal, whereas MT1/MT2 signaling produces context-dependent cellular effects. Chronobiotic activity may modify circadian phase but does not independently establish microglial regulation or improved encephalitis outcomes [54,94].

At pharmacological exposure, melatonin may also influence inflammation, oxidative balance, and mitochondrial function. Its effects on NF-κB-associated responses in brain slices and human microglial cells support mechanistic plausibility but, because nonviral stimulation was used, do not demonstrate protection during CNS infection [39,48].

Melatonin reduced selected viral, inflammatory, or neurological outcomes in some flaviviral models but suppressed macrophage antiviral responses and partly aggravated infection in an encephalomyocarditis virus model. Its effects are therefore not uniformly protective and may depend on virus, dose, and disease stage [45,47].

Stage-dependent benefit remains a testable hypothesis. Early and delayed administration should be compared using infectious viral burden, interferon signaling, microglial functional states, and neurological outcomes.

Exogenous melatonin is not equivalent to endogenous nighttime secretion. Studies should report dose, formulation, route, bioavailability, and timing relative to biological phase, and should not treat immediate- and prolonged-release formulations as interchangeable [55,56].

8.4. A Potentially Self-Reinforcing Pathological Cycle

Inflammation and sleep or circadian disruption may form a self-reinforcing interaction, although the complete cycle has not been demonstrated in one encephalitis model. Viral effects on clock machinery and inflammation-induced disruption of clock-gene and microglial rhythms support plausibility but not causality in human viral encephalitis [34,80].

Melatonin might modify this interaction through chronobiotic, receptor-mediated, antioxidant, or mitochondrial actions. However, reduced inflammatory or oxidative markers alone indicate anti-inflammatory or cytoprotective activity, not restoration of the integrated axis.

8.5. Testable Predictions

The model yields six falsifiable predictions (Figure 2): phase-dependent infection outcomes; effects of circadian disruption; consequences of microglia-specific clock perturbation; phase- and stage-dependent melatonin effects; dependence on microglial clocks or MT1/MT2 signaling; and the relationship between rhythmic recovery, inflammatory resolution, and neurological recovery.

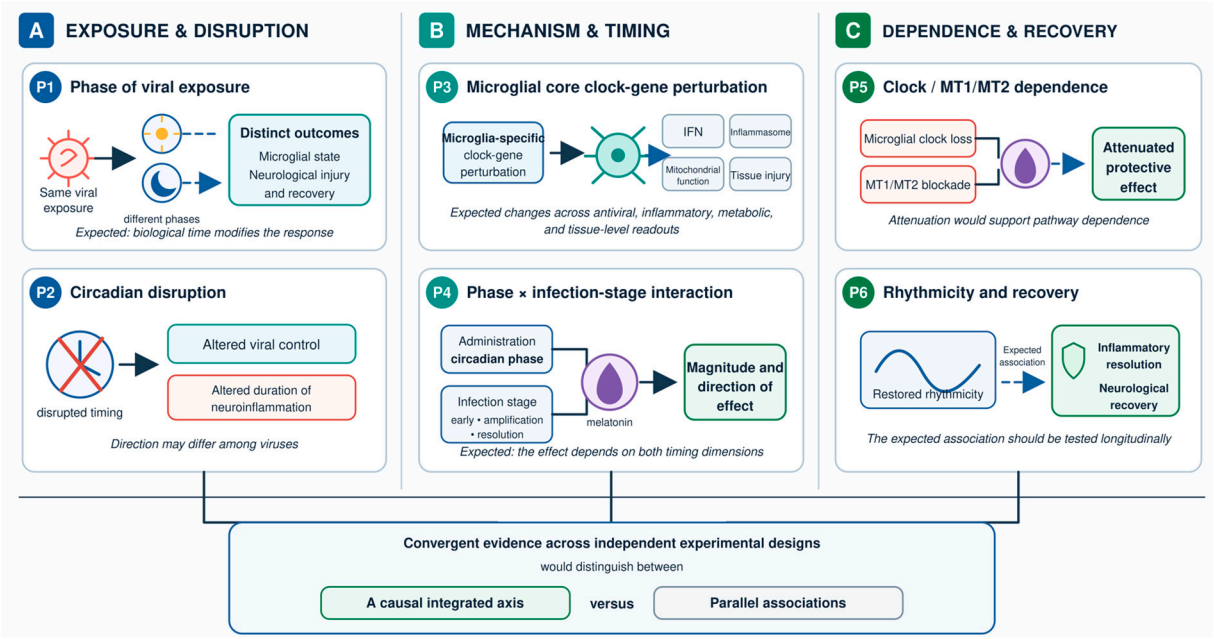


Figure 2. Experimentally testable predictions derived from the proposed chrono-immunological axis. (**A**) Effects of the circadian phase of viral exposure (P1) and circadian disruption (P2). (**B**) Effects of microglial clock-gene perturbation (P3) and interactions between the circadian phase of melatonin administration and infectionstage (P4). (**C**) Dependence of melatonin-associated protective effects

on microglial clocks or MT1/MT2 signaling (P5), and associations between restored rhythmicity, inflammatory resolution, and neurological recovery (P6). Convergent evidence across these complementary designs would help distinguish a causal integrated axis from parallel associations. Arrows indicate experimental manipulations or hypothesized relationships rather than an established integrated mechanism.

Testing these predictions requires phase-controlled, longitudinal experiments with repeated sampling and simultaneous measurement of infectious viral burden, microglial functional states, and neurological outcomes [88,89].

9. Translational Challenges and Future Directions

The proposed axis may inform treatment timing, mechanistic monitoring, and patient stratification but is not a validated therapeutic, diagnostic, or prognostic framework. Circadian abnormalities may contribute to disease, reflect illness severity, or result from intensive-care exposures. The following implications should therefore be regarded as research priorities rather than recommendations for clinical practice.

9.1. Melatonin as an Investigational Adjunctive Treatment

Management of suspected viral encephalitis must remain centered on timely etiological investigation, pathogen-directed therapy, and neurological and critical-care support. Empirical intravenous acyclovir should be initiated promptly while herpes simplex and varicella-zoster viruses are being evaluated. Melatonin cannot replace established treatment and should currently be considered only an investigational adjunct [95].

The rationale for melatonin derives primarily from mechanistic and preclinical evidence. Selected flaviviral models report effects on oxidative injury, inflammation, viral or tissue-injury endpoints, and neurological outcomes, but differences in virus, host, dose, route, and timing limit translation. These findings do not establish efficacy in human encephalitis or necessarily indicate direct antiviral activity [42,47].

Stage-dependent benefit remains hypothetical. Excessive immunomodulation during early infection could impair viral control, as suggested by an encephalomyocarditis virus study in which melatonin suppressed antiviral responses. Although later treatment may have different effects, its proposed benefit has not been clinically demonstrated [45].

Future studies should measure infectious viral burden together with inflammatory, neurological, and circadian outcomes and report dose, formulation, timing, disease stage, concomitant therapy, and immune status. Until controlled clinical evidence is available, melatonin should not be described as an effective treatment for viral encephalitis.

9.2. Chronotherapeutic Timing

Chronotherapeutic timing should be defined relative to biological phase rather than civil time. Because the same clock hour may represent different circadian phases, fixed schedules should be described as time-of-day dosing unless alignment with biological phase is established [54,55].

In physiologically stable individuals, dim-light melatonin onset based on serial sampling is a validated phase marker. Sleep diaries, actigraphy, and light-exposure records are indirect proxies, while a single melatonin measurement cannot define phase or amplitude. Baseline assessment should preferably precede exogenous administration [96].

Phase assessment is more difficult during acute encephalitis because fever, inflammation, seizures, medications, ventilation, irregular feeding, immobility, nocturnal procedures, and abnormal light exposure may disrupt physiological rhythms. Temperature, cortisol, activity, or sleep estimates should therefore not be interpreted individually as reliable phase markers [97].

Immediate- and sustained-release formulations produce different peak concentrations and exposure durations. Identical doses given at the same time are therefore not pharmacologically equivalent, particularly when circadian phase shifting is intended [56].

Studies should distinguish fixed clock-time, estimated phase-aligned, and biomarker-confirmed phase-aligned dosing and document relevant sleep, light, activity, feeding, sedation, and ventilation schedules. Circadian phase should be analyzed separately from disease stage and antiviral-treatment timing.

9.3. Biomarkers and Clinical Monitoring

No biomarker has been validated to quantify the proposed axis, diagnose circadian dysregulation in viral encephalitis, or identify candidates for melatonin. Research should therefore combine longitudinal multimodal monitoring with established etiological, neurological, electrophysiological, and imaging assessments.

Circadian phase, amplitude, and rhythmicity require serial assessment using measures such as melatonin or urinary 6-sulfatoxymelatonin, cortisol, body temperature, rest–activity, sleep, and light exposure. Sampling time, sleep, feeding, fever, medications, organ function, and exogenous melatonin should also be documented [96,97].

Pathogen-specific monitoring should distinguish viral nucleic acid from ongoing infectious replication where feasible [8]. Viral burden should be interpreted with immune and neural-injury markers. Neurofilament light chain may support severity or longitudinal assessment but is not specific to viral etiology, microglial activity, or circadian dysfunction [98].

Blood and cerebrospinal fluid provide complementary but non-equivalent information. Peripheral measures are influenced by systemic factors, whereas cerebrospinal fluid better reflects the central compartment but is unsuitable for frequent sampling. Paired measurements should be obtained only when clinically justified.

Translocator-protein positron-emission tomography may provide spatial information about neuroinflammation but is not microglia-specific and cannot distinguish antiviral, inflammatory, phagocytic, or reparative states. It should therefore remain an exploratory research method in this context [99].

9.4. Implications for Clinical Trials

Initial clinical studies should prioritize safety, feasibility, pharmacokinetics, and proof of mechanism. Melatonin should be evaluated only as an adjunct to standard care in clearly defined, preferably laboratory-supported viral encephalitis. Direct encephalitis should not be pooled with post-infectious immune-mediated encephalitis or systemic encephalopathy.

Randomization or prespecified analyses should account for viral cause, disease stage, neurological severity, age, immune status, and intensive-care requirement. Trials should define one primary outcome and assess viral burden, mortality, seizures, functional disability, cognition, sleep–wake organization, quality of life, and long-term recovery using standardized measures [100].

Intensive-care treatments and environmental exposures should be recorded and balanced because they may obscure circadian and clinical outcomes. In mechanically ventilated patients, 0.3 mg nightly melatonin produced a more favorable pharmacokinetic profile than 3 mg but did not improve delirium or major outcomes. Although not an encephalitis trial, this finding shows that pharmacokinetic optimization does not necessarily provide clinical benefit [101].

Trials comparing administration times should estimate biological phase where feasible and report dose, formulation, route, timing, duration, and exposure. A chronotherapeutic mechanism would require a reproducible change in circadian phase, amplitude, or rhythmicity associated with subsequent virological or neurological improvement; reduced cytokines alone would be insufficient.

9.5. Methodological and Translational Limitations

Most studies examine only one component relationship. Consequently, reductions in cytokines, oxidative stress, or tissue injury after melatonin treatment cannot establish circadian resynchronization or direct regulation of microglial clocks and may instead reflect immunomodulation, antioxidant activity, altered viral replication, or mitochondrial protection [42,102,103].

The evidence base is predominantly preclinical. Cell systems permit pathway-specific manipulation but do not reproduce interactions among neural, vascular, immune, and systemic circadian components. Human-derived organoids improve cellular complexity but remain limited by maturation, vascularization, immune-cell representation, and batch variability [104].

Animal models preserve whole-organism interactions but differ from humans in viral tropism, immunity, sleep–activity organization, and melatonin physiology. Mouse strains vary in endogenous melatonin synthesis, and exogenous treatment in melatonin-deficient strains represents pharmacological exposure rather than physiological restoration. Findings from nocturnal rodents also cannot be mapped directly onto human biological phases [91].

Comparisons are limited by differences in viral species and strain, inoculation, host characteristics, brain region, experimental model, and disease stage. Findings from attenuated viruses, neonatal or immunodeficient animals, or single brain regions should not be generalized to wild-type infection or human disease [105,106].

Circadian studies should use consistent terminology, sample multiple biological phases, estimate phase and amplitude, and use clock perturbation or constant conditions where feasible. Light, feeding, temperature, activity, infection, treatment, and sampling schedules should be reported [107].

Melatonin studies vary in dose, formulation, route, vehicle, duration, timing, and pharmacokinetic exposure. Physiological signaling and high pharmacological concentrations are not equivalent, and pharmacological effects should not be described as circadian restoration without demonstrated changes in phase, amplitude, or rhythmicity [54–56].

Small M1/M2 marker panels and morphology alone cannot define microglial function. Studies should distinguish resident microglia from infiltrating macrophages and combine cell-resolved profiling with assessments of viral restriction, cytokine release, phagocytosis, metabolism, and effects on neural cells [108].

The biological onset of infection is rarely identifiable, and viral replication, immune activation, blood–brain barrier dysfunction, and neuronal injury may coexist at diagnosis. Because outcomes also depend on viral etiology, host characteristics, neurological severity, electroencephalography, and neuroimaging, a uniform therapeutic window is unlikely [100].

In intensive care, fever, seizures, medications, ventilation, artificial lighting, irregular feeding, immobility, and nocturnal procedures may disrupt or mask circadian rhythms. Altered melatonin, cortisol, temperature, or activity profiles may therefore reflect encephalitis, critical illness, treatment, or their combined effects. Peripheral biomarkers and neuroinflammation imaging do not directly measure microglial clock function [97–99].

Viral encephalitis is biologically heterogeneous, and an intervention that limits inflammation in one infection or disease stage may impair antiviral defense in another. Clinical translation must therefore remain virus-specific and stage-sensitive [106].

9.6. Research Priorities

Research should proceed through four complementary levels: longitudinal clinical characterization, causal preclinical testing, validation in human-relevant models, and

staged clinical development. These levels should not be treated as interchangeable forms of evidence.

First, prospective studies should determine whether reproducible circadian disruption occurs in laboratory-confirmed viral encephalitis and how it relates temporally to viral burden, inflammation, and neurological function. Serial assessments should include circadian phase and amplitude, sleep–wake organization, light exposure, temperature, endogenous melatonin, treatment, and acute and long-term outcomes [89].

Second, causal preclinical studies should combine virus-specific models with microglia-selective clock-gene manipulation and MT1/MT2 perturbation. Endogenous and exogenous melatonin should be evaluated separately, with viral burden, microglial states, inflammatory and mitochondrial pathways, and neurological outcomes measured across biological phases and disease stages [93,94].

Third, induced pluripotent stem cell-derived microglia, multicellular co-cultures, and microglia-containing brain organoids should complement animal models. These systems should demonstrate stable cellular rhythmicity, and their donor background, maturation, microglial integration, batch variability, and incomplete vascular, endocrine, and immune inputs should be reported [109].

For reproducibility, studies should report lighting, feeding, biological phase, host characteristics, viral inoculation, and melatonin administration. Randomization, blinding, exclusion criteria, and sample-size justification should follow established reporting standards [110].

Finally, clinical development should follow reproducible target engagement and acceptable virological safety. Early studies should assess pharmacokinetics, tolerability, circadian effects, and pathogen control, while subsequent trials should distinguish clock-time from phase-aligned dosing and evaluate viral, neurological, sleep-related, and long-term functional outcomes [111].

The recommended variables and essential reporting requirements for future studies are summarized in Table 2.

Table 2. Recommended variables for investigating the chrono-immunological axis in viral encephalitis.

Domain	Recommended Variables	Essential Reporting Requirement	Scientific Purpose	Reference(s)
Circadian context	Light–dark cycle, sleep–wake activity, body temperature, feeding schedule, and sampling phase	Report Zeitgeber or circadian time for infection, treatment, and sample collection	Determine whether outcomes vary according to biological time	[112]
Molecular clock	BMAL1, CLOCK, PER, CRY, REV-ERB, and ROR expression or activity	Use repeated measurements sufficient to estimate phase and amplitude	Distinguish rhythmic regulation from isolated changes in clock-gene expression	[107]
Melatonin signaling	Endogenous melatonin rhythm, MT1 and MT2 activity, administered dose, formulation, route, and timing	Separate physiological nighttime signaling from pharmacological exposure	Identify receptor-dependent, circadian, and dose-related effects	[39]
Virological status	Viral RNA, infectious titre, viral antigen, tissue distribution, and stage of infection	Measure viral burden together with inflammatory outcomes	Distinguish antiviral activity from host-directed neuroprotection	[106]

Table 2. *Cont.*

Domain	Recommended Variables	Essential Reporting Requirement	Scientific Purpose	Reference(s)
Microglial state	Cytokine production, morphology, phagocytosis, inflammasome activity, metabolism, and transcriptional profile	Avoid classification based only on a small number of M1 or M2 markers	Characterize dynamic and functionally relevant microglial states	[3]
Cellular stress	Reactive oxygen species, antioxidant capacity, mitochondrial function, and cell death	Assess these variables at comparable disease and circadian phases	Determine whether melatonin limits secondary cellular injury	[113]
Neurological injury	Neuronal survival, synaptic integrity, blood–brain barrier function, seizure activity, and glial injury markers	Combine molecular measures with functional outcomes	Establish whether biological changes translate into neuroprotection	[12]
Host and model factors	Species, strain, age, sex, immune status, brain region, and viral inoculation route	Report factors known to affect circadian and immune physiology	Improve reproducibility and comparison across studies	[110]
Clinical outcomes	Consciousness, neurological disability, cognition, sleep organization, intensive care duration, and mortality	Include both acute and long-term follow-up	Evaluate clinical relevance beyond short-term biomarker changes	[100]

10. Conclusions

Viral encephalitis requires an effective antiviral response, but prolonged or excessive neuroinflammation can extend neuronal injury beyond the period of active viral replication. Microglia are central to this balance because they contribute to viral recognition, immune-cell recruitment, inflammatory amplification, debris clearance, and tissue repair.

Circadian clocks may influence when and how these microglial functions are activated. Viral infection can simultaneously disrupt sleep, melatonin secretion, and cellular clock activity, creating a bidirectional cycle between circadian dysfunction and neuroinflammation. Melatonin may interrupt this cycle through its combined chronobiotic, immunomodulatory, antioxidant, and mitochondrial effects.

The available evidence therefore supports the possibility that melatonin and circadian clocks can modulate microglial responses during viral encephalitis. However, this modulation should not be interpreted as a simple transition from an M1 to an M2 phenotype. Microglial states are dynamic and depend on viral species, brain region, circadian phase, disease stage, and local cellular conditions.

Direct evidence for the complete chrono-immunological axis remains insufficient. Few studies have simultaneously examined circadian phase, endogenous melatonin, microglial clock function, viral burden, and neurological outcomes. The therapeutic effects of melatonin are also likely to depend on dose and administration time.

The chrono-immunological axis should therefore be regarded as a testable mechanistic framework rather than an established clinical pathway. Carefully timed experimental studies and longitudinal clinical investigations are needed to determine whether circa-

dian assessment and melatonin-based interventions can improve viral control, promote inflammatory resolution, and reduce long-term neurological injury.

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