Review

Neuroimmune Reaction to Sleep Deprivation: A Mechanistic Review

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Neuroimmune Reaction to Sleep Deprivation: A Mechanistic Review

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Abstract

Sleep deprivation (SD) has long been linked to neuroinflammation, yet a unified mechanistic framework integrating cytokine, microglial, oxidative stress, and neuroendocrine pathways remains underdeveloped. This PRISMA-informed review synthesizes evidence across human, rodent, and in vitro models to identify a multi-axis mechanism through which sleep loss drives inflammatory signaling in the brain. A search of Google Scholar and ResearchRabbit (1995-July 2025) **identified 29 eligible studies, including 13 human studies, 14 rodent studies, and 2 in vitro investigations.** Human work primarily quantified systemic inflammatory cytokines, while rodent and cell-based studies provided microglial, ATP-purinergic, and transcriptional pathway data. Across species, sleep deprivation consistently increased Tumor Necrosis Factor-α (TNF-α), Interleukin-1β (IL-1β), and Interleukin-6 (IL-6) signaling and activated canonical inflammatory cascades such as Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-κB) and Mitogen-activated protein kinase (MAPK). Rodent studies demonstrated convergent microglial remodelling, including P2X purinoceptor 7 (P2RX7) and Purinergic Receptor P2Y (P2Y12) dependent phagocytic activation, ATP-driven process motility, and enhanced synaptic engulfment. Oxidative stress markers and HPA-axis hormones were variably elevated, indicating broader neuroimmune engagement. Across axes, a shared upstream signature emerged: extracellular ATP accumulation, microglial activation, cytokine amplification, and glucocorticoid sensitive modulation of inflammatory tone. These pathways converge on microglial and cytokine networks that collectively reshape synaptic and circuit function during sleep loss. This review highlights mechanistic motifs across models but also identifies limitations, including heterogeneous protocols, limited human microglial data, and lack of formal bias assessment. Together, the synthesized evidence supports a multi-axis neuroimmune model that explains how sleep deprivation induces neuroinflammation.

Keywords: TNF-α; sleep deprivation; microglia; inflammation; cytokines; cytokines

Introduction

Sleep is a physiological state essential for maintaining the health of our brain and our body. It is characterized by the cyclic change in state between Non-Rapid Eye Movement (NREM) Sleep and Rapid Eye Movement (REM) Sleep every 80-110 minutes over the duration of sleep [4]. NREM stages are associated with restoration, synaptic pruning, and clearance of neurotoxic materials such as β-amyloid [32,48], whereas REM Sleep contributes to emotional regulation, memory consolidation, and neuroplasticity [4].

Sleep deprivation (SD) is a major public health concern with growing prevalence in all age groups. Chronic sleep loss is linked to adverse effects such as an increased risk of Alzheimer's disease [8,34], anxiety disorders [29,48], cognitive decline [39,46,49], cardiovascular dysfunction, and other systemic inflammatory conditions [7,9,44].

The emerging evidence shows that sleep is not only a neurobehavioral phenomenon but a critical modulator of immune, endocrine, and autonomic function. Even partial sleep restriction has shown to disrupt circadian homeostasis, elevates sympathetic drive, and induces a persistent low-grade inflammatory state. These changes are associated with the dysregulation of pro-inflammatory

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cytokines, particularly Tumor Necrosis Factor-α (TNF-α), Interleukin-1β (IL-1β), and Interleukin-6 (IL-6), Hypothalamic-pituitary-adrenal axis **(**HPA axis) dysregulation and altered activity of microglia. This makes sleep deprivation an immune stressor, not just a behavioral variable.

Given these findings, it is critical to understand the molecular mechanisms through which sleep deprivation drives inflammatory pathology. Accordingly, *this PRSIMA-informed review aims to identify and synthesize mechanistic neuroimmune evidence linking sleep deprivation to inflammatory signaling, rather than to estimate pooled effect sizes*.

Methods

This study was conducted as a PRISMA-informed review with a hybrid design, combining systematic study identification and screening with a primarily mechanistic and narrative synthesis with a particular focus on microglial activation and TNF-α signaling in mice and humans. Owing to substantial heterogeneity in sleep deprivation paradigms, biomarkers, and outcome measures, quantitative meta-analysis and formal risk-of-bias scoring were not appropriate; instead, evidence was synthesized to identify reproducible neuroimmune pathways and cross-study mechanistic convergence. All screening and extraction were performed by a single reviewer due to scope constraints. This review follows PRISMA 2020 reporting guidelines where applicable to a mechanistic narrative synthesis, without claiming full systematic review compliance.

Search Strategy:

A systematic search was performed to identify relevant scientific articles on Google Scholar, and ResearchRabbit from 1995 to July 30th 2025, using the following keywords in titles and abstracts: *("sleep deprivation" OR "sleep fragmentation") AND ("microglia" OR "TNF-α") AND ("inflammation")*. Due to platform limitations, exact reproducible queries could not be exported from Google Scholar or ResearchRabbit. This limitation is acknowledged, and results should be interpreted accordingly. Searches were limited to English-language articles only.

Eligibility Criteria:

Experimental studies (in vivo or in vitro) assessing neuroimmune or inflammatory outcomes following sleep deprivation, and mechanistic reviews providing molecular or cellular insights into TNF-α and microglial pathways were included. Human experimental or observational studies were also eligible when they measured systemic or neural inflammatory markers following sleep deprivation, even if they did not include microglial endpoints. Clinical trials, animal studies, and in vitro studies were all treated equally throughout the process. Mechanistic reviews were included only when they contributed essential molecular context not accessible from primary data alone. Studies had to mention at least two of the following core terms: *sleep deprivation or sleep fragmentation, microglia, TNF-α, inflammation,* to be included. Studies relying solely on self-reported sleep measures or not meeting the minimum keyword threshold were excluded from this review.

Study Selection:

A total of 97 records were identified through database searching across Google Scholar and ResearchRabbit (literature discovery platform). After title and abstract screening, 47 records were excluded for not meeting the inclusion criteria. To ensure consistency, a random sample of 20% of the excluded records at the title/abstract stage (n = 9 out of 47) was re-screened one week later by the same author to confirm the initial decision, demonstrating high intra-rater reliability. The full texts of 50 articles were assessed for eligibility, and 29 were included in the final synthesis. Study selection was conducted by a single reviewer. The PRISMA flow diagram detailing identification, screening, and inclusion steps is shown in Figure 1.

 doi:10.20944/preprints202512.1753.v2

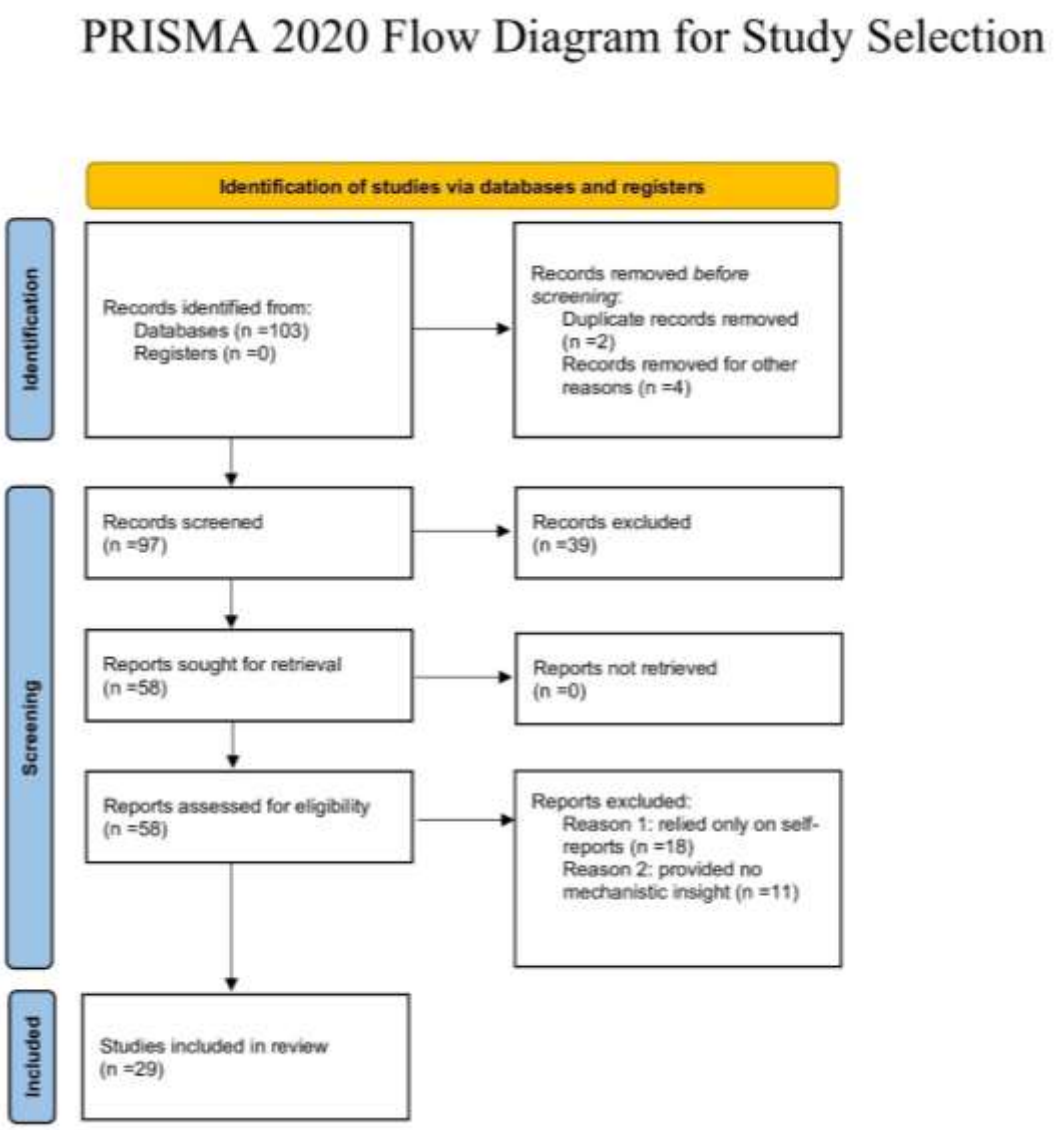

Figure 1. PRISMA flow diagram illustrating the search strategy and study selection process for mechanistic studies on neuroimmune responses to Sleep Deprivation.

Data Extraction and Assessment:

Key study characteristics (species, SD method and duration, brain region, TNF-α and microglial outcomes, assay methods) were extracted using a standardized chart. Although no formal risk-of-bias tool was applied, as the included studies were primarily mechanistic and heterogeneous in design, the studies were informally evaluated for clarity of experimental design, consistency of SD protocols, and transparency of outcome reporting. Qualitative evaluation for methodological transparency and internal consistency considered study clarity, reproducibility of SD protocols, appropriateness of outcome assays, and general methodological transparency.

Synthesis:

Although a structured thematic systematic review or meta-analysis was considered, due to the heterogeneity in study designs, species, and outcome measures, a narrative synthesis was performed. Findings were grouped by species (mouse vs human) and type/duration of sleep deprivation (acute vs chronic), with emphasis on TNF-α modulation, microglial activation, and associated signaling pathways. Human studies primarily contributed cytokine and systemic inflammatory data, while microglial mechanisms were derived mainly from rodent and in vitro models. Species-specific differences were retained, but conserved pathways (TNF-α signaling, ATP-mediated microglial activation, HPA-axis effects) were synthesized together when appropriate. Some studies contributed

 doi:10.20944/preprints202512.1753.v2

data to multiple mechanistic categories (cytokines, microglia, HPA axis, oxidative stress). These were analyzed under each relevant domain.

Results

Cytokine Aspect

A total of 23 studies met inclusion criteria for the Cytokine Axis, comprising 13 human studies, 9 murine models, and 1 in vitro investigation. Across methodologies, a consistent pattern was observed in which sleep loss, circadian misalignment, or stress exposure induced elevations in pro-inflammatory cytokines including IL-6, TNF-α and IL-1β. These cytokine shifts were observed in both central and peripheral compartments, indicating that sleep stress perturbations engage conserved inflammatory pathways across species. This axis is mechanistically important because these cytokines serve as upstream initiators and amplifiers of neuroimmune signaling that subsequently alter microglial activation states, synaptic homeostasis, and sleep-regulatory circuits.

Evidence Synthesis

This section summarizes the characteristics and principal findings of the included studies shown in Fig.1. Across the 23 included studies, 13 were human investigations, 9 were murine experimental models, and 1 used an in-vitro microglial system. Human studies comprised experimental sleep-manipulation paradigms, cross-sectional observational analyses, and behavioral or lifestyle interventions. Experimental paradigms most often employed total or partial sleep deprivation to induce changes in inflammatory signaling [1,7,13,18,23,25,33,36,42,44,47]. Cross-sectional work focused on sleep-dependent redistribution of circulating immune cells [7], diurnal cytokine rhythms [18,23,45], and inflammatory profiles during circadian misalignment or chronic sleep disruption [36,47]. Several studies incorporated polysomnography [1,25,44,45] or dense sampling protocols [33,42] to relate sleep architecture with circulating IL-6, TNF-α, IL-10, cortisol, and soluble TNF receptors. Two human trials tested interventions: weight-loss programs [1] and behavioral sleep therapies (CBT-I, Tai Chi Chih, sleep-seminar control) [22], both reporting measurable immunomodulatory effects.

Murine studies uniformly used controlled experimental manipulations to examine mechanistic links between sleep, microglial activation, and cytokine signaling. These included chronic sleep fragmentation [28], acute or total sleep deprivation [9,37,39], genetic perturbations of microglial receptors [46], TNF-α or P2RX7 knockouts [39], and LPS-induced neuroinflammation to probe cytokine transport across the Blood-Brain-Barrier (BBB) [35]. Additional studies used transcriptomic analyses to identify neuroinflammatory pathway enrichment [29] or characterized sleep-deprivation-induced microglial changes in specific brain regions such as the midbrain raphe [32]. Rodent models consistently quantified TNF-α, IL-1β, IL-6, Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-κB)/Mitogen-activated protein kinase (MAPK) activity, oxidative stress markers, and synaptic proteins using qPCR, ELISA, Western blotting, and immunohistochemistry.

The sole in-vitro study [30] examined NF-κB and MAPK activation under inflammatory stimulation, measuring IκB degradation, p38 MAPK phosphorylation, and microglia-derived cytokine release.

Across human, murine, and in-vitro evidence, a consistent pattern was observed: sleep deprivation, circadian disruption, or immune challenge reliably increased pro-inflammatory signaling. Human studies [1,7,13,18,22,23,25,33,36,42,44,45,47] repeatedly observed elevations in IL-6 and TNF-α following sleep loss, in some cases persisting beyond recovery sleep [25] or manifesting as disrupted diurnal rhythmicity in insomnia [45]. Murine studies [9,28,29,32,35,37,39,46] showed parallel activation of central and peripheral cytokine pathways, with receptor-specific requirements demonstrated in models using TNF-α or P2RX7 deletion [39] and BBB-transport mechanisms identified through LPS models [35]. Lifestyle factors such as voluntary exercise modulated

 doi:10.20944/preprints202512.1753.v2

neuroinflammatory responses [9]. The in-vitro findings [30] supported pathway-level mechanisms, confirming microglial NF-κB and p38 MAPK activation and pharmacological suppression of cytokine release. A detailed summary table of the included cytokine studies is presented in Table 1.

Overall, human studies detailed systemic inflammatory responses, whereas murine and in-vitro models provided mechanistic resolution, identifying microglial and receptor-level pathways that drive cytokine changes. Across evidence domains, the findings consistently support a strong association between sleep disruption and activation of microglia-dependent cytokine signaling.

Table 1. Overview of mechanistic cytokine studies examining how sleep deprivation alters inflammatory signaling pathways (e.g., IL-6, TNF-α) across animal, human, and in vitro models.

Author, Year	Kaushal et al., 2021 [28]	Chennaoui et al., 2015 [9]	Periasamy et al., 2015 [37]
System	TNFR1-/- mice, wild-type controls, and wild-type mice treated with TNF-α neutralizing antibody.	Mice were assigned to four experimental groups: (1) exercised mice under normal sleep conditions, (2) sedentary mice under normal sleep conditions, (3) exercised mice subjected to sleep deprivation, and (4) sedentary mice subjected to sleep deprivation.	Male C57BL/6J mice subjected to 0-72 hours sleep deprivation using the modified multiple platform method; blood and multiple organs analyzed.
Mechanism/ Measured outcome studied	TNFR-1 pathway studied using Polysomnography (PSG).	Hippocampal and cortical tissues, along with blood plasma samples, were collected for analysis, and cytokine mRNA expression levels were quantified to evaluate neuroinflammatory responses.	Inflammatory markers (TNF-α, IL-1β, IL-6) were measured in tissue and serum samples.
Condition	Sleep fragmentation	Chronic Sleep deprivation	72-hour Total Sleep Deprivation
Key Findings	Wild-type (WT) mice exhibited increased delta power and a robust rebound response following sleep fragmentation, indicating that TNF-α is required for normal sleep-homeostatic regulation.	In sedentary sleep-deprived rats, hippocampal IL-1β mRNA, IL-1β protein, and TNF-α protein levels were significantly increased, whereas voluntary exercise attenuated these SD-induced elevations. TNF-α mRNA levels remained unchanged across conditions. In the periphery, plasma IL-6 and TNF-α concentrations were elevated in sedentary sleep-deprived rats but not in the exercised group.	Sleep-deprived mice showed a significant increase in serum IL-6 on Day 3 of the protocol and an increase in serum IL-1β on Day 1, indicating a time-dependent peripheral inflammatory response.
Limitations	Although TNF-α signaling was inferred from genetic and antibody manipulations, no cytokine quantification (e.g., ELISA, qPCR) was performed	Only male rodents were included, which limits generalizability because sleep–inflammation interactions and TNF-α/IL-1β	Only male rodents were included, which limits generalizability because TNF-α/IL-1β signaling

 doi:10.20944/preprints202512.1753.v2

	to directly validate changes in inflammatory markers.	signaling exhibit well-documented sex differences.	exhibit well-documented sex differences.
Author, Year	Al-Sharif et al., 2021 [1]	Irwin et al., 2015c [25]	Wright et al., 2015 [47]
System	Humans with chronic primary insomnia.	Partial sleep deprivation (03:00-07:00) vs. baseline sleep and recovery sleep in healthy adults	Endocrine and Immune response of healthy adults
Mechanism/ Measured outcome studied	Lifestyle-induced weight loss reduces systemic inflammation, which may improve sleep regulation through decreased circulating pro-inflammatory cytokines (e.g., IL-6, TNF-α) and increased anti-inflammatory cytokines (IL-10).	Activation of spontaneous pro-inflammatory cytokine production and intracellular STAT signaling pathways. IL-6, TNF-α, STAT1, STAT3, and STAT5 were measured in peripheral immune cells alongside polysomnographic (PSG) sleep parameters.	Plasma inflammatory markers (TNF-α, IL-10, and CRP) were evaluated across three contrasts: baseline compared with acute sleep deprivation, baseline compared with chronic circadian misalignment, and misaligned participants compared with synchronized control groups.
Condition	6-months of weight loss intervention	Partial Sleep Deprivation	Chronic circadian misalignment for 25 days and Acute total sleep deprivation (40h)
Key Findings	No significant changes in sleep or cytokines of control group. Reduced IL-6 and TNF-α and elevated IL-10 indicates reduced systemic inflammation.	Spontaneous IL-6 and TNF-α production increased after PSD ($p < .02$). This elevation persisted even after recovery sleep ($p < .01$), showing lasting inflammatory activation.	Chronic circadian misalignment was associated with significant increases in circulating IL-10, TNF-α, and CRP.
Limitations	No long-term follow-up to assess durability of improvements.	Due to single night of PSD, it is unclear if effects generalize to repeated or chronic partial sleep loss.	Inflammatory markers measured infrequently (two time points), unlike cortisol which was sampled frequently.
Author, Year	Brás et al., 2020 [6]	Patel et al., 2009 [36]	Irwin et al., 2010 [18]
System	Primary rat mixed glia and N9 murine microglial cell line	Human adults (n = 614) from the Cleveland Family Study	Human adults (n=26)
Mechanism/ Measured outcome studied	TNF-α–induced microglial activation and Cytokine secretion (TNF-α, IL-1β, IL-6, IL-10, MIP-2, IL-4, IL-12) were measured.	Circulating inflammatory biomarkers: CRP, IL-6, TNF-α, IL-1, IL-10 were measured.	LPS-stimulated monocyte production of IL-6 and TNF-α across the day.
Condition	TNF-α stimulation of primary rat microglia or N9 microglial cells (20 ng/mL)	PSG-measured sleep duration on the night prior to blood draw	Early-night partial sleep deprivation (PSD): awake from 23:00 to 03:00, followed by the remaining sleep opportunity; compared against baseline

 doi:10.20944/preprints202512.1753.v2

			nights of uninterrupted full sleep.
Key Findings	TNF-α robustly activates microglia via NF-κB, increasing TNF-α, and IL-1β expression without altering IL-6 or IL-10.	Self-reported sleep duration: IL-6 increased by 7% per additional hour; PSG sleep duration: TNF-α increased by 8% for each 1-h decrease.	Both males and females show marked increases in LPS-stimulated IL-6 and TNF-α relative to baseline on the morning after PSD. Females show sustained increases in IL-6 and TNF-α production (early & late evening).
Limitations	Mixed species system (rat microglia, mouse neurons, murine N9 cell line) may limit translational relevance.	Self-reported sleep duration may overestimate or misrepresent habitual sleep patterns.	Small sample size (N=26, only 11 females) limits generalizability of the findings.
Author, Year	Irwin et al., 2006 [23]	Pan et al., 2008 [35]	Irwin et al., 2014 [22]
System	Human adults (n=30)	Mice (in vivo) CD1 (wild-type), TNF ligand-KO, TNFR1/2 double-KO, and background control strain.	Human clinical population (N=123)
Mechanism/ Measured outcome studied	Inflammatory responses were evaluated by repeatedly measuring LPS-stimulated monocyte intracellular IL-6 and TNF-α production, complemented by molecular analyses of IL-6 and TNF gene transcription.	The study quantified how LPS-induced neuroinflammation alters receptor-mediated cytokine transport across the blood-brain barrier, with a specific focus on determining whether TNF/TNFR signaling mediates the LPS-evoked increase in LIF transport.	Inflammatory markers were measured in comparison of CBT vs. Tai Chi Chih (TCC) vs. Sleep Seminar education (SS control)
Condition	Early-night partial sleep deprivation (23:00–03:00)	LPS-induced neuroinflammation (5 mg/kg, intraperitoneal; 48 h post-injection)	Chronic primary insomnia in older adults Randomized to CBT, TCC, or SS for 4 months, with assessments at 7 and 16 months.
Key Findings	PSD led to a significant morning increase in LPS-stimulated monocyte production of IL-6 and TNF-α compared to rested baseline. PSD produced a >3-fold increase in IL-6 mRNA and approximately 2-fold increase in TNF mRNA, indicating transcriptional activation of inflammatory pathways.	LPS increased TNFR1 and TNFR2 expression in microvessels. gp130 upregulation after LPS was absent in TNF ligand-KO and TNFR double-KO mice. Therefore, TNF/TNFR signaling is required for LPS-induced gp130 upregulation.	CBT showed the highest insomnia remission versus TCC and SS ($P < 0.01$). CBT reduced the risk of high CRP (>3.0 mg/L) at 16 months. PSG measures showed no significant change across interventions.
Limitations	Short-term PSD only; chronic effects not addressed.	Single time point (48 h after LPS); dynamics of early/late changes not assessed. Only male mice and a specific LPS dose; different doses or	PSG outcomes did not change, limiting objective confirmation of sleep improvements. CRP was the only inflammatory

 doi:10.20944/preprints202512.1753.v2

		chronic inflammation not tested.	marker assessed, restricting interpretation of broader immune effects.
Author, Year	Shearer et al., 2001 [42]	Liu et al., 2017 [30]	Vgontzas et al., 2004 [44]
System	58 healthy adult men (final analyzed N = 42 for cytokines)	In vitro microglial model; BV2 or primary microglia exposed to intermittent hypoxia	Healthy young human adults (n = 25; 12 males and 13 females).
Mechanism/ Measured outcome studied	IL-6, TNF-α, IL-10, soluble TNF-α receptor I (sTNF-αRI), soluble TNF-α receptor II (sTNF-αRII), soluble IL-2 receptor were measured through blood sampling every 6 hours across 5 days.	Microglial inflammatory signaling was assessed through NF-κB pathway activation, indicated by IκB degradation and p38 MAPK phosphorylation measured via Western blot, together with quantification of pro-inflammatory cytokines. TNF-α and IL-6 expression was evaluated at the mRNA level using qRT-PCR and at the protein level through ELISA.	Quantified 24-hour secretion profiles of IL-6, TNF-α, and cortisol, assessed daytime sleepiness using the Multiple Sleep Latency Test (MSLT), evaluated psychomotor performance with the Psychomotor Vigilance Test (PVT), and measured sleep architecture through polysomnography.
Condition	Total Sleep Deprivation (TSD) for 88 hours vs Partial Sleep Deprivation (PSD) with two 2-hour naps per 24 hours.	Intermittent hypoxia consisted of alternating exposures between 1% and 21% O_2 in 400-second cycles for a total duration of 8 hours. Propofol was administered as a pre-treatment at concentrations of 0, 25, 50, or 100 μM, applied 30 minutes before the onset of intermittent hypoxia. Control cultures were maintained under normoxic conditions without propofol exposure.	Participants underwent mild chronic sleep restriction in which time in bed was reduced from 8 to 6 hours per night for one week, and outcomes were compared against a baseline full-sleep condition.
Key Findings	TSD produced significant increases in sTNF-αRI and IL-6, with linear increases over days. PSD did not show increases in sTNF-αRI or IL-6; IL-6 even trended downward. Findings suggest dose-dependent cytokine activation proportional to sleep pressure, with sTNF-αRI and IL-6 being particularly sensitive.	IH caused strong p38 MAPK phosphorylation and marked reduction of IκB (4–8 h), indicating NF-κB pathway activation. IH significantly upregulated TNF-α and IL-6 mRNA and increased TNF-α and IL-6 secretion (ELISA). 50–100 μM propofol significantly decreased TNF-α mRNA, and 100 μM reduced IL-6 mRNA. Propofol counteracts IH-induced microglial inflammation by inhibiting the NF-κB/p38 MAPK	One week of sleep restriction resulted in a significant increase in IL-6 secretion across 24 hours in both sexes and an increase in TNF-α secretion restricted to males. The findings indicate that even modest sleep loss induces measurable increases in proinflammatory cytokine activity.

 doi:10.20944/preprints202512.1753.v2

		signaling axis, leading to reduced production of major pro-inflammatory cytokines.	
Limitations	All participants were healthy young men, limiting generalizability.	Acute IH model (8 h) only; no chronic/intermittent time-course assessment; Cytokine protein was measured in culture supernatant; intracellular stores not assessed.	The short duration of the intervention limits inference about chronic effects. The sleep laboratory environment may not accurately represent real-world sleep behavior.
Author, Year	Vgontzas et al., 2002 [45]	Mullington et al., 2000 [33]	Metaxas et al., 2018 [32]
System	Human subjects with chronic insomnia (n = 11; 6 males, 5 females) and matched healthy controls (n = 11; 8 males, 3 females).	Healthy young adult male humans (n = 19; each participant studied under both placebo and endotoxin conditions).	APPswe/PS1dE9 mouse model (midbrain raphe)
Mechanism/ Measured outcome studied	The study quantified 24-hour plasma secretion patterns of IL-6 and TNF using serial blood sampling and assessed sleep architecture across multiple nights of polysomnography to evaluate associations between cytokine rhythmicity and insomnia-related fatigue.	Circulating pro-inflammatory cytokines (TNF-α, IL-6), soluble TNF receptors (sTNFR-p55, sTNFR-p75), and the anti-inflammatory IL-1 receptor antagonist (IL-1ra) were quantified via serial blood sampling every 30 minutes from evening to the following midday. Cytokine-driven effects on sleep were assessed through changes in NREM sleep amount and EEG slow-wave (delta) power.	Neuroinflammatory activation in the midbrain raphe despite absence of local amyloid or tau pathology; cytokine-related microglial activation
Condition	Participants with chronic insomnia were compared with age- and BMI-matched healthy controls under controlled laboratory conditions during four consecutive nights of monitored sleep, with cytokine sampling performed across the fourth 24-hour period.	Acute, experimentally induced immune activation using intravenous endotoxin (0.2, 0.4, or 0.8 ng/kg) administered immediately before nocturnal sleep onset and compared to placebo in a balanced, single-blind crossover design.	Transgenic vs. wild-type mice at 12 months
Key Findings	While overall 24-hour mean IL-6 and TNF secretion did not differ between groups, insomniacs showed a significant increase in IL-6 from mid-afternoon to evening and a marked phase shift in IL-6 rhythmicity, with	Endotoxin produced a clear, dose-dependent increase in circulating TNF-α, IL-6, sTNFR-p55, sTNFR-p75, and IL-1ra. The lowest dose (0.2 ng/kg) significantly increased cytokine and soluble receptor release without activating	TNF-α mRNA increased approximately two-fold relative to controls; IL-1β and IL-6 mRNA assessed but showed only modest or nonsignificant elevation; pronounced microglial, but not

 doi:10.20944/preprints202512.1753.v2

	the primary peak occurring in the evening rather than during the night. TNF exhibited a circadian rhythm in controls with a peak near sleep offset, but insomniacs lacked this rhythm and instead showed a distinct 4-hour ultradian TNF periodicity during the daytime. These findings indicate that chronic insomnia is associated with a redistribution of IL-6 and TNF secretion toward the daytime period.	cortisol or altering body temperature, yet was sufficient to enhance deep NREM sleep and increase EEG delta, theta, and alpha power, indicating increased sleep intensity driven by cytokine signaling. Higher doses continued to amplify cytokine responses but produced sleep disruption rather than NREM enhancement.	astrocytic, immunoreactivity consistent with localized cytokine signaling; TNF-α mRNA levels positively correlated with SERT mRNA loss, suggesting cytokine-linked serotonergic disruption.
Limitations	The observational design limits causal inference about whether cytokine phase shifts drive insomnia symptoms or arise secondarily from disturbed sleep. Cortisol measures referenced by the authors were not directly analyzed in this cohort.	Cytokine effects cannot be disentangled from downstream mediators (e.g., vagal, neuroendocrine pathways). Dose timing was fixed to immediate pre-sleep administration, which may not model all infection states.	Cytokine expression limited to mRNA without parallel protein quantification; assessment confined to a single age and brainstem region; small sample size (n=6/group).
Author, Year	Born et al., 1997 [7]	Liu et al., 2022 [29]	Vicente et al., 2023 [46]
System	Healthy adult men	Mouse (C57BL/6J), prefrontal cortex (PFC)	Rat STZ-induced sporadic AD model; locus coeruleus (LC)
Mechanism/ Measured outcome studied	Sleep-associated redistribution of circulating immune cells with selective modulation of adaptive cytokine production; interaction between sleep–wake state and circadian phase on immune responsiveness.	Bioinformatic analyses across multiple datasets identified enriched inflammatory pathways, with emphasis on TNF-related signaling.	Minocycline reduces microglial density/morphology but shows minimal effect on LC cytokine transcription.
Condition	Comparison of normal nocturnal sleep vs. sustained nocturnal wakefulness across 24-51h; cytokine production assessed after ex vivo stimulation (LPS for monocytes; PHA for T cells)	6-hour acute partial sleep deprivation	STZ vs. STZ + minocycline (30 mg/kg, 5 days)
Key Findings	Sleep did not alter LPS-induced IL-1β or TNF-α production and did not change plasma IL-6 concentrations; cytokine effects occurred independently of cortisol;	Acute PSD upregulated TNF-pathway associated genes including Ptgs2 (COX-2) and Socs3, both implicated in inflammatory amplification and cytokine signaling modulation. Casp3 was	STZ increased microglial density and inflammatory morphology in the LC. Minocycline reduced microglial recruitment but did not significantly alter STZ-induced expression of

 doi:10.20944/preprints202512.1753.v2

	circadian rhythmicity of cytokine-producing PBMC subsets preserved under both sleep and sustained wakefulness.	downregulated at the protein level, potentially reflecting altered apoptotic signaling during neuroinflammatory activation.	IL-10, IL-1β, or MCP-1 mRNA. A strong trend toward reduced TNF-α expression was observed but did not reach significance.
Limitations	Cytokine responses measured only in ex vivo stimulated whole blood rather than in vivo circulating cytokines; small sample size with only men (n=10) limits generalizability	Study focused on gene-level correlates rather than direct cytokine quantification in brain tissue; plasma cytokines were not assessed, limiting systemic–central comparison. Only male mice were included, restricting generalizability across sexes.	Cytokines measured only at mRNA level; lack of protein quantification limits interpretation. Trend-level TNF-α change may reflect insufficient statistical power. Short treatment duration (5 days) may be inadequate to detect full cytokine modulation.
Author, Year	Pinto et al., 2024 [39]		Frey et al., 2007 [13]
System	Microglial TNF-α knockout mice vs wild-type; microglial depletion; P2RX7 KO; sleep vs sleep deprivation (ZT6 vs ZT18).		19 Healthy Men and Women
Mechanism/ Measured outcome studied	The study quantified several synaptic and molecular outcomes to determine how microglial signaling influenced inhibitory and excitatory synaptic regulation across cortical layers. Synaptic $GABA_A$ receptor subunits γ2 and α1 were measured in layers 1 and 5 to assess inhibitory synapse composition, while GluA2 levels were used to index changes in excitatory postsynaptic content. CaMKIIα phosphorylation at Thr286 was evaluated as a readout of activity-dependent synaptic plasticity. In addition, performance on sleep-dependent memory tasks was assessed to determine whether microglia-mediated synaptic modifications translated into functional behavioral outcomes.		Plasma concentrations of inflammatory biomarkers, including IL-1β, IL-6 and IL-1ra were analyzed to study alterations in circulating inflammatory signaling.
Condition	Sleep vs sleep deprivation at different circadian phases (ZT6 sleep phase, ZT18 wake phase)		40-hours of Total Sleep Deprivation
Key Findings	$GABA_A$ receptor enrichment at inhibitory synapses is normally observed at ZT6, which corresponds to the sleep phase. This enrichment is absent when animals undergo sleep deprivation. Experimental disruption of microglial signaling, including microglial depletion, microglial TNF-α knockout, TNF-α neutralization, or P2RX7 knockout, prevents both $GABA_A$ receptor enrichment and CaMKIIα phosphorylation. Loss of microglial TNF-α also impairs sleep-dependent memory consolidation, demonstrating that microglia-derived TNF-α is required for synaptic remodeling and cognitive benefits associated with sleep.		One night of total sleep deprivation induced a mixed inflammatory response, activating both pro- and anti-inflammatory pathways. Increased IL-1β and IL-1ra was observed, indicating a stress-dependent immune modulation.

 doi:10.20944/preprints202512.1753.v2

Limitations	Cytokine source inferred from prior datasets, not measured in this study. Peripheral cytokine levels were not measured.	Small sample size (N = 19), limiting statistical power and generalizability.

Mechanistic Narrative

1. Purinergic Receptor Activation (P2RX7 and P2Y12) as Initiators

During sleep deprivation, wake-associated neural activity increases extracellular ATP accumulation in cortical and subcortical circuits [31,39]. Microglia are the primary sensors of this danger-associated ATP signal [39]. Two major purinergic receptors: P2X7 and P2Y12**,** mediate distinct but complementary inflammatory and sleep-regulatory responses.

Elevated extracellular ATP binds to P2X7, a low-affinity ligand-gated ion channel expressed on microglia. P2X7 activation produces a rapid K^+ efflux that triggers assembly of the NLRP3 inflammasome, leading to cleavage and release of IL-1β, a cytokine strongly linked to sleep pressure and homeostatic slow-wave activity [3,39,50].

Simultaneously, P2X7 stimulation promotes microglial TNF-α release. TNF-α then binds on TNFR-1, activating intracellular cascades such as CaMKII phosphorylation. CaMKII modifies synaptic availability of $GABA_AA$ receptors and glutamatergic components, facilitating a shift toward increased slow-wave sleep (SWS), a compensatory response that aids synaptic enrichment and metabolic clearance following sleep loss [39,50].

In the context of sleep loss, P2Y12 signaling induces controlled Ca^{2+} oscillations within microglia, facilitates ATP-to-adenosine conversion via microglial enzymatic pathways. Adenosine accumulates extracellularly and acts on neuronal A1 receptors to suppress wake-promoting circuits and enhance restorative sleep [31].

2. NF-κB as a Primary Amplifier in Microglial Pro-inflammatory Signaling

Sleep deprivation increased microglial release of TNF-α and IL-1β, which in turn activated their respective receptors (TNFR1 and IL-1R1) on microglia. TNF-α–TNFR1 signaling rapidly promoted NF-κB nuclear translocation, while IL-1β amplified this effect through NLRP3-dependent maturation and MyD88-mediated signaling [14,28,30,33,35,49]. This stimulation rapidly engaged the canonical NF-κB pathway, leading to IκBα degradation and nuclear translocation of p65/p50. The resulting NF-κB activation boosted transcription of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6), creating a self-reinforcing loop that sustained microglial activation [6,30,33,35]. Because both TNF-α and IL-1β converged on the same NF-κB dependent program, their combined action amplified inflammatory signaling and likely contributed to the observed effects on synaptic regulation and increased sleep pressure. A visual summary of this pathway is provided in Figure 2.

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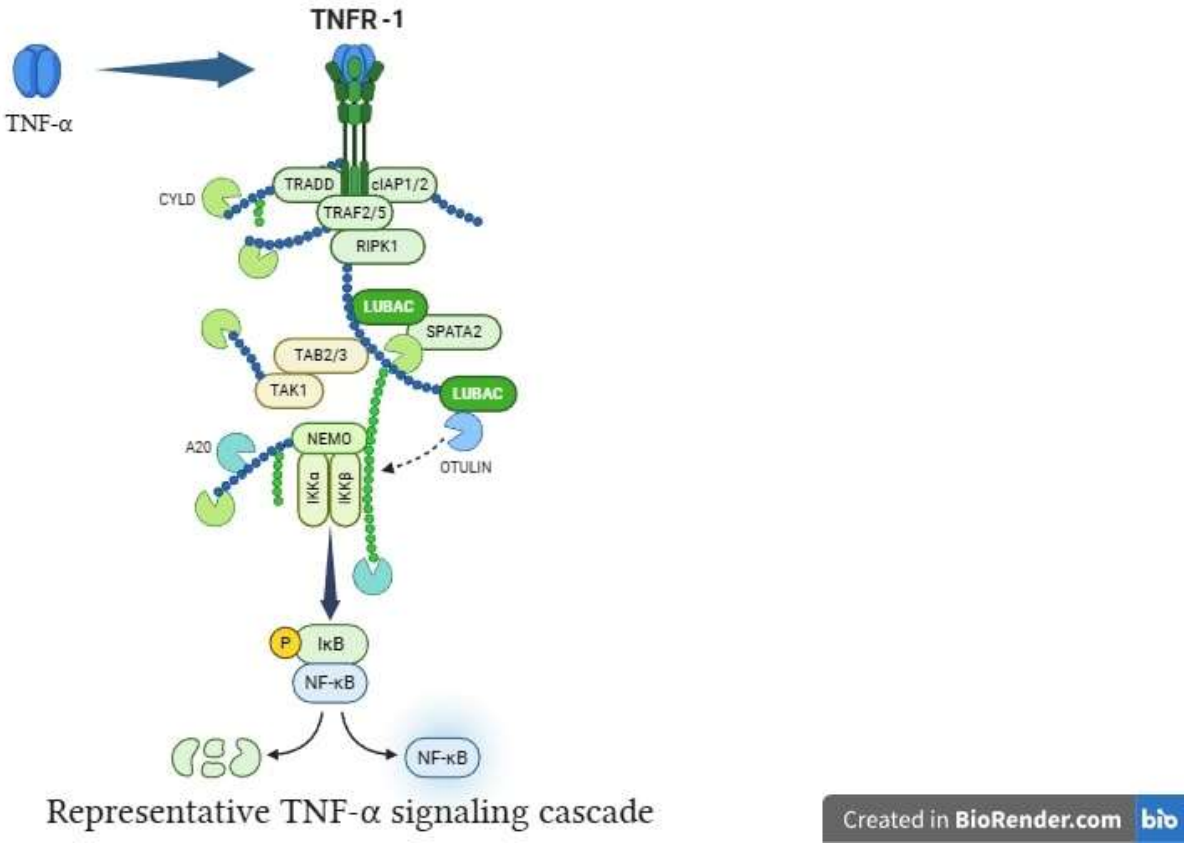

Figure 2. TNF-α–TNFR signaling pathway activating NF-κB, including IκB degradation, NF-κB nuclear translocation, and transcriptional activation.

3. STAT proteins and MAPK as Secondary Amplifiers in Microglial Pro-inflammatory Signaling

Pro-inflammatory signaling is further amplified through STAT and MAPK cascades downstream of cytokine and TNF-family receptors. IL-6 and IFN-γ activate their receptors (IL-6R/GP130), leading to JAK-mediated phosphorylation of STAT1/STAT3 [25,38], which then dimerize and enter the nucleus to induce secondary pro-inflammatory genes (e.g., IL-6, CCL2, iNOS) and reinforce NF-κB dependent programs [2,25].

TNF-α binding to TNFR1 initiates TRADD-dependent assembly of Complex I (TRAF2, RIPK-1, LUBAC), which engages MAPK pathways [26]. These MAPK signals further increase transcription of TNF-α, IL-1β, and IL-6, thereby acting as parallel amplifiers that sustain and escalate microglial activation [30].

Oxidative Stress-HPA Axis

Sleep deprivation engages oxidative-stress pathways and disrupts hypothalamic-pituitary-adrenal (HPA) axis regulation, forming a secondary but critical arm of the neuroimmune response. Across animal and human models, SD induces metabolic stress, elevates reactive oxygen species, alters autophagy-lysosomal trafficking, and produces shifts in cortisol rhythmicity. These processes interact with microglial activation and inflammatory cytokine signaling, amplifying the overall neuroimmune impact of sleep loss.

Evidence Synthesis

Across the oxidative-stress and HPA-axis evidence base, four studies were murine experimental models and one was a human circadian-disruption paradigm. Murine investigations examined chronic sleep fragmentation and its effects on oxidative-stress linked intracellular degradation pathways involving Rab5, Rab7, LC3B, and autophagy regulators [48]; acute microglial activation or

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inhibition to assess Ca^{2+} dynamics and neuromodulatory changes in norepinephrine and adenosine signaling during sleep wake regulation [31]; miRNA-mediated inflammatory amplification via miR-342 and NF-κB activation with downstream effects on neuronal viability in microglia neuron co-cultures [6]; and extended total sleep deprivation (72 h) to quantify oxidative-stress markers in brain and serum [37]. The sole human study used 25 days of circadian misalignment combined with 40 h of total sleep deprivation, demonstrating altered cortisol rhythmicity and increased systemic oxidative stress [47]. Outcomes across studies included molecular assays (Rab5, Rab7, LC3B, autophagy proteins, NOS-2, MDA, NO), neuronal-viability assays, hormonal profiling (cortisol), and behavioral sleep metrics. Together, these studies indicate that sleep and circadian disruption converge on microglia-linked oxidative-stress pathways and HPA-axis dysregulation, with murine models providing mechanistic insights and the human model confirming physiological relevance.

Across murine and cellular models, all studies consistently reported oxidative-stress-linked disruptions to intracellular homeostasis. Chronic sleep fragmentation impaired endosome-autophagosome-lysosome trafficking via Rab5, Rab7, and LC3B dysregulation and increased amyloid-β accumulation [48]. Microglial activation amplified oxidative and metabolic load through Ca^{2+} dynamics and neuromodulatory shifts that promote sleep pressure and arousal suppression [31]. TNF-α-activated microglia increased NOS-2 expression, nitrite release, and miR-342-dependent neurotoxicity [6]. Acute sleep deprivation produced systemic oxidative injury with elevated MDA and NO and multi-organ biochemical dysfunction [37]. The human study showed that extreme sleep disruption acutely activated the HPA axis via elevated evening-night cortisol during total sleep deprivation, whereas circadian misalignment suppressed overall cortisol output [47]. Together, these findings demonstrate that sleep disruption and inflammatory activation reliably induce oxidative-stress responses and modulate stress-axis output.

Murine models provided mechanistic resolution, revealing disrupted autophagy-lysosomal trafficking [48], microglia-driven nitric-oxide overproduction [6,37], neuronal vulnerability, and Ca^{2+}/adenosine-mediated modulation of sleep arousal circuits [31]. The human model highlighted physiologically measurable HPA-axis consequences: acute cortisol elevation during sleep loss and reduced output during circadian misalignment, without cellular-level mechanistic access [47]. A detailed summary of the included oxidative stress and HPA-axis studies is presented in Table 2.

Overall, the evidence is strengthened by convergence between mechanistic rodent data and physiologically relevant human endocrine alterations. Despite methodological heterogeneity, the evidence supports a consistent association between sleep disruption, microglia-linked oxidative stress, and HPA-axis dysregulation.

Table 2. Summary of mechanistic studies assessing oxidative stress and HPA-axis responses to sleep deprivation across animal, human, and in vitro models.

Author, Year	Xie et al., 2020 [48]	Ma et al., 2024 [31]	Brás et al., 2020 [6]
System	Wild-type mouse model exposed to chronic sleep fragmentation for two months.	Mouse model with chemogenomic and pharmacological manipulation of microglial Gi-coupled GPCR signaling; two-photon imaging of microglial Ca^{2+} and cortical norepinephrine	Primary rat mixed glia and N9 murine microglial cell line
Mechanism/ Measured outcome studied	Oxidative-stress–linked intracellular degradation pathways through Rab5, Rab7, LC3B and autophagy-regulatory proteins.	Intracellular Ca^{2+} dynamics, norepinephrine suppression, adenosine-linked modulation of arousal pathways; sleep-wake regulation quantified	miRNA microarray identification; functional dissection of miR-342; NF-κB pathway activation; downstream inflammatory mediator release; neuronal

 doi:10.20944/preprints202512.1753.v2

		after microglial activation or inhibition.	viability in microglia-neuron co-cultures.
Condition	Chronic sleep fragmentation inducing long-term sleep insufficiency	Acute experimental modulation of microglial signaling to assess their role in sleep regulation under normal physiological conditions	Acute inflammatory stimulation of microglia with TNF-α to model sustained neuroinflammatory states and microglia-driven neuronal toxicity
Key Findings	Chronic sleep fragmentation increased Rab5, Rab7, LC3B and positive autophagy-regulating factors, indicating disrupted endosome-autophagosome-lysosome clearance pathways associated with oxidative stress burden. Intracellular amyloid-β accumulation rose in cortex and hippocampus, consistent with impaired degradation under stress.	Activation of microglial Gi signaling increased intracellular Ca^{2+} and robustly promoted sleep, while P2Y12 blockade reduced sleep. Microglia-dependent suppression of cortical norepinephrine and increased adenosine contributed to the transition from wake to sleep. Because norepinephrine levels strongly influence hypothalamic arousal centers that interact with the HPA axis, these findings suggest that microglial signaling can modulate sleep arousal states partly through pathways that converge on stress-related neuroendocrine systems.	TNF-α stimulation upregulated classical inflammatory markers (NOS-2, TNF-α, IL-1β). In co-cultures, TNF-α–activated or miR-342–overexpressing microglia significantly reduced neuronal viability and increased nitrite levels in the supernatant, reflecting elevated nitric oxide production. Because nitric oxide overproduction promotes oxidative stress and can synergize with inflammatory cytokines to damage neurons, the data indicate that miR-342 amplifies a TNF-α driven inflammatory-oxidative cycle in microglia that propagates oxidative stress linked neurotoxicity.
Limitations	Continuous physiological measures of oxidative load were not included, restricting mechanistic resolution.	It used acute manipulations rather than chronic sleep disruption, limiting conclusions about longer term neuroendocrine adaptation.	Oxidative stress was inferred primarily through nitrite accumulation: direct measurements of ROS/RNS, antioxidant pathways, mitochondrial redox state, or lipid/protein oxidation were not performed.
Author, Year	Wright et al., 2015 [47]		Periasamy et al., 2015 [37]
System	Healthy adult humans (N = 17; males and females) undergoing baseline assessment, a 40-h total sleep deprivation constant routine, and a 25-day circadian misalignment entrainment protocol with frequent plasma sampling.		Male C57BL/6J mice subjected to 0-72 h sleep deprivation using the modified multiple platform method; blood and multiple organs analyzed
Mechanism/ Measured outcome studied	HPA-axis activity via cortisol rhythms		Oxidative stress markers measured in tissue and serum samples.
Condition	Chronic circadian misalignment for 25 days and Acute total sleep deprivation (40h)		72-hour Total Sleep Deprivation
Key Findings	Acute sleep deprivation caused a marked rise in cortisol, especially in the early evening and night,		Sleep deprivation produced significant oxidative stress responses,

 doi:10.20944/preprints202512.1753.v2

	indicating acute HPA-axis activation. Circadian misalignment led to an overall reduction in cortisol levels despite stable sleep duration.	with elevated MDA and NO levels. Oxidative stress was accompanied by mild-to-moderate liver and lung injury and biochemical evidence of liver, heart, kidney, and pancreatic dysfunction.
Limitations	Cortisol was not continuously sampled over full circadian cycles, restricting assessment of complete HPA rhythm changes.	Study did not isolate causal pathways linking oxidative stress to specific organ damage. Only male mice were used, reducing generalizability.

Mechanistic Narrative

Across included studies, sleep deprivation consistently triggered early oxidative stress responses, reflecting one of the most rapid physiological consequences of sustained wakefulness. SD increased Nitric Oxide generation in neural and peripheral tissues [6,37]. This burden of oxidative stress disrupted endosomal-autophagy pathways, as reflected by elevated Rab5, Rab7, LC3B, and other autophagy-related markers, indicating impaired clearance of damaged proteins and organelles under prolonged sleep disruption [48]. This redox imbalance is mechanistically important because oxidative stress acts as an upstream amplifier of inflammatory signaling, mitochondrial dysfunction, and synaptic vulnerability.

In parallel, sleep deprivation activated the HPA axis, leading to elevated cortisol levels [47]. Glucocorticoid elevations further exacerbated oxidative load by promoting mitochondrial dysfunction and weakening antioxidant systems, thereby amplifying OS-driven cellular stress. Together, these findings indicate that sleep loss initiates a dual oxidative pathway, jointly contributing to cumulative oxidative and metabolic strain in sleep-disrupted conditions.

Microglial Dynamics

A total of 11 studies met inclusion criteria for the Microglial Axis, comprising 9 murine models and 2 in vitro investigations. Across paradigms involving stress exposure, a convergent pattern emerged in which microglia transitioned toward a more reactive or phagocytic state. These studies consistently reported increased expression of activation-associated markers (e.g., CD68, CD16/32) and enhanced synaptic contact, surveillance, or engulfment behaviors. Collectively, these findings indicate that sleep stress perturbations reliably recruit microglial pathways that modulate synaptic architecture and contribute to downstream changes in sleep homeostasis.

Evidence Synthesis

Among the included microglia studies (n = 11), none used human cohorts, nine employed rodent models, and two used in vitro systems. Study designs were predominantly experimental and interventional, spanning acute sleep deprivation [3,29], chronic sleep fragmentation [48], maternal sleep deprivation [49], intermittent hypoxia [30], inflammatory challenge with LPS [41], and disease-relevant stressors such as STZ-induced sporadic Alzheimer's disease [46]. Interventions also included pharmacological manipulation (minocycline [29,46,49], propofol [30], PLX5622-mediated microglial depletion [11,41]) and genetic or chemogenomic perturbations (microglial TNF-α knockout, P2RX7 KO, CX3CR1 deficiency, Gi-coupled GPCR activation [11,31,39]). Microglial outcomes were quantified through immunohistochemistry and morphology (Iba1, CD68, CD16/32, CD206) [29,48,49], ultrastructural imaging (serial block-face EM, TEM) [3], two-photon Ca^{2+} imaging [31], electrophysiology [11,39], transcriptomic or microRNA analysis [6], and biochemical assays of microglial activation or secretory activity [6,30]. These methods consistently revealed sleep- or challenge-induced microglial alterations such as enlarged somata, increased phagocytic vesicles [3], miR-342 dependent inflammatory signaling [6], shifts toward M1-like (pro-inflammatory) activation

 doi:10.20944/preprints202512.1753.v2

after maternal sleep deprivation [49], and Gi-mediated modulation of sleep via microglial Ca^{2+} dynamics [31].

The dominant pattern across these studies is that manipulations disrupting sleep, inducing systemic or local inflammatory challenge, or directly perturbing microglial signaling produce measurable changes in microglial morphology, functional state, or engagement with synapses. 10/11 (91%) studies reported measurable microglial changes in response to sleep perturbation, inflammatory challenge, hypoxia, or direct manipulation of microglial signaling [3,6,29–31,39,41,46,48,49]. A consistent pattern of increased microglial activation or altered morphology emerged across paradigms, including greater phagocytic activity after sleep deprivation [3], NF-κB and p38 MAPK activation under intermittent hypoxia [30], increased Iba1 and M1 markers in offspring following maternal sleep deprivation [49], and heightened CD68/CD16/32 expression after chronic sleep fragmentation [48]. Studies targeting specific microglial pathways (P2RX7, TNF-α, CX3CR1, Gi-coupled GPCRs) converged on the finding that sleep or its disruption reliably engages microglial mechanisms that influence synaptic structure or function [11,31,39]. Both in vitro studies (2/2, 100%) replicated core features of microglial inflammatory activation, including NF-κB and MAPK pathway induction, increased TNF-α and IL-6 production, and microglia-induced neuronal toxicity [6,30]. A subset of 6/11 (55%) studies explicitly reported increases in canonical activation markers or secreted inflammatory mediators following the experimental manipulation (for example TNF-α/IL-6 increases with intermittent hypoxia, upregulation of CD68/CD16/32 with chronic fragmentation, and elevated Iba1 and M1 markers after maternal sleep deprivation) [6,29,30,46,48,49]. Although methodological heterogeneity existed, the dominant pattern indicated a consistent directional shift toward increased microglial activation, heightened cytokine-linked signaling, and enhanced microglial engagement with synaptic substrates following sleep disruption or related stressors.

Rodent models provided the clearest systems-level evidence linking microglial state to sleep and circuit function, showing alterations in NREM sleep duration [11], slow-wave parameters [39], cortical norepinephrine levels [31], hippocampal synaptic transmission [11], and sleep-dependent memory performance [39,46,49]. In vitro systems (primary microglia and microglial cell lines) reproduced cell-autonomous mechanisms such as TNF-α induced NF-κB activation, miR-342 regulated cytokine secretion [6], and hypoxia-triggered inflammatory signaling [30], supporting mechanistic plausibility but lacking behavioral endpoints. Studies involving microglial depletion and repopulation revealed altered baseline sleep and exaggerated or dysregulated responses to repeated inflammatory challenge, indicating that microglial history and state strongly shape sleep immune coupling [11,41]. Genetic and chemogenomic approaches further demonstrated pathway specificity: P2RX7–TNF-α signaling was required for sleep-associated GABAAR enrichment at inhibitory synapses [39], CX3CR1 regulated phase-dependent synaptic transmission and NREM sleep [11], and Gi-coupled GPCR activation promoted sleep via Ca^{2+}-dependent suppression of cortical norepinephrine [31]. The body of evidence provides moderate support for a causal contribution of microglia to sleep synapse interactions. This conclusion is grounded in convergent interventional data showing that targeted disruption of microglial pathways (for example microglial TNF-α deletion [39], P2RX7 blockade [39], CX3CR1 deficiency [11], microglial depletion [11,41], Gi-pathway activation [31]) produces predictable changes in sleep architecture, synaptic receptor composition, electrophysiological responses, or behavior. Overall, findings across the included studies consistently suggest that microglia respond to and influence sleep-related processes, with moderate-strength evidence supporting a functional and mechanistic role. The full evidence summary table for the cytokine axis is shown in Table 3.

 doi:10.20944/preprints202512.1753.v2

Table 3. Summary of microglia-focused mechanistic studies evaluating neuroimmune mechanisms of sleep deprivation.

Author, Year	Bellesi et al., 2017 [3]	Brás et al., 2020 [6]	Zhao et al., 2015 [49]
System	Mouse cortex; microglia-mediated synaptic remodeling across sleep, wake, and sleep deprivation	Primary rat mixed glia and N9 murine microglial cell line	Pregnant Wistar rats and their prepuberty male offspring; hippocampal microglia, and cognition
Mechanism/ Measured outcome studied	Microglial phagocytosis via CX3CR1, complement, and vesicle engulfment; ultrastructural synaptic changes measured by serial block-face and TEM	TNF-α–induced microglial activation; miRNA microarray screening; RT-qPCR validation; gain- and loss-of-function manipulation of miR-342.	Maternal sleep deprivation effects on microglial activation states (M1/M2 markers) and minocycline modulation of microglial activation.
Condition	Sleep, spontaneous wake, and 8 h sleep deprivation	Primary microglia stimulated with TNF-α; miR-342 overexpression or inhibition; co-culture with hippocampal neurons.	72-hour maternal sleep deprivation during late gestation; offspring assessed prepuberty with or without intraperitoneal minocycline.
Key Findings	Microglia showed increased phagocytic activity after sleep deprivation, with larger somata, more phagocytic vesicles, and upregulated markers of activation. Sleep did not induce these changes. The authors report that microglia are capable of regulating both excitatory and inhibitory synaptic elements, and that prolonged wakefulness shifts microglia toward greater synaptic engulfment.	TNF-α stimulation increased phosphorylated NF-κB p65 levels in microglia. Inhibition of miR-342 reduced cytokine release in TNF-α-stimulated microglia. Both TNF-α-activated and miR-342-overexpressing microglia reduced neuron viability in co-culture. The study identifies miR-342 as a key mediator of TNF-α-driven microglial activation and associated neurotoxicity.	Maternal sleep deprivation increased Iba1+ microglia in offspring hippocampus, reduced hippocampal neurogenesis, and impaired spatial learning and memory. Offspring showed elevated M1-associated markers and reduced M2-associated markers. Minocycline treatment improved Morris Water Maze performance, reduced Iba1+ microglia, and restored M2 markers. The study concludes that disrupted balance of microglial pro- and anti-inflammatory activation contributes to impaired cognition in MSD offspring.
Limitations	Findings are limited to acute sleep deprivation; functional consequences on neural activity or behavior were not directly assessed; small sample size limits generalizability.	The study relies entirely on in vitro systems using primary rat microglia and the N9 microglial cell line, which may not fully replicate the complexity of microglial behavior in the intact brain.	Findings are limited to a rat model and may not generalize to humans. Sleep deprivation was acute and applied only in late gestation, so effects across other developmental windows were not assessed. Only male offspring were evaluated, restricting interpretation across sexes.
Author, Year	Liu et al., 2017 [30]	Liu et al., 2022 [29]	Xie et al., 2020 [48]

 doi:10.20944/preprints202512.1753.v2

System	In vitro microglial model; BV2 or primary microglia exposed to intermittent hypoxia	Prefrontal cortex microglia of mice assessed with imaging and molecular assays	Wild-type mouse model exposed to chronic sleep fragmentation for two months.
Mechanism/ Measured outcome studied	Propofol effects on microglial inflammatory signaling; cell viability under different propofol concentrations.	Microglial activation and morphology; effects of minocycline on microglial activation and behavior.	Spatial learning and memory, anxiety-like behavior, and microglial activation markers (CD68, CD16/32, CD206)
Condition	Intermittent hypoxia consisted of alternating exposures between 1% and 21% O_2 in 400-second cycles for a total duration of 8 hours. Propofol was administered as a pre-treatment at concentrations of 0, 25, 50, or 100 μM, applied 30 minutes before the onset of intermittent hypoxia. Control cultures were maintained under normoxic conditions without propofol exposure.	6-hour acute partial sleep deprivation	Chronic sleep fragmentation inducing long-term sleep insufficiency
Key Findings	Intermittent hypoxia activated NF-κB and p38 MAPK pathways in microglia and significantly increased TNF-α and IL-6 expression and secretion. The study concludes that propofol suppresses microglial inflammatory responses induced by intermittent hypoxia.	Microglia in the prefrontal cortex showed activated morphology with increased soma area and reduced branching. Minocycline attenuated anxiety-like behavior and partially reduced microglial activation.	Chronic sleep fragmentation impaired spatial learning and memory and increased anxiety-like behavior. Microglia showed enhanced activation with increased CD68, CD16/32, and CD206 expression. The study concludes that chronic sleep insufficiency initiates microglia-mediated neuroinflammation.
Limitations	Experiments were conducted entirely in vitro, limiting applicability to in vivo CNS physiology. The study focused on NF-κB and p38 MAPK pathways, so broader signaling effects of propofol were not assessed.	Microglial activation was inferred primarily from morphology without phenotype-specific markers. Findings are acute and may not generalize to chronic sleep loss.	Microglial activation markers did not clarify functional phenotypes or cytokine output.
Author, Year	Vicente et al., 2023 [46]	Ma et al., 2024 [31]	Corsi et al., 2022 [11]
System	Streptozotocin (STZ; 2 mg/kg, ICV) rat model of sporadic Alzheimer's disease	Mouse model with chemogenomic and pharmacological manipulation of microglial Gi-coupled GPCR signaling; two-photon imaging of	Male mice with microglial depletion using CSF-1R antagonist PLX5622; cx3cr1GFP/GFP mice; ex vivo hippocampal synaptic transmission recordings

 doi:10.20944/preprints202512.1753.v2

		microglial Ca^{2+} and cortical norepinephrine	
Mechanism/ Measured outcome studied	Cognitive performance, chemoreflex responses to hypercapnia and hypoxia, total sleep time, locus coeruleus microglial density and morphology,	Microglial regulation of sleep via Gi-coupled GPCRs, intracellular Ca^{2+} signaling, P2Y12 receptor activity, norepinephrine suppression, and adenosine modulation.	Microglial regulation of sleep/wake cycle and hippocampal excitatory neurotransmission via CX3CR1 receptor signaling and response to light/ATP.
Condition	STZ-induced sporadic AD model with or without minocycline (30 mg/kg, IP) treatment for five days.	Chemogenomic activation of microglial Gi signaling, pharmacological blockade of P2Y12 receptors, and natural wake-to-sleep transitions	Microglial depletion (PLX5622) and genetic CX3CR1 deficiency; assessment across light and dark phases.
Key Findings	Minocycline treatment improved learning and memory and normalized microglial density and morphology in the LC. It restored daytime sleep-wake patterns but did not reverse STZ-induced increase in CO2 sensitivity during wakefulness. The study indicates that minocycline can reduce microglial activation and partially recover cognitive and sleep deficits in the STZ-AD model.	Activation of microglial Gi signaling strongly promoted sleep, while blockade of P2Y12 receptors reduced sleep. Gi-induced sleep increase required intracellular Ca^{2+} elevation in microglia. Microglial activation decreased cortical norepinephrine levels partly through adenosine elevation. Natural wake-to-sleep transitions were associated with increased microglial Ca^{2+}. The study demonstrates that microglia can regulate sleep via reciprocal interactions with norepinephrine transmission and intracellular Ca^{2+} signaling.	Near-complete microglial depletion increased NREM sleep duration and reduced hippocampal excitatory neurotransmission. CX3CR1 signaling is essential, as CX3CR1GFP/GFP mice showed similar sleep and synaptic changes. Microglial CX3CR1 expression varied with the light phase and decreased in response to ATP stimulation. Microglia modulate sleep and synaptic activity in a phase-dependent manner through CX3CR1-mediated signaling.
Limitations	Treatment duration was short (five days), limiting understanding of long-term effects.	Mechanistic pathways linking Ca^{2+} signaling, norepinephrine suppression, and adenosine production remain partially unresolved. Findings are limited to cortical microglia, potentially excluding region-specific microglial contributions.	Study focused on male mice, limiting generalization across sexes. Effects of long-term microglial depletion on overall brain function and behavior were not assessed.
Author, Year	Pinto et al., 2024 [39]		Rowe et al., 2022 [41]
System	Microglial TNF-α knockout mice vs wild-type; microglial depletion; P2RX7 KO; sleep vs sleep deprivation (ZT6 vs ZT18).		Central nervous system – microglial depletion and repopulation in relation to sleep regulation following inflammatory challenge.
Mechanism/ Measured	The work examines how microglia influence synapse density, synaptic pruning, and activity-dependent remodeling. It also		Microglial presence, morphology, and functional involvement in sleep regulation after systemic LPS-induced inflammatory

 doi:10.20944/preprints202512.1753.v2

outcome studied	evaluates physiological or molecular readouts that reflect microglial engagement with neuronal circuits, including synaptic markers, electrophysiological changes, and gene-expression profiles.	challenge; quantitative skeletal morphology analysis; sleep recording via piezoelectric monitoring.
Condition	Baseline sleep, sleep deprivation, microglial TNF-α deletion, P2RX7 inhibition, and TNF-α neutralization	PLX5622-induced microglial depletion in mice, followed by LPS administration (0.4 mg/kg), microglial repopulation period, and a second LPS challenge.
Key Findings	The study found that neuronal ATP activates microglial P2RX7, leading to release of soluble TNF-α. This TNF-α increased CaMKIIα phosphorylation and promoted enrichment of $GABA_AR$s at inhibitory synapses in cortical layer 1 during sleep. Loss of microglial TNF-α or P2RX7 signaling reduced inhibitory synaptic $GABA_A$ receptor levels, altered NREM slow-wave parameters, and impaired performance on sleep-dependent memory tasks. The study also notes that microglia influence both excitatory and inhibitory synaptic regulation, and this work identifies a sleep-specific role in controlling inhibitory synapse strength.	Microglial depletion altered baseline sleep. Following the first LPS injection, microglia-depleted mice showed enhanced sleep relative to controls, indicating dysregulated sleep–immune coupling. Repopulated microglia exhibited reactive morphology distinct from controls. Across LPS exposures, control mice displayed temporal shifts in sleep architecture without changes in cumulative sleep time, whereas mice with repopulated microglia showed reduced dark-phase cumulative sleep, indicating altered microglial functional integration. Findings support that intact microglia are required for appropriate sleep modulation following inflammatory challenge.
Limitations	Primarily mouse data; restricted to cortical layer 1; the study does not account for potential developmental changes or long-term compensatory adaptations in knockout or chronically manipulated models; reliance on pharmacologic and knockout models with limited temporal specificity.	Microglial depletion was global rather than region-specific, limiting anatomical inference. Only acute LPS exposure was tested, and mechanistic molecular markers beyond morphology were limited.

Mechanistic Narrative

According to the literature, extracellular ATP accumulation during sleep deprivation due to heightened neuronal activity was found to act as a key trigger for microglial state transitions [50]. ATP engagement of P2X7 reliably increased microglial phagocytic activity [3,10], reflected by elevated CD68, and CD16/32 consistent with a phagocytic profile, and coincided with enhanced synaptic pruning responses [10,48,49]. This P2X7-driven shift occurred alongside inflammasome-dependent cytokine release [39], indicating that ATP functions as both a danger signal and a pruning cue in sleep-loss relevant conditions.

Simultaneously, ATP sensing through P2Y12, together with parallel CX3CR1 signaling, supported microglial process motility and targeted engulfment of synaptic elements. ATP-driven P2Y12 activation, occurring alongside CX3CR1-mediated signaling promoted process extension toward active synapses and maintained similar phagocytic profile, indicating coordinated receptor-specific contributions to microglial engulfment behavior [3,11,31]. Together, these results demonstrate that ATP engages two separable microglial pathways: a purinergic mechanism and a CX3CR1 pathway, each contributing differentially to microglial modulation of synaptic architecture.

 doi:10.20944/preprints202512.1753.v2

Discussion

This systematic synthesis identifies convergent neuroimmune and cellular pathways activated by sleep deprivation, spanning cytokine signaling, microglial state transitions, oxidative stress responses, and HPA-axis activity. Together, these results delineate a coordinated multi-system response linking sleep loss to neuroinflammatory and synaptic alterations. Across 29 included studies, we found consistent activation of pro-inflammatory cytokines, convergent microglial morphological and functional changes, and reproducible alterations in sleep pressure following targeted perturbations. Although methodological heterogeneity was present, a common directional pattern emerged in which sleep disruption caused an inflammatory state in the brain and peripheral tissue. Findings across the cytokine, microglial, and oxidative stress-HPA axes converged on a shared set of upstream processes involving extracellular ATP [3,31,39,50], inflammasome activation [48,50], and glucocorticoid or catecholamine dependent modulation of inflammatory tone [15,16,18,19,31,47]. Elevated ATP consistently activated P2RX7/P2Y12-mediated microglial pathways, resulting in altered phagocytic profiles and increased engagement with synaptic substrates. In parallel, TNF-α and IL-1β signaling activated canonical (e.g., NF-κB / MAPK) pathways, shaping downstream transcriptional responses that aligned with observed morphological changes [2,6,25,26,30,33,35]. Together, these mechanistic threads point toward a coordinated multi-axis response rather than isolated pathway activation. The patterns identified in this review are broadly consistent with prior work demonstrating immune involvement in sleep regulation, microglial contributions to synaptic remodeling, and the sensitivity of cytokine networks to physiological stressors. The observed modulation of TNF-α during sleep disruption aligns with longstanding evidence linking these cytokines to sleep intensity and homeostatic regulation. The convergent role of P2RX7 and CX3CR1 signaling in microglial process dynamics resembles findings from developmental synaptic pruning and disease models, indicating that sleep loss induced mechanisms leverage conserved microglial pathways [3,11,39]. However, relative to earlier descriptive work, the present synthesis more clearly separates purinergic, cytokine, and neuroendocrine contributions and highlights their intersection in shaping sleep synapse coupling. This distinction may refine theoretical models describing how immune signals reorganize neural activity during sleep loss. A key strength of the included literature is the use of multimodal approaches, combining ultrastructural imaging; electrophysiology; genetic perturbation; and molecular profiling, to dissect microglial and cytokine mechanisms with high resolution. Interventional designs such as microglial depletion, pathway-specific knockouts, and targeted receptor manipulation provide causal leverage that strengthens mechanistic inference. The reproducible directionality of cytokine and microglial responses across diverse paradigms suggests robustness of the core biological signal. Future work should include standardized sleep-disruption paradigms to improve cross-study comparability, alongside longitudinal designs that identify how microglial and cytokine dynamics evolve over time. Expanded investigation of the oxidative stress and HPA-axis, including glucocorticoid resistance and β-adrenergic contributions, would strengthen mechanistic models of neuroimmune integration. Ideally, translational work utilizing human biosamples or polysomnography-linked inflammatory [8] profiling will be needed to establish clinical relevance and refine therapeutic targets.

Limitations

This review has several methodological and conceptual limitations that should be considered when interpreting the synthesized evidence. First, the **literature included displays substantial heterogeneity in experimental paradigms**, with studies differing in outcome measures, tissue sources, and analytical platforms. Such variability constrains direct comparison and complicates efforts to draw unified mechanistic conclusions. A second limitation is the **predominant reliance of animal and in vitro studies on interpreting mechanistic insights and translational value of conclusions**, particularly with respect to microglial activation states, oxidative stress pathways, and systems-level neuroimmune interactions. Relatedly, there remains limited human data on microglial

 doi:10.20944/preprints202512.1753.v2

dynamics and oxidative stress axes in the context of sleep deprivation, which restricts the ability to evaluate whether the mechanisms observed in preclinical models accurately reflect human neurobiology. Finally, this review employed **single-reviewer screening and did not conduct a formal risk-of-bias assessment**, which may introduce selection bias and limits the methodological rigor compared to systematic reviews that adhere to multi-reviewer and validated appraisal protocols. As a result, the conclusions presented should be interpreted as integrative trends rather than definitive causal statements. These methodological choices reflect an intentional trade-off prioritizing biological interpretation over quantitative precision.

Conclusions

This review demonstrates that sleep disruption consistently engages cytokine, microglial, and neuroendocrine mechanisms that converge on synaptic and circuit-level processes. Across models, elevated ATP signaling, cytokine activation, and microglial remodeling represent robust biological pathways through which sleep loss shapes neural function. Although further work is needed to establish translational relevance, the integrated evidence base supports a multi-axis framework for understanding how immune processes regulate sleep and its associated cognitive functions.

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