Chronic Circadian Disruption as a Driver of Microglial NLRP3-Mediated Tau Pathology in Alzheimer's Disease: A Research Proposal

La perturbation chronique du rythme circadien comme moteur de la pathologie tau médiée par l'inflammasome NLRP3 microglial dans la maladie d'Alzheimer : une proposition de recherche

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Abstract | Résumé

Alzheimer's disease (AD) affects more than 55 million people worldwide and has no approved disease-modifying treatment. One known risk factor is disruption of the circadian rhythm — the body's internal 24-hour clock — but exactly how this disruption contributes to the buildup of toxic tau protein in the brain is unknown. Brain immune cells called microglia have their own internal clocks, controlled by the BMAL1/CLOCK protein complex. When this clock is disrupted, microglia become chronically inflamed. Separately, activation of a microglial inflammatory pathway called the NLRP3 inflammasome causes tau to become abnormally phosphorylated — a key step in forming the neurofibrillary tangles (NFTs) that kill neurons in AD. This proposal identifies an untested mechanistic link between these findings. It is hypothesized that chronic jet lag (CJL), a laboratory model of circadian disruption, would impair microglial BMAL1 rhythmicity and thereby drive unchecked NLRP3 activation and accelerated tau pathology in P301S tauopathy mice. Five groups would be compared, using the pharmacological NLRP3 blocker MCC950 as a rescue approach to confirm causality. These findings would identify a modifiable lifestyle factor as a direct driver of AD and point to microglial NLRP3 as a promising therapeutic target.

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La maladie d'Alzheimer (MA) touche plus de 55 millions de personnes dans le monde, et il n'existe toujours pas de traitement approuvé capable de modifier directement l'évolution de la maladie. Un facteur de risque connu est la perturbation du rythme circadien, c'est-à-dire l'horloge interne de 24 heures du corps. Cependant, on ne sait pas encore exactement comment cette perturbation contribue à l'accumulation de la protéine tau toxique dans le cerveau. Les cellules immunitaires du cerveau, appelées microglies, possèdent aussi leur propre horloge interne. Cette horloge est contrôlée par le complexe de protéines BMAL1/CLOCK. Lorsque cette horloge est perturbée, les microglies peuvent rester dans un état d'inflammation chronique. De façon séparée, l'activation d'une voie inflammatoire microgliale appelée inflammasome NLRP3 peut rendre la protéine tau anormalement phosphorylée. Cette étape est importante dans la formation des enchevêtrements neurofibrillaires, qui contribuent à la mort des neurones dans la MA. Cette proposition met donc en évidence un lien mécanique qui n'a pas encore été testé directement. L'hypothèse est que le décalage horaire chronique, utilisé en laboratoire comme modèle de perturbation circadienne, pourrait affaiblir le rythme de BMAL1 dans les microglies. Cela pourrait ensuite entraîner une activation non contrôlée de NLRP3 et accélérer la pathologie tau chez des souris P301S, un modèle de tauopathie. Cinq groupes seraient comparés, et le bloqueur pharmacologique de NLRP3, MCC950, serait utilisé comme approche de "sauvetage" afin de confirmer le lien de causalité. Ces résultats pourraient montrer qu'un facteur de mode de vie modifiable contribue directement à la MA et que NLRP3 dans les microglies est une cible thérapeutique prometteuse.

Keywords: Alzheimer's disease; circadian rhythm disruption; NLRP3 inflammasome; microglia; tau hyperphosphorylation; MCC950; tauopathy

Background and Rationale

AD accounts for 60–70% of all dementia cases globally (1). As shown in Figure 2, World Bank data on the proportion of people aged 65 and over shows growth from 6.9% in 2000 to 9.8% in 2022. Statistical modelling projects this will reach 17.8% globally — and 28.5% in high-income countries — by 2050. Because AD risk rises sharply with age, this demographic shift means the disease burden is accelerating rapidly, yet there is still no way to slow or reverse it.

Disrupted circadian rhythms are an increasingly recognized and potentially modifiable risk factor for AD. Sleep disruption and circadian misalignment in midlife consistently raise the risk of developing AD, and circadian behavioural changes — including sundowning — appear years before obvious memory loss (2). Tau protein has been found to accumulate in neurons connecting to the suprachiasmatic nucleus (SCN), the brain's master clock, creating a self-reinforcing loop where tau pathology worsens circadian function (2). Whether the reverse is also true — whether circadian disruption itself accelerates tau buildup — remains unanswered.

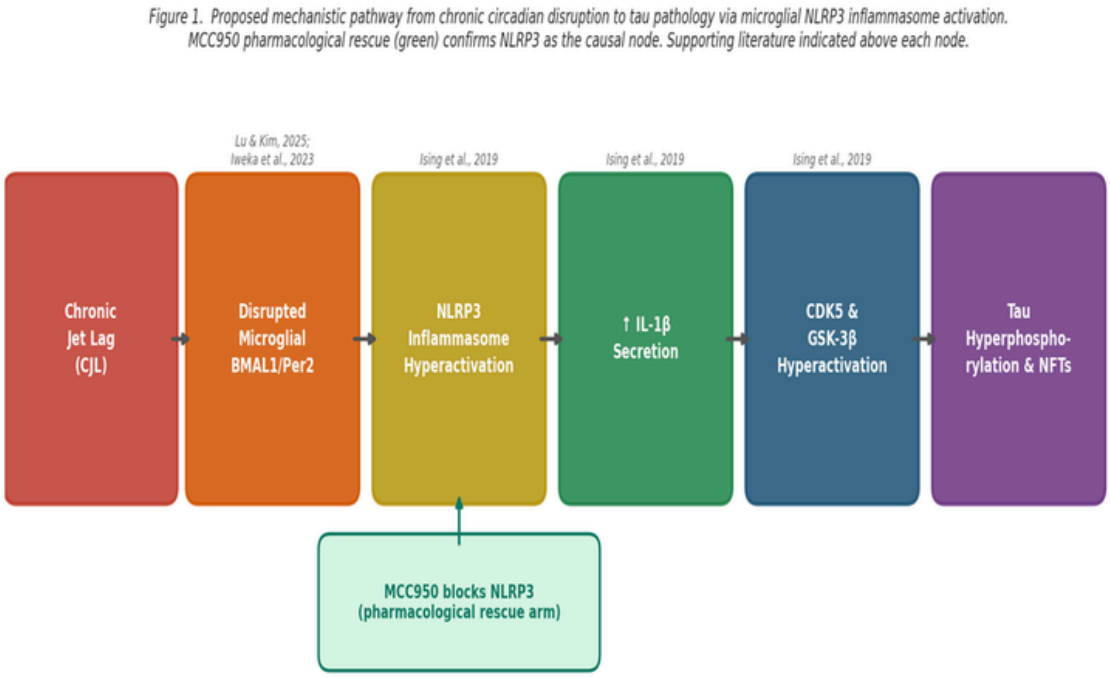

Figure 1. Proposed mechanistic pathway from chronic circadian disruption to tau pathology via microglial NLRP3 inflammasome activation. Each arrow represents a proposed causal step supported by the cited literature. The green box shows where MCC950 would intervene to confirm NLRP3 as the key causal node. BMAL1 = brain and muscle ARNT-like 1; Per2 = period circadian regulator 2; NLRP3 = NOD-like receptor protein 3; IL-1β = interleukin-1 beta; CDK5 = cyclin-dependent kinase 5; GSK-3β = glycogen synthase kinase 3 beta; NFTs = neurofibrillary tangles.

Microglia have their own internal circadian clocks driven by the brain and muscle ARNT-like 1 (BMAL1)/CLOCK molecular feedback loop. Deleting BMAL1 specifically in immune cells accelerates cognitive aging in mice through chronic inflammatory activation and impaired synaptic pruning (3). External neuroinflammation has also been shown to shift the phase of microglial BMAL1, locking microglia in a pro-inflammatory state (4). This raises the possibility that circadian disruption could constitutively activate microglia.

Crucially, activation of the microglial NLRP3 inflammasome drives tau hyperphosphorylation by triggering release of interleukin-1 beta (IL-1β), which activates the tau kinases cyclin-dependent kinase 5 (CDK5) and glycogen synthase kinase 3 beta (GSK-3β) while suppressing the phosphatase PP2A in the Tau22 tauopathy model (5). Together with Figure 1, these findings converge on an untested chain — CJL → impaired microglial BMAL1 → uncontrolled NLRP3 → IL-1β → accelerated tau phosphorylation and NFT formation — that this proposal is designed to test directly

Research Question and Objectives

Primary Research Question: Would chronic circadian disruption worsen microglial NLRP3 inflammasome activation and tau phosphorylation in a tauopathy mouse model, and would blocking NLRP3 pharmacologically reverse these effects?

Specific Aim 1
To determine whether CJL disrupts BMAL1 and period circadian regulator 2 (Per2) expression rhythms in microglia of P301S mice compared to normally entrained controls.

Hypothesis 1: CJL-exposed P301S mice would show significantly reduced BMAL1 amplitude, and disrupted Per2 oscillation timing compared to normally entrained P301S controls, reflecting loss of microglial circadian gating.

Specific Aim 2
To assess whether disrupted microglial clock function is associated with elevated NLRP3 inflammasome activation and IL-1β release in the hippocampus of CJL-treated P301S mice.

Hypothesis 2: NLRP3, apoptosis-associated speck-like protein containing a CARD (ASC), and cleaved IL-1β would be significantly higher in P301S+CJL mice than in normally entrained P301S controls, correlating inversely with BMAL1 amplitude.

Specific Aim 3
To test whether CJL-driven NLRP3 activation accelerates tau phosphorylation and memory impairment in P301S mice, and whether MCC950 reverses these effects.

Hypothesis 3: P301S+CJL mice would show greater AT8 (phospho-Ser202/Thr205) and PHF1 (phospho-Ser396/Ser404) tau phosphorylation and worse Morris Water Maze (MWM) performance than P301S+NLC mice, with both outcomes significantly reduced by MCC950.

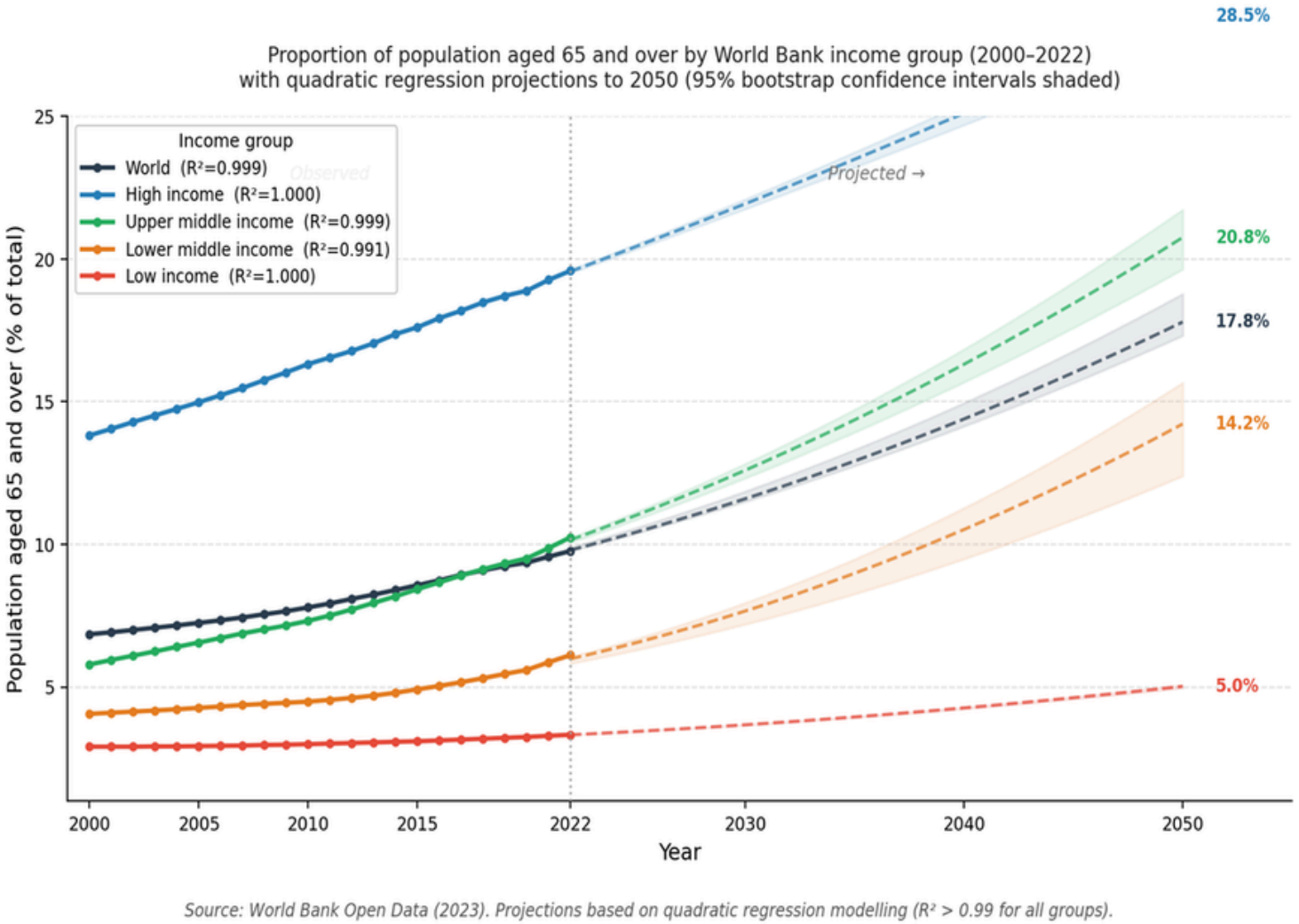

Figure 2. Proportion of population aged 65 and over by World Bank income group (2000–2022), with quadratic regression projections to 2050 and 95% bootstrap confidence intervals (shaded). Solid lines = observed data; dashed lines = model-based projections. $R^2 > 0.99$ across all income groups. Projections assume continuation of historical aging trends and do not account for potential future disruptions. Source: World Bank Open Data (2023) (8).

Proposed Methodology

Study design

Five groups of P301S (PS19) transgenic mice (Jackson Laboratories #008169) would be established at 3 months of age, before overt tau pathology: (1) wild-type (WT) + normal light-dark cycle (NLC); (2) WT + CJL; (3) P301S + NLC; (4) P301S + CJL; (5) P301S + CJL + MCC950. A total of n=55 animals (n=11 per group with equal sex distribution, including 10% over-recruitment for attrition) would be used. Power calculations indicate n=10 per group achieves 82% power to detect a medium effect size (Cohen's d=0.8) at α=0.05, based on published tau phosphorylation variability in this model. Animals would be randomly assigned using a computer-generated sequence stratified by sex. Any animal meeting pre-defined exclusion criteria (>20% body weight loss unrelated to the experiment) would be excluded and documented.

Circadian disruption

CJL would be induced by shifting the light phase forward by 8 hours every 3 days for 12 weeks — an established protocol that disrupts both central and peripheral circadian synchrony without total sleep deprivation (6). Locomotor activity would be continuously recorded by infrared actigraphy to confirm entrainment disruption.

Pharmacological rescue

Group 5 would receive MCC950 (50 mg/kg i.p., every other day), a selective NLRP3 inhibitor with established central nervous system (CNS) penetrance in mice (7). Group 4 would receive an equivalent vehicle.

Primary endpoints

At endpoint (15 months of age), microglia would be isolated from

brain tissue by CD11b+ magnetic-activated cell sorting (MACS). Clock gene expression (BMAL1 and Per2) would be measured by quantitative reverse transcription polymerase chain reaction (qRT-PCR) from cells collected at Zeitgeber Time (ZT) 0, 6, 12, and 18; cosinor rhythmometry would quantify amplitude and phase. NLRP3, ASC, and cleaved IL-1β would be measured by Western blot and Luminex multiplex immunoassay in brain tissue and cerebrospinal fluid (CSF). Tau phosphorylation (AT8, PHF1 antibodies) and upstream kinase activity (active GSK-3β at pTyr216; CDK5/p25 complex) would be assessed by Western blot. NFT burden would be quantified by AT8 immunohistochemistry and Gallyas silver staining. MWM and novel object recognition (NOR) tasks would assess spatial and recognition memory, conducted blind to group between ZT4–ZT8. Histological analyses would be performed by investigators blinded to group assignment throughout.

Statistical analysis

One-way ANOVA with Tukey's post-hoc test; Bonferroni correction applied across multiple endpoints. Sex was included as a covariate throughout. Within-group Pearson correlations (or Spearman's if normality is violated per the Shapiro-Wilk test) would assess the mechanistic linkage between IL-1β and tau phosphorylation. Missing data would be reported transparently; sensitivity analyses would be conducted if attrition exceeds 15%.

Feasibility and Justification

All proposed methods are well established in the field. P301S mice are commercially available, widely used, and well characterized. The CJL protocol requires only programmable lighting cabinets and has been implemented in numerous published studies. MACS isolation, qRT-PCR, Western blot, and Luminex assays are routine techniques in university neuroscience facilities. MCC950 is commercially available at an appropriate purity. An 18-month timeline — 3 months of colony setup, 12 months for the main cohort, and 3 months for analysis — is realistic under faculty supervision.

Expected Outcomes and Impact

P301S+CJL mice would likely show stronger NLRP3 activation, increased tau phosphorylation, and poorer memory performance compared with P301S+NLC controls. If MCC950 were able to largely reverse these effects, this would suggest that circadian disruption plays a direct pathomechanistic role in Alzheimer's disease, rather than simply being associated with it. This would represent an important conceptual shift. From a clinical perspective, identifying microglial NLRP3 activation as the causal link would support future trials of NLRP3 inhibitors in AD patients with documented sleep problems, a large group that remains relatively underserved. Several NLRP3-targeting therapies are already moving into Phase II testing, making this a realistic therapeutic direction.

Limitations and Risk Assessment

The P301S model carries a rare inherited tau gene mutation (MAPT) absent in most sporadic AD cases; however, the CDK5/GSK-3β kinase pathway under study is mutation-independent and expected to generalize across tauopathies. The CJL protocol disrupts peripheral circadian rhythms — including the hypothalamic-pituitary-adrenal (HPA) axis stress response — in addition to brain clocks, which could confound CNS-specific interpretation. Plasma corticosterone measurements and continuous actigraphy monitoring would be used to characterize systemic effects. Because MCC950 inhibits NLRP3 across all cell types, a conditional microglial Nlrp3 knockout (Cx3cr1-Cre; Nlrp3fl/fl) is planned as a confirmatory follow-up. Sex-stratified analysis is incorporated throughout, given documented differences in tau pathology progression between male and female P301S mice.

Future Directions

Confirmatory experiments using microglial-specific Bmal1 conditional knockout mice (Cx3cr1-Cre; Bmal1fl/fl) would establish whether the CJL effect is specifically driven by the microglial clock rather than peripheral inflammatory signals. Validation in the MAPT H1-GR humanized knock-in model would extend findings beyond the overexpression system. Translational studies measuring plasma p-tau217, serum IL-1β, and objective sleep metrics in early-stage AD patients stratified by circadian dysfunction severity would provide the first human biomarker evidence for this mechanistic axis and inform the design of NLRP3-targeting clinical trials.

Ethical Considerations

Any study of this design would require Institutional Animal Care and Use Committee (IACUC) approval and compliance with Canadian Council on Animal Care (CCAC) guidelines. The 3Rs principles would guide all decisions. Replacement: *in vitro* microglial models would be used first to refine hypotheses before moving to animal work. Reduction: group sizes are set by power analysis to minimize animal use. Refinement: the CJL protocol avoids total sleep deprivation; humane endpoints would be defined in advance (>20% body weight loss; severe motor impairment); animals would be euthanized by approved methods. No human participants or biological materials would be involved.

Abbreviations

AD = Alzheimer's disease; ASC = apoptosis-associated speck-like protein containing a CARD; BMAL1 = brain and muscle ARNT-like 1; CCAC = Canadian Council on Animal Care; CDK5 = cyclin-dependent kinase 5; CJL = chronic jet lag; CNS = central nervous system; CSF = cerebrospinal fluid; GSK-3β = glycogen synthase kinase 3 beta; HPA = hypothalamic-pituitary-adrenal; IACUC = Institutional Animal Care and Use Committee; IL-1β = interleukin-1

beta; MACS = magnetic-activated cell sorting; MAPT = microtubule-associated protein tau; MWM = Morris Water Maze; NFT = neurofibrillary tangle; NLC = normal light-dark cycle; NLRP3 = NOD-like receptor protein 3; NOR = novel object recognition; Per2 = period circadian regulator 2; PP2A = protein phosphatase 2A; p-tau217 = phosphorylated tau-217; qRT-PCR = quantitative reverse transcription polymerase chain reaction; SCN = suprachiasmatic nucleus; WT = wild-type; ZT = Zeitgeber Time

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