

Neuroinflammation modulates circadian rhythm robustness in a mouse model of tauopathy

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Background

The disruption of circadian rhythm (chronodisruption) is an early and progressive feature of AD, that often precedes and predicts cognitive decline and pathological progression [1]. Moreover, circadian disruption can worsen amyloid- β and tau pathology [2,3]. In parallel, neuroinflammation driven by the NLRP3 inflammasome is a key driver of AD: NLRP3-deficient mice show reduced amyloid- β load and cognitive decline [4], and loss of NLRP3 also reduces tau hyperphosphorylation and aggregation [5].

Yet, whether inflammatory signalling contributes *causally* to chronodisruption during AD progression remains unknown.

Aim & Hypothesis

We tested whether NLRP3 inflammasome signalling shapes the robustness and age-trajectory of circadian rhythms during tau-driven neurodegeneration. Because NLRP3-mediated neuroinflammation drives tau pathology [5], and chronodisruption is an early, common feature of AD that often precedes and predicts cognitive decline [1], we crossed *Nlrp3* deletion onto the pR5 (P301L) tauopathy model [6] to dissociate inflammatory from tau-intrinsic effects on rhythm strength. Four genotypes, pR5 (Tg/0) *Nlrp3* (+/+), pR5 (0/0) *Nlrp3* (+/+), pR5 (Tg/0) *Nlrp3* (-/-) and pR5 (0/0) *Nlrp3* (-/-), allow the effects of tau pathology and inflammasome deletion to be separated.

Hypothesis: inflammasome signalling modulates circadian robustness in an age- and disease-dependent manner, influencing the temporal trajectory of rhythm decline rather than its eventual endpoint.

Methods

Animal model & design

P301L tau-expressing pR5 mice, a well-established tauopathy model [6], were crossed with *Nlrp3* deletion to impair inflammasome activation, a pathway strongly implicated in amyloid and tau pathology [4,5]. Four genotypes were analysed to dissociate tau pathology from inflammasome signalling: pR5 (Tg/0) *Nlrp3* (+/+); pR5 (0/0) *Nlrp3* (+/+); pR5 (Tg/0) *Nlrp3* (-/-); and pR5 (0/0) *Nlrp3* (-/-). This 2 $\times$ 2 design isolates the main effects of, and the interaction between, tau expression and inflammasome loss.

Circadian phenotyping

- Locomotor (wheel-running) activity rhythms monitored longitudinally over ~1 year under both 12 h light : 12 h dark (LD) and intermittent constant darkness (DD).
- Circadian amplitude and period quantified under DD, in the absence of an external zeitgeber, providing an endogenous readout of clock output independent of environmental light cues.

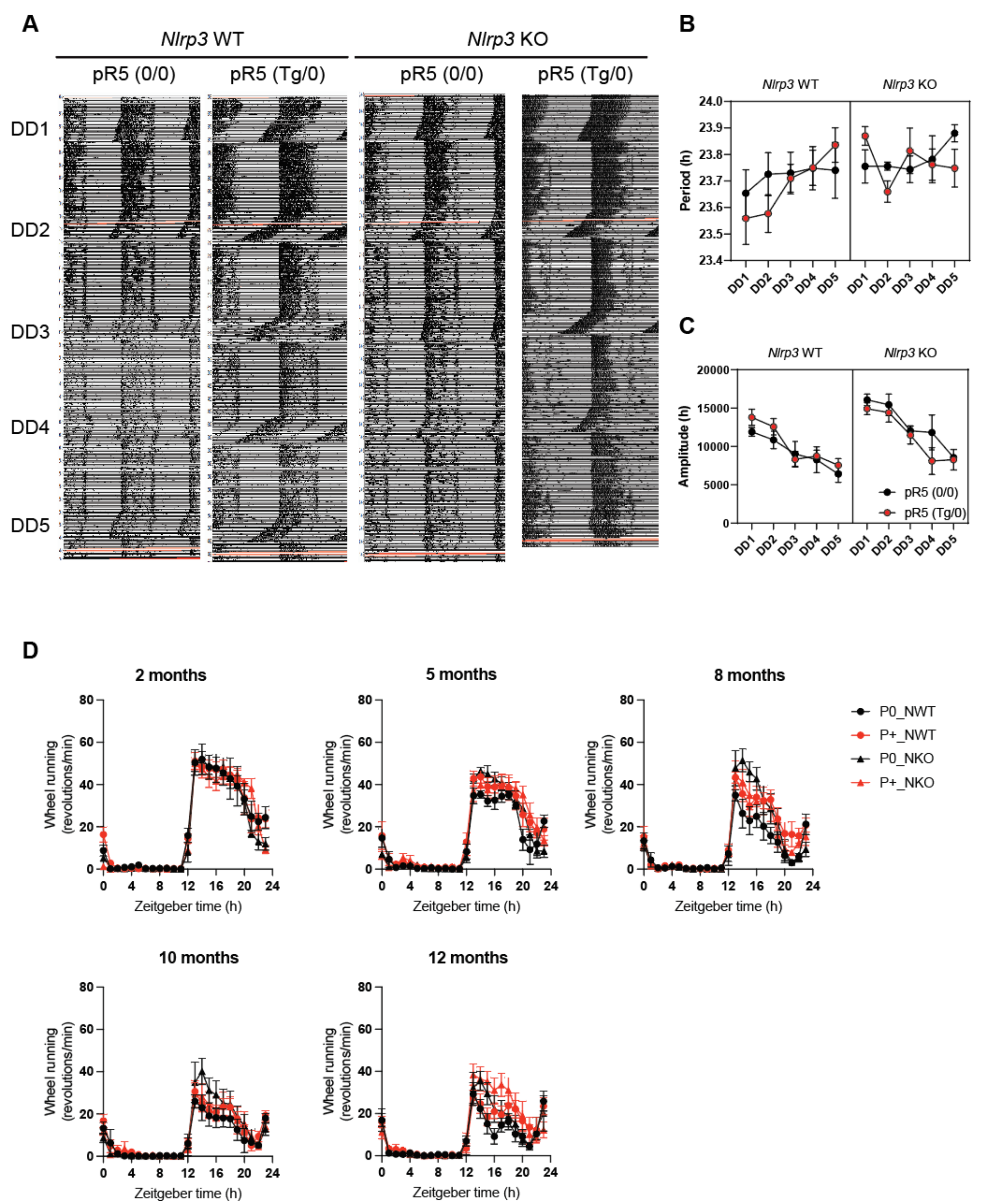
Transcriptomics

- Bulk RNA sequencing (BRB-seq) on the first cohort at 13 months of age; N = 3 per time point; four tissues: cerebellum, cerebral cortex, liver and skeletal muscle. All samples collected at ZT3.

References

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Result 1: Circadian phenotyping



Locomotor activity of pR5 $\times$ NLRP3 KO mouse. (A) Representative double-plotted actograms showing locomotor activity for male mice of the indicated genotypes exposed to a 12-hour light/dark cycle (LD) followed by free running conditions in constant darkness (DD) across five sequential 3-week intervals (DD1 to DD5). Red lines mark periods where recording was interrupted due to technical issues. (B) Circadian period length and (C) rhythm amplitude during DD, measured under constant darkness in the absence of an external zeitgeber. N = 5 to 7 per genotype. (D) Locomotor activity profile across a 24-hour window for indicated genotype and age under LD.

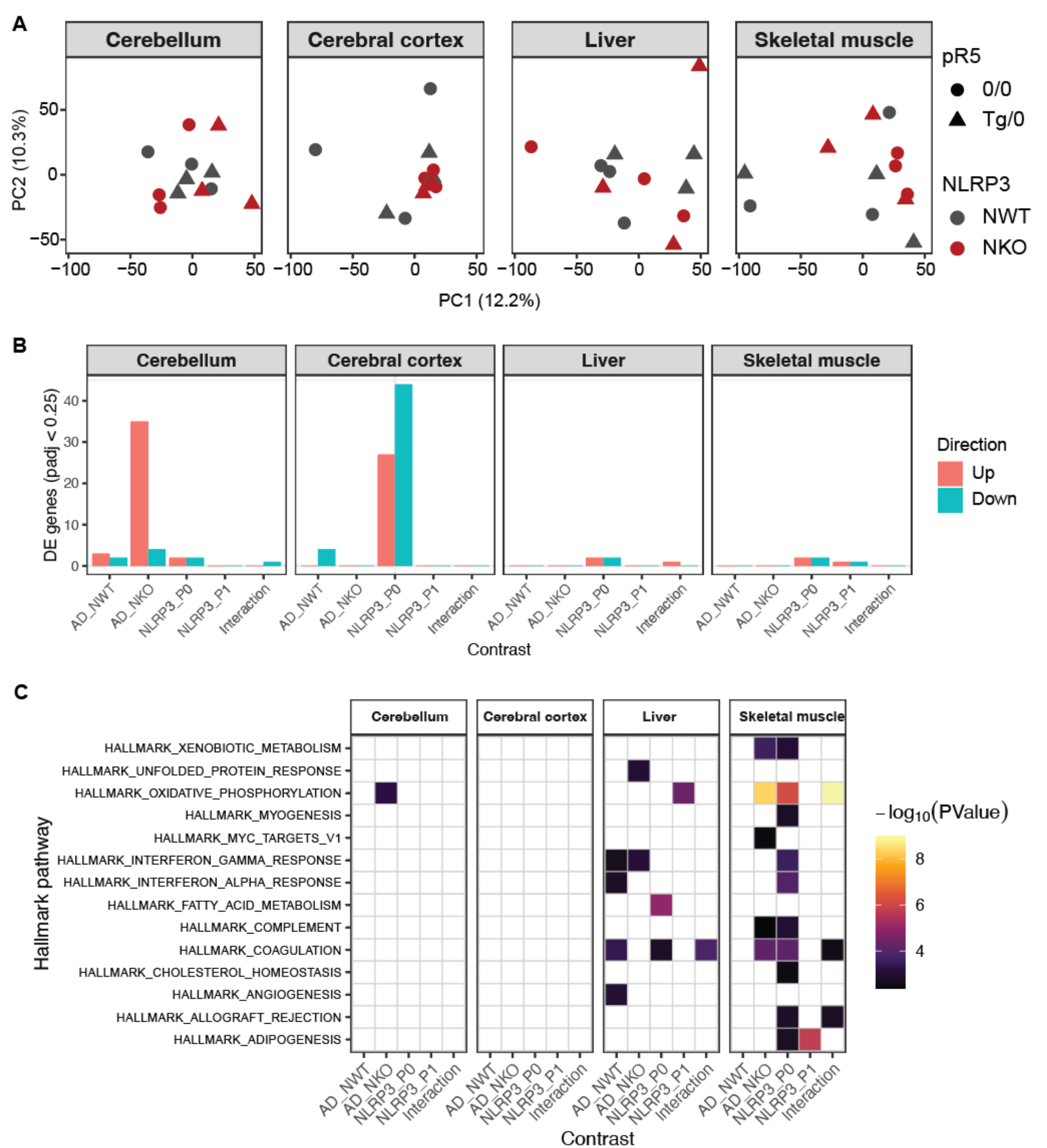
Amplitude declines with age; inflammasome status shapes the trajectory

Across all four genotypes, wheel-running rhythm amplitude declined progressively with age under both LD and DD (Fig. 1), consistent with the age-related weakening of circadian output [7]. Under LD, two-way repeated-measures ANOVA (LD1–LD4) revealed no significant main effect of genotype, neither *Nlrp3* deletion nor pR5 status altered diurnal locomotor patterns overall, yet significant time $\times$ genotype interactions emerged at 8 months ($P = 0.0053$) and 12 months ($P < 0.0001$), indicating subtle divergence between groups as the AD phenotype progressed.

Under constant darkness, *Nlrp3* loss raises amplitude but may hasten early decline

- Amplitude decreased with age under DD (mixed linear model, main effect of age $P < 0.0001$), confirming intrinsic, zeitgeber-independent rhythm deterioration (Fig. 1).
- *Nlrp3* (-/-) mice showed generally higher amplitude than *Nlrp3* (+/+) counterparts (main effect $P = 0.0071$), independent of pR5 status (pR5 main effect $P = 0.7298$), mice with an intact inflammasome exhibited overall lower rhythm robustness across age.
- While pR5 (Tg/0) and pR5 (0/0) did not differ on the *Nlrp3* (+/+) background, pR5 (Tg/0) *Nlrp3* (-/-) mice showed an earlier amplitude-reduction tendency at 10 months versus pR5 (0/0) controls (unadjusted $P = 0.0328$, a tendency only), with all groups converging by 12 months, suggesting inflammasome signalling may help delay, rather than prevent, the age-dependent decline in circadian output in this preliminary first cohort.
- Circadian period under DD remained largely stable (age $P = 0.0474$; *Nlrp3* $P = 0.1942$; pR5 $P = 0.6741$), with slightly greater period variability in pR5 (Tg/0) mice on the *Nlrp3* (-/-) background.

Result 2: Tissue transcriptomics



Tissue-specific differential expression (13 months, ZT3; BRB-seq). (A) PCA of cerebellum, cerebral cortex, liver and skeletal muscle. (B) Up-/down-regulated DE-gene counts per contrast. (C) Hallmark pathway enrichment (camera); OXPHOS recurs across tissues.

Bulk RNA sequencing (BRB-seq) at 13 months (ZT3; N = 3 per time point) across cerebellum, cerebral cortex, liver and skeletal muscle revealed subtle, tissue-specific transcriptomic remodelling. Differentially expressed (DE) gene counts were modest, consistent with the small sample size.

Transcriptional changes were strongest in brain and far weaker in periphery. In cerebellum, most DE genes were upregulated in pR5 (Tg/0) versus (0/0) on the *Nlrp3* (-/-) background, an AD effect emerging only in the absence of a functional inflammasome. Cortex showed both up- and down-regulation, strongly *Nlrp3*-dependent when comparing (-/-) with (+/+) in pR5 (0/0) animals.

Competitive gene-set analysis (*camera*) flagged **oxidative phosphorylation (OXPHOS) as a recurrent theme**, dysregulated in cerebellum, in skeletal muscle (AD $\times$ *Nlrp3*, including interaction effects), and in liver (*Nlrp3* effect confined to pR5 (Tg/0)). Coagulation pathways were enriched in peripheral tissues. Thus, in this first, preliminary cohort, AD genotype and inflammasome status interact in a tissue-specific manner, converging on OXPHOS.

Conclusions

- Inflammasome signalling **modulates the temporal trajectory** of circadian disruption in tauopathy rather than preventing its eventual decline.
- *Nlrp3* loss raises baseline rhythm amplitude but, on the tau background, brings the age-dependent decline forward before convergence by 12 months.
- AD genotype and *Nlrp3* status interact tissue-specifically, converging on a recurrent OXPHOS signature.
- Next: higher-powered, full circadian-resolution RNA-seq is underway.

Funding



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