

# The circadian clock and its neuro-protective role during aging

## **INTRODUCTION**

- ⑦ Circadian clocks generate daily rhythms in sleep/activity, physiological, metabolic and cellular processes.
- Provide the second s 24h and are entrained (set) to local time by external Zeitgebers (time-givers) such as day/night cycles.
- Disrupted circadian rhythms lead to agerelated pathologies, and may reduce lifespan [1,2].
- **We demonstrated that fruitflies without a** functional clock gene period (per<sup>01</sup>) are more susceptible to oxidative stress during aging [3].
- **W** Neurodegeneration is often associated with accumulation of oxidative damage in the nervous system.

### Hypothesis

The circadian clock gene period (per) may contribute to cellular homeostasis by curbing oxidative damage in the nervous system.

### Fruitflies as model organisms

- Single copy of every clock gene
- Short lifespan (~75 days)  $(\mathbf{N})$
- Ease of maintenance  $(\mathbf{N})$
- Fly and mammalian clocks conserved
- Aging symptoms similar to humans

# **METHODS**

Fly rearing and strains: 12h light: 12h dark (LD),

<u>Wild type</u> (Controls): Canton S (CS) yellow white (y w)

Single mutants: period null (per<sup>01</sup>) sniffer loss of function (sni<sup>1</sup>)

Double mutants: *per*<sup>01</sup> *sni*<sup>1</sup> lines 1 & 2

Locomotor activity analysis: Drosophila Activity Monitor Fast Fourier Transform (FFT) analysis using ClockLab [4]. Lifespan analysis:



Neuronal degeneration: Paraffin-embedded sectioning as described [5]. **Rapid Iterative Negative** Geotaxis (RING) assay Vertical mobility [3,5]

Cohorts of 100 flies housed in 8oz polypropylene bottles inverted over culture dishes with diet replaced daily [5].

**Gene Expression**: aRT-PCR,  $2^{-\Delta\Delta Ct}$  method, normalized to the gene *rp4*9 at ZT 4 [3,4].

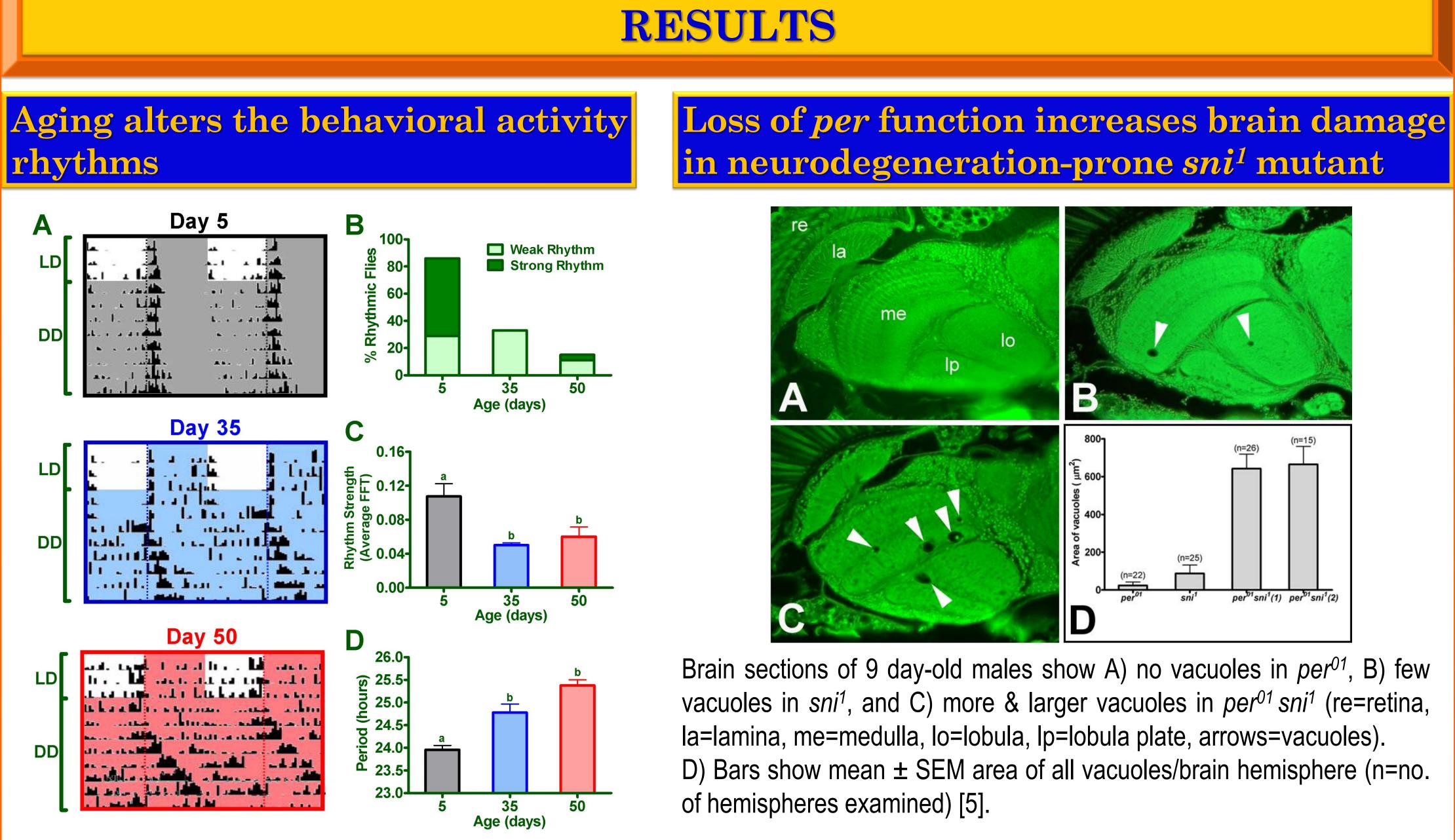


Oxidative damage assay: Protein carbonyls in heads measured at 370nm after reaction with 2,4-DNPH [5].

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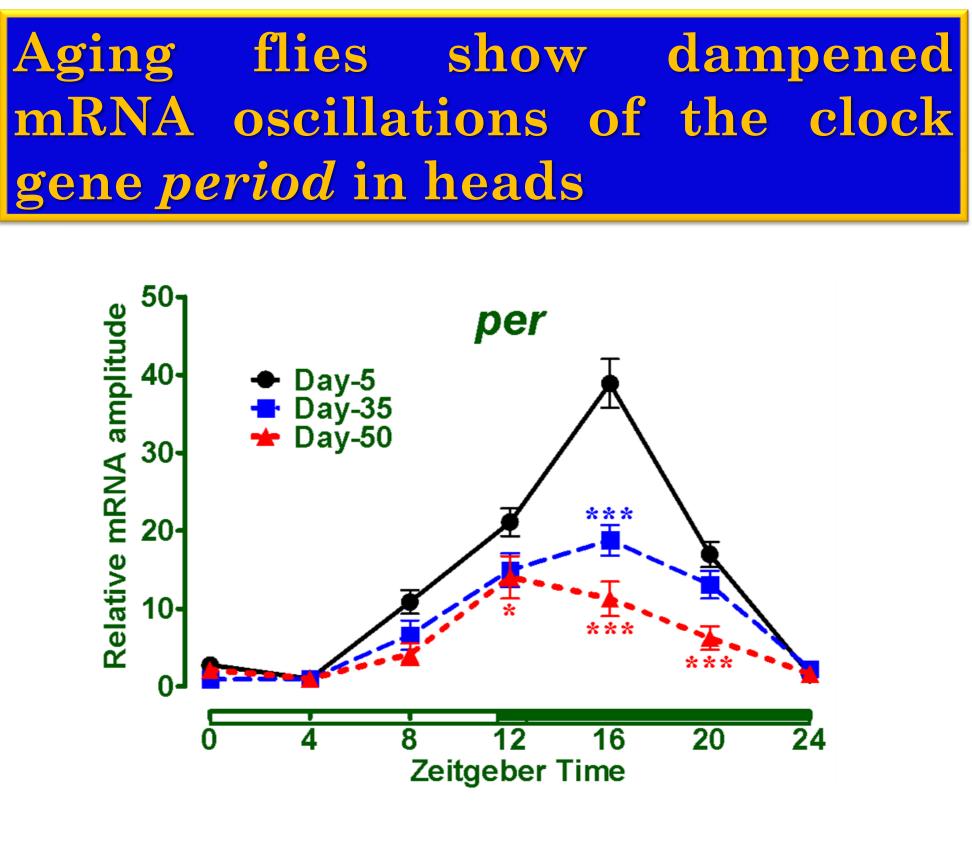
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A) Locomotor activity profiles of young (day 5), middle-aged (day 35) and old (day 50) CS males, monitored in 12h light: dark (LD) for 3 days, followed by 7 days in DD at 25°C. Shaded areas represent periods of dark.

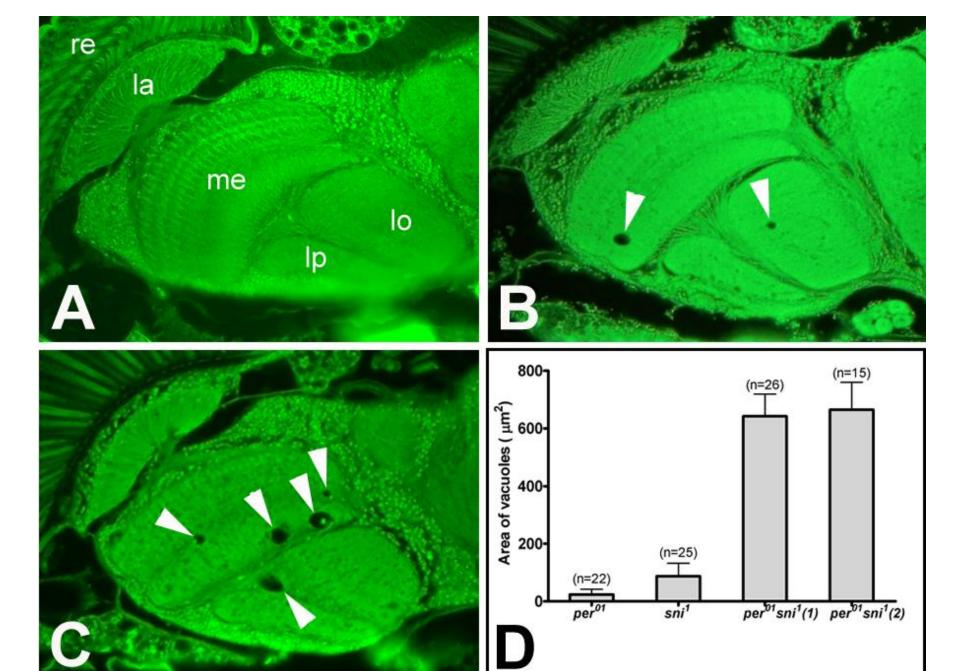
Bar graphs depict B) percentage of rhythmic flies, C) average rhythm strength, and D) average free-running period of locomotor activity. Bars with different letters are significantly different at p<0.01 [4].



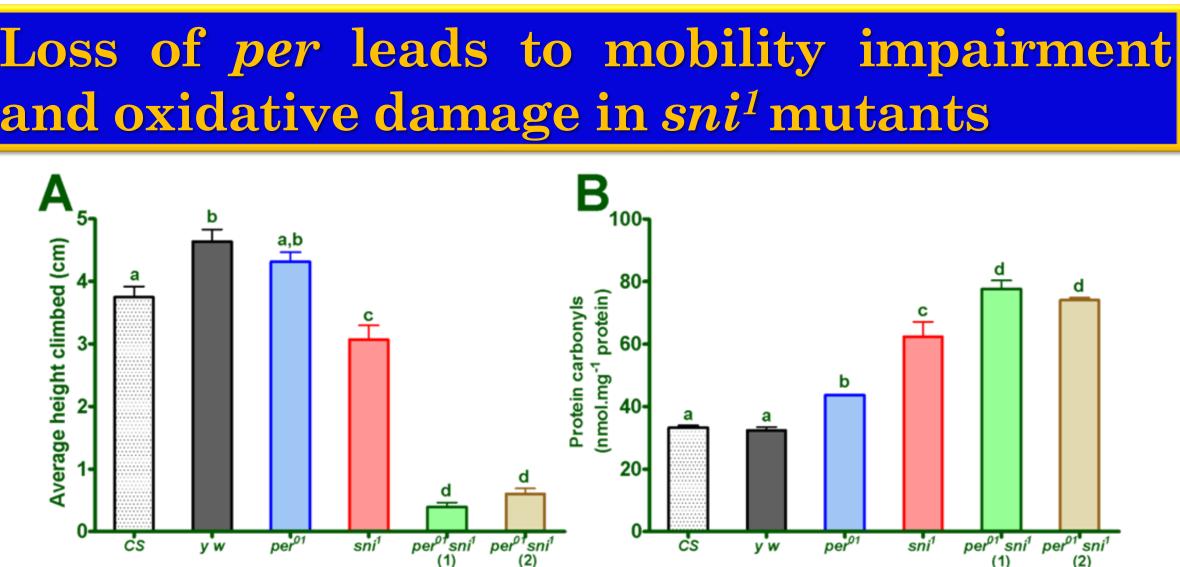
Daily mRNA profile of *per* in heads of CS males. White and shaded horizontal bars mark periods of light and dark, respectively. Statistical significance is denoted by \*\*\* = p < p0.001 and \* = p < 0.05 [4].

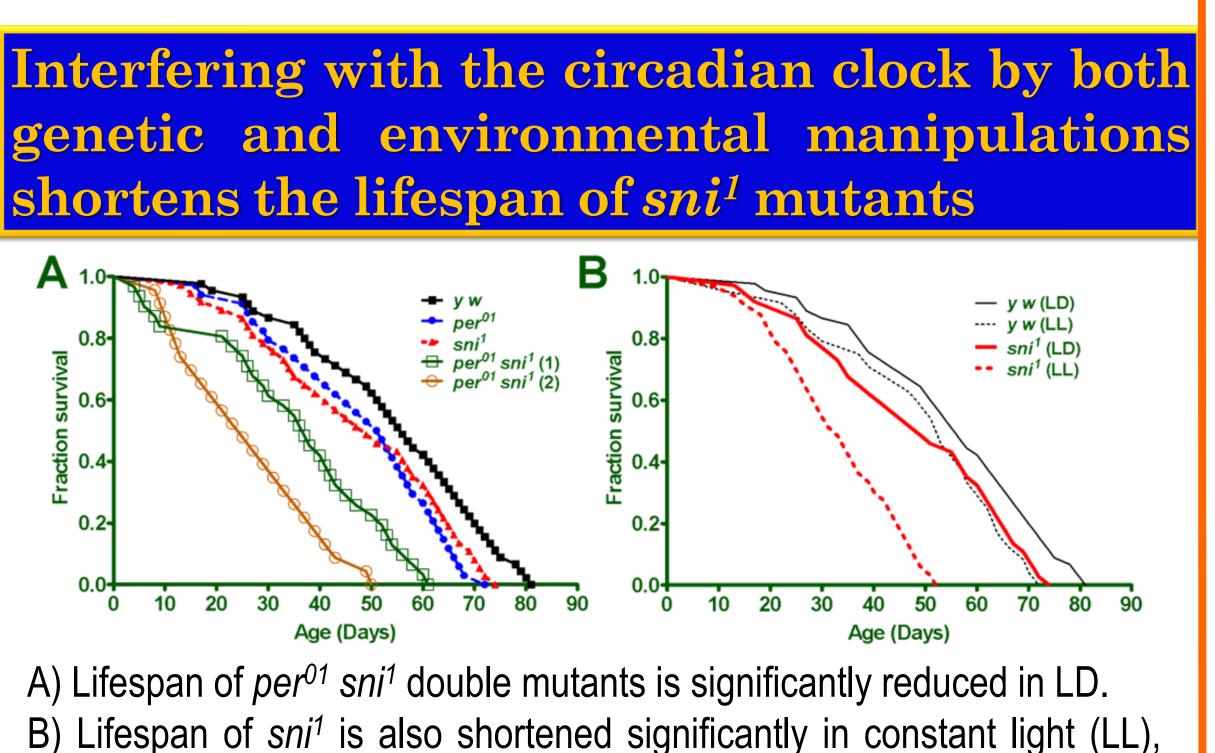






Brain sections of 9 day-old males show A) no vacuoles in *per<sup>01</sup>*, B) few vacuoles in sni<sup>1</sup>, and C) more & larger vacuoles in per<sup>01</sup> sni<sup>1</sup> (re=retina, la=lamina, me=medulla, lo=lobula, lp=lobula plate, arrows=vacuoles). D) Bars show mean  $\pm$  SEM area of all vacuoles/brain hemisphere (n=no.





A) Climbing ability is significantly reduced, and B) protein carbonyl levels in heads are greatly elevated in 10 day-old per<sup>01</sup> sni<sup>1</sup>.double mutants. Bars with different letters are significantly different at p<0.01 [5].

which disrupts the circadian clock function [5].







## CONCLUSIONS

- Aging is associated with reduced strength of rest/activity rhythms, and dampening of molecular circadian oscillations.
- (i) Loss of clock function is associated with brain damage, loss of climbing ability, and oxidative stress.
- (i) Disruption of circadian rhythms by both genetic and environmental manipulations reduces the lifespan of neurodegenerationprone mutants.
  - Intact circadian clocks may help prevent damage to the nervous system during aging.

### SIGNIFICANCE

- Sleep disorders, observed during aging, are pronounced in neurodegenerative diseases like Alzheimer's and Parkinson's.
- Our results demonstrate that loss of clock function contributes to brain damage.
- Our study which suggests that a robust circadian system has neuro-protective effects, may serve as a starting point for translational research in humans.

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