

## INTRODUCTION

- Circadian clocks generate daily rhythms in sleep/activity, physiological, metabolic and cellular processes.
- Rhythms have an endogenous periodicity of 24h and are entrained (set) to local time by external Zeitgebers (time-givers) such as day/night cycles.
- Disrupted circadian rhythms lead to age-related 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].
- 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)
- Ease of maintenance
- Fly and mammalian clocks conserved
- Aging symptoms similar to humans

## METHODS

**Fly rearing and strains:**  
12h light: 12h dark (LD).  
**Wild type** (Controls): Canton S (CS)  
yellow white (*y w*)  
**Single mutants:** *period* null (*per<sup>01</sup>*)  
*sniffer* loss of function (*snif<sup>1</sup>*)  
**Double mutants:** *per<sup>01</sup> snif<sup>1</sup>* lines 1 & 2

**Locomotor activity analysis:**  
*Drosophila* Activity Monitor  
Fast Fourier Transform (FFT) analysis using ClockLab [4].  
**Lifespan analysis:**  
Cohorts of 100 flies housed in 8oz polypropylene bottles inverted over culture dishes with diet replaced daily [5].  
**Gene Expression:**  
qRT-PCR, 2<sup>-ΔΔCt</sup> method, normalized to the gene *rp49* at ZT 4 [3,4].

**Neuronal degeneration:**  
Paraffin-embedded sectioning as described [5].  
**Rapid Iterative Negative Geotaxis (RING) assay:**  
Vertical mobility [3,5].  
**Oxidative damage assay:**  
Protein carbonyls in heads measured at 370nm after reaction with 2,4-DNPH [5].

## RESULTS

### Aging alters the behavioral activity rhythms

**A**  
Day 5  
LD  
DD

**B**  
% Rhythmic Flies  
Age (days)  
Weak Rhythm  
Strong Rhythm

**C**  
Day 35  
LD  
DD  
Rhythm Strength (Average FFT)

**D**  
Day 50  
LD  
DD  
Period (hours)

### Loss of *per* function increases brain damage in neurodegeneration-prone *snif<sup>1</sup>* mutant

**A**  
re  
la  
me  
lo  
lp

**B**

**C**

**D**  
Area of vacuoles (μm<sup>2</sup>)

### Loss of *per* leads to mobility impairment and oxidative damage in *snif<sup>1</sup>* mutants

**A**  
Average height climbed (cm)

**B**  
Protein carbonyls (nmol/mg<sup>1</sup> protein)

### Aging flies show dampened mRNA oscillations of the clock gene *period* in heads

**A**  
*per*  
Day-5  
Day-35  
Day-50  
Relative mRNA amplitude  
Zeitgeber Time

### Interfering with the circadian clock by both genetic and environmental manipulations shortens the lifespan of *snif<sup>1</sup>* mutants

**A**  
Fraction survival  
Age (Days)  
*y w*  
*per<sup>01</sup>*  
*snif<sup>1</sup>*  
*per<sup>01</sup> snif<sup>1</sup> (1)*  
*per<sup>01</sup> snif<sup>1</sup> (2)*

**B**  
Fraction survival  
Age (Days)  
*y w* (LD)  
*y w* (LL)  
*snif<sup>1</sup>* (LD)  
*snif<sup>1</sup>* (LL)

## CONCLUSIONS

- Aging is associated with reduced strength of rest/activity rhythms, and dampening of molecular circadian oscillations.
- Loss of clock function is associated with brain damage, loss of climbing ability, and oxidative stress.
- Disruption of circadian rhythms by both genetic and environmental manipulations reduces the lifespan of neurodegeneration-prone 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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