CIRCADIAN CLOCK DISRUPTION PROMOTES THE DEGENERATION OF DOPAMINERGIC NEURONS IN MALE DROSOPHILA

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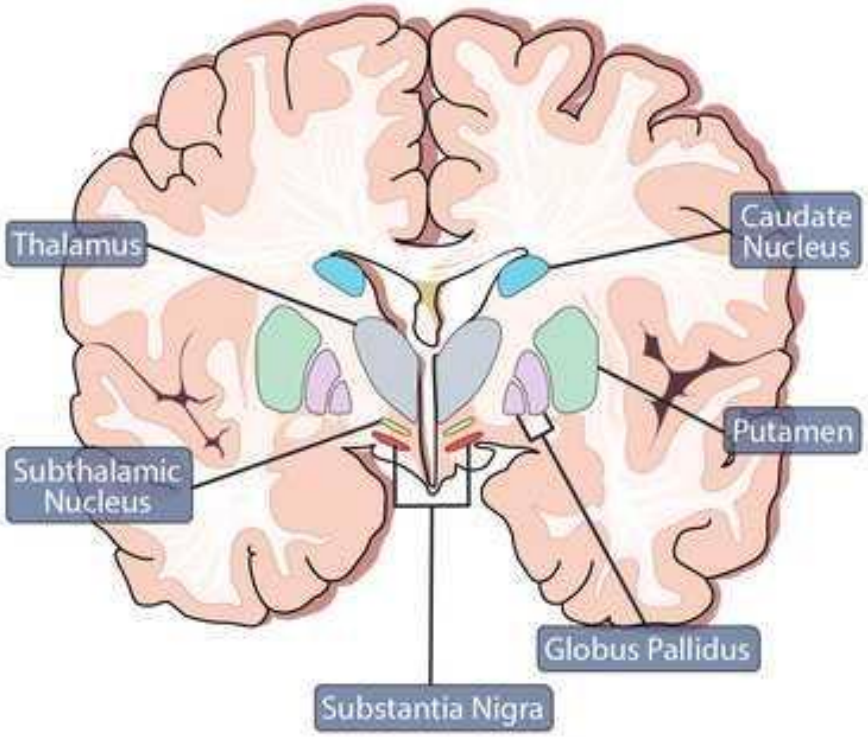
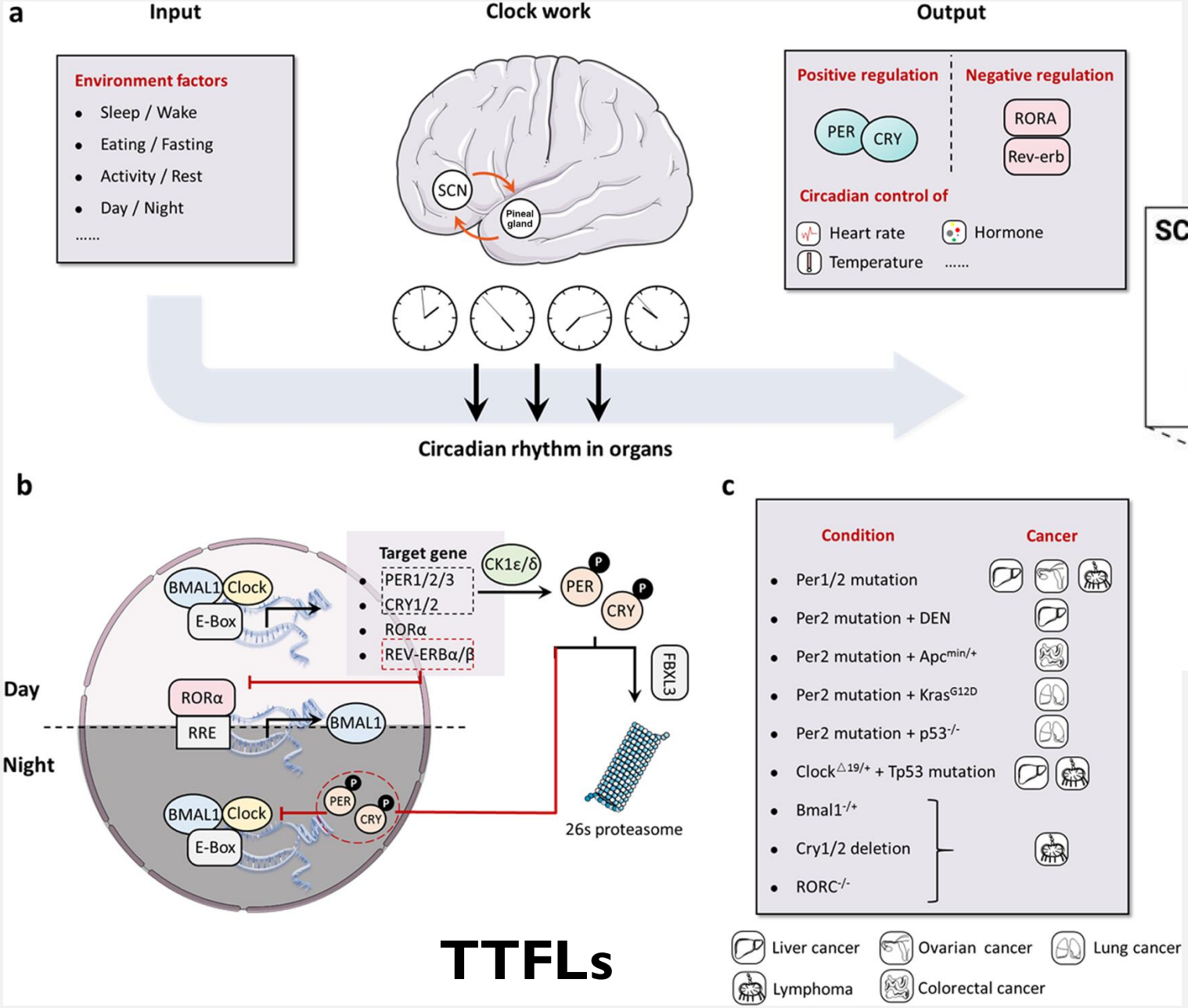
By Tran Hong Thuan

I. Introduction

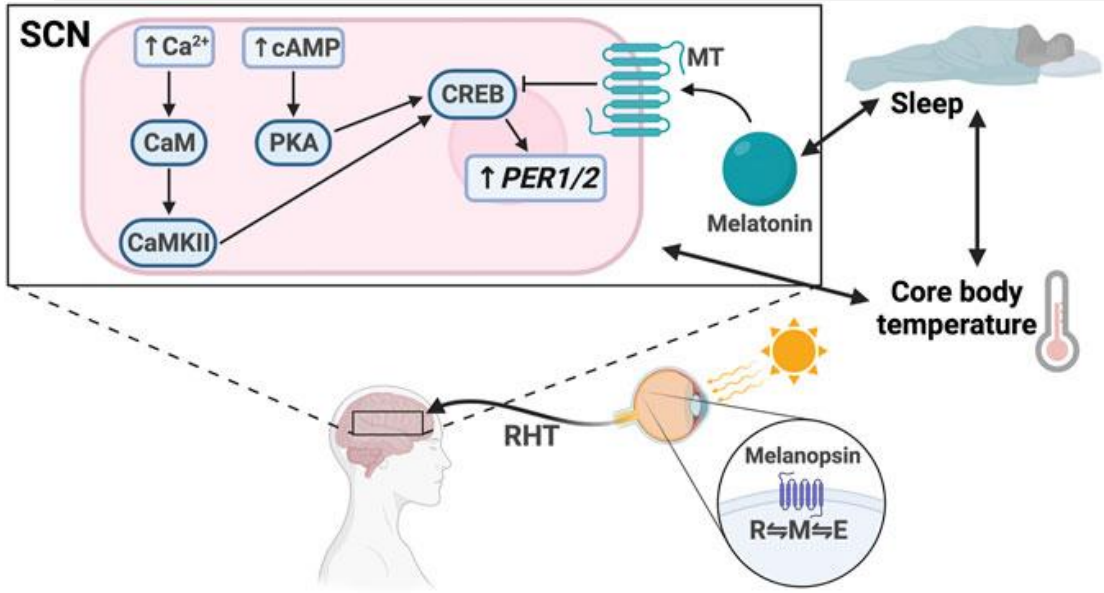
Sleep and circadian rhythm disruptions → loss of dopaminergic (DA) neurons in the substantia nigra. → Parkinson's disease (PD) (The motor system is interrupted)



I. Introduction

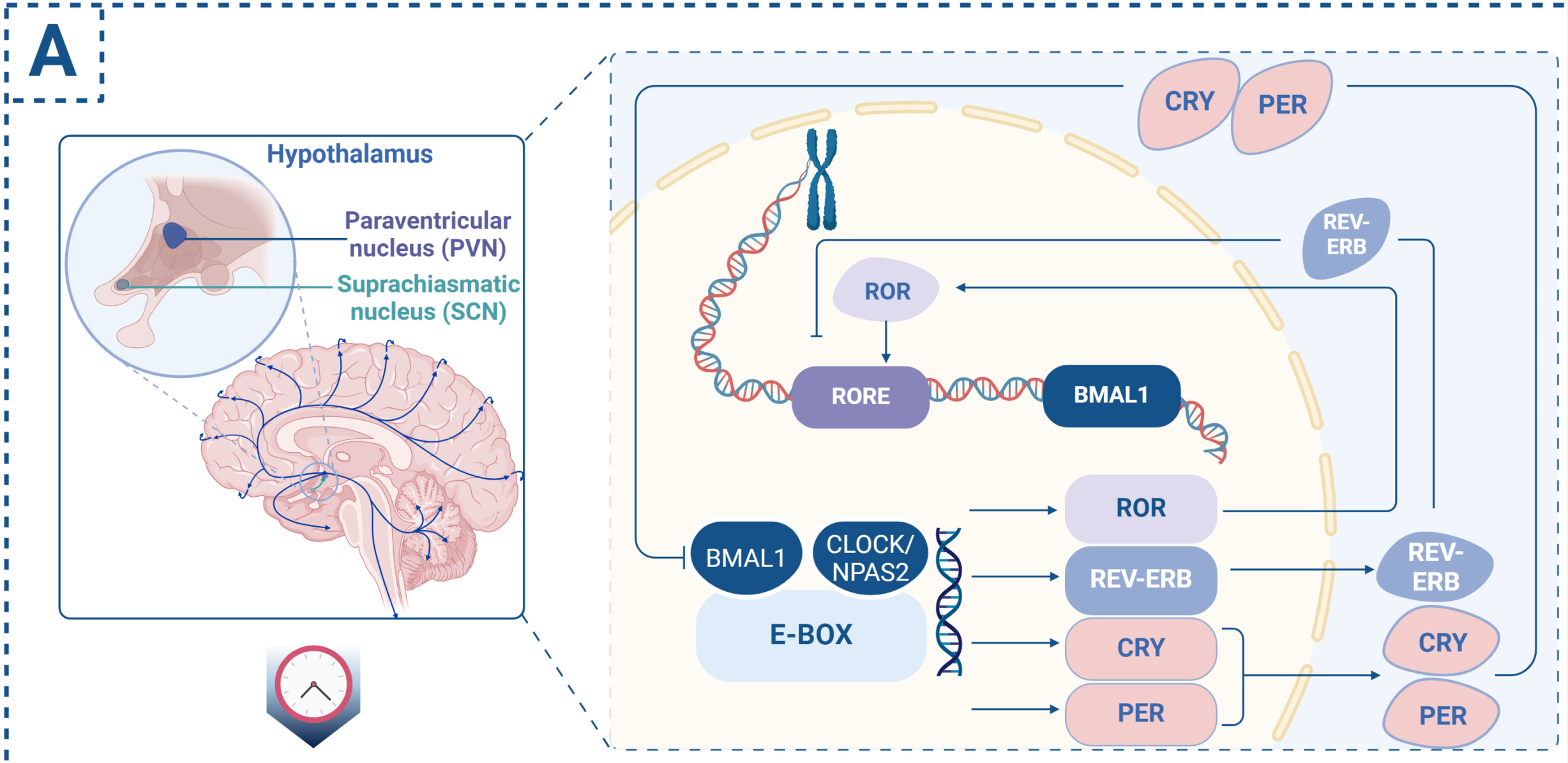


TTFLs



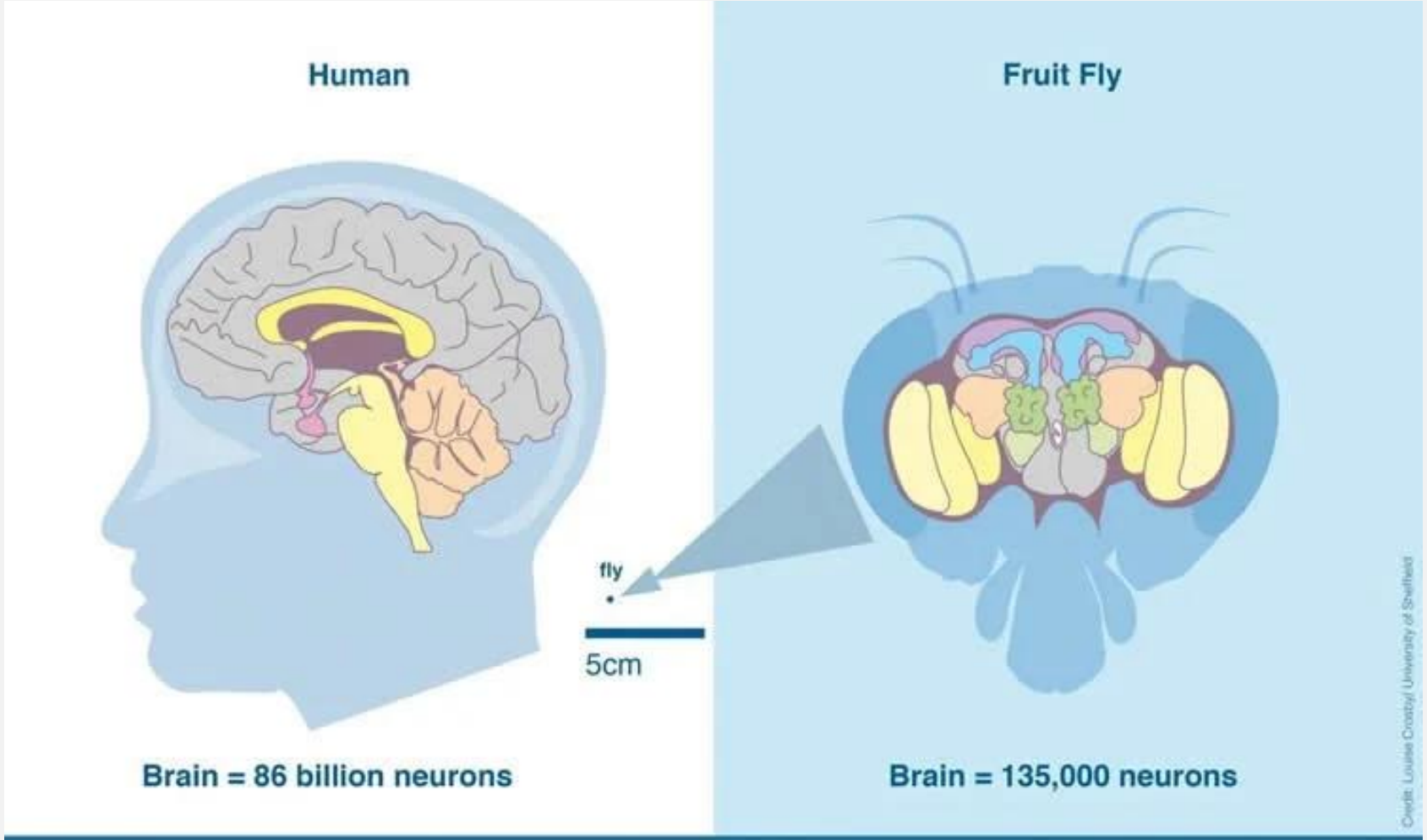
Kelly L. Healy, Andrew R. Morris and Andrew C. Liu, 2021

Circadian clockworks drive the hormone secretion

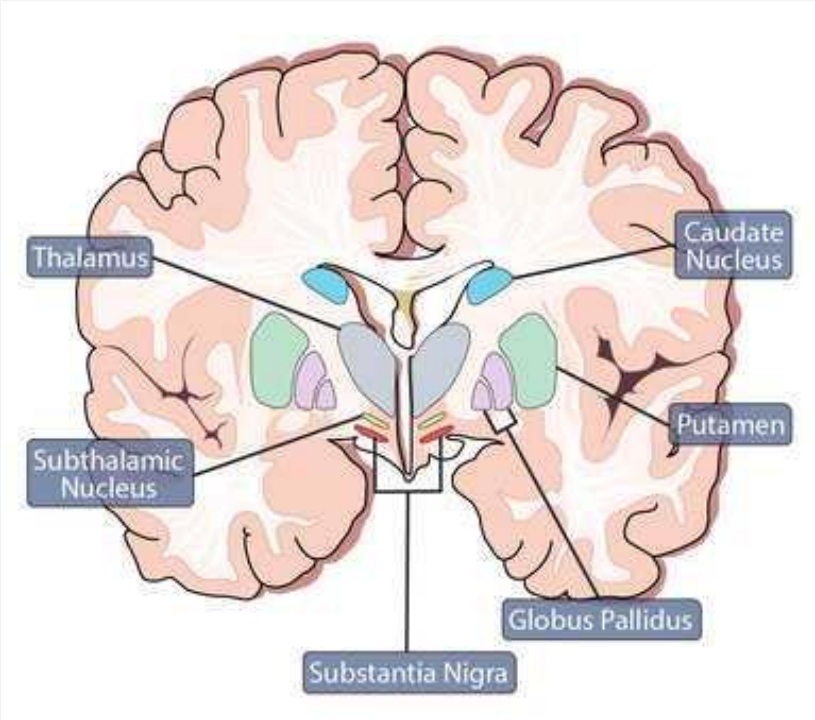


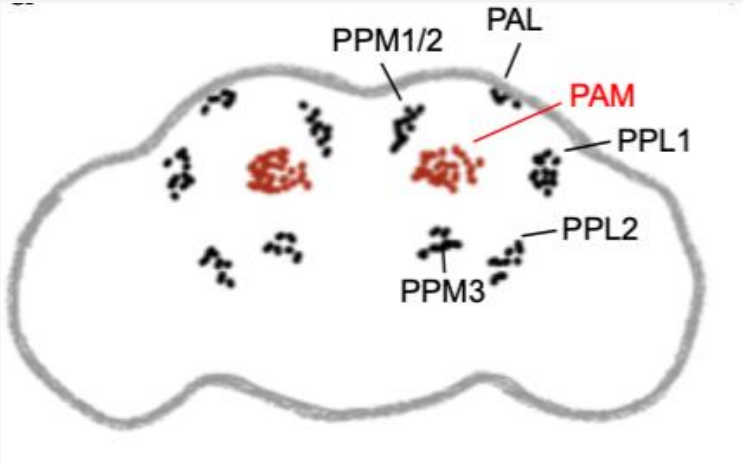
Tran., et al. 2024

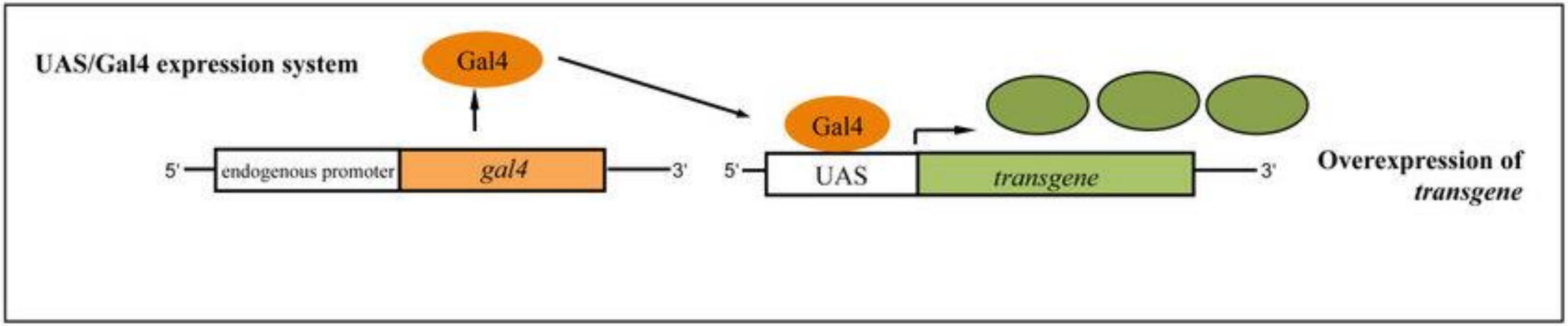
Fly genes are almost homologs to the human brain

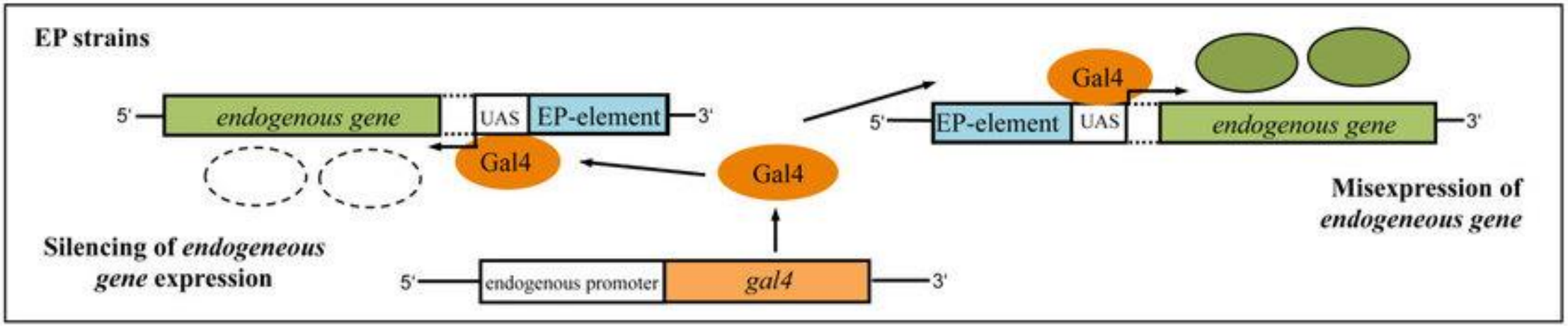


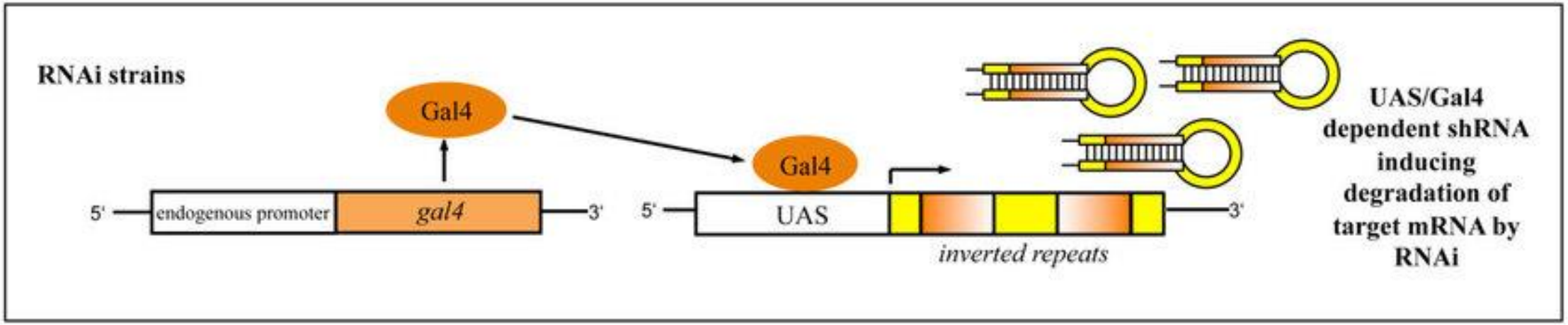
"Fruit flies share nearly 60% of our genes, so the neural circuits in their brains – despite the huge size difference – are likely to be similar to ours. A model of the fruit fly brain – which is achievable – will tell us a lot about the human brain." Daniel Coca, University of Sheffield





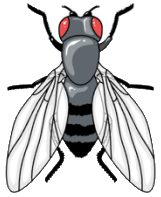

UAS/Gal4 expression system
Gal4
5'
endogenous promoter
gal4
3'
Gal4
5'
UAS
transgene
3'
Overexpression of
transgene


EP strains
5'
endogenous gene
UAS
EP-element
3'
Gal4
Gal4
5'
EP-element
UAS
endogenous gene
3'
Gal4
Misexpression of
endogeneous gene
Silencing of endogeneous
gene expression
5'
endogenous promoter
gal4
3'


RNAi strains
Gal4
5'
endogenous promoter
gal4
3'
Gal4
5'
UAS
3'
inverted repeats
UAS/Gal4
dependent shRNA
inducing
degradation of
target mRNA by
RNAi

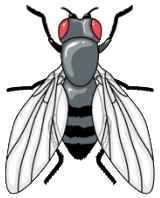
Generation a knockdown flies by using UAS-gal4

♂♂*UAS-VALIUM20/22-Target-RNAi*



UAS promoter optimized for ovarian expression

♀♀ **mat^{67};mat^{15}** (virgin)



4 maternal tubulin promoters expressing GAL4 drivers

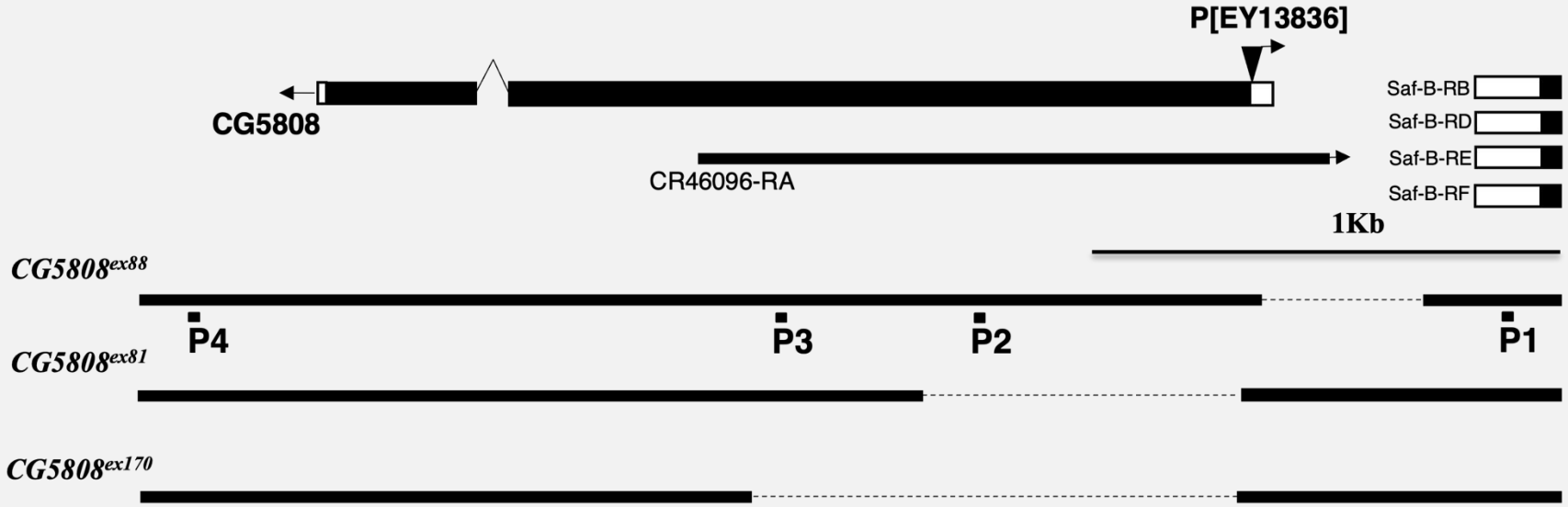
Target-*RNAi* expressed in ovaries, n=65

Collect Embryos at Cellularization Stage

Stain with anti-Zip (Myosin Heavy Chain)

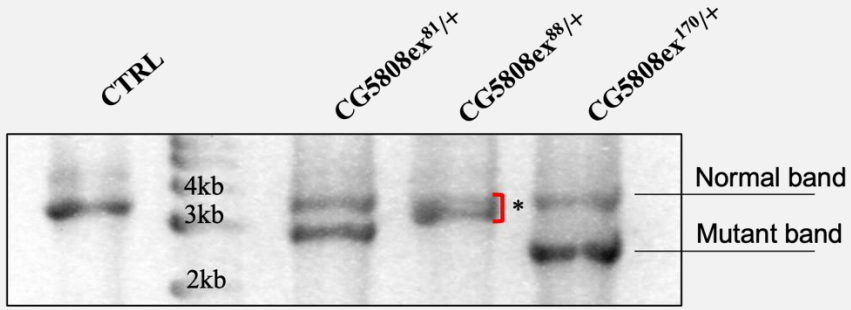
Analyze Microfilament Ring Circularity

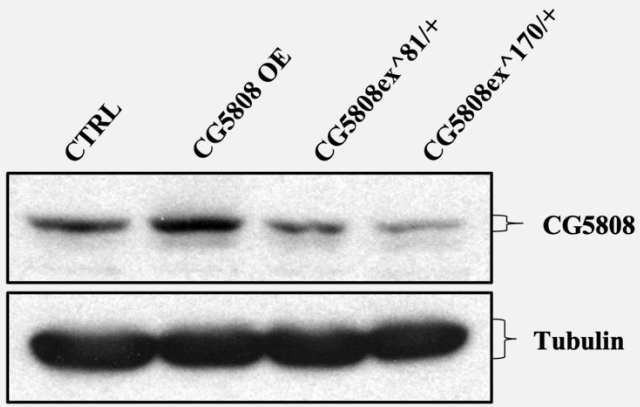
Generation CG5808 mutant fly using P-element



Deleted fragment

- Background clearance was applied
- CG5808 excision lines with TM6B chromosome balancer





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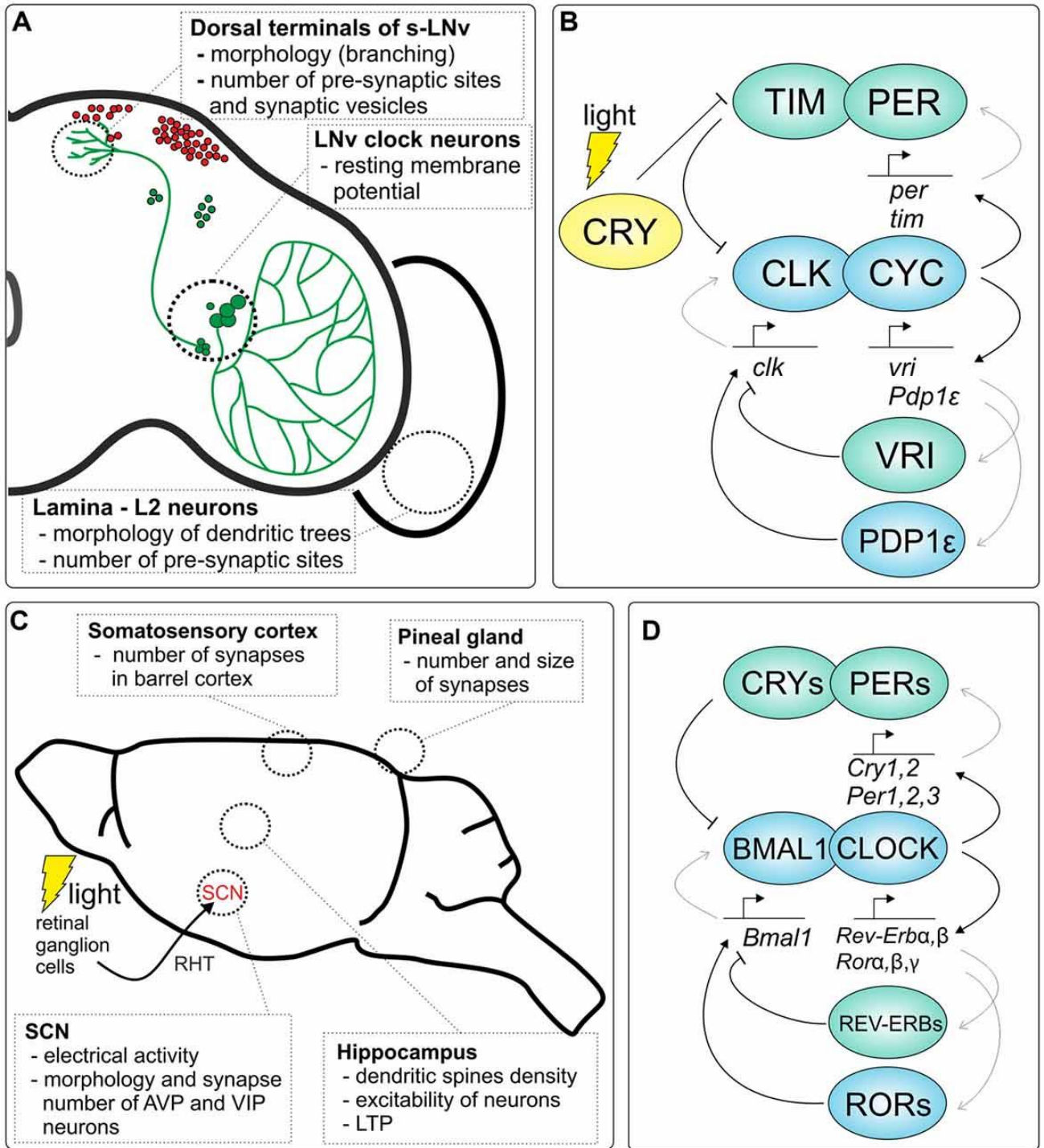
Neuronal circuits of circadian clocks in Drosophila and mammalian brains



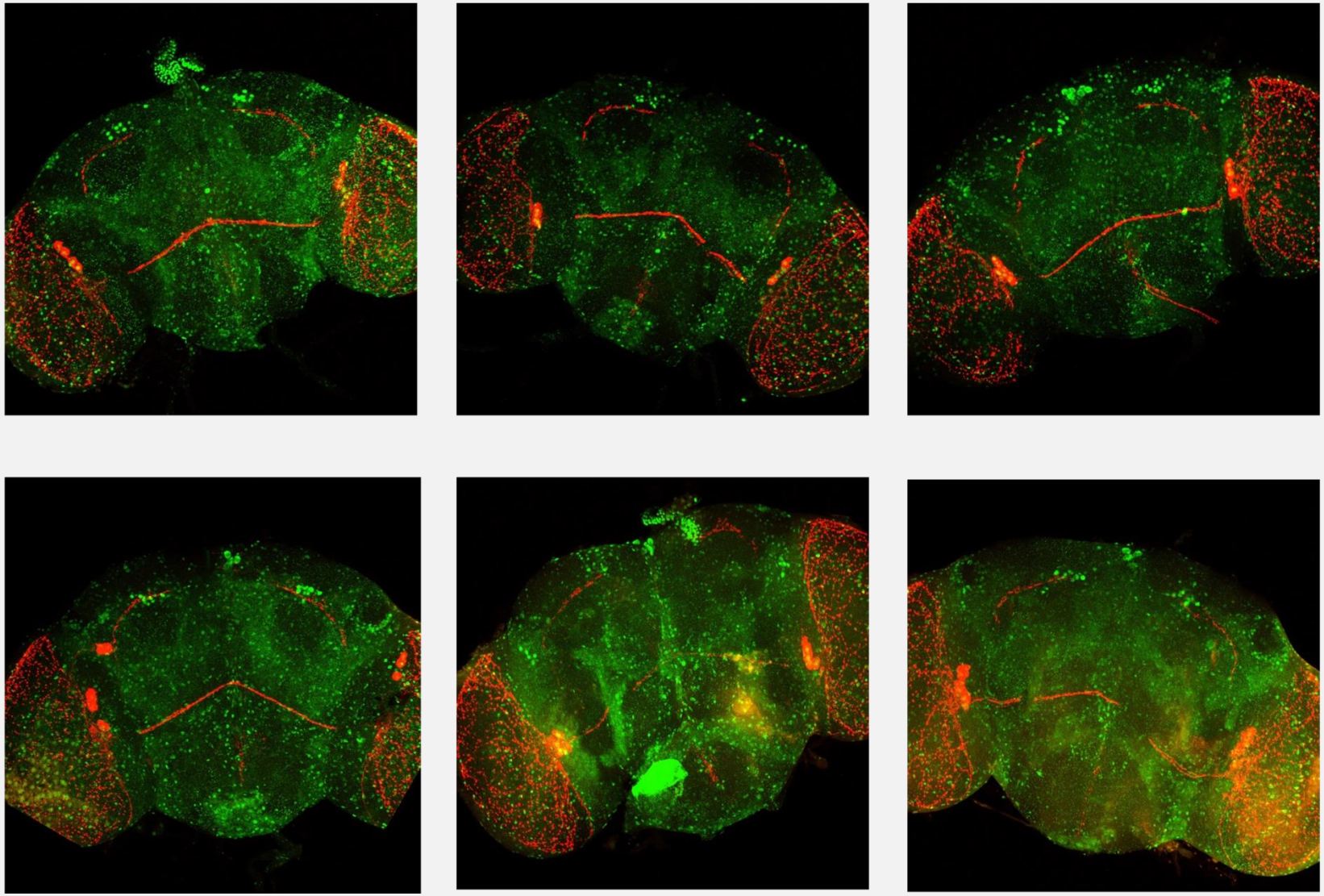
Fly brain dissection

ZT22

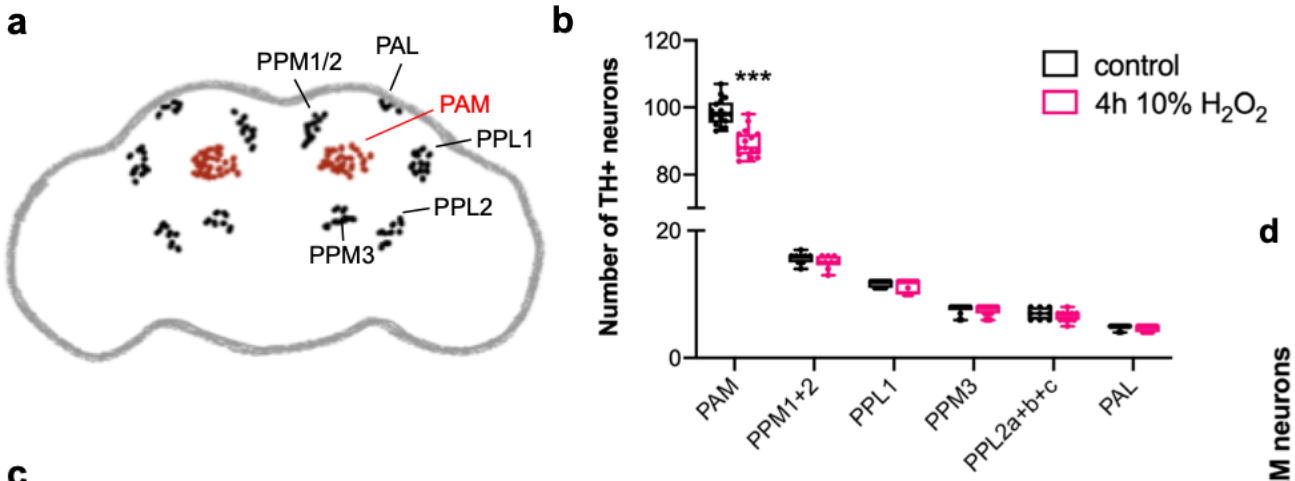
PER/PDF

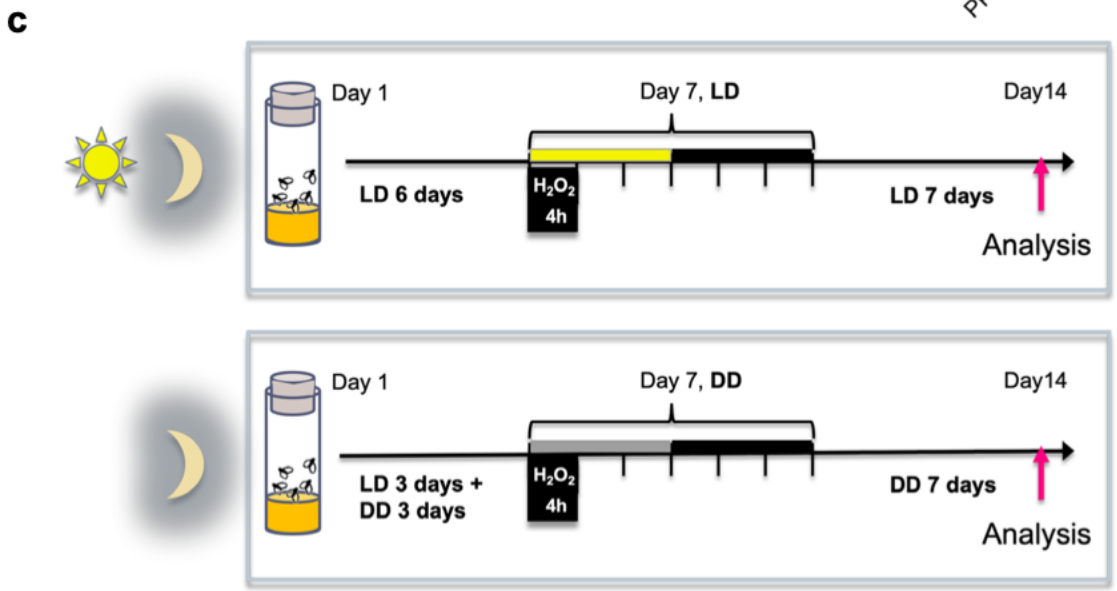
control

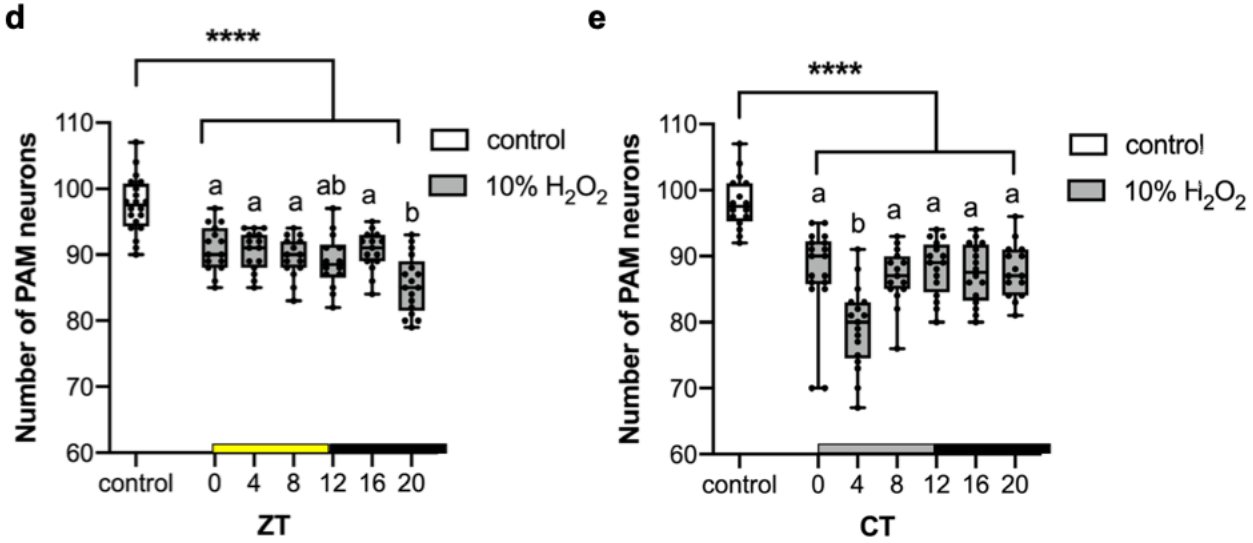
CG5808Ri



Circadian vulnerability of DA neurons to oxidative insults

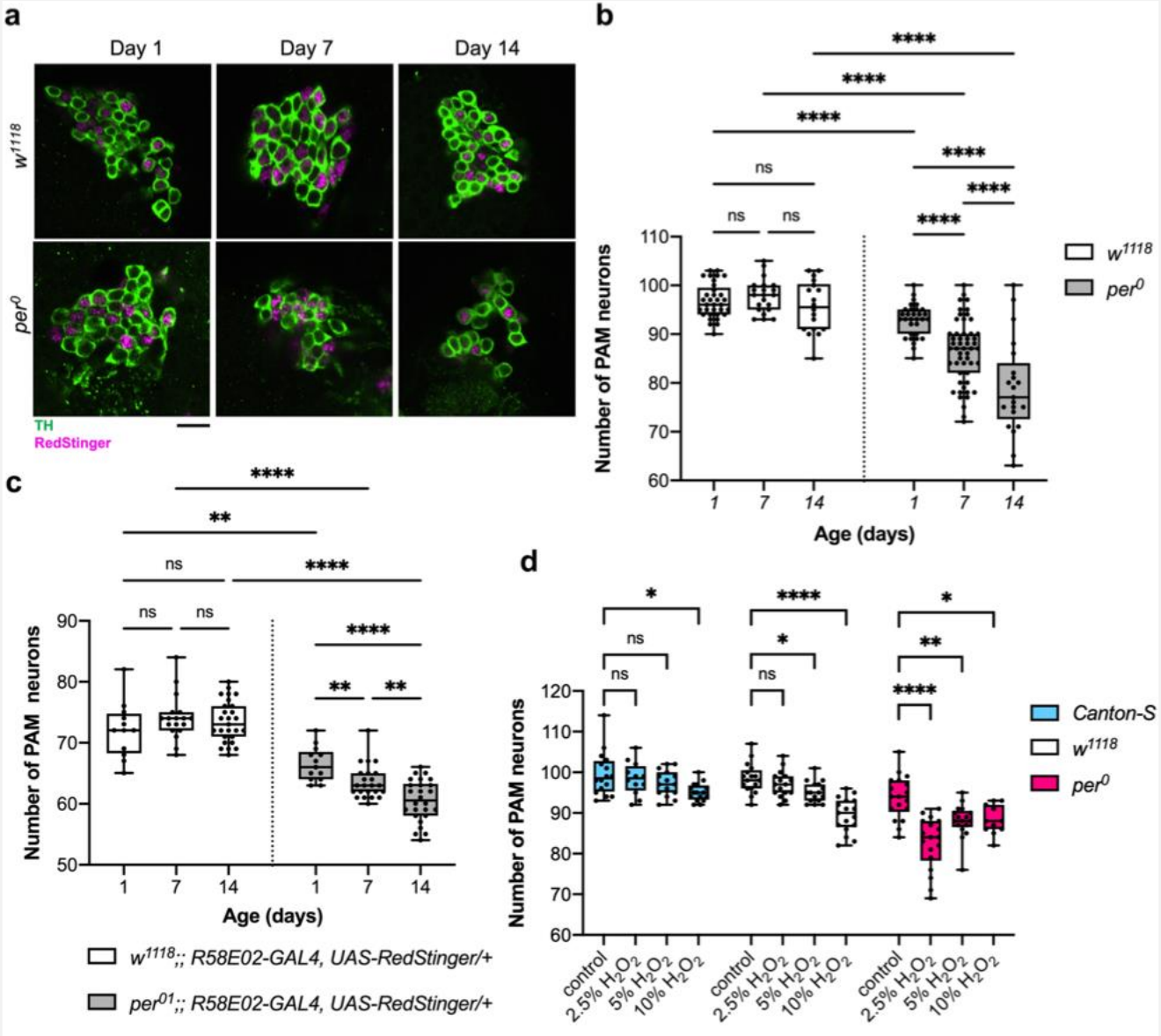


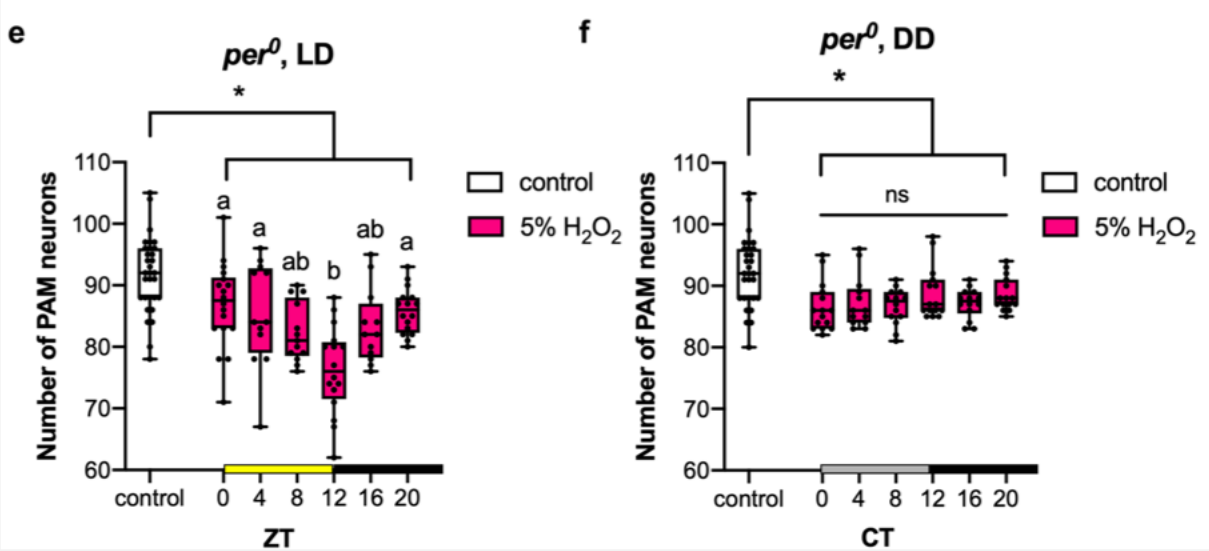




→ intrinsic rhythmicity in the vulnerability of PAM neurons to oxidative stress modulated by light

Circadian vulnerability of DA neurons to oxidative insults

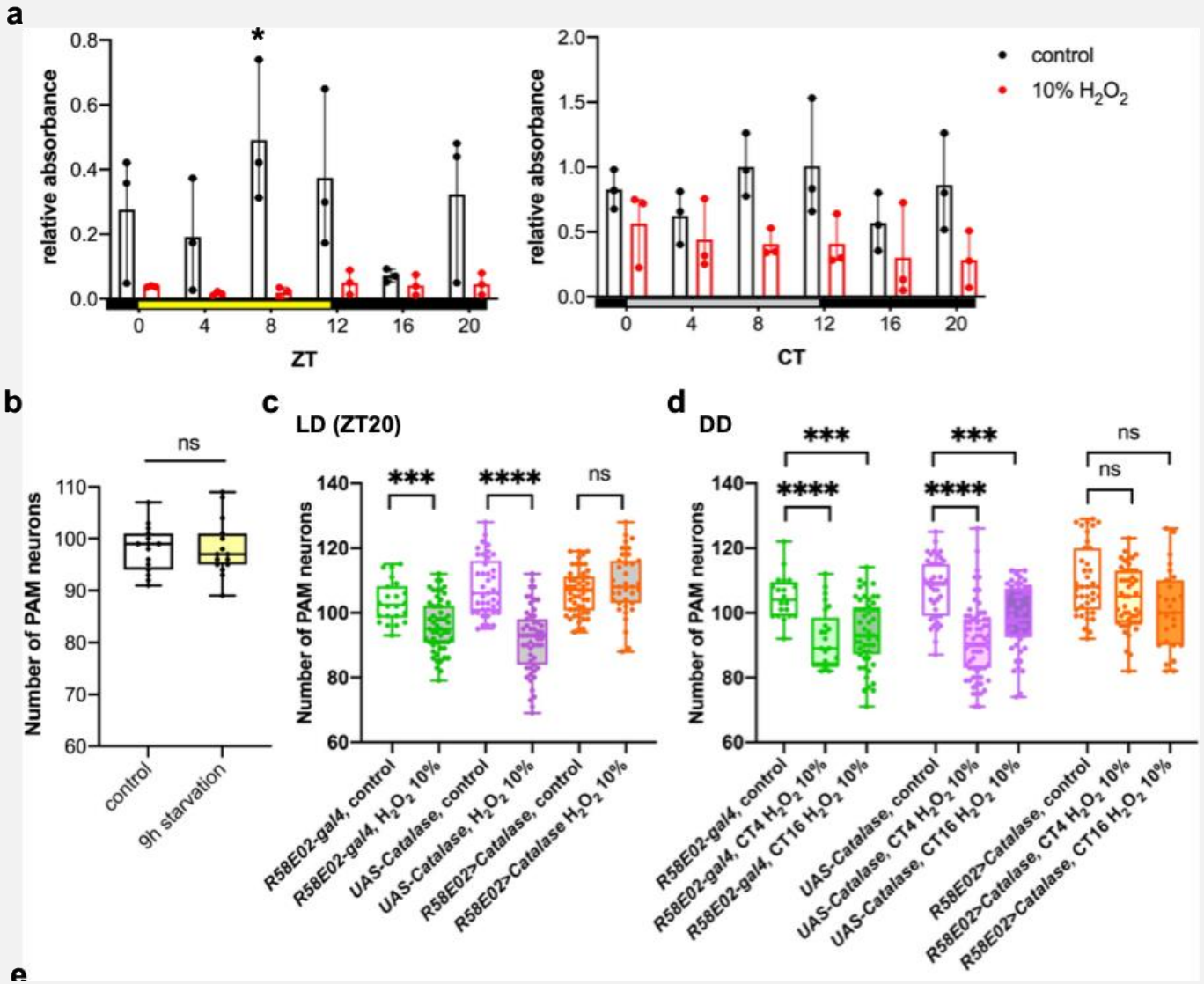


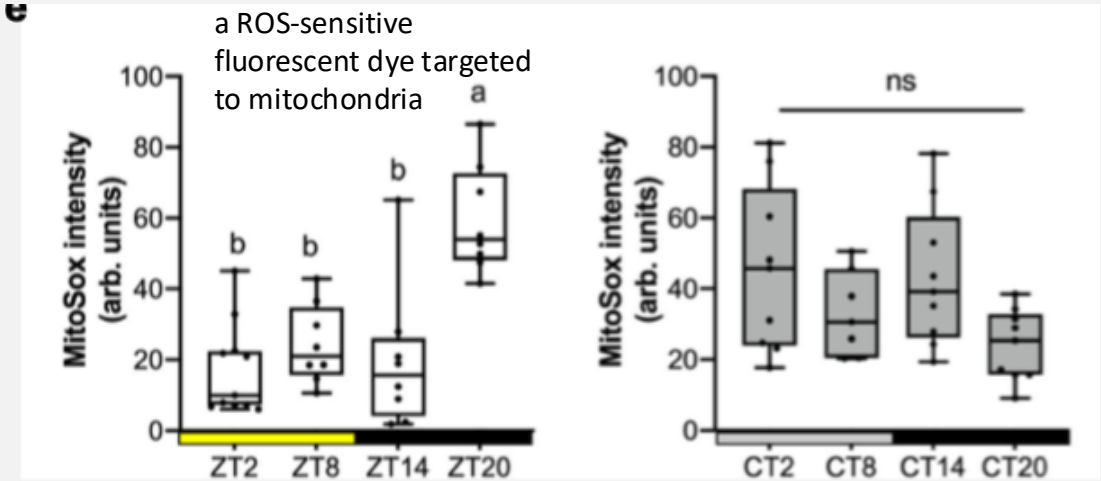


→ disruption of circadian clocks interferes with the development of DA neurons in the PAM cluster and accelerates their age-dependent degeneration

→ loss of per null mutation enhances the vulnerability of PAM neurons to oxidative stress-induced degeneration

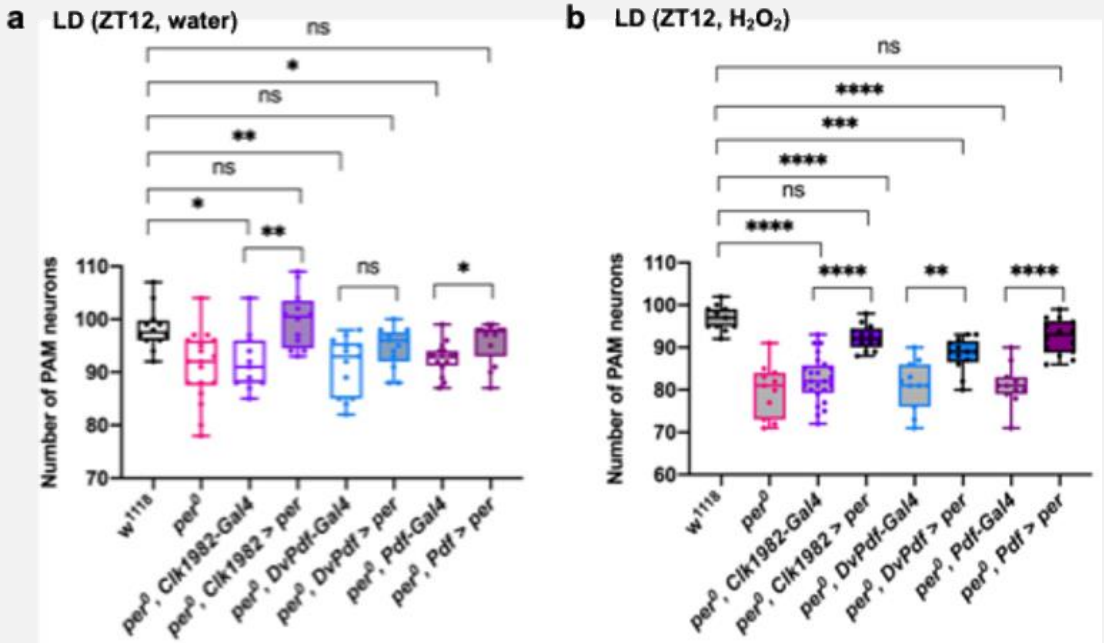
The circadian neural circuit controls the rhythmic vulnerability of PAM neurons

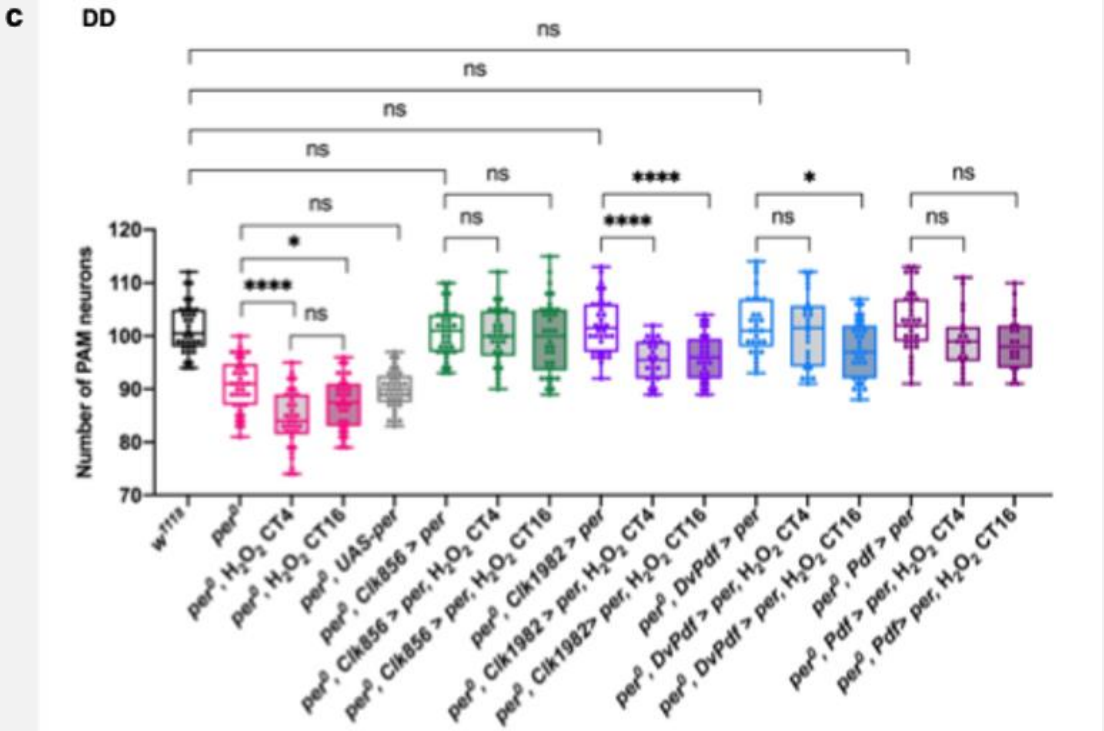


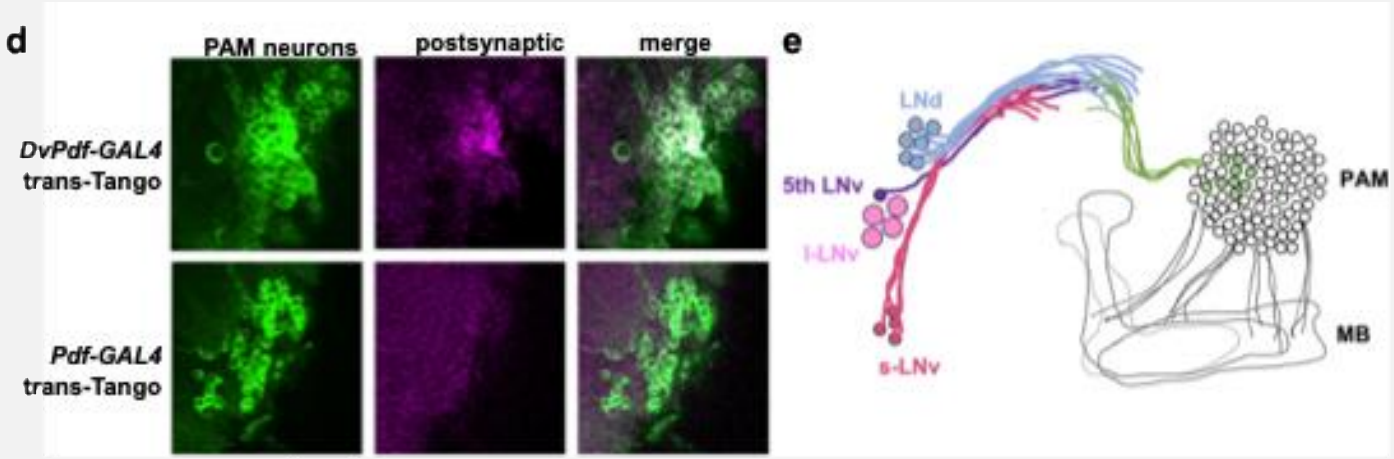


The circadian neural circuit controls the rhythmic vulnerability of PAM neurons

A subset of clock neurons are presynaptic to PAM neurons







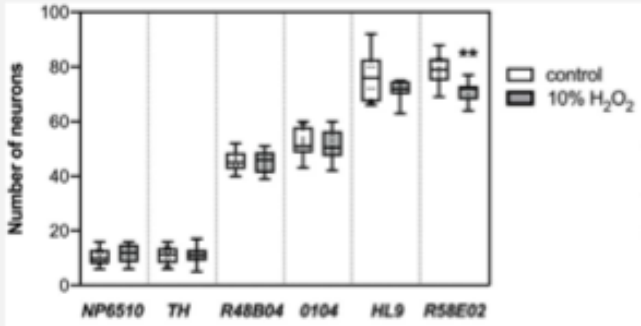
using trans-Tango, an anterograde transsynaptic tracing tool

→ the three CRY-negative LNds, one CRY/ITP double-positive LNd, and the fifth LNv) are presynaptic to PAM neurons

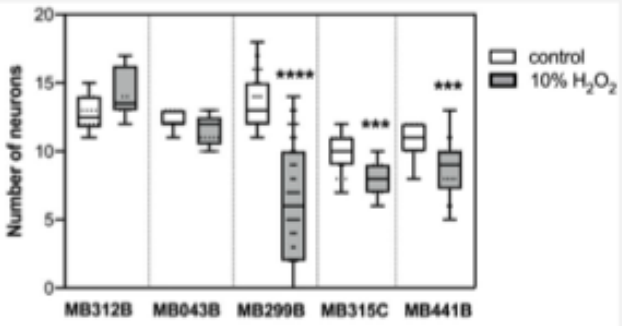
→ these findings suggest that PER expression within the PDF-positive LNvs plays a significant role in the regulation of survival of PAM neurons following oxidative insults

Identification of the vulnerable subpopulation of PAM neurons

a



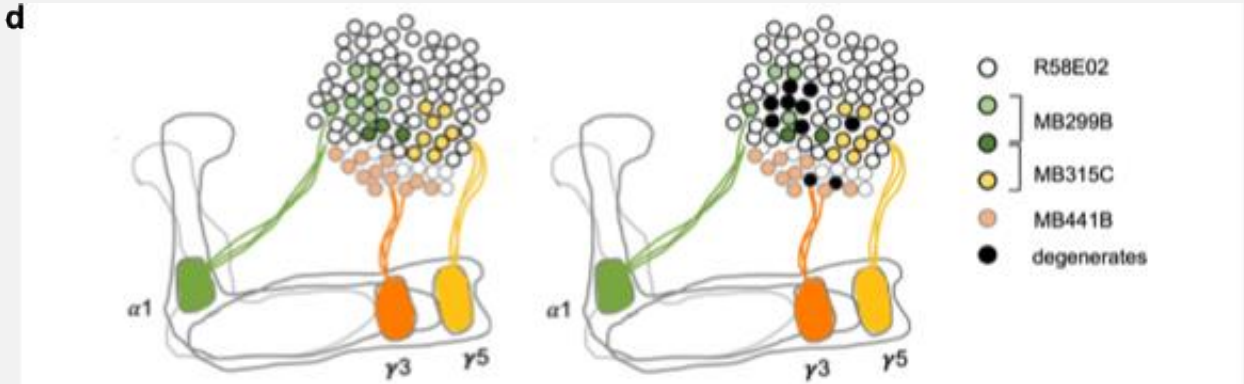
b



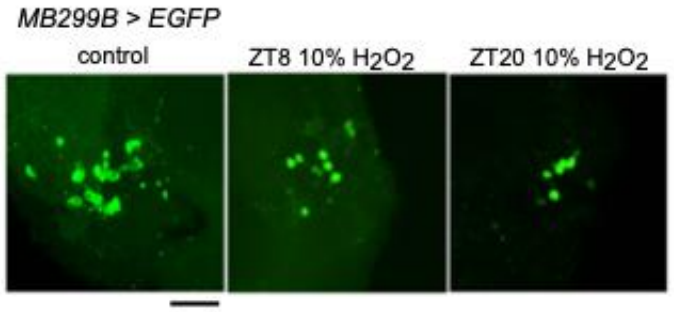
c

Detection method			Number of cells	Number of lost cells	*p*-value
TH staining			98 (93-107)	13 (10-16)	< 0.0001
GAL4					
NP6510			10	ns	
TH			12	ns	
R48B04			45	ns	
104			50	ns	
HL9			76	ns	
R58E02			78	8 (5-10)	0.0035
split-GAL4	AD	DBD			
MB312B	R58-E02	R10-G03	13	ns	
MB043B	R58-E02	R32-D11	11	ns	
MB299B	R58-E02	R37-E10	13 (11-18)	7 (6-13)	< 0.0001
MB315C	R58-E02	R48-H11	10 (7-12)	2 (1-3)	0.0006
MB441B	R30-G08	R48-B03	11 (8-12)	2 (1-3)	0.0006

d



e



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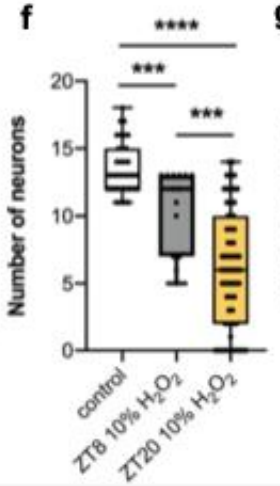
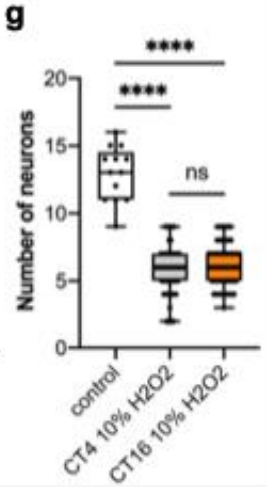
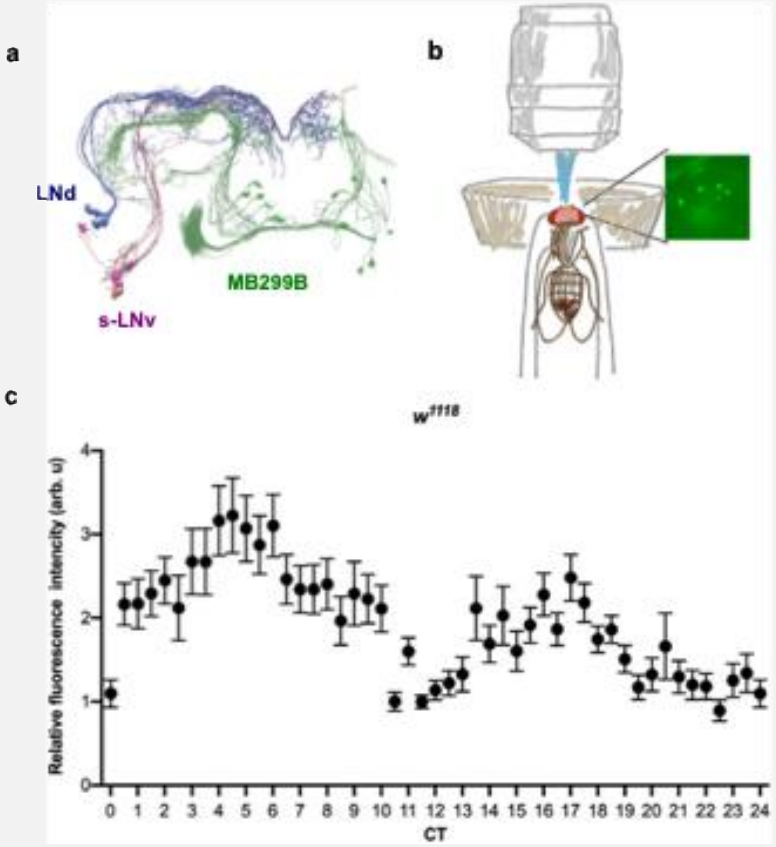
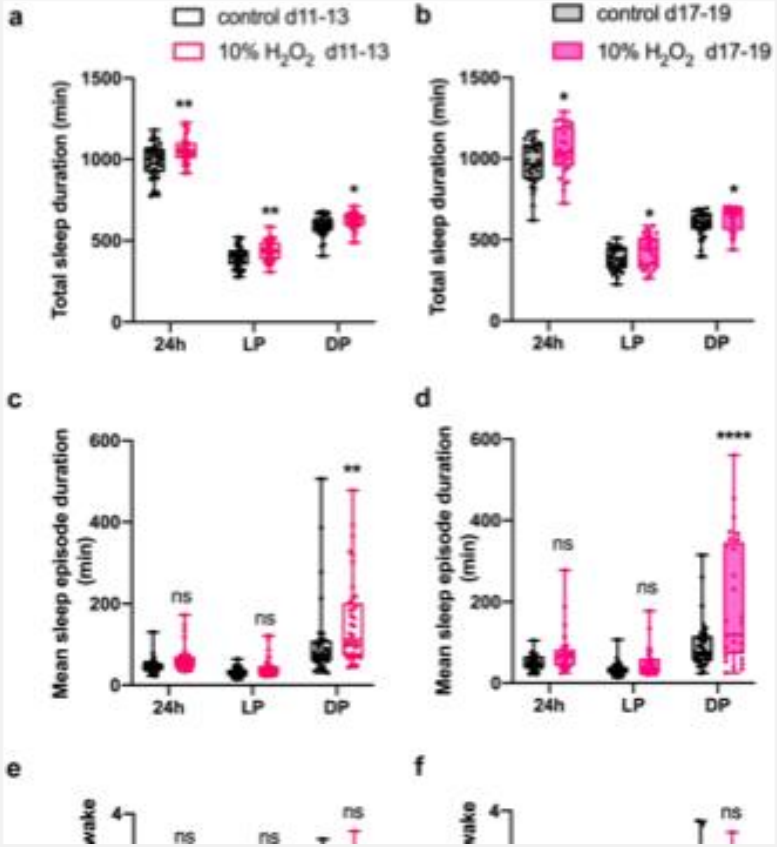


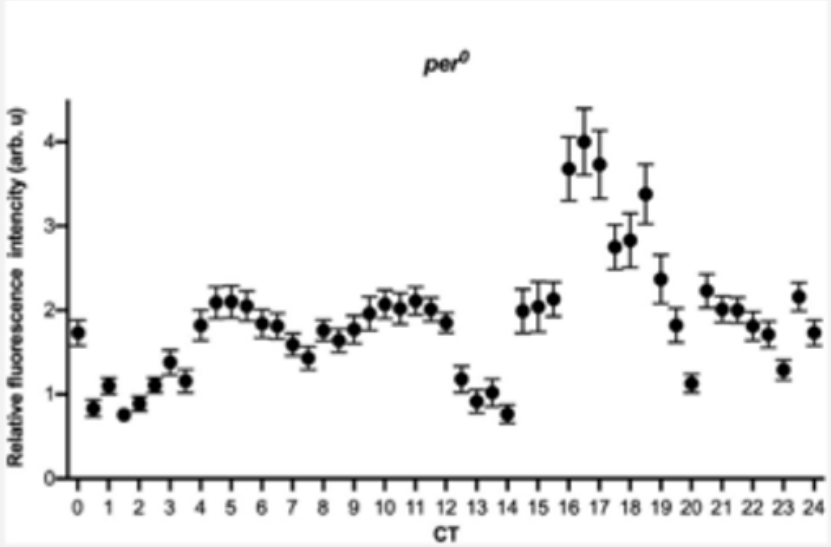
g

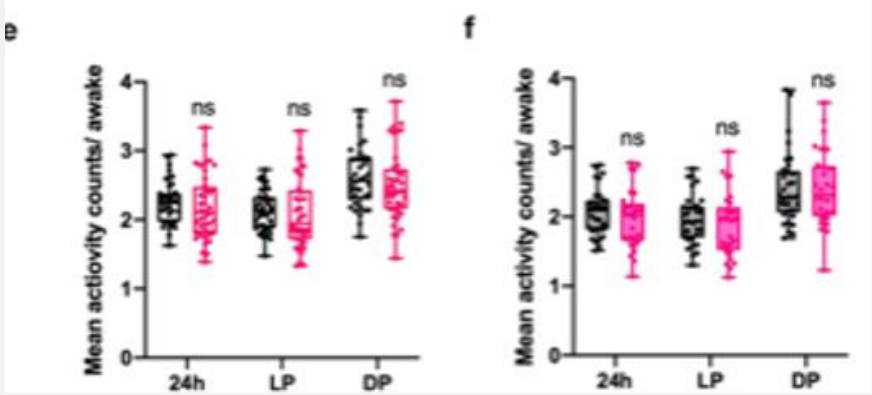


Consequences of PAM-α1 neurodegeneration on motor and non- motor functions



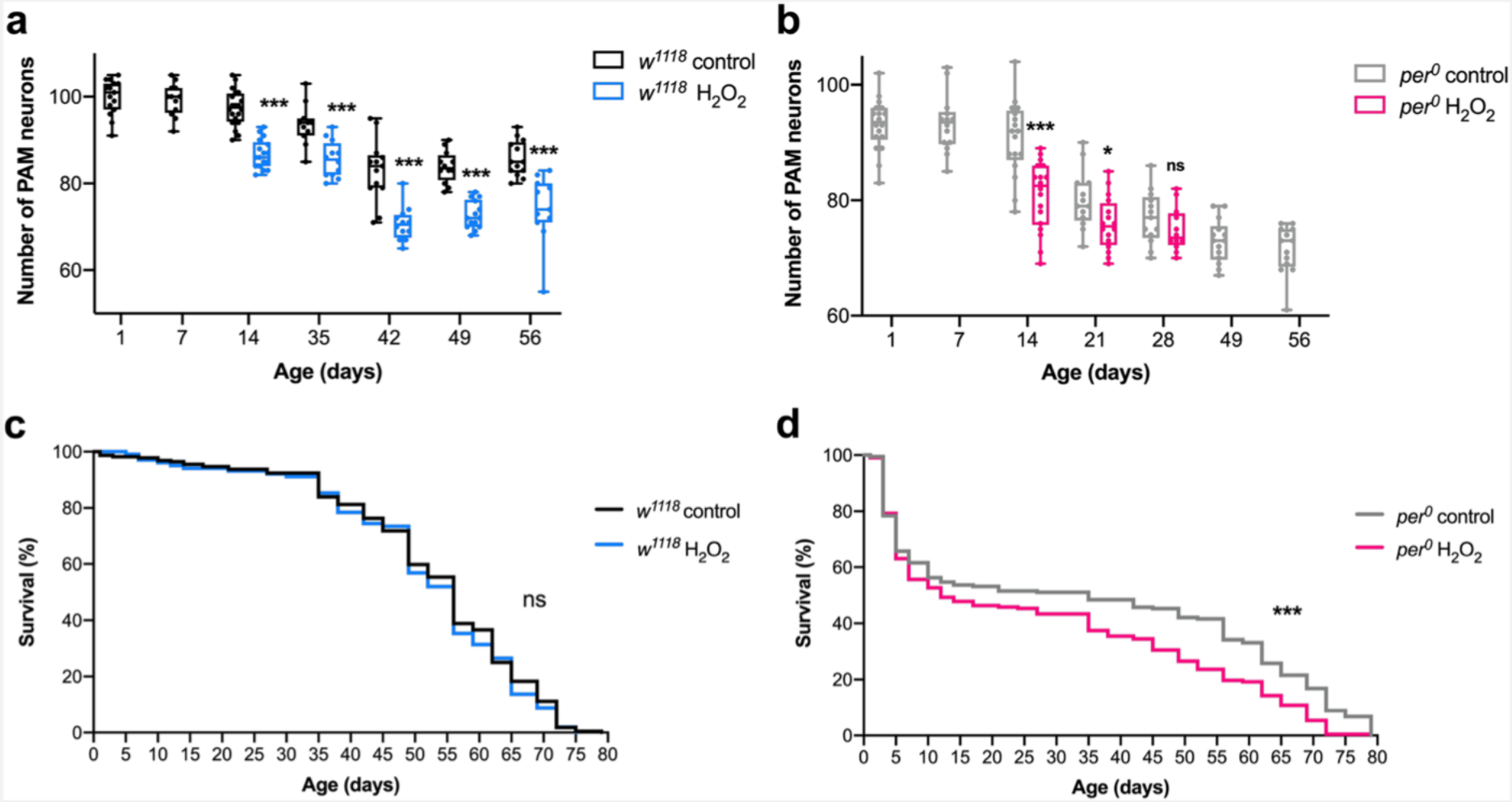






These findings indicate that a 4-h H_2O_2 treatment increases sleep but not hypoactivity

The implication of the multiple-hit hypothesis for Parkinson's disease



These results demonstrate that the combination of an oxidative insult and the per null mutation causes premature death of the animal

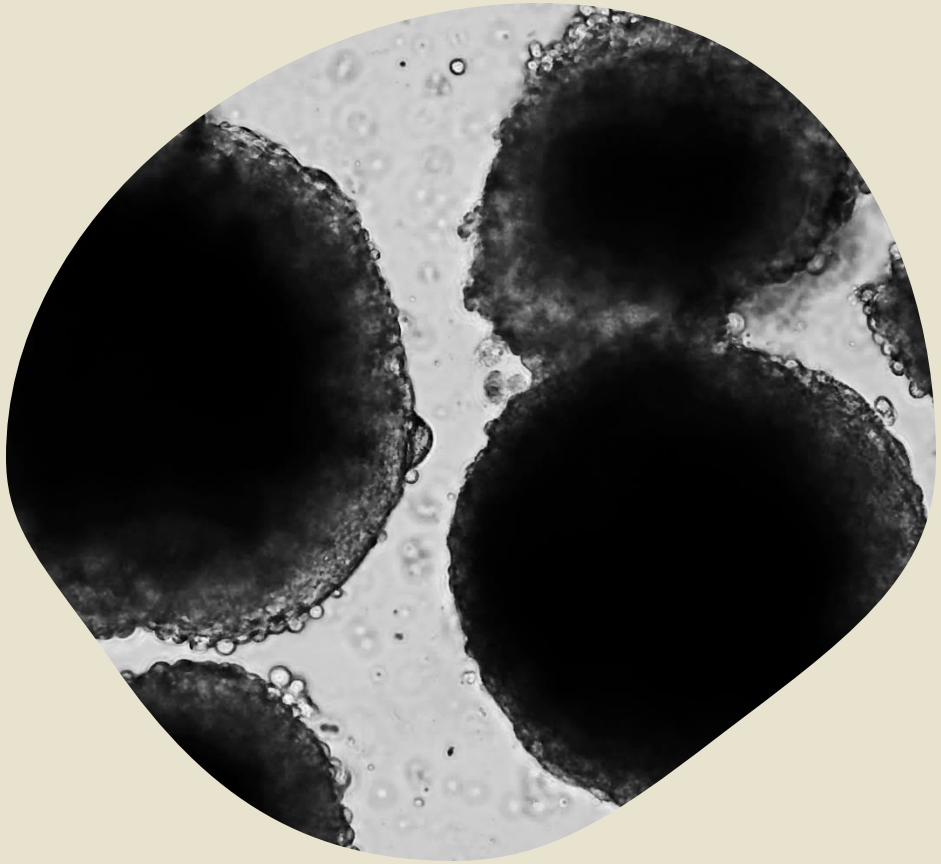
Summary

- Circadian clocks regulate the rhythmicity and magnitude of the vulnerability of DA neurons to oxidative stress in male Drosophila

- Circadian pacemaker neurons are presynaptic to a subset of DA neurons and rhythmically modulate their susceptibility to degeneration

- The arrhythmic period (per) gene null mutation exacerbates the age-dependent loss of DA neurons and, in combination with brief oxidative stress, causes premature animal death

➔ These findings suggest that circadian clock disruption promotes dopaminergic neurodegeneration.



THANKS FOR YOUR ATTENTION ☺