Journal Club

Circadian Clock in *Drosophila*

GC VG ZH

2018-10-26

Circadian Clock in *Drosophila*

- Biological clock: the molecular and neuronal mechanism. (GC)
- Resetting the clock by light, temperature, etc. (ZH)
- Circadian regulation of behaviors and physiology. (VG)

Part I

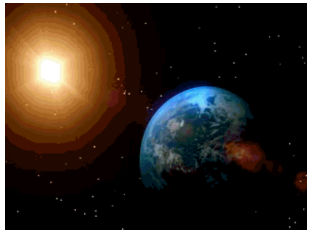
Biological clock:

molecular and neuronal mechanism

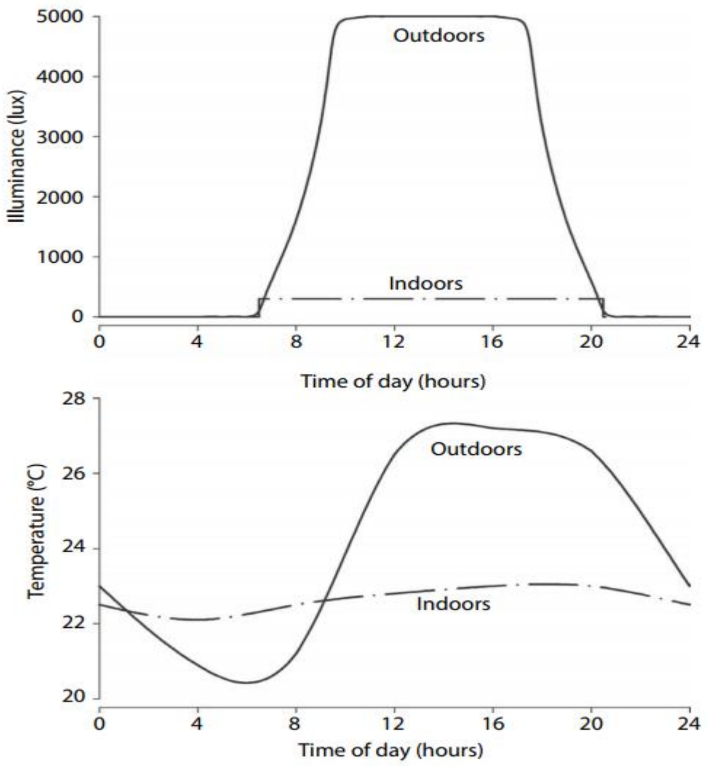
GC

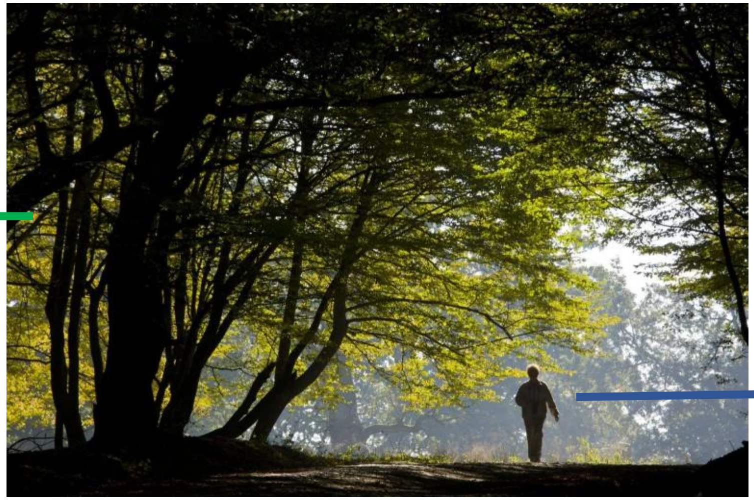
Cir–ca–di–an (sər–kā′dē–ən) *adj. Biol.*
Of or exhibiting approximately 24-hour periodicity.
[<Lat. *circā*, around + *diēs*, day.]

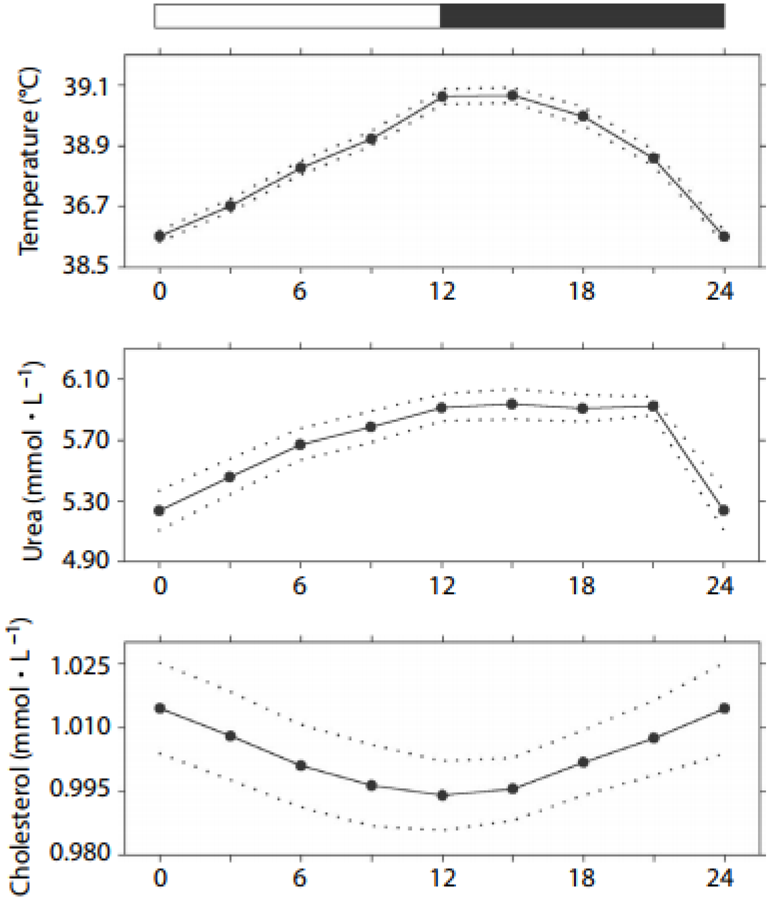
The American Heritage Dictionary of the English Language, Houghton Mifflin, New York, 1994.)



Circadian cycling of physical environment and physiology/behaviors of organisms on earth

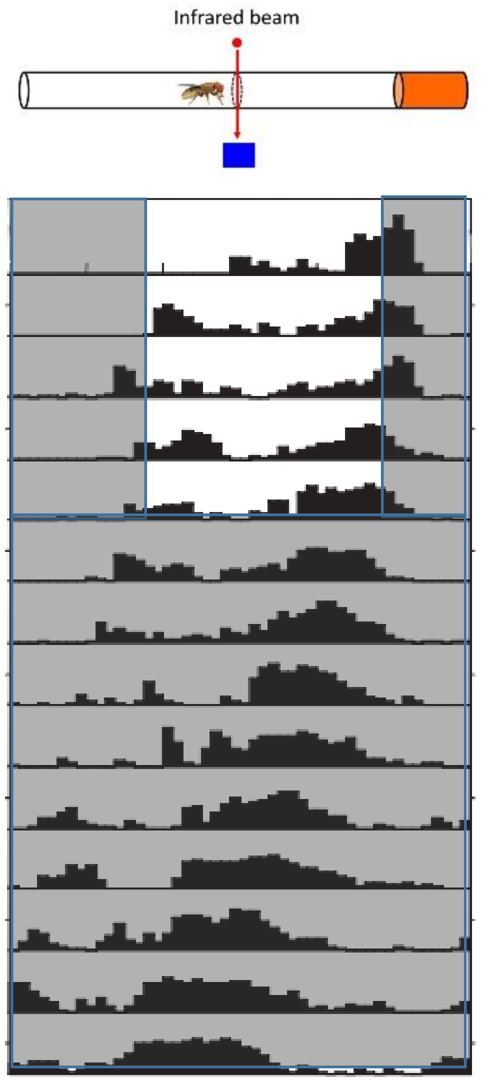


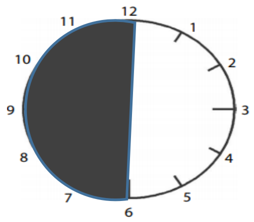




Refnetti, R. (2016). Circadian Physiology, Taylor & Francis Group, LLC.

Circadian rhythm in Chronobiology





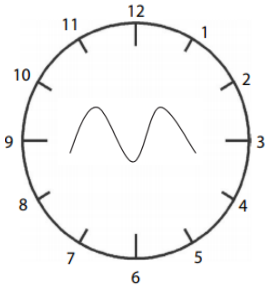
zeitgeber ("timegiver")

zeitgeber time (ZT)

DD

Constant condition

Free running



circadian time (CT)

endogenous
oscillator

A circadian cycle was first observed in the 18th century in the movement of plant leaves

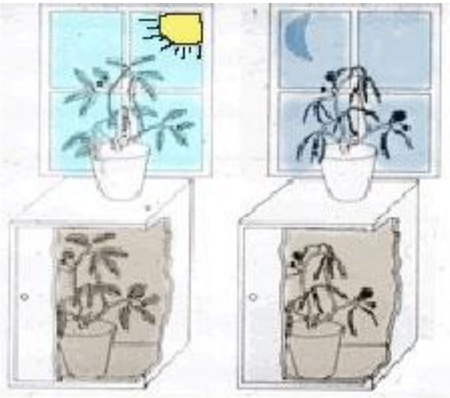
DES SCIENCES. 35

OBSERVATION BOTANIQUE.

ON ſçait que la Senſitive eſt *heliotrope*, c'eſt-à-dire que ſes rameaux & ſes feüilles ſe dirigent toûjours vers le côté d'où vient la plus grande lumiére, & l'on ſçait de plus qu'à cette propriété qui lui eſt commune avec d'autres Plantes, elle en joint une qui lui eſt plus particuliére, elle eſt Senſitive à l'égard du Soleil ou du jour, ſes feüilles & leurs pédicules ſe replient & ſe contractent vers le coucher du Soleil, de la même maniére dont cela ſe fait quand on touche la Plante, ou qu'on l'agite. Mais M. de Mairan a obſervé qu'il n'eſt point néceſſaire pour ce phénoméne qu'elle ſoit au Soleil ou au grand air, il eſt ſeulement un peu moins marqué lorſqu'on la tient toûjours enfermée dans un lieu obſcur, elle s'épanoüit encore très-ſenſiblement pendant le jour, & ſe replie ou ſe reſſerre réguliérement le ſoir pour toute la nuit. L'expérience a été faite ſur la fin de l'Eté, & bien répétée. La Senſitive ſent donc le Soleil ſans le voir en aucune maniére; & cela paroît avoir rapport à cette malheureuſe délicateſſe d'un grand nombre de Malades, qui s'apperçoivent dans leurs Lits de la différence du jour & de la nuit.

Il ſeroit curieux d'éprouver ſi d'autres Plantes, dont les feüilles ou les fleurs s'ouvrent le jour, & ſe ferment la nuit, conſerveroient comme la Senſitive cette propriété dans des lieux obſcurs; ſi on pourroit faire par art, par des fourneaux plus ou moins chauds, un jour & une nuit qu'elles ſentiſſent; ſi on pourroit renverſer par là l'ordre des phénomenes du vrai jour & de la vraye nuit, &c. Mais les occupations ordinaires de M. Mairan ne lui ont pas permis de pouſſer les expériences juſque-là, & il ſe contente d'une ſimple invitation aux Botaniſtes & aux Phiſiciens, qui pourront eux-mêmes avoir d'autres choſes à ſuivre. La marche de la véritable Phiſique, qui eſt l'Expérimentale, ne peut être que fort lente.

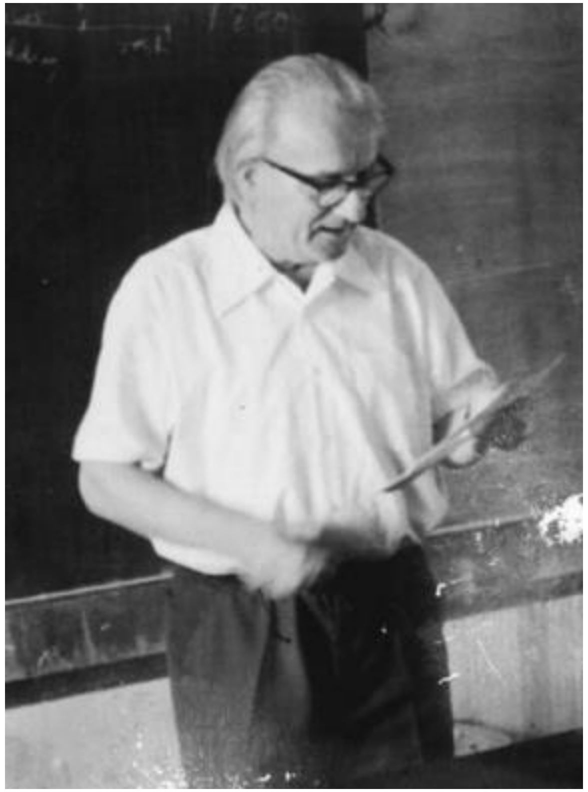
E ij



Credit: National Library of Medicine

De Mairan, J.J.O. 1729. Observation Botanique, Histoire de l'Academie Royale des Sciences, Paris, p.35.

Founders of modern Chronobiology

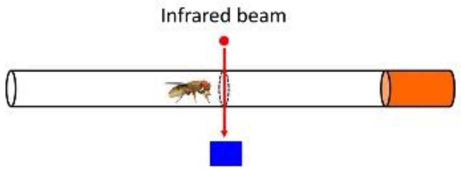


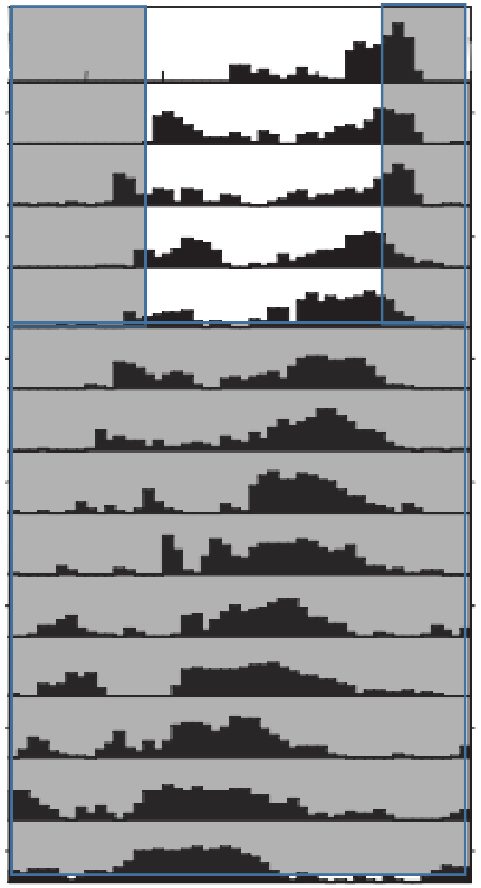
Erwin Bünning
endogenous circadian rhythms
governing plant photoperiodism

Jürgen Aschoff
Zeitgeber, zeitgeber time

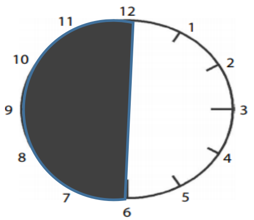
Colin Pittendrigh
Entrainment to local light-dark cycles
temperature compensation

Circadian rhythm in Chronobiology



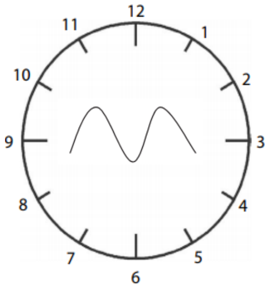


DD
Constant condition
Free running



zeitgeber (“timegiver”)
zeitgeber time (ZT)

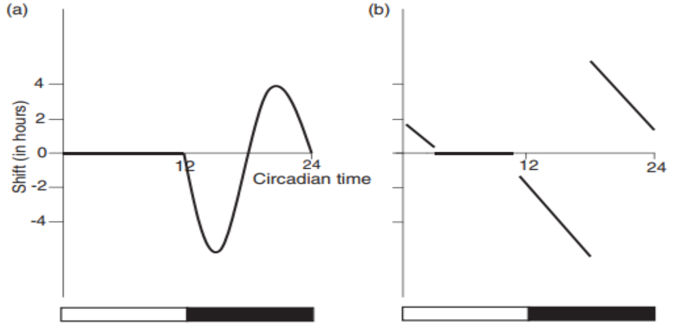
Entrainment

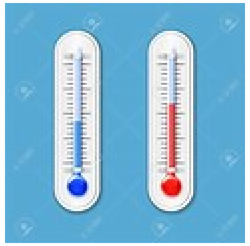


endogenous oscillator

circadian time (CT)

phase response curves (PRCs)





temperature compensated

Drosophila as a model for chronobiologists

- 1930s Kalmus and Bunning demonstrated a circadian rhythm in *Drosophila* eclosion.
- 1950s Colin Pittendrigh employed *Drosophila pseudoobscura* to explore the formal properties of phase resetting and to formulate the general circadian principle of temperature compensation.
- 1971, Ronald Konopka and Seymour Benzer isolated the first single gene “clock” mutants in *Drosophila melanogaster*, thereby initiating genetic analysis of the clock mechanism.

Proc. Nat. Acad. Sci. USA
Vol. 68, No. 9, pp. 2112–2116, September 1971

Clock Mutants of *Drosophila melanogaster*

(eclosion/circadian/rhythms/X chromosome)

RONALD J. KONOPKA AND SEYMOUR BENZER

Division of Biology, California Institute of Technology, Pasadena, Calif. 91109

Contributed by Seymour Benzer, July 2, 1971

ABSTRACT Three mutants have been isolated in which the normal 24-hour rhythm is drastically changed. One mutant is arrhythmic; another has a period of 19 hr; a third has a period of 28 hr. Both the eclosion rhythm of a population and the locomotor activity of individual flies are affected. All these mutations appear to involve the same functional gene on the X chromosome.

Rhythmic variations in behavior are displayed by many organisms, ranging from single cells to man (1). When the rhythm persists under constant conditions, and has a period of around one day, depending little on temperature, the rhythm is called circadian (2). Many experiments have attempted to probe the mechanism (3), but the nature of the underlying oscillation remains unknown (4). Perturbations by inhibitors of RNA or protein synthesis suggest that such molecules are involved (5–8). Biochemical systems that oscillate with much shorter periods have been demonstrated

dark period. In a few bottles, males emerged in approximately equal numbers during day and night. Each mutant candidate was examined in more detail by raising pupae in LD 12:12, then monitoring the adult eclosion rhythm in constant darkness. From a total of about 2000 F_1 males, three rhythm mutants were obtained.

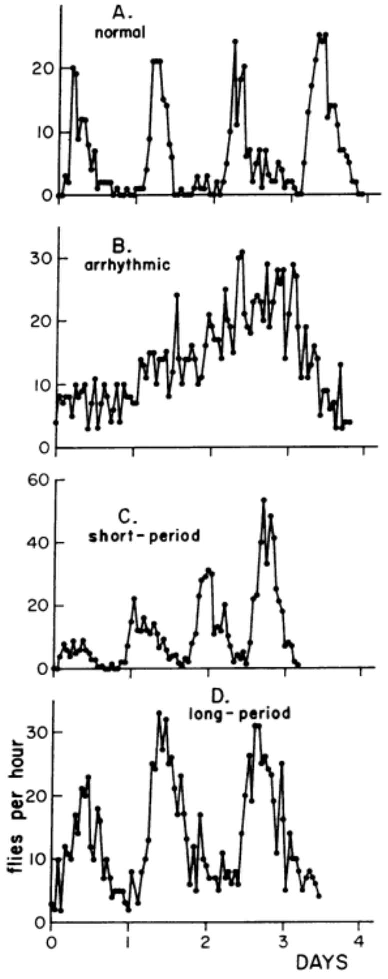
Determination of eclosion and locomotor activity rhythms

Eclosion rhythms, free-running in constant darkness, were determined with automatic "bang boxes" (20), generously loaned by Dr. Colin Pittendrigh. Several hundred pupae, raised in LD 12:12, were transferred to the apparatus at the end of a light cycle. The apparatus was thereafter maintained in constant darkness. Fractions were collected every hour, yielding an eclosion profile.

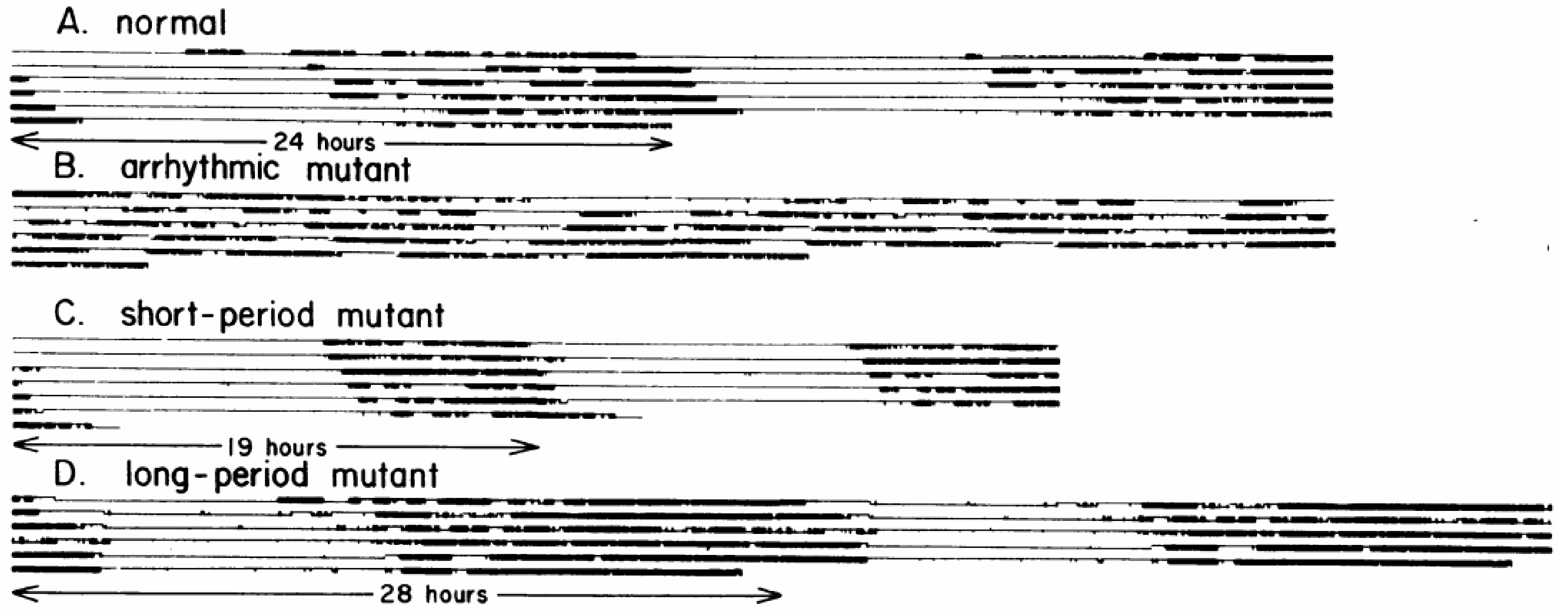
Locomotor activity of individual adult flies was measured

Eclosion rhythms and locomotor activity rhythms in constant darkness for populations of rhythmically normal and mutant flies

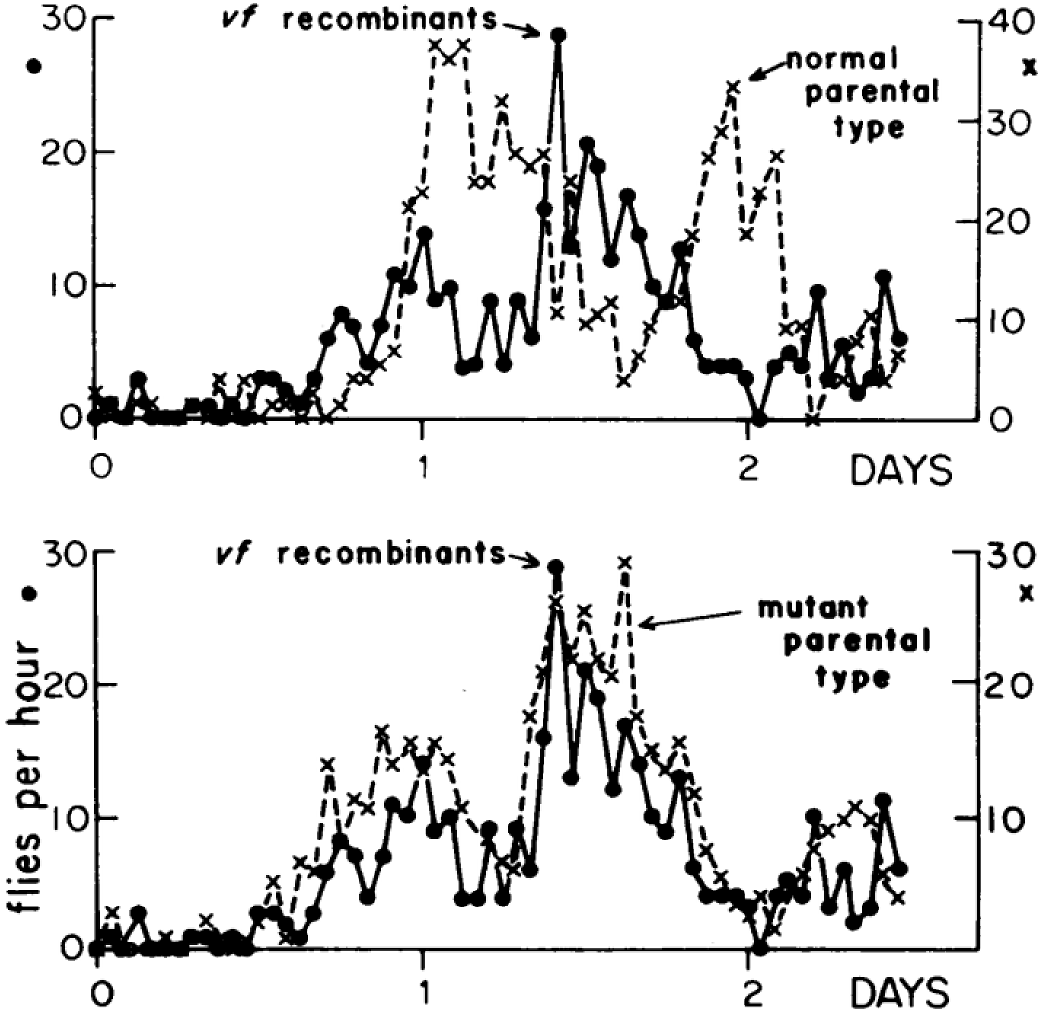
Eclosion

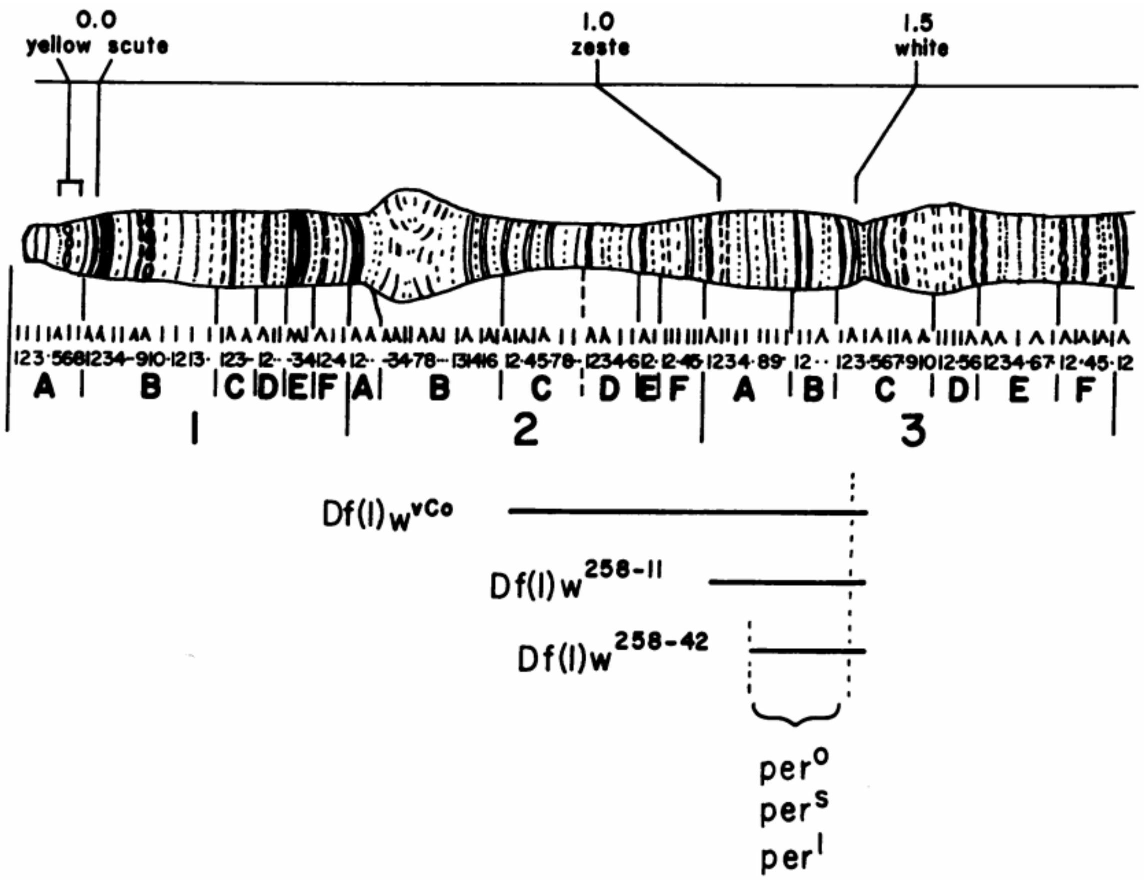


Locomotor activity



Mapping *per* to 3B1-3B2 on X chromosome



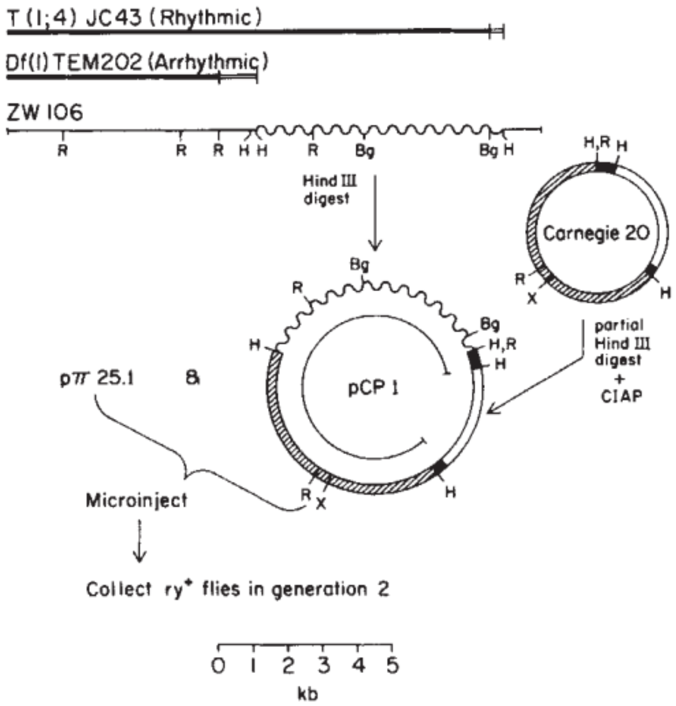


Cloning *per* - the first clock gene

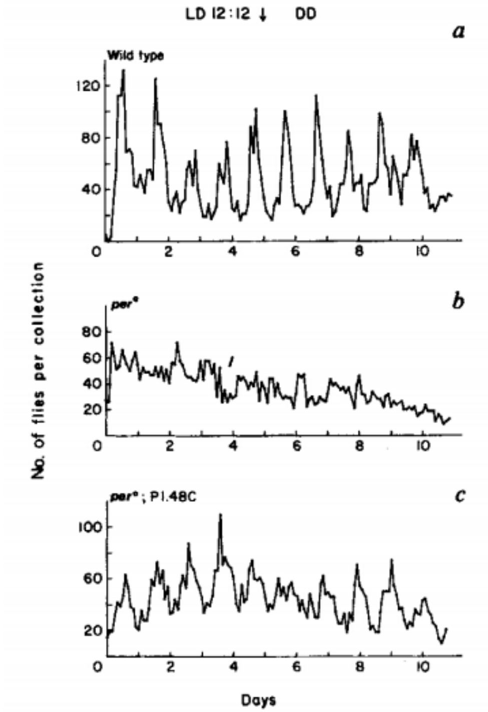
Bargiello, T. A., F. R. Jackson and M. W. Young (1984). "Restoration of circadian behavioural rhythms by gene transfer in Drosophila." Nature 312(5996): 752-754.

Reddy, P., W. A. Zehring, D. A. Wheeler, V. Pirrotta, C. Hadfield, J. C. Hall and M. Rosbash (1984). "Molecular analysis of the period locus in Drosophila melanogaster and identification of a transcript involved in biological rhythms." Cell 38(3): 701-710.

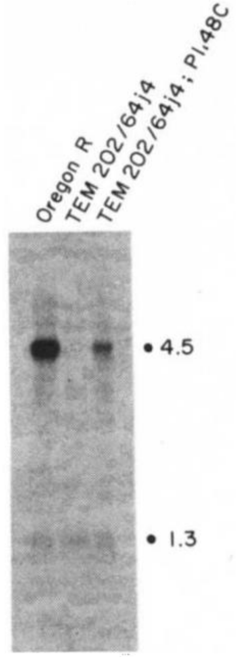
Zehring, W. A., D. A. Wheeler, P. Reddy, R. J. Konopka, C. P. Kyriacou, M. Rosbash and J. C. Hall (1984). "P-element transformation with period locus DNA restores rhythmicity to mutant, arrhythmic Drosophila melanogaster." Cell **39**(2 Pt 1): 369-376.



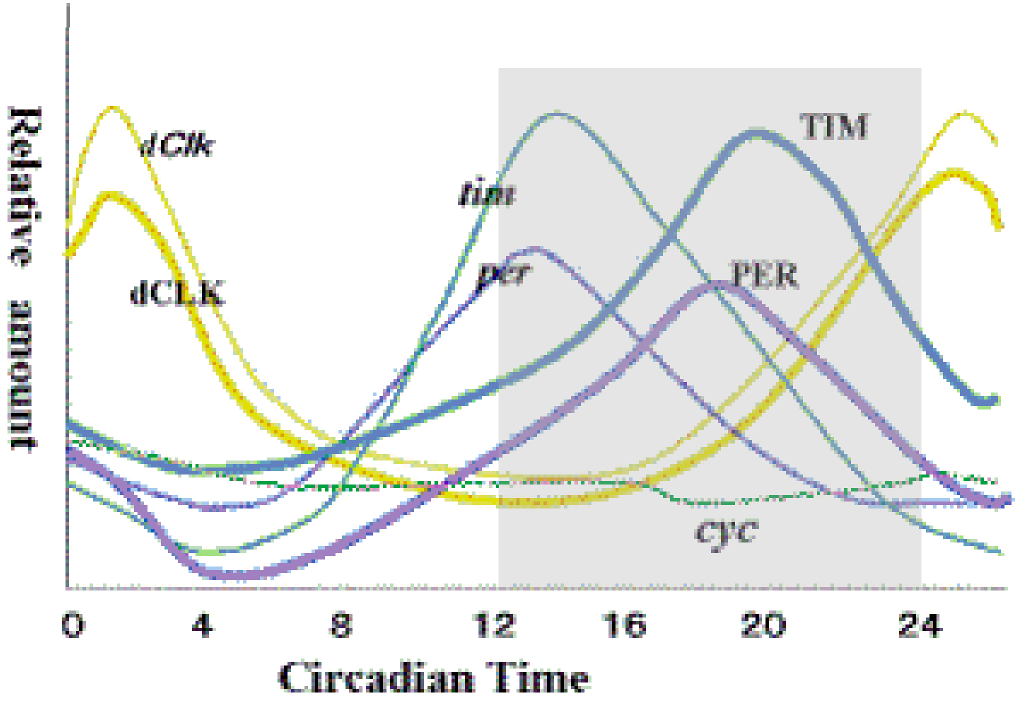
Physical map of per region DNA, and construction of transforming DNA pCP1

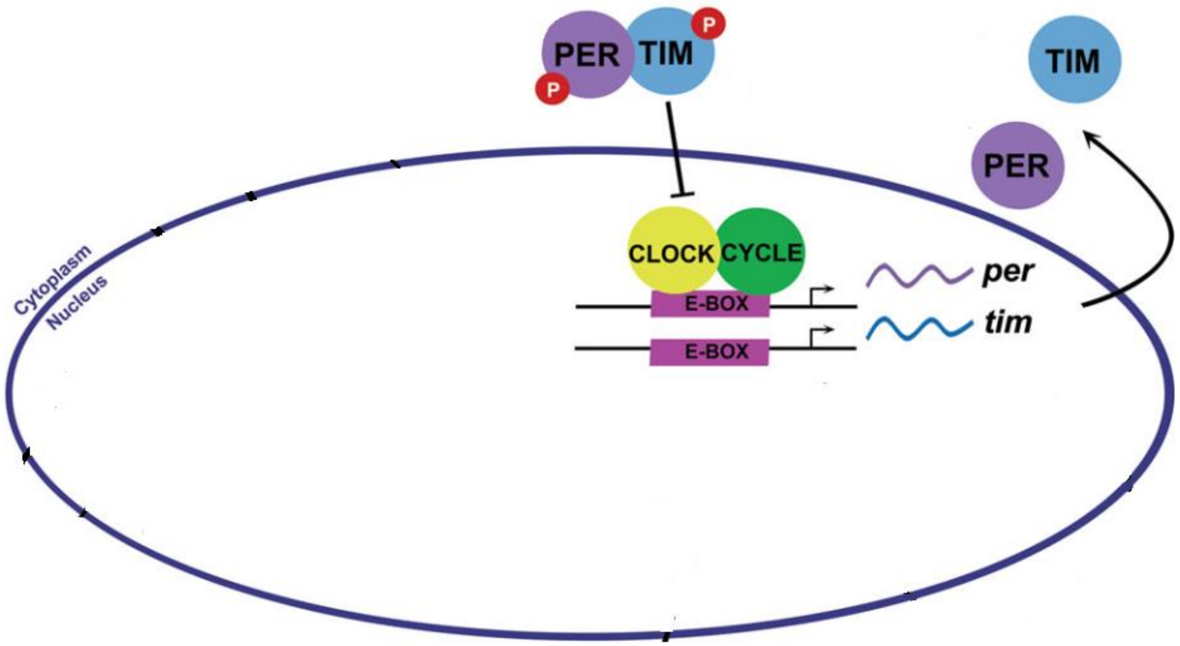


Temporal profile of eclosion

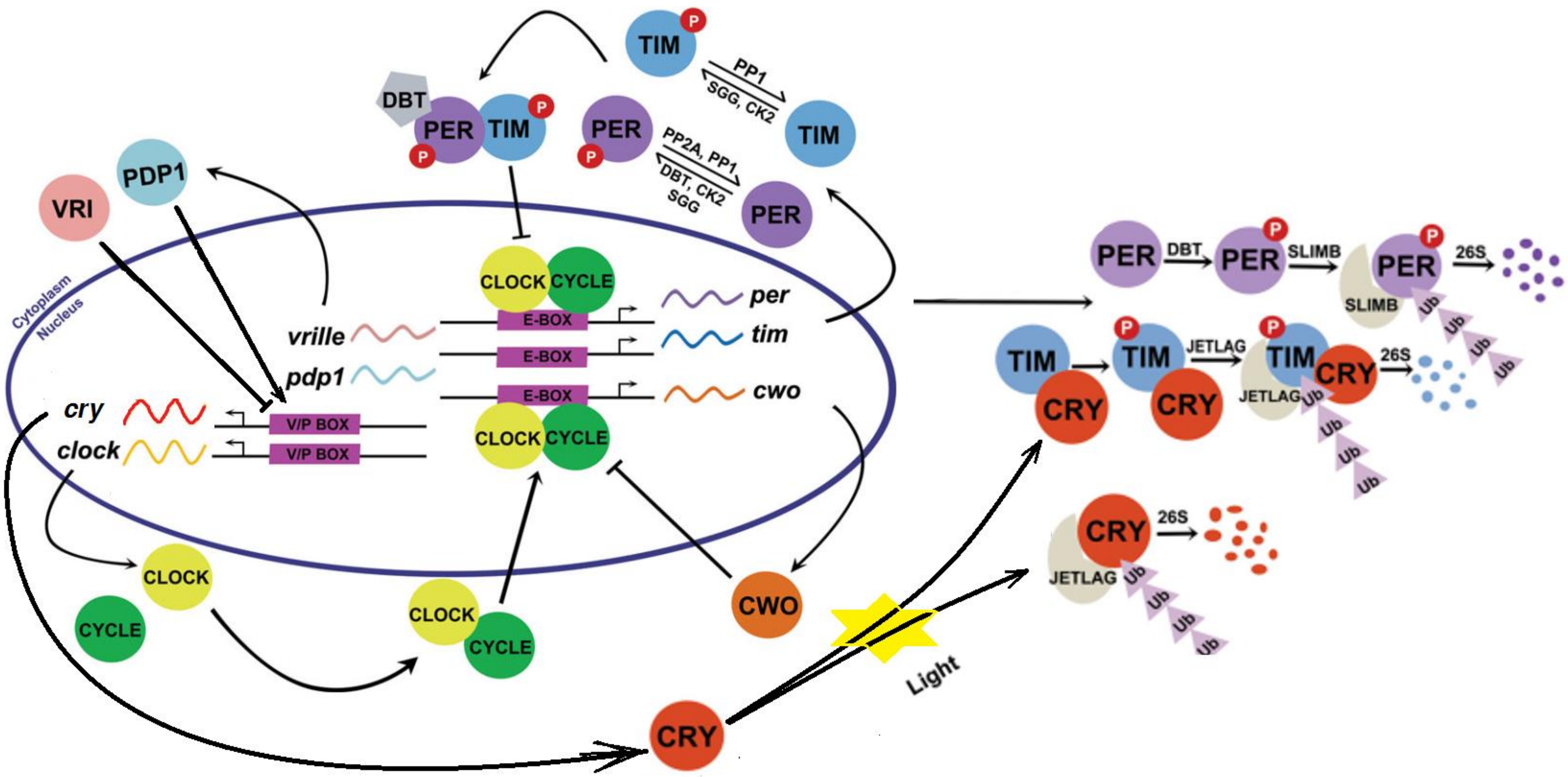


The core transcription/translation feedback loop





The circadian molecular network

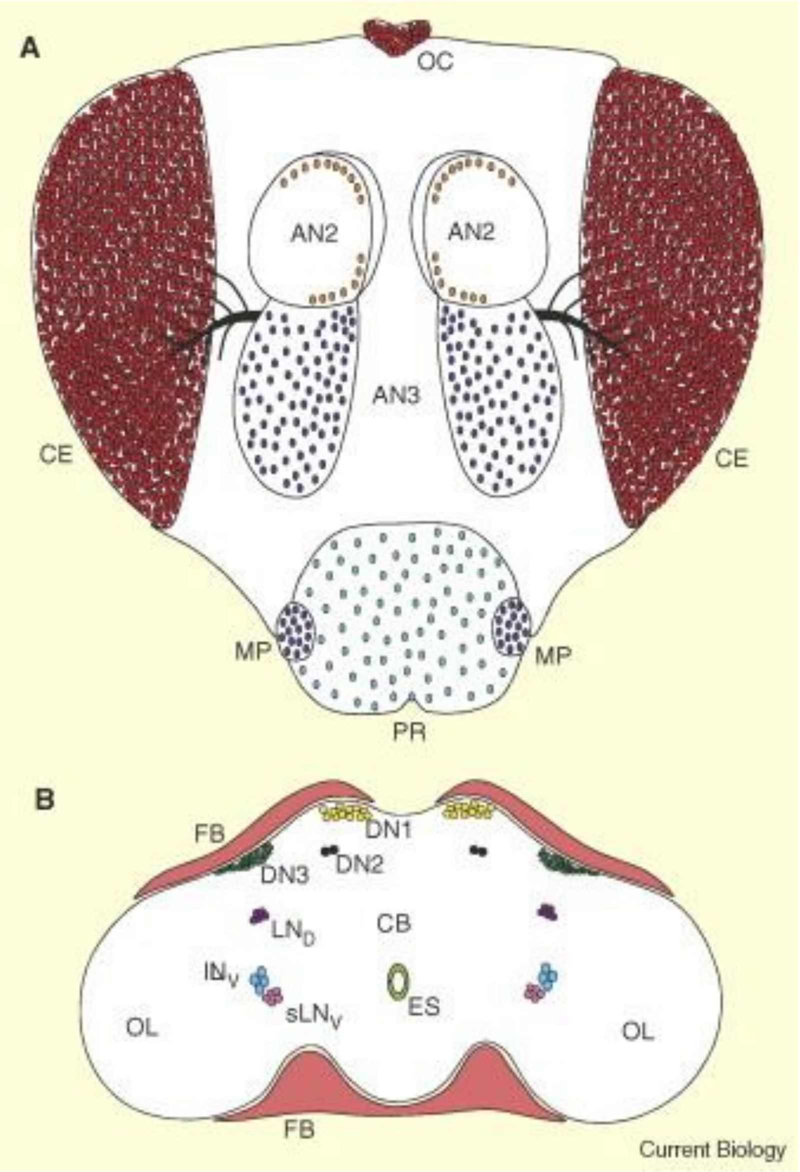


Mendoza-Viveros, L. et al. 2017

Circadian genes

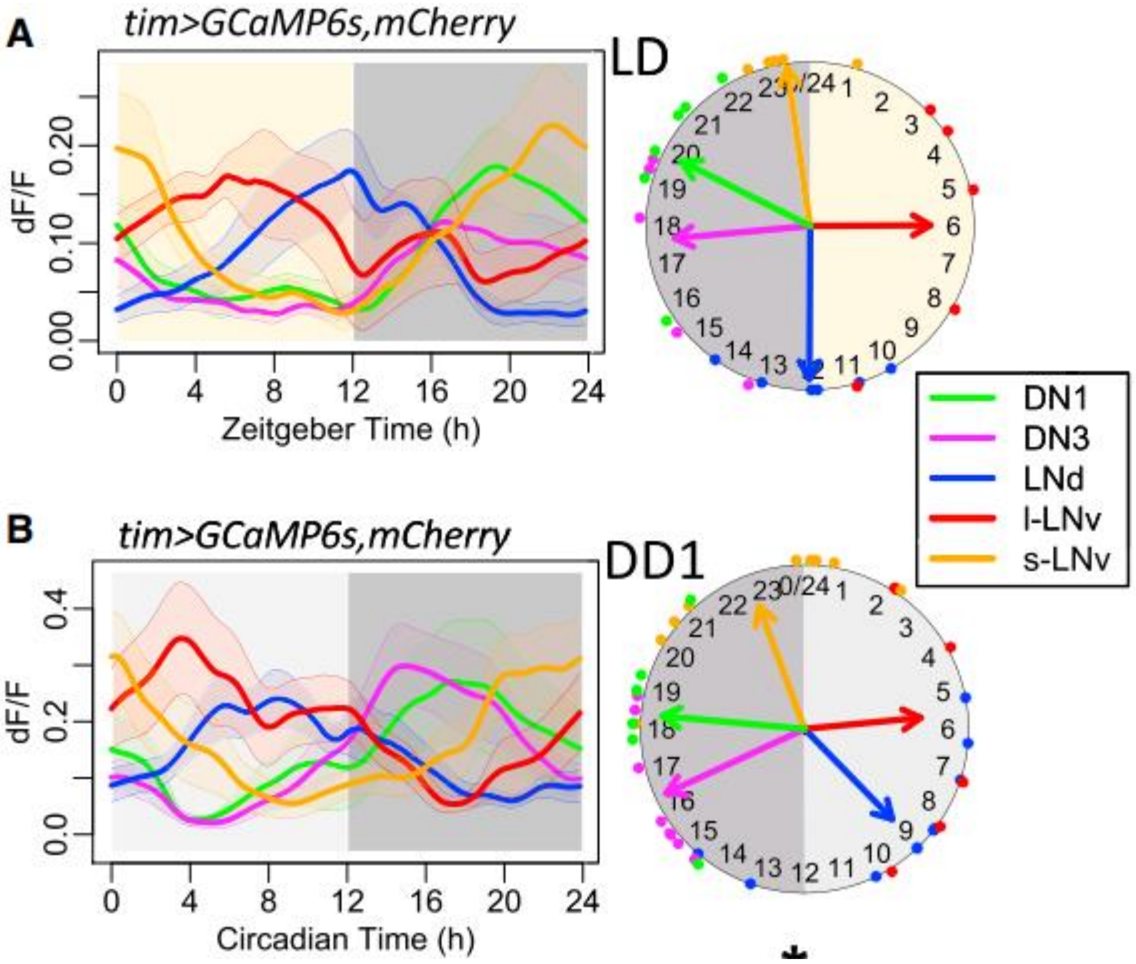
gene	Circadian function	Molecular action	Circadian system
period	ar, short, or long circadian behavioral and molecular rhythms	Interacts with TIM, dCLK and CYC to negatively regulate dCLK/CYC	Oscillator
sgg	Shaggy, Short or long circadian behavioral and molecular rhythms	Glycogen synthase kinase III ortholog which phosphorylates TIM to regulate the timing of TIM's light sensitivity and nuclear feedback	Oscillator
tim	timeless	Dimerizes with Per proteins to inhibit Cycle/Clock transcriptional activity	Oscillator
Clk	ar circadian behavioral and molecular rhythms	Dimerizes with Cycle to bind E-box and activate transcription of Per and Tim	Oscillator
Pdf	Pigment-dispersing factor		Oscillator
cyc	Cycle ar circadian behavioral and molecular rhythms	Interacts with dCLK to positively regulate *per* and *tim* mRNA and to negatively regulate *dClk* mRNA; both types of regulation repressed by PER/TIM	Oscillator
Doubletime	ar, short, or long circadian behavioral and molecular rhythms	Phosphorylates Per proteins Casein kinase I ortholog which interacts with PER to produce phosphorylation of PER; regulates stability and timing of nuclear feedback by PER	Oscillator
slmb	supernumerary limbs		Oscillator
vri	Vrille, Short, long, and damped circadian behavioral and molecular rhythms	Transcription factor which is positively regulated by dCLK/CYC and which negatively regulates *per*, *tim* and *dClk* mRNA	Oscillator
cry	Cryptochrome, Arrhythmic and high levels of PER and TIM in most tissues; rhythmic behavior and molecular oscillations in lateral neurons.	Light-activated Cry binds Tim, allows its phosphorylation by Double-time, with ensuing degradation and clock resetting	Input; peripheral Oscillator
CkII	casein kinase IIα	phosphorylates and destabilizes PER and TIM, inhibit CLK,	
CrebB	Cyclic-AMP response element binding protein	cAMP response element binding protein transcription factor	

Circadian oscillators in *Drosophila* heads



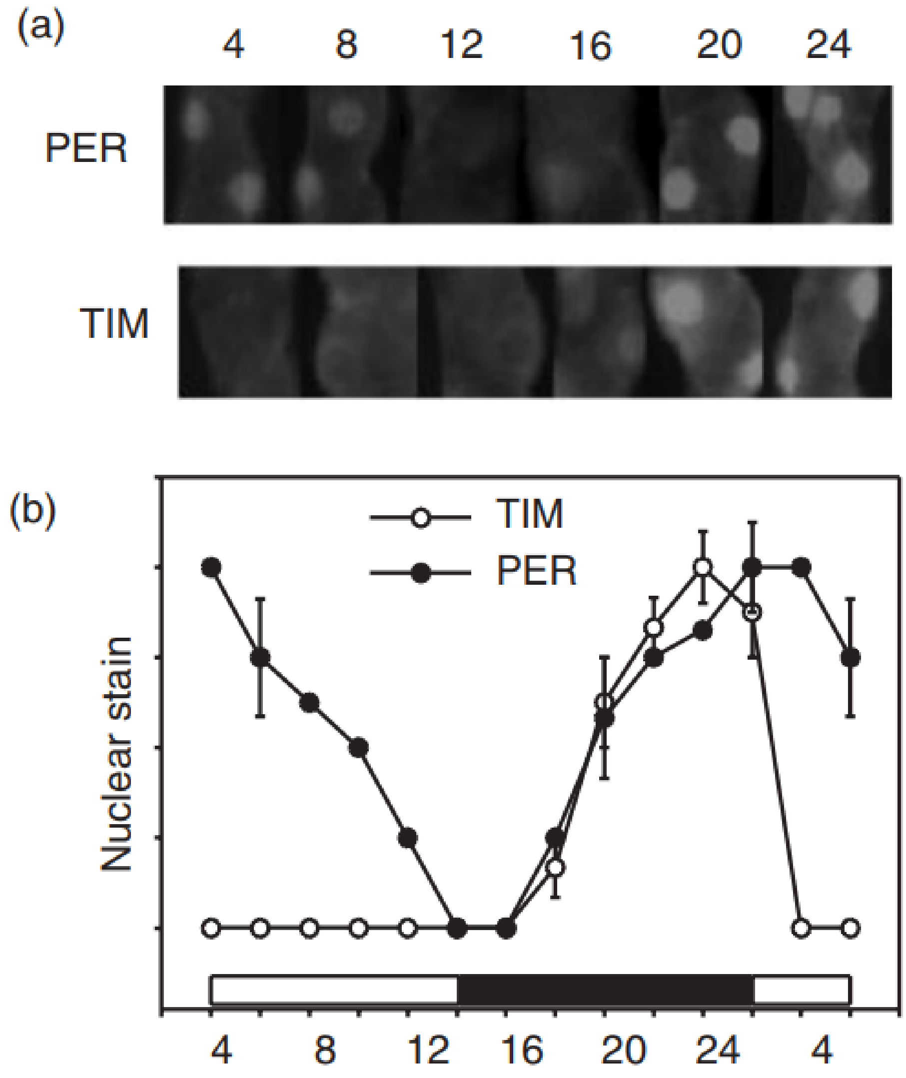
Paul E. Hardin , 2005

Cycling in circadian circuits



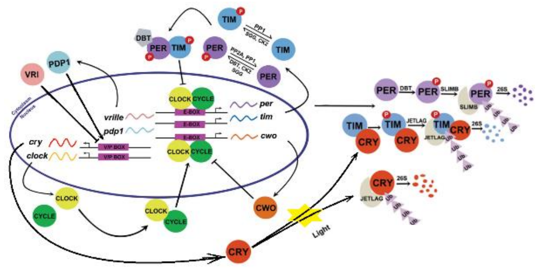
Liang, X et al. (2017)

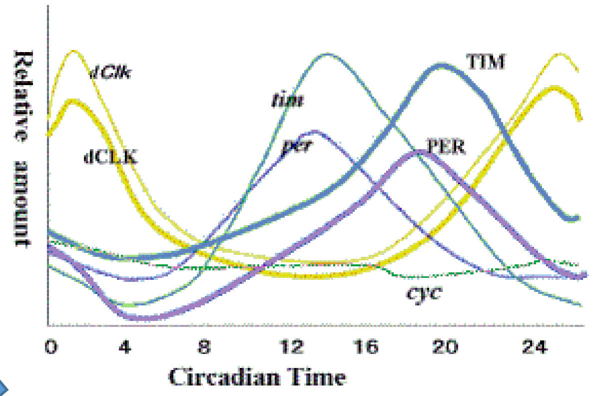
Circadian clock in Malpighian tubules

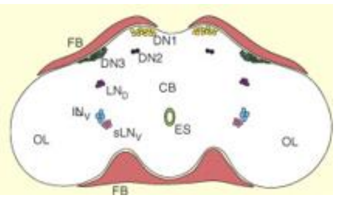


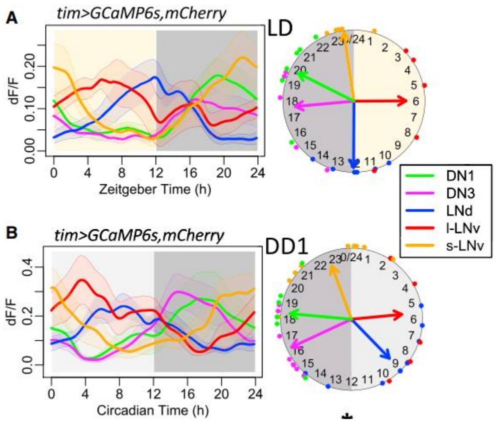
from Ivanczenko et al.(2001)

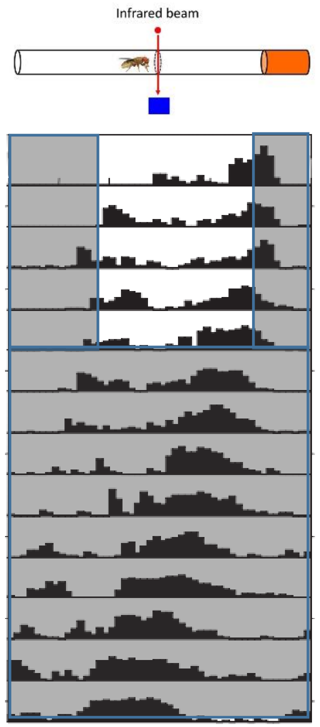
Circadian rhythm in *Drosophila*

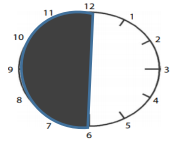




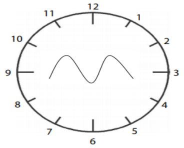








zeitgeber



endogenous oscillator

Questions

- More clock genes
- How clocks communicate
- Temperature compensation

Part II

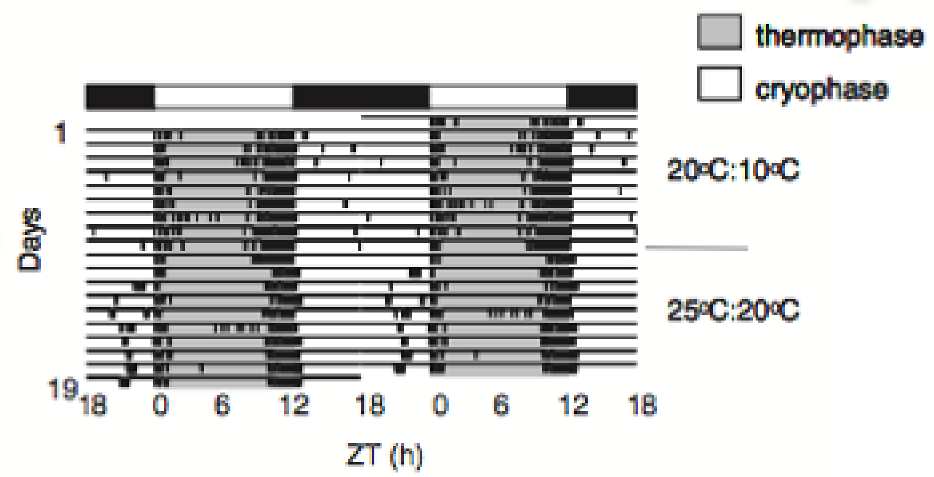
Resetting the clock

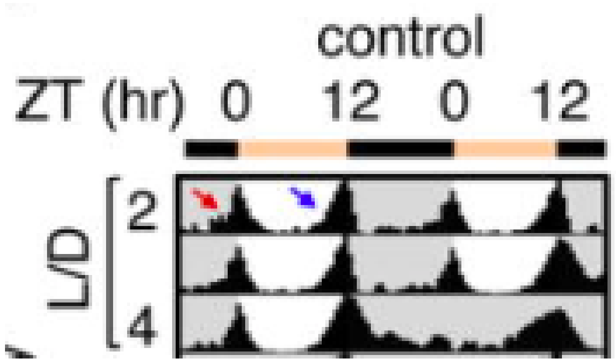
light & temperature

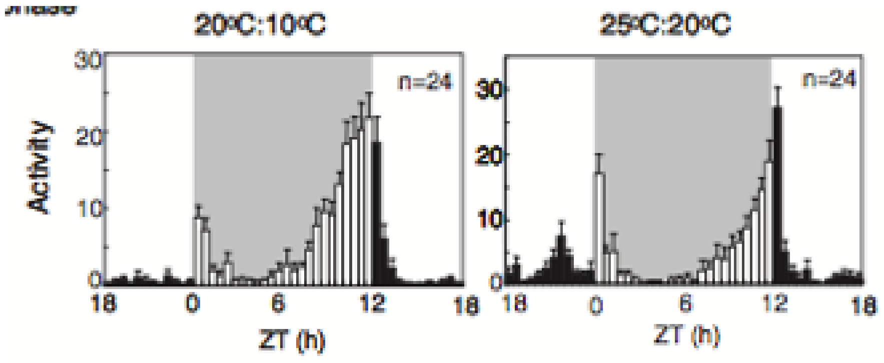
ZH

- Animals have a set of intrinsic strong oscillating pacing mechanisms. What synchronize their cycles with the outside world?
- Light is the most direct clue of the environment, how does the entrainment of circadian clock respond to light?
- Temperature is another clue of the external environmental. It is also one of the important conditions affecting the biochemical reaction. How does the circadian clock respond to the temperature shifts?
- Are there any other elements that influence the entrainment of circadian clock？

Natural entrainment of circadian clock

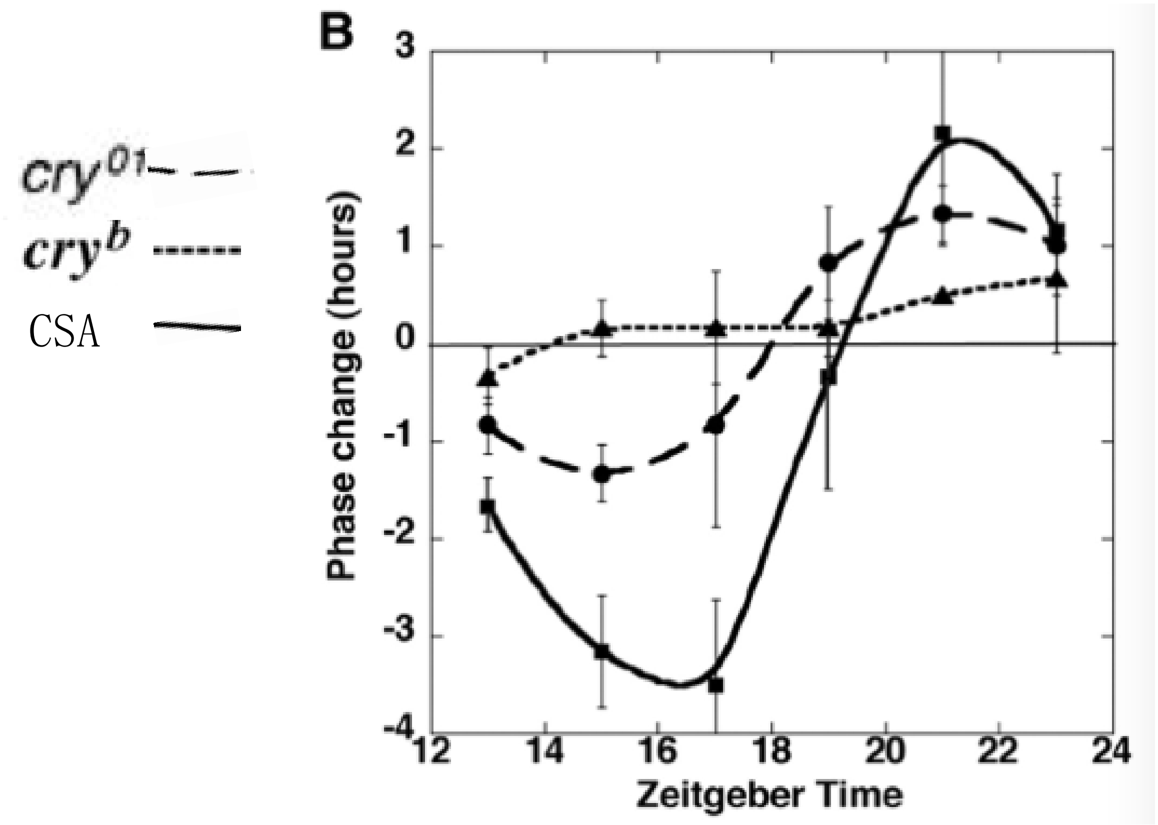






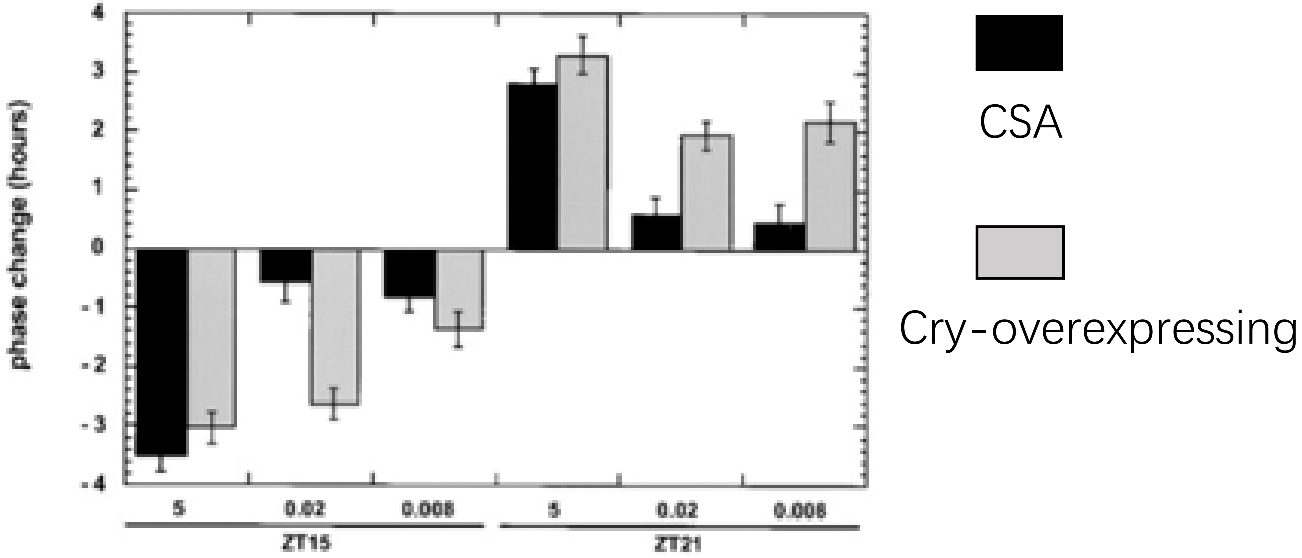
Miyasako Y.2007

Cryptochrome: a Circadian Photoreceptor in *Drosophila*



Circadian photoresponses in *cry^m* flies

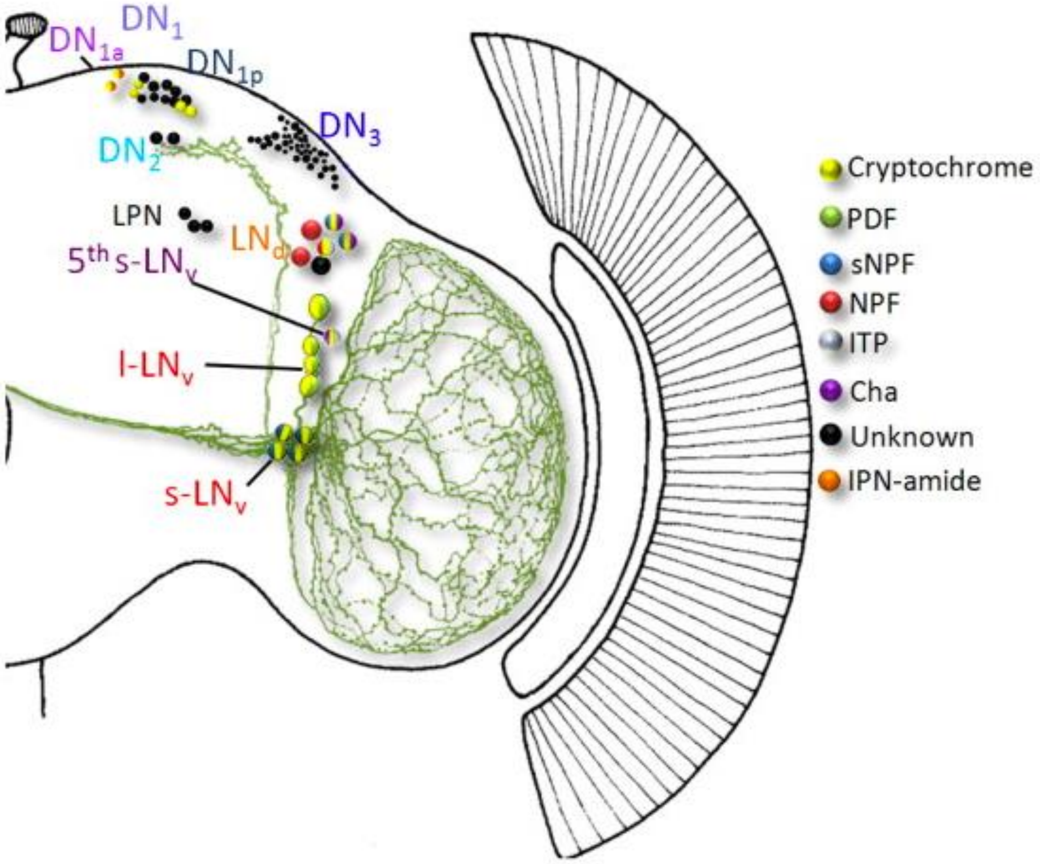
Busza. 2004

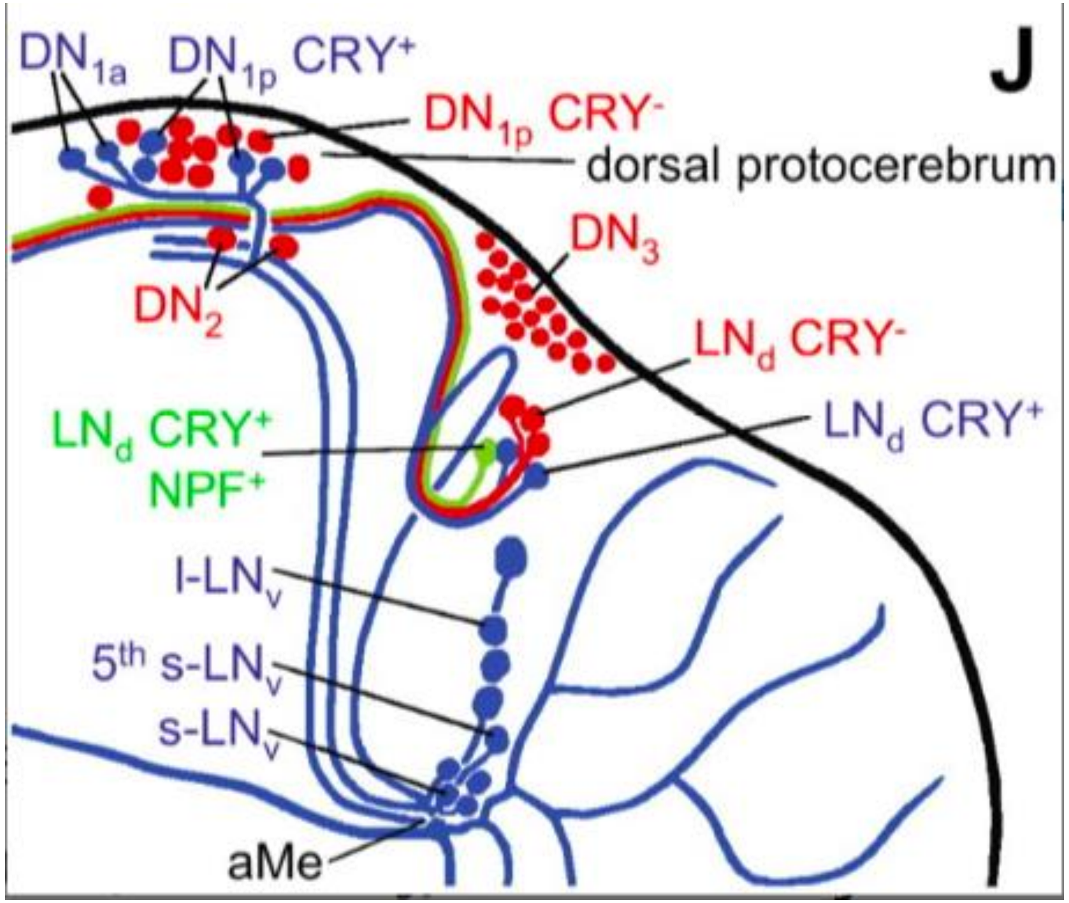


cry-Overexpressing Flies Manifest Hypersensitive Circadian Responses to Light

Emery. 2000

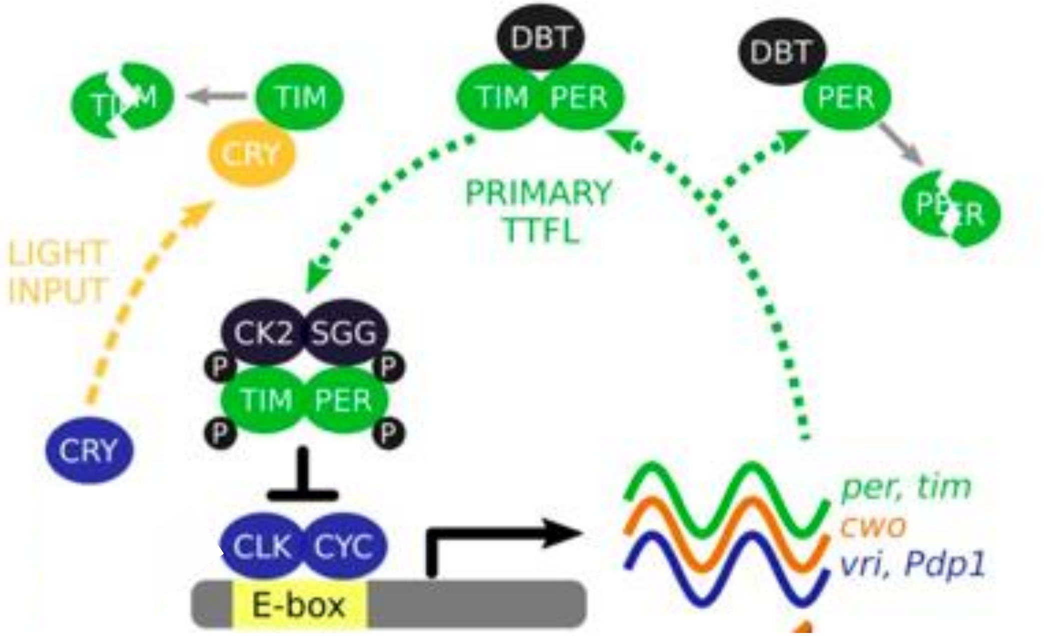
Pattern of CRY expression



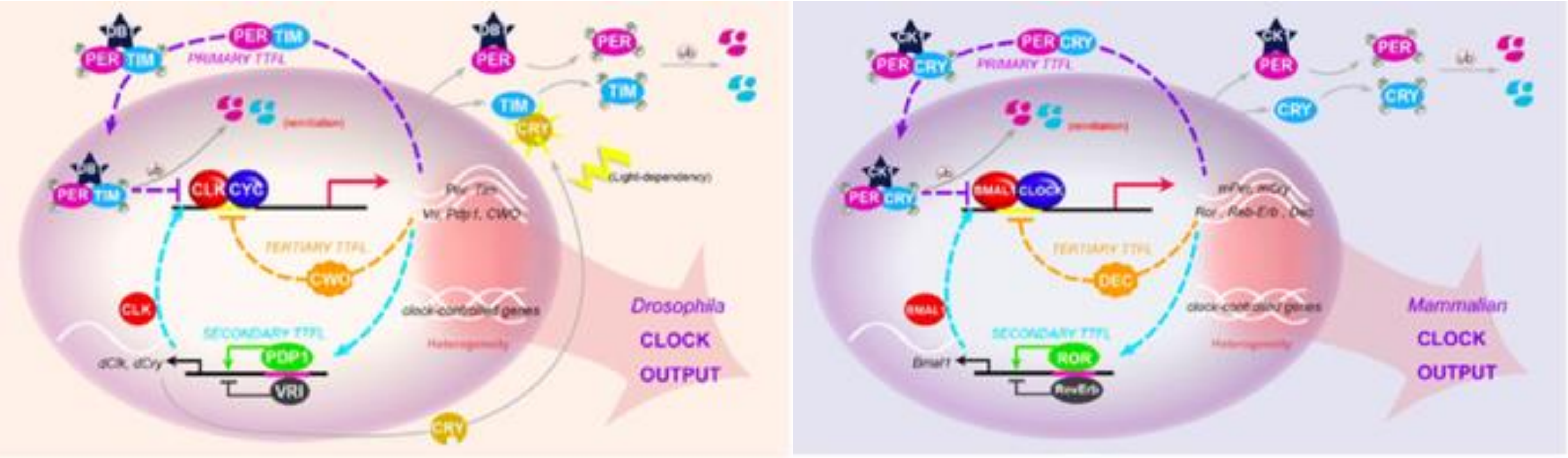


Yoshii T. J Comp Neurol. 2008

The mechanism of CRY working on circadian feedback loop

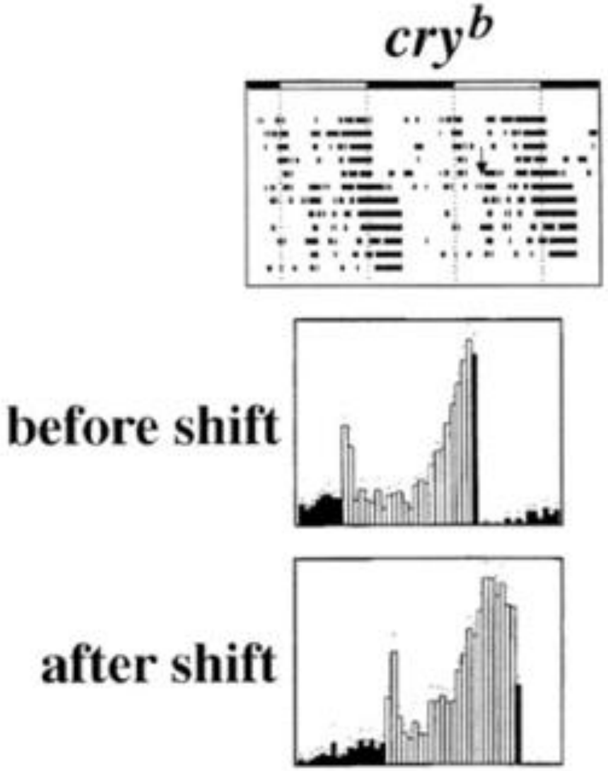


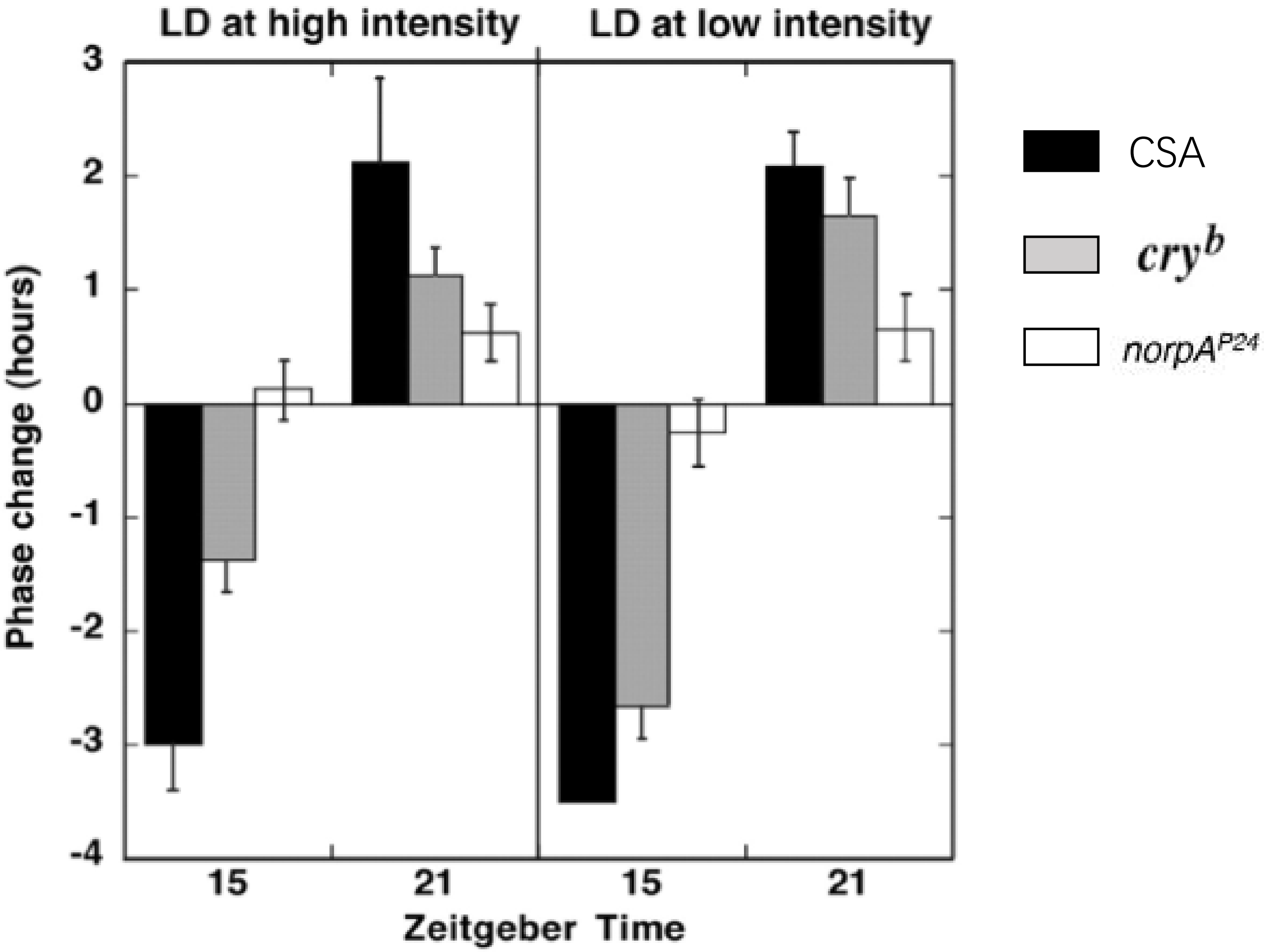
Compare *Drosophila* with mammalian



Yoonhee Ki.2015

Cry is not sufficient for circadian photoentrainment

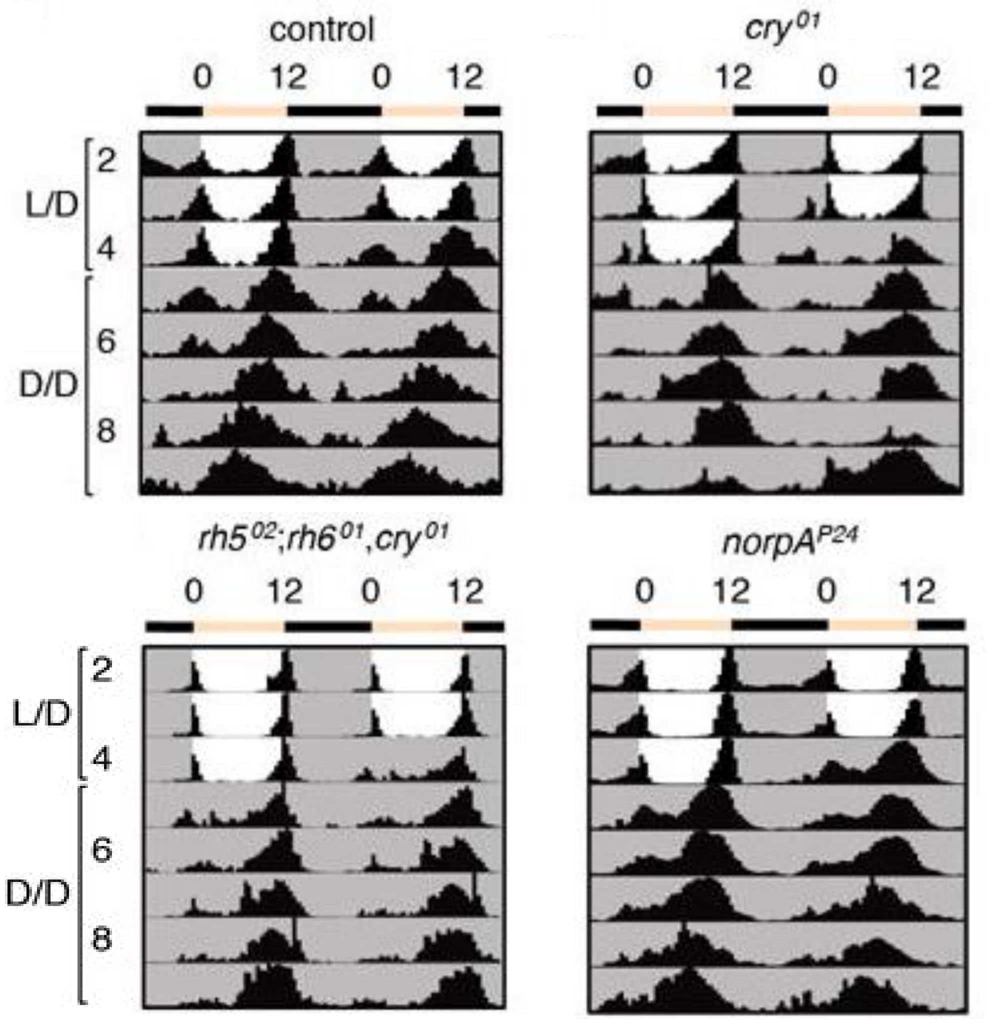


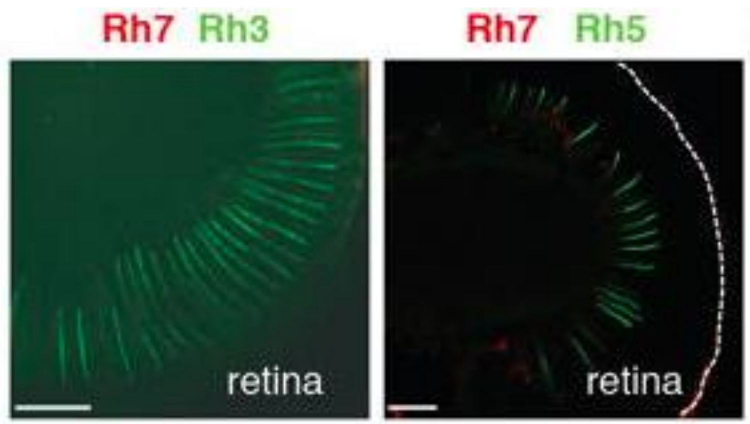


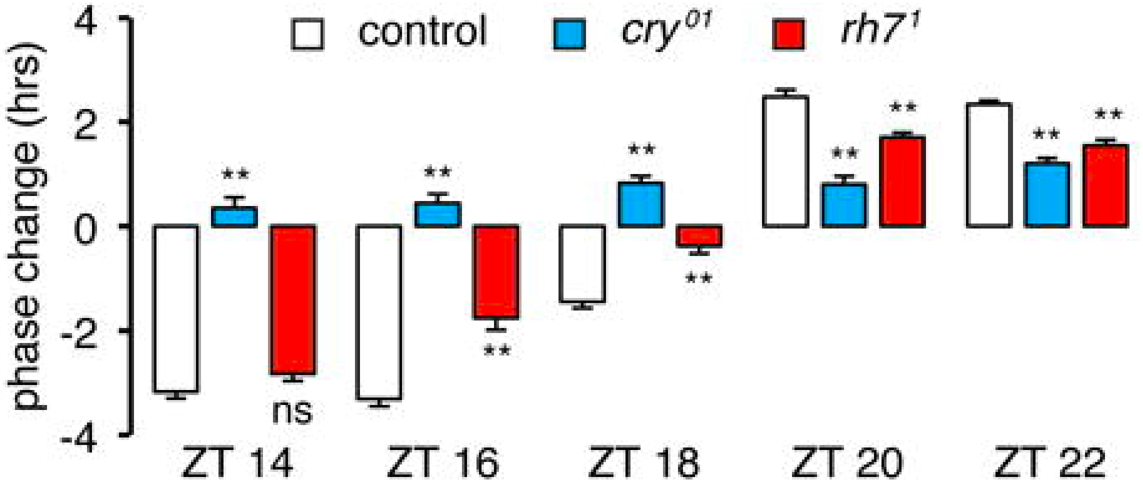
Stanewsky. 1998

Busza.ect 2004

Rh7:A rhodopsin functions in circadian photoentrainment

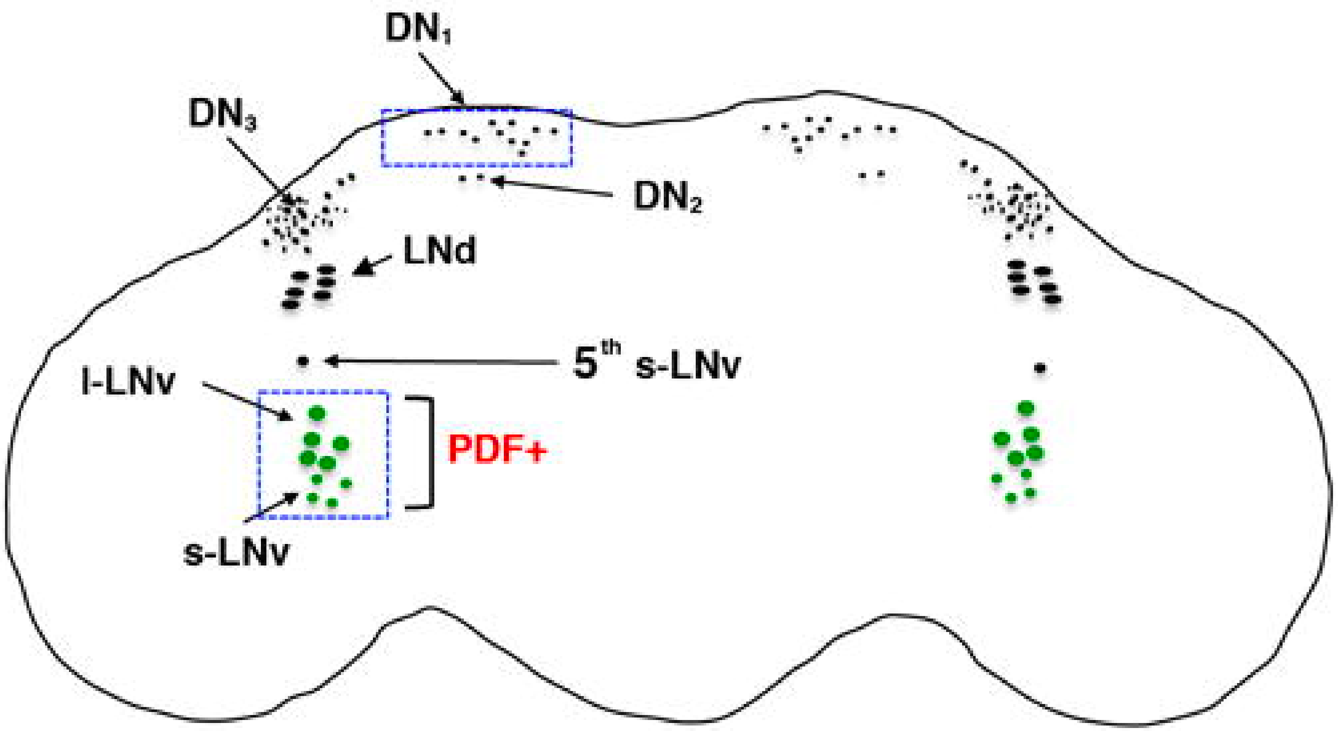




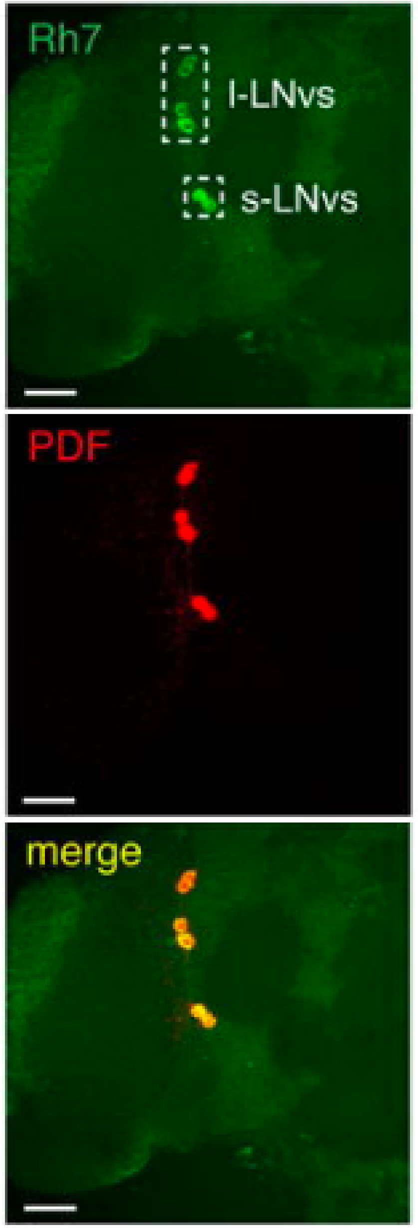


Jinfei D. Ni.2017

Pattern of Rh7 expression

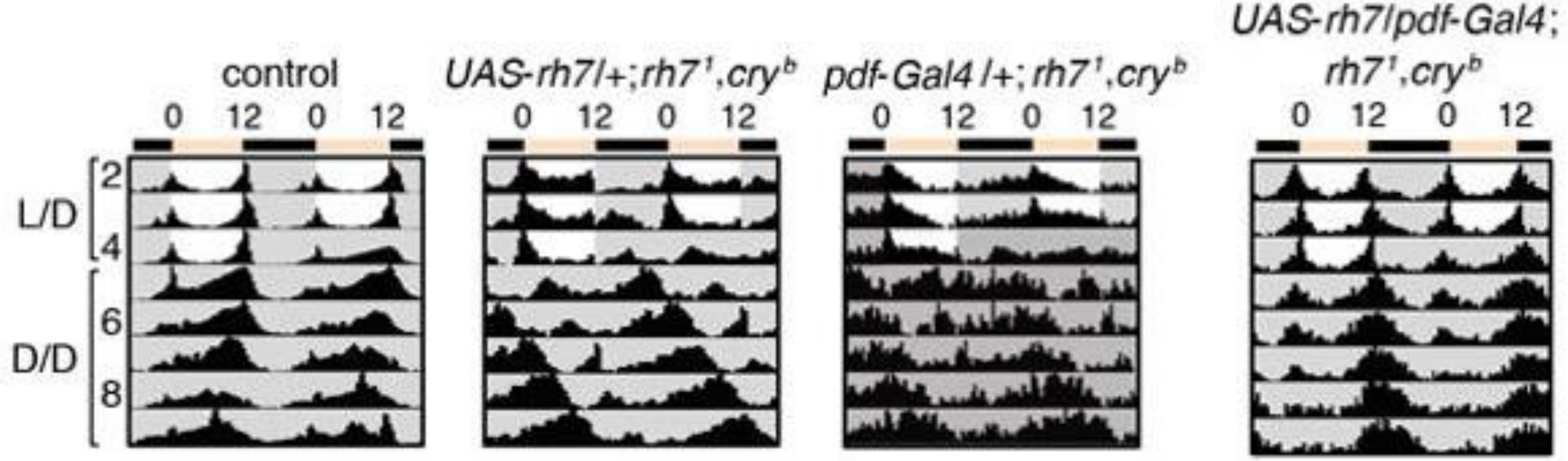


Different groups of clock neurons. The boxed areas indicate locations of two groups of Rh7-positive cells.



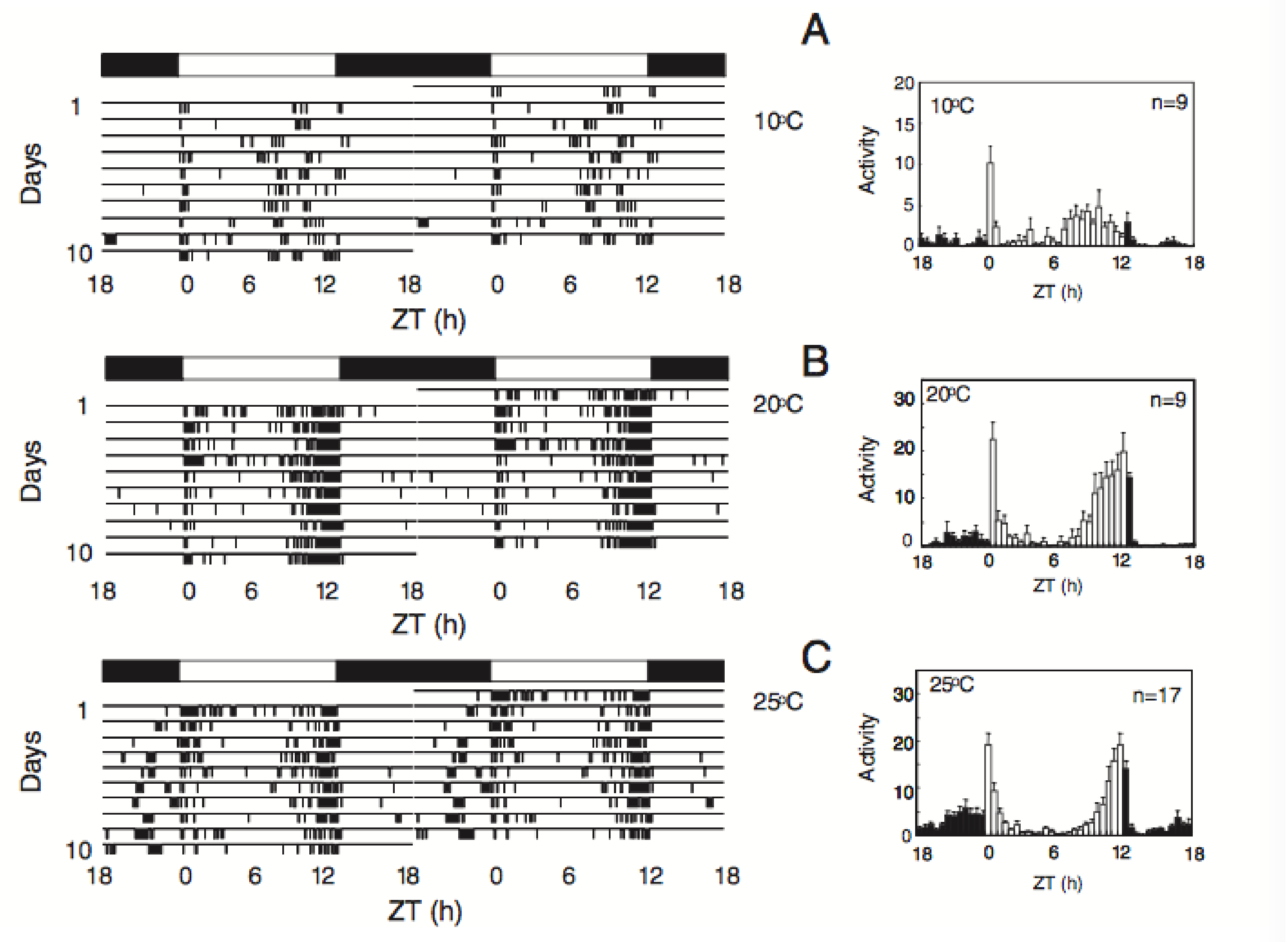
Jinfei D. Ni.2017

Rescue of the *rh71,cry*^{b} photoentrainment defect by expression of *rh7* in pacemaker neurons



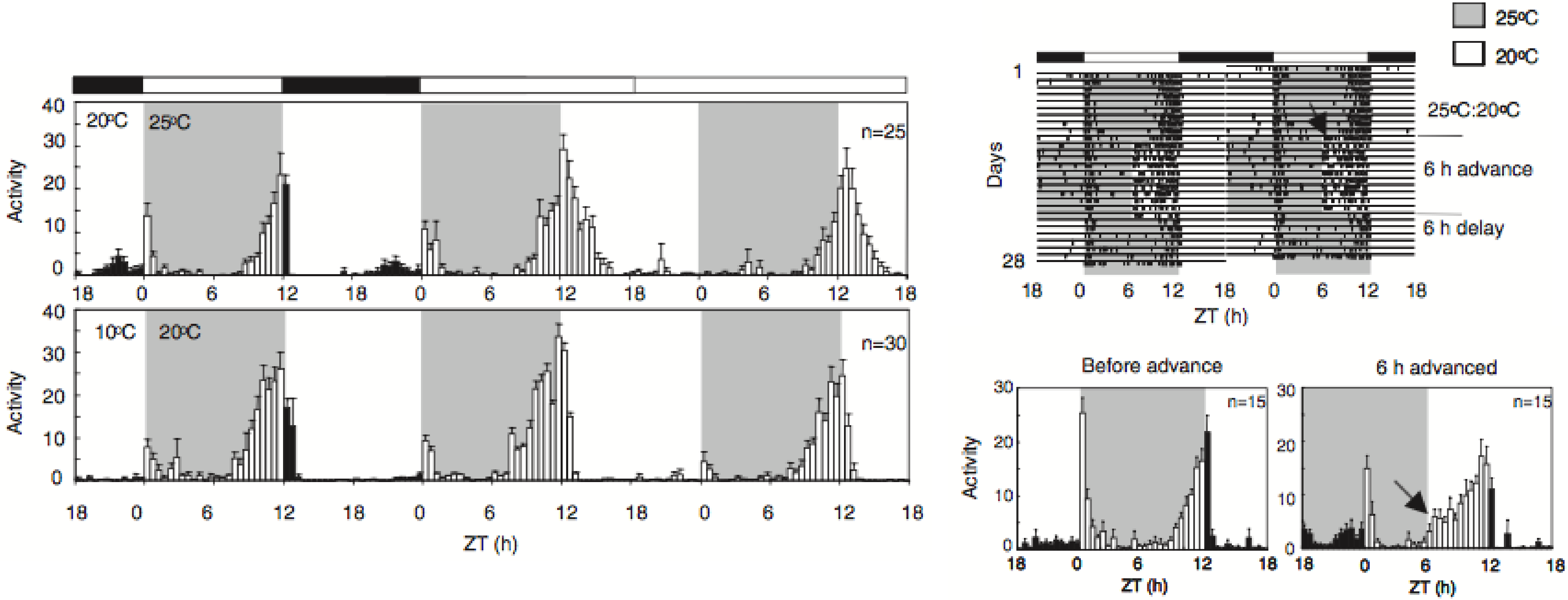
Jinfei D. Ni.2017

Effect of temperature on circadian locomotion in *Drosophila*



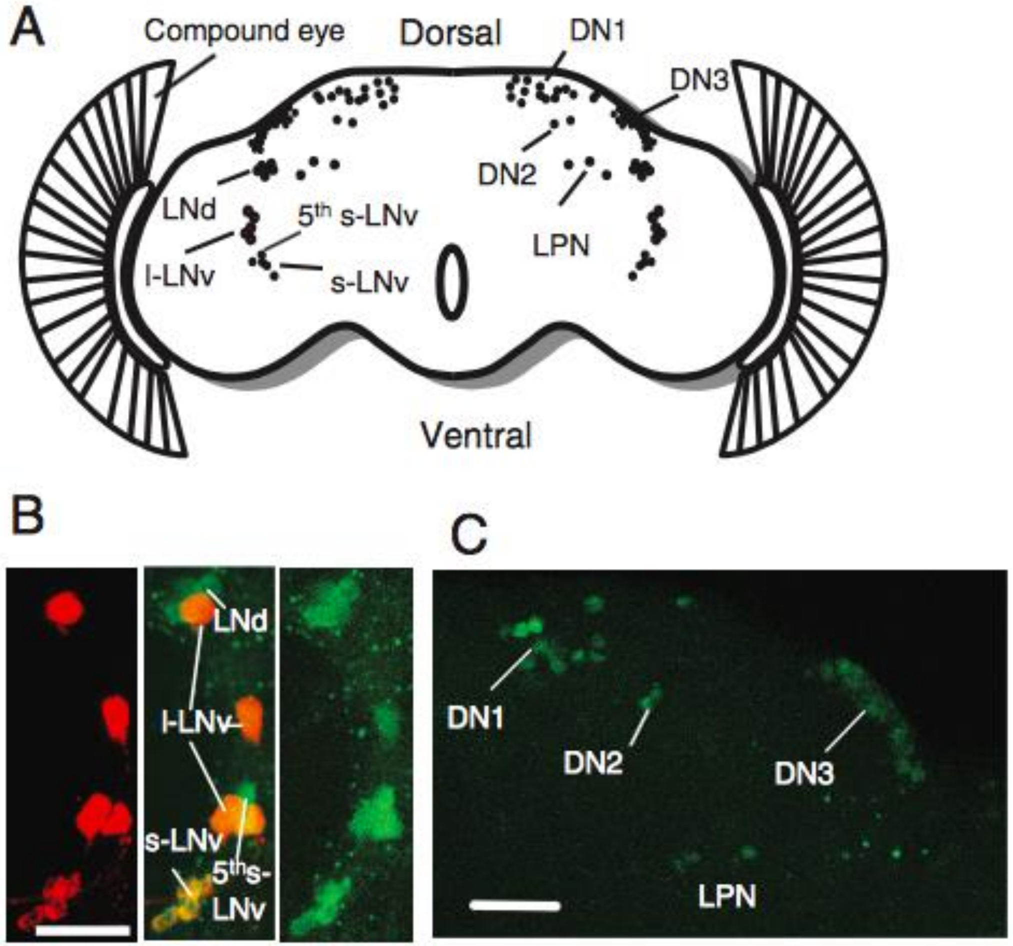
Miyasako Y.2007

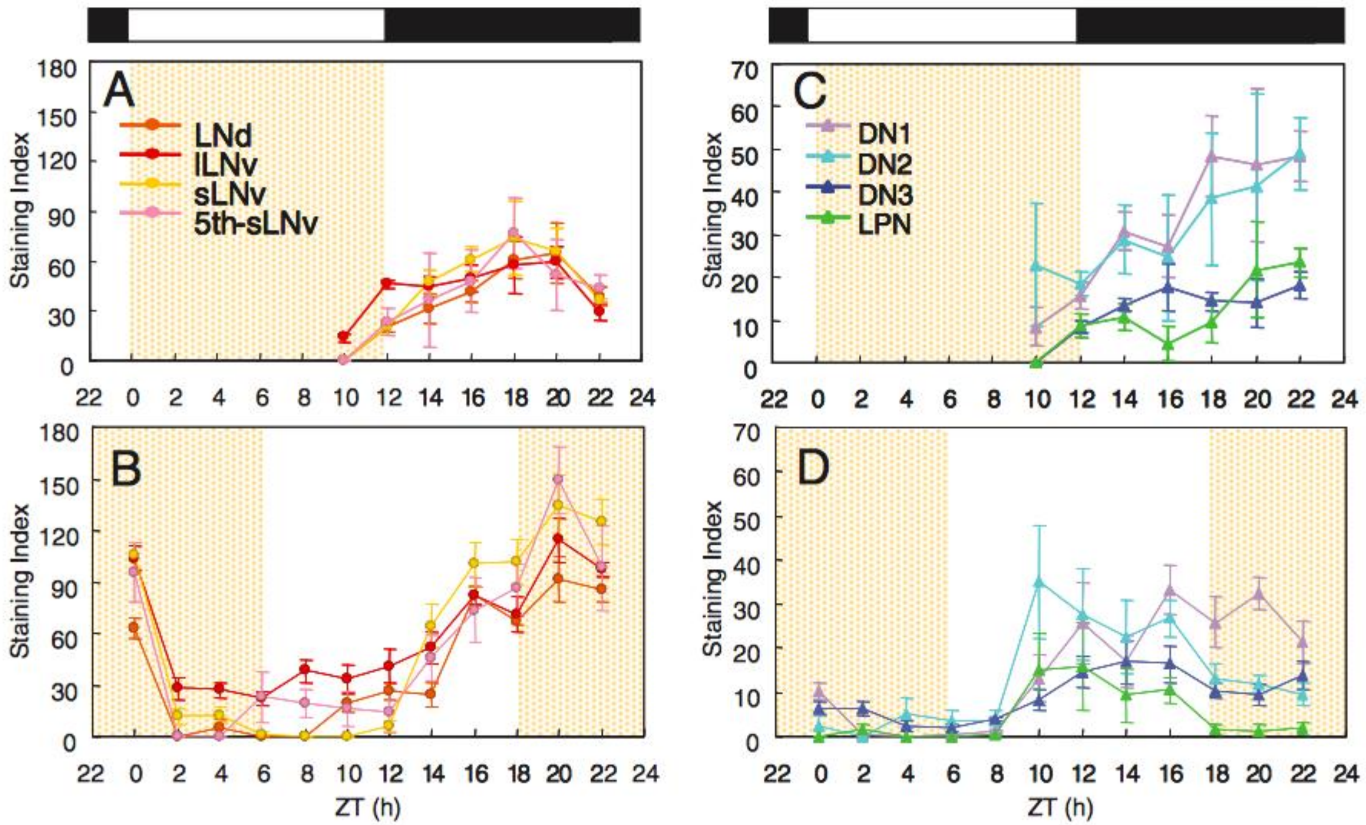
Effect of temperature on circadian locomotion in *Drosophila*



Miyasako Y.2007

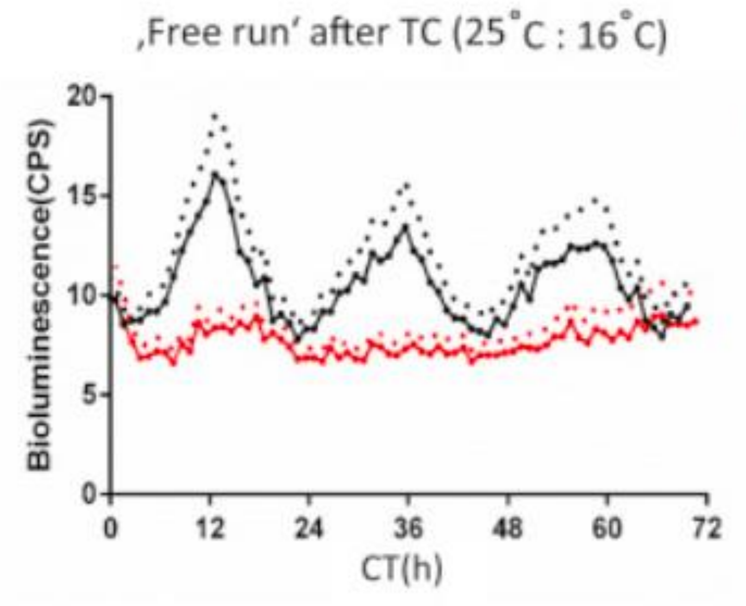
The circadian neurons response to temperature

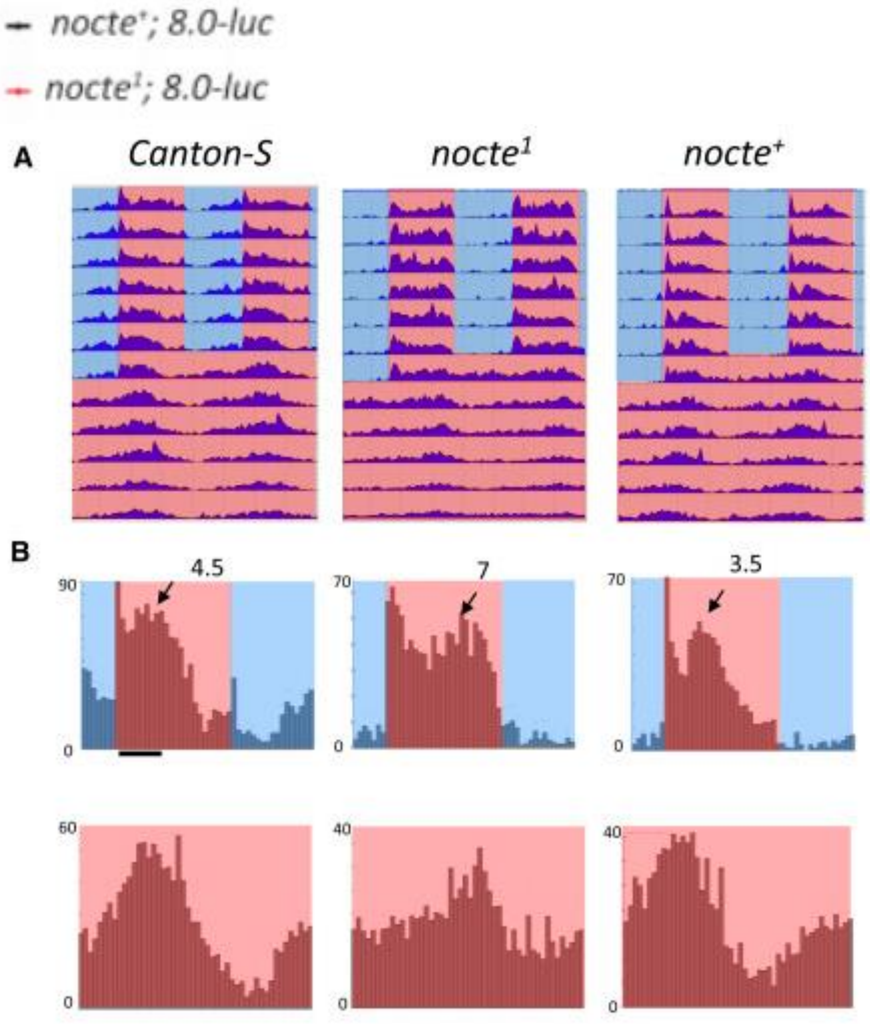


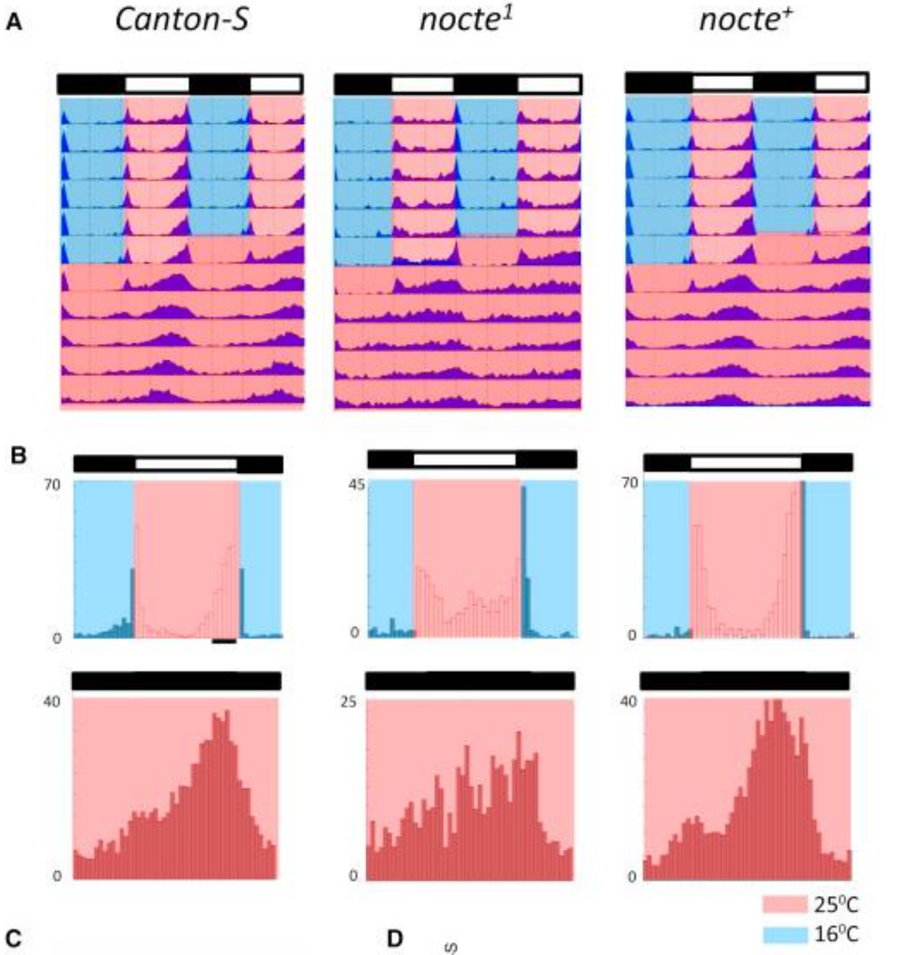


Miyasako Y.2007

nocte mediates molecular temperature synchronization in clock neurons

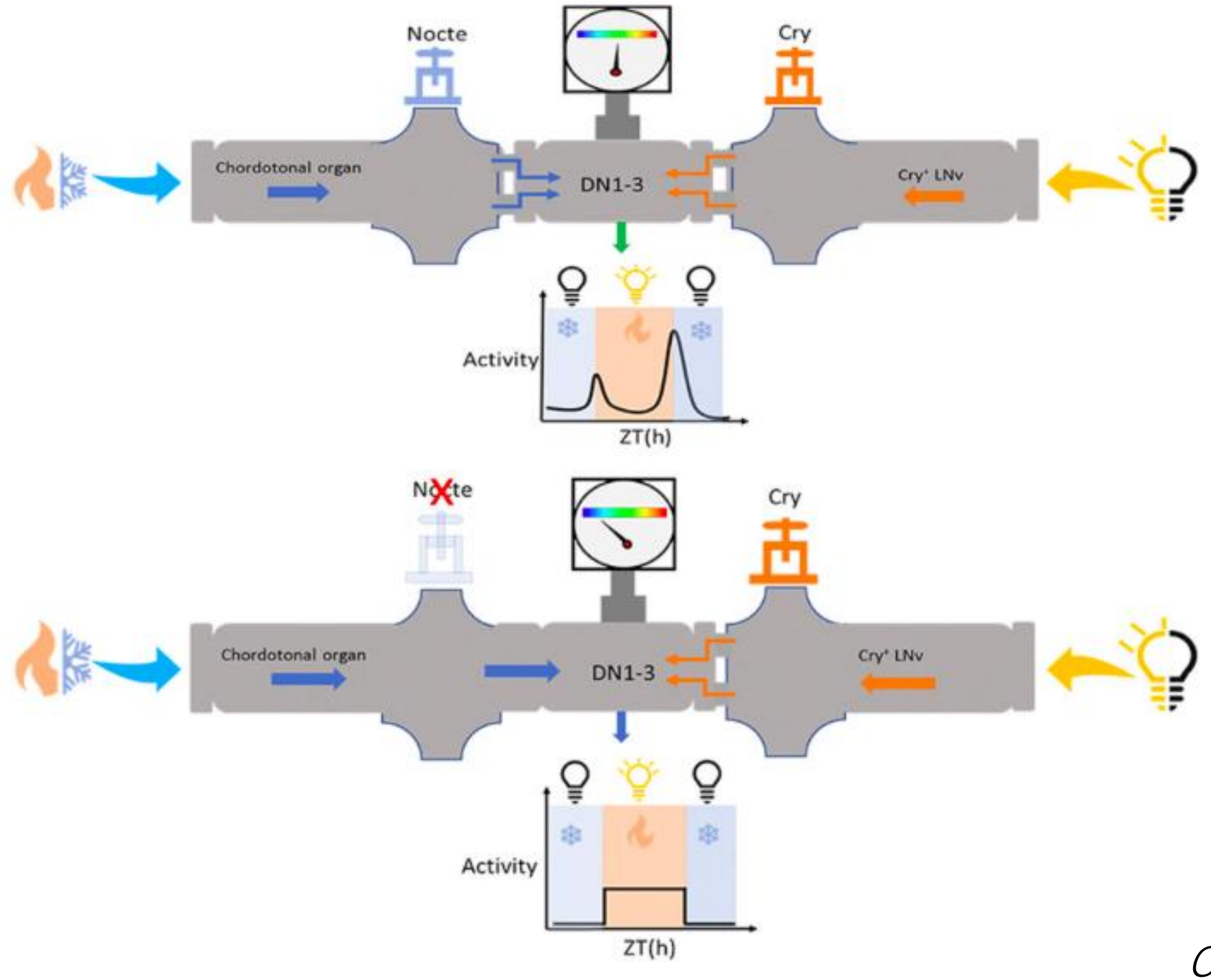






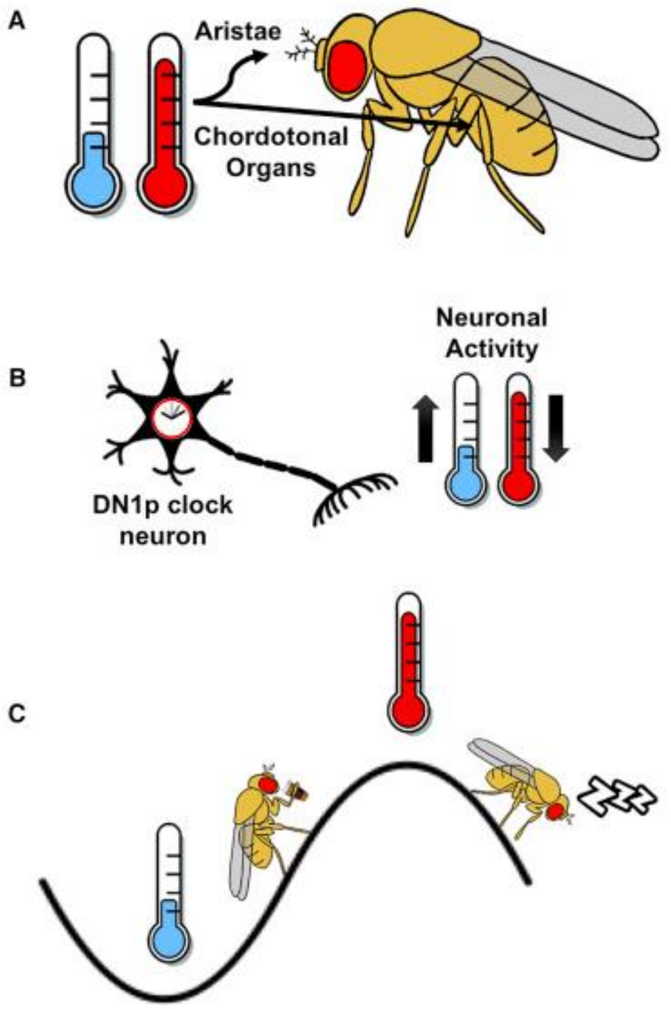
Chenghao Chen.2018

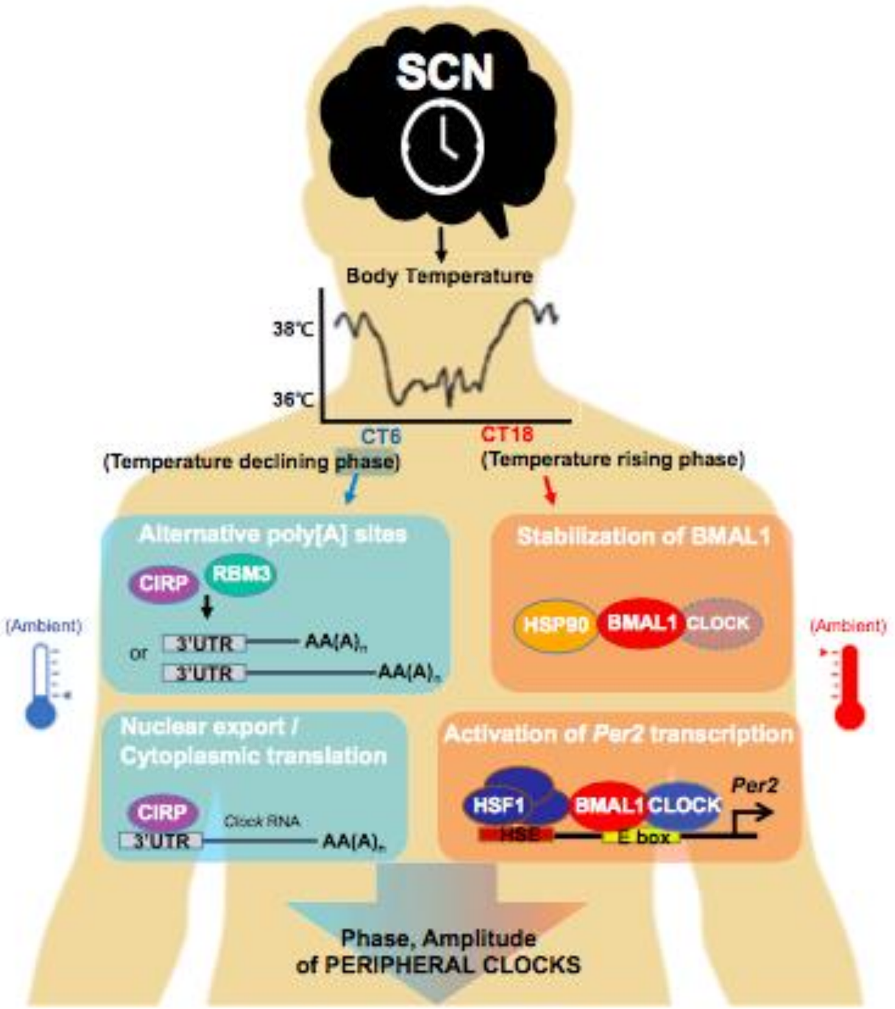
Nocte integrate light and temperature inputs in circadian clock neurons



Chenghao Chen.2018

Compare *Drosophila* with human

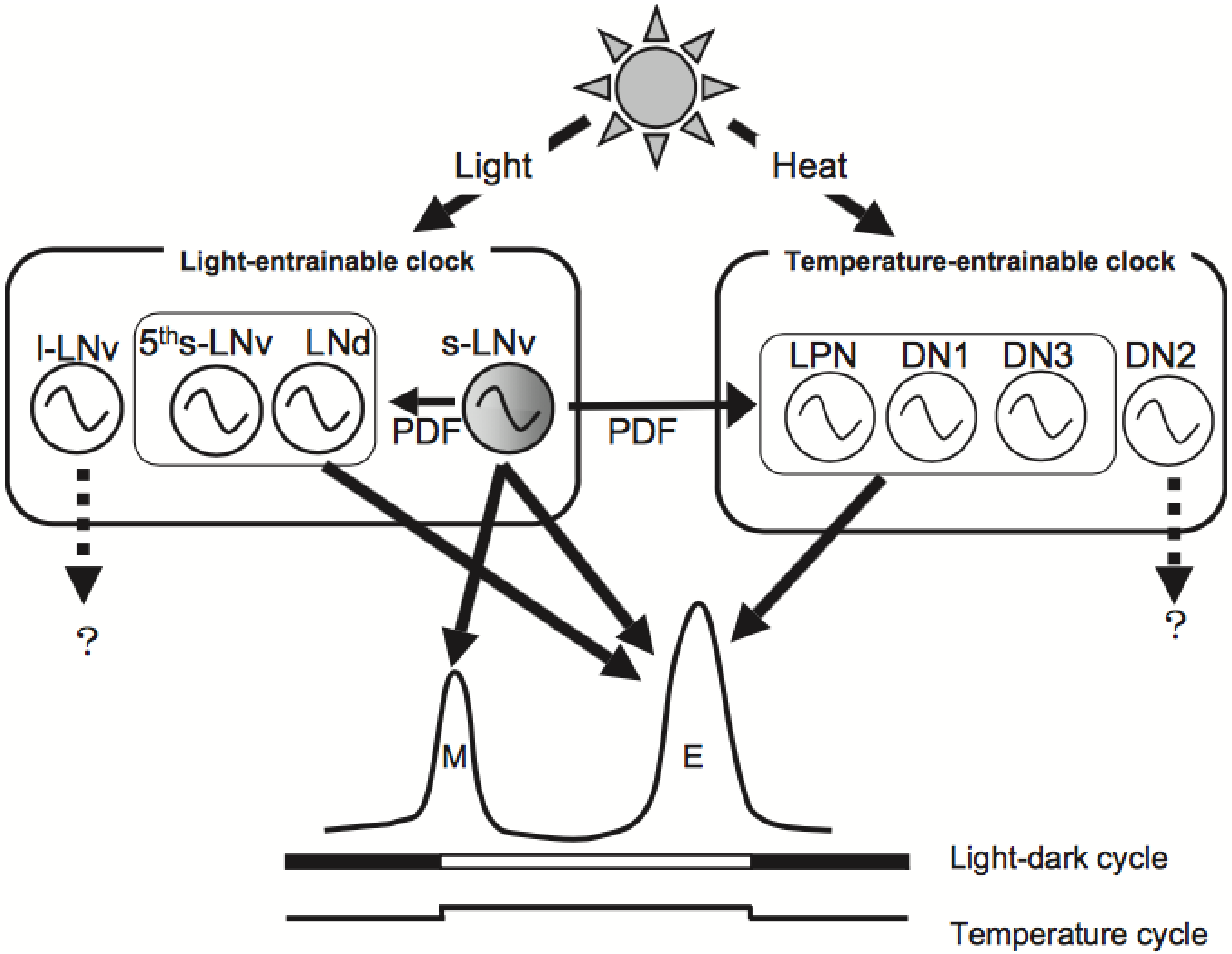




Barber AF.2018

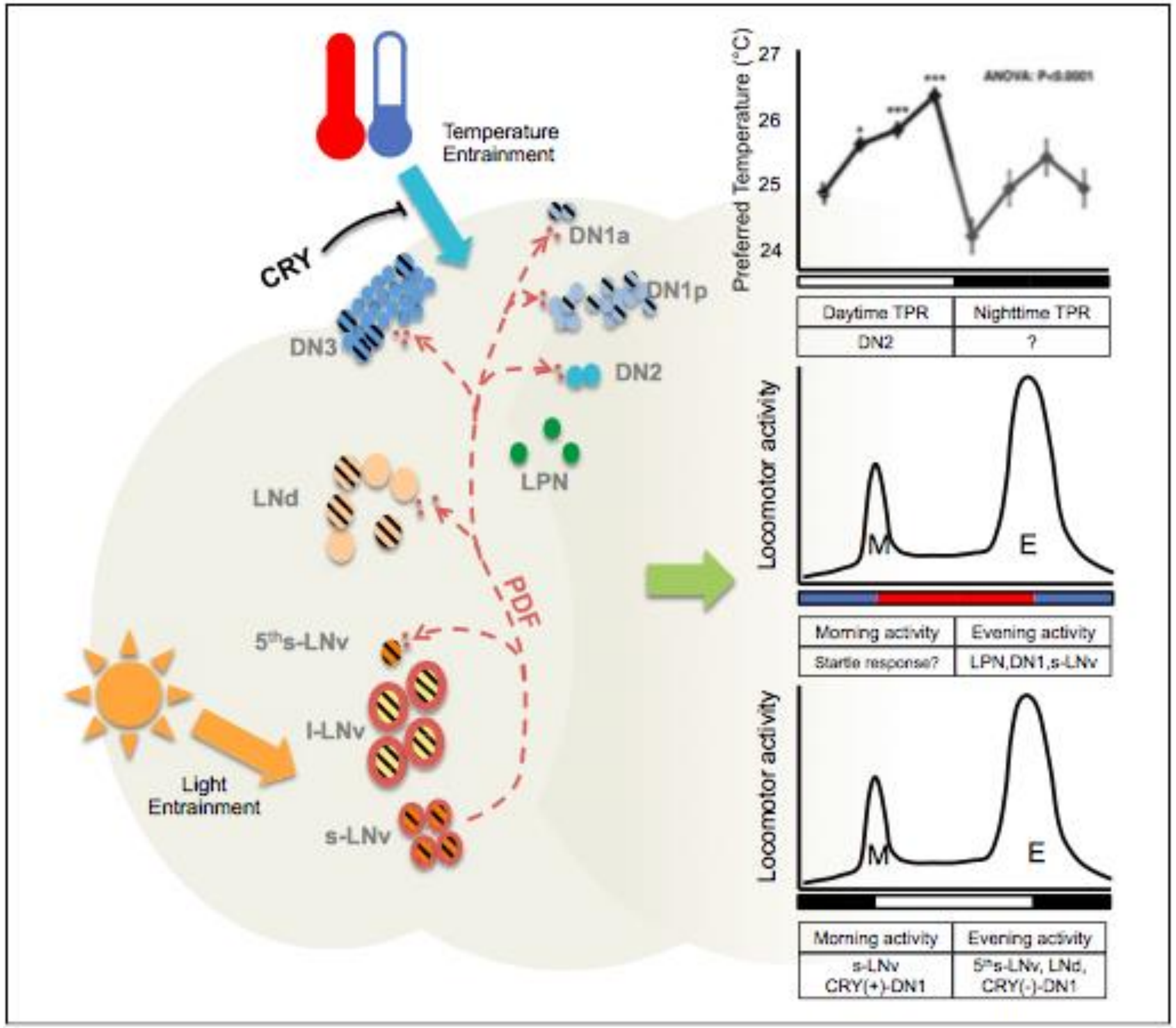
Yoonhee Ki.2015

A model of *Drosophila* circadian entrainment



Miyasako Y.2007

A model of *Drosophila* circadian entrainment



Yoonhee Ki.2015

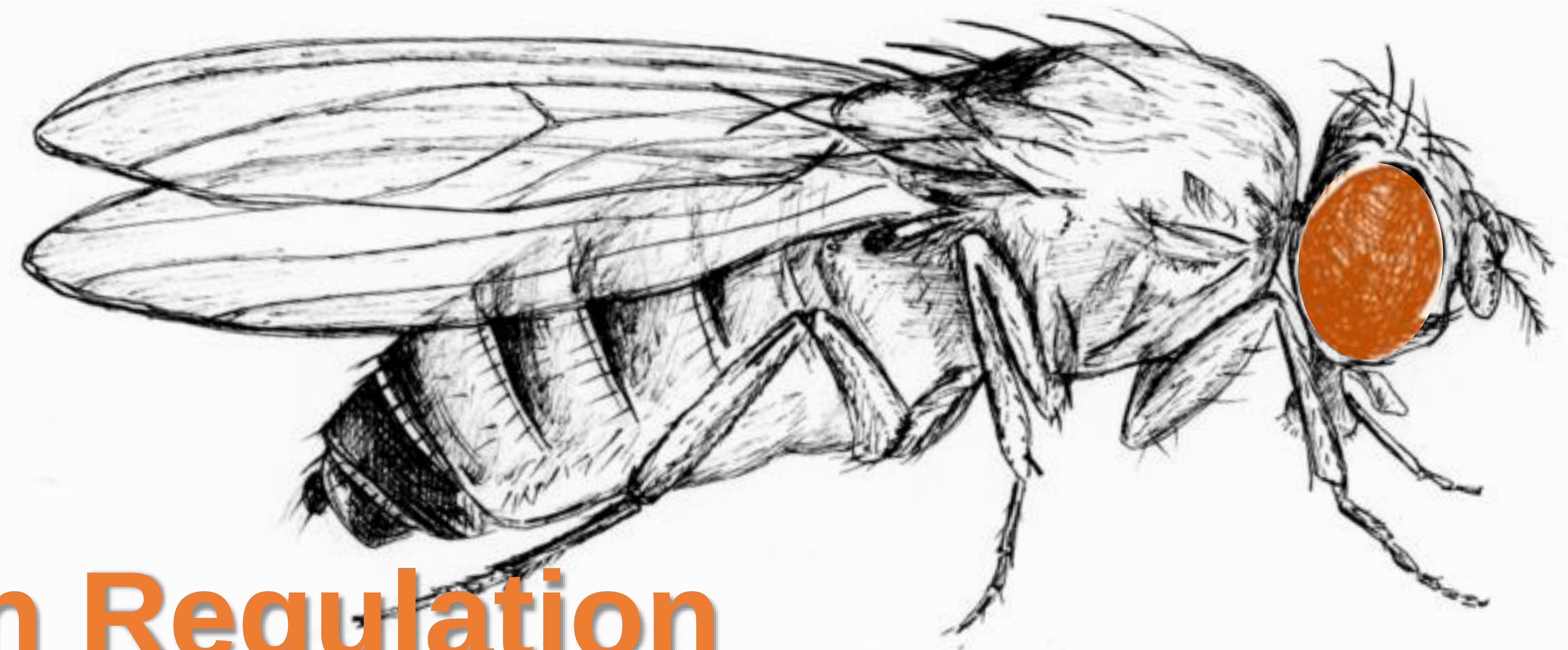
Other ways influencing circadian entrainment

- Feeding
- Social experience
- ……

Questions

- Is there a temperature-dependent adjustment mechanism independent of light?
- How does the synchronization molecular mechanism in response to temperature work?
- Is there a different pathway mediates protein degradation other than TIM?
- How do other behaviors and conditions affect the entrainment of circadian clock?

Part III

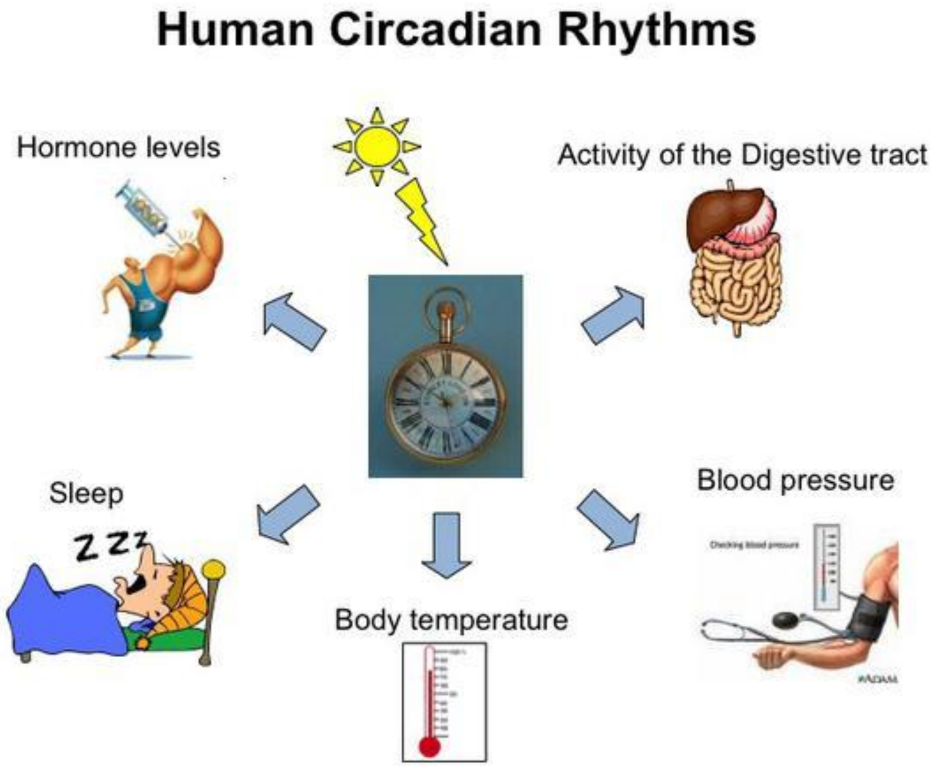


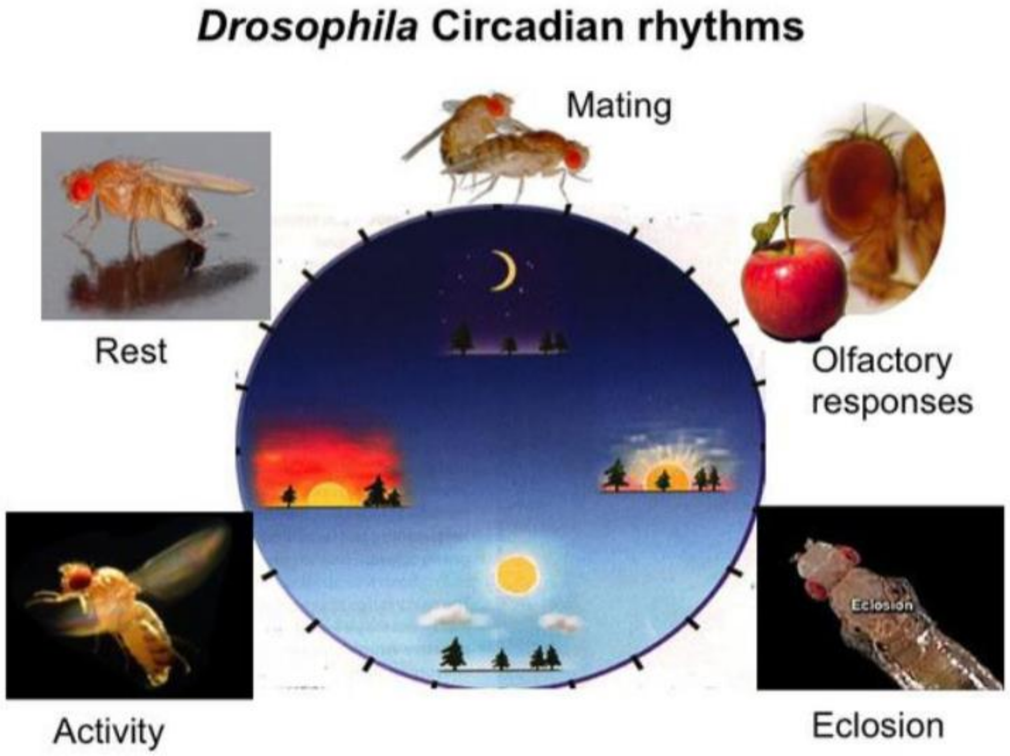
Circadian Regulation

Behaviors & Physiology

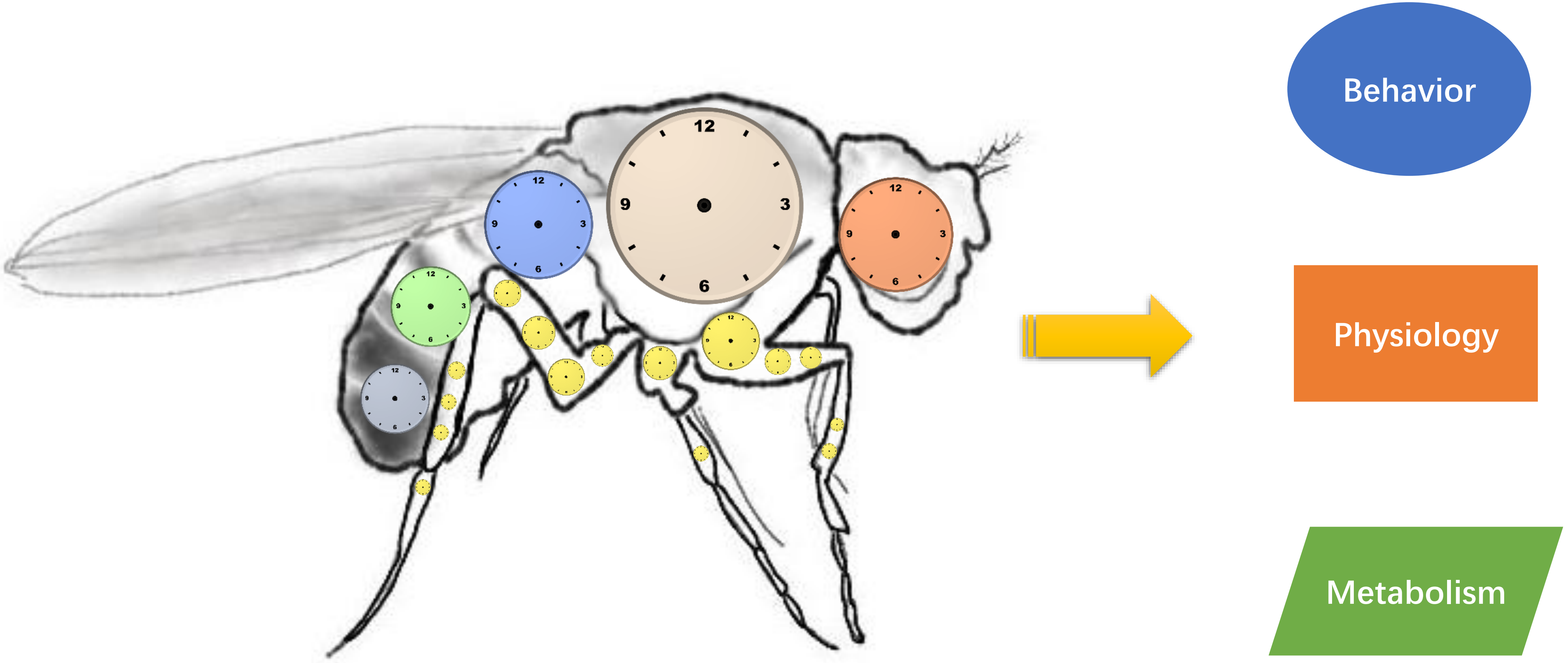
V.G

Rhythmic behaviors & physiology phenomenon in human and *Drosophila*





https://genev.unige.ch/research/laboratory/Emi-Nagoshi


Behavior
Physiology
Metabolism

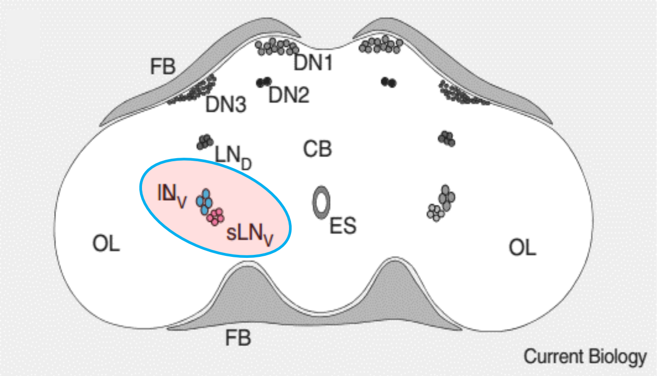
Behaviors regulated by circadian rhythmic outputs

- **Regulation of Locomotor Activity Rhythms**
- **Regulation of Egg-Laying**
- **Regulation of Temperature Preference**
- **Regulation of Feeding Behavior**
- **Regulation of Olfaction Rhythms**
- **Eclosion as a “One Time Only” Output of the Clock**

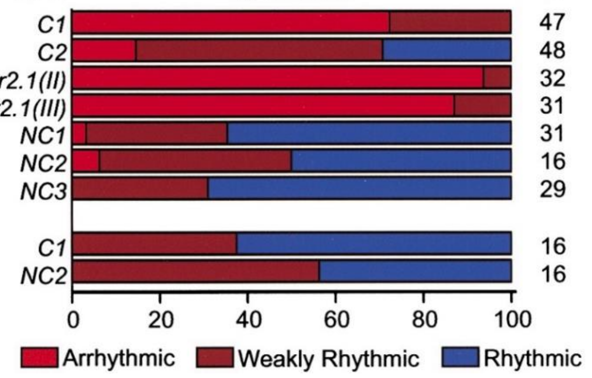
Regulation of locomotor activity rhythms

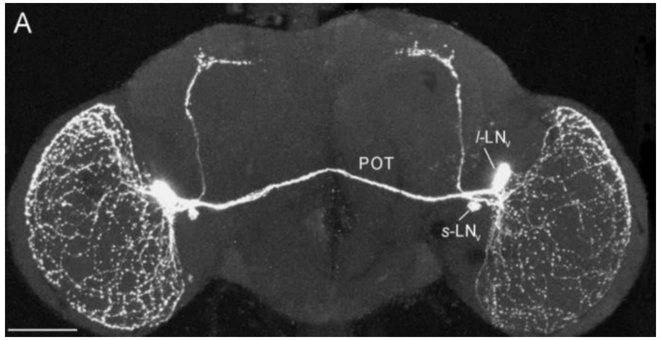
RONALD J. KONOPKA AND SEYMOUR BENZER , 1971

Large and small LNvs are necessary for free-running (DD) locomotor activity rhythms

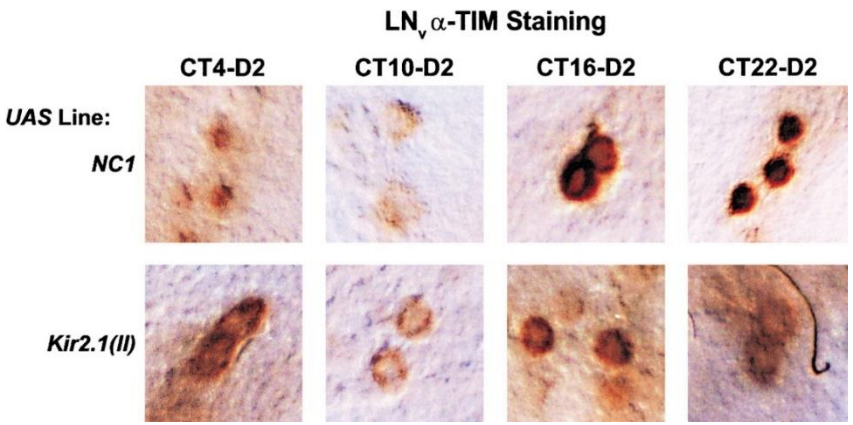


Driver	UAS	n	Period (h)	Activity(min^{-1})	Power
pdf-GAL4	*C1*	47	24.2±0.29	0.54±0.02	81±2
pdf-GAL4	*C2*	48	24.3±0.04	0.47±0.02	131±7
pdf-GAL4	*Kir2.1(II)*	32	—	0.38±0.02	76±1
pdf-GAL4	*Kir2.1(III)*	31	—	0.60±0.03	77±2
pdf-GAL4	*NC1*	31	24.7±0.04	0.62±0.03	174±9
pdf-GAL4	*NC2*	16	24.5±0.08	0.77±0.07	149±13
pdf-GAL4	*NC3*	29	24.5±0.04	0.44±0.02	165±9
y w	*C1*	16	23.5±0.05	0.52±0.03	160±17
y w	*NC2*	16	23.4±0.10	0.45±0.03	147±12



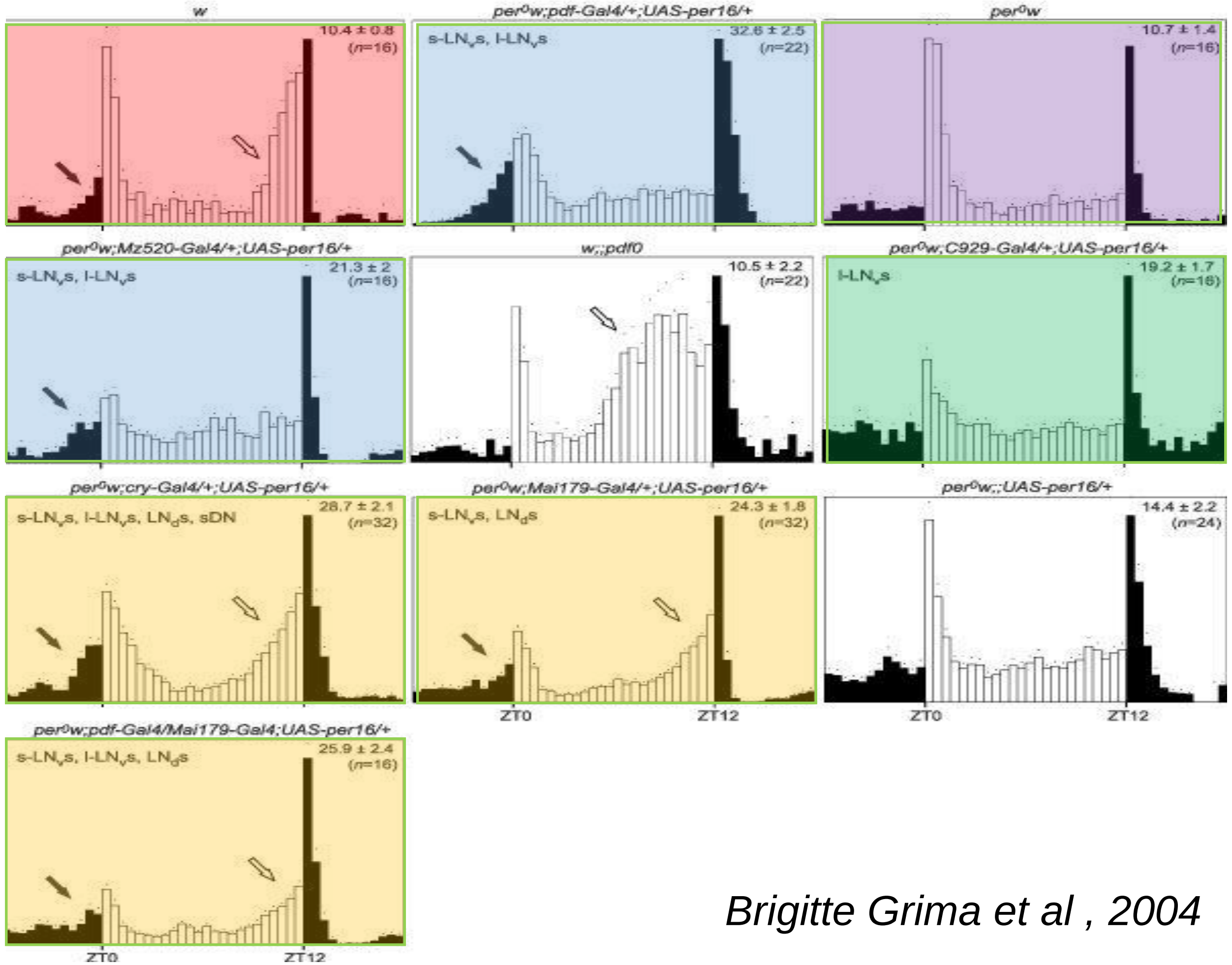


pdf-Gal4>UAS-GFP



Michael Nitabach et al , 2002

Morning and evening peaks of activity rely on different clock neurons of the *Drosophila* brain



WT

per mutant

s-LNvs , l-LNvs

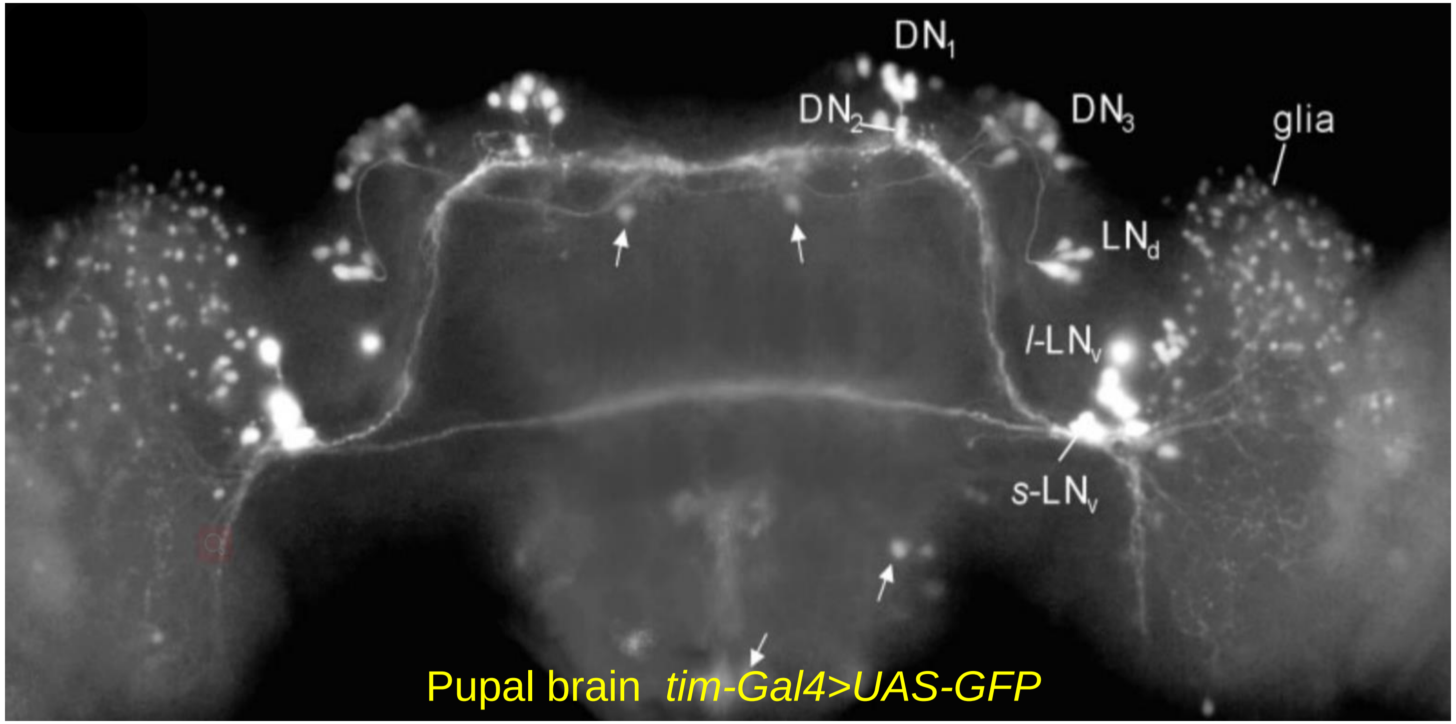
l-LNvs

含 LNds

As l-LNvs do not sustain oscillator function in DD , s-LNvs alone appear to be sufficient for this rhythmic output

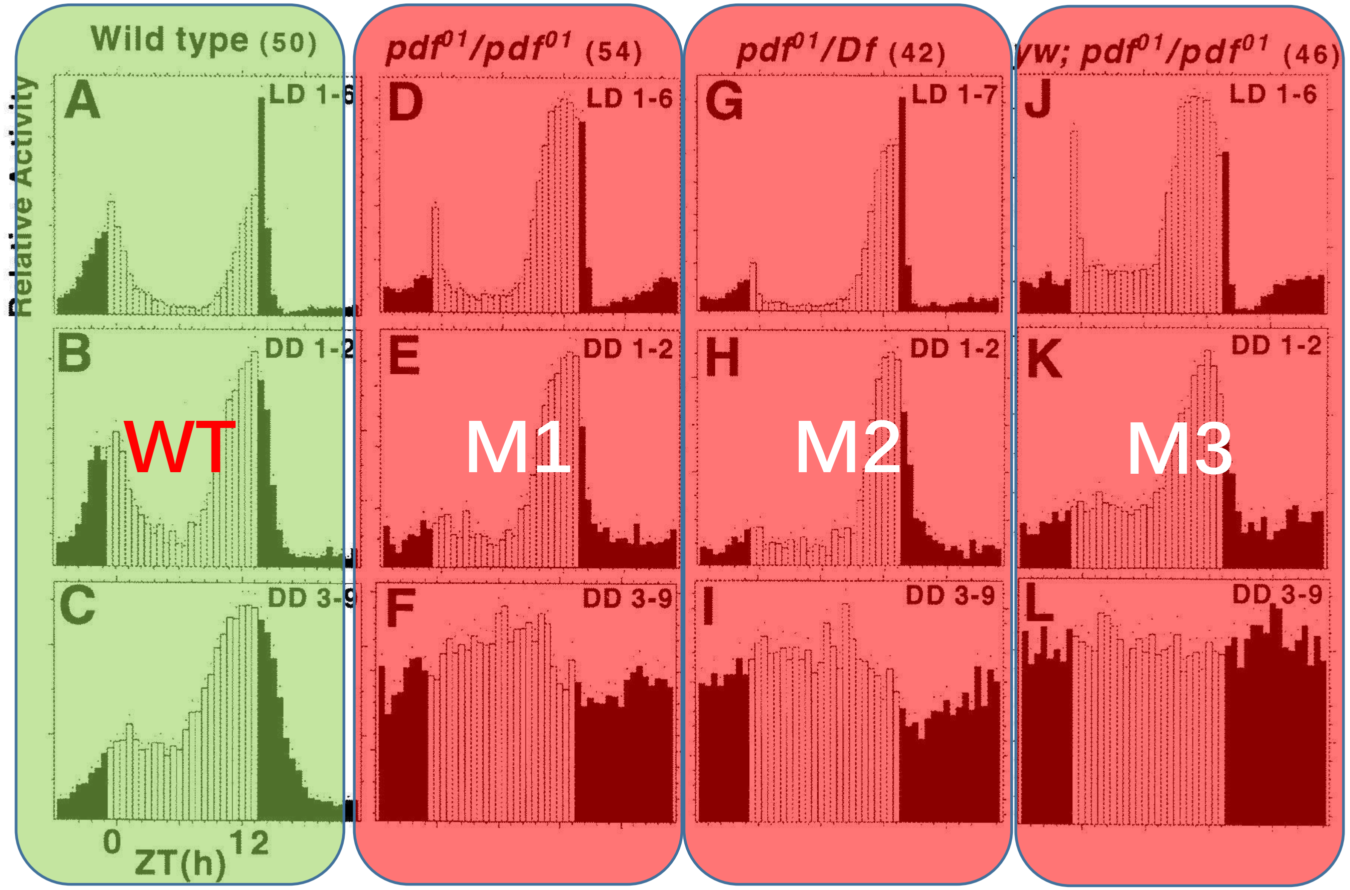
Brigitte Grima et al , 2004

s-LNvs project to dorsal brain



s-LNvs is pdf+ neuron

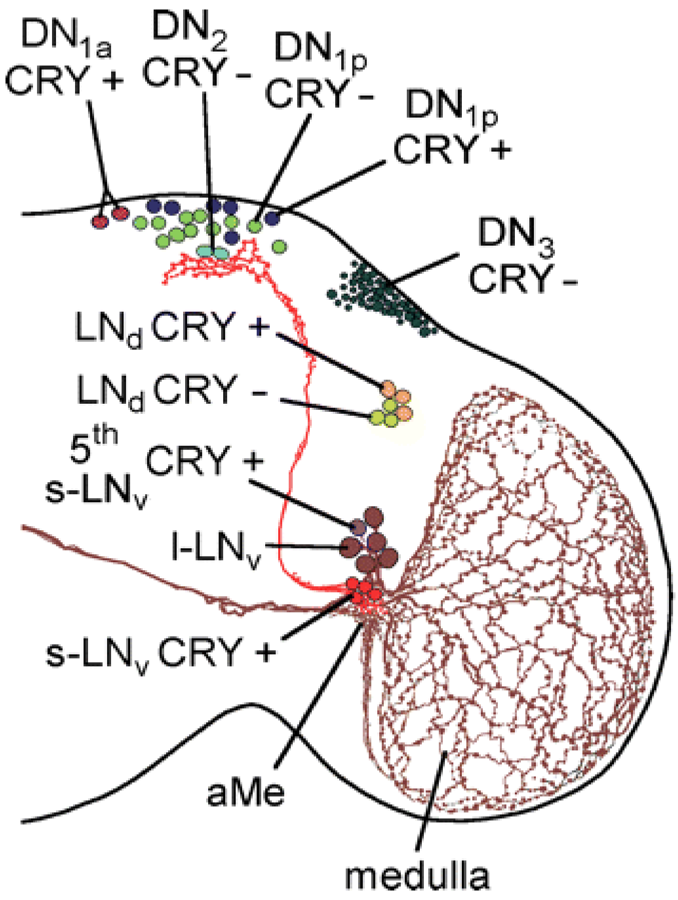
PDF is required for free running locomotor activity rhythms in flies



		DD 1-9				DD 3-9			LD Phases (ZT	
Genotype	n	n Rhythmic (%)	τ (h)	SNR	Events/ Bin	n Rhythmic (%)	τ (h)	SNR	Morning	Even
UAS-*pdf*	16	15 (94)	24.1 ± 0.1	1.1 ± 0.09	20 ± 2	15 (94)	24.1 ± 0.1	1.15 ± 0.11	0.8 ± 0.1	11.6 : 0.2
UAS-*pdf*; *pdf*[01]	21	5 (24)	21.5 ± 0.4	0.28 ± 0.02	14 ± 1	1 (5)	22.5	0.32 ± 0.02	0.1 ± 0.2	10.6 0.1
pdf-GAL4	16	16 (100)	24.6 ± 0.1	1.5 ± 0.15	16 ± 1	16 (100)	24.8 ± 0.1	1.52 ± 0.2	0.5 ± 0.1	12 ±
pdf-GAL4; *pdf*[01]	17	0 (0)	NA	0.26 ± 0.01	18 ± 2	0 (0)	NA	0.33 ± 0.03	0.6 ± 0.2	10.5 0.2
UAS-*pdf*/*pdf*-GAL4; *pdf*[01]	32	31 (97)	23.8 ± 0.1	0.77 ± 0.11	19 ± 1	26 (81)	23.7 ± 0.1	0.64 ± 0.09	23.7 ± 0.2	10.9 0.1

Renn SC et al, 1999

PDF (pigment dispersing factor) —— a bridge between different clock neurons



Taishi Yoshii et al, 2009

Neuron, Vol. 48, 267–278, October 20, 2005, Copyright ©2005 by Elsevier Inc. DOI 10.1016/j.neuron.2005.08.025

Drosophila GPCR Han Is a Receptor for the Circadian Clock Neuropeptide PDF

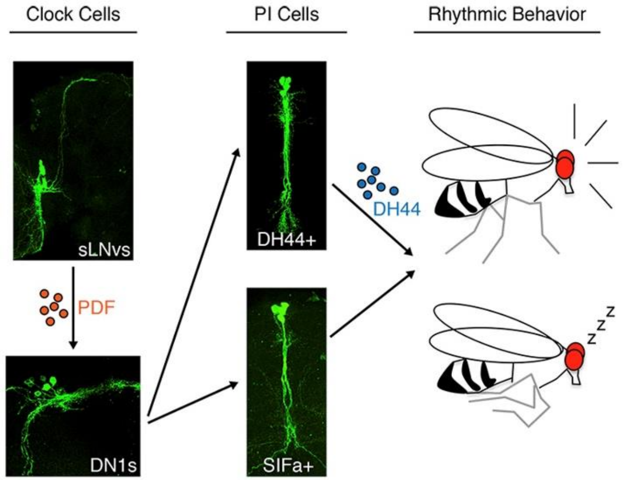
Neuron, Vol. 48, 221–227, October 20, 2005, Copyright ©2005 by Elsevier Inc. DOI 10.1016/j.neuron.2005.09.008

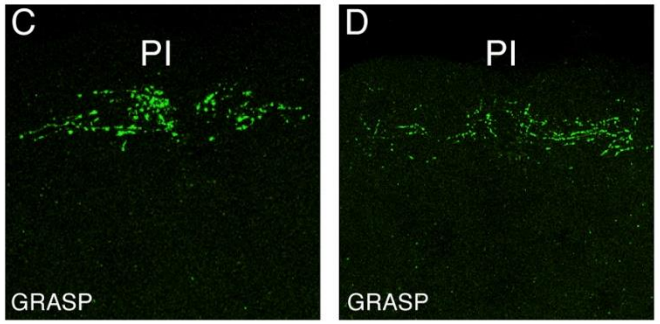
A G Protein-Coupled Receptor, _groom-of-PDF_, Is Required for PDF Neuron Action in Circadian Behavior **Report**

Neuron, Vol. 48, 213–219, October 20, 2005, Copyright ©2005 by Elsevier Inc. DOI 10.1016/j.neuron.2005.09.009

PDF Receptor Signaling in _Drosophila_ Contributes to Both Circadian and Geotactic Behaviors **Report**

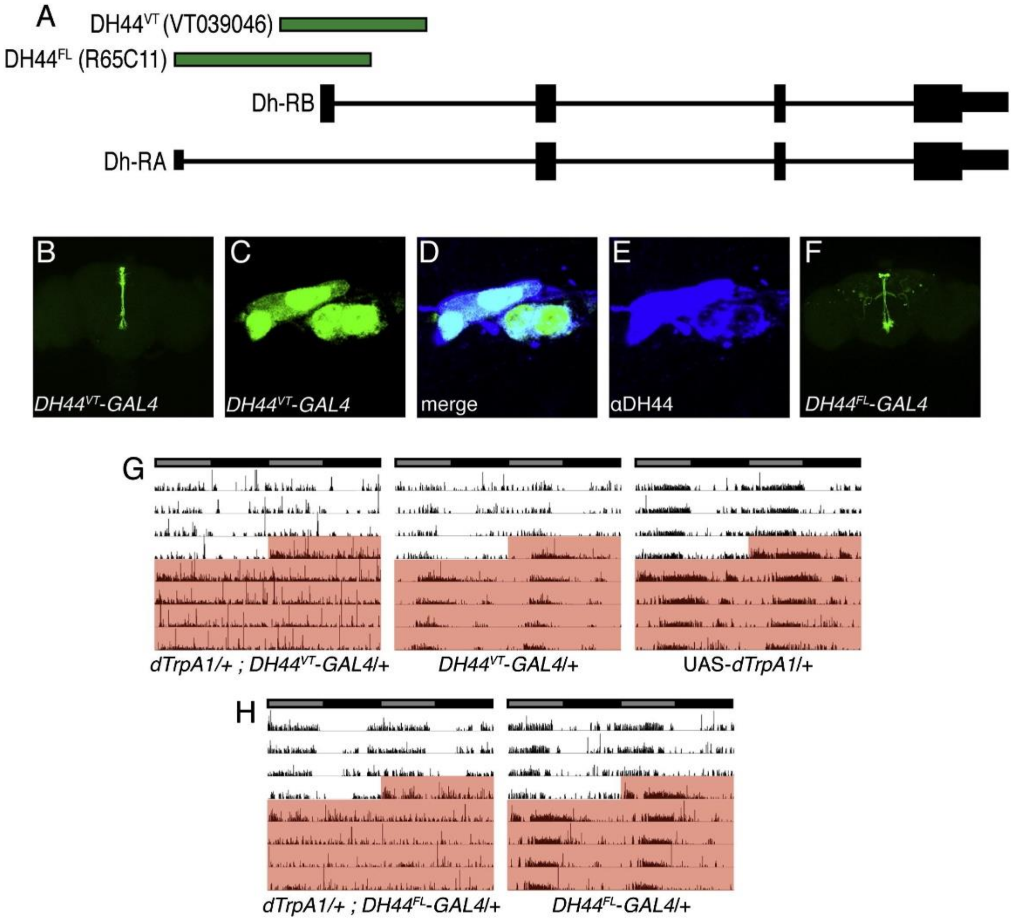
DN1s contact the diuretic hormone 44 (DH44)+ neurons in the Pars Intercerebralis (PI)





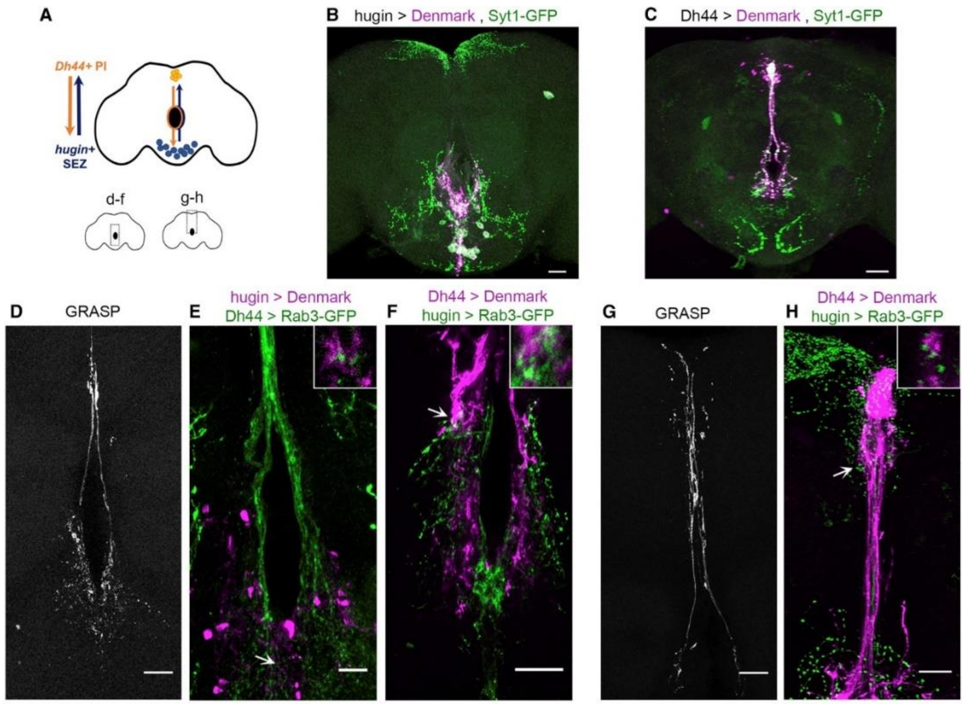
C LexAop-*GFP11*/+ ; *DH44^{VT}-GAL4*,*Clk*4.1LexA/UAS-*GFP1-10*

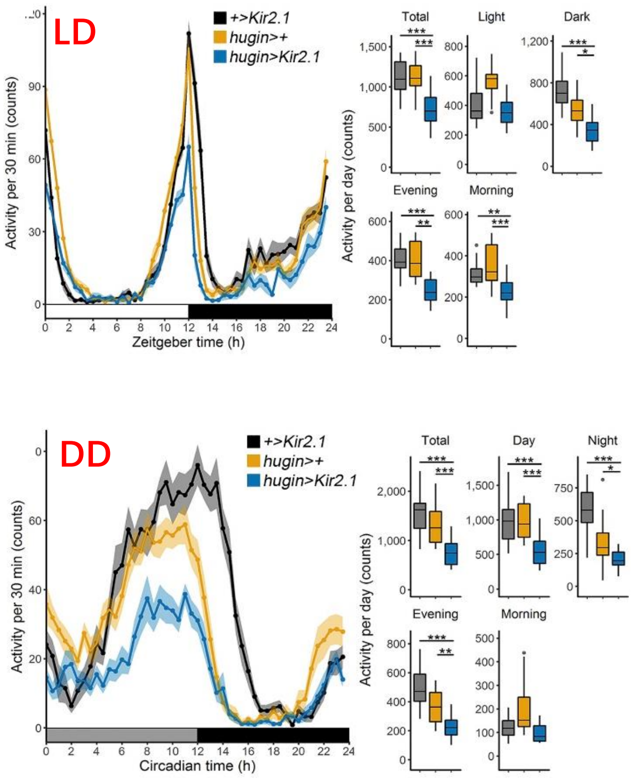
D *SIFa-GAL4/LexAop-GFP11 ; Clk4.1LexA /UAS-GFP1-10 brain*



Daniel J. Cavanaugh et al, 2014

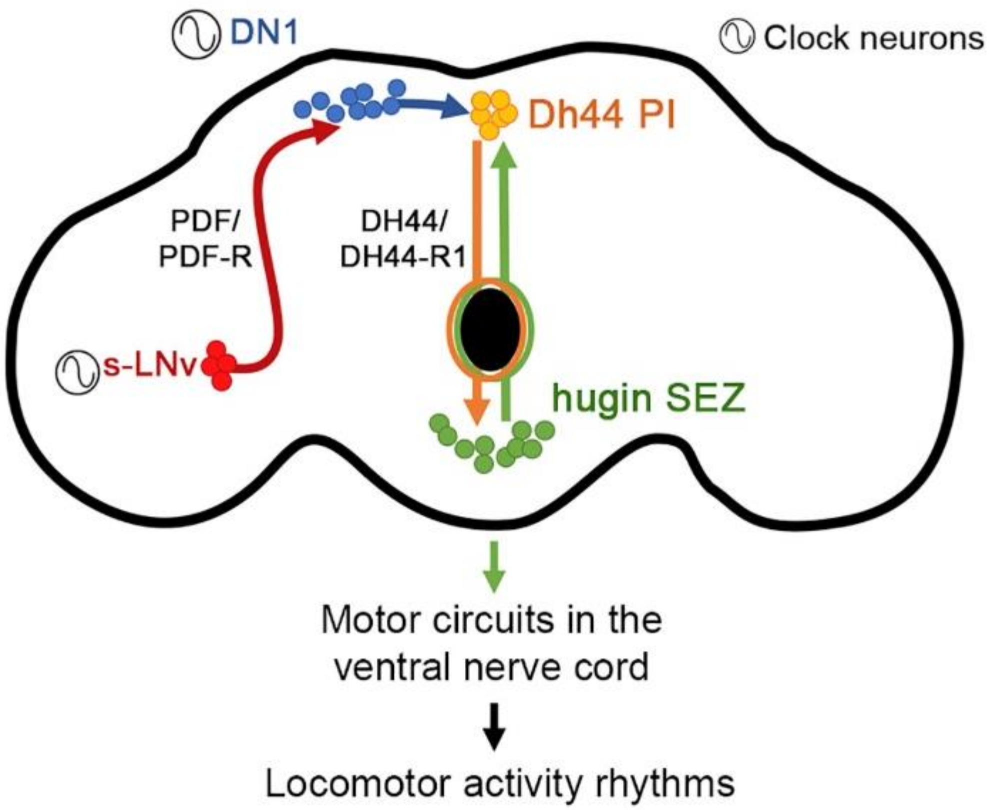
DH44 contact Hugin neuron which is necessary for locomotor activity rhythms



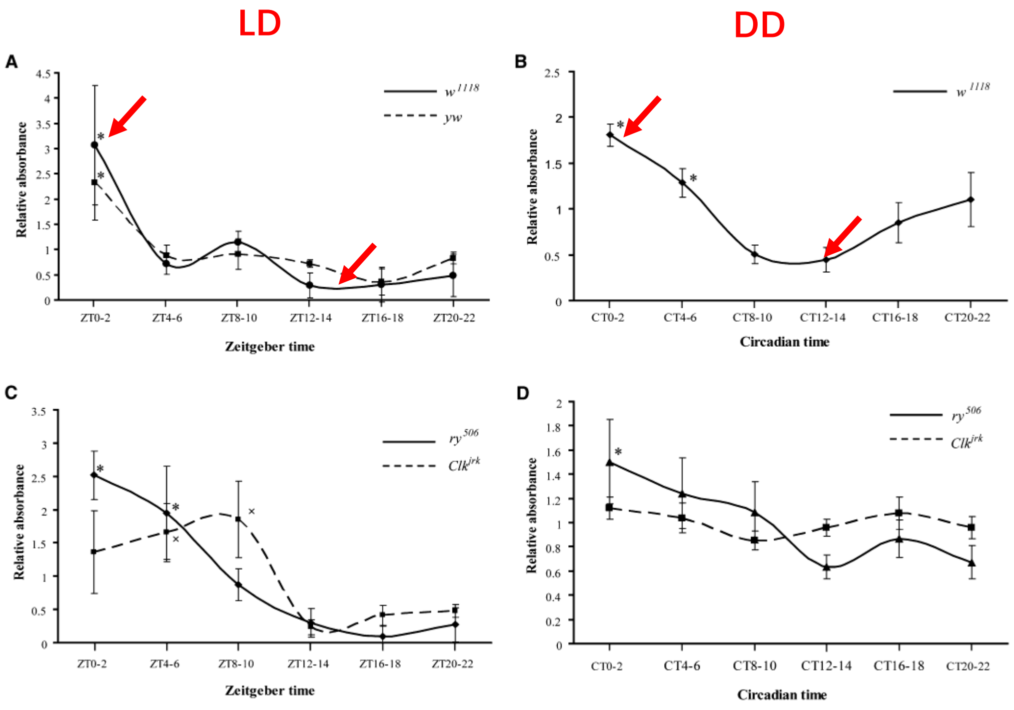


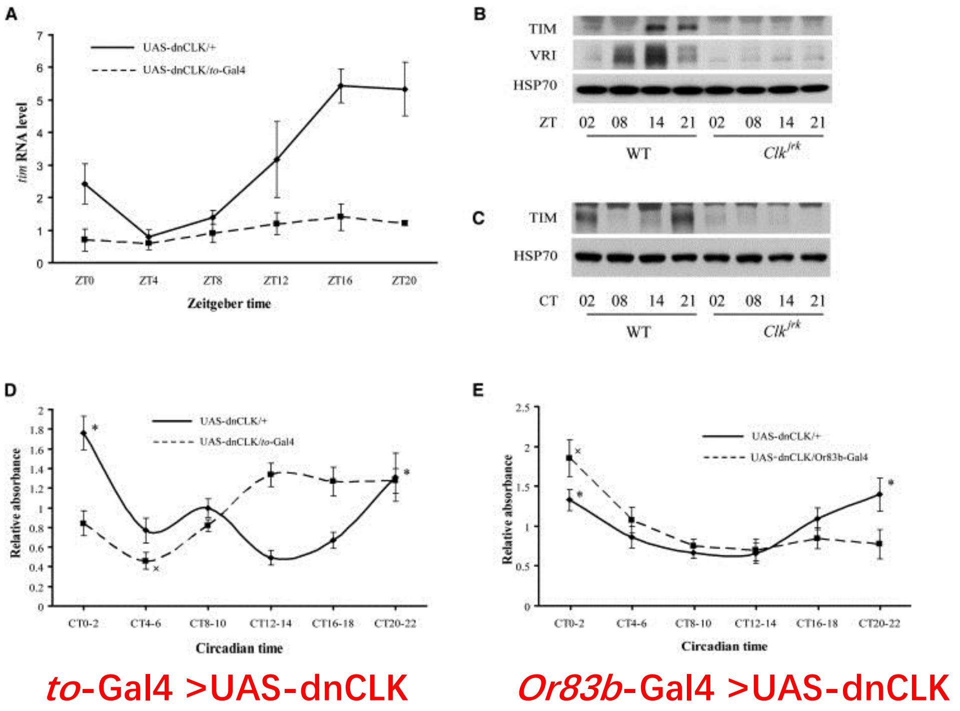
Anna N. King et al, 2017

An s-LNvs →DN1→DH44 PI→Hugin SEZ→VNC circuit links the clock to motor output



Regulation of feeding behavior

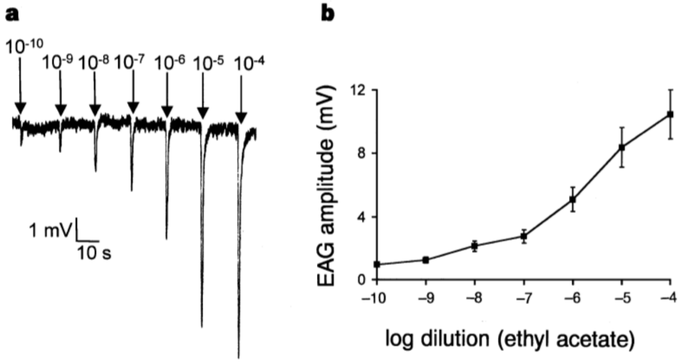




Xu K et al, 2008

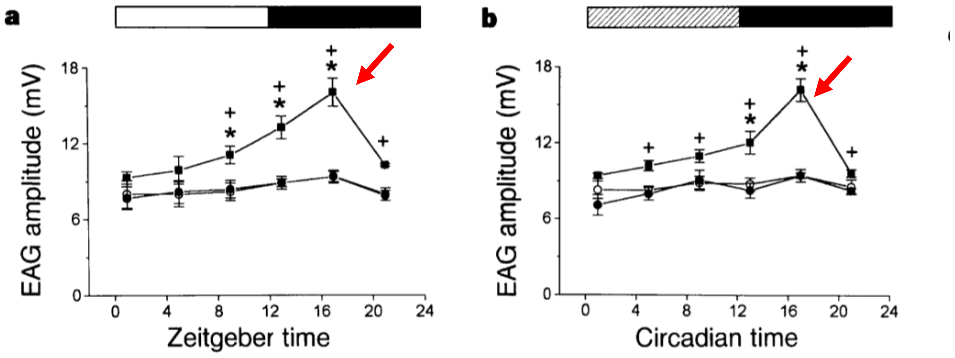
Drosophila feeding behavior displays a 24 hr circadian rhythm that is regulated by clocks in digestive/metabolic tissues

Circadian rhythms in olfactory responses



LD

DD



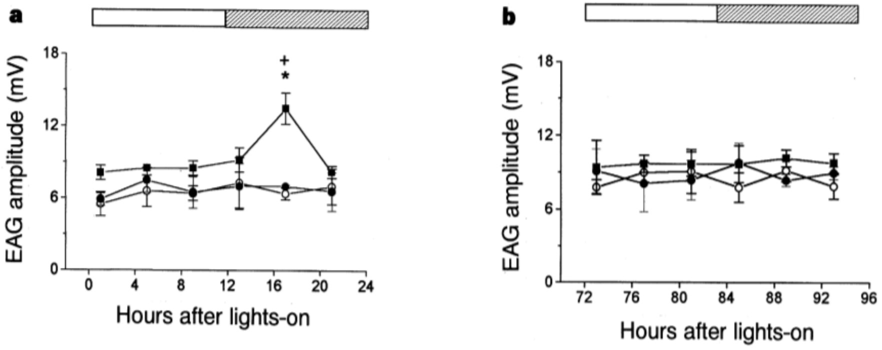
EAG：electroantennogram responses to odorants

Detection of predators ?

Opportunistic feeding ?

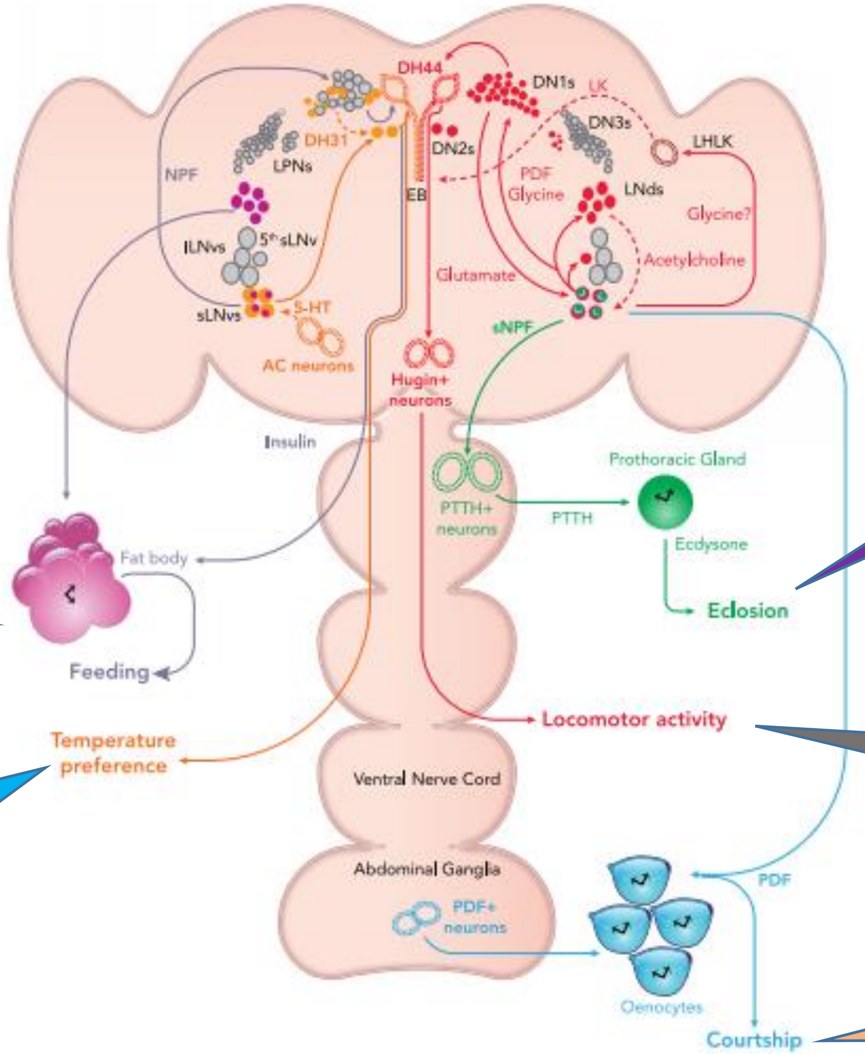
Day1 LL

Day4 LL



Time of day needs to be carefully considered in the design and interpretation of experiments on olfactory learning.

Central to peripheral connectivity



Feeding

Temperature preference

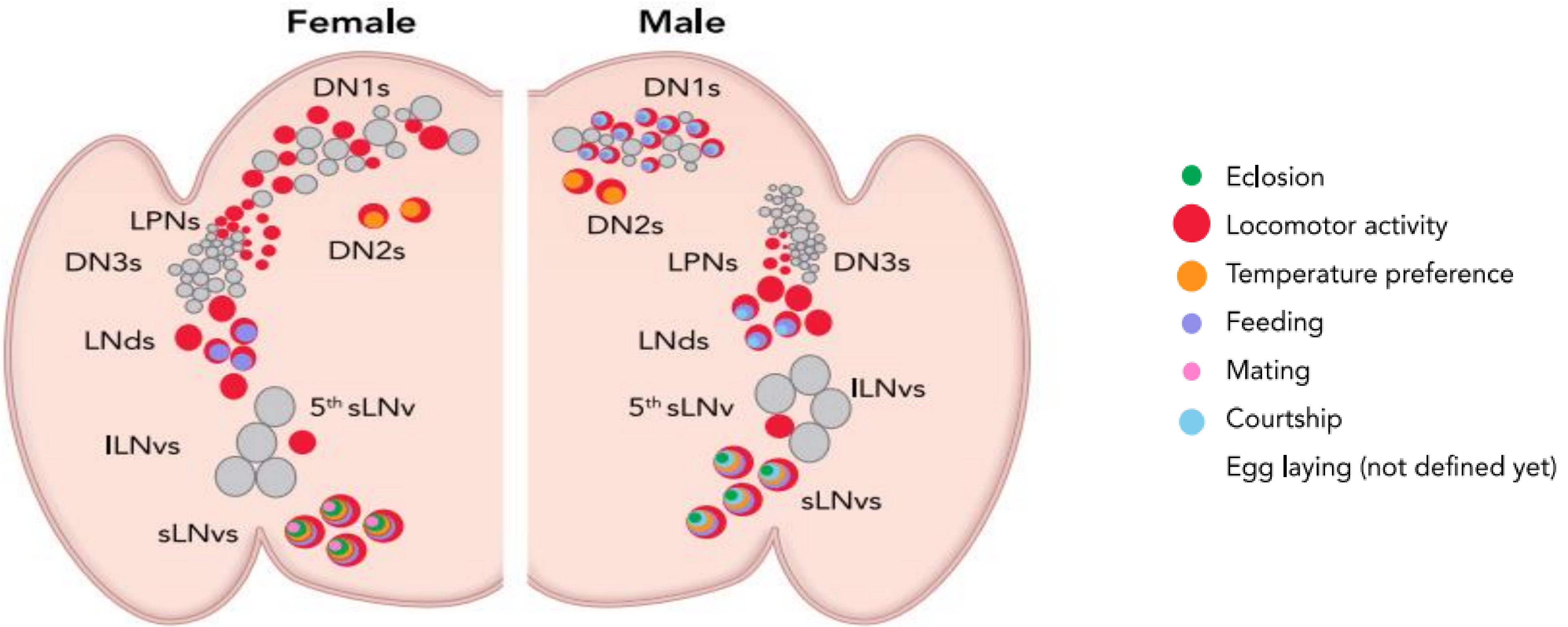
Eclosion

Locomotor activity

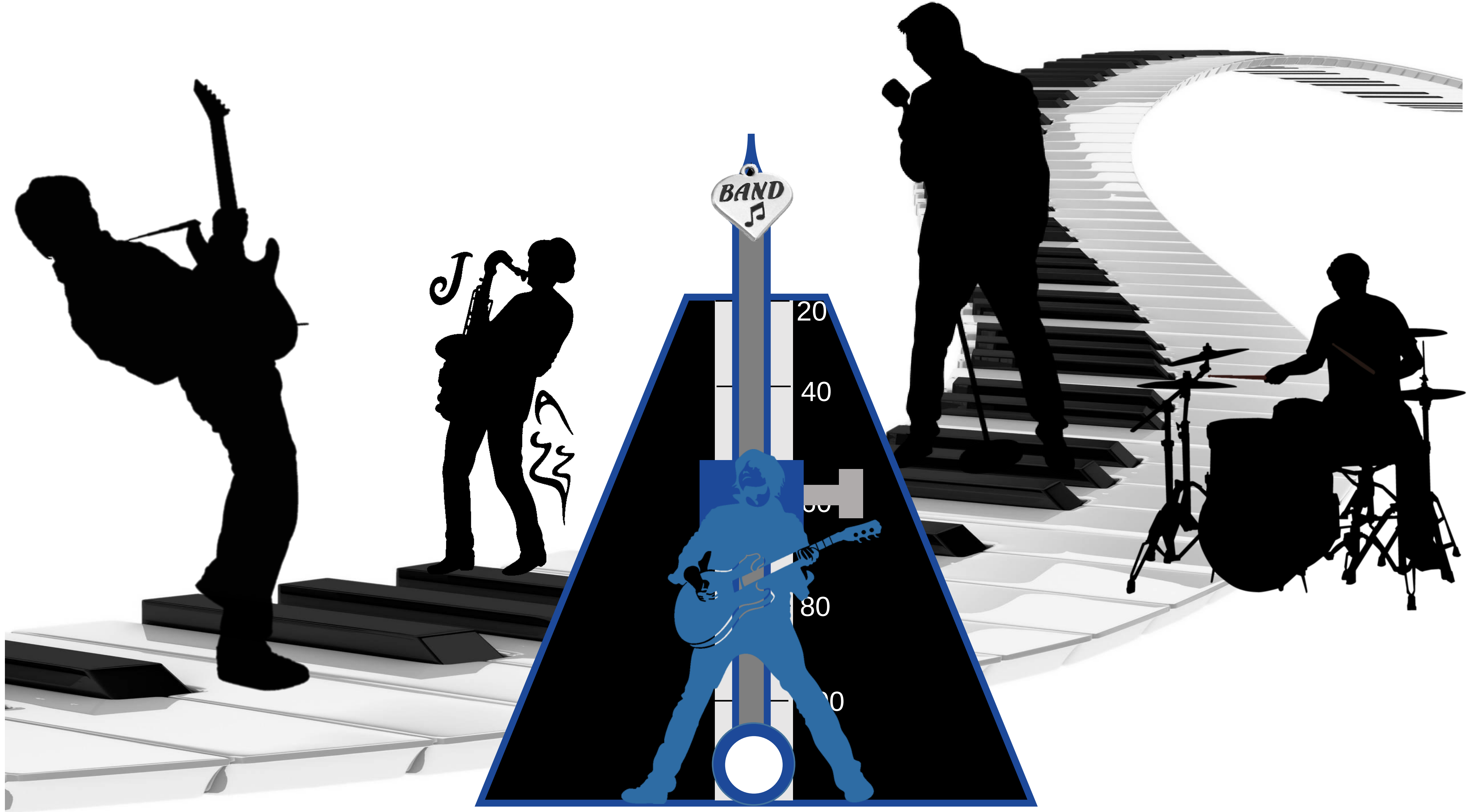
Courtship

D. Lorena Franco et al, 2017

Circadian neurons drive rhythmic behaviors



Questions & Perspectives


BAND
J
20
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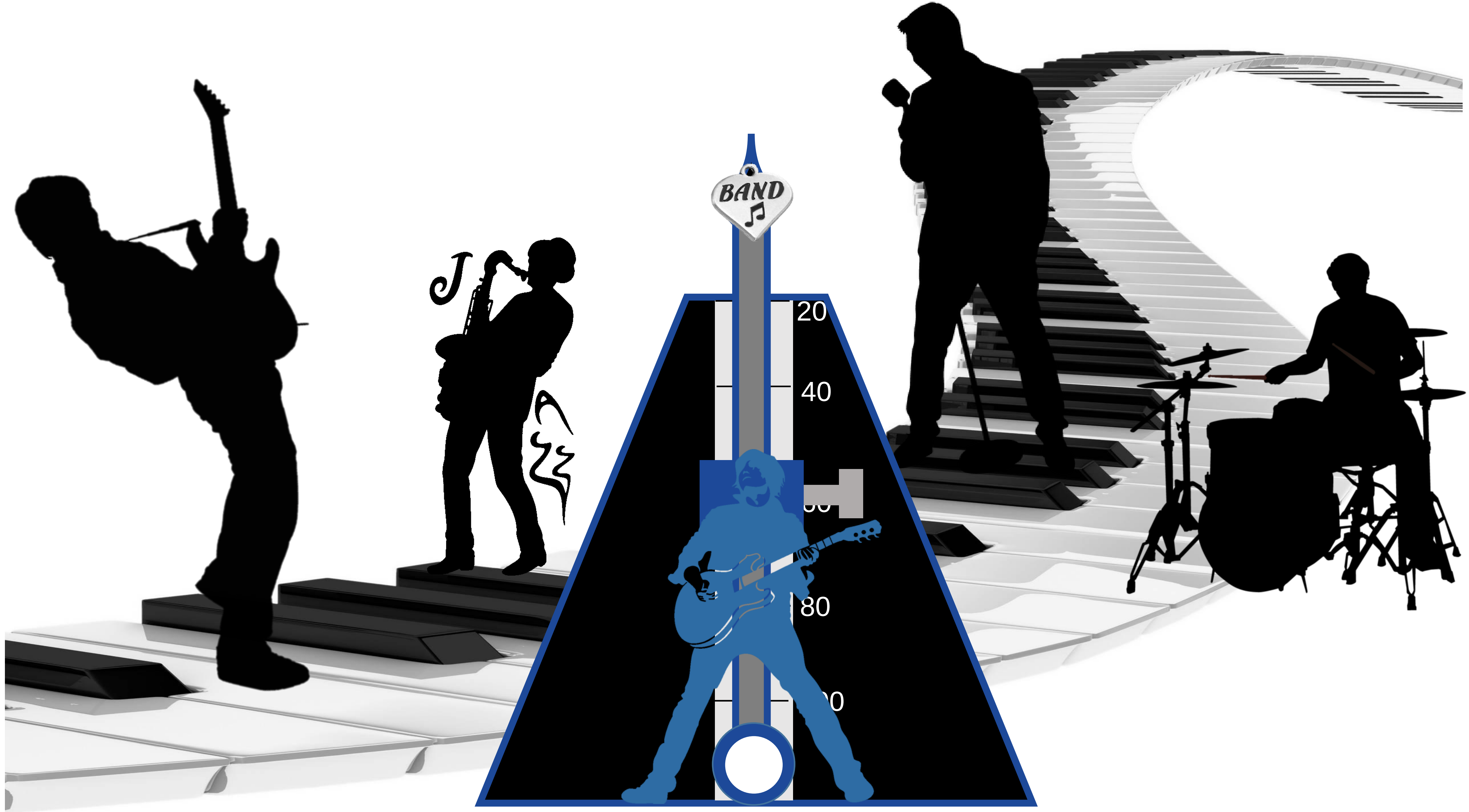
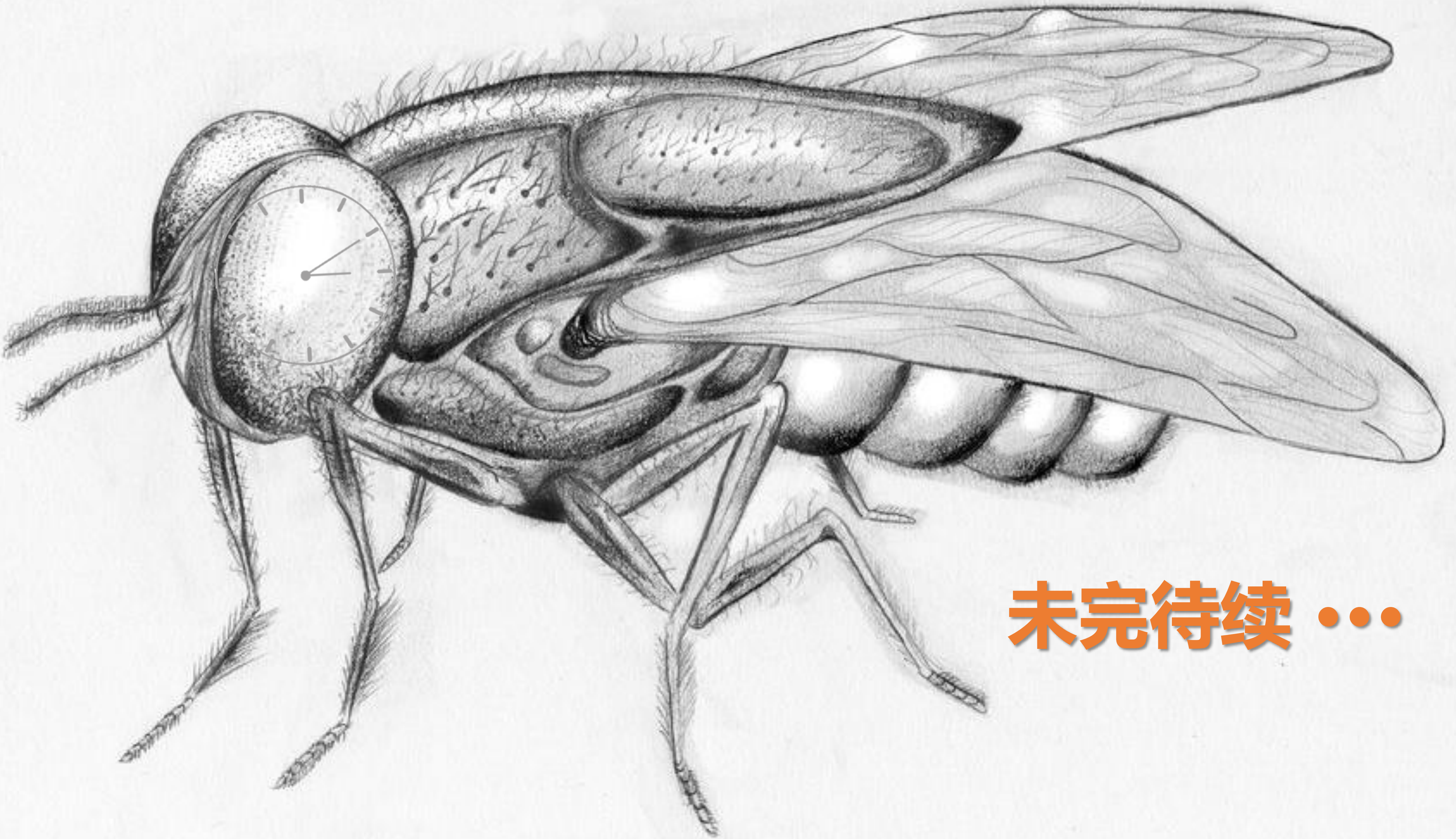

BAND
20
40
80

- 内源性 timekeeping 机制主要是进化过程中产生，是动物自身对地球上昼夜交替环境的一种预知和适应性调整；

- 就 *Drosophila* 来看，其自身的 timekeeping **周期小于 24 h**，所以并不是完美契合地球的昼夜交替，故其内在的环路需要受到外界环境的调整 (resetting)，主要是光照和温度两个因素的影响。 所以目前来看 *Drosophila* 的内源性 timekeeping 周期是一个很完美的周期还是说只是处于进化中的某一阶段，以后还会继续的变化？（地球上会出现昼夜长短变化的现象）；

- 能否通过基因改造，产生出节律加快或节律变缓的果蝇，它们的行为会有哪些影响？



未完待续 …

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Thank You