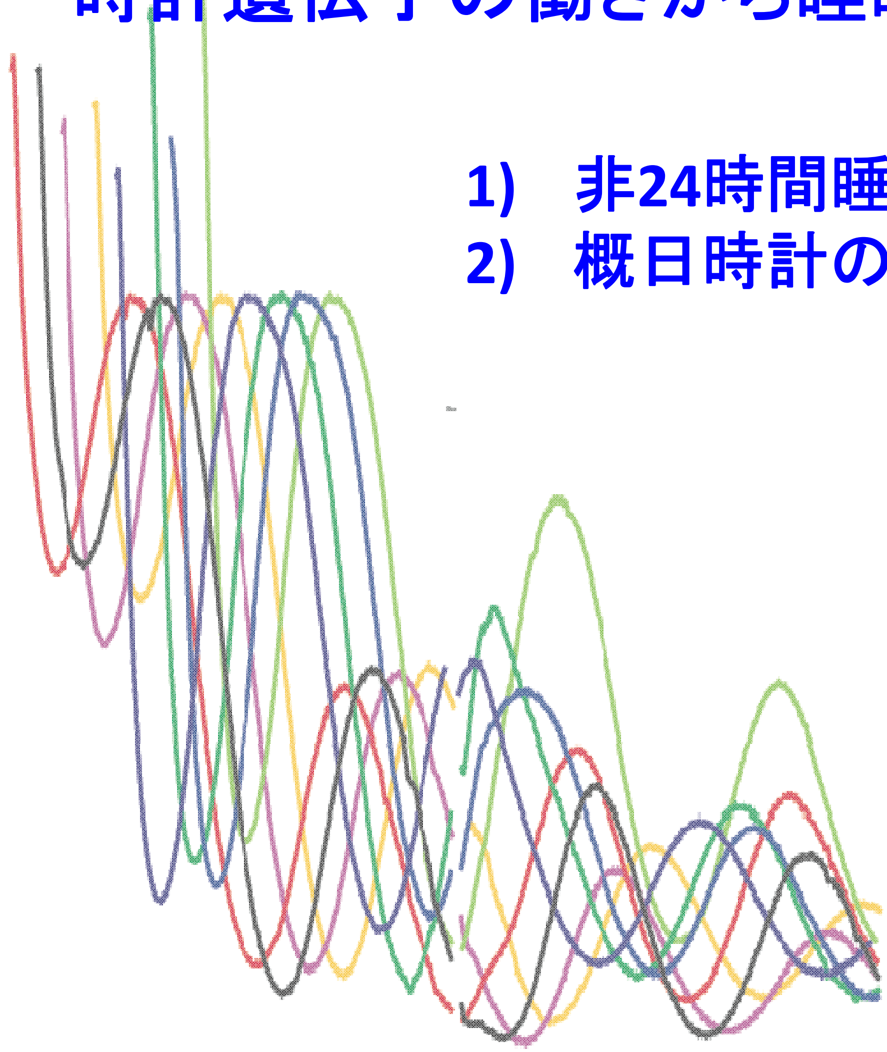
180712 電気通信大学BLSCセミナー

生物時計のサイエンス:
時計遺伝子の働きから睡眠障害の理解まで



1) 非24時間睡眠覚醒症候群マウス
2) 概日時計の分子発振メカニズム

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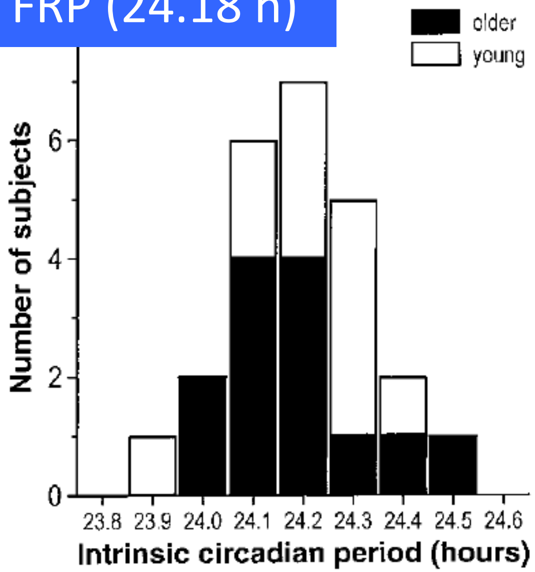
Terminology

Human FRP (24.18 h)



Zeitgeber: German for "Time giver".
Environmental stimulus that can entrain clock

Free running period, FRP: Period length of clock under no zeitgeber signals

T: Period length of zeitgeber cycle

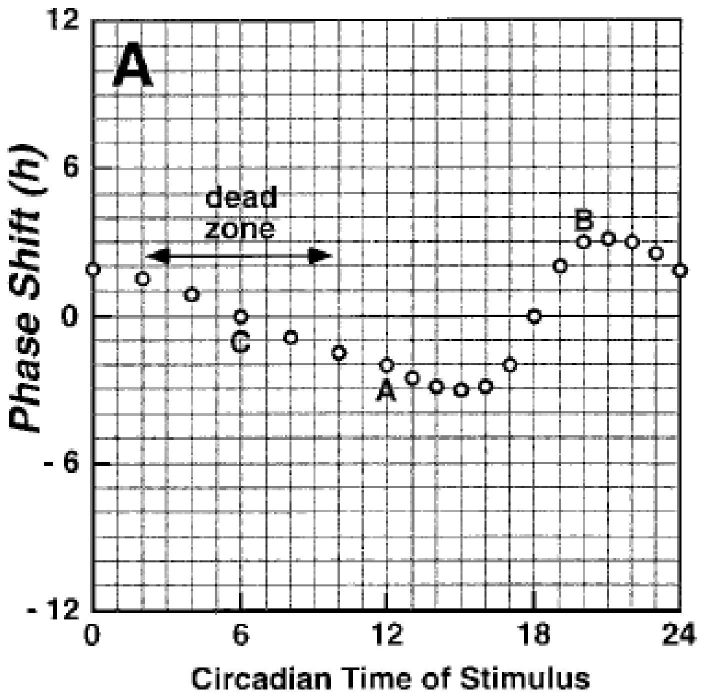
Phase relationship: Relationship between phase (time) of clock and that of zeitgeber cycle

Entrain: From French *entrainer*, "to carry along".
T = FRP with appropriate phase relationship.

Science, 284, 2177 (1999); Chronobiol Int, 20, 741 (2003)

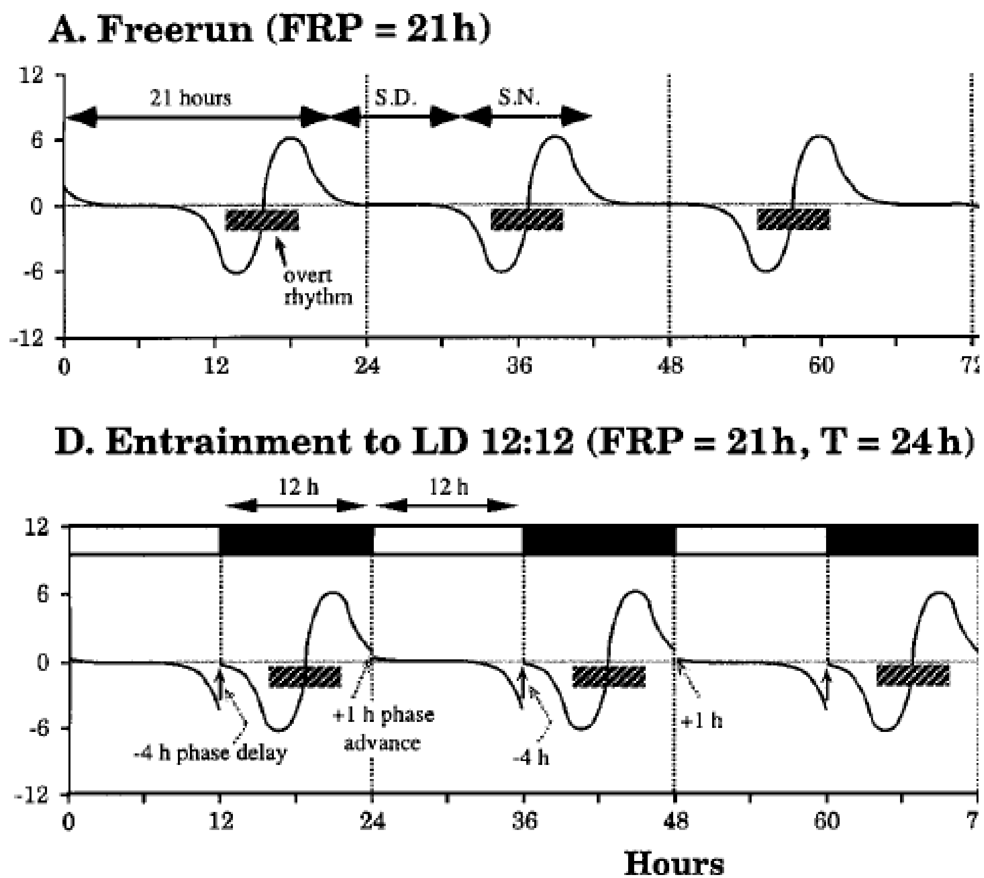
Entrainment is explained by phase shift of clock

Phase response curve (PRC)



→ Phase-dependent response to single zeitgeber pulse

Under entrained condition



Daily phase shift = FRP - T

Sleep disorder is representative problem caused by abnormal clock

International Classification of Sleep Disorders (ICSD-3, 2014)

Table 8 General criteria for circadian rhythm sleep–wake disorder (adapted from ICSD-3)

Criteria A–C must be met

A. A chronic or recurrent pattern of sleep–wake rhythm disruption due primarily to alteration of the endogenous circadian timing system or misalignment between the endogenous circadian rhythm and the sleep–wake schedule desired or required by an individual's physical environment or social/work schedules

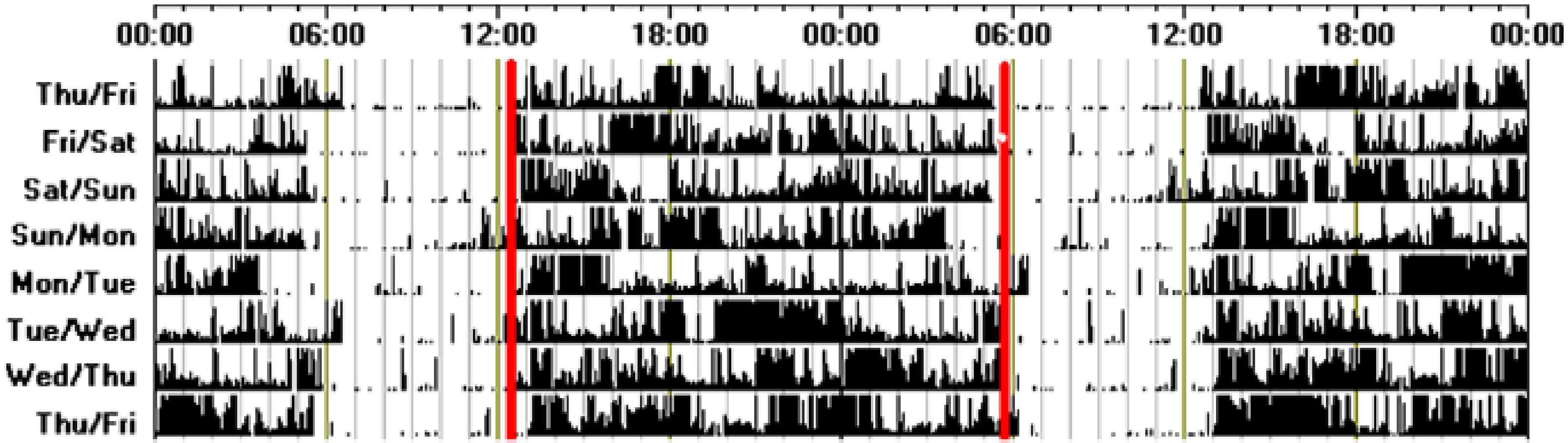
B. The circadian rhythm disruption leads to insomnia symptoms, excessive sleepiness or both

C. The sleep and wake disturbances cause clinically significant distress or impairment in mental, physical, social, occupational, educational or other important areas of functioning

1. Delayed sleep–wake phase disorder
2. Advanced sleep–wake phase disorder
3. Irregular sleep–wake rhythm disorder
4. Non-24-h sleep–wake rhythm disorder
5. Shift work disorder
6. Jet lag disorder
7. Circadian sleep–wake disorder not otherwise specified (NOS)

Delayed sleep phase syndrome, DSPS

Activity watch rhythm



Features

- 3-6 hours delay relative to desired or socially acceptable schedules
- Sleep onset insomnia

Prevalence

- 0.13-0.17% in general population
- 7-16 % in adolescent (13-17 years old)
- 7% of patients with chronic insomnia in sleep clinics

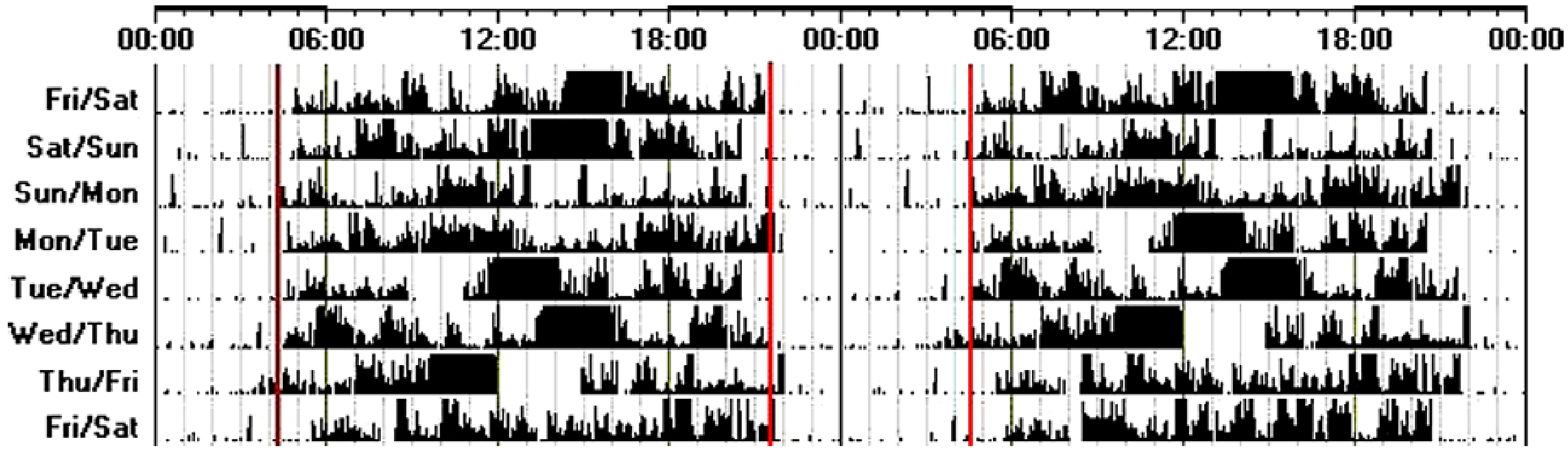
Pathophysiology (Unclear)

- Normal FRP in general DSPS patients (Kitamura et al., 2013)
- Long FRP (0.5 hr) in familial DSPS with *Cry1* mutation (Patke et al, 2017)
- More sensitive melatonin suppression to evening light (Aoki et al., 2001)

Sleep Med **8, 566 (2007)**

Advanced sleep phase syndrome, ASPS

Activity watch rhythm



Features

- Several hours early relative to conventional and desired time
- Early morning awakenings and sleepiness in late afternoon

Prevalence

- Unclear
- Increase with age (1 % in middle-aged and older adults)

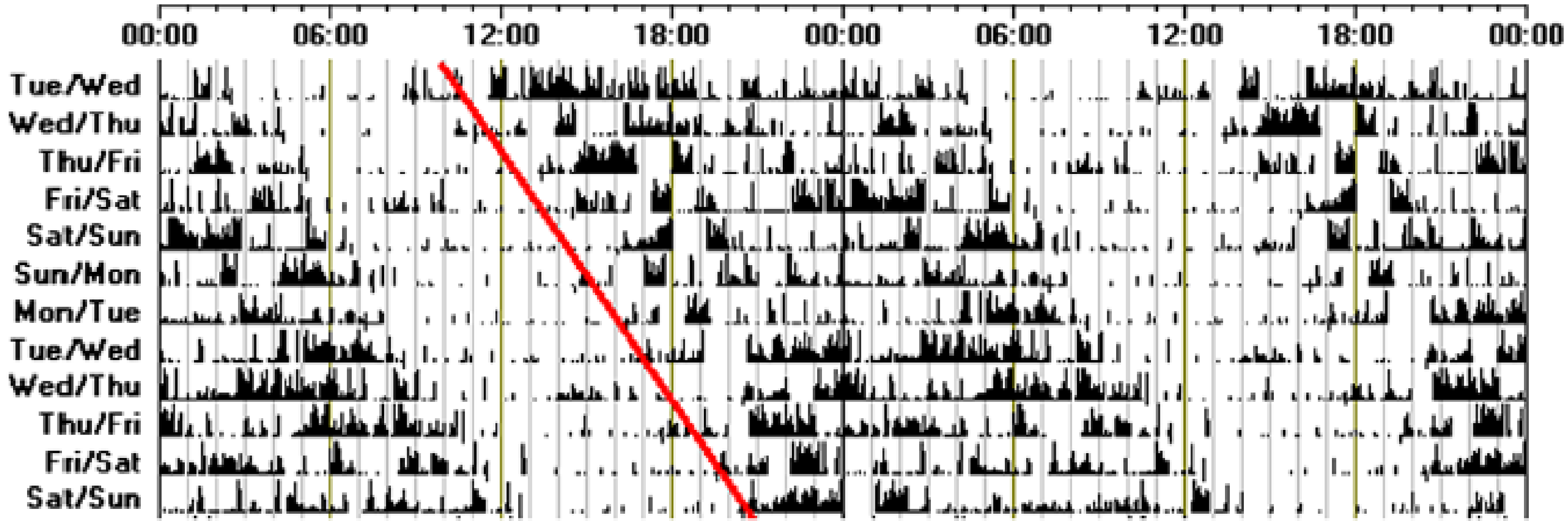
Pathophysiology (Unclear)

- Short FRP in familial ASPS

Sleep Med 8, 566 (2007)

Non-24-h sleep-wake syndrome (Non-24)

Activity watch rhythm



Features

・Daily drift of sleep and wake times

Prevalence

・50% of total blind people

・Although rare, observed in sighted people (> 10 years old)

Pathophysiology (Unclear)

・Long free running period (0.31hr)

・In sighted non-24, 26% patients shows DSPD before the onset

Sleep Med 8, 566 (2007)

Non-24 with normal visual function

Clinical Analyses of Sighted Patients with Non-24-Hour Sleep-Wake Syndrome: A Study of 57 Consecutively Diagnosed Cases

Tatsuro Hayakawa, MD[1]; Makoto Uchiyama, MD, PhD[2]; Yuichi Kamei, MD, PhD[1]; Kayo Shibui, MD, PhD[2]; Hirokuni Tagaya, MD, PhD[2]; Takashi Asada, MD, PhD[3]; Masako Okawa, MD, PhD[4]; Jujiro Urata, MD[1]; Kiyohisa Takahashi MD, PhD[5]

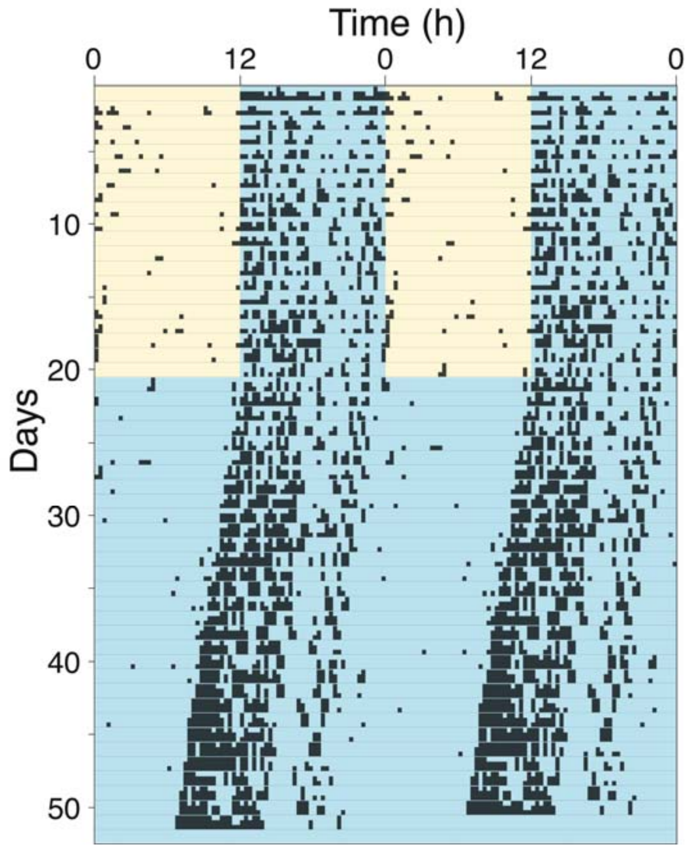
Sleep 28, 945 (2005)

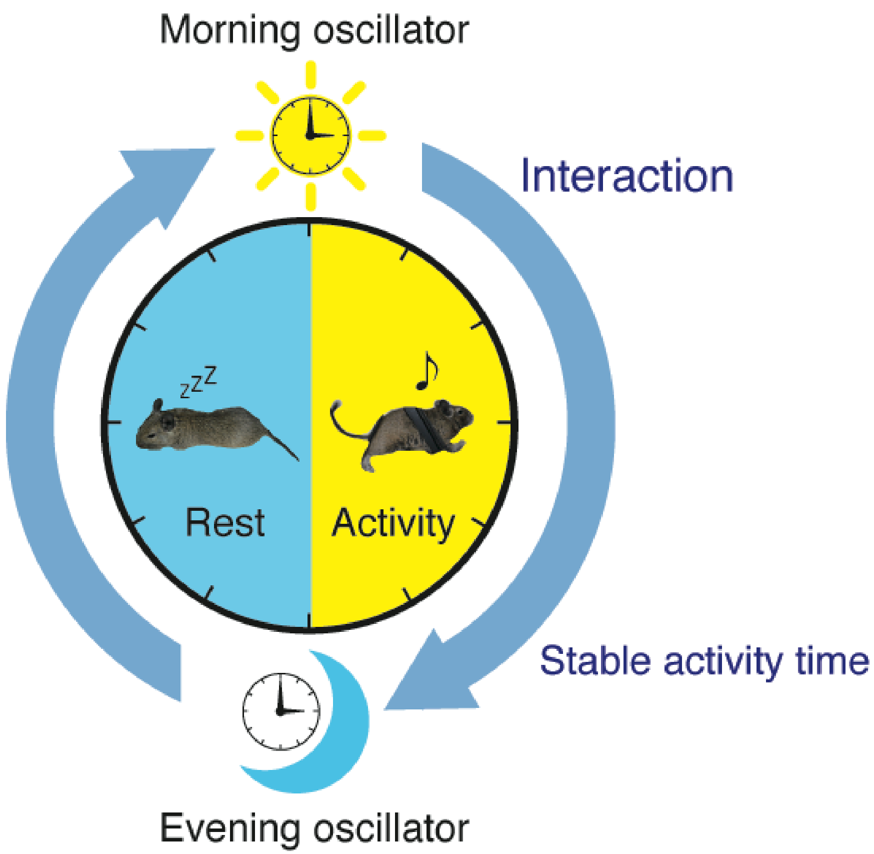
Table 1— Characteristics of 57 Consecutive Patients Diagnosed With Non-24 Hour Sleep-Wake Syndrome*

Characteristic	No. (%)
Sex	
Men	41 (72)
Women	16 (28)
Age at onset, y	
mean ± SD	20.2 ± 7.0
< 10	0 (0)
10-19	36 (63)
20-29	13 (23)
30-39	6 (11)
40-49	2 (3)
Marital status	
Married	6 (11)
Unmarried	51 (89)
Presence of family or roommate	
Yes	45 (79)
No	12 (21)
Social status at first visit	
Student	20 (35)
Employed	12 (21)
Part-time worker	3 (5)
Unemployed	22 (39)
Premorbid status	
Psychiatric problems	16 (28)
Physical problems	1 (2)
Delayed sleep-phase syndrome	15 (26)
Family history of mental, sleep, or neurologic disorder	
Yes	5 (9)
No	52 (91)

*Data are presented as number (%) unless otherwise indicated.

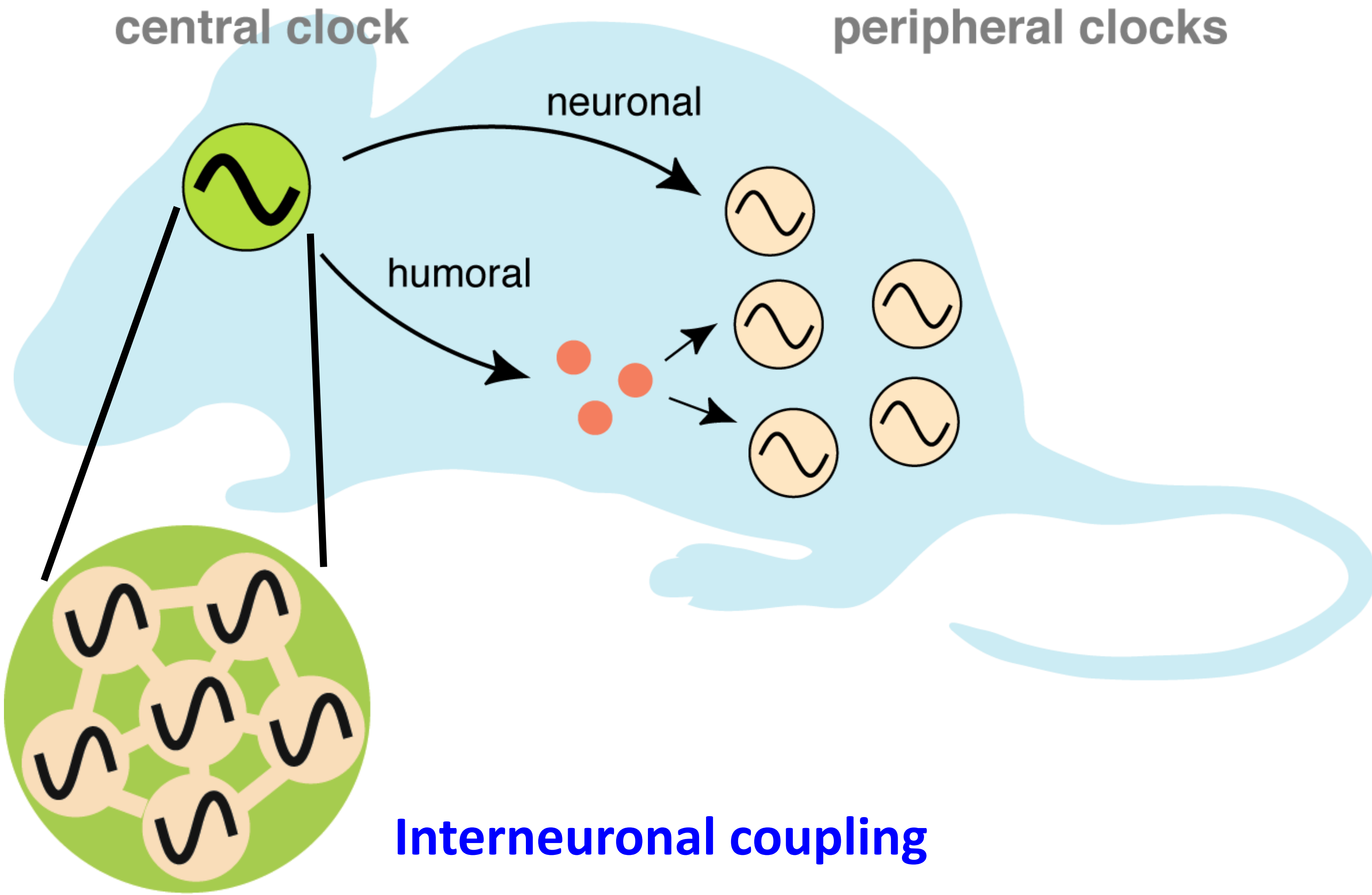
Circadian clock generates behavioral rhythm





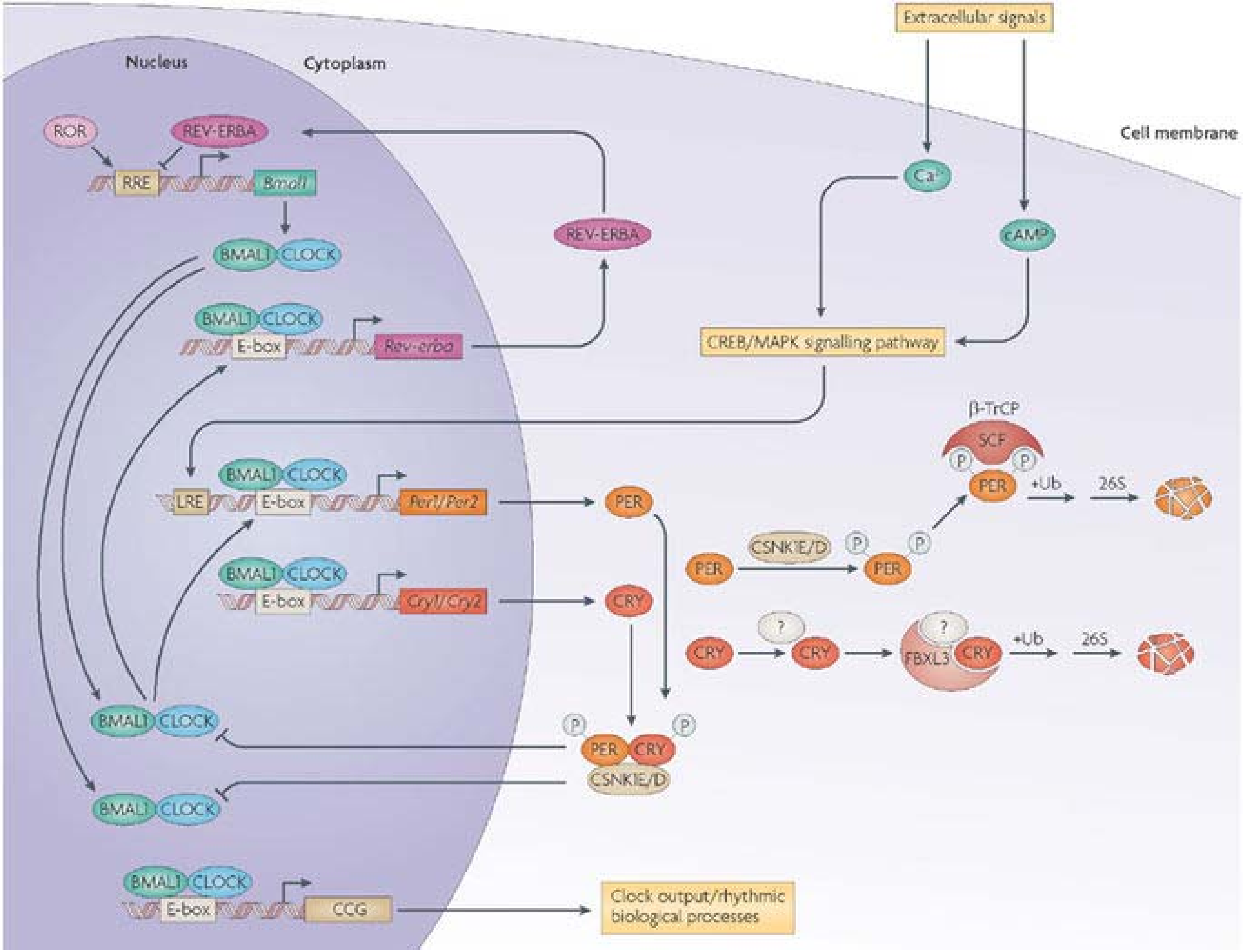
Tight-coupling of morning and evening oscillators establishes temporal organization of daily activities

Central clock in the SCN controls behavioral rhythms and peripheral clock



Interneuronal coupling

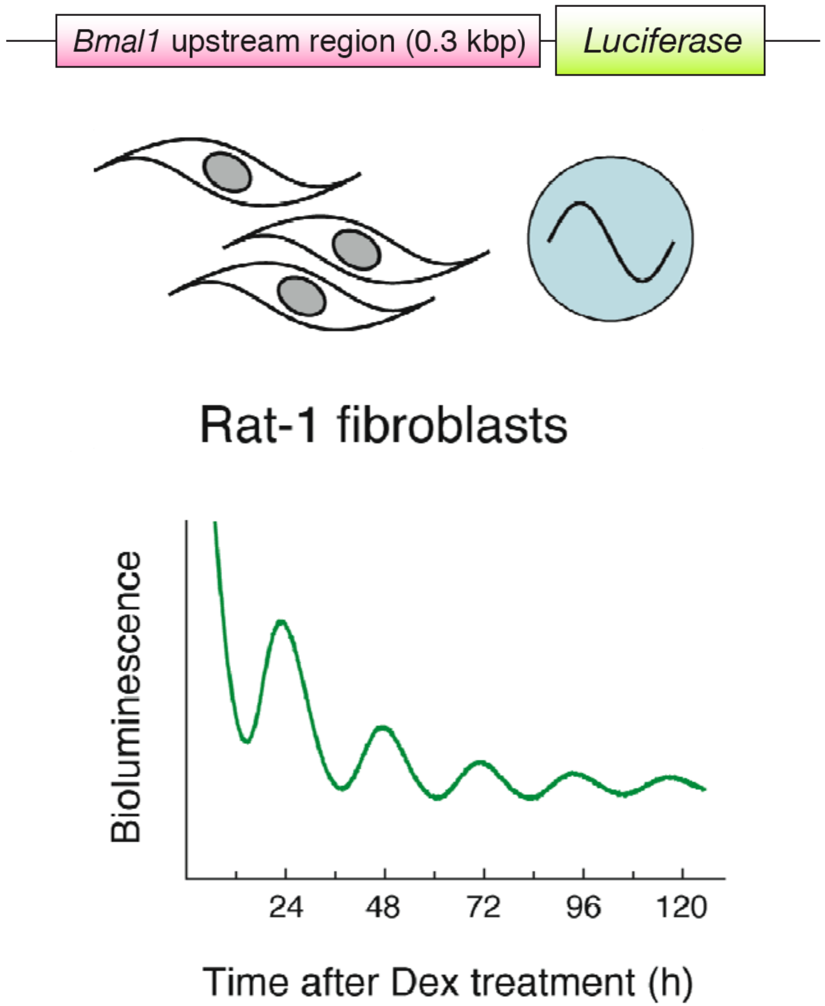
Transcriptional feedback loop for oscillation



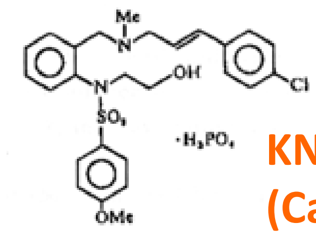
Nature Reviews Genetics 9, 764 (2008)

Exploring of bioactive molecule affecting cellular clock

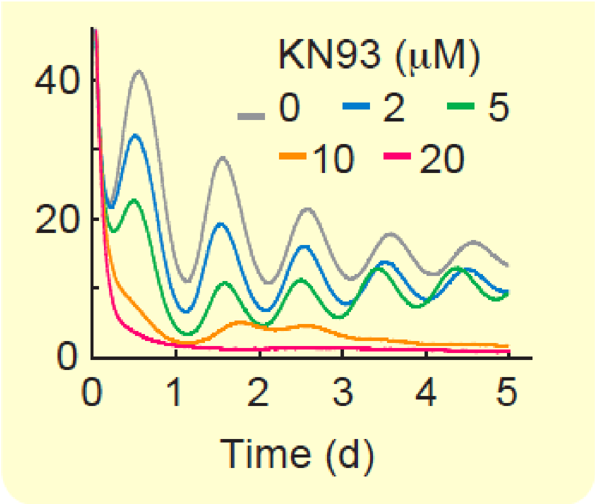
Monitoring of cellular rhythm



Effect of KN93 on rhythm



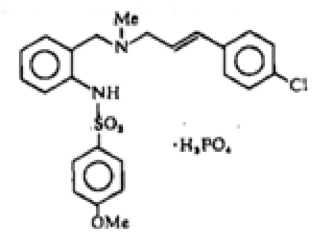
KN93
(CaMKII inhibitor)



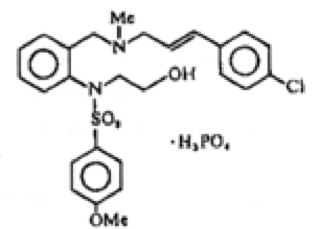
Kon *et al*., *Communicative & Integrative Biology*, 2015.

CaMKII activity is essential for cellular clock

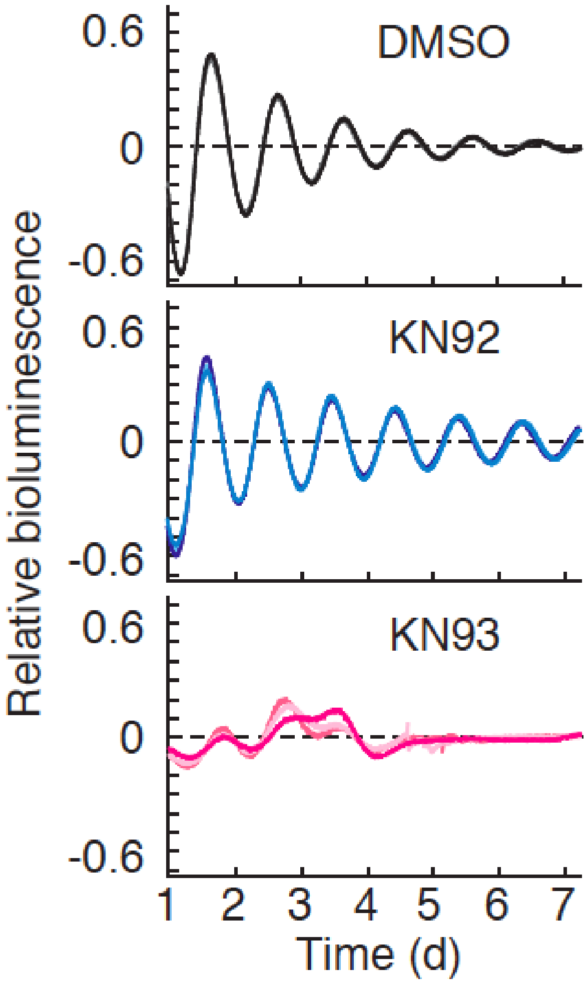
Small-molecule inhibitor of CaMKII



KN-92
(Inactive analog)

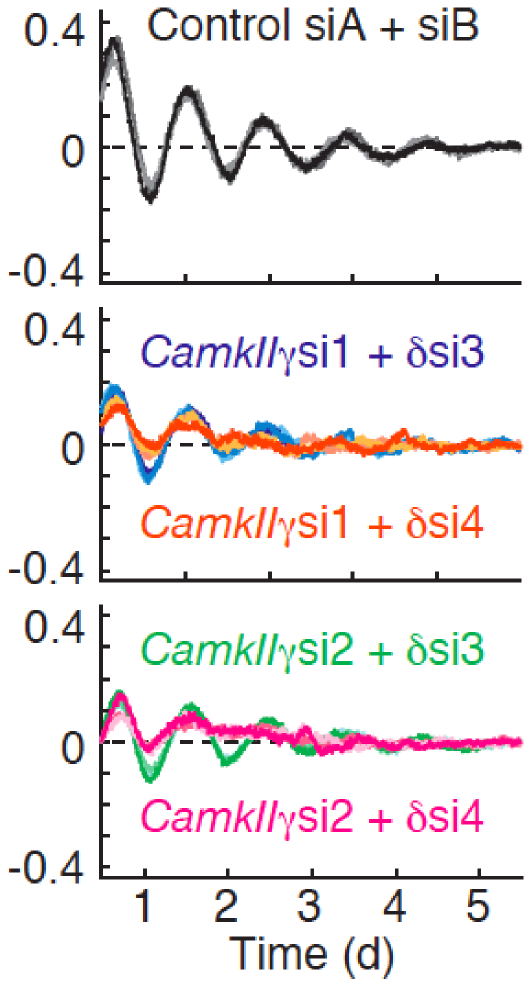


KN-93
(CaMKII inhibitor)



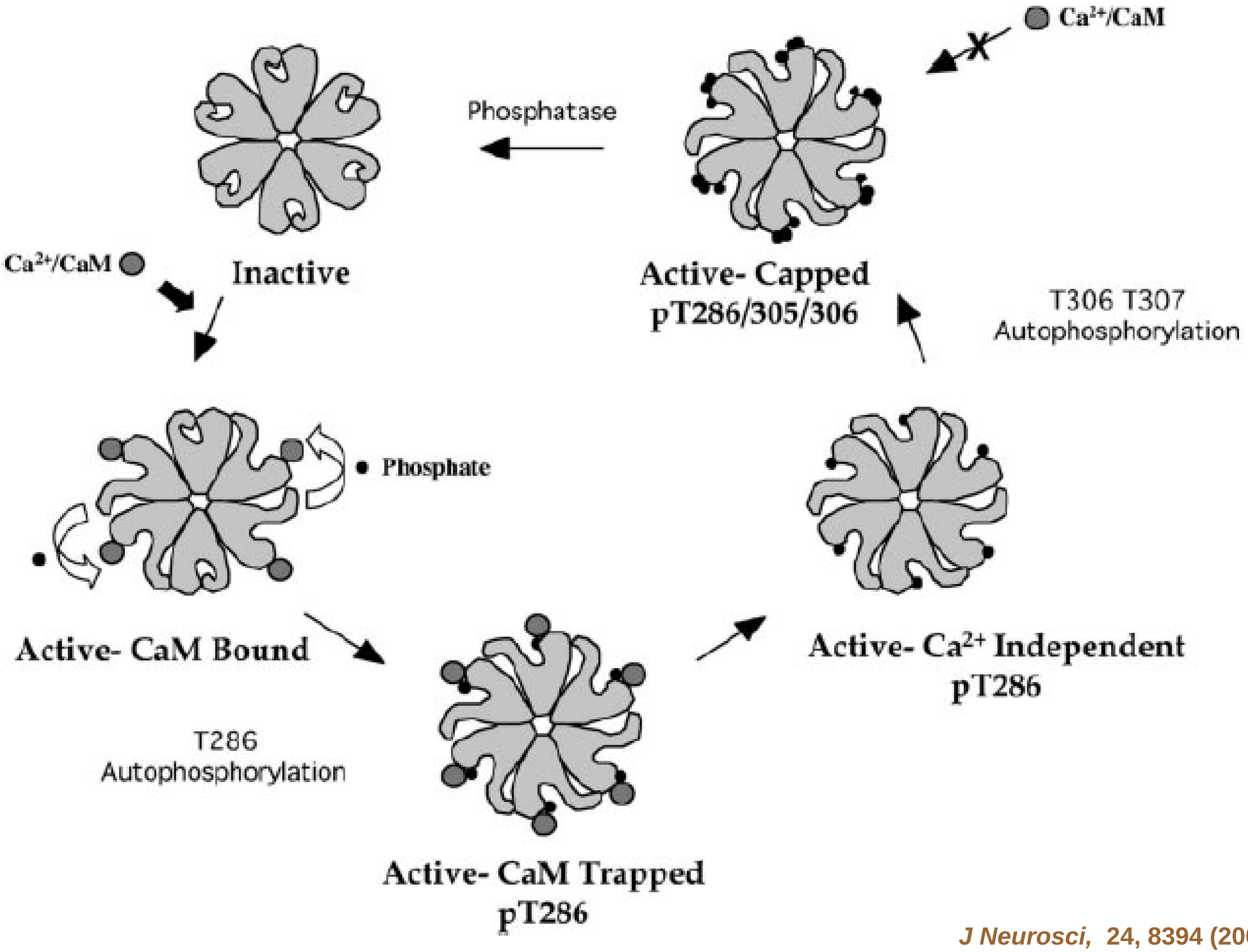
Rat-1 *Bmal1*-luc cells

Knock-down of CaMKIIγ/δ



NIH3T3 *Bmal1*-luc reporter

CaMKII is Ca^{2+}-dependent molecular switch



J Neurosci, 24, 8394 (2004).

CaMKII is essential for brain function

De Novo Mutations in Protein Kinase Genes *CAMK2A* and *CAMK2B* Cause Intellectual Disability

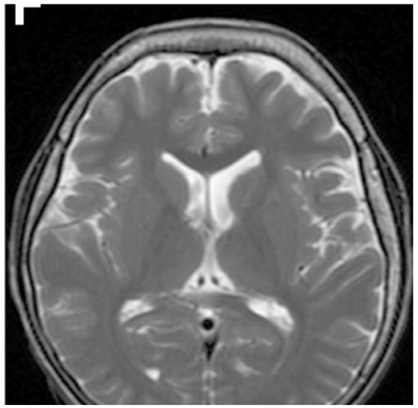
The American Journal of Human Genetics *101*, 768–788, November 2, 2017

De novo variants in *CAMK2A* and *CAMK2B* cause neurodevelopmental disorders

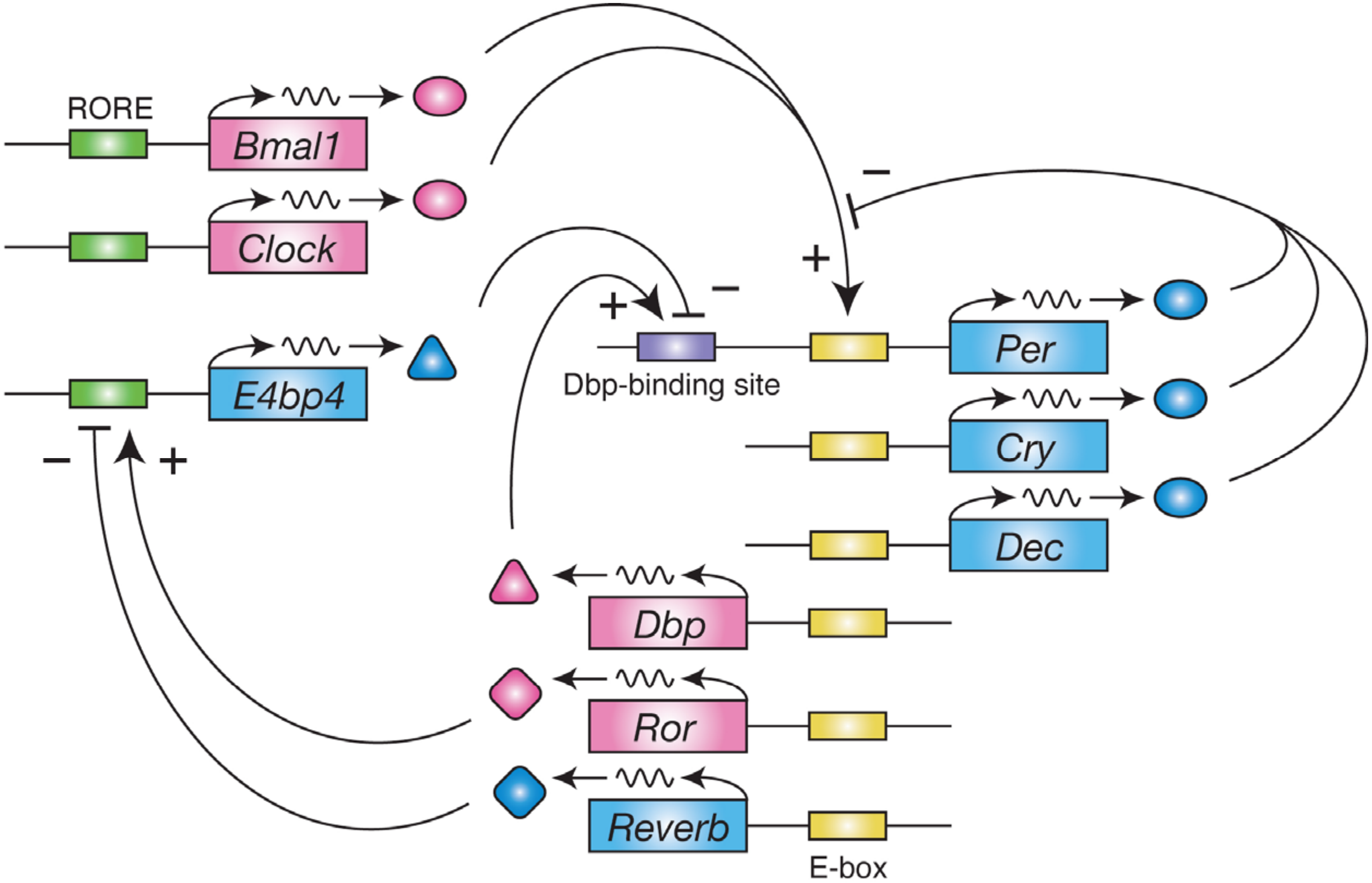
Tenpei Akita[1,a], Kazushi Aoto[2,a], Mitsuhiro Kato[3,a], Masaaki Shiina[4], Hiroki Mutoh[1], Mitsuko Nakashima[2,5], Ichiro Kuki[6], Shin Okazaki[6], Shinichi Magara[7], Takashi Shiihara[8], Kenji Yokochi[9,10], Kaori Aiba[10], Jun Tohyama[7], Chihiro Ohba[5], Satoko Miyatake[5], Noriko Miyake[5], Kazuhiro Ogata[4], Atsuo Fukuda[1], Naomichi Matsumoto[5] & Hirotomo Saitsu[2]

Annals of Clinical and Translational Neurology 2018; 5(3): 280–296

- Intellectual disability
- Epileptic seizure
- Ataxia



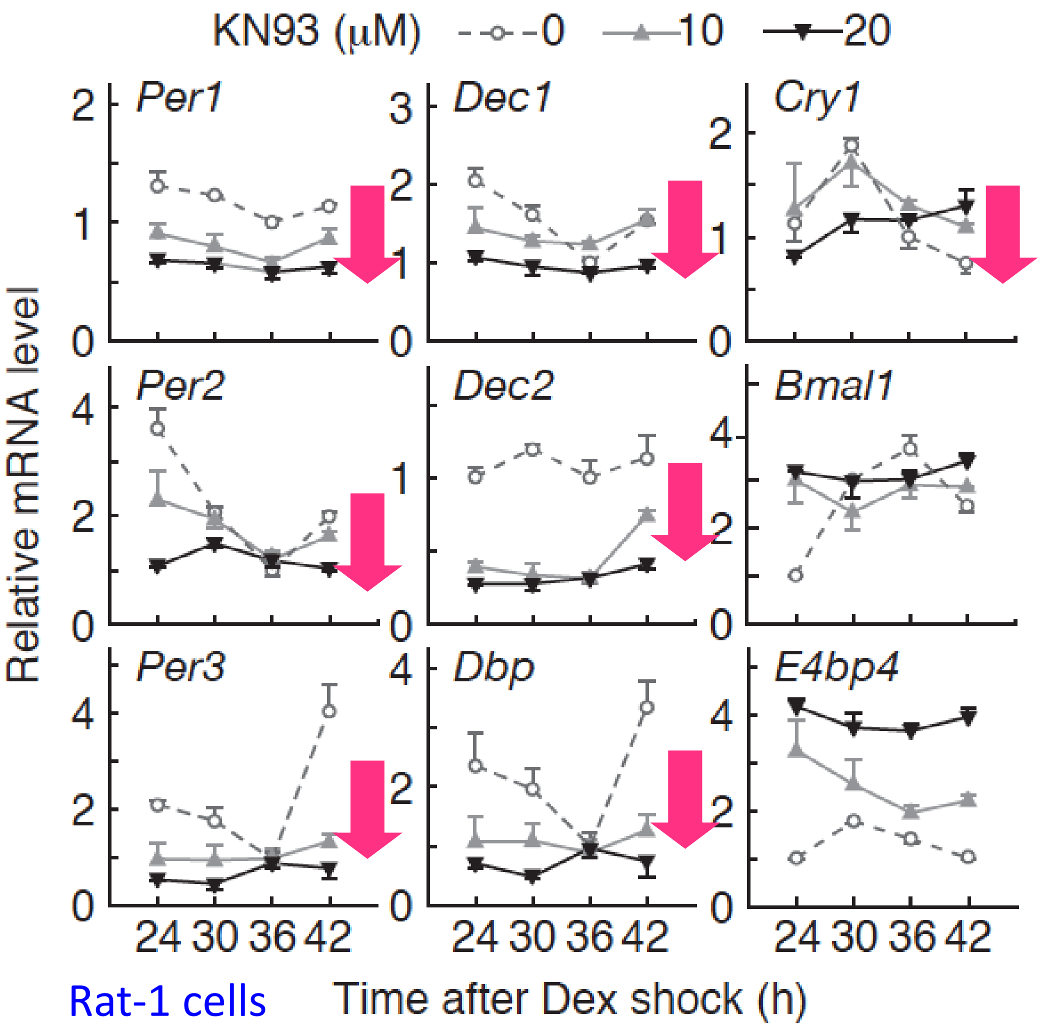
Transcriptional feedback loops for cellular clock



What component(s) is affected by CaMKII?

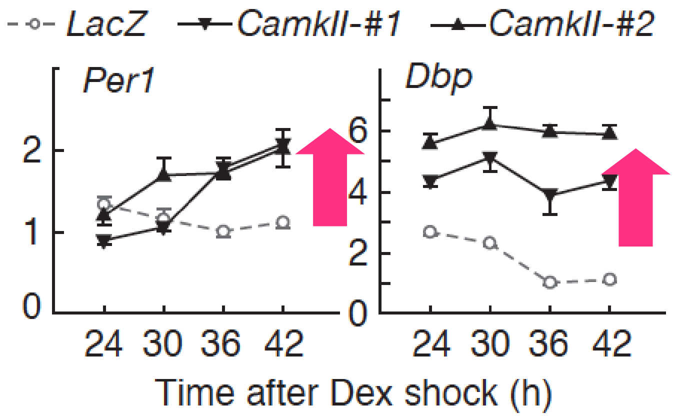
CaMKII activity is essential for E-box-dependent gene expression

Chronic treatment of CaMKII inhibitor



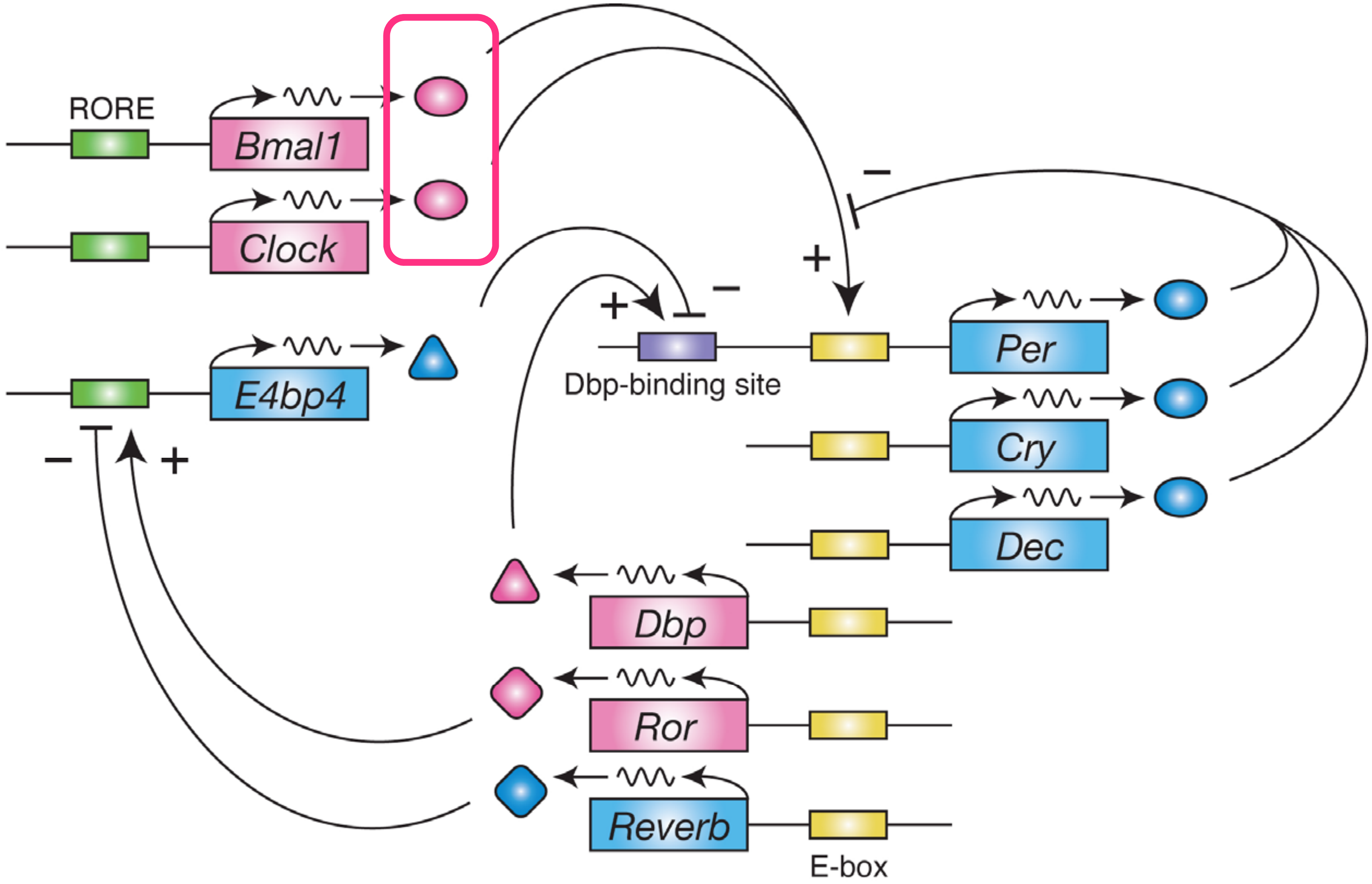
Rat-1 cells

Overexpression of CaMKII



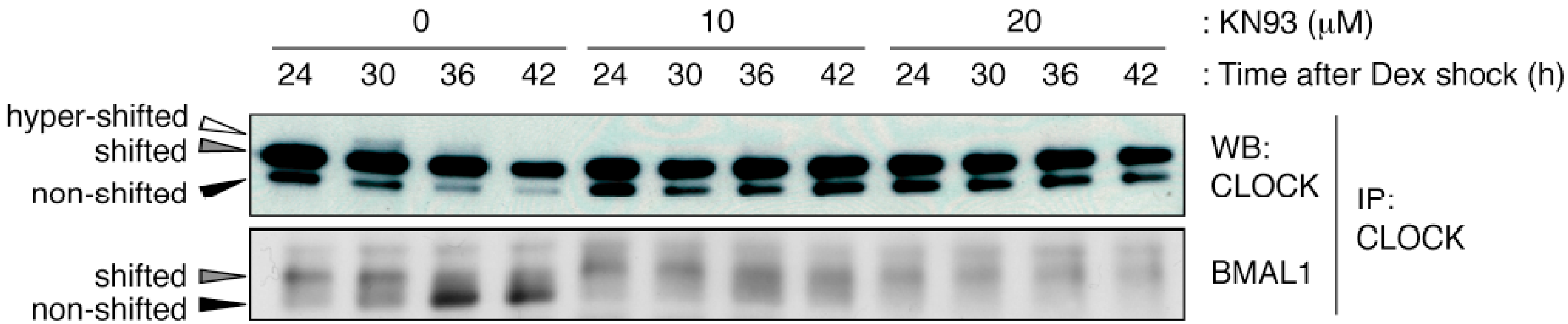
NIH3T3 cells

E-box-dependent transcription may be a target of CaMKII

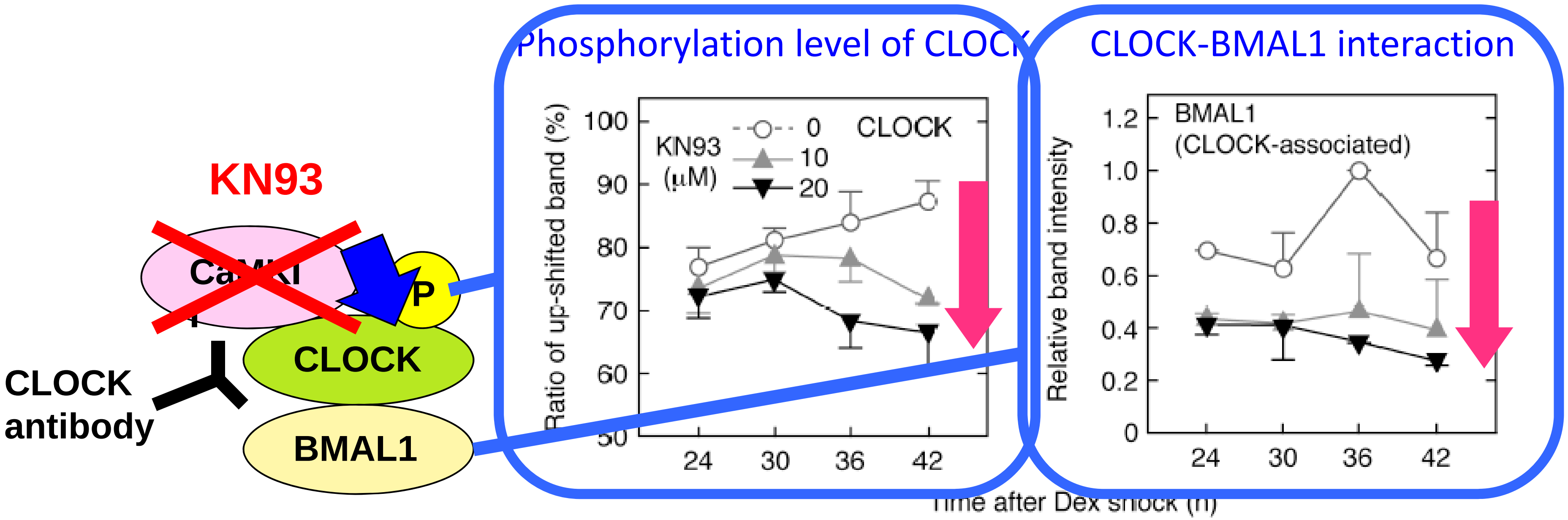


CaMKII Inhibition decreases phosphorylation of CLOCK and heterodimerization of CLOCK and BMAL1

Immunoprecipitation by anti-CLOCK antibody

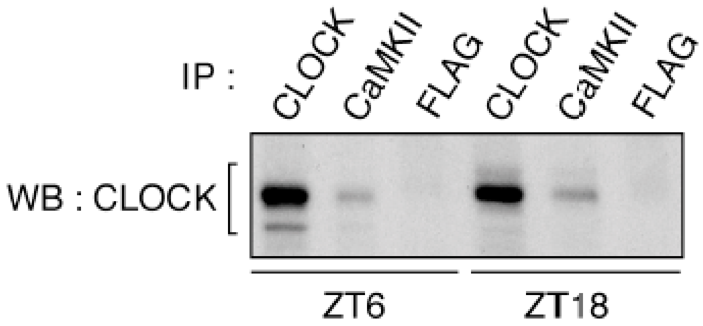


Synchronized Rat-1 cells



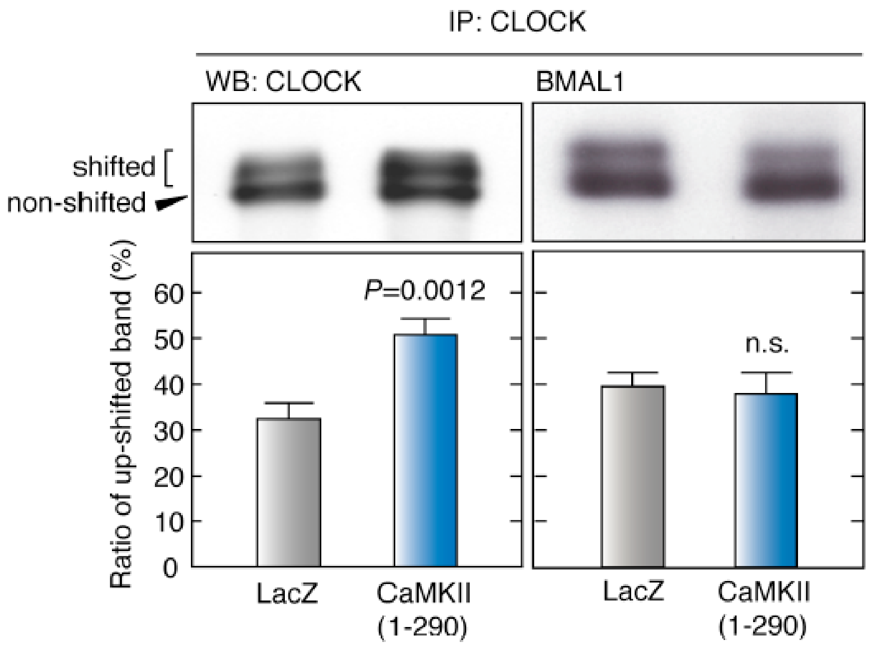
CaMKII phosphorylates CLOCK

Co-immunoprecipitation assay



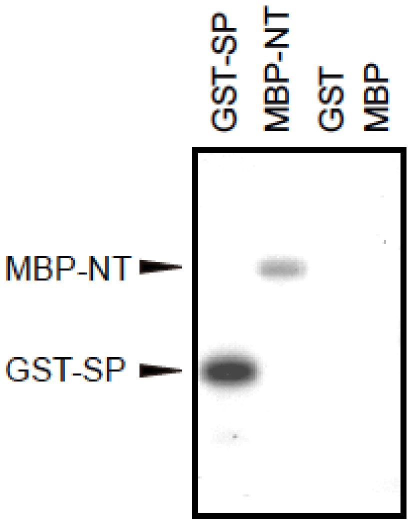
Nuclear lysate of mouse liver

In cell phosphorylation assay

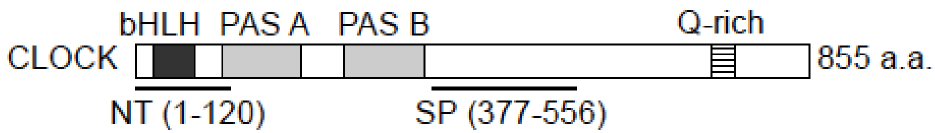


CLOCK and BMAL1
expressed in HEK293T cell

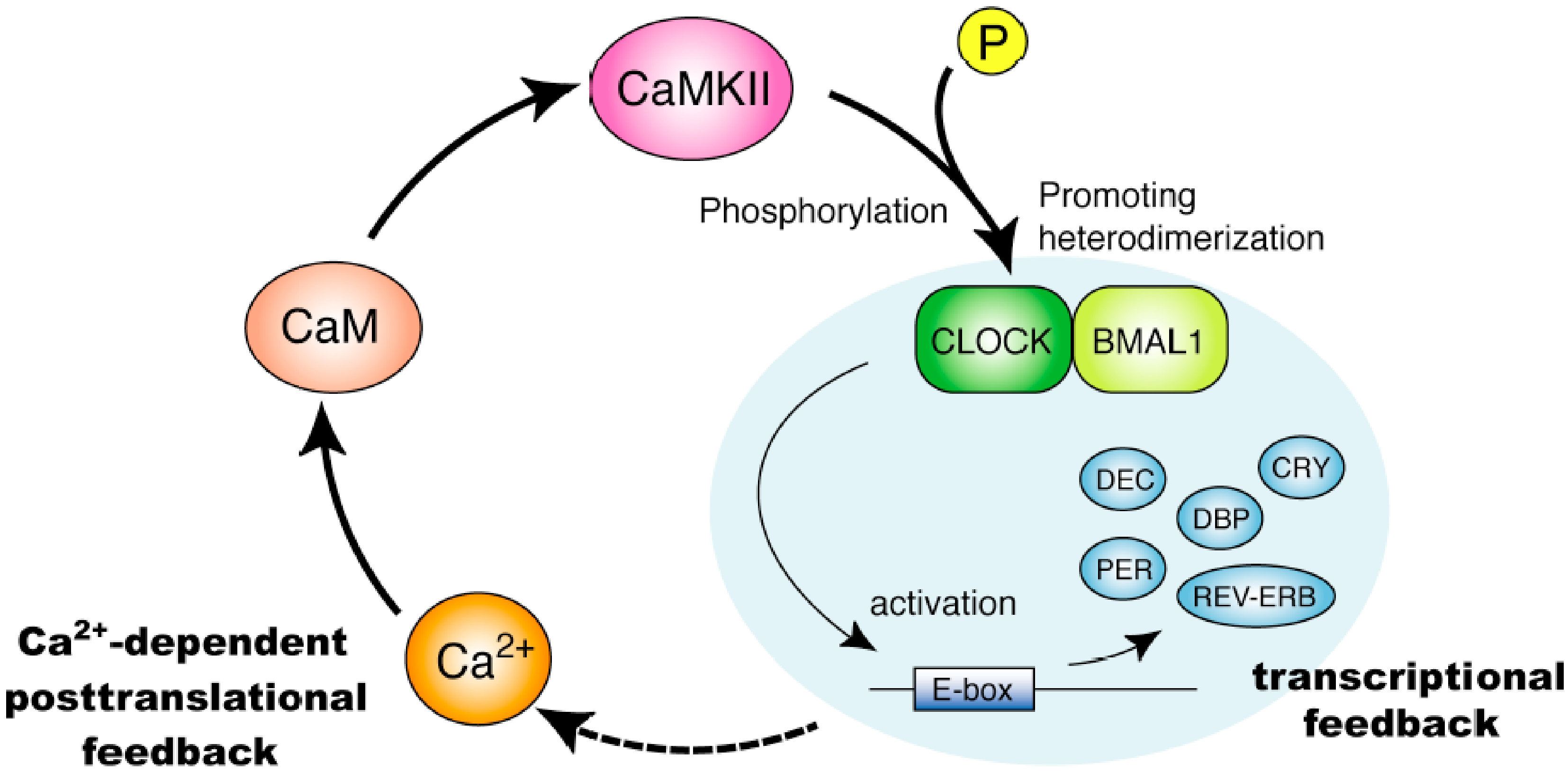
In vitro kinase assay



Partial fragment of CLOCK



Is CaMKII a linker between two oscillation mechanism?

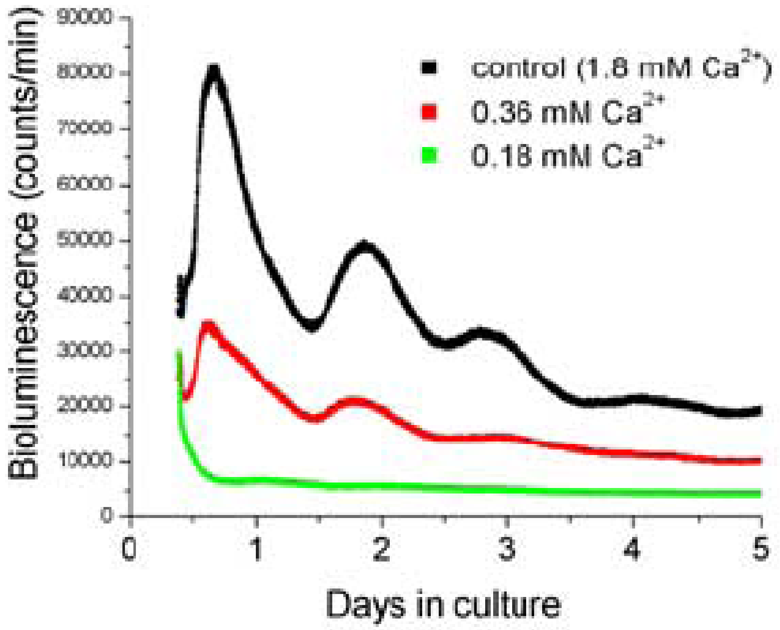


7682 • The Journal of Neuroscience, August 17, 2005 • 25(33):7682–7686

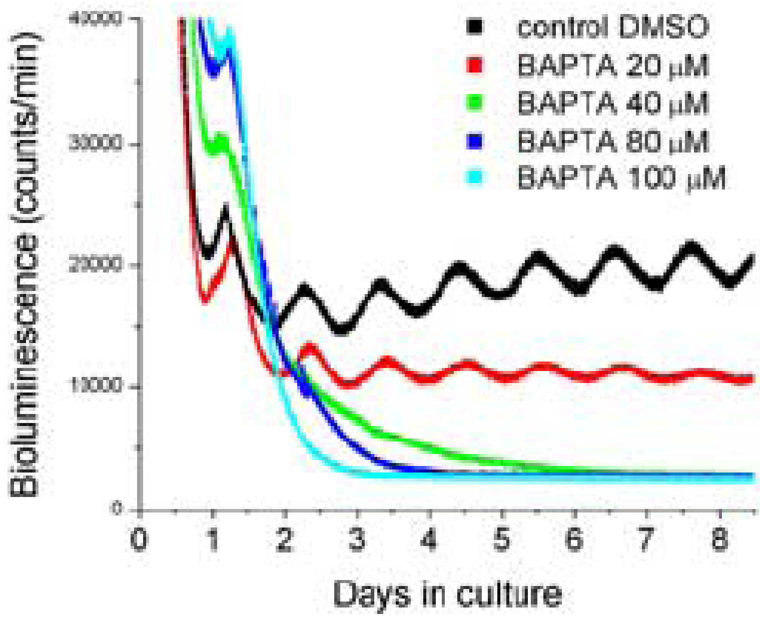
A Calcium Flux Is Required for Circadian Rhythm Generation in Mammalian Pacemaker Neurons

Gabriella B. Lundkvist,[1] Yongho Kwak,[1] Erin K. Davis,[1] Hajime Tei,[2] and Gene D. Block[1]
[1]Center for Biological Timing, Department of Biology, University of Virginia, Charlottesville, Virginia 22903, and [2]Research Group of Chronogenomics, Mitsubishi Kagaku Institute of Life Sciences, Machida, Tokyo 194-8511, Japan

Liver from Per1-luc. TG mice

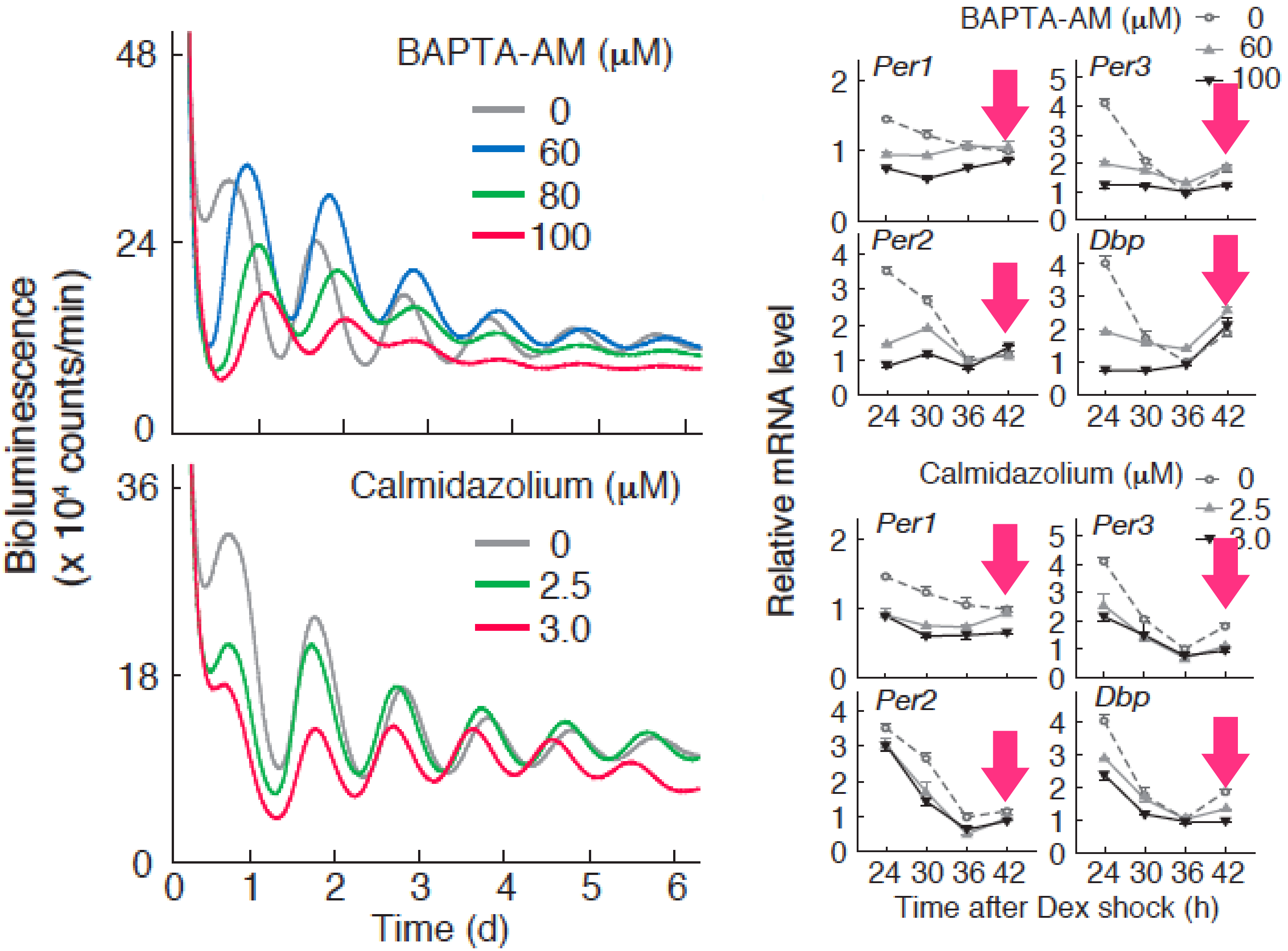


SCN from Per1-luc. TG mice



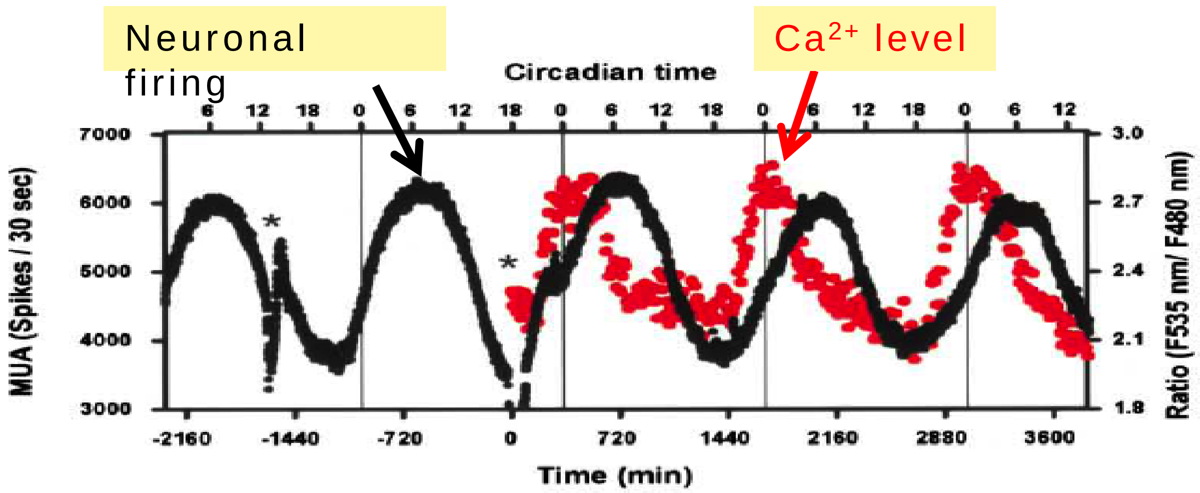
Ca^{2+} is essential for the transcriptional feedback loops.

Roles for upstream regulators of CaMKII

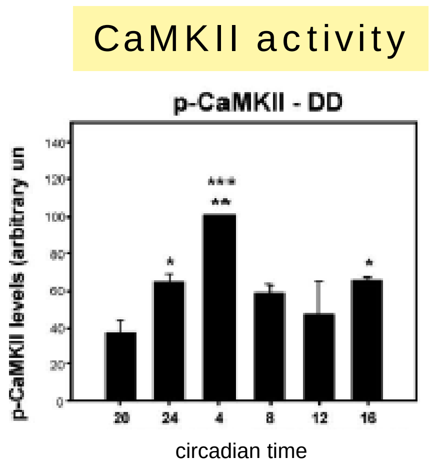


Ca^{2+}/CaM is important for cellular rhythm and E-box gene expression

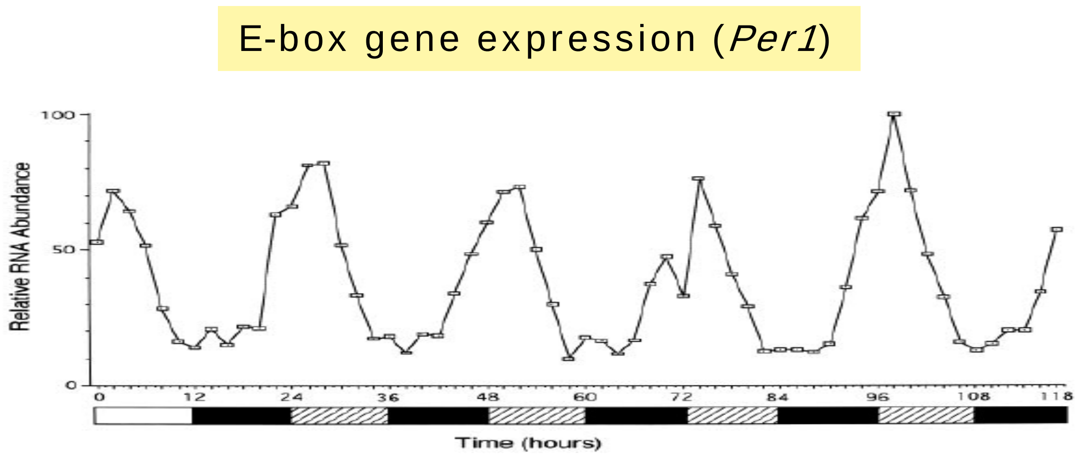
Morning activation of Ca^{2+}-CaMKII-E-box gene expression in SCN



***Neuron*, 38, 253, (2003).**



***Neurochemistry Int*., 44, 617, (2004).**



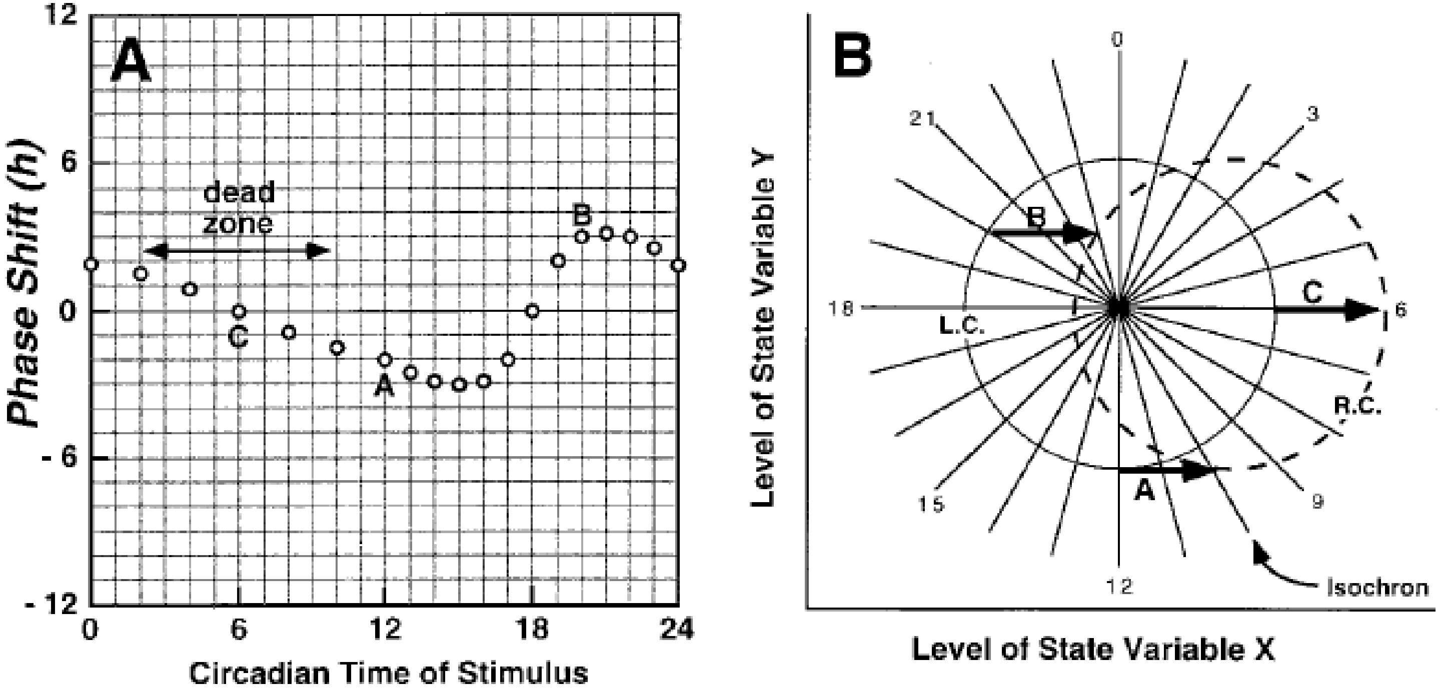
***Cell*, 91, 1043, (1997).**

Do CaMKII activity levels determine phase of clock?

State variables define the state of of the oscillation

The fundamental modeling concept is that components underlying rhythm generation change rhythmically in time.

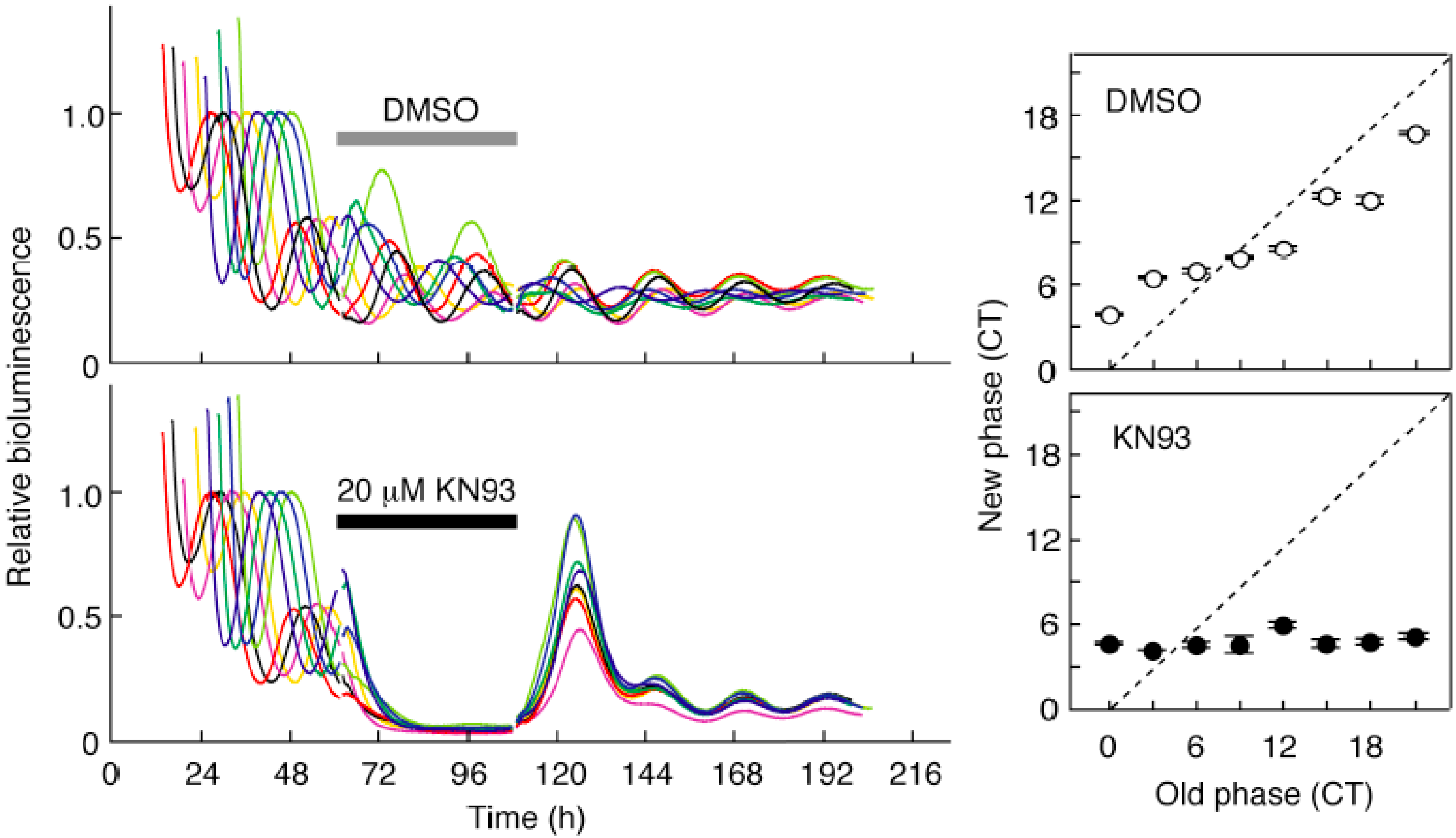
Chronobiology: Biological Timekeeping Jay C. Dunlap, Jennifer J. Loros, and Patricia J. DeCoursey



Chronobiol Int, 20, 741 (2003)

If CaMKII activity is a state variable, modulation of the level might reset phase of clock.

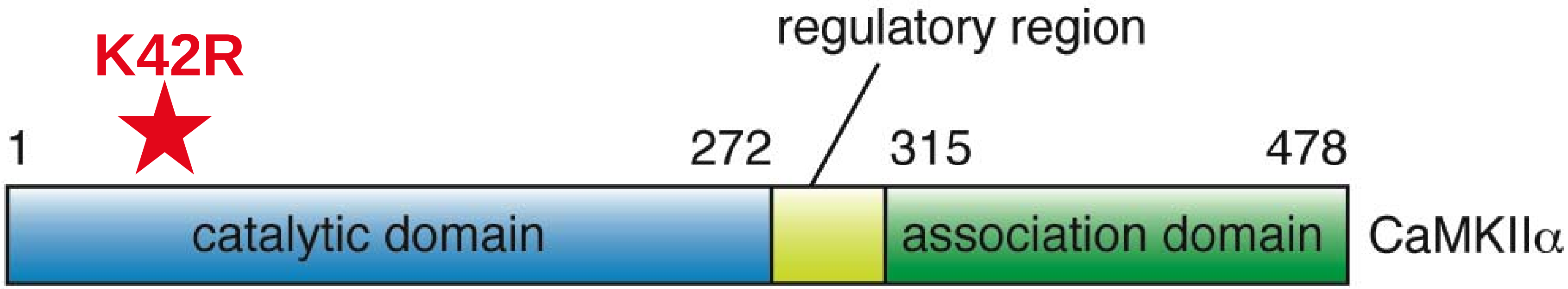
Setting CaMKII activity to trough level leads the oscillator to trough level of E-box gene expression



Rat-1 *Bmal1*-luc cells

Kon *et al.*, *Genes and Development*, 2014.

Behavioral analysis of CaMKIIα kinase-dead knock-in mice



CaMKIIαK42R

K42R prevents ATP binding for phosphorylation

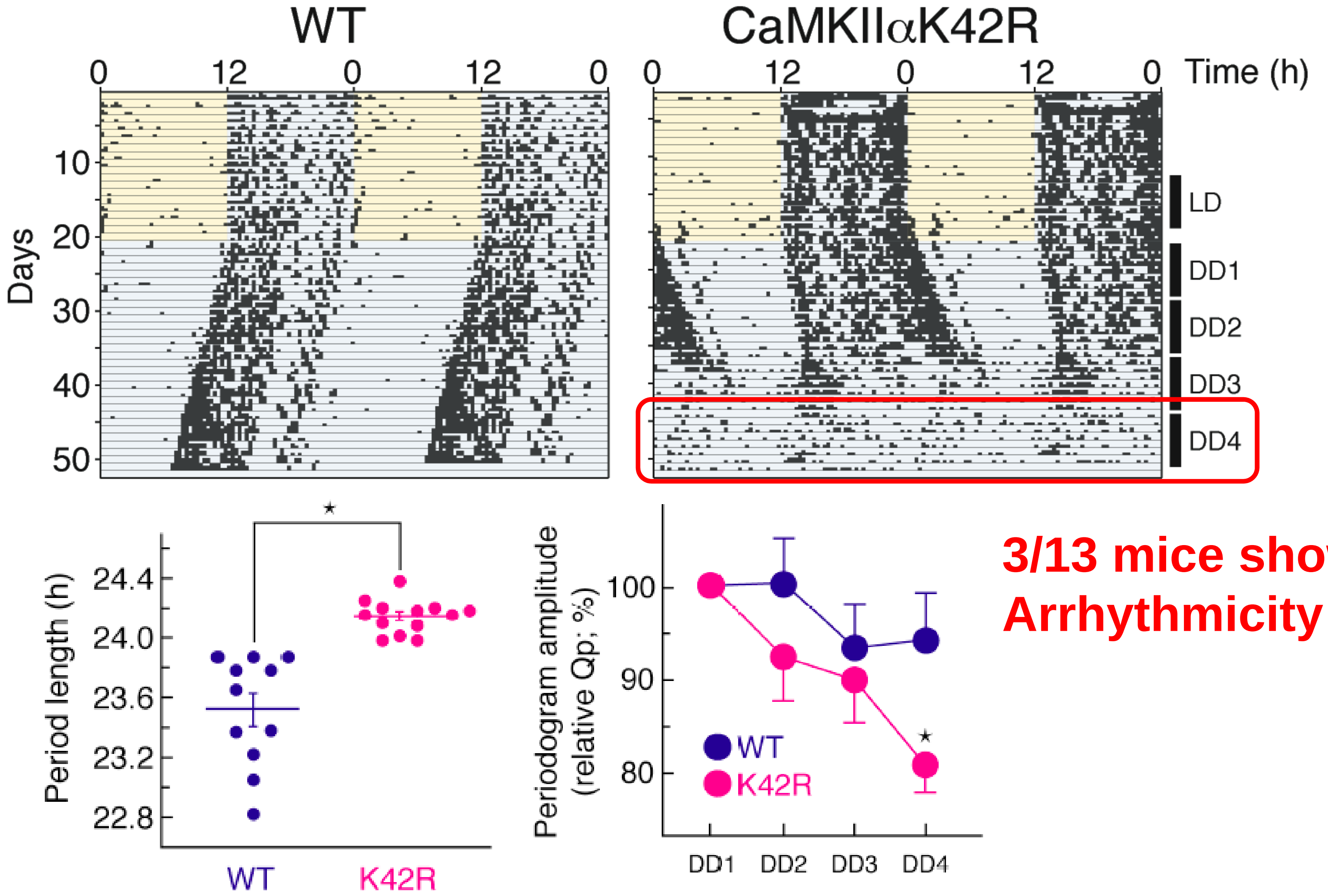
Table 2. Relative CaMKII kinase activity in brain homogenates from homozygous and heterozygous CaMKIIα (K42R) mice compared with wild-type mice

	Forebrain (% of WT)		Cerebellum (% of WT)	
	K42R	K42R(+/−)	K42R	K42R(+/−)
Total activity	41.0 ± 1.6	75.9 ± 2.9	106.5 ± 8.0	104.8 ± 6.9
Autonomous activity	31.9 ± 2.7	75.6 ± 4.1	96.6 ± 17.3	105.2 ± 14.7

CaMKII kinase activity in mutant mice shown in Figure 2C is expressed as a percentage of that in wild-type (WT) mice in the same preparation group ($n = 5$).

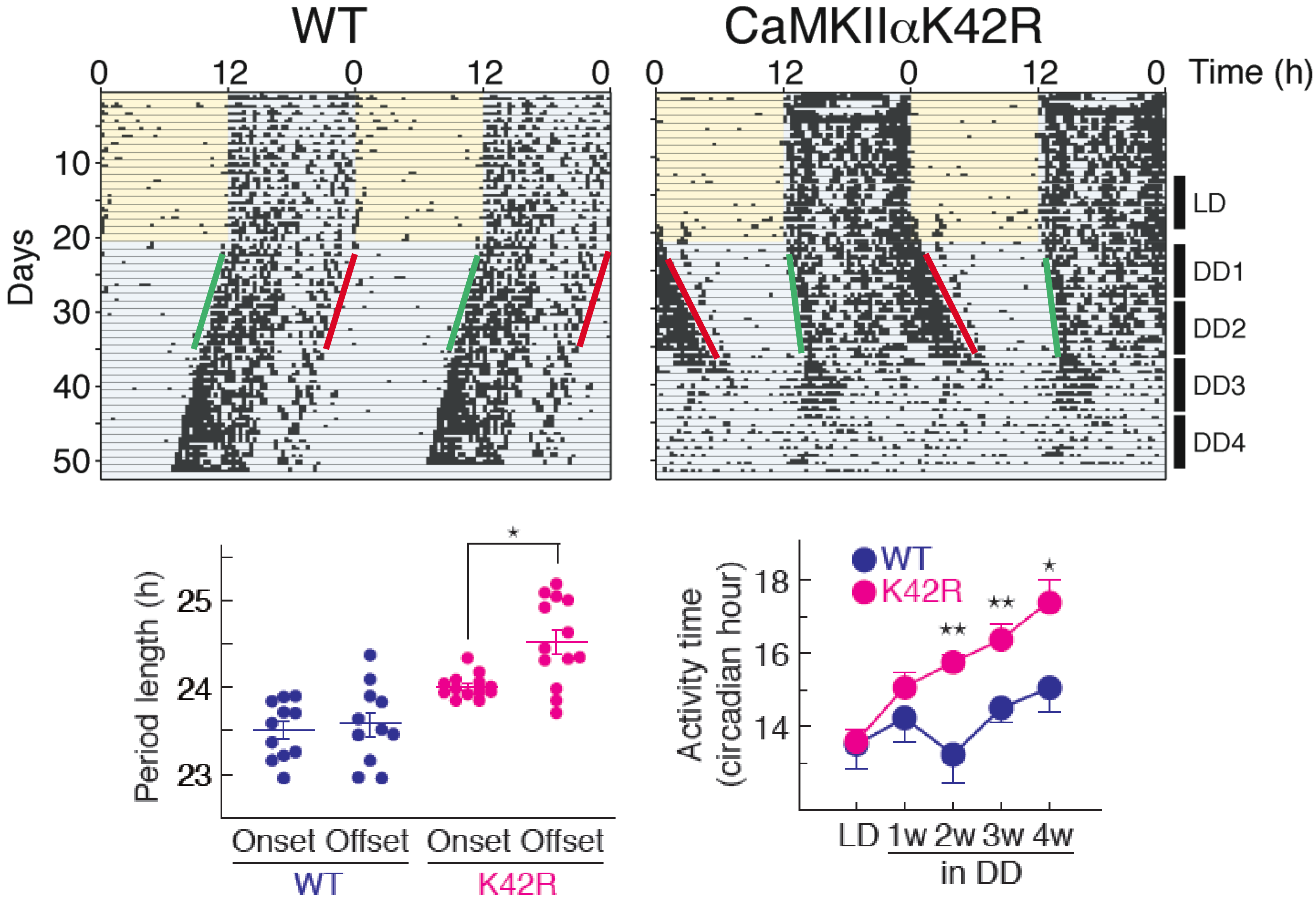
Yamagata *et al*., *J. Neurosci.* 29, 7607 (2009)

CaMKIIα mutants show long period and temporal decrease in periodogram amplitude

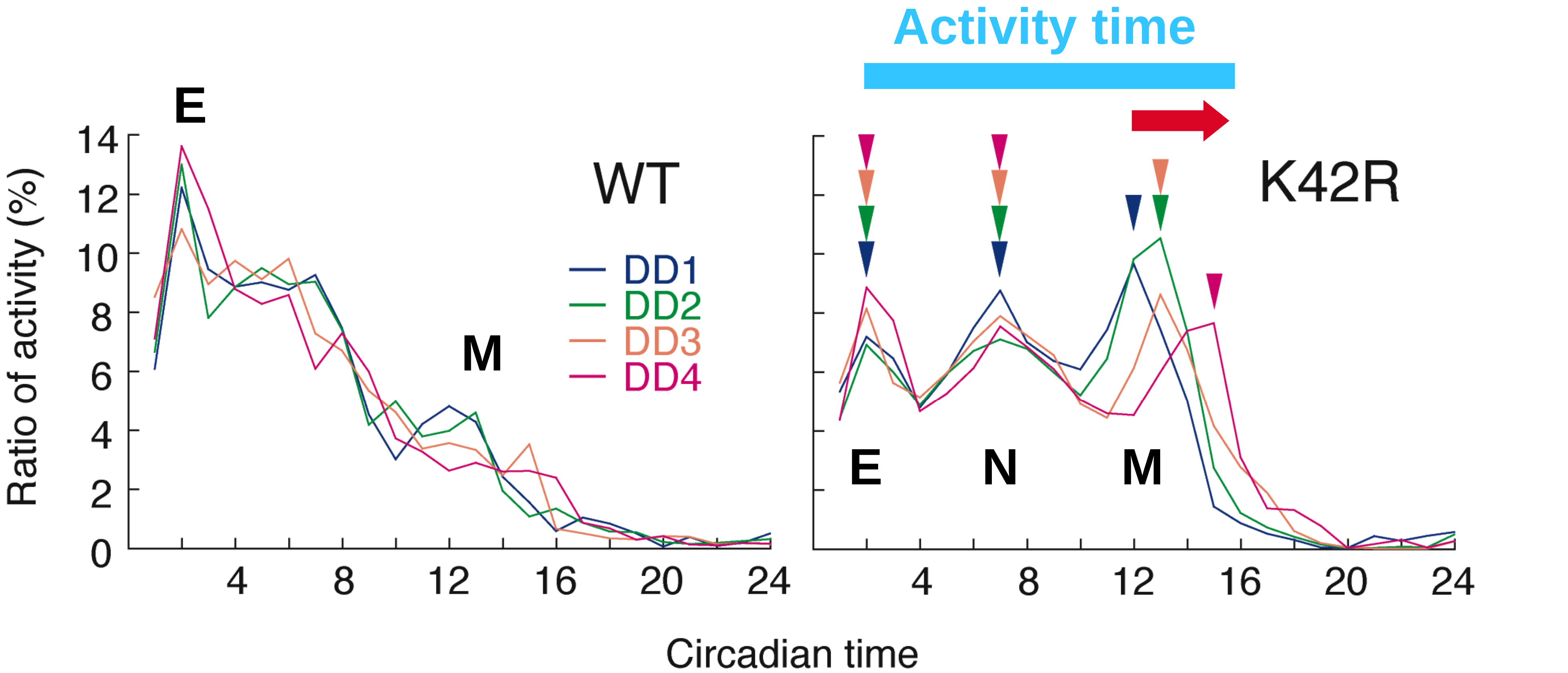


3/13 mice show Arrhythmicity

CaMKIIα mutants show decoupling of onset and offset activity rhythms, resulting in prolongation of activity time.



Temporally delaying morning activity prolongs activity time in CaMKIIαK42R mice

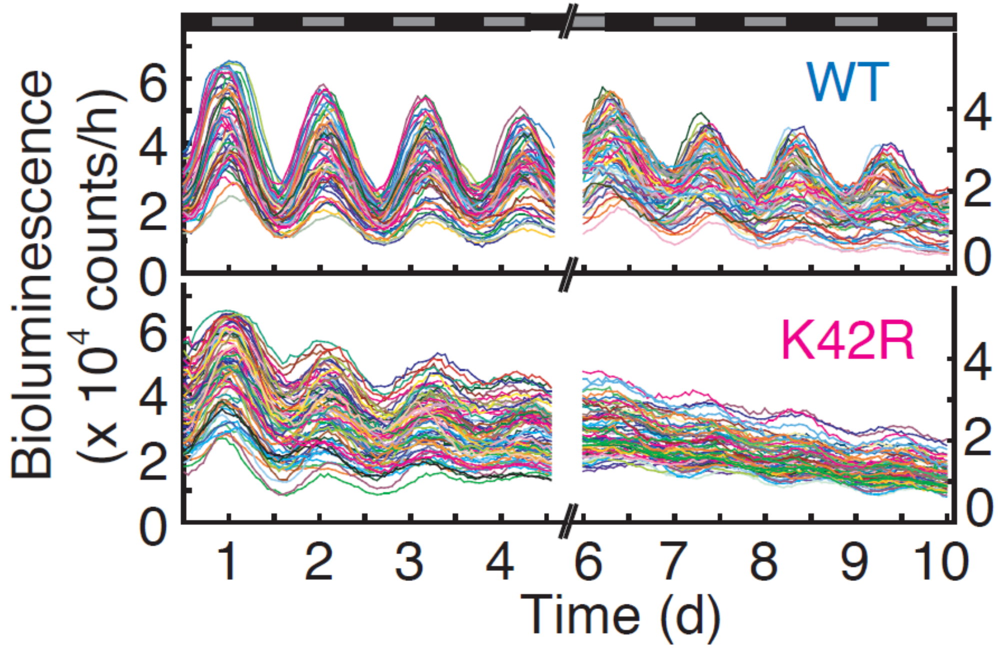


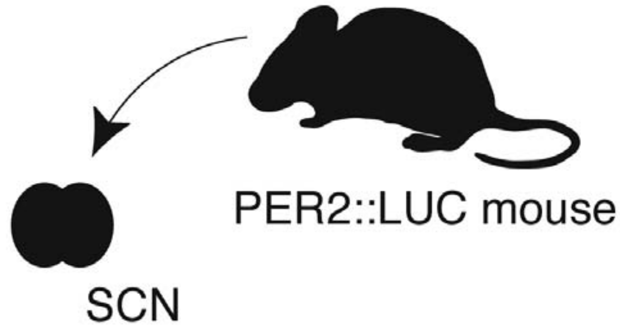
E; evening peak N; night peak M; morning peak

CaMKII mediates coupling of morning and evening rhythms

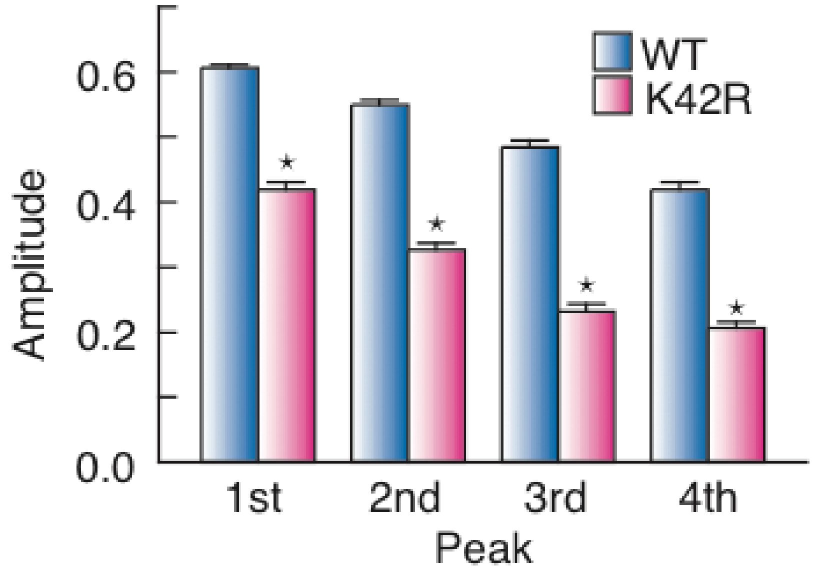
Attenuated amplitude of individual cellular oscillation in the SCN of CaMKIIαK42R mice

Single neuronal rhythms of SCN



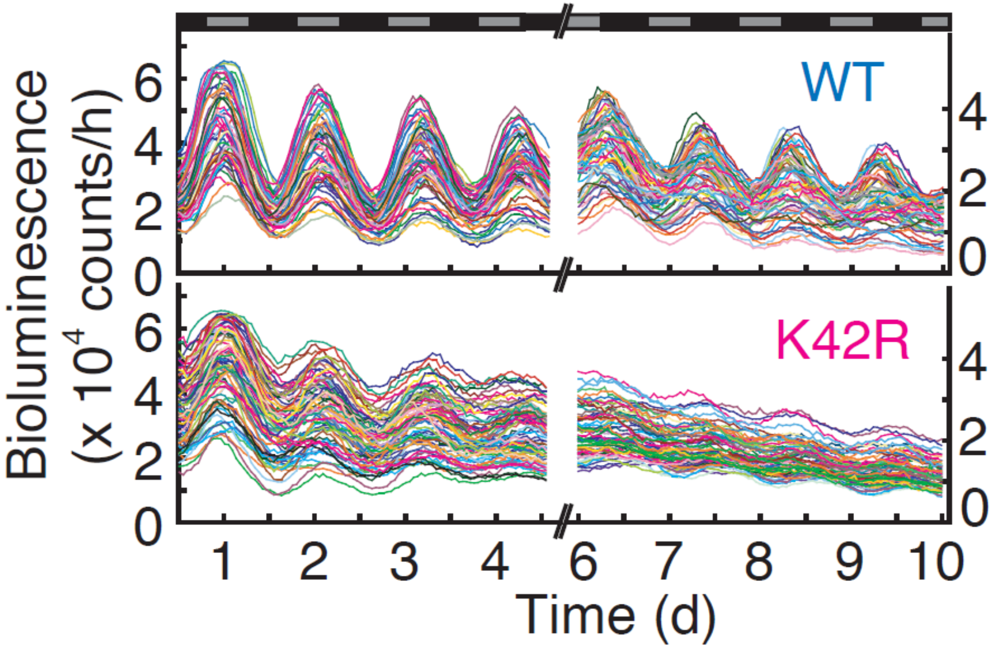


Amplitude of individual rhythms

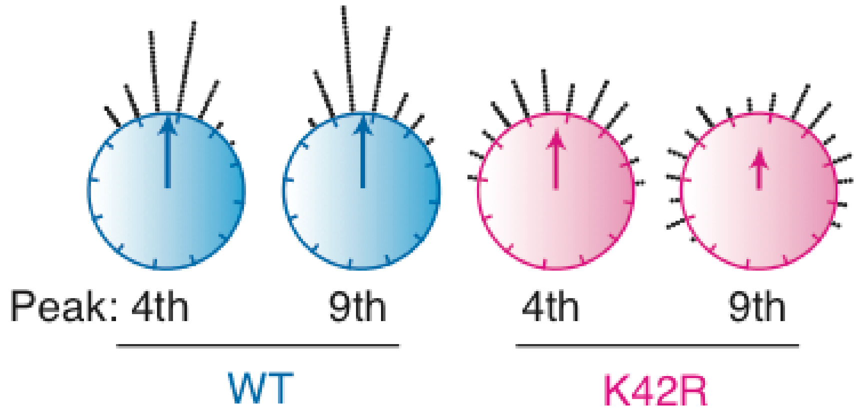


Desynchronization of individual cellular oscillation in the SCN of CaMKIIαK42R mice

Single neuronal rhythms of SCN

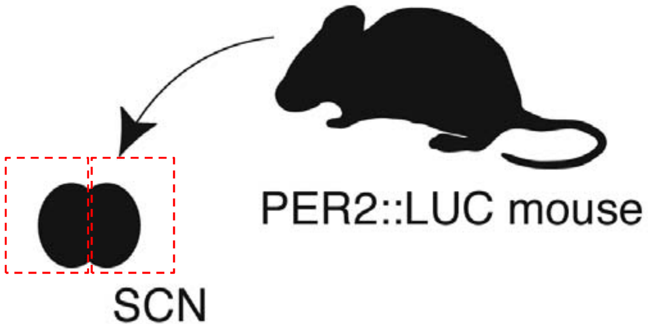


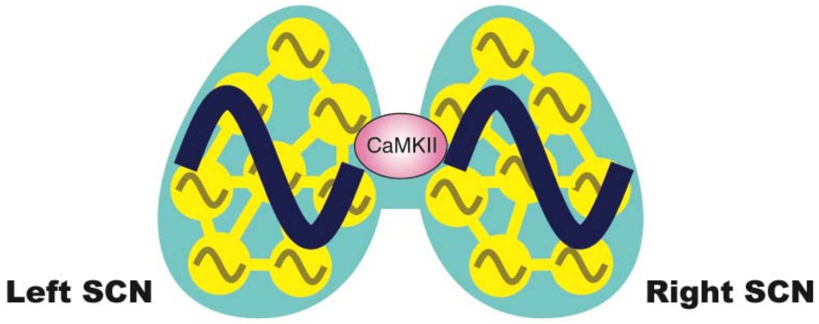
Phase plotting of individual rhythms

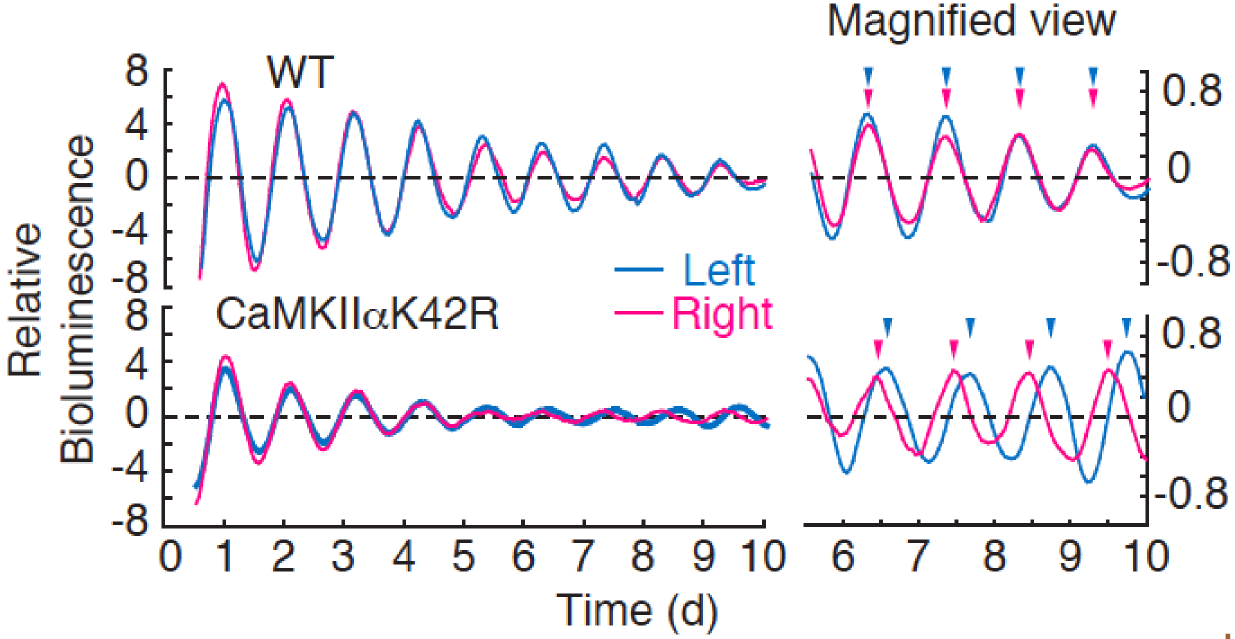


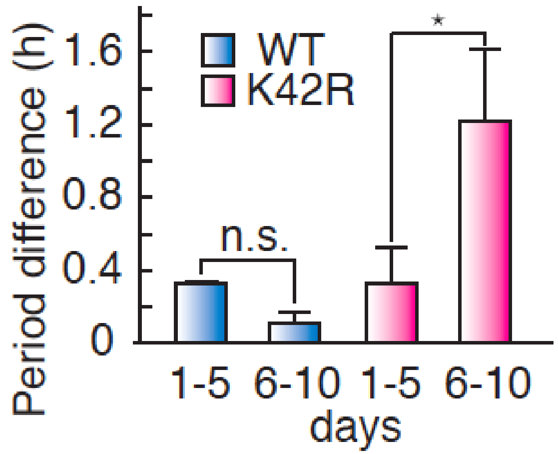
Decoupling of oscillations between left and right SCN in CaMKIIαK42R mice

Bioluminescence from left or right SCN nuclei





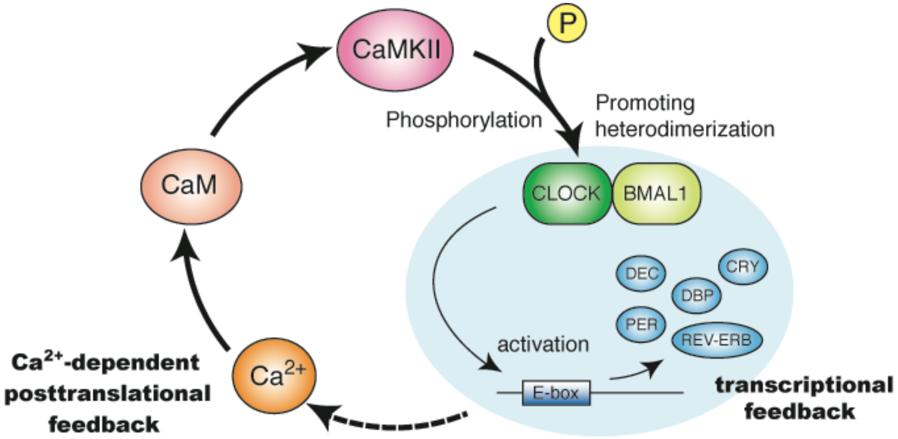




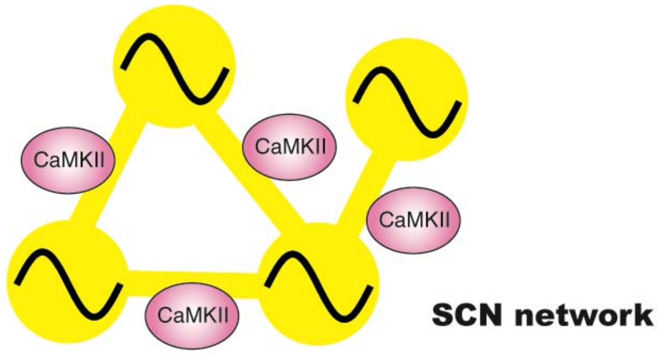
Kon *et al*., *Genes and Development*, 2014.

CaMKII regulates circadian clock at multiple levels

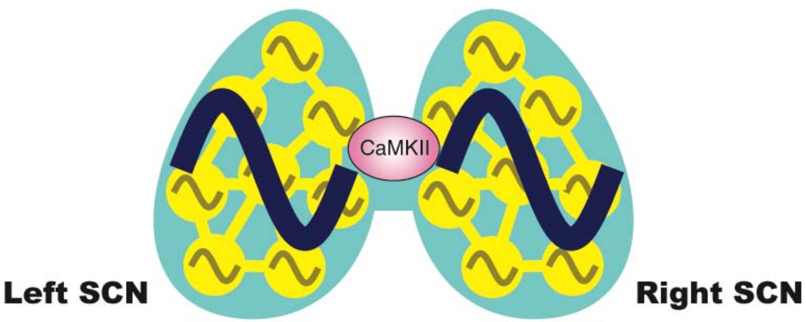
Cellular Oscillation



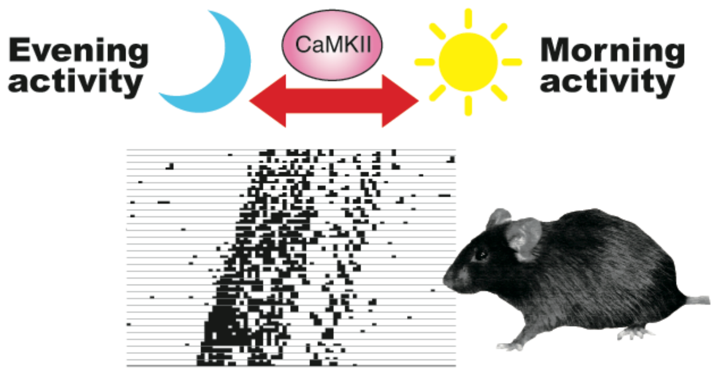
SCN Network



Left-Right SCN coupling



Evening-Morning behavioral Coupling



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