

생체리듬과 정서조절 메카니즘



김 경 진

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대구경북과학기술원(DGIST) 뇌인지과학전공**

SpatioTemporal Dimension

Internet



GPS



Smart phone



Space

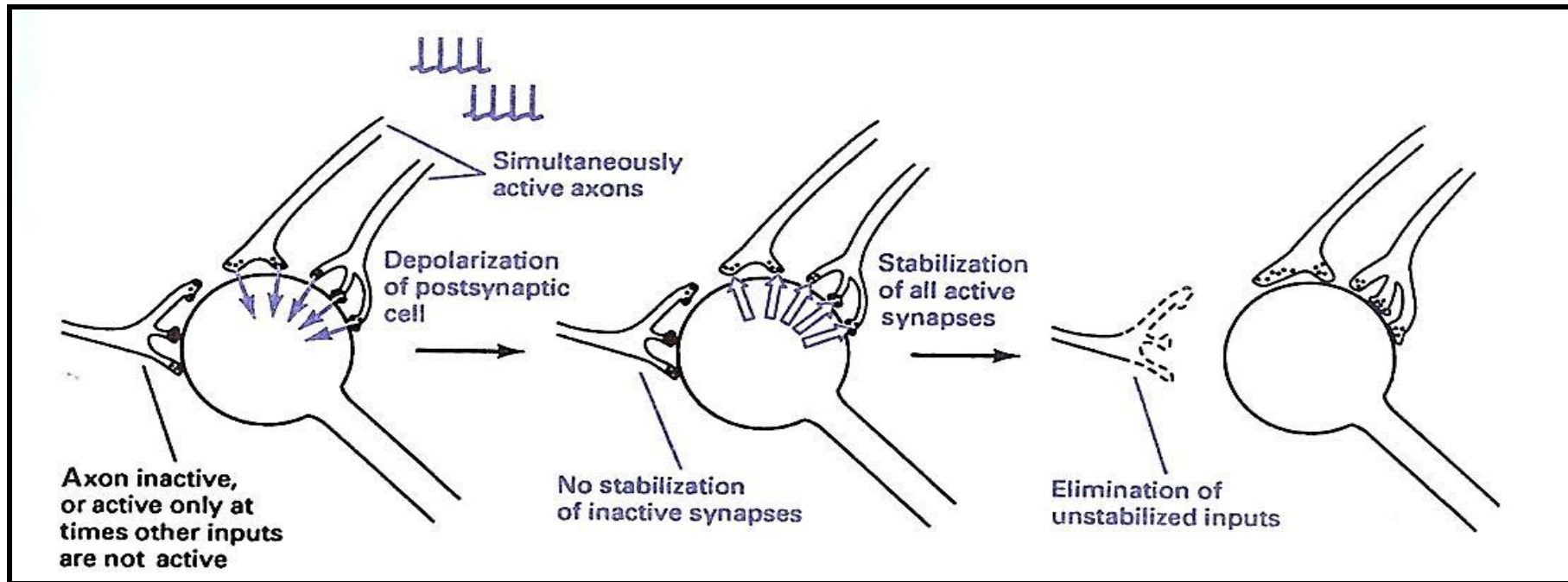
Time

Chronobiology

Fire Together, Wire Together



Donald O. Hebb



■ 신경세포의 동시적 활성화 ➔ 신경망의 구조적 · 기능적 변화 (시냅스 가소성)

Synaptic Plasticity

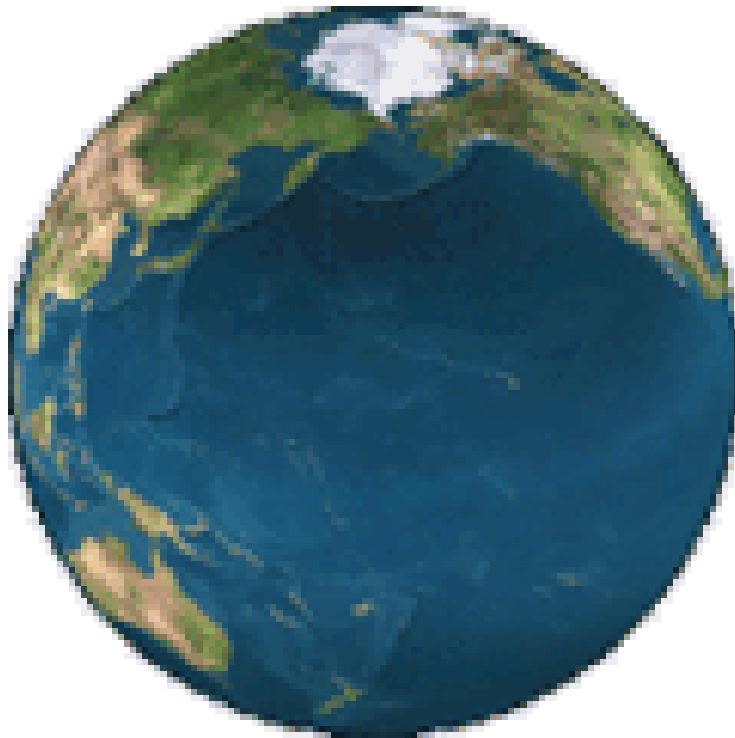


Basic : Circadian Rhythm / Molecular Clock



Mood Regulation Links to Circadian Timing System

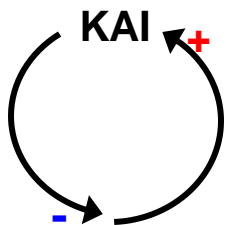




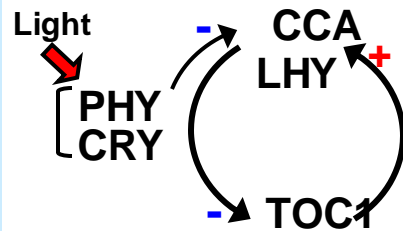
Time-keeping Genetic Device in the Genome

Evolution of molecular clock

Bacteria

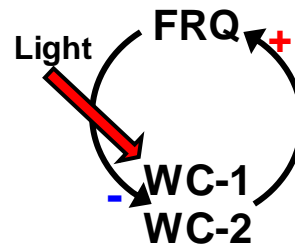


Plants



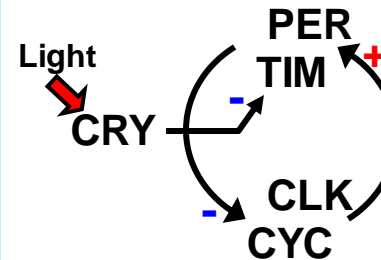
PHY: phytochrome
CRY: cryptochrome
CCA: circadian clock associated
LHY: late elongated hypocotyl
TOC1: timing of cap expression 1

Fungi



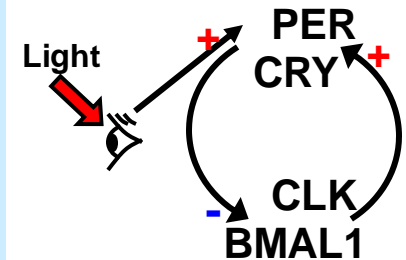
FRQ: frequency
WC: white collar

Invertebrate animals



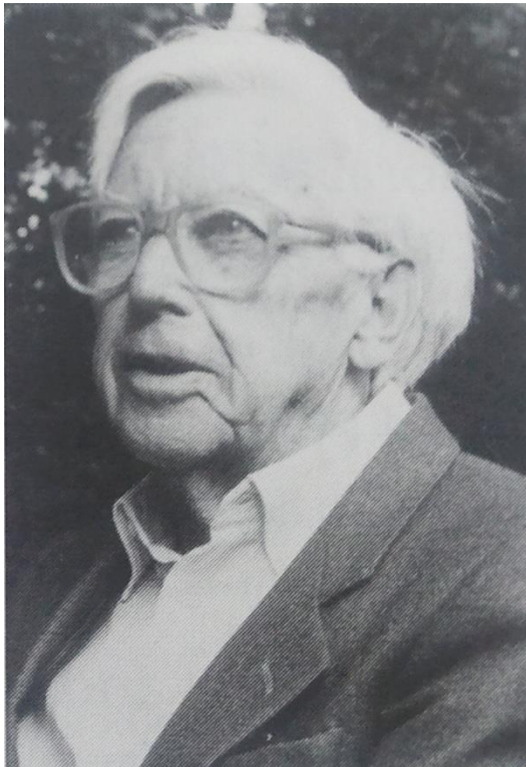
PER: period
CRY: cryptochrome
TIM: timeless
CLK: clock
CYC: cyclin

Vertebrate animals

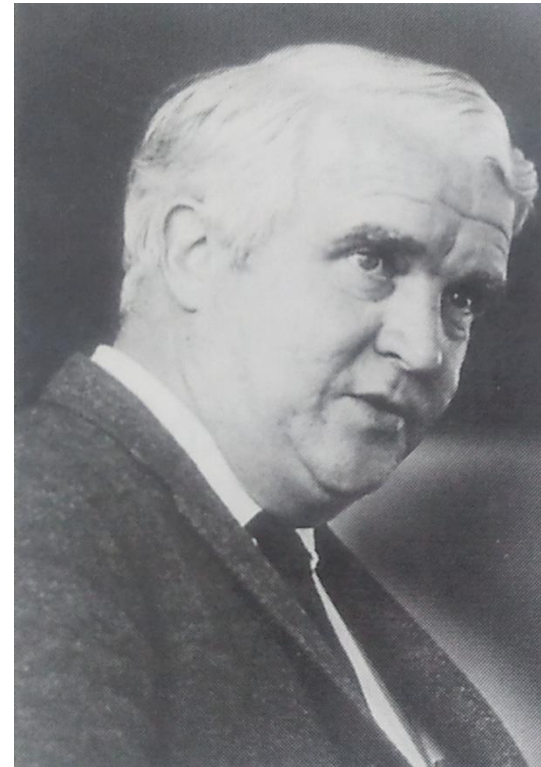


PER: period
CRY: cryptochrome
CLK: clock
BMAL1: brain muscle ARNT like 1

Founders of Chronobiology



Juergen Aschoff [1913–1998]
(MPI for Behavioral Physiology)



Colin S. Pittendrigh [1919–1996]
(Stanford University)

Discovery of *Period* mutants in *Drosophila*



Seymour Benzer
(1921~2007)



Ronald J. Konopka

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

Clock Mutants of *Drosophila melanogaster*

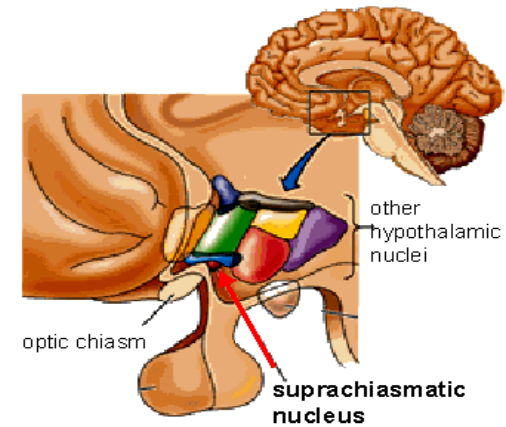
(eclosion/circadian/rhythms/X chromosome)

RONALD J. KONOPKA AND SEYMOUR BENZER

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

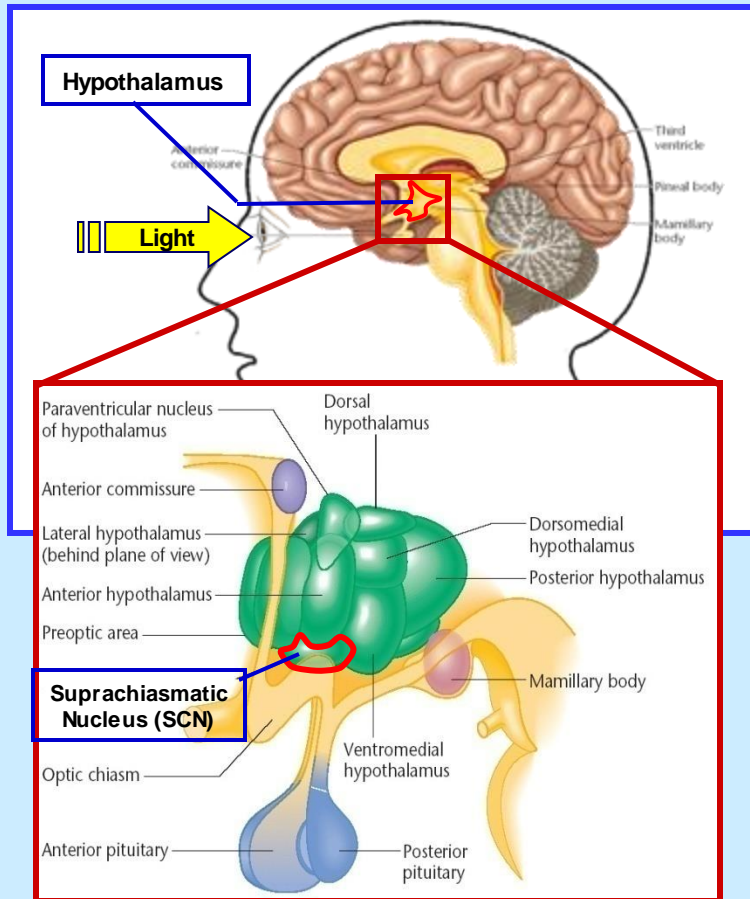
Properties of circadian clock

- What is circadian rhythm?
 - *circa*: approximately
 - *dies*: day
 - Biological rhythms with a period of about 24hrs.
- Properties
 - In mammals, **suprachiasmatic nucleus (SCN)** serves as the master circadian clock.
 - Rhythmicity is **endogenous** and self-sustaining.
 - Rhythmic environmental cues such as **light-dark** reset the phase to entrain the rhythm to exactly 24 hours.
 - Period length under constant conditions is **genetically** determined.
 - Oscillation activity can be measured at the level of **a single cell**.

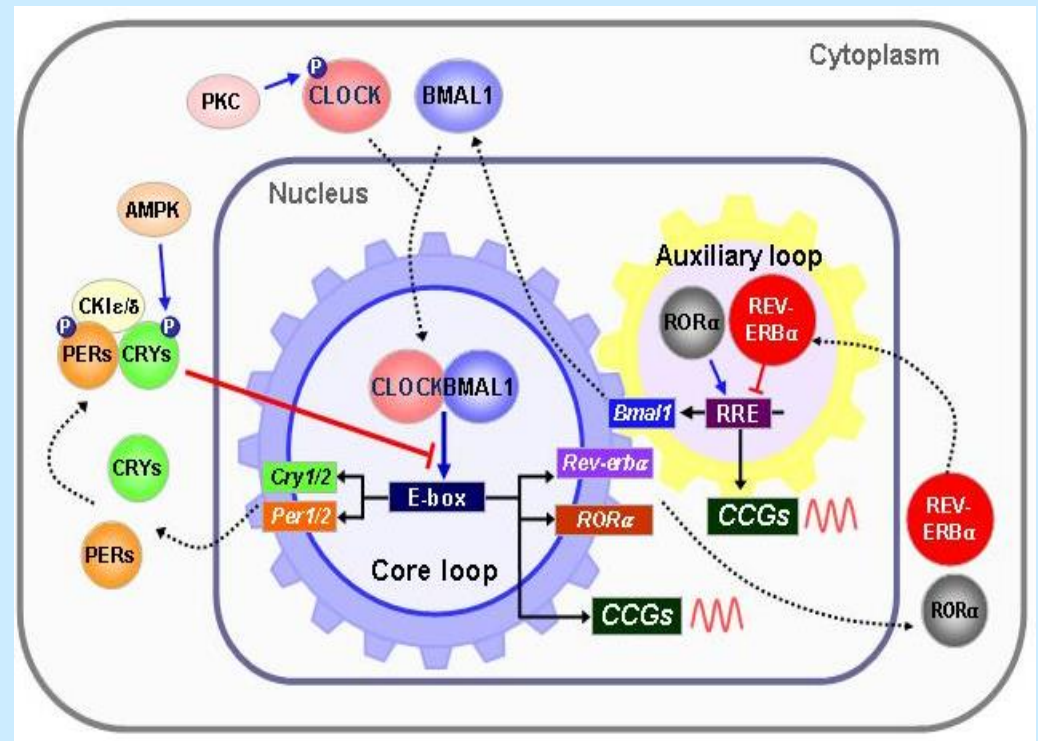


Mammalian molecular circadian clock

■ SCN



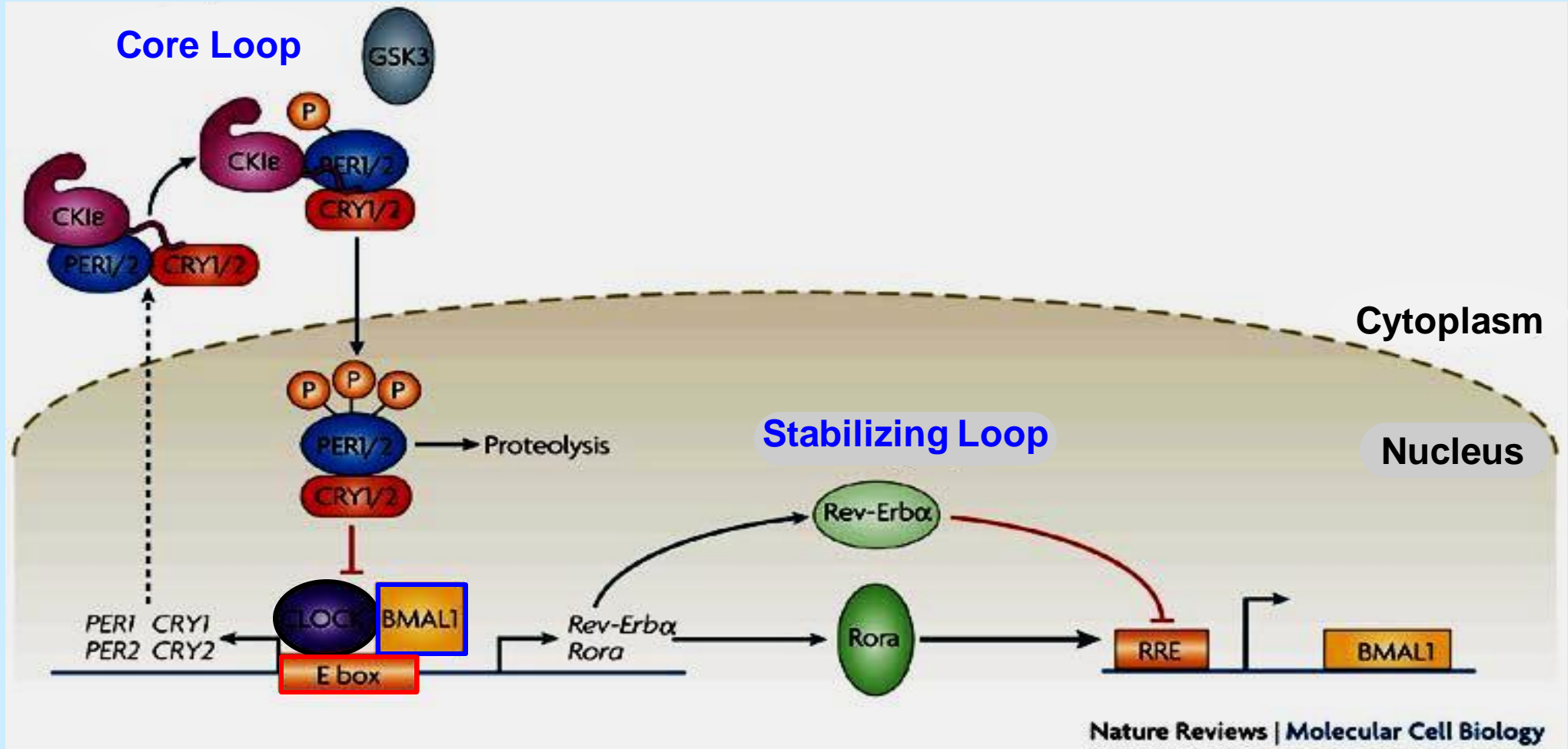
■ Molecular clockworks



Transcription-translational feedback loop

Son et al., 2011. Front Neuroendocrinol 32:451

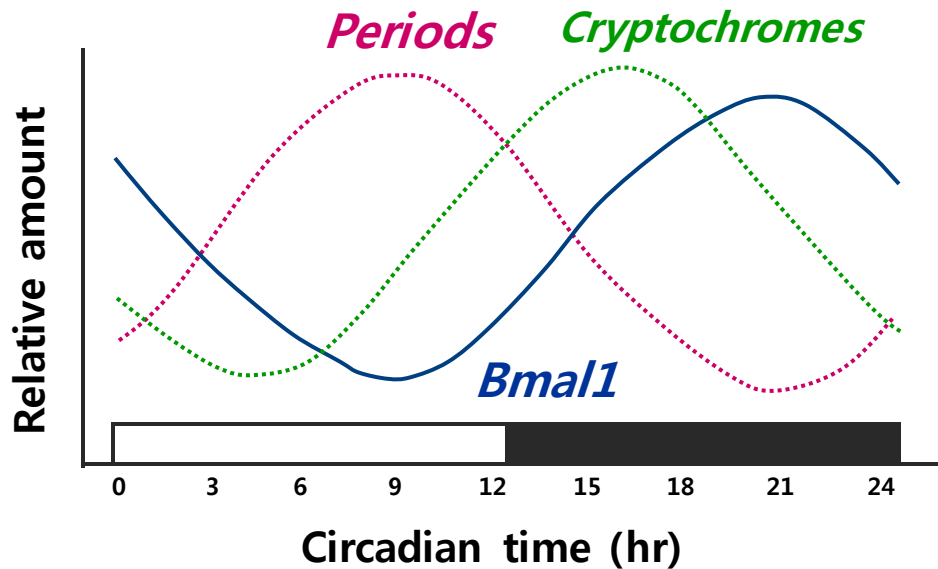
Mammalian molecular circadian clock



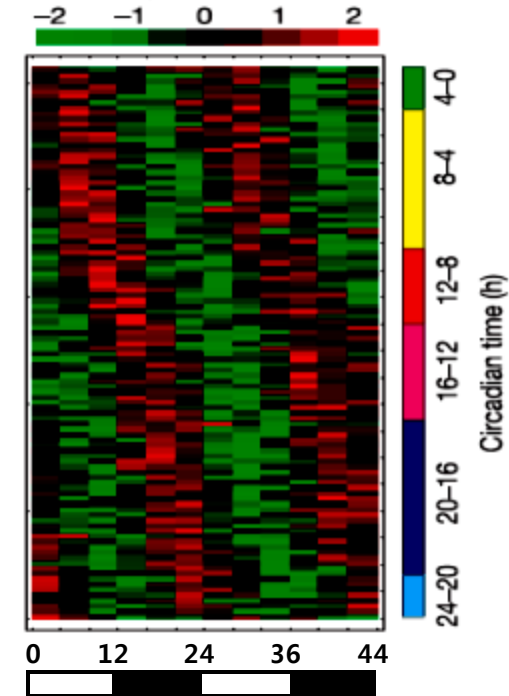
(Gallego and Virshup, 2007, Nat Rev Mol Cell Biol)

Molecular clock gene profiles

■ Mammalian molecular clock



■ Circadian gene expression - Microarray data of SCN

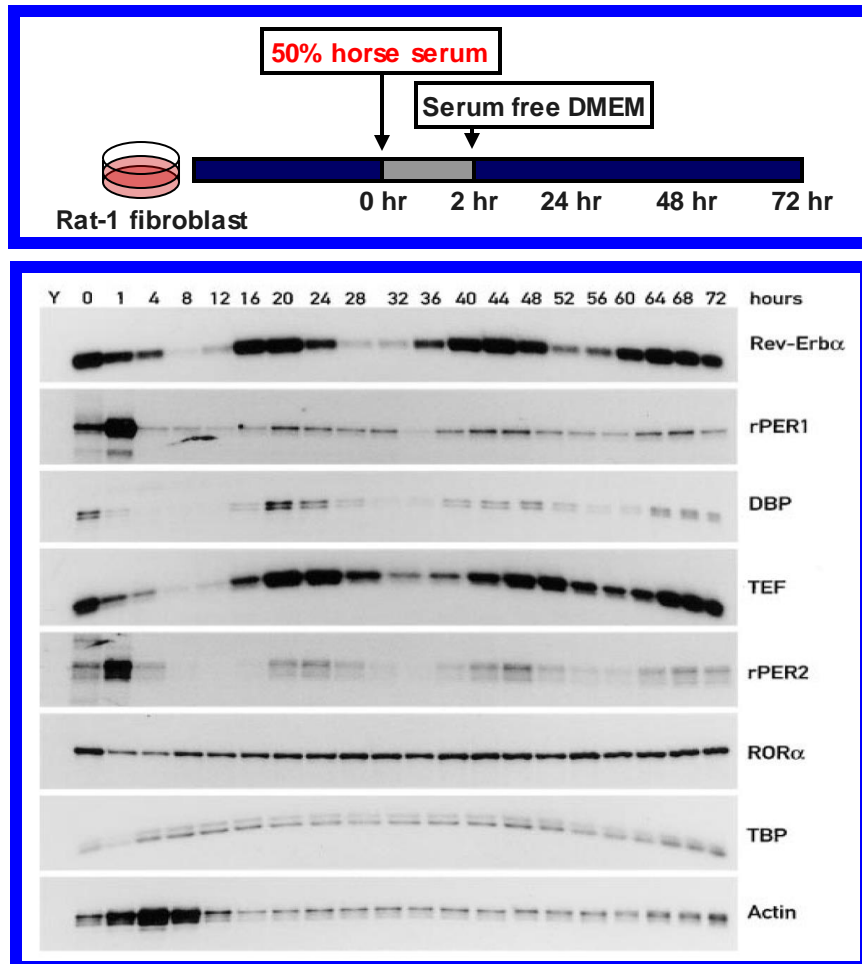


Ueda et al., 2003, Nature

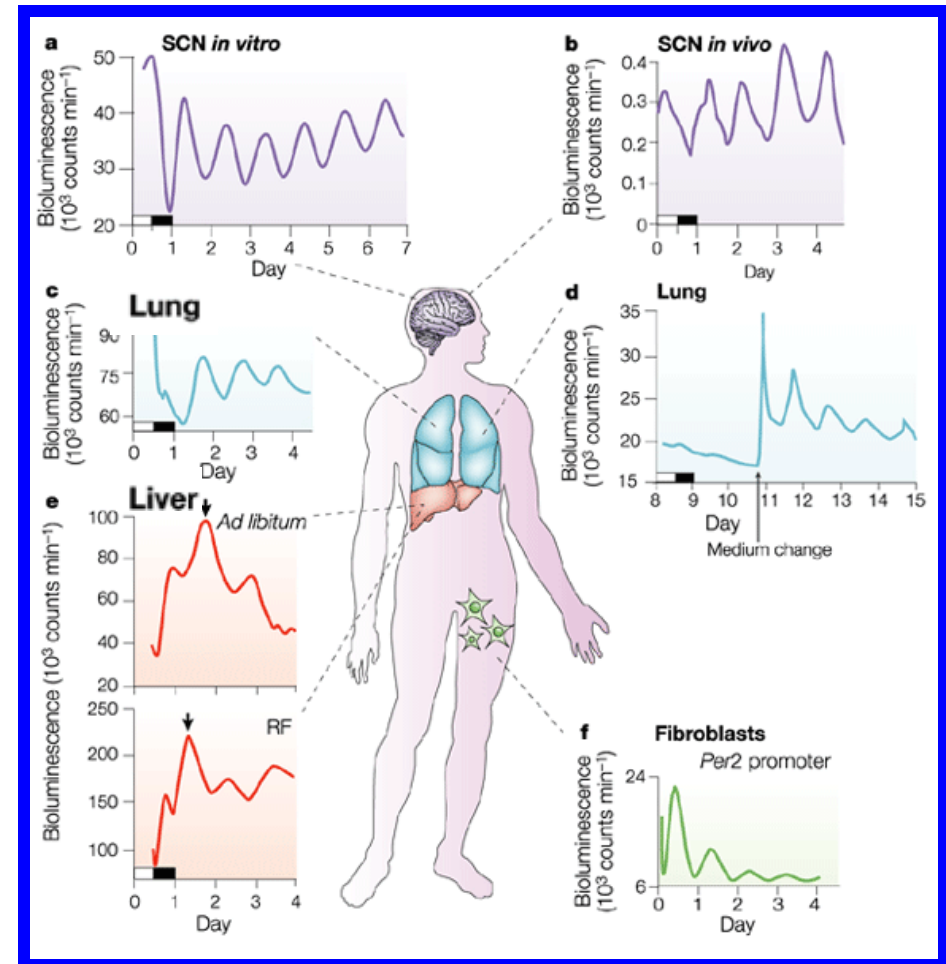
Clock-controlled genes (CCG)

- 5~30% of transcripts show daily rhythms

Peripheral circadian clocks

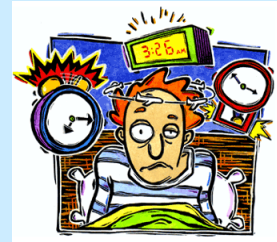
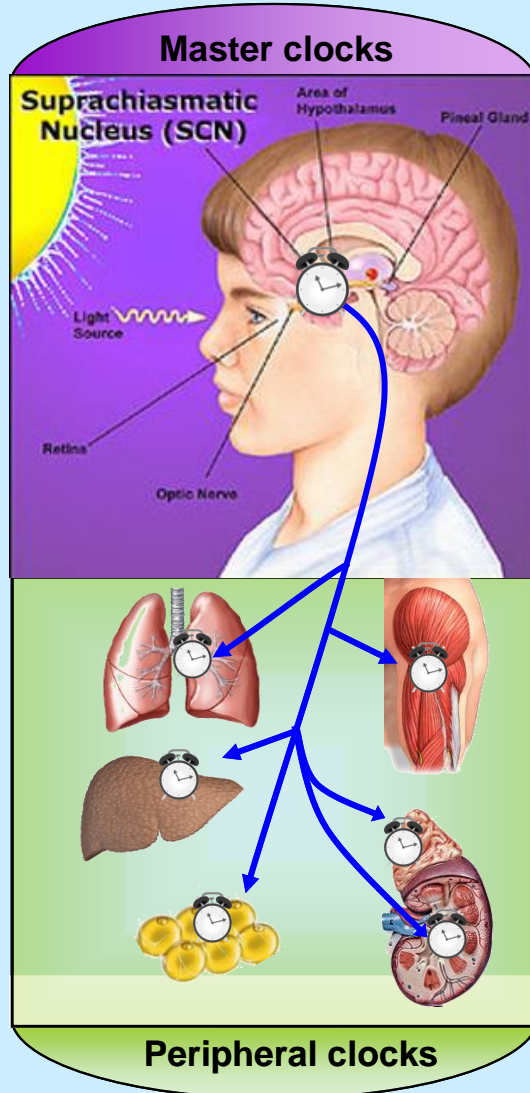


(Balsalobre et al., Cell, 1998)

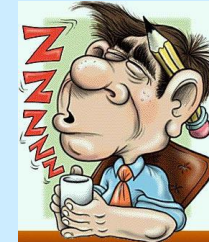


(Hasting et al., Nat. Neurosci. Rev., 2003)

Circadian clock-related disorders



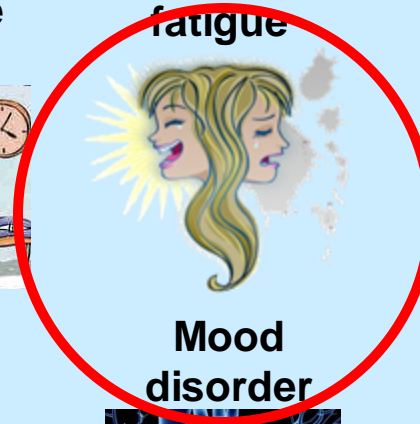
Jet-lag Syndrome



Shift work fatigue



Sleep disorder



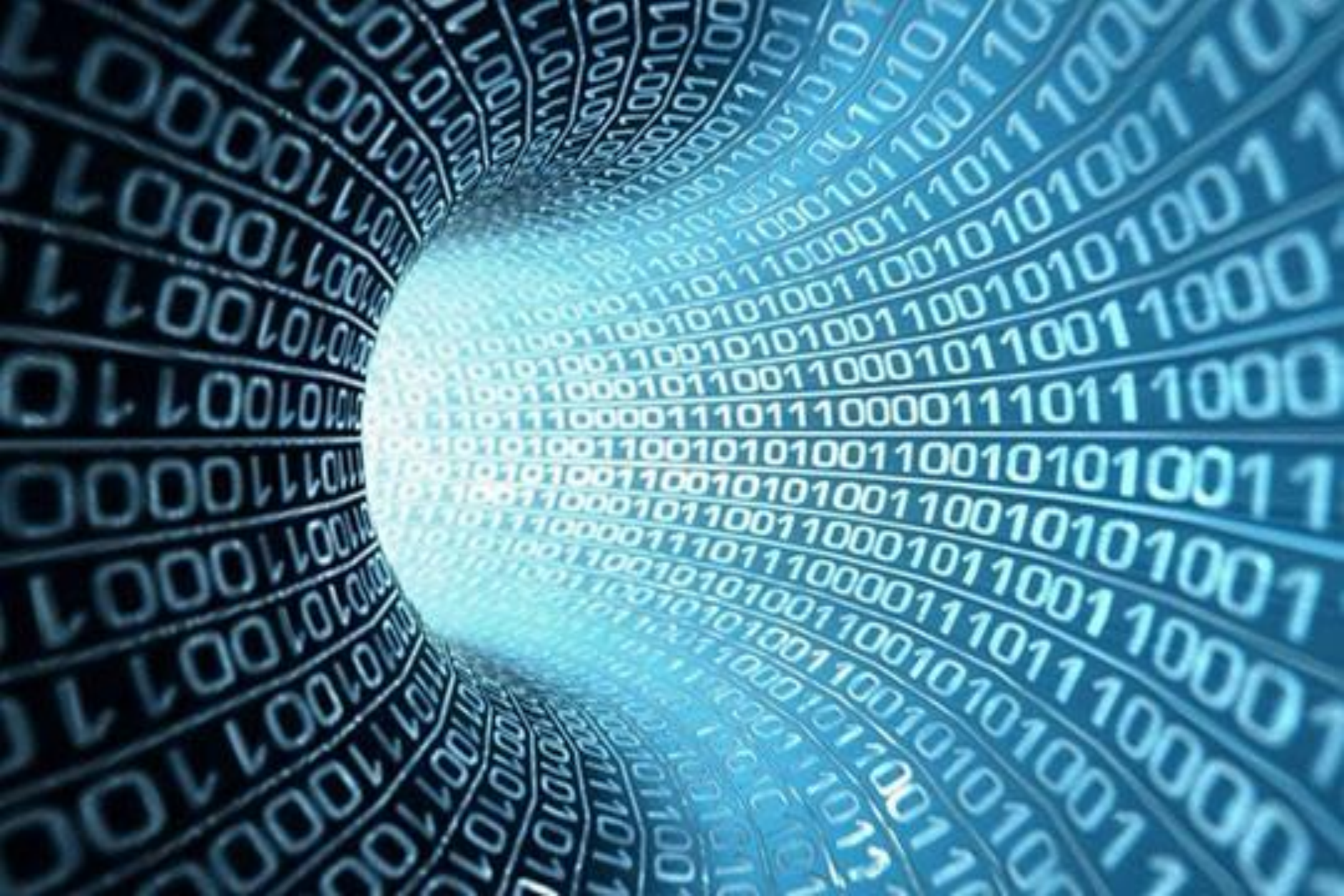
Mood disorder



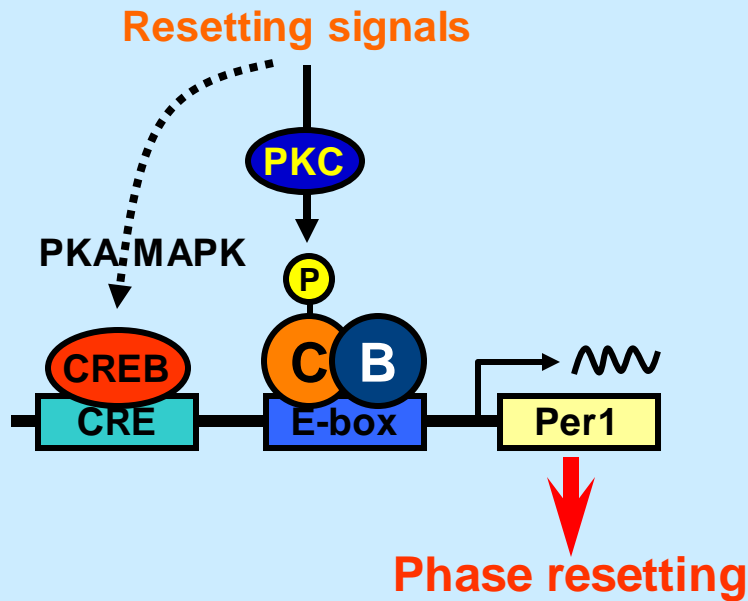
Metabolic syndrome



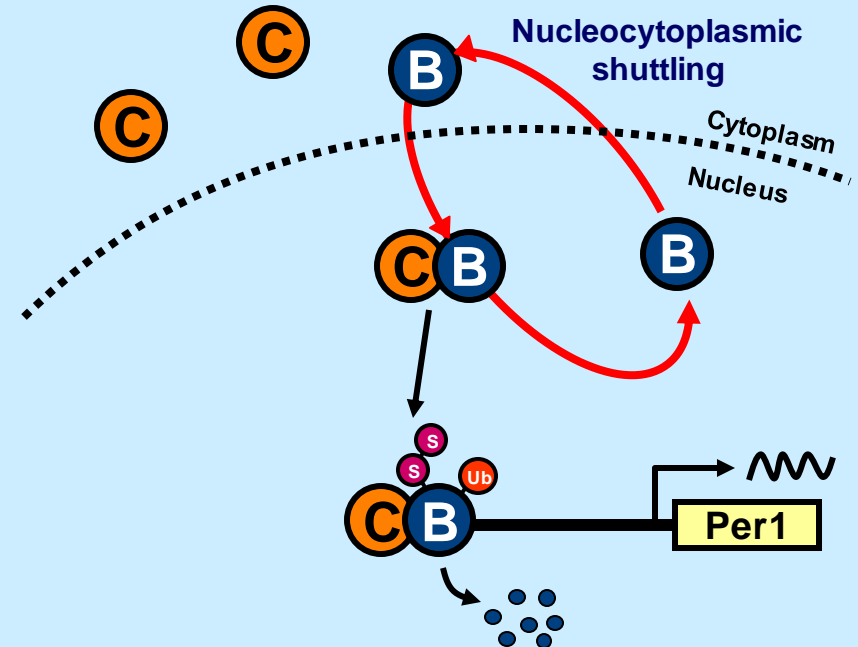
Cardiovascular disease



BioClock : C:B Heterodimers



Jung et al. (2003) Neuroreport 14:15
Shim et al. (2007) EMBO Rep 8:366
Lee et al. (2010) J Cell Sci 123:3547

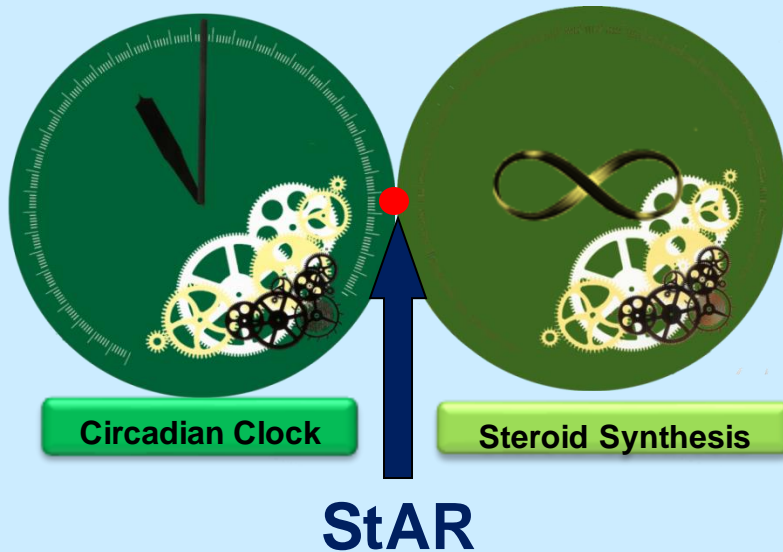


Transactivation
& Degradation (Sumoylation / Ubiquitination)

Kwon et al. (2006) Mol Cell Biol 26:7318
Lee et al. (2008) Mol Cell Biol 28:6056

■ Circadian rhythm of glucocorticoid (GC)

- StAR: Link b/w glucocorticoid synthesis in adrenal gland and circadian rhythm



PNAS (2008) 105:20970

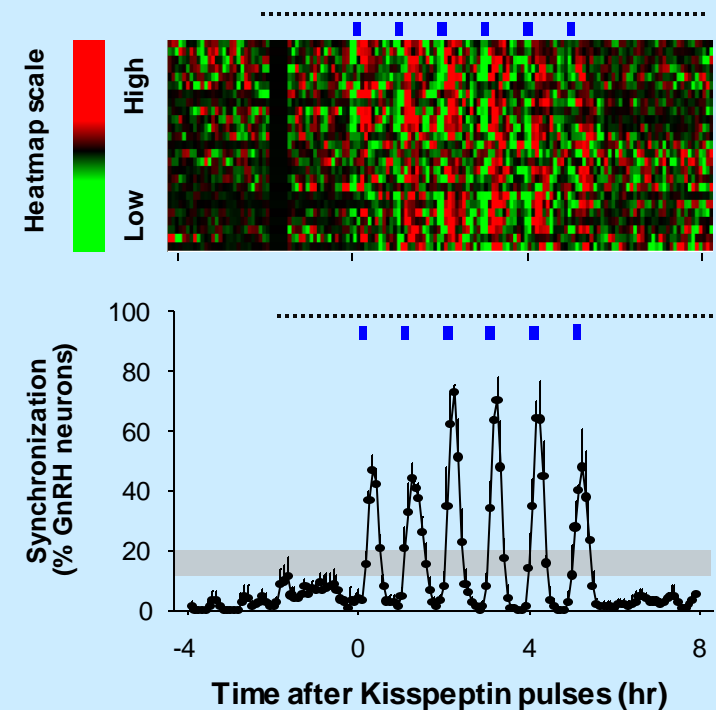
BBA Rev (2011) 1812:581

Front Neuroendocrinol (2011) 32:451

- GC : Synchronizer of peripheral clock, Per2

Nucleic Acid Res (2013) 41:6161

■ Ultradian rhythm : GnRH pulse generator



PNAS (2013) 110:5677

■ Identification of Cry inhibitors by cell-based assay

ACS Chem Biol (2014) 9(3): 703



Basic : Circadian Rhythm / Molecular Clock



Mood Regulation Links to Circadian Timing System



정신질환(Mental Illness)

“조승희, 영혼에 구멍이 나...
폭력적 충동 가뒤흔 뚜껑 열린 것”



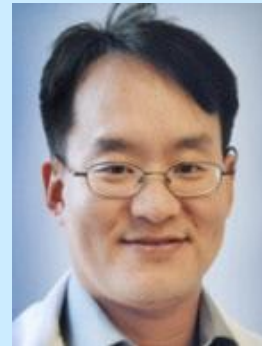
후안 메시취 회장
(세계정신의학회)



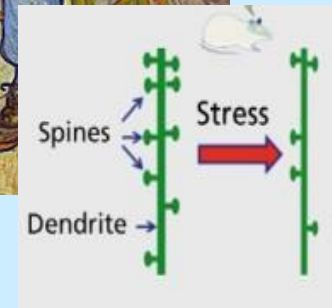
Psychopath

- 2007. 4. 16
미 버지니아 공대 총기난사사건
- 조선 인터뷰 기사 2007. 4. 22

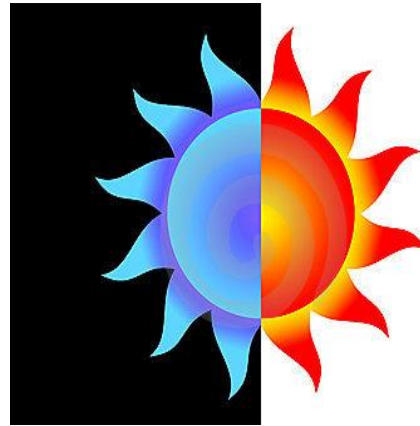
우울해서 한잔,
슬개면 더 우울 ‘도파민의 덫’



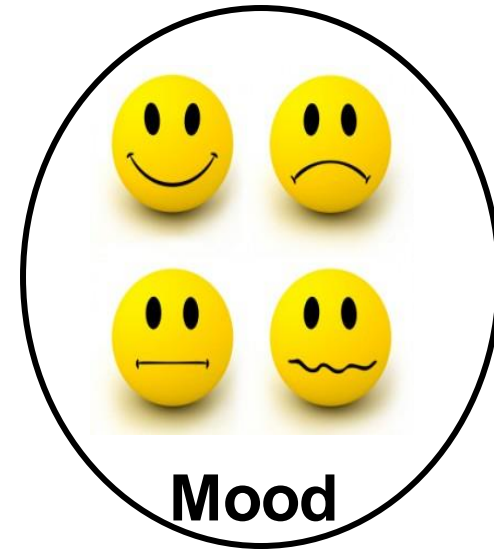
구자욱 박사
(한국뇌연구원)



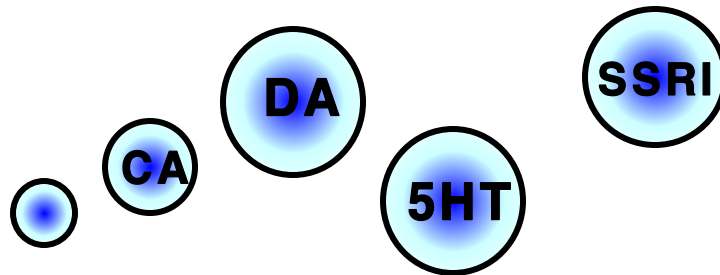
- 우울증과 알코올 중독
- 뇌의 비밀(경향신문 기사) 2016. 12. 22



Circadian rhythm



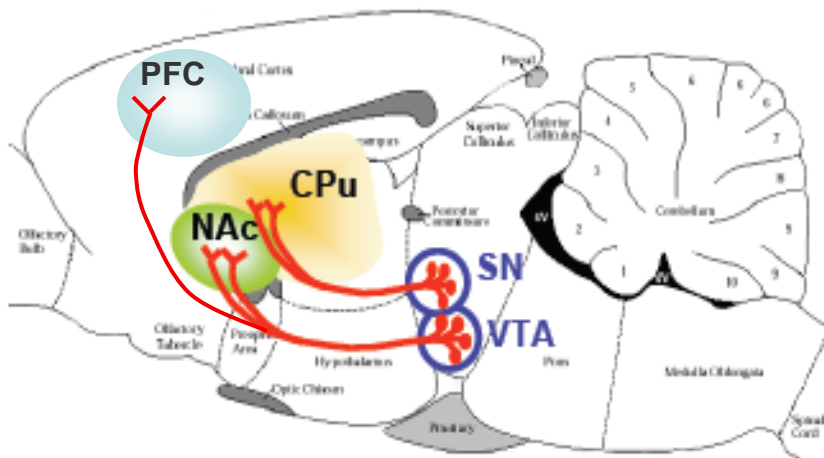
Mood



- Many neurotransmitters involved in mood regulation
- Neural circuits of mood regulation is complex
- Circadian features of affective disorders

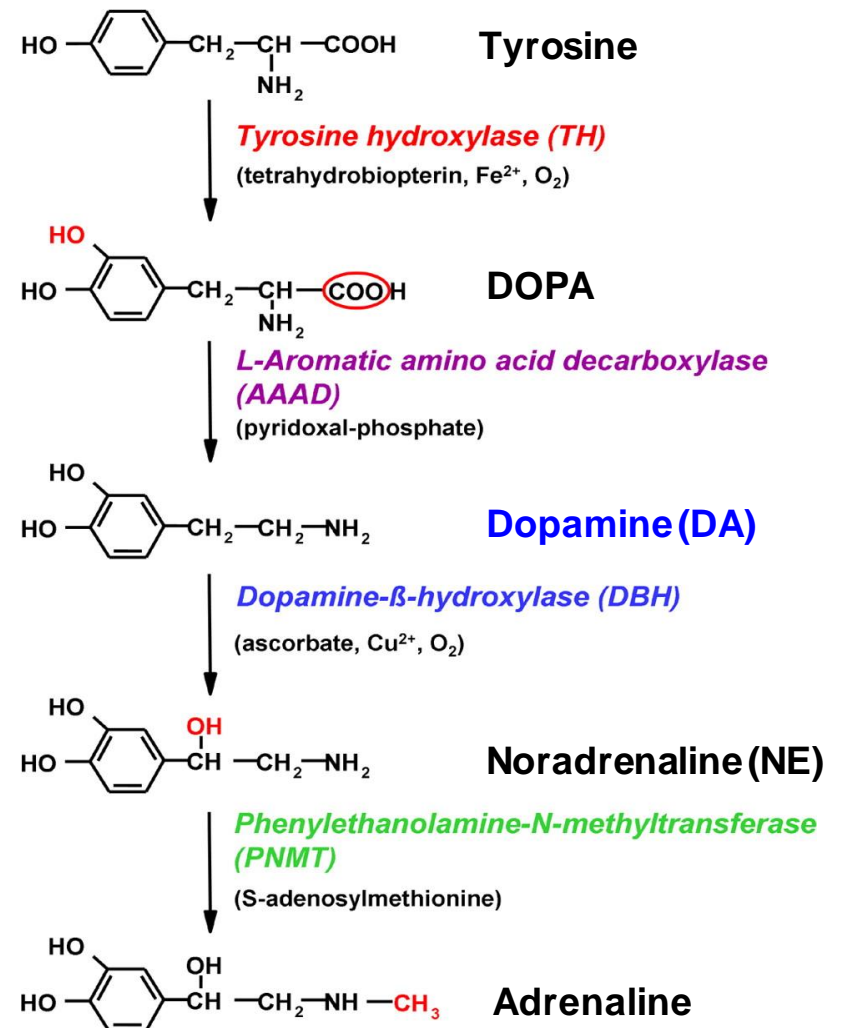
Circadian rhythm and midbrain DAergic system

■ Midbrain DAergic system



- ▶ **Nigrostriatal pathway**
: Controlling motor behaviors
- ▶ **Mesolimbic pathway**
: Feelings of reward and desire
- ▶ **Mesocortical pathway**
: Emotional responses and motivation

■ Biosynthetic pathway of CA



Circadian rhythm and mood disorders

■ Disruption of Clock genes ➡ Behavioral abnormalities

■ Clock $\Delta 19$ mutant mouse

Mania-like behavior induced by disruption of *CLOCK*

Kole Roybal*, David Theobald*, Ami Graham*, Jennifer A. DiNieri†, Scott J. Russo*, Vaishnav Krishnan*, Sumana Chakravarty*, Joseph Peevey*, Nathan Oehrlein‡, Shari Birnbaum*, Martha H. Vitaterna§, Paul Orsulak‡, Joseph S. Takahashi¶, Eric J. Nestler*, William A. Carlezon, Jr.†, and Colleen A. McClung*||

Roybal et al., 2007. PNAS 104:6406

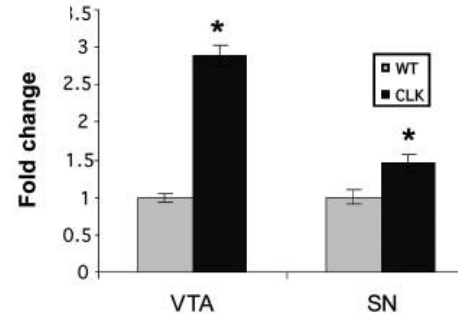
■ KD of Clock gene in VTA of WT mouse

Knockdown of *Clock* in the Ventral Tegmental Area Through RNA Interference Results in a Mixed State of Mania and Depression-Like Behavior

Shibani Mukherjee, Laurent Coque, Jun-Li Cao, Jaswinder Kumar, Sumana Chakravarty, Aroumougame Asaithamby, Ami Graham, Elizabeth Gordon, John F. Enwright III, Ralph J. DiLeone, Shari G. Birnbaum, Donald C. Cooper, and Colleen A. McClung E-mail: colleen.mcclung@utsouthwestern.edu.

Mukherjee et al., 2010. Biol Psychiatry 68:503

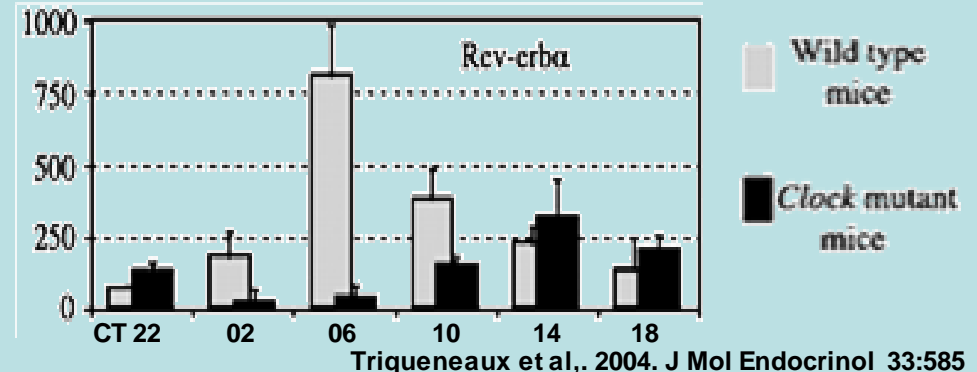
■ Clock ➡ DA neurotransmission



↑TH mRNA in the VTA of Clock $\Delta 19$ mutant mouse

McClung et al., 2005. PNAS 102:9377

■ Clock $\Delta 19$ mutant mouse : ↓ Rev-erba mRNA level



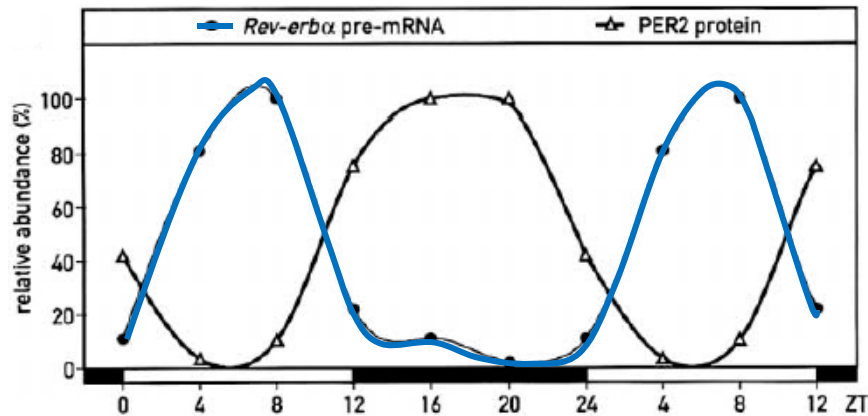
Triqueneaux et al., 2004. J Mol Endocrinol 33:585

Question: Rev-erba may mediate circadian control of DA biosynthesis through transcriptional regulation of TH gene expression.

Rev-erb α (NR1D1)

■ Rev-erb α knockout mouse

- Rev-erb α is a nuclear receptor, which acts mainly as a repressor.



Preitner et al., 2002, Cell 110:251

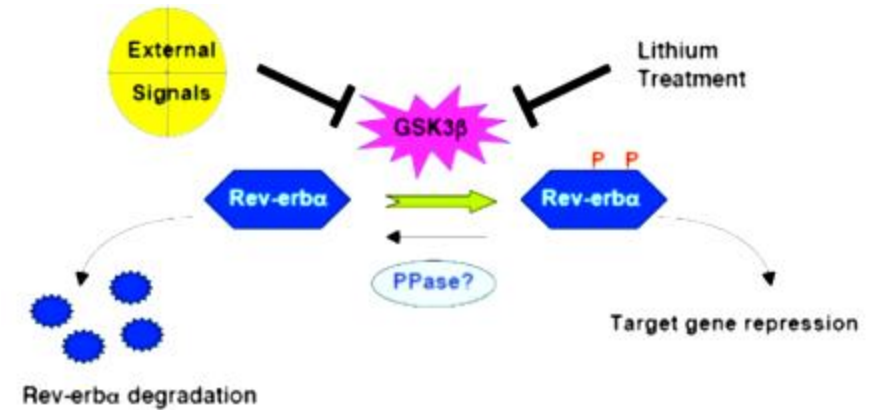
■ Rev-erb α : Integrator of circadian rhythm and metabolism

- Cho H. (2012) Nature 485: 123
- Bass J. (2012) Nature 491: 348

■ Synthetic Rev-erb α drugs

- Kojetin D. et al. (2011) ACS Chem Biol 6: 131
- Solt L.A. et al. (2012) Nature 485: 62

■ Li-responsive clock component

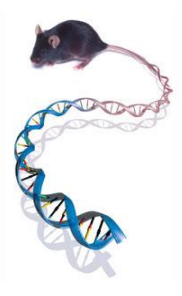


Yin et al., 2006, Science 311:1002

■ Genetic association with mood disorders

Single nucleotide polymorphism in Rev-erb α gene loci in mood disorder patients

- rs939347 + rs12941497 (p=0.040)
(Serverino et al., 2009, Bipolar Disorders 11:215)
- rs2314339 (p=0.0005)
(Kripke et al., 2009, J Circadian Rhythms 7:2)
- rs2071427
(McCarthy et al., 2011, Genes Brain Behav 10:852)



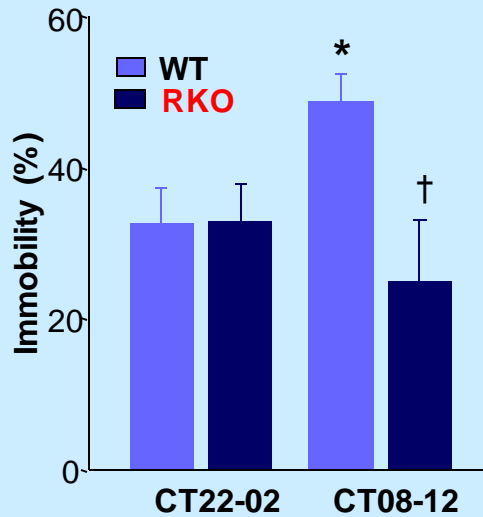
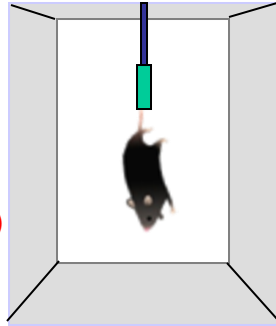
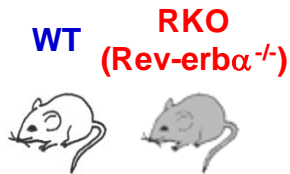
Question:

What is a role of Rev-erb α in mood regulation?

Multimodal Approach: From Gene To Behavior

■ Tail suspension test

Measuring the
immobile time



Behavior

Behavioral

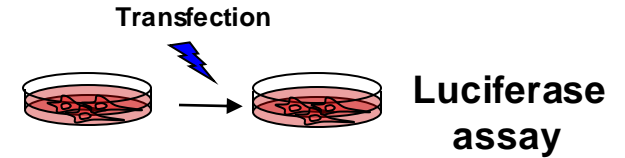
Anatomical

Physiological
/ Pharmacological

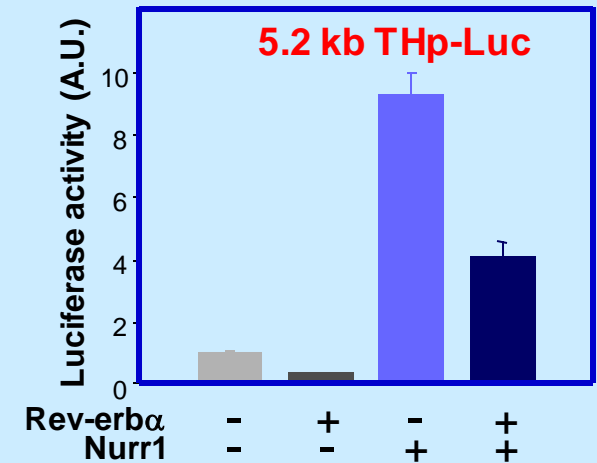
Electrophysiological

Biochemical
/ Molecular

■ Promoter assay



■ Nurr1/Rev-erb α -TH promoter



Molecular biology

Experimental scheme

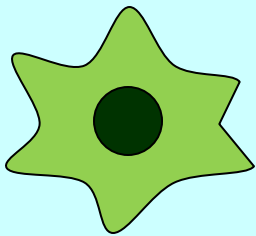
■ Mouse Model



Wild Type mouse



Rev-erba KO mouse



Cath.a cell

■ Multimodal Approaches

- *From Gene to Behavior*
- *From Cell to Animal*

Behavioral

- Tail suspension test
- Forced swim test
- Elevated plus maze
- Open field test
- Fear conditioning test
- Predator odor test
- Aggressive behavior test

Anatomical

- Immunohistochemistry

Electrophysiological

- In vivo recording

Physiological / Pharmacological

- In vitro DA release from striatal tissues
- In vivo microdialysis for DA release
- DA and its metabolites by HPLC-ECD
- Local microinjection to the VMB region

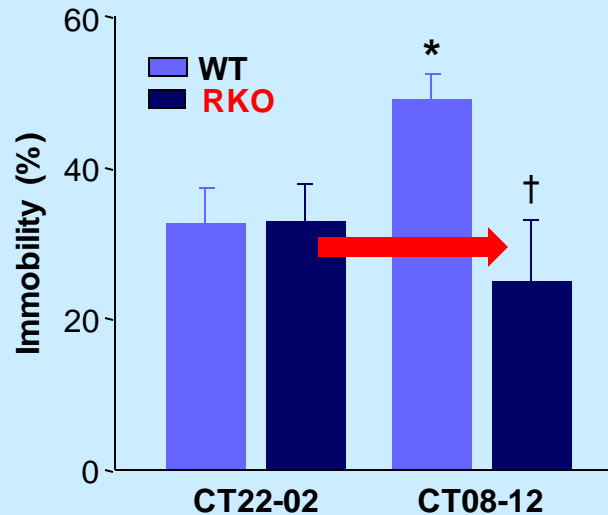
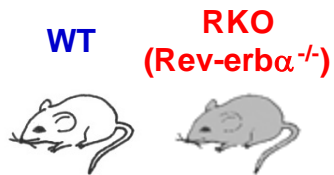
Biochemical / Molecular

- Northern blot
- Western blot
- Q-PCR
- Microarray
- Promoter assay
- Site-directed mutagenesis
- Kinetic ChIP assay

Despair-based behaviors of Rev-erb α KO mice

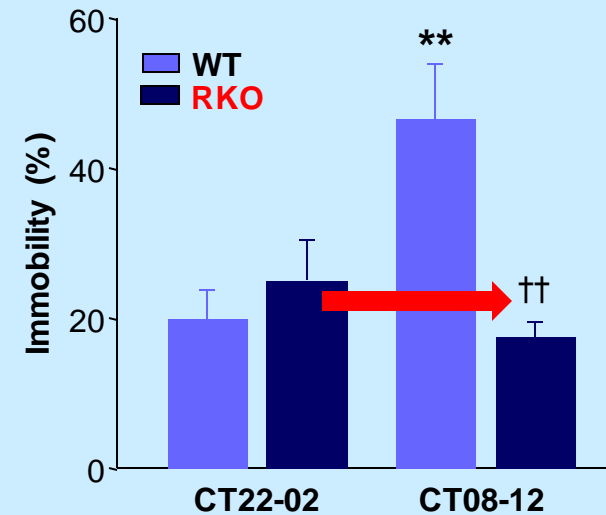
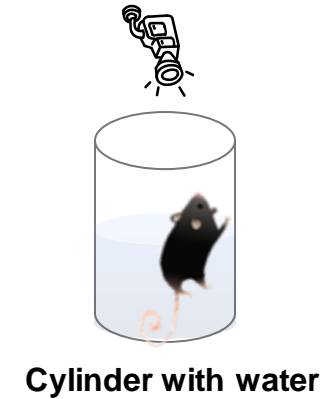
■ Tail suspension test

Measuring the
immobile time



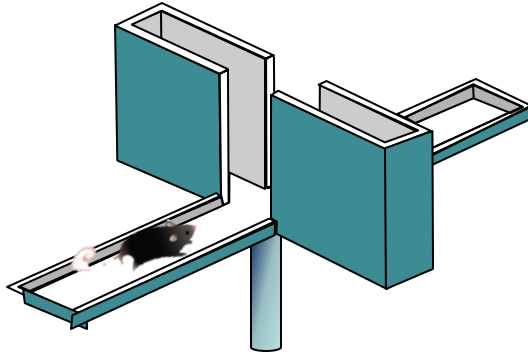
■ Forced swim test

Measuring the
immobile time



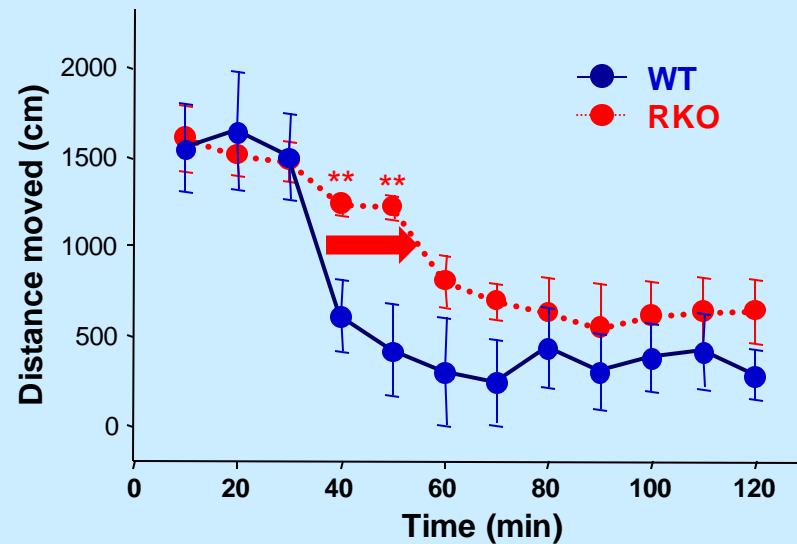
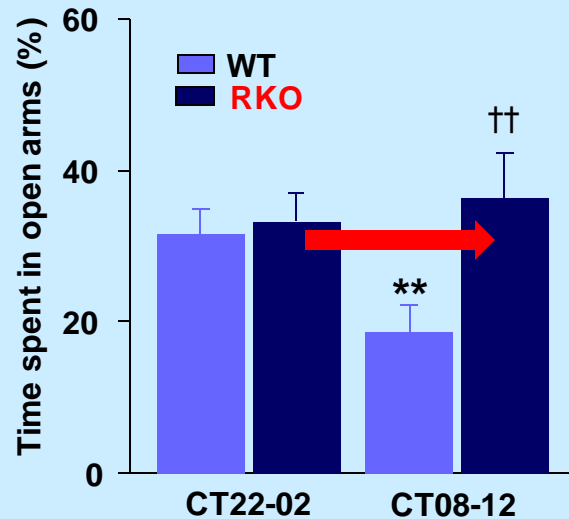
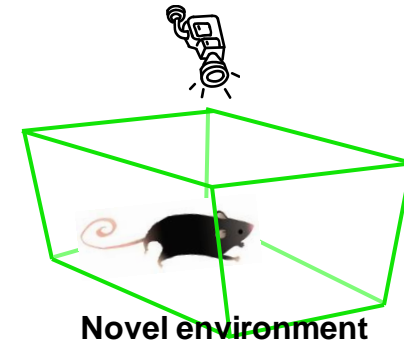
Anxiety behaviors of Rev-erb α KO mice

Elevated plus maze



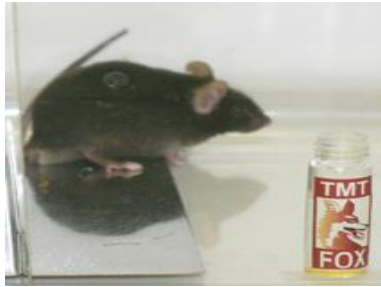
Open field test

Detection of
motility for 2 hrs



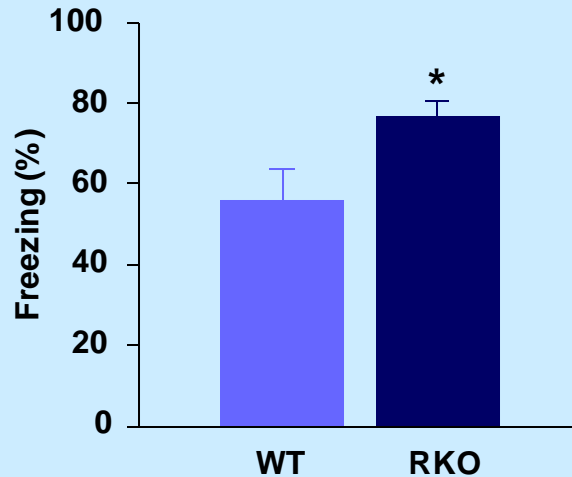
Predator odor test

■ Predator odor test



TMT
(2,5-Dihydro-2,4,5-trimethylthiazoline;
 $C_6H_{11}NS$)
-Synthetic fox feces
odor which evokes
freezing in rodents

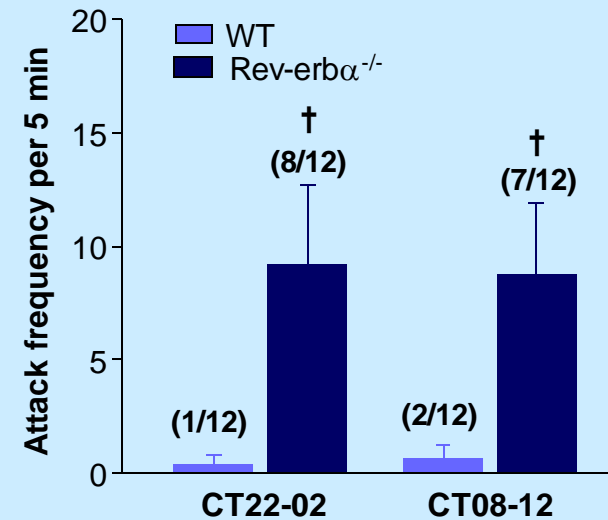
Innate fear



Aggressive behavior

WT

Rev-erb $\alpha^{-/-}$



Summary: Behavioral Studies



Rev-erba KO mouse

Depression	▼
Anxiety	▲
Risk-taking	▲
Innate fear	▲
Aggression	▲

RKO mouse exhibits emotional instability in a mixed state of depression and mania-like behaviors



Question:

Are there alterations in DA neurotransmission in Rev-erba KO mice?

Experimental approach

■ Which target ?

● Microarray experiment

- WT vs RKO → TH

● Circadian profile

- Northern blot / Q-PCR / Western blot
- IHC → Quantitation by FACS

■ DA and metabolites by HPLC-ECD

● DA release in vitro

● DA release in vivo by microdialysis

■ Constitutive expression of hTH in the VTA of WT mouse

→ Behavioral assessment

■ Pharmacological inhibition of DA neurotransmission

● Haloperidol, AMPT

→ Behavioral assessment

■ In vivo electrophysiological recording

● SR8278 (Rev-erb α antagonist)

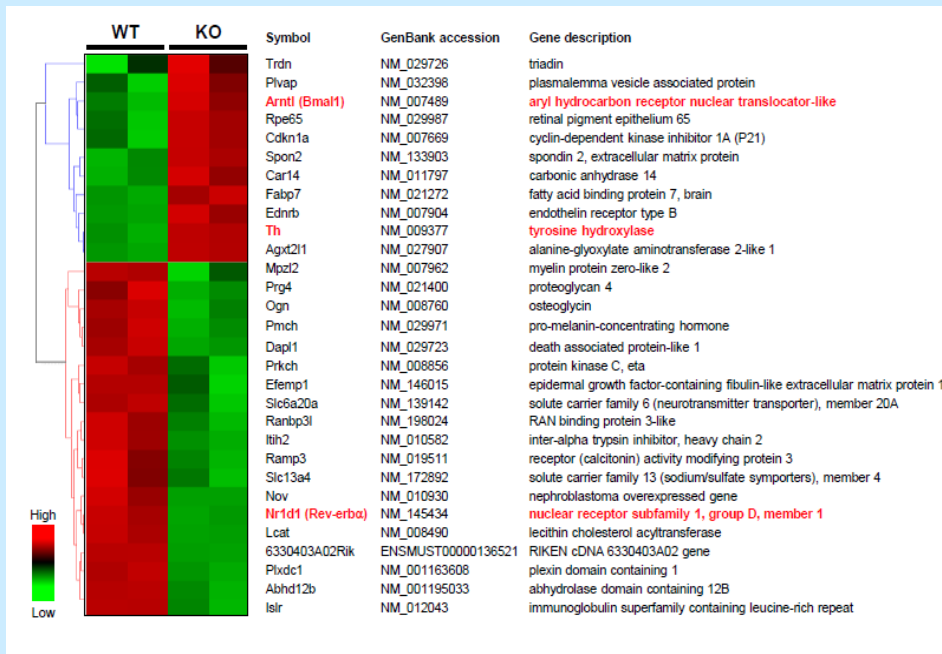
■ Rev-erb α antagonism by SR8278

● Microinjection to VMB

→ Behavioral assessment

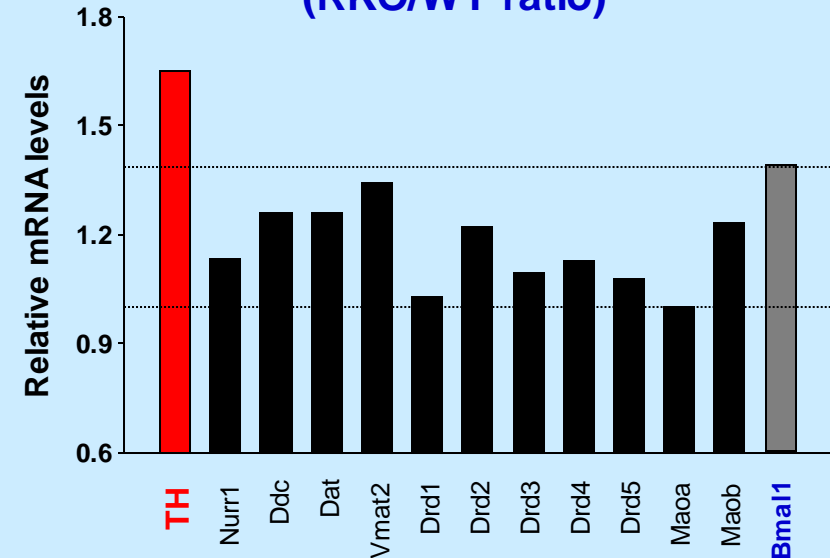
Transcriptome analyses on vantral midbrain of Rev-erb α KO mice

Microarray analysis of WT and RKO VMB (CT08)



Up-regulated genes: **BMAL1 and TH**

Expression profiles of genes involved in DA biosynthesis and function (RKO/WT ratio)

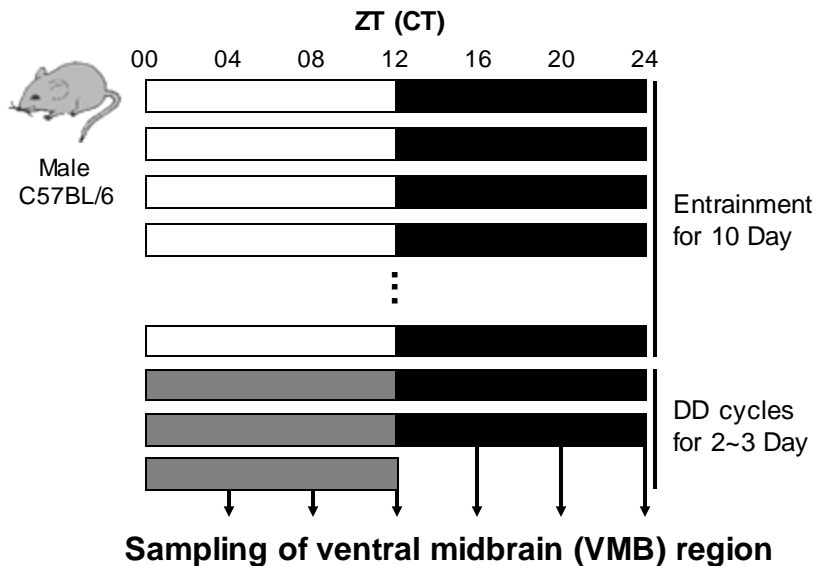


TH: tyrosine hydroxylase
DDC: DOPA decarboxylase
DAT: DA transporter

VMAT2: vesicular monoamine transporter 2
DRD: dopamine receptor
MAO: monoamine oxygenase

Circadian TH mRNA expression in ventral midbrain

Experimental scheme



TH: tyrosine hydroxylase

DA: dopamine

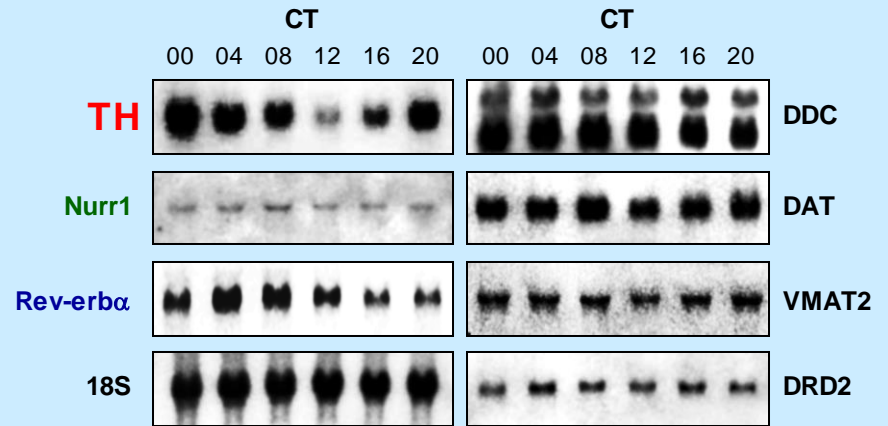
DAT: DA transporter

DDC: DOPA decarboxylase

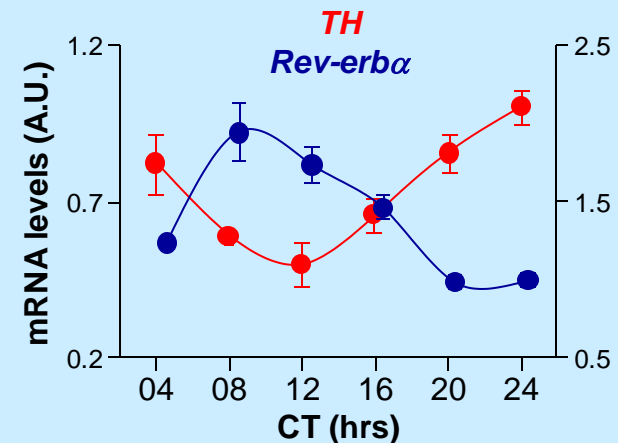
VMAT2: vesicular monoamine transporter 2

DRD2: DA receptor 2

Northern blot

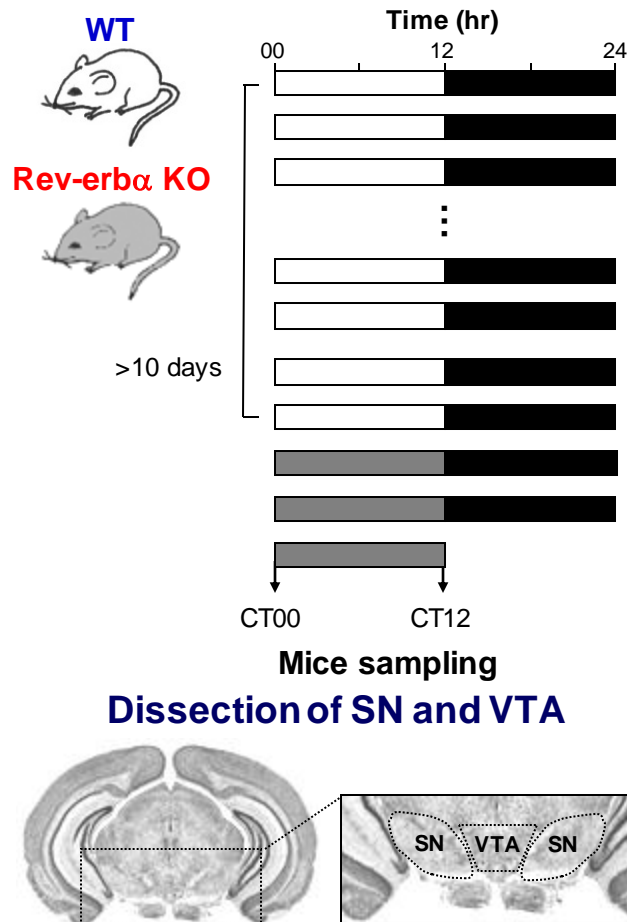


Q-PCR

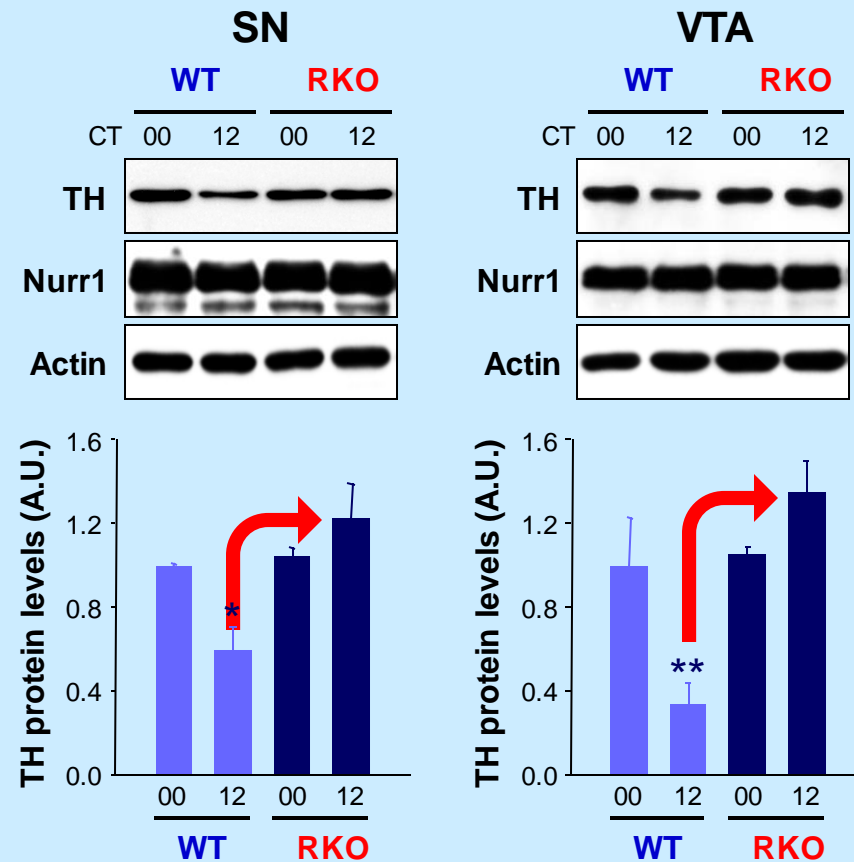


TH expression in SN and VTA of Rev-erb α KO mice

Experimental scheme



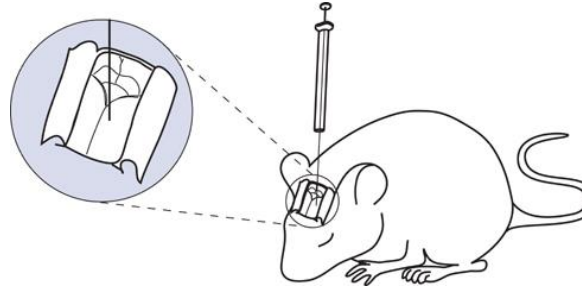
TH and Nurr1 protein levels in SN and VTA



Effect of local administration of Rev-erb α antagonist

■ Experimental scheme

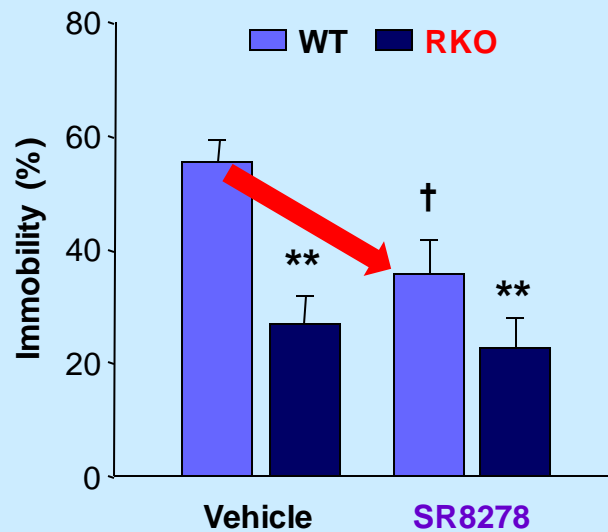
Local infusion of
Rev-erb α antagonist
to ventral midbrain



WT mouse
Rev-erb α KO mouse

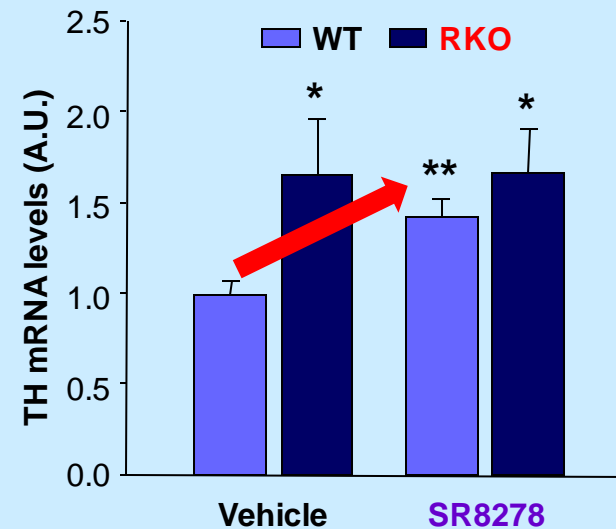
CT 08-10

■ Tail suspension test



*: $p < 0.05$ and **: $p < 0.01$ vs. vehicle injected WT group

■ TH mRNA levels



*: $p < 0.05$ and **: $p < 0.01$ vs. vehicle injected WT group

Summary

■ Which target ?

- Rev-erb α KO vs WT → TH
- Microarray →
 - Northern blot
 - Q-PCR
 - Western blot
 - IHC

■ DA and metabolites by HPLC-ECD

- DA release in vitro
- DA release in vivo by microdialysis

■ Constitutive hTH expression → Behaviors

■ Pharmacological inhibition of DA

- Behavioral tests
 - Haloperidol and AMPT

■ In vivo electrophysiological recording

- Firing rate
- SR8278 (Rev-erb α antagonist)

■ Rev-erb α antagonism by SR8278

- Micro injection of SR8278 to VMB
- Behaviors

**Hyper DA activity
affects mood-related
behaviors**



Question:

What are the underlying molecular mechanisms?

- How does Rev-erb α regulate TH expression?**