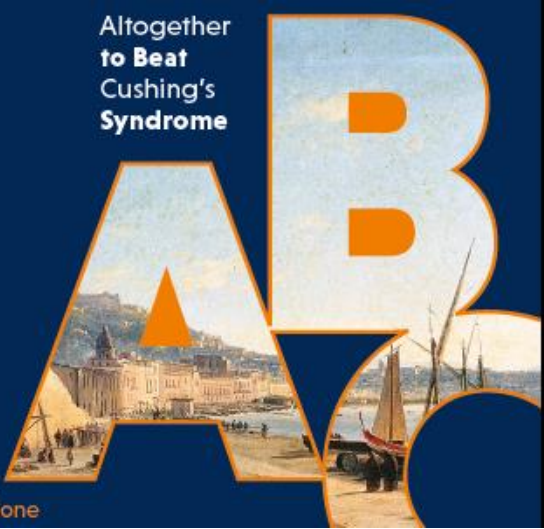


Altogether
to Beat
Cushing's
Syndrome



5ª Edizione

Viaggio alla (ri)scoperta della **Sindrome di Cushing**

Napoli, 10-12 Aprile 2017

Centro Congressi Federico II - Via Partenope, 36

Coordinatori Scientifici

Annamaria Colao, Rosario Pivonello

Il ritmo circadiano del cortisolo

Andrea M. Isidori

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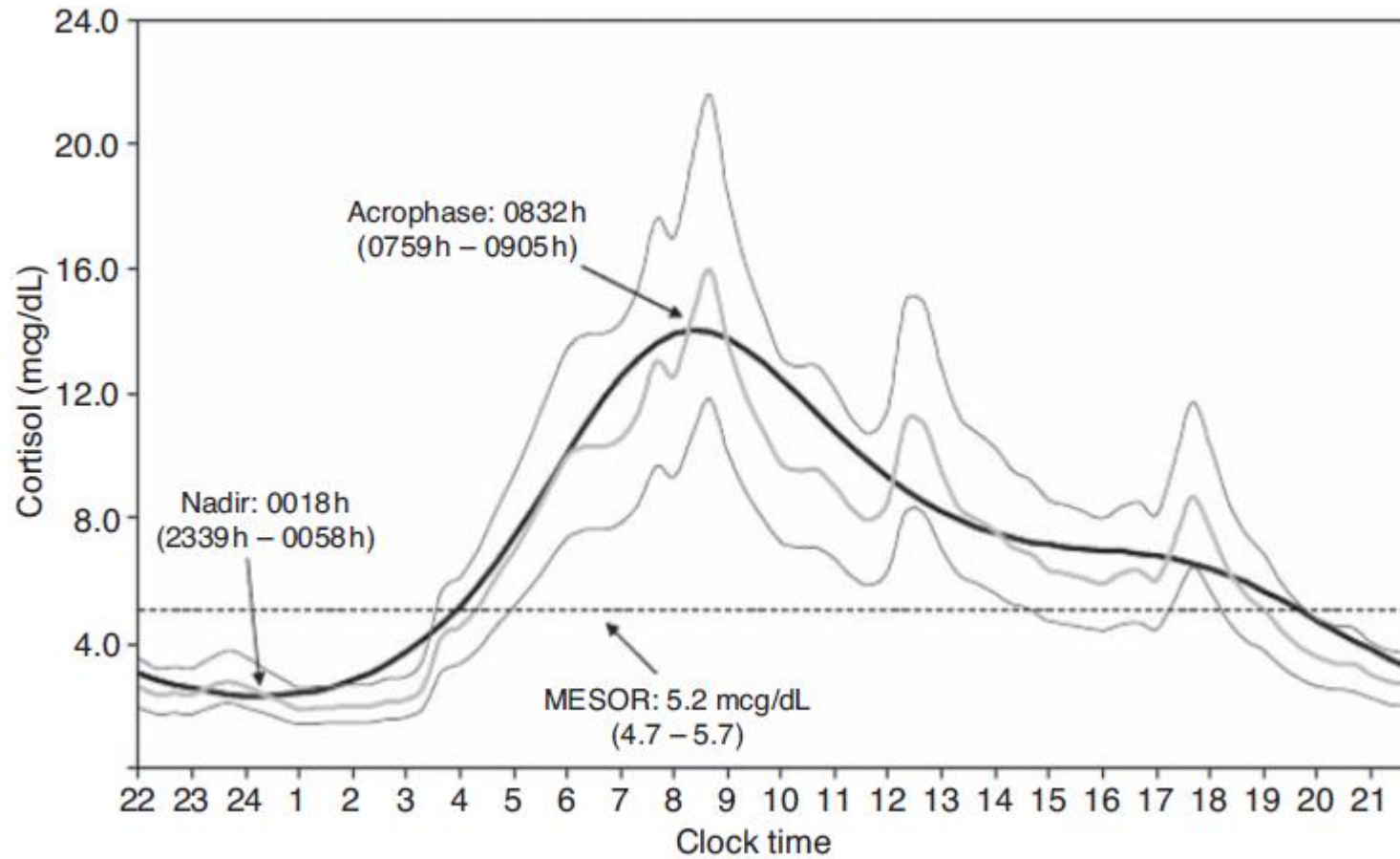
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Circadian rhythm of cortisol



Circadian rhythm of cortisol in 33 individuals with 20-minute cortisol profiling

A circadian gene expression atlas in mammals: Implications for biology and medicine

Ray Zhang^{a,1}, Nicholas F. Lahens^{a,1}, Heather I. Ballance^a, Michael E. Hughes^{b,2}, and John B. Hogenesch^{a,2}

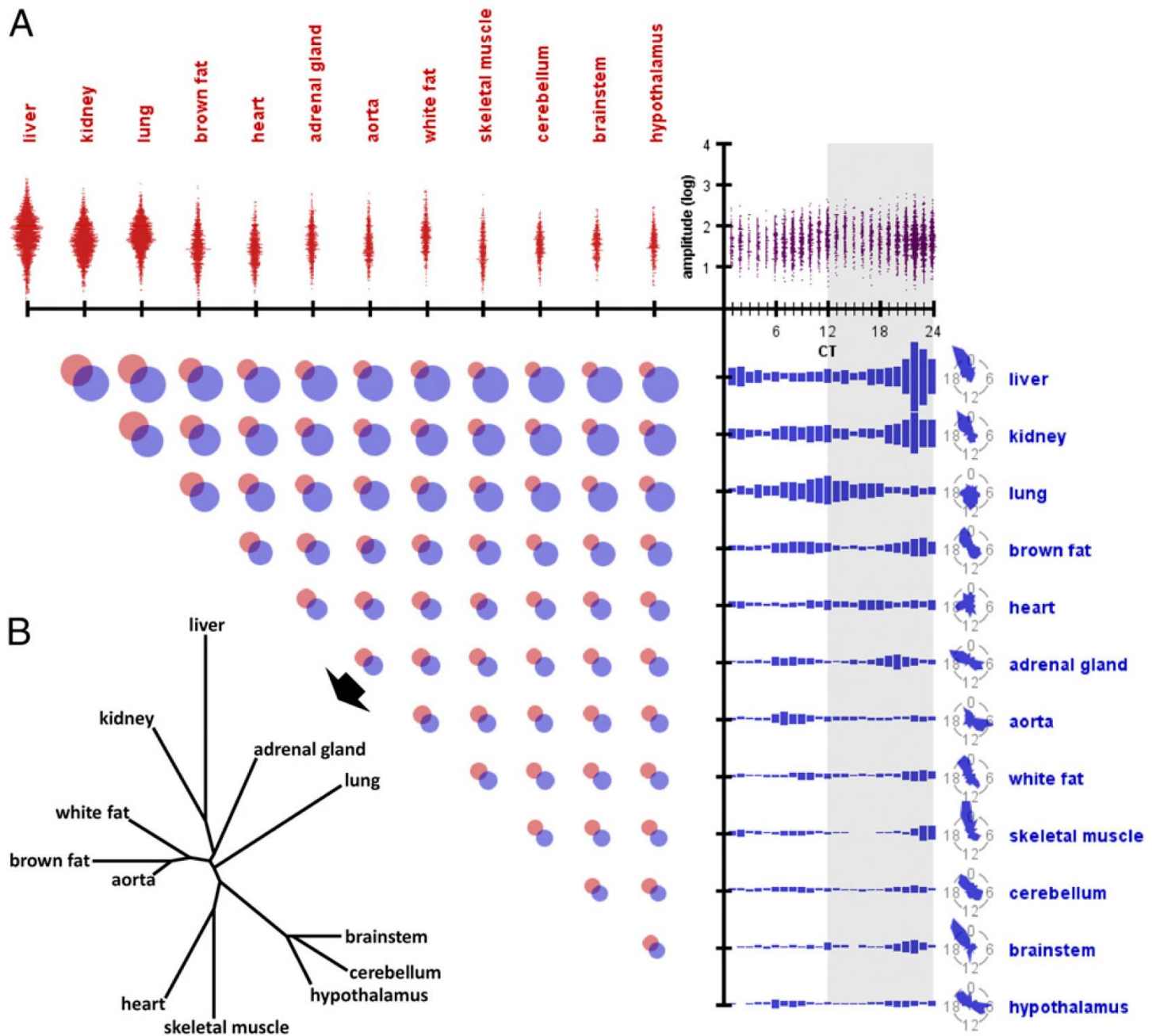
^aDepartment of Pharmacology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104; and ^bDepartment of Biology, University of Missouri, St. Louis, MO 63121

- About 10% of the entire genome is under circadian control.
- More than 50% of all genes in the mouse genome oscillate with circadian rhythm somewhere in the body.

A circadian Implication

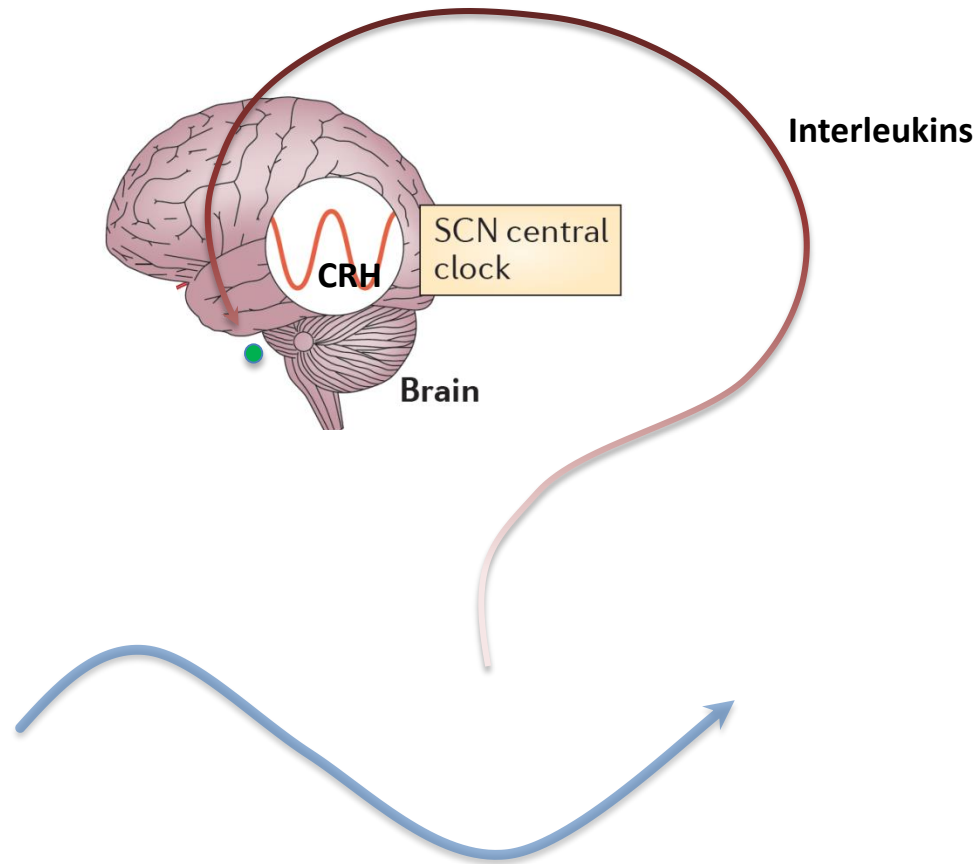
Ray Zhang^{a,1}, Nik

^aDepartment of Pharr
Missouri, St. Louis, MO





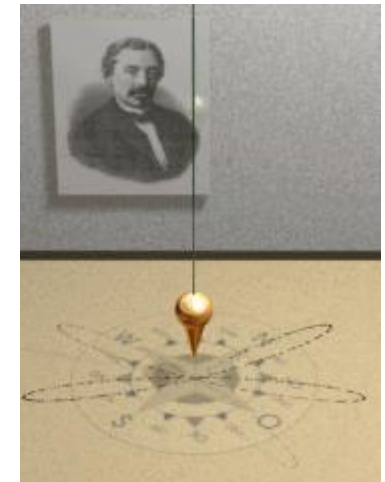
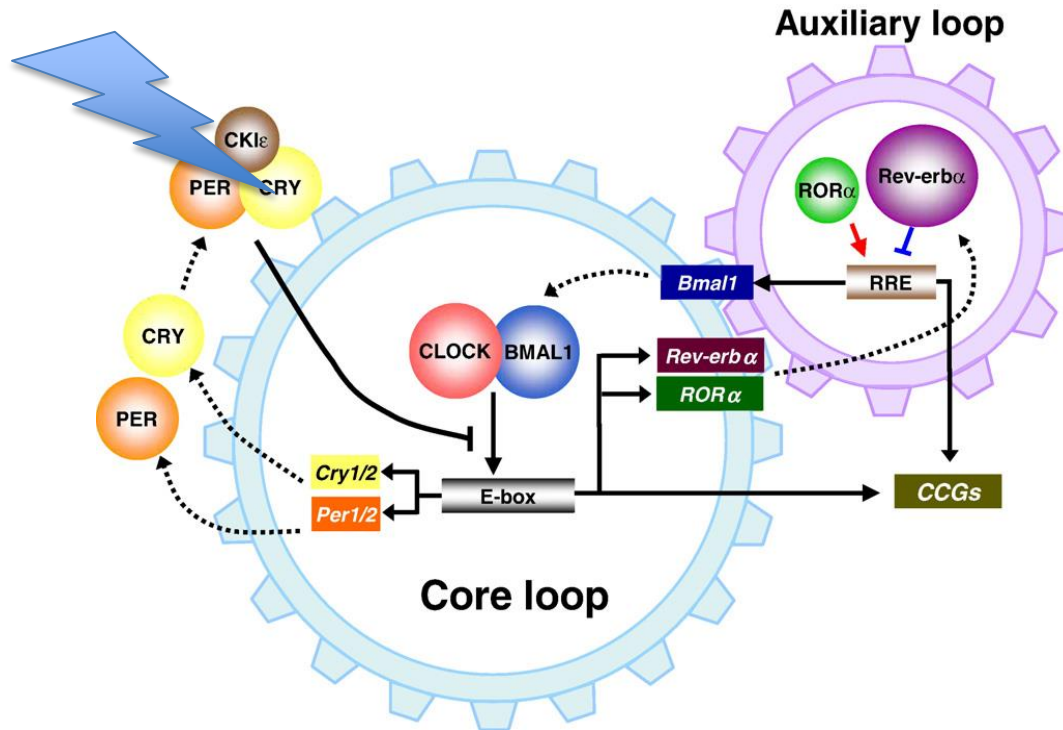
HPA control of immune circadian rhythm



ACTH, adrenocorticotrophic hormone; SNS, sympathetic nervous system;
MIF, macrophage inhibitory factor; CRH, Corticotropin-releasing hormone;
SCN, suprachiasmatic nuclei

Molecular circadian clockwork

Principal or core feedback loop CLOCK and BMAL1, form heterodimers to activate the transcription of their target genes containing E-box element

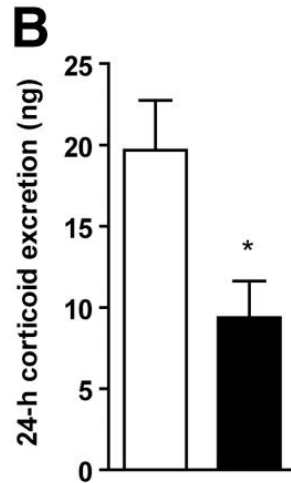
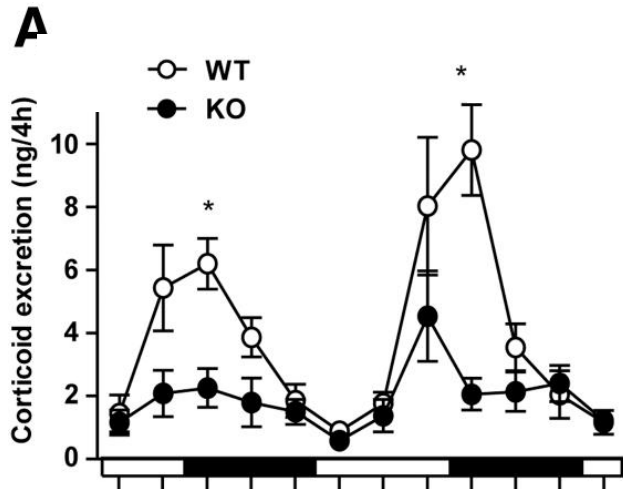


These target genes include their negative regulators the Periods (PERs: PER1, PER2 and PER3) and the Cryptochromes (CRYs: CRY1 and CRY2).

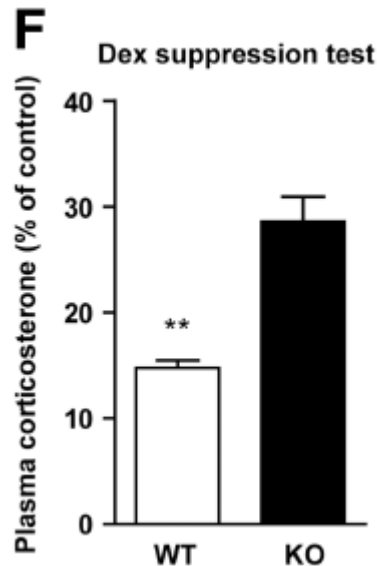
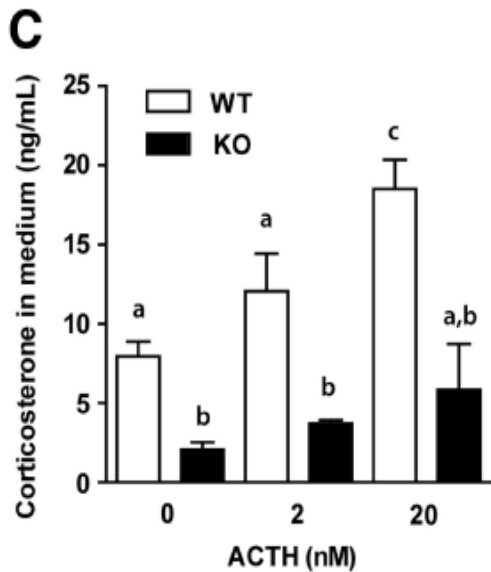
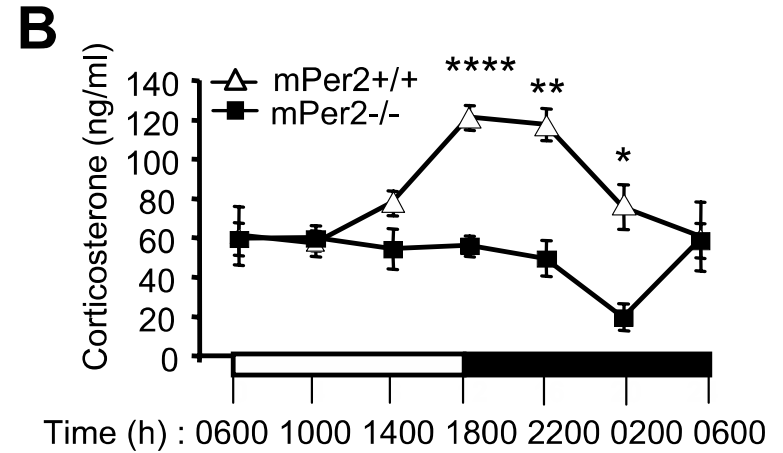
The concentration of BMAL1 is adjusted by an auxiliary or stabilizing feedback loop formed by the clock-controlled nuclear receptors REVERB α

Clock genes influence GC rhythm and release

BMAL1 (arntl -/-) ko mice



Per ko mice



Alexei Leliavski, Anton Shostak, Jana Husse, and Henrik Oster Impaired Glucocorticoid Production and Response to Stress in Arntl-Deficient Male Mice *Endocrinology* 155: 133–142, 2014

Shutong Yang et al The Role of mPer2 Clock Gene in Glucocorticoid and Feeding Rhythms *Endocrinology* 150: 2153–2160, 2009

Cortisol synchronizes peripheral clocks

Science

AAAS

Resetting of Circadian Time in Peripheral Tissues by Glucocorticoid Signaling

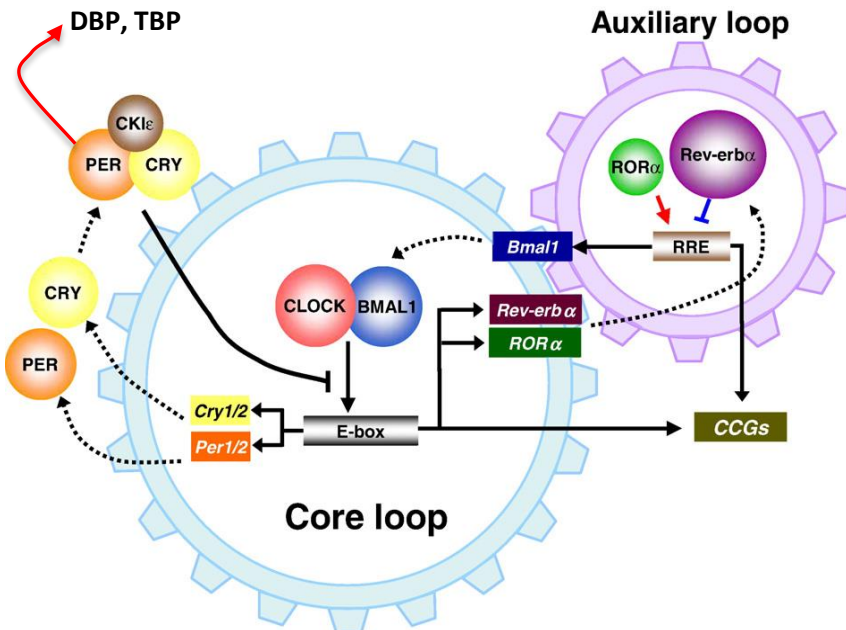
Aurélio Balsalobre *et al.*

Science **289**, 2344 (2000);

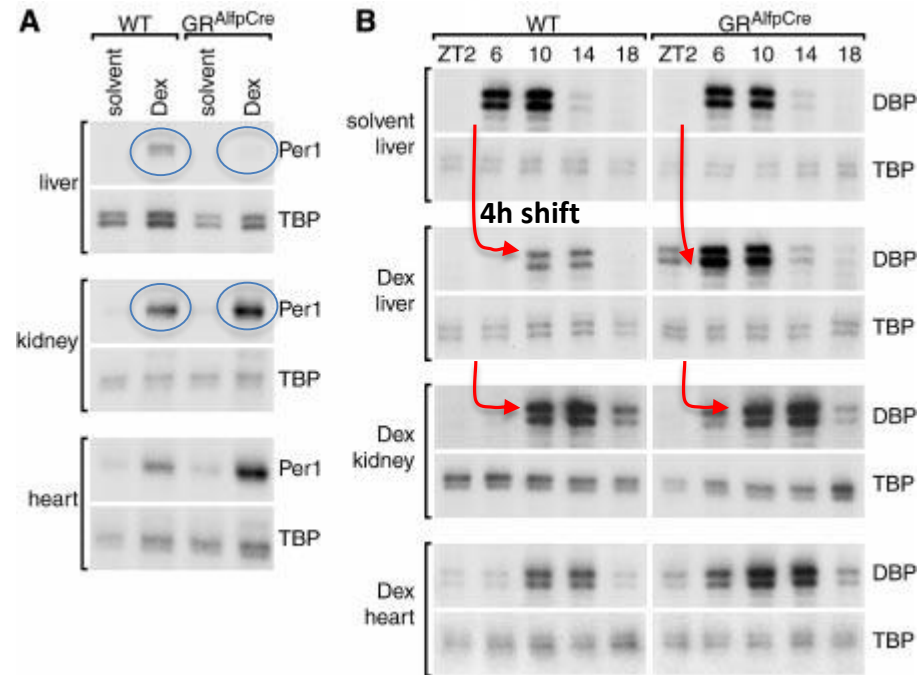
DOI: 10.1126/science.289.5488.2344



Dexamethasone induces circadian gene expression¹



The glucocorticoid receptor (GR) is required for dexamethasone-induced *Per1* phase shifting¹

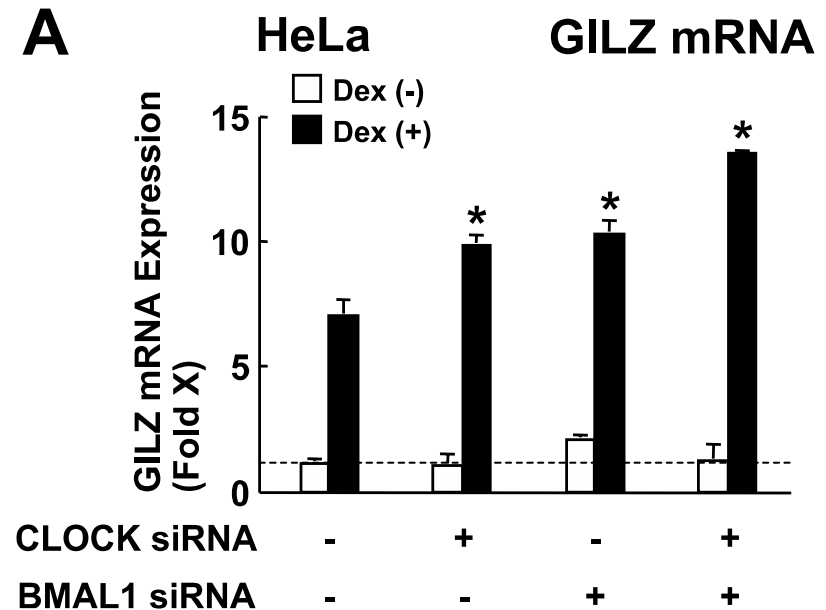


GR^{AlfpCre} mice with a liver-specific disruption of the GR gene

1. Balsalobre A et al. *Science* 2000;289:2344-2347; 2. Gi Hoon Son et al. *Frontiers in Neuroendocrinology* 2011;32:451-465.

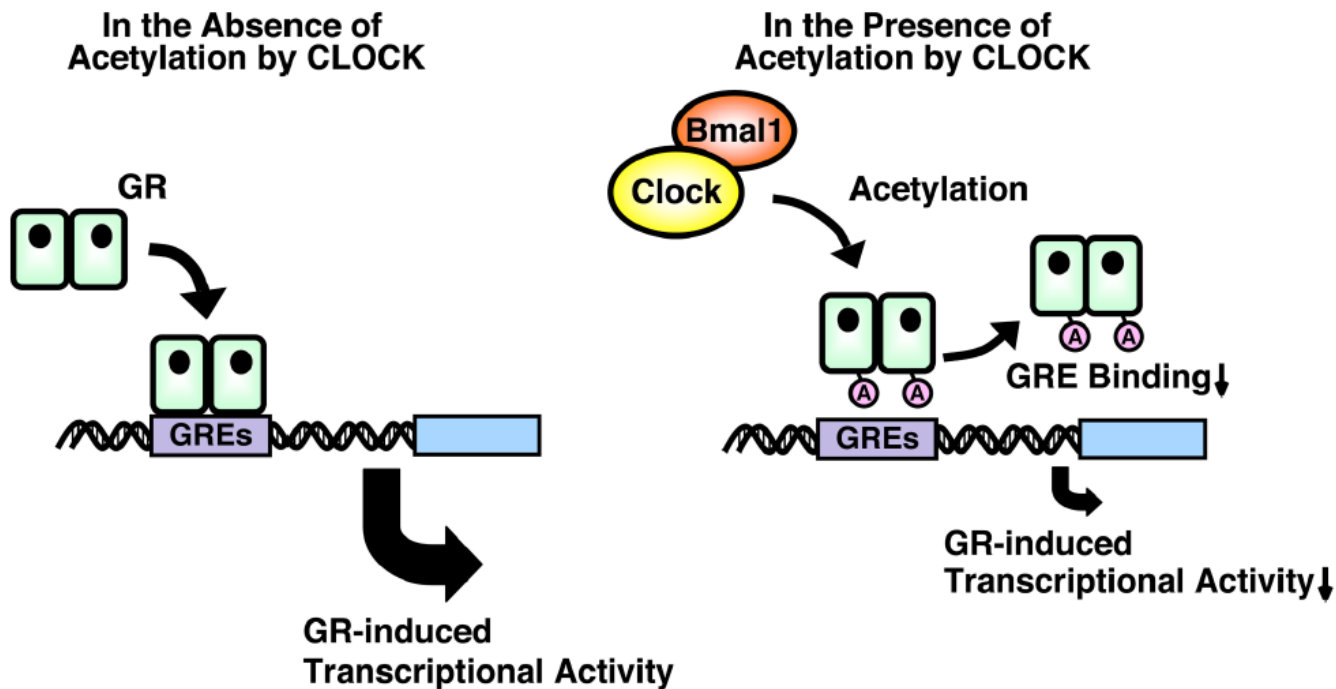
The Clock-HPA axis counter-regulatory feedback (2)

The clockwork influence
responsiveness to
glucocorticoids



The clockwork influence responsiveness to GC

Clock/Bmal1 physically interact with the ligand-binding domain of the GR through a region enclosed in the C-terminal part of the Clock protein, and suppressed GR-induced transcriptional activity

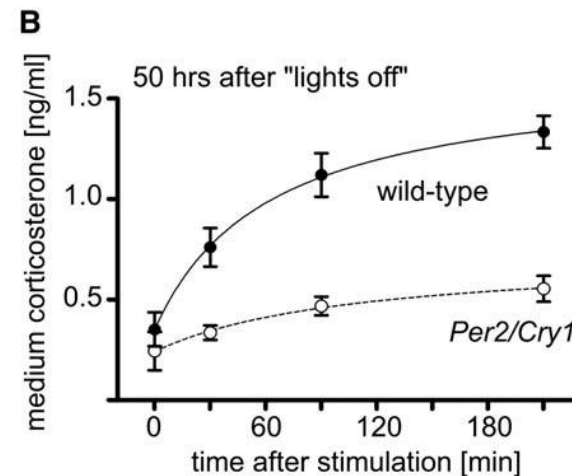
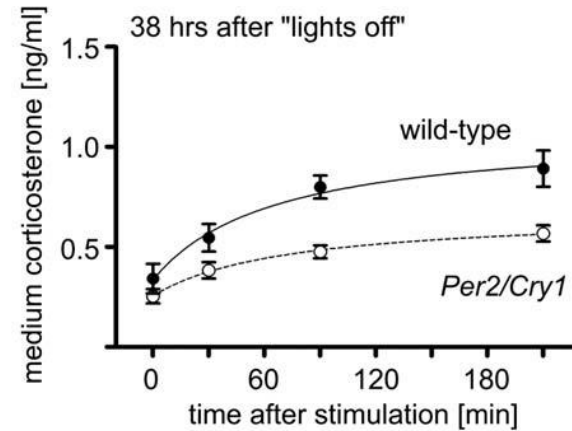


➔ By disrupting the clock -> we can alter the efficacy (**side effects**) of GC

Adrenal intrinsic mechanisms: the involvement of adrenal oscillator

“Gating mechanism”: the local clock machinery in the adrenal gland contributes to the diurnal rhythm of GC by controlling the daily variation in the adrenal sensitivity to ACTH.

I geni clock regolano
La sensibilità del
surrene all'ACTH

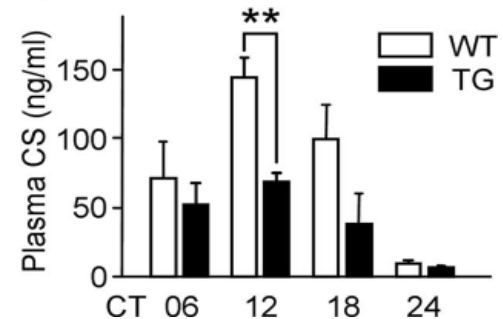
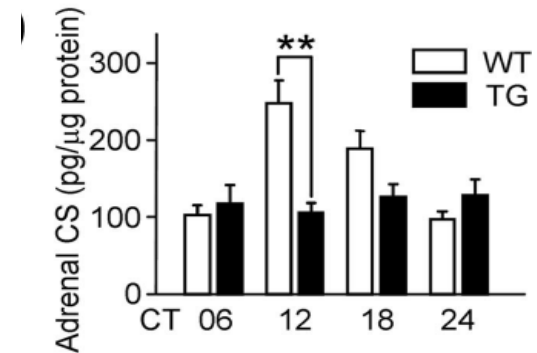
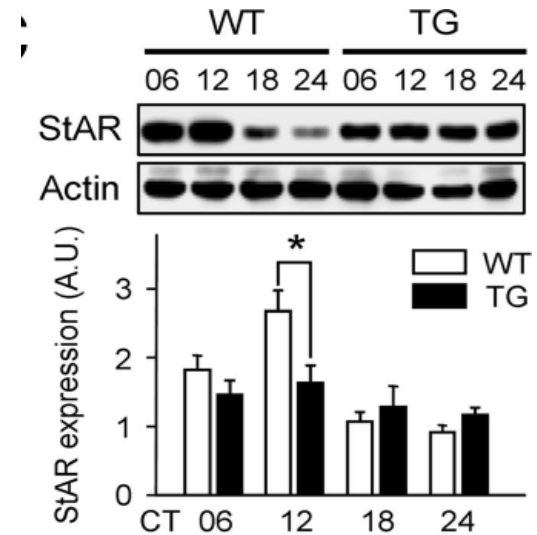


Adrenal intrinsic mechanisms: the involvement of adrenal oscillator

Geni clock controllano la produzione dei glucocorticoidi modulando l'espressione StAR

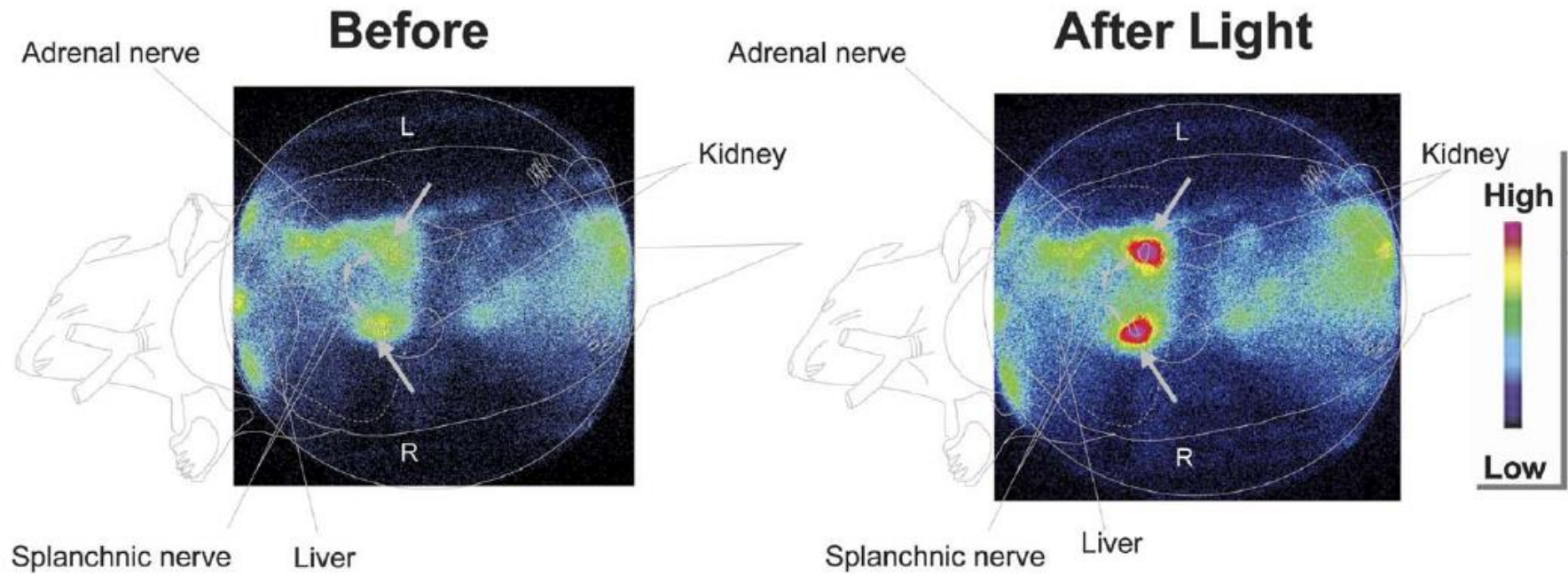
Examination of mice with adrenal-specific knockdown of the canonical clock protein BMAL1 reveals that the adrenal clock machinery is required for circadian GC production

Son GH et al Proc Natl Acad Sci U S A. 2008 Dec 30;105(52):20970-5 Adrenal peripheral clock controls the autonomous circadian rhythm of glucocorticoid by causing rhythmic steroid production



HOW?

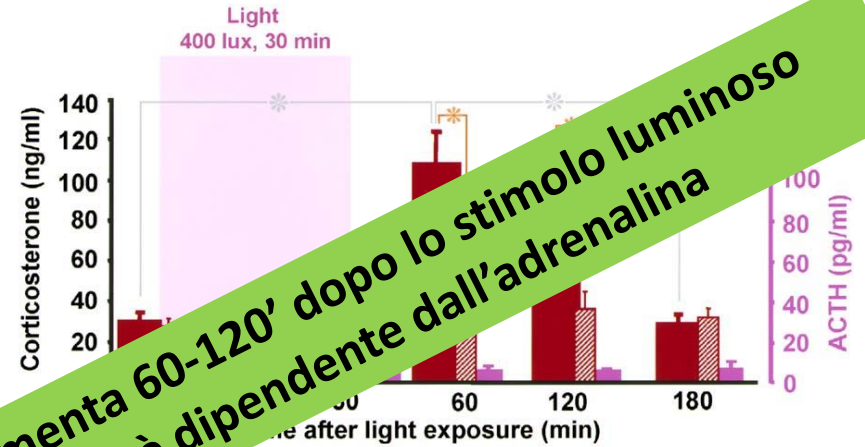
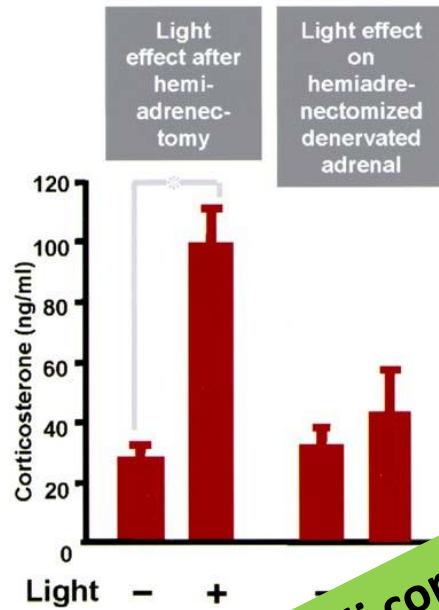
Light activates the adrenal gland



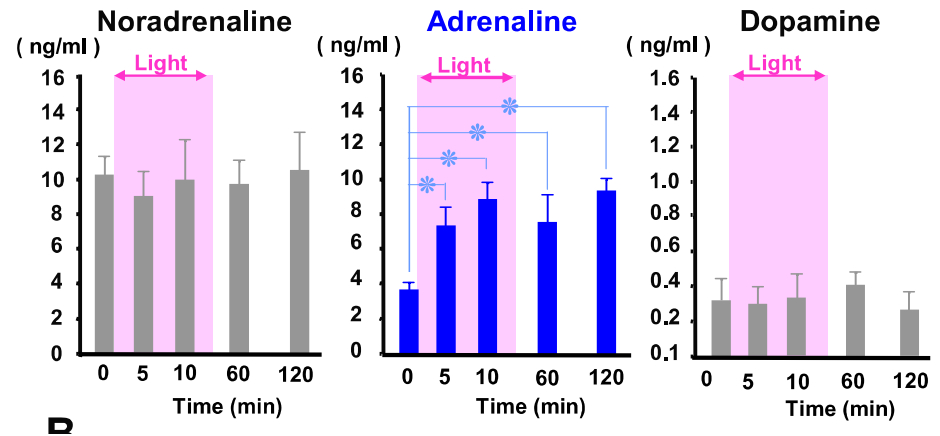
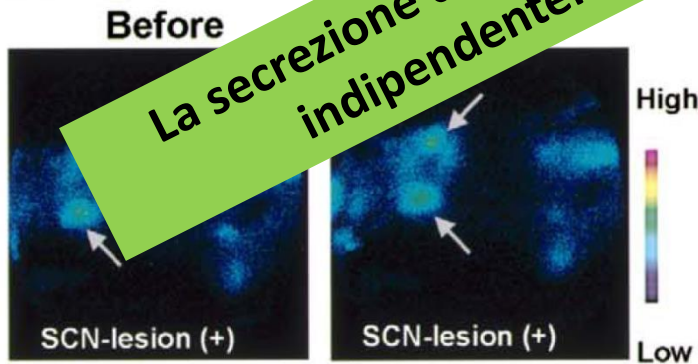
Light-induced *Per1-luc* luminescence in visceral organs

A. Ishida, T. Mutoh, T. Ueyama, H. Bando, S. Masubuchi, D. Nakahara, G. Tsujimoto, H. Okamura, Light activates the adrenal gland: timing of gene expression and glucocorticoid release, *Cell Metab.* 2 (2005) 297–307.

Light activates the adrenal gland through SCN activation and splanchnic innervation of the gland



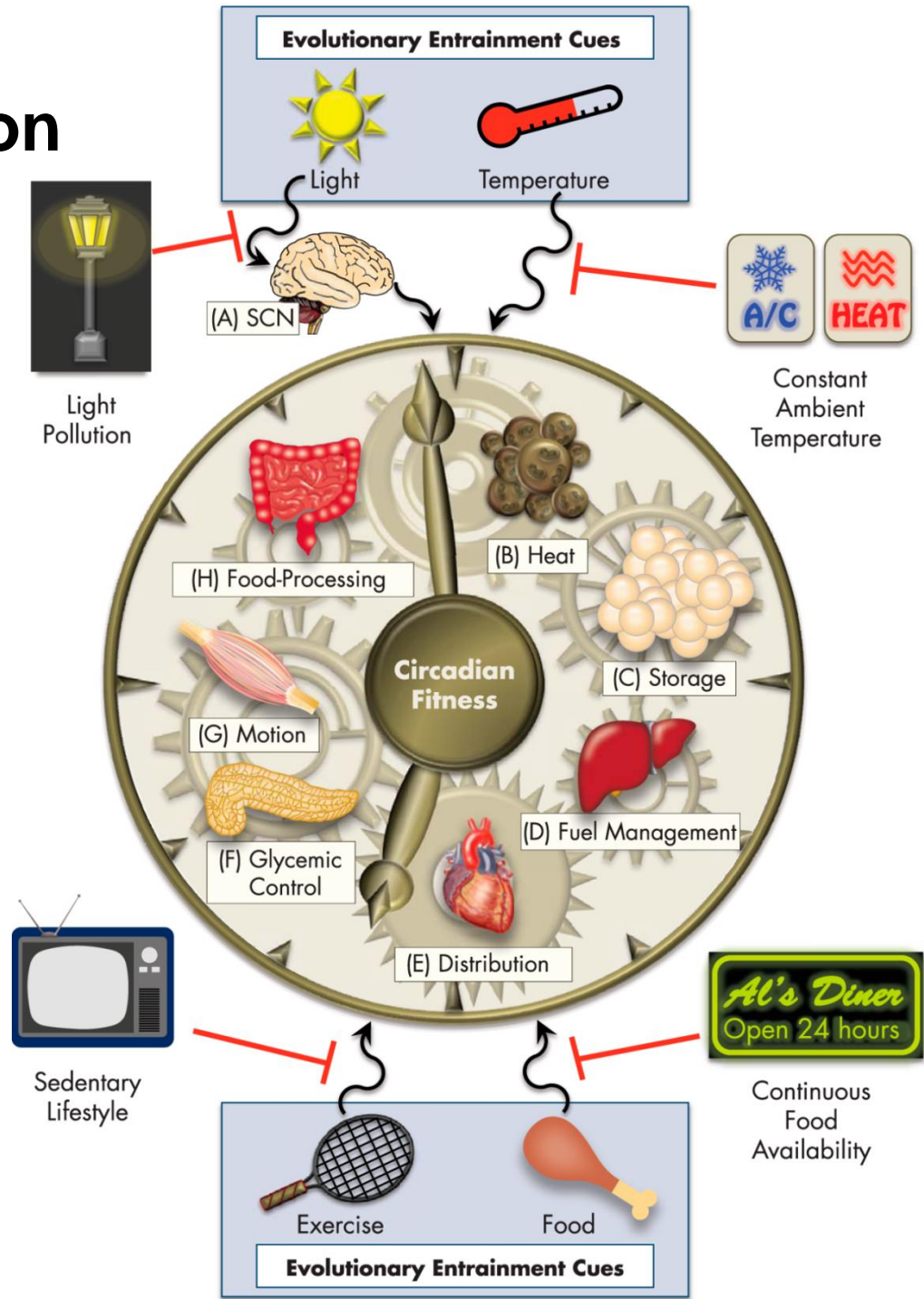
La secrezione di corticosterone aumenta 60-120' dopo lo stimolo luminoso indipendentemente dall'ACTH ed è dipendente dall'adrenalina



Circadian Metabolism in the Light of Evolution

Endocr Rev. 2015;36(3):289-304

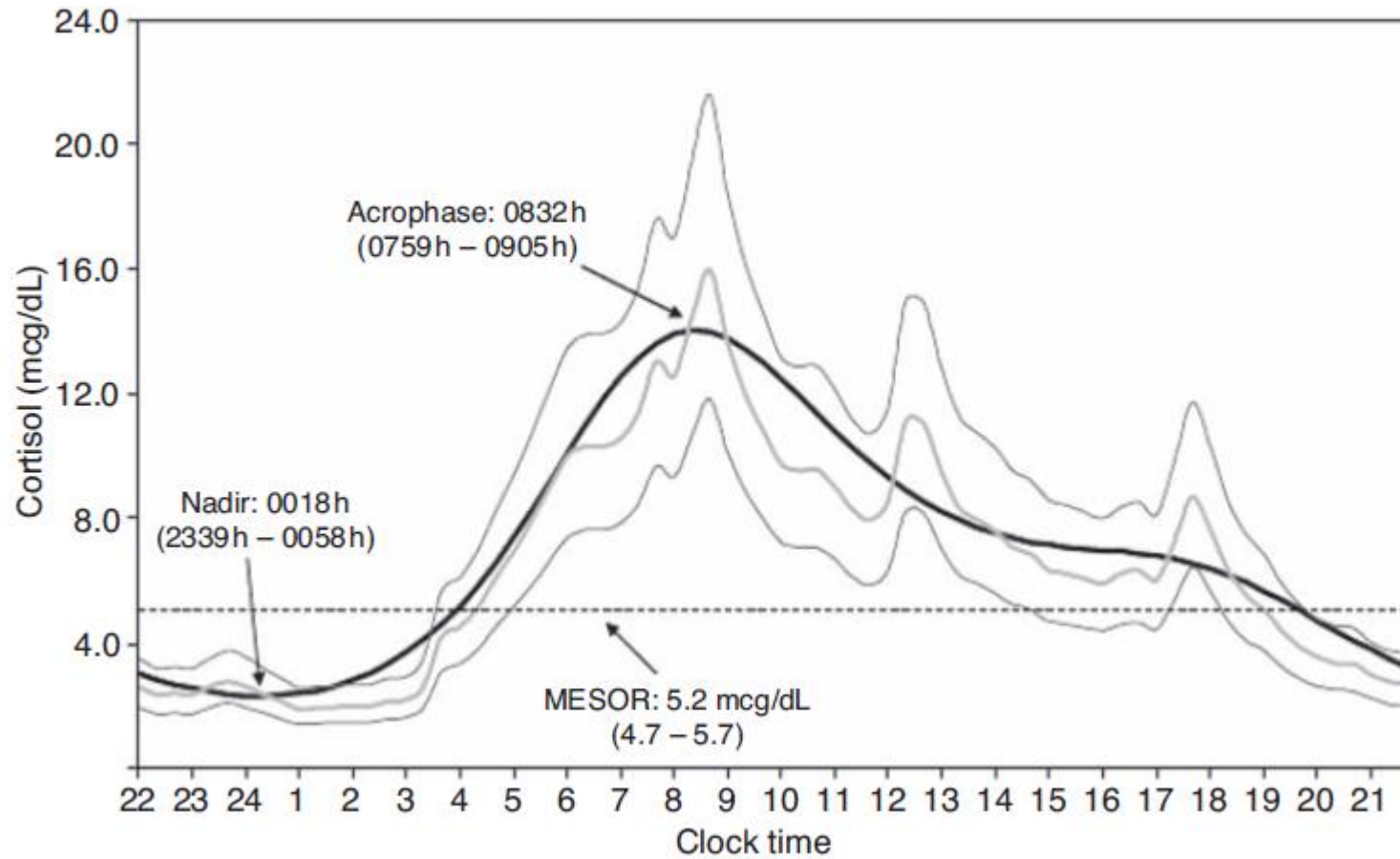
Sunlight, temperature, physical activity, and food intake serve as basic entraining cues, or zeitgebers, to reset the master clock (A) in the hypothalamic SCN each day.



Phase shifts

- The phase-response can be **phase-advances** or **phase delays** according to when the external zeitgeber is perceived.
- In humans, the phase delay region is generally longer than the phase advance region, and phase delays. As a consequence the “average” human clock can be more easily phase delayed than phase advanced.

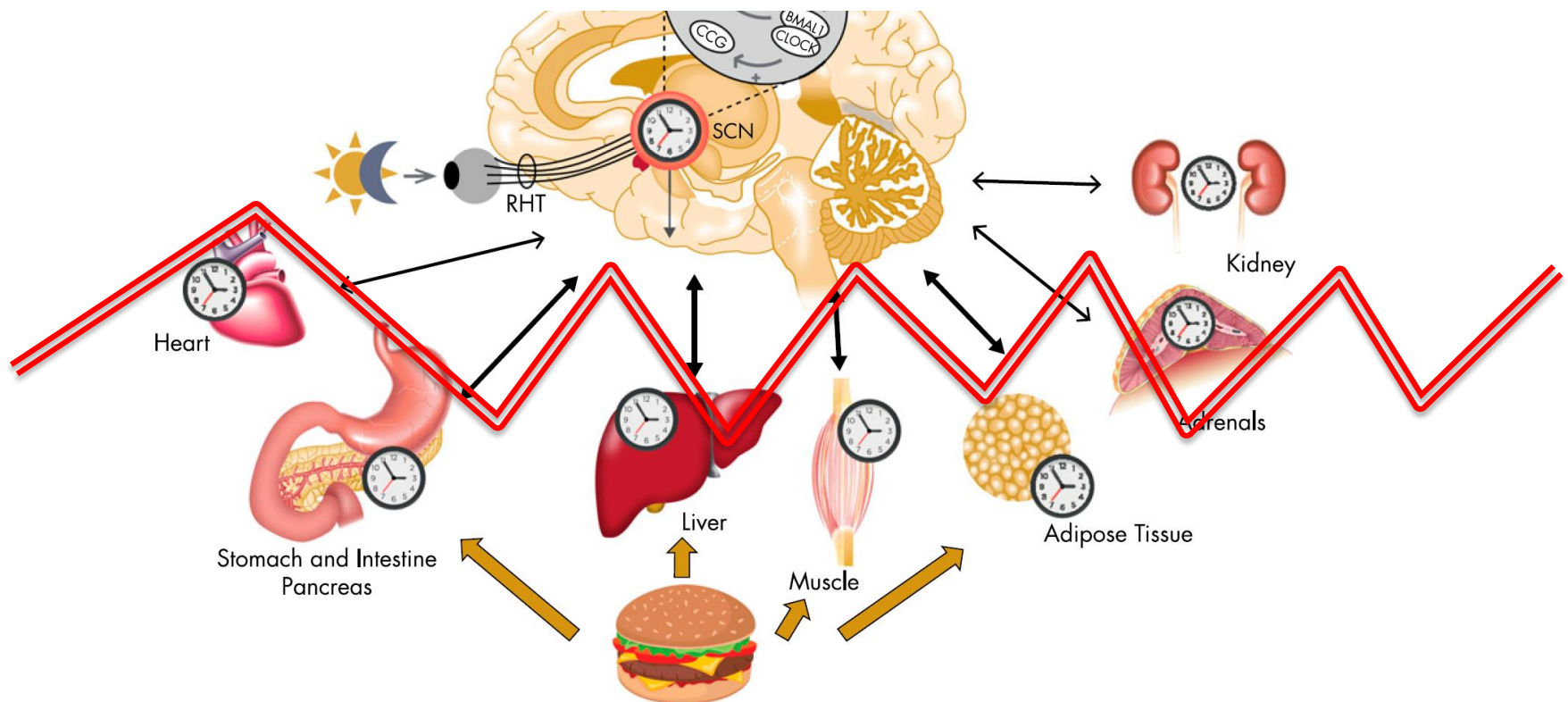
Circadian rhythm of cortisol



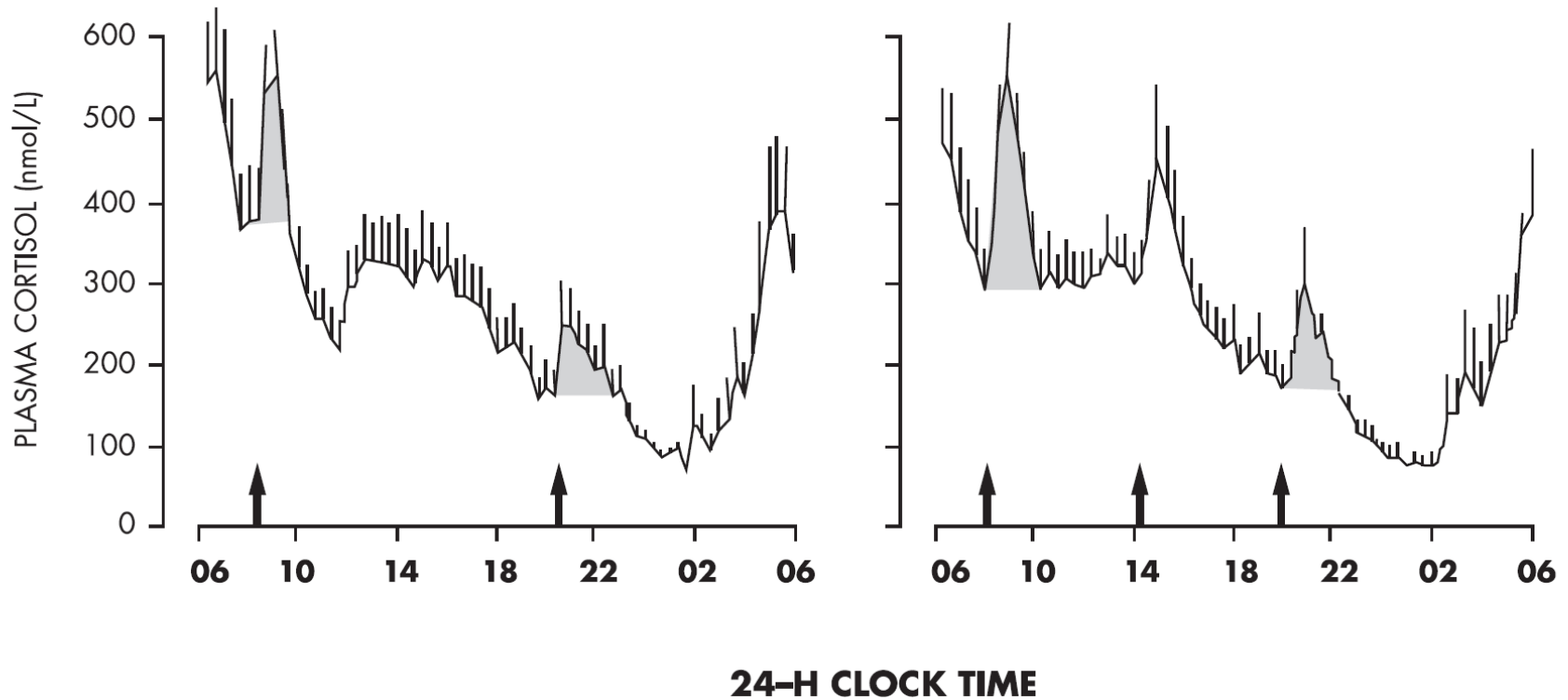
Circadian rhythm of cortisol in 33 individuals with 20-minute cortisol profiling

Phase shifts

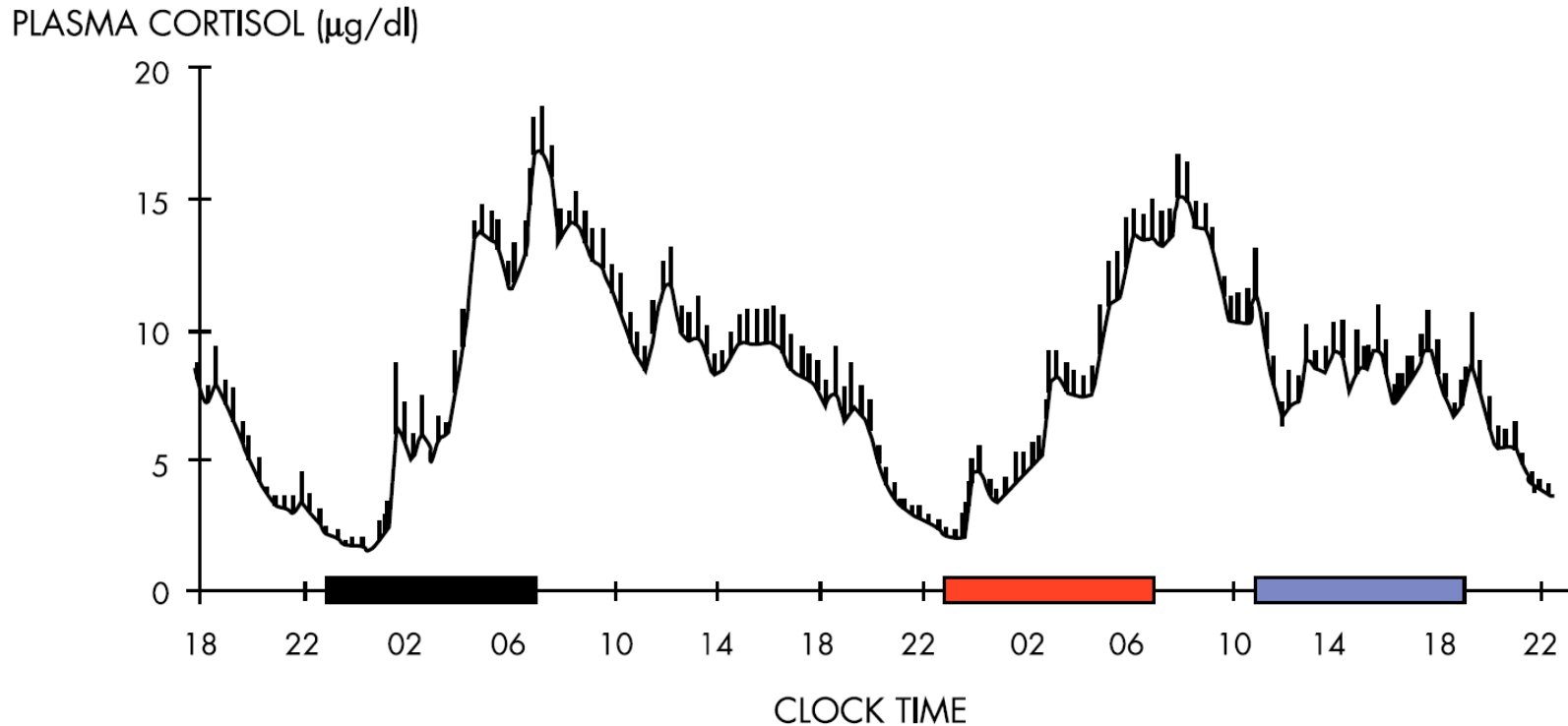
- Restricted feeding can uncouple the phase of peripheral clocks from the SCN, which stays phase-locked to the light-dark cycle.



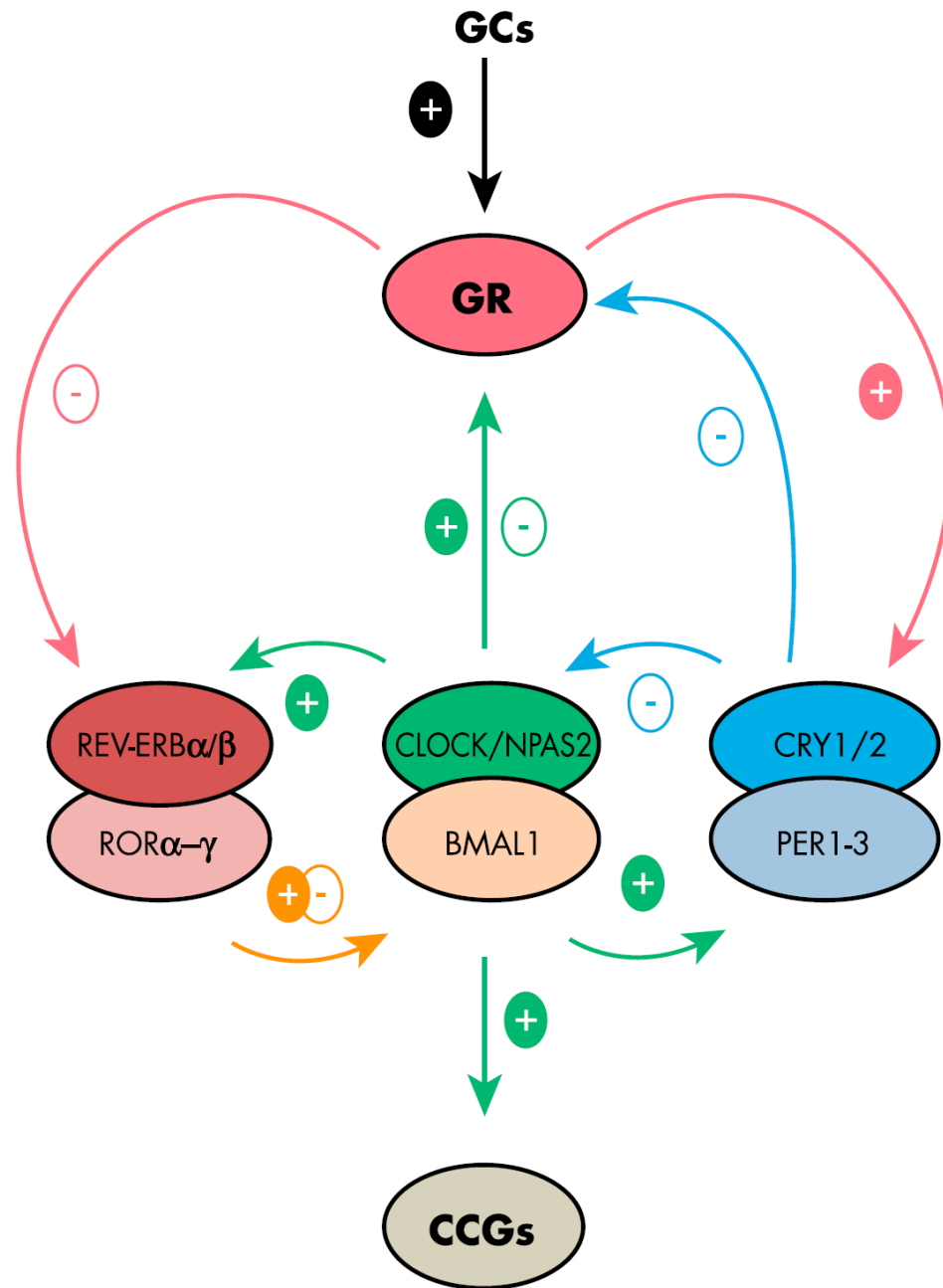
2 vs 3 Carbohydrate Meals / day



Acute Sleep Deprivation



- NOCTURNAL SLEEP
- NOCTURNAL SLEEP DEPRIVATION
- DAYTIME SLEEP



Conditions with altered HPA axis activity

Although the precise significance of the daily GC rhythm is not yet well established, a growing body of evidence does point to its clinical importance.

Alterations in its rhythmicity are frequently found in many human diseases

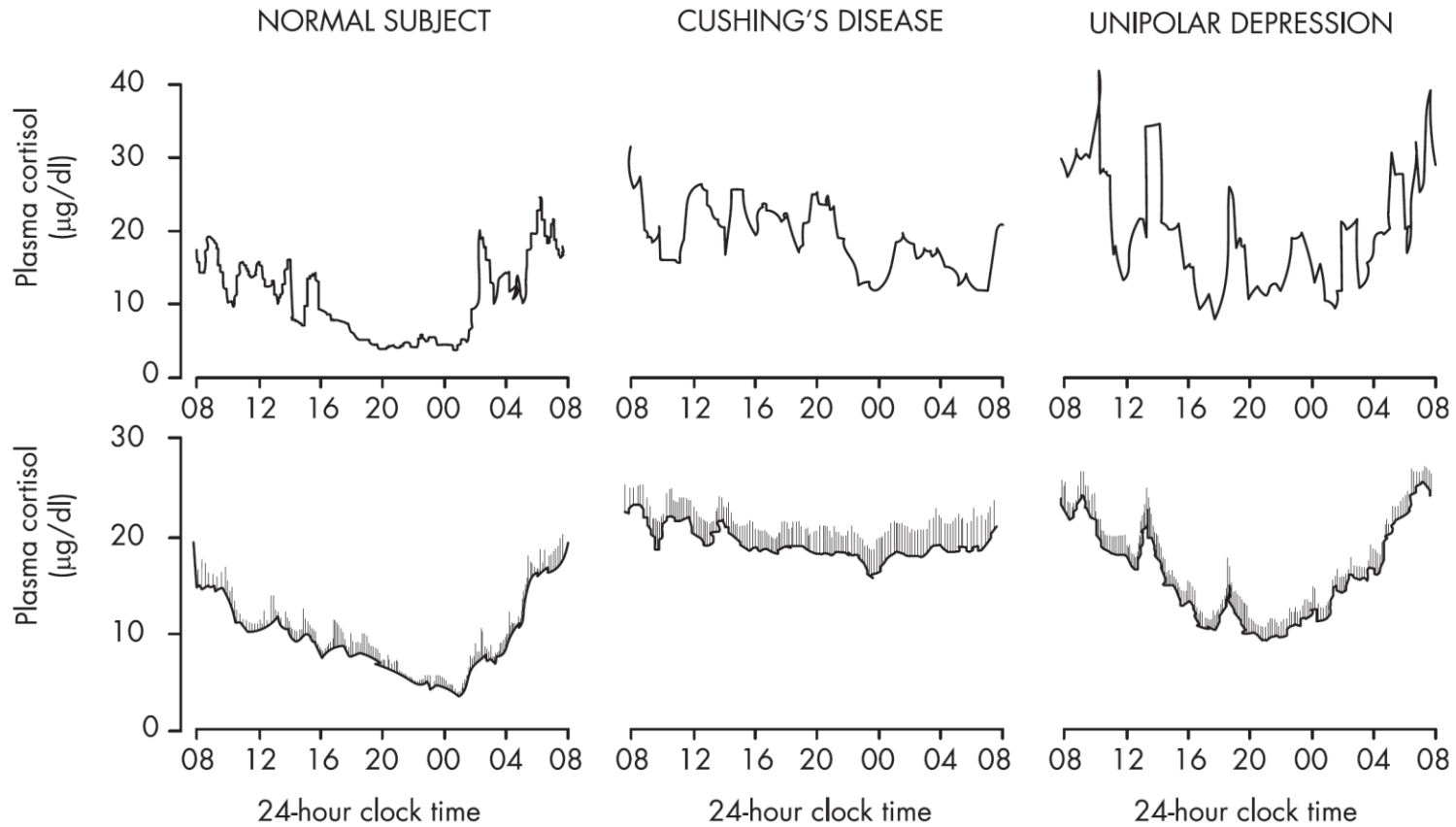
Increased activity of the HPA axis

- Cushing syndrome
- Chronic stress
- Melancholic depression
- Anorexia nervosa
- Obsessive–compulsive disorder
- Panic disorder
- Excessive exercise (obligate athleticism)
- Chronic, active alcoholism
- Alcohol and narcotic withdrawal
- Diabetes mellitus
- Central obesity (metabolic syndrome)
- Post-traumatic stress disorder in children
- Hyperthyroidism
- Pregnancy

Decreased activity of HPA axis

- Adrenal insufficiency
- Atypical/ seasonal depression
- Chronic fatigue syndrome
- Fibromyalgia
- Premenstrual tension syndrome
- Climacteric depression
- Nicotine withdrawal
- Following cessation of glucocorticoid therapy
- Following Cushing syndrome cure
- Following chronic stress
- Postpartum period
- Adult post-traumatic stress disorder
- Hypothyroidism
- Rheumatoid arthritis
- Asthma, eczema

Cushing's Disease



Cushing's Disease

- Overall circadian modulation is absent , but a low amplitude circadian variation may persist in some patients with pituitary-dependent Cushing's disease.
- Cortisol pulsatility is blunted in about 70% of patients with Cushing's disease
- However, in about 30% of these patients, the magnitude of the pulses is instead enhanced.
- In adrenal Cushing's data a more pronounced loss of circadian rhythm seems to occur compared to pituitary Cushing's.



Perspectives: toward better **Chronopharmacology**

A circadian gene expression atlas: implications in medicine

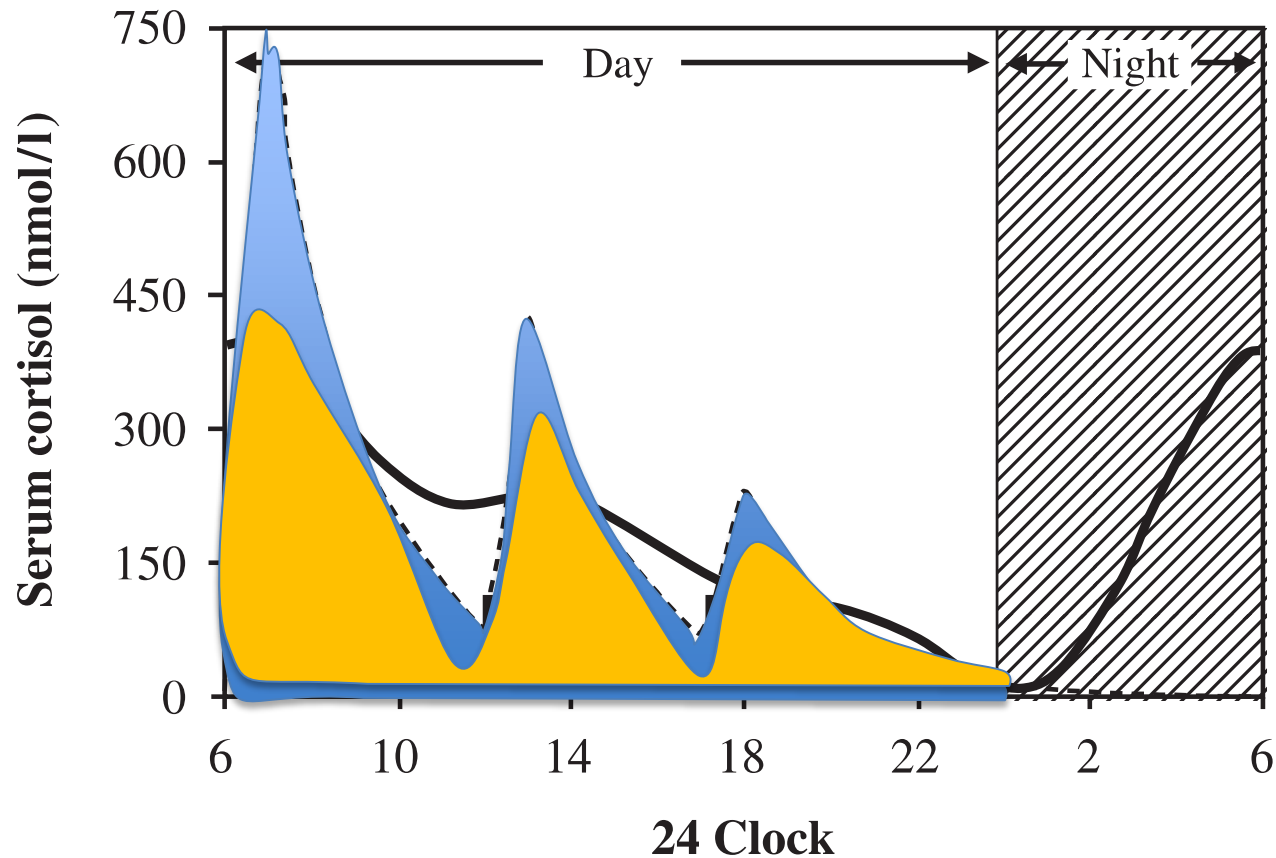
Table 1. Drugs of the top-100 best-seller list that target circadian genes and have half-life < 6h

Rank	Sales, \$	Trade name	Indications	Circadian-gene targets	Organs in which targets oscillate
2	1.46 b	Nexium	Gastritis, GERD, Esophagitis	<i>Atp4a</i>	L
5	1.28 b	Advair Diskus	Asthma, Chronic obstructive pulmonary di...	<i>Serpina6, Pgr, Nr3c2, Adrb2, Pla2g4a</i>	Lu, H, L, K, S, A
11	794 m	Rituxan	Rheumatoid arthritis, Non-Hodgkin's lymph...	<i>Fcgr2b, Ms4a1, Fcgr3</i>	L, K, S
20	538 m	Diovan	Hypertension, Heart failure	<i>Slc22a6, Agtr1a, Slco1b2, Car4, Kcnma...</i>	H, AG, L, K, S
27	431 m	Vyvanse	Attention deficit hyperactivity disorder	<i>Adra1b</i>	L
32	392 m	Tamiflu	Influenza	<i>Neu2, Neu1, Ces1g, Slc22a8, Slc15a1, ...</i>	Lu, L, BF, K, C
33	383 m	Ritalin	Attention deficit hyperactivity disorder	<i>Slc6a4</i>	AG, K
37	348 m	AndroGel	Hypogonadism	<i>Slc22a4, Slc22a3, Ar, Cyp1a1, Cyp2b10...</i>	Lu, H, BS, WF, AG...
38	346 m	Lidoderm	Pain	<i>Slc22a5, Cyp2b10, Egfr, Abcb1a</i>	Lu, H, AG, BF, L,...
44	304 m	Seroquel XR	Bipolar disorder, Major depressive disor...	<i>Htr2c, Htr1b, Htr2a, Chrm2, Drd4, Adr...</i>	Lu, H, BS, WF, AG...
45	289 m	Viagra	Erectile dysfunction	<i>Cyp1a1, Pde6g, Abcc5, Abcc10, Pde5a, ...</i>	Lu, H, BS, WF, AG...
47	281 m	Niaspan	Hyperlipidemia	<i>Slco2b1, Slc22a5, Qprt, Slc16a1</i>	Lu, H, BS, AG, WF...
48	279 m	Humalog	Diabetes mellitus T2	<i>Igf1r</i>	K
49	274 m	Alimta	Mesothelioma, Nonsmall cell lung cancer	<i>Tyms, Atic, Gart, Slc29a1</i>	Lu, H, BS, BF, L,...
54	267 m	Combivent	Asthma, Chronic obstructive pulmonary di...	<i>Slc22a5, Slc22a4, Chrm2, Adrb1, Adrb2</i>	Lu, H, BS, BF, K,...
56	262 m	ProAir HFA	Asthma, Chronic obstructive pulmonary di...	<i>Adrb1, Adrb2</i>	Lu, K, S
62	240 m	Janumet	Diabetes mellitus T2	<i>Slc47a1, Slc22a2, Prkab1, Abcb1a, Dpp4</i>	H, BS, AG, Hy, L,...
66	236 m	Toprol XL	Hypertension, Heart failure	<i>Slc22a2, Adrb1, Adrb2, Abcb1a</i>	Lu, H, AG, BF, L,...
71	220 m	Vytorin	Hyperlipidemia	<i>Hmgcr, Cyp2b10, Soat1, Abcc2, Anpep, ...</i>	Lu, H, BS, AG, BF...
78	209 m	Aciphex	Gastritis, GERD, Esophagitis	<i>Cyp1a1, Atp4a, Abcg2</i>	Lu, H, BS, WF, L,...
90	189 m	Lunesta	Insomnia	<i>Ptgs1, Tspo, Gabra3</i>	Lu, H, AG, K
98	173 m	Prilosec	Gastritis, GERD, Esophagitis	<i>Cyp1a1, Atp4a, Abcg2, Cyp1b1, Abcb1a</i>	Lu, H, BS, WF, AG...
99	171 m	Focalin XR	Attention deficit hyperactivity disorder	<i>Slc6a4</i>	AG, K

Rank and sales are based on USA 2013 Q1 data from Drugs.com. A, aorta; AG, adrenal gland; BF, brown fat; BS, brainstem; C, cerebellum; H, heart; Hy, hypothalamus; K, kidney; L, liver; Lu, lung; S, skeletal muscle; WF, white fat.

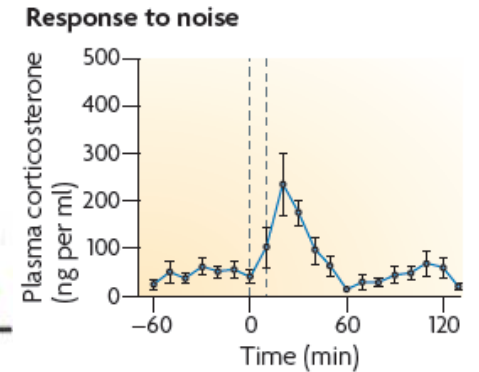
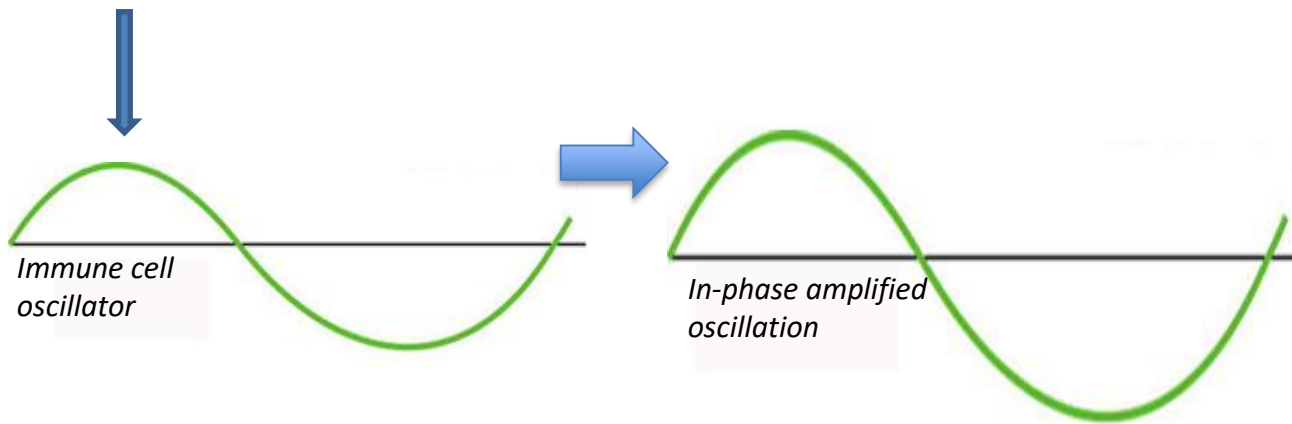


Modification of GC replacement should be undertaken with care



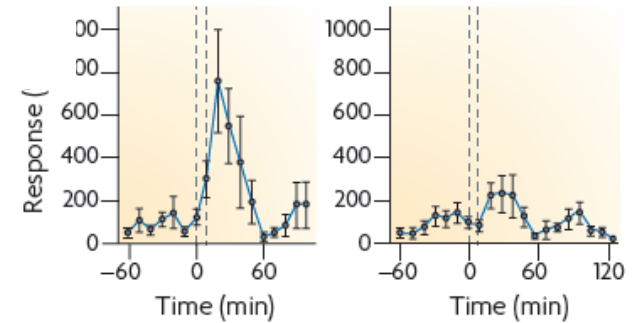
Stress response superimposed on cortisol rhythm

Single Pulse GC



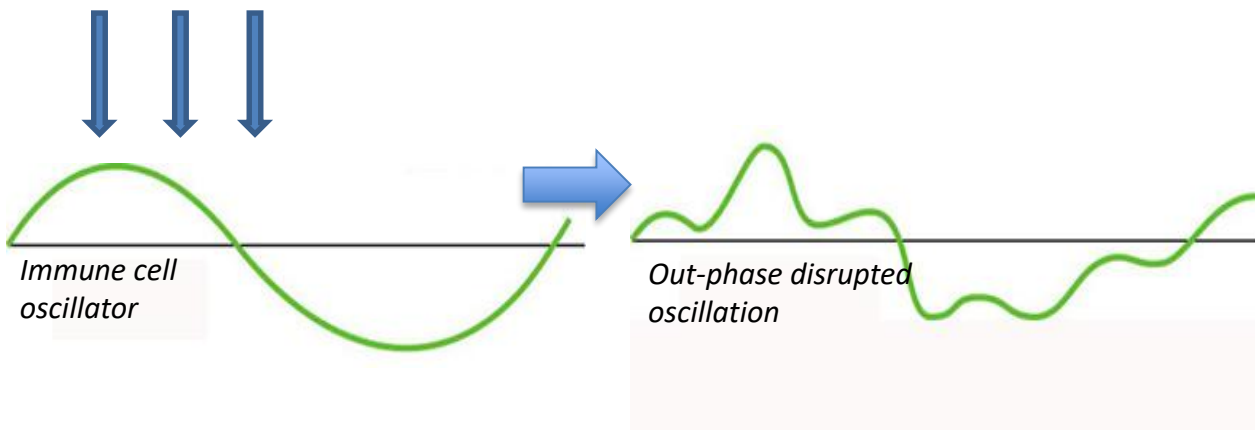
Rising

Falling



A stress coincident with the rising/falling (secretory) phase

Multiple Pulses GC



Open clinical issues?

Targeting rhythm in CS?

Patients feeding and HC

Night rise? Relevance ?
Early night vs. Late night

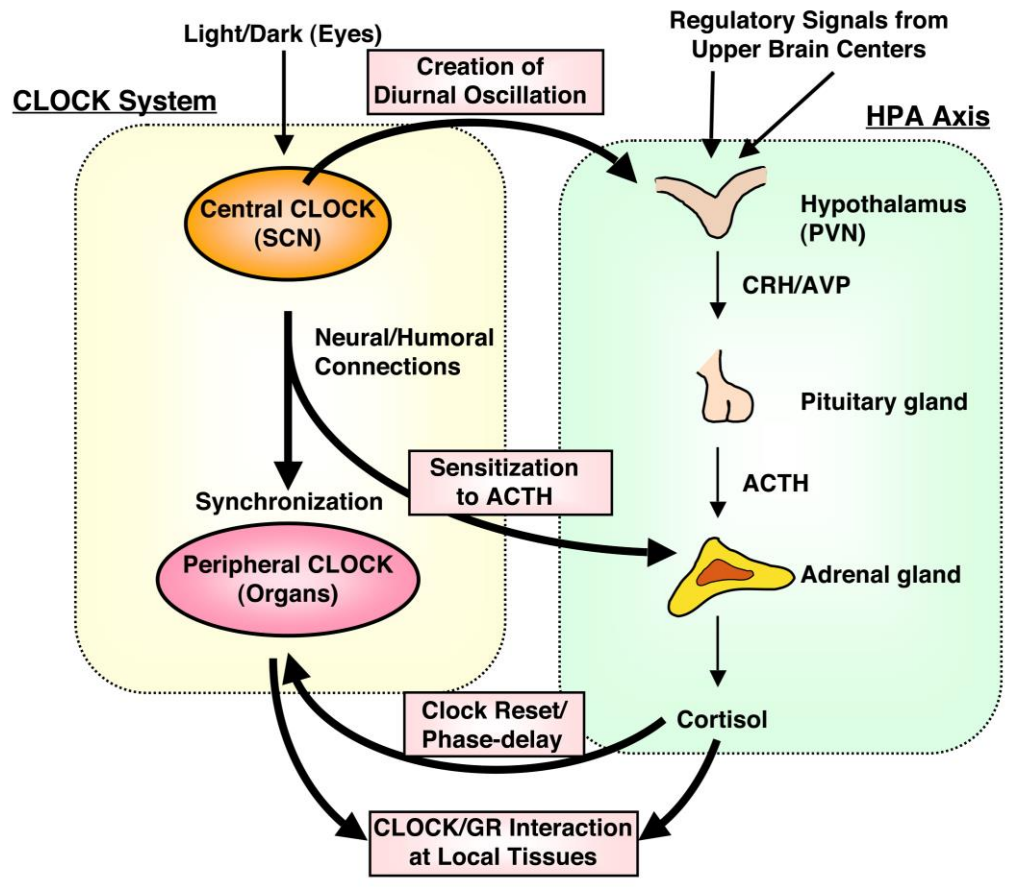
Mineralocorticoid administration?
Reverse rhythm?

Other Pharmacological zeitgebers?

Night shift vs. Jet Lag in AI patients?

Week-end time shift?

BEARING IN MIND THAT GC ARE THE POTENT SYNCHRONIZERS OF CENTRAL AND PERIPHERAL CLOCK



Thanks to all my LAB:

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Chiara Graziadio

Emilia Sbardella

Daniela Fiore

Valeria Hasenmajer

Elisa Giannetta

Daniele Gianfrilli

Riccardo Pofii

Giulia Puliani

Marianna Minnetti



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