Cirkadiánní Rytmus a Imunitní Odpověď

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Ostrava 30.ledna 2018

DISCLOSURE ……NONE

£825,000 prize shared between American scientists Jeffrey C Hall, Michael Rosbash and Michael W Young for work on the internal clock of living organisms

The American scientists Jeffrey C Hall, Michael Rosbash and Michael W Young, who have won this year's prize. Illustration: NobelPrize.org

In 1984, Jeffrey Hall and Michael Rosbash at Brandeis University in Waltham, Massachusetts, studied the period gene and the protein the body makes from it. They showed that the protein, named PER, built up in cells overnight before being broken down in the daytime

In 1994, Michael Young at Rockefeller University showed that this kind of feedback loop was indeed at work. He discovered a second body clock gene that is used to make a protein called TIM. When TIM proteins come across PER proteins in cells, the two stick together, move into the nucleus, and shut the period gene down.

Rosbash heard he had won the prize when the Nobel committee chair called about 5am local time. **"I am very pleased for the fruit fly," he said. "When the landline rings at that hour, normally it is because someone died."**

Octomilka obecná

Drosophila melanogas

FIGURE 1

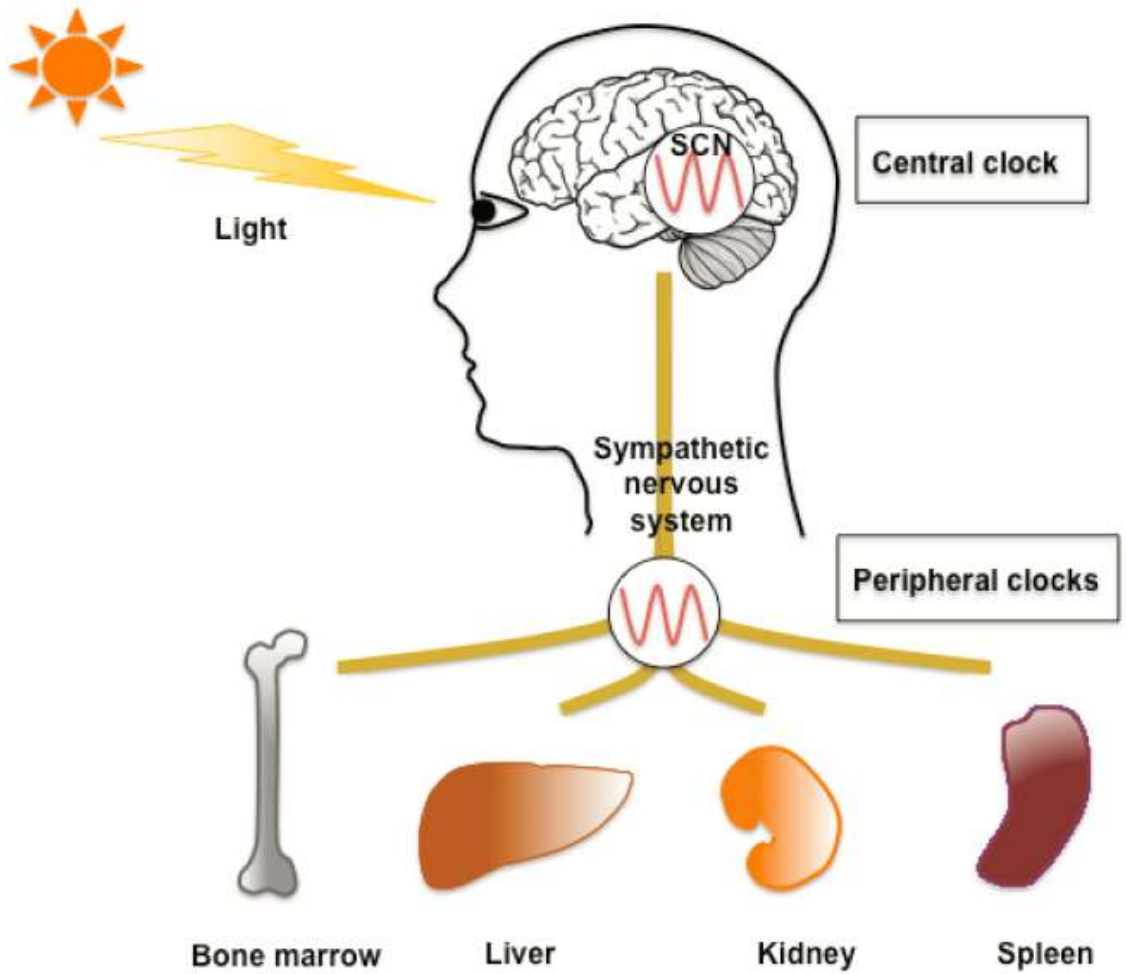
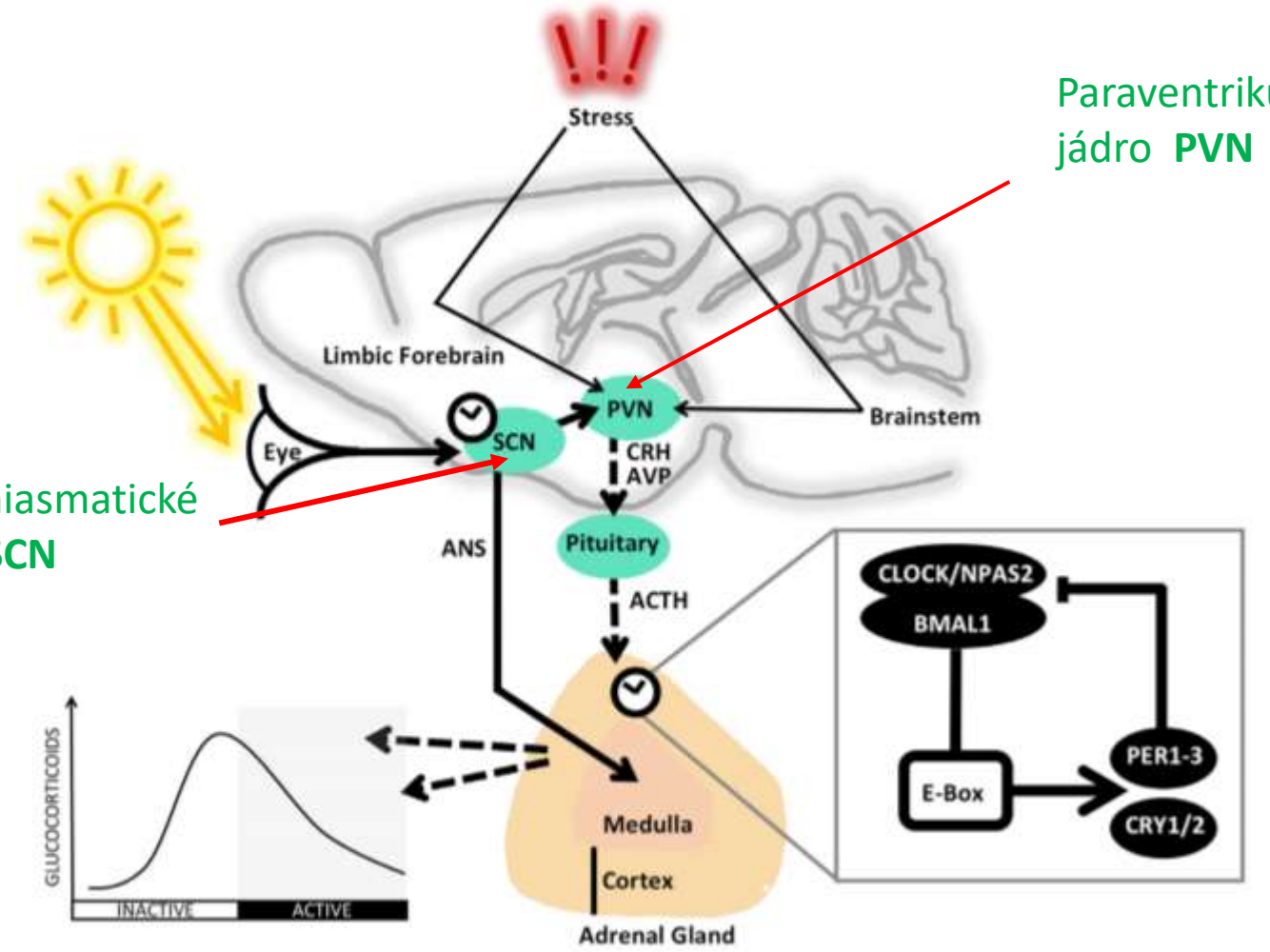


FIG. 1: Synchronization of internal biological rhythms by external cues. Light is the predominant environmental cue and is processed vial the retina, leading to the synchronization of rhythms in hypothalamic suprachiasmatic nuclei (SCN), which constitute the master clock of the organism. Humoral and neural output systems then modulate clocks in peripheral tissues.

Centrální a periferní hodiny

30% genomu je pod kontrolou cirkadiánních rytmů



Paraventrikulární jádro **PVN**

Suprachiasmatické jádro **SCN**

CLOCK
circadian locomotor output cycles kaput

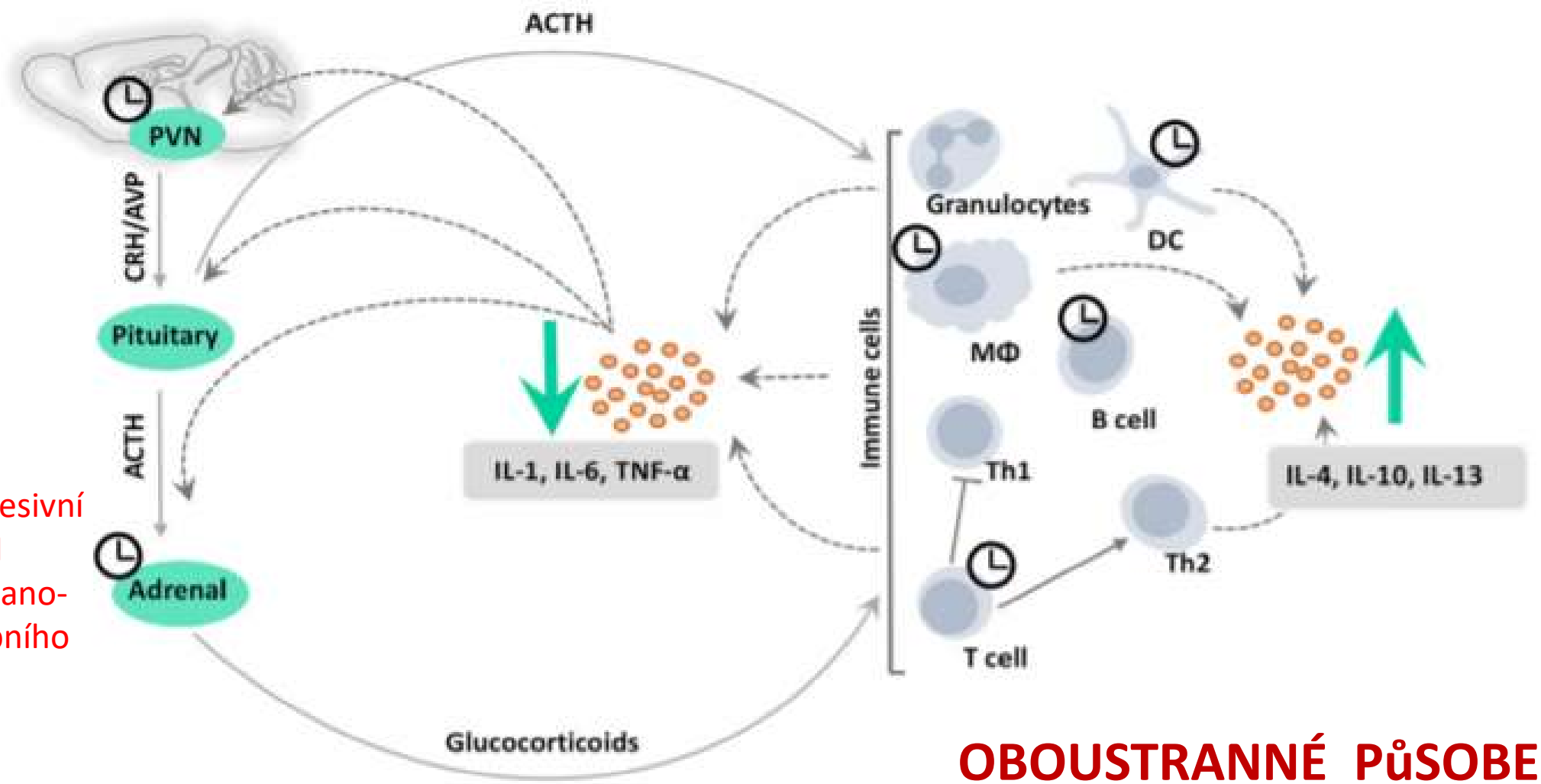
NPAS2
neuronal PAS domain-containing protein 2

BMAL1
brain and muscle aryl hydrocarbon receptor nuclear translocator-like 1

PER1-3 *Period genes*
CRY1/2 *Chlyctochrome genes*

FIGURE 1 | The rhythmic control of the HPA axis is regulated at several levels. The master clock residing in the suprachiasmatic nucleus (SCN) is synchronized by light information received *via* the retinohypothalamic tract from the eye in order to exert autonomic (ANS) and hormonal influence on the clocks and rhythms of downstream tissues of the body. In addition to the direct innervation of the adrenal, the SCN influences the paraventricular nucleus (PVN) to secrete corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP), which reach the pituitary *via* the blood portal system to stimulate secretion of adrenocorticotropic hormone (ACTH), which activates production and release of glucocorticoids. In addition, local adrenal clocks are thought to regulate responsiveness to ACTH in a circadian fashion. The baseline circadian rhythm of circulating glucocorticoids peaks just before the beginning of the active phase (day in humans and night in rodents). Stress-induced stimulation of the HPA axis acts *via* afferent signals from the limbic forebrain and brainstem to the PVN. Inset: the core transcriptional–translational feedback loop (TTL) that makes up the molecular circadian clockwork. In the positive arm of the clock, CLOCK or NPAS2 form a complex with BMAL1 and bind to *E-Box* elements in the gene promotors of PERs and CRYs, which make up the negative arm and act to inhibit the activity of CLOCK–BMAL1 or NPAS2–BMAL1, with a cycle of roughly 24 h. For further detail, see the main text.

Přímý imunosupresivní efekt ACTH cestou melano-kortikotropního systému



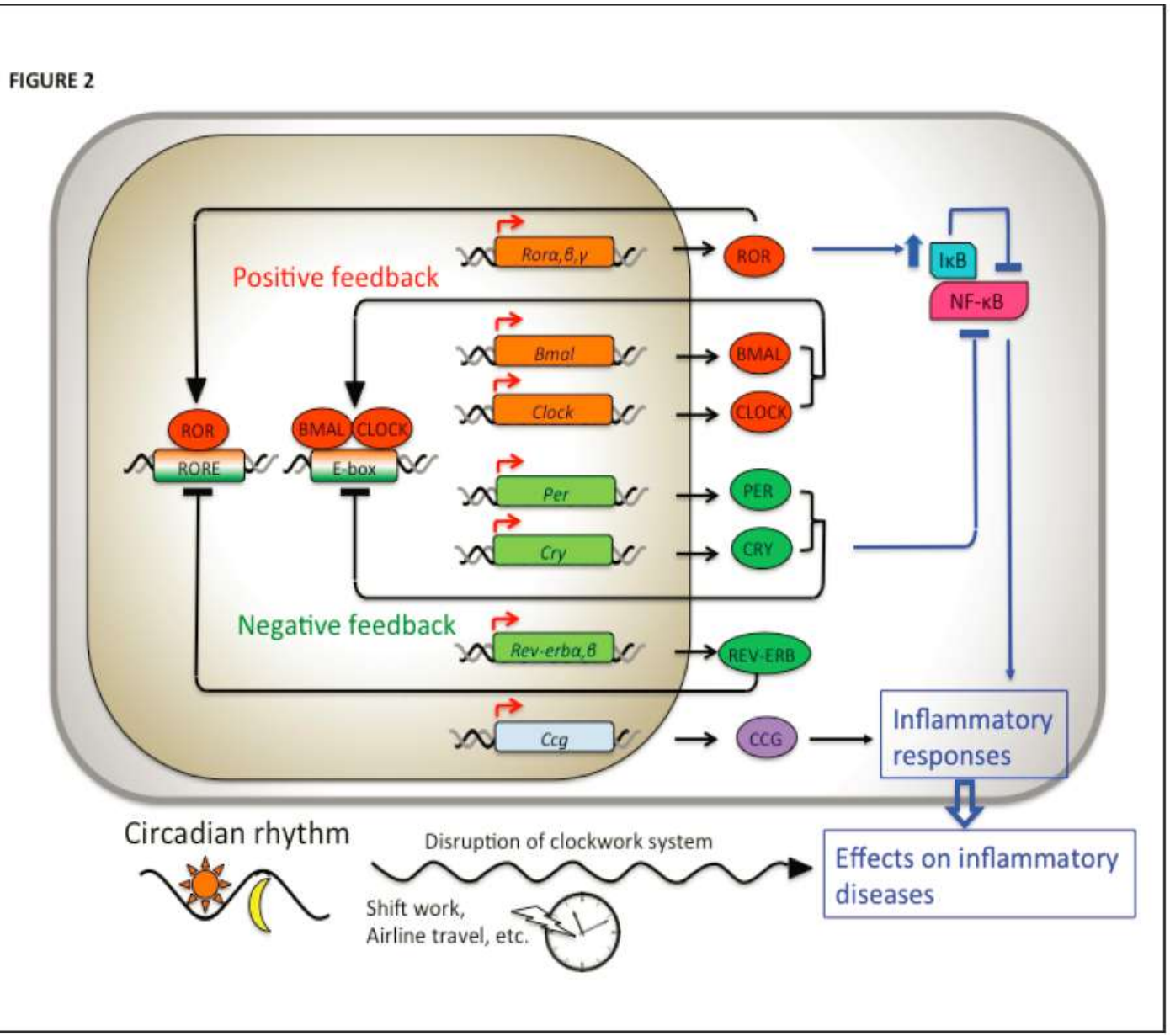
OBOUSTRANNÉ PůSOBENÍ

FIGURE 2 | Circadian clocks in HPA axis-immune system crosstalk. Immune cells can activate the HPA axis *via* cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukins (IL-1/6) at the level of the paraventricular nucleus (PVN) of the hypothalamus as well as at the pituitary and adrenal, stimulating the production of glucocorticoids. Glucocorticoids in turn act on the receptors on the surface or in the cytoplasm of immune cells to suppress the induction of pro-inflammatory responses, and to promote a shift from T helper cell type 1 (Th1) toward T helper cell type 2 (Th2)-mediated humoral immunity. This inhibits the production of pro-inflammatory cytokines, while promoting the production of anti-inflammatory cytokines, such as interleukin-4, interleukin-10, and interleukin-13 (IL-4/10/13) by various immune cells. In addition, ACTH exerts direct anti-inflammatory and immune-modulating effects *via* the melanocortin system. CRH, corticotropin-releasing hormone; AVP, arginine vasopressin; DC, dendritic cell; MΦ, macrophage.

HPA osa a regulace stresu

- GKS – jejich produkce hraje klíčovou roli v systémové koordinaci cirkadiánních rytmů resetováním buněčných hodin na úrovni suprachiasmatického jádra (SCN)
- Negativní feedback GKS působí na hladinu CRH v hypothalamu a ACTH v hypofýze
- PVN CRH exprese je nepřímo kontrolována prostřednictvím SCN
- GR- R – exprimovány v mozku a periferních orgánech, ale nejsou v SCN
- GR vážou glucocorticoid response elements (GRE or nGRE) s následnou regulací transkripce cílových genů
- GRE jsou přítomny v promotorové oblasti „clock genů“ Per1, Per2, Npas2, a tak jsou tyto geny účastny prostřednictvím extracelulárních GKS a intracelulárního cAMP nebo hladiny Ca v synchronizaci cirkadiánních rytmů

Autoregulace BMAL a CLOCK komplexu cestou interakce S PER (Period genes) a CRY (Clyctochrome genes)



Proteiny CLOCK a BMAL1 se vážou na E elementy v genech kodujících *CRY, PER a REV-ERBA*.

PER a CRY poté translokují do jádra a způsobují represi jejich vlastní exprese interferencí s BMAL1/CLOCK komplexem v promotorové oblasti genu.

RORs(retinoic acid receptor-related orphan receptors) společně s *Erb α/β (REV-ERB*) což jsou jaderné receptory rovněž translokují do jádra a ovlivňují expresi BMAL1 RORs aktivují , REV-ERBs dělají represi exprese BMAL1

Takže *ROR, BMAL a CLOCK* vykazují pozitivní feedback na genovou expresi BMAL a CLOCK, zatímco *PER, CRY a REV-ERBs* vykazují negativní feedback

Kromě toho *CRY* limituje *phosphorylaci p65 a NFkappa B aktivace,* čímž reguluje expresi zánětlivých parametrů

BMAL1/CLOCK komplex kontroluje expresi clock-controlled genes (CCG), které jsou spojeny se zánětlivými funkcemi

Lokální regulace cirkadiánního rytmu

- „Cirkadiánní hodiny“ v nadledvinkách hrají roli v regulaci HPA osy prostřednictvím produkce GKS a jejich citlivosti vůči ACTH
- the lokální „adrenal clock“ jsou důležité pro regulaci cirkadiánního glukokortikoidního rytmu nezávislém na systémových faktorech jako je např. stres.

frontiers in Cellular and Infection Microbiology

MINI REVIEW
published: 10 May 2016
doi: 10.3389/fcimb.2016.00054

Circadian Control of Antibacterial Immunity: Findings from Animal Models

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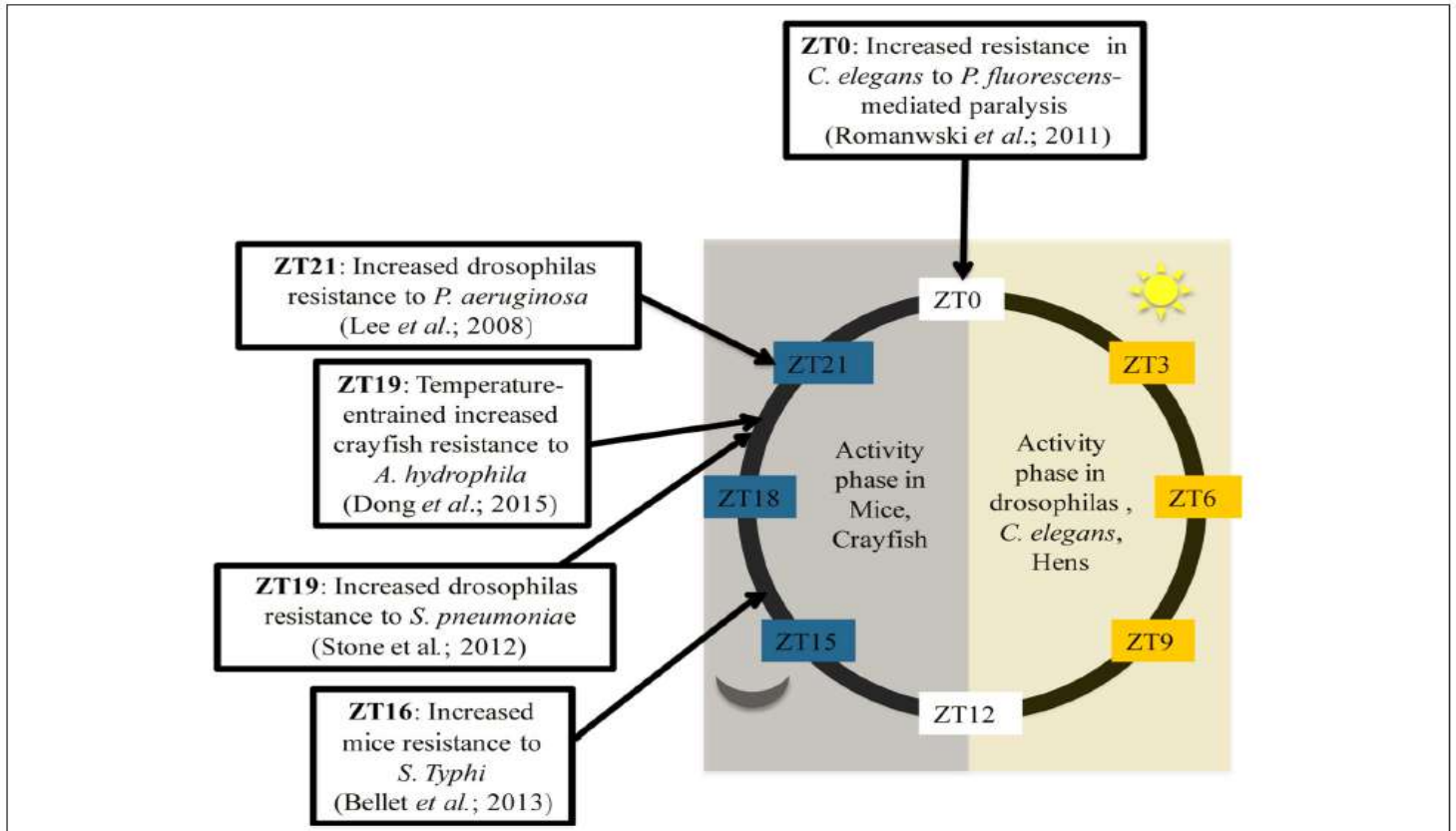
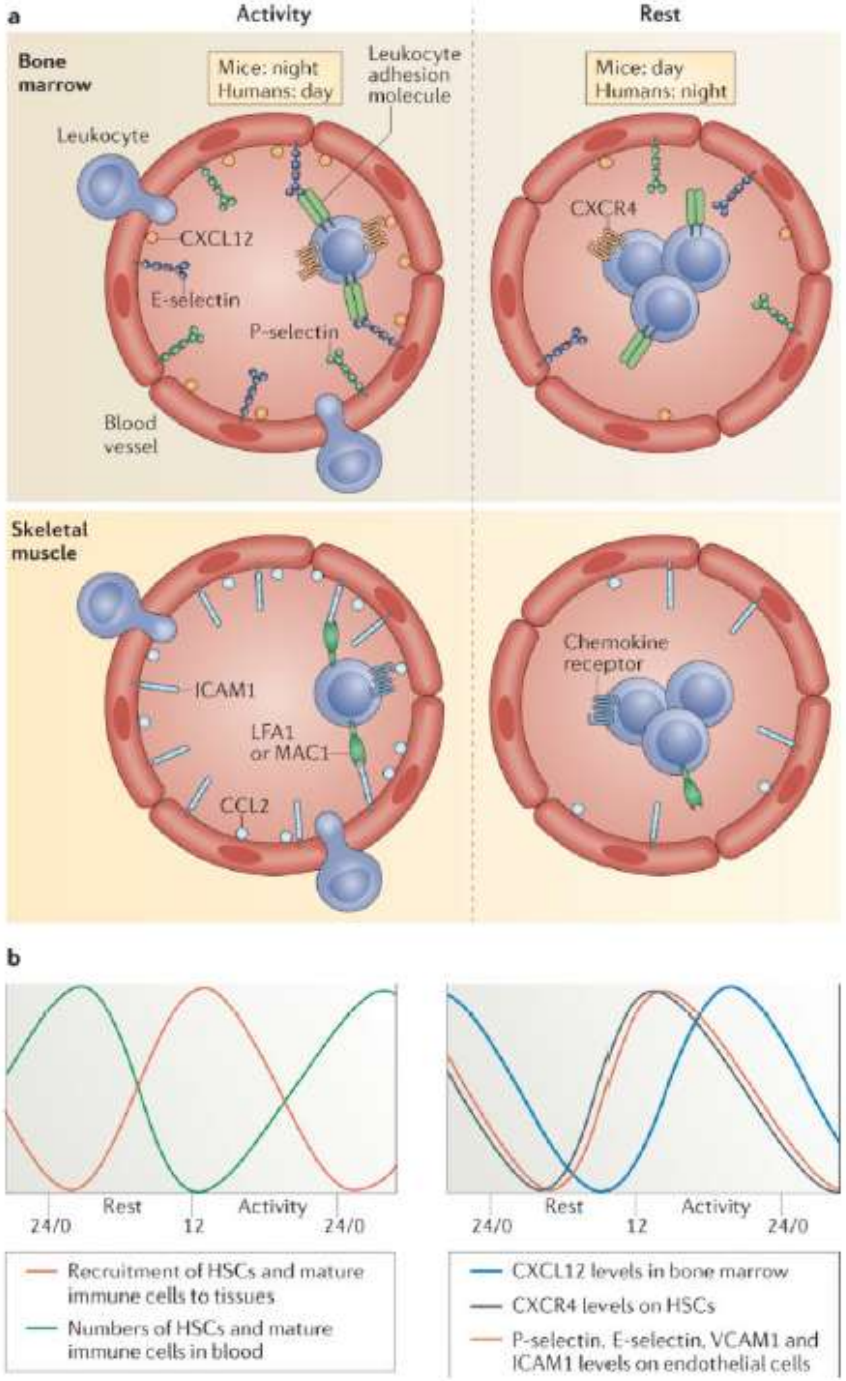


FIGURE 1 | Time of day higher tolerance to bacterial infection in mice, Drosophilas, *C. elegans*, and crayfish. ZT is corresponding to an experimental time referring to the onset of a zeitgeber (light or temperature). ZT0 is the transition time from dark to light while ZT12 is the transition time from light to dark. Activity phase is corresponding to the phase where movements or displacements are most recorded.



Rhythms in immune cell function

Species	Source	Oscillating parameter	Acrophase	Trough	Refs
Acrophase during the resting phase					
Human	Blood	Neutrophils	CT20	CT8	25
		Naive, central memory, effector memory $CD4^+$ and $CD8^+$ T cells	CT2	CT14	26
		HSCs	CT20	CT8	29
		HSC mobilization from the BM	Afternoon	Morning	
Mouse	Blood	Neutrophils, monocytes, lymphocytes, eosinophils	ZT5	ZT13	7
		HSCs	ZT5	ZT13	28
		HSC mobilization from the BM			29
	Spleen	Macrophages, B cells	CT8	CT16	
Rat	Spleen/lymph node	Splenocyte proliferation	CT9-13	CT21-1	30
Acrophase during the active phase					
Human	Blood	Effector $CD8^+$ T cells	CT14	CT2	26
		Cortisol, epinephrine, norepinephrine	CT8-11	CT1-5	26
	Naive, central memory $CD4^+$ and $CD8^+$ cells	CXCR4	CT9	CT21	26
	Mature CD8+ cells	CX3CR1	CT9	CT21	26
Mouse	Bone marrow Muscle/liver	Neutrophil/HSC homing Monocyte/neutrophil recruitment	ZT13	ZT1-5	7
	Bone marrow	CXCL12	ZT21	ZT9	28
	Bone marrow ECs Muscle ECs	P-selectin, E-selectin, VCAM-1 ICAM-1, *Ccl2*	ZT13	ZT5	7
	HSCs	CXCR4	ZT13	ZT5	29
	Splenic B cells/ Macrophages	TLR9	ZT19	ZT7	9
	Splenic macrophages	*Tnf, Ccl2*	CT16-20	CT24	8
Mouse/ Rat	Neutrophils	Phagocytic activity	CT3-4	CT10-16	40
Rat	NK cells	Granzyme, Perforin, IFNv, TNF	ZT14-24	ZT2-6	39
	Muscle	Neutrophil recruitment	CT17	CT11	35

CT: actual circadian time in hours (e.g. CT6 = 6AM); ZT: Zeitgeber time: time after the onset of light with lights on at ZT0/24 and off at ZT12; EC: endothelial cells; HSC: hematopoietic stem cell; BM: bone marrow.

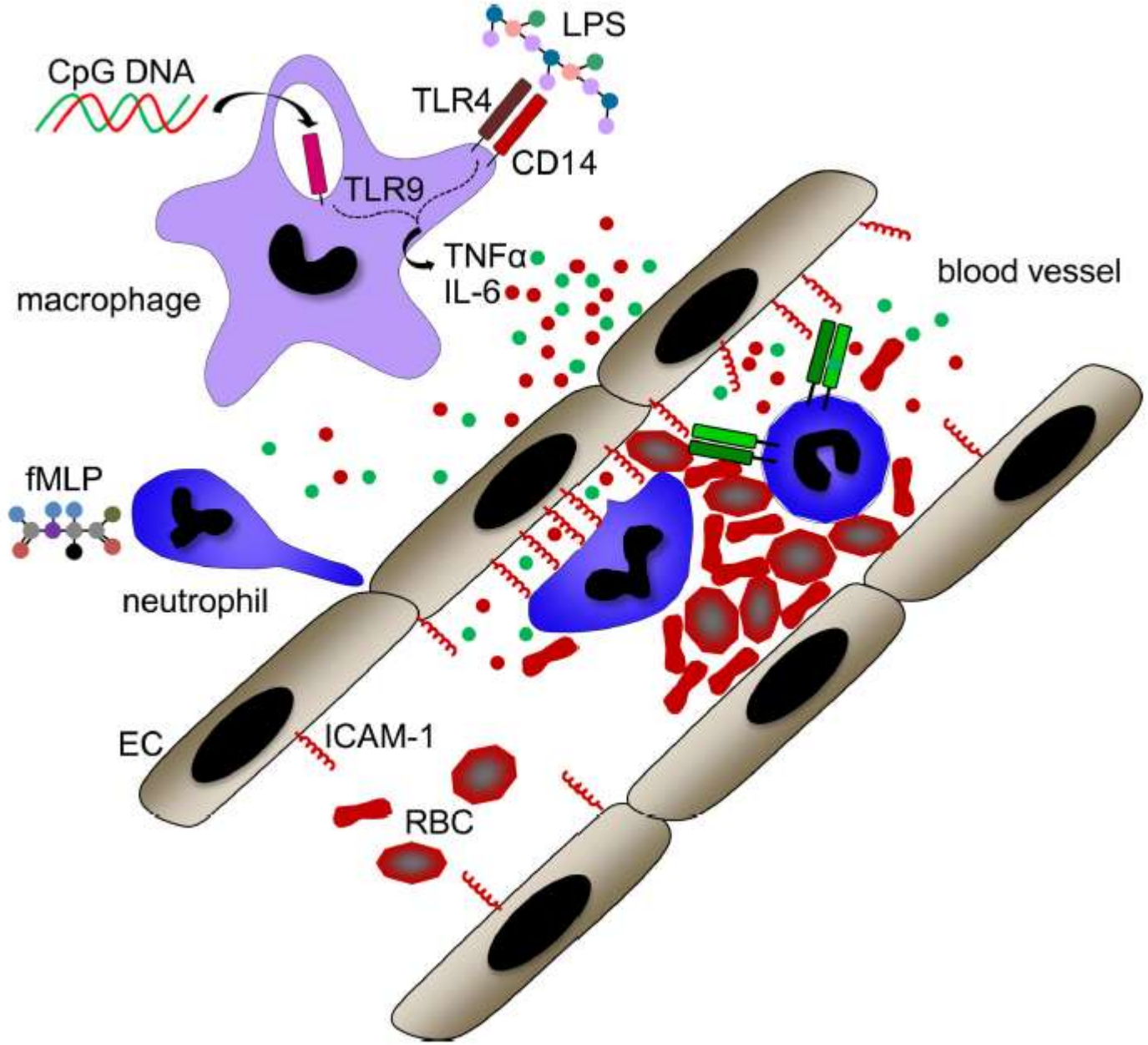


Figure 3. Contributing factors in circadian disease onset

Migrace leukocytů je pod kontrolou cirka-diánních rytmů jednak při jejich uvolnění z kostní dřeně a následně při jejich recruitmentu do tkání.

Cirkadiánní systém a „hodinové“ molekulární mechanismy

působí na vrozenou i adaptivní imunitu

Vrozená : monocyty a makrofágy, NK buňky
- Interakce PRRs s bakteriálními motivy
- Exprese cytokinů v důsledku působení LPS
- Fagocytóza
- Recruitment leukocytů do tkání

Adaptivní : Lymfocyty B a T

In vitro a in vivo T buněčná odpověď

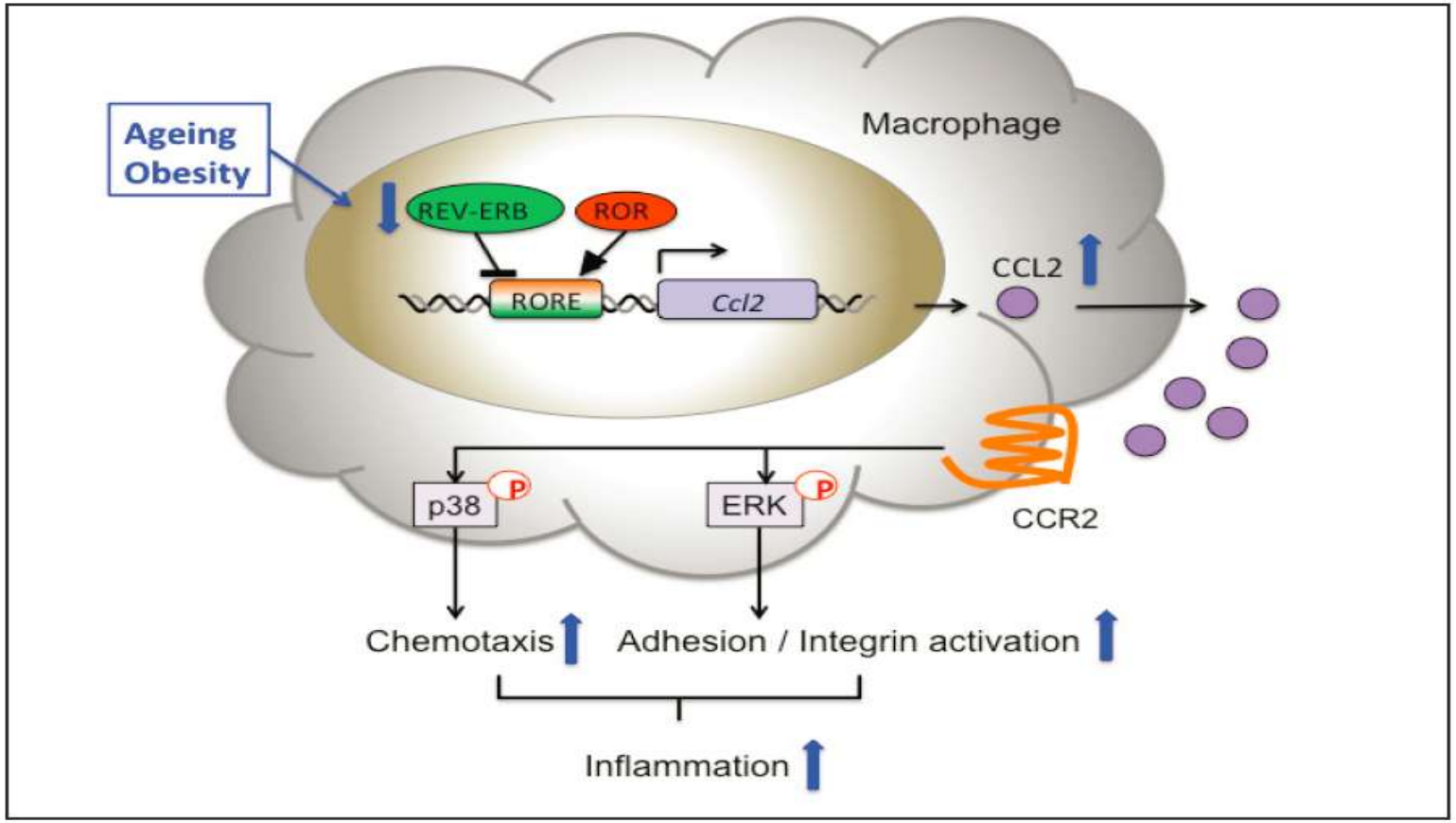


FIG. 3: Regulation of the murine *Ccl2* promoter activity and modulation of the inflammatory functions of macrophages by *Rev-erbα* and *RORα*. REV-ERBα represses *Ccl2* expression via a RORE in its promoter. As a result, inflammatory functions of macrophages, such as adherent and migratory activities, are suppressed by decreased secretion of CCL2. In contrast, RORα enhances Ccl2 expression via a RORE in its promoter. As a consequence, the inflammatory functions of macrophages are promoted by increased secretion of CCL2. Aging and obesity, known as chronic and systemic inflammatory conditions, dampen *Rev-erbα* gene expression in murine peritoneal macrophages, suggesting that REV-ERBα plays a potential role in the regulation of inflammatory function of macrophages. CCR2: CC chemokine receptor 2.

Role „hodinových“ molekul u zánětu – BMAL1

brain and muscle aryl hydrocarbon receptor nuclear translocator-like 1

- Jedna z hlavních molekul
- Nepřítomnost (knock out myši) vede k alteraci funkce „hodin“ a ztrátě kontroly zánětu
- BMAL1 deficience byla zjištěna u diety bohaté na tuky…chronická zánětlivá odpověď tkáně, insulinová resistence a hyperglykémie
- Exprese TLR9 má rovněž cirkadiánní charakter – přímá regulace přes CLOCK/BMAL1 komplex a jeho vazbu na E box přítomný v promotorové oblasti genu pro TLR9

Role hodinových molekul u zánětu - CLOCK

- *circadian locomotor output cycles kaput*
- Vykazuje aktivitu histon acetyl transferázy a inhibuje vazbu GKSR na cílový gen acetylací tohoto receptoru
- Jeho absence je škodlivá u systémové infekce (experimenty s mutovanými formami CLOCK)

Cirkadiánní rytmus a sepse (Period genes)

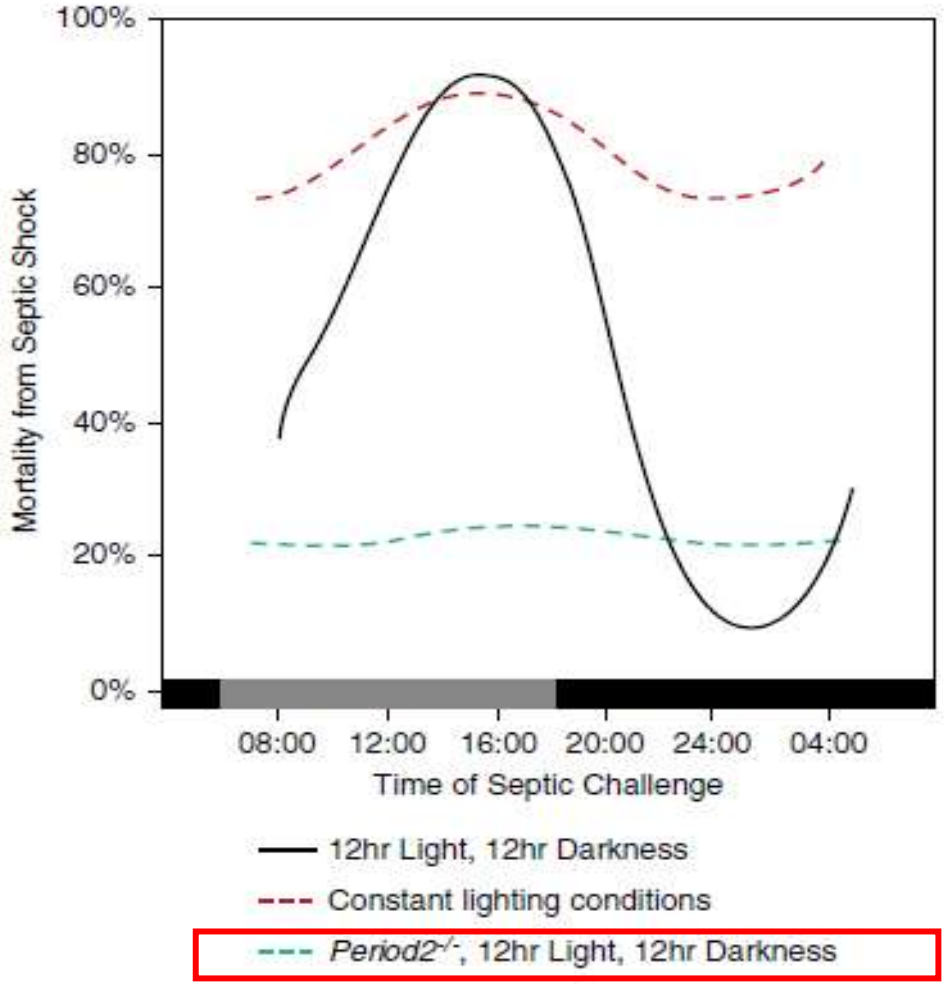
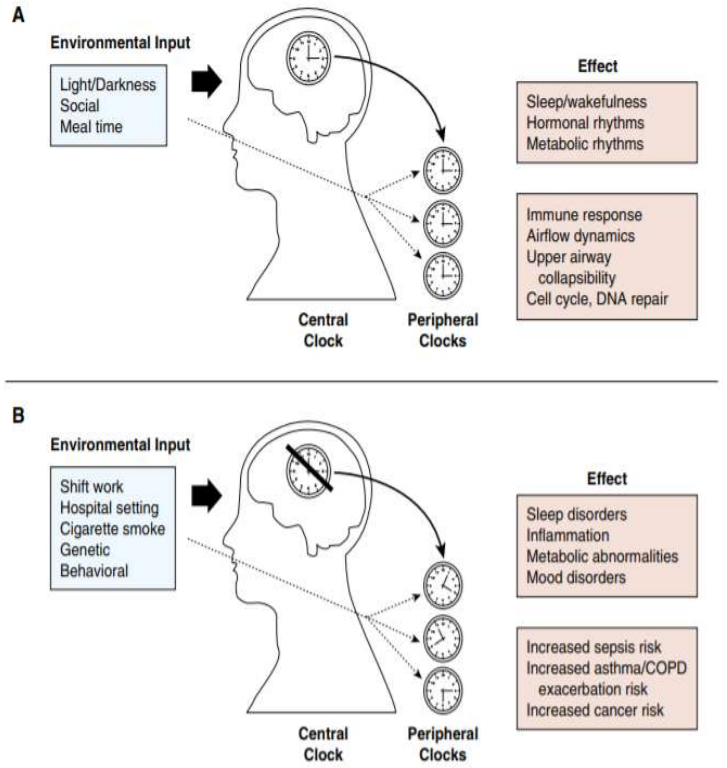


Figure 2. Circadian effect on mortalities from animal model of septic shock. Mortality rate from septic shock exhibited a strong diurnal pattern dependent on the timing of the septic challenge (*black line*) (31). The diurnal variation diminished when subjects were housed in conditions with constant lighting (*red dashed line*) (39). Knockout of *Period 2* also abolished the diurnal variation even when the subjects were housed in normal 12-hour light/12-hour dark conditions (26).



SOUHRN

- Cirkadiánní rytmy ovlivňují vrozený i adaptivní systém imunity
- Imunologické mechanismy – buněčné i humorální ovlivňují cirkadiánní rytmy
- Leukocyty oscilují s maximem množství ve fázi odpočinku, zatímco recruitment do tkání nastává v době aktivity – změny jsou mediovány chemokiny a adhesivními molekulami
- Odpověď organismu k zánětlivým podnětům včetně infekce je vázána na cirkadiánní oscilaci. Mechanismy jsou kombinací migrace leukocytů, expresí patogen – sensitivních receptorů a fagocytární aktivity leukocytů.
- Tato obousměrnost dává předpoklad k cílenému terapeutickému působení u řady onemocnění včetně sepse