Biorhythms - studied by chronobiology

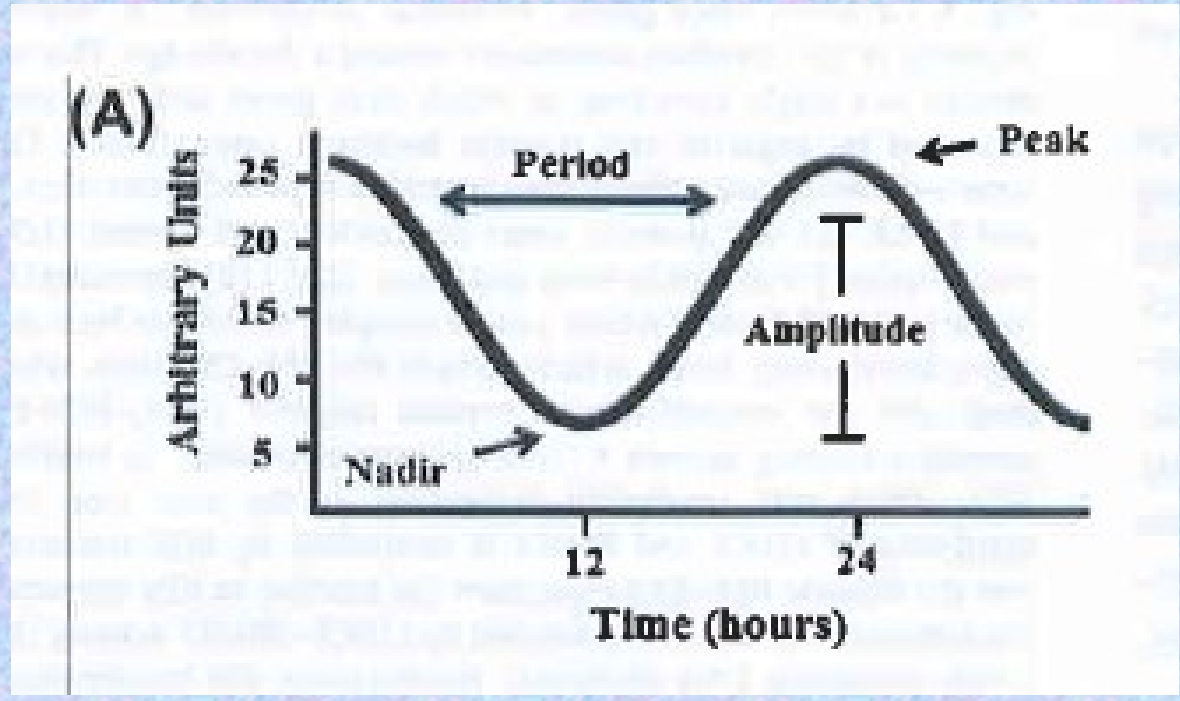
as a special branch of physiology research

In humans and other mammals, processes such as sleep/awake and feed/fast cycles, body temperature oscillations, hormonal secretions and metabolic events occur in a circadian manner and are ruled by biological clock

rhythm period: the time that elapses

before a biological variable enters the same phase

- most often described as sinusoidal curve



Periods of rhythms (a frequency with which the parametr is repeated):

Ultradian period – period is shorter than 24hours (e.g.:10sec rhythm in breathing)

Circadian period – period is 24h (sleep/wakefulness…..from Latin *circa diem* -about a day)

Infradian period – period is longer than 24h (the menstrual cycle)

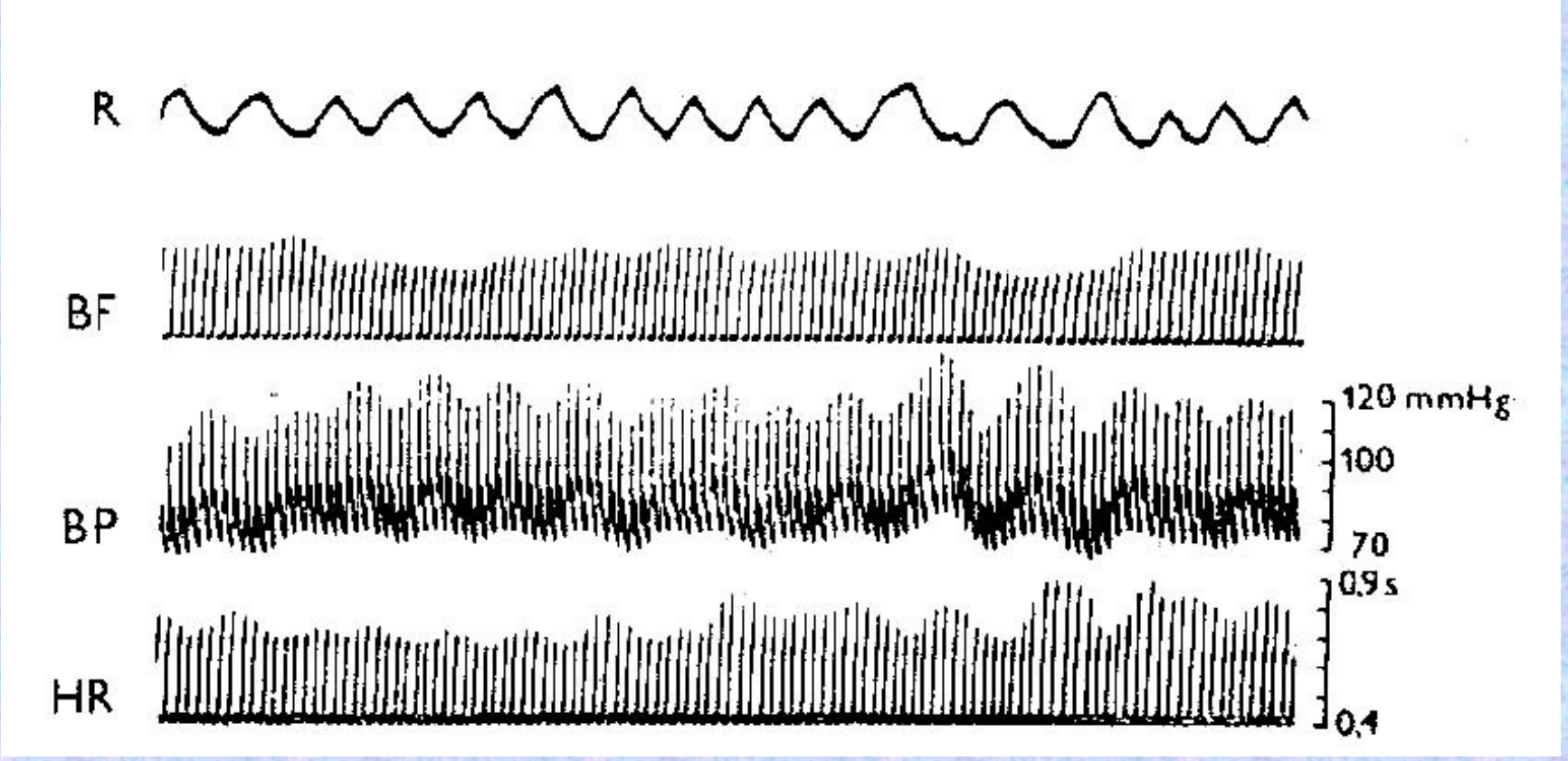
Information from previous lectures:

Type of secretion of hormones - summary:

- **Constant secretion** – hormones of glandula thyreoidea
- **Pulsatile secretion** – GnRH (gonadoliberin)
- **circadian secretion** (latin: circa diem = „approximately“/“around“ 24h) – hormons of adrenal cortex
- **monthly fluctuation** – estrogens, testosteron in saliva
- **„en demande“** (according to need - demands) – e.g. Insuline and regulation blood glucose

Records of breathing and circulatory parameters waves

(from plethysmomanometer - Peňáz method)



„Our internal clock“ – how it works

The molecular clockwork

- Period genes (PER) was the first discovered clock gene which is conversed from fruit flies to humans……1971 (Konopka and Benzer)
- In the past decades our knowledge of the molecular clockwork has been significantly expanded
- **Transcriptional-translation feed-back loop -** simplified explanation
  - Two helix-loop-helix transcription factors:

    CLOCK – circadian locomotor output cycles kaput

    + BMAL1 (ARNTL) – brain and muscle aryl hydrocarbon receptor nuclear translocator.

    Both form heterodimers via their PAS domein – PER-ARNTL-SIM binding.

    Activates E-box-element containing gens.

    Complexes CLOCK+BMAL1 activate transcription of PER and CRY genes during the day. PERs and CRYs translocate into the nucleus and forms inhibitory complexes, PER/CRY complexes accumulate and does their inhibitory effect on CLOCK-BMAL1 activity, shutting down Per and Cry transcription during the night.

- PER – period gene
- CLOCK – circadian locomotor output cycles kaput
- BMAL1 (ARNTL) – brain and muscle aryl hydrocarbon receptor nuclear translocator
- PAS domena – PER-ARNTL-SIM
- E-Box –controlled genes
- CRY genes – cryptochrome

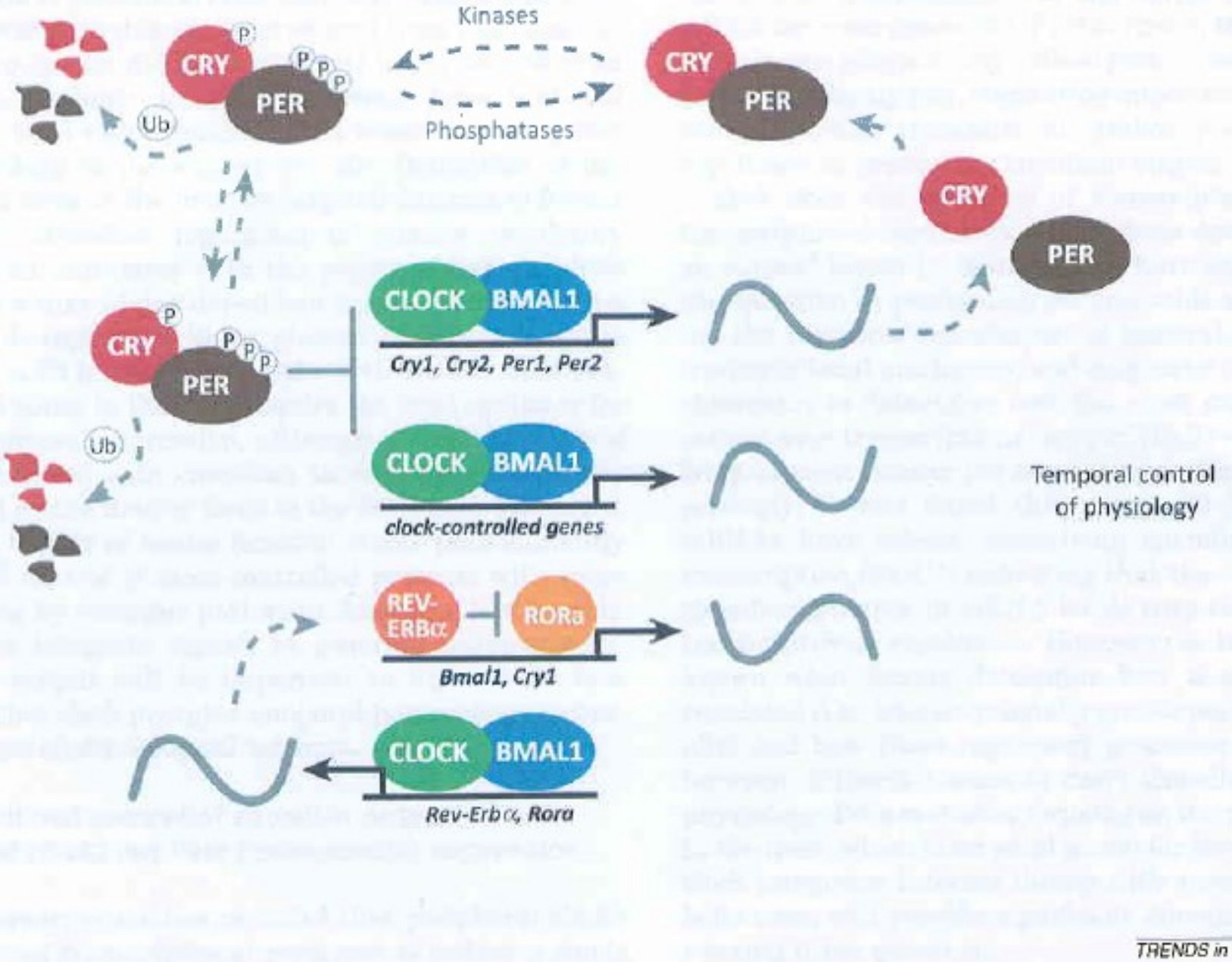


Figure I. Temporal control of physiology via four integral clock proteins: two activators (CLOCK, circadian locomotor output cycles kaput; and BMAL1, brain and muscle ARNT-like 1) and two repressors (period, PER; and cryptochrome, CRY).

Box 1. The molecular clock in mammals

The cell-autonomous molecular clock in mammals is generated by two interlocking transcription/translation feedback loops (TTFL) that function together to produce robust 24 h rhythms of gene expression. The core TTFL is driven by four integral clock proteins: two activators (CLOCK and BMAL1) and two repressors (PER and CRY), as well as by kinases and phosphatases that regulate the phosphorylation (P) and thereby localization and stability of these integral clock proteins (kinases: CKIα, CKIδ, and CKIε; phosphatases PP1, PP5). CLOCK and BMAL1 are subunits of the heterodimeric basic helix-loop-helix-PAS (PER-ARNT-SIM) transcription factor CLOCK:BMAL1 [59], which activates transcription of the repressor *Per* and *Cry* genes, as well as other clock-controlled output genes. PER and CRY proteins heterodimerize in the cytoplasm and translocate to the nucleus to interact with CLOCK:BMAL1, inhibiting further transcriptional activation. As PER and CRY proteins are degraded through ubiquitin (Ub)-dependent pathways [72,73,109–111], repression on CLOCK:BMAL1 is relieved and the cycle begins again with ~24 h periodicity (Figure I). The casein kinases CKIδ and CKIε play an

Where our internal clock is located ?
Master circadian pacemaker

- Information about the external light-dark cycle was passed via the **retinohypothalamic tract** – sensory input integrating centers in the thalamus, but also to the hypothalamic **suprachiasmatic nucleus**
- SCN – is a bilaterally paired structure with high cell body density located to the third ventricle and directly atop the optic chiasm.
  - It comprises about 50 000 neurons in humans (in rodents 20 000)
  - Plays critical role in generation of mammalian circadian rhythms
  - (in experiments – animal with ablated SCN become behaviorally and physiologically arrhythmic. Critically, transplanting isolated SCN tissue into SCN-lesioned animals restores circadian rhythmicity)

In humans: circadian rhythm

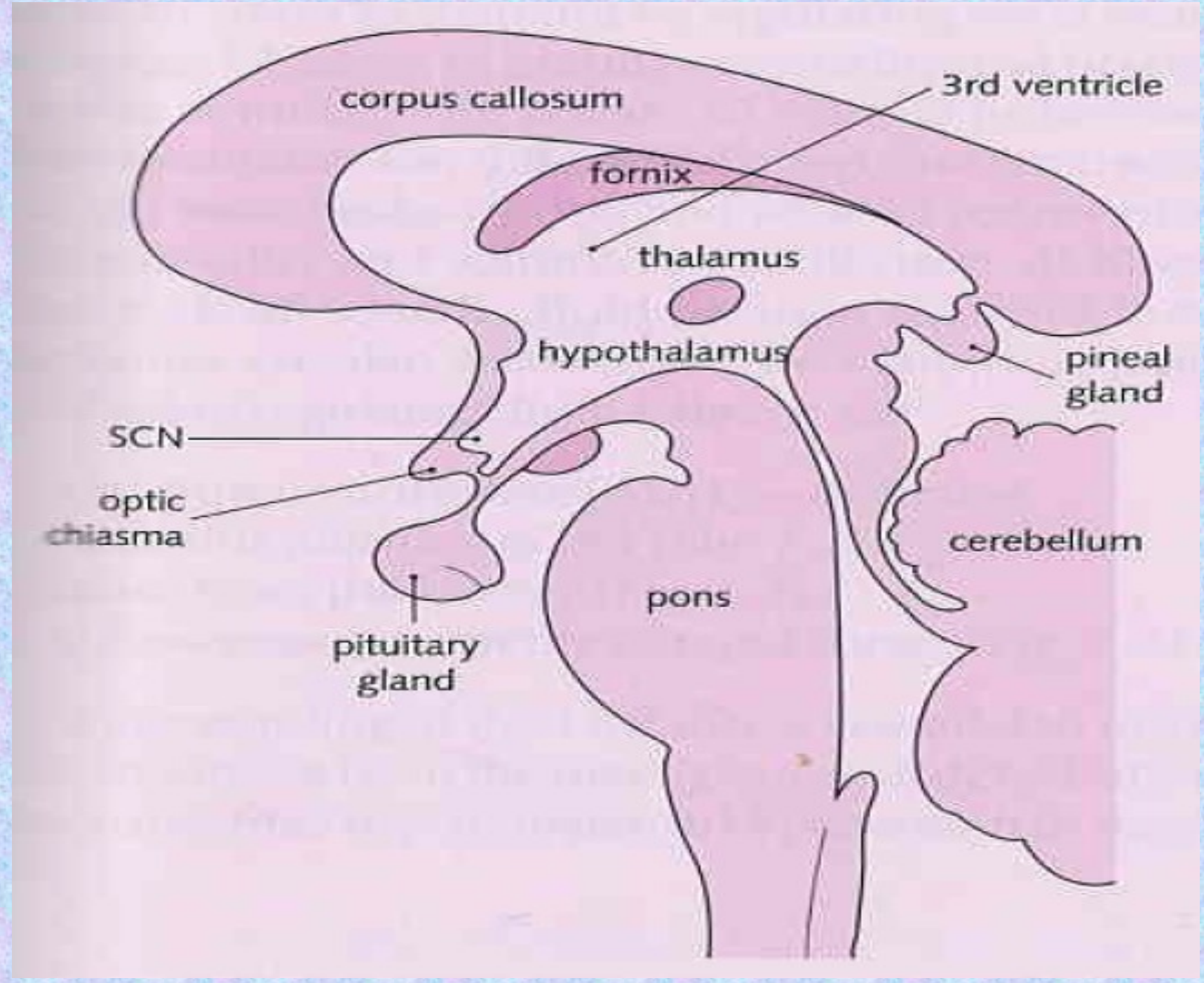
- Perioda of rhythm 25±1.5 hours

- Synchronization via external conditions:
- E.g. Light/dark phase of day
- Or fluctuation of external temperature (e.g. in blind people)
- Or cycle of food intake (experiment in caves)
- Or social stimulus

- Entry: retinal ganglionic cells – photopsine – melanopsin (blue wavelenght)- SCN – main oscilator
- Pathway: neuronal and humoral

Pineal gland

- The pineal gland coordinates circadian (daily) rhythms of dark/light (day-night) cycles by secreting the hormone melatonin. Darkness stimulates its release.



Macrostructure:

-it is a small gland found of posterior end of the corpus callosum, forming a section of the roof in the posterior wall of the third ventricle

repetition is the mother of wisdom

- Microstructure of pineal gland

: - is composed of 2 types of neural cells: pinealocytes (specialized secretory neurons) + glial support cells

: - it has a very rich blood supply that forms a network of capillaries surrounded by the pinealocytes

: - it receives innervation from many parts of the brain, but the main connections are with:

Suprachiasmatic nuclei (SCN)

Retina

Sympathetic system (**multisynaptic sympathetic way**: paraventricular nucleus in hypothalamus + upper sympathetic cervical ganglion – SCG; releas norepinephrin-beta adrenergic receptors –stimulate cAMP-activate gene expression for gen coding AA-NAT=arylalkylamin-N-acetyltranspherase)

Pineal gland - Function:

- The pineal gland synthesizes and secrets the hormone MELATONIN

  (NOT melanin – the brown skin pigment)

Melatonin is modified form of the aminoacid tryptophan (4steps of biosynthesis - serotonin – enzyme AA-NAT=arylalkylamin-N-acetyltranspherase : activity on the night, light – inhibited – acetylation –methylation ….melatonin

Effects of melatonin – pleiotropic effect

Melatonin has 3 main effects:

- It resets the SCN
- It induces sleep (hypnotic effect)
- It influences the hypothalamus, especially the reproductive function

Circadian rhythms **influence almost every cell in the body**. Hormones are secreted from the hypothalamus, pituitary gland and gonads with a circadian rhythm-e.g.: CRH, ACTH-peak early in the morning

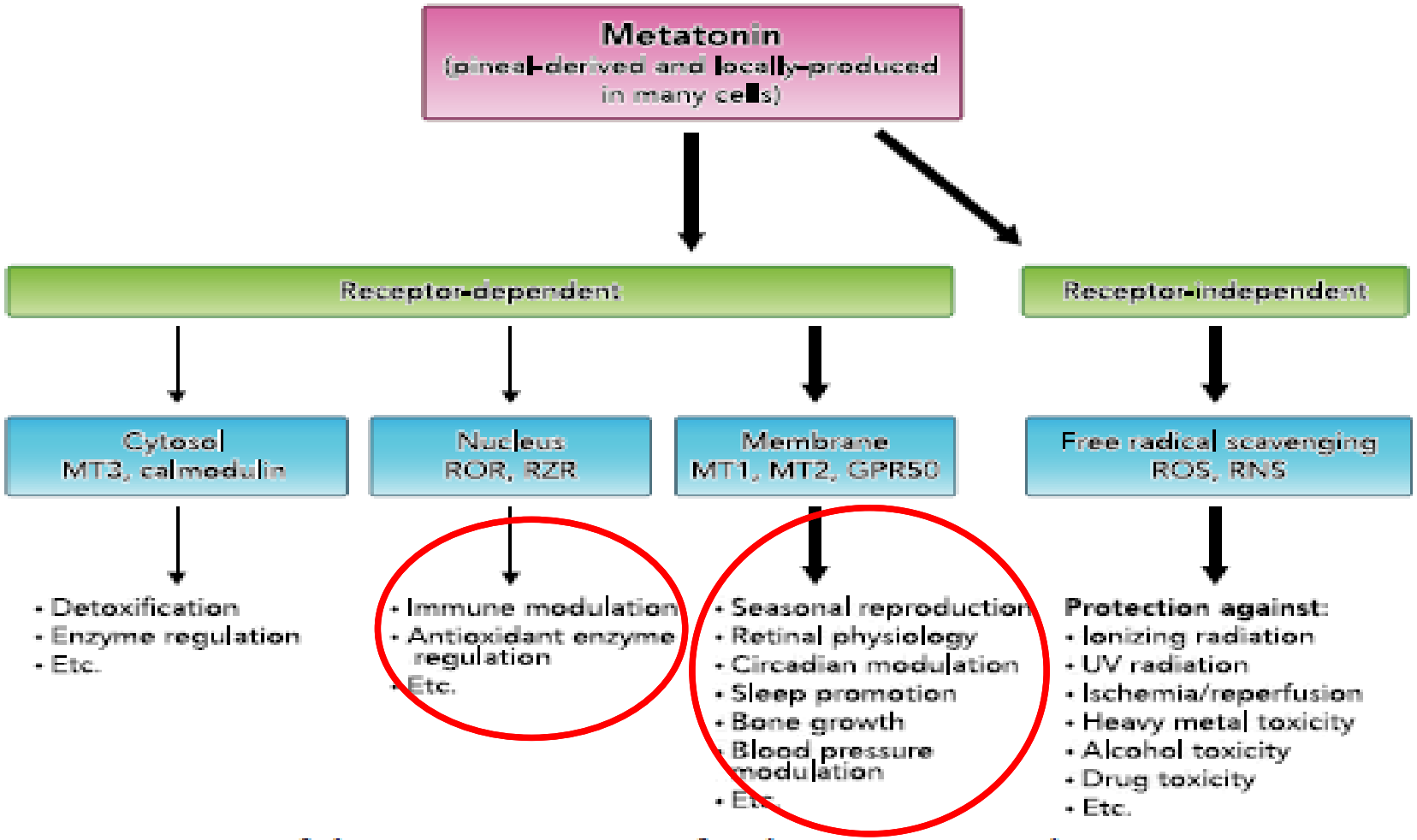


FIGURE 4. **Some of the numerous actions of melatonin in mammals**
Melatonin has both receptor-dependent and receptor-independent actions. The indole binds to well known membrane receptors (MT1 and MT2) and, via several signal transduction pathways, influences a host of physiological effects. MT1 and MT2 may homo- and/or heterodimerize in some cases, and they may interact with nuclear receptors (binding sites). There is considerable debate regarding the existence/function of the orphan nuclear melatonin receptors ROR and RZR. The cytosolic receptor, MT3, is a detoxifying enzyme, quinone reductase 2. Receptor-independent actions are mediated by the ability of melatonin and its metabolites to scavenge reactive oxygen (ROS) and reactive nitrogen species (RNS). These actions allow melatonin to protect against a wide variety of toxins and processes that generate highly toxic reactants. Any cell can simultaneously respond to melatonin by both its receptor-mediated and receptor-independent actions. Many of the documented physiological and molecular actions of melatonin are not listed in this figure. Additionally, the figure does not include melatonin functions in nonvertebrates or in plants.

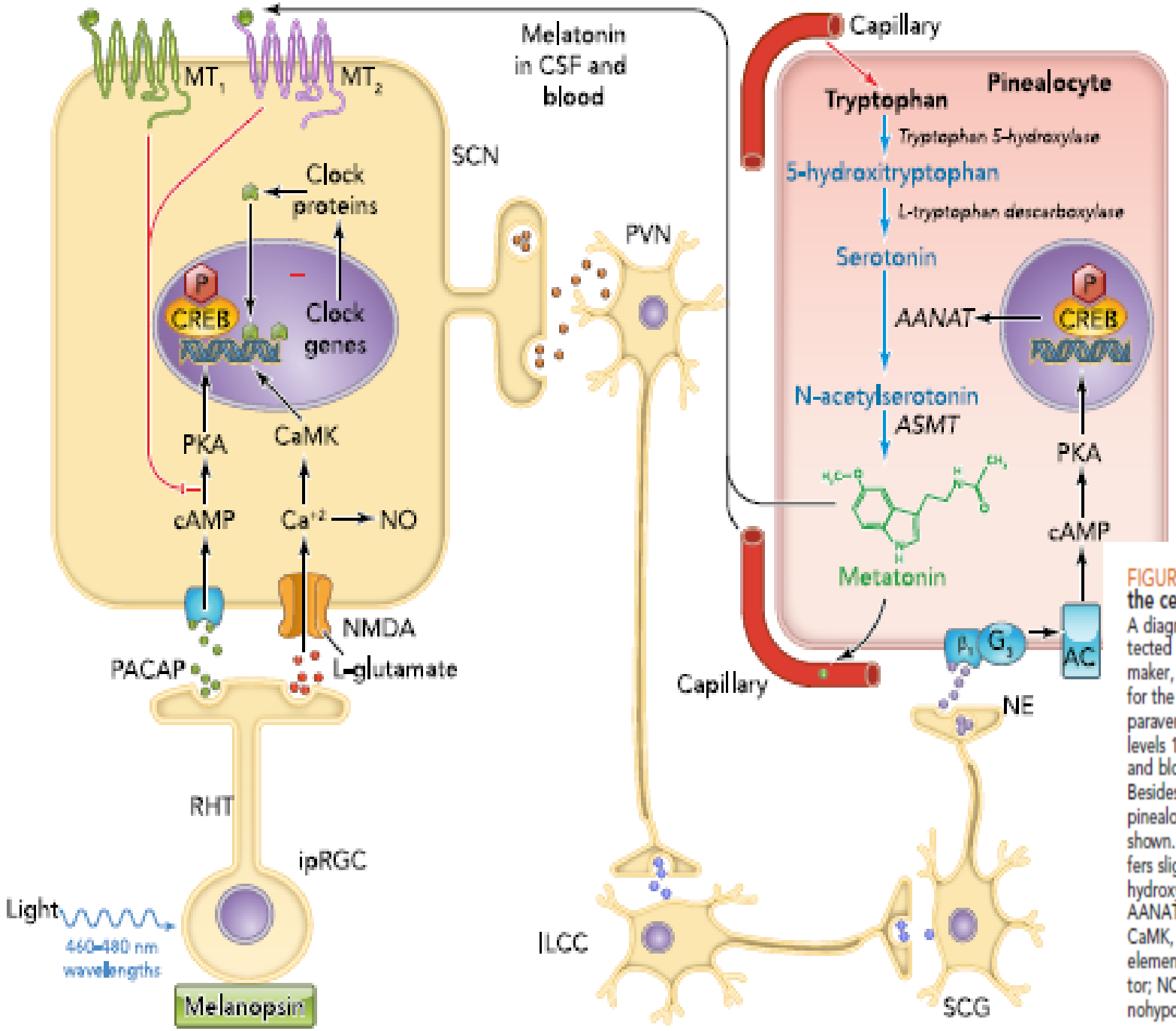


FIGURE 1. **A diagrammatic representation of the means by which light wavelengths regulate the central circadian pacemaker**

A diagrammatic representation of the means by which 460- to 480-nm light wavelengths (blue light), detected by the intrinsically photoreceptive retinal ganglion cells (*ip*RGC), regulate the central circadian pacemaker, the suprachiasmatic nucleus (SCN), and the transfer of photoperiodic information to the pineal gland for the regulation of melatonin synthesis from tryptophan. This vertebrate pathway involves synapses in the paraventricular nucleus (PVN) of the hypothalamus, the intermediolateral cell column (ILCC) at thoracic cord levels 1 and 2, and the superior cervical ganglia (SCG). Melatonin released into the cerebrospinal fluid (CSF) and blood act on the SCN to modulate its circadian activity and on circadian oscillations in peripheral tissues. Besides influencing circadian rhythms, melatonin has many other functions, as summarized in the text. In the pinealocyte depicted at the *top right*, melatonin synthesis as it occurs in the mammalian pineal gland is shown. Based on currently available data, the synthetic pathway for melatonin production in plant cells differs slightly from that in animals. In plants, tryptophan is initially decarboxylated to tryptamine, which is then hydroxylated to form serotonin. The final two steps are similar to those in the mammalian pineal gland. AANAT, arylalkylamine *N*-acetyltransferase; AC, adenylate cyclase; ASMT, acetylserotonin methyl transferase; CaMK, calcium/calmodulin protein kinase; cAMP, cyclic adenosine monophosphate; CREB, cAMP response element-binding protein; CSF, cerebrospinal fluid; NE, norepinephrine; NMDA, *N*-methyl-D-aspartate receptor; NO, nitric oxide; PACAP, pituitary adenylate cyclase-activating peptide; PKA, protein kinase A; RHT, retinohypothalamic tract.

Conclusion

- The suprachiasmatic nucleus in the hypothalamus serves as an „intrinsic clock“ which interacts with an external rhythm stimulus, in this case light, to coordinate melatonin release with the external day-night cycle

- This system allows the conversion of inhibitory light stimuli into a hormonal stimulus that can regulate:

  : Day-night (circadian rhythm)

  : Seasonal breeding rhythms – sexual life of animals (conception of future pups) (e.g. deer, reindeer, birds)

Health issues

Jet lag syndrome

The pineal gland has evolved to allow adaptation to changing day length:

- When people leaves his/her home country the SCN and pineal gland are synchronized: at night, darkness and SCN activation stimulate melatonin production, inducing sleep
- If the person flies across time zones, the SCN continues to oscillate in accordance with the previous time zone, which means that the timing of melatonin production (and, therefore, tiredness) does not change
- At a rate of adjustment of a couple of hours a day, the SCN adapts to a new time zone

Jat leg syndrome - treatment

- Taking oral melatonin can shorten the period of jet lag
- Melatonin should be taken at the times of darkness in the new time zone whilst on the plane and for several days at the destination
- For shift work, the melatonin should be taken during the period of desired sleep
- The SCN is reset more quickly and the body becomes resynchronized

Seasonal affective disorder

- Effect of melatonin also on an anual rhythm

- season rhythm - in winter – a little light, elevated of concentration of melatonin – internal dyssynchronization …….. increased incidence of depression

Sleep disorder

- **Sleep delay** (problem to sleep at night - delayed sleep) –problem: sleep at night, wake up in the morning is wrong. Treatment: administered melatonin when he wants to sleep

- **Phase advance** – go to sleep without any problems, but the waking up too early in the morning . Treatment: bright light exposure at a time when he wants to sleep, but it should still be awake)

Health problems are coming

- transition to summer or winter time
- shift work (porter, in hospital – nurse, doctor – working during the night time)

- Resynchronisation internal clock system with the external part – acompanied with symptoms/diseases:
  - fatique (tiredness) - sleep disruption - lack of appetite – gastric ulcers – stress – hypertension – obesity – behavioral changes

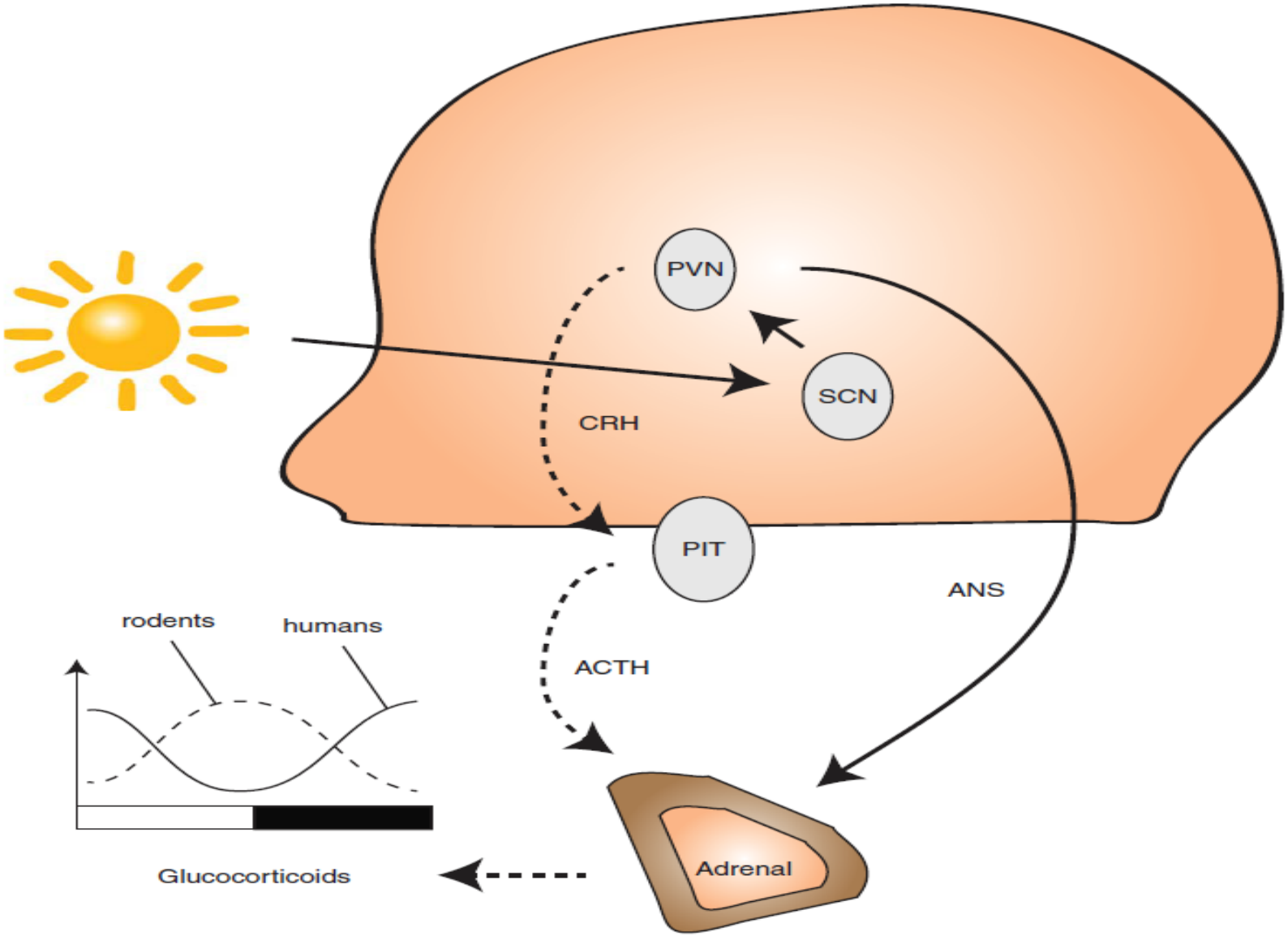


Figure 2

Interaction of central and peripheral clocks in the regulation of GC secretion. The SCN innervates the PVN from where rhythmic CRH release triggers secretion of ACTH from the pituitary (PIT). At the same time autonomic innervation (ANS) of the adrenal resets adrenocortical clocks regulating sensitivity of the steroidogenic machinery to ACTH. Synchrony between HPA axis activity and adrenal ACTH gating results in high amplitude and robust circadian GC rhythms. GC rhythms are phase-shifted between nocturnal and diurnal species indicating differential interpretation of SCN signals at downstream targets.

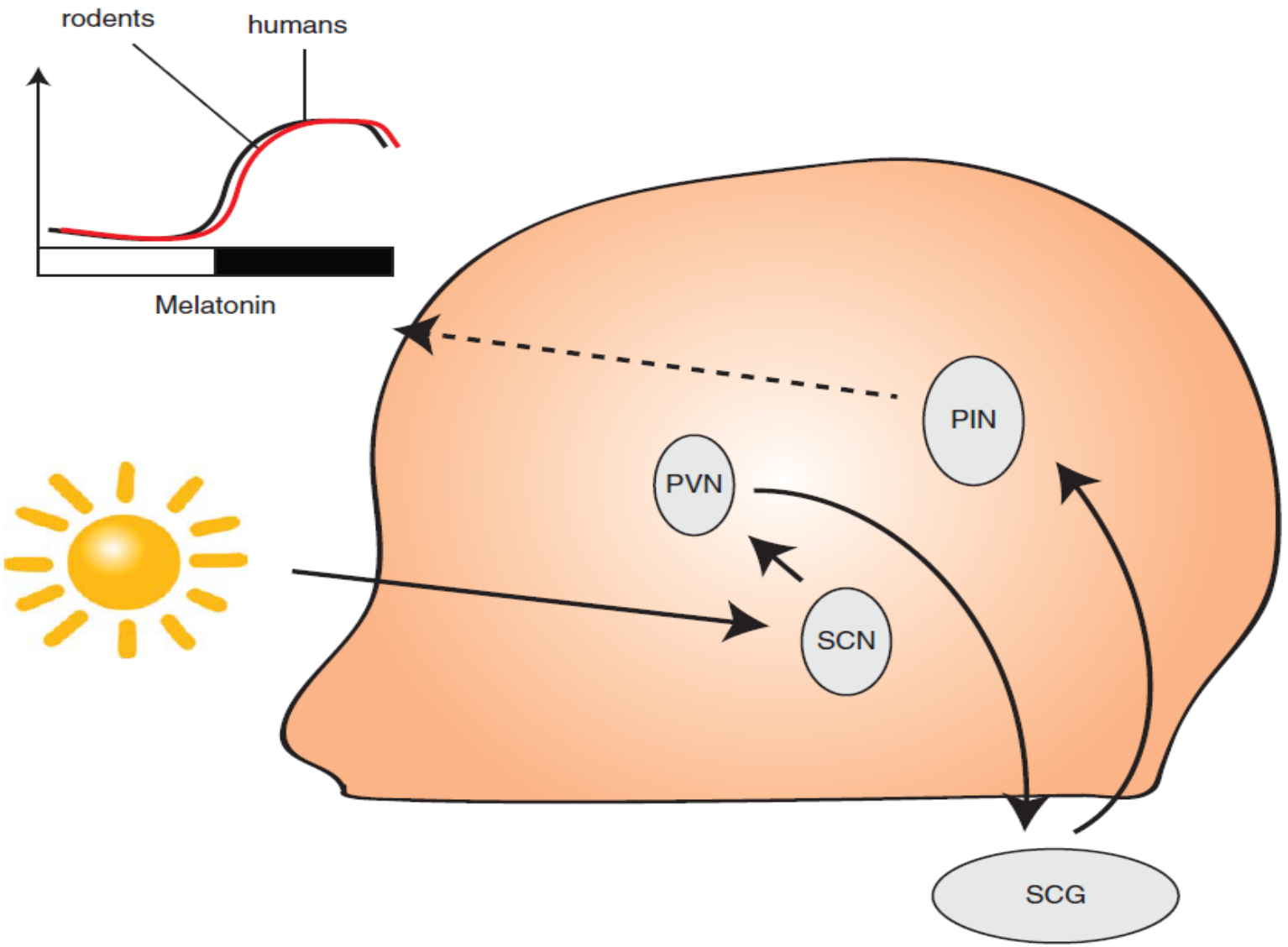


Figure 3

Melatonin release from the pineal is driven by the SCN pacemaker. The SCN innervates the PVN from where autonomous fibres descend into the spinal cord and out via the superior cervical ganglia (SCG) to reach the pineal gland (PIN). Clock genes are expressed in the pineal, but a functional contribution of a potential pineal clock to melatonin production has not been demonstrated. Unlike GCs, melatonin secretion is always confined to the dark phase, regardless of the activity profile of the animal.

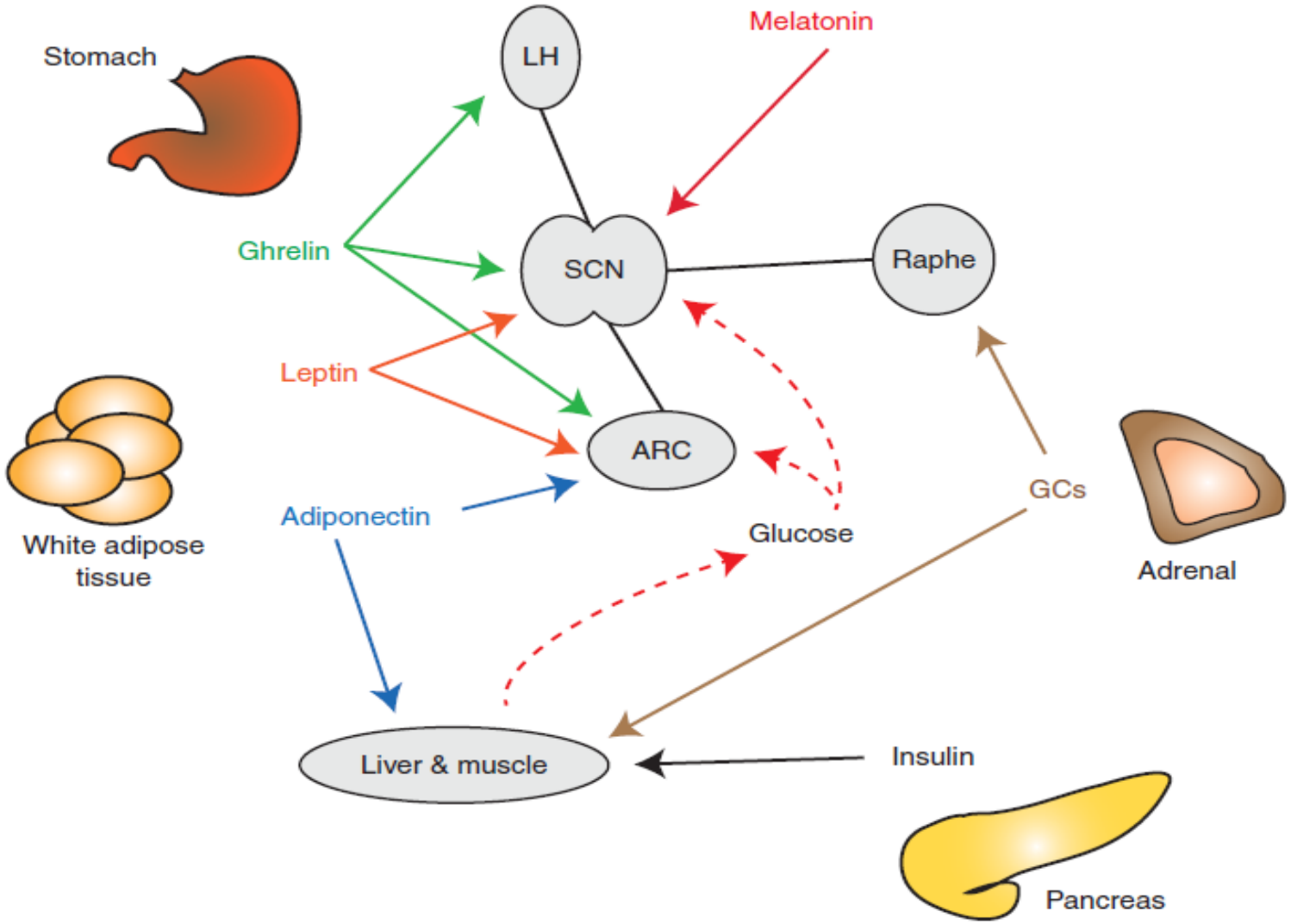


Figure 4

Endocrine feedback to the circadian clock. Various hormones can directly or indirectly feedback on central and peripheral clock function. In the brain endocrine targets with connections to the SCN include the orexinergic neurons of the lateral hypothalamus (LH), the arcuate nucleus (ARC), and the raphe nuclei of the brainstem. Other endocrine effects may be mediated via peripheral tissues and clocks such as the liver and muscle. For details see text.

Anthony H Tsang et al.

Journal of Molecular Endocrinology (2014) 52, R1–R16

The Nobel Prize in Physiology or Medicine 2017

Photo: Brian Summers

Jeffrey C. Hall

Prize share: 1/3

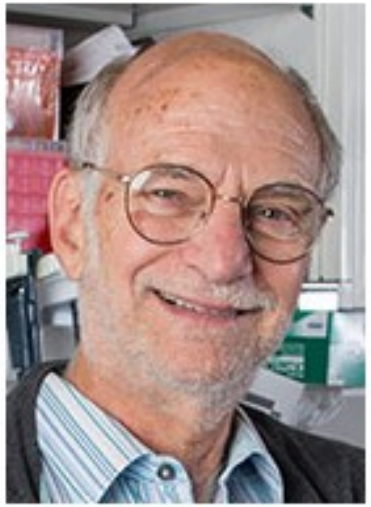


Photo: Scott Eisen, HHMI

Michael Rosbash

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Photo: Mario Morgado

Michael W. Young

Prize share: 1/3

The Nobel Prize in Physiology or Medicine 2017 was awarded jointly to Jeffrey C. Hall, Michael Rosbash and Michael W. Young *"for their discoveries of molecular mechanisms controlling the circadian rhythm"*.