V2: circadian clocks – Noble prize in physiology or medicine 2017

Jeffrey C. Hall
*1945

Michael Roshbash
*1944

Michael W. Young
*1949

„for their discoveries of molecular mechanisms controlling the circadian rhythm”

https://www.nobelprize.org/nobel_prizes

Noble prize in physiology or medicine 2017

During the 1970's, Seymour Benzer and Ronald Konopka tried to **identify genes** that **control** the **circadian rhythm** in fruit flies.

fruit fly

They showed that **mutations** in an unknown gene disrupt the circadian clock of flies. They named this gene ***period***. How can this gene influence the circadian rhythm?

In 1984, Jeffrey Hall and Michael Rosbash, working in close collaboration at Brandeis University in Boston, and Michael Young at the Rockefeller University in New York, succeeded in **isolating** the *period* gene.

Jeffrey Hall and Michael Rosbash then discovered that PER, the protein encoded by *period,* accumulated during the night and was degraded during the day.

Thus, PER **protein levels oscillate** over a 24-hour cycle, in synchrony with the circadian rhythm.

https://www.nobelprize.org/nobel_prizes

eol.org

Noble prize in physiology or medicine 2017

The next key goal was to understand how such circadian oscillations could be generated and sustained.

Jeffrey Hall and Michael Rosbash hypothesized that the PER protein blocked the activity of the *period* gene.
They reasoned that by an **inhibitory feedback loop**, PER protein could prevent its own synthesis and thereby regulate its own level in a continuous, cyclic rhythm.

The model was tantalizing, but a few pieces of the puzzle were missing.
To block the activity of the *period* gene, PER protein, which is produced in the cytoplasm, would have to **reach** the **cell nucleus,** where the genetic material is located.

Jeffrey Hall and Michael Rosbash had shown that PER protein builds up in the nucleus during night, but how did it get there?

https://www.nobelprize.org/nobel_prizes

Noble prize in physiology or medicine 2017

In 1994 Michael Young discovered a second clock gene, ***timeless***, encoding the TIM protein that was required for a normal circadian rhythm.

He showed that when **TIM bound to PER**, the two proteins were able to enter the cell nucleus where they blocked *period* gene activity to close the inhibitory feedback loop.

https://www.nobelprize.org/nobel_prizes

Noble prize story of Michael Roshbash: competition

„I graduated from Caltech in 1965 with a BS in Chemistry.

There I worked on nucleic acids in the laboratories of

Norman Davidson and then Robert Sinsheimer. ….

Then I attended graduate school at Massachusetts Institute of Technology (MIT). Although my PhD from there was officially in biophysics, I worked in the laboratory of Sheldon Penman; he was an ex-physicist turned cell physiologist with an intense interest in the messenger RNA (mRNA) of higher cells.

I then did a 3-year postdoc at the University of Edinburgh in the laboratory of John Bishop, who was a young faculty member in the Department of Epigenetics.

I arrived at Brandeis in the fall of 1974 as a newly minted assistant professor.

I was 30 years old, and 9 years had passed since I graduated from Caltech.

This was a standard trajectory in those days, when graduate work and postdocs were much shorter than they are today.”

Cold Spring Harb Perspect Biol doi:10.1101/cshperspect.a032516 (2017)

Noble prize story of Michael Roshbash: failures on the road

"In "the good old days", many prominent new professor instructors (PIs) had no publications during their postdocs, or their papers were published considerably after they took their first faculty jobs and often without the names of their postdoc mentors.

….

I was **denied tenure** in the Rosenstiel Center, where my laboratory was located in the 1970s and early 1980s. … my laboratory was forced to move to the only available Biology Department space, which was adjacent to Jeff 's laboratory.

… this proximity, including a shared conference room where we had joint laboratory meetings for many years, catalyzed our collaborative efforts.

… I had a serious health crisis in the summer of 1982. … this crisis lowered the energy barrier to making serious changes to my life.

They included deciding to work on the cloning of *period* as soon as someone appeared who was interested."

Cold Spring Harb Perspect Biol doi:10.1101/cshperspect.a032516 (2017)

Noble prize story of Michael Roshbash

“I gave the *period* **cloning project** to the second-year graduate student Pranitha Reddy, and this is how my collaborative work with Jeff Hall on circadian rhythms began in the early fall of 1982.

We were locked in an intense battle for primacy with the Young laboratory at Rockefeller for the first few years, and the cloning and rescue of *period* was performed independently in both places.

Mike and his colleagues deserve high marks for their accomplishments. Although unpleasant, the competition contributed to a fast-paced focus, which probably contributed to some of our successes.”

Cold Spring Harb Perspect Biol doi:10.1101/cshperspect.a032516 (2017)

Effect of sleep duration on humans?

30% of civilian adults in the US sleep less than 6 hours per day …

reasons: work, habits, studies …

Importantly, **short sleep** duration (< 6 hours/day) has been associated with **negative health outcomes**!

Short sleep increases: overall mortality, obesity, diabetes, cardiovascular diseases …

→ What happens on the molecular level?

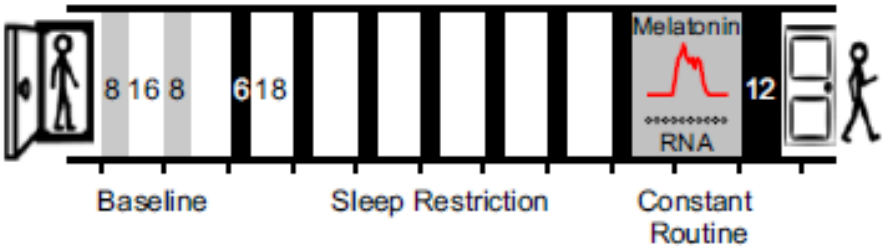
Effects of insufficient sleep on circadian rhythmicity and expression amplitude of the human blood transcriptome

PNAS (2013) 110, E1132-E1141

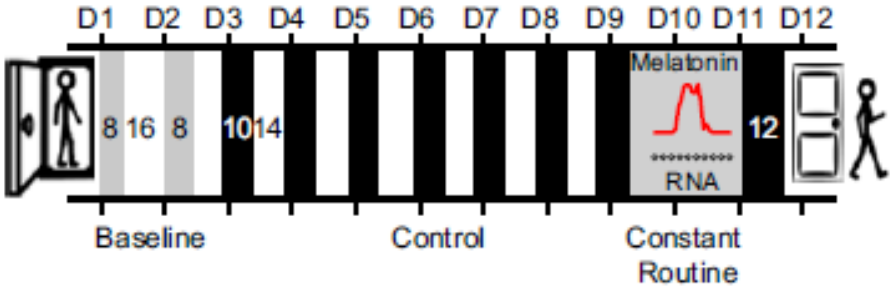
Carla S. Möller-Levet[1], Simon N. Archer[1], Giselda Bucca[1], Emma E. Laing, Ana Slak, Renata Kabiljo, June C. Y. Lo, Nayantara Santhi, Malcolm von Schantz, Colin P. Smith[1], and Derk-Jan Dijk[1,2]

Cross-over design study

26 participants (volunteers) were first put into **sleep-restricted conditions** with only 6 hours of sleep opportunity per night (dark bars)



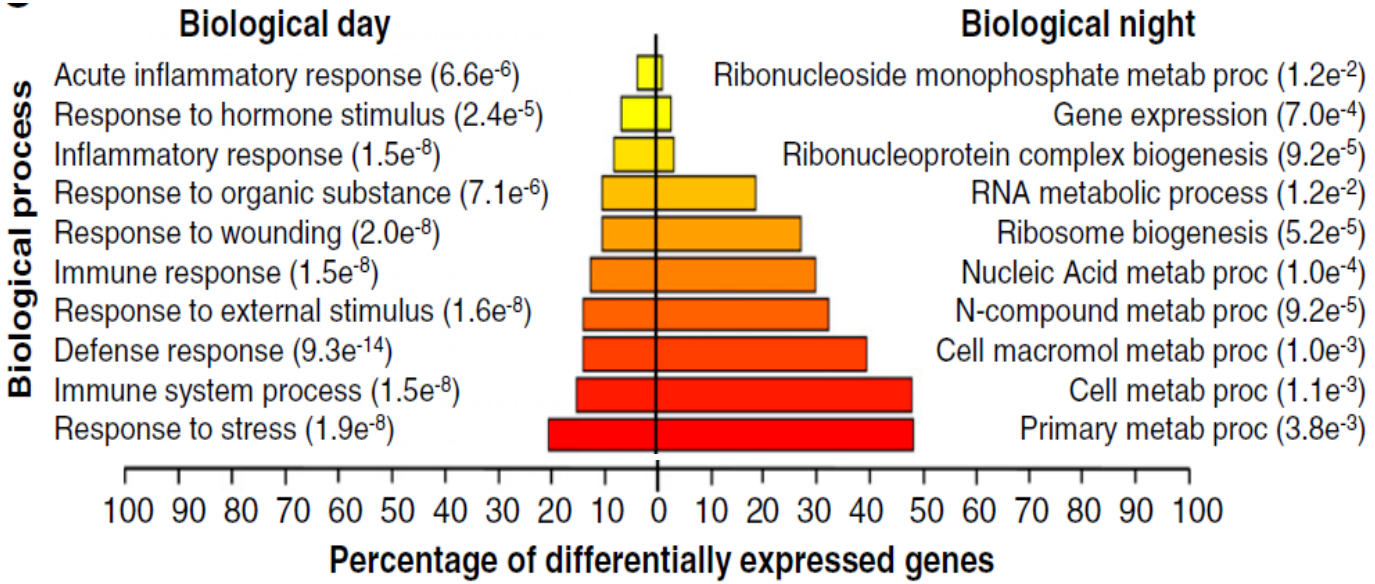
and then into conditions of **sufficient sleep** with 10 hours of sleep opportunity.
-> effects of genetic pre-disposition are mimimized by using „matched samples“



D1 to D12: day 1 to day 12

PNAS 110, E1132 (2013)

Gene functions of „normal“ circadian genes



Top 10 enriched GO BPs within the circadian gene list of the control condition using the human genome as a background

Enrichment p-values are given in brackets.

Immune, defense, stress and inflammatory responses, cytokine receptor activity, IL-1 receptor activity, NF-κB signaling are more prominent during day time.

(Also found for rodents, taking into account that they are night-active).

Night time processes: “normal” maintenance + growth processes …

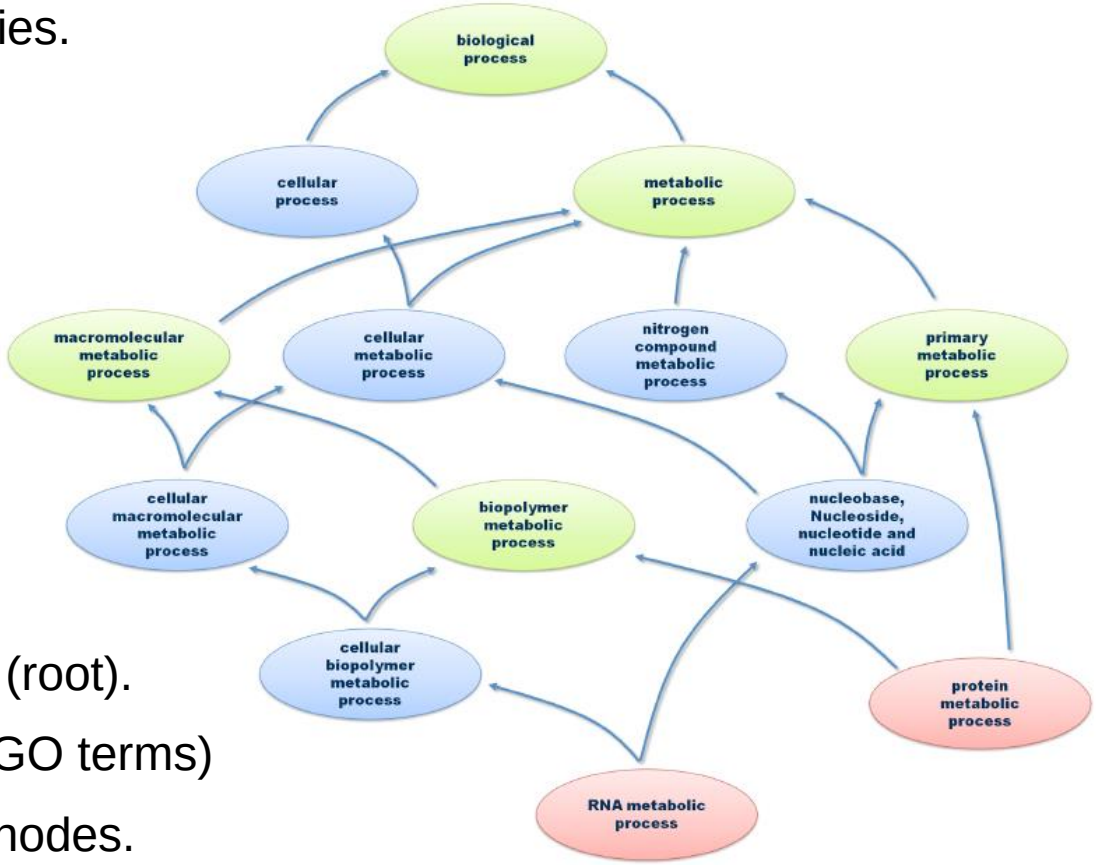
Gene Ontology (GO)

Ontologies are structured vocabularies.

The **Gene Ontology** has 3 tracks:

- biological process (BP)
- molecular function (MF)
- cellular component (lokalisation).

Shown here is a part of the BP tree.



At the top: most general expression (root).

Red: leafs of the tree (very specific GO terms)

Green: common ancestors of 2 red nodes.

Blue: other nodes.

Lines: „Y is contained in X“- relationships

Dissertation Andreas Schlicker (UdS, 2010)

Over-representation analysis (WebGestalt)

Suppose that we have *n* genes in a "**gene set of interest**" (A)
and *m* genes in the **reference gene set** (B).

Suppose further that there are *k* genes in A and *j* genes in B
that belong to a particular functional category (C)
(e.g. a GO category, a KEGG pathway, a BioCarta pathway etc.).

Based on the reference gene set, the expected proportion k_{exp} would be

$$k_{exp} = (n/m) \times j$$

If *k* exceeds the above expected value,
category C is said to be **enriched**,
with a **ratio of enrichment** (*r*) given by $r = k/k_{exp}$.

Zhang, Kirov, Snoddy (2013)
Nucl Ac Res *33: W741-W748*

Over-representation analysis (WebGestalt)

If B represents the population from which the genes in A are drawn, WebGestalt uses the **hypergeometric test** to evaluate the significance of enrichment for category C in gene set A,

$$P = \sum_{i=k}^{n} \frac{\binom{m-j}{n-i}\binom{j}{i}}{\binom{m}{n}}$$

Interpretation: draw $i = k$ genes for A that belong to category C from the j genes from B that belong to C.
→ The other $n - i$ genes in A do not belong to C. They are drawn from the $m - j$ genes in B that do not belong to C.
Normalization is done by the total number of possibilities to draw n genes from m genes.

If A and B are two independent gene sets, WebGestalt uses **Fisher's exact test** instead,

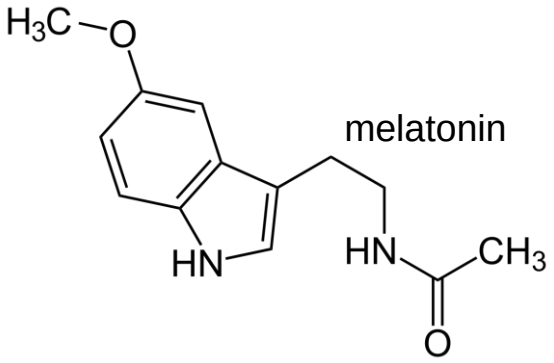
$$P = \sum_{i=k}^{n} \frac{\binom{n}{i}\binom{m}{j+k-i}}{\binom{m+n}{j+k}}$$

Zhang, Kirov, Snoddy (2013)
Nucl Ac Res *33: W741-W748*

Effects of sleep deprivation on melatonin (SCN marker)

Melatonin is a hormone that regulates sleep-wake cycles.

On D10 + D11, melatonin **peaked significantly later** after sleep restriction:



melatonin

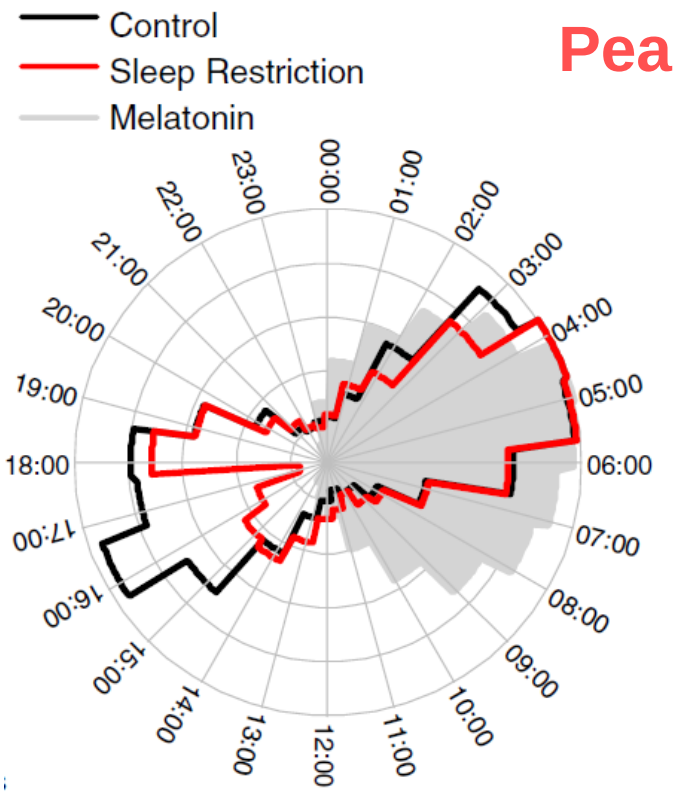
04:15 am ± 19 min →	05:01 am ± 19 min
Control	sleep restriction

But duration of melatonin secretion was only **insignificantly shortened:**

9:53 ± 12 min →	9:35 ± 11 min

PNAS 110, E1132 (2013)
wikipedia.de

Peak times of expression

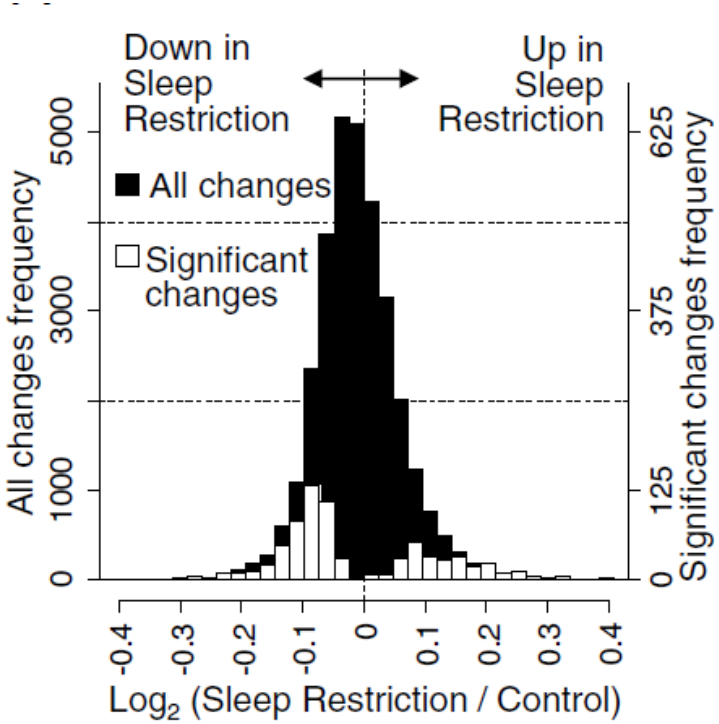


Shown are phase histogram of the peak times of prevalent circadian genes following sleep restriction or control.

The profiles of different individuals are aligned by their personal melatonin peaks.

Clear reduction (> 50%) of the # of genes that peak during day time!

Global overview: changes open sleep deprivation



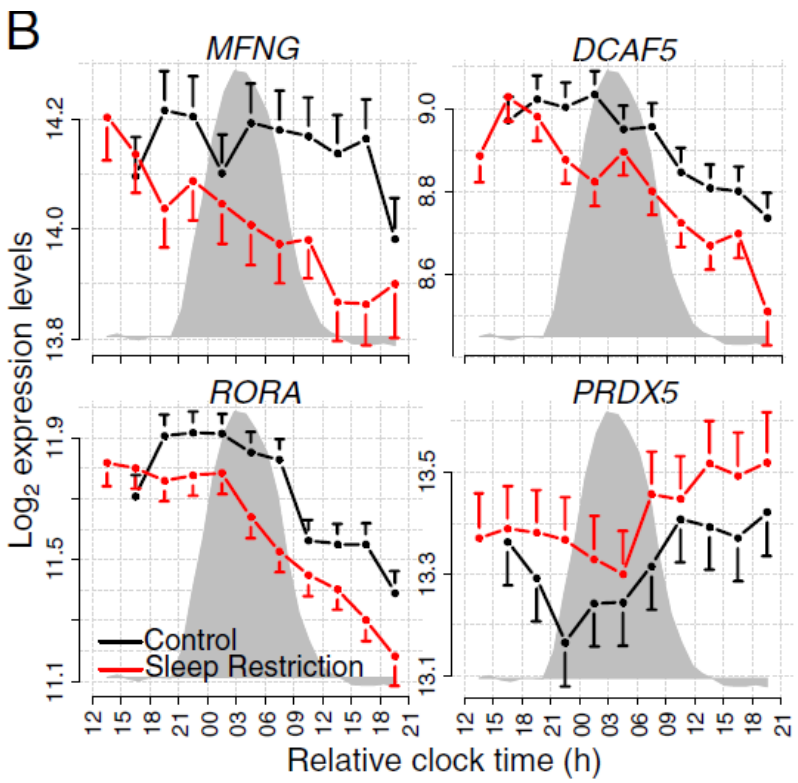
Frequency distribution of expression fold-changes after sleep restriction relative to control.
Filled area: Histogram of changes in all transcripts (31,685 probes that target 22,862 genes)

Open area: changes in transcripts identified as having a statistically significant (FDR-corrected p-value < 0.05) main effect of sleep condition (744 transcripts that target 711 genes).

444 genes are **down-regulated** upon sleep restriction (including the circadian rhythm related genes RORA, IL6, PER2, PER3, TIMELESS, CAMK2D)

267 genes are **up-regulated** (including several circadian-rhythm related genes)

Examples of genes with significant effect of Sleep Condition



Most affected genes: $p < 10^{-6}$

MFNG: O-fucosylpeptide 3-beta-N-acetylglucosaminyltransferase

DCAF5: is a protein-coding gene …

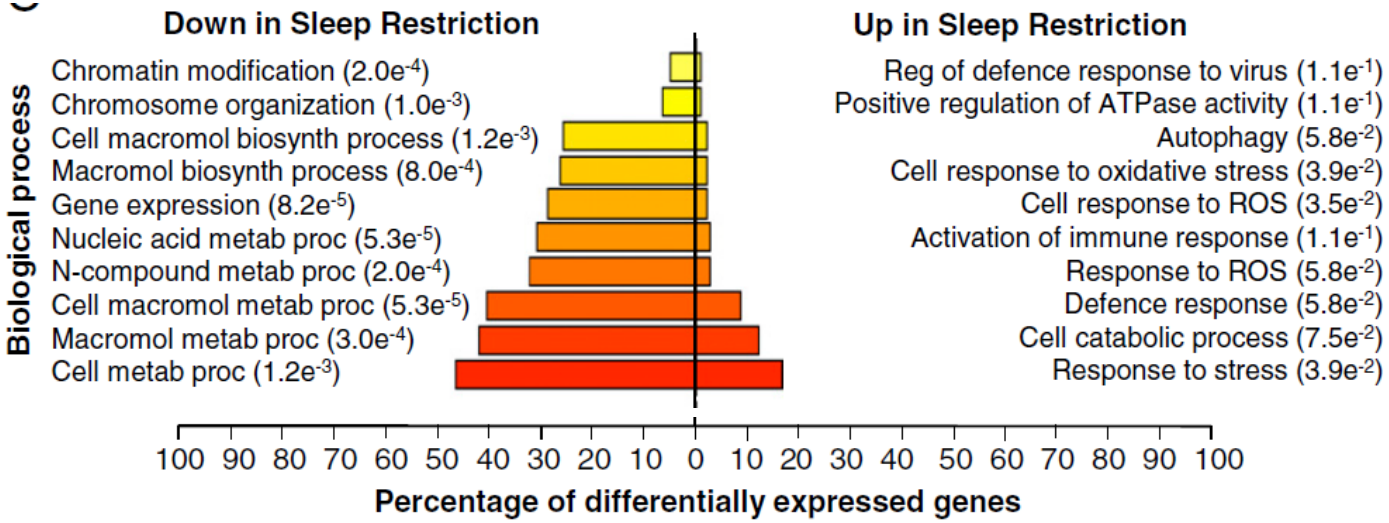
RORA: retinoic acid receptor-related orphan receptor alpha is a nuclear hormone receptor – associated with circadian rhythms

PRDX5: peroxiredoxin 5

Greyed areas: melatonin profile averaged for the two conditions.

Individual data were aligned relative to the individual melatonin rhythm and sorted into discrete circadian phase bins.

What sort of genes are differentially expressed upon sleep restriction?



Top 10 enriched GO biological processes within the statistically significant differentially expressed gene list as identified by WebGestalt when using the human genome as background.

p-values are corrected by Benjamini-Hochberg method for multiple testing.

Down-regulation: chromatin modification and organization, metabolism

Up-regulation: cellular response to oxidative stress and reactive oxygen

This does not sound healthy!

Question in V1: circadian rhythm of blind people?

The master clock plays a vital role in the regulation of the circadian rhythms of several major biological processes. Its natural period is slightly longer than 24 h and requires **daily synchronization** with the solar cycle by exposure to morning light. …

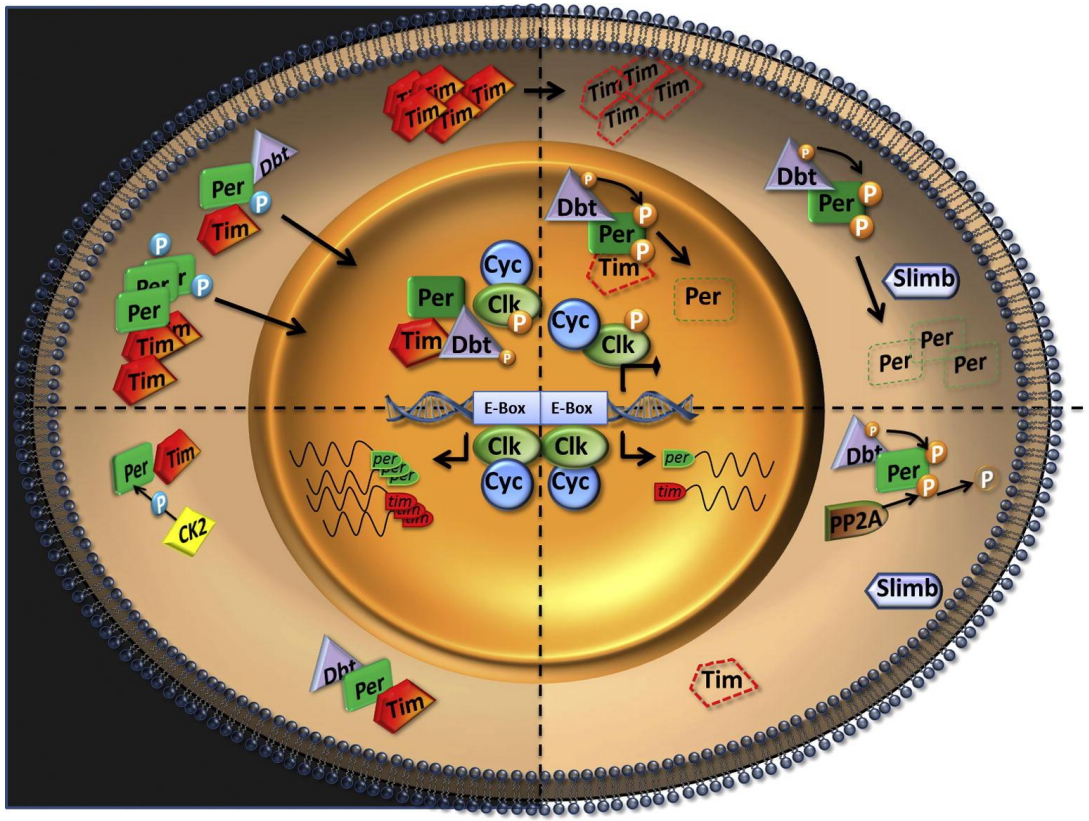
In totally **blind people**, the absence of light impairs circadian synchronization. In some this leads to gradual drift of their circadian rhythms....
This gradual desynchronization leads to **non-24 SWRD (**sleep–wake rhythm disorder) with cyclical periods of severe insomnia and excessive daytime sleepiness.
The resulting social and professional handicap may be severe and lead to social isolation and psychological difficulties.

Rare in the general population and considered an orphan disease, non-24 SWRD is common in the totally blind.
Treatments that have been shown to be safe and effective in this disorder include melatonin and the melatonin agonist Tasimelteon.
Tasimelteon is to date the only treatment approved by the FDA and the EMA for non-24 SWRD in totally blind persons.

https://www.frontiersin.org/articles/10.3389/fneur.2017.00686/full

Molecular mechanisms of the Drosophila circadian clock.

night day



Inner circle: nucleus
Outer circle: cytoplasm of a neuron.

Each quarter of the cell represents 6 hours of the circadian day.

P: phosphorylation

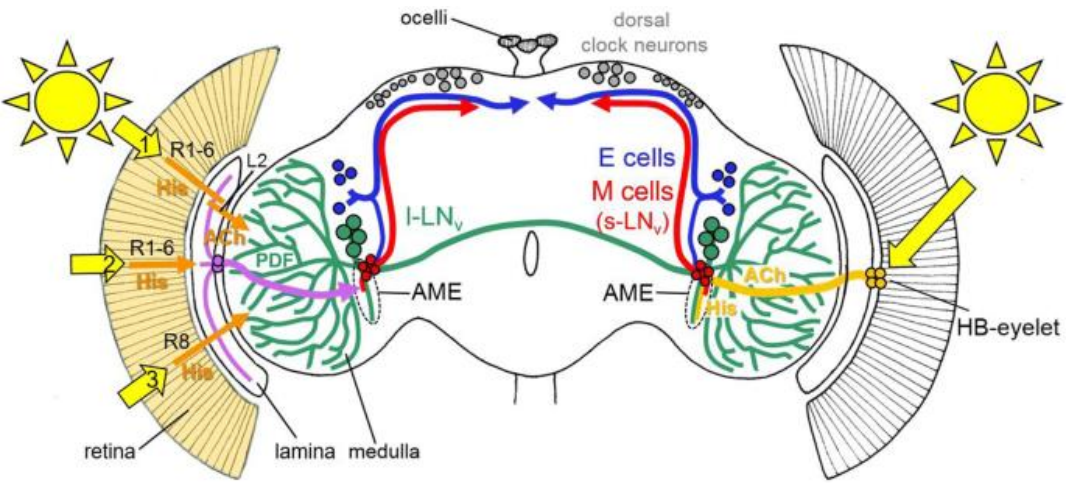
Dashed protein symbols: proteo-somal degradation.
Sinous lines: mRNA transcription

At midday, the transcription factors clock (Clk) and cycle (Cyc) bind as heterodimers to E-Box sequences (CACGTG) to the promoter regions of period (per) and timeless (tim) and activate their transcription.

N. Peschel, C. Helfrich-Förster (2011)
FEBS Letters 585, 1435-1442

https://febs.onlinelibrary.wiley.com/doi/full/10.1016/j.febslet.2011.02.028#

Circadian rhythm in fruit flies

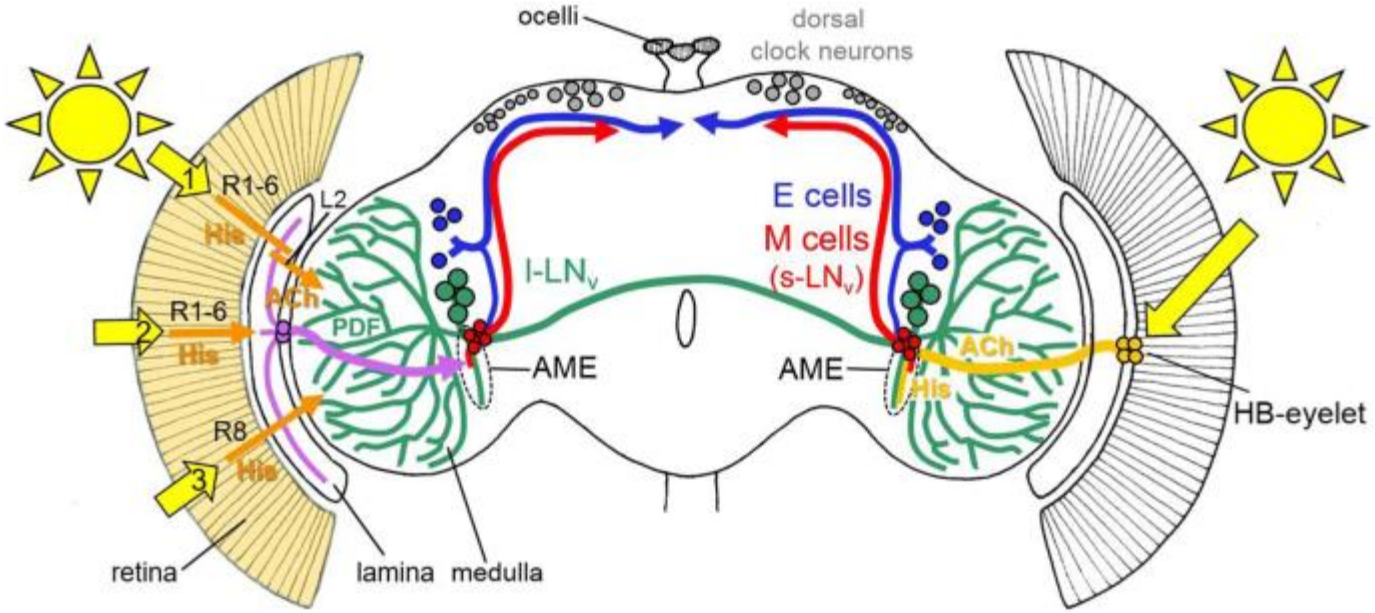


Schematic representation of the light input pathways from the eyes in *Drosophila melanogaster*. Light reaches the circadian lateral clock neurons [M cells (= s-LNv), E cells (mainly LNd), and the large ventrolateral neurons (l-LNv)] through the compound eyes (left) and the Hofbauer–Buchner (HB)-eyelets (right).

All receptor cells of the compound eyes use histamine (His) as a neurotransmitter, whereas the HB eyelets utilize histamine and acetylcholine (ACh).
The HB eyelets project into the accessory medulla (AME) and signal via histamine to the l-LNv and via ACh to the M cells.
The l-LNv and the M cells (s-LNv) express the neuropeptide PDF (pigment-dispersing factor). The PDF fibres are indicated in green and red.

C. Helfrich-Förster (2020)
J Compar Physiol A 206, 259–272

Circadian rhythm in fruit flies



From the compound eyes, there are 3 putative input pathways to the clock neurons.

(1) Receptor cells 1–6 (R1–6) signal via His to the lamina monopolar cells (L2). L2 cells express ACh and signal in the distal medulla to the l-LNv.

(2) R1-6 signal to wide-field fibres arborizing in the lamina and stemming from two peptidergic interneurons (AstC/CcapR in lilac) that are located between lamina and medulla. These neurons send axons into the AME, where they contact most clock neurons.

(3) Rh6-positive R8 cells that appear to play an integrative role in the light input from all other receptor cells, signal indirectly to the circadian clock neurons.

C. Helfrich-Förster (2020)
J Compar Physiol A 206, 259–272

Current Biology Report

Next paper for you …

Circadian Regulation of Light-Evoked Attraction and Avoidance Behaviors in Daytime- versus Nighttime-Biting Mosquitoes

NO introduction (very uncommon) – BUT partly replaced by general „Summary“

Methods section: 4 paragraphs (at the end of paper)

(1) How are mosquitos kept?

(2) Light attraction behavior

(3) Immunocytochemistry – 2 antibodies; second AB attached to fluorophore Alexa 488

(4) Statistics

Results section:

Fig. 1 attraction to day/night UV light exposure

Fig. 2 circadian neural circuits in mosquito brains

Fig. 3 PER expression in diurnal/nocturnal mosquitos

Fig. 4 constant UV light exposure - immuno-cytochemistry + light attraction

see https://www.sciencedirect.com/science/article/pii/S0960982220308265