

Metronomes that Regulate the Day

Ludwig II of Bavaria is a particularly striking example of how differently people's internal clocks can tick. According to historical sources, the monarch usually conducted his government business at night and slept during the day. Whether the Fairy Tale King had a disorder that disrupted his sleep-wake rhythm is a matter even **Gregor Eichele** can only speculate about. Nevertheless, Eichele and his team at the **Max Planck Institute for Biophysical Chemistry** in Göttingen have gained much new insight into how the body's natural timekeepers work.

TEXT **KLAUS WILHELM**

The sleep-wake cycle is intimately linked with our internal clock," says Gregor Eichele, who heads the Genes and Behaviour Department at the Max Planck Institute in Göttingen. Eichele knows first-hand how the internal clock can affect wellbeing: he commuted regularly between Germany and the US for many years. The circadian clock has long been one of his scientific passions.

Every day, millions of people experience how their sleep patterns are affected by their internal clock when they cross several time zones within the space of a few hours. This frequently results in their internal clocks getting out of sync. Some already complain of sleep disturbances when the clocks are turned back just one hour when daylight saving time ends. Even in the case of such small adjustments, it can take several days for the internal

and external clocks to re-synchronize before the affected individual is able to sleep as usual.

"Although sleep and the internal clock are related, they are fundamentally different. Whereas sleep is a function of the body as a whole, the internal clock is a property of individual cells," Gregor Eichele emphasizes. At the same time, the two influence each other. For example, neurons and sleep-regulating substances are controlled by the 24-hour circadian clock, which is thus responsible for ensuring that we fall asleep at the right time.

THE INTERNAL CLOCK RUNS A BIT SLOW

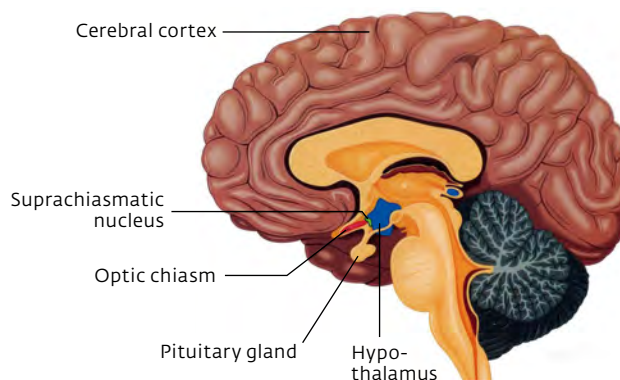
The word "circadian" is derived from the Latin *circa* (approximately) and *diem* (day). It expresses the fact that the internal clock only roughly follows a 24-hour rhythm. An individual can have a circa-

dian clock with a rhythm of, say, 24.7 hours. If that person lived for several weeks in a room that was always illuminated, he or she would fall asleep every day 42 minutes later than on the previous day. Environmental conditions – mainly light – act as timekeepers to continuously recalibrate the internal clock to a rhythm of exactly 24 hours.

This seemingly complex system exists because the daily cycle of day and night is not sufficient to synchronize the biological processes in our body. Consider, for example, the light-dark rhythm of modern life. If our physiological rhythms were merely a response to the presence or absence of light, any prolonged evening with all its artificial light sources would have catastrophic effects on our metabolism and sleep-wake rhythm. Instead, our internal clock recognizes these external time signals as spurious and keeps the body chronologically stable. >



The life rhythm of most animals follows an internal clock. In mice, for example, the timekeeper is naturally set in such a way that the animals are active at night and rest during the day.



Left Gregor Eichele is fascinated by clocks – especially the internal clocks that synchronize processes in our cells and organs. He has elucidated important elements of the molecular mechanism that regulates the circadian rhythm of cells.

Above The suprachiasmatic nucleus is the master clock in the human brain. Measuring just a few millimeters across, it is located above the site where the two optic nerves cross, and receives information they carry from the eyes about light conditions. The pituitary also produces hormones that affect the body's internal clocks.

The circadian clock probably arose at the dawn of evolution. The very first single-celled organisms in the primordial seas may have benefited from an ability to anticipate the sunrise and to descend into deeper waters to avoid it. In this way, they escaped from the UV radiation of the Sun, which at the time was still essentially unfiltered. In the darkness of the deep sea, the clock then signalled to the microbes when it was time to surface again.

Since they evolved, virtually all life forms have retained the internal circadian clock. It is beneficial for plants to carry out photosynthesis only during the day. In diurnal mammals such as humans, body temperature rises before waking. The release of the stress hormone cortisol peaks in the morning to boost physical and mental performance. Metabolism, muscle tone, renal function and concentration fluctuate over the course of a day.

There is a molecular clock for every cell, every tissue, every organ – whether the liver, kidneys, heart, gut, immune system or skin – and for the body as a whole. “We have an entire shop full

of clocks,” says Eichele. Being Swiss, you might say he is predestined to analyze such mechanisms. In order for all the clocks to display the same time, they must all be continuously synchronized with the 24-hour light-dark cycle of the environment – all the cell clocks and the organ clocks, as well as the body as a whole.

THE BRAIN NUCLEUS SETS THE RHYTHM

The most important clock, the suprachiasmatic nucleus, is located in the brain. Within this nucleus are 50,000 interlinked neurons that are also connected to neurons in other brain regions. The nucleus receives signals through nerve fibers from specialized sensory cells in the eyes. When light strikes a light-sensitive pigment in the sensory cells of the retina, they generate an electrical signal, which is relayed to the suprachiasmatic nucleus.

Without the suprachiasmatic nucleus, hamsters, for example, lose their daily rhythm. Scientists measured this by placing a treadmill in the cage and

recording the rotations of the wheel as a measure of the hamsters’ activity. Hamsters are normally active primarily between sunset and sunrise. Without a suprachiasmatic nucleus, however, they were just as likely to exercise during the day as during the night – yet the animals didn’t sleep more than usual.

This finding suggests that the nucleus, as the master clock, relays information to all the other clocks in the body’s cells, tissues and organs, and synchronizes them both with the day and with each other. Recent research, however, has called this theory into question: Eichele’s team modified mice genetically in such a way that the important clock gene *Bmal1* is inactive in the suprachiasmatic nucleus. Their experiments differ from the hamster studies in that the connections to and from the nucleus are left intact. Nevertheless, according to the theory, the animals’ internal clocks should go haywire.

But that doesn’t happen! “We found that the other circadian clocks remain synchronized even without the master clock in the suprachiasmatic nucleus,” Eichele explains – at least when light

and dark alternate in a 24-hour rhythm. However, if mice lacking the clock gene are kept in permanent darkness, chaos ensues and they have problems keeping their internal clocks in sync.

The body thus needs the natural light-dark cycle as a timekeeper. Although food intake can calibrate the circadian clock to a precise 24-hour rhythm, it results in only semi-synchronized internal clocks. Evidently the clock system is organized like a federal country that is able to keep the individual regional governments running even if the federal government sometimes

grows weak. "This system is ultimately more stable than one that relies exclusively on the suprachiasmatic nucleus," Eichele says.

But how do internal clocks synchronize without the master pacemaker in the brain? One possibility is that the body's clocks receive light-dark information from the suprachiasmatic nucleus. Researchers have shown that light can activate clock genes in organs such as the liver via the autonomous nervous system.

If the nucleus is absent, light signals travelling from the eyes into the body

also peter out. Light then no longer has an effect on the autonomous nervous system or the body's clocks. As the scientists in Göttingen only switch off a single clock gene and not the entire neural nucleus, light signals are still able to reach and synchronize the other clocks in the body via the nucleus. The signals evidently don't have to be pre-processed in the clock cells of the nucleus.

However, it is also possible that other important clocks in the brain stand in for the suprachiasmatic nucleus and synchronize the body's clocks. A likely candidate would be the pituitary gland,

DO CILIA IN OUR BRAIN REGULATE OUR SLEEP?

There is a cavity system located deep within the human brain: four cavities, called ventricles, are connected to each other via channels that act as conduits for cerebrospinal fluid. This fluid contains, among other things, neuropeptides, which ensure, for example, that we become tired. The suprachiasmatic nucleus (see text), believed to be the seat of the internal clock, is located near one of the ventricles. Scientists at the Max Planck Institute for Biophysical Chemistry and the Max Planck Institute for Dynamics and Self-Organization recently discovered that eyelash-like processes,

called cilia, on the wall cells of the ventricles can change their direction of motion and thus alter the direction in which the cerebrospinal fluid flows. At certain times of the day, they even produce eddies that act as barriers. It is still not definitively known whether the distribution of the fluid, and consequently the sleep-inducing neuropeptides, actually follow a circadian rhythm. The researchers may have discovered an entirely new mechanism that is based, not on the activity of neurons, but on the activity of wall cells in the ventricles.



which also receives light signals from the eyes. Located at the base of the brain, the gland releases the hormone ACTH into the bloodstream. It is then transported to the adrenal glands, where it triggers the release of cortisol, adrenalin and noradrenalin.

These stress hormones are known to be important pacemakers for the internal clocks. Eichele and his team discovered that mice with a defective clock gene rhythmically release the hormone corticosterone in the course of the day in sync with other body clocks – almost as in normal mice. This hormone is analogous to cortisol in humans. “It’s possible that corticosterone synchronizes the body’s clocks if the suprachiasmatic nucleus fails as a timekeeper,”

Eichele concludes. This suggests that the internal clock in the adrenal glands is almost as important as the clock in the suprachiasmatic nucleus.

CHRONOTYPE DETERMINES WHEN YOU GO TO BED

But the clocks in the body’s tissues and organs are influenced not only by light, but also by sleep. “You have to be relaxed, free of stress and able to sleep when you want, meaning in accordance with your personal chronotype, which determines whether you go to bed early or late and tend to sleep for short or long periods,” says Henrik Oster of the

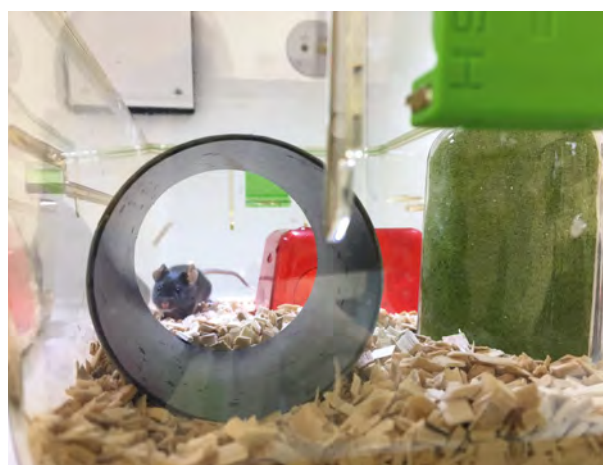
University of Lübeck, who led a research group at the Max Planck Institute in Göttingen until the end of 2012.

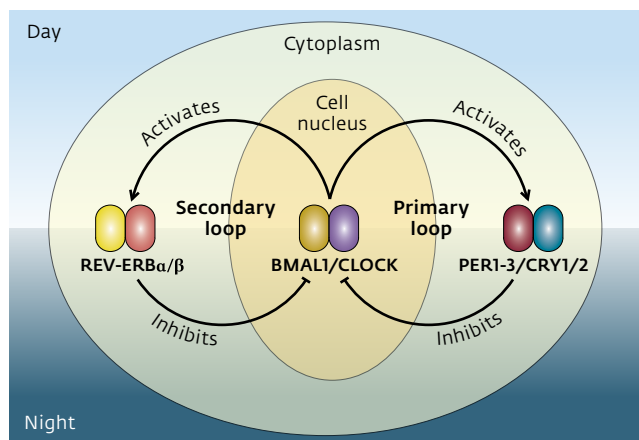
Since Oster’s time in Göttingen, he and his colleagues have been studying the relationships between sleep, the internal clock and metabolism. They observed, for example, that the liver and fat cells of mice with sleep disorders no longer operate in sync. The researchers want to determine whether the rhythm of the cells of other organs, such as the kidneys, is also decoupled.

A lot of evidence suggests that sleep disorders can also alter metabolism via the internal clock. For example, Oster and his colleagues at the Max Planck Institute knocked the sleep rhythm and

Left Henrik Oster keeps his mice under precisely controlled light-dark conditions. The cages are in cabinets in which the light duration and intensity can be programmed, allowing the animals’ sleep-wake rhythm to be controlled.

Right top, bottom Mice are very curious animals – so much so that their curiosity even stops them from sleeping. The cages thus contain unfamiliar objects for the mice to explore to their heart’s content – a particularly stress-free form of sleep deprivation.





Two feedback loops control a cell's circadian rhythm through an interplay of gene activation and gene inhibition. Every morning, the BMAL1 and CLOCK proteins in the cell nucleus stimulate the production of cryptochrome (CRY) and period (PER) proteins. These proteins accumulate in the cell plasma and migrate over the course of the afternoon and evening back into the cellular nucleus, where they block the BMAL1 and CLOCK proteins. During the night, levels of CRY and PER in the cell decrease to the extent that the blockade of CLOCK and BMAL1 ceases. In the morning, a new round of CRY and PER production starts again. This cycle is stabilized by another feedback loop in which BMAL1 and CLOCK stimulate the production of REV-ERB proteins, which progressively switch off the Bmal1 and clock genes over the course of the day. As a result, the production of the REV-ERB proteins decreases so that BMAL1 and CLOCK can be produced again in the early morning.

thus the internal clock out of sync in mice. They prevented the animals from sleeping in the morning by placing toys in their cages. After a few days, they found that the disrupted sleep pattern had an impact on the internal clocks of peripheral organs, which were then no longer able to switch important metabolic genes on and off.

One example of such a metabolic disturbance is hormone-sensitive lipase. Normally, the circadian rhythm ensures that this lipid-cell enzyme is active during the sleep phase, when it breaks down stored fats that the body needs to bridge the period without food. However, because lipase activity is lower in the case of sleep disorders, little fat is released into the body. "This causes blood glucose levels to fall, an energy emergency results, and the animals get hungry," Oster says. The mice start to eat, which then really disrupts their sleep patterns. The result is a vicious circle that causes the animals to gain more and more weight. To complicate matters, hormones in the stomach reset the liver's clock if the mice eat when they should actually be asleep. Consequently, the liver's metabolism becomes increasingly imbalanced.

Is the body somehow able to compensate for this metabolic chaos? The answer is yes, in some circumstances. Oster's team disturbed sleeping mice and provided access to food only during their normal waking phase, but allowed them to eat as much as they wanted. "That normalized activation of the clock genes in the liver," Oster

says. "So it appears that the time of food intake is a very important factor in the development of obesity and metabolic diseases."

The researchers in Lübeck also observed that clock genes cause metabolic changes in sleep-deprived humans, as well. Whether that really can lead to obesity and diabetes hasn't been established. However, studies of shift workers suggest that that is, in fact, the case.

In any case, the mice experiments clearly show that the correct synchroni-

zation of sleep and food intake can compensate for many metabolic imbalances – and perhaps even reverse some of them. For this and other reasons, Oster believes that stabilizing the internal rhythm can be an important factor in the treatment of metabolic diseases. After all, these disorders follow a pronounced daily rhythm and are influenced by stress. Sleep plays a key role here. "If you get sufficient sleep, and get it at the right time," Oster says, "you'll be less susceptible to these disorders." ◀

TO THE POINT

- Sleep and the internal clock are closely associated: if the internal clock gets out of sync, sleep problems can develop. And individuals who sleep poorly or irregularly disrupt their internal clock.
- Cells and organs follow their own internal clock. The suprachiasmatic nucleus, a cluster of neurons in the brain, is the master timekeeper for other clocks in the body. However, they can also function without the nucleus. Some of them receive light/dark information directly from the eyes.
- Sleep disorders can trigger metabolic disorders by throwing the activity of clock genes into confusion, disrupting metabolic processes.

GLOSSARY

Nucleus: A cluster of neurons within the central nervous system. The cells of a nucleus usually have the same or at least similar tasks. Nuclei represent another pattern of how neurons can be arranged in the brain besides in layers. In vertebrates, there are hundreds of such nuclei located in deep-lying regions of the brain, where they are surrounded by a type of tissue known as white matter, through which nerve fibers run.

Circadian rhythm: Some biological processes follow an approximately 24-hour rhythm. This rhythm is regulated by genes whose activity controls metabolic processes in cells, organs and the body as a whole, and thus also behavior. The rhythm is self-regulating, meaning that it requires no external timekeeper. However, outside factors can recalibrate the rhythm of the body's clocks. In diurnal organisms, the circadian rhythm is usually somewhat longer than 24 hours, while it is somewhat shorter in nocturnal animals (Aschoff rule).