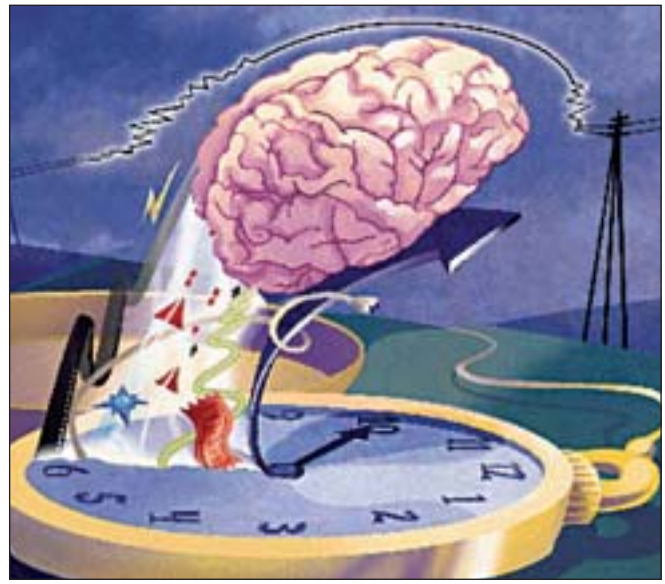
Biological rhythms in Leiden

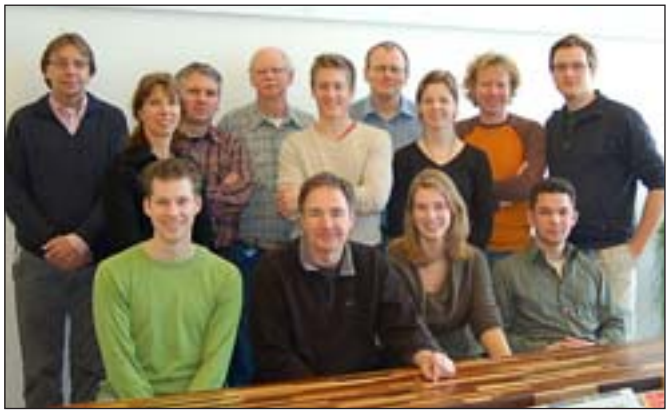
Ticking Clocks and Changing Times



The circadian pacemaker encodes the body's response to seasonal change. Johanna Meijer and her group have a twisting tale of how their work on pacemaker cells revealed exceptions to the limit cycle oscillator theory. As we arrive at a consensus, do the results have more profound importance?

What do the thirsty cries of a migratory swallow, the summer mating call of an oyster toadfish, the squeaks of a nocturnal Syrian Hamster and the groan of a tired globe-trotter have in common? Their internal, rhythmic molecular clocks. Biological clocks present a way of fine-tuning the physiological functions of an organism to suit its environment or, more precisely, the amount of daylight in its area. Molecular clocks endow migratory birds and polar animals with the ability to respond to changing seasons, allow nocturnal fauna to sense day-light and, of course, help humans to organize their work shifts and recover from jet lag.

With the strong foothold provided by studies on the molecular mechanisms underlying circadian rhythms, recent research in this field has applied techniques in neurophysiology to address the function of the "pacemaker" for newer insights from a different level of organization. Johanna Meijer's group has for over a decade been caught up with the question of how the suprachiasmatic nuclei (SCN), the mammalian version of the circadian pacemaker, respond to changes in day length. Their counterintuitive findings, coupled with studies from other groups, have given a new direction to the understanding of biological rhythms.



'They 've got rhythm': Johanna H. Meijer (2nd from left) and her group

Ticking in all organisms alike

From single-cell organisms to mammals and humans, the circadian clock serves the same function: to equip an organism with anticipatory behaviour towards approaching changes in seasons and thus offer a cue for various physiological functions, such as metabolism, mating and reproduction and defence from predators. The "pacemaker", which receives input mainly in the form of light, has evolved into the sole structure responsible for driving circadian rhythms. The electrical activity of the pineal gland in lizards, the optic lobes in *Drosophila*, the retina in molluscs and the SCN in mammals are thus modulated by light.

The first episodes

Having completed her doctoral thesis with Gerard A. Groos at Leiden University and Benjamin Rusak at Dalhousie University, Canada, in 1989, Johanna Meijer undertook post-doctoral training in Leiden and at the Biological Center in Haren near Groningen (NL). Currently a professor of Neurophysiology at the Department of Molecular Cell Biology of the Leiden University Medical Center (LUMC), Johanna and her research group have focused on elucidating the contribution of individual neurons in the SCN to the electrical activity of the functioning pacemaker. Their first observations came from extracellular recordings of electrical activity in SCN slices. "Extracellular or *ex vivo* recordings are simple and non-invasive but require extremely low noise levels", admits Johanna. This technique enabled the simultaneous quantification of the circadian discharge pattern of many neuronal subpopulations within the SCN. The results showed discrepancies during the activity peaks of small neuronal subpopulations. While large phase differences were conspicuous among very small groups of neurons, within subpopulations the neurons were mutually synchronized.

It came as a surprise when Johanna and colleagues, using cluster analysis, determined the activity profile of single cells from the multiunit signal of the SCN ensemble. The single cells of the 24-hour molecular clock showed only short durations (5-7h) of increased neuronal activity. "Our results did not comply with the 'half-conscious' expectation that SCN neurons are active throughout the day and our manuscript was at first rejected by many journals," recalls Johanna. "Only with the timely help of Joseph Takahashi from the University of Texas Southwestern Medical Center at Dallas, the paper finally made its appearance in *PNAS*", she sighs, recollecting the relief (*PNAS* vol. 100(26): 15994-99).

Despite the controversy, the determined group proceeded to confirm their hypothesis that, "changes in phase distribution among oscillating neurons is the most effective mechanism to code for the response

of the SCN to day-length". They began their studies with simulations (*J. Biol. Rhythms* vol. 21(4): 301-13) and thereafter took *in vitro* and *in vivo* approaches to make multi-unit subpopulation and single-unit recordings and confirmed the plausibility of their prediction (*Current Biol.* vol. 17: 468-73). Individual SCN cells peak at different times of the day with small periods of neuronal activity. The activity peak of single neurons, as both simulations and single-cell recordings revealed, is condensed into shorter day-lengths, implying a higher degree of synchronization among the contributing neurons. Conversely, oscillating neurons in longer days are desynchronized, producing a broader peak of activity for the whole group. The response to day-length is thus, in every way, a network property.

Working in the opposite way again

Even as the group was coming to a conclusion about the 'intrinsic' network property of the SCN and its response to day length, they were forced to make another tough hypothesis about phase shifts and photoperiodicity (*Plos ONE* vol. 4(3): e4976). It has long been believed that high-amplitude rhythms show reduced phase shifting responses than do low-amplitude rhythms. But again, in the Meijer group it had worked in exactly the opposite way! They observed that the high-amplitude rhythms characteristic of short days (when neurons are highly synchronized resulting in a high frequency of multiunit activity) exhibit a large overall shift in response to a light input signal. They excluded a lack of response to NMDA as a factor for decreased phase-shifting capacity among neurons from long days, as the group observed no difference in the acute response to increased concentrations of the neurotransmitter. Though their observations vividly illustrated the photoperiodic modulation of the phase shifting capacity of the circadian rhythm, Johanna and colleagues had to fight hard to proclaim their discovery owing to general disconcertion about their challenge to the classical limit cycle oscillator theory. "The theory holds valid in explaining rhythms in single-cell organisms, but complex neuronal networks are governed by different rules," affirms Johanna. She adds that phase-shifting responses are not explained by merely accounting for responsiveness to light but that it also takes neuronal network analysis to decipher the mechanism.

Johanna is now pleased that there are many other groups that have confirmed her observations using molecular and neurophysiological approaches and have now come to a consensus about the short-term activity of single cells and their effect on the concomitant output of the SCN. Despite the challenges that she was confronted with, Johanna explains that the heart of her interest in studying how behavioural traits are encoded by the brain kept her spirit alive and lent her persistence. "What makes biological rhythm research attractive," she adds with excitement, "is that it is here that one can quantify rhythms in terms of period or revolution time, shifts in phase along the X-axis and amplitudes and this enables a comparative physiological research across different species. Moreover, even within the same organism it allows comparison of different levels of organization, from molecular and cellular to networks and all the way up to the behavioural level". Johanna confirms, that despite the reductionist approach in science, it is very worthwhile looking at higher levels of organization, since many attributes, including those of the circadian system, originate here and not all properties are encoded at the genetic level.

Untrodden paths

The Meijer group has a sound combination of technical expertise, with specialists in extracellular electrophysiology in live animals and slices, patch clamping, sleep recordings and mathematical modelling. Johanna and her colleagues also collaborate with international scientists, such as Gene Block and Chris Colwell at the University of California in Los Angeles and Bill Schwartz at the University of Massachusetts Medical School in Worcester.

The inquisitive team is now all set to apply their studies to addressing the question 'is ageing associated with those changes in the SCN that are observable during long days?' The similarities, they theorise, between ageing and longer day length include a greater degree of de-synchronization between neurons, hence a decreased phase-shifting capability and a decrease in the amplitude of rhythms. One cannot help but agree with their rationale. Ageing is indeed manifested by increased difficulties in sleeping at night and staying awake during the day, accompanied by problems with adjusting to new time zones and working shifts. Does the circadian rhythm, as we age, go awry? Do the contributing neurons stop acting in harmony? Does the SCN become refractory to seasonal changes? The Meijer group are in a great position to crack these problems.

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