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Left: Prof.dr. Joke Meijer, Leiden University Medical Center
Right: Henk Tjebbe van der Leest MA, Leiden University Medical Center

4 How our biological clock tells the time of the year

Before it's time to wake up, a well-timed release of cortisol prepares our bodies for action by raising blood pressure and glucose levels. Similarly, animals become reproductive at a certain time of the year, so that off-spring is born when food is available and temperatures are optimal. Our biological clock is essential for survival, but the way it works has long been misunderstood.

'It has been known for some time that the suprachiasmatic nuclei (SCN), two minuscule balls at the centre of the mammalian brain, function as a biological clock for 24-hour rhythms as well as for seasonal rhythms,' says Joke Meijer, professor of neurophysiology at Leiden University Medical Center. 'This clock generates electrical signals during daytime and is silent at night. Since the days in summer are longer, the clock can tell the time of year by the duration of the activity peak.' Now her group recently found that surprisingly, the mutual timing of individual nerve cell activity is the driving force behind this seasonal encoding.

In the past ten years, Meijer and her colleagues developed electro-physio-logical recording techniques to measure the activity of individual nerve cells in the biological clock. 'We record their action potentials with electrodes in mouse brain slices *ex vivo*. Interestingly, these cells continue to produce a 24-hour rhythm autonomously as long as you feed them.'
To widespread astonishment, including their own, they found in 2003 that each cell is only active for four to five hours. 'Some are active at the beginning of the day, others at the end or the middle. Together they explain the activity of the clock as a whole. We were the first ones to look at individual cells. Everyone in the field just assumed they would all act simultaneously throughout the day. Then we hypothesised that our findings might also explain seasonal rhythms. That's when we applied for the N*W*O programme grant.'

Weak signals

In a small lab down the hall, a microscope focused upon a brain slice attached to electrodes stands in a Faraday cage. 'We have to block out all disturbances from outside, such as mobile phones, lights and all the equipment in this room,' says Henk Tjebbe van der Leest. He is the PhD-student who was assigned to study how the seasonal rhythm of the biological clock is encoded. Van der Leest explains that the SCN nerve cells are some of the smallest cells in the brain and emit very weak signals.
The minute electrodes, which he had to make himself, are connected to a device with many controls and a small screen showing a live graph of the electrical activity that is being measured. 'My first task was to optimize the recordings and the computer analysis techniques. The recorded tissue signal is composed of many electrical pulses of around a hundred cells. On the computer you extract the individual cells from the wave pattern. I had to try different approaches to get a higher success rate.'

Identical firing patterns

During the project, mice were kept under two different light regimes, mimicking long summer and short winter days. Meijer explains: 'When days are long and nights are short, like in summer, the clock is active for sixteen hours at our latitude. In winter, the clock is only active for around eight hours. We thought this difference could be based on the change in distribution of the 4-5 hour single cell activity peaks.'
Van der Leest found that indeed there were no differences between the individual firing patterns of nerve cells of mice that were kept under short or long days regime. 'The only thing that differed was their distribution over 24 hours. For instance, in winter all cells are active around the same time, which results in a narrower peak of activity with a higher amplitude.'

He worked closely together with Jos Rohling from the Leiden Institute of Advanced Computer Science (LIACS), who did the simulations based on these recordings. 'From behind his computer, Jos found out that this was in fact the most effective way to code for day length, rather than by individual cells varying the length of their activity periods over long and short days.

That was however a very counterintuitive result. Back then, people still thought that each cell had a clock that coded both for 24-hour and seasonal effects all by itself.'

According to Meijer, they would not have come this far without the NWO grant. 'The project really required a full-time PhD student for the neurophysiological work. But also the collaboration with the LIACS contributed a lot to the quality of the papers and would never have been as intensive without this project.'

Winter depressions

Van der Leest's first paper got a lot of attention in the field and has been cited frequently. His latest one came out in March, and dealt with seasonal differences in phase shifting capacity of the clock. He plans to continue this research in a postdoc project by looking at gene expression in the clock cells. 'Although this work is quite fundamental, it is interesting to see that it has links to lots of everyday things, like night shift work, jetlags and winter depressions.'