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LENScience Senior Biology Seminar Series

Circadian Rhythms: Keeping Time

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How often do you have difficulty getting up in the morning?

Do you sleep later during the school holidays?

Despite what your parents may say about your need for sleep, there is actually a biological reason for teens to wake later and it's all based around your body clock or more precisely your **circadian rhythms.**

The rotation of the Earth around the Sun, and the Moon around the Earth, produce the cycles that we know as night and day, lunar months, and years. Animals and plants have rhythms that match these cycles. A simple example of this is activity linked to day and night. Kiwi are active at night; behaviour that is described as nocturnal. Pūkeko are active during the day; behaviour that is described as diurnal. Daily rhythms such as these are called **circadian rhythms**.

Circadian Rhythms

Circadian rhythms are found in most living things, including plants, animals and many microorganisms. These rhythms are the repeating patterns that we see in the biochemical, physiological, and behavioural processes. The rhythms follow a roughly 24-hour cycle linked to the patterns of light and dark in the environment around the organism. Humans are no exception, with processes and behaviours such as sleep, cell division, and alertness following this 24-hour cycle. A fascinating fact about circadian rhythms is that when we take away the environmental cues such as light and dark, or the temperature changes that occur during the day and night, the rhythms keep going. If you place a person in a room where there is always light and the temperature never changes, they will still follow a roughly 24-hour pattern of sleeping and waking. This led scientists to suspect that the rhythm is controlled by a biological clock inside the organism.

Dr Guy Warman is a Senior Lecturer in Anaesthesiology at the University of Auckland. Guy leads a team of researchers from the Faculty of Medical and Health Sciences and the School of Biological Sciences, working on understanding human biological clocks, in particular sleep timing and circadian rhythms, and the similarities between sleep and anaesthesia. The team works on both understanding the fundamental biology of circadian rhythms, and applied research in humans.

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Human Circadian Rhythms

Circadian rhythms in humans can be investigated by measuring the rhythms of peak performance for physiological and behavioural processes. Figure 1 shows examples of processes in humans that display these rhythms. Notice that in most cases, there is a period of peak activity or peak performance during the 24-hour cycle. Look at the example of skin cell division (mitosis) which peaks after midnight for about a 2 hour period.

<u>Human Circadian Rhythms</u>

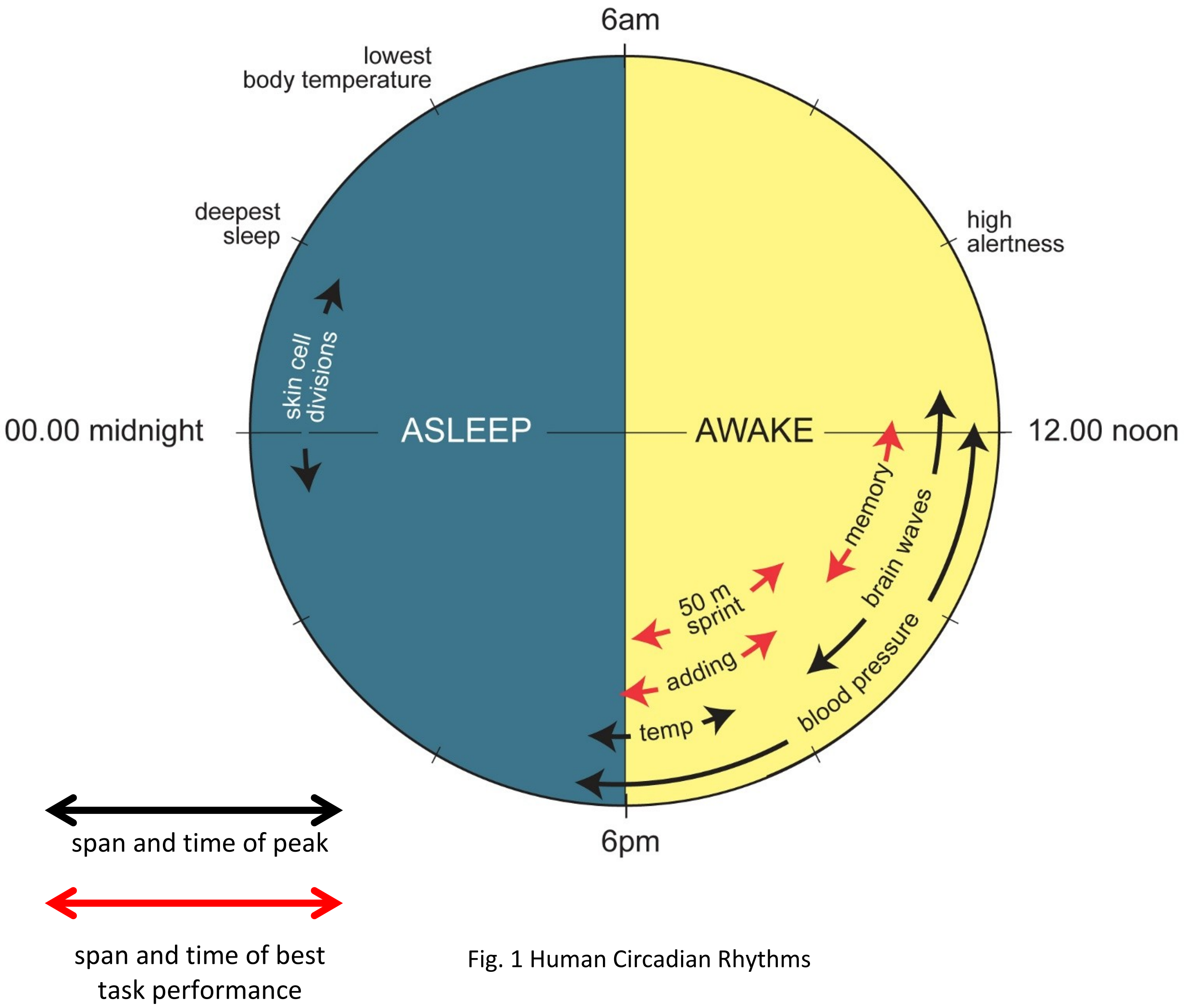


Fig. 1 Human Circadian Rhythms

The time that it takes for a circadian rhythm to run one cycle is called the *period* of the rhythm. In most organisms, the natural or innate period of the circadian rhythm is not exactly 24-hours, however the regular changes in light and temperature that we experience in our environment help us to adjust our biological clock to a 24-hour day. This called **entrainment**.

Experiments have clearly demonstrated that the human biological clock ticks with an innate period of 24.3 hours, slightly longer than the 24-hour day. This was measured by putting people into constant environmental conditions where light and temperature did not change at all over a period of several months, allowing their biological clocks to 'free-run'. Scientists measured when activity started and stopped in the people taking part in the experiment. These measurements are graphed to show when the subject are active. The graphs of the activity patterns are called **actograms.** A typical set of results is shown in the actogram in Figure 2 on page 3.

Interpreting an actogram showing human rhythms

The actogram below shows the results of activity measurements for one human subject over a period of 87 days (almost 3 months). Look carefully at the X-axis. It covers a period of 48 hours. Each day is shown side by side with the next day so that you can see clearly when the activity patterns start and end.

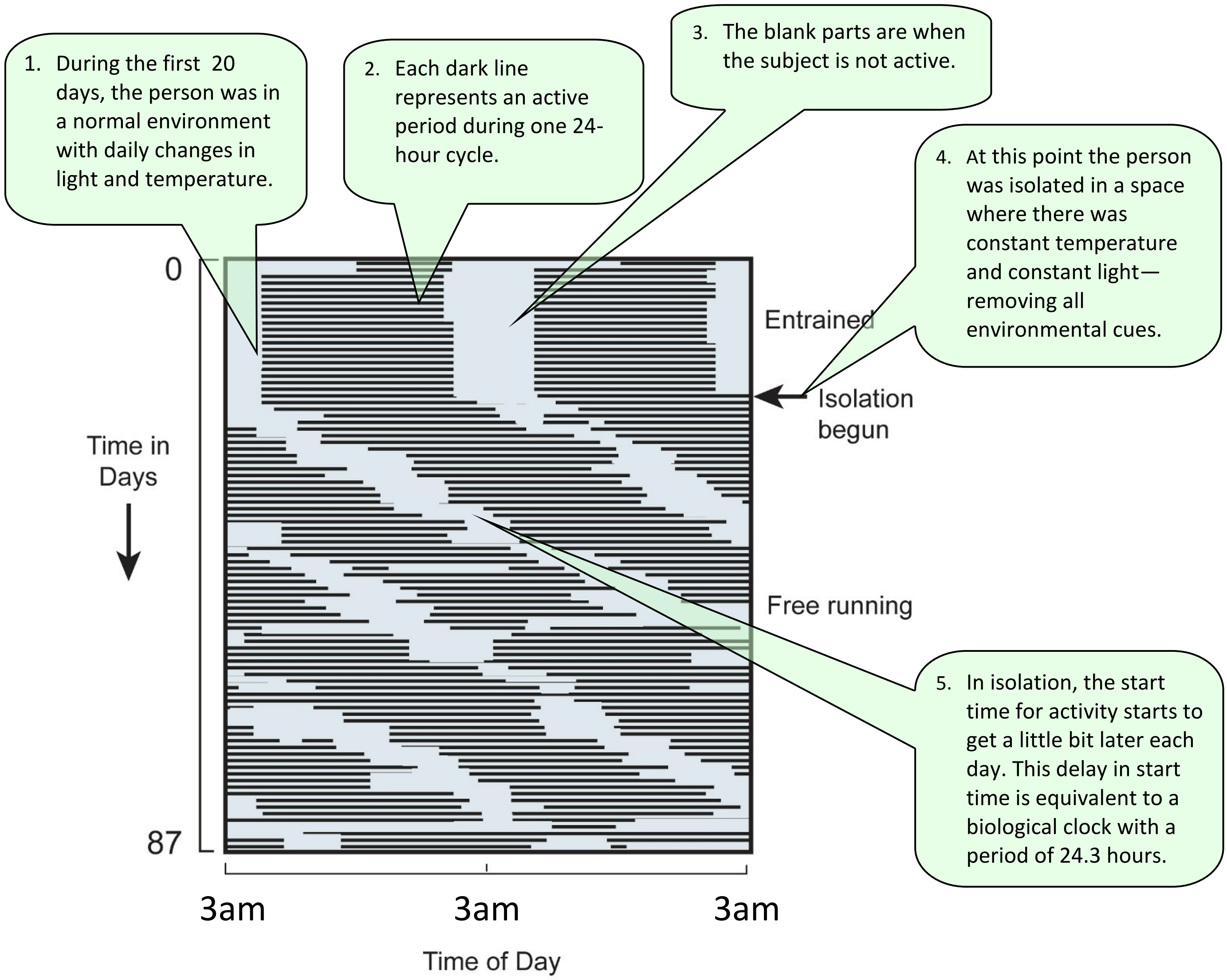


Fig. 2: Activity/time graph for a human.

The human biological clock is adjusted (or entrained) to the 24-hour day on a daily basis by light sensed through the eyes. Morning light 'phase advances' the clock to shift it to an earlier time zone, whereas evening light 'phase delays' the clock to shift it to a later time zone. As our clock ticks with a period slightly longer than 24 hours, it is the morning light that is essential to keep our daily rhythms adjusted to the 24-hour day.

Effect of blindness on circadian rhythms

As the light that entrains the human biological clock is perceived through our eyes, you can imagine the potential problems that blind people may experience. People without eyes or with a reduced light perception can have problems entraining their clocks to a 24-hour day. This can result in their clocks literally 'free-running'. This means that each day the time their clock wakes them up will get later and later (following the innate 24.3 hour period). Guy and his team have just finished a study of this in New Zealand. They interviewed 300 blind people throughout the country and the findings suggest that up to 25% of blind people may suffer from sleep-timing problems (Warman et al., unpublished).

Jet Lag and Biological Clocks

Knowing that humans have a biological clock gives us a better understanding of what causes jet lag and how we can help alleviate it. Jet lag results from travel so far east or west that our biological clocks are not immediately able to adjust. If you travel to California for example, it is approximately four hours earlier than here. This means that when you arrive your biological clock is four hours out of sync. Consequently in the first few days it can be very hard to get up in the morning because our clocks are adjusted to New Zealand time so are telling our bodies that it's not time to wake up. Eventually our biological clock does adjust but we can speed this up by using knowledge of how light affects human biological clocks. By getting light in the morning and avoiding it in the evening we will assist our circadian clocks to 'phase advance' to California time. By contrast, getting light in the evening and not in the morning in California will make the jet lag last longer.

Social Jet Lag and teenagers' sleep patterns

Just like travel, puberty and adolescence can also cause jet-lag of a sort where teenagers find it very difficult to wake up in the morning. Some scientists call this 'social jet-lag'.

Between childhood and adulthood the timing of sleep changes drastically. The diagram in Figure 3 shows the self reported sleep timing (or chronotype) of people from the ages of five to 90. As you can see, when we are young we wake up early but between the ages of 15 and 20 we start to sleep until later and later in the day. Then between the ages of 20 and 30 people drift back to sleeping earlier again. These changes cannot be attributed to genetic change as the genes controlling our clocks remain the same throughout life. The differences in waking time appear to result from a combination of behavioural and physiological changes that occur during puberty and adolescence.

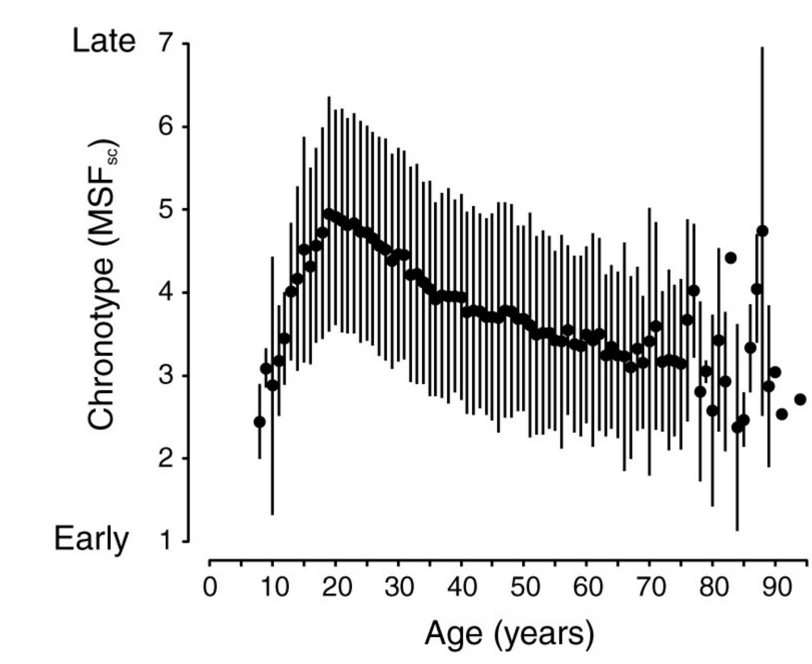


Fig. 3 Age dependent changes in chronotype.
From: Roenneberg et al., 2004, reprinted with permission from Elsevier

As a result of data like this some high schools around the world (including one in Wellington) have changed school times to starting and ending later in the day. Many are now reporting less students falling asleep in class! One important thing to remember is that these changes in sleep timing are not just laziness. If you find that you are struggling to get up in the morning, a good way to combat this is to avoid staying up too late at night. It is also important to avoid high levels of light exposure late in the evening (which will phase delay your clock) and to maximize your light exposure in the early morning (which will advance your clock). So sleep with your curtains open and go for a morning walk.

The workings of the biological clock

Animals and plants possess endogenous biological clocks whose innate period is linked closely to the period of the relevant geophysical cycle such as day length or year length. We say a biological rhythm is endogenous if it continues even when external cues such as day length are removed. By observing behaviour and physiological processes, we can clearly see these rhythms. The biological clock that controls circadian rhythms in mammals consists of two groups of nerve cells in the brain called the Suprachiasmatic Nuclei (SCN). The SCN are found in the hypothalamus just above the optic chiasm—the part of the brain where the optic nerves partially cross (Figure 4).

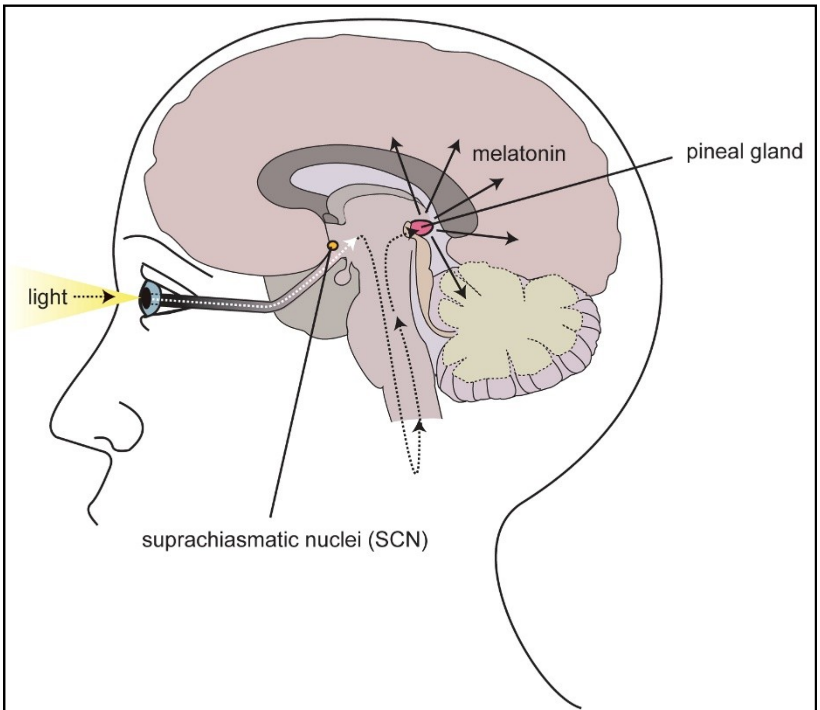


Fig. 4. Location of Suprachiasmatic Nuclei

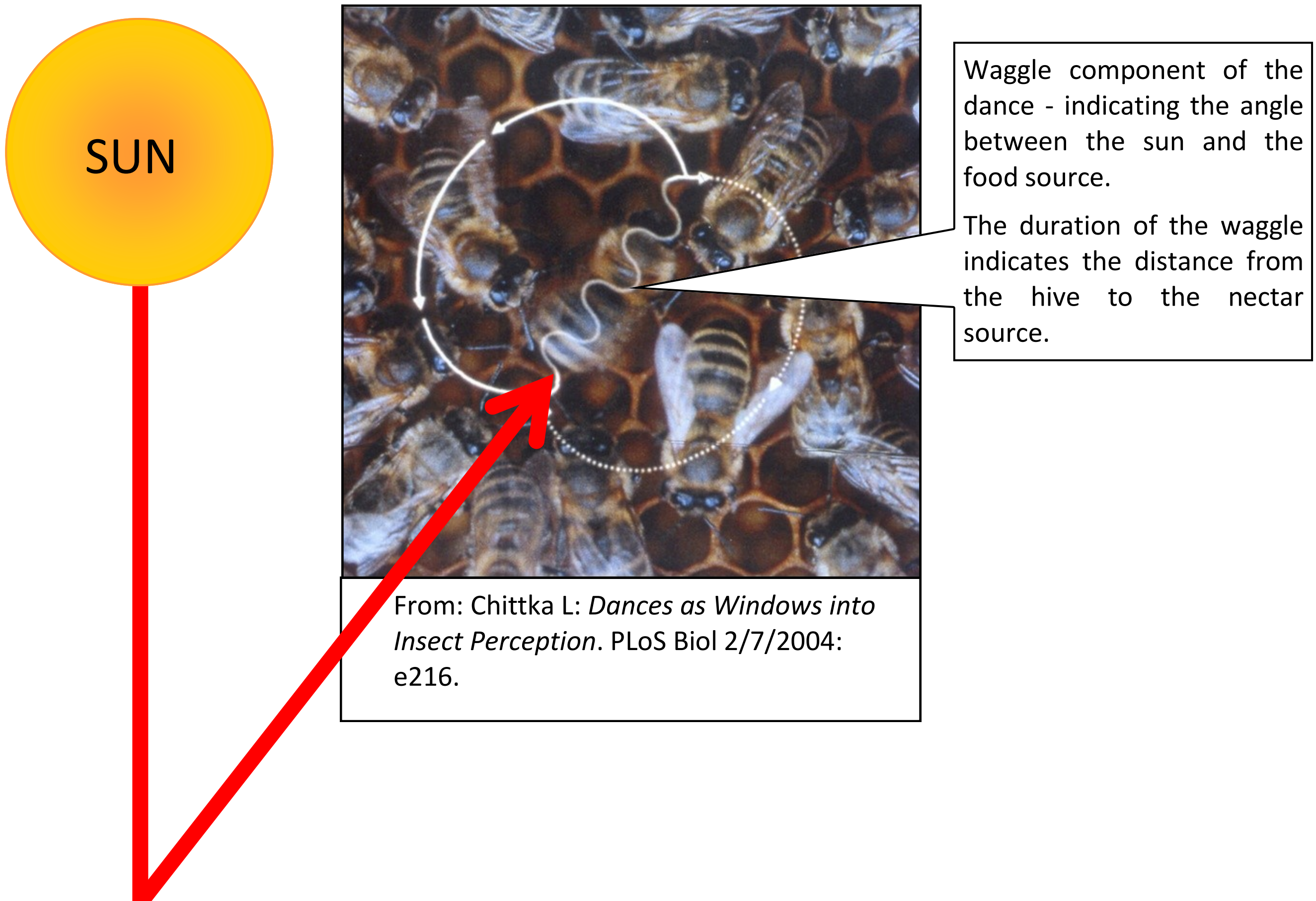
Investigating the differences between sleep and anaesthesia

When people wake up from a general anaesthetic they often seem confused about what time of the day it is, especially how much time has passed. This suggests that general anaesthesia somehow affects our sense of time. Guy's research team are interested in studying this phenomenon in order to potentially better manage the care of patients who undergo a general anaesthetic.

As you have seen in previous LENScience seminars (See Using Animal Models to Understand Aging), significant advances in understanding of how biological systems work can be made by studying model organisms. The classic model organism is the fruit fly *Drosophila melanogaster* but it is not the only one. Guy's research group uses the honey bee *Apis mellifera,* to investigate how general anaesthesia may affect our perception of time.

The honey bee is unique in the animal kingdom in that they have a 'continuously consulted clock'. This means that bees are able to 'consult' their clock at any time to very accurately determine the exact time of the day. This clock forms the basis of their time compensated sun-compass. Bees navigate using this sun compass. They know where the sun should be at any time of the day and use this to determine the compass directions of a nectar source.

On finding a good nectar source the bee will fly back to the hive and perform a waggle dance in the darkness of the hive. This dance informs other forager bees in the hive of the direction and distance of the nectar source. The angle of the 'waggle' component of the dancing bee (with respect to the vertical) is exactly equal to the angle between the sun and the food source.



From: Chittka L: *Dances as Windows into Insect Perception*. PLoS Biol 2/7/2004: e216.

As the sun moves across the sky over the course of the day, the dancing bees change the angle of their dance so that they are still communicating the correct direction of the food source. They do this by consulting their internal clock and adjusting the angle of the dance accordingly.

Guy and his colleagues Dr James Cheeseman and Dr Craig Millar (from the University of Auckland) together with collaborators from the Free University of Berlin, used the fact that bees have a continuously consulted clock to model how general anaesthesia affects our sense of time.

Aim: To test how general anaesthesia affects sense of time in an insect model.

Method

Dr Cheeseman and Dr Millar anaesthetised honey bees for a six hour period using the human anaesthetic agent isoflurane. The bees were then fitted with a harmonic radar transponder (see picture on right) which enables them to track the direction of the bees' flight with a modified ships radar. The direction of the flight is used to determine what time of the day the bees think it is when they wake up from their anaesthetic. The bees had already been trained to get nectar from a source at a known direction from the hive. After the bees were anaesthetised, they were released and their path was tracked (See Figure 5).

Bee fitted with a harmonic radar transponder

Results

In Figure 5, **Diagram *a*** shows the paths of the bees as tracked by the radar.
The grey track shows the path of the bees that haven't been anaesthetised. When released they set off in a direction that effectively heads straight for the hive.

The blue track shows the path of bees that had been anaesthetised for 30 min. They were a little more confused but did head in much the same direction as the unanaethetised bees.

The bees that were anaesthetised for 6 hours headed off in completely the wrong direction (red track).

Diagram *b* shows the same data but this time the direction each group of bees headed off in has been plotted against compass direction.

These results show that on recovering from a six hour anaesthetic, bees show an angle of orientation which is consistent with the bees perceiving that it is earlier in the day than it really is, ie., that time has not passed during their anaesthetic.

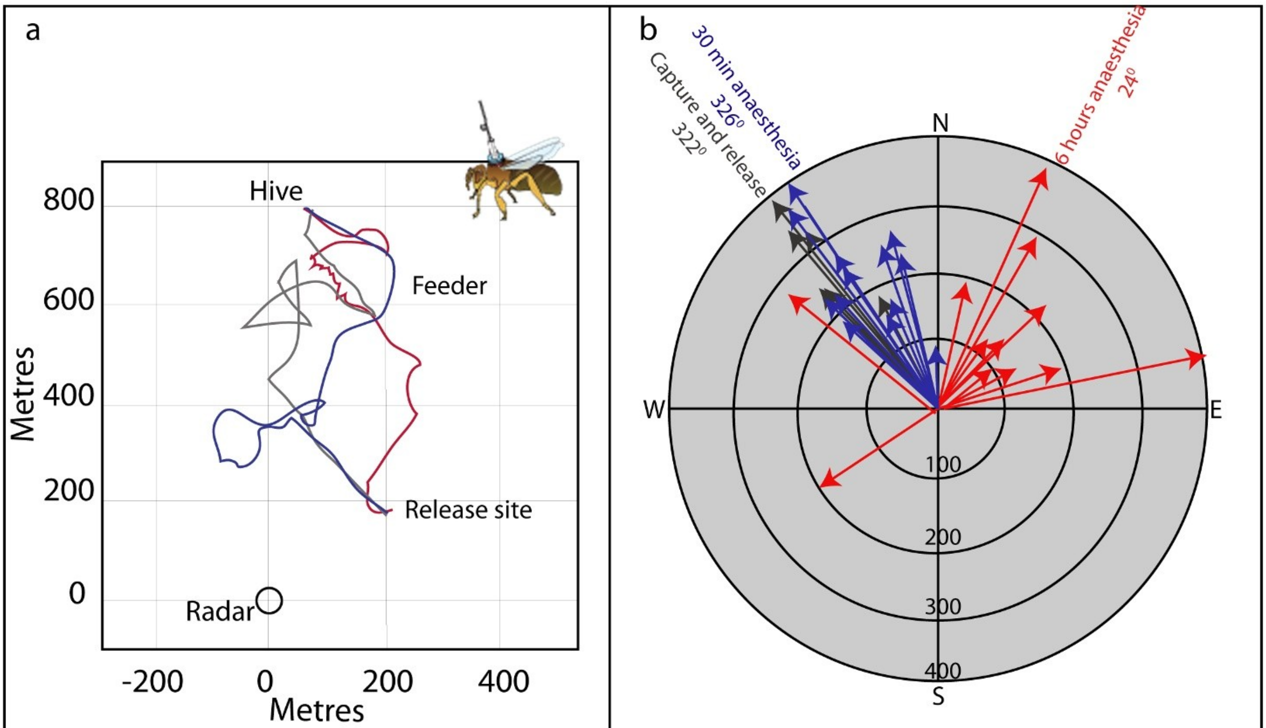


Fig. 5. Effect of anaesthesia on bees' sense of direction
From Cheeseman et al., unpublished data

The next hypothesis the researchers wanted to test was whether this change in orientation is due to the bees' biological clocks being stopped by the anaesthetic. A PhD student working in Guy's group (Ms Eva Winnebeck) used behavioural recording of bee activity and the molecular analysis of the expression of two clock genes (*period* and *cryptochrome)* to test this hypothesis.

Eva's results

After a six hour anaesthetic with isoflurane the circadian rhythms of activity of an entire hive of bees appeared to be shifted to a later time zone (ie., phase delayed) by 4-5 hours (See Figure 6).

The molecular basis for the behavioural phase shift was established by using real time quantitative PCR to measure the expression of the clock genes. Eva found that the anaesthetic resulted in the rhythms of expression of *cryptochrome* mRNA being damped (ie., the oscillations have a smaller amplitude) and phase delayed (See Figure 7). This shows that anaesthetic effectively stops the biological clock.

Over 230 million people undergo anaesthesia annually; more than the number of children born each year. These findings have implications for such patients and their caregivers as they provide a scientific explanation of why, on waking from an anaesthetic, many patients feel that they have only just 'gone to sleep'.

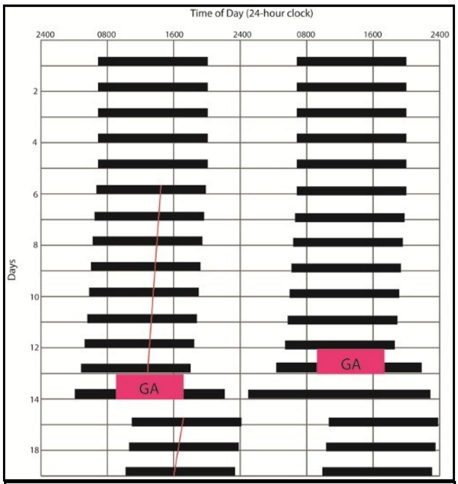


Fig 6. Circadian rhythms of bees before and after treatment with general anaesthetic (GA)
Redrawn from: Winnebeck et al., unpublished data

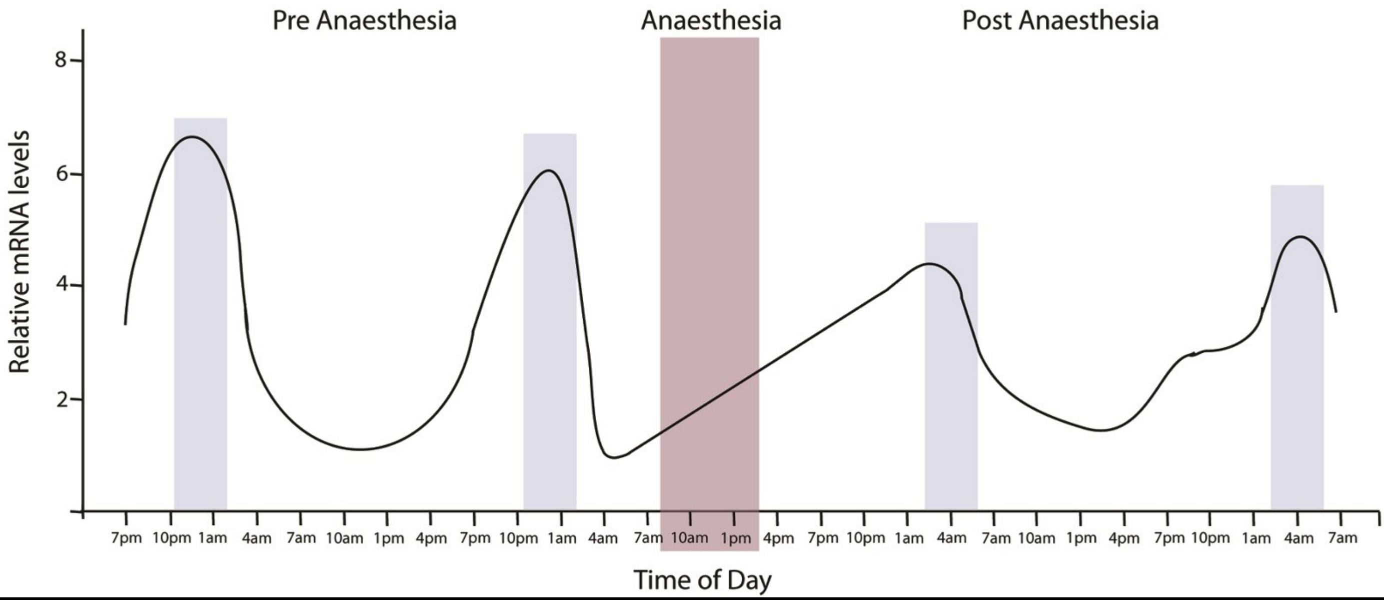


Fig. 7. Expresssion levels of mRNA from Cryptochrome pre and post treatment with anaesthesia.
Redrawn from: Winnebeck et al., unpublished data

Conclusion

Chronobiology or the study of biological rhythms and the clocks that control them is a unique area of biology because of the direct link that can be made between what is happening at the sub-cellular molecular level and the behaviour of an organism or even an entire population. This, and that fact that chronobiology research has direct application to human health and sleep makes it a fascinating area of research.

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