

# Untangling the Clock: Towards a Network View of the *Drosophila* Circadian System

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Circadian networks generate endogenous rhythms that optimise organism behaviour for a periodic environment. However, surrounding environments are themselves intrinsically flexible. A good circadian pacemaker must therefore possess the capacity to resynchronize its oscillations to external cues (entrainment).

What happens when these cues disagree?

## Circadian Locomotor Behaviour in *Drosophila*

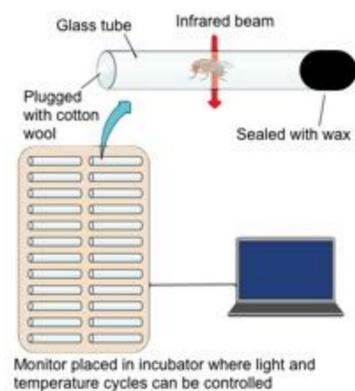
- Locomotor activity is under strict circadian control. It is a powerful behavioural marker of clock output.
- *Drosophila* entrain to both **LIGHT** and **TEMPERATURE** cycles.



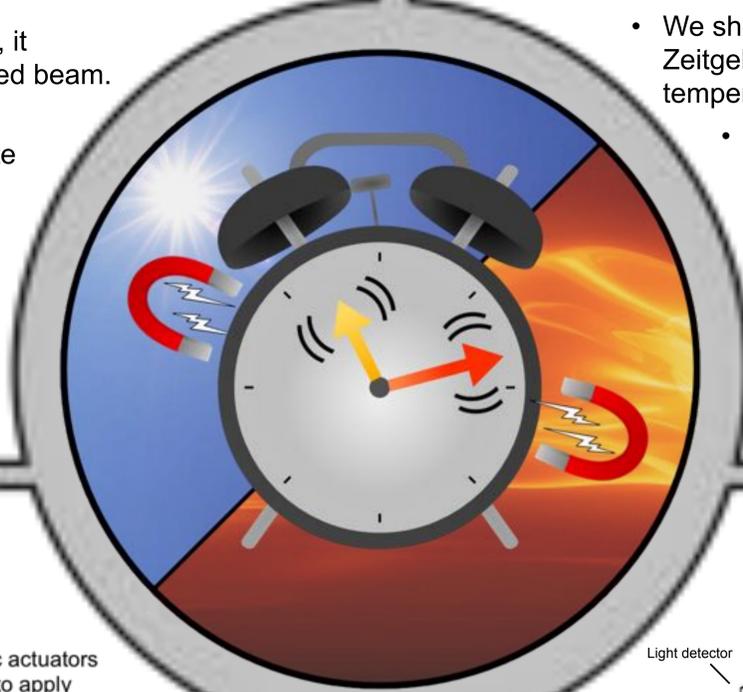
We see anticipatory 'ramping' of activity prior to Zeitgeber onset (ZT0) and offset (ZT12)

Figure adapted from Yoshi 2009<sup>1</sup>. A) Entrainment to light-dark cycles in constant temperature. B) Entrainment to temperature cycles in constant darkness.

## Measuring Fly Behaviour



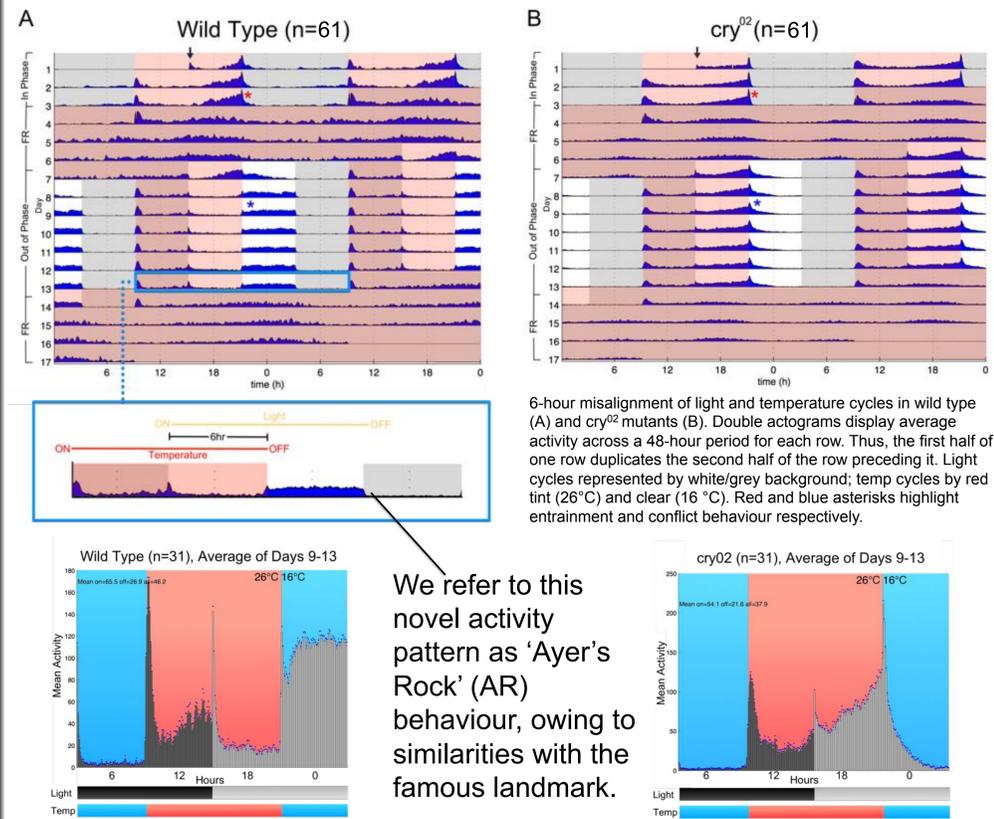
- As the fly moves, it breaks the infrared beam.
- Number of beam breaks per minute recorded.
- We use a modified setup with 9 infrared beams per tube to increase resolution.



- Cryptochrome mutants (*cry*<sup>02</sup>) do not entrain well to light<sup>2</sup>.
- We show that *cry*<sup>02</sup> mutants are largely unaffected by Zeitgeber misalignment, and appear to entrain only to temperature cycles.
- This supports the view that AR behaviour is a result of sensory interference within the clock network.

AR behaviour was not seen with misalignments of 12hr or 3hr. The novel phenotype is restricted to a discrepancy of 6hr.

## 6hr Misalignment of Light and Temperature Cycles Generates Novel Behaviour in Wild Type Flies

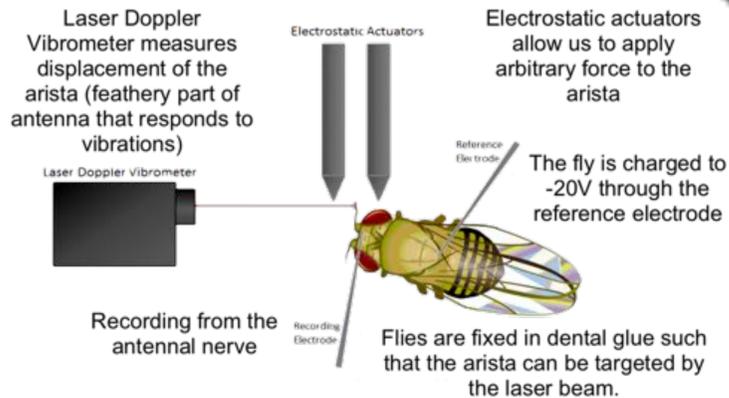


6-hour misalignment of light and temperature cycles in wild type (A) and *cry*<sup>02</sup> mutants (B). Double actograms display average activity across a 48-hour period for each row. Thus, the first half of one row duplicates the second half of the row preceding it. Light cycles represented by white/grey background; temp cycles by red tint (26°C) and clear (16°C). Red and blue asterisks highlight entrainment and conflict behaviour respectively.

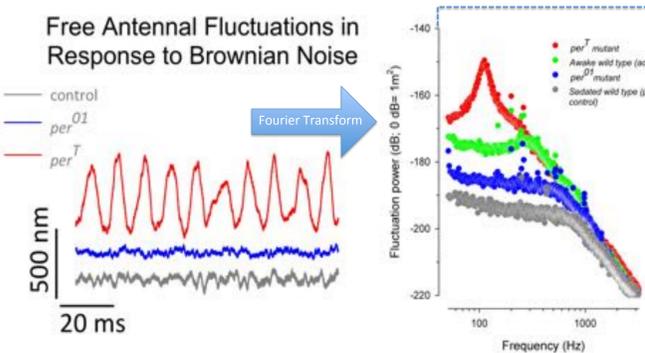
We refer to this novel activity pattern as 'Ayer's Rock' (AR) behaviour, owing to similarities with the famous landmark.

## A Separate Role of PER in Mechanotransduction?

### Measuring *Drosophila* Hearing

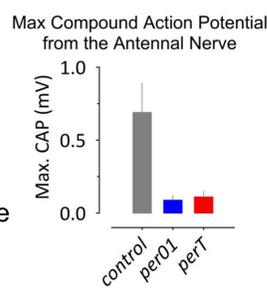


dB scale proportional to energy in the system

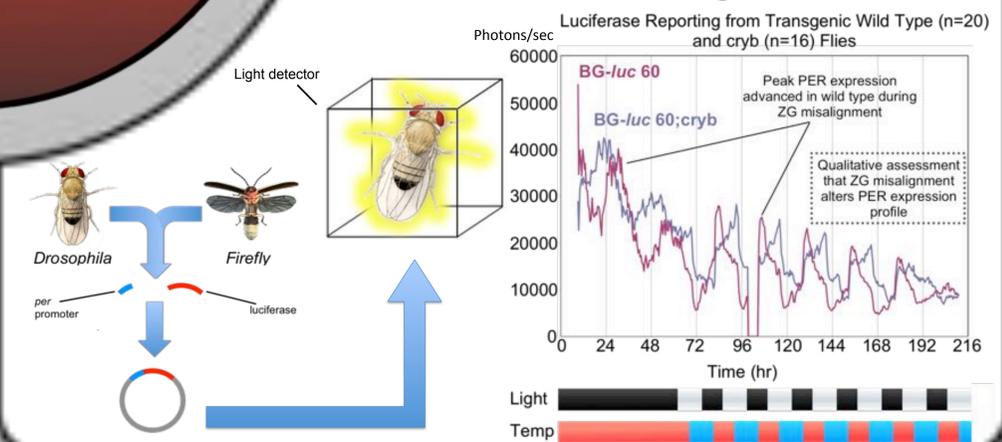


- Fluctuation data reveal differences in the sensitivity of the hearing apparatus between wild type and PER mutants. This could reflect a role of PER in mechanotransduction.

- Electrophysiological recordings further support a role of PER in hearing.



## PER Expression Profile Appears Altered in Conflicting Conditions



Our investigation of Zeitgeber misalignment supports a growing view of the clock as a network of independent oscillatory subunits, inviting the idea that these may become dissociated. We are currently constructing a mathematical model to further test this hypothesis.

The possible role of PER in mechanotransduction hints at a circadian influence in sensory gating, blurring the distinction between output and input within the circadian network.

## References

- 1) Yoshii T, Vanin S, Costa R, Helfrich-Förster C. Synergic entrainment of *Drosophila*'s circadian clock by light and temperature. *J Biol Rhythms*. 2009 Dec;24(6):452-64. doi: 10.1177/0748730409348551.
- 2) Yoshii T, Hermann C, Helfrich-Förster C. Cryptochrome-positive and -negative clock neurons in *Drosophila* entrain differentially to light and temperature. *J Biol Rhythms*. 2010 Dec;25(6):387-98. doi: 10.1177/0748730410381962.
- 3) Brandes C, Plautz JD, Stanewsky R, Jamison CF, Straume M, Wood KV, Kay SA, Hall JC. Novel features of *Drosophila* period transcription revealed by real-time luciferase reporting. *Neuron*. 1996 Apr;16(4):687-92.

