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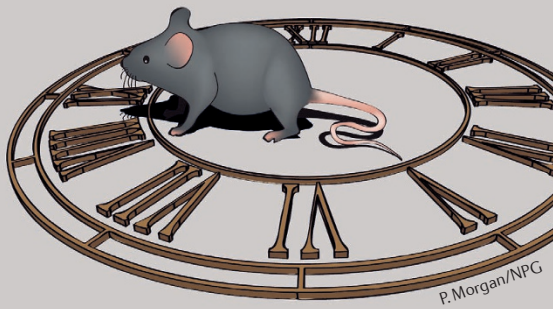
CIRCADIAN GENETICS

Tick Tock—keep your eyes on the clock



Researchers have developed a new technique for the real-time imaging of gene expression *in vivo* and used it to simultaneously monitor circadian gene expression in multiple body tissues of freely moving mice.

Circadian rhythms are under the control of 'clock' genes that are expressed in an oscillating manner with 24 h rhythmicity. However, little was known about the patterns of clock gene expression in unrestrained animals, owing to the challenge of measuring reporter gene expression in moving targets.

Hamada *et al.* set out to monitor clock gene expression in six body regions in the mouse, including the ears, skin and parts of the brain. To enable the localization of these regions of interest (ROIs) in moving mice, they attached fluorescent scintillators to sites on the body and head and placed the animals in a dark recording cage containing two highly sensitive cameras. Using tracking software based on pattern matching, they were able to determine the 3D coordinates of the scintillators—and hence the location of the ROIs—by combining the 2D coordinates of the fluorescence detected by the cameras.

To facilitate visualization of clock gene expression, the authors used mice in which the promoter regions of the clock genes *Per1* or *Bmal1* were fused to the bioluminescent reporter genes *Luc* or *ELuc*, encoding luciferase. The required substrate, luciferin, was supplied through a pump. A set of algorithms was used to calibrate bioluminescent signal intensity according to distance from the cameras using the scintillator coordinates.

Mice that had been reared with cycles of 12 h light and 12 h darkness were then housed in continual darkness in the recording cage and monitored for several days. In all body regions, *Per1–Luc* expression was observed to peak in the evening and reach a trough in early morning, whereas *Bmal1–Luc* displayed the opposite pattern; this observation was recapitulated in *ex vivo* analyses of brain slices.

Exposure of *Per1–Luc* mice to a single 8 h light pulse resulted in a phase shift of both locomotor activity and *Per1–Luc* expression. In all six body regions, the rhythmicity of *Per1–Luc* expression exhibited a phase delay; however, this effect differed between regions, occurring one day after light exposure in the olfactory bulb (a structure in the brain associated with smell) and two days after exposure in the other five ROIs. In addition, these five regions displayed a small extra peak on the second day after light exposure, suggestive of desynchronization of circadian rhythms between tissues, comparable with that seen in humans with jet lag.

As well as providing new insights into the kinetics of circadian rhythms across body parts *in vivo*, the techniques developed in this study could, with adjustments, be applied to studies of non-circadian genes.

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ORIGINAL ARTICLE Hamada, T. *et al. In vivo* imaging of clock gene expression in multiple tissues of freely moving mice. *Nat. Comms.* http://dx.doi.org/10.1038/ncomms11705 (2016)