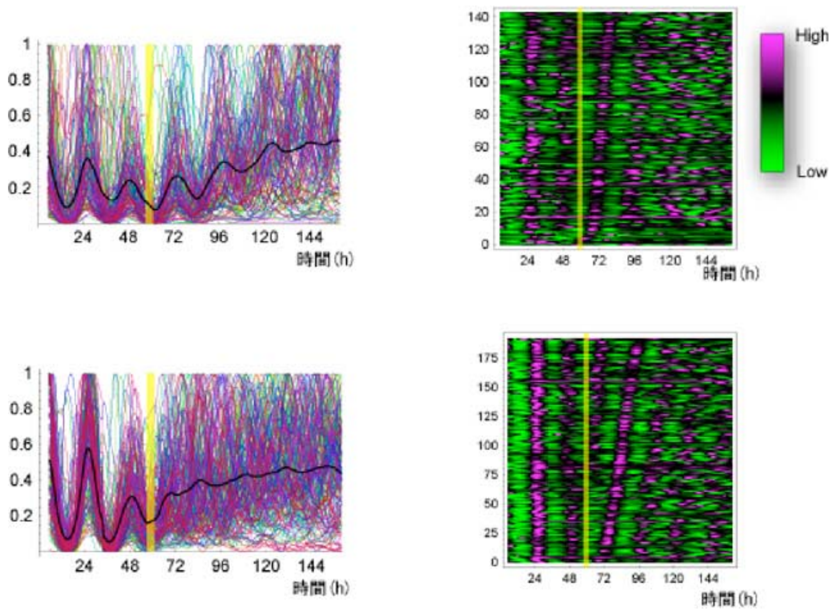
## Shedding light on singularity

*October 26, 2007* – Many of the mammalian body's cyclical activities, from sleep to the secretion of hormones, follow schedules set by biological clocks. Such clocks are subject to regulation by both environmental cues and intrinsic signaling determined by a complex network of genes. Under normal day-light conditions, biological clocks provide a reliable timetable for a wide spectrum bodily functions, but they are also susceptible to what is known as "singularity behavior," the loss of robust periodicity when challenged by critical stimuli, such as a pulse of bright light during the night hours. The mechanisms underlying such singularities, however, have remained unclear despite decades of intensive study.




In non-photo-responsive cells, oscillations gradually degrade over time independent of exposure to a light pulse (top left) and rhythmicity of individual cells is not lost; while in photo-responsive cells, the amplitude of the oscillations in the multi-cell sample (bottom left) is reduced dramatically although the individual cell maintains its periodicity.

Now, a study by Hideki Ukai and Tetsuya J. Kobayashi of the Laboratory for Systems Biology (Hiroki R. Ueda; Team Leader) has revealed the likely means by which singularity is triggered in the mouse. Using exogenous melanopsin, a photosensitive molecular receptor, to render cultured cells responsive to light stimuli, the team determined that singularity involves the desynchronization of individual cellular clocks, challenging one popular model which suggested that it is the result of the arrhythmicity or suppression of clocks. This work, conducted in collaboration with scientists from Kinki and Nagoya Universities, was published in the online edition of *Nature Cell Biology*.

Reckoning that a photoinducible system *in vitro* would allow them to study singularity in a simpler, more controlled fashion, Ukai transfected melanopsin, which is normally only expressed in retinal cells, into mouse fibroblasts, exposed them to a 6-hour pulse of light and measured the effect on biological clock activity by monitoring changes in the expression of *PER-2*, a clock gene intrinsic to these cells. Melanopsin-positive cells exposed to a light pulse showed changes in both the phase and amplitude of *PER-2* expression, while untransfected cells did not, indicating that the melanopsin protein had made the fibroblasts photo-responsive. A series of tests

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using light pulses delivered at different time-points yielded one critical pulse capable of inducing singularity *in vitro*.

They looked next at the individual cells to decipher how singularity manifests itself, and found that although each cell generally maintained their intrinsic rhythmicity, but that they cycled out of step with other cells. This stands in contrast to several previous studies that had suggested that multi-cell singularity is the result of loss of rhythmicity in individual cells. So whether desynchronization would be observed *in vivo* remained an important question.

The team entrained rats in a laboratory under conditions known to induce singularity in humans, and studied a section of their brains, the suprachiasmatic nucleus (SCN), known to be the master of the body's biological clocks. The expression of clock genes *Per1* and *Per2* showed a dramatic decrease in total amplitude, as well as a pattern of expression that correlated to the desynchronization seen *in vitro*, suggesting that a similar mechanism may be at work *in vivo* as well. The model is not without its caveats, though, as the SCN cells appear to lack cell-cell coupling and the induction of singularity *in vivo* occurs indirectly, via the effect of light pulses on retinal neurons, while the effect is direct on the melanopsin-transduced fibroblasts *in vitro*.

Nonetheless, this work by Ukai, Kobayashi et al., represents an important new insight into a question now more than 30 years old. The phenomenon of singularity is of fundamental interest not only for its biological significance, but for the role it plays in some forms of sleep disturbance inked with late-night exposure to artificial, an increasingly common occurrence in the modern world. "It's interesting what this has revealed about how well-balanced a system the biological clock is," say the authors. "The individual cells keep each other in a steady cycle that is robust in the face of most environmental stimuli."