Introduction to Chronobiology

Chronobiology (chrónos, meaning "time") is that field of biology that examines periodic (cyclic or time related) changes in living organisms and their adaptation to environmental rhythms. These cycles are known as biological rhythms.

We live in a highly rhythmic world, and humans have developed a number of strategies for marking the passage of time by counting rhythms. Perhaps the most obvious is the 24 hour cycle of light and dark that corresponds to the time it takes for the earth to make a full rotation along its axis ("one day"). From a single point on earth, we experience it as the predictable rising and setting of the sun. The time required for the earth to make a full revolution of around the sun is called "one year" – tracked as the cycling through the seasons. The time for the moon to revolve around the earth is roughly 29 days, and is the approximate basis for measuring a "month" – this rhythmic process induces observable alterations of high and low tide, as well as the changing phases of the moon.

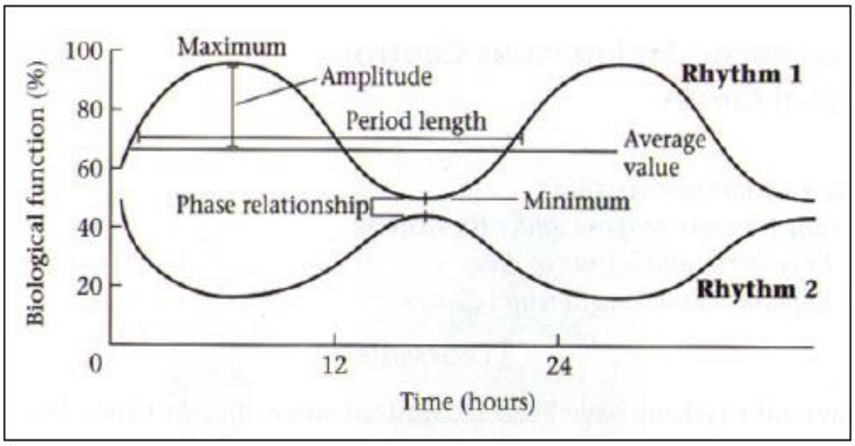
Based on these rhythmic changes, there are different types of biological rhythms in nature. For example:

- circadian (circa = "approximately", dian = "a day"): A rhythm with a periodicity of 24 h
- circannual : Period of ~365 days
- circatidal: Period of ~12.4 h
- circalunar: Period of ~29.4 days

To further simplify, the rhythms are particularly classified as ultradian rhythms (rhythms with a period very less than 24 hours e.g. blinking of eyes), circadian rhythms (e.g. sleep wake cycles) and infradian rhythms (Rhythms with a period much greater than 24 hours e.g. menstrual cycles in human females).
Modern time-keeping methods, like the atomic clock, rely on physical principles that were only understood in the middle of the 20th century. But long before we were able to build an atomic clock, humans (and many other organisms) benefitted from having a biological mechanism, a circadian clock, which also enabled them to keep rather precise time from day to day. A circadian clock enables an organism to reliably track a length of time which is about the length of a day – the term comes from the Latin circa (about) + diem (day).

The evolutionary logic of why an organism might benefit from having a circadian clock:
With a functioning clock, the organism can "plan ahead" for environmental changes that reliably recur, instead of just "waiting to see" what will happen and only taking action then. If a photosynthetic organism like a tree, for example, could predict when the sun would rise, then it could get ready in advance, initiating metabolic processes to prepare for photosynthesis. If instead the tree starts up these processes only once the sun has risen, it will miss out on the opportunity to harness the energy of the sun during this preparation interval and thereby reduce nutritional efficiency. Likewise, the "early bird" benefits from being able to predict when worms will be at the surface.

Components of a circadian clock:



(i) **Period**: refers to the amount of time it takes a cyclical process to return to the same phase (e.g, from one sunset to the next day's sunset, or from an organism's waking to the next day's waking) (ii) **Frequency**: cycles per unit of time (iii) **Amplitude**: maximum change above/below the average value (iv) **Phase**: a point on the rhythm relative to some objective time point during the cycle.

Characteristic Properties of a circadian rhythm

For any rhythm to be qualified as a circadian rhythm, it must be

(i) Rhythmic with a period of approximately 24 hours

(ii) Endogenous: A rhythms should be self-sustained and should be persistent under constant conditions A rhythm that is intrinsically generated and can continue to show a rhythmic pattern for numerous repititions without external influences (or in a constant environment)

(iii) Entrainable: The rhythm should be able to coupling to a Zeitgeber resulting in shared period.

Telling Time in Chronobiology

Chronobiologists have developed a number of relatively standardized formats and definitions for presenting data in support of the function of a circadian clock.

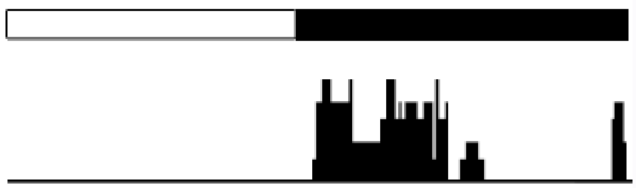
1. Light dark cycle: "LD" cycle (L for light and D for dark).
2. Constant darkness (shortened as "DD") and constant light ("LL").
3. Circadian time (CT): A quantification of time as defined by an organism's endogenous circadian clock, without reference to any environmental cue or *zeitgeber*.
4. Actogram: A graphical representation of an organism's phases of activity and rest over the course of a day.
5. Double-Plotted (Actogram): An actogram in which the values on the x-axis range over two Zeitgeber-cycles (usually, 24h+24h=48h), resulting in a duplication of displayed data.
6. Entrainment: The coupling of an observable rhythm in an organism to a *zeitgeber* resulting in shared period, where this change is caused by an alteration of the endogenous clock that schedules the observable rhythm.
7. Free running: An organism that is in constant conditions (LL or DD) and not exposed to any exogenous time cues

8. Free-Running Period (FRP): The length of time it takes for an organism's endogenous rhythm to repeat (return to the same phase) in the absence of environmental time cues.
9. Onset of Activity: The time at which an organism's passive (e.g., sleep) phase ends, and daily activities (e.g., locomotion) begin.
10. Period: Refers to the amount of time it takes a cyclical process to return to the same phase (e.g, from one sunset to the next day's sunset, or from an organism's waking to the next day's waking).
11. Phase-Shift: A change (either an advance or a delay) in the phase of an organism's free-running circadian rhythm, usually in response to a stimulus.
12. Zeitgeber: From the German "time giver" or "synchronizer", a Zeitgeber is any external time cue that is effective in entraining an organism.
13. Zeitgeber Time (ZT): A quantification of time, defined with reference to environmental regularities or zeitgebers.

Data collection

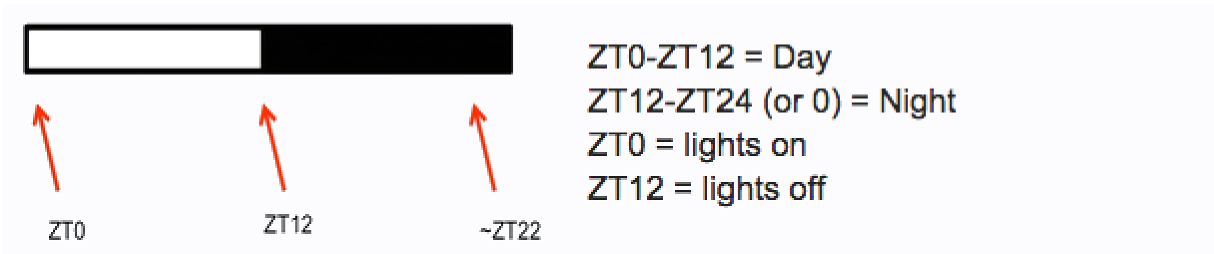
Based on the organism that you wish to study, there are different setups for studying activity rest rhythms. For testing Drosophila activity rest pattern, the flies are kept in glass vials with an infrared motion sensor on the side to detect the general movement. Same is true for reptiles and birds, but they are maintained in big cages. For rodents, the wheel running activity is recorded. In case of human, actimetry (recording of activity using a motion logger that looks like a wrist watch) is used to record the daily activity rest patterns.

Reading an actogram



A simplified zoomed-in graphical conventions used in actograms.

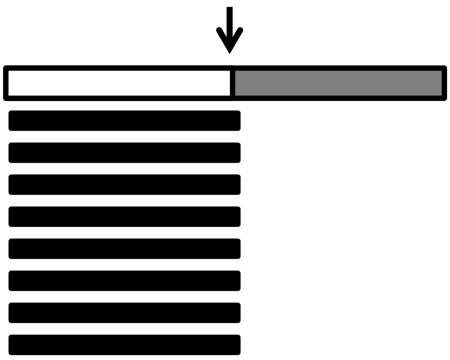
The white and black bars are actually a conventional label used to indicate the light-dark cycle of ***zeitgeber* time (ZT)**. The white bar represents the period of "lights on," and the black bar represents the period of "lights off." The two bars are the same length because our lighting schedule (LD:12:12) involves equal durations of lights on and lights off. So simply by including these bars at the top of the graphic, we've indicated that a full day's worth of data is being plotted, and we've given some information about our lighting schedule. Because in zeitgeber time, ZT0 is anchored to the beginning of the light phase, we can read off some key timepoints of ZT from this simple pair of bars, and can make a reasonable estimate of other timepoints. Underneath this pair of white and black bars, there is a little bar graph, showing how many times the wheel was spun at each time of day. The higher the black bar, the more times the wheel was spun at that time. Since the mouse is nocturnal, there's a long period during the day (at left, under the white bar) where no wheel-running is recorded. Instead, all the wheel running occurs during the night (though there are different "bouts" of wheel-running throughout the night).



How to read some timepoints in ZT from light-dark bars

<u>**Entrained and Free running actograms**</u>

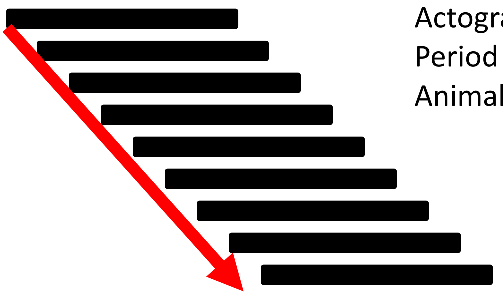
In LD cycle: Entrained condition



Activity aligned to the previous day. The circadian period is equal to the T-cycle (24 hours)

In constant conditions: Free running

Case 1:



Actogram is going towards right
Period (Tau) >24 h
Animal needs to phase advance to entrain to a 24 h LD cycle

Case 2:

Actogram is going towards left
Period (Tau) >24 h
Animal needs to phase advance to entrain to a 24 h LD cycle