Mechanisms and Delineating Circadian Phenomena

...a rose is not necessarily and unqualifiedly a rose…it is a very different biochemical system at noon and at midnight.
— Colin Pittendrigh, 1965.

Nomological vs. Mechanistic Explanations

- Under the influence of paradigmatic examples in physics, in the 1950s Carl Hempel advanced an account of explanation in terms of laws

  Laws (e.g., Boyle-Charles gas law)
  <u>Initial conditions</u>
  ∴Description of phenomenon to be explained (e.g., increase in temperature of gas)
- Laws: true universal generalizations with counter-factual import
  - Specify what would happen if initial conditions were satisfied
- Problem: there aren't many examples of such laws in biology, but lots of explanations
  - These offer accounts of mechanisms claimed to produce the phenomenon

Conceptions of Mechanism

- Machamer, Darden, and Craver [MDC] (2000)
  - "Mechanisms are entities and activities organized such that they are productive of regular changes from start or set-up to finish or termination conditions"
- Bechtel and Abrahamsen (2005, cf. Bechtel and Richardson, 1993):
  - "A mechanism is a structure performing a function in virtue of its component parts, component operations, and their organization. The orchestrated functioning of the mechanism is responsible for one or more phenomena"
- Besides the incidental differences in vocabulary, the major difference involves the last phrase of MDC—imposing an order from start to termination conditions

Features of Mechanistic Explanations

- Dualism of Entities and Activities
  - Activities (operations) are the producers of changes
    - Types of causings
  - Entities (parts) are the things that engage in activities
- Organization: "Entities often must be appropriately located, structured, and oriented, and the activities in which they engage must have a temporal order, rate, and duration"
  - Productive continuity: operations must link entities into a continuous network

Contrasts with Nomological Account

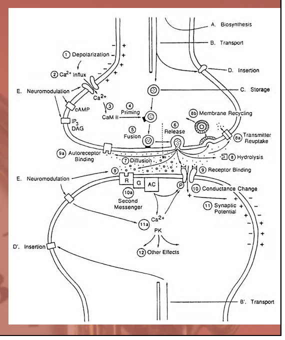
- Both nomological and mechanistic explanations can be concerned with causal phenomena—something happens which brings about something else
  - Nomological explanations focus on the regularity in the change itself
  - Critical feature of mechanistic accounts is that they focus on the system in which change is occurring and ask what is going on inside to produce its behavior
- Nomological explanations emphasize linguistic representations and logic
  - Logic is the glue that relates laws to actual cases
- Mechanistic explanations focus on the entities and activities
  - What are the operations performed that together bring about the effect?

Mentally Imagining Mechanisms

- An early example in Machamer, Darden, and Craver:
  - In the mechanism of chemical neurotransmission, a presynaptic neuron transmits a signal to a post-synaptic neuron by releasing neurotransmitter molecules that diffuse across the synaptic cleft, bind to receptors, and so depolarize the post-synaptic cell
- The account has the form of a narrative—relating a sequence of happenings
- Each of these occurs at a place and in a relative time order
- This narration invites one to visually imagine the events and to see them happening in a connected fashion
  - As one might imagine the activities in a human-made device

Visualizing Mechanisms

- Often mechanisms are explicitly presented visually in diagrams
- Iconic shapes or text labels are used to designate parts
- Often arrows are used to indicate activities such as transport of substances or reactions
  - Sometimes arrows are labeled with text
- In this case the sequence of steps is noted with numbers
  - But a viewer does not have to follow the sequence



Hierarchy of Mechanisms

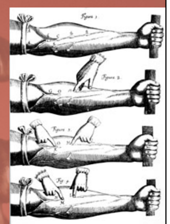
- The entities (parts) of a mechanism may themselves be mechanisms
  - One explains how they perform the activity (operation) in virtue of the parts and operations within them
  - Important to note that there is now a new explanatory goal—explain the operation within the previous mechanism
- Explanatory hierarchies bottom out in activities left unexplained
  - In molecular biology: geomechanical activities (e.g., opening), electro-chemical (attracting), energetic (diffusion), electro-magnetic (conduction of charge)

Delineating the Phenomenon

- An important first step in mechanistic explanation is specifying exactly what the phenomenon is
  - What happens and under what conditions does it happen
    - People have vivid memories of how they learned of major events such as the 9/11 attacks
    - Bees signal the location of food through a "dance"
    - DNA is transcribed into mRNA, which is translated into a protein
- Not just data reports but characterizations of what happens in the world
- Often it is important to characterize phenomena quantitatively, for it is the detailed behavior that the mechanism must explain

Phenomena and Experiments

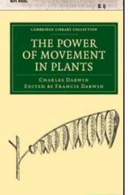
- Although some phenomena are easily identified by anyone, many require experiments to discover and characterize them
  - Recall Harvey—he had to demonstrate that blood circulates
    - Only then did it make sense to explain what made it circulate—how the muscles and valves of the heart, organized in the right way, served to circulate the blood
  - To demonstrate that it circulates, experimentation was required
- Goal: to determine what happens and what are the factors that affect its happening



The Phenomenon (a?) of Circadian Rhythms

- Endogenously generated rhythms of approximately 24 hours (circa [about] + dies [day])
  - In fact, the variability is seen as crucial—if the rhythms were under exogenous (environmental) control, their period should be exactly one day
- Entrainable by cues to the time in the environment (Zeitgebers)
  - Daylight, temperature, feeding, etc.
- Temperature compensated—rhythms have nearly the same period at different temperatures
  - Typically biochemical reactions are temperature sensitive—faster at higher temperatures

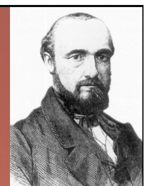
De Mairan's *Mimosa* Experiment



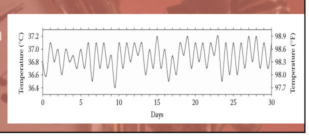
- In 1729 de Mairan, a French astronomer, not only noted the regular opening and closing of the leaves of a mimosa plant, but to determine whether this was just a response to sunlight, confined the plant to darkness
  - Its leaves still opened and closed on a daily cycle
- Investigation taken up 150 years later by Darwin who developed instrument for measuring leaf movements, and quantified and graphed the results

Sleep Activity of *Oxalis* Plant

First Clues to Circadian Rhythms in Humans

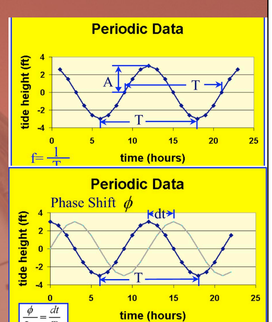


- In 1868 Carl Reinhold August Wunderlich conducted a study of body temperature involving more than 25,000 individuals
  - Recorded temperature several times during the day
  - Between 2 AM and 8 AM, mean temperature was 36.2°C / 97.2° F
  - Between 4 PM and 9 PM, mean temperature was 37.5° C / 99.5° F
  - Mean of over 1 million reports: 37°C / 98.6°F



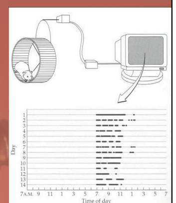
Representing Oscillations

- A common way of representing circadian or other oscillations is by placing time on the x-axis and the variable measured on the y-axis
- The distance between two troughs is the period (τ) of oscillation
  - Freq=1/period
- The difference between the mean value and the maximum is the amplitude
- Two oscillations with the same wave-form but shifted in time are phase-shifted



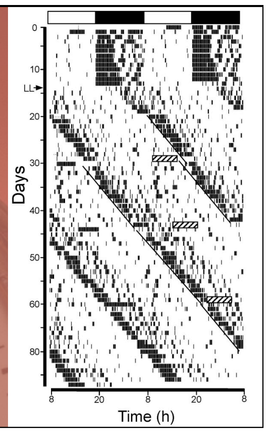
Recording Circadian Behavior: Actograms

- Researchers have developed techniques to make manifest the pattern of circadian behavior in animals
  - Record each time a behavior occurs and show it as a hash mark across a 24 (or 48 hour) line
  - Or number of behaviors within a time bin by height of line



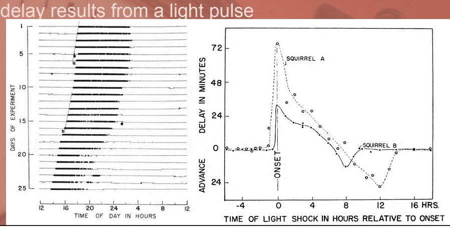
Double Plotting

- Showing each day twice, first on the right of one line and again on the left of the next, allows for deciphering patterns of change in both active and inactive phases



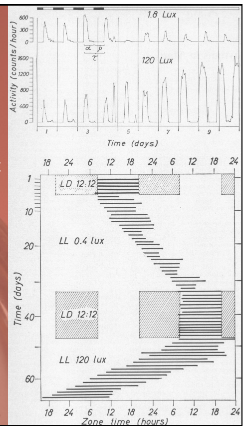
Entrainment and Phase Response Curves

- The change in circadian rhythms in response to Zeitgebers depends upon the time at which they are administered
- Phase response curve shows how much advance or delay results from a light pulse



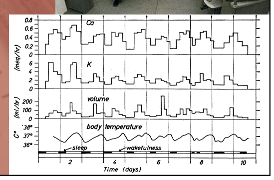
Oscillations are Endogenous

- Free running: behavior in the absence of time cues (Zeitgebers)
  - Birds in constant dim or bright light
  - Period (τ) varies from 24 hours
- "These two facts, the undamped continuation of the rhythm and the deviation of the period from 24 hours, allow only one plausible explanation: that this rhythm is not imposed on the organism by the environment but is truly endogenous" Aschoff



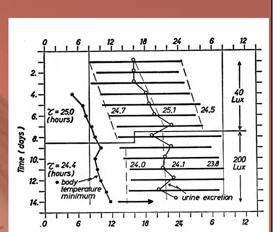
Aschoff's Cave Experiment

- Volunteers spent several days/weeks in an underground bunker from WWII where they had no indication of external time
- They chose when to have lights on, when to eat, etc.
- Their bodily functions were regularly monitored
- Their pattern of activity varied somewhat from 24 hours



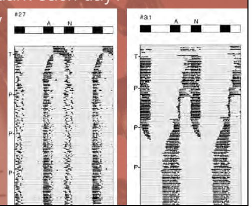
Effect of Brightness of Light

- Since light is a Zeitgeber, individuals might be resetting their own clocks as a result of their activities
- Since subjects would not agree to no light, the best alternative was to leave light under their control but vary its brightness
- With brighter light, their rhythms were closer to 24 hours



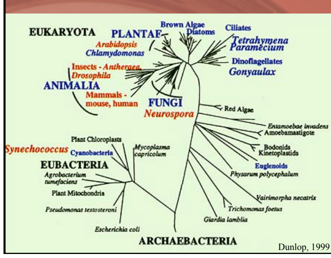
Entraining to Unusual Environments

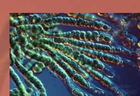
- While circadian rhythms can be entrained to day-night cycles somewhat different than 24 hours (e.g., 21 or 27 hours), they cannot be entrained to 19 or 29 hour cycles
- What about two bouts of light:dark each day?
  - Most hamsters successfully trained (left) but some (right) did not (Gorman, 2001)



The Phenomenon is Found in Many Species

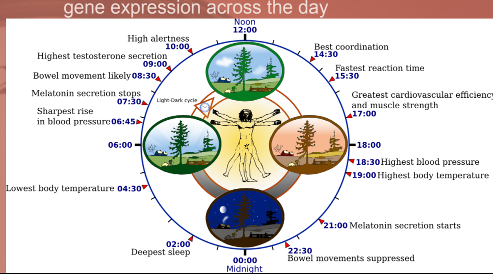
- The major limit in studying circadian rhythms in different species is finding one or more behaviors whose periodic changes can be identified



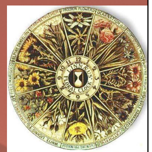


Circadian Rhythms Regulate Many Activities of Life

- This is just a small sample
  - More recently it has been possible to measure gene expression across the day



The Importance of Preparing in Advance



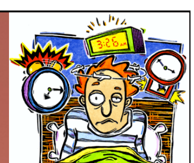
- Importance of plants spreading their leaves and orienting to the sun before sunlight arrives
  - Linnaeus designed a flower clock of species that open and close at different times of day
- Adjusting eyes to light (for fish it can take 20 minutes, plenty of time to become someone's meal)
  - Horseshoe crab: for 350 millions years it has changed its receptivity to light 1,000,000 fold over the course of the day

Abnormal Sleep Patterns

- Familial advanced sleep phase syndrome
  - Screen of patients within a family revealed involvement of CK1δ, which phosphorylates PER2
    - Mouse studies showed that Ser662Gly mutation in *Per2* results in shortening of period, mimicking sleep shift
- Delayed sleep phase syndrome
  - Gene polymorphism studies have linked it to *Per3* and *Clock*
- Irregular patterns of sleep
- Failure to entrain sleep patterns

26

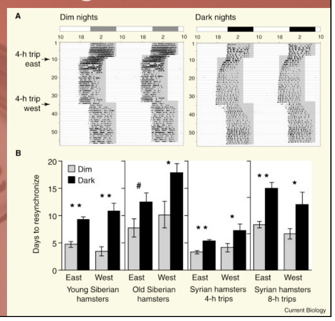
Jet Lag



- As a result of crossing multiple time zones, an individual's endogenous sense of time may be several hours different than the local day-night cycle
  - Sleep, body temperature, hormones, digestive enzymes may all be produced at inappropriate times, resulting in fatigue, insomnia, headaches, depression
  - Depending on number of time zones crossed, several days may be required to recover from jet lag
  - Eastbound travel is usually more disruptive than westbound travel

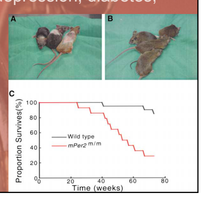
Using Dim Light to Ameliorate Jet Lag?

- Simulating a 4 hour time zone change in hamsters, Evans et al. (2009) found that when nights were dimly lit, animals responded more rapidly to new time



Shift-work/Jet Lag and Cancer

- Epidemiological studies show disruption of circadian rhythms (due to shift work or regular time zone change) is associated with a variety of cancers—level of risk proportional to cumulative exposure to circadian stressor
  - Breast cancer
  - Prostrate cancer
  - Non-Hodgkin's lymphoma
  - Also associated with sleep disorders, depression, diabetes, cardiovascular problems and obesity
- Linkage is likely to be the disruption of the cell cycle, leading to abnormal and unregulated growth (tumorigenesis)
  - First study: Fu et al. (2002) identified abnormal DNA damage response after γ-radiation in *Per2* null mutant



Rhythms on Different Time Scales

- Ultradian: milliseconds to hours
  - Action potentials in neurons
  - Heart beats
  - 90 minute sleep cycles (from stage I to REM)
- Circadian: circa (approximately) + dies (day)
- Infradian
  - Estrous cycles (28 days in humans)
  - Annual cycles of migration, hibernation
  - Multi-year cycles (cycads that emerge from larvae 13 or 17 years after eggs were laid)

Many Fields of Biology Contributed to Understanding Circadian Rhythms

- Center for Chronobiology at UCSD involves
  - Cell and molecular biologists
  - Experimental psychologists
  - Psychiatrists
  - Sleep researchers
  - Cancer researchers
  - Biological engineers
  - and even a philosopher