Circadian Rhythms Journal Club: Spring 2004

Formal Analysis of Circadian Rhythms and their Entrainment by Light.

Dunlap, Loros and DeCoursey, Chronobiology: Biological Timekeeping, Sinauer, 2003
Circadian Rhythms are endogenously generated

• Most behaviors and physiological events occur at a particular time of day
• Locomotor behavior is particularly easy to monitor
• The pacemaker can be synchronized by light, but rhythm persists in DD
Some definitions

- **Phase**: an event which can be recognized in each cycle; *e.g.*, full moon, estrus, activity onset (CT12 if nocturnal animal)
- **Period**: the interval between successive recurrences of the same event in consecutive cycles. (Note frequency is inverse of period).
- **Phase angle**: temporal relationship of cycle to outside world
  
  Conservation of phase angle is the *Key function* of circadian clocks - natural selection acts on this

- **Amplitude**: a characteristic of the output; need not be a feature of the oscillator
Criteria of Circadian Rhythms

- persist in constant conditions with *period* of 24h
- temperature compensated (\(Q_{10}\) close to 1)
  \[ Q_{10} = \text{rate at higher} / \text{rate at lower temp, 10° apart} \]
  non-temp compensated timekeepers are of limited use in time measurement
  temp compensation is not same as temp insensitivity
- can be *entrained* to exactly 24h by environmental cues.
Some other features of Circadian Rhythms

- *permissive range* (conditionality) of light, temperature at which rhythms persist
- *Transients*: period, phase unstable immediately after perturbation
- resetting can produce *aftereffects*: long-lasting changes in period
- *endogenous nature*: not controlled by “subtle geophysical cues”
  
  FRPs differ between individuals in same constant environment
  can select for period
  period mutants: several genes now identified
Johnson’s Figure 1: the Phase Response Curve

- Phase shift (advance or delay, Y axis) is a function of phase at which light is presented (circadian time - subjective day or subjective night, X axis)
- Light in subjective day elicits no shift
- Light in early subjective night causes phase delay
- Light in late subjective night causes phase advance
Two different models of entrainment

- Parametric (continuous): suggested by observation that light intensity can regulate period (velocity of the pacemaker?)
- Nonparametric (discrete): Brief pulses of light can shift the phase of the pacemaker and mimic effects of whole light cycles
Phase response and phase transition curves

A

Type 0 Resetting

Type 1 Resetting

Phase shift (hrs)

New circadian phase

Initial circadian phase
Effect of light intensity on PRC type

A

B

Phase shift (h)

Stimuli of increasing strength

Circadian time (h)

discontinuity
Phase advances and delays: Johnson, Fig. 4

- Shown for diurnal animal: activity (overt rhythm) is expressed during subjective day (dead zone of PRC)
- PRC reflects behavior of the oscillator, but can only be assessed by presenting a second pulse of light
- Light pulse is shown as instantaneously shifting the pacemaker’s phase
Johnson, Fig. 4

**A. Phase Advance**

- Light Pulse
- Shifted PRC
- Control PRC
- Overt rhythm

**B. Phase Delay**

- Light Pulse
- Control PRC
- Shifted PRC
- Overt rhythm
Figure 3. Representative double-plotted actograms of wheel-running activity of Syrian hamsters held in continuous dim red light and exposed to brief pulses of light (15 min, asterisks) at one pulse only at CT13 (a), paired pulses first at CT13 and a second 2 hr later (b), at CT20 and a second 2 hr later (c), and at CT18 and a second 2 hr later (d). Lines on left side indicate phase shift of activity onset. Pulses in a, b, 15 min, 50 μW/cm²; pulses in c, d, 15 min, 200 μW/cm².
A "Two-Pulse" Experiment shows the oscillator shifts phase very quickly!


Figure 4. a, PRCs (mean ± SEM) of the circadian activity rhythm of Syrian hamsters held in continuous dim red light and exposed to a brief pulse of light (15 min) of 50 (filled symbols; n = 84) or 200 (open symbols; n = 45) μW/cm². The dotted line about the abscissa is the SEM for control dark pulses (mean shift, −0.01 hr; n = 15). b, Predicted and observed resetting to paired pulses in delaying phase of PRC. Shifts to individual pulses (15 or 30 min, 50 μW/cm²) delivered at CT13, CT14, or CT15. NO, Predicted shift if no resetting within 2 hr of first pulse; YES, predicted composite shift if delay arising from pulse at CT13 is completed before second pulse is given; OBS, observed data. n = 11–25; n = 99. **p < 0.01, pairwise comparison. c, d, Predicted and observed resetting to paired pulses in advancing phase of PRC. Shifts to individual pulses (15 min, 200 μW/cm²) delivered at CT18, CT20, CT22, CT23, or CT24. NO, Predicted shift if no resetting within 2 hr of first pulse; YES, predicted composite shift if advance arising from pulse at CT20 or CT18 is completed before second pulse is given; OBS, observed data. n = 6–11; n = 74. **p < 0.01, pairwise comparison.
Why bother with PRCs? Because they explain entrainment

- In real world, cr’s can be *entrained* by environmental cues (*zeitgebers*) within a range of periods close to 24h
- To demonstrate entrainment:
  1. period of rhythm equals that of zeitgeber
  2. upon release to constant conditions, free run starts from entrained phase. Otherwise, light has a *masking* effect

- Light is the most effective zeitgeber; brief pulses can set phase
- Entrainment need not be synchrony; appropriate phase angle established (remember the *key function* of cr’s!)
How does entrainment work?
Johnson, p. 16:

“The basic premise of the model is that an entrained circadian pacemaker is in equilibrium with an LD cycle consisting of repetitive light pulses (the zeitgeber) when each pulse falls at that phase in which the light pulse elicits a phase shift which is equal to the difference between the free running period (FRP, $\tau$) and the period of the entraining cycle.”
The only equation you need to know: \( D_f = t - T \)

- If \( T=24 \) and \( t=24.2 \), the animal must achieve a phase advance \( (+D_f) = 0.2 \text{h/cycle} \).
- There is only one phase at which light will elicit the appropriate shift.
- The phase response curve (Fig. 1) allows us to predict the phase angle of entrainment.
Entrainment by one pulse/day (Johnson, Fig. 5)

- $\tau=21\text{h}$, $T=24\text{ h}$
- For entrainment, need 3h phase delay each cycle
- Animal adopts a phase angle such that light falls in early subjective night to achieve appropriate shift each cycle.
Johnson, Fig. 5

A. Freerun (FRP = 21 hours)

B. One-pulse Entrainment (FRP = 21 hours, T = 24 hours)
Entrainment is possible for a range of T’s and t’s (Johnson, Fig. 6)

- Phase angle is a function of t and T
- Activity may lag or lead the zeitgeber, and is predicted by the PRC
- Shift of oscillator phase is shown as shift of PRC - need to test with second light pulse
Johnson, Fig. 6

A. FRP = 27 h; T = 24 h; Rhythm = day active

- PRC model:
  - Timing of light pulse to give: +3 h phase shift
  - In DD: 27 h, 24 h
  - Activity: +3 h

- Actogram:
  - Timing of light pulse: after shift
  - Duration: 24 h

B. FRP = 21 h; T = 24 h; Rhythm = day active

- PRC model:
  - Timing of light pulse to give: -3 h phase shift
  - In DD: 21 h, 24 h
  - Activity: -3 h

- Actogram:
  - Timing of light pulse: after shift
  - Duration: 24 h

C. FRP = 25 h; T = 22 h; Rhythm = day active

- PRC model:
  - Timing of light pulse to give: +3 h phase shift
  - In DD: 25 h, 22 h
  - Activity: +3 h

- Actogram:
  - Timing of light pulse: after shift
  - Duration: 24 h

D. FRP = 23 h; T = 26 h; Rhythm = day active

- PRC model:
  - Timing of light pulse to give: -3 h phase shift
  - In DD: 23 h, 26 h
  - Activity: -3 h

- Actogram:
  - Timing of light pulse: after shift
  - Duration: 24 h
Phase angle (\(\phi\)) depends upon \(T\) and \(\tau\).

Figure 7. Phase angle differences between entrained circadian systems and zeitgeber with different periods \(T\) in seven studies. Note the systematic increase in \(\psi\) with \(T\) and the negative association between the steepness of this increase and the range of entrainment. (From Aschoff and Pohl, 1978.)
Entrainment is stable (phase angle is unique) if □ differs from T

- Dead zone of PRC often spans several hours
- If FRP=T, light can fall anywhere in subjective day
- Adaptive value of PRC: light in early night should cause a delay, in late night should case an advance, to achieve reset to appropriate phase.
Stable entrainment favored by unequal to $T$
Entrainment by two pulses (Johnson, Fig. 8)

• Advances and delays are summed to give net $D$ required to compensate for difference between $t$ and $T$.

• PRC shows oscillator is reset by both dawn and dusk pulses
A. Two-pulse Entrainment (FRP = 21 hours, T = 24 hours)

B. Entrainment to LD 12:12 (FRP = 21 hours, T = 24 hours)
Modeling entrainment using the PRC (Johnson, Fig. 9)

- Organisms respond to *skeleton photoperiods* much as they do to light cycles in the real world - a triumph for the discrete entrainment model.

- Organisms take the short interval between pulses as day-phase phase angle jumps when this interval exceeds about 13h

- Range of bistability where animal can see either pulse as “dawn”.

- Thus there *is* a role for light during the day to help maintain the appropriate phase angle!
Limit cycle analysis of circadian oscillations

State variables: 2 (or more) interdependent variables defining phase space.

Isochrons: lines predicting phase at which oscillation will return to limit cycle after perturbation (displacement).

Singularity: phaseless point (may be stable or unstable) where oscillation stops.
A

$x_{\text{min}}$ $x_{\text{max}}$

$x'(t) > 0$ $x'(t) < 0$

\[-1 \quad 0 \quad +1 \quad X(t)\]

B

position "a" position "b"

\[-1 \quad 0 \quad +1 \quad X(t)\]

C

position "a" position "b"

\[-1 \quad 0 \quad +1 \quad X(t)\]
Fig. 1. (a) Illustration of successive censuses of fox and rabbit populations. (b) Plot of trajectories approaching a limit cycle. (c) Segments of zero-population-growth curves. (d), (e) Trajectories near a stable and an unstable equilibrium point.
Singularities

A
- Isochron
- Limit cycle
- Population of oscillators
- Effect of light
- Singular region
- Critical pulse

B
- Recovery to various phases
Oscillations may run on different limit cycles in LL and DD.