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Time microscopy for all kinds of data including circadian clock biology

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Abstract

Data showing a rhythm to the naked eye prominently, barely or not at all were described as kinds A, B and C, respectively. Here, we document good agreement between estimates of maxima and minima with eyeballing and with the addition of point and interval estimates of parameters in kind A data. We also construct a chart that provides estimates of uncertainties that can be obtained objectively while they are more difficult to quantify subjectively; again there is agreement. Interval as well as point estimates of rhythm characteristics and parameter comparisons are useful in charting all kinds of data and become indispensable as we proceed from kind A to kind C data. Illustrations included herein from molecular biology apply equally to all aspects of transdisciplinary science.

Keywords

Time microscopy; Rhythm characteristics; Molecular biology

1. Introduction

The standardization of all circumstances of observation, even under the somewhat controlled conditions of a modern laboratory, with some degree of adjustable temperature, relative humidity and lighting, is not always feasible [1,2] or can be interfered with by disturbance, such as a magnetic storm [3,4]. In many time series, as usually obtained in the clinic or field, rhythms and other time structural elements, such as trends and probabilistic chaos [5] can be further blurred by other variations. As a first approximation, certain physiological time series may be classified according to the prominence of their circadian rhythms [6] (cf. [2]):

- Kind A data: periodicity stands out clearly; a plot against time readily demonstrates it; it involves statistically significant changes, which are reasonably reproducible from one study to the next.
- Kind B data: periodicity can be recognized, although it is distorted by noise. The changes found in any one study may be statistically significant but their timing with respect to external or internal reference points may vary greatly among studies done under presumably identical conditions.
- Kind C data: periodicity, if any, is completely masked by other variations.

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When faced with time series of Kind B or Kind C, one can choose the best compromise between 1. Using computational procedures for unmasking a possible circadian periodic component from interference, e.g. by autocorrelograms [7; cf. 2], power spectra [6], among other procedures of linear–nonlinear rhythmometry [8–11]; and 2. Removing interfering variations, to some extent at least, by standardized procedures.

2. Materials and methods

Cyclic sequences of molecular events related to the clock genes, i.e. RNAs and proteins, published earlier by others [12,13], were quantitatively evaluated by interval as well as point estimates obtained by chronobiological microscopic methods [9–11]. The statistical significance of intervals among events following each other in time was estimated [14] to start maps at all levels of organization [15–21]. We here focus on reviews on “Molecular analysis of mammalian circadian rhythms” [12] and “The neurobiology of sleep: genetics, cellulax physiology and subcortical networks” [13]; referring to the scheme of events at the molecular level both in the cytoplasm and the nucleus, presented as the sequence in activity of different genes, RNAs and proteins responsible for the coordination of circadian rhythms, perhaps by feedsidwards yet to be analyzed [20]. Concentrations of those substances were recorded around the clock 6–12 times per day and corresponding graphs were plotted in the original publications [12,13]. The abscissas and ordinates of these plots were taken off and the data were fitted with 24-h cosine curves, computing the acrophases and their 95% confidence intervals by least squares (cosinor) approximations [8–11]. By parameter tests [14], *P*-values were obtained for the test of equality of acrophases of all rhythms investigated.

3. Results

Analyses are summarized in Table 1 and Fig. 1. A comparison of the original reviewers’ inferences with results of quantitative chronobiological analysis is presented in Table 2. In Table 1, the difference in time is shown among the peaks of activity of all components in the metabolic cyclicality tested, which were illustrated in [12] and [13]. In the boxes located to the right and above from the main diagonal (empty cells), the time intervals are shown in hours and minutes. In corresponding boxes to the left of and below the main diagonal, the *P*-values from the test of equality of acrophase pairs corresponding to the respective rows and columns are shown. For example, a difference between the activity of *per1* and *per3* might be found in row #1 and column #3 (02:12 L, where L means later or delay); the probability of this difference is given as *P* = 0.0065). In the last column of this table, acrophases and their 95% confidence limits for every component of the system are shown, except for *Cry1*, for which these limits could not be computed since the zero-amplitude assumption was not rejected.

In Table 2, a comparison of data published in [13] and [14] with quantitative results computed herein shows that for most variables, there is good agreement for the point estimates and confidence limits. The latter are more precise than the naked eye, and what seems critical, the limits will be the same for different investigators. Time-microscopic estimates were taken as the basis for the graphic presentation of the sequence of events in Fig. 1. The chain of arrows shows this sequence from *Bmal1* RNA above to the same substance below (closing the loop of the cycle). The next cycle might be presented as a continuation of the graph to the right and/or as a double plot.

4. Discussion

Quantitative analyses confirm the original reviewers’ conclusions regarding the timing of the expression of RNAs and proteins involved in molecular mechanisms of circadian rhythms. Additional point and 95% confidence intervals, to evaluate the uncertainties of the acrophases,

enable the mapping of a chart in Fig. 1 and the statistical testing of the equality of acrophases. The usefulness of the approach here described is the greater, the larger the background noise. When, for instance, any wobbly periodisms, are being examined in the solar wind, as in ([21], Fig. 2 therein), the time-microscopy becomes indispensable.

5. Conclusion

The cartography of chronomes (time structures) is best accompanied by interval as well as point estimates of parameters in molecular biology as in all fields involving cyclicities and hypothesis tests of the validity of cycles.

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ACROPHASE (ϕ) CHART OF PUTATIVE MOLECULAR MECHANISMS OF CIRCADIAN RHYTHMS*

NOTE:

1. Difference in ϕ between *Bmal1* and *Per1* ($P < 0.0001$);
2. Difference in ϕ s of RNA from *Per1*, *Per2*, *Per3* to *Cry1* shows progressive advance ($P < 0.001$);
3. Synchronization of circadian ϕ s of proteins (*Per1*, *Per2*, *Cry1*, *Cry2*) ($P > 0.50$);
4. Difference in ϕ between RNA and proteins for *Per1* vs *Per1* ($P < 0.001$), *Per2* vs *Per2* ($P < 0.001$), but not *Cry1* vs *Cry1* ($P > 0.10$); P-values from parameter tests of grouped or paired series.

* Data from: S.M.Reppert and D.R.Weaver. Annu. Rev. Physiol. 2001, 63: 647-76.

Fig. 1.

ACROPHASE (ϕ) CHART OF PUTATIVE MOLECULAR MECHANISMS OF CIRCADIAN RHYTHMS*

P-values (left, bottom) and time differences ($\Delta\phi$, -right, top) from comparison of circadian acrophases of clock-gene, RNA and protein expression in the suprachiasmatic nuclei of mice on LD12:12

RNAs	PROT	#	Per1	Per2	Per3	Crv1	Per1	Per2	Crv1	Crv2	BMAL1	BMAL1	Φ (95% CI)
			1	2	3	4	5	6	7	8	9	10	
Per1		1		01:52 L	02:12 L	04:44 L	06:24 L	06:20 L	0 6:2 8 L	06:44 L	10:48 L	08:24 E	-93 ° (-77, -109)
Per2		2	.0856		00:20 L	02:52 L	04:32 L	04:28 L	04:36 L	04:52 L	08:56 L	10:16 E	-121 ° (-81, -152)
Per3		3	.0065	.7230		02:32 L	04:12 L	04:18 L	04:16 L	04:32 L	08:36 L	10:36 E	-126 ° (-106, -143)
Crv1		4	.0120	.0473	.0489		01:40 L	01:36 L	01:44 L	02:00 L	06:04 L	10:52 L	-164 °
	Per1	5	<.0001	.0064	.0002	.1219		00:04 E	00:04 L	00:20 L	04:24 L	09:12 L	-189 ° (-173, -206)
	Per2	6	<.0001	.0068	.0002	.1401	.8893		00:08 L	00:24 L	04:28 L	09:16 L	-188 ° (-171, -204)
	Crv1	7	.0001	.0064	.0003	.1299	.9738	.8745		00:16 L	04:20 L	09:08 L	-190 ° (-170, -209)
	Crv2	8	<.0001	.0096	.0002	.0806	.5744	.4713	6532		04:04 L	08:52 L	-194 ° (-184, -204)
BMAL1		9	<.0001	.0002	.0001	.0026	.0019	.0018	.0030	.0026		04:48 L	-255 ° (-227, -283)
	BMAL1	10	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001		-327 ° (-319, -336)

* Original data from figures in: S.M. Reppert and D.R. Weaver, Annu Rev. Physiol. 2001, 63: 647-76.

$\Delta\phi$ given in hr:min as column# vs. row#, L = later or delay, E = earlier or advance.

LD: regimen of 12 hours of light alternating with 12 hours of darkness; Φ : circadian acrophase expressed in (negative) degrees, with 360° = 24 hours, 0° = light onset. 95%CI: 95% confidence interval (for series where circadian rhythm is detected at P<0.05). PROT = Proteins.

P-values from parameter tests (C. Bingham et al., Chronobiologia, 1982, 9: 397-439) comparing data series normalized to have MESOR of zero and circadian amplitude of one.

Table 2
Comparison of original reviewers' conclusions with results of quantitative inferential statistical analyses

Circadian oscillations of	Reviewer's report	Results of analyses herein
1. mPER and mCRY RNAs in the suprachiasmatic nuclei		
<i>mPer1</i> peaking from CT	4–6	06:12 (05:08–07:16) *
<i>Mper3</i>	4–8	08:04 (05:24–10:08)
<i>Mper2</i>	At 8	08:24 (07:04–09:32)
<i>Mcry1</i>	At 10	10:56
2. mPER and mCRY PROTEINS		
mPer1	12	12:36 (11:32–13:44)
mPer2	12	12:32 (11:24–13:36)
mCry1	12	12:40 (11:20–13:56)
mCry2	12	12:56 (12:16–13:36)
3. <i>Bmal1</i> RNA	15–18	17:00 (15:08–18:52)
4. <i>Bmal1</i> PROTEIN rhythm is delayed with respect to <i>Bmal1</i> RNA	4–6	21:48 (21:16–22:54)
Quantitative analyses have shown also statistical significance of phase shifting: Difference in acrophases	among RNAs <i>Per1</i> , <i>Per2</i> , <i>Per3</i> to <i>Cry1</i>	shows progressive advance in acrophase ($P < 0.001$)
	between RNA and proteins for <i>Per1</i> vs. <i>Per1</i>	($P < 0.001$)
	between RNA and proteins for <i>Per2</i> vs. <i>Per2</i>	($P < 0.001$)
No difference	between RNA and proteins for <i>Cry1</i> vs. <i>Cry1</i>	($P > 0.10$)

* 95% confidence intervals in (); not available if $P > 0.05$ from test of zero-amplitude assumption (9–12). All comparisons are given in hours after light onset (HALO), which under conditions of light (L) alternating with darkness (D) at 12-h intervals is equivalent to circadian time (CT) in the original publication (1).