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Metachronanalysis of Circannual and Circasemiannual Characteristics of Human Suprachiasmatic Vasopressin-Containing Neurons*

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Abstract. *We here test for and detect anticipated about-yearly (circannual) changes in the volume and number of vasopressin-containing neurons in the human suprachiasmatic nucleus. We then resolve inferential statistical parameters quantifying the extent and timing (the amplitude and acrophase) of the circannual rhythm previously missed by data inspection and classical biometry. We parametrize about-half-yearly changes previously validated by non-parametric statistical tests. New dynamic circannual and circasemiannual endpoints thus become available for basic investigation and the assessment of disease risk elevation and/or chronoprotopathology. It was earlier demonstrated that the circannual rhythms of prolactin and TSH are prominent classifiers of individuals at high versus low familial and other risk for developing breast or prostate cancer. Any neurocrine or neural mechanisms contributing to this classification are now amenable to study, on a population basis, with the dynamic hypothalamic rhythm characteristics yielded by this metachronanalysis.*

*Dedicated to the memory of Erna Harberg

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Abbreviations: N, number; V, volume; D, density; SCN, suprachiasmatic nucleus; M, MESOR; A, amplitude; ϕ, acrophase.

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Introduction

Parameter estimations. Components of a time structure, a chronome, built into most physiologic variables include, as a rule, with prominent circadians, also circannual features [1-3]. The details of this temporal makeup can (but need not) be apparent to the unaided eye, i.e, to time-macroscopy. In either case it is desirable to quantify parameters, such as the amplitude-acrophase pair and to do so by inferential statistics. Along this time-microscopic line, the mapping of circadian rhythms is proceeding at a high rate, e.g., by cosinor analysis [1,2], accompanying the inspection of data in chronograms and the application of procedures documenting a time effect without deriving parameters [3]. The parameter estimation, briefly parametrization, however, constitutes a procedure for isolating the characteristics of a rhythm and is a *sine qua non* for the use of new temporal endpoints needed in research and practice.

Parametrization for disease assessment. For instance, the application of circadian parameters has led to the recognition of a new disease entity, circadian amplitude-hypertension [4,6]. A relative large circadian amplitude of neonatal blood pressure can be a gauge of both a positive family history of high blood pressure [7] and of the in utero exposure to betamimetics of infants [8,9] and adolescents [10]. Most recently, the circadian blood pressure amplitude has been recognized, on a population basis, as the major risk factor for human ischemic stroke and nephropathy [11].

Important risk information also has been yielded by the quantification of circannuals [12-15], Figure 1, and of other infradians with a frequency lower than 1 cycle in 28 hours [16]. As Aschoff put it in 1981 [3]: 'Temporal order in synchrony with the seasons may eventually turn out to be as profound as

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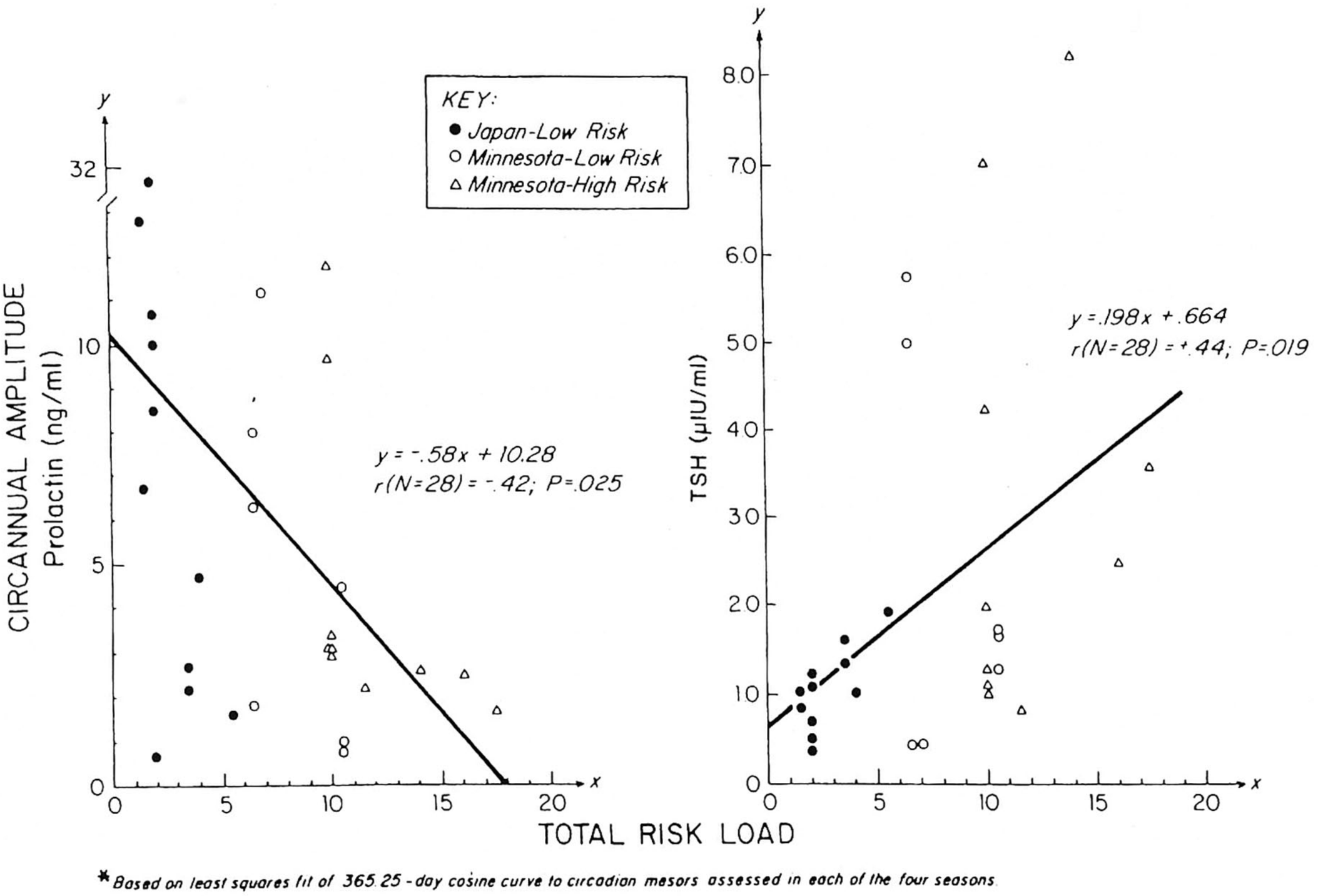


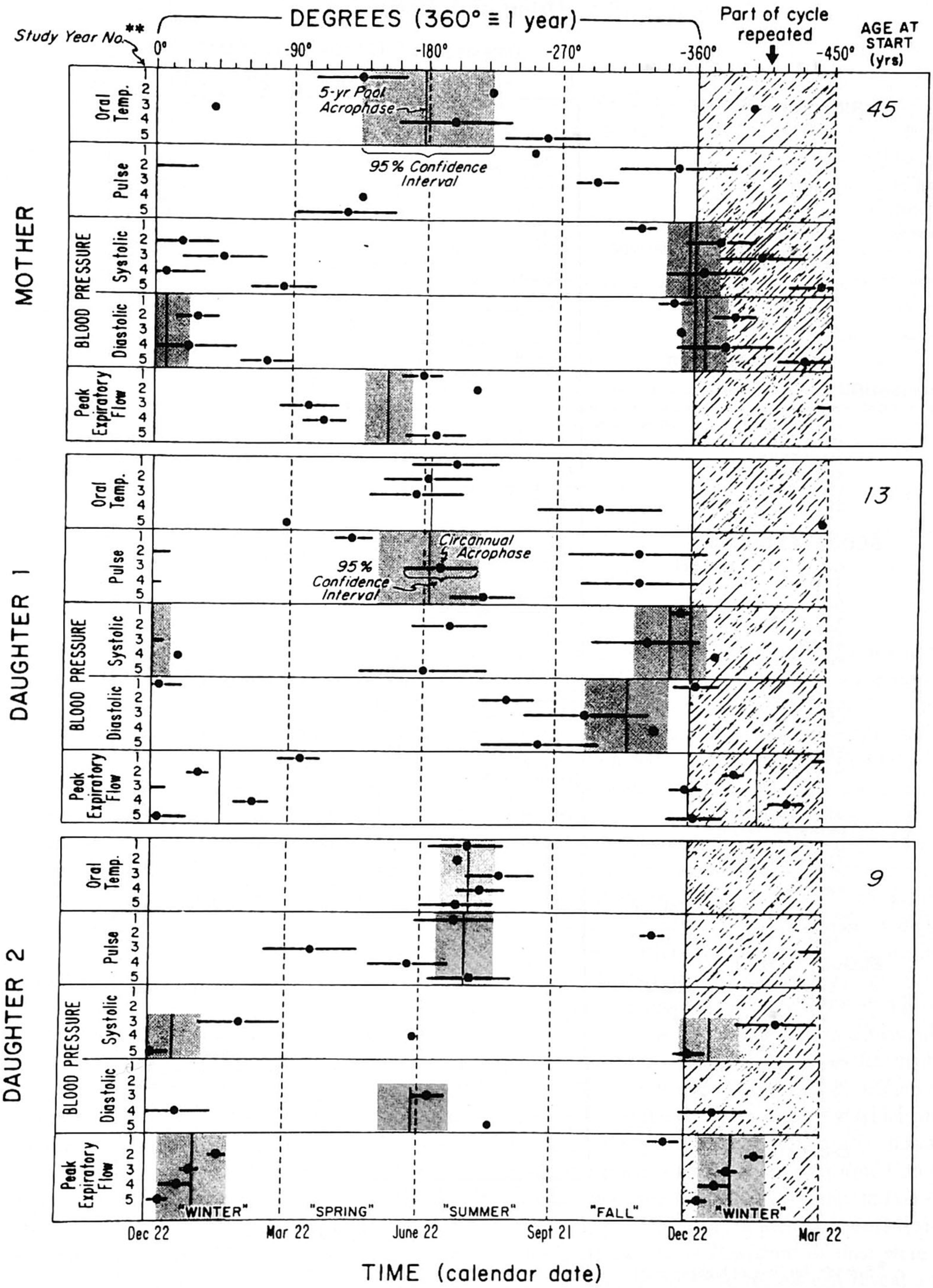
Figure 1. *Relation of the circannual amplitude of circulating prolactin and TSH to the risk of developing breast cancer [12].*

the circadian temporal order ...'; two years earlier [13] and again in 1981 [12] it was already abundantly and quantitatively documented that circannual rhythm alteration can be informative when the circadian system as yet is unaltered. Circannuals among other infradians are ubiquitous and critical components of the broader chronome, Figures 2-4 [12-25].

Ubiquitous circannual parameters. Circannual variations in many hormones, such as circulating cortisol, aldosterone, testosterone, TSH and prolactin, are demonstrated and their importance for a population has been quantified [17] and discussed [3,12,15,16,21]. From the viewpoint of human ecology, Johnston [26] regards circannuals as '...a basic component of the ecosystems in which our ancestors have evolved, one that has left its mark indelibly upon the makeup of our biology and behaviour'. Some 'seasonal' variations persist in the absence of any periodic changes in the daily light fraction and even in the absence of light: thus, the about-yearly change in the case of the ovarian size of the Jamuna River catfish, *Heteropneustes fossilis* from about 16 g (when the monsoon blows) to 1 g or so (about half a year later) persists in continuous light or on a fixed photofraction and even in continuous darkness [20], Figure 5.

Why parametrize the hypothalamus? Changes of particular interest are those at body sites that coordinate the chronomes of other structures, such as the adrenal cortex [12,22] and the brain [23-25]. The hypothalamus not only receives directly but also utilizes photic information from the eyes for the synchronization of cyclic physiological processes [23] with the alternation of light and darkness; specifically the suprachiasmatic nuclei (SCN) [24,25] influence the circadian amplitude and/or acrophase of most variables exhibiting changes recurring at intervals of about 24-hours [16][1]. The SCN, like most other structures, themselves show circadian rhythms. Circadians in

[1]In 1993 alone about 1,000 publications contained the term 'circadian' in their title and/or abstract and many more referred to circadians only in the body of the publication. Circadians not only are ubiquitous but their parameters are clinically important [11]. Hence, it has become desirable to map their characteristics on a large scale. They have become an essential quantitative reference standard [1,2].



*Gauged by fit of 365.25 day cosine curve, separately to each of 3 to 5 series per variable, each series covering a one-year span (with several measurements on most days) on the subjects' usual routine. Acrophase = timing of peak in fitted curve as lag from Dec. 22 of year preceding start of study.

**Year 1 began in 1965; year 5 ended in 1970.

Figure 2. *Extent of synchronization of circannual component of the chronomes of five variables studied during 5 consecutive years, shown separately for each year by dots bracketted by their 95% confidence intervals, shown as horizontal lines. Shaded area corresponds to 95% confidence interval of the 5-year summary when, for the set of 5-yearly series, the assumption of no circannual rhythm can be rejected, as is the case for 5 variables in daughter 2, 4 of them in the mother and 3 in daughter 1. This familial autorhythmometry suggested an apparent drifting, if not free-running, of the circannual rhythm in oral temperature of the series from the mother, in the face of a reasonable synchronization of the same variable in at least one of the daughters. Also note differences or drifts in the acrophase of other variables, such as peak expiratory flow. Several systemic circannual rhythms are rather loosely synchronized with their environs.*

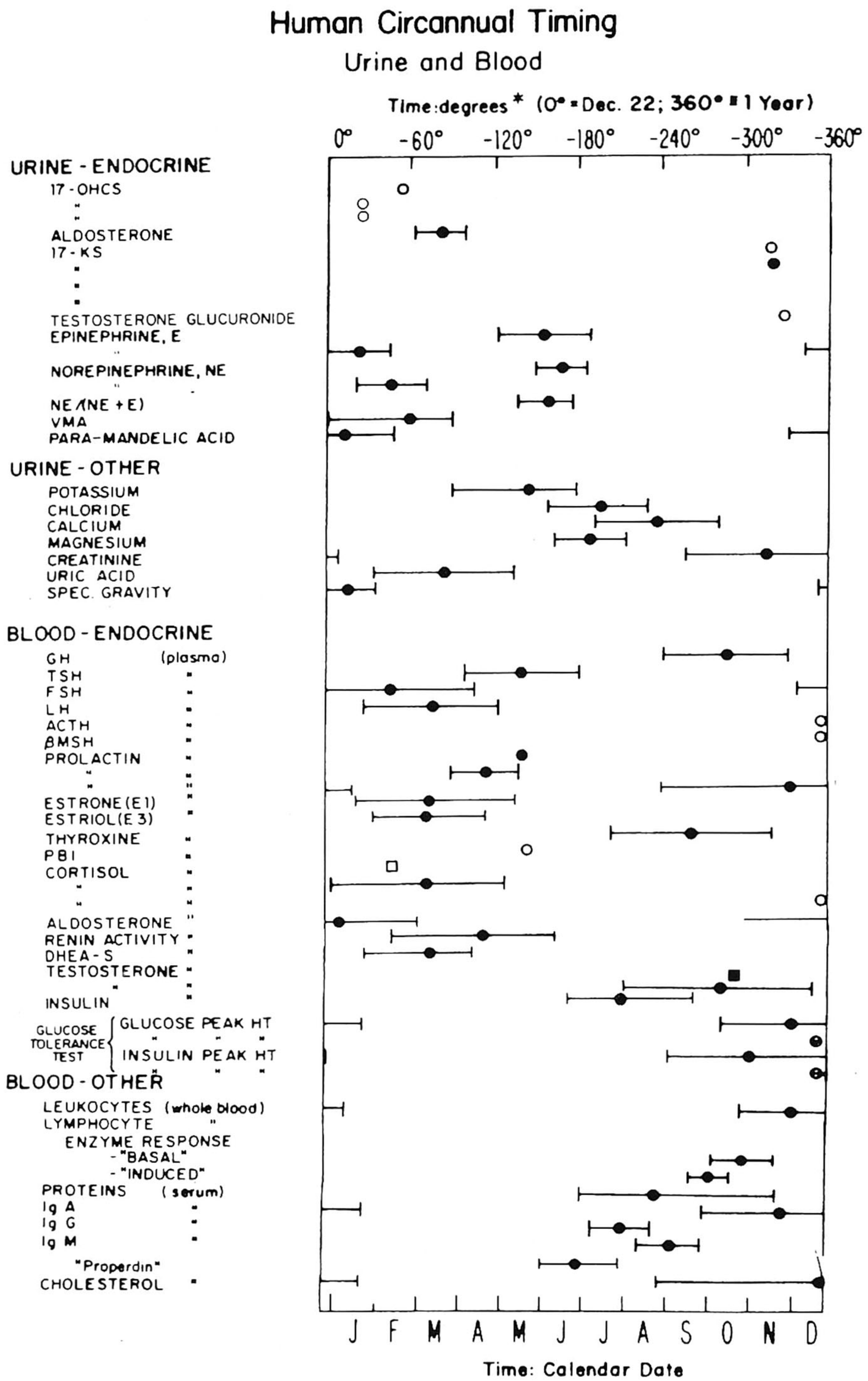


Figure 3. *Ubiquity of human circannual rhythms in urine and blood yielding endpoints for the assessment of the risk of developing diseases of our civilization (see Figure 1).*

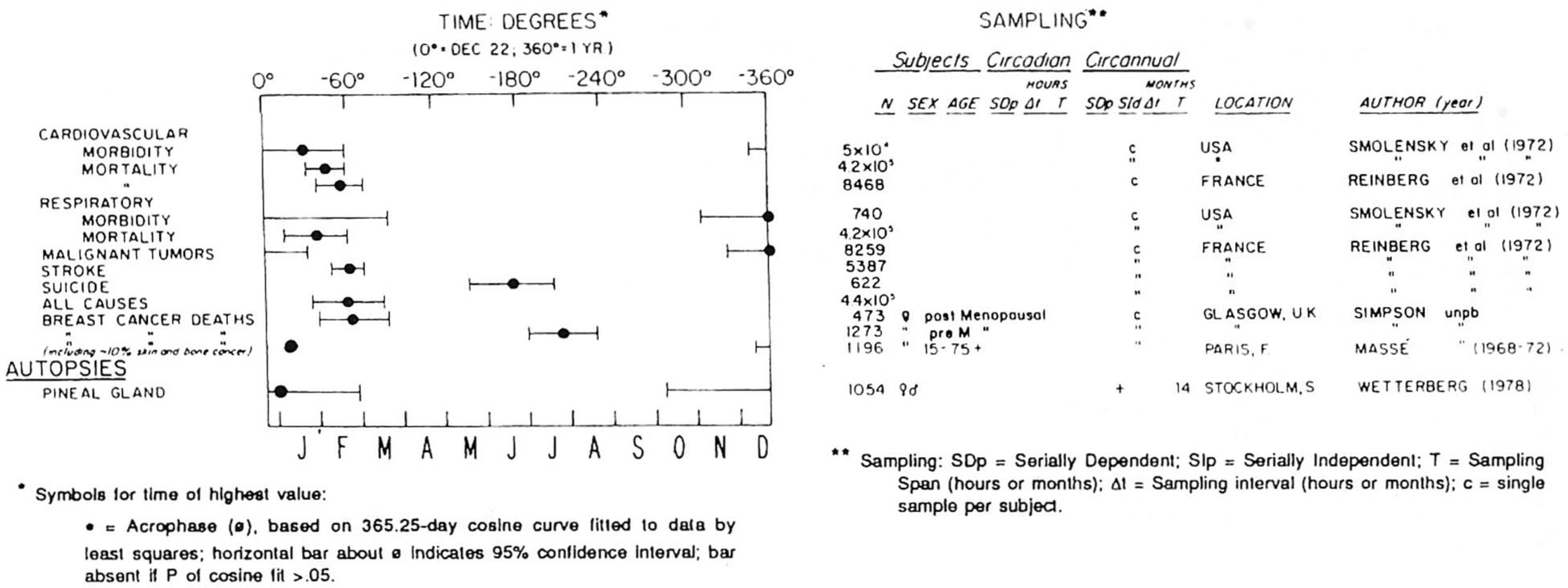


Figure 4. *Data on morbidity and mortality as circannual stage-dependent phenomena, greatly extended in scope by results obtained subsequently on over 6 million diagnoses associated with a call for an ambulance (not shown) [57].*

vasopressin content of plasma, of cerebrospinal fluid and of the SCN, have been reported *in vivo* and *in vitro* [27-33].

Against this background, variations along the scale of a year in the population of vasopressin containing neurons in the human SCN reported earlier time-macroscopically [34,35] as showing (only) an about half-yearly change deserve a meta-analysis. Before the reported data are interpreted as representing only a frequency-multiplied circannual rhythm (exhibiting 2 rather than a single cycle/year), chronobiometry [1,2] here reported provides inferential statistical circannual as well as circasemiannual parameters.

Analytical procedures

Parametric chronobiometry was performed on the mean values based on the study of 48 brains of individuals who had no known neurological or psychiatric disorder and had died with different diagnoses during the span 1981-1989. Data on the volume (V), number (N) and density (D) of vasopressin neurons related to time of death had been grouped into eight annual sections of 1.5 months each, based on the time of death, starting on the first of January. These data, more or less uniformly distributed over the year, are here analyzed by cosinor methods [1,2] involving the fit, jointly (and also separately, for didactic purposes only), of a 1-year and 0.5-year cosine curve. Thus, an overall rhythm-adjusted mean, the **m**idline-estimating statistic **o**f **r**hythm or MESOR (M) is obtained. In addition for each component of the composite model, a double amplitude (2A) and an acrophase (ϕ) are computed as rhythm characteristics. The double amplitude assesses the total change contributed by the given component in the model. Both the MESOR and amplitude are expressed in original units. The acrophase is the lag from a reference time of a component's crest-time. It is usually expressed in (negative) degrees, with 360° equated to the period length (1 or 0.5 year, in this particular analysis). For the circannual and circasemiannual components, the reference time (0°) is December 22, chosen as the day with the longest night, on the average [19].

Chronobiologic analyses also provide a P-value from the zero amplitude (no rhythm) test. The proportion of the total variance accounted for by the fitted model is given by the Percent Rhythm (PR). The analyses herein are limited by being based on data stacked over a single (idealized) year. This prevents the investigation of the extent of reproducibility from year to year of the circannual patterns; it can also result in the underestimation of the amplitude if the rhythm is free-running. Moreover, the stacking also prevents any investigation of periodicities different from that over which the stacking was done, i.e, one cycle per year or its precise harmonics. Furthermore, the present analyses are performed on (and hence the uncertainty of the parameters is computed on the basis of) the previously published folded mean values [34] since the original (unfolded) values as a function of time were unavailable. A cosinor analysis based on means can reveal a statistically significant rhythm while one based on the original data does not (e.g., in the presence of a large inter-individual variability) and *vice versa*.

With respect to the choice of a period for the multiple regression by the cosinor methods, the different considerations for 1) hypothesis testing versus 2) parameter estimation

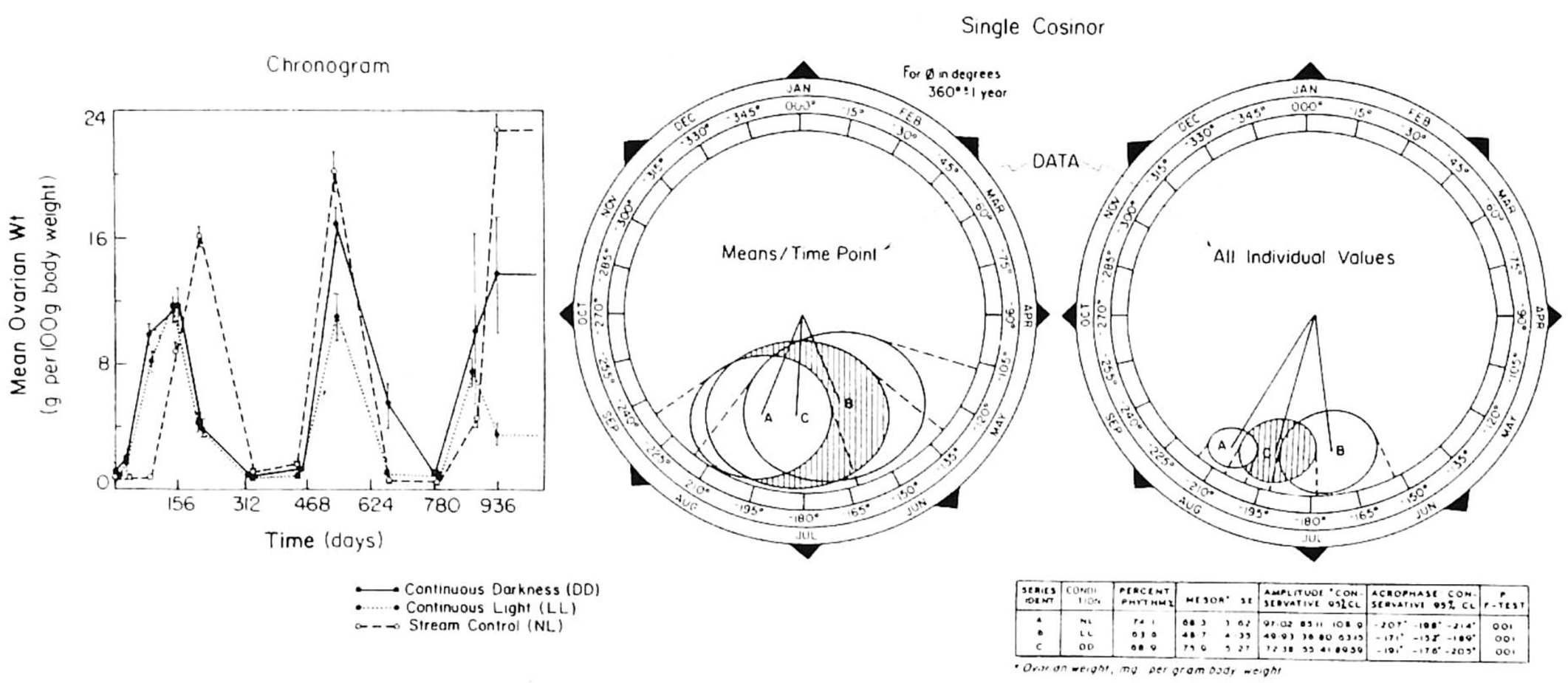


Figure 5. *Demonstration by serially independent sampling of the persistence in controlled ambient temperature of circannual rhythmicity in continuous light or continuous darkness as well as in controls exposed to the daily and yearly alternations of light/darkness and ambient temperature.*

deserve added comment. The cosinor uses curve-fitting for both aims 1) and 2), yet according to distinctly different criteria.

1) For hypothesis testing, the fit of a precise 1.0- or 0.5-year cosine curve serves to examine the no-circannual rhythm (or zero-circannual-amplitude) assumption [1]. For hypothesis testing, i.e., for rhythm detection, no period other than the anticipated 1.0- or 0.5-year cosine curve can be used, unless there is circumstantial evidence for a free-run and preferably also for the likely period of the free-run. For example, for individuals in social isolation the period can be anticipated to differ from exactly 24 hours, even if one does not know the precise period. In this case, when the time series is very long and/or the deviation from precisely 24 hours is large the fit of the precise environmental period may not allow the demonstration of a free-run. In this case a spectral window bracketting a trial period of 24 hours is justified. The ideal case, however, is one based on prior evidence for a free-run with a known average period. This is available for instance, for the circadian temperature rhythm of the blinded Bagg albino mouse, the original model for which free-running was first quantified with its uncertainties in repeated lifespan studies covering the decade of the fifties [36-38]. Thus, in the blinded mouse, the zero-circadian-amplitude assumption can be tested by fitting a 23.4-hour cosine curve, since one knows not only that this free-running period differs from the 24.0±0.1-h period of sham-operated controls with eyes. One knows also the likely average length and further that the sham-operated control mice with eyes can be synchronized by the alternation of 12 h of light with 12 h of darkness but not the blind mice, at least not for several postoperative months.

While quantitative parameter estimations of free-running circannual periods are available from the experimental laboratory [20], no such evidence of a desynchronization is available for any free-running human circannual rhythm. To the contrary, in the circannual case, there is abundant, extensively reviewed evidence that a one-year synchronized circannual, Figures 1-4 [2,17], or a circasemiannual [39] rhythm characterizes human populations under ordinary socio-ecologic conditions in Western civilizations.

2) In turning to parameter estimation, it can be kept in mind that the fit of a precise 1.0 -year or 0.5-year period to test the zero-amplitude assumption has as its alternate hypothesis that the data are characterized by a rhythm with the period fitted or one near that period [40]. Prior evidence to the contrary notwithstanding (i.e., studies on other variables all showing the 1-year synchronization of human circannual rhythms), one is free (for aims differing from a test of the occurrence of a rhythm) to fit a period differing from precisely 1.0 (or 0.5) year. Accordingly, for parameter estimation but not for deciding the issue of whether or not there is a desynchronization, a better fit to the data might be obtained with the fit of a period different from precisely one year or one-half year, as compared to the result of the fit of a cosine curve with a precise 1.0 or 0.5-year period. The finding of a period different from precisely one year requires data series collected longitudinally over several cycles.

In the case of interest herein, the V and N of vasopressin neurons, each value for a given variable stems from a different individual, i.e, we are dealing with a transverse data series. In other words, in the case of a truly longitudinal data series, further curve-fitting can be done in a window around the year (or

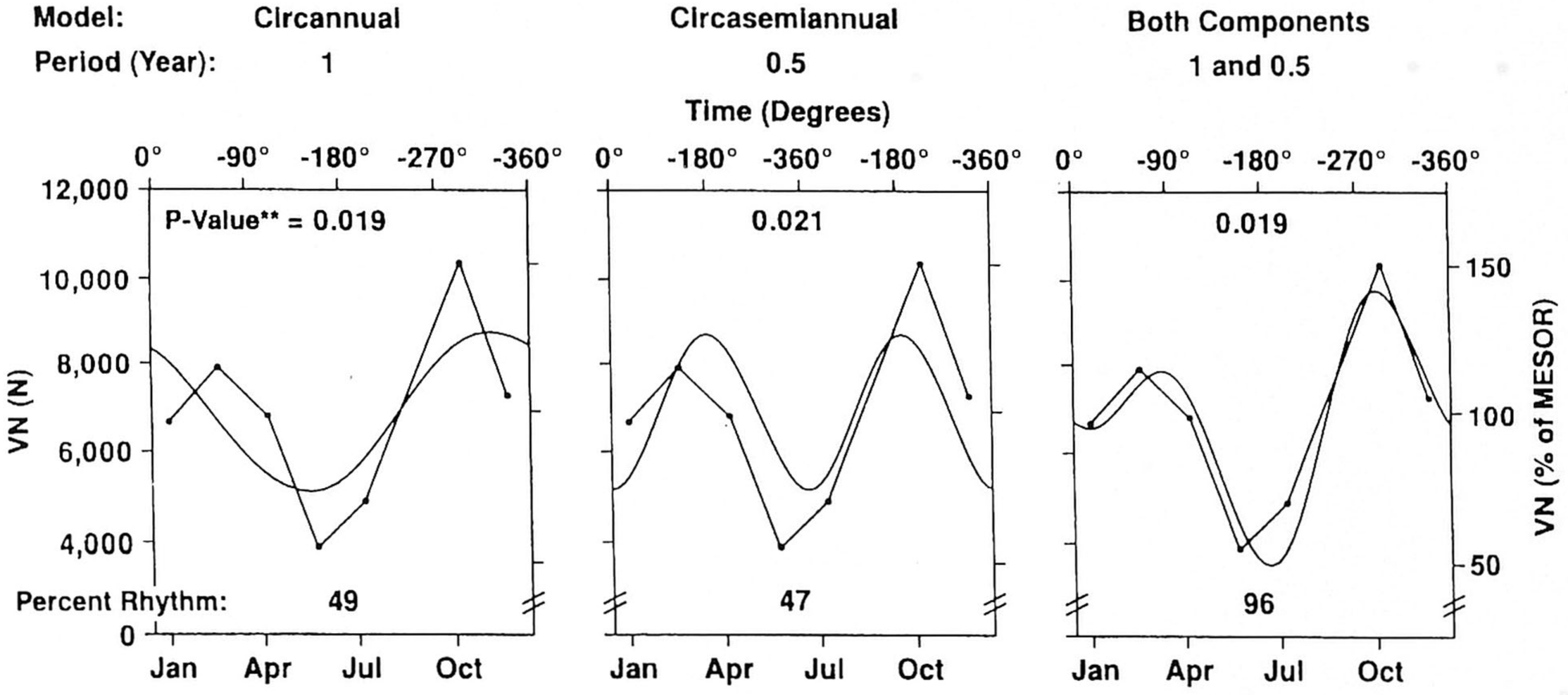


Figure 6. *Demonstration of circannual rhythm in human hypothalami. Whereas the data suggest a 'bimodal' pattern to the unaided eye, inspection of the results from curve-fitting reveals that the separate fit of a one-yearly cosine curve (left) or that of a half-yearly cosine curve (middle) is not as good as the fit of the joint model (right). P-values listed are from the concomitant fit; when a 1.0- and 0.5-year cosine curve each is fitted separately, the corresponding P-value is 0.189 and 0.210, respectively (Table I).*

the half-year) once the zero-circannual and/or circasemiannual amplitude assumption has been rejected. But, as in the case of the (non-24-h) 23.4-h average circadian period of the circadian temperature rhythm in blinded Bagg albino mice [2,16], the result can be taken at its face value only after a successful replication explicitly aimed at reproducing a particular period different from 1.0 and 0.5 years in the case of circannuals and circasemiannuals, respectively.

Taking these considerations into account, the best-fitting period is here not sought, even if, in support of a possible transient "free-run", phase-drifts have been observed by us in humans living with air-conditioning in the summer and heating in the winter, Figure 2 [17], yet it would be most surprising if humans studied at different times of different years would all exhibit the same free-running period, modulations by other cycles with still longer or shorter periods than l-year notwithstanding.

Also as an added argument against a free-run, is the answer to another question: Does the analysis of a single idealized cycle suffice to demonstrate a circannual or circasemiannual rhythm rather than merely a non-recurrent pattern? In this context, it is pertinent that the original results, before stacking, cover a span longer than a single calendar year. Indeed, in order to reject the no circannual- and/or no-circasemiannual-amplitude assumptions, a majority of the 48 patients had to have rhythms with a similar period and phase. It follows that the result of the analyses summarized herein is based on much more than a single cycle.

Another methodologic point deserves emphasis. With respect to the relative merits of the arithmetic mean vs. the MESOR, the latter is usually superior [6], except when the sampling times depart drastically from an equidistribution over the cycle length investigated, in which case the MESOR may be less representative than the arithmetic mean, or even misleading; but this argument applies only to most unequidistant data. In the overwhelming majority of the cases examined, when dense time series with identical mean and MESOR are decimated in different ways, the MESOR remains consistent with the original midline-estimating statistic of rhythm, whereas the arithmetic mean will depend on the location of the data remaining after decimation[6]. As compared to the arithmetic mean the MESOR estimate is usually more accurate when the data are unequidistant and more precise when the data are equidistant. The latter point will be illustrated under results.

Results

The concomitant least squares fit of cosine curves with 1.0- and 0.5-year periods allows the rejection of the 'zero-amplitude' assumption for both the V and N of vasopressin neurons.

Table I. *Circannual and circasemiannual aspects of vasopressin-containing neurons in thehuman suprachiasmatic nucleus*.*

Variable (unit)	Period (τ) fitted (year)		PR	P	M± SE	2A ± SE	φ ± SE
Volume (mm^3)	1	s	45	0.223	0.248 ± 0.009	0.116±0.056	-334° ± 28°
	0.5	s	46	0.212		0.118 ± 0.056	-181° ± 27°
	1	j	46	0.050		0.118 ± 0.026	-333° ± 13°
	0.5	j	47	0.049		0.118 ± 0.026	-181° ± 13°
	overall	j	93	0.047			
Cell number (x 10^3)	1	s	49	0.189	6.939 ± 0.206	3.590 ± 1.640	-338° ± 26°
	0.5	s	46	0.210		3.518 ± 1.666	-203° ± 27°
	1	j	49	0.019		3.630 ± 0.580	-337° ± 9°
	0.5	j	47	0.021		3.560 ± 0.578	-203° ± 9°
	overall	j	96	0.019			
Cell density (x $10^3/mm^3$)	1	s	12	0.717	28.445 ± 0.552	2.994 ± 3.542	-25° ± 68°
	0.5	s	78	0.023		7.452 ± 1.794	-279° ± 14°
	1	j	12	0.311		2.926± 1.554	-24° ± 30°
	0.5	j	78	0.039		7.424 ± 1.570	-279° ± 12°
	overall	j	90	0.076			

* Resolved by the separate (s) or joint (j) fit of 1.0-year and/or 0.5-year cosine curves. PR=Percent Rhythm (proportion of total variance accounted for by fitted model); P=P-value from zero-amplitude (no-rhythm) test; M=MESOR (**m**idline-**e**stimating **s**tatistic **o**f **r**hythm), a rhythm-adjusted mean; 2A=double amplitude, a measure of the extent of predictable change within one cycle; φ=acrophase, a measure of the timing of overall high values recurring in each cycle; φ expressed in (negative) degrees, with 360°=period length (1.0 or 0.5 year); 0°=December 22; SE=Standard Error.

For N, Figure 6 visualizes each component separately and the composite model. On the left, the fit of a 1-year cosine curve allows rejection of the zero-amplitude assumption (P=0.019). The model, however, does not account for the two macroscopic peaks in the data. The fit of a 0.5-year cosine curve is shown in the middle. Whereas it is also statistically significant (P=0.021), the model does not account for the fact that the second peak in October is larger than the first one in FebruaryMarch. The concomitant fit of both components shown on the right provides a better approximation of the waveform.

Table I reports the numerical results for V, N and D. The double amplitudes of the circannual and circasemiannual components of V and N are similar. D shows a larger circasemiannual than circannual component of variation: only the former can be validated with statistical significance.

There is a good agreement between the circannual acrophases of all three variables, as it can be anticipated since they measure similar characteristics of the vasopressin neuron content of the human SCN. Since 360° are equated to 1 year, one month corresponds roughly to 30°; therefore -330° represent the end of November and -25° mid-January. A similar agreement between the circasemiannual acrophases is observed among the variables studied.

It is of methodologic interest that the standard errors of the more precise MESOR vs. those of the arithmetic mean are, respectively, in the case of V, 0.009 vs. 0.023; for N, 0.206 vs. 0.685; and for D, 0.552 vs. 1.135.

Discussion and Conclusion

For the description of a pattern, time-macroscopy and classical statistical procedures are highly desirable, but they are no substitute for the time-microscopy whereby new endpoints are provided for the analysis of ubiquitous and critically important features of a time structure. Chronobiologists isolate the parameters of anticipated rhythms with their uncertainties for eventual basic or clinical use, just as chemists seek a specific melting point and other characteristics to determine whether they are dealing with a pure compound. In a first demonstration as reported herein, with methodologic detail, in the isolation of a circannual rhythm in the vasopressin-containing neurons both hypothesis testing and parameter estimations are indispensable, even if eventually in follow-up work, the hypothesis testing may be dispensed for the sake of direct parameter estimation.

By the use of simple mathematical procedures, anticipated circannual and circasemiannual components can be estimated in the vasopressin neurons of the human SCN. The extent and

Table II. *Approximation of circannual rhythm in vasopressin-containing neurons requires only two-component model while that in ulcer incidence requires three-component model*.*

	Period(year)	PR	P	M±SE	2A±SE	ϕ±SE
SCN vasopressin-containing neurons						
Volume (mm^3)	1	46	0. 141	0.248 ±0.006	0.116 ±0.016	-333° ±8°
	0.5	47	0.139		0.118 ±0.016	-181° ±8°
	0.33	7	0.357		0.044 ±0.016	-209° ±22°
	overall	99	0.180			
2-component model**		93	0.047			
Cell number 0.5 (x 10^3)	1	49	0.185	6.938 ±0.241	3.624 ±0.680	-337° ±11°
	47	0. 190			3.552 ±0.676	-203° ±11°
	0.33	2	0.676		0.748 ±0.672	-217° ±53°
	overall	98	0.246			
2-component model**		96	0.019			
Cell density (x $10^3/mm^3$)	1	12	0.289	28.451 ±0.316	2.956 ±0.890	-24° ±17°
	0.5	78	0.118		7.464 ±0.900	-279° ±7°
	0.33	8	0.330		2.576 ±0.882	-42° ±20°
	overall	99	0.196			
2-component model**		90	0.076			
Gastrointestinal ulcer incidence						
N of ulcers	1	29	0.119	15.4 ±1.3	8.8 ±2.6	-56° ±24°
	0.5	37	0.078		9.8 ±2.6	-142° ±21°
	overall	66	0.078			
	1	29	0.039	15.4 ±0.8	8.7 ±2.4	-56° ±16°
	0.5	37	0.024		9.8 ±2.4	-142° ±14°
	0.33	24	0.054		7.8 ±2.4	-190° ±17°
	overall	89	0.025			

* Resolved by the concomitant fit of 1- and 0.5-year and 1-, 0.5- and 0.33-year cosine curves. PR=Percent Rhythm (proportion of total variance accounted for by fitted model); P=P-value from zero-amplitude (no-rhythm) test; M=MESOR (**m**idline-**e**stimating **s**tatistic **o**f **r**hythm), a rhythm-adjusted mean; 2A=double amplitude, a measure of the extent of predictable change within one cycle; ϕ=acrophase, a measure of the timing of overall high values recurring in each cycle; ϕ expressed in (negative) degrees, with 360° ≡ period length (1, 0.5 or 0.33 year); 0°=December 22; SE=Standard Error.

** See Table I for complete summary of 2-component fit.

timing of predictable change along the scale of the year in V, N or D of SCN cells is estimated by their double amplitudes and acrophases, respectively. Vasopressin neurons can be as low as 50% (V, N) or 74% (D) of the yearly mean at certain circannual stages and as high as 150% (V, N) or 126% (D) of the yearly mean at other circannual stages. With respect to D, a qualification is in order. If two variables change periodically in a roughly similar fashion, their ratio will be time-invariant. This consideration applies to D, which depends on V and N, and shows only a circasemiannual but not a circannual component with statistical significance. The extent of similarity in the time courses of change in V and N may have been sufficient to eliminate the circannual component of D, but not the circasemiannual feature.

The composite model described herein applies to the population of patients studied, since the data are serially independent, that is each individual contributed only one set of values. Hence, a high extent of synchronization among individuals must have been present for the circannual rhythm to be demonstrated as a group phenomenon. (If the individuals' circannual rhythms scattered all year round, even if each individual's rhythm was perfectly sinusoidal, an overall circannual rhythm could not have been demonstrated). The circannual population rhythm is quite non-sinusoidal, however, its 'bimodal' waveform can be approximated with statistical significance by a composite model consisting of only two cosine curves, with periods of 1.0 and 0.5 year.

The relation of the periodicity in vasopressin neurons to animal reproduction has been very extensively discussed elsewhere [34,35]. If such a relation can eventually be established,

this would be another example where investigations on human material can serve those concerned with nonhuman species, to return the favor extended by so many other animals providing information for human use. Studies on mink, however, provide a caveat to premature generalization. The absence of vasopressin immunoreactive perikarya within the mink's SCN [41], in the face of circannual (seasonal) behavior in the reproduction of these animals, suggests that the SCN may not be a universal, if any, pacemaker of internal circannual rhythms. For the time being, the vasopressin neurons constitute a group of cells with its own circannual and circasemiannual rhythmicity, synchronized by external factors, such as perhaps the changes in the daily photofraction during the year. The occurrence of a 24-hour periodic pineal melatonin synthesis in the vasopressin-deficient Brattleboro rat [42] limits the indispensability of these vasopressin neurons in the SCN also as a circadian mechanism. By the same token, their periodicity notwithstanding, at least insofar as vasopressin neurons are concerned, there is no evidence (as yet?) that they play a role as a mechanism of components of the chronome other than circadian.

SCN removal brings about, as a rule, an advance in circadian phase and a reduction in circadian amplitude. To the naked eye, notably in the presence of noise, the amplitude reduction may be so pronounced as to simulate a disappearance of the circadian rhythm. A complete rhythm obliteration has often been reported [24]. In some cases the data are too sparse, in others the method of analysis was not specified or was inappropriate, as reviewed elsewhere [43,44]. In data of sufficient density cosinor analysis allows the rejection of zero-circadian-amplitude assumption, revealing a damping but not disappearance of rhythm; this applies to the circadians in the murine core temperature [45-47], serum corticosterone, cell division [48,49] and DNA synthesis of different tissues such as tongue, esophagus, colon and cornea [49], when these variables are investigated by the use of time-microscopic techniques.

Different effects of suprachiasmatic nuclear lesions upon different variables are also reported [50-54]. Sometimes the extent of the effect of the lesion upon the same variable differs and the circadian amplitude decrease in one case, can be so great in another that a rhythm is not detected by one investigator but is demonstrated with statistical significance by another. When chronobiometry is applied to the data the bilateral ablation of the SCN leads to the obliteration of only a few and certainly not of most circadian rhythms [16,25]. What seems certain is that the SCN are not the master oscillator responsible for all circadian rhythmicity [16,25].

A strict link, such as a frequency demultiplication, between circadian and circannual rhythms has been ruled out, at least for female squirrels; the manipulation of light-dark cycles spanning 23, 24 or 25 hours with documented synchronization by these environmental cycles of the circadian activity rhythm did not lead to corresponding differences in the length of the about-yearly cycle in body weight [55]. Circannual rhythms intermodulate by feedsidewards [2] with other components of each variable's chronome, its genetically anchored time structure. In such chronomes, circannuals may be the component to be altered early in the presence of disease risk elevation [2,12,15,56].

This finding leads to the question as to what a metachronoanalysis can offer. Does it contribute more than the recognition that there is a larger V and N of vasopressin neurons at certain times of year? [34,35]. It can only be suggested by precedent that one purpose of chronobiology is to measure the earliest abnormality in the heretofore indivisible normal range [16,56] by measuring the dynamic characteristics of change in one or another component of a variable's chronome. Chronome parameters, the chrones in a set of multifrequency rhythms and trends can then be tested for various applications in biomedicine. Apart from the fact that the MESOR has its merits as compared to the arithmetic mean, the dynamic parameters such as the amplitude and acrophase themselves have already served to assess earliest disease risk, notably in relation to the circulation, where circadian amplitude-hypertension has been shown to precede MESOR-hypertension and where the circannual amplitude of aldosterone was shown to correlate with both the familial cardiovascular disease risk determined by questionnaire and with the circadian MESOR of blood pressure measured automatically at 10-minute intervals for 24 hours in each season [12].

By the same token, one may look for associations between the parameters here quantified and overt pathology such as gastrointestinal ulcers identified by endoscopy. From this viewpoint, Table II complements Table I; its results are separated from Table I in order to allow the merit of a 2-component fit to the V and N data analyzed to stand out in clear view of all. Table II shows first that a 3-component model does not add to the analysis of the data from the suprachiasmatic nucleus: overall, the 3-component model does not reach statistical significance (Table II) when the 2component model does so, at least for V and N (Table I). By contrast, as shown for the number of ulcers in Table II, the 'bimodal' waveform of these results from endoscopy is better approximated by the 3-component model ($P \leq 0.05$ for each component of the composite model and for the model overall), whereas statistical significance is not reached with the 2-component model (Table II).

It is noteworthy that the circannual acrophases of V and N (Table I) lead that for the incidence of ulcers (Table II). Such phase differences (whether time-microscopic or -macroscopic) may possibly be interpreted as the result of (partly) different mechanisms or as a lag between two phenomena that may or may not be related.

As the most important example in the context of carcinogenesis, several different approaches converge to suggest that the circannual rhythm of circulating prolactin in women may be a classifier for the risk of developing breast cancer. First, an 'obliteration' of the circannual prolactin rhythm was found in women with fibrocystic mastopathy, whereas women without this condition had such a rhythm [13]. Second, for a group of women who actually had breast cancer but who had prolactin determined in a sample of blood drawn while they were in apparent good health, the circannual group prolactin amplitude was much lower than that in a group consisting of women who did not develop breast cancer [14]. Third, the longitudinally assessed circannual amplitude of prolactin was lower in women at a high vs. that in women at a low familial risk of developing breast cancer in Minnesota [12]. Fourth, this dif-

ference holds in a geographic comparison of Japanese women (at a relatively low breast cancer risk) and Minnesotan women (at a higher such risk): the circannual prolactin amplitude was much higher in the former low risk group than in the latter [12]. By contrast, the circannual amplitude of TSH was higher in women at high vs. low familial breast cancer risk [12]. Fifth, the same two circannual rhythms, namely that in prolactin and in TSH, were classifiers in human prostatic cancer [15].

The circannual and circasemiannual parameters here offered for vasopressin-containing neurons—their volume and number—could be of a chronoepidemiologic use similar to that of the several hormones mentioned in the above examples. They contribute a new dimension for the study of covert as well as overt predisease and chronopathology, respectively. The volume, number and density of vasopressin-containing neurons of the SCN should be compared in clinically healthy accident victims who were at a high versus low familial (or other) risk of developing one or the other disease. Routine questionnaires could be given to relatives and/or friends to seek on a population basis differences between the risk groups thus established with respect to these chronome components. This approach could lead to a better understanding of any hypothalamic mechanisms of (time-structure related) chronorisk. The same suggestion applies to other structures collected at autopsy, such as the pineal (cf bottom Figure 4). Further work exploring any putative association of human disease or risk states and the pattern of V and N investigated herein must be viewed with the caveat in mind that temporal relations alone can never be interpreted as causal; they provide only leads. Such leads could be particularly useful as gauges of human neuroendocrine chronome development in relation to malignancy.

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