COSINOR ANALYSIS OF CIRCADIAN RHYTHM OF TRANSCRIPTION IN NERVE CELL POPULATIONS IN VARIOUS PARTS OF THE RAT NERVOUS SYSTEM

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UDC 616.8-018.1-092.9-07

KEY WORDS: cortical, bulbar, and spinal nerve cell populations; activity of endogenous RNA-polymerases, nucleoplasm, nucleoli.

The intensity of transcription, as a parameter of gene expression, varies at different levels of activity of nerve cells. Biorhythms and reactivity are closely interconnected, for endogenous rhythms have an effect on the formation of adaptive reactions taking place in the body in response to exposure to various environmental factors [1].

The aim of this investigation was to study the circadian rhythm of template activity of chromatin in cortical, bulbar, and spinal populations of rat neurons.

EXPERIMENTAL METHOD

The experimental animals, 45 male Wistar rats weighing 150-200 g, and adapted beforehand for 2 weeks to the lighting conditions (light:darkness = 12 h: 12 h; light from 8 a.m. to 8 p.m.) were kept in an insulated room.

The test object consisted of nerve cells from the cranial cervical sympathetic ganglion (CCSG) spinal ganglion L5 (SG), Purkinje cells (PC) of the cerebellum, cells of the suprachiasmatic nucleus of the hypothalamus (SCH), spinal motoneurons (SM), and also neurons of the sensomotor and visual regions of the cortex (SMC, VC; layers III and IV). Five animals were killed at 1, 7, and 10 a.m., and 1, 4, 7, and 10 p.m. for 2 days and 100 cells of each organ were studied. Template activity of chromatin was characterized by autoradiographic determination of activity of endogenous RNA-polymerases in fixed cells [5]. At each time point the state of transcription was evaluated as the intensity of labeling of the nuclei, which was taken to be the number of grains of reduced silver counted visually, above the nucleus and nucleoplasm separately.

The results were analyzed by computer by the "Cosinor" program, revealing mesors, amplitudes, and calculated acrophases of rhythms within a 95% interval of significance [4]. The degree of correlation between individual parameters in the course of the circadian cycle was evaluated by calculation of the linear correlation coefficients.

EXPERIMENTAL RESULTS

The data in Figs. 1 and 2 and Table 1 show that acrophases of endogenous RNA-polymerase activity in the nucleoplasm of neurons of SCH (9.01 p.m.), SG (9.40 p.m.), CCSG (9.04 p.m.), SM (9.21 p.m.) and PC (4.41 a.m.) were observed in the period of darkness. Maximal values of nucleolar labeling also were observed in the dark period, at about the same times as the acrophases of nucleoplasmic labeling: SCH 9.13 p.m., SG 8.36 p.m., CCSG 10.47 p.m., and PC 7.26 a.m. Moreover, the largest number of inscription regions of chromatin observed in the above nerve cell populations at night was approximately 40-110% higher than their minimal value during the daytime.

Department of Biology, N. I. Pirogov Second Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 109, No. 3, pp. 301-303, March, 1990. Original article submitted April 25, 1989.

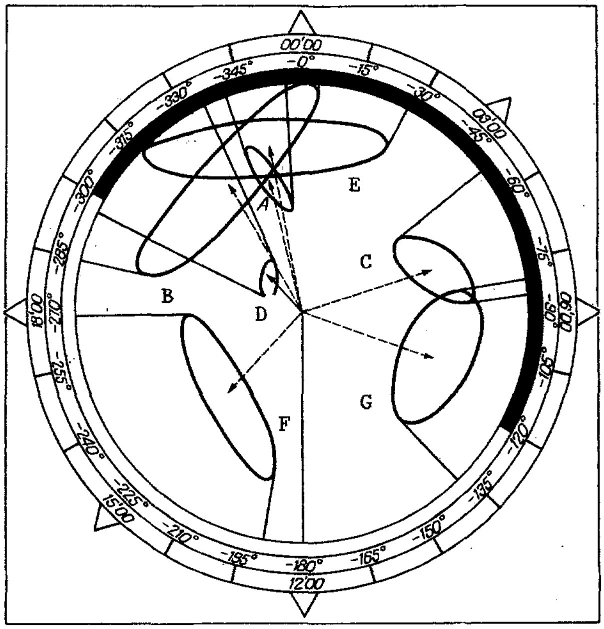

Fig. 1. Cosinor diagram of circadian rhythm of endogenous RNA-polymerase activity in nucleoplasm of various populations of nerve cells in rats (360° = 24 h). Direction of radial vectors on diagram indicates position of acrophases (calculated maxima) of circadian rhythms of template activity. Amplitudes of vectors correspond to mesor (mean level of rhythm for the 24-h period). Ellipses of errors indicate statistical significance with 95% level of probability. a) CCSG, b) SG, c) PC, d) SCH, e) SM, f) SMC, g) VC.

Thus differential analysis of the intensity of nucleoplasmic and nucleolar labeling demonstrates the similarity between the circadian rhythm of expression of structural and ribosomal genes. Realization of genetic information in the cells of eukaryotes is effected by synthesis of specific RNase, and is controlled to a certain degree by activity of the corresponding RNA-polymerases. In the populations of nerve cells investigated, the mechanisms regulating polymerase activity during the 24-h period are evidently similar in character in the cases of polymerase I, polymerase II, and polymerase III.

Acrophases of template activity of cerebellar PC (Figs. 1 and 2) were shifted by 5-6 h relative to the acrophases of SCH, SG, CCSG, and SM, the ellipses of errorof which overlapped. However, coefficients of correlation of the time series of endogenous RNA-polymerase activity in the nuclei of nerve cells of SM, SG, CCSG, SCH, and PC demonstrate a varied level of positive correlation (Fig. 3), evidence of their synchronization during the circadian cycle. It can thus be tentatively suggested that there is a circadian system, formed by a set of populations, the maximal levels of whose gene activity correspond to times of an enhanced level of activity of the rats which, according to data in the literature, is observed during the period of darkness [2].

Analysis of the nucleoplasmic and nucleolar labeling of neurons in VC and SMC of the rat brain shows that the number of transcription sites in the genome varies with the time of day. Expression of ribosomal and structural genes, as in the populations examined above, moreover, is similar in character. In VC the acrophase was observed at 7.24 a.m. for the nucleoplasm and 7.44 a.m. for the nucleolus, evidently in connection with the impending reception of daylight at 8.00 a.m. These calculated peaks are out of phase with the acrophases of bulbar and spinal neuron populations (for example, the internal acrophases of VC and SMC were 8.03 a.m. and 8.59 a.m., $r = -0.4$). In the somatosensory zone acrophases of endogenous RNA-polymerase activity of nucleoplasm and nucleolus were observed at 3.21 p.m., and 2.42 p.m. Internal acrophases with similar values of SM parameters occurred at 4.00 p.m. and 3.57 p.m., respectively, with a coefficient of correlation of −0.38. Ellipses of error of acrophases of template activity of chromatin of VC and SMC neurons do not cross on the diagrams, evidence of the absence of their synchronization in the circadian rhythm (coefficient of correlation −0.4).

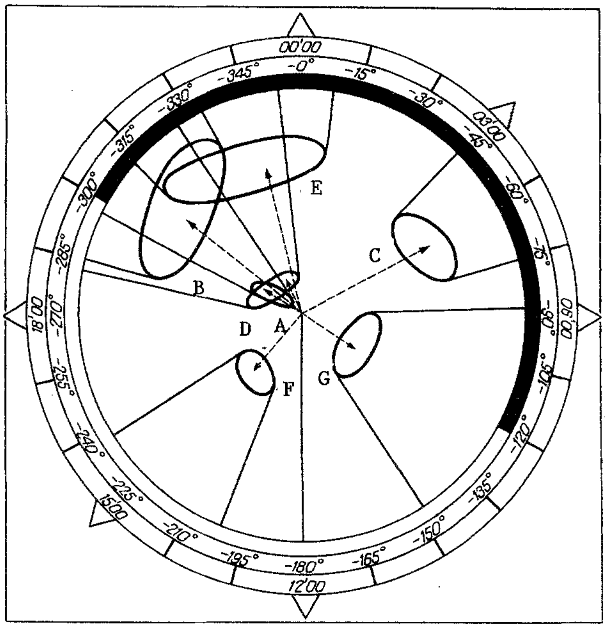

Fig. 2. Cosinor diagram of circadian changes in endogenous RNA-polymerase activity of cell nuclei of various populations in the rat nervous system (360° C = 24 h). Legend as to Fig. 1.

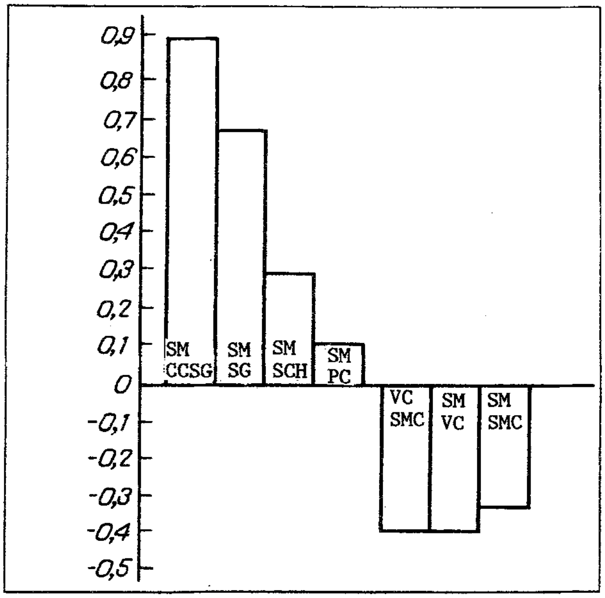

Fig. 3. Coefficients of correlation of circadian rhythm of endogenous RNA-polymerase activity in nucleoplasm of cell populations from different parts of the rat nervous system.

Thus the observed position of acrophases of template activity of the chromatin in cell populations in different parts of the rat nervous system is evidence, first, of the dynamic multioscillatory regulatory system which helps to maintain the functional integrity of the organism, and second, it confirms the view that photoperiodicity is an important synchronizer of biological rhythms, and finally, that the activating effect of increased function at the transcription level of the genetic apparatus may be manifested at different times of day depending on specific time signals from the external environment.

TABLE 1. Circadian Rhythm of Endogenous RNA-Polymerase Activity in Nucleoplasm (NP) and Nucleoli (NL) of Cell Populations in Different Parts of the Rat Nervous System (mean number of grains of reduced silver)

Neuron population	Mesor ($M \pm m$)	Amplitude		Acrophase*	
		A	95% confidence interval		95% confidence interval
SCH:					
NP	13,8±0,7	1,7	1,3—2,1	21.01	19.47—22.15
NL	2,7±0,2	0,33	0,4—0,2	21.13	18.48—23.38
SG:					
NP	31,1±0,7	8,4	7,8—9,0	21.40	19.08—0.12
NL	20,5±0,4	5,3	5,1—5,5	20.36	18.52—22.20
CCSG:					
NP	32,09±0,9	7,4	7,2—7,6	23.04	22.37—23.31
NL	3,1±0,3	0,4	0,3—0,5	20.47	19.46—21.48
SM:					
NP	41,9±0,6	10,1	9,5—10,7	23.21	20.58—1.44
NL	3,1±0,3	4,4	4,1—4,7	22.45	21.03—0.27
PC:					
NP	32,1±0,7	7,5	7,2—7,8	4.41	3.35—5.47
NL	23,4±0,5	4,1	4,0—4,2	7.26	2.58—11.54
VC:					
NP	21,9±0,7	6,6	6,2—7,0	7.24	5.29—9.19
NL	13,6±0,3	3,4	3,2—3,6	7.44	5.47—9.41
SMC:					
NP	21,2±0,5	4,2	4,0—4,4	15.21	12.45—17.57
NL	16,5±0,3	4,2	4,0—4,4	14.42	13.29—15.55

Legend. Asterisk indicates time in hours and minutes.

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