15
INFLUENCE OF ELECTROMAGNETIC FIELDS OF EXTREMELY DIFFERENT FREQUENCY DIAPASON ON INFRADIAN RHYTHMS OF PHYSIOLOGICAL PROCESSES

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1. INTRODUCTION

The beginning of the modern electromagnetic (EM) field theories in biology appeared only in 1970, with A.M. Presman`s report of the pioneering work of Soviet Bioelectromagnetics researchers, which also contained the first outline of a holistic EM field theory of the organism and its relationships with the environment. Since then, there is ample evidence of bioeffects of EM fields and endogenous EM fields. It is now established that organisms react sensitively to the impact of electromagnetic fields, including very weak ones; effects of various types of endogenous physical fields on cellular organization and morphogenesis are very similar. We also know that several kinds of electromagnetic fields, including microwaves and optical frequencies (biophotons), are emitted from living beings.

It is known that weak extremely low frequency (ELF) dominates in the spectrum of variable magnetic fields that can be registered on the surface of the earth and, perhaps, are utilized by living organisms as means of information exchange, as an internal clock time gauge in a wide spectrum of periods[1,2]. In particular, it has been proven that 8 Hz, 5 μT EMR alters infradian rhythmicity of the studied parameters in epiphysectomized[2, 3] and hypokinetic[4] animals.

Unlike ELF electromagnetic radiation (EMR), ambient low intensity, ultra-high frequency (UHF) EMR reaches minimal value due to increased absorption in the upper atmosphere layers. This leads to a decrease in the background levels 100- and 1000-fold and prevented evolutionary adaptation to EMR in this frequency range[5]. Therefore, it is precisely in this frequency range that one would expect to see increased biological and therapeutical activity, and this has been confirmed in numerous experimental and clinical inventions[6]. However, the

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ability of UHF EMF to affect temporal organization and, in particular, infradian rhythmicity of biological systems, remains practically not studied.

It has been established that the degree of synchronization of physiological parameters is not the same under different circumstances. Thus it could be used to assess stability of physiological systems affected by various factors[7]. Adaptation to stress-factors is accompanied by changes in the rhythmicity of function of various systems, and desynchronization, i.e., discordance in nervous and endocrine regulatory mechanisms, remains an essential component of a general adaptation syndrome[8] and leads to the weakening of an organism's resistance to noxious factors[9,10]. Therefore, one of the most important tasks of contemporary physiology is to search for the effective methods of optimization of a biorhythmic state of an organism, and of promoting its adaptation capacities.

The search for the adequate tests for performing such a task is not easy. Physiological systems of an increased sensitivity to any internal and external changes include, first and foremost, blood and, in particular, bactericidal, hydrolytic and energy systems in neutrophils. Thus, by studying these systems one could adequately evaluate the reaction that an organism may produce in response to factors of various origins.

The goal of this project was to study the effects of week UHF and ULF EMR on infradian rhythmicity of neutrophils' functional activity.

2. MATERIALS AND METHODS

The study was conducted on 120 wild-type male rats of 180-200 g weight. The individual characteristics were identified by the "open field" test that allowed for quick and confident selection of animals with similar constitutional characteristics[11]. On the basis of this test, we identified animals with low mobile activity (LMA), medium (MMA) and high mobile activity (HMA), which displayed distinct differences in the horizontal and vertical mobility.

Rats selected into each mobility category were further divided into two equal sub-groups of 20. The first sub-group included animals housed in the usual conditions of our vivarium (biological control group, C). The second sub-group consisted of animals that were exposed for 3 hours daily to 8 Hz, 5 μT VMF for 32 days.

In the present study, the selection of VMF parameters was based upon assessment of their physiological and geophysical importance. The basis for this selection and methodology is described in Ref. 12.

This study was conducted on 80 wild-type, white male rats weighing 200-250 g. We selected animals of the same age, with a medium level of mobile activity and low emotionality determined by the "open field" test. This provided the selection of homogenous groups of animals, that developed a typical reaction in response to extraneous stimuli, including EMR.

In further experiments, all animals were distributed among four equal groups. The first group was composed of rats maintained under the usual vivarium conditions (biological control, C). The second group was composed of the rats maintained under stress conditions due to imposed limitations on mobility (hypokinesia, HK)[4]. HK was produced by placing the rats in the specially constructed Plexiglass cassettes for 22 hours per day[13]. The third group was composed of animals exposed to UHF EMR. The fourth group was composed of animals that were kept in HK conditions and were exposed to UHF EMR (HK+UHF). The exposure to UHF EMR was daily effected for 30 min exposures on the occipital area during 45 days. The "Luch KVCh-01" generator that was used for this purpose was set for wavelength of 7.1mm at 0.1mW/cm^2.

A blood taken for analysis was obtained during 45 days at the same time once per day by means of a phlebotomy of the tail vein. Blood smears were studied by cytochemistry for bactericidal agents (peroxidase, PO), cation proteins (CP), hydrolytic enzymes (acid phosphatase, AP) and proteases (PR)[14]. Qualitative assessment of the studied parameters was performed by calculating the cytochemical index of content (CIC) according to the L. Kaplow's principle[14]. Average content of oxidative-reducing enzymes in neutrophiles (succinate- and α-glycerophosphatedehydrogenase, SDG and α-GPDG) was determined according to Nartsissov[15].

Phase-amplitude characteristics of the studied processes were calculated by cosinor-analysis, which completely represents the structure of physiological rhythms. The computer program for cosinor-analysis was developed by V. Martynyuk, P. Grigoriev and N. Zuyev in the Crimean Scientific Center of the Academy of Sciences of Ukraine. The using of cosinor-analysis affords an opportunity to reveal periodic components in number rows, as well as to obtain and amplitude and fluctuation phase of any periods that form the total rhythmicity of a particular parameter. For each animal, each parameter was plotted as a periodgram (spectrum) covering from 2.2 to 30.0 days periods divided to 0.5 day steps. By this technique we identified the periods (local maxima) that contribute to the rhythmicity of animals in the corresponding group. Latent co-relations between the studied parameters were established by cluster analysis, which is an adequate tool of assessing multifactorial reactions of an organism[16]. The use of agglomerative strategy of analysis allows one to construct a dendrogram of all parameters by means of hierarchical combination of them into groups (clusters) of greater commonality based on the minimal distance criterion in the spectrum of variables that describe given parameters.

3. RESULTS AND DISCUSSION

3.1. Specific Characteristics of Infradian Rhythmicity of Physiological Parameters in Rats with Different Individual Characteristics

Human beings are known to be divided into three types of biological rhythms based upon different diapasons and this effect would be commensurated with our understanding of the endogenous origins of biological rhythms. As will be shown by our data, the extent of changes in the parameters of infradian rhythms in rats expoused to different noxious stimuli is rather variable (Table 1).

Table 1. Specific characteristic of infradian rhythmicity of physiological parameters in rats with different individual characteristics

Parameter	Groups of rats		
	Low Mobile Activity	**Moderate Mobile Activity**	**High Mobile Activity**
Vertical motility			
Range of periods identification	2.2 – 11.8 days	2.2 – 20.6 days	2.2 – 22.8 days
Number of periods in the spectrum	7	10	13
No periods	≈7[d]	–	–
High amplitude periods	≈10[d]	–	≈19[d]
Horizontal mobility			
Range of periods identification	2.7 – 14.7 days	2.2 – 22.1 days	2.7 – 21.8 days
Number of periods in the spectrum	9	13	12
No periods	≈7[d],6	≈14[d],7	–
High amplitude periods	≈11[d],5	–	≈19[d]
Neutrophils acid phosphatase content			
Range of period identification	2.4 – 15.2 days	2.4 – 18 days	2.4 – 21.9 days
Number of periods in the spectrum	11	13	12
No periods	–	–	≈15[d], ≈18[d],4
High amplitude periods	–	–	–
Low amplitude periods	9[d]	–	2[d],4
Lymphocyte α-GPDG			
Range of period identification	2.4 – 22.3 days	2.4 – 14.1 days	2.4 – 14.4 days
Number of periods in spectrum	7	6	7
No periods	4[d],0	4[d],0	–
High amplitude periods	–	–	–
Low amplitude periods	–	–	–

3.2. Changes in Blood Lymphocyte Dehydrogenase Infradian Activity in Rats with Different Individual Characteristics Exposed to Extremely Low Frequency Magnetic Fields

The results of this study show that the exposure of rats with different mobile activity to 8 Hz, 5 μT MF leads to a shift in infradian rhythmic functional activity of blood lymphocyte and neutrophils.

ELF EMF causes polydirectional changes of infradian rhythmicity of the lymphocyte functional state in rats with different individual characteristics, i.e., the effect of VMF on rhythmic processes depends on the baseline state (Fig. 1). This data is corroborated by the previously reported results about the effects of ELF MF upon animals with different kinds of desynchronosis. These effects are accompanied by the restoration of the baseline temporal organization of physiological systems[17, 18].

3.3. Modification of Infradian Rhythmicity of Neutrophil Functional Activity by Low Intensity Ultra-High Frequency Electromagnetic Fields

UHF EMR is also capable to change the temporal organization of physiological systems. However, the character and direction of these changes depend upon the functional state of an organism.

The most expressive reconstructive processes were noticed in LMA rats. Perhaps, rats with LMA are more susceptible to ELF VMF. The results pertaining to individual sensitivity of animals to ELF VMF are in accordance with those from[19] which demonstrated polydirectional changes of non-specific resistivity in LMA and HMA rats.

Intact animals exposed to UHF EMR exhibit just a slight trend towards changes in the infradian activity of the studied parameters. For instance, no

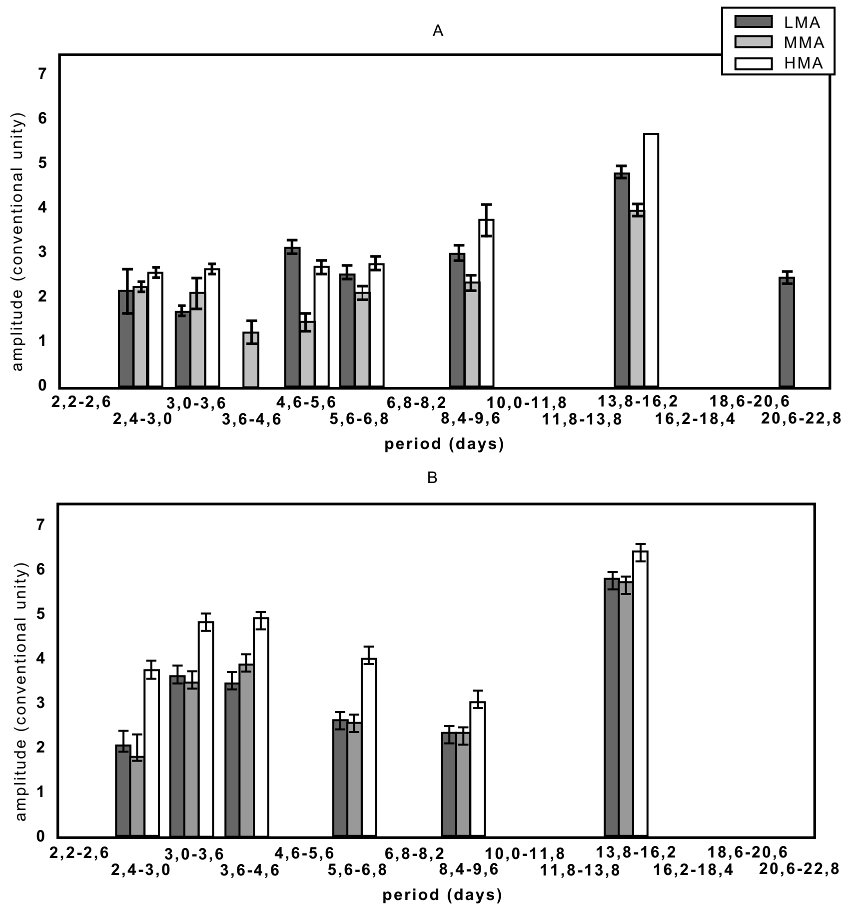

Figure 1. Average α-GPDG activity power spectra of blood lymphocytes in intact rats (A) and rats exposed to ELF VMF (B) exhibiting different mobile activity.

changes were revealed in the phase-amplitude characteristics of CIC PO and CP and the average activity of SDG and α-GPDG. At the same time, in ≈7-day rhythm an obvious increase in CIC AP ($p < 0.05$), in ≈14.0-day period no less obvious decrease in the amplitude of protease activity ($p<0.05$) (Fig. 2) and in ≈22-day period a 178^0 phase shift in the α-GPDG/SDG ($p<0.05$) were registered. Such changes in the rhythms of the studied parameters are not accompanied with either intra- or inter-functional synchronization (Fig. 3, and 6). Thus, the analysis of interphase correlations revealed the decrease in the difference between the phases of the average activity of SDG and α-GPDG, CIC CP and PO, CIC AP and PR, as compared to the data from cosinor-analysis of animals subjected to hypokinesia. However, in all the periods there were stable baseline phase correlations between the rhythms of the studied systems, indicating the increase of synchronization. The general distribution order of the parameters turned out to be clustered and the extent of connections was the same as in the control group (Fig. 4).

Therefore, UHF EMR induced changes in the structure of infradian rhythmicity considerably differ from the changes observed under ELF EMF exposure.

A stress reaction performed by hypokinesia leads to considerable alterations of the infradian activity of all the physiological parameters of the neutrophiles, as expressed by an extensive amplitude changes and the phase shifts, as compared with the control animals (Figs. 2, 5, 6). It was also found that hypokinesia leads to the establishment of new correlations between the functional parameters, as demonstrated by the breakdown of phase correlations between average activities of SDG, α-GPDG, CIC CP and PO, CIC AP and PR in the neutrophils of hypokinetic animals. Moreover, along with the changes in

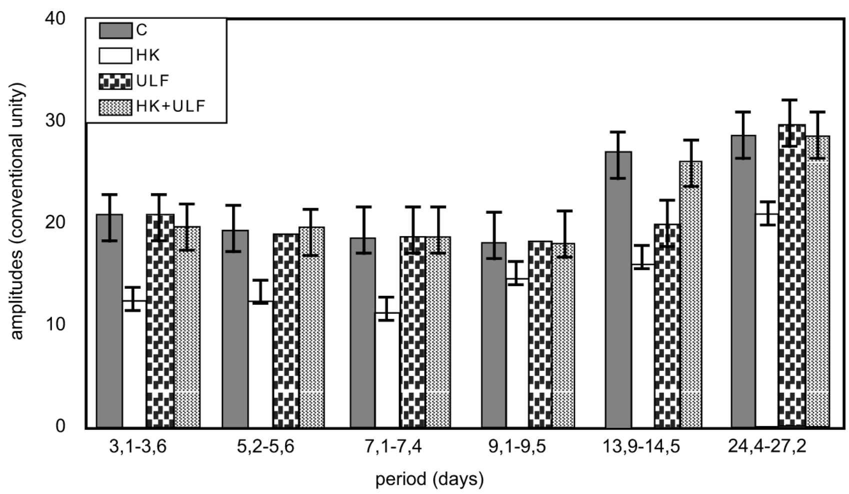

Figure 2. Amplitudes of integral rhythms of rat blood neutrophils protease activity under various conditions. C – control; HK – hypokinesia; ULF – ULF EMR; HK+ULF EMR – hypokinesia combined with ULF EMR.

intrafunctional correlations, cosinor-analysis revealed also a change in inter-functional co-relations (Fig. 3). With the help of cluster analysis (Fig. 4), inter-functional changes were also registered, as expressed by a rearrangement of the studied parameters in neutrophils, increase in the number of clusters (up to 4) and increase in the correlations lengths not only within individual clusters, but also between them. Such rearrangements demonstrate a hypokinesia-induced functional strain exerted upon the studied systems.

Therefore, a hypokinesia-induced stress reaction yields considerable influence on the temporal organization of the studied systems of neutrophils as reflected by a considerable change of amplitudes of the studied rhythms, by the phase shifts in all the identified periods, and by disturbances in the intra- and inter-functional correlations. All of this demonstrates the weakening of the coordination of enzymatic activity [20] and a progressive desynchronosis.

The analysis of results of the action of UHF EMR on the rats subjected to the limitations in mobility revealed considerable changes in the infradian activity

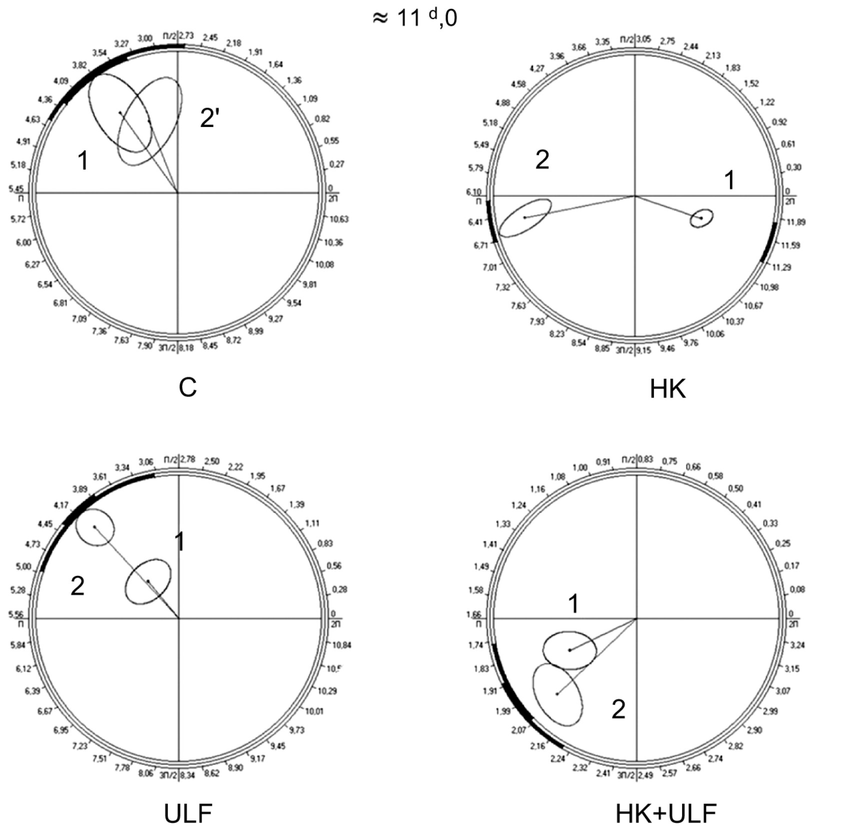

Figure 3. Cosinorograms of integral rhythms of hydrolytic enzyme ratios (CPC AP to CPC PR) and bactericidal enzymes (CPC PO to CPC CP) in blood neutrophiles of control rats (C) and in rats exposed to various stimuli: HK – hypokinesia; ULF – ULF EMR; HK+ULF EMR – hypokinesia combined with ULF EMR в in ≈11d.0 day period, where 1 is co-efficient of CPC AP to CPC PR, and 2 is co-efficient of CPC PO to CPC CP.

of the studied parameters comparing to hypokinetic rats. Thus, the exposure to UHF EMR in animals already subjected to hypokinesia had a reconstructive effect on the amplitudes of the identified rhythms in all the studied parameters, although their values did not always reach the control level (Fig. 2). Moreover, the action of UHF EMR on animals with limited mobility led to a substantial phase shift of the identified rhythms, bringing them closer to the values typical for the control group. Thus, it seems that a reconstruction of baseline phase/amplitude characteristics of hydrolytic, bactericidal and redox systems of neutrophils indicates normalization of the intra- and inter-functional correlations disturbed by hypokinesia (Fig. 2, 3, 5, 6). These data are corroborated by the results of cluster analysis (Fig. 4).

It should be noted that the combined action of UHF EMR and hypokinesia revealed changes in infradian rhythmicity of the studies parameters that were more significant that those produced under the influence of the latter factor alone. This is another proof of the correlation between the efficacy of UHF EMR exposure and the baseline state of an organism.

Thus, under conditions of desynchronosis caused by stress-reaction to limitations in mobility, a daily exposure to UHF EMR produces corrective, synchronizing action, which leads to the normalization of infradian rhythmicity of the studied parameters. These data are in agreement with previous works[21], in which it was shown that UHF EMR assists in the normalization of conditions

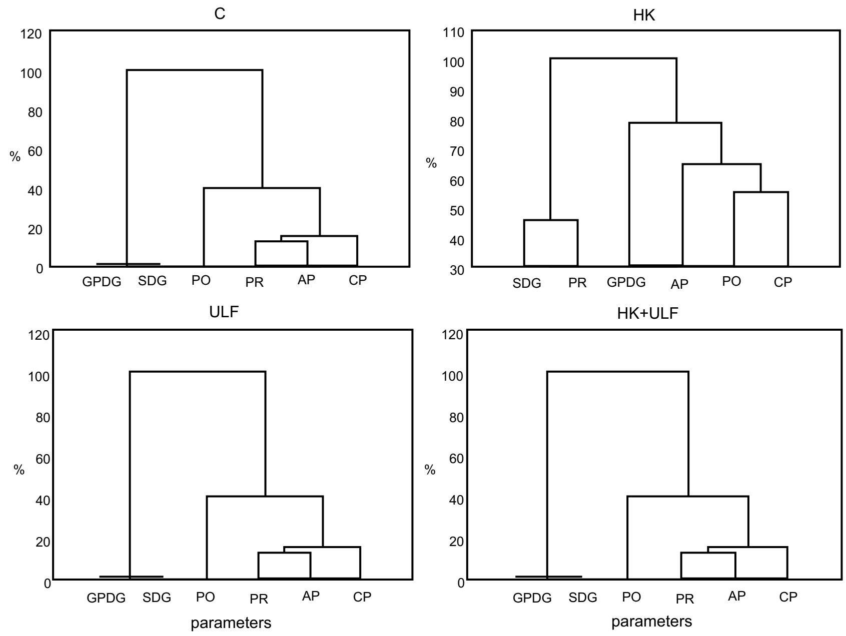

Figure 4. Cluster analysis dendrograms of blood neutrophil activity in control rats (C) and in rats subjected to various stimuli: HK – hypokinesia; ULF – ULF EMR; HK+ULF EMR – hypokinesia combined with ULF EMR, where PO – peroxidase, CP – cation proteins, AP – acid phosphatase, PR – protease, SDG – succinatedehydrogenase, α-GPDG – α-glycerophosphatedehydrogenase in blood neutrophils.

that accompany hypokinetic stress. This also provides the evidence of UHF EMR's ability to synchronize physiological processes, which may be one of the mechanisms of the anti-stress action.

According to the current understanding, a healthy organism maintains strict, but flexible correlation between different processes that comprise homeostasis, whereas various pathological processes reveal some degree of desynchronosis[9, 22]. In order to achieve synchronization of the endogenous processes, a very weak signal would suffice, such as UHF EMR, and this would bring about a "narrowing" of the near frequency[7, 23]. This phenomenon agrees with the concept of the "biological action of microdoses" of various physical and chemical agents[24].

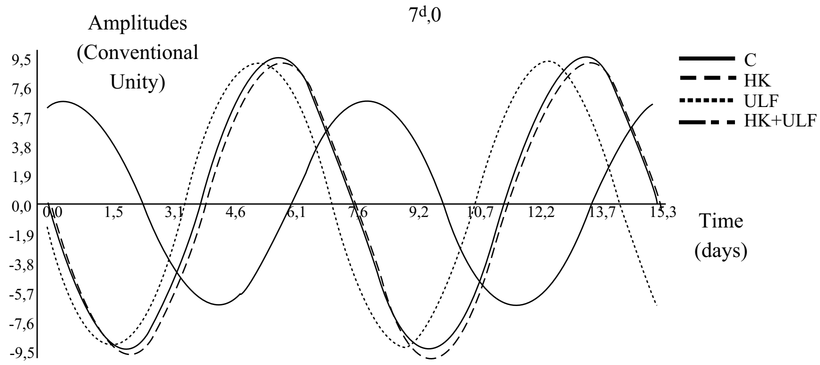

Figure 5. Phase co-relations of CPC PO in blood neutrophils in rats subjected to various stimuli: HK – hypokinesia; ULF – ULF EMR; HK+ULF EMR – hypokinesia combined with ULF EMR in ≈7^d.0 period.

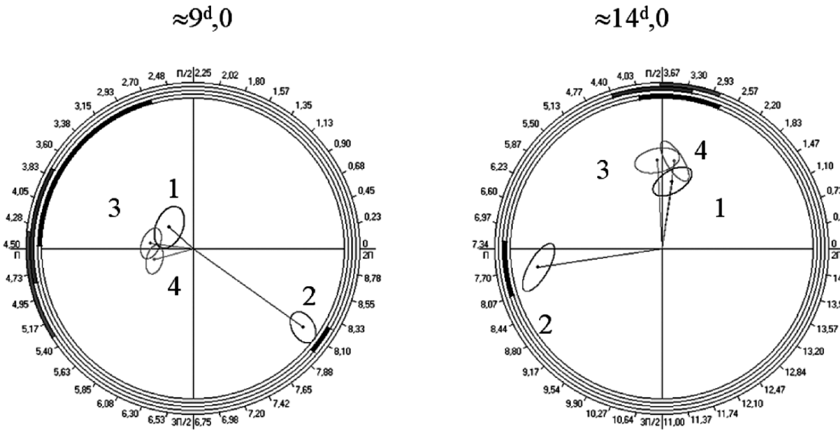

Figure 6. Cosinor.grams of integral rhythms of CPC AP in blood neutrophils in rats subjected to various stimuli: 1 – control (C); 2 – hypokinesia (HK); 3 – exposure to UHF EMR (UHF); 4 – combined action of UHF EMR and hypokinesia (HK+UHF EMR) in ≈9^d.0 and ≈14^d.0 day periods.

Our data allow to conclude that among the mechanisms of biological activity of UHF EMR an important role is played by its ability to modify the temporal organization of physiological systems. It is supposed that APUD-system elements localized in the skin play an important role in the mechanisms of receptivity and response to UHF EMR[25]. APUD-system structures that are located in various organs and tissues of an organism and synthesize biogenic amines and peptide hormones, including melatonin and serotonin[26], may also possess pace-maker properties and can play a role of potentially independent oscillators that are interconnected between themselves and the biosphere. These facts male it possible to use UHF EMR of the above described parameters as an external synchronizer, or a "time gauge", in the different types of desynchronoses.

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