Features of EEG & HRV in 1997 in humans exposed to the factors of the accident at the Chornobyl nuclear power plant in 1986

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Abstract

Background. The dramatic events of the Russian-Ukrainian war around the Chornobyl’ nuclear power plant (NPP) once again opened up the healed wound inflicted by radionuclides on the human body not only in Ukraine, but also in Belarus and the border regions of Russia. The drama of the situation was further intensified by the events surrounding the Zaporizhzhia NPP. This prompted us to turn to the archive of the Truskavetsian Scientific School of Balneology and process the results of research that, due to certain technical and ethical circumstances, remained unanalyzed. Material and methods. The cohort of observation in 1997 were men and women who were exposed to pathogenic factors of the accident at the Chornobyl nuclear power plant during the liquidation of its consequences in 1986. The subject of the study was EEG and HRV parameters. The survey was conducted twice - on admission and after two weeks of rehabilitation at the Truskavets’ Spa. Results. Screening of the registered parameters revealed a pronounced sympathotonic shift of the autonomic balance, as well as a statistically significant deviation from the norm of 18 EEG parameters (increase in 8 and decrease in 10). Under the influence of two-week balneotherapy, 5 increased and 5 decreased EEG parameters normalized, but the remaining abnormal EEG and HRV parameters were resistant to the influence of balneofactors. Conclusion. Neuropsychological disorders, detected even 11 years after the effects of the factors of the Chornobyl’ disaster, differ both in severity and sensitivity to the sanogenic effect of the adaptogenic factors of the Truskavets’ Spa.

Keywords: EEG, HRV, liquidators of the accident at ChNPP, Truskavets’ Spa.
INTRODUCTION

The dramatic events of the Russian-Ukrainian war around the Chornobyl' nuclear power plant (NPP) once again opened up the healed wound inflicted by radionuclides on the human body not only in Ukraine, but also in Belarus and the border regions of Russia [5,8,43]. The drama of the situation was further intensified by the events surrounding the Zaporizhzhia NPP. The reaction of the leading Ukrainian researchers did not take long [15,29]. This prompted us to turn to the archive of the Truskavet Scientific School of Balneology (which at one time successfully dealt with the problems of rehabilitation of accident liquidators and residents of radiation-contaminated territories in the Truskavets’ Spa [1,2,12,23,37,38]) and process the results of research that, due to certain technical and ethical circumstances, remained unanalyzed.

MATERIAL AND METHODS

For the research the database of the Truskavetsian Scientific School of Balneology was used. The cohort of observation in 1997 were 19 men (26±61 years) and 3 women (38, 40 and 47 years) with urate urolithiasis and chronic pyelonephritis who were exposed to pathogenic factors of the accident at the ChNPP during the liquidation of its consequences in 1986. According to the documents, the total effective radiation dose was 10±25 cGy, which is most typical for this contingent [1,8,12,23,.]. The survey was conducted twice: on admission and after two weeks of standard rehabilitation at the Truskavets’ Spa: bioactive water Naftussya by 3 mL/kg for 1 hour before meals three times a day; baths with mineral water (Cl-SO42- Na-Mg2+ containing salt concentration 25 g/L; t° 36-37°C during 8-10 min) [23,38].

Systolic (Ps) and diastolic (Pd) blood pressure was measured by tonometer “Omron M4-I” (Netherlands) in a sitting position.

Then recorded electrocardiogram in II lead to assess the parameters of heart rate variability (HRV) (software and hardware complex "CardioLab+HRV"), "KhAI-MEDICA", Kharkiv, Ukraine). For further analysis the following parameters HRV were selected: the square root of the mean of the sum of the squares of differences between adjacent NN intervals (RMSSD); power spectral density (PSD) of low-frequency (LF, range 0,15-0,04 Hz) and high-frequency (HF, range 0,4-0,15 Hz) bands as well as LFnu=100*LF/(LF+HF) as markers of vagal and sympathetic tones respectively [3,16]. Kerdoe's Vegetative Index was also calculated [11,20].

Simultaneously EEG recorded during 25 sec a hardware-software complex “NeuroCom Standard” (KhAI Medica, Kharkiv) monopolar in 16 loci (Fp1, Fp2, F3, F4, F7, F8, C3, C4, T3, T4, P3, P4, T5, T6, O1, O2) by 10-20 international system, with the reference electrodes A and Ref on the earlobes. Among the options considered the average EEG amplitude (µV), average frequency (Hz), frequency deviation (Hz), index (%), absolute (µV²/Hz) and relative (%) PSD of basic rhythms: β (35±13 Hz), α (13±8 Hz), θ (8±4 Hz) and δ (4±0,5 Hz) in all loci, according to the instructions of the device.

In addition, calculated for HRV and each locus of EEG the Entropy (h) of normalized PSD using Popovych’s IL [14,44] formulas based on classic Shannon’s CE [47] formula: hEEG = - [PSDα•log2PSDα+PSDβ•log2PSDβ+PSD0•log2PSD0+PSDδ•log2PSDδ]/log2 4; hHRV = - [PSDFH•log2PSDFH+PSDLF•log2PSDLF+PSDVLF•log2PSDVLF+PSDULF•log2PSDULF]/log2 4.

Statistical processing performed using a software package “Microsoft Excell” and “Statistica 64 StatSoft Inc”.

Reference values of HRV are taken from the instructions for the device. Since there are no reference values for EEG, we used the database of the Truskavetsian Scientific School of Balneology (the sample contains 112 normal EEGs).
RESULTS AND DISCUSSION

Screening of variables registered upon admission to rehabilitation revealed 21, the levels of which differed significantly or on the border of significance from the reference ones.

In order to make a correct comparison, the individual actual values of the variables (V) were transformed into Z-scores according to the classical formula: \( Z = \frac{V - \mu}{\sigma} \).

For the convenience of further analysis, the variables deviating from the norm were divided into two main clusters, taking into account the nature of the deviation, and in each cluster, in turn, the variables that remained abnormal or normalized after balneotherapy were highlighted.

A noticeable increase in sympathetic tone and relative PSD of the beta rhythm was found in three loci of the scalp, which remained stable during repeated testing. Instead, the initially elevated levels of the beta-rhythm frequency, its absolute PSD in the O2 locus, as well as PSD entropy in three loci after two weeks of exposure to balneofactors did not differ from the reference ones (Table 1 and Fig. 1).

On the other hand, a noticeable stable decrease of two HRV markers of vagal tone was noted, which was accompanied by a stable decrease in the levels of indices of \( \alpha \)-and \( \beta \)-rhythms, relative PSD F7-0 and P4-\( \alpha \) as well as PSD entropy in O2 locus. At the same time, the initially reduced levels of the absolute PSD of \( \beta \)-rhythm in the other three loci, as well as the relative PSD of \( \delta \)-rhythm in O2 locus were completely normalized under the influence of balneofactors (Table 2 and Fig. 2).

Table 1. Increased HRV and EEG variables, and their sensitivity to the influence of balneofactors

<table>
<thead>
<tr>
<th>Variables</th>
<th>Parameters</th>
<th>Pre (22)</th>
<th>Post (22)</th>
<th>Reference Values (112)</th>
<th>Student’s statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>LFnu, %</td>
<td>M±SE</td>
<td>80,6±1,4</td>
<td>77,8±1,8</td>
<td>M±SE 63,2±2,7, 0,230</td>
<td>t 5,85 p 4,59</td>
</tr>
<tr>
<td></td>
<td>Z±SE</td>
<td>+1,20±0,09</td>
<td>+1,00±0,12</td>
<td></td>
<td>&lt;10⁻³ &lt;10⁻³</td>
</tr>
<tr>
<td>PSD O2-( \beta ), %</td>
<td>M±SE</td>
<td>38,8±5,5</td>
<td>35,2±5,3</td>
<td>M±SE 24,4±1,2, 0,531</td>
<td>t 2,57 p 1,98</td>
</tr>
<tr>
<td></td>
<td>Z±SE</td>
<td>+1,11±0,42</td>
<td>+0,83±0,41</td>
<td></td>
<td>&lt;0,05 &lt;0,05</td>
</tr>
<tr>
<td>PSD P4-( \beta ), %</td>
<td>M±SE</td>
<td>31,2±3,5</td>
<td>31,7±3,6</td>
<td>M±SE 22,8±1,1, 0,503</td>
<td>t 2,27 p 2,39</td>
</tr>
<tr>
<td></td>
<td>Z±SE</td>
<td>+0,73±0,31</td>
<td>+0,77±0,31</td>
<td></td>
<td>&lt;0,05 &lt;0,02</td>
</tr>
<tr>
<td>PSD P3-( \beta ), %</td>
<td>M±SE</td>
<td>29,4±3,2</td>
<td>29,9±3,4</td>
<td>M±SE 22,7±1,1, 0,514</td>
<td>t 1,98 p 2,00</td>
</tr>
<tr>
<td></td>
<td>Z±SE</td>
<td>+0,57±0,27</td>
<td>+0,61±0,29</td>
<td></td>
<td>&lt;0,05 &lt;0,05</td>
</tr>
<tr>
<td>PSD O2-( \beta ), ( \mu )V/Hz</td>
<td>M±SE</td>
<td>133±16</td>
<td>106±19</td>
<td>M±SE 90±5, 0,555</td>
<td>t 2,59 p 0,82</td>
</tr>
<tr>
<td></td>
<td>Z±SE</td>
<td>+0,87±0,32</td>
<td>+0,32±0,38</td>
<td></td>
<td>&lt;0,02 &lt;0,2</td>
</tr>
<tr>
<td>Frequency ( \beta ), Hz</td>
<td>M±SE</td>
<td>20,9±0,9</td>
<td>18,7±0,9</td>
<td>M±SE 17,9±0,4, 0,244</td>
<td>t 2,98 p 0,75</td>
</tr>
<tr>
<td></td>
<td>Z±SE</td>
<td>+0,68±0,21</td>
<td>+0,16±0,20</td>
<td></td>
<td>&lt;0,01 &lt;0,2</td>
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<tr>
<td>PSD P3 Entropy</td>
<td>M±SE</td>
<td>0,84±0,020</td>
<td>0,83±0,025</td>
<td>M±SE 0,802±0,013, 0,167</td>
<td>t 1,98 p 1,04</td>
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<td></td>
<td>Z±SE</td>
<td>+0,34±0,15</td>
<td>+0,21±0,19</td>
<td></td>
<td>&lt;0,01 &lt;0,2</td>
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<tr>
<td>PSD F4 Entropy</td>
<td>M±SE</td>
<td>0,89±0,016</td>
<td>0,84±0,019</td>
<td>M±SE 0,851±0,011, 0,139</td>
<td>t 2,00 p -0,48</td>
</tr>
<tr>
<td></td>
<td>Z±SE</td>
<td>+0,33±0,14</td>
<td>-0,09±0,16</td>
<td></td>
<td>&lt;0,05 &lt;0,5</td>
</tr>
<tr>
<td>PSD T5 Entropy</td>
<td>M±SE</td>
<td>0,866±0,017</td>
<td>0,807±0,038</td>
<td>M±SE 0,825±0,012, 0,156</td>
<td>t 1,94 p -0,48</td>
</tr>
<tr>
<td></td>
<td>Z±SE</td>
<td>+0,32±0,13</td>
<td>-0,14±0,30</td>
<td></td>
<td>&gt;0,05 &gt;0,5</td>
</tr>
</tbody>
</table>

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1. Variable: \( \delta \)-rhythm
2. HRV: Heart Rate Variability
3. EEG: Electroencephalography
4. PSD: Power Spectral Density
5. \( \alpha \)-rhythm: Alpha rhythm
6. \( \beta \)-rhythm: Beta rhythm
7. \( \mu \)-V: Microvolts
8. \( \delta \)-rhythm: Delta rhythm
9. \( \mu \)-Hz: Microhertz
10. \( \mu \)-Entrop: Microentropy
11. \( \mu \)-SE: Microstandard error
12. \( \mu \)-Cv: Microcoefficient of variation
13. \( \mu \)-t: Microt-test
14. \( \mu \)-p: Microprobability
15. \( \mu \)-Student’s statistics: MicroStudent's statistics
16. \( \mu \)-F: MicroF-test
17. \( \mu \)-R: Microreadings
18. \( \mu \)-Post/Pre: Micropost/pre
Fig. 1. Patterns of initially increased parameters, resistant (left) and sensitive (right) to the influence of balneofactors. In each pair of columns, the first is before, the second is after balneotherapy

Table 2. Decreased HRV and EEG variables, and their sensitivity to the influence of balneofactors

<table>
<thead>
<tr>
<th>Variables</th>
<th>Parameters</th>
<th>Pre</th>
<th>Post</th>
<th>Reference Values</th>
<th>Student’s statistics</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(22)</td>
<td>(22)</td>
<td>(112)</td>
<td>Pre/R         Post/R</td>
</tr>
<tr>
<td>PSD T4-β, µV²/Hz</td>
<td>M±SE</td>
<td>61±6</td>
<td>86±26</td>
<td>80±6</td>
<td>t -2.09       p &lt;0.05</td>
</tr>
<tr>
<td></td>
<td>Z±SE</td>
<td>-0.29±0.10</td>
<td>+0.11±0.40</td>
<td>0.798</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td>PSD F4-β, µV²/Hz</td>
<td>M±SE</td>
<td>60±8</td>
<td>76±17</td>
<td>83±7</td>
<td>t -2.28       p &lt;0.05</td>
</tr>
<tr>
<td></td>
<td>Z±SE</td>
<td>-0.33±0.11</td>
<td>-0.10±0.23</td>
<td>0.854</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td>PSD C3-β, µV²/Hz</td>
<td>M±SE</td>
<td>71±9</td>
<td>88±20</td>
<td>94±6</td>
<td>t -2.04       p &lt;0.05</td>
</tr>
<tr>
<td></td>
<td>Z±SE</td>
<td>-0.33±0.13</td>
<td>-0.08±0.29</td>
<td>0.733</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td>PSD C4-β, µV²/Hz</td>
<td>M±SE</td>
<td>70±8</td>
<td>87±17</td>
<td>96±6</td>
<td>t -2.63       p &lt;0.02</td>
</tr>
<tr>
<td></td>
<td>Z±SE</td>
<td>-0.39±0.12</td>
<td>-0.14±0.26</td>
<td>0.691</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td>PSD O2-δ, %</td>
<td>M±SE</td>
<td>15.0±2.3</td>
<td>23.2±4.6</td>
<td>22.8±1.6</td>
<td>t -2.81       p &lt;0.01</td>
</tr>
<tr>
<td></td>
<td>Z±SE</td>
<td>-0.47±0.14</td>
<td>+0.02±0.28</td>
<td>0.720</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td>Index β, %</td>
<td>M±SE</td>
<td>88.3±1.9</td>
<td>88.7±4.1</td>
<td>93.9±1.1</td>
<td>t -2.59       p &lt;0.02</td>
</tr>
<tr>
<td></td>
<td>Z±SE</td>
<td>-0.49±0.17</td>
<td>-0.45±0.36</td>
<td>0.121</td>
<td>&gt;0.2</td>
</tr>
<tr>
<td>PSD P4-α, %</td>
<td>M±SE</td>
<td>35.8±3.9</td>
<td>38.0±3.6</td>
<td>44.8±1.8</td>
<td>t -2.04       p &lt;0.05</td>
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<tr>
<td></td>
<td>Z±SE</td>
<td>-0.47±0.21</td>
<td>-0.36±0.19</td>
<td>0.428</td>
<td>&gt;0.1</td>
</tr>
<tr>
<td>PSD F7-θ, %</td>
<td>M±SE</td>
<td>8.1±0.8</td>
<td>8.1±0.7</td>
<td>10.0±0.4</td>
<td>t -2.04       p &lt;0.05</td>
</tr>
<tr>
<td></td>
<td>Z±SE</td>
<td>-0.42±0.17</td>
<td>-0.42±0.16</td>
<td>0.458</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>PSD O2 Entropy</td>
<td>M±SE</td>
<td>0.700±0.025</td>
<td>0.712±0.032</td>
<td>0.776±0.013</td>
<td>t -2.72</td>
</tr>
<tr>
<td></td>
<td>Z±SE</td>
<td>-0.55±0.18</td>
<td>-0.46±0.23</td>
<td>0.178</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Index α, %</td>
<td>M±SE</td>
<td>33±7</td>
<td>39±7</td>
<td>51±3</td>
<td>t -2.48       p &lt;0.02</td>
</tr>
<tr>
<td></td>
<td>Z±SE</td>
<td>-0.65±0.24</td>
<td>-0.43±0.25</td>
<td>0.560</td>
<td>&gt;0.1</td>
</tr>
<tr>
<td>PSD HF, msec²</td>
<td>M±SE</td>
<td>102±29</td>
<td>117±36</td>
<td>446±62</td>
<td>t -5.07       p &lt;10⁻³</td>
</tr>
<tr>
<td></td>
<td>Z±SE</td>
<td>-0.99±0.07</td>
<td>-0.99±0.10</td>
<td>0.754</td>
<td>&lt;10⁻³</td>
</tr>
<tr>
<td>RMSSD, msec</td>
<td>M±SE</td>
<td>14.7±1.8</td>
<td>14.6±1.7</td>
<td>33.4±2.5</td>
<td>t -5.59       p &lt;10⁻³</td>
</tr>
<tr>
<td></td>
<td>Z±SE</td>
<td>-1.16±0.12</td>
<td>-1.20±0.10</td>
<td>0.472</td>
<td>&lt;10⁻³</td>
</tr>
</tbody>
</table>
Fig. 2. Patterns of initially decreased parameters, sensitive (left) and resistant (right) to the influence of balneofactors. In each pair of columns, the first is before, the second is after balneotherapy

Placing the profiles of all four clusters on a common plane gives the impression that the EEG parameters, which, as a rule, were minimally deviated from the norm for the sample, were susceptible to balneotherapy, while more noticeable pathological manifestations are not object to the favorable influence of balneofactors (Fig. 3).

The identified regularity is more clearly manifested after calculating the average values (Fig. 4). So, the normalization of neurophysiological parameters under the influence of balneofactors of Truskavets’ Spa occurs according to the good old Wilder's JF law of initial level [49]. By the way, back in 1993 we discovered that according to this law parameters of water-salt exchange react to the use of Naftussya bioactive water [4].

It is known that the law of the initial level is valid only with sufficient capacity of regulatory (obviously, neuro-endocrine-immune) mechanisms, which is preserved in functional pathological conditions. The second requirement is a sufficient strength of the sanogenic/adaptogenic factor(s) [2,4,13,23,35,36,41,42].

Fig. 3. Profiles of Z-scores (Z±SE) of EEGs and HRVs variables, resistant (extreme) or sensitive (intermediates) to the influence of balneofactors. In each pair of columns, the first is before, the second is after balneotherapy
Regarding our cohort, two interpretations are possible. Either the stability of the pathological deviations of 11 HRV&EEG parameters is evidence of their organic (morphological) nature, or the sanogenic (normalizing) effect of the applied balneofactors is not strong enough and is manifested only in relation to less pronounced functional deviations of the other 10 neurophysiological parameters.

The results of model experiments on animals and clinical observations indicate that post-radiation encephalopathy has both morphological and functional components [5,6,8,15,21,24-29,31-34,43,46].

On the other hand, research in this direction of the Truskavetsian Scientific School of Balneology showed that the sanogenic influence of the resort's adaptogenic balneofactors on the parameters of the autonomic nervous system, immunity, hemostasis, erythron and metabolism of rats poisoned with $^{137}$Cs, residents of radiation-contaminated territories and liquidators, although pronounced, but not completely normalizing, and requires additional use of phytoadaptogens and pharmacons [1,2,23,38,42].

One of the probable mechanisms of insensitivity/resistance of sympatho-vagal imbalance to the sanogenic effect of balneofactors is a violation of the regulation of the autonomic nervous system by the central part. In this cohort, only two pairs of connections were found, moreover, at the limit of significance (Figs. 5 and 6), while in healthy volunteers [39] and even similar urological patients without radiation exposure [40,36,42], the connections between EEG and HRV parameters are more numerous and stronger.
It is time to move on to the probable mechanism of the neurotropic effect of balneofactors. The main therapeutic factor of the Truskavets’ Spa is Naftussya bioactive water. Unlike classic mineral waters, its physiological and therapeutic activity is determined by organic substances and autochthonous microbes [17,19,35,36]. Approximately 2/3 of the mass of organic substances in Naftussya water is leached from water-bearing petroleum rock, which is reflected in its name (naphta/ναφθα means petroleum in Greek), and 1/3 are the products of their biotransformation by hydrocarbon-oxidizing, sulfate-reducing and thione microbes [9,17,19]. Another source of organic substances, in particular phenols, are fallen leaves on the surface of the deposit [18].
At least some of the organic substances (alkylbenzene, alkenylbenzene, alkynaphthalenes, alkyl phenols, esters of aromatic acids, polyaromatic hydrocarbons) are, obviously, agonists of aryl hydrocarbon receptors (AhR), which are expressed by almost all types of cells of living organisms, starting from unicellular. The activation of AhR by endogenous and environmental factors has important physiologic effects, including the regulation of the endocrine and immune response [10,30].

Kimura E & Töhyama C [22] shown that the mRNA of AhR was expressed in the hippocampus, cerebral cortex, cerebellum, olfactory bulb of the mouse brain. Although AhR expression decreases from the embryonic period into adult life, several physiological functions remain in the adult brain, which include the regulation of neurotransmitter levels, blood-brain barrier functions, and immune responses [7,48]. Therefore, the ability of Naftussya water organic substances to directly affect CNS neurons via AhR is very real. Another possible mechanism of effect of Naftussya water on the brain is AhR irritation of afferent terminals n. vagus in the intestinal mucosa. By the way, similar AhR agonists are also present in mineral water for baths, so it is likely that they irritate the receptors of the afferent nerves of the skin (more about this in the review: Popovych IL [36]).

Therefore, both the chemical factors of mineral bath applied to the skin and the chemical as well as bacterial (antigenic) factors of Naftussya bioactive water applied to the mucosa of the digestive tract eventually change the activity of the nervous structures, which, in turn, modulate the state of the endocrine and immune systems and metabolism [36,42].

In conclusion - about priorities. In previous studies, EEG assessment was only descriptive [21,25,32,33]. We are aware of only one cross-sectional quantitative electroencephalogram study (1996–2001) among Chornobyl accident survivors [26]. However, despite the high methodological level, the study concerns survivors, who had confirmed acute radiation sickness and were irradiated in dose of 1−5 Gy, while the cohort analyzed by us received a much smaller (10−25 cGy), but the most characteristic dose. The authors revealed the neurophysiological markers of ionizing radiation: left fronto-temporal dominant frequency reduction; absolute δ-power lateralization to the left (dominant) hemisphere; relative δ-power increase in the fronto-temporal areas; absolute θ-power decrease in the left temporal region; absolute and relative α-power diffusive decrease, which may reflect cortico-limbic dysfunction lateralized to the left, dominant hemisphere, with the fronto-temporal cortical and hippocampal damage. Quantitative electroencephalogram proposed for differentiation of radiation and nonradiation brain damages and as a new biological dosimetry method.

Our data are consistent only with respect to a decrease in relative α-power, but not a diffuse one, only in the right parietal locus. However, judging by the scheme of Winkelmann T et al. [50], we found a decrease in the electrical activity of β-rhythm-generating neurons of right caudal anterior cingulate cortex (F4), right (C4) and left (C3) precentral gyrus, right superior temporal gyrus (T4) as well as δ-rhythm-generating neurons of right lingual gyrus (O2) and left superior frontal gyrus (F7) and α-rhythm-generating neurons of right supramarginal gyrus (P4), the thickness of which are positively correlated (r=0.43±0.55) with vagally mediated HRV (HF band), which is consistent with a decrease in vagal tone in our cohort. At the same time, the activity of β-rhythm-generating neurons of right supramarginal gyrus (P4) and lingual gyrus (O2) turned out to be increased, which apparently led to an increase in sympathetic tone.

Instead of a conclusion, we note that both the deviation and the reaction to balneofactors of nervous structures were related to the state and dynamics of the parameters of immunity, hemostasis, erythron and metabolism, which will be the subject of subsequent publications.
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ACCORDANCE TO ETHICS STANDARDS

Tests in patients are carried out conducted in accordance with positions of Helsinki Declaration 1975. During realization of tests from all participants the informed consent is got and used all measures for providing of anonymity of participants.

REFERENCES

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