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INTERRELATIONS BETWEEN CHANGES IN PARAMETERS OF HRV, EEG AND CELLULAR IMMUNITY AT PATIENTS WITH CHRONIC PYELONEPHRITIS AND CHOLECYSTITIS

Igor L Popovych¹, Andriy B Kul'chyns'kyi^{2,3}, Tetyana A Korolyshyn¹, Walery Zukow⁴

¹Department of Immunophysiology, OO Bohomolets' Institute of Physiology,
Kyiv, Ukraine i.popovych@biph.kiev.ua

²Communal Hospital, Truskavets', Ukraine akul@i.ua

³Ukrainian Scientific Research Institute of Transport Medicine Ministry of Health,
Odesa

⁴Department of Spatial Management and Tourism, Faculty of Earth Sciences, Nicolaus
Copernicus University, Torun, Poland w.zukow@wp.pl

Abstract

Background. Previously we have shown within the immunological homunculus conception that take place causal relationships between induced by balneotherapy changes in parameters of EEG and HRV, on the one hand, and the parameters of **humoral** immunity, on the other hand. The purpose of this study is to find out the relationships between induced by balneotherapy changes in parameters of EEG and HRV, on the one hand, and the parameters of **cellular** immunity, on the other hand. **Methods.** In basal conditions in 33 men and 10 women with chronic pyelonephritis and cholecystitis in remission, we recorded twice, before and after balneotherapy at the spa Truskavets', EEG ("NeuroCom Standard") and HRV ("Cardiolab+VSR"). In blood estimated routine parameters of cellular immunity. **Results.** We detected that changes in relative level CD4⁺ T helper lymphocytes upregulated by parasympathetic outflows and β -rhythm generating structures that are projected onto the loci T3, T4 and P4, while downregulated by sympathetic outflows and β -rhythm generating structures that are projected onto the locus F7 as well as α -rhythm generating structures onto T6 and P4 loci. Level of CD8⁺ T cytolytic lymphocytes vice versa downregulated by parasympathetic outflows as well as δ -rhythm generating structures onto locus F7 and θ -rhythm generating structures onto the locus T3, while upregulated by θ -rhythm generating structures onto the locus T4. Level of CD16⁺ natural killers upregulated by sympathetic outflows and β -rhythm generating structures that are projected onto the loci T4, T5 and C3 as well as α -rhythm generating structures onto the locus T4, while downregulated by parasympathetic

outflows and β -rhythm generating structures that are projected onto the loci Fp1 and F4 as well as α -rhythm generating structures onto the loci P4, O1 and O2, θ -rhythm generating structures onto the loci F7, T5, T6 and O2 as well as δ -rhythm generating structures that are projected onto the loci F7, T5 and T6. Level of 0-lymphocytes upregulated by sympathetic outflows and δ -rhythm generating structures that are projected onto the loci F7 and T5 as well as α -rhythm generating structures onto the loci P4 and O2, while downregulated by β -rhythm generating structures that are projected onto the loci T5 and O1. **Conclusion.** The results are supplemented and refined Tracey's immunological homunculus conception.

Keywords: HRV, EEG, CD4⁺, CD8⁺, CD16⁺ Lymphocytes, correlations.

INRODUCTION

Previously, we have shown within the immunological homunculus conception [16] that take place causal relationships between induced by balneotherapy changes in parameters of EEG and HRV, on the one hand, and the parameters of **humoral** immunity, on the other hand. We detected that changes in relative level CD22⁺ B-Lymphocytes upregulated by parasympathetic outflows and β -rhythm generating structures that are projected onto the loci O2, Fp1, F3 and F4, while downregulated by sympathetic outflows and θ -rhythm generating structures that are projected onto the locus T5. Serum level of IgA upregulated by parasympathetic outflows and α -rhythm generating structures that are projected onto the loci T3 and C3 as well as δ -rhythm generating structures onto loci F7 and T5, while downregulated by sympathetic outflows and θ -rhythm generating structures that are projected onto the loci T5, F7, Fp2, F3, F8 and F4. Serum level of CIC upregulated by parasympathetic outflows and α -rhythm generating structures that are projected onto the loci T4, Fp2, T3, F8 and C3 as well as θ -rhythm generating structures onto the loci T3 and T6, while downregulated by sympathetic outflows and β -rhythm generating structures that are projected onto the loci Fp1, F3 and P4 as well as δ -rhythm generating structures onto T4. Unlike the previous parameters serum level of IgM downregulated by parasympathetic outflows and α -rhythm generating structures that are projected onto the loci T3 and T6 as well as β -rhythm generating structures onto the locus T5, while upregulated by sympathetic outflows and θ -rhythm generating structures that are projected onto the loci F3, Fp2, T5, F4, O1 and F8. Serum level of IgG downregulated by parasympathetic outflows, while upregulated by sympathetic outflows and δ -rhythm generating structures that are projected onto the loci O2 and F8 as well as α -rhythm generating structures onto C3 [9]. The purpose of this study is to find out the relationships between induced by balneotherapy changes in parameters of EEG and HRV, on the one hand, and the parameters of **cellular** immunity, on the other hand.

MATERIAL AND METHODS

The object of observation were 33 men and 10 women aged 24-70 years old, who came to the spa Truskavets' (Ukraine) for the treatment of chronic pyelonephritis combined with cholecystitis in remission. The survey was conducted twice, before and after balneotherapy.

We recorded electrocardiogram in II lead to assess the parameters of HRV [1-3] (software and hardware complex "CardioLab+HRV" production "KhAI-MEDICA", Kharkiv, Ukraine). For further analysis the following parameters heart rate variability (HRV) were selected. Temporal parameters (Time Domain Methods): the standart deviation of all NN intervals (SDNN), coefficient of variation (Cv), the square root of the mean of the sum of the squares of differences between adjacent NN intervals (RMSSD), the percent of interval differences of successive NN intervals greater then 50 ms (pNN₅₀); heart rate (HR), the moda (Mo), the amplitude of moda

(AMo), variational sweep (MxDMn) as well as triangular index (TI). Spectral parameters (Frequency Domain Methods): spectral power density (SPD) bands of HRV - high-frequency (HF, range 0,4÷0,15 Hz), low-frequency (LF, range 0,15÷0,04 Hz), very low-frequency (VLF, range 0,04÷0,015 Hz) and ultra low-frequency (ULF, range 0,015÷0,003 Hz). Expectant as classical indexes: LF/HF, $LF_{nu}=100\% \cdot LF/(LF+HF)$ and Baevskiy's Stress Index (BSI= $AMo/2 \cdot Mo \cdot MxDMn$) as well as Baevskiy's Activity Regulatory Systems (BARS) [1].

Then EEG recorded a hardware-software complex "NeuroCom Standard" (KhAI Medica, Kharkiv, Ukraine) 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 tassels on the ears. Among the options considered the average EEG amplitude (μV), average frequency (Hz), frequency deviation (Hz), index (%), coefficient of asymmetry (%) as well as absolute ($\mu V^2/Hz$) and relative (%) SPD 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 Laterality Index (LI) for SPD each Rhythm using formula [11]:

$$LI, \% = \Sigma [200 \cdot (\text{Right} - \text{Left}) / (\text{Right} + \text{Left})] / 8$$

We calculated also for HRV and each locus EEG the Entropy (h) of normalized SPD using formula C Shannon [cit. by: 12]:

$$hHRV = - [SPD_{HF} \cdot \log_2 SPD_{HF} + SPD_{LF} \cdot \log_2 SPD_{LF} + SPD_{VLF} \cdot \log_2 SPD_{VLF} + SPD_{ULF} \cdot \log_2 SPD_{ULF}] / \log_2 4$$

$$hEEG = - [SPD_{\alpha} \cdot \log_2 SPD_{\alpha} + SPD_{\beta} \cdot \log_2 SPD_{\beta} + SPD_{\theta} \cdot \log_2 SPD_{\theta} + SPD_{\delta} \cdot \log_2 SPD_{\delta}] / \log_2 4$$

For phenotyping subpopulations of lymphocytes used the methods of rosette formation with sheep erythrocytes on which adsorbed monoclonal antibodies against receptors CD3, CD4, CD8 and CD16 from company "Granum" (Kharkiv) with visualization under light microscope with immersion system. Subpopulation of T cells with receptors high affinity determined by test of "active" rosette formation [10].

Results processed by methods of correlation and canonical analyses, using the software package "Statistica 5.5".

RESULTS AND DISCUSSION

As can be seen from Table 1, the canonical root of nerve regulation receives predominantly **negative** factor loadings that reflect the **stimulating** effect on $CD3^+CD4^+$ T helper Lymphocytes blood level by the side of HRV markers of vagal tone as well as β -rhythm generating structures that are projected onto the both right and left temporalis anterior loci (amygdala? [15]) and right parietalis locus. **Positive** factor loadings reflect the **inhibiting** effect by the side of HRV markers of sympathetic tone and β -rhythm generating structures that are projected onto the left frontalis lateralis locus as well as α -rhythm generating structures that are projected onto the right temporalis posterior and parietalis loci. In addition, inhibiting effect makes right-side Lateralization of θ -rhythm.

Table 1. Factor structure of canonical Roots representing Neural parameters and CD3⁺CD4⁺ T helper Lymphocytes blood level

Right set	R
VLF HRV SPD, ms ²	-,509
RMSSD HRV, ms	-,478
SDNN HRV, ms	-,386
T4-β SPD, %	-,419
T3-β SPD, μV ² /Hz	-,417
T4-β SPD, μV ² /Hz	-,372
P4-β SPD, %	-,402
Bayevskiy Stress Index, ln	,384
AMo HRV, %	,343
HR, beats/min	,372
F7-β SPD, μV ² /Hz	,407
P4-α SPD, μV ² /Hz	,391
T6-α SPD, μV ² /Hz	,364
θ-rhythm Laterality, %	,344
Left set	R
CD4 ⁺ Th	-1

Judging by the coefficient of canonical correlation, changes in the nervous regulation determine changes in the level of T helper lymphocytes by 64,5% (Fig. 1).

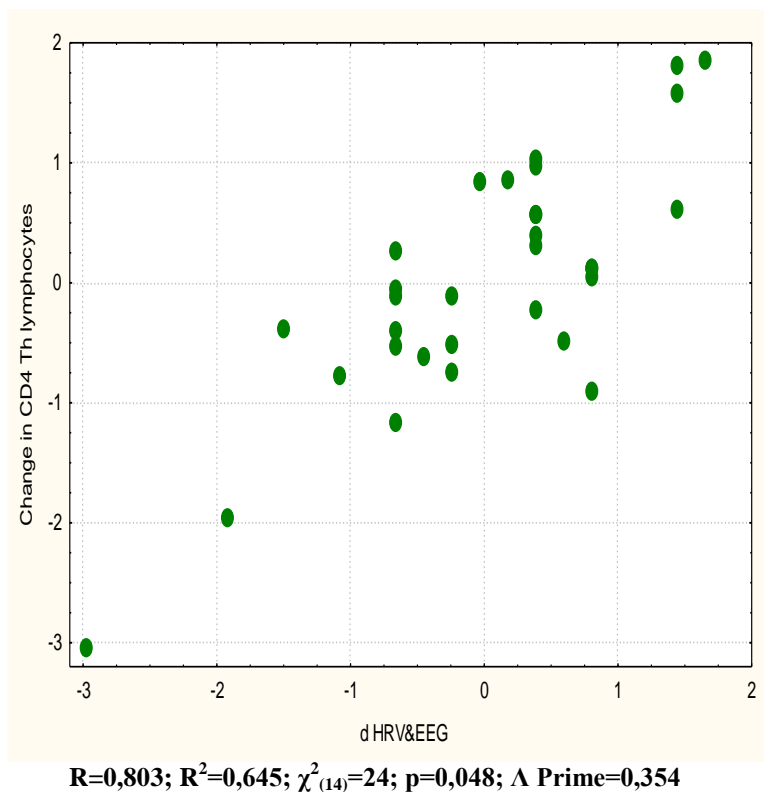


Figure 1. Canonical correlation between changes in parameters of HRV and EEG (line X) and blood CD3⁺CD4⁺ T helper lymphocytes level (line Y)

Among mentioned parameters HRV and EEG a regression model with stepwise exclusion included the following 8 only (Table 2).

Table 2. Regression Summary for Dependent Variable: change in CD3⁺CD4⁺ T helper Lymphocytes

R=0,782; R²=0,612; Adjusted R²=0,477; F_(8,2)=4,5; p=0,002; SE of estimate: 3,5 %

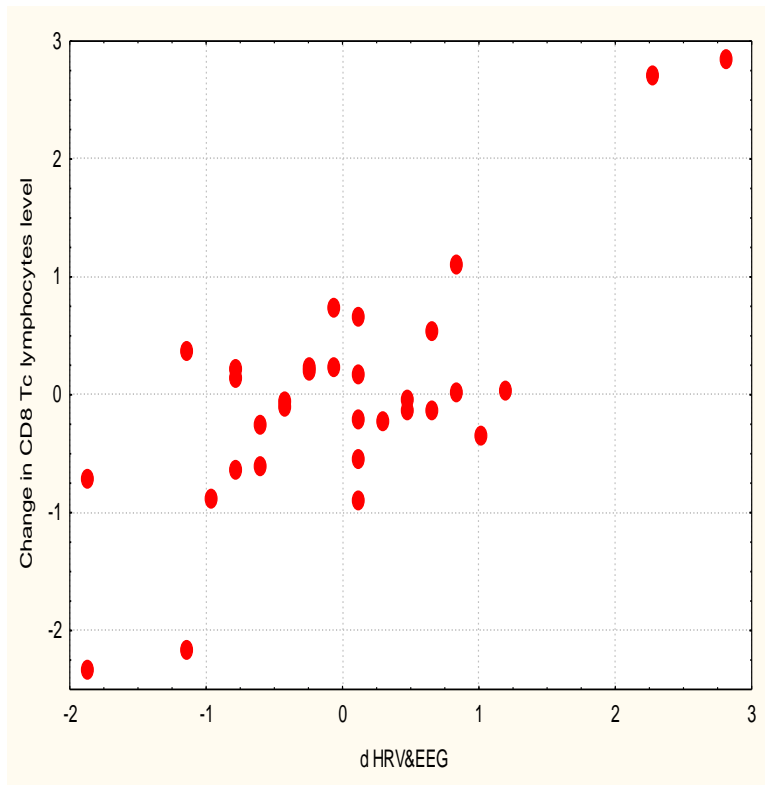
		Beta	St. Err. of Beta	B	St. Err. of B	n=32 t ₍₂₃₎	p-level
Change in Variables	r		Intercept	2,104	,7406	2,84	,009
VLF HRV SPD, ms ²	0,41	,634	,303	,0030	,0014	2,09	,048
RMSSD HRV, ms	0,38	,519	,250	,2400	,1155	2,08	,049
SDNN HRV, ms	0,31	-,939	,369	-,2805	,1102	-2,55	,018
T4-β SPD, %	0,34	,238	,149	,0635	,0396	1,60	,123
T4-β SPD, μV ² /Hz	0,30	,433	,134	,0326	,0101	3,22	,004
F7-β SPD, μV ² /Hz	-0,33	-,236	,147	-,0261	,0162	-1,61	,121
P4-α SPD, μV ² /Hz	-0,31	-,237	,144	-,0059	,0036	-1,65	,113
HR, beats/min	-0,30	-,282	,152	-,2081	,1121	-1,86	,076

Level of CD3⁺CD8⁺ T cytolytic lymphocytes vice versa **downregulated** by parasympathetic outflows as well as δ-rhythm generating structures onto left lateralis frontalis locus and θ-rhythm generating structures onto the left temporalis anterior locus, while **upregulated** by θ-rhythm generating structures onto the right temporalis anterior locus. In addition, enhancing effect makes right-side Lateralization of θ-rhythm. The impression that the left amygdala through the parasympathetic mechanisms reduces the level of T cytolytic lymphocytes, while the right through sympathetic mechanisms increases them.

Table 3. Factor structure of canonical Roots representing Neural parameters and CD3⁺CD8⁺ T helper Lymphocytes blood level

Right set	R
VLF HRV SPD, ms ²	,624
RMSSD HRV, ms	,519
SDNN HRV, ms	,469
HRV TI, units	,431
LF HRV SPD, ms ²	,397
T3-θ SPD, %	,501
F7-δ SPD, μV ² /Hz	,409
α-rhythm Index, %	,379
P4-α SPD, %	,373
T4-θ SPD, %	-,424
θ-rhythm Laterality, %	-,379
Left set	R
CD8 ⁺ Tc	-1

In general changes in the nervous regulation determine changes in the level of T cytolytic lymphocytes by 56,5% (Fig. 2).



$R=0,752$; $R^2=0,565$; $\chi^2_{(11)}=20$; $p=0,040$; $\Lambda \text{ Prime}=0,435$

Figure 2. Canonical correlation between changes in parameters of HRV and EEG (line X) and blood CD3⁺CD8⁺ T cytolytic lymphocytes level (line Y)

Among mentioned parameters HRV and EEG a regression model with stepwise exclusion included the following 4 only (Table 4).

Table 4. Regression Summary for Dependent Variable: change in CD3⁺CD8⁺ T cytolytic Lymphocytes

$R=0,684$; $R^2=0,468$; Adjusted $R^2=0,390$; $F_{(4,3)}=5,9$; $p=0,0014$; SE of estimate: 4,4%

		Beta	St. Err. of Beta	B	St. Err. of B	n=32 $t_{(27)}$	p-level
Change in Variables	r		Intercept	-,0445	,8820	-,05	,960
VLF HRV SPD, ms ²	-0,47	-,407	,145	-,0022	,0008	-2,81	,009
T3- θ SPD, %	-0,38	-,257	,145	-,2924	,1642	-1,78	,086
T4- θ SPD, %	0,32	,263	,141	,1967	,1056	1,86	,073
F7- δ SPD, $\mu V^2/Hz$	-0,31	-,315	,141	-,0018	,0008	-2,24	,033

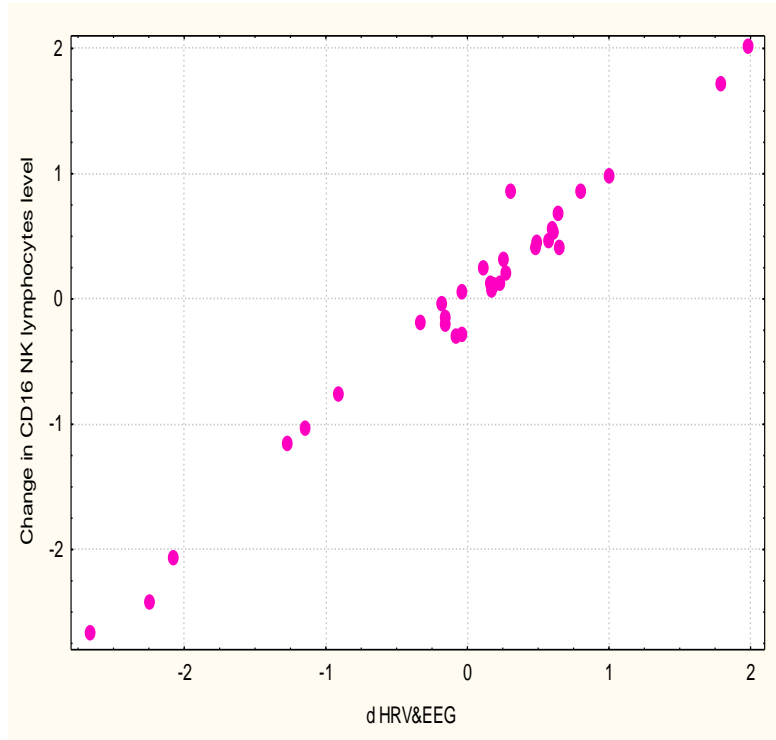
The level of natural killers, as well as T-killers, also **decreases** under the influence of vagal mechanisms and **increases** under the sympathetic impulses (Table 5).

Table 5. Factor structure of canonical Roots representing Neural parameters and CD16⁺ Natural Killer Lymphocytes

Right set	R
VLF HRV SPD, %	,352
O2- θ SPD, $\mu V^2/Hz$,592
O2- θ SPD, %	,384
O2- α SPD, $\mu V^2/Hz$,533
O1- α SPD, $\mu V^2/Hz$,376
T6- θ SPD, %	,402
T6- θ SPD, $\mu V^2/Hz$,353
T6- δ SPD, $\mu V^2/Hz$,377
T5- θ SPD, $\mu V^2/Hz$,371
T5- δ SPD, $\mu V^2/Hz$,404
T4- δ SPD, %	,368
P4- α SPD, $\mu V^2/Hz$,428
F7- θ SPD, $\mu V^2/Hz$,388
F7- δ SPD, $\mu V^2/Hz$,389
Fp1- β SPD, $\mu V^2/Hz$,377
F4- β SPD, $\mu V^2/Hz$,325
α -rhythm Amplitude, μV	,373
β -rhythm Index, %	,335
LF HRV, nu	-,357
ULF HRV PSD, %	-,362
T4- α SPD, %	-,354
T5- β SPD, %	-,331
T4- β SPD, %	-,317
C3- β SPD, %	-,299
β -rhythm Deviation, Hz	-,356
θ -rhythm Asymmetry, %	-,437
F7 Entropy	-,324
Left set	R
CD16	-1

A **common** sources of **inhibitory** influences are **also** the δ -rhythm generating structures that are projected onto the left frontalis lateralis locus as well as α -rhythm generating structures that are projected onto the right parietalis locus. However, the **additional** sources of impulses to reduce the level of natural killers are structures that are projected onto the right and left occipitalis and temporalis posterior, right parietalis and frontalis medialis as well as left frontalis anterior and lateralis loci generating θ -, α - and δ -rhythms. A **common** source of **enhancing** influences on both types of killers are structures of right amygdala, but, in relation to natural killers, they are associated with β - and α -rhythms, whereas an increase in T-killers level is realized through θ -rhythms. The **additional** sources of impulses to **increase** the level of natural killers are β -rhythm generating structures that are projected onto the left centralis and temporalis posterior loci. In addition, **increase** in level of natural killers are associated with Deviation of β -rhythm, Asymmetry of α -rhythm and Entropy of rhythms in left frontalis lateralis locus.

In general changes in the nervous regulation determine changes in the level of CD16⁺ lymphocytes by 97,9% (Fig. 3).



$R=0,989$; $R^2=0,979$; $\chi^2_{(27)}=63$; $p<10^{-4}$; Λ Prime=0,021

Figure 3. Canonical correlation between changes in parameters of HRV and EEG (line X) and blood CD16⁺ Natural Killer lymphocytes level (line Y)

Among mentioned parameters HRV and EEG a regression model with stepwise exclusion included the following 18 (Table 6).

Table 6. Regression Summary for Dependent Variable: change in CD16⁺ Natural Killer Lymphocytes
R=0,967; R²=0,936; Adjusted R²=0,848; F₍₁₈₎=10,6; p<10⁻⁴; SE of estimate: 1,3%

		Beta	St. Err. of Beta	B	St. Err. of B	n=32 t ₍₁₃₎	p-level
Change in Variables	r		Intercept	1,128	,4094	2,76	,016
O2-θ SPD, μV ² /Hz	-0,59	,577	,210	,0275	,0100	2,74	,017
O2-α SPD, μV ² /Hz	-0,53	-,695	,127	-,0120	,0022	-5,47	,0001
P4-α SPD, μV ² /Hz	-0,42	,540	,152	,0092	,0026	3,55	,004
T5-δ SPD, μV ² /Hz	-0,40	-3,049	,564	-,0086	,0016	-5,41	,0001
F7-δ SPD, μV ² /Hz	-0,39	2,256	,520	,0077	,0018	4,34	,0008
O2-θ SPD, %	-0,38	-,327	,122	-,2250	,0837	-2,69	,019
Fp1-β SPD, μV ² /Hz	-0,37	,550	,196	,0522	,0186	2,81	,015
T5-θ SPD, μV ² /Hz	-0,37	-,822	,180	-,0542	,0119	-4,57	,0005
T6-δ SPD, μV ² /Hz	-0,37	1,424	,277	,0093	,0018	5,14	,0002
T4-δ SPD, %	-0,36	-,932	,221	-,1209	,0287	-4,21	,0010
F4-β SPD, μV ² /Hz	-0,32	-,585	,187	-,0557	,0178	-3,12	,008
LF HRV, nu	0,35	,254	,116	,0691	,0315	2,19	,047
θ-rhythm Asymmetry, %	0,43	,398	,108	,0424	,0115	3,69	,003
β-rhythm Deviation, Hz	0,35	,558	,129	1,799	,4158	4,33	,0008
T5-β SPD, %	0,33	-,604	,154	-,1008	,0257	-3,92	,002
F7 Entropy	0,32	,770	,150	12,47	2,43	5,13	,0002
T4-β SPD, %	0,31	-,495	,197	-,0903	,0358	-2,52	,026
C3-β SPD, %	0,30	,737	,161	,1818	,0396	4,59	,0005

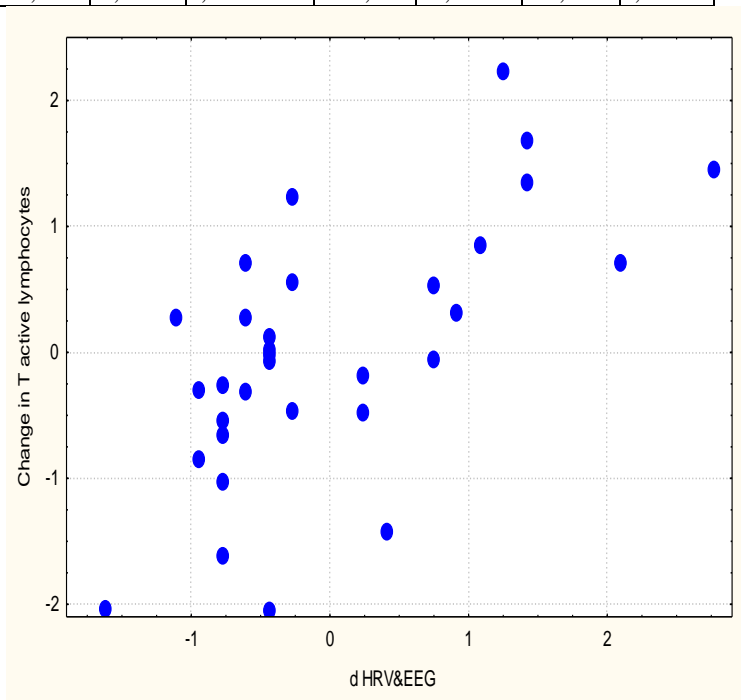
The level of T active Lymphocytes also **decreases** under the vagal influence while **increases** under the sympathetic influence, however, the measure of neurogenic determination is much weaker (Tables 7 and 8) and is only 42,1% (Fig. 4).

Table 7. Factor structure of canonical Roots representing Neural parameters and T active Lymphocytes blood level

Right set	R
HRV TI, units	,691
VLF HRV SPD, ms ²	,585
T5 Entropy	,597
AMo/Mo, units	-,642
AMo HRV, %	-,593
HF HRV SPD, %	-,572
O2-δ SPD, μV ² /Hz	-,518
Left set	R
T active Lymphocytes	-1

Table 8. Regression Summary for Dependent Variable: change in T active Lymphocytes
R=0,630; R²=0,397; Adjusted R²=0,332; F_(3,3)=6,1; p=0,002; SE of estimate: 4,9 %

		Beta	St. Err. of Beta	B	St. Err. of B	n=32 t ₍₂₈₎	p-level
Change in Variables	r		Intercept	,303	,891	,34	,737
HRV TI, units	-0,45	-,301	,159	-,547	,288	-1,90	,068
HF HRV SPD, %	0,37	,302	,159	,223	,117	1,90	,067
T5 Entropy	-0,39	-,386	,149	-10,59	4,10	-2,59	,015



R=0,649; R²=0,421; $\chi^2_{(7)}$ =14,5; p=0,043; Λ Prime=0,579

Figure 4. Canonical correlation between changes in parameters of HRV and EEG (line X) and blood T active lymphocytes level (line Y)

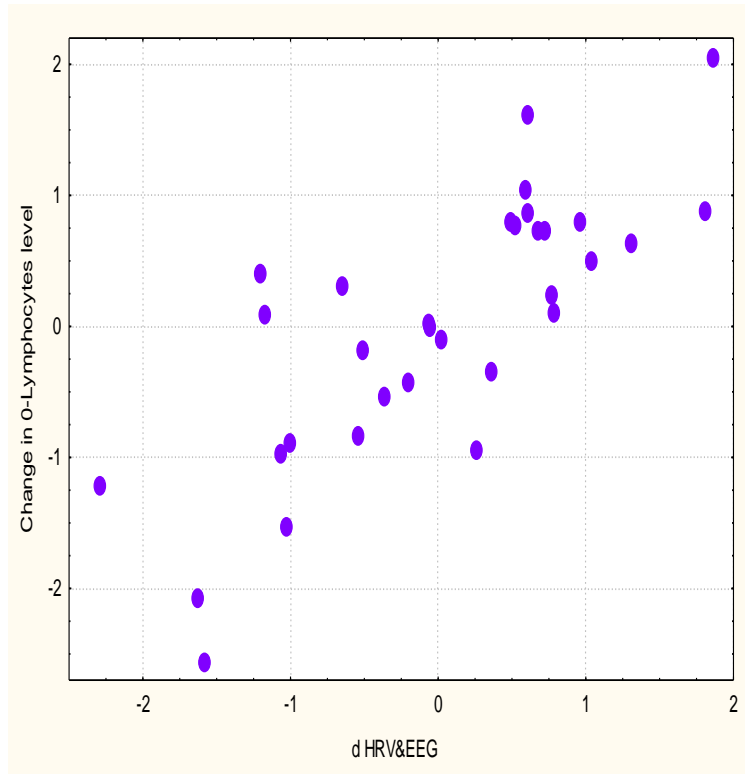
The last object of the correlation analysis was the level of 0-lymphocytes, that is, those that do not express CD3⁺, CD4⁺, CD8⁺, CD16⁺ and CD22⁺ receptors. It turned out that their level **upregulated** by sympathetic outflows and α -rhythm generating structures onto the right parietalis and occipitalis loci as well as δ -rhythm generating structures that are projected onto the left frontalis lateralis and temporalis posterior loci, while **downregulated** by β -rhythm generating structures that are projected onto the left temporalis posterior and occipitalis loci (Table 9).

Table 9. Factor structure of canonical Roots representing Neural parameters and 0 Lymphocytes blood level

Right set	R
AMo HRV, %	-,459
AMo/MxDMn	-,420
P4- α SPD, $\mu\text{V}^2/\text{Hz}$	-,571
O2- α SPD, $\mu\text{V}^2/\text{Hz}$	-,480
T5- δ SPD, $\mu\text{V}^2/\text{Hz}$	-,425
T5- δ SPD, %	-,414
F7- δ SPD, $\mu\text{V}^2/\text{Hz}$	-,405
β -rhythm Index, %	-,466
α -rhythm Amplitude, μV	-,450
β -rhythm Deviation, Hz	,545
T5- β SPD, %	,480
O1- β SPD, %	,444
C3 Entropy	,462
Left set	R
0-Lymphocytes	-1

In addition, **decrease** in level of 0-Lymphocytes are associated with Deviation of β -rhythm and Entropy of rhythms in centralis locus.

In general changes in the nervous regulation determine changes in the level of 0-lymphocytes by 61,6% (Fig. 5).



$R=0,785$; $R^2=0,616$; $\chi^2_{(13)}=23$; $p=0,048$; $\Lambda \text{ Prime}=0,384$

Figure 5. Canonical correlation between changes in parameters of HRV and EEG (line X) and blood 0-Lymphocytes level (line Y)

Among mentioned parameters HRV and EEG a regression model with stepwise exclusion included the following 7 only (Table 6).

Table 10. Regression Summary for Dependent Variable: change in 0-Lymphocytes
 $R=0,761$; $R^2=0,579$; Adjusted $R^2=0,456$; $F_{(7,2)}=4,7$; $p=0,002$; SE of estimate: 6,4%

		Beta	St. Err. of Beta	B	St. Err. of B	n=32 $t_{(24)}$	p-level
Change in Variables	r		Intercept	-5,98	1,21	-4,93	10^{-4}
β -rhythm Deviation, Hz	-0,43	-,394	,139	-3,31	1,17	-2,84	,009
T5- β SPD, %	-0,38	-1,07	,442	-,465	,192	-2,42	,023
C3 Entropy	-0,36	-,407	,141	-20,0	6,93	-2,88	,008
O1- β SPD, %	-0,35	,244	,202	,143	,118	1,21	,240
AMo HRV, %	0,36	,321	,149	,234	,109	2,15	,042
T5- δ SPD, $\mu V^2/Hz$	0,33	,313	,206	,0023	,0015	1,52	,142
T5- δ SPD, %	0,32	-,850	,456	-,215	,115	-1,87	,074

In the last article of this cycle [4-8,13,14], the relationships between changes in the nervous regulation and phagocytosis parameters will be analyzed and a general discussion will be held.

ACKNOWLEDGMENT

We express our sincere gratitude to administration JSC “Truskavets’kurort” for help in carrying out immune tests and recording EEG and HRV.

ACCORDANCE TO ETHICS STANDARDS

Tests in patients are conducted in accordance with positions of Helsinki Declaration 1975, revised and complemented in 2002, and directive of National Committee on ethics of scientific researches. During realization of tests from all participants the informed consent is got and used all measures for providing of anonymity of participants.

For all authors any conflict of interests is absent.

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