Popovych Igor L, Kul'chyns'kyi Andriy B, Gozhenko Anatoliy I, Zukow Walery, Kovbasnyuk Marta M, Korolyshyn Tetyana A. Interrelations between changes in parameters of HRV, EEG and phagocytosis at patients with chronic pyelonephritis and cholecystitis. Journal of Education, Health and Sport. 2018;8(02):135-156. eISNN 2391-8306. DOI http://dx.doi.org/10.5281/zenodo.1172164 http://ojs.ukw.edu.pl/index.php/johs/article/view/5280

The journal has had 7 points in Ministry of Science and Higher Education parametric evaluation. Part b item 1223 (26.01.2017).
1223 Journal of Education, Health and Sport eissn 2391-8306 7

The authors declare that there is no conflict of interests regarding the publication of this paper.
Received: 05.01.2018. Revised: 10.01.2018. Accepted: 12.02.2018.

Interrelations between changes in parameters of HRV, EEG and phagocytosis at patients with chronic pyelonephritis and cholecystitis

Igor L Popovych[1], Andriy B Kul'chyns'kyi[2,3], Anatoliy I Gozhenko[3], Walery Zukow[4], Marta M Kovbasnyuk[1], Tetyana A Korolyshyn[1]

[1]Department of Immunophysiology, OO Bohomolets' Institute of Physiology, Kyïv, Ukraine i.popovych@biph.kiev.ua
[2]Communal Hospital, Truskavets', Ukraine akul@i.ua
[3]Ukrainian Scientific Research Institute of Transport Medicine, Odesa prof.gozhenko@gmail.com
[4]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 and cellular 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 **phagocytosis**, on the other hand. **Material and 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 parameters of phagocytic function of neutrophils against Staphylococcus aureus and Escherichia coli. **Results.** Judging by the coefficients of canonical correlation, the maximal subordinate nervous regulation is the completeness of phagocytosis of E. coli (R=0,944), whereas the influence of the nervous system on the completeness of phagocytosis Staph. aur. is minimal (R=0,607). With regard to the intensity of phagocytosis, the differences in the strength of nerve influence are expressed less (0,893 vs 0,744), and in relation to the activity of phagocytosis as well as the bactericidal ability of neutrophils, there are no differences between R (0,858 vs 0,857 and 0,831 vs 0,834, respectively). However, significant differences were found between profiles of correlation coefficients between EEG and HRV parameters, on the one hand, and phagocytosis parameters, on the other hand, for representatives of Gram-negative and Gram-positive microbes. **Conclusion.** In the nervous regulation of

phagocytosis by neutrophils of the Gram-negative and Gram-positive microbes are involved the structures that are localized in different parts of the CNS.

Keywords: EEG, HRV, neutrophils, phagocytosis, Staphylococcus aureus, Escherichia coli.

INRODUCTION

In the course of implementing the project "The role of the nervous system in the mechanism of immunotropic effects of balneotherapy in the spa Truskavets'", we first analyzed the relationships between the parameters of EEG and HRV, on the one hand, and immunogram, leukocytogram as well as phagocytosis - on the other hand [12-14,19,20]. At the second stage, the object of the study was the relationships between the induced by balneotherapy **changes** in the parameters of the nervous regulation, on the one hand, and humoral and cellular immunity - on the other hand [8,9,11,15,18]. This article contains the results of analysis in the same channel of parameters of phagocytosis by neutrophils of Gram-negative and Gram-positive microbes.

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 (software and hardware complex "CardioLab+HRV" production "KhAI-MEDICA", Kharkiv, Ukraine). For further analysis the following parameters heart rate variability (HRV) were selected [1,2,6]. 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 (pNN_{50}); heart rate (HR), the moda (Mo), the amplitude of moda (AMo), variational sweep (MxDMn) as well as triangulary index (TINN). Spectral parameters (Frequency Domain Methods): spectral power (SP) 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). On the basis of these parameters were calculated classical indexes: LF/HF, LFnu=100%•LF/(LF+HF), Baevskiy's Stress Index (BSI=AMo/2•Mo•MxDMn) and Baevskiy's Activity Regulatory Systems Index (BARSI) [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 (μV^2/Hz) and relative (%) spectral power density (SPD) in the standard frequency bands: 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 [16]:

$$LI, \% = \Sigma [200 \bullet (Right - Left)/(Right + Left)]/8$$

We calculated also for HRV and each locus EEG the Entropy (h) of normalized SPD using formula CE Shannon [22]:

$hHRV = -[SPD\ HF \bullet \log_2 SPD\ HF + SPD\ LF \bullet \log_2 SPD\ LF + SPD\ VLF \bullet \log_2 SPD\ VLF + + SPD\ ULF \bullet \log_2 SPD\ ULF]/\log_2 4;$

$hEEG = -[SPD\alpha \bullet \log_2 SPD\alpha + SPD\beta \bullet \log_2 SPD\beta + SPD\theta \bullet \log_2 SPD\theta + SPD\delta \bullet \log_2 SPD\delta]/\log_2 4$

Parameters of phagocytic function of neutrophils estimated as described by SD Douglas and PG Quie [5] with our (MM Kovbasnyuk) moderately modification. To do this, 5 drops of blood immediately after collection, made in glass centrifuge tubes with 2 ml of 4% solution of sodium citrate. Blood samples were stored in a refrigerator at a temperature of 4^0C. Further samples were centrifuged (5000 rev/min for 5 min). The supernatant was removed with the help of the Pasteur's pipette. We used a fraction of leukocytes with traces of erythrocytes. The objects of phagocytosis served daily cultures of Staphylococcus aureus (ATCC N 25423 F49) as typical specimen for Gram-positive Bacteria and Escherichia coli (O55 K59) as typical representative of Gram-negative Bacteria. Both cultures obtained from Laboratory of Hydro-Geological Regime-Operational Station JSC "Truskavets'kurort". To prepare the suspension microbes did wipes with relevant shoals sterile saline, immersed tubes in boiling water for 3 seconds, cooled to room temperature. Integrity microbes controlled with the aid of a microscope. To do this, drop the suspension of microbes applied to skimmed substantive piece of glass, fixed in alcohol lamp flame. Ready preparations stained by Papenheim, microscoped during immersion, lense h90, eyepiece x10. The test samples were prepared as follows. In Vidal's plastic tubes made in the following order of 0,05 mL of heparin, 0,05 mL of sterile saline, 0,1 mL suspension of leukocytes, 0,05 mL suspension of microbial bodies. Samples shaked and placed in thermostat at 37^0C for 30 min, shaking them with every 10 mins. Then, to stop phagocytosis, the sample was cooled under running water for 10 min. In further samples are centrifuged (5000 rev/min, for 5 min), the supernatant removed with the help of the Pasteur's pipette. From the suspension of leukocytes (with traces of red blood cells) prepared strokes, dried in air at room temperature and stained by Papenheim. Microscoped during immersion lens h90, x10 eyepiece. Take into account the following parameters of phagocytosis: activity (percentage of neutrophils, in which found microbes - Hamburger's Phagocytic Index), intensity (number of microbes absorbed one phagocytes - Microbial Count or Right's Index) and completeness (percentage of dead microbes - Killing Index). Microbial number and index their digestion is determined for each phagocyte and fixed in phagocytic frame.

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

RESULTS AND DISCUSSION

According to the classic formula:

$$|r| \geq \{exp[2t/(n-1,5)^{0,5}] - 1\}/\{exp[2t/(n-1,5)^{0,5}] + 1\},$$

for a sample of 32 full observations critical value of correlation coefficient module at $p<0,05$ ($t>2,04$) is 0,35; at $p<0,01$ ($t>2,75$) is 0,46; at $p<0,001$ ($t>3,64$) is 0,58.

Nevertheless, in regressive models with step-by-step exclusion, there were separate indices (variables) with the values |r| less than critical level.

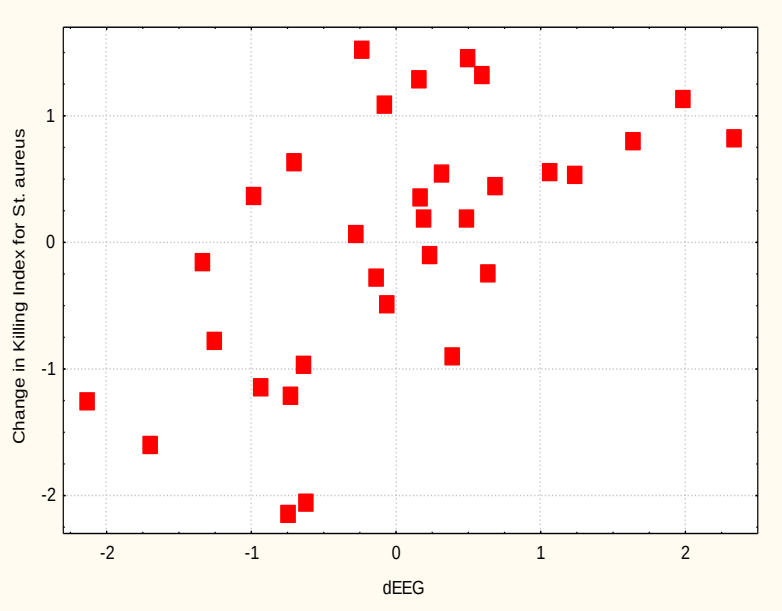
In particular, changes in the Killing Index for Staph. aureus upregulated by changes in the Deviation of θ-rhythm while downregulated by changes in the Deviation of δ-rhythm as well as in β-rhythm Frequency and α-rhythm Index. Changes in these parameters of EEG in aggregate

determines the changes in the Completeness of phagocytosis Staph. aureus only 27,5%, but statistically significant (Table 1 and Fig. 1).

Table 1. Regression Summary for Dependent Variable: change in Killing Index for Staph. aureus
R=0,607; R^2=0,369; Adjusted R^2=0,275; $F_{(4,3)}$=3,9; p=0,012; SE of estimate: 6,8 %

		Beta	St. Err. of Beta	B	St. Err. of B	n=32 $t_{(27)}$	p-level
Changes in Variables	r		Intercpt	3,397	1,252	2,71	,011
θ-rhythm Deviation, Hz	0,42	,365	,161	4,888	2,148	2,28	,031
β-rhythm Frequency, Hz	-0,37	-,187	,165	-,272	,240	-1,13	,268
δ-rhythm Deviation, Hz	-0,32	-,225	,158	-4,565	3,209	-1,42	,166
α-Rhythm Index, %	-0,27	-,276	,154	-,081	,045	-1,79	,084

We consider it important to pay attention to the absence of an attachment to any EEG locus.



R=0,607; R^2=0,369; $\chi^2_{(4)}$=12,9; p=0,012; Λ Prime=0,631
Fig. 1. Scatterplot of canonical correlation between changes in parameters EEG and HRV (X-line) and Completeness Phagocytosis of Staphylococcus aureus (Y-line)

In return changes in the Killing Index for E. coli equally noticeable downregulated by changes in SPD of δ-rhythm in Left Temporalis Anterior (Fig. 2) as well as Right Temporalis Posterior (Fig. 3) loci.

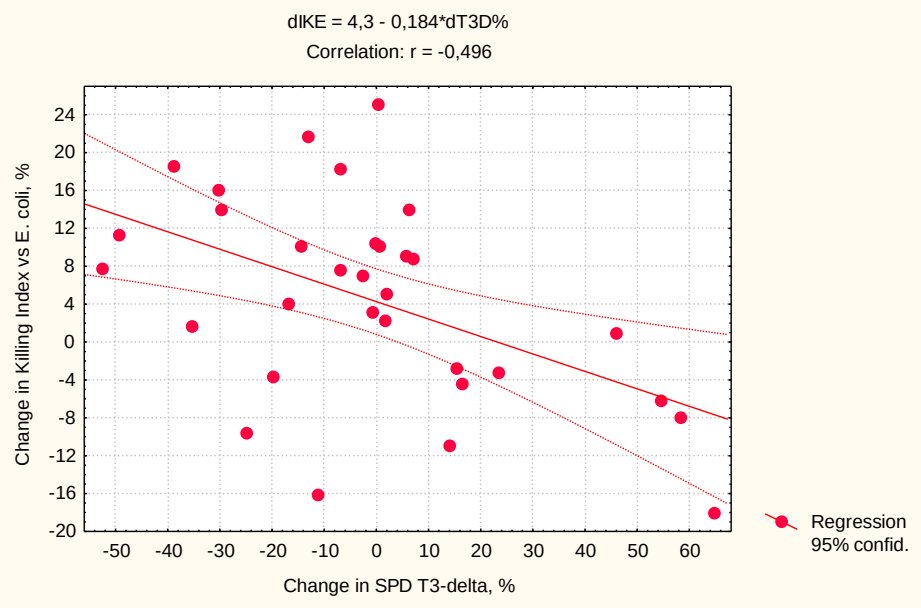


Fig. 2. Scatterplot of relationship between changes in relative SPD of δ-rhythm in locus T3 (line X) and Completeness Phagocytosis of E. coli (line Y)

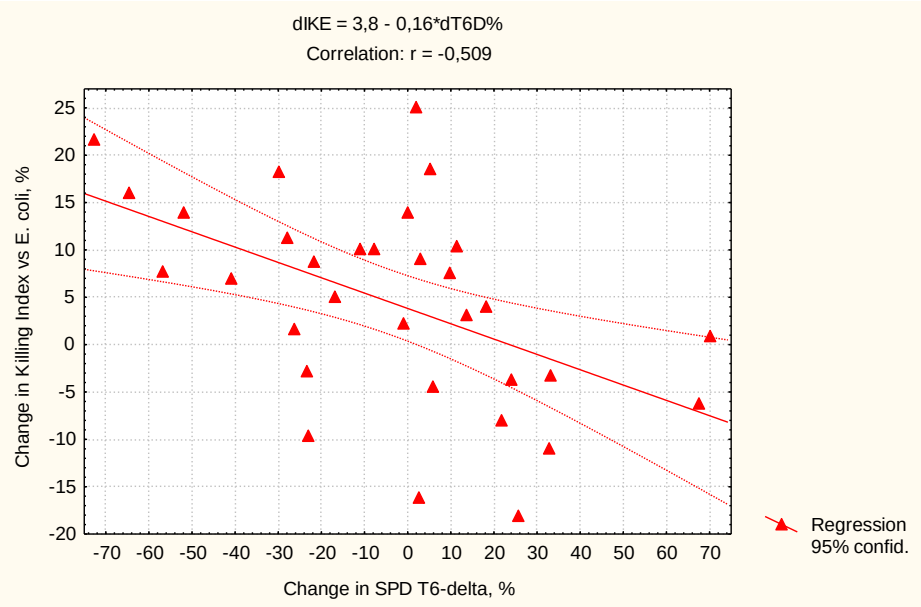
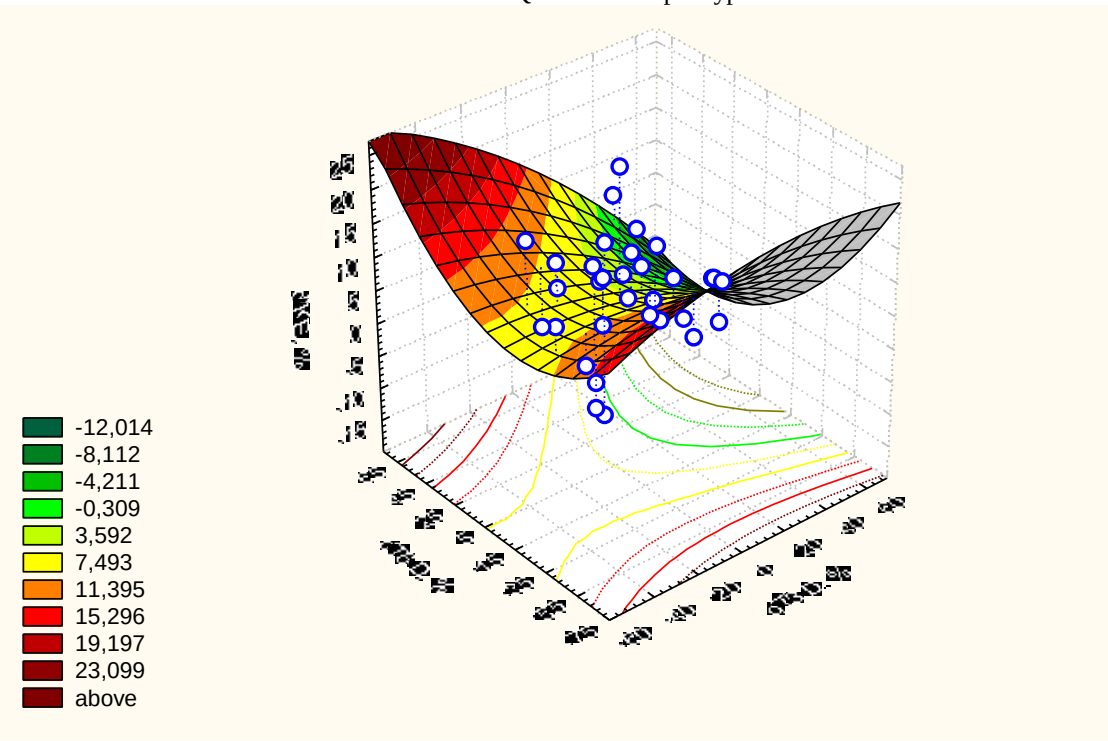


Fig. 3. Scatterplot of relationship between changes in relative SPD of δ-rhythm in locus T6 (line X) and Completeness Phagocytosis of E. coli (line Y)

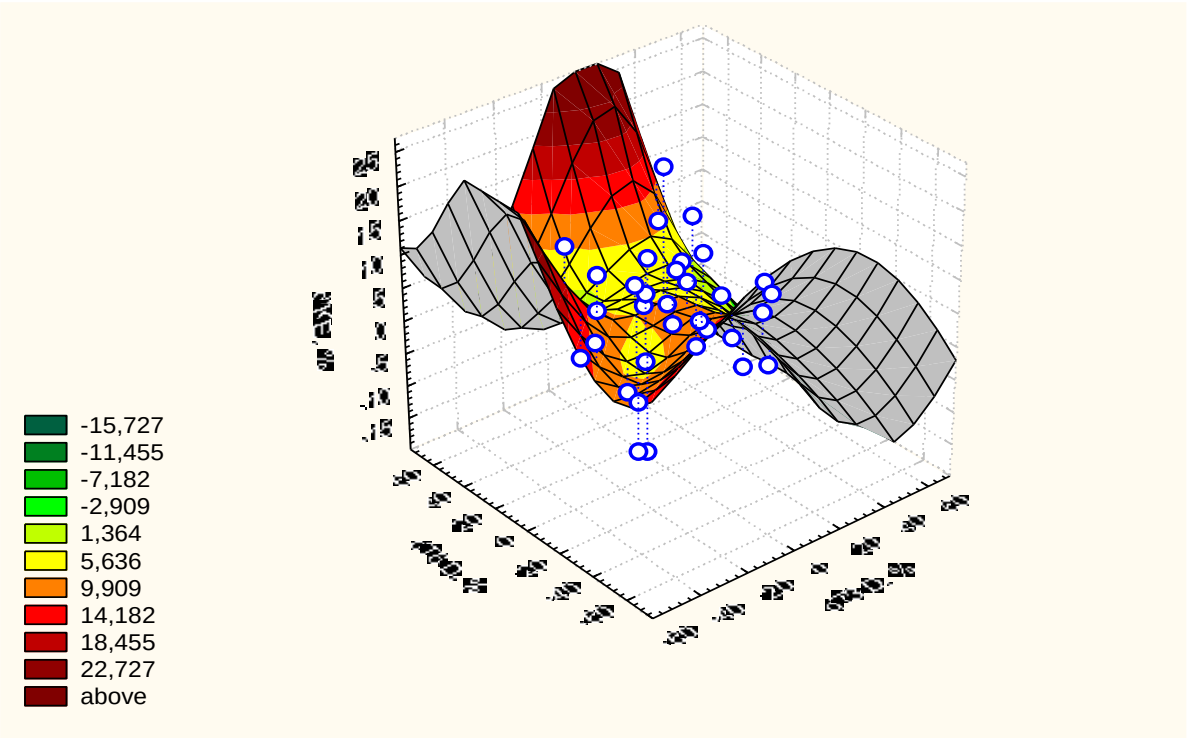
We draw attention to the fact that the changes caused by balneotherapy both EEG and Killing Index take place both in increase and decrease, that is, they are ambivalent. This is consistent with the concept of multivariable effects of the balneofactors of the spa Truskavets' on the functional parameters of the autonomic nervous, endocrine, immune, cardiovascular and digestive systems, which is due to the individual reactivity of the organism and is subject to reliable prediction [3,6,20,29].

Interestingly, the combined regulatory effect of impulses from both loci does not at all outweigh the effects of each individual loci (Fig. 4), and none of them was included in the final regression model with step-by-step exclusion (Table 2).

Quadratic Graph Type



Least Square Graph Type



dIKE(%)=3,93-0,110•dT3-δ SPD(%)-0,105•dT6-δ SPD (%)
R=0,561; R^2=0,314; Adjusted R^2=0,267; $F_{(2,3)}$=6,6; p=0,004; SE of estimate: 9,1%

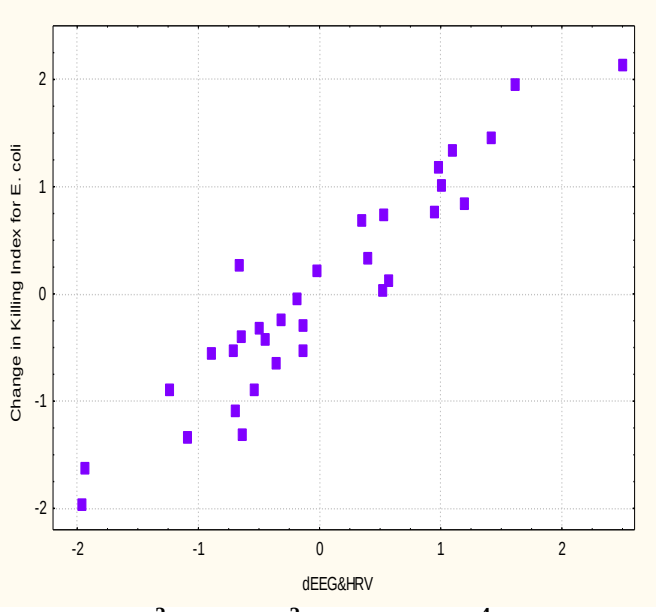
Fig. 4. Interrelations between changes in relative SPD of δ-rhythm in locus T3 (line X), T6 (line Y) and Completeness Phagocytosis of E. coli (line Z)

Changes in these included parameters of EEG and HRV in aggregate determines the changes in the Completeness of phagocytosis E. coli on 79% (Table 2 and Fig. 5).

Table 2. Regression Summary for Dependent Variable: change in Killing Index for E. coli

R=0,944; R^2=0,892; Adjusted R^2=0,791; $F_{(15)}$=8,8; $p<10^{-4}$; SE of estimate: 4,9%

		Beta	St. Err. of Beta	B	St. Err. of B	n=32 $t_{(16)}$	p-level
Changes in Variables	r		Intercpt	6,84	1,28	5,36	10^{-4}
F8-α SPD, %	0,46	,686	,345	,460	,231	1,99	,064
T3-α SPD, %	0,44	,455	,116	,273	,070	3,91	,001
T4-α SPD, %	0,38	,512	,146	,432	,123	3,50	,003
F7-β SPD, %	0,38	,900	,271	,430	,129	3,33	,004
F8-β SPD, %	0,37	1,583	,358	,718	,162	4,43	10^{-3}
SP ULF band HRV, sec^2	0,32	-,383	,145	-,022	,008	-2,64	,018
F8-δ SPD, %	-0,40	2,738	,645	,783	,184	4,25	10^{-3}
F7-δ SPD, %	-0,36	,955	,336	,295	,104	2,85	,012
F4-δ SPD, %	-0,32	,472	,168	,180	,064	2,80	,013
O2-δ SPD, %	-0,35	-,699	,207	-,237	,070	-3,37	,004
O2-δ SPD, μV^2/Hz	-0,33	,410	,160	,004	,001	2,56	,021
Baevskiy Stress Index, un.	-0,37	-,284	,137	-,024	,011	-2,08	,054
θ-rhythm Frequency, Hz	0,40	,548	,116	3,562	,756	4,71	10^{-3}
O2 Entropy	0,41	1,042	,139	57,97	7,74	7,48	10^{-6}
HRV Entropy	0,24	,358	,131	33,18	12,11	2,74	,014



R=0,944; R^2=0,892; $\chi^2_{(15)}$=50; $p<10^{-4}$; Λ Prime=0,108

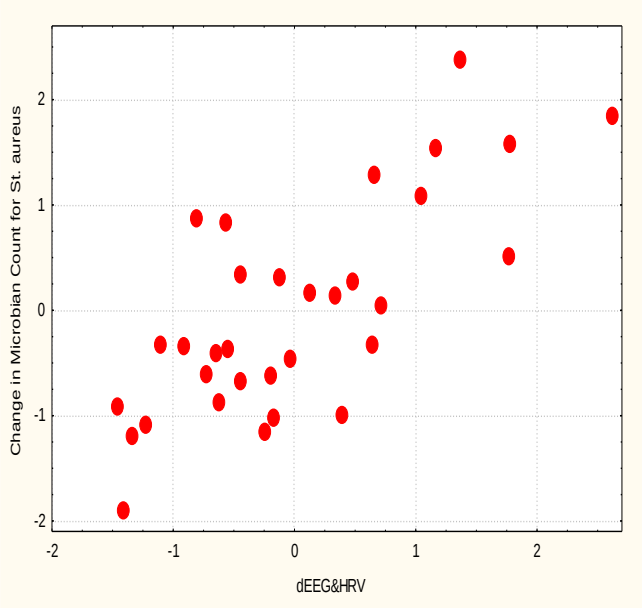
Fig. 5. Scatterplot of canonical correlation between changes in parameters EEG and HRV (X-line) and Completeness Phagocytosis of E. coli (Y-line)

Changes in the Microbial Count for Staph. aureus upregulated by changes in the Index of θ-rhythm and SPD of α-rhythm in Right Frontalis Anterior locus while downregulated by changes in the Index of δ-rhythm and Vagal tone. Changes in these parameters in aggregate determines the changes in the Intensity of phagocytosis Staph. aureus on 49% (Table 3, Fig. 6).

Table 3. Regression Summary for Dependent Variable: change in Microbial Count for Staph. aureus

R=0,744; R^2=0,554; Adjusted R^2=0,488; $F_{(4,3)}$=8,4; p=0,0002; SE of estimate: 5,5 Bac/Phag

		Beta	St. Err. of Beta	B	St. Err. of B	n=32 $t_{(27)}$	p-level
Changes in Variables	r		Intercpt	1,927	1,060	1,82	,080
θ-Rhythm Index, %	0,41	,422	,132	,074	,023	3,20	,003
Fp2-α SPD, %	0,29	,201	,131	,113	,074	1,53	,137
δ-Rhythm Index, %	-0,39	-,311	,131	-,050	,021	-2,38	,025
pNN_{50}, %	-0,45	-,447	,131	-,445	,130	-3,42	,002



R=0,744; R^2=0,554; $\chi^2_{(4)}$=22,6; p=0,0002; Λ Prime=0,446

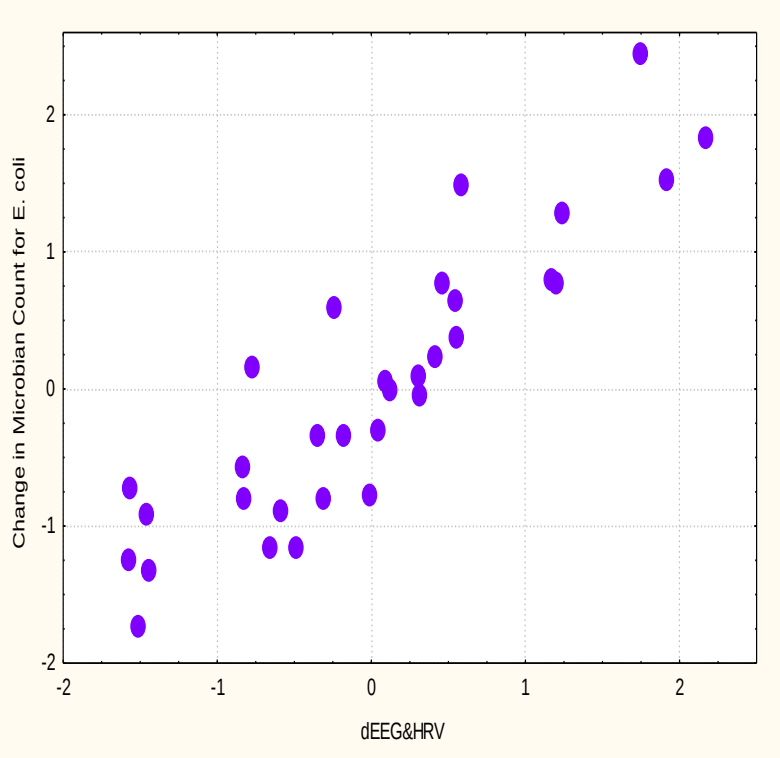
Fig. 6. Scatterplot of canonical correlation between changes in parameters EEG and HRV (X-line) and Intensity Phagocytosis of Staphylococcus aureus (Y-line)

The Intensity of Phagocytosis of Escherichia coli is also more susceptible to regulatory neural influences than Staphylococcus aureus. Changes in its upregulated by changes in the Deviation of δ-rhythm while downregulated by changes in the SPD of β-rhythm in Left Frontalis Medialis and Right Occipitalis loci as well as SPD of α-rhythm in Left Temporalis Anterior and Posterior loci and its Asymmetry as well as Vagal tone. Changes in these parameters in aggregate determines the changes in the Intensity of phagocytosis of E. coli on 74% (Table 4, Fig. 7).

Table 4. Regression Summary for Dependent Variable: change in Microbial Count for E. coli

R=0,893; R^2=0,798; Adjusted R^2=0,739; $F_{(7,2)}$=13,5; $p<10^{-5}$; SE of estimate: 5,8 Bac/Phag

		Beta	St. Err. of Beta	B	St. Err. of B	n=32 $t_{(24)}$	p-level
Changes in Variables	r		Intercpt	2,76	1,18	2,34	,028
δ-rhythm Deviation, Hz	0,40	,491	,095	14,0	2,7	5,19	10^{-4}
F3-β SPD, %	-0,40	-,259	,123	-,2021	,0964	-2,10	,047
O2-β SPD, %	-0,34	-,314	,127	-,2376	,0957	-2,48	,020
T5-α SPD, μV^2/Hz	-0,37	-,332	,097	-,0246	,0072	-3,41	,002
T3-α SPD, %	-0,33	-,369	,103	-,2339	,0653	-3,58	,002
α-rhythm Asymmetry, %	-0,36	-,265	,098	-,1721	,0636	-2,70	,012
SP HF band HRV, sec^2	-0,29	-,177	,100	-,0074	,0041	-1,78	,087



R=0,893; R^2=0,798; $\chi^2_{(7)}$=42,3; p<10^{-6}; Λ Prime=0,202

Fig. 7. Scatterplot of canonical correlation between changes in parameters EEG and HRV (X-line) and Intensity Phagocytosis of E. coli (Y-line)

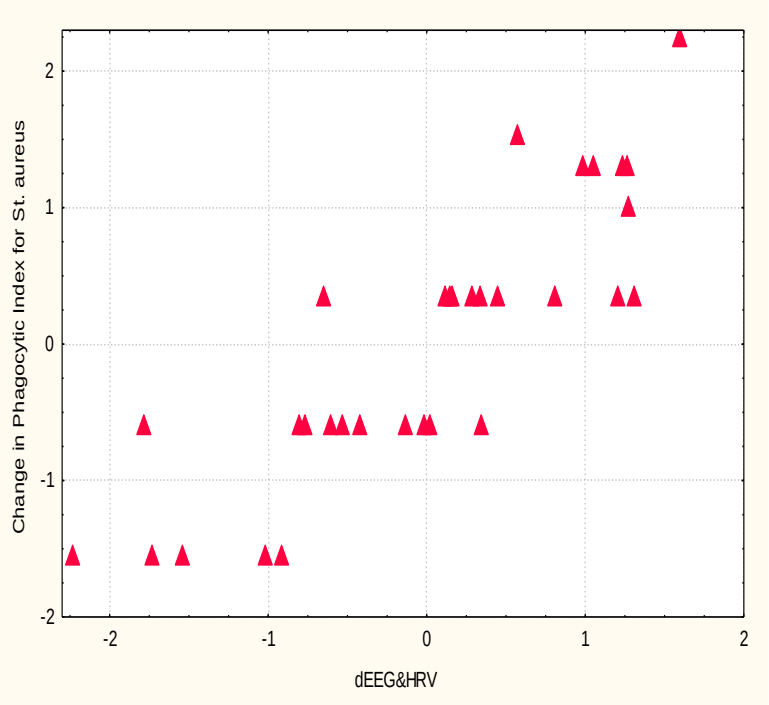
In return Activity of Phagocytosis both Staphylococcus aureus and Escherichia coli subordinate to nervous regulatory influences to approximately the same extent. Determination rate is 63% and 57% respectively.

For both bacteria, activation is carried out through changes in parameters of α-rhythm while inhibition through changes in parameters of β-rhythm as well as Sympathetic tone. However, the factor structure for both bacteria is different (Table 5 and Fig.8 vs Table 6 and Fig. 9).

Table 5. Regression Summary for Dependent Variable: change in Phagocytic Index for Staph. aureus

R=0,857; R^2=0,735; Adjusted R^2=0,627; $F_{(9,2)}$=6,8; p=0,0001; SE of estimate: 0,6%

		Beta	St. Err. of Beta	B	St. Err. of B	n=32 $t_{(22)}$	p-level
Changes in Variables	r		Intercpt	,457	,137	3,34	,003
P4-α SPD, %	0,44	-,386	,239	-,029	,018	-1,61	,121
P4-α SPD, μV^2/Hz	0,34	,230	,142	,001	,001	1,62	,119
T6-α SPD, %	0,40	,454	,171	,026	,010	2,66	,014
α-rhythm Index, %	0,39	,342	,199	,013	,008	1,72	,099
α-rhythm Asymmetry, %	-0,32	-,309	,136	-,019	,008	-2,26	,034
θ-rhythm Frequency, Hz	0,32	,337	,174	,219	,113	1,94	,066
β-rhythm Deviation, Hz	-0,36	-,269	,135	-,280	,141	-1,99	,059
O1-β SPD, %	-0,35	-,286	,136	-,021	,010	-2,10	,047
Heart Rate, beats/min	-0,32	-,324	,118	-,053	,019	-2,75	,012



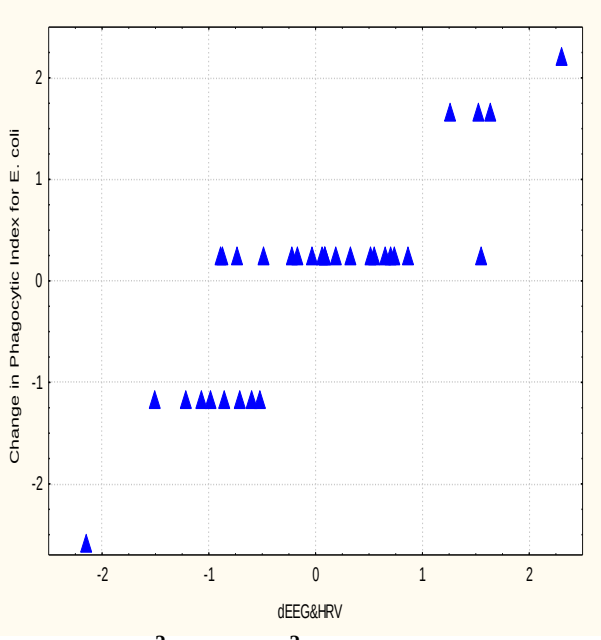
R=0,857; R^2=0,735; $\chi^2_{(9)}$=33,9; p<10^{-4}; Λ Prime=0,265

Fig. 8. Scatterplot of canonical correlation between changes in parameters EEG and HRV (X-line) and Activity of Phagocytosis of Staphylococcus aureus (Y-line)

Table 6. Regression Summary for Dependent Variable: change in Phagocytic Index for E. coli

R=0,858; R^2=0,737; Adjusted R^2=0,571; $F_{(12)}$=4,4; p=0,002; SE of estimate:0,5%

		Beta	St. Err. of Beta	B	St. Err. of B	n=32 $t_{(19)}$	p-level
Changes in Variables	r		Intercpt	,059	,115	,51	,615
O1-β SPD, %	-0,44	-,513	,195	-,025	,009	-2,64	,016
F7-β SPD, %	-0,42	,332	,219	,011	,007	1,51	,147
P3-β SPD, %	-0,38	,539	,237	,036	,016	2,27	,035
C3-β SPD, %	-0,38	-,899	,318	-,048	,017	-2,82	,011
T6-β SPD, %	-0,36	-,422	,312	-,015	,011	-1,35	,192
O2-β SPD, %	-0,36	,541	,223	,026	,011	2,43	,025
F3-β SPD, %	-0,35	,400	,296	,020	,015	1,35	,193
θ-rhythm Index, %	-0,39	-,296	,158	-,005	,003	-1,87	,077
θ-rhythm Frequency, Hz	0,28	,460	,173	,200	,075	2,66	,015
α-rhythm Index, %	0,34	,605	,235	,016	,006	2,58	,018
Fp1 Entropy	0,32	,538	,165	1,844	,566	3,26	,004
SP LF band HRV, sec^2	-0,31	-,247	,145	-,000	,000	-1,71	,103



R=0,858; R^2=0,737; $\chi^2_{(12)}$=32,0; p=0,0014; Λ Prime=0,263

Fig. 9. Scatterplot of canonical correlation between changes in parameters EEG and HRV (X-line) and Activity Phagocytosis of E. coli (Y-line)

On the basis of the recorded partial parameters of Phagocytosis, taking into account the Neutrophils content of 1 L blood, we calculated the integral parameter - Bactericidal Capacity of Neutrophils (BCCN) by the formula [13]:

BCCN (10^9 Bact/L) = N (10^9/L)•PhI (%)•MC (Bact/Phag)•KI (%)•10^{-4}

Changes in BCCN against Staph. aur. downregulated by changes in SPD of θ-rhythm in Left Frontalis Lateralis (Fig. 10) and Occipitalis loci as well as in Frequency of β-and α-rhythms and Entrory SPD in Temporalis Posterior locus (Fig. 10, Table 7).

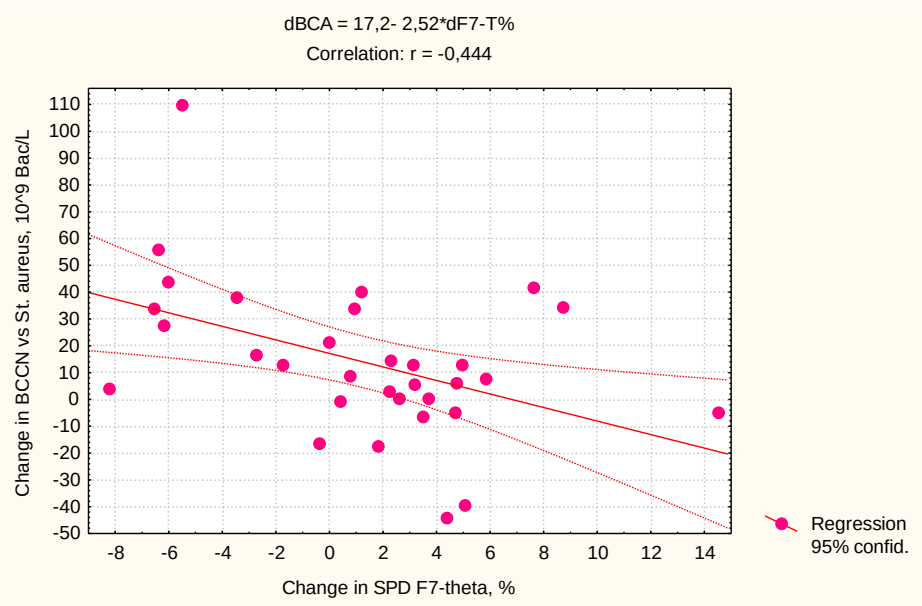
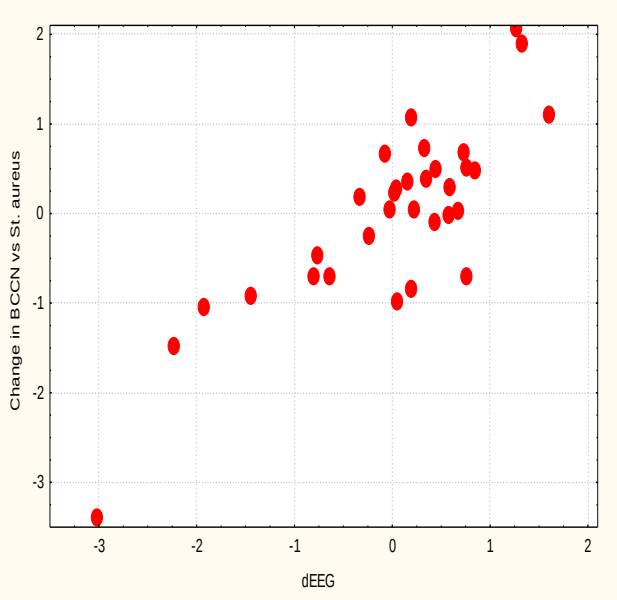


Fig. 10. Scatterplot of correlation between changes in relative SPD θ-rhythm in locus F7 (X-line) and Bactericidal Capacity Neutrophils against Staphylococcus aureus (Y-line)

Table 7. Regression Summary for Dependent Variable: change in Bactericidal Capacity vs Staph. aureus
R=0,834; R^2=0,696; Adjusted R^2=0,590; $F_{(8,2)}$=6,6; p=0,0002; SE of estimate: 18,4•10^9 Bact/L

		Beta	St. Err. of Beta	B	St. Err. of B	n=32 $t_{(23)}$	p-level
Changes in Variables	r		Intercpt	21,0	3,59	5,85	10^{-5}
F7-θ SPD, %	-0,44	-,571	,152	-3,245	,862	-3,77	,001
O1-θ SPD, %	-0,34	-,195	,135	-1,217	,845	-1,44	,163
β-rhythm Frequency, Hz	-0,37	-,479	,138	-2,498	,717	-3,48	,002
α-rhythm Frequency, Hz	-0,34	-,264	,127	-9,96	4,81	-2,07	,050
T6 Entropy	-0,33	,483	,182	68,9	26,0	2,65	,014
O2-δ SPD, μV^2/Hz	0,47	,763	,324	,018	,007	2,36	,027
F8-δ SPD, μV^2/Hz	0,37	-,524	,311	-,027	,016	-1,68	,105
F3-α SPD, μV^2/Hz	0,31	,337	,130	,144	,056	2,59	,016

Instead, the changes in BCCN are unified with changes in SPD of δ-rhythm in Right Frontalis Lateralis and Left Frontalis Medialis loci. In general, the measure of neurogenic determination BCCN against Staph. aureus is 59% (Table 7, Fig. 11).



R=0,834; R^2=0,696; $\chi^2_{(8)}$=30,9; p=0,0001; Λ Prime=0,304
Fig. 11. Scatterplot of canonical correlation between changes in parameters EEG (X-line) and Bactericidal Capacity Neutrophils against Staphylococcus aureus (Y-line)

Changes in BCCN against E. coli most of all downregulated by changes in Baevskiy's Activity Regulatory Systems Index (Fig. 12) and Asymmetry of α-rhythm (Fig. 13). The combined effect of both factors determines the change of this parameter by 36,5% (Fig. 14).

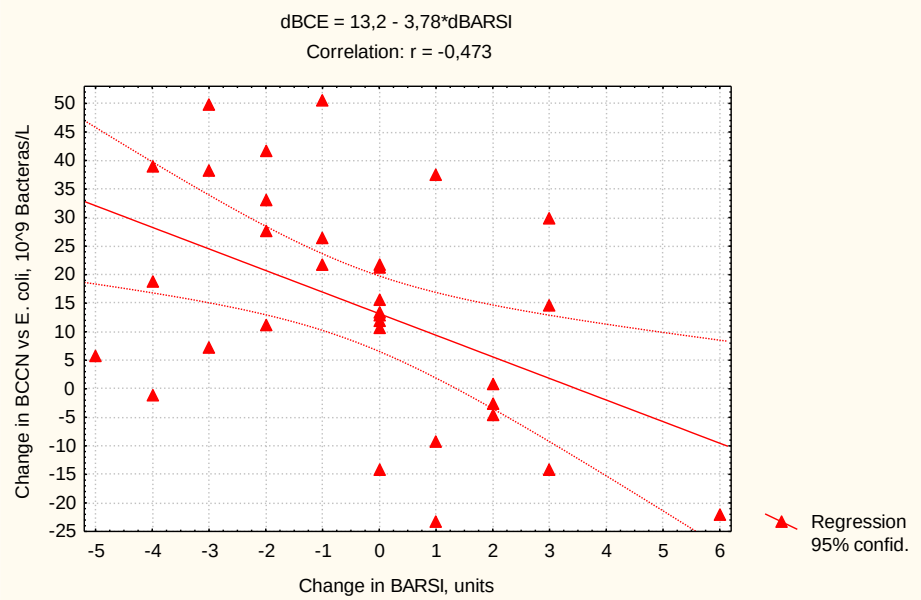


Fig. 12. Scatterplot of correlation between changes in Baevskiy's Activity Regulatory Systems Index (X-line) and Bactericidal Capacity Neutrophils against E. coli (Y-line)

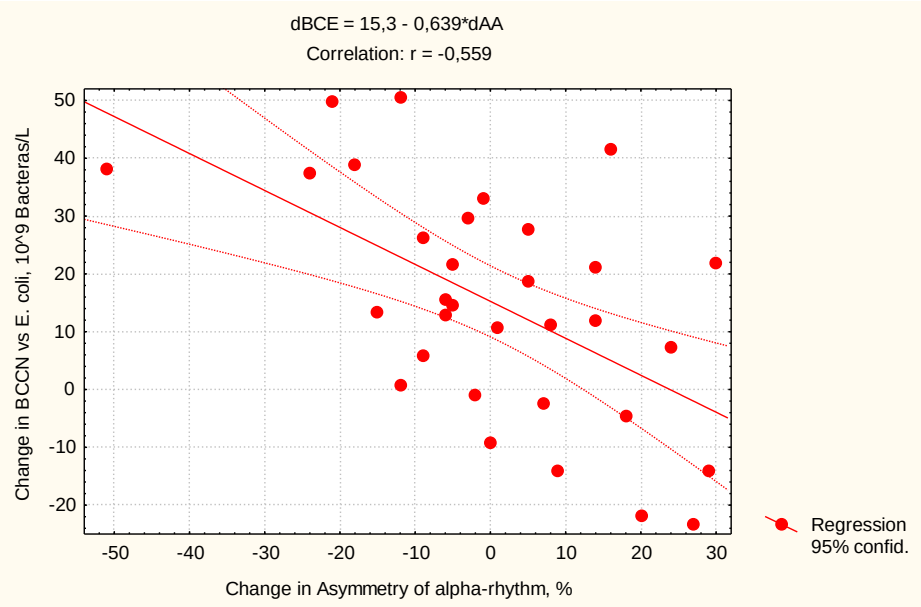
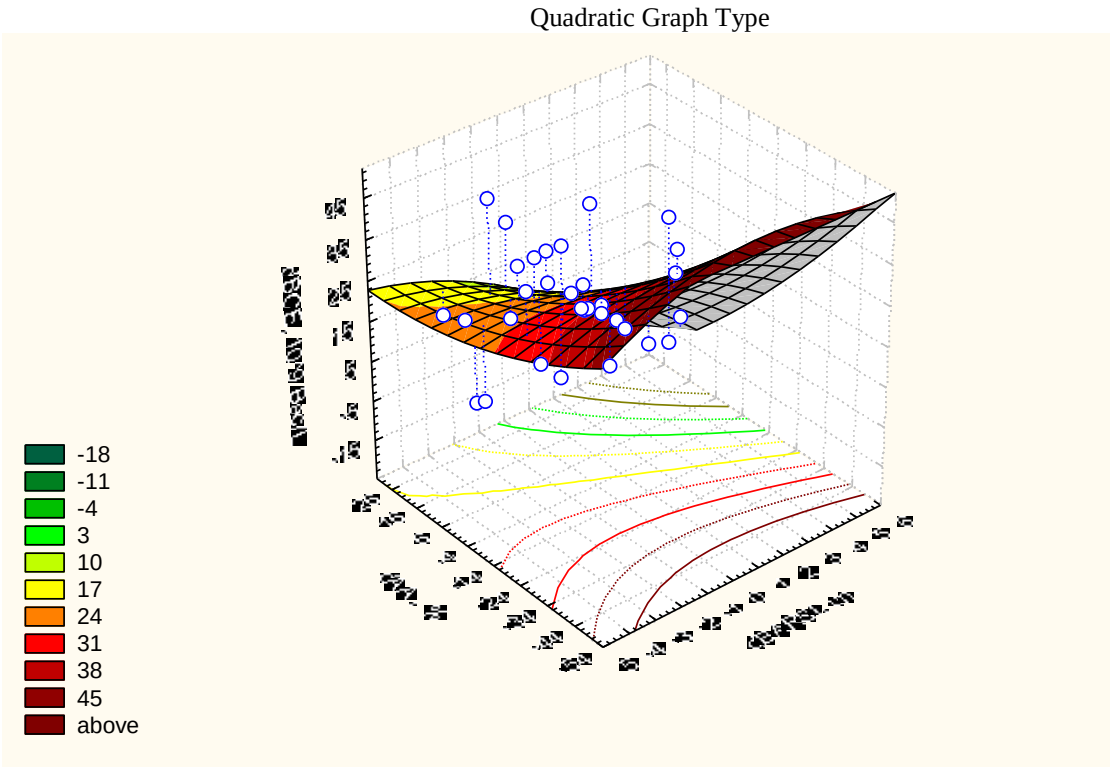
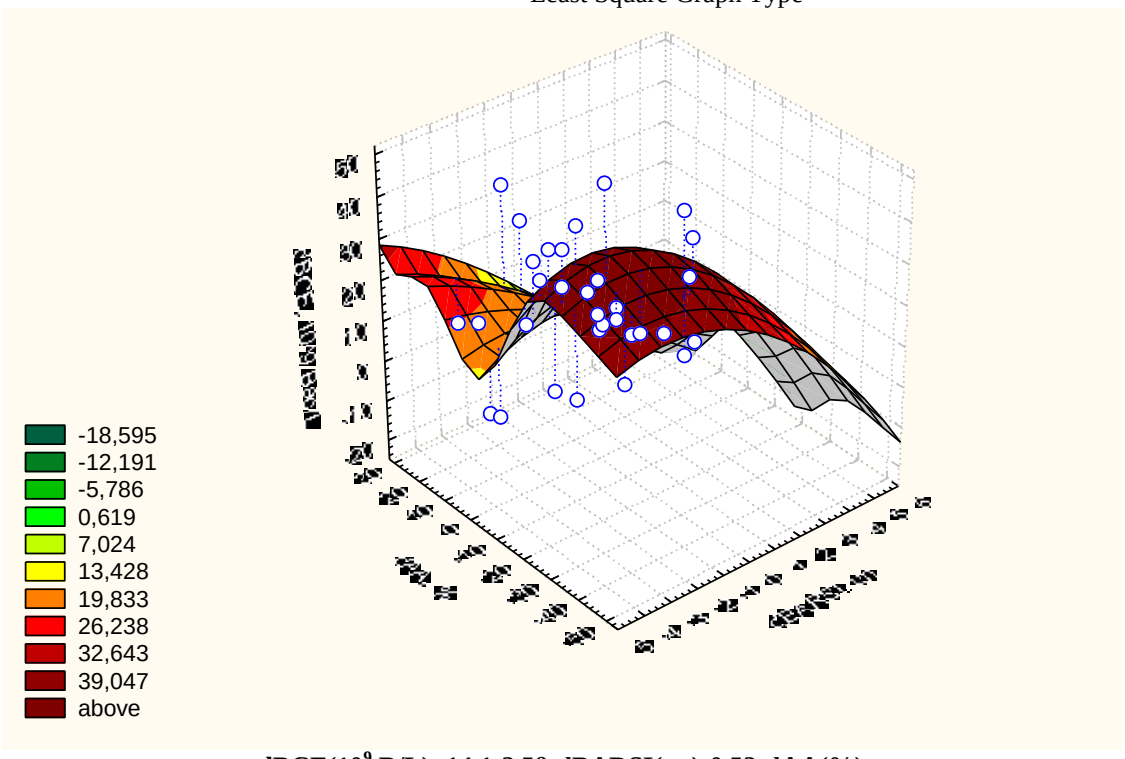


Fig. 13. Scatterplot of correlation between changes in Asymmetry of α-rhythm EEG (X-line) and Bactericidal Capacity Neutrophils against E. coli (Y-line)





dBCE(10^9 B/L)=14,1-2,59•dBARSI(un)-0,52•dAA(%)
R=0,637; R^2=0,406; Adjusted R^2=0,365; $F_{(2,3)}$=9,9; $p<10^{-3}$; SE of estimate: 16 10^9 B/L

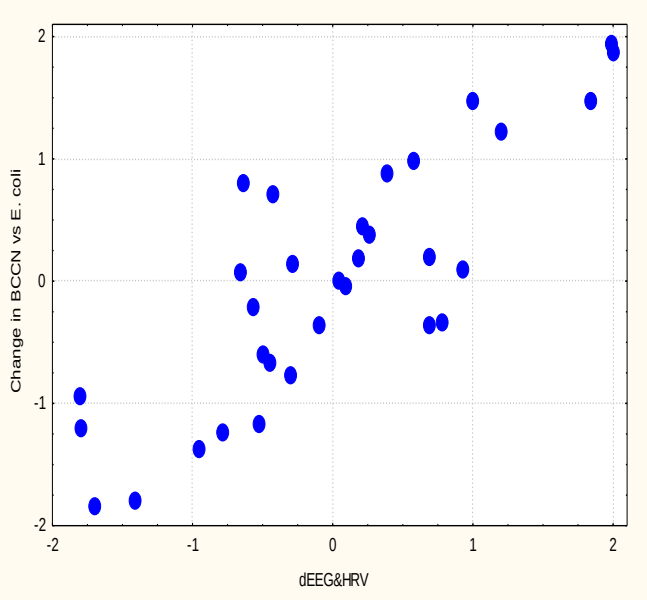
Fig. 14. Interrelations between changes in Baevskiy's Activity Regulatory Systems Index (line X), Asymmetry of α-rhythm EEG (line Y) and Bactericidal Capacity of Neutrophils against E. coli (line Z)

Taking into account included in the model of other parameters of EEG and HRV, the measure of neurogenic determination of changes in Bactericidal Capacity vs. E. coli increases to 62% (Table 8, Fig. 15).

Table 8. Regression Summary for Dependent Variable: change in Bactericidal Capacity vs E. coli

R=0,831; R^2=0,690; Adjusted R^2=0,616; $F_{(6,3)}$=9,3; $p<10^{-4}$; SE of estimate:12,3•10^9 Bact/L

		Beta	St. Err. of Beta	B	St. Err. of B	n=32 $t_{(25)}$	p-level
Changes in Variables	r		Intercpt	14,3	2,27	6,28	10^{-6}
α-rhythm Asymmetry, %	-0,56	-,436	,119	-,499	,136	-3,66	,001
β-rhythm Frequency, Hz	-0,41	-,300	,116	-1,081	,416	-2,60	,016
O1-β SPD, μV^2/Hz	-0,32	-,211	,114	-,084	,045	-1,86	,075
Baevskiy ARS Index, un.	-0,47	-,268	,127	-2,145	1,015	-2,11	,045
HRV Triangulary Index	-0,40	-,198	,131	-1,188	,788	-1,51	,144
SP ULF band HRV, sec^2	-0,29	-,173	,135	-,018	,014	-1,28	,212



R=0,831; R^2=0,690; $\chi^2_{(6)}$=31,6; $p<10^{-4}$; Λ Prime=0,310

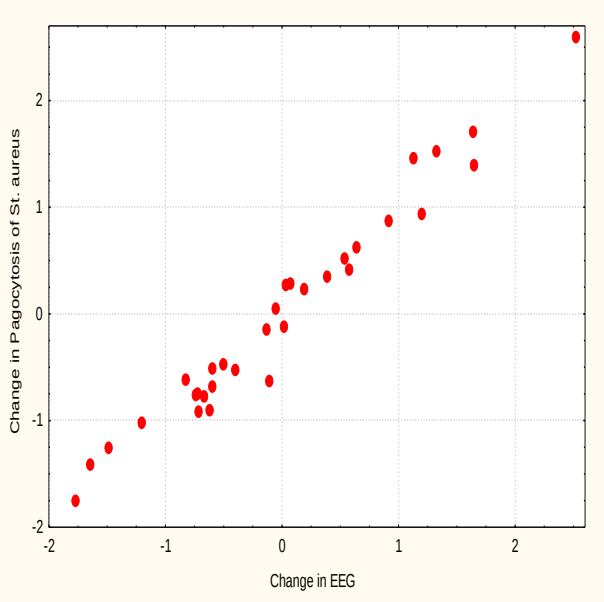
Fig. 15. Scatterplot of canonical correlation between changes in parameters EEG and HRV (X-line) and Bactericidal Capacity Neutrophils against E. coli (Y-line)

And now, let's turn to a **complete** canonical correlation analysis, that is, the clarification of the relationship between changes in regulating neurogenic influences, on the one hand, and the parameters regulated by them phagocytic function of neutrophils in relation to two types of bacteria, on the other hand.

Regarding Staphylococcus aureus, the program has identified two almost equally pairs of canonical roots. The phagocytic root of the first pair receives a dominant factor load from changes in the Activity of phagocytosis and a very moderate load from changes in the Intensity of phagocytosis (Table 9). The neurogenic root represents **one-sided** (**upregulating**) changes in Index of α-rhythm and its SPD in Right Temporalis Posterior, Parietalis and Frontalis Anterior loci as well as in Frequency of θ-rhythm.

Table 9. Factor structure of canonical Roots representing parameters of Neural regulation and Phagocytic function of Neutrophils against Staphylococcus aureus

Right set	**Root 1**	**Root 2**
T6-α SPD, %	**-,411**	,353
P4-α SPD, %	**-,394**	,159
P4-α SPD, μV^2/Hz	**-,261**	-,025
α-rhythm Index, %	**-,362**	,035
θ-rhythm Frequency, Hz	**-,263**	,255
Fp2-α SPD, %	**-,223**	,236
α-rhythm Asymmetry, %	**,369**	,144
β-rhythm Deviation, Hz	**,331**	-,273
Heart Rate, beats/min	**,322**	-,187
O1-β SPD, %	**,277**	-,099
F7-θ SPD, %	-,068	**,505**
O1-θ SPD, %	,188	**,322**
α-rhythm Frequency, Hz	-,135	**,378**
δ-rhythm Deviation, Hz	-,055	**,359**
T6 Entropy	-,047	**,326**
β-rhythm Frequency, Hz	,208	**,295**
β-rhythm Index, %	,016	**,215**
θ-rhythm Deviation, Hz	-,093	**-,437**
O2-δ SPD, μV^2/Hz	,039	**-,442**
F8-δ SPD, μV^2/Hz	-,088	**-,311**
F3-α SPD, μV^2/Hz	-,247	**-,256**
Left set	**Root 1**	**Root 2**
Phagocytic Index for Staph. aur.	**-,912**	,277
Microbial Count for Staph. aur.	**-,388**	-,206
Killing Index for Staph. aureus	,058	**-,659**
Bactericidal Capacity vs St. aur.	-,211	**-,969**

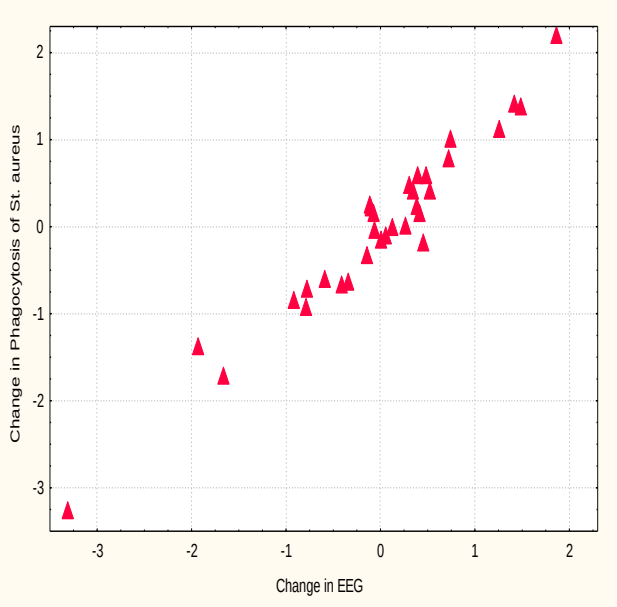


R=0,983; R^2=0,966; $\chi^2_{(96)}$=150; p=0,0004; Λ Prime=0,0001

Fig. 16. Scatterplot of correlation between first pair of canonical Roots reflecting changes in parameters EEG (X-line) and Phagocytic function of Neutrophils against Staphylococcus aureus (Y-line)

Instead, **opposite-directed** changes in Asymmetry of α-rhythm, Deviation of β-rhythm and its SPD in Left Occipitalis locus as well as in Heart Rate as marker of Sympathetic tone represents **downregulating** influences. As a result, the measure of neurogenic determination is 96,6% (Fig. 16).

The phagocytic root of the second pair receives a dominant factor load from changes in the Bactericidal Capacity and moderate load from changes in the Completeness of phagocytosis (Table 9). The neurogenic root represents **opposite-directed (downregulating)** changes in SPD of θ-rhythm in Left Frontalis Lateralis and Occipitalis loci as well as in Deviation of δ-rhythm while **one-sided** (**upregulating**) changes in SPD of δ-rhythm in similar Right loci as well as in Deviation of θ-rhythm. Additional **downregulating** factors are Frequency of α- and β-rhythms. As a result, the measure of neurogenic determination is 94,6% (Fig. 17).



R=0,972; R^2=0,946; $\chi^2_{(69)}$=94; p=0,0237; Λ Prime=0,0033

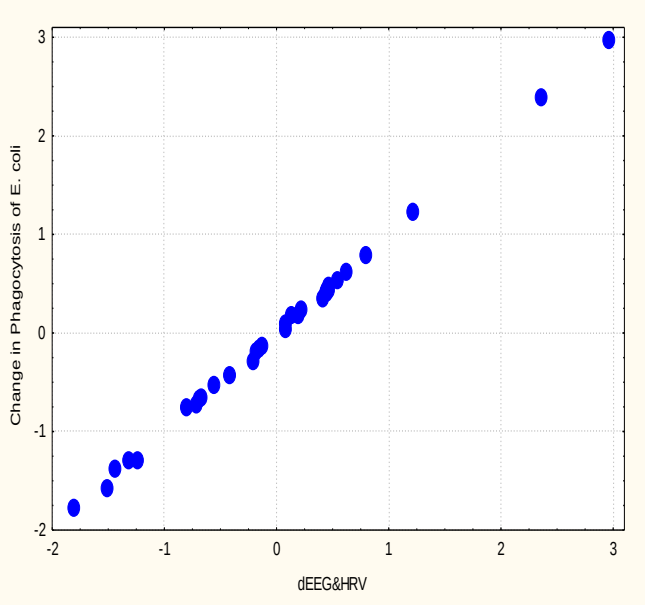
Fig. 17. Scatterplot of correlation between second pair of canonical Roots reflecting changes in parameters EEG (X-line) and Phagocytic function of Neutrophils against Staphylococcus aureus (Y-line)

Regarding Escherichia coli the phagocytic root of the first pair receives a moderate negatively factor loads from changes in the Activity and Completeness of phagocytosis while moderate positively loads from changes in the Intensity of phagocytosis and BCCN (Table 10). The neurogenic root represents both one-sided and one-sided changes in parameters of HRV and EEG that collectively determine changes in phagocytosis parameters by 99,9% (Fig. 18).

The phagocytic root of the second pair, unlike the first pair, receives a significant factor only from changes in BCCN (positively) and in the Intensity of phagocytosis (negatively). The measure of their neurogenic determination is 98,4% (Fig. 19).

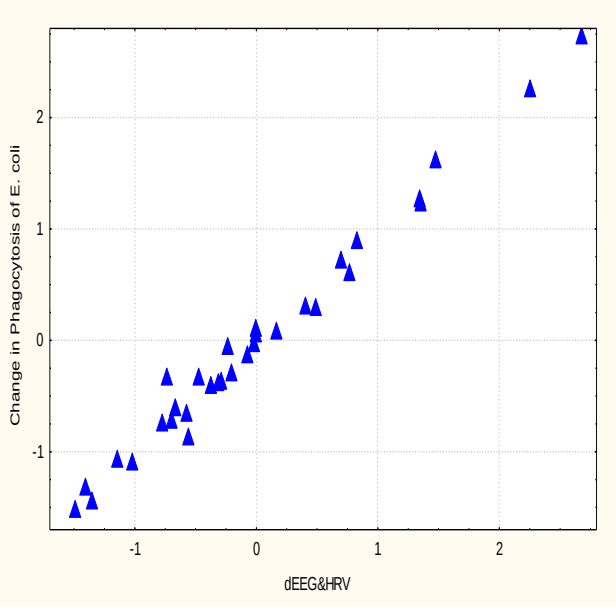
Table 10. Factor structure of canonical Roots representing parameters of Neural regulation and Phagocytic function of Neutrophils against Escherichia coli

Right set	**Root 1**	**Root 2**
SP ULF band HRV, sec^2	**-,505**	,003
T3-α SPD, %	**-,417**	,304
θ-rhythm Frequency, Hz	**-,303**	-,115
HRV Triangulary Index	**-,150**	-,213
θ-rhythm Index, %	**,429**	-,273
T3-δ SPD, %	**,303**	-,072
α-rhythm Asymmetry, %	**-,251**	-,020
P3-β SPD, %	**,216**	-,149
O1-β SPD, %	**,197**	,011
Baevskiy Stress Index, un.	**,155**	,105
δ-rhythm Deviation, Hz	,096	**-,541**
T5-α SPD, $\mu V^2/Hz$	-,153	**,489**
O2-δ SPD, $\mu V^2/Hz$	,063	**,478**
O2-δ SPD, %	,033	**,338**
O2 Entropy	-,244	**-,291**
C3-β SPD, %	,023	**-,211**
Left set	**Root 1**	**Root 2**
Phagocytic Index for E. coli	**-,467**	-,035
Killing Index for E. coli	**-,465**	,180
Microbial Count for E. coli	,465	**-,580**
Bactericidal Capacity vs E. coli	,350	**,425**



R=0,999; R^2=0,999; $\chi^2_{(104)}$=237; p<10^{-6}; Λ Prime<10^{-6}

Fig. 18. Scatterplot of correlation between first pair of canonical Roots reflecting changes in parameters EEG (X-line) and Phagocytic function of Neutrophils against Escherichia coli (Y-line)



R=0,992; R^2=0,984; $\chi^2_{(75)}$=131; p<10^{-4}; Λ Prime=0,0002

Fig. 19. Scatterplot of correlation between second pair of canonical Roots reflecting changes in parameters EEG (X-line) and Phagocytic function of Neutrophils against Escherichia coli (Y-line)

The critical volume of the article forces us to postpone a detailed discussion of the following. And in this we confine ourselves to the reference to the known significant differences between mechanisms of phagocytosis of gram-positive and gram-negative bacteria [23] as well as about anti-inflammatory effects both sympathetic/adrenergic and cholinergic pathways [3,25-28].

ACKNOWLEDGMENT

We express our sincere gratitude to administration JSC "Truskavets'kurort" for help in conducting 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.

References

1. Baevskiy RM, Ivanov GG. Heart Rate Variability: theoretical aspects and possibilities of clinical application [in Russian]. Ultrazvukovaya i funktsionalnaya diagnostika. 2001; 3: 106-127.
2. Berntson GG, Bigger JT jr, Eckberg DL, Grossman P, Kaufman PG, Malik M, Nagaraja HN, Porges SW, Saul JP, Stone PH, Van der Molen MW. Heart Rate Variability: Origines, methods, and interpretive caveats. Psychophysiology. 1997; 34: 623-648.
3. Chavan SS, Tracey KJ. Essencial Neuroscience in Immunology. J Immunol. 2017; 198: 3389-3397.
4. Chebanenko OI, Chebanenko LO, Popovych IL. Multivariate Balneoeffects of Factors of Spa Truskavets' and Forecasting [in Ukrainian]. Kyiv: UNESCO-SOCIO. 2012: 496 p.

5. Douglas SD, Quie PG. Investigation of Phagocytes in Disease. Churchil. 1981. 110 p.
6. Heart Rate Variability. Standards of Measurement, Physiological Interpretation, and Clinical Use. Task Force of ESC and NASPE. Circulation. 1996; 93(5): 1043-1065.
7. Kozyavkina OV, Kozyavkina NV, Gozhenko OA, Gozhenko AI, Barylyak LG, Popovych IL. Bioactive Water Naftussya and Neuroendocrine-Immune Complex [in Ukrainian]. Kyiv: UNESCO-SOCIO. 2015: 349 p.
8. Kul'chyns'kyi AB. The relationships between changes in parameters of Electroencephalogram and Immunogram in the conditions balneotherapy on spa Truskavets' [in Ukrainian]. In: Valeology: current status, trends and persrectives of development. Abstracts. XIV Intern. scient. and practical. conf. (Kharkiv-Drohobych, 14-16 April 2016). Kharkiv: VN Karazin KhNU. 2016: 322-323.
9. Kul'chyns'kyi AB. Correlations between parameters EEG, HRV and phagocytosis at patients with chronic pyelonephrite and cholecystite. In: XVI International Conference "The current status and approaches to development of physical and rehabilitation medicine in Ukraine according to international standards" (15-16 December 2016, Kyiv). Kyiv, 2016: 163.
10. Kul'chyns'kyi AB**,** Gozhenko AI, Popovych IL**.** Relationships between autonomic nervous and immune systems (review) [in Ukrainian]. Actual problems of transport medicine. 2017; 3(49): 7-25.
11. Kul'chyns'kyi AB, Gozhenko AI, Zukow W, Kovbasnyuk MM, Korolyshyn TA, Popovych IL. Immune responses to balneotherapy at the spa Truskavets' at patients with chronic pyelonephritis and cholecystitis, their neuro-endocrine mechanism and associated changes in metabolism, intestinal and urine microbiocenosis and cholekinetics. In: Proceedings X Scientific Conference "Issues of pathology in conditions of extreme factors action on the body" (Ternopil', 5-6 October 2017). Ternopil', 2017: 57-58.
12. Kul'chyns'kyi AB**,** Gozhenko AI, Zukow W, Popovych IL. Neuro-immune relationships at patients with chronic pyelonephritis and cholecystitis**.** Communication 3. Correlations between parameters EEG, HRV and Immunogram. Journal of Education, Health and Sport. 2017; 7(3): 53-71.
13. Kul'chyns'kyi AB, Kovbasnyuk MM, Korolyshyn TA, Kyjenko VM, Zukow W, Popovych IL. Neuro-immune relationships at patients with chronic pyelonephrite and cholecystite. Communication 2. Correlations between parameters EEG, HRV and Phagocytosis. Journal of Education, Health and Sport. 2016; 6(10): 377-401.
14. Kul'chyns'kyi AB**,** Kyjenko VM, Zukow W, Popovych IL. Causal neuro-immune relationships at patients with chronic pyelonephritis and cholecystitis**.** Correlations between parameters EEG, HRV and white blood cell count. Open Medicine. 2017; 12(1): 201-213.
15. Kul'chyns'kyi AB**,** Zukow W, Korolyshyn TA, Popovych IL. Interrelations between changes in parameters of HRV, EEG and humoral immunity at patients with chronic pyelonephritis and cholecystitis. Journal of Education, Health and Sport. 2017; 7(9): 439-459.
16. Newberg AB, Alavi A, Baime M, Pourdehnad M, Santanna J, d'Aquili E. The measurement of regional cerebral blood flow during the complex cognitive task of meditation: a preliminary SPECT study. Psychiatry Research: Neuroimaging Section. 2001; 106: 113-122.
17. Popovych IL. The concept of neuro-endocrine-immune complex (review) [in Russian]. Medical Hydrology and Rehabilitation. 2009; 7(3): 9-18.
18. Popovych IL, Kul'chyns'kyi AB**,** Korolyshyn TA, Zukow W**.** Interrelations between changes in parameters of HRV, EEG and cellular immunity at patients with chronic pyelonephritis and cholecystitis. Journal of Education, Health and Sport. 2017; 7(10): 11-23.
19. Popovych IL, Kul'chyns'kyi AB, Kovbasnyuk MM, Korolyshyn TA, Barylyak LG, Tkachuk SP. Neuro-immune relationships in patients with chronic pyelonephritis and cholecystitis in conditions for balneotherapy on spa Truskavets'. In: Proceedings VIII Scientific Conference "Issues of pathology in conditions of extreme factors action on the body" (Ternopil', 1-2 October 2015). Ternopil'. 2015: 73-74.
20. Popovych IL, Kul'chyns'kyi AB, Kovbasnyuk MM, Korolyshyn TA, Barylyak LG, Zukow W. Neuro-immune relationships and homunculus conception. In: Pathophysiology and Pharmacy: ways of integration: Abstracts VII National Congress pathophysiologists Ukraine with international

participation (5-7 October 2016). Kharkiv: Publishing National Pharmaceutic University. 2016: 21.
21. Popovych IL, Vis'tak (Markevych) HI, Humega MD, Ruzhylo SV. Vegetotropic Effects of Bioactive Water Naftussya and their Neuroendocrine-Immune, Metabolic and Hemodynamic Accompaniments [in Ukrainian]. Kyiv: UNESCO-SOCIO. 2014: 162 p.
22. Shannon CE. Works on the theory of informatics and cybernetics [transl. from English to Russian]. Moskwa: Inostrannaya literatura. 1963: 329 p.
23. Straub RH, Pongratz G, Weidler C, Linde H-J, Kirschning CJ, Glück T, Schölmerich J, Falk W. Ablation of the Sympathetic Nervous System Decreases Gram-Negative and Increases Gram-Positive Bacterial Dissemination: Key Roles for Tumor Necrosis Factor/Phagocytes and Interleukin-4/Lymphocytes. J Infect Dis. 2005; 192(4): 560-572.
24. Sydoruk NO, Chebanenko OI, Popovych IL, Zukow W. Comparative Investigation of Physiological Activity of Water Naftussya from Truskavets' and Pomyarky Deposits [in Ukrainian]. Kyiv: UNESCO-SOCIO; 2017: 216 p.
25. Thayer JF, Sternberg EM. Neural aspects of immunomodulation: Focus on the vagus nerve. Brain Behav Immun. 2010; 24(8): 1223-1228.
26. Tracey K.J. Physiology and immunology of the cholinergic antiinflammatory pathway. J. Clin. Invest.-2007.-117(2).-P. 289-296.
27. Tracey KJ. Understanding immunity requires more than immunology. Nature Immunology. 2010; 11(7): 561-564.
28. Tracey KJ. Physiology and immunology of the cholinergic antiinflammatory pathway. J Clin Invest. 2007; 117(2): 289-296.
29. Yaremenko MS, Ivassivka SV, Popovych AI, Bilas VR, Yasevych HP, Zahorodnyuk VP et al. Physiological Bases of Curative Effect of Water Naftussya [in Russian]. Kyiv: Naukova dumka. 1989. 144 p.