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# FEATURES OF THE NEUROTROPIC EFFECTS OF PARTIAL COMPONENTS OF THE BALNEOTHERAPEUTIC COMPLEX OF SPA TRUSKAVETS'

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## Abstract

**Background.** In the previous article we outlined the results of comparative evaluation of immunotropic effects of partial components of the balneotherapeutic complex of spa Truskavets'. In the previous article we outlined the results of comparative evaluation of neurotropic effects of partial components of the complex. **Material and methods.** The object of observation were the same 41 men and 10 women aged 24-70 years old, who came to the spa Truskavets' for the treatment of chronic pyelonephritis combined with cholecystitis in remission. We recorded simultaneously parameters of HRV and EEG before and after course of balneotherapy. 23 patients drank bioactive water Naftussya (BAWN); 7 others volunteers drank Ozokerite extract; 8 patients in the third group received BAWN and baths with mineral water and for the other 13 patients in the balneotherapeutic complex included additionally application of Ozokerite on the lumbar region **Results.** It has been shown that the use of the water solution of Ozokerite dramatically increases the vagus tone and reduces the reciprocally the sympathetic tone, which is accompanied by an increase in the Amplitude of both β- and δ-rhythms as well as in the Frequency of the latter, on the one hand, and a decrease in the Index and the Deviation of θ-rhythm as well as the Amplitude of α-rhythm, on the other hand. At the same time, there is a left-sided shift in the Laterality of β- and α-rhythms. Instead, the Microbiota of Naftussya water, together with the transformed by microbes of organic substances that are related to Ozokerite, have the same pronounced but opposite effect on the listed parameters of HRV and EEG. As a result, BAWN, which contains both neurogenic antipodes in its composition, has a very moderate neurotropic effect on the listed parameters of HRV and EEG. Mineral baths activate other vagus tone markers and suppress the sympathetic marker, coupled with a decrease in the Entropy of HRV bands and β-rhythm Frequency, as well as a significant left-sided shift in Laterality both θ- and δ-hythms. The consistent use of BAWN somewhat weakens the listed effects of Baths due to

the slight opposite effect of its organic substances, but not Microbes. Instead, the application of organic substances to the skin causes a much more pronounced opposing neurotropic action. Organic substances of Ozokerite, applied to the skin, causes a increase in Bayevskiy's ARS Index as well as vagal tone and a decline in Bayevskiy's Stress Index coupled with a decrease in Indexes both β- and α-rhythms. A similar, but much weaker effect makes contact with the skin of mineral water, while, as native and transformed by microbes organic matter on the side of the mucous of the digestive tract has the opposite effects on the listed parameters of HRV and EEG. **Conclusion.** The hypothesis that as native and transformed by microbes organic matter related to Ozokerite on the side of the gut activate chemoreceptors of vagus terminals and/or TL-Receptors of Macrophages of GALT. Activated Macrophages release cytokines, which too activate vagus afferents. When applying Ozokerite or taking Baths, organic substances and mineral salts activate the skin nerve terminals and/or TL-Receptors of Langerhans cells (as variety of Macrophages of SALT) which also release cytokines.

**Key words:** Spa Truskavets'; balneotherapeutic complez; HRV; EEG.

## INTRODUCTION

In the previous article we outlined the results of comparative evaluation of immunotropic effects of partial components of the balneotherapeutic complex of spa Truskavets'. It is shown that the most pronounced as stimulating (blood level of total, active and cytolytic T-lymphocytes, Circulating Immune Complexes, IgA and Microbial Count for Staph. aureus) and suppressor (blood level of helper T-lymphocytes and Neutrophils, theirs Killing Index and Bactericydity vs both Staph. aureus and E. coli) action are the organic substances of Ozokerite that contact the surface of the skin, whereas their contact with the mucous of the digestive tract causes less pronounced immunotropic effect. Naftussya water has a stronger effect than the water solution of Ozokerite, apparently due to the additional effects of microbes and organic matter produced by them. In contrast, the bath factors affect the immune parameters of the opposite influences. Microbiota has the most pronounced enhancing effects on the Phagocytose Index of Neutrophils vs Staph. aureus and blood level of Natural Killers as well as Entropy of Leukocytogram. Instead, organic substances of Ozokerite have the same tangible but opposite effects on these parameters [12]. It is known about the role of the autonomic and central nervous systems in regulation of immunotropic action of balneotherapy at the Truskavets' spa [4-6,9-11,13,15,16,18-22]. Based on this, the aim of this study was to compare the neurotropic effects of partial components of the balneotherapeutic complex.

## MATERIAL AND METHODS

The object of observation were the same 41 men and 10 women aged 24-70 years old, who came to the spa Truskavets' for the treatment of chronic pyelonephritis combined with cholecystitis in remission.

We recorded for 7 min electrocardiogram in II lead to assess the parameters of heart rate variability (HRV) (hardware-software complex "CardioLab+HRV" "KhAI-MEDICA", Kharkiv).

For further analysis the following parameters HRV were selected. Bayevskiy's parameters: heart rate (HR), the moda (Mo), the amplitude of moda (AMo), variational sweep (MxDMn), Stress Index (BSI=AMo/2•Mo•MxDMn) as well as Activity Regulatory Systems Index (BARSI) [1]. Temporal parameters (Time Domain Methods): the standard deviation of all NN intervals (SDNN), 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 ($pNN_{50}$), triangulary index (TNN). Spectral parameters (Frequency Domain Methods): SP of HRV bands: 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) [2].

Simultaneously we recorded for 25 sec EEG a hardware-software complex "NeuroCom Standard" (production 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 on tassels of the ears. Among the options considered the average EEG amplitude (μV), modal frequency (Hz), frequency deviation (Hz), index (%), coefficient of asymmetry (%), absolute ($\mu V^2$/Hz) and relative (%) spectrum power density (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:

LI, % = Σ [200•(Right – Left)/(Right + Left)]/8.

The survey was conducted twice, before and after 7-10-days balneotherapy.

After first testing 23 patients drank bioactive water Naftussya (BAWN); 7 others volunteers drank Ozokerite extract; 8 patients in the third group received BAWN and baths with mineral water and for the other 13 patients in the balneotherapeutic complex included additionally application of Ozokerite on the lumbar region [12].

Results processed using the software package "Statistica 5.5".

## RESULTS AND DISCUSSION

The initial state of our patients (see Tables 3 and 4) is characterized by a moderately elevated sympathetic tone in combination with a moderately reduced vagal tone, which eventually manifests itself in an increased Bayevskiy's stress-index and activity of regulatory systems index. In addition to the quantitative deviations from the norm, it has been established the reduction in entropy of normalized spectral power (SP) of HRV bands calculated by classical CE Shannon's formula:

$$h = -[SPHF \cdot \log_2 SPHF + SPLF \cdot \log_2 SPLF + SPVLF \cdot \log_2 SPVLF + SPULF \cdot \log_2 SPULF]/\log_2 4$$

Moderate stress is accompanied by a small but significant increase in the amplitude of the δ-rhythm and the β-rhythm index in conjunction with the same decrease in its amplitude and frequency as well as the amplitude of α-rhythm and the deviation of the θ-rhythm frequency.

Given the known neuro-immune interactions [9,14,15,18,19,21,22] we assume that precisely such deviations in neurodynamics have led to a deviation from the norm of a number of immune parameters described in the previous article [12].

The application of the discriminant analysis method made it possible to reveal precisely the parameters of HRV and EEG, the changes of which the neurotropic effects of the balneofactors differ from each other. The forward stepwise program [8] is included in the discriminant model

the 26 parameters (Table 1), including 13 parameters of the **HRV**, 3 parameters of **δ-rhythm**, 3 parameters of **θ-rhythm,** 3 parameters of **α-rhythm** as well as 4 parameters of **β-rhythm**.

**Table 1. Summary of Stepwise Analysis of partial neurotropic effects of balneofactors**

| **Variables currently in the model** | F to enter | p-le-vel | Lam-bda | F-va-lue | p-level |
|---|---|---|---|---|---|
| **β-Amplitude, μV** | 4,87 | ,005 | ,763 | 4,9 | ,005 |
| **Moda, msec** | 4,22 | ,010 | ,598 | 4,5 | $10^{-3}$ |
| **ULF, msec²** | 3,81 | ,016 | ,477 | 4,3 | $10^{-4}$ |
| **α-Amplitude, μV** | 3,54 | ,022 | ,384 | 4,2 | $10^{-5}$ |
| **β-Index, %** | 4,19 | ,011 | ,298 | 4,4 | $10^{-5}$ |
| **VLF, msec²** | 4,13 | ,012 | ,230 | 4,5 | $10^{-6}$ |
| **θ-Deviation, Hz** | 1,97 | ,133 | ,201 | 4,2 | $10^{-6}$ |
| **δ-Amplitude, μV** | 2,19 | ,104 | ,172 | 4,0 | $10^{-6}$ |
| **ARSI, units** | 1,77 | ,169 | ,152 | 3,8 | $10^{-6}$ |
| **β-Laterality, %** | 1,68 | ,187 | ,134 | 3,7 | $10^{-6}$ |
| **HR, beats/min** | 2,15 | ,110 | ,114 | 3,6 | $10^{-6}$ |
| **RMSSD, msec** | 1,85 | ,156 | ,099 | 3,5 | $10^{-6}$ |
| **θ-Laterality, %** | 1,51 | ,230 | ,088 | 3,4 | $10^{-6}$ |
| **α-Index, %** | 1,02 | ,396 | ,080 | 3,2 | $10^{-6}$ |
| **TNN, units** | 2,57 | ,072 | ,057 | 3,3 | $10^{-6}$ |
| **LF, msec²** | 1,31 | ,287 | ,050 | 3,2 | $10^{-6}$ |
| **δ-Frequency, Hz** | 1,59 | ,213 | ,043 | 3,1 | $10^{-6}$ |
| **LF, %** | 1,29 | ,296 | ,038 | 3,0 | $10^{-6}$ |
| **α-Laterality, %** | 1,24 | ,313 | ,034 | 3,0 | $10^{-5}$ |
| **θ-Index, %** | 2,97 | ,049 | ,025 | 3,1 | $10^{-6}$ |
| **δ-Laterality, %** | 2,65 | ,070 | ,019 | 3,3 | $10^{-6}$ |
| **Entropy HRV** | 1,68 | ,197 | ,016 | 3,3 | $10^{-6}$ |
| **AMo, %** | 1,42 | ,263 | ,014 | 3,2 | $10^{-6}$ |
| **Stress Index, un** | 1,05 | ,390 | ,012 | 3,1 | $10^{-6}$ |
| **MxDMn, ms** | 1,17 | ,343 | ,010 | 3,1 | $10^{-5}$ |
| **β-Frequence, Hz** | 1,87 | ,165 | ,008 | 3,1 | $10^{-5}$ |

The discriminant information is condensed in 3 canonical roots (Table 2). In particular , the first root contains as much as 91% of discriminative properties while the second root has 5% and the third root 4% only.

The calculation of the discriminant root values for each patient as the sum of the products of raw coefficients (Table 2) to the individual values of discriminant variables together with the constant enables the visualization of each patient in the information space of the roots (Fig. 1-2).

**Table 2. Standardized and Raw Coefficients and Constants for Discriminant Variables as well as Chi-Square Tests with Successive Roots Removed**

| Coefficients | Standardized | | | Raw | | |
|---|---|---|---|---|---|---|
| Variables currently in the model | Root 1 | Root 2 | Root 3 | Root 1 | Root 2 | Root 3 |
| β-Amplitude, μV | -2,202 | -,152 | -,950 | -1,070 | -,074 | -,462 |
| Moda, msec | -3,909 | ,443 | -,137 | -,0457 | ,0052 | -,0016 |
| ULF, msec$^2$ | 3,929 | ,391 | ,457 | ,0092 | ,0009 | ,0011 |
| α-Amplitude, μV | 1,692 | ,533 | 1,433 | ,3961 | ,1248 | ,3355 |
| β-Index, % | ,293 | -,115 | -,847 | ,0204 | -,0080 | -,0589 |
| VLF, msec$^2$ | -3,374 | -,504 | ,587 | -,0036 | -,0005 | ,0006 |
| θ-Deviation, Hz | ,320 | ,062 | -,433 | ,6221 | ,1200 | -,8398 |
| δ-Amplitude, μV | -1,147 | ,132 | ,491 | -,0401 | ,0046 | ,0172 |
| ARSI, units | 1,849 | -,034 | ,359 | ,7647 | -,0143 | ,1484 |
| β-Laterality, % | 2,685 | -1,506 | -1,345 | ,0786 | -,0441 | -,0394 |
| HR, beats/min | -3,181 | -,102 | ,654 | -,4013 | -,0128 | ,0825 |
| RMSSD, msec | 1,835 | -1,750 | -,538 | ,1386 | -,1322 | -,0407 |
| θ-Laterality, % | 4,494 | ,012 | -2,007 | ,0947 | ,0003 | -,0423 |
| α-Index, % | -,920 | -,314 | -1,323 | -,0397 | -,0136 | -,0572 |
| TNN, units | -1,241 | -,797 | -1,709 | -,3636 | -,2336 | -,5008 |
| LF, msec$^2$ | -1,213 | 2,000 | 1,070 | -,0009 | ,0015 | ,0008 |
| δ-Frequency, Hz | -,658 | ,564 | ,294 | -3,626 | 3,110 | 1,623 |
| LF, % | 1,759 | -1,356 | -,198 | ,1051 | -,0810 | -,0118 |
| α-Laterality, % | -3,380 | 1,029 | 2,871 | -,0941 | ,0286 | ,0799 |
| θ-Index, % | 1,834 | -,140 | -,764 | ,0433 | -,0033 | -,0180 |
| δ-Laterality, % | -2,483 | -,106 | ,284 | -,0428 | -,0018 | ,0049 |
| Entropy HRV | -1,797 | ,419 | ,388 | -14,82 | 3,455 | 3,197 |
| AMo, % | 1,762 | -,996 | -1,129 | ,1408 | -,0795 | -,0902 |
| Stress Index, un | 1,246 | -,461 | 1,434 | ,0097 | -,0036 | ,0112 |
| MxDMn, ms | -1,930 | 2,337 | -,893 | -,0295 | ,0357 | -,0136 |
| β-Frequence, Hz | -,879 | ,492 | ,021 | -,1904 | ,1065 | ,0045 |
| | Constants | | | 2,311 | -,352 | ,464 |
| Eigenvalues | 24,16 | 1,26 | 1,14 | | | |
| Canonical R | ,980 | ,747 | ,730 | | | |
| Wilks' Λ | ,008 | ,207 | ,467 | | | |
| $\chi^2$ | 166 | 54 | 26 | | | |
| Degree of Freedom | 81 | 52 | 25 | | | |
| p-level | $<10^{-6}$ | 0,383 | 0,395 | | | |

**Table 3. Discriminant Function Analysis Summary of partial neurotropic effects of balneofactors**

Step 26, N of vars in model: 26; Grouping: 4 grps
Wilks' Lambda: 0,0082; approx. $F_{(82)}$=3,13; $p<10^{-5}$

| Variables currently in the model | Norm | Cv | Ba-sal level (51) | Change after course of | | | | Wil ks' Λ | Par-tial Λ | F-re-mo-ve | p-le-vel | Tole-ran-cy |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | O drink (7) | NB (8) | NBO ap (13) | N (23) | | | | | |
| **LF, msec²** | 627 | 0,528 | 955 | **+1337** | +222 | +84 | **-309** | ,010 | ,821 | 1,5 | ,236 | ,042 |
| **VLF, msec²** | 1384 | 0,576 | 1258 | **+481** | +412 | +45 | **-504** | ,017 | ,470 | 7,9 | ,001 | ,047 |
| **β-Amplit, μV** | 13,6 | 0,313 | 12,3 | **+3,2** | -0,4 | -0,3 | 0,0 | ,024 | ,338 | 13,7 | $10^{-4}$ | ,128 |
| **Moda, msec** | 870 | 0,116 | 843 | **+71** | +10 | -69 | -31 | ,020 | ,418 | 9,7 | $10^{-3}$ | ,039 |
| **δ-Freque, Hz** | 1,08 | 0,170 | 1,07 | **+0,14** | -0,10 | +0,06 | -0,02 | ,010 | ,836 | 1,4 | ,278 | ,257 |
| **δ-Amplit, μV** | 13,3 | 0,442 | 21,8 | **+15,6** | -0,9 | -8,7 | +2,9 | ,012 | ,661 | 3,6 | ,031 | ,242 |
| **ULF, msec²** | 122 | 1,021 | 169 | **-438** | -93 | +116 | -21 | ,014 | ,584 | 5,0 | ,009 | ,028 |
| **HR, beat/min** | 68,9 | 0,120 | 71,6 | **-6,0** | -0,2 | +5,0 | +1,1 | ,014 | ,579 | 5,1 | ,008 | ,042 |
| **LF, %** | 25,3 | 0,414 | 32,8 | **-2,6** | +1,1 | +2,2 | -0,3 | ,013 | ,637 | 4,0 | ,022 | ,090 |
| **θ-Deviat, Hz** | 1,06 | 0,647 | 0,89 | **-0,36** | +0,10 | -0,13 | **+0,11** | ,009 | ,903 | ,8 | ,532 | ,485 |
| **θ-Index, %** | 25 | 1,679 | 22 | **-36** | -20 | -17 | **0** | ,019 | ,440 | 8,9 | $10^{-3}$ | ,158 |
| **α-Amplit, μV** | 22,1 | 0,657 | 18,3 | **-2,6** | -2,4 | -2,0 | **+1,0** | ,013 | ,642 | 3,9 | ,023 | ,090 |
| **β-Lateral, %** | -8±3 | | -6±4 | **-25** | 0 | -7 | -10 | ,019 | ,428 | 9,4 | $10^{-3}$ | ,063 |
| **α-Lateral, %** | -2±2 | | -4±5 | **-21** | -18 | -17 | **-11** | ,018 | ,468 | 8,0 | $10^{-3}$ | ,033 |
| **RMSSD, msec** | 27,6 | 0,486 | 28,3 | +7 | **+10** | +1 | **-4** | ,012 | ,670 | 3,5 | ,035 | ,065 |
| **TNN, units** | 11,2 | 0,217 | 11,1 | +1,3 | **+2,2** | -0,6 | **-0,8** | ,011 | ,738 | 2,5 | ,088 | ,077 |
| **AMo, %** | 36,5 | 0,250 | 44,7 | -2,7 | **-5,4** | -0,2 | **+2,7** | ,011 | ,773 | 2,1 | ,136 | ,054 |
| **β-Freque, Hz** | 19,2 | 0,179 | 17,9 | -1,9 | **-4,8** | +2,4 | +1,4 | ,010 | ,789 | 1,9 | ,165 | ,241 |
| **Entropy HRV** | 0,788 | 0,127 | 0,703 | -0,02 | **-0,04** | +0,05 | +0,03 | ,013 | ,633 | 4,1 | ,020 | ,112 |
| **θ-Lateral, %** | -5±3 | | -8±5 | -18 | **-48** | -22 | **-3** | ,022 | ,372 | 11,8 | $10^{-4}$ | ,029 |
| **δ-Lateral, %** | +1±4 | | -6±5 | -16 | **-23** | -4 | **+5** | ,015 | ,565 | 5,4 | ,007 | ,073 |
| **β-Index, %** | 87,9 | 0,197 | 94,0 | +3 | +8 | **-15** | -1 | ,009 | ,885 | ,9 | ,452 | ,244 |
| **α-Index, %** | 50 | 0,625 | 55 | -9 | -4 | **-15** | **+5** | ,011 | ,722 | 2,7 | ,072 | ,155 |
| **ARSI, units** | 0÷3 | | 3,4 | -0,6 | -0,2 | **+0,8** | **-1,4** | ,020 | ,412 | 10,0 | $10^{-3}$ | ,176 |
| **MxDMn, ms** | 260 | 0,293 | 212 | +15 | +6 | **+15** | +5 | ,010 | ,786 | 1,9 | ,159 | ,030 |
| **Stress Ind, un** | 137 | 0.280 | 189 | -32 | -20 | -33 | -38 | ,010 | ,783 | 1,9 | ,154 | ,080 |

**Table 4. Factor Structure Matrix (Correlations Variables-Canonical Roots), Roots Means, Z-scores of Basal levev and Changes in Variables**

| | Root 1 | Root 2 | Root 3 | Ozok. drink | Naft+ Baths | N+B+ Oz app | Naftu-ssya | Basal level (n=51) |
|---|---|---|---|---|---|---|---|---|
| **Root 1 (91%)** | | | | **-11,5** | -0,16 | +1,12 | +2,92 | |
| LF, Z | **-,083** | -,022 | ,049 | **+2,27** | +0,49 | +0,32 | **-1,08** | +0,94±0,38 |
| VLF, Z | **-,069** | -,159 | ,135 | **+0,68** | +0,43 | +0,07 | **-0,64** | -0,19±0,17 |
| β-Amplitude, Z | **-,110** | ,112 | -,063 | **+0,75** | -0,09 | -0,06 | -0,01 | -0,30±0,11 |
| Moda HRV, Z | **-,081** | -,035 | -,183 | **+0,69** | +0,10 | -0,69 | -0,31 | -0,16±0,22 |
| δ-Frequency, Z | **-,059** | ,144 | ,119 | **+0,78** | -0,54 | +0,34 | -0,12 | -0,07±0,13 |
| δ-Amplitude, Z | **-,035** | ,047 | -,123 | **+2,70** | -0,16 | -1,49 | +0,50 | +1,44±0,41 |
| ULF, Z | **,077** | ,008 | ,089 | **-3,52** | -0,75 | +0,93 | -0,32 | +0,38±0,54 |
| HR, Z | **,062** | -,016 | ,181 | **-0,54** | -0,01 | +0,44 | +0,10 | +0,13±0,17 |
| LF %, Z | **,018** | -,005 | ,016 | **-0,25** | +0,11 | +0,21 | -0,03 | +0,72±0,20 |
| θ-Deviation, Z | **,059** | -,045 | -,111 | **-0,52** | +0,15 | -0,18 | **+0,17** | -0,25±0,09 |
| θ-Index, Z | **,054** | ,057 | -,088 | **-0,84** | -0,47 | -0,40 | **0,00** | -0,07±0,11 |
| α-Amplitude, Z | **,051** | ,127 | -,170 | **-0,18** | -0,17 | -0,14 | **+0,07** | -0,26±0,10 |
| β-Laterality, % | **,031** | -,087 | ,030 | **-25** | 0 | -7 | -10 | -6±4% |
| α-Laterality, % | **,019** | ,027 | -,043 | **-21** | -18 | -17 | **-11** | -4±5% |
| **Root 2 (5%)** | | | | +0,55 | **-2,48** | +0,23 | +0,57 | |
| RMSSD, Z | -,054 | **-,190** | ,081 | +0,67 | **+0,80** | +0,27 | **-0,58** | +0,42±0,33 |
| TNN, Z | -,041 | **-,161** | -,013 | +0,53 | **+0,91** | -0,26 | **-0,32** | +0,17±0,33 |
| AMo, Z | ,032 | **,129** | -,065 | -0,37 | **-0,58** | -0,11 | **+0,29** | +1,04±0,33 |
| β-Frequency, Z | ,052 | **,266** | ,089 | -0,54 | **-1,40** | +0,69 | +0,42 | -0,39±0,19 |
| Entropy HRV, Z | ,029 | **,121** | ,070 | -0,16 | **-0,44** | +0,55 | +0,37 | -0,84±0,17 |
| θ-Laterality, % | ,018 | **,189** | -,097 | -18 | **-48** | -22 | **-3** | -8±5% |
| δ-Laterality, % | ,023 | **,087** | -,028 | -16 | **-23** | -4 | **+5** | -6±5 |
| **Root 3 (4%)** | | | | -0,32 | -0,31 | **+1,72** | -0,77 | |
| β-Index, Z | -,027 | -,137 | **-,287** | +0,17 | +0,47 | **-0,85** | -0,05 | 0,35±0,07 |
| α-Index, Z | ,035 | ,044 | **-,220** | -0,30 | -0,13 | **-0,47** | **+0,16** | +0,17±0,13 |
| ARSI, units | -,017 | -,085 | **,243** | -0,6 | -0,2 | **+0,8** | **-1,4** | 3,4±0,4 |
| MxDMn, Z | -,009 | ,003 | **,045** | +0,25 | +0,10 | **+0,27** | +0,09 | -0,74±0,22 |
| Stress Index, Z | ,001 | -,027 | **,061** | -0,78 | -0,55 | -0,75 | -0,93 | +1,22±0,64 |

Note. Changes in Laterality can not be mathematically calculated in the Z-score; Bayevskiy's ARSI is by definition a Z.

The extremal left-side localization along the axis of the first root of clusters of patients who received water solution of Ozokerite reflects the maximum **increase** of 6 parameters that correlate with this root **inversely** as well as maximum **decrease** of others 6 parameters that correlate with this root **directly** (Table 4). Instead, the rightmost zone of the axis occupies cluster whose members drank BAWN. The intermediate position is occupied by patients who received two or three balneofactors. In this case, the members of the cluster are mixed together.

Instead, along the axis of the second root, both clusters are clearly delineated. The extreme lower position of patients receiving BAWN and Baths reflects the maximal increase in vagal tone and reciprocal loss of sympathetic tone, coupled with a decrease in the entropy of HRV bands and β-rhythm frequency, as well as a significant left-sided shift in Laterality both θ- and δ-hythms.

Finally, along the third root axis, the highest localized patients who received all three balneofactors, which caused a decrease in Indexes both β- and α-rhythms coupled with an increase in Bayevskiy's ARS Index as well as vagal tone and a decline in Bayevskiy's Stress Index.

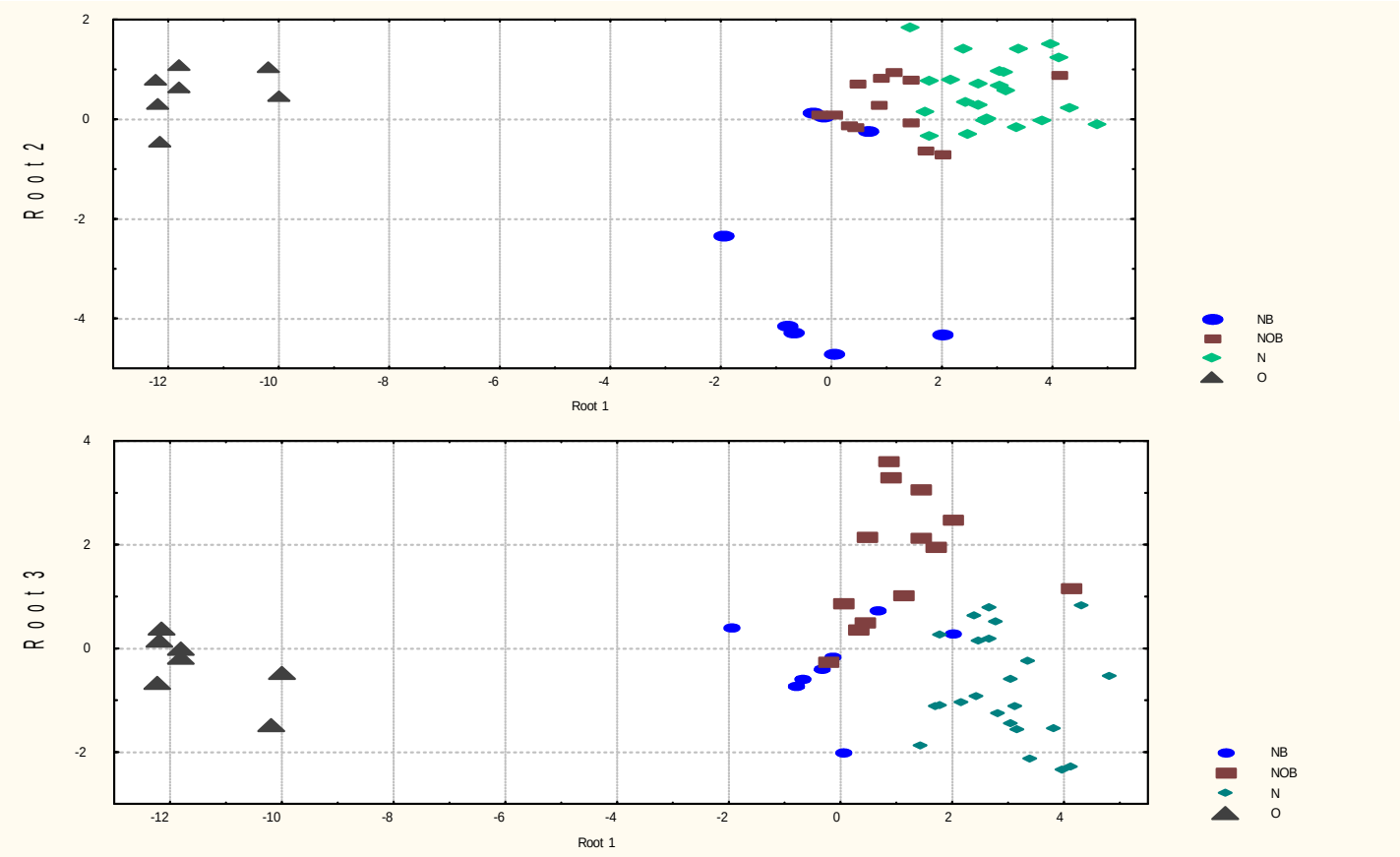


**Fig. 1. Individual sizes of canonical discriminatory roots of changes in HRV and EEG caused by balneofactors**

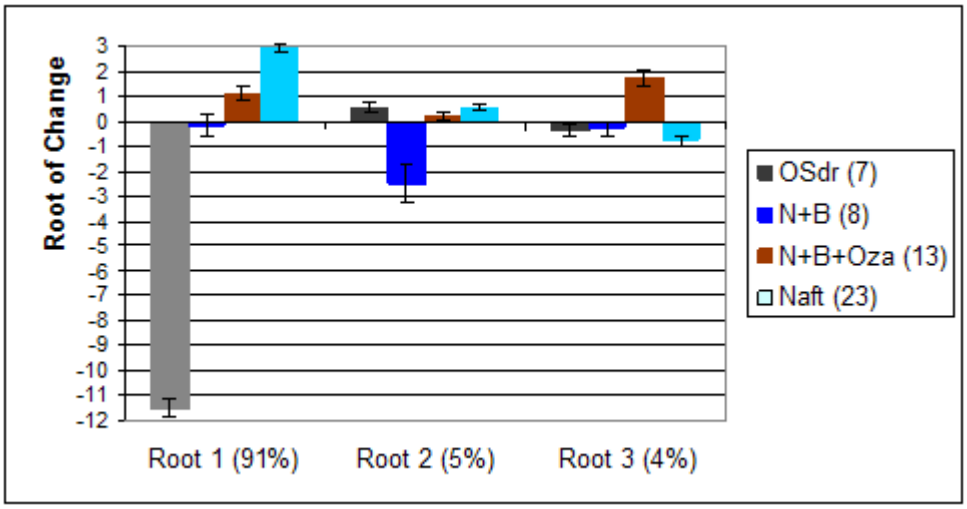


**Fig. 2. Means of canonical discriminatory roots of changes in HRV and EEG caused by balneofactors**

The calculation of Squared Mahalanobis Distances (Table 5) shows the unambiguous uniqueness of the effects of the use of the water solution of Ozokerite, while the delimitation of other clusters is statistically insignificant. Nevertheless, the accuracy of the classification (see Tables 6 and 7) was unmistakable also with respect to drinking BAWN as well as high in relation to the use of a full range of factors.

**Table 5. Squared Mahalanobis Distances (over diagonal), F-values (under diagonal) and p-levels (in brackets) between immunotropic effects of balneofactors**

| Balneofactors | Naft+ Baths | N+B+ Oz app | Naftu -ssya | Ozok. drink |
|---|---|---|---|---|
| Naftussya + Baths | 0 | 14,2 | 20,6 | 149 |
| Naftussya + Baths + Oz application | 1,04 (0,470) | 0 | 10,4 | 177 |
| Naftussya only | 1,81 (0,084) | 1,33 (0,253) | 0 | 225 |
| Ozokerite drink | 7,97 ($10^{-5}$) | 11,71 ($10^{-6}$) | 17,57 ($10^{-6}$) | 0 |

**Table 6. Coefficients and Constants for Classification Functions**

| Balneofactors | Naft+ Baths | N+B+ Oz app | Naftu-ssya | Ozok. drink |
|---|---|---|---|---|
| Variables | p=,157 | p=,255 | p=,451 | p=,137 |
| **β-Amplitude, μV** | 3,723 | 1,219 | ,417 | 15,63 |
| **Moda HRV, msec** | ,102 | ,055 | -,022 | ,635 |
| **ULF, msec²** | -,035 | -,019 | -,004 | -,137 |
| **α-Amplitude, μV** | -2,078 | -,553 | -,633 | -6,191 |
| **β-Index, %** | -,025 | -,140 | ,041 | -,279 |
| **VLF, msec²** | ,011 | ,007 | -,002 | ,050 |
| **θ-Deviation, Hz** | ,143 | -,443 | 2,807 | -6,525 |
| **δ-Amplitude, μV** | ,033 | ,030 | -,084 | ,502 |
| **ARSI, units** | -2,204 | -,965 | ,037 | -10,91 |
| **β-Laterality, %** | -,067 | -,166 | ,059 | -1,090 |
| **HR, beats/min** | ,903 | ,523 | -,408 | 5,407 |
| **RMSSD, msec** | ,088 | -,176 | ,130 | -1,882 |
| **θ-Laterality, %** | -,234 | -,198 | ,077 | -1,305 |
| **α-Index, %** | ,239 | ,035 | ,101 | ,648 |
| **TNN, units** | 1,959 | -,155 | ,359 | 5,375 |
| **LF, msec²** | -,004 | ,001 | -,003 | ,011 |
| **δ-Frequency, Hz** | -6,955 | ,130 | -9,386 | 43,52 |
| **LF, %** | -,100 | -,209 | -,018 | -1,535 |
| **α-Laterality, %** | ,071 | ,191 | -,168 | 1,222 |
| **θ-Index, %** | -,102 | -,092 | ,030 | -,602 |
| **δ-Laterality, %** | ,146 | ,096 | ,006 | ,625 |
| **Entropy HRV** | 42,70 | 39,61 | 6,160 | 220,9 |
| **AMo, %** | -,044 | -,263 | ,188 | -1,878 |
| **Stress Index, units** | -,052 | -,027 | -,038 | -,173 |
| **MxDMn, msec** | ,074 | ,105 | ,098 | ,516 |
| **β-Frequency, Hz** | ,413 | ,468 | ,150 | 2,892 |
| **Constants** | -8,814 | -4,387 | -3,509 | -99,19 |

**Table 7. Classification Matrix**
Rows: Observed classifications
Columns: Predicted classifications

| | Percent correct | N+B | N+B+ O app | Naftus-sya | Ozokerite drink |
|---|---|---|---|---|---|
| | | p=,157 | p=,255 | p=,451 | p=,137 |
| Naftussya + Baths | 62,5 | **5** | **3** | 0 | 0 |
| Naftussya + Baths + Oz application | 92,3 | 0 | **12** | **1** | 0 |
| Naftussya only | 100 | 0 | 0 | **23** | 0 |
| Ozokerite drink | 100 | 0 | 0 | 0 | **7** |
| **Total** | 92,2 | 5 | 15 | 24 | 7 |

The application of the algorithm outlined in the previous article [12] makes it possible to indirectly evaluate the partial neurotropic effects of each active ballenofactor.

It has been shown (Fig. 3) that the use of the water solution of Ozokerite dramatically increases the vagus tone and reduces the reciprocally the sympathetic tone, which is accompanied by an increase in the Amplitude of both β- and δ-rhythms as well as in the Frequency of the latter, on the one hand, and a decrease in the Index and the Deviation of θ-rhythm as well as the Amplitude of α-rhythm, on the other hand. At the same time, there is a left-sided shift in the Laterality of β- and α-rhythms. Instead, the Microbiota of Naftussya water, together with the transformed by microbes of organic substances that are related to Ozokerite, have the same pronounced but opposite effect on the listed parameters of HRV and EEG. As a result, BAWN, which contains both neurogenic antipodes in its composition, has a very moderate neurotropic effect on the listed parameters of HRV and EEG.

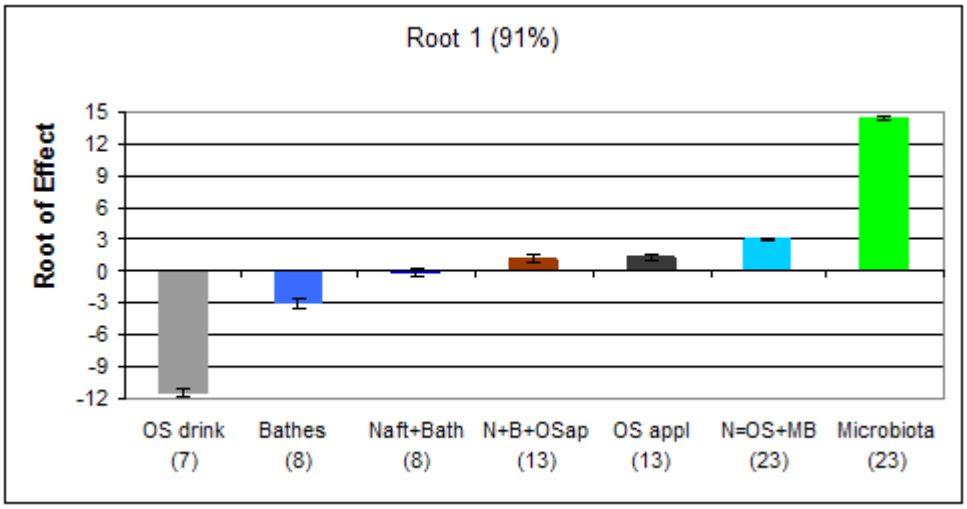


**Fig. 3. Actual and calculated means of Root 1 of changes in HRV and EEG caused by balneofactors**

Mineral baths activate other vagus tone markers and suppress the sympathetic marker (Fig. 4), coupled with a decrease in the Entropy of HRV bands and β-rhythm Frequency, as well as a significant left-sided shift in Laterality both θ- and δ-hythms. The consistent use of BAWN somewhat weakens the listed effects of Baths due to the slight opposite effect of its organic substances, but not Microbes. Instead, the application of organic substances to the skin causes a much more pronounced opposing neurotropic action.

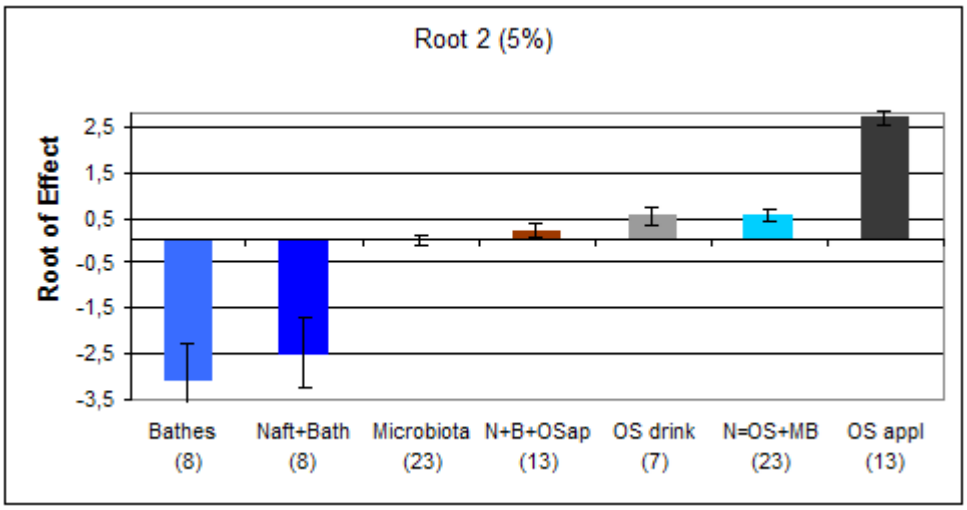


**Fig. 4. Actual and calculated means of Root 2 of changes in HRV and EEG caused by baln**

Organic substances of Ozokerite, applied to the skin, causes a increase in Bayevskiy's ARS Index as well as vagal tone and a decline in Bayevskiy's Stress Index coupled with a decrease in Indexes both β- and α-rhythms. A similar, but much weaker effect makes contact with the skin of mineral water, while, as native and transformed by microbes organic matter on the side of the mucous of the digestive tract has the opposite effects on the listed parameters of HRV and EEG (Fig. 5).

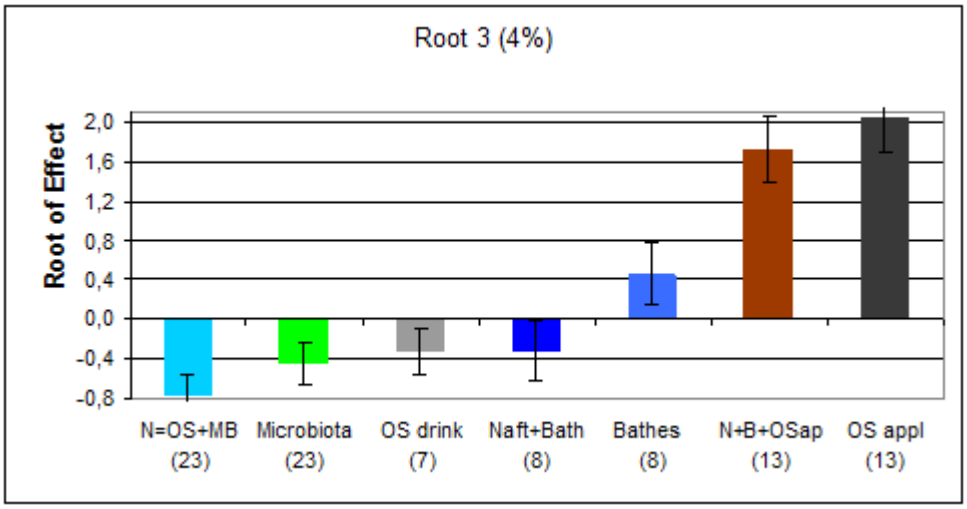


**Fig. 5. Actual and calculated means of Root 3 of changes in HRV and EEG caused by balneofactors**

## CONCLUSION

On the basis of the results as well as modern ideas [3,7,14,15,21] we put forward the hypothesis that as native and transformed by microbes organic matter related to Ozokerite on the

side of the gut activate chemoreceptors of vagus terminals and/or TL-Receptors of Macrophages of GALT. Activated Macrophages release cytokines, which too activate vagus afferents. When applying Ozokerite or taking Baths, organic substances and mineral salts activate the skin nerve terminals and/or TL-Receptors of Langerhans cells (as variety of Macrophages of SALT) which also release cytokines.

Detailed justification of the hypothesis will be the subject of the following article.


## ACKNOWLEDGMENT

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## 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.

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