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Andriy B Kul'chyns'kyi, Valeriy M Kyjenko, Walery Zukow*, Igor L Popovych

Causal neuro-immune relationships at patients with chronic pyelonephritis and cholecystitis. Correlations between parameters EEG, HRV and white blood cell count

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Abstract: We aim to analyze in bounds KJ Tracey's immunological homunculus conception the relationships between parameters of electroencephalogram (EEG) and heart rate variability (HRV), on the one hand, and the parameters of bhite blood cell count, on the other hand.

Methods. In basal conditions in 23 men, patients with chronic pyelonephritis and cholecystitis in remission, recorded EEG ("NeuroCom Standard", KhAI Medica, Ukraine) and HRV ("Cardiolab+VSR", KhAI Medica, Ukraine). In portion of blood counted up white blood cell count.

Results. Revealed that canonical correlation between constellation EEG and HRV parameters form with blood level of leukocytes 0.92 ($p<10-5$), with relative content in white blood cell count stubnuclear neutrophiles 0.93 ($p<10-5$), segmentonucleary neutrophiles 0.89 ($p<10-3$), eosinophiles 0.87 ($p=0.003$), lymphocytes 0.77 ($p<10-3$) and with monocytes 0.75 ($p=0.003$).

Conclusion. Parameters of white blood cell count significantly modulated by electrical activity some structures of central and autonomic nervous systems.

Keywords: HRV; EEG; White blood cell count; Correlations

1 Introduction

It is believed that the first immunologist who raised the problem of the relationship between the nervous and immune systems was Pfeiffer; he wrote that understanding the nature of immunity must penetrate the nature of physiological processes in brain neurons [1].

Although there are still some disagreements about specific aspects of neural-immune communications, much evidence to supports the existence of numerous interactions between the central nervous system, the peripheral nervous system (both sympathetic and parasympathetic divisions), and immune systems [2-9].

Whereas some studies have shown that catecholamines and activation of sympathetic nervous system enhance immune and inflammatory responses [10, 11], most studies indicate the inhibitory effects on inflammatory responses [12]. In vitro, norepinephrine mediates its immunosuppressive effects on dendritic cells and monocytes via inhibition of production of proinflammatory cytokines, including TNF-α, IL-1, IL-6 i IL-12, whereas products under these conditions, inflammatory cytokines such as IL-10 activated these cells. Rather, a pharmacological blockade of adrenergic receptors potentiates the secretion of IL-6 and inhibits the secretion of IL-10 in vivo. Noradrenalin also increases the secretion of chemokines by fibroblasts and increases the migration of natural killer cells, macrophages, and monocytes, but inhibits the migration of dendritic cells in vitro. Norepinephrine inhibits in vitro chemotactic response of dendritic cells in chemokines (important for the migration of dendritic cells from the site of antigen to the regional lymph nodes) by activating the regulation of production of anti-inflammatory IL-10 [12]. However, it has been shown that depletion

***Corresponding author: Walery Zukow,** Faculty of Physical Education, Health and Tourism, Kazimierz Wielki University, Bydgoszcz, Poland, E-mail: w.zukow@ukw.edu.pl
Andriy B Kul'chyns'kyi, Communal Hospital, Truskavets', Ukraine
Valeriy M Kyjenko, Igor L Popovych, Laboratory of Experimental Balneology, OO Bogomoletz Institute of Physiology NAS, Kyiv, Ukraine

of norepinephrine reduces resistance to some bacterial infections [13-15]; according to EM Sternberg [3], the role of sympathetic nervous system in responses to bacterial resistance of the organism remains uncertain.

In the review by DM Nance and VM Sanders [2] it is noted that in vitro experiments with macrophages isolated from spleen and lymph nodes, and noradrenaline by β-adrenergic receptors may dramatically (indicative) inhibit the production and secretion of TNF-α in response to LPS. Less permanent (consistency) results were observed for production of IL-1β, but on the whole noradrenaline is considered an inhibitor of this cytokine. Noted as the inhibitory and activating effect on the products of IL-6, the direction of response IL-6 to noradrenaline may depend on simultaneous (competitive) presence or absence of LPS. The same in vitro studies also show that activation of α-adrenergic receptor agonists specifically exercises a stimulating effect on TNF-α production by macrophages in response to LPS. However, in vivo activation of the sympathetic nervous system by stress or central inflammatory stimulus inhibits the function of splenic macrophages, indicating the dominant influence of β-adrenergic mechanisms. The authors conclude that activation of the sympathetic nervous system (noradrenergic nerves and adrenal medully) has powerful anti-inflammatory effects on the innate immune system. Among the pro-inflammatory cytokines produced by macrophages, TNF-α is the principal product and its release is regulated by sympathetic nervous system.

Interestingly, these same inflammatory cytokines have a prominent place in the "cholinergic anti-inflammatory hypothesis" of the KJ Tracey laboratory. He and his colleagues [5-8, 16-20] believe that the crucial role in immunomodulation is played by the parasympathetic nervous system, both afferent and efferent. We know that most vagally fibers (80%) are sensory by nature and provide effective coverage of the body to detect damage (invasions). Many species of animals and humans show links to the vagus afferents from the heart, lungs, esophagus, liver, and other organs. The nature of this "wandering" way is such that the body vagus nerves are uniquely structured to provide an effective early warning system and detection of pathogens, as well as a source of negative feedback from the immune system after exposure to a pathogen [21].

Currently, there is a discussion in the literature about the role in the regulation of inflammation of sympathetic and parasympathetic parts of the autonomic nervous system. On one hand, KJ Tracey et al. [5-8, 16-20] proved that efferent portion vagus nerve, and thus the parasympathetic nervous system, plays a unique and powerful role in the regulation of systemic and localized inflammation, mainly through inhibition of production of TNF-α by macrophages. They showed that vagally efferent stimulation may inhibit endotoxin-induced sepsis and production of TNF-α, as well as localized inflammation induced in the dermal air bag. However, the adrenal medullary zone and sympathetic nerves also inhibit production of TNF-α by macrophages and systemic inflammation [2, 3, 14, 22]. SY Yoon et al. [23] also showed that inhibition of localized inflammation in models of the air bag-mediated sympathoadrenal mechanism. It seems that sympathetic and parasympathetic nervous systems mediate inhibition of TNF-α production and inflammation, but no evidence has been produced of an anti-inflammatory role for efferents of vagus nerves independent of the sympathetic nervous system. DM Nance and VM Sanders [2] argue that to date no there is neuroanatomic evidence for vagal efferent entrance in immune organs and body regions outside the respiratory and alimentary tract and internal visceral organs such as the heart and pancreas. Moreover, the absence of a vesicular acetylcholine transporter in the fibers that innervate lymphoid organs indicates the absence of a parasympathetic entrance to the immune system [24]. However, these facts came as a surprise to some authors; for some reason, other researchers generally ignored them. The KJ Tracey Research laboratory focused on α7 nicotinic cholinergic receptors mainly as mediators of anti-inflammatory signal transmitted through efferentes vagus nerve [20]. Acetylcholine binds to two major subtypes of receptors—nicotinic and muscarinic cholinergic receptors—each of which consists of many different subunits that provide cellular and tissue specificity cholinergic effects. Both of those receptors are found on immune cells, but nicotine receptors specifically mediate cholinergic anti-inflammatory effects on macrophages. Cholinergic receptors mainly expressed on macrophages are α7-subunit nicotinic acetylcholine receptosr [20]. Activation of this receptor on macrophages inhibits NF-κB signaling, thereby inhibiting the production and release of pro-inflammatory cytokines [18, 20]. Acetylcholine also inhibits the endotoxin-induced release of pro-inflammatory cytokines (IL-1, IL-6 i TNF-α), but no anti-inflammatory cytokines (IL-10) by macrophages [21, 20]. The effects of the parasympathetic nervous system on the migration of white blood cells are less clear. Thus, acetylcholine increasing production CCL2 by monocytes, by stimulation of the vagus nerve and acetylcholine agonist and acting through the α7-subunit, inhibit recruitment of leukocytes to endothelial cells, suppressing expression of vascular cell adhesion molecule I (VCAMI) [19]. Taken together, these parasympathetic mechanisms form the

so-called "cholinergic anti-inflammatory mechanism" [5-8].

However, nicotinic receptors containing the α7-subunit mediate the relationship between spinal preganglionic sympathetic cholinergic neurons and produce catecholamine neurons localized in sympathetic ganglia and adrenal medulla zone [25]. Thus, nicotine stimulates the release of catecholamines through activation of nicotinic receptors localized in the peripheral postganglionic sympathetic neurons and adrenal medulla [3, 26].

Infectious agents (bacteria and viruses as well as their toxins and cell membrane components) and vacated damaged cell material (DNA, HMGB1, uric acid) are able to activate the TL-receptors in the cell membrane of tissue macrophages, dendritic and epithelial cells, T- and B-lymphocytes GALT (Gut-associated lymphoid tissue). [5, 27] Activating the TL-receptors of immune cells leads to release of their pro-inflammatory cytokines (TNF, IL-1, IL-4) and HMGB1. Paraganglia glomus cells located in the vagal ganglia express IL-1 receptors [28, 29]. Therefore, information about the presence of cytokines, including IL-1, on the periphery is passed through chemosensitive glomus cells and afferent fibers in the nervus vagus to structures in the central nervous system, one of the most important of which is the nucleus tractus solitarius (NTS).

NTS is a large relay station for neuro-immune communication [3]. On the afferent side vagally terminals in somatotopical manner entering the functional units NTS [30]. This somatotopical organization allows a high degree of localization and specificity of immune–brain communication. This is important because the anatomical location of the pathogens transmits the information necessary to establishof local specificity, and thereby a more effective immune response. Additionallhy, NTS has direct and indirect links to a wide range of nervous structures, giving the vagus nerve ability to influence a wide range of processes [31].

From NTS—more precisely, its dorsal medial portio (dmNTS)— in the integrative set point center is divided into the parasympathetic and sympathetic branches. For parasympathetic branch neurons dmNTS are projected to the nucleus dorsalis motoris n. vagus and nucleus ambiguous (NA), whose axons innervate the intramural plexus, including the heart and ganglion coeliacum. On the other hand, the sympathetic branch projects to the caudal ventro-lateral medulla, and then to the rostral ventro-lateral medulla (RVLM), which is the signal from nuclei that goes back to a ganglion coeliacum and hence to the N-cholinergic receptors organs associated with the immune system, including the heart, liver, and gastrointestinal system. In addition, impulses from RVLM activate the adrenal cortex, increasing the release of glucocorticoids [3, 32].

Signals ascending from NTS in the vagus reaches M1-cholinergic neurons of the parabrachial nucleus, the thalamus, the paraventricular nucleus, the central nucleus of the amygdala, the insula cortex, and in animals, the intralimbic cortex including the homologous sites in humans of the anterior cingulate cortex (ACC) and the medial prefrontal cortex (MPFC) [8, 32, 33].

The most important subject of regulatory influence the vagus spleen as "the most important organ for antibacterial and antifungal immune reactivity." These vagally signals through N-cholinergic receptors (or rather their α7-subunit) macrophages inhibit the release of pro-inflammatory cytokines. In addition, these neural signals also modulate the ability to traffic circulating neutrophils and monocytes, affecting their ability to recruit regions inflammation in peripheral tissues [8].

Objects that regulatory influence parasympathetic and sympathetic divisions of the autonomic nervous system and the hypothalamic-pituitary-adrenocortical axis, or rather their neurotransmitters acetylcholine, noradrenaline, and glucocorticoids, are other than spleen organs of the immune system as thymus, lymph nodes, bone marrow, or rather their macrophages/monocytes, microphages/neutrophils, NK-, T- and B-lymphocytes, and epitheliocytes gastroduodenal mucosa as components the classic triad of stress [H Selye, 3].

EA Korneva in 1993 launched the hypothesis of the existence of a "indivisible immune-neuro-endocrine complex, which is involved in ensuring the constancy of internal environment" [35]. This suggestion was developed by IG Akmayev [36] and PN Uchakin et al. [37]. IL Popovych [38] proposed changing the term to "neuroendocrine-immune complex"; this has been widely accepted [39].

The results of research ties immediate reactions to nonantigen stimuli (exercise, hypoxia and hyperthermia) indicators of immunity and nonspecific resistance, on the one hand, with indicators of neuroendocrine regulation on the other hand, are summarized in the monograph by TI Kolyada et al. [1].

The results of research in this vein using the bioactive water Naftussya, which is the carrier as nonantigen (organic substances water-bearing rocks [40]) and antigen (autochthonous microflora [40]) stimuli, are summarized in the articles [38, 41-49] and monographs [50-53] of the Truskavetsian Balneology Scientific School.

According to KJ Tracey [5], there is a structured, organized somatotopically neural network that controls the specific components of the immune response through

communication input and output. This theoretical organization, similar to the classic homunculus, demonstrates that specific area of the brain exercise control specific parts of the body and that in the future, it will be possible to construct a "immune homunculus." Like the classic map of the brain that is associated with somatotopically specific neural structure and has specific effects on the periphery, an immunological homunculus can show that there are specific areas of the brain associated with modulation of specific immune functions. For example, one region of the brain can control the cytokine response in the liver, and another, the activation of T-cells in the spleen or lymph nodes. Some centers can integrate information about the presentation of antigens, while others influence the maturation of dendritic cells. Some neurological domains in the CNS can regulate the overall state of readiness to respond to innate immunity to pathogens or injury.

At this timepoint, data on the somatotopical organization of immune functions are scarce and unstructured. In this regard, LA Henderson et al. [54], applying functional magnetic resonance imaging (fMRI), showed that pain from various locations as well as from various tissues is represented in the cortex of insula somatotopically. T Weiss et al. [55], using the same method, showed that different vagal nerve afferent fibers (e.g., Aδ against C) represented differentially in the cortex of man.

According to JF Thayer and EM Sternberg [4], advances in neuroimaging and other related technologies, such as transcranial magnetic stimulation (TMS), will allow mapping of the neural satellites of a number of immune functions, so that a certain area of the brain may be associated with the control reactions of cytokines in the liver, while another region could map the distribution of NK-lymphocytes.

In the present report we begin a series of articles about neuro-immune relationships in healthy humans and patients with chronic diseases. The goal of this series is the development and concretization of the concept of the immunological homunculus. However, we hope to recruit specialists in medical rehabilitation, adaptology, physical education, and other related areas to conduct their research in line with the aforementioned concept.

2 Material and methods

Our observations are based on 23 men aged 24 to 70 years (mean 49.1±2.5) who came to the spa Truskavets' (Ukraine) for the treatment of chronic pyelonephritis combined with cholecystitis in remission. We first recorded a 7-minute electrocardiogram in the II lead to assess the parameters of HRV (hardware-software complex "CardioLab+HRV" production "KhAI-MEDICA", Kharkiv). For further analysis, the following parameters of heart rate variability (HRV) were selected: 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 (pNN50); triangulary index (HRV TI) [56-58]; heart rate (HR); moda (Mo) and the amplitude of moda (AMo); and variational sweep (MxDMn) [56]. Spectral parameters (Frequency Domain Methods) were: power spectral density (PSD) bands (components) 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). Expressed as classical indexes: LF/HF where LFnu=100%•LF/(LF+HF) and Centralization Index (CI=(VLF+LF)/HF); Baevskiy's Stress Index (BSI=AMo/2•Mo•MxDMn) as well as Baevskiy's Activity Regulatory Systems Index (BARS) [2], both in supine and orthostatic positions.

Next, during a 25-second EEG recorded by 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 parameters considered were the average EEG amplitude (μV), average frequency (Hz), frequency deviation (Hz), index (%), coefficient of asymmetry (%), raw (μV2/Hz) and relative (%) PSD of basic rhythms: β (35÷13 Hz), α (13÷8 Hz), θ (8÷4 Hz), and δ (4÷0,5 Hz) in all loci, according to the manufacturer's instructions for the device. In addition, we calculated the Laterality Index (LI) for PSD of each rhythm using the formula [58-63]:

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

After ECG and EEG recording, we assessed the white blood cell count in a portion of the capillary blood.

Quantification of the Laterality/Asymmetry electrical activity of brain due to various deposits is necessary: the right and left side cortical structures that regulate the autonomic nervous system.

Using functional magneto-resonance visualization, it was found that both dorsal and ventral parts of the anterior cingulate cortex are significantly involved in autonomic control [64, 65]. Activation of the ventral anterior part of the cingulate cortex significantly correlates with

the HF HRV component; i.e., these cortical regions probably control parasympathetic autonomic activity [65]. The subgenual anterior cingulate cortex has tight functional and anatomical connections with centers of the autonomic control; these connections are stronger than those of the dorsal anterior cingulate cortex. The activity of the subgenual cortex is related to a greater extent to the parasympathetic autonomic nervous system subdivisions than to the sympathetic ones [66].

Back in 1996 SM Oppenheimer et al. [67] reported, that left insular cortex is responsible mostly for realization of the parasympathetic effects, whereas the right insular cortex, most probably, induces sympathetic reactions. S Vanneste and D de Ridder [68] in the study of tinnitus patients also indicated that the dorsal and subgenual anterior cingulate, as well as the left and right insula, are important in the central control of HRV. Although the sympathovagal balance is controlled by the subgenual and pregenual anterior cingulate cortex, the right insula controls sympathetic activity and the left insula, the parasympathetic activity. However, T Winkelmann et al. [69] shown that in a healthy young adult population the amount of resting HRV was positively correlated with the cortical thickness of an area within the right anterior midcingulate cortex, that was associated with degree of parasympathetic regulation of the heart rate. The correlation coefficient between thickness of this cortical structure and the HF component of HRV was 0.63. The authors concluded that cortical structures forming the central autonomic network inhibited limbic and brainstem structures that are sympathoexcitatory.

Previously, IL Popovych et al. had studied relationships between the parameters of EEG and HRV in men with chronic renal disease [70] compared with those in healthy men [71]. According to the results of canonical correlation analysis, it seemed that that a sympathotonic shift in autonomic homeostasis demonstrates relations mostly with the power/amplitude of EEG α activity in the left occipital and parietal cortical areas and with th δ activity in the occipital/posterior temporal areas and frontomedial suprasegmental structures. In contrast, a vagotonic shift is determined by the θ rhythm in the right lateral and medial frontal areas and left frontal cortico-subcortical structures [70]. Positive correlations were found between indicators of the parasympathetic tone RMSSD and PSD P4-θ (r=0.46), normalized PSD HF band and PSD F4-θ (r=0.38) as well as P4-θ (r=0.45), which is on the right side. However, the correlation between relative PSD HF and PSD O1-θ was negative (r=-0.42) [71]. Yi-Yuan Tang et al. [72], in their study of healthy young males, detected positive correlations between the changes in HFnu and frontal midline θ power (related to generators in the anterior cingulate cortex [73]): Fz-θ (r=0.566), FCz-θ (r=0.551) and Cz-θ (r=0.575).

GE Prinsloo et al. [74] in the study for healthy males found that less pronounced changes in HRV, due to work-related stress, accompanied by higher relative PSD Fz-θ, Pz-θ and Cz-θ, lower fronto-central relative β power and higher θ/β ratio. It is consistent with IL Popovych et al. [53] data on a negative correlation indicators of sympathetic tone LFnu and LF/HF with PSD in right loci F4-θ, P4-θ, F8-θ and positive with PSD F7-β and F8-β - on the one hand, and a positive correlation PSD HF with Fp1-θ and P4-θ and negative with P4-β - on the other side.

AR Subhani et al. [67] in the study of healthy participants showed a significant rise in the power Fz-θ/Pz-α ratio during mental stress. PSD LFnu and LF/HF ratio were significantly increased and HFnu sank during video games. On the other hand, the decrease in healthy elderly individuals LFnu accompanied by a fall in α-wave proportion of EEG [75], whereas in healthy young volunteers during mental arithmetic task were found positive correlation between the percent change from the baseline in slow α-power and that in LF/HF ratio [76]. Instead, IL Popovych et al [71] found a negative correlation between PSD LFnu and F4-θ (r=-0.38), and P4-θ (r=-0.45) and positive correlation between PSD LFnu and P4-α (r=0.41), and O2-α (r=0.32), the amplitude of α-rhythm (r=0.35) and the index α-rhythm (r=0.46).

Such conclusions with respect to both localization and, especially, lateralization of the above-mentioned influences do not fully agree with the data published earlier. Therefore, the need to quantify the laterality/asymmetry remains valid.

2.1 Statistical analisis

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

Preliminary results have been published as abstracts [77, 78].

Ethical standard. The research related to human use complied with all the relevant national regulations, institutional policies, and was in accordance with the tenets of the Helsinki Declaration (http://www.wma.net/en/30publications/10policies/b3/index.html). The study protocol was approved by the Ethical Committee of OO Bogomoletz Institute of Physiology NAS, Kyiv, Ukraine and directive of National Committee on ethics of scientific research [89].

During realization of tests, all participants provided informed consent and used all measures for maintaining anonymity of participants.

3 Results

We believe that the analysis of relationships should begin with the blood level of total leukocytes, found to be most closely associated with deviation (variation) of θ-rhythm frequency (Figue 1).

Weaker and thus negatively affecting the blood level of leukocytes is the PSD of θ-Rhythm in left anterior temporal locus and PSD of the β-Rhythm in the right central locus. Interestingly, that PSD of δ-Rhythm correlated with this variable also negatively in Left Parietal locus while positively in Right Anterior Temporal locus (Table 1).

Among parameters of HRV revealed to be negatively correlation with markers of vagal tone are RMSSD (r=-0.44), pNN_{50} (r=-0.43) and PSD HF (r=-0.44), while there is positive correlation with the marker of sympathetic tone normalized PSD LF (r=0.41) as well as with the Baevskiy Stress Index (r=0.30).

On the other hand, the PSD of β-Rhythm in the right central locus correlated negatively with raw PSD LF (r=-0.30), which provides a basis for assumptions about realization inhibitory outflow β-rhythm-generating structures of right hippocampus via inhibition of activating sympathetic outflow. The assumption is based on the assertion that PSD LF reflects mainly sympathetic outflow [57]. However, it is believed that PSD LF reflects both sympathetic and parasympathetic origin [4, 57] and in the supine position reflects almost exclusively parasympathetic influences [4].

JF Thayer and JE Fischer [79], in a sample of 611 apparently healthy adults, also found that both RMSSD and pNN_{50} were inversely associated with white blood cell counts (as well as with C-reactive protein as a marker of inflammation), whereas overnight urinary norepinephrine as a marker of sympathetic activity was positively related to leukocyte blood level.

Recently, in our laboratory, we have been shown that in chronic experiments an increase in vagal tone is accompanied by a decrease blood level leukocytes in female rats [48, 51, 53], but an increase in the male rats [42, 51]. Increase in sympathetic tone by this condition does not change the leukocyte blood level. Whereas in another experiment, the day following an acute (4 h) stress there was an increase in leukocyte blood level in "sympathotonic" male and female, rats but not in "vagotonic" rats [51].

In summary, a model of multiple regression with stepwise excluding, there are currently 5 parameters of EEG only, which together determine the blood level of leukocytes on 82% (Table 1).

We now proceed to analysis of neurogenic regulation of individual elements of the white blood cell count, which revealed that the relative content major fraction of segmentonucleary neutrophiles correlated positively with normalized PSD LF HRV (Figure 2).

Slightly weaker positive correlation with this element of the white blood cell count LF/HF ratio (r=0.51) and Baevskiy Stress Index in orthostase (r=0.36) as well as asymmetry of θ-rhythm while moderate negatively its PSD in central loci (Table 2) as marker activity of hippocampus [80, 81]. In turn, PSD in the central loci correlated negatively with PSD LFnu, but positively with relative PSD HF. and stronger on the right side (r makes -0.41 and -0.32 as

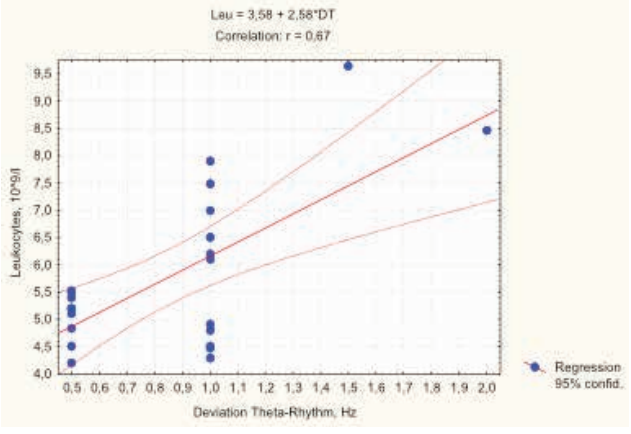

Figure 1: Relationship between deviation of θ-Rhythm (X-line) and blood level of Leukocytes (Y-line)

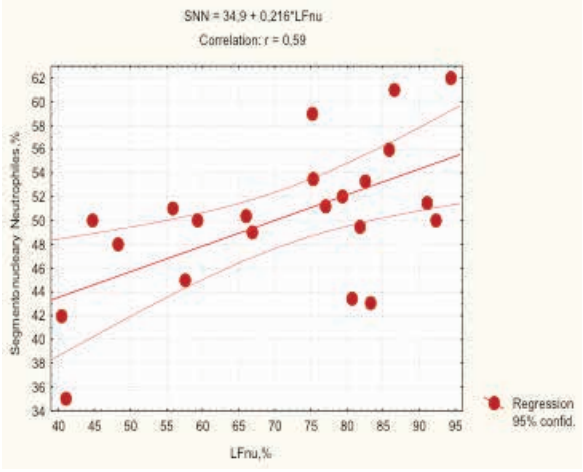

Figure 2: Relationship between normalized power LF HRV (X-line) and blood level of Segmentonucleary Neutrophiles (Y-line)

Table 1: Regression Summary for Dependent Variable: Leukocytes
R=0.930; R^2=0.864; Adjusted R^2=0.822; $F_{(5,2)}$=20.4; p<10^{-5}; Std. Error of estimate: 0.62•10^9/l

		Beta	St. Err. of Beta	B	St. Err. of B	n=22 $t_{(16)}$	p-level
Variables	r	Intercpt		5.76	.484	11.9	10^{-6}
θ-rhythm Deviation, Hz	0.67	.557	.102	2.14	.392	5.45	10^{-4}
PSD T4-δ, μV²/Hz	0.43	.294	.108	.003	.001	2.73	.015
PSD T3-θ, %	-0.49	-.401	.099	-.088	.022	-4.05	10^{-3}
PSD C4-β, μV²/Hz	-0.36	-.199	.104	-.005	.003	-1.91	.074
PSD P3-δ, %	-0.35	-.274	.109	-.038	.015	-2.52	.023

Table 2: Regression Summary for Dependent Variable: Segmentonucleary Neutrophiles
R=0.893; R^2=0.797; Adjusted R^2=0.695; $F_{(7,1)}$=7.8; p<10^{-3}; Std. Error of estimate: 3.4%

		Beta	St. Err. of Beta	B	St. Err. of B	n=22 $t_{(14)}$	p-level
Variables	r	Intercpt		37.9	5.5	6.82	10^{-5}
PSD LFnu, %	0.59	.644	.159	.236	.058	4.06	.001
θ-rhythm Asymmetry, %	0.51	.336	.190	.111	.063	1.77	.099
δ-rhythm Asymmetry, %	0.34	-.329	.184	-.110	.062	-1.79	.096
PSD C3-θ, %	-0.40	-1.351	.435	-1.416	.456	-3.11	.008
PSD T5-β, %	-0.39	-.386	.126	-.132	.043	-3.05	.009
θ-rhythm Laterality, %	-0.35	-.547	.145	-.072	.019	-3.77	.002
PSD C4-θ, %	-0.34	1.509	.446	1.312	.387	3.39	.004

Table 3: Regression Summary for Dependent Variable: Monocytes
R=0.754; R^2=0.568; Adjusted R^2=0.473; $F_{(4,2)}$=5.9; p=0.003; Std. Error of estimate:1.4%

		Beta	St. Err. of Beta	B	St. Err. of B	n=23 $t_{(18)}$	p-level
Variables	r	Intercpt		2.72	1.78	1.53	.144
θ-rhythm Deviation, Hz	0.46	.401	.156	2.05	.80	2.56	.020
PSD LFnu, %	0.42	.235	.191	.026	.021	1.24	.233
PSD F7-β, μV²/Hz	0.37	.378	.156	.0127	.005	2.42	.026
pNN_{50}, %	-0.45	-.320	.189	-.051	.030	-1.69	.108

well as 0.42 and 0.35 respectively) as well as asymmetry of θ-rhythm with LF (r=0.31).

The blood level of monocytes (Table 3) correlated positively with markers of sympathetic tone while negatively with markers of vagal tone RMSSD (r=-0.49), HRVTI (r=-0.37), HF (r=-0.34) as well as with PSD VLF (-0.38).

The information above suggests that the blood level of segmentonucleary neutrophiles as well as of monocytes is upregulated by sympathetic structures of the brain stem (probably caudal and rostral ventro-lateral medulla RVLM) or downregulated by parasympathetic structures of the brain stem (probably nucleus ambiguous NA),which respectively are down-or up-regulated by θ-rhythm-generating structures of the hippocampus, more right side.

It seems that the right to exist has both assumptions based on the long-known facts [82] that increased sympathetic effector outflows on β1-adrenergic postsynaptic membranes are accompanied by reciprocal weakening of parasympathetic effects on the postsynaptic membrane through β2- and, possibly, α2-adrenergic presynaptic membrane parasympathetic terminals that reduces acetylcholine release. Conversely, increased parasympathetic effector effects on postsynaptic M-cholinergic receptors associated with reciprocal weakening of sympathetic influences through M-cholinergic receptors adrenergic

Table 4: Regression Summary for Dependent Variable: Subnuclear Neutrophiles
R^2=0.934; R^2=0.872; Adjusted R^2=0.812; $F_{(7,2)}$=14.6; p<10^{-5}; Std. Error of estimate: 0.4%

		Beta	St. Err. of Beta	B	St. Err. of B	n=23 $t_{(15)}$	p-level
Variables	r	Intercpt		4.046	.522	7.75	10^{-6}
PSD O2-β, %	-0.66	-1.176	.259	-.068	.015	-4.54	10^{-3}
β-rhythm Frequency, Hz	-0.56	-.395	.129	-.083	.027	-3.05	.008
PSD O2-β, μV^2/Hz	-0.49	-.453	.110	-.012	.003	-4.14	10^{-3}
PSD O1-β, %	-0.46	.752	.218	.046	.013	3.45	.004
PSD T6-β, %	-0.45	.602	.188	.029	.009	3.21	.006
θ-rhythm Asymmetry, %	-0.42	-.227	.103	-.011	.005	-2.21	.043
α-rhythm Index, %	0.47	.381	.127	.013	.004	3.01	.009

presynaptic membrane of nerve endings release them through inhibition of norepinephrine.

The blood level of subnuclear neutrophiles correlated negatively with β-rhythm frequency as well as its PSD in both occipital and right posterior temporal loci and θ-rhythm asymmetry, but positively with the α-rhythm index (Table 4).

Among parameters of HRV that revealed negative correlation with RMSSD (r=-0.32), pNN_{50} (r=-0.32) and PSD LF (r=-0.33) but positive correlation with 1/Mo•MxDMn ratio (r=0.38) as an inversely marker of vagal outflow and with relative PSD VLF (r=0.36).

The physiological interpretation of VLF (0.04÷0.015 Hz) and ULF (0.015÷0.003 Hz) components of HRV needs further elucidation. There is speculation that the formation of oscillation in the range of 0.007÷0.003 Hz is associated with the activity of the hypothalamic centers of suprasegmentary autonomic control; these centers generate rhythms transmitted to the heart (as well as immune structures) via the sympathetic nervous system. It is supposed that there is a correlation between the PSD

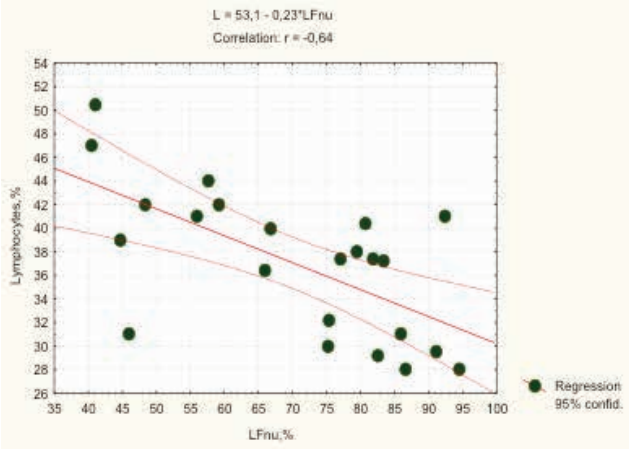

Figure 3: Relationship between normalized LF HRV (X-line) and Lymphocytose (Y-line)

of VLF component with thermoregulatory influences provided by the hypothalamus. The are rhythms presumably associated with oscillation blood level of renin (0.04 Hz), epinephrine (0.025 Hz), norepinephrine (0.002 Hz), 17-oxycorticosteroids (0.0019 Hz) [cit by: 57, 83]. There is also speculation that the VLF (0.06÷0.01 Hz) component of HRV is associated with changes in sympathetic activity [84].

Both β-rhythm frequency and asymmetry of the θ-rhythm correlated positively with PSD LF (r=0,31), in addition, β-rhythm Frequency correlated positively with another vagal marker Moda (r=0,34) as well as with raw PSD VLF (r=0,40). PSD O2-β correlated little positively with raw PSD ULF (r=0,25). On the other hand, the index of α-rhythm correlated negatively with LH/HF Ratio (r=-0,32) as marker of sympatho-vagal balance. Hence the assumption that blood level of subnuclear neutrophiles downregulated by cerebral structures generates β-rhythm, while upregulated by α-rhythm-generating structures via enhanced cholinergic or weakened adrenergic outflows.

D Tolkunov et al. [85] found that there is a rather strong negative correlation (r=−0,61) of a scalar EEG parameter, namely the PSD of β oscillations in the amygdala, with the HRV indices within the awakened state. As was concluded, slow limbic regulatory effects are translated from the amygdalar complex via descending connections, and this factor influences “slow” autonomic regulation. This was interpreted as a proof in the favor of existence of tight relations between disregulatory limbic efferent influences and their autonomic consequences.

A Haensel et al. [86] reviewed 13 studies involving healthy adults and patients with various disorders, including cardiovascular and renal diseases. The authors concluded that most studies found an inverse assotiation between vagally mediated HRV and inflammatory markers, and that the range of observed correlations was between -0,02 and -0,40.

Blood level of eosinophiles were downregulated by generating θ-rhythm structures projected in the left anterior temporal and parietal loci (amygdala?), but were while upregulated by generating δ-rhythm structures projected in the right lateral frontal and occipital loci (Table 5).

Relationships for the blood level of lymphocytes appeared opposed to the ties of segmentonucleary neutrophiles concerning sympathetic tone (Fig. 4), θ-rhythm asymmetry (r=-0.34), PSD T5-β (r=0.35), and LF/HF ratio (r=-0.51). This is consistent with an inverse relationship with their blood level (r=-0.87). Also, we found a positive correlation with RMSSD (r=0,49) and PSD VLF (r=0,42). But in the summary model of multiple regression with stepwise excluding there are currently only 3 parameters, which together determine the blood level of lymphocytes on 77% (Table 6).

4 Discussion

Vagally mediated HRV is considered a biomarker of adaptability and health [69]. The white blood cell count also contains information on adaptability and health. SV Petsyukh et al. [87] have shown that the factors that favorably influence the adaptive capacity of the body, measured by the leukocytary Popovych's Adaptation Index, may be considered the sympathetic tone and activity of neural structures generating the δ-Rhythm in the left parietal locus, whereas activity of neural structures generate the β-rhythm in the right occipital locus, and the θ-rhythm in right medial frontal and entral loci affect unfavourable adaptation.

As already mentioned experiments our laboratory have been shown that in the male rats increased sympathetic tone is accompanied by a decreased blood cell count of lymphocytes but in change in monocytes. Neutrophiles and eosinophiles increase vagal tone accompanied by an increase in eosinophiles only, without a change in cell count of other elements of the white blood cell count [43, 51]. Instead, the female rats revealed an increased blood cell count for lymphocytes and decreased monocytes by increasing both sympathetic and vagal tone as well as a decrease in eosinophiles and stubnuclear neutrophiles by an increase in sympathetic tone [48, 51, 55]. In other experiments after 24 h after an acute (4 h) stress revealed a decreased blood cell count for lymphocytes, eosinophiles, and monocytes but an increase in neutrophiles. Under these circumstances, decreased lymphocytes and eosinophiles were more expressed in "sympathotonic" rats then in "vagotonic" rats, whereas the decrease in monocytes as well as an increase in neutrophiles was approximately equal [51].

Table 5: Regression Summary for Dependent Variable: Eosinophiles
R=0,873; R^2=0,763; Adjusted R^2=0,627; $F_{(8,1)}$=5,6; p=0,003; Std. Error of estimate: 1,0 %

		Beta	St. Err. of Beta	B	St. Err. of B	n=23 $t_{(14)}$	p-level
Variables	r	Intercpt		5,56	,85	6,54	10^{-5}
PSD T5-θ, %	-0,54	-,685	,188	-,1992	,055	-3,64	,003
δ-rhythm Asymmetry, %	-0,44	-,482	,193	-2,3658	,950	-2,49	,026
PSD P3-θ, %	-0,35	,320	,197	,1185	,073	1,63	,126
δ-rhythm Laterality, %	0,51	,845	,321	,0260	,010	2,63	,020
PSD O2-δ, μV^2/Hz	0,42	1,881	,789	,0022	,001	2,38	,032
PSD F8-δ, μV^2/Hz	0,40	-1,867	,816	-,0054	,002	-2,29	,038
α-rhythm Laterality, %	0,39	,662	,407	,0434	,027	1,63	,126
θ-rhythm Laterality, %	0,39	-1,113	,542	-,0367	,018	-2,05	,059

Table 6: Regression Summary for Dependent Variable: Lymphocytes
R=0.773; R^2=0.598; Adjusted R^2=0.534; $F_{(3.2)}$=9.4; p<10^{-3}; Std. Error of estimate: 4.3%

		Beta	St. Err. of Beta	B	St. Err. of B	n=23 $t_{(19)}$	p-level
Variables	r	Intercpt		53.3	4.46	11.9	10^{-6}
PSD LFnu, %	-0.64	-.596	.146	-.213	.052	-4.08	10^{-3}
θ-rhythm Deviation, Hz	-0.36	-.298	.146	-4.96	2.43	-2.04	.056
PSD T5-β, %	0.35	.315	.146	.107	.050	2.16	.043

The blood level of immunocytes is the result of relationships between processes of apoptosis, proliferation, and distribution of cells between blood and immune organs. In terms of our observation, it is impossible to identify the role a given process plays in changes of blood level of immunocytes. It is therefore advisable to analyze the results of experiments on animals.

K Schauenstein et al. [88] in a rat model showed that an increased norepinephrine plasma level (after subcutaneous implantation tablets) leads to a significant shift in the differential white blood cell count; that is, an increase in neutrophiles and a drop in total lymphocytes. This decrease of lymphocytes was, likewise, observed in the thymus, spleen, and lymph nodes and accompanied by increased apoptosis in lymphoid organs.

Experiments in our laboratory have shown that in the male rats, an increased sympathetic tone is accompanied by decreased mass of the spleen and total cell count of lymphocytes, neutrophils, and eosinophiles while the relative content of macrophages/monocytes increased, whereas the increase in vagal tone was accompanied by an increase in its mass and total content of these cells [43]. Post-stressory rats revealed a decrease mass of the spleen and total cell count of lymphocytes and neutrophiles were more expressed in "sympathotonic" rats then in "vagotonic" rats. The mass of the thymus and total cell count of lymphocytes decreased in "sympathotonic" rats only [51].

5 Conclusion

This research aimed to analyze the boundaries of KJ Tracey's immunological homunculus conception by the relationships between electroencephalogram and heart rate variability parameters, on the one hand, and the parameters of white blood cell count, on the other hand.

Our research revealed that canonical correlation between constellation EEG and HRV parameters form with blood level of leukocytes 0.92 ($p<10^{-5}$), with relative content in white blood cell count stubnuclear neutrophils 0.93 ($p<10^{-5}$), segmentonucleary neutrophiles 0.89 ($p<10^{-3}$), eosinophiles 0.87 ($p=0.003$), lymphocytes 0.77 ($p<10^{-3}$) and with monocytes 0.75 ($p=0.003$). Thus, parameters of the white blood cell count are significantly modulated by electrical activity in some structures of the central and autonomic nervous systems.

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