Bombushkar, Igor, Gozhenko, Anatoliy, Korda, Mykhaylo, Żukow, Xawery, Popovych, Igor. Relationships between plasma levels of nitrogenous metabolites and some psycho-neuro-endocrine parameters. Journal of Education, Health and Sport. 2022;12(6):365-383. eISSN 2391-8306. DOI http://dx.doi.org/10.12775/JEHS.2022.12.06.036
https://apcz.umk.pl/JEHS/article/view/40198
https://zenodo.org/record/7111720

The journal has had 40 points in Ministry of Education and Science of Poland parametric evaluation. Annex to the announcement of the Minister of Education and Science of December 1, 2021. No. 32343. Has a Journal's Unique Identifier: 201159. Scientific disciplines assigned: Physical Culture Sciences (Field of Medical sciences and health sciences); Health Sciences (Field of Medical Sciences and Health Sciences).

Punkty Ministerialne z 2019 - aktualny rok 40 punktów. Załącznik do komunikatu Ministra Edukacji i Nauki z dnia 1 grudnia 2021 r. Lp. 32343. Posiada Unikatowy Identyfikator Czasopisma: 201159. Przypisane dyscypliny naukowe:Nauki o kulturze fizycznej (Dziedzina nauk medycznych i nauk o zdrowiu); Nauki o zdrowiu (Dziedzina nauk medycznych i nauk o zdrowiu).

Received: 16.05.2022. Revised: 15.06.2022. Accepted: 30.06.2022.

RELATIONSHIPS BETWEEN PLASMA LEVELS OF NITROGENOUS METABOLITES AND SOME PSYCHO-NEURO-ENDOCRINE PARAMETERS

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Abstract

Background. It was recently shown by our group that bilirubin has a neurotropic activity. It is also shown that endogenous uric acid exerts a noticeable modulating effect on neuro-endocrine adaptation factors. In this study, we supplemented the constellation both of subjects of influence with other nitrogenous metabolites - urea and creatinine, and the objects of influence - with plasma levels of the main adaptation hormones and the severity of trait and reactive anxiety. **Materials and Methods**. The object of observation were almost healthy volunteers: 30 females (30÷76 y) and 31 males (24÷69 y). In basal conditions determined plasma levels of nitrogenous metabolites as well as cortisol, aldosterone, testosterone, triiodothyronine and calcitonin, estimated the severity of the trait and reactive anxiety, recorded the ongoing HRV and EEG. After 4 or 7 days, repeated testing was performed. **Results**. By constructing regression models, it was found that direct bilirubin determines the levels of psycho-neuro-endocrine parameters by 66,0%, free bilirubin by 56,7%, uric acid by 40,8%, creatinine by 37,6%, and urea - by 31,1%. Taken together, nitrogenous plasma metabolites determine the severity of trait, but not reactive anxiety, plasma levels of testosterone, cortisol and triiodothyronine, but not calcitonin and aldosterone, as well as a number of HRV and EEG parameters by 70,6%. **Conclusion.** Nitrogenous plasma metabolites, even in the absence of uremia, are able to influence the state of the psyche, autonomic and central nervous and endocrine systems, apparently through aryl hydrocarbon and adenosine receptors of neurons and endocrinocytes and/or directly.

Keywords: plasma bilirubin, uric acid, urea, creatinine, cortisol, testosterone, aldosterone, triiodothyronine, calcitonin, ongoing EEG, HRV, anxiety, men, women, relationships.

INTRODUCTION

It was recently shown by our group that even normal plasma bilirubin has a neurotropic activity: downregulation of theta and delta rhythm-generating nuclei and vagal tone, while upregulation of sympathetic tone and beta rhythm-generating nuclei [30]. Another study of ours shows that uricemia correlates negatively with markers of vagal tone and the plasma level of triiodothyronine while positively with markers of sympathetic tone, sympatho-vagal balance and parathyroid activity; uricosuria correlates positively with a marker of sympatho-vagal modulation. The rate of urico-neuroendocrine determination is 38%. Changes in the parameters of uric acid metabolism caused by a course of adaptogenic balneotherapy are accompanied by inverse changes in the Kerdoe autonomic index, cortisolemia, and calcitoninemia, while unidirectional changes in HRV indices of sympatho-vagal balance, vagal tone, testosterone, and parathyroid activity, determining the dynamics of the neuro-endocrine constellation by 53% [9].

In this study, we supplemented the constellation both of subjects of influence with other nitrogenous metabolites - urea and creatinine, and the objects of influence - with plasma levels of the main adaptation hormones and the severity of trait and reactive anxiety.

MATERIAL AND METHODS

The object of observation were employees of the clinical sanatorium "Moldova" and PrJSC "Truskavets' Spa": 30 females (30÷76; 49±13 y) and 31 males (24÷69; 47±12 y). The volunteers were considered practically healthy (without a clinical diagnosis), but the initial testing revealed deviations from the norm in a number of parameters of the neuro-endocrine-immune complex (details follow) as a manifestation of dys(mal)adaptation. Testing was performed twice with an interval of 4 ("Moldova") or 7 ("Truskavets' Spa") days.

We determined the plasma levels of the direct (conjugated) and free (unconjugated) Bilirubin (by diazoreaction using the Jedrashik-Kleghorn-Grof method), Uric acid (by uricase method), Urea (by urease method by reaction with phenol hypochlorite) and Creatinine (by Jaffe's color reaction by Popper's method) as well as main adaptation hormones Cortisol, Testosterone, Aldosterone, Triiodothyronine and Calcitonin (by the ELISA with the use of corresponding sets of reagents from "Алкор Био", XEMA Co. Ltd, and DRG International Inc.).

The analyzes were carried out according to the instructions described in the manual [19]. The analyzers "Pointe-180" ("Scientific", USA), "Reflotron" (Boehringer Mannheim, BRD) and "RT-2100C" (PRCh) were used.

To assess the parameters of heart rate variability (HRV) we recorded during 7 min electrocardiogram in II lead (software-hardware complex "CardioLab+HRV", KhAI-MEDICA, Kharkiv). For further analysis the following parameters HRV were selected [5,8,23,59]. 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 than 50 msec (pNN_{50}). Spectral parameters (Frequency Domain Methods): power spectrum density (PSD) 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 ultralow-frequency (ULF, range 0,015÷0,003 Hz). Derived indices were calculated: Baevskiy's Activity Regulatory Systems (BARSI), LF/HF, HFnu.

Simultaneosly EEG recorded a hardware-software complex "NeuroCom Standard" (KhAI MEDICA, Kharkiv) monopolar in 16 loci (Fp1, Fp2, F3, F4, F7, F8, C3, C4, T3, T4, P3, P4, T5, T6, O1, O2) by 10-20 international system, with the reference electrodes A and Ref on tassels the ears. The duration of the epoch was 25 sec. Among the options considered the average EEG amplitude (μV), average frequency (Hz), frequency deviation (Hz) as well as absolute (μV^2/Hz) and relative (%) PSD of basic rhythms: β (35÷13 Hz), α (13÷8 Hz), θ (8÷4 Hz) and δ (4÷0,5 Hz) in all loci, according to the instructions of the device.

In addition, we calculated coefficient of Asymmetry (As) and Laterality Index (LI) for PSD each Rhythm using formulas [40]:

As, % = 100•(Max – Min)/Min; LI, % = Σ [200•(Right – Left)/(Right + Left)]/8.

We calculated for HRV and each locus of EEG the Entropy (h) of normalized PSD using Popovych's IL [20,55] formulas based on classic Shannon's CE [57] formulas:

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

$hHRV = -[PSHF \bullet \log_2 PSHF + PSLF \bullet \log_2 PSLF + PSVLF \bullet \log_2 PSVLF + PSULF \bullet \log_2 PSULF]/\log_2 4$

At last volunteers filled a questionnaire with the purpose of estimation of level of the trait and reactive anxiety by STAI of Spielberg ChD in modification of Khanin YL [44].

Results processed by using the software package "Statistica 64".

RESULTS AND DISCUSSION

According to the algorithm of the Truskavetsian Scientific School of Balneology [20], at the first stage of the analysis, a matrix of correlations was created between plasma levels of nitrogenous metabolites, on the one hand, and psycho-neuro-endocrine parameters, as well as age and sex, on the other hand (Table 1, appendix).

First of all, we note the absence of a connection with age and a significant correlation with the sex-index (M=1, F=2). Therefore, the well-known sexual dimorphism in the neurotropic effects of caffeine [2,3], as well as of uric acid, revealed in our previous study [47], also takes place in relation to urea and creatinine and will be the subject of a separate study. But in this article, the neurotropic effects of nitrogenous metabolites are analyzed regardless of gender.

At the next stage, following the algorithm, regression models were built by step-by-step exclusion of the variant until the maximum value of Adjusted was reached R^2.

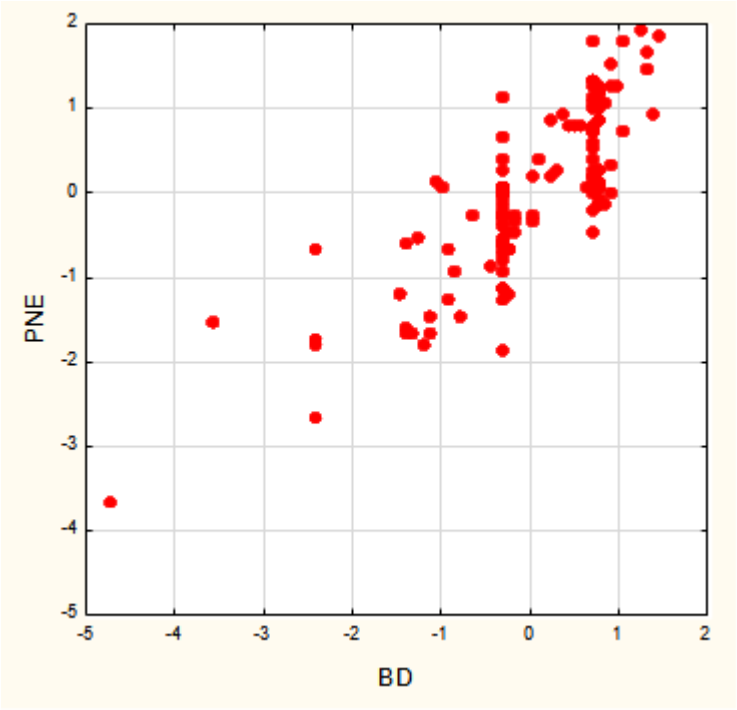
The pseudostaining variant included in the model for direct bilirubin (Table 2) shows that the most numerous are the EEG loci to which **theta-rhythm** generating neurons are projected, followed by **beta-rhythm** generating neurons, while **alpha-rhythm** generating neurons are represented by only three loci, in the absence of **delta-rhythm** generating neurons in the model.

Table 2. Regression Summary for Bilirubin direct, µM/L
R=0,812; R^2=0,660; Adjusted R^2=0,571; $F_{(26)}$=7,4; $p<10^{-5}$

N=122		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(96)}$	p-level
Variables	r		Intercpt	0,982	0,656	1,50	0,138
T5-θ PSD, %	**-0,37**	-0,502	0,115	-0,107	0,025	-4,35	10^{-4}
C4-θ PSD, µV²/Hz	**-0,32**	0,604	0,201	0,0099	0,0033	3,01	0,003
F3-θ PSD, µV²/Hz	**-0,31**	-0,566	0,161	-0,0109	0,0031	-3,51	0,001
T3-θ PSD, µV²/Hz	**-0,30**	-0,271	0,160	-0,0056	0,0033	-1,69	0,094
C3-θ PSD, µV²/Hz	**-0,30**	-0,378	0,251	-0,0073	0,0048	-1,51	0,135
Amplitude θ, µV	**-0,27**	0,226	0,131	0,051	0,029	1,72	0,088
T6-θ PSD, µV²/Hz	**-0,23**	0,370	0,158	0,0071	0,0030	2,35	0,021
T3-θ PSD, %	**-0,22**	0,182	0,096	0,042	0,022	1,89	0,061
Fp2-θ PSD, µV²/Hz	**-0,19**	0,181	0,131	0,0046	0,0033	1,38	0,170
P3-θ PSD, %	**-0,18**	-0,192	0,100	-0,038	0,020	-1,92	0,057
P4-β PSD, µV²/Hz	**-0,34**	-1,173	0,239	-0,0172	0,0035	-4,90	10^{-5}
O2-β PSD, µV²/Hz	**-0,33**	0,238	0,118	0,0038	0,0019	2,02	0,046
T6-β PSD, µV²/Hz	**-0,29**	-0,268	0,140	-0,0057	0,0030	-1,92	0,058
O1-β PSD, µV²/Hz	**-0,27**	-0,292	0,128	-0,0042	0,0018	-2,27	0,025
C3-β PSD, µV²/Hz	**-0,25**	0,372	0,161	0,0048	0,0021	2,31	0,023
Amplitude β, µV	**-0,20**	0,280	0,124	0,071	0,031	2,26	0,026
C3-α PSD, µV²/Hz	**-0,19**	0,454	0,173	0,0020	0,0007	2,63	0,010
F7-α PSD, µV²/Hz	**-0,19**	0,332	0,154	0,0024	0,0011	2,15	0,034
T5-α PSD, %	**-0,17**	-0,228	0,095	-0,011	0,005	-2,41	0,018
Entropy T5	**-0,21**	0,143	0,104	0,819	0,594	1,38	0,171
Trait anxiety, points	**-0,19**	-0,200	0,078	-0,024	0,009	-2,57	0,012
T4-β PSD, %	**0,29**	0,340	0,080	0,023	0,005	4,25	10^{-4}
T6-β PSD, %	**0,18**	0,222	0,097	0,014	0,006	2,28	0,025
LFnu HRV PSD, %	**0,27**	0,269	0,069	0,021	0,005	3,92	10^{-4}
ULF HRV PSD, %	**0,24**	0,210	0,069	0,028	0,009	3,03	0,003

The listed neurons, as well as trait anxiety, are **downregulated** by direct bilirubin, on the other hand, beta-rhythm generating neurons that project to T4 and T6 loci, as well as sympathetic tone (marker - LFnu) are object to **upregulation**. The physiological essence of the ULF band remains a subject of debate. It is speculated that ULF band (0,015÷0,003 Hz) associated with oscillation blood level of norepinephrine (0,002 Hz) and 17-OKS (0,0019 Hz) [31]. In this study, we did not find a relationship between the level of direct bilirubin, either with the level of cortisol, or with Mode HRV as a marker of circulating catecholamines. At the same time, a significant relationship with the level of testosterone (r=0,20) was found, which, however, was outside the regression model.

The constellation of parameters included in the model is determined by the level of direct bilirubin in the plasma by 66,0% (Table 2 and Fig. 1).



R=0,812; R^2=0,660; $\chi^2_{(25)}$=116; $p<10^{-6}$; Λ Prime=0,340

Fig. 1. Scatterplot of canonical correlation between Bilirubin direct (X-line) and Psychoneuronal parameters (Y-line)

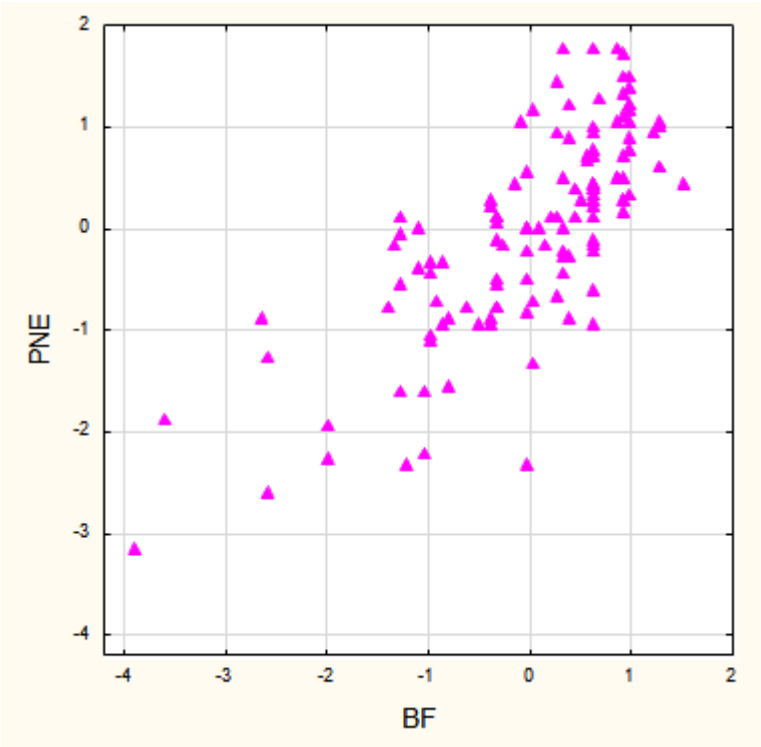
The constellation of parameters subject to the regulatory influence of free bilirubin is similar, but not identical (Table 3). The **beta-rhythm** was the most representative. **Delta-rhythm** frequency, combined (VL+UL)F HRV band (0,040÷0,003 Hz) and triiodothyronine were included in the model, but again without testosterone, despite a significant correlation (r=0,22). It should be clarified that in our device the ULF band is the final link of the VLF band of other devices.

There are opinions that VLF band (0,040÷0,003 Hz) directly reflects both vagal and sympathetic tone [1] or vagal tone only [65] as well as saliva testosterone level [66] while inversely - renin-angiotensin-aldosterone system activity [1,66]. It was reported that low VLF power has been correlated with low levels of testosterone, while other biochemical markers, such as those mediated by the hypothalamic–pituitary–adrenal axis (e.g., cortisol), have not [66]. While Del Valle-Mandragon L et al [16] showing that angiotensin II has a positive correlation with VLF (r=0,390) and with LF/HF ratio (r=0,359).

The constellation of parameters included in the model is determined by the level of direct bilirubin in the plasma by 56,0% (Table 3 and Fig. 2).

Table 3. Regression Summary for Bilirubin free, µM/L
R=0,753; R^2=0,567; Adjusted R^2=0,466; $F_{(24)}$=5,6; $p<10^{-5}$

N=122		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(98)}$	p-level
Variables	r		Intercpt	11,22	2,52	4,45	10^{-4}
T6-β PSD, µV²/Hz	**-0,33**	0,267	0,107	0,062	0,025	2,49	0,015
O2-β PSD, µV²/Hz	**-0,33**	-0,223	0,140	-0,0115	0,0072	-1,59	0,115
P4-β PSD, µV²/Hz	**-0,32**	0,405	0,139	0,0281	0,0096	2,92	0,004
O1-β PSD, µV²/Hz	**-0,27**	-0,766	0,202	-0,0402	0,0106	-3,80	10^{-3}
Amplitude β, µV	**-0,21**	0,385	0,150	0,350	0,136	2,57	0,012
Fp2-β PSD, µV²/Hz	**-0,20**	0,140	0,114	0,0080	0,0065	1,23	0,223
T3-β PSD, µV²/Hz	**-0,17**	-0,592	0,156	-0,0407	0,0108	-3,78	10^{-3}
T5-θ PSD, %	**-0,19**	0,450	0,186	0,027	0,011	2,42	0,017
C3-θ PSD, µV²/Hz	**-0,18**	0,228	0,085	0,0544	0,0203	2,67	0,009
C4-θ PSD, µV²/Hz	**-0,18**	0,511	0,212	0,0353	0,0147	2,41	0,018
T6-θ PSD, µV²/Hz	**-0,18**	-0,334	0,156	-0,0254	0,0119	-2,13	0,035
Deviation θ, Hz	**-0,17**	-0,145	0,072	-0,864	0,430	-2,01	0,047
Fp2-α PSD, µV²/Hz	**-0,19**	-0,536	0,231	-0,0161	0,0069	-2,32	0,022
F3-α PSD, µV²/Hz	**-0,18**	0,557	0,270	0,0127	0,0062	2,07	0,041
Frequency δ, Hz	**-0,19**	-0,233	0,083	-2,925	1,046	-2,80	0,006
Trait anxiety, points	**-0,19**	-0,124	0,082	-0,053	0,035	-1,50	0,136
Triiodothyronine, nM/L	**-0,16**	-0,267	0,121	-0,517	0,234	-2,21	0,029
T4-β PSD, %	**0,24**	-0,155	0,105	-0,007	0,005	-1,48	0,143
T6-β PSD, %	**0,17**	-0,388	0,082	-0,298	0,063	-4,75	10^{-5}
ULF HRV PSD, %	**0,23**	0,182	0,078	0,088	0,038	2,33	0,022
LFnu HRV PSD, %	**0,22**	0,210	0,071	0,059	0,020	2,96	0,004
(UL+VL)F PSD, %	**0,20**	0,130	0,079	0,024	0,014	1,65	0,101



R=0,753; R^2=0,567; $\chi^2_{(23)}$=91; $p<10^{-6}$; Λ Prime=0,433

Fig. 2. Scatterplot of canonical correlation between Bilirubin free (X-line) and Psychoneuroendocrine parameters (Y-line)

It is well known that the main generator of theta-rhythm is the hippocampus [10] - a favorite object of experimental studies of neurotoxicity of bilirubin [15]. It is believed that the hippocampus is projected at the C3 and C4 loci [53]. Based on this position, we found that **direct** bilirubin downregulates the activity of neurons in both the right (r=-0,32) and left (r=-0,30) hippocampus, which generate theta-rhythm, as well as, to a lesser extent, beta-rhythm generating neurons of the hippocampus (r=-0,27 and -0,25 respectively). On the other hand, the influence of **free** bilirubin on the theta-rhythm generating neurons of the hippocampus is insignificant (r=-0,18 and -0,18 respectively), and on the beta-rhythm generating neurons it is weaker than the influence of its direct fraction (r=-0,23 and -0,23 respectively). Importantly, none of the listed parameters is included in the regression model for free bilirubin.

However, the inhibitory effect of direct bilirubin on theta-rhythm generating neurons is not limited to the hippocampus, because significant negative correlations also occur for other loci. Among them, loci F3 (r=-0,31), F4 (r=-0,31) and T3 (r=-0,30) are of particular interest.

Iseger TA et al [24] applied trains of transcranial magnetic stimulation (rTMS) over 7 cortical regions aiming to identify which regions would affect heart rate. They found that F3 and F4 expressed the largest heart rate deceleration, in line with studies suggesting these are the best sites to target the dorsolateral prefrontal cortex (DLPFC). On the individual level, 20-40% subjects expressed the largest heart rate deceleration at FC3 or FC4, indicating individual differences as to the 'optimal site for stimulation'. Interestingly, stimulation of the C3, C4 and Pz loci showed opposite effects. After real HF-rTMS over the left DLPFC the physiological stress response was diminished, as indicated by a significant increase in HRV. No effects were found in the sham or right side stimulation condition. This is consistent with the provision that left-sided (dominant hemisphere) forebrain structures appear to be predominantly involved in vagal regulation, whereas homotopic right (non-dominant) forebrain regions seem to primarily control sympathetic tone and responses [22,71]. However, the lateralization model of autonomic control of the heart remains controversial [11,72]. In a TMS/fMRIS, Vink JJT et al [69] found that only 4 under 9 participants had the subgenual cingulate cortex activated by stimulation of the DLPFC. One single session of excitatory transcranial direct current stimulation (tDCS) over the left DLPFC reduced HR and favored a larger vagal prevalence prior to stress exposure, moderated stress-induced HR acceleration and sympathetic activation/vagal withdrawal [12]. Similar results were found with bifrontal tDCS [41] which raises again the question about the effect of laterality when stimulating the DLPFC with the aim to increase vagus nerve activity.

Montenegro RA et al [38] assessed the effects of anodal tDCS over the T3 scalp position (aims to reach the insular cortex) on measures of cardiac autonomic control. The authors found that the parasympathetic activity (HF(log)) increased and the sympathetic activity (LF(log)) and sympatho-vagal balance (LF/HF(log)) decreased in athletes but not in untrained individuals. No significant changes in HRV indexes were provoked by sham stimulation in both groups. The authors attributed the specific results to neuroanatomical and functional changes in the brain induced by long-term exercise training. Furthermore, Piccirillo G et al [46] demonstrated that anodal tDCS over T3 scalp position reduced sinus sympathetic activity and increased vagal sinus activity and baroreflex sensitivity in older, but not younger individuals. Taking together, those studies suggest that stimulation of the left dorsolateral prefrontal or the insular cortex with rTMS or tDCS increase vagal activity [review: 6,25].

Previously we [4,49,50] also found positive correlations between HFnu and F4-θ and P4-θ, while negative correlation LFnu and LF/HF with F4-θ, P4-θ.

In our study, the situation is the mirrored: the inhibition of cortical theta-rhythm generating neurons that project to the F3, F4 and T3 loci is accompanied by an increase in sympathetic tone and decrease in vagal tone (r=-0,27 with HFr). However, the positive correlation of the bilirubin level with beta-rhythm generating neurons projecting to the T4 locus (r=0,29 and 0,24 for direct and free bilirubin, respectively), which reflects the activity of the amygdala [53], leads us to an alternative interpretation of the mechanism of increase sympathetic tone.

According to the concept of “central autonomic network (CAN)” [7,43,64] it include following cortical, subcortical, and medullary structures: the anterior cingulate, insular, orbitofrontal, and ventromedial cortices; the **central nucleus of the amygdala** (**CeA**); the paraventricular and related nuclei of the hypothalamus; the periaqueductal gray matter; the nucleus of the solitary tract; the nucleus ambiguous; the ventrolateral medulla; the ventromedial medulla and the medullary tegmental field (Fig. 3).

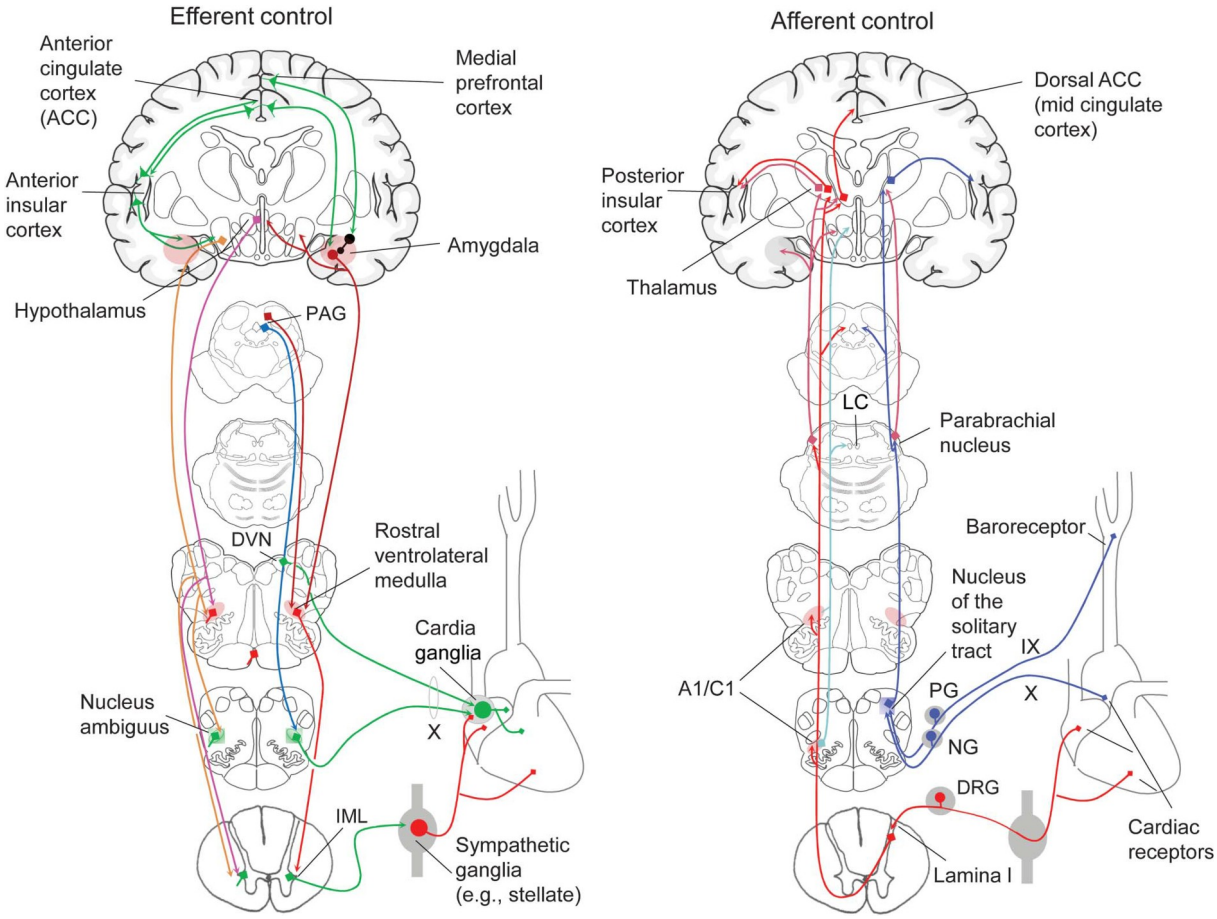


Fig. 3. Efferent and afferent control of cardiac function [43]

The primary output of the CAN is mediated through the preganglionic sympathetic and parasympathetic neurons, which exert control over the heart via the stellate ganglia and the vagus nerve, respectively. The prefrontal, cingulate, and insula cortices form an interconnected network with bi-directional communication with the amygdala. The amygdala is under tonic inhibitory control via prefrontal vagal pathways to intercalated cells in the amygdala. The activation of the CeA inhibits the nucleus of the solitary tract (NTS) which in turn inhibits inhibitory caudal ventrolateral medullary (CVLM) inputs to the rostral ventrolateral medullary (RVLM) sympathoexcitatory neurons, and simultaneously inhibits vagal motor neurons in the nucleus ambiguus (NA) and the dorsal vagal motor nucleus (DVN). In addition, the CeA can directly activate the sympathoexcitatory neurons in the RVLM [43]. Indeed, inhibition of prefrontal activity leads to disinhibition of sympathoexcitatory circuits [56,64,67,68], with a resultant increase in heart rate and HRV-markers of sympathetic tone.

The presence among objects of bilirubin's influence of anxiety is naturally connected with its effect on the amygdala. There is a wealth of evidence for the involvement of amygdala in anxiety disorders. Furthermore, the magnitude of amygdala activation is correlated with symptom severity, such that hyperactivation actually decreases or even normalizes following successful treatment of anxiety disorders [61,62].

It is time to find out the mechanism of the neurotropic action of bilirubin. Only three decades after the discovery of the aryl hydrocarbon receptor (AhR), bilirubin was added to its list of numerous agonists.

Phelan D et al [45] thought so. Although no endogenous physiological ligand for the AhR has yet been described, persistent expression of hepatic CYP1A1 gene expression (an AhR-dependent response) in congenitally jaundiced Gunn rats indirectly supports the existence of such a ligand(s) in these animals. High plasma levels of the heme degradation product bilirubin in these animals prompted authors to evaluate whether bilirubin is an endogenous AhR agonist. Expression of dioxin responsive element (DRE)-driven luciferase gene expression in stably transfected mouse, guinea pig, rat, and human cells was induced by treatment with physiological concentrations of bilirubin. Biliverdin, the metabolic precursor of bilirubin, also induced luciferase activity in all species. Both chemicals not only stimulated AhR transformation and DRE binding in vitro and in cells in culture, but competitive inhibition of [^{3}H]TCDD-specific binding to the cytosolic AhR revealed that these chemicals are AhR ligands. The significantly greater inducing potency of these chemicals in intact cells,

compared to their ligand binding and AhR transformation potency in vitro, suggests that bilirubin and biliverdin may also be converted within the cell to a more potent activator(s).

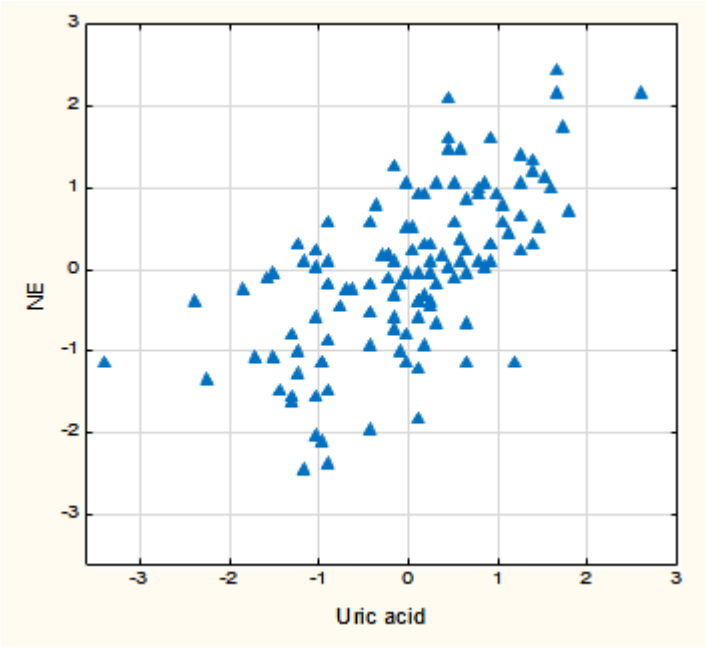
Recently, the AhR, an ancient protein that possesses highly conserved functions across various species, has been associated with brain aging and age-associated diseases [18,42]. Apart from its well-described role in xenobiotic metabolism, AhR plays a critical role in the developing nervous system of invertebrates and vertebrates. Kimura E & Tohyama C [28] analyzed AhR mRNA expression in the brains of mice. The mRNA was expressed in the hippocampus, cerebral cortex, cerebellum, olfactory bulb. These results reveal temporal and spatial patterns of AhR mRNA expression in the mouse brain, providing the information that may contribute to the elucidation of the physiologic and toxicologic significance of AhR in the developing brain. Although AhR expression decreases from the embryonic period into adult life [28], several physiological functions remain in the adult brain, which include the regulation of neurotransmitter levels, blood-brain barrier functions, and immune responses [13,70]. By the way, immunotropic effects of bilirubin were found in previous studies of our laboratory [32-36,48].

AhR activation can regulate several genes involved in multiple aspects of synaptic plasticity and neurogenesis after brain development. The administration of classic AhR exogene agonist dioxin (TCDD) in the adult brain upregulates the genes required for synaptic plasticity and neuronal activities [14]. 6-formylindolo [3,2-b] carbazole (FICZ), an endogenous ligand of AhR, showed positive effects on the fate of neuronal stem/progenitor cells by upregulating the genes necessary for neuronal differentiation in the SGZ area of the adult mouse hippocampus [27]. Additionally, AhR activation by FICZ improves hippocampal-dependent memory and learning tasks, which [21] was reversed following treatment with the AhR antagonist, CH22319 [27].

The level of plasma uric acid upregulates the levels of testosterone and cortisol in it, but downregulates the heart rate, the **beta-rhythm** generating neurons, apparently, of the hippocampus and the left amygdala, as well as the **delta-rhythm** generating neurons of the right prefrontal cortex. A positive correlation with the asymmetry of the electrical activity of the brain reflects its rightward shift. This neuro-endocrine constellation is determined by uric acid by 40,8% (Table 4 and Fig. 4).

Table 4. Regression Summary for Uricemia, µM/L
$R=0,639$; $R^2=0,408$; Adjusted $R^2=0,349$; $F_{(11)}=6,9$; $p<10^{-5}$

N=122		Beta	St. Err. of Beta	B	SE of B	$t_{(110)}$	p-level
Variables	r		Intercpt	285,7	44,4	6,43	10^{-6}
Testosterone, nM/L	**0,39**	0,350	0,079	3,577	0,809	4,42	10^{-4}
Cortisol, nM/L	**0,17**	0,183	0,079	0,113	0,049	2,33	0,022
Laterality δ, %	**0,22**	0,228	0,080	0,482	0,170	2,83	0,005
Asymmetry θ, %	**0,21**	0,184	0,079	0,644	0,278	2,31	0,023
Asymmetry β, %	**0,18**	0,136	0,084	0,616	0,381	1,62	0,108
C4-β PSD, %	**-0,24**	-0,245	0,079	-1,558	0,505	-3,09	0,003
T3-β PSD, µV²/Hz	**-0,18**	-0,164	0,110	-0,1554	0,1040	-1,49	0,139
C3-β PSD, µV²/Hz	**-0,18**	0,392	0,167	0,3718	0,1584	2,35	0,021
P4-β PSD, µV²/Hz	**-0,17**	-0,280	0,131	-0,3035	0,1413	-2,15	0,034
Fp2-δ PSD, µV²/Hz	**-0,17**	-0,212	0,078	-0,018	0,006	-2,74	0,007
Heart Rate, beats/min	**-0,21**	-0,136	0,077	-0,868	0,495	-1,75	0,082



R=0,639; R^2=0,408; $\chi^2_{(11)}$=60; $p<10^{-6}$; Λ Prime=0,592

Fig. 4. Scatterplot of canonical correlation between Uricemia (X-line) and Neuro-endocrine parameters (Y-line)

The data we received about the effect of uric acid on the EEG, as far as we can judge from the absence of such data on PubMed and PMC resources, are of priority. Li X et al [37] observed significantly lower serum uric acid levels in patients with acute central nervous system virus infections who had neurological abnormalities, seizures, abnormal EEG results than in patients who did not have these conditions.

True, the neurotropic activity of uric acid has been discussed for a long time. Sofaer JA & Emery AF [60] as well as Efroimson VP [17] considered hyperuricemia to be one of the factors of increased mental activity (even genius), based on the abnormally high incidence of gout and urolithiasis among prominent individuals.

In neurons, A_{2A} adenosine receptors have been identified both pre- and post-synaptically, where they control neurotransmitter release and neuronal stimulation, respectively [52,54,58,63].

The similarity of the molecule of uric acid (**2,6,8**-tri**oxipurine**) to the molecules of methylxanthines: caffeine (**2,6**-di**oxi**-1,3,7-trimethyl**purine**) and theophylline (**2,6**-di**oxi**-1,3-dimethyl**purine** or 1,3-dimethyl**xantine**), which in turn are a structural homolog of adenosine [(2R,3R,4R,5R)-2-(6-amino**purine**-il)-5-(hydroximethyl) oxolan-3,4-diol)] and capable of 0,2 mM/L at blocking adenosine A_1- and A_{2A} receptors [51] back in 2004 led our laboratory [26] to hypothesize that uric acid, the level of which in plasma of the same order (normal range: 0,12÷0,58 mM/L), is also an ***endogenous non-selective adenosine receptor antagonist***.

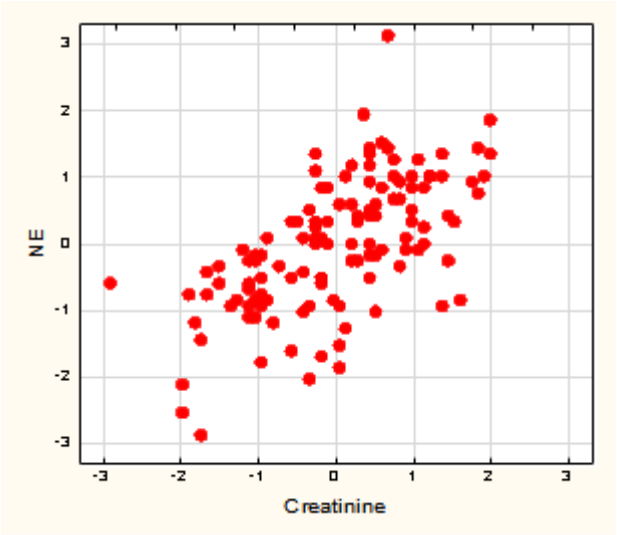
In the excellent review of Morelli M et al [39] hypothesized that urate is not only the end product of the metabolism of purines like adenosine, but even more a ***novel target for neuroprotection***. Authors visualized their hypothesis about the neuroprotective effect of urate and caffeine in the following scheme. Adenosine A_{2A} antagonists (including caffeine) and urate have emerged as realistic candidate neuroprotectants. The schematic suggests a possible homeostatic mechanism linking an adenosinergic neurodegenerative influence with an offsetting neuroprotective influence of urate.

Creatinine, like uric acid, upregulates testosterone level, as well as the ULF band of HRV, which, as mentioned, reflects testosterone level too [66], but downregulates cortisol level. Neurons of the prefrontal cortex and hippocampus, generating theta and beta rhythms, are also subject to downregulation. This neuro-endocrine constellation is determined by creatinine by 37,6% (Table 5 and Fig. 5).

Urea upregulates power HRV in total, as well as its VLF band (r=0,24), which is accompanied by a decrease in the entropy of HRV bands and an increase in the activity of the autonomic nervous system. The influence on the central nervous system is manifested in the downregulation of the delta-rhythm generating neurons of the right hippocampus and the variability of the beta-rhythm, on the one hand, instead of the upregulation of the delta-rhythm generating neurons of the left prefrontal cortex and the variability of the alpha-rhythm, on the other hand. The determination rate of this constellation by urea is 31,1% (Table 6 and Fig. 6).

Table 5. Regression Summary for Creatinineemia, µM/L
R=0,613; R^2=0,376; Adjusted R^2=0,320; $F_{(10)}$=6,7; $p<10^{-5}$

N=122		Beta	St. Err. of Beta	B	SE of B	$t_{(111)}$	p-level
Variables	r		Intercpt	94,68	5,00	18,9	10^{-6}
Testosterone, nM/L	**0,40**	0,340	0,078	0,640	0,147	4,36	10^{-4}
ULF HRV PSD, msec²	**0,22**	0,209	0,077	0,0048	0,0018	2,73	0,007
Cortisol, nM/L	**-0,30**	-0,227	0,077	-0,026	0,009	-2,93	0,004
T5-θ PSD, %	**-0,26**	-0,153	0,094	-0,445	0,273	-1,63	0,106
Fp1-θ PSD, %	**-0,24**	-0,144	0,107	-0,360	0,268	-1,35	0,181
Fp2-θ PSD, %	**-0,19**	-0,139	0,109	-0,366	0,288	-1,27	0,206
C4-θ PSD, %	**-0,18**	0,155	0,115	0,480	0,355	1,35	0,178
C3-β PSD, µV²/Hz	**-0,23**	-0,185	0,083	-0,0324	0,0144	-2,23	0,028
F7-β PSD, %	**-0,16**	-0,197	0,093	-0,168	0,079	-2,12	0,036
Entropy F7	**-0,17**	0,135	0,102	8,162	6,192	1,32	0,190

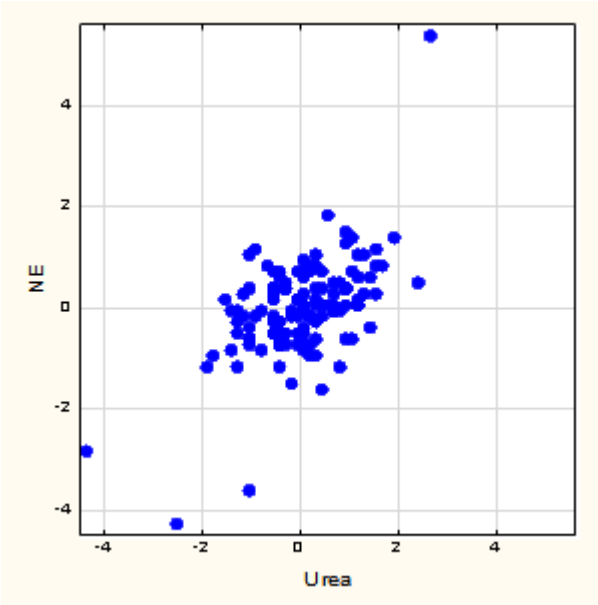


R=0,613; R^2=0,376; $\chi^2_{(10)}$=54; $p<10^{-6}$; Λ Prime=0,624

Fig. 5. Scatterplot of canonical correlation between Creatinineemia (X-line) and Neuroendocrine parameters (Y-line)

Table 6. Regression Summary for Urea plasma, mM/L
R=0,558; R^2=0,311; Adjusted R^2=0,269; $F_{(7,1)}$=7,4; $p<10^{-5}$

N=122		Beta	St. Err. of Beta	B	SE of B	$t_{(114)}$	p-level
Variables	r		Intercpt	6,302	0,528	11,9	10^{-6}
C4-δ PSD, µV²/Hz	**-0,26**	-0,292	0,081	-0,00038	0,00010	-3,61	0,0005
Deviation-β, Hz	**-0,23**	-0,203	0,079	-0,2499	0,0980	-2,55	0,012
Entropy HRV	**-0,22**	-0,164	0,080	-1,357	0,661	-2,05	0,042
Total Power HRV, msec²	**0,24**	0,182	0,083	0,00004	0,00002	2,20	0,030
Baevskiy's ARSI, units	**0,24**	0,160	0,085	0,060	0,032	1,89	0,061
F7-δ PSD, µV²/Hz	**0,18**	0,241	0,080	0,00014	0,00004	3,01	0,003
Deviation-α, Hz	**0,16**	0,181	0,079	0,3485	0,1516	2,30	0,023



R=0,558; R^2=0,311; $\chi^2_{(7)}$=43; $p<10^{-6}$; Λ Prime=0,689

Fig. 6. Scatterplot of canonical correlation between Urea plasma (X-line) and HRV&EEG parameters (Y-line)

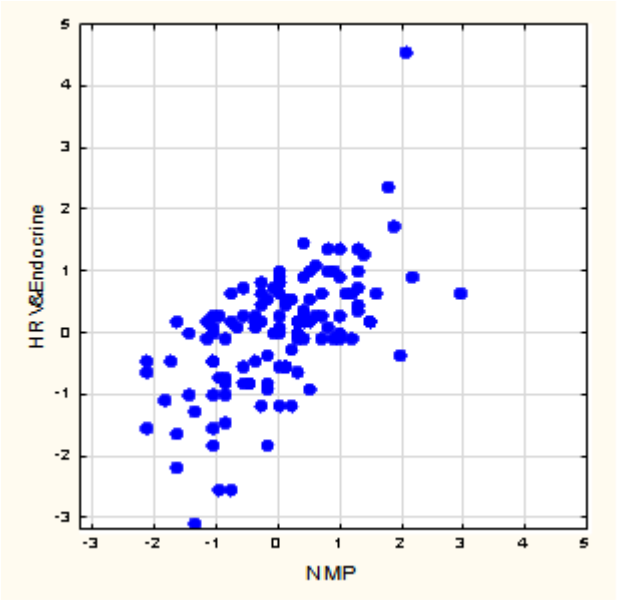
Since all nitrogenous plasma metabolites interact with the nervous and endocrine systems not individually, but simultaneously, which allows both synergism and antagonism, it is the canonical correlation between nitrogenous and neuro-endocrine constellations that is most adequate for this situation.

First, let's analyze the canonical correlation between nitrogenous metabolites and parameters of the endocrine and autonomic nervous systems. The program selected two pairs of canonical roots. The nitrogenous root of the first pair receives the maximum factor load from creatinine and the minimum one-way load from urea, on the other hand, the loads from uric acid and free bilirubin are opposite in sign (Table 7).

Table 7. Factor structure of first pair of canonical Roots representing the plasma nitrogenous metabolites and HRV&endocrine parameters (n=122)

Left side	**R1**
Creatinine	**0,759**
Urea	**0,133**
Uric acid	**-0,303**
Bilirubin free	**-0,214**
Right side	**R1**
Cortisol	**-0,710**
ULF HRV PSD, ms²	**0,353**
Testosterone	**0,226**
Baevskiy's ARSI	**0,326**
Entropy HRV	**0,299**
Heart Rate	**0,384**
Triiodothyronine	**0,192**
(UL+VL)F PSD, %	**-0,457**

The endocrine-autonomous root is represented by variables that are subject to influences of different directions, the integral measure of which is 36,2% (Fig. 7).



R=0,602; R^2=0,362; $\chi^2_{(60)}$=146; $p<10^{-6}$; Λ Prime=0,272

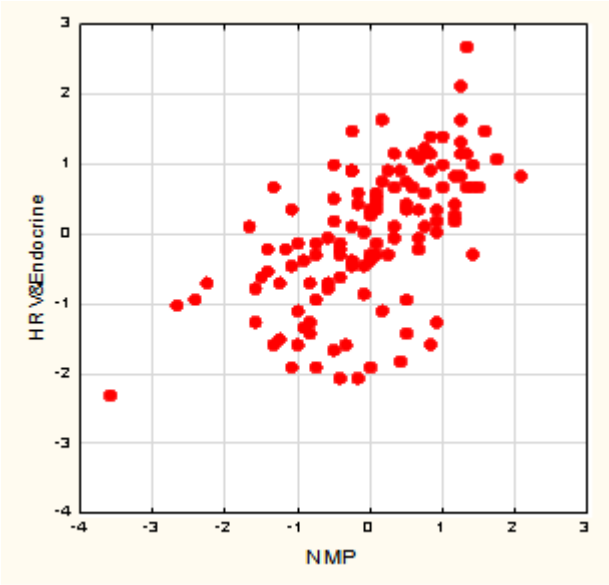
Fig. 7. Scatterplot of canonical correlation between plasma nitrogenous metabolites (X-line) and HRV&Endocrine parameters (Y-line). First pair of Roots

The nitrogenous root of the second pair is equally represented by uric acid, creatinine, and both fractions of bilirubin, while urea gives a minimal and oppositely directed factor loading (Table 8).

Table 8. Factor structure of second pair of canonical Roots representing the plasma nitrogenous metabolites and HRV&endocrine parameters (n=122)

Left side	**R2**
Uric acid	**-0,692**
Bilirubin free	**-0,679**
Bilirubin direct	**-0,617**
Creatinine	**-0,520**
Urea	**0,107**
Right side	**R2**
Testosterone	**-0,777**
ULF HRV PSD, %	**-0,412**
(UL+VL)F PSD, %	**-0,214**
LFnu HRV PSD, %	**-0,202**
Trait anxiety	**0,375**
Heart Rate	**0,297**
Triiodothyronine	**0,277**
Entropy HRV	**-0,260**
Baevskiy's ARSI	**0,245**
Total Power HRV	**0,151**

The regulated root is represented by variables subject to **upregulation** or **downregulation** by uric acid, bilirubin and creatinine, as well as **downregulation** or **upregulation** by urea, the integral measure of which is 35,3% (Fig. 8).



R=0,594; R^2=0,353; $\chi^2_{(44)}$=95; p=10^{-5}; Λ Prime=0,427

Fig. 8. Scatterplot of canonical correlation between plasma nitrogenous metabolites (X-line) and HRV&Endocrine parameters (Y-line). Second pair of Roots

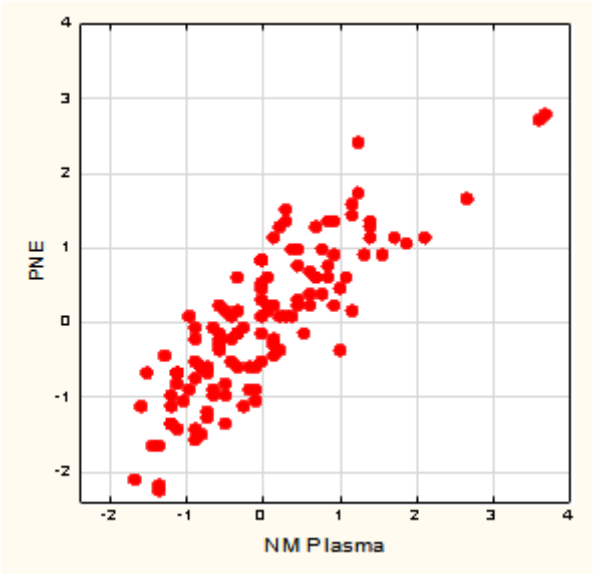
At the final stage, EEG parameters were included in the canonical analysis. Two pairs of roots are created again. The nitrogenous root receives the maximum factor loads from both fractions of bilirubin, to a lesser extent from uric acid and the minimum from creatinine, in the absence of a noteworthy load from urea (Table 9).

Table 9. Factor structure of first pair of canonical Roots representing the plasma nitrogenous metabolites and psychoneuroendocrine parameters

Left side	**R1**
Bilirubin direct	**0,831**
Bilirubin free	**0,802**
Uric acid	**0,520**
Creatinine	**0,375**
Right side	**R1**
Trait anxiety	**-0,280**
F3-θ PSD, μV²/Hz	**-0,329**
T5-θ PSD, %	**-0,320**
C3-θ PSD, μV²/Hz	**-0,316**
T3-θ PSD, μV²/Hz	**-0,305**
C4-θ PSD, μV²/Hz	**-0,262**
C4-θ PSD, %	**-0,219**
Amplitude θ, μV	**-0,215**
P4-β PSD, μV²/Hz	**-0,424**
O2-β PSD, μV²/Hz	**-0,368**
C3-β PSD, μV²/Hz	**-0,365**
T6-β PSD, μV²/Hz	**-0,364**
T3-β PSD, μV²/Hz	**-0,261**
Triiodothyronine	**-0,209**
Testosterone	**0,467**
ULF HRV PSD, %	**0,305**
LFnu HRV PSD, %	**0,213**
(UL+VL)F PSD, %	**0,175**
Asymmetry β	**0,293**
T4-β PSD, %	**0,240**

The regulated root is represented by trait anxiety, θ- and β-rhythm generating neurons, which are **downregulated** by direct and free bilirubin, as well as triiodothyronine. Another part of the factor structure

consists of testosterone as well as HRV and EEG parameters, subject to upregulation by nitrogenous metabolites. This psycho-neuro-endocrine constellation is determined by bilirubin, uric acid and creatinine by 70,6% (Fig. 9).



$R=0,840$; $R^2=0,706$; $\chi^2_{(190)}=361$; $p<10^{-6}$; Λ Prime=0,026

Fig. 9. Scatterplot of canonical correlation between plasma nitrogenous metabolites (X-line) and psycho-neuro-endocrine parameters (Y-line). First pair of Roots

Urea, albeit with a minimal load, is included in the factor structure of the nitrogenous root of the second pair (Table 10). Accordingly, HRV&EEG parameters subject to **upregulation** or **downregulation** appeared in the regulated root.

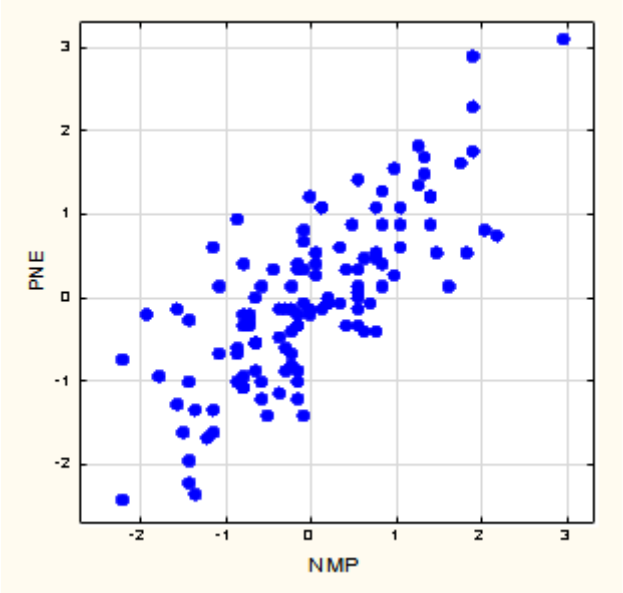
The top positions in the factor structure are occupied by creatinine and uric acid, but with loads opposite in sign. Cortisol, which is **downregulated** by creatinine, leads the factor structure of the oppositional root, followed by theta-rhythm generating neurons, which are **downregulated** by creatinine and direct bilirubin, as well as two HRV parameters, which are **upregulated** by them. Parameters **downregulated** or **upregulated** by uric acid, form another cluster.

Table 10. Factor structure of second pair of canonical Roots representing the plasma nitrogenous metabolites and neuro-endocrine parameters

Left side	**R2**
Creatinine	**0,577**
Bilirubin direct	**0,274**
Uric acid	**-0,552**
Urea	**0,207**
Right side	**R2**
Cortisol	**-0,529**
T5-θ PSD, %	**-0,481**
Fp1-θ PSD, %	**-0,378**
T3-θ PSD, μV²/Hz	**-0,288**
C4-θ PSD, %	**-0,287**
Amplitude θ, μV	**-0,274**
Fp2-θ PSD, %	**-0,244**
F3-θ PSD, μV²/Hz	**-0,241**
C4-θ PSD, μV²/Hz	**-0,240**
C3-θ PSD, μV²/Hz	**-0,221**
Entropy F7	**-0,344**
F7-β PSD, %	**-0,289**
ULF HRV PSD, ms²	**0,293**
LFnu HRV PSD, %	**0,238**
Heart Rate	**0,319**
C4-β PSD, %	**0,238**

Fp2-δ PSD, μV²/Hz	0,220
(UL+VL)F PSD, %	-0,332
Asymmetry θ	-0,264
Laterality δ	-0,152
Asymmetry β	-0,055
Baevskiy's ARSI	0,229
F7-δ PSD, μV²/Hz	0,183
Total Power HRV	0,050
Deviation-α	0,048
Entropy HRV	-0,230
Deviation-β	-0,200
C4-δ PSD, μV²/Hz	-0,137

Such a constellation of endocrine and neural parameters is determined by nitrogenous metabolites by 59,4% (Fig. 10).



R=0,771; R^2=0,594; $\chi^2_{(148)}$=240; $p<10^{-5}$; Λ Prime=0,089

Fig. 10. Scatterplot of canonical correlation between plasma nitrogenous metabolites (X-line) and neuroendocrine parameters (Y-line). Second pair of Roots

Therefore, bilirubin and uric acid realize their neuro-endocrine effects, with a high probability, through aryl hydrocarbon and adenosine receptors of neurons and endocrinocytes, respectively. On the other hand, in relation to creatinine and uric acid, the question remains open due to the lack of literature data on the existence of the corresponding receptors. It is possible that there is a direct toxic effect.

Acknowledgment. We express sincere gratitude to administration of clinical sanatorium "Moldova" and PrJSC "Truskavets' Spa" as well as TA Korolyshyn and VV Kikhtan for help in carrying out this investigation.

Accordance to ethics standards. Tests in patients are carried out in accordance with positions of Helsinki Declaration 1975 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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APPENDIX

Table 1. Matrix of correlations between nitrogenous metabolites and psycho-neuro-endocrine parameters

Variable	Correlations (n=122)				
	B ilD	B ilF	C reat	U rea	UA
B D	1,00	0,80	0,20	0,25	0,02
B F	0,80	1,00	0,19	0,22	0,24
CR	0,20	0,19	1,00	0,32	0,32
UREA	0,25	0,22	0,32	1,00	-0,02
UA	0,02	0,24	0,32	-0,02	1,00
C alcitonin	0,06	0,05	0,18	0,01	-0,06
Ald osterone	0,10	0,07	0,03	0,06	-0,02
Trait anxiety	-0,19	-0,19	-0,11	-0,10	-0,19
React anx	-0,08	-0,02	-0,13	-0,14	-0,12
S ex	-0,31	-0,30	-0,37	-0,13	-0,27
Age	-0,03	0,04	-0,11	-0,04	0,08
Testosteron e	0,20	0,22	0,40	0,05	0,39
VLFs%	0,09	0,20	-0,12	-0,00	0,16
HHRV	0,09	0,06	0,13	-0,22	-0,04
Cor tisol	-0,03	0,06	-0,30	-0,01	0,17
T3	-0,13	-0,16	0,01	0,04	-0,12
BARSI	-0,07	-0,02	0,15	0,24	-0,13
M ode	0,04	0,11	-0,09	-0,15	0,18
HR	-0,04	-0,13	0,10	0,16	-0,21
SDNN	0,01	-0,01	0,04	0,10	-0,04
RMSSD	-0,07	-0,08	0,06	0,06	0,01
PNN50	-0,10	-0,09	0,10	0,07	-0,01
TP	0,01	0,01	0,04	0,24	-0,04
ULF	0,13	0,10	0,22	0,14	-0,05
VLF	-0,01	0,00	-0,04	0,24	-0,02
LF	0,08	0,03	0,13	0,15	-0,09
HF	-0,13	-0,11	0,07	0,04	-0,00
LFNU	0,27	0,22	0,12	0,12	-0,08

Note. According to calculations by the formula:
$|r| = \{\exp[2t/(n-1,5)^{0,5}]-1\}/\{\exp[2t/(n-1,5)^{0,5}]+1\}$
for a sample of n=122 critical value |r| at $p<0,05$ ($t>2,00$) is 0,18; at $p<0,01$ ($t>2,66$) is 0,24; at $p<0,001$ ($t>3,46$) is 0,30.