Polovynko Ilona S, Zajats Lyubomyr M, Zukow Walery, Yanchij Roman I, Popovych Igor L. Quantitative evaluation of integrated neuroendocrine and immune responses to chronic stress in rats male. Journal of Education, Health and Sport. 2016;6(8):154-166. eISSN 2391-8306. DOI http://dx.doi.org/10.5281/zenodo.60023
http://ojs.ukw.edu.pl/index.php/johs/article/view/3745

The journal has had 7 points in Ministry of Science and Higher Education parametric evaluation. Part B item 755 (23.12.2015).
755 Journal of Education, Health and Sport eISSN 2391-8306 7

The authors declare that there is no conflict of interests regarding the publication of this paper.
Received: 02.07.2016. Revised 25.07.2016. Accepted: 28.07.2016.

QUANTITATIVE EVALUATION OF INTEGRATED NEUROENDOCRINE AND IMMUNE RESPONSES TO CHRONIC STRESS IN RATS MALE

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Abstract

Background. Previously we have been analysed neuro-endocrine-immune relationships by chronic restraint stress at male rats. The **purpose** of this study - to carry out integrated quantitative estimation of neuroendocrine and immune responses to chronic restraint stress at male rats. **Material and research methods.** The experiment is at 50 white male rats. Of these 10 animals not subjected to any influences and 40 within 7 days subjected to moderate stress by daily 30-minute immobilization. The day after the completion of stressing in rats of both groups determined parameters of heart rate variability, urinary excretion of 17-ketosteroids, plasma levels of corticosterone, testosterone, thyroxine and triiodothyronine as well as sodium, potassium, calcium and phosphates. The same portion of the blood immunological parameters were determined by tests I and II levels of WHO. The spleen and thymus did smears for counting spleno- and thymocytograms. In sections of the adrenal glands was measured the thickness of glomerular, fascicular, reticular and medullar zones. **Results.** The method of discriminant analysis found that distinctive endocrine signs of chronic stress is increasing the thickness of Fasciculary Zone whereas decreasing thickness of Glomerulary Zone of Adrenal Cortex as well as plasma $(Ca/P)^{0,5}$ ratio as Parathyrine Activity. Other signs of chronic stress such as increasing plasma levels Corticosterone, Testosterone and Triiodethyronine, Sympathetic tone, Heart Rate and thickness of Reticular Zone of Adrenals as well as decreasing Vagal Tone and plasma $(Na/K)^{0,5}$ ratio as Mineralocorticoid Activity currently not in the discriminant model. Canonical Neuroendocrine Roots for Intact and Stressed Males Rats averages +0,99±0,40 and -0,25±0,15 respectively (Squared Mahalanobis Distance=1,61; F=3,76; p=0,017). Among the parameters of Immunity characteristic of chronic stress appeared to increase Thymus Massa Index, level in Thymocytogram of Macrophages and Reticulocytes, in Splenocytogram of Macrophages and Eosinophils, while Monocytes in Leukocytogram of Blood as well as Entropy of

Leukocytogram and Splenocytogram whereas decrease both Intensity and Activity of Phagocytose by Neutrophils, levels of Endotheliocytes in Thymocytogram, Neutrophils in Splenocytogram, NK-Lymphocytes, Stub Neutrophils and Basophils in Leukocytogram. Canonical Immune Roots for Intact and Stressed Males Rats averages -3,41±0,40 and +0,85±0,15 respectively (Squared Mahalanobis Distance=19,0; F=5,44; $p<10^{-4}$). Canonical correlation between Neuroendocrine and Immune parameters is very strong: R=0,976; $\chi^2_{(297)}=432$; $p<10^{-6}$. **Conclusion.** The components of the autonomic nervous, endocrine and immune systems interact closely within the triune neuroendocrine-immune complex and changing them exposed to integrated evaluation. The author's approach can be used to quantify the integrated assessment of intensity of stress factors and to integral assessing the effectiveness of stresslimiting factors.

Keywords: chronic stress, autonomic regulation, adaptive hormones, adrenals, thymocytogram, splenocytogram, leukocytogram and immunocytogram of blood, relationships, male rats.

INTRODUCTION

As summarized in the lovely review FS Dhabhar [4], stress is known to suppress immune function and increase susceptibility to infections and cancer. Paradoxically, stress is also known to exacerbate asthma, and allergic, autoimmune and inflammatory diseases, although such diseases should be ameliorated by immunosuppression. Moreover, the short-term fight-or-flight stress response is one of nature's fundamental defense mechanisms that enables the cardiovascular and musculoskeletal systems to promote survival, and it is unlikely that this response would suppress immune function at a time when it is most required for survival (e.g. in response to wounding and infection by a predator or aggressor). These observations suggest that stress may suppress immune function under some conditions while enhancing it under others. The effects of stress are likely to be beneficial or harmful depending on the type (immunoprotective, immunoregulatory/inhibitory, or immunopathological) of immune response that is affected. Studies have shown that several critical factors influence the direction (enhancing vs. suppressive) of the effects of stress or stress hormones on immune function: (1) *Duration (acute vs. chronic) of stress:* Acute or short-term stress experienced at the time of immune activation can enhance innate and adaptive immune responses. Chronic or long-term stress can suppress immunity by decreasing immune cell numbers and function and/or increasing active immunosuppressive mechanisms (e.g. regulatory T cells). Chronic stress can also dysregulate immune function by promoting proinflammatory and type-2 cytokine-driven responses. (2) *Effects of stress on leukocyte distribution:* Compartments that are enriched with immune cells during acute stress show immunoenhancement, while those that are depleted of leukocytes, show immunosuppression. (3) *The differential effects of physiologic versus pharmacologic concentrations of glucocorticoids, and the differential effects of endogenous versus synthetic glucocorticoids:* Endogenous hormones in physiological concentrations can have immunoenhancing effects. Endogenous hormones at pharmacologic concentrations, and synthetic hormones, are immunosuppressive. (4) *The timing of stressor or stress hormone exposure relative to the time of activation and time course of the immune response:* Immunoenhancement is observed when acute stress is experienced at early stages of immune activation, while immunosuppression may be observed at late stages of the immune response. Author propose that it is important to study and, if possible, to clinically harness the immunoenhancing effects of the acute stress response, that evolution has finely sculpted as a survival mechanism, just as we study

its maladaptive ramifications (chronic stress) that evolution has yet to resolve. In view of the ubiquitous nature of stress and its significant effects on immunoprotection as well as immunopathology, it is important to further elucidate the mechanisms mediating stress-immune interactions and to meaningfully translate findings from bench to bedside.

Previously we [13] have been analyzed neuro-endocrine-immune relationships by chronic restraint stress at male rats. It is detected considerable (R=0,67) canonical correlation between autonomous regulation parameters and thymocytogram. Thymic canonical radical receives negative factor loading on the relative weight of the thymus gland and levels there macrophages, endothelial cells and Gassal corpuscules, while positive factor loading on radical give lymphoblasts and lymphocytes. The canonical correlation between vegetative parameters and splenocytogram very strong (R=0,94). Splenic canonical radical receives negative factor loading of macrophages and reticulocytes and positive - of the mass of the spleen and the contents therein neutrophils, lymphocytes and eosinophils. Revealed a strong (R=0,79) canonical correlation between autonomous regulation parameters and immune parameters of blood. In this immune root is represented B-lymphocytes, plasmacytes, basophils, eosinophils, neutrophils (stub and segmented), completeness of neutrophil phagocytosis, phagocytic activity of monocytes, leukocytosis and general lymphocytosis. We found a close relationship (R=0,89) between the endocrine and immune parameters. Endocrine canonical radical right represented relative adrenal weight, thickness of fascicular, glomerular and reticular zones, excretion of 17-ketosteroids, plasma level of triiodothyronine and inverse represented plasma level of corticosterone. Immune radical receives positive factor loading on the relative weight of the thymus and spleen, content in the last lymphoblasts and neutrophils, content in blood leukocytes, completeness and intensity of phagocytosis of neutrophils. Instead, the negative loadings on the immune radical given level of splenic fibroblasts, macrophages, reticulocytes, thymic epithelial cells and Gassal corpuscules, level in blood of NK-lymphocytes, plasmacytes, basophils and monocytes.

The purpose of this study - to carry out integrated quantitative estimation of neuroendocrine and immune responses to chronic restraint stress at male rats.

MATERIAL AND METHODS

The experiment is at 50 white male rats Wistar line weighing 240-280 g. Of these 10 animals not subjected to any influences (intact), accounting for the control group, and the remaining 40 within 7 days subjected to moderate stress by daily 30-minute immobilization [14]. The day after the completion of stressing in rats of both groups took samples of peripheral blood (through a cut tail) to analyze leukocytogram. An hour under light ether anesthesia for 15-20 s recorded ECG in standard lead II (introducing needle electrodes subcutaneously) to determine parameters of heart rate variability (HRV) [1,2]. Then the rats were placed in individual chambers with perforated bottom to collect daily urine, in which determined the concentration of 17-ketosteroids (by method colorimetric reaction with m-dinitrobenzene [5]), followed by calculation of the daily excretion. The next day, the animals were decapitated, for the purpose of collecting blood plasma, in which was determined concentration of adaptive hormones corticosterone, testosterone, thyroxine and triiodothyronine (by ELISA [7]). In the same portion of the blood immunological parameters were determined by tests I and II levels of WHO [9,11] and the previously developed algorithm [14]. After a blood sample was removed spleen, thymus and adrenal glands and weighed them. Since the spleen and thymus did smears for counting spleno-

and thymocytograms [14]. In sections of the adrenal glands was measured under a microscope the thickness of glomerular, fascicular, reticular and medullar zones.

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RESULTS AND DISCUSSION

When compared to traditional neuroendocrine parameters in rats subjected to chronic stress, with intact animals revealed an increase Corticosterone plasma level by 20,8±10,0% ($p<0,05$), Sympathetic tone (evaluated by AMo HRV) by 20,1±6,5% ($p<0,01$), Heart Rate by 9,6±3,2% ($p<0,01$), thickness of reticular zone of adrenals by 17,1±5,6% ($p<0,01$), Testosterone plasma level by 16,9±8,7% ($p>0,05$), thickness of fascicular zone of adrenals by 12,6±3,6% ($p<0,01$) as well as Triiodethyronine plasma level by 6,2±3,8% ($p>0,10$) while Vagal Tone (evaluated by ΔX HRV), thickness of glomerular zone of adrenals, Mineralocorticoid Activity (evaluated by plasma $(Na/K)^{0,5}$ ratio) as well as Parathyrin Activity (evaluated by plasma $(Ca/P)^{0,5}$ ratio) decreased by 27,5±8,6% ($p<0,01$), 12,5±2,6% ($p<0,001$), 5,6±1,8% ($p<0,01$) and 3,7±1,9% ($p>0,05$) respectively.

According recommendation by IL Popovych [14] variables obtained after Chronic Stress (SV) expressed as Z-score calculated by formula:

$Z=(SV/IV – 1)/Cv$, where

IV is Initial (Control) Variable, Cv is Coefficient its variation at intact rats.

This approach allows us to estimate variables expressed in various units (μM, %, nM/L, msec etc) not just in one scale, and taking into account their variability as physiological cost 1% change stable setting higher than this parameter fluctuation whose normally wider. As shown in Fig.1, ranking caused by chronic stress changes in neuroendocrine parameters by Z-scores differs of ranking by %.

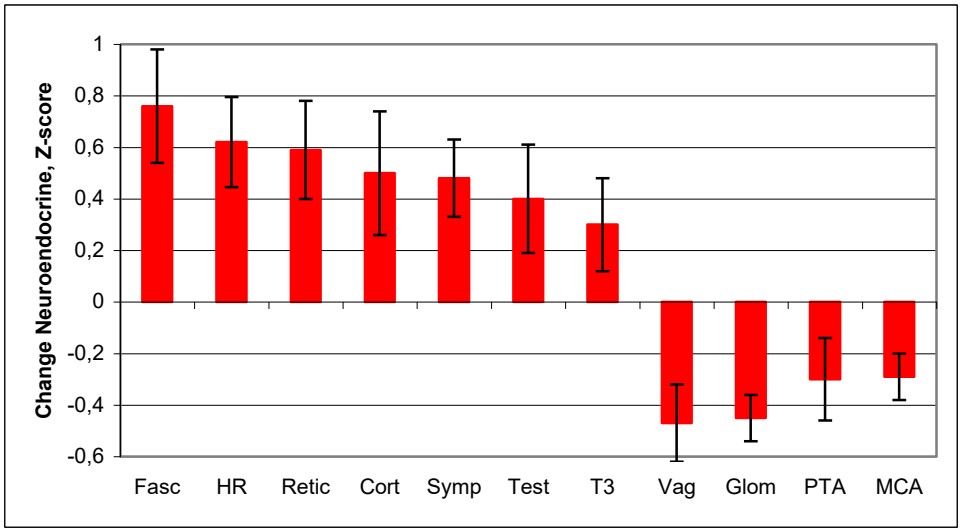

Fig. 1. Ranking caused by chronic stress changes in neuroendocrine parameters

Overall, our findings are consistent with a classic conception about the leading role in neuro-endocrine manifestations of Chronic Stress Corticoadrenal and Autonomic Nervous Systems and about functional antagonism between Glucocrticoide and Mineralocorticoide as well as between Sympathetic and Vagal Activities. However, our data support the discussion about the nature of changes by Chronic Stress in other endocrine glands [2,3,4,10,17,21,25].

If we take as an integrated quantitative measure of neuro-endocrine manifestations of Chronic Stress mean of modules of Z-Scores, it will be 0,47±0,04 σ (or Euklidian units).

Another approach to identify the parameters (variables, options) set of which neuro-endocrine status intact and stressed rats significantly different is discriminant (recognizing) analysis. In applying method forward stepwise variables currently in the model turned out three only (Tables 1 and 2), while other earlier marked variables currently not in the discriminant model.

Table 1. Discriminant Function Analysis Summary for Neuro-endocrine Variables
Step 3, N of variables in model: 3; Grouping: 2 groups;
Wilks' Λ: 0,795; approx. $F_{(3,5)}$=3,95; p=0,014.

Variables currently in the model	Initial level (Control)	After Chronic Stress	Stressory change as Z-score	Wilks' Λ	Partial Λ	F-remove	p-level	Tolerancy
Fasciculary ZAC, μM	218±11	245±8	+0,76±0,22	,925	,859	7,5	,009	,748
Glomerulary ZAC, μM	129±11	113±3	-0,45±0,09	,899	,884	6,0	,018	,885
$(Cap/Pp)^{0,5}$ as PTA, units	1,66±0,06	1,59±0,03	-0,30±0,15	,838	,949	2,5	,124	,836

Variables currently not in the model	Initial level (Control)	After Chronic Stress	Stressory change as Z-score	Wilks' Λ	Partial Λ	F to enter	p-level	Tolerancy
Heart Rate, beats/min	336±20	375±11	+0,62±0,18	,789	,992	,346	,56	,86
Reticulary ZAC, μM	20,0±1,8	23,5±1,1	+0,59±0,20	,788	,991	,415	,52	,69
Corticosterone, nM/L	340±44	411±34	+0,50±0,24	,778	,979	,966	,33	,86
Sympathotone (AMo), %	55,6±7,4	66,8±3,6	+0,48±0,15	,782	,983	,764	,39	,83
Testosterone, nM/L	34,6±4,6	40,4±3,0	+0,40±0,20	,793	,997	,143	,71	,94
Triiodethyronine, nM/l	2,50±0,17	2,66±0,10	+0,30±0,18	,795	1,00	,015	,90	,94
Vagal Tone (ΔX), msec	42±8	30±4	-0,47±0,15	,786	,988	,532	,47	,84
$(Nap/Kp)^{0,5}$ as MCA, un	6,10±0,37	5,76±0,11	-0,29±0,09	,787	,989	,480	,49	,93

Table 2. Summary of Stepwise Analysis and Chi-Square Tests with Successive Root Removed for Neuro-endocrine Variables

Variables currently in the model	F to enter	p-level	Lambda	F-value	p-level
Fasciculary Zone of Adrenal Cortex	5,35	,025	,838	4,56	,015
Glomerulary Zone of Adrenal Cortex	3,45	,069	,933	3,45	,069
$(Cap/Pp)^{0,5}$ as Parathyrine Activity	2,45	,124	,795	3,95	,014
Canonical R=0,453; Wilks' Λ=0,795; $\chi^2_{(3)}$=10,7; p=0,014					

Table 3. Standardized, Structural and Raw Coefficients and Constant for Canonical Variables

	Coefficients		
Variables currently in the model	Standardized	Structural	Raw
Fasciculary Zone of Adrenal Cortex	-0,958	-0,47	-0,021
Glomerulary Zone of Adrenal Cortex	0,799	0,53	0,033
$(Cap/Pp)^{0,5}$ as Parathyrine Activity	0,544	0,23	2,818
Eigenvalue	0,258	**Constant**	-3,396

Information about these 3 variables condensed in canonical root which correlated with thickness of Fascicular zone of adrenals negatively instead positively with this of Glomerular

zone as well as Parathyrine Activity. The calculation of individual Root values based on Raw Coefficients for discriminant variables and Constant (Table 3) allows to visualize the status of each rat (Fig. 1).

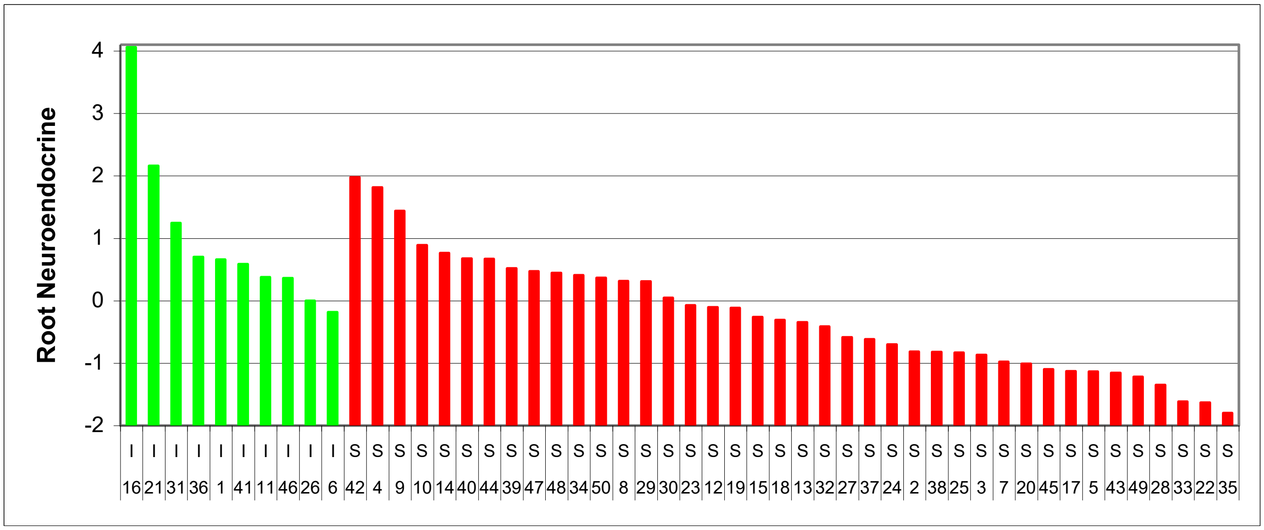

Fig. 1. Individual Root Neuroendocrine values for intact (I) and stressed (S) rats (below the specified number of rat)

Despite widespread individual values difference between status of intact and subjected stress rats is much significantly (Fig. 2). Canonical Neuroendocrine Roots for Intact and Stressed Males Rats averages +0,99±0,40 and -0,25±0,15 respectively (Squared Mahalanobis Distance=1,61; F=3,76; p=0,017).

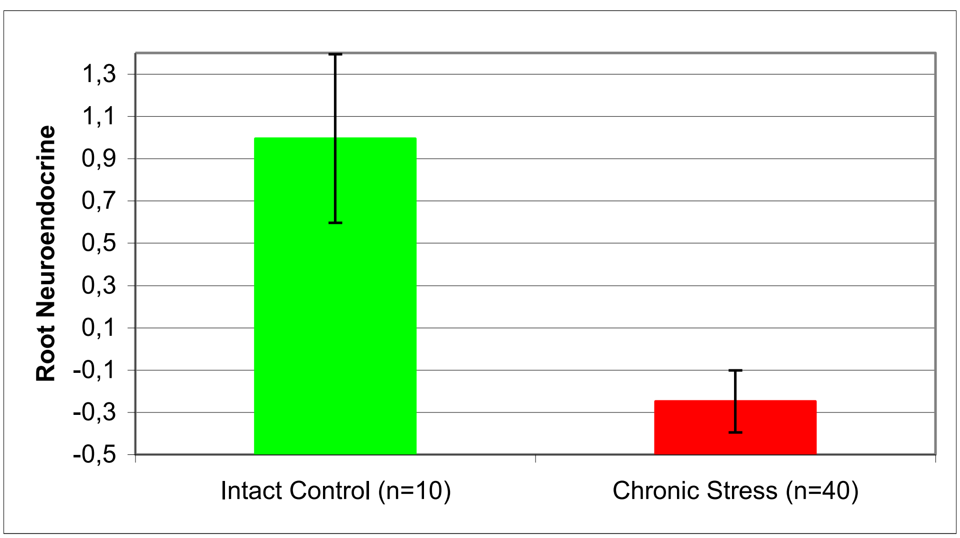

Fig. 2. Means of Root Neuroendocrine for intact and stressed rats

Among the parameters of Immunity characteristic of chronic stress appeared (Table 4) to increase Thymus Massa Index, level in Thymocytogram of Macrophages and Reticulocytes, in Splenocytogram of Macrophages and Eosinophils, while Monocytes in Leukocytogram of Blood as well as Entropy of both Leukocytogram and Splenocytogram whereas decrease both

Intensivity and Activity of Phagocytose by Neutrophils Staphylococcus aureus, levels of Endotheliocytes in Thymocytogram, Neutrophils in Splenocytogram, NK-Lymphocytes, Stub Neutrophils and Basophils in Leukocytogram.

Table 4. Discriminant Function Analysis Summary for Immune Variables
Step 17, N of variables in model: 17; Grouping: 2 groups
Wilks' Lambda: 0,248; approx. $F_{(17,3)}=5,7$; $p<10^{-5}$

Variables currently in the model	Initial level (Control)	After Chronic Stress	Stressory change as Z-score	Wilks Λ	Partial Λ	F remove	p-level	Tolerancy
Macrophages of Thymus	4,70±0,21	6,13±0,28	+2,12±0,42	,402	,617	19,9	,000	,352
Macrophages of Spleen	5,50±0,65	7,35±0,31	+0,90±0,15	,356	,696	14,0	,001	,254
Thymus Massa Index	0,295±0,022	0,340±0,013	+0,67±0,19	,278	,892	3,9	,058	,512
Eosinophils of Spleen	1,80±0,25	2,18±0,16	+0,48±0,21	,325	,763	10,0	,003	,353
Monocytes of Blood	4,20±0,73	5,18±0,29	+0,42±0,13	,256	,967	1,1	,304	,426
Entropy Splenocytogram	0,588±0,007	0,597±0,004	+0,37±0,19	,269	,921	2,7	,108	,358
Entropy LCG of Blood	0,350±0,007	0,356±0,005	+0,26±0,24	,346	,716	12,7	,001	,050
Reticulocytes of Thymus	5,20±0,63	5,51±0,29	+0,16±0,14	,307	,806	7,7	,009	,355
Lymphocytes of Blood	60,4±1,4	60,2±1,0	-0,05±0,21	,314	,789	8,6	,006	,075
Killing Ind Neut. of Blood	54,7±2,0	54,3±0,7	-0,06±0,11	,347	,715	12,7	,001	,283
Phagocyt Ind Neutr Blood	82,3±0,7	81,9±0,6	-0,31±0,17	,274	,906	3,3	,077	,286
Basophils of Blood	0,30±0,15	0,15±0,06	-0,31±0,12	,302	,821	7,0	,013	,380
Stub Neutroph. of Blood	3,50±0,17	3,27±0,17	-0,43±0,32	,264	,937	2,1	,153	,290
NK-Lymphocyt of Blood	10,4±0,6	9,4±0,2	-0,54±0,12	,261	,949	1,7	,198	,592
Neutrophils of Spleen	11,5±0,5	10,6±0,4	-0,55±0,24	,257	,965	1,2	,287	,532
Endotheliocytes of Thym	7,40±0,43	6,15±0,25	-0,92±0,19	,268	,925	2,6	,117	,620
Microb Count Neutr Bloo	8,14±0,07	7,91±0,08	-1,10±0,39	,316	,785	8,7	,006	,295

Variables currently not in the model Df for all F-tests: 1,31	Initial level (Control)	After Chronic Stress	Stressory change as Z-score	Wilks Λ	Partial Λ	F to enter	p-level	Tolerancy
Microb Count Monoc Bloo	2,8±0,1	3,6±0,3	+2,55±0,82	,245	,989	,349	,56	,346
Bactericid. Mon., 10^6 M/l	81±14	141±30	+1,36±0,67	,245	,989	,340	,56	,340
0-Lymphocytes of Blood	29,9±1,5	33,8±0,8	+0,82±0,17	,245	,989	,336	,57	,507
Leukocytes of Blood	9,57±0,54	10,20±0,34	+0,37±0,20	,244	,985	,466	,50	,475
Epitheliocytes of Tymus	19,9±0,7	20,6±0,4	+0,33±0,18	,247	,998	,056	,81	,616
Blasttransform T-Lym Blo	65,8±3,7	62,0±1,7	-0,33±0,15	,247	,998	,058	,81	,390
Lymphocytes of Thymus	55,6±1,0	54,3±0,7	-0,41±0,23	,246	,992	,240	,63	,219
Reticulocytes of Spleen	14,3±0,6	13,6±0,3	-0,41±0,17	,248	1,00	,004	,95	,675
Entropy ICG of Blood	0,522±0,004	0,517±0,003	-0,41±0,19	,247	,996	,126	,72	,570
Th-Lymphocytes of Blood	32,3±0,8	30,9±0,4	-0,57±0,17	,247	,998	,071	,79	,454
Phagoc Ind Monoc Blood	7,8±1,1	5,6±0,3	-0,64±0,10	,248	,999	,046	,83	,289

However, noteworthy is the number of immune parameters currently not in the model, namely the increase Microbial Count for Monocytes of Blood and their Bactericidal Capacity against Staphylococcus aureus (despite the decrease in their Phagocytose Index) as well as level of 0-Lymphocytes in Blood in return decrease level of Th-Lymphocytes in Blood and their Blasttransformation induced by Phytohemaglutinin.

Emerges pattern of inhibition in chronic stress subjected rats NK- and Th-Lymphocytes as well as Neutrophils/Microphages in combination with activation Monocytes/Macrophages.

Information about 17 variables currently in discriminant model condensed in canonical root which is poorly structured and correlated poorly positively with Macrophages of Spleen and Thymus instead negatively with Endotheliocytes of Thymus and NK-Lymphocytes of Blood. The calculation of individual Root values based on Raw Coefficients for discriminant variables and Constant (Table 5) allows to visualize the immune status of each rat (Fig. 3).

Table 5. Summary of Stepwise Analysis as well as Standardized, Structural and Raw Coefficients and Constant for Canonical Variables

Variables currently in the model	F to enter	p-level	Λ	F-value	p-level	Coefficients		
						Standardized	Structural	Raw
Macrophages of Spleen	6,93	,011	,874	6,93	,012	1,260	,218	,634
Macrophages of Thymus	4,16	,047	,617	7,00	10^{-3}	1,204	,207	,747
Thymus Massa Index	3,24	,081	,273	6,04	10^{-5}	,530	,133	65,50
Monocytes of Blood	1,09	,304	,248	5,71	10^{-4}	-,321	,118	-,165
Eosinophils of Spleen	2,40	,129	,407	5,67	10^{-4}	,945	,088	,951
Entropy of Splenocytogram	2,15	,152	,256	5,98	10^{-5}	-,541	,075	-20,77
Entropy of LCG of Blood	2,96	,093	,479	5,58	10^{-4}	2,746	,043	87,69
Reticulocytes of Thymus	3,48	,070	,342	5,93	10^{-4}	-,852	,040	-,462
Endotheliocytes of Thymus	7,11	,010	,759	7,46	,002	-,402	-,189	-,261
NK-Lymphocytes of Blood	5,83	,020	,674	7,43	10^{-3}	-,339	-,155	-,224
Microbes Count of Neutr of Blood	1,66	,206	,327	5,70	10^{-4}	-,983	-,115	-2,075
Neutrophils of Spleen	3,39	,073	,374	5,78	10^{-4}	-,297	-,091	-,132
Basophils of Blood	2,01	,164	,513	5,69	10^{-4}	-,792	-,091	-2,044
Stub Neutrophils of Blood	3,73	,060	,568	6,68	10^{-4}	-,537	-,052	-,534
Phagocytose Ind of Neutr of Blood	3,27	,079	,299	5,86	10^{-5}	,663	-,023	,173
Killing Ind of Neutroph. of Blood	2,45	,125	,538	6,16	10^{-4}	1,157	-,017	,236
Lymphocytes of Blood	4,26	,046	,433	5,83	10^{-4}	1,940	-,009	,331
Canonical R=0,867; Wilks' Λ=0,248; $\chi^2_{(17)}$=55; p<10^{-5}							**Constant**	-51,41

Immune status intact and subjects chronic stress rats are very different. Canonical Immune Roots for Intact and Stressed Males Rats averages -3,41±0,40 and +0,85±0,15 respectively (Squared Mahalanobis Distance=19,0; F=5,44; p<10^{-4}).

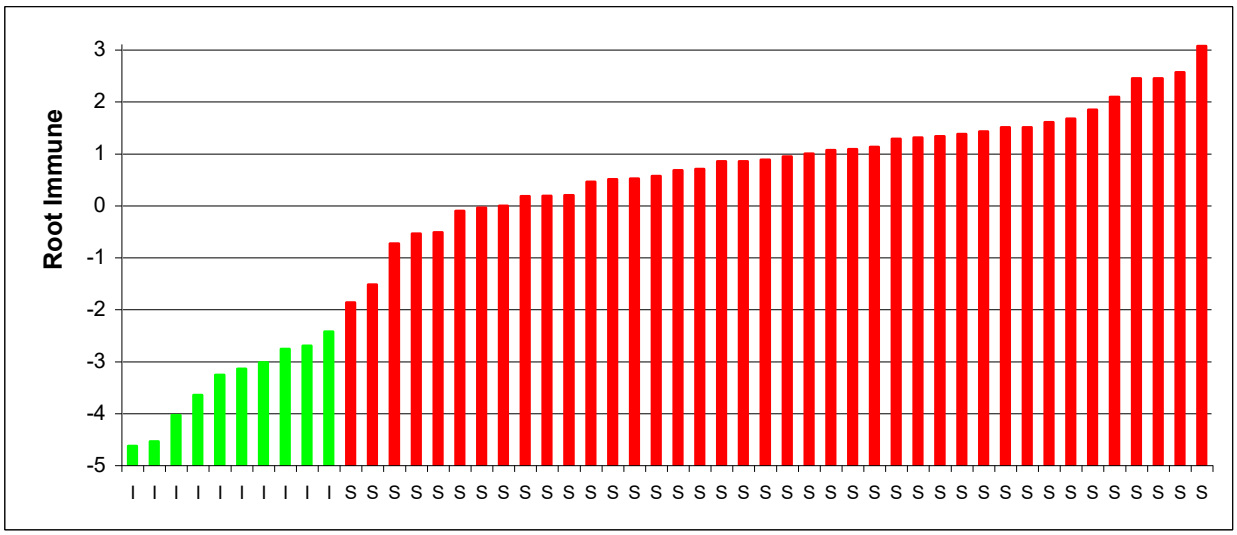

Fig. 3. Individual Root Immune values for intact (I) and stressed (S) rats

Calculation of Classification Functions based Coefficients and Constant (Table 6) allows retrospectively recognize intact rats without mistakes and stressed rats accurately to within 97,5% (one error).

Table 6. Coefficients and Constant for Classification Functions

Variables currently in the model	Intact Rats	Stressed Rats
Macrophages of Spleen, %	22,56	25,27
Endotheliocytes of Thymus, %	1,32	0,20
NK-Lymphocytes of Blood, %	-8,28	-9,23
Macrophages of Thymus, %	54,14	57,33
Stub Neutrophils of Blood, %	-1,44	-3,72
Killing Index of Neutrophils of Blood, %	22,31	23,32
Basophils of Blood, %	-166,8	-175,5
Entropy of Leukocytogram of Blood	11272	11646
Lymphocytes of Blood, %	52,36	53,78
Eosinophils of Spleen, %	61,29	65,35
Neutrophils of Spleen, %	-19,47	-20,04
Reticulocytes of Thymus, %	-22,04	-24,01
Microbes Count of Neutr of Blood, Bac/Phag	4,38	-4,48
Phagocytose Index of Neutrophils of Blood, %	7,20	7,94
Thymus Massa Index, ‰	1087	1367
Entropy of Splenocytogram	874,3	785,6
Monocytes of Blood, %	-33,30	-34,01
Constant	-4692	-4905

Finally, we have analyzed the canonical link between Neuroendocrine parameters, on the one hand, and parameters of Immunity, on the other. The program provided two noteworthy pair of canonical roots. Neuroendocrine Root of first pair (Table 7) receives a significant positive factor loading from Sympathetic Tone and caused him Heart Rate and weak load from Corticosteronemia as a marker of situational (morning) Glucocorticoid activity. Opposite the nature significant factor loading give Vagal Tone and Parathyrine Activity and weak load give the thickness zones of Adrenal Cortex as markers non situational activity but their potential functional capacity.

Table 7. Factor Structure for Neuroendocrine Roots (right set)

Variables	Root 1	Root 2
AMo as Sympathetic Tone	**,84**	,44
Heart Rate	**,81**	,10
Corticosteronemia	**,24**	,05
ΔX as Vagal Tone	**-,85**	-,35
$(Cap/Pp)^{0,5}$ as Parathyrine Activity	**-,49**	,17
Glomerulary Zone of Adrenal Cortex	**-,34**	,13
Reticulary Zone of Adrenal Cortex	**-,23**	-,05
Fasciculary Zone of Adrenal Cortex	**-,18**	-,12
$(Nap/Kp)^{0,5}$ as Mineralocorticoid Activity	,08	**,47**
Testosteronemia	,17	**-,37**
Triiodethyroninemia	,06	**-,08**

Immune Root of first pair (Table 8) receives a maximal positive factor loading from Macrophages of Spleen and significant loads from Macrophages and Endotheliocytes of Thymus as well as from Entropy of Immunocytogram of Blood and Splenocytogram (as markers their structural reserves [14]). Significant negative factor loading give total Leukocytes of Blood and parameters of Phagocytose both Neutrophils/Microphages and Monocytes/Macrophages. Canonical correlation between first pair of Neuroendocrine and Immune parameters is very strong: R=0,976; $\chi^2_{(297)}$=432; $p<10^{-6}$ (Fig. 4 upper).

Neuroendocrine Root of second pair (Table 7) receives a significant positive factor loading from marker of situational Mineralocorticoid activity while negative from Testosteronemia and very weak from Triiodethyroninemia. These endocrine factors associated with blood levels of Th- and NK-Lymphocytes as well as with Entropy of Leukocytogram of Blood as marker its structural reserves [14]. Canonical correlation between second pair of Neuroendocrine and Immune parameters is very strong too: R=0,961; $\chi^2_{(260)}$=342; $p<10^{-3}$ (Fig. 4 lower).

Table 8. Factor Structure for Immune Roots (left set)

Variables	Root 1	Root 2
Macrophages of Spleen	**,87**	,21
Entropy of Immunocytogram of Blood	**,41**	,01
Entropy of Splenocytogram	**,34**	-,16
Macrophages of Thymus	**,27**	-,03
Endotheliocytes of Thymus	**,27**	,23
Blasttransformation of T-Lymphocytes of Blood	**,26**	-,17
Basophils of Blood	**,24**	,16
Stub Neutrophils of Blood	**,23**	,16
Microbial Count for Neutrophils of Blood	**,12**	,11
Reticulocytes of Spleen	**,14**	,15
Phagocytose Index of Neutrophils of Blood	**,07**	,01
Total Leukocytes of Blood	**-,34**	-,26
Killing Index of Neutrophils of Blood	**-,32**	-,22
Phagocytose Index of Monocytes of Blood	**-,30**	,00
0-Lymphocytes of Blood	**-,28**	-,14
Neutrophils of Spleen	**-,28**	-,29
Reticulocytes of Thymus	**-,22**	-,13
Lymphocytes of Thymus	**-,20**	-,02
Microbial Count for Monocytes of Bloo	**-,13**	-,04
Th-Lymphocytes of Blood	,02	**,35**
Entropy of Leukocytogram of Blood	,12	**,34**
NK-Lymphocytes of Blood	,12	**,24**
Monocytes of Blood	-,00	**,08**
Epitheliocytes of Tymus	,05	**,07**
Eosinophils of Spleen	-,13	**-,34**
Total Lymphocytes of Blood	-,21	**-,29**
Thymus Massa Index	,10	**-,17**

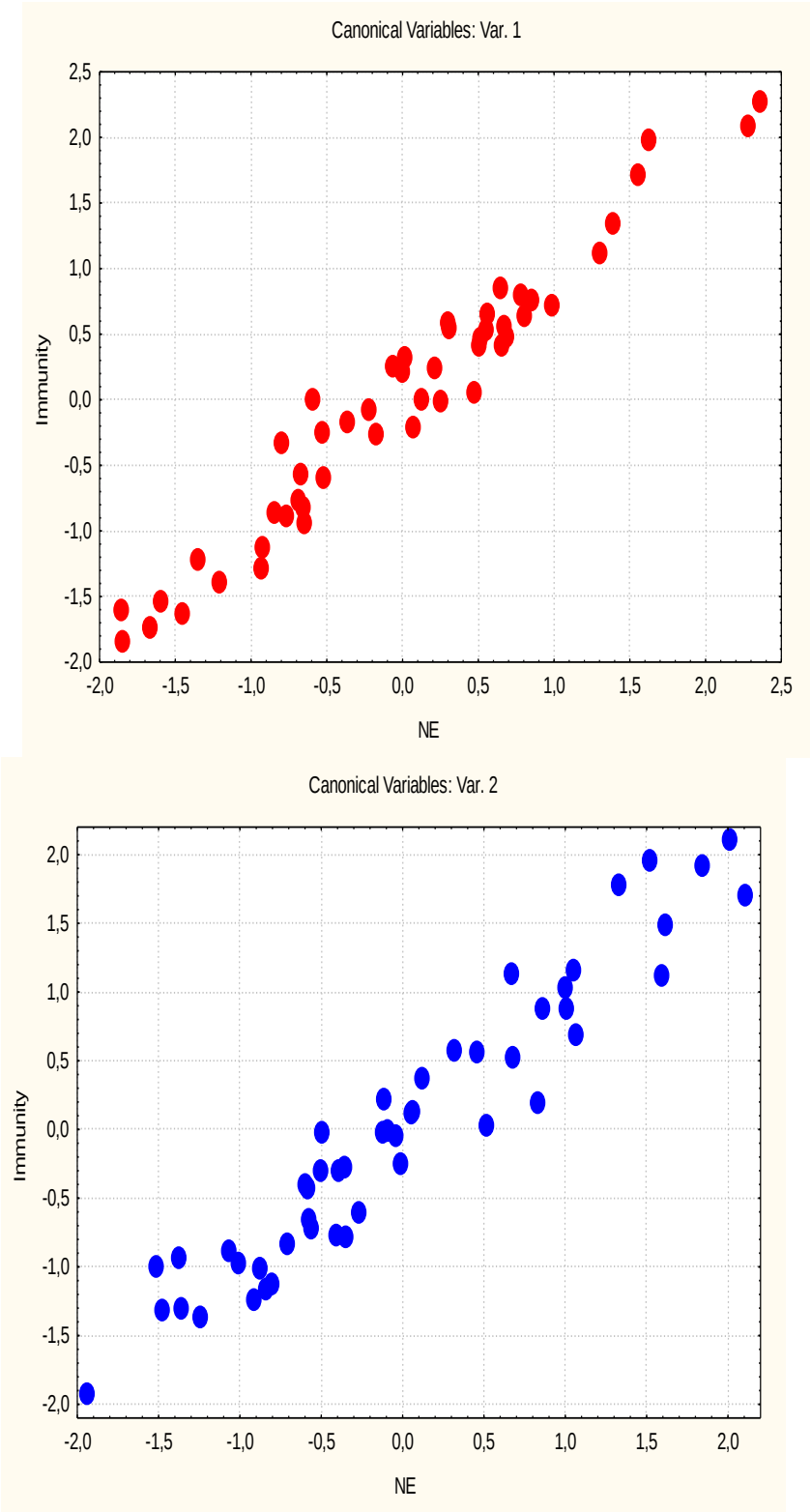

Fig. 4. Two pairs of canonical relationships between Neuroendocrine (axis X) and Immune (axis Y) parameters

Our findings are consistent with the concept of a triune neuroendocrine-immune complex [6,14-16,26] as well as provisions of the important role of autonomic nervous system in regulation of immunity [12,15,17-20,22-24]. Our approach can be used to quantify the integrated assessment of intensity of stress factors and to integral assessing the effectiveness of stresslimiting factors.

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