PLYSKA, Oleksandr, VOROBIENKO, Alyona, YANCHII, Roman and ŻUKOW, Xawery. Neuroendocrine-immune relationships at rats regardless of sex and exposure to stressors or adaptogens. Journal of Education, Health and Sport. 2023;47(1):133-154. eISSN 2391-8306. https://dx.doi.org/10.12775/JEHS.2023.47.01.013
https://apcz.umk.pl/JEHS/article/view/39622
https://zenodo.org/records/10401350

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 03.11.2023 No. 32318. Has a Journal's Unique Identifier: 201159. Scientific disciplines assigned: Health Sciences (Field of medical and health sciences); Medical sciences (Field of medical and health sciences); Cultural and religious studies (Field of humanities); Physical culture sciences (Field of medical and health sciences); Socio-economic geography and spatial management (Field of social sciences); Pedagogy (Field of social sciences); Earth and Environmental Sciences (Field of exact and natural sciences).
Punkty Ministerialne z 2019 - aktualny rok 40 punktów. Załącznik do komunikatu Ministra Edukacji i Nauki z dnia 03.11.2023 Lp. 32318. Posiada Unikatowy Identyfikator Czasopisma: 201159. Przypisane dyscypliny naukowe: Nauki o zdrowiu (Dziedzina nauk medycznych i nauk o zdrowiu); Nauki medyczne (Dziedzina nauk medycznych i nauk o zdrowiu); Nauki o kulturze i religii (Dziedzina nauk humanistycznych); Nauki o kulturze fizycznej (Dziedzina nauk medycznych i nauk o zdrowiu); Geografia społeczno-ekonomiczna i gospodarka przestrzenna (Dziedzina nauk społecznych); Pedagogika (Dziedzina nauk społecznych); Nauki o Ziemi i środowisku (Dziedzina nauk ścisłych i przyrodniczych).

The authors declare that there is no conflict of interests regarding the publication of this paper.
Received: 14.10.2023. Revised: 11.11.2023. Accepted: 07.12.2023. Published: 07.12.2023.

Neuroendocrine-immune relationships at rats regardless of sex and exposure to stressors or adaptogens

Oleksandr I. Plyska[1], Alyona A. Vorobienko[1], Roman I. Yanchii[2], Xawery Żukow[3]

[1]Mykhajlo Dragomanov Ukrainian State University, Kyïv, Ukraine
plys2005@ukr.net; https://orcid.org/0000-0001-7002-7637
a.a.vorobiinko@npu.edu.ua; https://orcid.org/0009-0008-4982-1551
[2]OO Bohomolets' Institute of Physiology of NAS of Ukraine, Kyïv, Ukraine
tas@biph.kiev.ua; https://orcid.org/0000-0001-7129-7698
[3]Medical University of Bialystok, Bialystok, Poland
xaweryzukow@gmail.com; https://orcid.org/0000-0001-5028-7829

Abstract

Introduction and aim. Since the discovery of the famous Selye triad, studies of the connections between adaptation hormones and the immune system have remained relevant. We formulated the aim of the future study: in the mode of synchronicity, to reveal sexual differences in the parameters of the neuroendocrine-immune complex and the state of neuroendocrine-immune relationships in intact rats and exposed to stressors and adaptogens. This article initiates the beginning of the movement towards achieving the goal.

Material and methods. The experiment is at 96 rats Wistar line: 48 males and 48 females. Over the 12 days, one male and female rat remained intact and 3 other pairs were exposed to chronic aversive stress for 6 days. We calculated the parameters of the HRV: Mode, Amplitude of the mode and Variational scope as markers of the circulating catecholamines, sympathetic and vagal tones respectively. Among endocrine parameters determined excretion of 17-Ketosteroides and serum levels of main adaptation hormones such as Corticosterone, Aldosterone, Testosterone, Triiodothyronine, as well as Parathyroid hormone and Calcitonin. The percentage of lymphocyte populations and the parameters of phagocytosis by neutrophils and monocytes of *Staphylococcus aureus* were determined

in the blood. The Thymus and Spleen were weighed and made smears-imprints for counting Thymocytogram and Splenocytogram.

Results. The canonical correlation between neuroendocrine and immune parameters was analyzed. The most pronounced immunomodulatory effect is exerted by catecholamines (R=0.928), sympathetic tone (R=0.849), and testosterone (R=0.829). Vagal tone (R=0.697), 17-ketosteroides (R=0.688), and aldosterone (R=0.686) moderately modulate immunity, while the immunomodulatory effects of triiodothyronine (R=0.610), corticosterone (R=0.584), PTH (R=0.510), and calcitonin (R=0.423) are the least pronounced. Three pairs of canonical roots were found. The neuroendocrine root of the first pair determines the parameters of immunity by 95.7%, the second pair by 86.5%, and the third pair by 69.2%. The elements of the Thymocytogram (in descending order: epitheliocytes, lymphocytes, endotheliocytes, lymphoblasts, and macrophages), the phagocytic activity of blood neutrophils and monocytes, the relative content of NK- and B-lymphocytes in the blood, as well as the percentage of lymphocytes and macrophages in the Splenocytogram turned out to be most influenced by neuroendocrine factors.

Conclusion. There is a close canonical correlation between registered neuroendocrine factors and immunity parameters in general. At the same time, both the severity of the immunomodulatory activity of individual neuroendocrine factors and the subjection to the regulatory influence of individual parameters of immunity differ significantly.

Keywords: adaptation hormones, HRV, thymus, spleen, immunocytes of blood, phagocytosis, rats.

Introduction

Since the discovery of the famous Selye's triad, studies of the connections between adaptation hormones and the immune system have remained relevant.[4,5,25,27] The discovery of the autonomous innervation of the immune system[21] initiated the study of neuro-immune connections.[12,13,16,21,22,28] New the stage was the formulation of the concept of a triune neuroendocrine-immune complex[23], in the course of which numerous experimental and clinical studies were conducted.[3,8,10,13,14,18,19,20,24,30] Meta-analysis of experimental studies shows the following. There are correlations of varying strength and direction between neuroendocrine factors, on the one hand, and immune parameters of the thymus, spleen, and blood, on the other hand. There are certain sexual differences in the strength of such connections. The levels of adaptation hormones and immune parameters are subject to the influence of both stressors and adaptogens. However, the effects of stressors and adaptogens on neuroendocrine-immune relationships remain unclear, as do sexual differences in such effects. In the cited studies, rats were subjected only to chronic stress, while significant differences are known between the effects of

chronic and acute stress on the neuroendocrine-immune complex.[4,5,20] Among the adaptogens, the reference adaptogen ginseng was not used. The levels of aldosterone, calcitonin, and parathyroid hormone were judged only by their specific effects on electrolyte metabolism. In our opinion, the design shortcoming of the cited studies is the lack of synchronicity of studies of animals of both sexes, which does not eliminate the influence of other factors: season, weather, solar activity, etc.

Based on the above, we formulated the aim of the future study: in the mode of synchronicity, to reveal sexual differences in the parameters of the neuroendocrine-immune complex and the state of neuroendocrine-immune relationships in intact rats and exposed to stressors and adaptogens.

This article initiates the beginning of the movement towards achieving the goal.

Material and methods

Ethics approval

All animals were kept in room having temperature 22±2ºC, and relative humidity of 44-55% under 12/12 hour light and dark cycle with standard laboratory diet and water given ad libitum. Studies have been conducted in accordance with the rules and requirements of the "General Principles for the Work on Animals" approved by the I National Congress on Bioethics (Kyïv, Ukraine, 2001) and agreed with the provisions of the "European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes" (Council of Europe No 123, Strasbourg 1985), and the Law of Ukraine "On the Protection of Animals from Cruelty" of 26.02.2006. The removal of animals from the experiment was carried out under light inhalation (ether) anesthesia by decapitation. The conduct of experiments was approved by the Ethics Committee of the Bohomolets' Institute of Physiology.

Participants

The experiment is at 96 rats Wistar line: 48 males (Weight Mean=256 g; SD=31 g) and 48 females (Mean=242 g; SD=23 g).

Study design and procedure

Over the 12 days, one male and female rat remained intact and 3 other pairs were exposed to chronic aversive stress[20] for 6 days by placing them in individual tight Plexiglas chambers for 30 min, which was preceded by injecting daily water (3 mL/200g) with a syringe through a curved needle with smooth olive into the esophagus. In other two groups, the classical adaptogen ginseng (alcohol tincture produced by Vishpha®) in a dose of 0.01 mL/200g or the Ukrainian phytocomposition "Balm Truskavets'" (produced by PRPE "Ukrainian Balms", Mykolaïv, Ukraine; ТУ У 15.8-24055046-005:2009) in a dose of 0.06 mL/200g was added to the water.

The day after completion the course of immobilization and water loads the animal were placed in individual chambers with perforated bottom for collecting for 12 hour nocturnal urine, in which determined the concentration of 17-ketosteroids (by color reaction with m-dinitrobenzene[6]).

The next morning, ECG under light ether anesthesia was recorded, by inserting needle electrodes under the skin of the paws. Right away the animals removed from the experiment by decapitation in order to remove the thymus and spleen, as well as collect the maximum possible amount of blood in which was determined some endocrine and immune parameters.

Based on about 120 R-R intervals we calculated the parameters of the HRV: Mode (Mo), Amplitude of the mode (AMo) and Variational scope (MxDMn) as markers of the circulating Catecholamines, Sympathetic and Vagal tones respectively.[1]

Among endocrine parameters determined serum levels of main adaptation hormones such as Corticosterone, Aldosterone, Testosterone, Triiodothyronine, as well as Parathyroid hormone and Calcitonin (by ELISA, with the use of analyzer "RT-2100C" and corresponding sets of reagents from "Alkor Bio", XEMA Co, Ltd and DRG International Inc).

Among the immune parameters of the blood, first of all, analysis of Leukocytogram (LCG), ie the percentage of lymphocytes (L), monocytes (M), eosinophils (Eo), basophils (Bas), rod-shaped (RN) and polymorphonucleary (PMNN) neutrophils was performed. Based on these data, the Entropy of the Leukocytogram (hLCG) was calculated according to the equation derived by Popovych[2,7,20,24] on the basis of the classical Shannon's[26] equation:

$$hLCG = - (L \bullet \log_2 L + M \bullet \log_2 M + Eo \bullet \log_2 Eo + Bas \bullet \log_2 Bas + RN \bullet \log_2 RN + PMNN \bullet \log_2 PMNN)/\log_2 6.$$

The percentage of theophylline-resistant (TR) and theophylline-susceptible (TS) T-lymphocytes, B-lymphocytes, plasma cells (Pla), natural killers (NK), and 0-lymphocytes were identified, as described in the manual.[17]

For these components the Entropy of the Immunocytogram (hICG) was calculated by Popovych[2,7,20] equation:

$$hICG = - (TR \bullet \log_2 TR + TS \bullet \log_2 TS + B \bullet \log_2 B + Pla \bullet \log_2 Pla + NK \bullet \log_2 NK + 0L \bullet \log_2 0L)/\log_2 6.$$

In addition, we tested the reaction of blast transformation of T-Lymphocytes to phytohemagglutinin.[17]

About the condition of the phagocytic function of neutrophils (microphages) and monocytes (macrophages) were judged by the phagocytosis index (percentage of cells, in which found microbes), the microbial count (number of microbes absorbed by one phagocyte) and the killing index (percentage of dead microbes) for *Staphylococcus aureus* (ATCC N25423 F49). Based on these parameters, taking into account the absolute content of neutrophils and monocytes, their bactericidal capacity (BCC N&M) was calculated.[3,19]

The Thymus and Spleen were weighed and made smears-imprints for counting Thymocytogram and Splenocytogram.[2,3,9] The components of the Thymocytogram (TCG) are lymphocytes (Lc), lymphoblastes (Lb), reticulocytes (Ret), macrophages (Mac), basophiles (B), endotheliocytes (En), epitheliocytes (Ep), and Hassal's corpuscles (H). The Splenocytogram (SCG) includes lymphocytes (Lc), lymphoblastes (Lb), plasma cells (Pla), reticulocytes (R), macrophages (Ma), fibroblasts (F), microphages (Mi), and eosinophils (Eo).

For them Shannon's Entropy was calculated too[2]:

$hTCG = - (Lc \bullet \log_2 Lc + Lb \bullet \log_2 Lb + Ret \bullet \log_2 Ret + Mac \bullet \log_2 Mac + B \bullet \log_2 B + En \bullet \log_2 En + Ep \bullet \log_2 Ep + H \bullet \log_2 H)/\log_2 8;$
$hSCG = - (Lc \bullet \log_2 Lc + Lb \bullet \log_2 Lb + Pla \bullet \log_2 Pla + R \bullet \log_2 R + Ma \bullet \log_2 Ma + F \bullet \log_2 F + Mi \bullet \log_2 Mi + Eo \bullet \log_2 Eo)/\log_2 8.$

Statistical analysis

Statistical processing was performed using a software package "Microsoft Excell" and "Statistica 6.4 StatSoft Inc" (Tulsa, OK, USA).

Results and discussion

In order to create a global picture, this article analyzed neuroendocrine-immune relationships at rats **regardless** of sex and exposure to stressors or adaptogens.

At the first stage, a correlation matrix was created (Table 1).

According to the equation: $|r|=\{\exp[2t/(n-1.5)^{0.5}]-1\}/\{\exp[2t/(n-1.5)^{0.5}]+1\}$, for a sample of n=96 critical value |r| at $p<0.05$ ($t>1.98$) is **0.201,** at $p<0.01$ ($t>2.62$) is **0.263,** at $p<0.001$ ($t>3.37$) is **0.333**.

The matrix included 8 thymus, 10 spleen, and 14 peripheral blood parameters.

Table 1. Correlation matrix for neuro-endocrine and immune parameters of all rats

Variables	Catechol amines	Sympa-thotone	Vagal tone	Testo sterone	17-KS	Cortico sterone	Aldo-sterone	T3	PTH	Calci-tonin
Thymus Mass	-.045	.141	-.110	**.227**	-.135	**-.302**	-.117	.193	-.086	.101
Hassal's corpusc T	**.213**	**.233**	-.169	.012	-.033	.079	.108	.019	**-.235**	-.108
Lymphocytes T	**.548**	-.150	**.228**	**-.733**	**.520**	**.508**	**.414**	**-.316**	**.261**	-.160
Lymphoblastes T	**.370**	-.147	.190	**-.615**	**.327**	**.358**	**.423**	**-.357**	**.250**	-.121
Epitheliocytes T	-.605	.113	-.193	**.773**	**-.512**	**-.477**	**-.431**	**.327**	-.279	.156
Macrophages T	**-.374**	.139	-.199	**.537**	**-.284**	**-.350**	**-.384**	**.312**	-.186	**.270**
Endotheliocytes T	**-.491**	.133	-.163	.666	**-.469**	**-.509**	**-.356**	**.254**	**-.244**	.180
Entropy TCG	**-.522**	.195	**-.229**	**.730**	**-.499**	**-.478**	**-.392**	**.308**	**-.288**	.193
Spleen Mass	.007	**-.228**	.084	-.047	.203	.060	.110	-.077	-.179	.111
Lymphocytes S	**-.586**	-.193	.045	.496	**-.414**	**-.363**	**-.385**	**.200**	-.105	-.029
Lymphoblastes S	**-.310**	-.188	.157	**.258**	-.065	-.160	**-.403**	**.226**	.183	.176
Plasmocytes S	**-.340**	-.126	.001	**.274**	-.169	-.124	**-.385**	.055	.126	**.274**
Reticulocytes S	**.324**	.185	-.123	**-.282**	**.207**	**.205**	**.308**	-.083	-.052	-.131
Fibroblastes S	**.297**	-.039	.098	-.371	**.439**	**.366**	**.379**	**-.334**	-.031	.065
Macrophages S	**.693**	**.708**	**-.434**	**-.259**	.125	.197	**.415**	-.068	-.184	-.025
Microphages S	**.260**	-.194	**.220**	**-.420**	**.237**	.178	.197	-.103	**.256**	-.104
Eosinophiles S	**-.215**	-.142	.044	**.267**	**-.235**	**-.229**	-.171	.098	-.107	-.095
Entropy SCG	**.408**	.129	-.037	**-.282**	**.305**	**.257**	.166	-.145	.126	.149
Leukocytes Blood	**.204**	.031	-.030	-.141	**.260**	.011	**.316**	-.041	.015	-.058
Eosinophiles B	**-.276**	-.036	**.355**	.013	-.003	**-.217**	.042	-.058	-.089	.051
Basophiles B	.068	.031	.173	-.148	.051	.041	**.270**	-.060	-.158	-.117
Entropy LCG	-.151	.083	.162	-.058	-.123	-.138	.031	-.139	-.116	-.172
Phagoc Index Mon	**-.545**	-.170	-.000	**.476**	**-.350**	**-.338**	**-.381**	**.248**	-.175	.042
Microb Coun Mon	.156	-.122	.034	**-.261**	**.278**	**.243**	.051	**-.343**	.010	.088

Phagoc Ind Neutr	**-.597**	.029	-.092	**.736**	**-.449**	**-.475**	**-.379**	**.341**	**-.203**	.195
BTR T-Lymph B	**.534**	.021	.121	**-.577**	**.327**	**.448**	**.331**	**-.262**	**.260**	-.160
TS T-Lymphoc B	**.259**	.012	.098	**-.252**	.088	.048	.123	-.195	.210	-.100
NK-Lymphocyte B	**.555**	.021	.182	**-.693**	**.417**	**.517**	**.452**	**-.385**	**.200**	**-.201**
B-Lymphocytes B	**.475**	.095	-.073	**-.396**	**.355**	.173	**.236**	-.193	**.292**	.048
Plasmocytes B	.110	**.270**	-.090	.044	-.156	-.066	.105	-.070	-.093	**-.226**
0-Lymphocytes B	**-.508**	-.075	-.010	**.517**	**-.356**	**-.325**	**-.339**	**.276**	**-.282**	.076
Entropy ICG	**.459**	.122	.138	**-.434**	.195	**.217**	**.361**	**-.277**	.059	**-.230**

Further, in order to assess the immunomodulatory effect of each neuroendocrine factor, regression models were built for it by stepwise exclusion of variables until the maximum Adjusted R^2 values were reached.

It was found (Table 2) that 1/Mode HRV, which is considered a marker of circulating Catecholamines (as well as some other humoral regulators of heart rhythm[1]), upregulates the content of Lymphoblasts in the Thymus, and Macrophages, Microphages, as well as other immunocytes with phagocytic ability, such as Reticulocytes and Fibroblasts, in the Spleen.

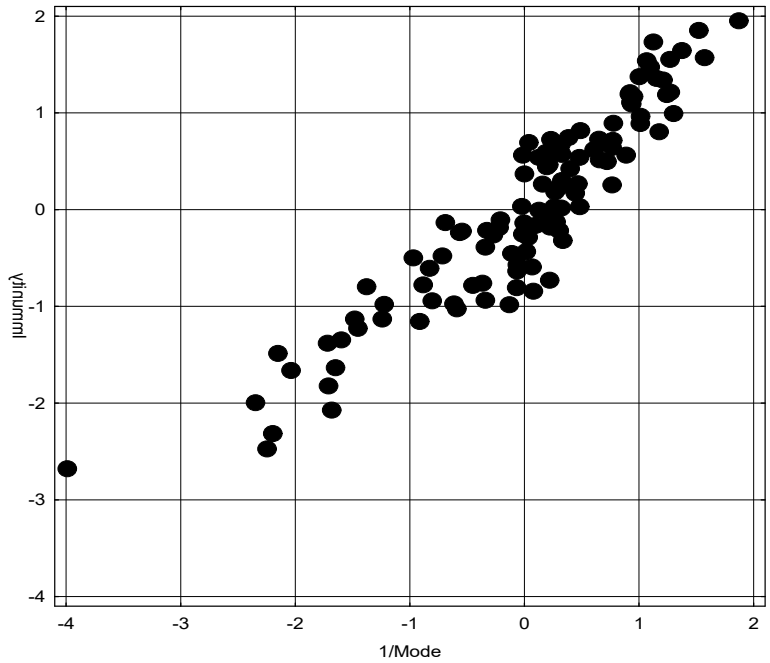
The negative correlation with the percentage of 0-Lymphocytes reflects a decrease in the share of immature lymphocytes, apparently due to an increase in the share of B- and/or TS-Lymphocytes due to the stimulation of the expression of the corresponding receptors.

While Catecholamines downregulates the content of Epitheliocytes and Endotheliocytes in the Thymus and Eosinophils in the Blood, as well as the phagocytic activity of Blood Neutrophils.

Judging by the coefficient of determination, the immunomodulatory effect of Catecholamines is 86.2% (Table 2 and Fig. 1).

Table 2. Regression Summary for 1/Mode HRV
$R=0.928$; $R^2=0.862$; Adjusted $R^2=0.848$; $F_{(11.0)}=61.7$; $p<10^{-6}$

N=96		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(99)}$	p-level
Variables	r		Intercpt	212	37.7	5.62	10^{-6}
Macrophages of Spleen, %	**0.693**	-0,657	0,043	-13,02	0,854	-15,2	10^{-6}
0-Lymphocytes of Blood, %	**-0.545**	0,123	0,050	0,568	0,231	2,46	0,016
Lymphoblastes of Thymus, %	**0.370**	0,077	0,059	2,522	1,908	1,32	0,189
Reticulocytes of Spleen, %	**0.324**	-0,063	0,041	-1,344	0,871	-1,54	0,126
Fibroblasts of Spleen, %	**0.297**	0,100	0,045	2,091	0,950	2,20	0,030
Microphages of Spleen, %	**0.260**	-0,121	0,043	-2,043	0,728	-2,81	0,006
Epitheliocytes of Thymus, %	**-0.605**	0,390	0,078	2,635	0,524	5,03	10^{-5}
Phagocytosis Index of Neutrophils, %	**-0.545**	-0,122	0,071	-0,681	0,396	-1,72	0,089
Endotheliocytes of Thymus, %	**-0.491**	0,181	0,065	3,247	1,172	2,77	0,007
Eosinophiles of Blood, %	**-0.276**	0,358	0,040	7,432	0,826	8,99	10^{-6}



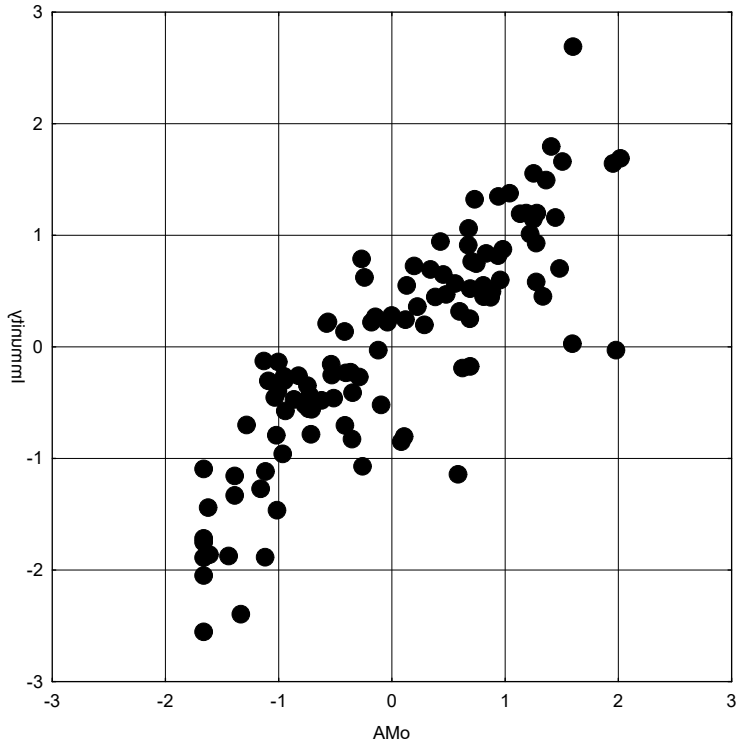
R=0.928; R^2=0.862; $\chi^2_{(10)}$=204; $p<10^{-6}$; Λ Prime=0,138

Fig. 1. Scatterplot of canonical correlation between 1/Mode HRV (X-line) and Immune parameters (Y-line) in all rats

AMo HRV as marker of Sympathetic tone[1] upregulates the content of Macrophages and Reticulocytes in the Spleen, Plasmocytes in the Blood, Hassal's corpuscles in the Thymus, as well as the Entropy of Thymocytogram, while downregulates the phagocytic activity of Blood Neutrophils and Spleen mass. In conclusion, the immunomodulatory effect of Sympathetic tone is 71.9% (Table 3 and Fig. 2).

Table 3. Regression Summary for AMo HRV, %
R=0.848; R^2=0.719; Adjusted R^2=0.700; $F_{(7.1)}$=37.3; $p<10^{-6}$

N=96		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(102)}$	p-level
Variables	r		Intercpt	-23.4	16.1	-1.45	0.149
Macrophages of Spleen, %	**0.708**	0,891	0,069	9,992	0,776	12,9	10^{-6}
Plasmocytes of Blood, %	**0.270**	-0,088	0,064	-2,433	1,762	-1,38	0,170
Hassal's corpuscles of Thymus, %	**0.233**	-0,093	0,069	-3,160	2,340	-1,35	0,180
Entropy of Thymocytogram	**0.195**	0,274	0,069	81,40	20,40	3,99	10^{-4}
Reticulocytes of Spleen, %	**0.185**	0,079	0,064	0,950	0,771	1,23	0,220
Spleen Mass, mg	**-0.228**	-0,364	0,059	-0,056	0,009	-6,19	10^{-6}
Phagocytosis Index of Monocytes, %	**-0.170**	0,105	0,077	0,996	0,730	1,36	0,176



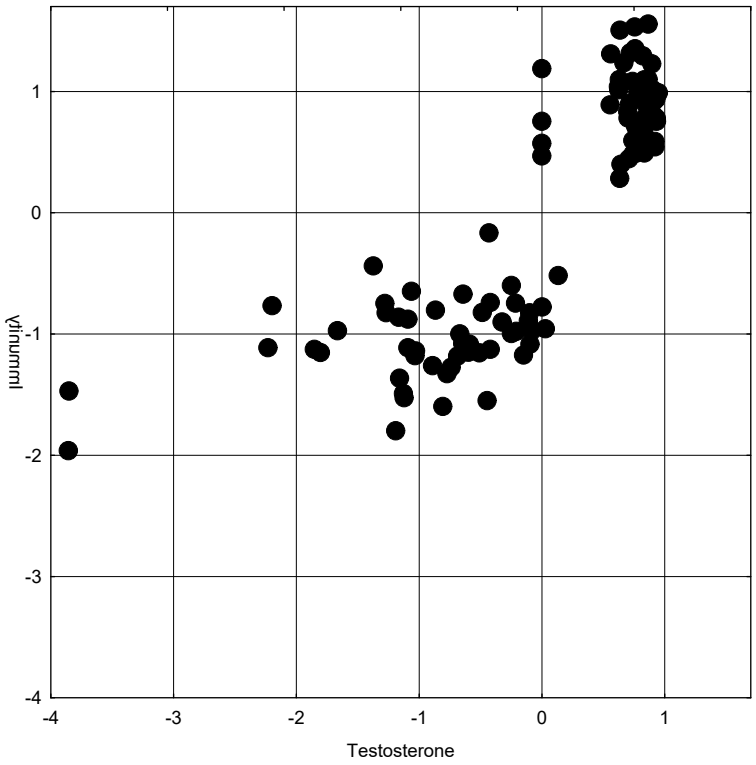
R=0.848; R^2=0.719; $\chi^2_{(7)}$=133; $p<10^{-6}$; Λ Prime=0,280

Fig. 2. Scatterplot of canonical correlation between AMo HRV (X-line) and Immune parameters (Y-line) in all rats

Serum Testosterone upregulates the content of Epitheliocytes in the Thymus, phagocytosis ativity of Blood Neutrophils, as well as the content of Plasmocytes and Eosinophils in the Spleen, while downregulates the content in it of other cells with phagocytic ability as well as Entropy of Splenocytogram. The immunomodulatory effect of Testosterone is 68.7% (Table 4 and Fig. 3).

Table 4. Regression Summary for Testosterone, nM/L
R=0.829; R^2=0.687; Adjusted R^2=0.659; $F_{(9.1)}$=24.4; $p<10^{-6}$

N=96		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(100)}$	p-level
Variables	r		Intercpt	-131.8	46.9	-2.81	0.006
Epitheliocytes of Thymus, %	**0.773**	0,503	0,095	1,708	0,321	5,32	10^{-6}
Phagocytosis Index of Neutrophils, %	**0.736**	0,280	0,095	0,781	0,264	2,96	0,004
Plasmocytes of Spleen, %	**0.274**	-0,222	0,117	-3,063	1,619	-1,89	0,062
Eosinophiles of Spleen, %	**0.267**	-0,090	0,082	-1,935	1,761	-1,10	0,275
Microphages of Spleen, %	**-0.420**	-0,277	0,091	-2,354	0,775	-3,04	0,003
Fibroblasts of Spleen, %	**-0.371**	-0,129	0,098	-1,360	1,031	-1,32	0,190
Reticulocytes of Spleen, %	**-0.282**	-0,170	0,079	-1,807	0,847	-2,13	0,035
Entropy of Splenocytogram	**-0.282**	0,311	0,134	215,2	93,15	2,31	0,023
Macrophages of Spleen, %	**-0.259**	-0,201	0,090	-2,01	0,896	-2,24	0,027



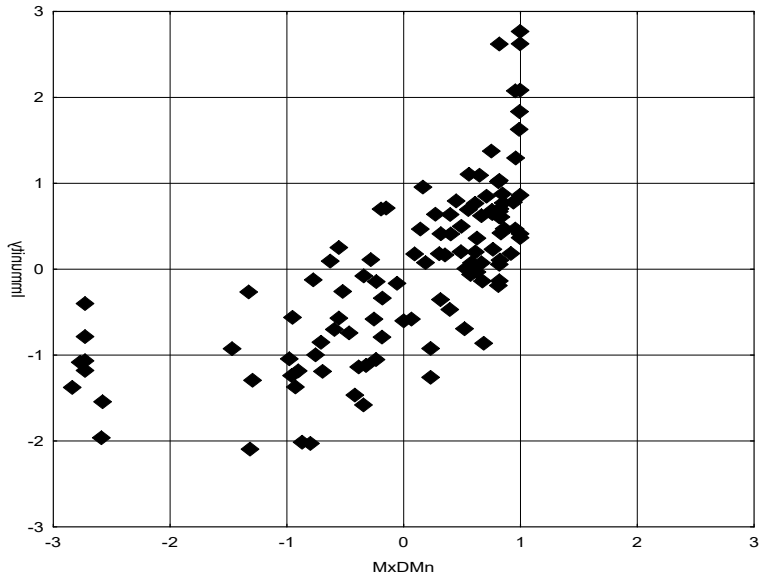
R=0.829; R^2=0.687; $\chi^2_{(9)}$=120; $p<10^{-6}$; Λ Prime=0,313

Fig. 3. Scatterplot of canonical correlation between serum Testosterone (X-line) and Immune parameters (Y-line) in all rats

Vagal tone, as opposed to Sympathetic tone and circulating Catecholamines, downregulates the content of Macrophages in the Spleen and Entropy of Thymocytogram, while upregulates the content of Eosinophiles (and Basophiles) in the Blood. However, in general, its immunomodulatory effect is much weaker than that of its functional antagonists and is only 48.2% (Table 5 and Fig. 4).

Table 5. Regression Summary for MxDMn HRV, msec
R=0.694; R^2=0.482; Adjusted R^2=0.462; $F_{(4.1)}$=24.4; $p<10^{-6}$

N=96		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(105)}$	p-level
Variables	r		Intercpt	159.8	21.2	7.53	10^{-6}
Macrophages of Spleen, %	**-0.434**	-0,571	0,073	-10,24	1,315	-7,79	10^{-6}
Entropy of Thymocytogram	**-0.229**	-0,305	0,071	-144,9	33,78	-4,29	10^{-4}
Eosinophiles of Blood, %	**0.355**	0,363	0,073	6,823	1,366	4,99	10^{-5}
Basophiles of Blood, %	**0.173**	0,202	0,074	18,07	6,648	2,72	0.008



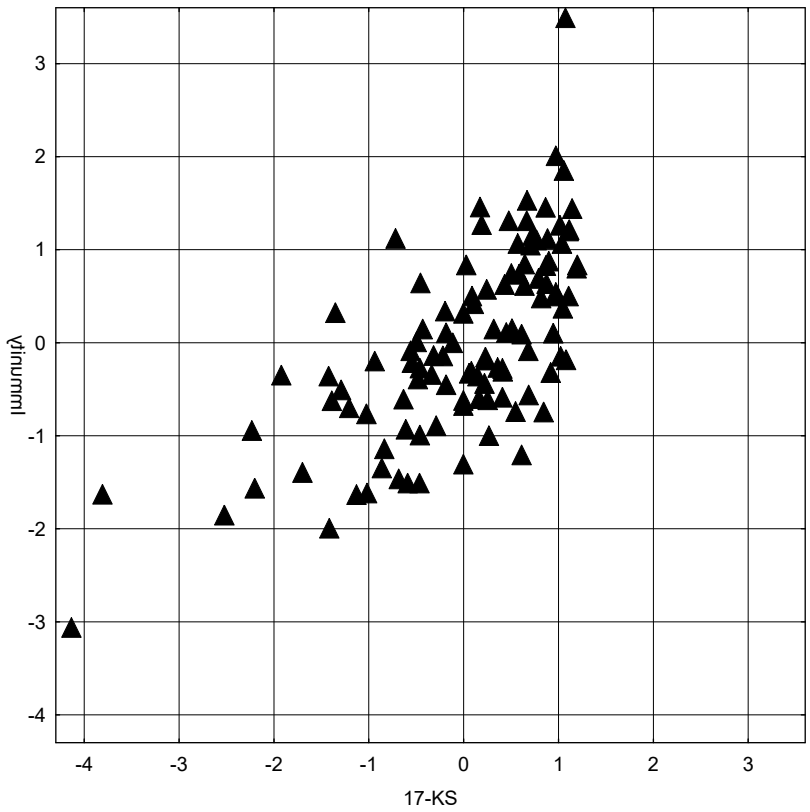
R=0.694; R^2=0.482; $\chi^2_{(4)}$=70; $p<10^{-6}$; Λ Prime=0,518

Fig. 4. Scatterplot of canonical correlation between MxDMn HRV (X-line) and Immune parameters (Y-line) in all rats

Urinary 17-Ketosteroides, which are metabolites, mainly of androgens of the reticular zone of the adrenal cortex (2/3 at males and 3/4 at females) and partly of glucocorticoids, upregulates the content of Lymphocytes in the Thymus, Fibroblasts, Microphages and Reticulocytes in the Spleen, Leukocytes in general and B-Lymphocytes in particular in the Blood as well as Entropy of Immunocytogram**.** While 17-Ketosteroides downregulates the content of Epitheliocytes, Endotheliocytes and Macrophages in the Thymus as well as Entropy of Thymocytogram. In conclusion, the immunomodulatory effect of 17-Ketosteroides is 47.3% (Table 6 and Fig. 5).

Table 6. Regression Summary for 17-KS, nM/100g•12h
R=0.688; R^2=0.473; Adjusted R^2=0.414; $F_{(12.0)}$=8.0; $p<10^{-6}$

N=96		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(98)}$	p-level
Variables	r		Intercpt	-318	172	-1.84	0.068
Lymphocytes of Thymus, %	**0.520**	1,148	0,365	5,412	1,720	3,15	0,002
Fibroblasts of Spleen, %	**0.439**	0,223	0,088	4,368	1,716	2,55	0,012
B-Lymphocytes of Blood, %	**0.355**	0,257	0,089	3,041	1,055	2,88	0,005
Leukocytes of Blood, 10^9/L	**0.260**	0,144	0,077	1,476	0,793	1,86	0,066
Microphages of Spleen, %	**0.237**	0,092	0,085	1,455	1,345	1,082	0,282
Reticulocytes of Spleen, %	**0.207**	0,128	0,087	2,537	1,723	1,47	0,144
Entropy of Immunocytogram	**0.195**	-0,155	0,095	-173,4	106,1	-1,63	0,105
Epitheliocytes of Thymus, %	**-0.512**	0,422	0,302	2,669	1,909	1,40	0,165
Entropy of Thymocytogram	**-0.499**	-0,322	0,294	-158,2	144,3	-1,10	0,275
Endotheliocytes of Thymus, %	**-0.469**	0,281	0,185	4,720	3,114	1,52	0,133
Macrophages of Thymus, %	**-0.284**	0,639	0,179	12,23	3,427	3,57	0,001



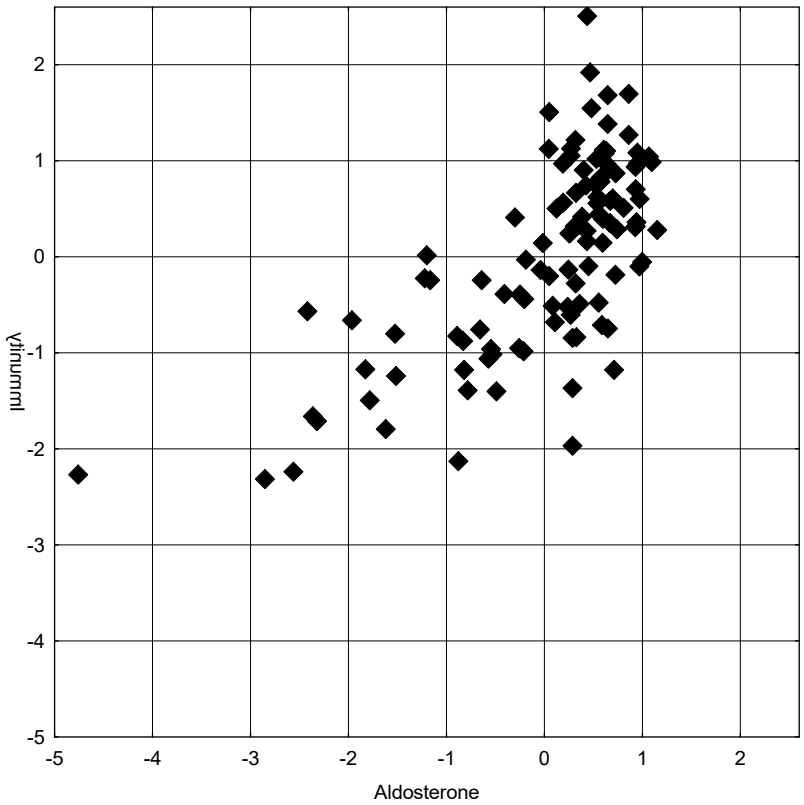
R=0.688; R^2=0.473; $\chi^2_{(11)}$=66; p<10^{-6}; Λ Prime=0,527

Fig. 5. Scatterplot of canonical correlation between excretion of 17-KS (X-line) and Immune parameters (Y-line) in all rats

Serum Aldosterone upregulates the content of Lymphoblastes in the Thymus, Macrophages and Fibroblasts in the Spleen, as well as Leukocytes in general and Basophiles in particular in the Blood, while downregulates the content of Lymphoblastes and Plasmocytes in the Spleen. The immunomodulatory effect of Aldosterone is 47.1% (Table 7 and Fig. 6).

Table 7. Regression Summary for Aldosterone, pM/L
R=0.686; R^2=0.471; Adjusted R^2=0.434; $F_{(7.1)}$=13.0; p<10^{-6}

N=96		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(102)}$	p-level
Variables	r		Intercpt	-711	359	-1.98	0.051
Lymphoblastes of Thymus, %	**0.423**	0,318	0,075	123,7	29,25	4,23	10^{-4}
Macrophages of Spleen, %	**0.415**	0,214	0,083	50,69	19,60	2,59	0,011
Fibroblasts of Spleen, %	**0.379**	0,107	0,082	26,88	20,46	1,31	0,192
Leukocytes of Blood, 10^9/L	**0.316**	0,200	0,076	26,14	9,940	2,63	0,010
Basophiles of Blood, %	**0.270**	0,150	0,075	176,2	88,61	1,99	0,049
Lymphoblastes of Spleen, %	**-0.403**	-0,112	0,085	-43,24	33,05	-1,31	0,194
Plasmocytes of Spleen, %	**-0.385**	-0,151	0,081	-49,55	26,50	-1,87	0,064



R=0.686; R^2=0.471; $\chi^2_{(7)}$=66; p<10^{-6}; Λ Prime=0,529

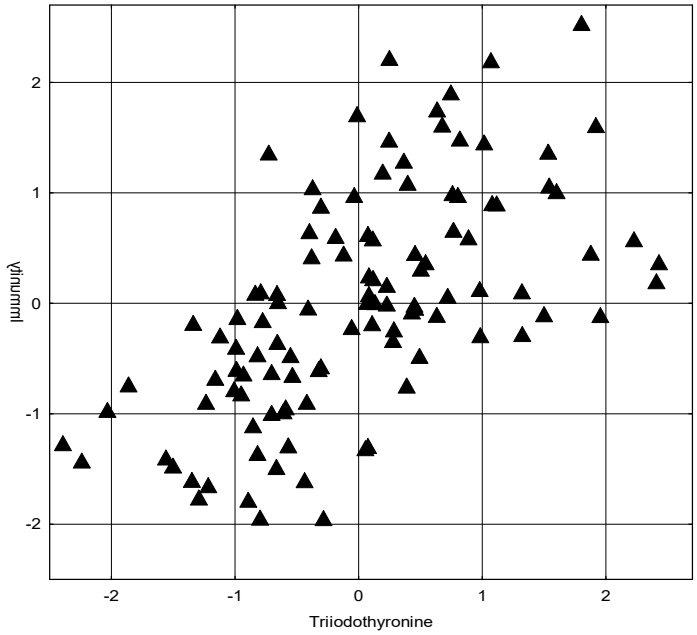
Fig. 6. Scatterplot of canonical correlation between serum Aldosterone (X-line) and Immune parameters (Y-line) in all rats

Serum Triiodothyronine downregulates the content of Lymphoblastes in the Thymus, Fibroblasts in the Spleen, NK-, B- and Theophylline-susceptible T-Lymphocytes in the Blood, which is accompanied by an increase in the proportion of 0-lymphocytes. While Triiodothyronine upregulates the content of Macrophages and Endotheliocytes in the Thymocytogram and its Entropy as well as the content of Lymphoblastes and Lymphocytes in the Spleen. It is interesting that the Triiodothyronine upregulates the activity while downregulates the intensity of phagocytosis of Blood Monocytes/Macrophages. The immunomodulatory effect of Triiodothyronine is 37.2% (Table 8 and Fig. 7).

Table 8. Regression Summary for Triiodothyronine, nM/L
R=0.610; R^2=0.372; Adjusted R^2=0.286; $F_{(14.0)}$=4.37; p<10^{-5}

N=96		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(96)}$	p-level
Variables	r		Intercpt	10.38	2.07	5.01	10^{-5}
NK-Lymphocytes of Blood, %	**-0.385**	-0,551	0,178	-0,080	0,026	-3,10	0,003
Lymphoblastes of Thymus, %	**-0.357**	-0,207	0,116	-0,088	0,049	-1,79	0,076
Microbial Count of Monoc., B/Ph	**-0.343**	-0,328	0,095	-0,095	0,028	-3,45	0,001
Fibroblasts of Spleen, %	**-0.334**	-0,280	0,114	-0,077	0,031	-2,46	0,016
0-Lymphocytes of Blood, %	**0.276**	-0,430	0,240	-0,026	0,015	-1,79	0,076
Theophylline-susceptible T-L, %	**-0.195**	-0,158	0,133	-0,025	0,021	-1,19	0,236

B-Lymphocytes of Blood, %	**-0.193**	-0,322	0,156	-0,053	0,026	-2,07	0,041
Macrophages of Thymus, %	**0.312**	0,319	0,154	0,085	0,041	2,07	0,041
Entropy of Thymocytogram	**0.308**	-0,431	0,234	-2,952	1,606	-1,84	0,069
Endotheliocytes of Thymus, %	**0.254**	-0,211	0,168	-0,049	0,039	-1,25	0,214
Phagocytosis Ind of Monocyt, %	**0.248**	0,163	0,120	0,036	0,026	1,36	0,176
Lymphoblastes of Spleen, %	**0.226**	0,106	0,089	0,045	0,038	1,19	0,237
Lymphocytes of Spleen, %	**0.200**	-0,364	0,138	-0,053	0,020	-2,64	0,010



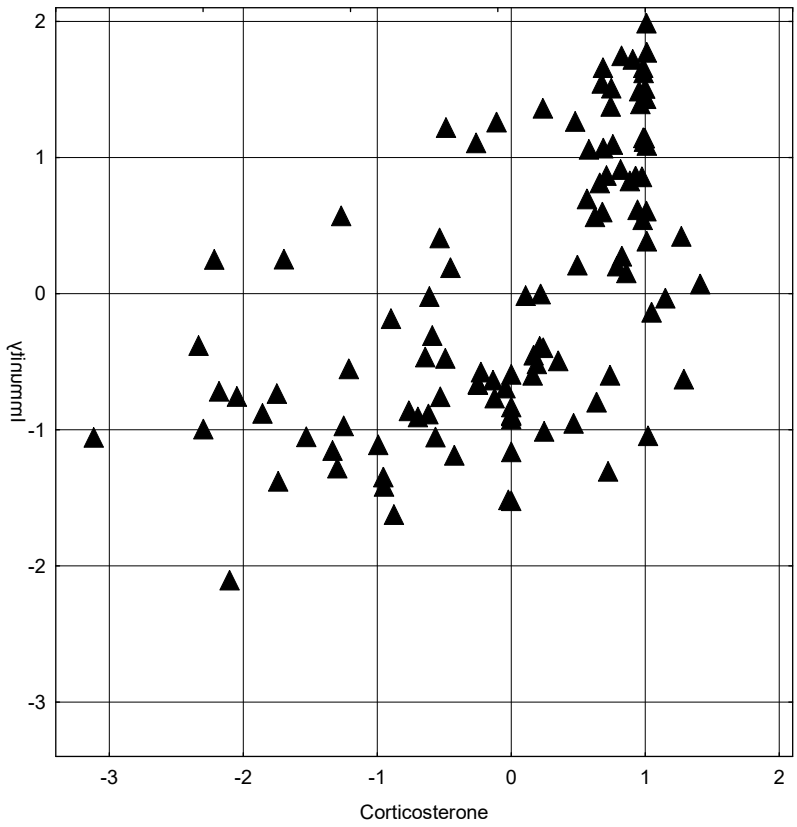
R=0.610; R^2=0.372; $\chi^2_{(13)}$=47; p<10^{-5}; Λ Prime=0,628

Fig. 7. Scatterplot of canonical correlation between serum Triiodothyronine (X-line) and Immune parameters (Y-line) in all rats

In the regression model of serum corticosterone, despite its numerous significant relationships with immune parameters, only three variables remained after stepwise elimination, probably due to redundancy/duplication of information. As a result, the immunomodulatory effect of corticosterone was, despite expectations, only 34.1% (Table 9 and Fig. 8).

Table 9. Regression Summary for Corticosterone, nM/L
R=0.584; R^2=0.341; Adjusted R^2=0.323; $F_{(3.1)}$=18.3; p<10^{-6}

N=96		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(106)}$	p-level
Variables	r		Intercpt	586	181	3.24	0,002
Endotheliocytes of Thymus, %	**-0.509**	-0,272	0,107	-33,97	13,34	-2,55	0,012
Thymus Mass, mg	**-0.302**	-0,167	0,082	-2,364	1,156	-2,04	0,043
NK-Lymphocytes of Blood, %	**0.517**	0,294	0,107	22,67	8,268	2,74	0,007



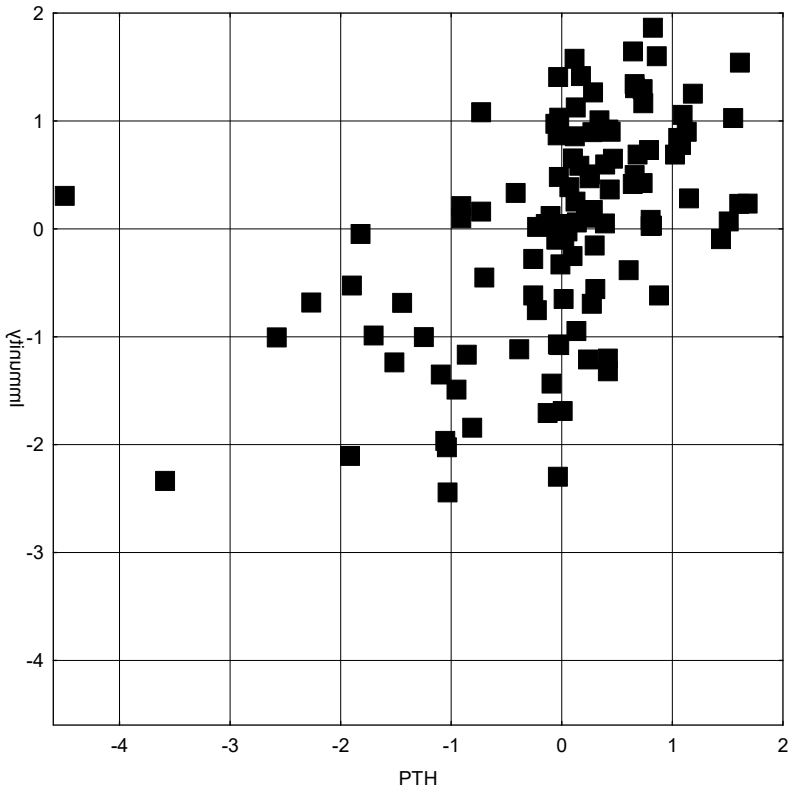
R=0.584; R^2=0.341; $\chi^2_{(3)}$=44; $p<10^{-6}$; Λ Prime=0,659

Fig. 8. Scatterplot of canonical correlation between serum Corticosterone (X-line) and Immune parameters (Y-line) in all rats

PTH is only weakly correlated with two thymus, three spleen, and two peripheral blood parameters. Accordingly, its immunoregulatory effect is only 26.0% (Table 10 and Fig. 9).

Table 10. Regression Summary for PTH, pg/L
R=0.510; R^2=0.260; Adjusted R^2=0.209; $F_{(7.1)}$=5.12; $p<10^{-4}$

N=96		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(102)}$	p-level
Variables	r		Intercpt	144	44.5	3.23	0,002
B-Lymphocytes of Blood, %	**0.292**	0,231	0,093	3,188	1,289	2,47	0,015
Blast Transformation T-Lym, %	**0.260**	0,138	0,113	0,419	0,344	1,22	0,225
Lymphoblastes of Spleen, %	**0.183**	0,123	0,098	4,354	3,484	1,25	0,214
Endotheliocytes of Thymus, %	**-0.244**	-0,149	0,114	-2,924	2,223	-1,32	0,191
Hassal's corpuscles of Thymus, %	**-0.235**	-0,198	0,091	-13,00	5,990	-2,17	0,032
Macrophages of Spleen, %	**-0.184**	-0,153	0,101	-3,310	2,189	-1,51	0,134
Spleen Mass, mg	**-0.179**	-0,129	0,089	-0,038	0,026	-1,46	0,148



R=0.510; R^2=0.260; $\chi^2_{(7)}$=31; p<10^{-4}; Λ Prime=0,740

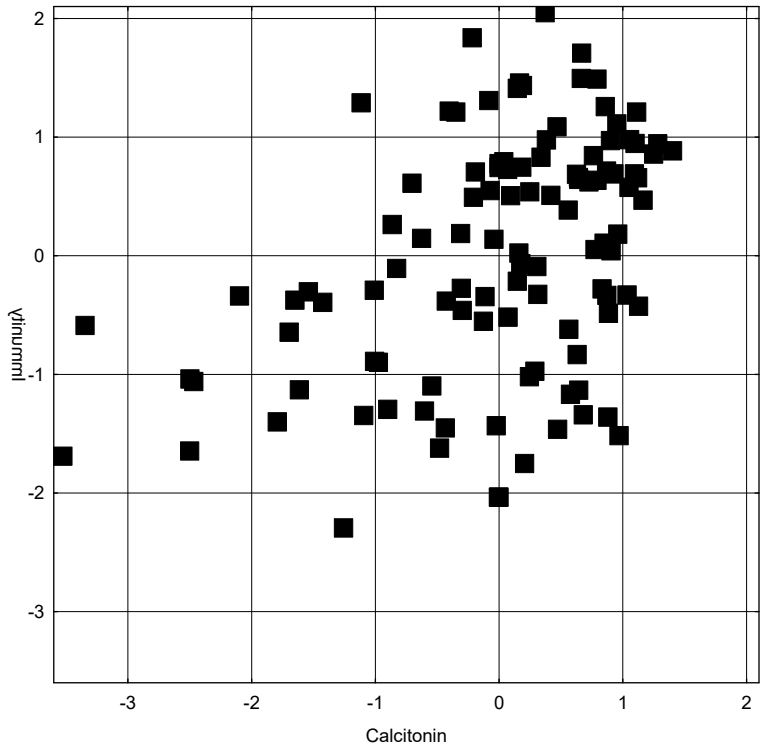
Fig. 9. Scatterplot of canonical correlation between serum PTH (X-line) and Immune parameters (Y-line) in all rats

Calcitonin is secreted mainly by the parafollicular C-cells of the thyroid gland. Other tissues capable of producing Calcitonin include the lungs, small intestine, thymus, liver, parathyroid glands.[11]

Calcitonin upregulates the content of Macrophages in the Thymus as well as Lymphoblastes and Plasmocytes in the Spleen, while downregulates the content of Plasmocytes in the Blood as well as Entropy of Leukocytogram. Accordingly, its immunoregulatory effect is only 17.9% (Table 11 and Fig. 10).

Table 11. Regression Summary for Calcitonin, ng/L
R=0.423; R^2=0.179; Adjusted R^2=0.139; $F_{(5.1)}$=4.53; p<10^{-3}

N=96		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(104)}$	p-level
Variables	r		Intercpt	34.6	16.6	2.08	0,040
Plasmocytes of Spleen, %	**0.274**	0.104	0.108	1.164	1.204	0.97	0.336
Macrophages of Thymus, %	**0.270**	0,245	0,094	2,035	0,778	2,62	0,010
Lymphoblastes of Spleen, %	**0.176**	0,113	0,098	1,491	1,292	1,15	0,251
Plasmocytes of Blood, %	**-0.226**	-0,194	0,101	-3,824	1,991	-1,92	0,057
Entropy of Leukocytogram	**-0.172**	-0,117	0,092	-33,58	26,42	-1,27	0,207



R=0.423; R^2=0.179; $\chi^2_{(5)}$=21; $p<10^{-3}$; Λ Prime=0,821

Fig. 10. Scatterplot of canonical correlation between serum Calcitonin (X-line) and Immune parameters (Y-line) in all rats

At the last stage, the canonical correlation between neuroendocrine parameters, on the one hand, and immunity parameters, on the other, was analyzed. According to the results of the analysis, three pairs of canonical roots with a significant correlation were found.

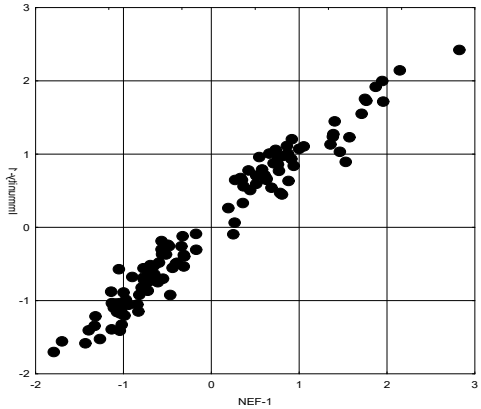
The neuroendocrine root of the first pair receives maximum factor loads from circulating catecholamines and testosterone, but with opposite signs, moderate loads from corticosterone, 17-ketosteroids and aldosterone, unidirectional with catecholamines. On the other hand, the load from the rest of the neuroendocrine factors is negligible.

Pseudostaining makes it possible to quickly find out that the most subject to neuroendocrine regulation are the immune parameters of the thymus and blood, while the immunocytes of the spleen provide the minimum factor load on the immune root. In general, this constellation of immune parameters was subject to neuroendocrine modulation by 95.7% (Table 12 and Fig. 11).

Table 12. Factor structure of first pair of Neuro-endocrine and Immune Roots

Left set	**Root 1**
1/Mode HRV as Catecholamines	**-0,920**
Testosterone	**0,772**
Aldosterone	**-0,581**
Corticosterone	**-0,531**
17-Ketosteroids	**-0,468**
Triiodothyronine	**0,278**

Calcitonin	**0,184**
Parathyroid hormone	**-0,181**
MxDMn HRV as Vagal tone	**0,174**
Right set	**Root 1**
Epitheliocytes of Thymus	**0,814**
Phagocytosis Index of Neutrophils	**0,777**
Lymphocytes of Thymus	**-0,764**
NK-Lymphocytes of Blood	**-0,761**
Entropy of Thymocytogram	**0,740**
Endotheliocytes of Thymus	**0,680**
Lymphocytes of Spleen	**0,667**
Phagocytosis Index of Monocytes	**0,643**
0-Lymphocytes of Blood	**0,628**
Macrophages of Spleen	**-0,604**
Lymphoblastes of Thymus	**-0,585**
Entropy of Immunocytogram	**-0,571**
Macrophages of Thymus	**0,564**
B-Lymphocytes of Blood	**-0,536**
Fibroblasts of Spleen	**-0,457**
Plasmocytes of Spleen	**0,397**
Microphages of Spleen	**-0,384**
Lymphoblastes of Spleen	**0,371**
Reticulocytes of Spleen	**-0,369**
Theophylline-susceptible T-Lymphocytes	**-0,322**
Eosinophiles of Spleen	**0,271**
Microbial Count of Monocytes	**-0,263**
Leukocytes of Blood	**-0,255**



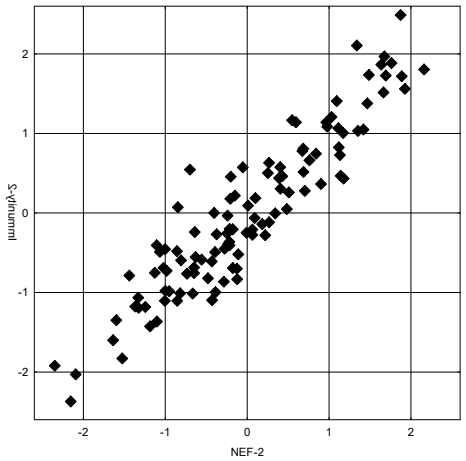
R=0.978; R^2=0.957; $\chi^2_{(340)}$=816; p<10^{-6}; Λ Prime<10^{-4}

Fig. 11. Scatterplot of canonical correlation between neuro-endocrine factors (X-line) and immune parameters (Y-line) at all rats. *First pair of Roots*

The factor structure of the neuroendocrine root of the second pair is mainly formed by sympathetic tone, to a lesser extent by vagal tone, while the rest of the factors are significantly inferior to the first in terms of factor loads. The immune set is led by macrophages of the spleen, but the upper zone of the list is occupied by parameters of the thymus. In general, this constellation of immune parameters was subject to neuroendocrine modulation by 86.5% (Table 13 and Fig. 12).

Table 13. Factor structure of second pair of Neuro-endocrine and Immune Roots

Left set	**Root 2**
AMo HRV as Sympathetic tone	**0,887**
MxDMn HRV as Vagal tone	**-0,604**
17-ketosteroids	**-0,348**
Testosterone	**0,297**
Parathyroid hormone	**-0,293**
1/Mode HRV as Catecholamines	**0,271**
Corticosterone	**-0,265**
Triiodothyronine	**0,254**
Right set	**Root 2**
Macrophages of Spleen	**0,633**
Entropy of Thymocytogram	**0,504**
Lymphocytes of Thymus	**-0,465**
Endotheliocytes of Thymus	**0,434**
Epitheliocytes of Thymus	**0,426**
Lymphoblastes of Thymus	**-0,381**
Macrophages of Thymus	**0,350**
Phagocytosis Index of Neutrophils	**0,332**
Microphages of Spleen	**-0,331**
Plasmocytes of Blood	**0,315**
Microbial Count of Monocytes	**-0,298**
Thymus mass	**0,282**
NK-Lymphocytes of Blood	**-0,276**
Blast Transformation T-lymphocytes, %	**-0,270**
Hassal's corpuscles of Thymus	**0,255**
Fibroblasts of Spleen	**-0,248**
Spleen Mass	**-0,247**



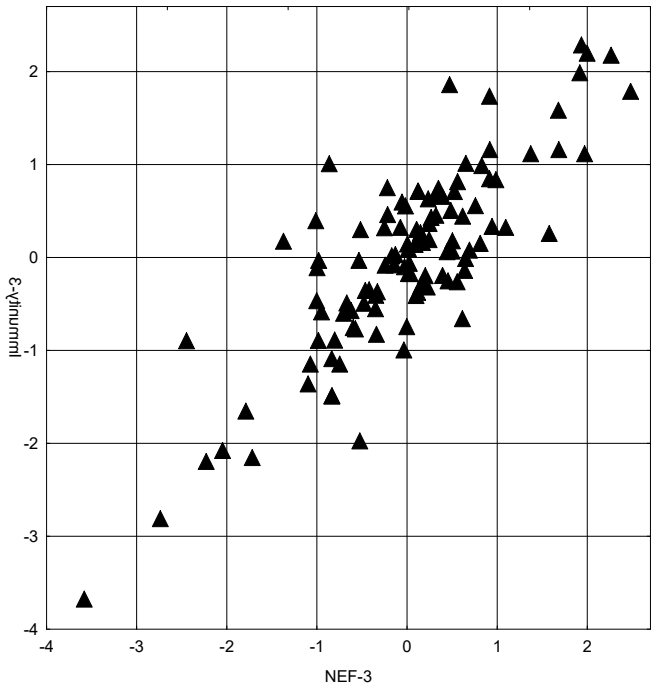
R=0.930; R^2=0.865; $\chi^2_{(297)}$=545; p<10^{-6}; Λ Prime=0.002

Fig. 12. Scatterplot of canonical correlation between neuro-endocrine factors (X-line) and immune parameters (Y-line) at all rats. *Second pair of Roots*

The neuroendocrine root of the third pair receives moderate loads from vagal tone and calcitonin, and the immune root is almost completely represented by blood parameters. The rate of immunomodulation is 69.2% (Table 14 and Fig. 13).

Table 14. Factor structure of third pair of Neuro-endocrine and Immune Roots

Left set	**Root 3**
MxDMn HRV as Vagal tone	**-0,529**
Calcitonin	**0,452**
Triiodothyronine	**0,246**
Aldosterone	**-0,216**
1/Mode HRV as Catecholamines	**0,199**
Testosterone	**0,191**
Right set	**Root 3**
Eosinophiles of Blood	**-0,594**
Entropy of Leukocytogram	**-0,539**
Basophiles of Blood	**-0,378**
NK-Lymphocytes of Blood	**-0,373**
Entropy of Immunocytogram	**-0,350**
Plasmocytes of Blood	**-0,278**
Plasmocytes of Spleen	**0,243**
Macrophages of Thymus	**0,237**
Spleen Mass	**0,216**



R=0.832; R^2=0.692; $\chi^2_{(256)}$=371; p<10^{-5}; Λ Prime=0.014

Fig. 13. Scatterplot of canonical correlation between neuro-endocrine factors (X-line) and immune parameters (Y-line) at all rats. *Third pair of Roots*

Conclusion

There is a close canonical correlation between registered neuroendocrine factors and immunity parameters in general. At the same time, both the severity of the immunomodulatory activity of individual neuroendocrine factors and the subjection to the regulatory influence of individual parameters of immunity differ significantly.

The following publications will analyze sexual differences in parameters in intact rats and those exposed to chronic stressors and adaptogens, as well as neuroendocrine-immune connections in each of the clusters.

Acknowledgments

We express sincere gratitude to PhD Volodymyra R. Bilas for help in carry out of immune analyses.

Declarations

Funding

No funding

Author contributions

Conceptualization, O.P.; Methodology, R.Y.; Software, A.V. and X.Ż.; Validation, O.P., R.Y. and A.V.; Formal Analysis, O.P., R.Y., A.V. and X.Ż.; Resources, A.V.; Data Curation, O.P., R.Y. and A.V.; Writing – Original Draft Preparation, A.V.; Writing – Review & Editing, O.P.,

R.Y. and A.V.; Visualization, A.V. and X.Ż.; Supervision, O.P. and R.Y.; Project Administration, O.P. and R.Y.; Funding Acquisition, A.V.

Conflicts of interest

The authors declare no competing interests.

Data availability

The datasets used and/or analyzed during the current study are open from the corresponding author on reasonable request.

REFERENCES

1. Baevsky RM, Berseneva AP. *Use KARDIVAR system for determination of the stress level and estimation of the body adaptability*. Standards of measurements and physiological interpretation. Moscow-Prague;2008:41.
2. Bilas VR, Popadynets' OO, Flyunt ISS, Sydoruk NO, Badiuk NS, Gushcha SG, Zukow W, Gozhenko AI, Popovych IL. Entropies of thymocytogram, splenocytogram, immunocytogram and leukocytogram in rats are regulated by sex and the neuroendocrine parameters while regulates immune parameters. *JEHS*. 2020;10(7):266-288.
3. Bilas VR, Popovych IL. Role of microflora and organic substances of water Naftussya in its modulating influence on neuroendocrine-immune complex and metabolism [in Ukrainian]. *Medical Hydrology and Rehabilitation*. 2009;7(1):68-102.
4. Dhabhar FS. The short-term stress response – mother nature's mechanism for enhancing protection and performance under conditions of threat, challenge, and opportunity. *Front Neuroendocrinol*. 2018;49:175–192.
5. Elsenbruch S, Enck P. The stress concept in gastroenterology: from Selye to today. F1000Res. 2017;6:2149. doi: 10.12688/f1000research.12435.1.
6. Goryachkovskiy AM. *Clinical biochemie* [in Russian]. Odesa: Astroprint,1998:608.
7. Gozhenko AI, Korda MM, Popadynets' OO, Popovych IL. *Entropy, Harmony, Synchronization and Their Neuro-Endocrine-Immune Correlates* [in Ukrainian]. Odesa: Feniks;2021:232.
8. Gozhenko AI, Korda MM, Smagliy SS, Badiuk NS, Zukow W, Klishch IM, Korda IV, Bombushkar IS, Popovych IL. *Uric Acid, Metabolism, Neuro-Endocrine-Immune Complex* [in Ukrainian]. Odesa: Feniks;2023:266. doi.org/10.5281/zenodo.7575158
9. Horizontov PD, Belousova BI, Fedotova MI. *Stress and the Blood System* [in Russian]. Moskva: Meditsina;1983:240.
10. Kozyavkina OV, Kozyavkina NV, Gozhenko OA, Gozhenko AI, Barylyak LG, Popovych IL. *Bioactive Water Naftussya and Neuro-Endocrine-Immune Complex* [in Ukrainian]. Kyïv: UNESCO-SOCIO;2015:349.
11. Kiriakopoulos A, Giannakis P, Menenakos E. Calcitonin: current concepts and differential diagnosis. Ther Adv Endocrinol Metab. 2022 May 21;13:20420188221099344. doi: 10.1177/20420188221099344.
12. Kul'chyns'kyi AB, Gozhenko AI, Zukow W, Popovych IL. Neuro-immune relationships at patients with chronic pyelonephrite and cholecystite. Communication 3. Correlations between parameters EEG, HRV and Immunogram. Journal of Education, Health and Sport. 2017;7(3):53-71.
13. Mel'nyk OI, Struk ZD, Zukow W, Popovych IL. Vegetative, endocrine and metabolic accompaniments of individual immune responses to adaptogenic balneotherapy. *JEHS*. 2019;9(12):207-229.
14. Mel'nyk OI, Zukow W, Hrytsak MV, Popovych DV, Zavidnyuk YV, Bilas VR, Popovych IL. Canonical analysis of neuroendocrine-metabolic and neuroendocrine-immune relationships at female rats. *JEHS*. 2021;11(5):356-369.
15. Nance DM, Sanders VM. Autonomic innervation and regulation of immune system (1987-2007). *Brain Behav Immun*. 2007;21(6):736-745.
16. Pavlov VA, Chavan SS, Tracey KJ. Molecular and functional neuroscience in immunity. *Annu Rev Immunol*. 2018;36:783-812.
17. Perederiy VG, Zemskov AM, Bychkova NG, Zemskov VM. *Immune Status, Principles of its*

Evaluation and Correction of Immune Disorders [in Russian]. Kyïv: Zdorovya;1995:211.
18. Polovynko IS, Zajats LM, Popovych AI, Popovych IL. Integral quantification of neuroendocrine and immune responses to chronic stress in male rats [in Ukrainian]. In: *Pathophysiology and Pharmacy: ways of integration: Abstracts VII National Congress pathophysiologists Ukraine with international participation (5-7 October 2016)*. Kharkiv: NPhU;2016:182-182.
19. Popovych IL, Gozhenko AI, Korda MM, Klishch IM, Popovych DV, Zukow W (editors). *Mineral Waters, Metabolism, Neuro-Endocrine-Immune Complex*. Odesa. Feniks;2022:252. doi.org/10.5281/zenodo.6604298
20. Popovych IL, Gozhenko AI, Zukow W, Polovynko IS. *Variety of Immune Responses to Chronic Stress and their Neuro-Endocrine Accompaniment*. Riga: Scholars' Press;2020:172. doi.org/10.5281/zenodo.3822074
21. Popovych IL, Kul'chyns'kyi AB, Gozhenko AI, Zukow W, Kovbasnyuk MM, Korolyshyn TA. Interrelations between changes in parameters of HRV, EEG and phagocytosis at patients with chronic pyelonephritis and cholecystitis. *JEHS*. 2018;8(2):135-156.
22. Popovych IL, Kul'chyns'kyi AB, Korolyshyn TA, Zukow W. Interrelations between changes in parameters of HRV, EEG and cellular immunity at patients with chronic pyelonephritis and cholecystitis. *JEHS*. 2017;7(10):11-23.
23. Popovych IL. The concept of neuroendocrine-immune complex (Review) [in Russian]. *Medical Hydrology and Rehabilitation*. 2009;7(3):9-18.
24. Popovych IL. *Stresslimiting Adaptogene Mechanism of Biological and Curative Activity of Water Naftussya* [in Ukrainian]. Kyïv: Computerpress;2011:300.
25. Selye H: A syndrome produced by diverse nocuous agents. 1936. *J Neuropsychiatry Clin Neurosci.* 1998;10(2):230–231. 10.1176/jnp.10.2.230a
26. Shannon CE. A mathematical theory of information. Bell Syst Tech J. 1948;27:379-423.
27. Szabo S, Yoshida M, Filakovszky J, Juhasz G. "Stress" is 80 Years Old: From Hans Selye Original Paper in 1936 to Recent Advances in GI Ulceration. Curr Pharm Des. 2017;23(27):4029-4041. doi: 10.2174/1381612823666170622110046.
28. Thayer JF, Sternberg EM. Neural aspects of immunomodulation: Focus on the vagus nerve. *Brain Behav Immun*. 2010;24(8):1223-1228.
29. Yoo BB, Mazmanian SM. The Enteric Network: Interactions between the Immune and Nervous Systems of the Gut. *Immunity*. 2017;46(6):910-926.
30. Zajats LM, Polovynko IS, Zukow W, Yanchij RI. Mysakovets' OG, Mel'nyk OI, Hrytsak YaL. Neuroendocrine-immune relationships in rat females. *JEHS*. 2017;7(10):59-78.