INTEGRATED QUANTITATIVE ASSESSMENT OF CHANGES IN NEURO-ENDOCRINE-IMMUNE COMPLEX AND METABOLISM IN RATS EXPOSED TO ACUTE COLD-IMMOBILIZATION STRESS

Nadiya O Sydoruk[1], Walery Zukow[2], Roman I Yanchij[3]

[1]JSC "Dnipro-Beskyd", Truskavets', Ukraine dnipro-b@bk.ru
[2]Faculty of Physical Education, Health and Tourism, Kazimierz Wielki University, Bydgoszcz, Poland w.zukow@ukw.edu.pl
[3]Department of Immunophysiology OO Bogomoletz Institute of Physiology NAS, Kyiv, Ukraine tas@biph.kiev.ua

Abstracts

Background. It is known that the reaction of the neuroendocrine-immune complex to acute and chronic stress are different. It is also known about sex differences in stress reactions. Previously we have been carry out integrated quantitative estimation of neuroendocrine and immune responses to chronic restraint stress at male rats. The **purpose** of this study - to carry out integrated quantitative estimation of neuroendocrine, immune and metabolic responses to acute stress at male and female rats. **Material and research methods.** The experiment is at 58 (28 male and 30 female) white rats Wistar line weighing 170-280 g (Mean=220 g; SD=28 g). The day after acute (water immersion restraint) stress determined HRV, endocrine, immune and metabolic parameters as well as gastric mucosa injuries and comparing them with parameters of intact animals. **Results.** Acute cold-immobilization stress caused moderate injuries the stomach mucosa as erosions and ulcers. Among the metabolic parameters revealed increased activity Acid Phosphatase, Asparagine and Alanine Aminotranspherase as well as Creatinephosphokinase. It was also found to reduce plasma Testosterone as well as serum Potassium and Phosphate probably due to increased Parathyrine and Mineralocorticoid activity and Sympathotonic shift of sympatho-vagal balance. Integrated quantitative measure manifestations of Acute Stress as mean of modules of Z-Scores makes for 10 metabolic parameters $0,75\pm0,10$ σ and for 8 neuro-endocrine parameters $0,40\pm0,07$ σ. Among immune parameters some proved resistant to acute stress factors, while 10 significant suppressed and 12 activated. Integrated quantitative measure poststressory changes makes $0,73\pm0,08$ σ. Found significant differences integrated status intact males and females, whereas after stress differences are insignificant. **Conclusion.** The approach to integrated quantitative assessment of neuroendocrine-immune complex and metabolism may be useful for testing the effectiveness streslimiting means.

INTRODUCTION

It is known that the reaction of the neuroendocrine-immune complex to acute and chronic stress are different. It is also known about sex differences in stress reactions [6,7,11,18,20,22,27,3339,41,42]. Previously we [29] have been carry out integrated quantitative estimation of neuroendocrine and immune responses to chronic restraint stress at male rats. 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. 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, 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. 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. Canonical correlation between Neuroendocrine and Immune parameters is very strong, thus components of the autonomic nervous, endocrine and immune systems interact closely within the triune neuroendocrine-immune complex.

The **purpose** of this study: to carry out integrated quantitative estimation of neuroendocrine, immune and metabolic responses to acute stress at male and female rats.

MATERIAL AND RESEARCH METHODS

The experiment is at 58 (28 male and 30 female) white rats Wistar line weighing 170-280 g (Mean=220 g; SD=28 g). It was created by 6 groups that were equivalent to about sex and body weight of both averages and their dispersion. 10 animals of the first group received tap water through a tube at a dose 2% of body weight once daily for seven days, that remained relatively intact. 48 rats were divided into 5 test groups and treated with tap water, distilled water as well as table water "Truskavets'ka" and bioactive waters Naftussya from Truskavets'and Pomyarky layers. This design explains another purpose of the study - comparing the effects of waters different layers [40].

A day after the end of course animals test groups subjected to water immersion restraint

stress by the method of J Nakamura et al. [26] as modified IL Popovych [29], which is to reduce the duration of stay of rats in cold water (t^o 20-21° C) from 8 to 4 hours.

The day after acute stress took samples of peripheral blood (through a cut tail) to analyze leukocytogram. An hour under light ether anesthesia for 15-20 sec recorded ECG in standard lead II (introducing needle electrodes subcutaneously) to determine parameters of heart rate variability (HRV) [3,22,33]. Then the animals were decapitated, for the purpose of collecting blood in which was determined some endocrine, immune and metabolic parameters.

Among endocrine parameters determined plasma concentration of corticosterone, testosterone and triiodothyronine (by ELISA, reagents from JSC "Alkor Bio", RF [16]).

Immune parameters were determined by tests I and II levels of WHO as described in the handbook [23] and the previously developed algorithm [32,33]. On the state of the phagocytic function of neutrophils (microphages) and monocytes (macrophages) judged by phagocytic index, microbial (phagocytic) number and index of killing regarding museum culture Staphylococcus aureus [5,8], with the calculation of derivative indices: microbial capacity (number of microbes that are able to absorb phagocytes contained in 1 L of blood) and bactericidal capacity (number of microbes that are able to neutralize neutrophils or monocytes contained in 1 L of blood) [5,32,33].

Among the parameters immunogram determined the relative amount of blood population of T-cells by spontaneous rosette test with sheep erythrocytes by M Jondal et al. [17], their theophylline resistant (T-helpers/inductors) and theophylline sensitive (T-supressors/killers) subpopulations (by test sensitivity rosette to theophylline by S Limatibul et al. [24]), the population of B-lymphocytes by test complementary rosette of sheep erythrocytes by Bianco as described in the handbook [23]. Natural killer identified as big containing granules lymphocytes.

On lipid metabolism judged by the level of plasma triacylglycerides (metaperiodate-acetylacetone colorimetric method), total cholesterol (direct method by reaction Zlatkis-Zach) and its distribution as part of α-lipoprotein (applied enzymatic method G Hiller [14] after precipitation nonα-lipoproteins using dextransulfate/Mg^{2+}) as well as nonα-lipoprotein (turbidometric method Burstein-Samay) as described in the handbook [13].

State of lipid peroxidation assessed the content in the serum its products: diene conjugates (spectrophotometry of heptane phase of lipids extract) [12] and malonic dyaldehid (test with tiobarbiture acid) [1], as well as the activity of antioxidant enzymes: catalase serum and red blood cells (by the speed of decomposition hydrogen peroxide) [21], superoxide dismutase erythrocytes (by the degree of inhibition of nitroblue tetrazolium recovery in the presence of N-methylphenazone metasulfate and NADH) [9,25].

On electrolyte metabolism judged by the level in the plasma of calcium (by the reaction with arsenazo III), phosphate (phosphate molibdate method), chloride (mercury rodanide method), sodium and potassium (flame photometry method) as described in the handbook [13].

Based on obtained data expected number of hormonal activities: mineralocorticoid MCA=(Na/K)^{0,5}, parathyrine PTA=(Ca/P)^{0,5} and calcitonine CTA=(1/Ca•P)^{0,5}, based on the classical principles and guidelines IL Popovych [30,33], as well as Ca/K ratio, which is considered a marker of sympatho-vagal balance [10].

Alanine and asparagine aminotranspherase, alkaline and acid phosphatase as well as creatinephosphokinase determined by uniform methods as described in the handbook [13].

Use analyzers "Tecan" (Oesterreich), "Pointe-180" ("Scientific", USA), "Reflotron" ("Boehringer Mannheim", BRD) and flame spectrophotometer.

After a blood sample was removed spleen, thymus, adrenal glands and stomach. Immune organs weighed and made them smears for counting of spleno- and thymocytograms [4,5]. The stomach was cut along the greater curvature, mounted it on gastroluminoscope and under a magnifying glass counted the number of ulcers and their length was measured, evaluated erosive and ulcerative damage on two scales: by Shatalov et al [38] and IL Popovych [30,31].

Given the fact that immune-, leuko-, spleno- and thymocytograms are closed systems of different formed elements certainly promising is the analysis from the standpoint of information theory [2]. Information analysis of cytograms allows using generalized index to assess the state of morpho-functional adaptive and protective systems, information which is contained in their cytograms [31,45].

Information analysis of cytograms conducted using the equation C Shannon [cit. by: 45] to calculate the value of H as entropy aggregate probabilities (synonyms: information entropy, uncertainty):

$$H = - \sum_{i=1}^{n} p_i \bullet \log_2 p_i,$$

where: i is the number of groups formed elements;

p is share of i-group of elements in cytogram.

Since entropy depends on the number of elements, for leveling this fact and allow for comparison of the number of different components calculated relative entropy index (h), that the share of the actual entropy (H) in maximum entropy (H_{max}) of system with n elements:

$H_{max} = \log_2 n$;

$h = H/H_{max}$.

Digital material it is processed using the package of softwares "Statistica 5.5".

RESULTS AND DISCUSSION

In this report we will analyze **common** to all 5 groups poststressory changes.

As expected, acute cold-immobilization stress caused injuries the stomach mucosa as erosions and ulcers. The severity of stress injuries assessed as moderate as a scale both VN Shatalov et al [38] (range 0÷4 points) (Fig. 1) and a scale IL Popovych [30] (range 0÷1 point): 0,28±0,04 points.
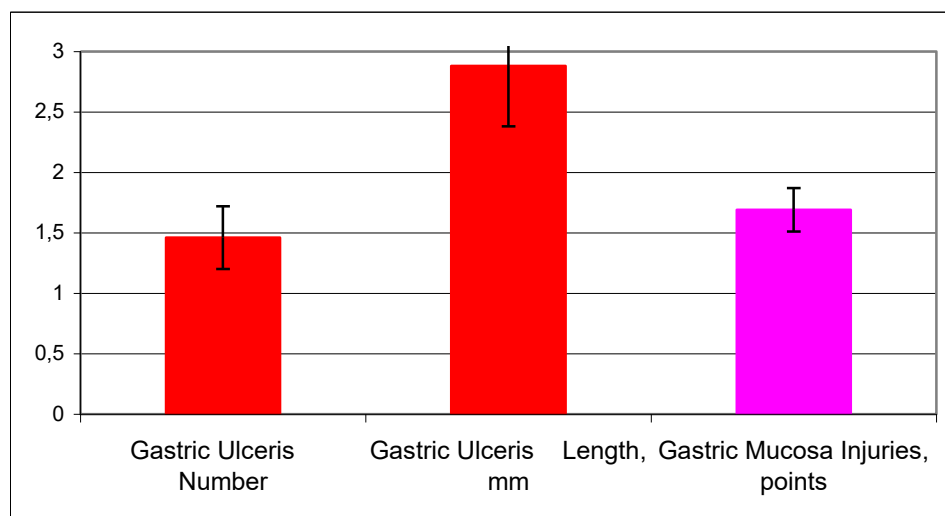

Fig. 1. Quantifying the damage caused by acute stress gastric mucosa

According recommendation by IL Popovych [32,33] variables obtained after Acute Stress (SV) expressed as Z-scores calculated by formula:

$Z = (SV/NV - 1)/Cv$, where

NV is Norm (obtained from intact rats) Variable, Cv is Coefficient its variation in intact rats.

This approach allows us to evaluate the variables expressed in different units (μKat, %, nM/L, msec etc) in one scale.

Among the metabolic parameters (Fig. 2) expected revealed increased activity Acid Phosphatase as marker labilization of lysosomes as well as Asparagine and Alanine Aminotranspherase and Creatinephosphokinase as markers cytolysis. However, contrary to expectations, the level of serum Diene Conjugates increased slightly, but Malonic Dyaldehide even declining. This can be explained by a significant decrease of serum Lipoproteines as object of lipid peroxidation. It was also found to reduce serum Potassium and Phosphate probably due to increased Parathyrine and Mineralocorticoid activity and Sympathotonic shift of sympatho-vagal balance (Fig 3).
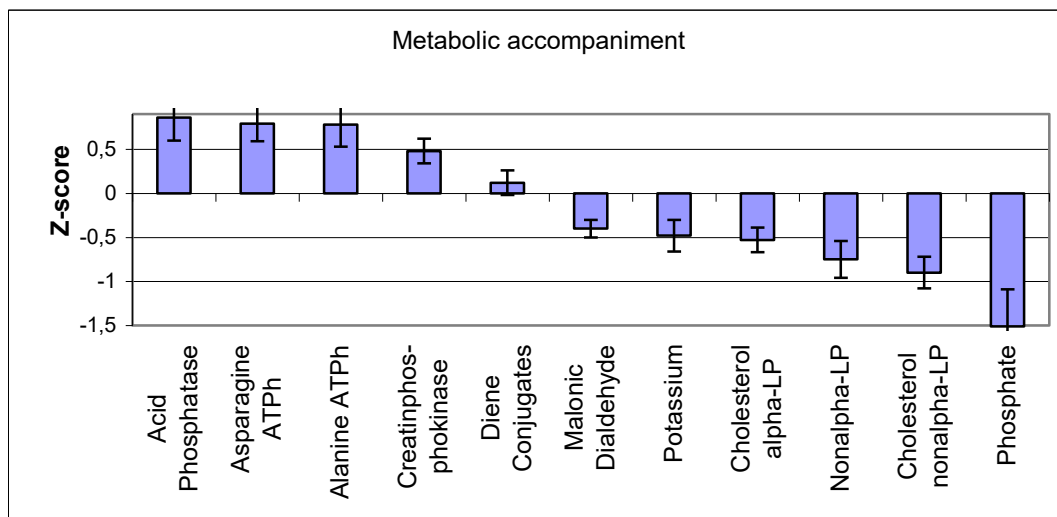

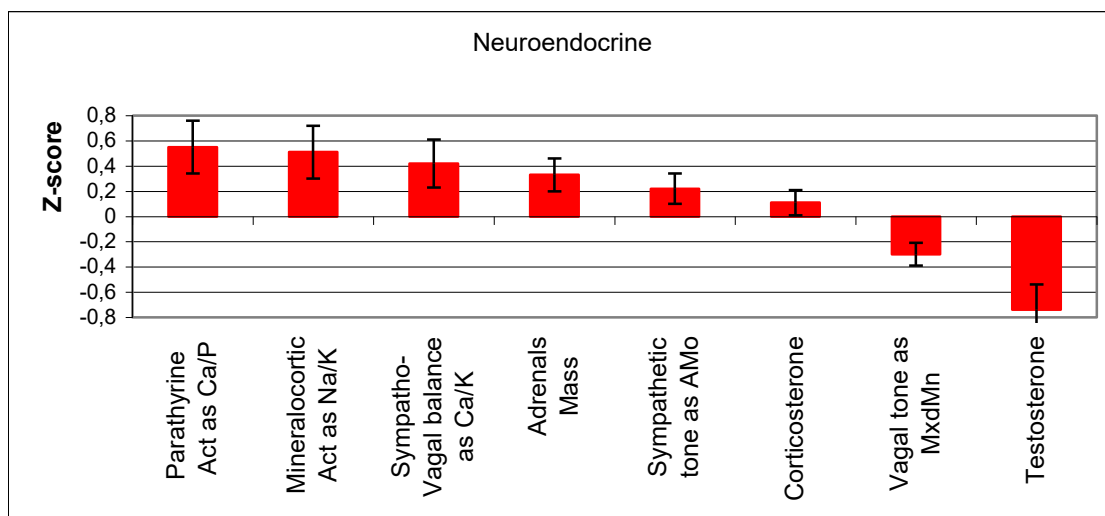**Fig. 2. Ranking caused by acute stress changes in metabolic parameters**

Fig. 3. Ranking caused by acute stress changes in neuroendocrine parameters

The level of plasma neither Corticosterone, nor Triiodthyronine naturally not changed. Looking ahead, we note that for the averages hides opposing poststressory changes in these hormones in different groups of rats, but it will in the next publication. Instead, revealed a significant decrease in testosterone levels.

Integrated quantitative measure manifestations of Acute Stress as mean of modules of Z-Scores makes for 10 metabolic parameters $0,75\pm0,10$ σ (or Euklidian units) and for 8 neuro-endocrine parameters $0,40\pm0,07$ σ.

Interestingly, in our previous experiment with **Chronic** Stress mean of modules of Z-Scores for 13 neuro-endocrine parameters was almost equal: $0,47\pm0,04$ σ [29].

Overall, our findings are consistent with a classic conception about the leading role in neuro-endocrine manifestations of Acute Stress Corticoadrenal and Autonomic Nervous Systems. However, our data support the discussion about the nature of changes by Acute Stress in other endocrine glands, specifically parathyroid and thyroid glands [6,7,11,15,18,20,22,27-30,32-37,39,41-44].

Among immune parameters some proved resistant to acute stress factors, while others significant suppressed (Fig. 4) or activated (Fig. 5).
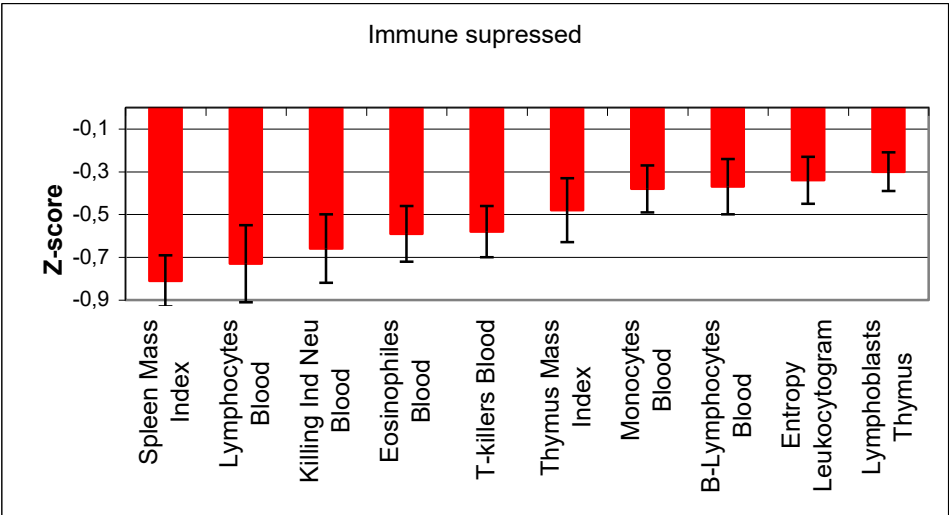

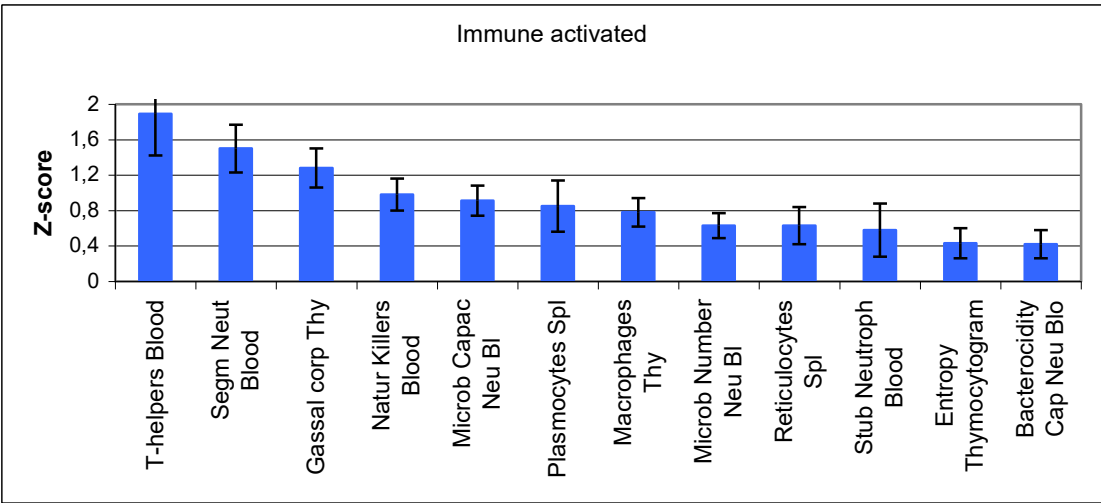**Fig. 4. Ranking caused by acute stress supressing changes in immune parameters**

Fig. 5. Ranking caused by acute stress activating changes in immune parameters

Integrated quantitative measure manifestations of **Acute** Stress makes for 22 immune parameters 0,73±0,08 σ versus 0,44±0,14 σ for 28 parameters in our previous experiment with **Chronic** Stress [29].

Our findings are consistent with the provisions, summarized in the perfect review FS Dhabhar [7], 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 immuno-pathological) 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.

Another approach to identify the variables set of which neuroendocrine-immune complex and metabolism intact and stressed rats significantly different is discriminant (recognizing) analysis. In applying method forward stepwise [19] variables currently in the model turned out 20 only (Table 1), while other earlier marked variables currently not in the discriminant model.

Table 1. Discriminant Function Analysis Summary. Recognized indices for Intact rats and exposed to Acute Stress rats. Step 20, N of vars in model: 20; Grouping: 2 grps.

Wilks' Lambda: 0,215; approx. $F_{(20)}=6,7$; $p<10^{-6}$

Variables currently in the model	Groups (n)		Parameters of Wilks' Statistics				
	Intact (10)	Stressed (48)	Wilks Λ	Parti al Λ	F rem	p	Tole-rancy
Injuries Gastric Mucosa, Sha-v's points	0	1,69±0,18	,309	,695	16	10^{-3}	,090
Injuries Gastric Mucosa, Popo-ch's poin	0	0,28±0,04	,247	,871	5,5	,025	,106
Hassal's corpuscules of Thymus, %	1,00±0,00	1,56±0,10	,360	,598	25	10^{-5}	,413
Macrophages of Thymus, %	5,39±0,50	6,62±0,25	,294	,731	14	10^{-3}	,411
Th-Lymphocytes of Blood, %	29,7±0,3	31,3±0,4	,258	,833	7,4	,010	,597
Asparagine Aminotranspherase, μKat/L	0,21±0,02	0,27±0,01	,236	,912	3,6	,067	,436
Microbial Capacity of Neutrophils,10^9/L	15,5±2,5	22,8±1,4	,232	,927	2,9	,096	,092
Bacterocidal Capacity Neutrophil, 10^9/L	7,54±1,39	9,37±0,69	,252	,854	6,3	,016	,089
Sympathetic tone as AMo HRV, %	58±8	64±3	,254	,845	6,8	,013	,241
Segmented Neutrophils of Blood, %	34,7±1,1	39,8±0,9	,222	,969	1,2	,282	,515
Diene conjugates, E^{232}/mL	1,47±0,11	1,51±0,05	,271	,793	9,7	,004	,346
Corticosterone for Males, nM/L	290±57	316±18	,245	,879	5,1	,030	,130
Corticosterone for Females, nM/L	1145±103	1146±55					
Spleen Mass Index, ‰	3,75±0,25	3,12±0,09	,217	,992	,3	,582	,562
Cholesterol nonα-Lipoproteines, mM/L	1,04±0,07	0,82±0,04	,221	,971	1,1	,301	,724
Nonα-Lipoproteines, units	4,47±0,28	3,82±0,18	,232	,927	2,9	,097	,544
Malonic dyaldehid, μM/L	63,5±5,6	56,5±1,7	,259	,829	7,6	,009	,394
Lymphoblastes of Thymus, %	7,50±0,96	6,58±0,23	,224	,961	1,5	,227	,538
Vagal tone as MxdMn HRV, msec	42±14	29±4	,270	,796	9,5	,004	,193
Thymus Mass Index, ‰	0,72±0,07	0,61±0,03	,216	,996	,2	,700	,420
Testosterone for Females, nM/L	5,88±0,28	5,29±0,30	,266	,809	8,7	,005	,103
Testosterone for Males, nM/L	67±2	61±1					

Variables currently not in the model	Groups (n)		Parameters of Wilks' Statistics				
	Intact (10)	Stressed (48)	Wilks Λ	Part Λ	F to ent	p	Tole ranc
Gastric Ulceris Number	0	1,46±0,26	,215	1,00	,00	,97	,145
Gastric Ulceris Length, mm	0	2,88±0,50	,215	1,00	,02	,89	,064
Adrenals Mass, mg	55±5	60±2	,212	,99	,47	,50	,299
Mineralocorticoide Activity as (Nap/Kp)^{0,5}	5,75±0,17	6,02±0,11	,215	1,00	,00	,96	,526
Sympatho-Vagal Balance as Cap/Kp	0,81±0,09	0,93±0,06	,215	1,00	,02	,90	,285
Parathyrine Activity as (Cap/Pp)^{0,5}	1,53±0,07	1,65±0,05	,215	1,00	,03	,86	,262
Acid Phosphatase, IU/L	31,4±1,9	36,5±1,5	,209	,97	,98	,33	,607
Alanine Aminotranspherase, μKat/L	0,53±0,05	0,65±0,04	,214	,99	,20	,66	,420
Creatinphosphokinase, μKat/L	1,68±0,10	1,84±0,05	,215	1,00	,00	,95	,479
Potassium, mM/L	4,10±0,20	3,79±0,12	,215	1,00	,03	,87	,503
Phosphate, mM/L	1,32±0,02	1,22±0,03	,214	,99	,18	,67	,375
Cholesterol of α-Lipoproteines, mM/L	0,84±0,05	0,76±0,02	,215	1,00	,00	,99	,493
Entropy of Leukocytogram, $•10^{-3}$	682±16	664±6	,215	1,00	,00	,98	,582
Eosinophiles of Blood, %	4,90±0,72	3,55±0,30	,215	1,00	,00	,95	,704
Monocytes of Blood, %	6,20±0,72	5,32±0,24	,212	,99	,53	,47	,403
Stub Neutrophils of Blood, %	2,20±0,25	2,66±0,24	,212	,98	,56	,46	,485
Microbial Number of Neutrophiles	5,5±0,3	6,2±0,2	,215	1,00	,01	,94	,274
Killing Index of Neutrophiles, %	47,5±2,9	41,4±1,5	,213	,99	,43	,52	,082
Total Lymphocytes of Blood, %	51,8±1,5	48,4±0,8	,215	1,00	,00	,99	,148
Ts-Lymphocytes of Blood, %	15,3±1,1	13,2±0,4	,215	1,00	,05	,82	,701
B-Lymphocytes of Blood, %	13,4±0,8	12,5±0,3	,215	1,00	,01	,92	,567
NK-Lymphocytes of Blood, %	5,3±0,3	6,4±0,2	,215	1,00	,06	,81	,218
Reticulocytes of Spleen, %	2,67±0,22	3,11±0,15	,215	1,00	,08	,78	,403
Plasmocytes of Spleen, %	1,67±0,22	2,27±0,20	,211	,98	,67	,42	,658
Entropy of Thymocytogram, $•10^{-3}$	596±15	617±8	,215	1,00	,02	,88	,344

Information about these 20 variables currently in discriminant model condensed in canonical root which is poorly structured and correlated with them in different ways (Table 2). The calculation of individual Root values based on Raw Coefficients for discriminant variables and Constant (Table 2) allows to visualize the status of each rat (Fig. 6).

Table 2. Discriminant Function Analysis Summary. Standardized, Raw and Structural Coefficients for Canonical Variables

| Variables currently in the model	Coefficients			Parameters of Wilks' Statistics				
	Standa-rdized	Raw	Struc-tural	F to enter	p-le-vel	Λ	F va-lue	p-le-vel
Injuries Gastric Mucosa by Shatalov	2,078	1,770	**,272**	15,1	10^{-3}	,787	15,1	10^{-3}
Injuries Gastric Mucosa by Popovych	-1,248	-5,553	**,239**	3,2	,078	,345	8,9	10^{-6}
Hassal's corpuscles of Thymus	1,113	1,905	**,189**	10,7	,002	,555	14,4	10^{-6}
Segmented Neutrophils of Blood	,278	,046	**,171**	1,2	,282	,215	6,8	10^{-6}
Microbial Capacity of Neutrophils	-1,005	-,106	**,155**	1,8	,184	,222	7,0	10^{-6}
Macrophages of Thymus	,913	,552	**,148**	10,1	,002	,666	13,8	10^{-4}
Th-Lymphocytes of Blood	,598	,240	**,127**	2,5	,118	,420	9,9	10^{-6}
Asparagine Aminotranspherase	,506	5,533	**,120**	1,6	,218	,233	7,1	10^{-6}
Bacterocidic Capacity of Neutrophils	1,443	,307	**,078**	7,3	,009	,488	13,9	10^{-6}
Sympathetic tone as AMo HRV	,905	,040	**,052**	2,0	,162	,297	8,0	10^{-6}
Diene conjugates	,873	2,561	**,024**	2,6	,114	,399	9,2	10^{-6}
Corticosterone	-1,087	-,002	**,005**	3,0	,089	,263	7,8	10^{-6}
Spleen Mass Index	-,137	-,206	**-,191**	2,9	,097	,463	12,1	10^{-6}
Cholesterol nonα-Lipoproteines	-,225	-,790	**-,150**	2,5	,120	,441	10,8	10^{-6}
Nonα-Lipoproteines	-,413	-,344	**-,109**	1,4	,246	,242	7,4	10^{-6}
Malonic dyaldehid	-,743	-,057	**-,108**	3,8	,056	,369	9,1	10^{-6}
Lymphoblastes of Thymus	-,305	-,164	**-,099**	2,5	,121	,311	8,3	10^{-6}
Thymus Mass Index	-,111	-,507	**-,094**	2,1	,157	,250	7,7	10^{-6}
Vagal tone as MxdMn HRV	1,160	,039	**-,088**	2,4	,126	,328	8,6	10^{-6}
Testosterone	-1,535	-,056	**-,039**	2,3	,136	,282	7,8	10^{-6}
Constant	-15,28							
Eigenvalue=3,65; Canonical R=0,886; Wilks'Λ=0,215; $\chi^2_{(20)}$=70; p<10^{-6}								
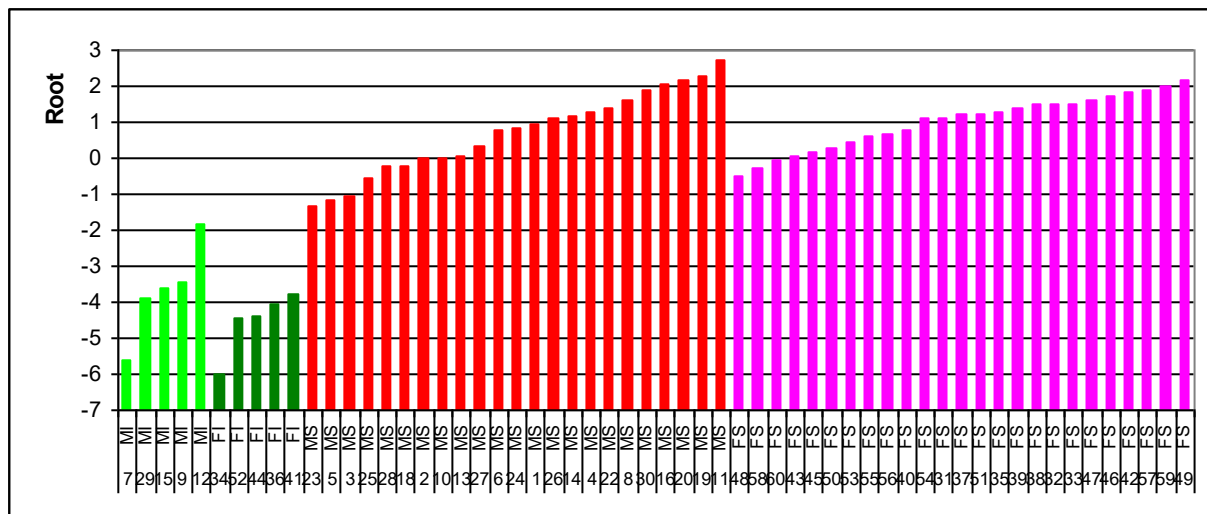
Fig. 6. Ranking individual Root values for Intact (I) and Stressed (S) Male (M) and Female (F) rats. The digits marked individual numbers rats

Squared Mahalanobis Distance between Intact and Stressed rats makes 26 (F=6,4; p<10^{-6}).

In calculating the averages turned out that integral status intact males and females differ significantly, while on the second day after acute stress differences are insignificant (Fig. 7).
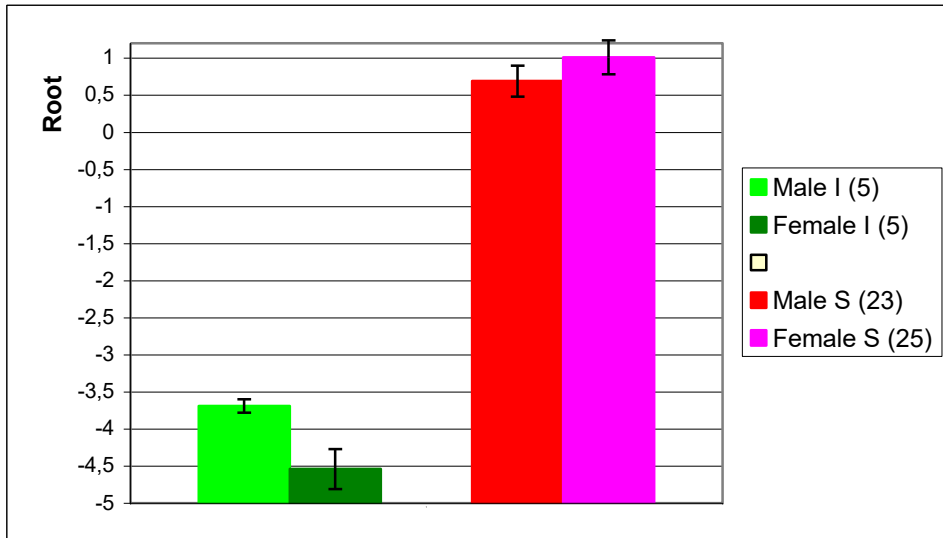
Fig. 7. Means±SE Root values for Intact (I) and Stressed (S) rats

Calculation of Classification Functions based Coefficients and Constants (Table 3) allows retrospectively recognize intact rats with one mistake (accuracy 90%) and stressed rats without mistake. Total accuracy of classification makes 98,3%.

Table 3. Coefficients and Constants for Classification Functions

Variables currently in the model	Intact Rats	Stressed Rats
Injuries Gastric Mucosa Index by Shatalov et al. Scale	22,3	31,1
Injuries Gastric Mucosa Index by Popovych Scale	-69,4	-97,0
Hassal's corpuscules of Thymus	24,8	34,2
Segmented Neutrophils	1,73	1,96
Microbial Capacity of Neutrophils	-2,68	-3,21
Macrophages of Thymus	13,4	16,1
Th-Lymphocytes of Blood	7,69	8,88
Asparagine Aminotranspherase	158,8	186,3
Bacterocidic Capacity of Neutrophils	6,75	8,27
Sympathetic tone as AMo HRV	1,06	1,26
Diene conjugates	40,2	52,9
Corticosterone	,001	-,011
Spleen Mass Index	12,1	11,0
Cholesterol nonα-Lipoproteines	24,0	20,1
Nonα-Lipoproteines	-8,01	-9,72
Malonic dyaldehid	-,117	-,399
Lymphoblastes of Thymus	-,648	-1,46
Thymus Mass Index	52,2	49,7
Vagal tone as MxdMn HRV	1,02	1,22
Testosterone	-,61	-,89
Constants	-317,5	-383,8

Conclusion. The approach to integrated quantitative assessment of neuroendocrine-immune complex and metabolism may be useful for testing the effectiveness streslimiting means. This is illustrated in our next publication.

733

ACKNOWLEDGMENT

REFERENCES

1. Andreyeva LI, Kozhemyakin LA, Kishkun AA. Modification of the method for determining the lipid peroxide in the test with thiobarbituric acid [in Russian]. Laboratornoye Delo. 1988; 11: 41-43.

2. Avtandilov GG. Medical Morphometry [in Russian]. Moskva: Meditsina. 1990. 384 p.

3. Baevskiy RM, Kirillov OI, Kletskin SZ. Mathematical Analysis of Changes in Heart Rate by Stress [in Russian]. Moskva: Nauka. 1984. 221 p.

4. Bazarnova MA. Cytology investigation punctates spleen. In: Guide to practical training in clinical laboratory diagnostics [in Russian]. Kyiv: Vyshcha shkola. 1988. 263-264.

5. 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.

6. Chrousos GP. The stress response and immune function: clinical implications. The 1999 Novera H Spector lecture. Neuroimmunomodulation. Perspectives at the new millenium. ANYAS. 2000; 917: 38-67.

7. Dhabhar FS. Enhancing versus Suppressive Effects of Stress on Immune Function: Implications for Immunoprotection and Immunopathology. Neuroimmunomodulation. 2009; 16(5): 300–317.

8. Douglas SD, Quie PG. Investigation of Phagocytes in Disease. Churchil. 1981. 110 p.

9. Dubinina YY, Yefimova LF, Sofronova LN, Geronimus AL. Comparative analysis of the activity of superoxide dismutase and catalase of erythrocytes and whole blood from newborn children with chronic hypoxia [in Russian]. Laboratornoye Delo. 1988; 8: 16-19.

10. Fajda OI, Drach OV, Barylyak LG, Zukow W. Relationships between Ca/K plasma ratio and parameters of Heart Rate Variability at patients with diathesis urica. Journal of Education, Health and Sport. 2016; 6(1): 295-301.

11. Garkavi LKh, Kvakina YeB, Kuz'menko TS. Antistress Reactions and Activation Therapy [in Russian]. Moskva: Imedis, 1998. 654 p.

12. Gavrilov VB, Mishkorudnaya MI. Spectrophotometric determination of plasma levels of lipid hydroperoxides [in Russian]. Laboratornoye Delo. 1983; 3: 33-36.

13. Goryachkovskiy AM. Clinical biochemi [in Russian]. Odesa: Astroprint. 1998. 608 p.

14. Hiller G. Test for the quantitative determination of HDL cholesterol in EDTA plasma with Reflotron ®. Klin Chem. 1987; 33: 895-898.

15. Hrytsak YaL, Barylyak LG, Zukow W, Popovych IL. Cluster analysis of hormonal constellation at women and men with harmonious and disharmonious general adaptation reactions. Journal of Education, Health and Sport. 2016; 6(4): 141-150.

16. Instructions for application for recruitment reagents for ELISA investigations hormones in the blood of humans [in Russian]. St. Petersburg: JSC "Alkor Bio". 2000.

17. Jondal M, Holm G, Wigzell H. Surface markers on human T and B lymphocytes. I. A large population of lymphocytes forming nonimmune rosettes with sheep red blood cells. J Exp Med. 1972; 136(2): 207-215.

18. Khaitov RM. Physiology of immune system [in Russian]. Moskva: VINITI RAN. 2005. 428 p.

19. Klecka WR. Discriminant Analysis [trans. from English in Russian] (Seventh Printing, 1986). In: Factor, Discriminant and Cluster Analysis. Moskva: Finansy i Statistika. 1989: 78-138.

20. Kolyada TI, Volyanskyi YL, Vasilyev NV, Maltsev VI. Adaptation Syndrome and Immunity [in Russian]. Kharkiv: Osnova. 1995. 168 p.

21. Korolyuk MA, Ivanova MI, Mayorova IG, Tokarev VYe. The method for determining the activity of catalase [in Russian]. Laboratornoye Delo. 1988; 1: 16-19.

22. Kozyavkina OV, Kozyavkina NV, Gozhenko OA, Gozhenko AI, Barylyak LG., Popovych IL. Bioactive Water Naftussya and Neuro-Endocrine-Immune Complex [in Ukrainian]. Kyiv: UNESCO-SOCIO. 2015. 349 p.

23. Lapovets' LYe, Lutsyk BD. Handbook of Laboratory Immunology [in Ukrainian]. Lviv. 2002. 173 p.

24. Limatibul S, Shore A, Dosch HM, Gelfand EW. Theophylline modulation of E-rosette formation: an indicator of T-cell maturation. Clin Exp Immunol. 1978; 33(3): 503-513.

25. Makarenko YeV. A comprehensive definition of the activity of superoxide dismutase and glutathione reductase in red blood cells in patients with chronic liver disease [in Russian]. Laboratornoye Delo. 1988; 11: 48-50.

26. Nakamura J, Takada S, Ohtsuka N, Heya T et al. An assessment of gastric ulcers in vivo: enhancement

of urinary recovery after oral administration of phenolsulfonphtalein in rats. J Parm Dyn. 1984; 7(7): 485-491.

27. Nance DM, Sanders VM. Autonomic innervation and regulation of the immune system. Brain Behav Immun. 2007; 21(6): 736-745.

28. Polovynko IS, Zayats LM, Zukow W, Popovych IL. Neuro-endocrine-immune relationships by chronic stress at male rats. Journal of Health Sciences. 2013; 3(12): 365-374.

29. Polovynko IS, Zajats LM, Zukow W, Yanchij RI, Popovych IL. Quantitative evaluation of integrated neuro-endocrine and immune responses to chronic stress rats male. Journal of Education, Health and Sport. 2016; 6(8): 154-166.

30. Popovych IL. The factor and canonical analysis parameters of neuro-endocrine-immune complex, metabolism and erosive-ulcerose injuries of mucous stomach at rats in conditions of acute water immersing stress [in Ukrainian]. Medical Hydrology and Rehabilitation. 2007; 5(2): 68-80.

31. Popovych IL. Information effects of bioactive water Naftyssya in rats: modulation entropic, prevention desynchronizing and limitation of disharmonizing actions water immersion stress for information components of neuro-endocrine-immune system and metabolism, which correlates with gastroprotective effect [in Ukrainian]. Medical Hydrology and Rehabilitation. 2007; 5(3): 50-70.

32. Popovych IL. Bioactive water Naftyssya in general like ginseng limits, negates, reverses neuro-hormonal, metabolic and immune pathogens manifestations and strengthens sanogens manifestations acute stress in rats without impacting significantly on performance, not subordinates stress action [in Ukrainian]. Medical Hydrology and Rehabilitation. 2007; 5(4): 7-29.

33. Popovych IL. Stresslimiting Adaptogenic Mechanisms of Biological and Therapeutic Activity of Water Naftussya [in Ukrainian]. Kyiv: Computerpress. 2011. 300 p.

34. Popovych IL. Functional interactions between neuroendocrine-immune complex in males rats [in Ukrainian]. Achievements of Clinical and Experimental Medicine. 2008; 2(9): 80-87.

35. Popovych IL. The concept of neuroendocrine-immune complex (Review) [in Russian]. Medical Hydrology and Rehabilitation. 2009; 7(3): 9-18.

36. Popovych IL, Vis'tak HI, Gumega MD, Ruzhylo SV. Vegetotropic Effects of Bioactive Water Naftussya and their Endocrine-Immune, Metabolic and Hemodynamic Accompaniments [in Ukrainian]. Kyiv: UNESCO-SOCIO. 2014. 163 p.

37. Schauenstein K, Felsner P, Rinner I, Liebmann PM, Stevenson JR, Westermann J, Haas HS, Cohen RL, Chambers DA. In vivo immunomodulation by peripheral adrenergic and cholinergic agonists/antagonists in rat and mouse models. Neuroimmunomodulation. Perspectives at the new millenium. ANYAS. 2000; 917: 618-627.

38. Shatalov VN, Polonskiy VM, Bulgakov SA, Vinogradov VA. The ligands of opiate receptors and experimental ulcer. In: Modern methods of diagnosis and treatment of internal diseases [in Russian]. Collection of Scientific Papers IV Main Administration under the Ministry of Health USSR. Moskva. 1980. 97-98.

39. Sternberg EM. Neural regulation of innate immunity: a coordinated nonspecific host response to pathogens. Nat Rev Immunol. 2006; 6(4): 318-328.

40. Sydoruk NO, Zukow W. Comparative investigation of immediate effects on neuro-endocrine-immune complex of bioactive water Naftussya from layers Truskavets', Pomyarky and Skhidnyts'a. Communication 1. Generic effects. Journal of Education, Health and Sport. 2016; 6(8): 85-101.

41. Thayer JF, Sternberg EM. Neural aspects of immunomodulation: Focus on the vagus nerve. Brain Behav Immun. 2010; 24(8): 1223-1228.

42. Uchakin PN, Uchakina ON, Tobin BV, Ershov FI. Neuroendocrine immunomodulation [in Russian]. Vestnik Ross AMN. 2007; 9: 26-32.

43. Vis'tak HI. Relationship between vegetotropic and endocrine, immunotropic as well as clinical effects of bioactive water Naftussya in women with thyroid hyperplasia [in Ukrainian]. Medical Hydrology and Rehabilitation. 2012; 10(2): 37-66.

44. Вісьтак Г.І., Попович І.Л. Вегетотропні ефекти біоактивної води Нафтуся та їх ендокринний і імунний супроводи у щурів-самок. Медична гідрологія та реабілітація. 2011.-9,№2.-С. 39-57. Vis'tak HI, Popovych IL. Vegetotropic effects of bioactive water Naftussya and their endocrine and immune support in female rats [in Ukrainian]. Medical Hydrology and Rehabilitation. 2011; 9(2): 39-57.

45. Yushkovs'ka OG. Using information theory to study adaptive responses in the body athletes [in Ukrainian]. Medical Rehabilitation, Kurortology, Physiotherapy. 2001;1(25): 40-43.