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VARIETY OF IMMUNE RESPONSES TO CHRONIC STRESS IN RATS MALE

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Abstract

Background. Previously we have been carry out integrated quantitative estimation of neuroendocrine and immune responses to chronic restraint stress in male rats. Revealed that the value of canonical discriminant roots rats subjected to chronic stress different not only on the values of intact animals (by definition), but also among themselves. So we set a goal retrospectively divided stressed rats into three homogeneous groups. **Material and 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 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. **Results.** The method of cluster analysis (k-means clustering) formed three groups-clusters. For further analysis selected 18 parameters that members of each cluster differing minimum and maximum are different from members of other clusters (η^2=0,73÷0,15; F=49,0÷3,26; p=10^{-6}÷0,05). We stated that in 16 rats from cluster III the deviation 16 parameters in either side of the average norm almost identical and are in an acceptable range of ±0,5σ. Thus, the immune status of 40% of the rats subjected to moderate chronic stress was resistant to its factors. For the immune status of the 15 (37,5%) rats cluster II typical moderate inhibition microphage, killer and T-cellular links in combination with a strong activation macrophage link. Poststressory changes in immunity in 9 rats (22,5%) from cluster I differ from those in cluster II both qualitatively and quantitatively. In particular, the rats in this cluster were found no deviations from the norm or reaction blast transformation T-cells nor NK-lymphocytes levels. However, other parameters of T-link and microhage link suppressed more and settings macrophage link appeared activated very significantly, and the area of activation, except thymus and blood, spread to the spleen. **Conclusion.** We assume that a variety of immune responses to chronic stress caused by a variety of reactions neuroendocrine factors.

Keywords: chronic stress, thymocytogram, splenocytogram, immunocytogram of blood, male rats.

INTRODUCTION

Previously we have been carry out integrated quantitative estimation of neuroendocrine and immune responses to **chronic** restraint stress at male rats. Revealed that the value of canonical discriminant roots rats subjected to chronic stress different not only on the values of intact animals (by definition), but also among themselves [33]. In another study, we found a wide variation in immune parameters in rats subjected to **acute** stress [43]. We also know about the wide range of state of the gastric mucosa in these conditions, namely no visible damage, erosions, solitary ulcer, multiple ulcers [3,23-26,37,38,43]. Since damage to gastric mucosa and lymphoid tissue, together with hypertrophy of the adrenal cortex, considered attributes stress (classic H Selye's triad) [reviews: 13,17,27,34,20,47], then it is expected poststressory variety of state immunity. So we set a goal retrospectively divided chronic stressed rats into three homogeneous for immunity while different one from the other groups, using cluster analysis.

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 [34]. The day after the completion of stressing in rats of both groups took samples of peripheral blood (through a cut tail) to analyze leukocytogram. The next day, the animals were decapitated, for the purpose of collecting blood, in which was determined immunological parameters by tests I and II levels of WHO as described in the handbook [21] and the previously developed algorithm [9,34]. 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 (ATCC N 25423 F49) [9,12], 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) [34].

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. [16], their theophylline resistant (T-helpers) and theophylline sensitive (T-cytotoxic) subpopulations (by test sensitivity rosette to theophylline by S Limatibul et al. [22]), the population of B-lymphocytes by test complementary rosette of sheep erythrocytes by C Bianco [8]. Natural killers identified as big containing granules lymphocytes. We set also induced by phytohemagglutinin blast transformation reaction T-Lymphocytes, as described in the handbook [21].

After a blood sample was removed spleen and thymus and weighed them. Since the spleen and thymus did smears for counting splenocytogram and thymocytogram [6,9,34]. For the latter, as well as to leukocytogram and immunocytogram we calculated entropy [34,43,48].

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

Abstracts of the results published in the materials of conferences [28,29,31].

RESULTS AND DISCUSSION

While routine methodical approach can only **turn** to analyze a **particular** sign of statistical sampling, the use of cluster analysis makes possible the **simultaneous** consideration of **all** the signs. Considering the totality of characteristics of objects undertaken in their

relationship and conditionality of some of these (derivatives) other (main determinants) allows to make a **natural** classification that reflects the nature of things, their essence. It is believed that knowledge of the essence of the object is to identify those of its quality properties that actually define the object, distinguish it from other [1].

Clustering by parameters of immunity implemented by iterative k-means method. In this method, the object belongs to the class, Euclidean distance of which is minimal. The main principle of the structural approach to the allocation of uniform groups is that the objects of one class close to each other, and different classes removed. In other words, a cluster (the image) is an accumulation of points in n-dimensional geometric space in which the distance between a point less than the average distance of the data points the rest.

Through this approach, formed three groups-clusters. For further consideration among 40 registered immunity parameters selected 18 parameters that members of each cluster differing minimum and maximum are different from members of other clusters (Table 1).

The maximum contribution to the division into clusters, according to the criterion η^2, which shows the share of inter-group variance in total variance, brings the ability of T lymphocytes to the transformation in blasts under the influence of mitogen. Much smaller, but significant ($p \leq 0,05$) η^2 values found on 7 parameters of lymphocytes, 5 of neutrophils and 4 options of monocytes/macrophages, as well as the total content of leukocytes in the blood.

Table 1. Analysis of Variance. The variables that make a significant contribution to the distribution of clusters

Variables	Between SS	Within SS	η^2	R	F	p
Blast transformation of T-Lymphocytes	3363	1271	**0,726**	**0,852**	**49,0**	**$<10^{-6}$**
Lymphocytes of Thymus	337	459	**0,424**	**0,651**	**13,6**	**$<10^{-4}$**
Macrophages of Thymus	45,9	74,4	**0,382**	**0,618**	**11,4**	**$=10^{-4}$**
Leukocytes of Blood	63,8	118	**0,351**	**0,593**	**10,0**	**$<10^{-3}$**
Lymphocytes of Blood	467	999	**0,319**	**0,565**	**8,65**	**$<10^{-3}$**
Macrophages of Spleen	47,9	103	**0,317**	**0,563**	**8,58**	**$<10^{-3}$**
T-helpers Lymphocytes of Blood	86,8	200	**0,303**	**0,551**	**8,05**	**$=10^{-3}$**
Lymphoblastes of Spleen	12,6	31,4	**0,287**	**0,536**	**7,45**	**,002**
Phagocytic Index Neutrophils of Blood	182	477	**0,276**	**0,526**	**7,06**	**,002**
Bactericidal Capacity Neutrophils of Blood	71,3	194	**0,269**	**0,519**	**6,81**	**,003**
Segmented Neutrophiles of Blood	211	640	**0,248**	**0,498**	**6,11**	**,005**
Microbial Capacity Neutrophils of Blood	180	558	**0,244**	**0,494**	**5,96**	**,006**
Lymphocytes of Spleen	110	347	**0,241**	**0,491**	**5,89**	**,006**
Fibroblastes of Spleen	22,7	101	**0,184**	**0,429**	**4,16**	**,023**
Microbial Count Monocytes of Blood	19,8	95,7	**0,171**	**0,414**	**3,82**	**,031**
NK-Lymphocytes of Blood	12,7	66,2	**0,161**	**0,401**	**3,54**	**,039**
0-Lymphocytes of Blood	159	887	**0,152**	**0,390**	**3,31**	**,048**
Microbial Count Neutrophiles of Blood	1,55	8,82	**0,149**	**0,387**	**3,26**	**,050**

Note. Parameters analysis of variance calculated by the following formulas:
$\eta^2=Sb^2/(Sb^2+Sw^2)$,
$R=\eta$,
$F=[Sb^2(n-k)]/[Sw^2(k-1)]$, where
Sb^2 is between SS;
Sw^2 is within SS;
n is number of animals (40);
k is number of groups (3).

The contributions of other parameters of immunity in animal distribution on clusters insignificant or negligible (Table 2). At noteworthy except that the content of eosinophils and neutrophils in the spleen, of epithelial cells in the thymus, of neutrophils in the blood and the degree of completeness of phagocytosis by them Staph. aureus.

Table 2. Analysis of Variance. The variables that provide insignificant or negligible contribution to the distribution of clusters

Variables	Between SS	Within SS	η^2	R	F	p
Neutrophiles of Spleen	32,9	188	0,149	0,386	3,23	,051
Epitheliocytes of Tymus	35,4	217	0,140	0,374	3,01	,06
Eosinophils of Spleen	5,62	36,2	0,135	0,367	2,88	,07
Stub Neutrophils of Blood	5,57	40,4	0,121	0,348	2,55	,09
Killing Index of Neutrophils of Blood	97	702	0,121	0,348	2,55	,09
Eosinophiles of Blood	16,2	139	0,104	0,323	2,16	*,13*
Reticulocytes of Thymus	13,2	115	0,103	0,321	2,13	*,13*
Phagocytic Index Monocytes of Blood	16,0	155	0,093	0,306	1,91	*,16*
Endotheliocytes of Thymus	8,66	88,4	0,089	0,299	1,81	*,18*
Monocytes of Blood	11,0	123	0,082	0,287	1,66	*,20*
Hassal corpuscles of Thymus	1,87	29,1	0,060	0,246	1,19	*,32*
Thymus Mass Index	,0001	,0026	0,051	0,227	1,01	*,37*
T-cytolytic Lymphocytes of Blood	18,1	375	0,046	0,215	,89	*,42*
Spleen Mass Index	0,003	0,055	0,043	0,208	,83	*,44*
Basophiles of Blood	0,21	4,89	0,041	0,203	,78	*,46*
Microbial Capacity Monocytes of Blood	0,006	0,147	0,039	0,197	,75	*,48*
Plasmocytes of Blood	1,52	50,8	0,029	0,170	,55	*,58*
Lymphoblastes of Thymus	0,37	24,6	0,015	0,122	,27	*,76*
Plasmocytes of Spleen	1,24	91,7	0,013	0,115	,25	*,78*
Reticulocytes of Spleen	0,49	135	0,004	0,060	,07	*,93*
B-Lymphocytes of Blood	0,28	225	0,001	0,035	,02	*,98*

Clusters clearly delineated, as evidenced by the Euclidean distance between them (Table 3), which far exceed the distance between members within each cluster.

Table 3. Euclidean Distances between Clusters
Distances below diagonal, Squared distances above diagonal

Cluster	No. 1	No. 2	No. 3
No. 1	0,00	10,17	6,83
No. 2	3,19	0,00	14,37
No. 3	2,61	3,79	0,00

In calculating the deviation from the norm of creating clusters immune parameters in the Z-units [33] proved the following (Table 4 and Fig. 1).

In 16 rats cluster III 16 parameters do not deviate from the norm (±0,5σ) and only 2 (content of macrophages in the spleen and lymphocytes in the thymus) moderately increased. For cluster II (15 rats) is characterized by a moderate reduction of 8 parameters (content of lymphoblasts in the spleen, lymphocytes in the thymus, segmented neutrophils and NK- and Th-lymphocytes in the blood, RBTL to PHA and intensity of phagocytosis by neutrophils) in combination with a noticeable increase of 5 parameters (content of macrophages in the thymus, intensity and activity of phagocytosis by macrophages as well as content in blood leukocytes, pan- and 0-lymphocytes), while within ±0,5σ remained only 5 parameters.

In 9 rats I cluster only 3 parameters are not deviated from the norm, while 5 significantly promoted (intensity of phagocytosis by macrophages, content of macrophages in the thymus

and spleen, of fibroblasts in the spleen, 0-lymphocytes in the blood), but 10 parameters significantly declined (blood levels of leukocytes, total lymphocytes and Th-lymphocytes, content of lymphocytes in the thymus and spleen, lymphoblasts in the spleen, activity and intensity of phagocytosis by neutrophils of blood, their microbial and bactericide capacity).

Table 4. Features abnormalities immune parameters in different clusters of rats subjected to chronic stress

Variables	Norm (n=10)	Cv	(V/N-1)/Cv as Z-scores for Clusters		
			III (n=16)	I (n=9)	II (n=15)
Microbial Count Neutrophils of Blood, Bac/Phag	8,1±0,1	0,026	**-0,16**	**-2,60**	**-1,20**
Lymphocytes of Thymus, %	55,6±1,0	0,057	**0,65**	**-1,77**	**-0,82**
Th-Lymphocytes of Blood, %	32,3±0,8	0,077	**0,13**	**-1,28**	**-0,90**
Lymphoblastes of Spleen, %	5,1±0,4	0,235	**0,02**	**-1,20**	**-0,58**
Phagocytic Index Neutrophils of Blood, %	82,3±0,7	0,028	**0,32**	**-1,10**	**-0,50**
NK-Lymphocytes of Blood, %	10,4±0,6	0,180	**-0,34**	**-0,25**	**-0,93**
Blasttransformation T-Lymphocytes of Blood, %	65,8±3,7	0,177	**0,46**	**-0,07**	**-1,31**
Microbial Capacity Neutroph Blood, 10^9 Bact/L	20,3±1,7	0,268	**0,32**	**-0,71**	**0,12**
Bactericidal Capacity Neutroph Blood, 10^9 Bac/L	11,2±1,1	0,325	**0,25**	**-0,72**	**0,12**
Lymphocytes of Spleen, %	53,1±0,9	0,055	**0,20**	**-1,21**	**0,08**
Segmented Neutrophiles of Blood, %	28,1±1,7	0,190	**0,11**	**0,34**	**-0,68**
Lymphocytes of Blood, %	60,4±1,4	0,074	**-0,52**	**-0,83**	**0,92**
Leukocytes of Blood, 10^9/L	9,57±0,54	0,179	**0,29**	**-0,80**	**1,16**
0-Lymphocytes of Blood, %	29,9±1,5	0,161	***0,33***	***1,34***	***1,02***
Fibroblastes of Spleen, %	5,9±0,4	0,203	**0,03**	**1,29**	**-0,36**
Macrophages of Spleen, %	5,50±0,65	0,376	**0,94**	**1,75**	**0,34**
Macrophages of Thymus, %	4,70±0,21	0,144	**0,44**	**4,89**	**2,42**
Microbial Count Monocytes of Blood, Bac/Phag	2,8±0,1	0,118	**0,19**	**5,59**	**3,26**
	Supressive effects		**-0,34 ±0,07 (4)**	**-1,07 ±0,18 (13)**	**-0,83 ±0,10 (10)**
	Enhancing effects		**+0,31 ±0,07 (14)**	**+2,77 ±1,04 (5)**	**+1,05 ±0,42 (8)**
	Modules of Deviation		|0,32| ±0,05 (18)	|1,54| ±0,35 (18)	|0,93| ±0,19 (18)

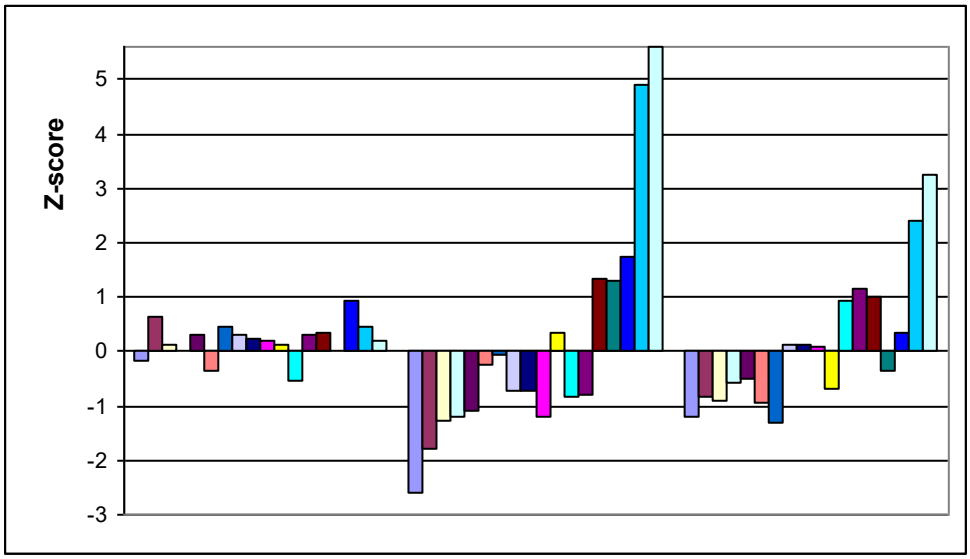

Fig. 1. Z-scores for immune parameters in III, I and II clusters of rats subjected to chronic stress

In calculating average values separately for the parameters that under stress increases or decreases, stated that in cluster III the deviation 16 parameters in either side of the average of

norm almost identical and are in an acceptable range of ±0,5σ (Fig. 2). Thus, the immune status of 40% of the rats subjected to moderate chronic stress are resistant to its factors.

For the immune status of rats cluster II is characterized moderate **inhibition** microphage, killers and T-cell links (an increase in blood *0-lymphocytes* indicates a loss of Th-lymphocytes $CD4^+$ receptors) in combination with a strong **activation** macrophage link

Poststressory changes in immunity in I cluster differ from those in cluster II both qualitatively and quantitatively. In particular, the rats in this cluster were found not deviations from the norm or the reaction of transformation of T-lymphocytes in the blasts nor the level of NK-lymphocytes. However, other parameters of T-link as well as of microphage link suppressed more and settings macrophage link appeared activated very significantly, and the area of activation, except thymus and blood, spread to the spleen.

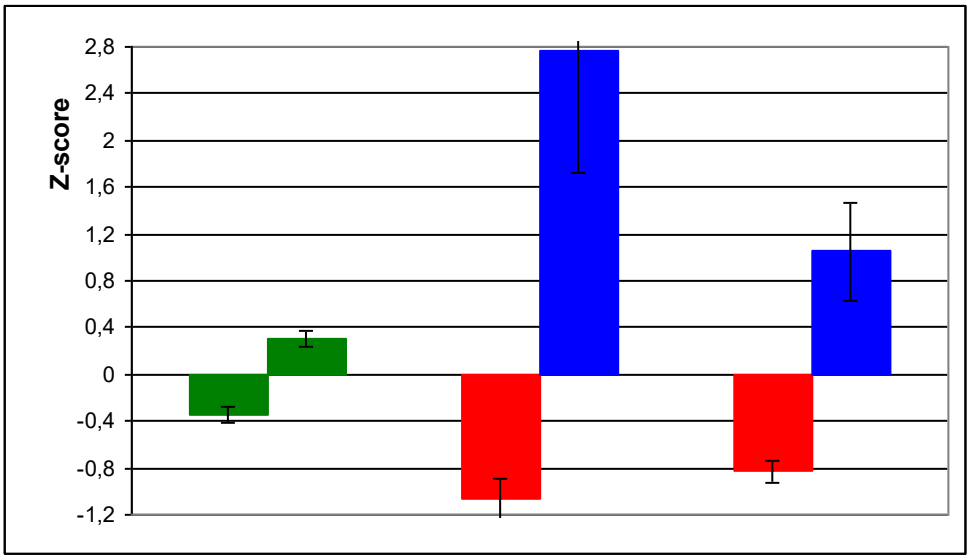

Fig. 2. Means of Z-scores for sets of immune parameters in III, I and II clusters of rats subjected to chronic stress

Module abnormal immune parameters as a measure of stress induced immune dysfunction is for cluster I 1,54±0,35σ, for Cluster II 0,93±0,19σ, while for cluster III only 0,32±0,05σ.

Another methodical approach to determine the characteristics of the immune clusters become discriminant analysis (method forward stepwise) [18]. For inclusion in the model program selected 12 parameters (Table 5).

Then the 12-dimensional space discriminant variables transformed into two-dimensional space of canonical discriminant functions (canonical roots), each of which is a linear combination of discriminant variables.

According to the theory of discriminant analysis, instead of checking statistical significance of the discriminant function considered the residual discriminant capacity of the system to determine the function. The residual discriminant ability is the ability of variables to distinguish between groups (clusters) if excluding information obtained using the previously calculated functions. Inverse measure of the differences between the groups in several discriminant variables is the Wilks' Λ-statistics. Very small quantities Wilks' Λ evidenced by the high resolution that is good separation centers of groups and much difference between them regarding the degree of spread within groups. The high level of significance for each group, calculated for χ^2 test, indicating that the results obtained from the general population with differences between groups and functions are statistically significant.

Table 5. Discriminant Function Analysis Summary
Step 12, N of vars in model: 12; Grouping: 3 grps
Wilks' Lambda: 0,0197; approx. $F_{(24,5)}$=13,3; $p<10^{-6}$

Variables currently in the model	Intact Male Rats (n=10)	Chronic Stressed Male Rats			Parameters of Wilks' Statistics				
		Cluster III (n=16)	Cluster I (n=9)	Cluster II (n=15)	Wilks Λ	Par-tial Λ	F-re-mo-ve	p-le-vel	To-lera-ncy
Blast transfor-mation T-Ly-mphocytes, %	65,8±3,7 1 0	71,1±1,4 1,08±0,02 +0,46±0,12	64,9±1,7 0,99±0,03 -0,07±0,15	50,5±1,6 0,77±0,02 -1,31±0,14	,088	,224	45,0	10^{-6}	,479
Segmented Neutrophiles of Blood, %	28,1±1,7 1 0	28,7±1,2 1,02±0,04 +0,11±0,23	29,9±1,3 1,06±0,04 +0,34±0,24	24,5±0,9 0,87±0,03 -0,68±0,16	,029	,664	6,57	10^{-3}	,432
Hassal corpuscles of Thymus, %	1,70±0,27 1 0	2,16±0,24 1,27±0,14 +0,53±0,28	1,88±0,23 1,10±0,14 +0,20±0,27	1,67±0,24 0,98±0,14 -0,04±0,28	,021	,916	1,19	,32	,542
Lymphoblastes of Thymus, %	5,50±0,17 1 0	5,25±0,23 0,95±0,04 -0,47±0,44	5,38±0,25 0,98±0,04 -0,24±0,47	5,47±0,19 0,99±0,03 -0,06±0,36	,023	,858	2,15	,14	,403
Macrophages of Thymus, %	4,70±0,21 1 0	5,0±0,3 1,06±0,07 +0,44±0,47	8,0±0,6 1,70±0,13 +4,89±0,87	6,3±0,3 1,35±0,07 +2,42±0,51	,025	,778	3,71	,04	,263
Entropy of Splenocyto-gram ($\bullet 10^3$)	588±7 1 0	590±7 1,00±0,01 +0,08±0,33	618±7 1,05±0,01 +1,32±0,32	591±5 1,00±0,01 +0,11±0,23	,023	,846	2,36	,11	,053
Fibroblastes of Spleen, %	5,90±0,38 1 0	5,94±0,45 1,01±0,08 +0,03±0,38	7,44±0,50 1,26±0,08 +1,29±0,42	5,47±0,40 0,93±0,07 -0,36±0,33	,0265	,744	4,48	,02	,532
Monocytes of Blood, %	4,20±0,73 1 0	5,06±0,49 1,21±0,12 0,38±0,21	6,11±0,42 1,46±0,10 +0,83±0,18	4,73±0,49 1,13±0,12 +0,23±0,21	,022	,902	1,41	,26	,716
Plasmocytes of Blood, %	0,68±0,28 1 0	0,77±0,27 1,13±0,41 +0,10±0,31	1,07±0,49 1,58±0,72 +0,45±0,55	0,55±0,27 0,81±0,40 -0,15±0,31	,022	,885	1,69	,20	,522
Lymphocytes of Thymus, %	55,6±1,0 1 0	57,7±1,1 1,04±0,02 +0,65±0,34	50,0±0,9 0,90±0,02 -1,77±0,30	53,0±0,7 0,95±0,01 -0,82±0,23	,020	,989	,15	,86	,287
Lymphocytes of Spleen, %	53,1±0,9 1 0	53,7±0,8 1,01±0,02 +0,20±0,29	49,6±0,8 0,93±0,02 -1,21±0,29	53,3±0,8 1,00±0,01 +0,08±0,26	,023	,857	2,16	,13	,063
Microb Capac. Neutro Blood, 10^9 Bact/L	20,3±1,7 1 0	22,0±1,1 1,22±0,06 +0,32±0,22	16,5±1,0 0,81±0,05 -0,71±0,18	20,9±1,6 1,03±0,08 +0,12±0,30	,028	,692	5,79	,01	,401

Note. In each column the first row: M±SE; second: M/N±SE; third: Z±SE

The first canonical root, by definition, has a maximum discriminatory (distinction) capacity: canonical correlation coefficient (R) as a measure of connectivity, degree of dependence between the clusters and the root is 0,948, and its share in dispersion, which explained by the distribution on clusters ($\eta^2=R^2$): 0,899 (Wilks' Λ=0,020; $\chi^2_{(24)}$=124; $p<10^{-6}$). The second root is characterized less significant values relevant parameters: R=0,898; η^2=0,806; Wilks' Λ=0,193; $\chi^2_{(11)}$=52; $p<10^{-6}$ (Table 6).

In Table 6 shows the complete structural coefficients, ie the coefficients of correlation between the roots and variables. The structural coefficient indicates how closely related variables and discriminant function, that is what portion of information about discriminant

function incorporated in this variable. Given the significantly coefficients roots can be interpreted as follows. The first describes the inverse way RBTL on PhHA, is crucial creating clusters immune parameter, and relative blood levels of neutrophils. The second root directly way reflects macrophage link of thymus, spleen and blood, while the inverse way microphage link of blood and content of lymphocytes in the thymus and spleen.

Table 6. Correlations Variables - Canonical Roots (Pooled-within-groups correlations). Chi-Square Tests with Successive Roots Removed. Z-scores for Variables at Poststressory Clusters

Variables currently in the model	Root 1	Root 2	**Z-scores for Variables**		
			Clu III	Clu I	Clu II
Blasttransformation T-Lymphocytes	**-,548**	-,013	+0,46	-0,08	**-1,31**
Segmented Neutrophiles of Blood	**-,175**	,120	+0,11	+0,33	**-0,68**
Hassal corpuscles of Thymus	**-,082**	-,033	+0,53	+0,20	**-0,04**
Lymphoblastes of Thymus	**,040**	,016	-0,47	-0,24	**-0,06**
Macrophages of Thymus	,101	**,355**	+0,44	**+4,89**	+2,42
Entropy of Splenocytogram	-,026	**,234**	+0,08	**+1,32**	+0,11
Fibroblastes of Spleen	-,072	**,207**	+0,03	**+1,29**	-0,36
Monocytes of Blood	-,046	**,131**	+0,37	**+0,83**	+0,23
Plasmocytes of Blood	-,038	**,065**	+0,10	**+0,45**	-0,15
Lymphocytes of Thymus	-,162	**-,347**	+0,65	**-1,77**	-0,82
Lymphocytes of Spleen	,018	**-,275**	+0,20	**-1,21**	+0,08
Microbial Capacity Neutroph of Blood	-,060	**-,264**	+0,32	**-0,71**	+0,12
Tests with Successive Roots Removed			**Means of Variables**		
Canonical R	,948	,898	Root 1		
Wilks' Lambda	,020	,193	-2,80	-0,99	3,58
Chi-Sqr.	124	52	Root 2		
Degree freedom	24	11	-1,45	3,59	-0,61
p-level	10^{-6}	10^{-6}			

In assessing the real usefulness of roots by the relative percentage (share of eigevalue in their amount) it is detected (Table 7), the first root contains 67,8% discriminant abilities, and the second contains 32,2%.

In the same Table 7 shows the standardized (normalized) and raw (current) coefficients of canonical variables.

Table 7. Summary of Stepwise Analysis

	Standardized Coefficients		Raw Coefficients		Parameters of Wilk's Statistics				
Variables currently in the model	Root 1	Root 2	Root 1	Root 2	F to enter	p-level	Λ	F-value	p-level
Blasttransformation T-Lymphocytes	-1,342	,046	-,229	,008	49,0	10^{-6}	,274	49,0	10^{-6}
Segmented Neutrophiles of Blood	-,831	,442	-,200	,106	9,52	10^{-3}	,114	22,9	10^{-6}
Hassal corpuscles of Thymus	-,387	-,159	-,436	-,180	2,62	,089	,033	17,0	10^{-6}
Lymphoblastes of Thymus	,266	,599	,326	,735	1,76	,191	,022	14,0	10^{-6}
Macrophages of Thymus	,424	,920	,299	,648	1,91	,167	,025	14,9	10^{-6}
Entropy of Splenocytogram	-1,084	1,506	-43,79	60,87	2,12	,138	,029	15,8	10^{-6}
Fibroblastes of Spleen	-,689	,262	-,417	,158	9,42	10^{-3}	,073	22,9	10^{-6}
Monocytes of Blood	-,219	,341	-,120	,187	3,78	,034	,038	18,1	10^{-6}
Plasmocytes of Blood	-,186	,484	-,159	,413	1,69	,204	,020	13,3	10^{-6}
Lymphocytes of Thymus	,211	,015	,060	,004	10,0	10^{-3}	,176	24,9	10^{-6}
Lymphocytes of Spleen	-1,529	,470	-,499	,153	3,83	,032	,048	19,0	10^{-6}
Microbial Capacity Neutroph Blood	-,022	-,976	-,006	-,251	3,90	,030	,059	20,5	10^{-6}
Constants			69,47	-52,84					
Discriminant Properties, %			67,8	32,2					

Standardized coefficient reflecting the relative contribution of variable independent of the measuring unit, instead coefficient in row form provides information on the absolute contribution of the variable to the value of function. These coefficients make it possible to identify those variables that make the largest contribution to the value of the discrimination function.

The amount of products of row coefficients on the value of discriminant variables together with the constant give discriminant function value for each animal, and the value of discriminant functions determined point in space discriminant functions (Fig. 3).

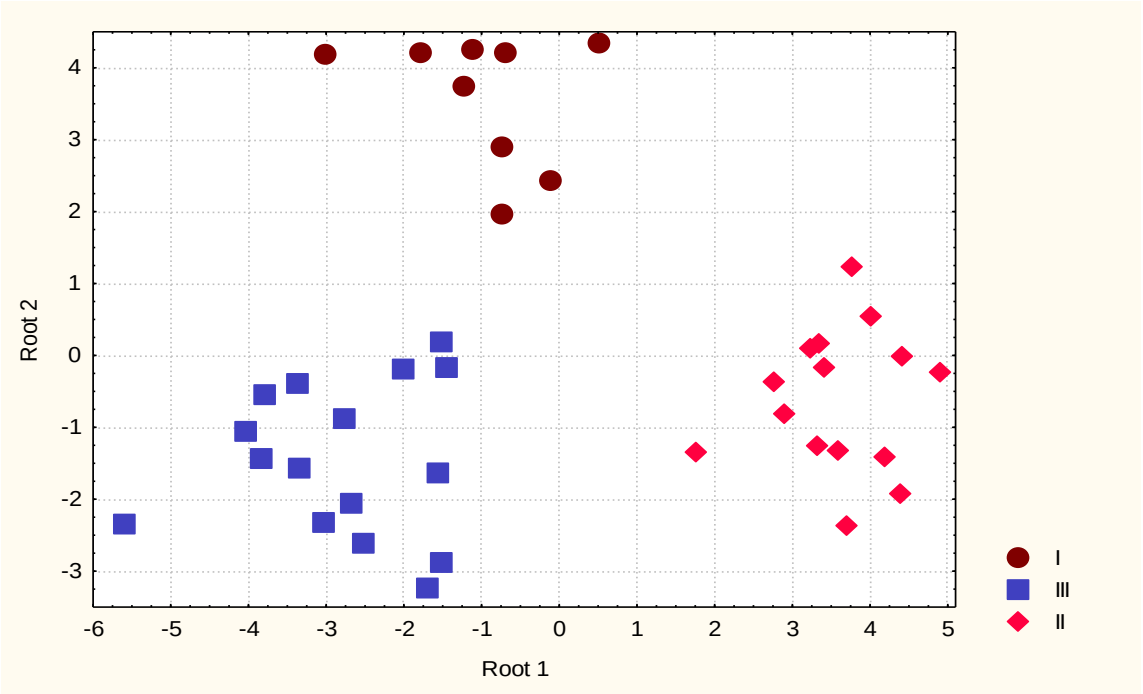

Fig. 3. Індивідуальні величини дискримінантних імунних коренів щурів-самців різних кластерів реакції імунітету на стрес

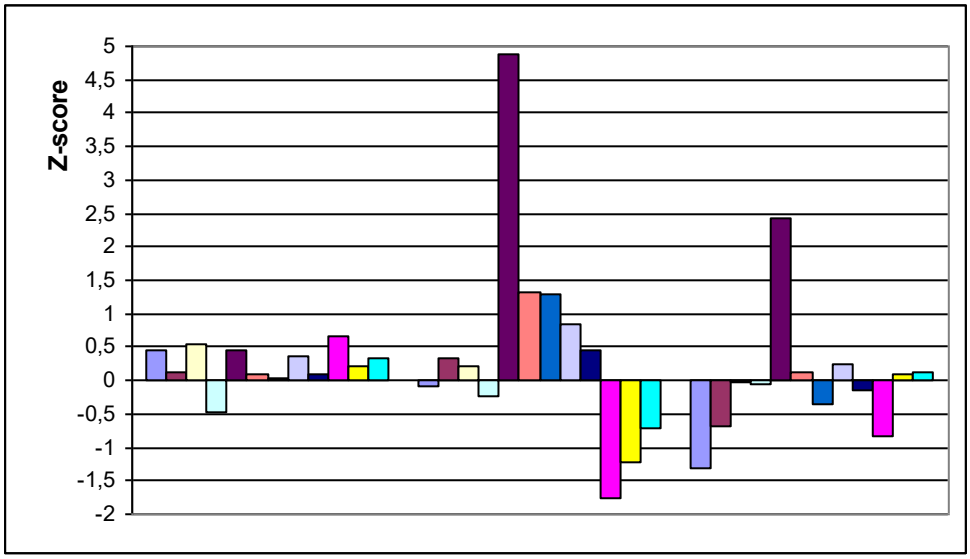

Fig. 4. Z-scores for discriminant immune parameters in III, I and II clusters of rats subjected to chronic stress

Figure 3 shows that the poststressory immune status of rats from three clusters significantly differ. The visual impression is confirmed by calculations Squared Mahalanobis Distances, who are between I and III clusters 30,9 ($F=9,4$; $p<10^{-6}$); between I and II clusters 41,6 ($F=12,4$; $p<10^{-6}$); between II and III clusters 44,7 ($F=18,9$; $p<10^{-6}$).

Comparison figures 4 and 1 shows the difference in the clarity of distribution for homogeneous groups by selecting parameters by analysis of variance and discriminant analysis.

Consequently, differences between poststressory immune status in various clusters exhaustively explained by the 12 parameters that reflects different links of immunity. The information contained in these parameters can be condensed into two distinctive roots. In other words, the selected parameters can be used for identification of type immune response to chronic stress. This goal is realized by using classification (discriminant) functions as special linear combinations for each group that maximize the differences between groups and minimize the variance within groups.

Coefficients for classification functions (Table 8) are not standardized, so not interpreted. The object belongs to a group with a maximum value of function calculated by summing the products of variable on coefficients for classification functions and constants.

Table 8. Coefficients and Constants for Classification Functions

Variables currently in the model	I	III	II
Blasttransformation T-Lymphocytes	15,40	15,78	14,33
Segmented Neutrophiles of Blood	9,71	9,53	8,35
Hassal corpuscles of Thymus	115,1	116,8	113,9
Lymphoblastes of Thymus	183,5	179,2	181,9
Macrophages of Thymus	98,81	95,01	97,45
Entropy of Splenocytogram	32692	32465	32237
Fibroblastes of Spleen	38,79	38,75	36,22
Monocytes of Blood	3,93	3,20	2,60
Plasmocytes of Blood	73,30	71,51	70,84
Lymphocytes of Thymus	27,25	27,12	27,50
Lymphocytes of Spleen	235,6	235,7	232,6
Microbial Capacity Neutroph of Blood	-25,37	-24,09	-24,34
Constants	**-18246**	**-18103**	**-17706**

In this case, we have achieved error-free classification rats on the immune status after stress.

As summarized in the review FS Dhabhar [11], 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, immuno-regulatory/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.

We consider it appropriate to complete the series of provisions that the direction and severity of the effects of stress or stress hormones on immune function were also caused by individual reactivity, which has a wide spectrum in each population. In particular, resistance to acute hypoxic hypoxia, estimated by time of occurrence of the second agonal breaths [7,27], in the same population averaged 142 sec with Cv=0,54 [3], in another population 132 sec with Cv 0,515 [37] so that the rats are divided into low, medium and highly resistant to hypoxia. Among children in a breath test Stange was found dispersion in the range of 44÷171% of the average rate [5]. The same broad variability take place regarding the tone of the autonomic nervous system, so that animals and people divided into sympathotonic, normotonic and vagotonic types [2,19,20,27,39,47].

In the cited experimental and clinical studies have found significant differences between the immune response to acute or chronic stress people from different functional groups. On the other hand, aware of the close relationship between the nervous, endocrine and immune systems [10,11,13,14,15,17,19,20,30,32-36,39,41,42,44-47].

The foregoing gives us reason to believe that the observed diversity of immune responses to chronic stress caused by the least variety of neuro-endocrine reactions. Verification of this hypothesis will be devoted our next article.

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