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FEATURES OF THE CONDITION OF THE NEUROENDOCRINE-IMMUNE COMPLEX IN DIFFERENT CONSTELLATIONS OF ENTROPIES OF MORPHO-FUNCTIONAL IMMUNE SUBSYSTEMS IN RATS

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Abstract

Background. We have previously shown that the Entropy (E) of the Thymocytogram (T), Splenocytogram (S) as well as Leukocytogram (L) and Immunocytogram (I) of the blood in rats are virtually independent of each other. The **purpose** of this study was to clarify the characteristics of the condition of the neuroendocrine-immune complex in different constellations of E(s) of these four morpho-functional immune subsystems. **Materials and methods.** Experiment was performed on 108 healthy Wistar rats (48 male and 60 female) weighing 240-290 g divided into 8 groups. Animals of the first group remained intact. Instead, the other rats received various balneofactors for 6 days. The day after the completion of the drinking/application course the parameters of neuroendocrine-immune complex were registered. **Results.** The method of cluster analysis created four homogeneous groups of rats, significantly different from each other in the constellation of E of four morpho-functional immune subsystems. The members of the first cluster (n=31) are characterized by elevated levels of E (Z±SE) of I (1,12±0,16), T (1,00±0,14) and L (0,88±0,12) at the normal E level of S (0,14±0,16). The members of the second cluster (n=21) are characterized by reduced E level of L (-1,59±0,16) in combination with increased levels of I (0,80±0,17) and T (0,81±0,15) at the normal E level of S (0,13±0,16). A characteristic feature of the members of the third cluster (n=28) is reduced E of T (-1,01±0,17) at normal E levels of S (-0,33±0,23), I (-0,19± 0,14) and L (0,35±0,20). The last cluster (n=28) is characterized by reduced E of I (-1,36± 0,14), while the E of other immune subsystems are at the borderline levels: L (-0,52±0,16), S (0,49±0,15), T (0,60±0,15). The method of discriminant analysis revealed 15 parameters of the neuroendocrine-immune complex, the set of which four clusters of Entropies differ from each other with an accuracy of 95%. **Conclusion.** Each constellation of independent Entropies of the thymocytogram, splenocytogram, immunocytogram and leukocytogram is accompanied

by a specific constellation of parameters of the neuroendocrine-immune complex which indicates their natural functional relationships.

Key words: Entropy of thymocytogram; splenocytogram; immunocytogram and leukocytogram; autonomic nervous; endocrine and immune parameters; female and male rats.

INTRODUCTION

We have previously shown that the Entropy of the Thymocytogram (TCG), Splenocytogram (SCG) as well as Leukocytogram (LCG) and Immunocytogram (ICG) of the blood in rats are virtually independent of each other [5], by confirming an already known position [19]. It is known about the functional relationships between the nervous, endocrine and immune systems within the triune neuroendocrine-immune complex [13,20,21]. The **purpose** of this study was to clarify the characteristics of the condition of the neuroendocrine-immune complex in different constellations of E(s) of these four morpho-functional immune subsystems.

MATERIAL AND METHODS

Experiment was performed on 108 healthy Wistar rats (48 male and 60 female) weighing 240-290 g divided into 8 groups. Animals of the first group (n=20) remained intact, using tap water from drinking ad libitum. Instead, the other rats received the same tap water (n=18) as well as mineral waters Naftussya (n=20), Sophiya (n=10), Hertsa (n=10) and its artificial salt analogue (n=10) through the tube at a dose of 1,5 mL/100 g of body mass for 6 days. Another group of rats received together with Naftussya water three applications on the tail of ozokerite (t^0 40-42^{0}C, duration 30 minutes, every other day) (n=10), and the last - only ozoketite applications (n=10). The choice of such balneofactors is based on their pronounced influence on the functional systems of the body [3,17,18,20].

The day after the completion of the drinking/application course in all rats, at first, a sample of peripheral blood (by incision of the tip of the tail) was taken for analysis of Leukocytogram (LCG), ie the relative content of lymphocytes (L), monocytes (M), eosinophils (Eo), basophils (Bas), rod-shaped (RN) and segmental (SN) neutrophils. Based on these data, the Entropy of the Leukocytogram (hLCG) was calculated according to the formula derived by IL Popovych [7,16,19,21] on the basis of the classical CE Shannon [24] formula:

$$hLCG = - [L \bullet \log_2 L + M \bullet \log_2 M + Eo \bullet \log_2 Eo + Bas \bullet \log_2 Bas + RN \bullet \log_2 RN + SN \bullet \log_2 SN] / \log_2 6.$$

Then they assessed the state of autonomous regulation. For this purpose, under an easy ether anesthesia, for 15-20 sec ECG was recorded in the lead II, inserting needle electrodes under the skin of the legs, followed by the calculation of the parameters of the HRV: mode (Mo), amplitude of the mode (AMo) and variational swing (MxDMn) as markers of the humoral channel of regulation (circulating catecholamines, steroides, glucagon etc), sympathetic and vagal tones respectively [2].

Animals were then placed in individual chambers with perforated bottom for collecting daily urine. The experiment was completed by decapitation of rats in order to collect as much blood as possible.

The plasma levels of the hormones of adaptation were determined: corticosterone, triiodothyronine and testosterone (by the ELISA [10]) as well as electrolytes: calcium (by

reaction with arsenase III), phosphate (phosphate-molybdate method), sodium and potassium (flamming photometry), electrolytes were also determined in daily urine. The latter also determined the concentration of 17-ketosteroids (by color reaction with m-dinitrobenzene). The analyzes were carried out according to the instructions described in the manual [8].

The analyzers “Tecan” (Oesterreich), “Pointe-180” ("Scientific", USA) and “Reflotron” (Boehringer Mannheim, BRD) were used with appropriate sets and a flamming spectrophotometer “СФ-47”.

According to the parameters of electrolyte exchange, hormonal activity was evaluated: parathyroide by coefficients $(Cap/Pp)^{0,5}$, $(Pu/Cau)^{0,5}$ and $(Cap•Pu/Pp•Cau)^{0,25}$, calcitonin by coefficients $(1/Cap•Pp)^{0,5}$, $(Cau•Pu)^{0,5}$ and $(Cau•Pu/Cap•Pp)^{0,25}$ as well as mineralocorticoid by coefficients $(Nap/Kp)^{0,5}$, $(Ku/Nau)^{0,5}$ and $(Nap•Ku/Kp•Nau)^{0,25}$, based on their classical effects and recommendations by IL Popovych [20,21].

In the blood, the parameters of immunity were determined, as described in the manual [14]: the relative content of the population of T-lymphocytes, their theophylline-resistant (T-helper) and theophyllin-susceptible (T-cytolytic) subpopulations; the population of B-lymphocytes, plasma cells (Pla) and Natural Killers. The content of zero-lymphocytes (0L) was calculated by the balance method. For these components the Entropy of the Immunocytogram (hICG) was calculated:

$$hICG = - [Th•\log_2 Th+Tc•\log_2 Tc+B•\log_2 B+Pla•\log_2 Pla+NK•\log_2 NK+0L•\log_2 0L]/\log_2 6.$$

The blast transformation reaction of T-lymphocytes to phytohemagglutinin was performed separately [14].

About the condition of the phagocytic function of neutrophils (microphages) and monocytes (macrophages) were judged by the phagocytosis index, the microbial count and the killing index for Staphylococcus aureus (ATCC N25423 F49). According to these parameters and the content of microphages and macrophages in the blood calculated their bactericidal ability [6].

After decapitation, the spleen, thymus and adrenal glands were removed from the animals. In the adrenal glands after weighing, the thickness of glomerular, fascicular reticular and medullar zones was measured under a microscope [6].

Immune organs weighed and made smears-imprints for counting Thymocytogram and Splenocytogram [4,5]. The components of the thymocytogram (TCG) are lymphocytes (Lc), lymphoblasts (Lb), reticulocytes (Ret), macrophages (Mac), endotheliocytes (En), epitheliocytes (Ep) and Hassal’s corpuscles (H). The Splenocytogram (SCG) includes lymphocytes (Lc), lymphoblasts (Lb), plasma cells (P), reticulocytes (R), macrophages (Ma), fibroblasts (F), microphages (Mi) and eosinophils (E).

For them Shannon’s entropy was calculated too:

$$hTCG = -[Lc•\log_2 Lc+Lb•\log_2 Lb+Ret•\log_2 Ret+Mac•\log_2 Mac+En•\log_2 En+Ep•\log_2 Ep+H•\log_2 H]/\log_2 7$$

$$hSCG = - [Lc•\log_2 Lc+Lb•\log_2 Lb+P•\log_2 P+R•\log_2 R+Ma•\log_2 Ma+F•\log_2 F+Mi•\log_2 Mi+E•\log_2 E]/\log_2 8$$

Digital material is statistically processed on a computer using the software package "Statistica 8.0".

RESULTS AND DISCUSION

The method of cluster analysis [1] (k-means clustering) created four **homogeneous** groups of rats, which is documented by the Euclidean distances of the members of each individual cluster from its centroid (Table 1). On the other hand, the clusters significantly **different** from each other in the constellation of Entropies of four morpho-functional immune subsystems (Fig. 1), which is documented by the Euclidean distances between them (Table 2).

Table 1. Members of Clusters and Distances from Respective Cluster Center

Cluster Number 3 contains 28 cases

Case	1	13	14	17	21	22	25	26	27	30	41	52	61	63	64	65	66
D	,55	,28	1,82	,95	1,61	,58	1,14	,79	,73	,59	,72	,84	1,06	,71	1,23	,86	,69

69	78	80	81	82	86	87	88	90	91	99
,79	,94	,71	,77	,57	1,16	1,77	,73	,41	,93	1,04

Cluster Number 4 contains 28 cases

Case	2	4	6	11	12	15	19	20	23	28	29	39	40	45	46	48	51
D	,67	,95	1,51	,89	069	,65	,40	1,02	,63	,37	,81	,91	1,03	067	,42	,77	,77

57	59	62	71	77	79	83	84	85	93	97
,70	,41	1,28	,29	,47	,70	,85	,81	,94	,25	,41

Cluster Number 2 contains 21 cases

Case	9	16	31	32	34	36	42	44	50	53	54	55	58	70	74	75	76
D	,58	,92	,34	,47	,68	,57	,65	,77	,83	,79	,95	,37	,58	,43	,36	,98	,88

95	107	109	110
,71	,97	,94	,52

Cluster Number 1 contains 31 cases

Case	3	5	7	8	10	18	24	33	35	37	38	43	47	49	56	60	67
D	,49	1,01	,76	,60	,99	,48	1,04	1,13	,59	,86	,58	1,10	,58	,58	,89	1,36	,40

68	72	73	89	92	94	96	98	100	101	103	105	106	108
,63	,83	,87	1,10	,70	,73	,88	,66	,68	,77	,62	,81	,75	,74

Table 2. Euclidean Distances between Clusters

Clusters	No. 1	No. 2	No. 3	No. 4
No. 1	0,00	1,52	1,56	2,09
No. 2	1,23	0,00	2,07	1,50
No. 3	1,25	1,44	0,00	1,33
No. 4	1,45	1,22	1,15	0,00

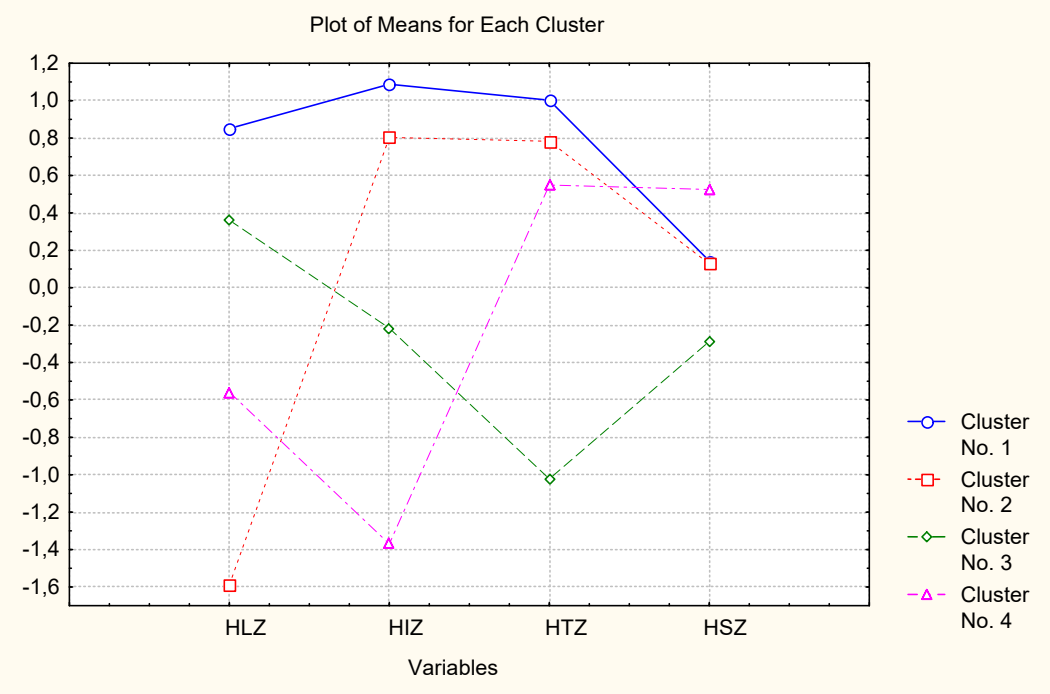

Fig. 1. The Normalized Means (Z) of Entropies (Y-Line) for each Cluster

Analysis of variance showed (Table 3) that the largest contribution to the distribution of the sample into clusters, judging by the eta-square criterion, is made by the Entropy of the Immunocytogram, and the smallest - the Entropy of the Splenocytogram.

Table 3. Analysis of Variance

Entropy of	Between SS	Within SS	η^2	R	F	signif. p
Immunocytogram	105,9	65,4	0,618	0,786	57,2	10^{-6}
Thymocytogram	72,7	65,6	0,526	0,725	39,1	10^{-6}
Leukocytogram	86,8	78,5	0,525	0,725	39,1	10^{-6}
Splenocytogram	9,6	95,3	0,092	0,303	3,6	,017

Based on the direction and degree of deviations of the Entropy of morpho-functional immune subsystems from the norm (±0,5 Z), the first cluster is denoted by the code **I+T+L+Sn**, the second **L-SnI+T+**, the third **T-SnInLn**, the fourth **I-LnSnTn**.

In order to visualize the members of each cluster of Entropies, discriminant analysis [12] (forward stepwise) was used. The distinguishing information contained in the four variables is condensed into three canonical discriminant roots (Tables 4 and 5). The first root contains 43,4% of discriminatory opportunities, representing the **Thymo**- and **Splenocytograms**, the second – 40,5%, representing the **Immunocytogram**, the third – 16,1%, representing the **Leukocytogram**.

Table 4. Discriminant Function Analysis Summary and Summary of Stepwise Analysis
Step 4, N of vars in model: 4; Grouping: 4 grps
Wilks' Lambda: 0,082; approx. $F_{(12)}$=34,9; $p<10^{-6}$

Entropy of	Wilks' Λ	Partial Λ	F-remove	p-level	Tolerancy	F to enter	p-level	Lambda	F-value	p-level
Immunocytogram	0,194	0,425	45,5	10^{-6}	0,937	54,8	10^{-6}	0,387	54,8	10^{-6}
Leukocytogram	0,180	0,459	39,6	10^{-6}	0,926	36,6	10^{-6}	0,187	45,0	10^{-6}
Thymocytogram	0,162	0,509	32,5	10^{-6}	0,950	35,1	10^{-6}	0,092	45,9	10^{-6}
Splenocytogram	0,092	0,895	4,0	0,010	0,909	4,0	0,010	0,082	34,9	10^{-6}

Table 5. Factor Structure Matrix and Means of Roots and Entropies

Variables (Entropy)	Correlations Variables-Canonical Roots			T-SnInLn III (28)	I+T+L+Sn I (31)	I-LnSnTn IV (28)	L-SnI+T+ II (21)
Root 1(43,4%)	**R1**	**R2**	**R3**	**-1,68**	-0,64	1,23	1,54
Thymocytogram	**0,509**	-0,493	0,631	**-1,01±0,17**	1,00±0,14	0,60±0,15	0,81±0,15
Splenocytogram	**0,196**	0,012	0,235	**-0,33±0,23**	0,14±0,16	0,49±0,15	0,13±0,16
Root 2(40,5%)	**R1**	**R2**	**R3**	0,94	**-1,44**	**1,45**	-1,06
Immunocytogram	-0,231	**-0,935**	-0,262	-0,19±0,14	**1,12±0,16**	**-1,36±0,14**	0,80±0,17
Root 3(16,1%)	**R1**	**R2**	**R3**	-0,64	0,77	0,64	**-1,14**
Leukocytogram	-0,649	-0,097	**0,727**	0,35±0,20	0,88±0,12	-0,52±0,16	**-1,59±0,16**

Table 6. Standardized and Raw Coefficients and Constants for Entropy Variables

Coefficients	Standardized			Raw		
Entropy of	**Root 1**	**Root 2**	**Root 3**	**Root 1**	**Root 2**	**Root 3**
Immunocytogram	-0,260	-0,903	-0,397	-0,329	-1,143	-0,503
Leukocytogram	-0,803	0,013	0,659	-0,933	0,015	0,766
Thymocytogram	0,673	-0,328	0,638	0,857	-0,418	0,813
Splenocytogram	0,389	-0,169	0,060	0,412	-0,178	0,063
			Constants	-0,406	0,234	-0,161
			Eigenvalues	1,765	1,649	0,655
			Cumul. Proport.	0,434	0,839	1,000

Next, using the raw coefficients for the variables and constants given in table 6, individual values of Entropies were transformed into individual values of discriminant roots, which made it possible to visualize each animal in the information field of these roots (Figs. 2 and 3).

Despite the visual impression of not very clear mutual delimitation of clusters, the calculation of the squares of the Mahalanobis distances between the clusters documents the statistical significance of the mutual delimitation (Table 7).

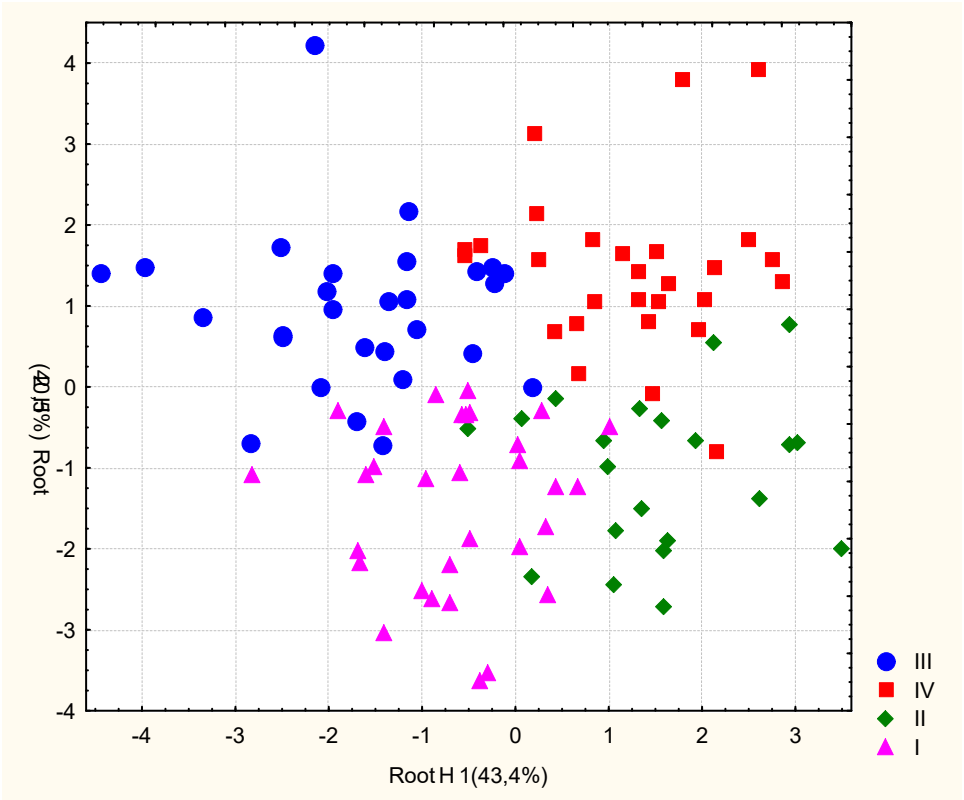

Fig. 2. Individual values of the first and second roots of the Entropies for each cluster

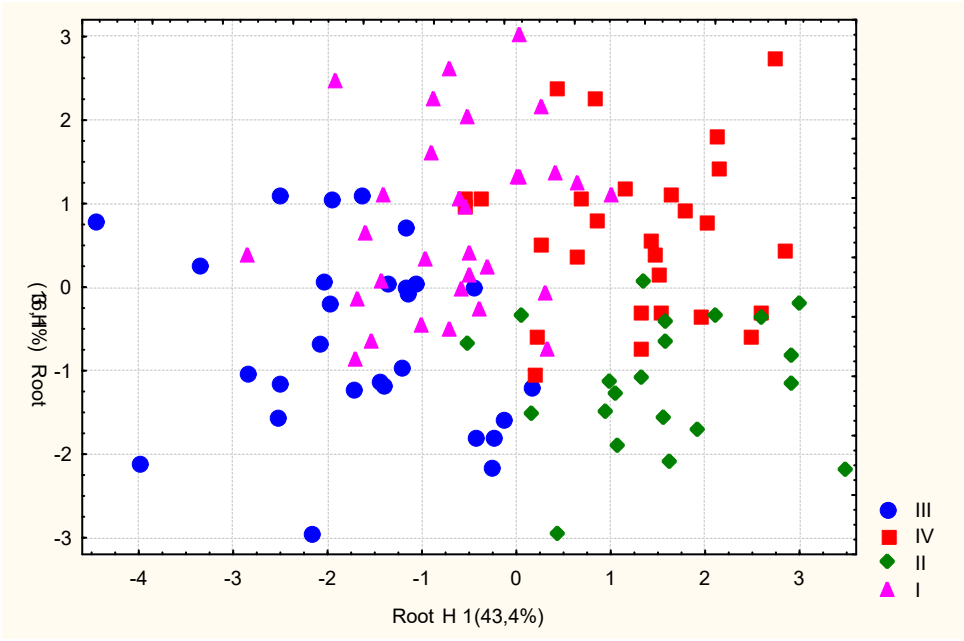

Fig. 3. Individual values of the first and third roots of the Entropies for each cluster

Table 7. Squared Mahalanobis Distances between Cluters (over diagonal) and F-values (under diagonal); for all p-level<10^{-6}

Cluters (n)	III (28)	IV (28)	II (21)	I (31)
T-SnInLn	**0,0**	10,7	15,2	9,1
I-LnSnTn	35,2	**0,0**	9,9	12,3
L-SnI+T+	42,3	27,6	**0,0**	8,8
I+T+L+Sn	31,3	42,5	23,8	**0,0**

The accuracy of retrospective recognition of the animal's belonging to a particular cluster by calculating the classification functions by the coefficients and constants given in Table 8, is 96,3% (Table 9).

Table 8. Coefficients and Constants for Classification Functions

Entropy of	III (,259)	IV (,259)	II (,195)	I (,287)
Immunocytogram	-0,156	-2,326	1,330	1,526
Leukocytogram	0,832	-0,899	-2,580	0,904
Thymocytogram	-1,772	1,549	1,413	1,254
Splenocytogram	-0,681	0,508	0,968	0,260
Constants	-2,522	-3,747	-4,837	-3,110

Table 9. Classification Matrix
Rows: Observed classifications, Columns: Predicted classifications

Cluters (n)	Percent	III (,259)	IV (,259)	II (,195)	I (,287)
T-SnInLn	96,4	**27**	0	**1**	0
I-LnSnTn	100	0	**28**	0	0
L-SnI+T+	85,7	0	**1**	**18**	**2**
I+T+L+Sn	100	0	0	0	**31**
Total	96,3	27	29	19	33

Discriminant analysis is also used to identify those parameters of the neuroendocrine-immune complex, the set of which Entropies clusters differ from each other. The program included in the model 15 parameters, in particular, by definition, the Entropies of immune subsystems, as well as one parameter of the **thymus**, 3 parameters of the **spleen**, 4 parameters of **blood** and 3 **neuroendocrine** parameters. Also noteworthy are a number of other parameters that emerged outside the model, apparently as carriers of redundant or duplicate discriminant information (Tables 10 and 11).

Table 10. Discriminant Function Analysis Summary for Variables of Entropy and Neuroendocrine-Immune Complex

Step 15, N of vars in model: 15; Grouping: 4 grps. Wilks' Λ: 0,043; approx. $F_{(45)}$=11,2; $p<10^{-6}$

Variables currently in the model	L-SnI+T+ (21)	I-LnSnTn (28)	I+T+L+Sn (31)	T-SnInLn (28)	Parameters of Wilk's Statistics				
					Wilks' Λ	Partial Λ	F-remove	p-level	Tolerancy
Root 1 (43,5%)	-1,64	-0,77	-0,47	**2,52**					
Entropy Thymocytogr	0,81±0,15	0,60±0,15	1,00±0,14	**-1,01±0,17**	0,080	0,543	25,3	10^{-6}	0,454
Entropy Splenocytogr	0,13±0,16	0,49±0,15	0,14±0,16	**-0,33±0,23**	0,047	0,921	2,59	0,058	0,766
Reticulocytes Spleen	0,27±0,18	0,46±0,19	0,27±0,16	**-0,68±0,18**	0,045	0,959	1,30	0,280	0,778
AMo as Sympathotone	0,35±0,29	0,53±0,20	0,47±0,22	**0,12±0,22**	0,045	0,956	1,39	0,251	0,645
Lymphocytes Thymus	-0,59±0,24	-0,68±0,22	-0,66±0,19	**0,24±0,25**	0,046	0,934	2,11	0,105	0,522
(Cap•Pu/Cau•Pp)^{0,25}	-1,05±0,28	-0,38±0,22	-0,91±0,21	**-0,19±0,14**	0,046	0,944	1,77	0,159	0,834
Spleen Mass	-0,28±0,10	-0,07±0,14	-0,19±0,09	**0,15±0,19**	0,045	0,958	1,30	0,279	0,755
Blasttransform T-Lym	-0,29±0,23	-0,18±0,19	-0,41±0,20	**-0,01±0,18**	0,045	0,962	1,19	0,318	0,513
Root 2 (35,8%)	-0,02	**-1,89**	**1,89**	-0,20					
Entropy Immunocytogr	0,80±0,17	**-1,36±0,14**	**1,12±0,16**	-0,19±0,14	0,082	0,527	26,9	10^{-6}	0,656
Plasmocytes Blood	0,47±0,20	**-0,31±0,19**	**1,60±0,25**	-0,15±0,16	0,047	0,921	2,57	0,059	0,578
Basophiles Blood	-0,29±0,16	**-0,32±0,13**	**0,94±0,24**	-0,32±0,14	0,045	0,958	1,31	0,276	0,523
Microphages Spleen	-0,33±0,28	-0,22±0,24	**-0,07±0,32**	-0,34±0,23	0,045	0,969	0,98	0,408	0,830
Root 3 (20,7%)	**-1,85**	0,95	0,80	-0,45					
Entropy Leukocytogra	**-1,59±0,16**	-0,52±0,16	0,88±0,12	0,35±0,20	0,080	0,538	25,8	10^{-6}	0,792
Testosterone	**1,28±0,46**	0,21±0,29	0,34±0,27	0,29±0,21	0,045	0,956	1,38	0,254	0,756
Microb Count Neutroph	**-0,18±0,20**	-1,25±0,45	-0,54±0,25	-0,39±0,26	0,047	0,917	2,71	0,050	0,732
Variables currently not in the model	**L-SnI+T+ (21)**	**I-LnSnTn (28)**	**I+T+L+Sn (31)**	**T-SnInLn (28)**	**Wilks' Λ**	**Partial Λ**	**F to enter**	**p-level**	**Tolerancy**
Endotheliocytes Thymus	0,23±0,18	-0,39±0,22	0,08±0,21	**-0,94±0,19**	0,043	0,984	0,47	0,701	0,607
Hassal corpuscles Thym	0,57±0,17	0,54±0,15	0,77±0,15	**-0,48±0,15**	0,043	0,990	0,29	0,831	0,565
Epitheliocytes Thymus	0,27±0,32	0,43±0,20	0,33±0,17	**-0,09±0,16**	0,042	0,981	0,56	0,642	0,551
Killing Index Neutroph	0,59±0,28	0,15±0,21	0,18±0,21	**0,17±0,16**	0,042	0,979	0,63	0,600	0,825
Macrophages Thymus	0,74±0,19	1,46±0,42	1,13±0,41	**0,33±0,32**	0,043	0,994	0,17	0,914	0,509
Sex Index	0,43±0,20	-0,06±0,19	0,36±0,17	**-0,21±0,19**	0,042	0,981	0,58	0,629	0,613
Reticulocytes Thymus	-0,07±0,11	0,01±0,14	-0,18±0,10	**0,56±0,21**	0,043	0,985	0,44	0,727	0,165
Monocytes Blood	-0,65±0,17	-0,14±0,16	0,16±0,13	**0,36±0,18**	0,042	0,980	0,61	0,609	0,759
Mode as Humoral chan	-0,83±0,31	-0,81±0,18	-0,33±0,24	**-0,21±0,19**	0,042	0,976	0,74	0,531	0,235
(Cau•Pu/Cap•Pp)^{0,25}	-2,39±0,60	-0,85±0,37	-1,62±0,47	**-0,84±0,39**	0,042	0,980	0,61	0,613	0,595
(Nap•Ku/Kp•Nau)^{0,25}	0,16±0,29	-0,11±0,17	**0,42±0,24**	0,15±0,18	0,042	0,974	0,79	0,502	0,872
Rodnucleary Neutrop B	**-1,14±0,31**	-0,48±0,26	0,52±0,22	-0,17±0,24	0,043	0,996	0,11	0,953	0,689
T-helper Lymphocytes	**-0,83±0,23**	0,14±0,31	-0,74±0,21	-0,25±0,23	0,042	0,977	0,70	0,554	0,673
Eosinophils Blood	**-0,69±0,11**	-0,36±0,15	0,29±0,20	0,16±0,19	0,043	0,994	0,19	0,902	0,674
Segmentonucl Neutr B	**-0,49±0,15**	-0,08±0,14	0,38±0,15	0,03±0,20	0,042	0,974	0,78	0,507	0,722
Lymphocytes Blood	**1,13±0,18**	0,34±0,14	-0,70±0,15	-0,22±0,23	0,043	0,983	0,50	0,685	0,379
T-cytolytic Lymphocyt	**0,59±0,19**	-0,59±0,22	0,34±0,16	0,04±0,21	0,043	0,983	0,50	0,681	0,614
Phagocytosis Ind Neutr	**0,06±0,26**	-0,52±0,36	-0,01±0,24	-0,17±0,22	0,042	0,972	0,87	0,460	0,559

Table 11. Summary of Stepwise Analysis for Variables of Entropy and Neuroendocrine-Immune Complex

Variables currently in the model	F to enter	p-level	Lambda	F-value	p-level
Entropy Immunocytogram	54,83	10^{-6}	0,387	54,8	10^{-6}
Entropy Leukocytogram	36,64	10^{-6}	0,187	45,0	10^{-6}
Entropy Thymocytogram	35,12	10^{-6}	0,092	45,9	10^{-6}
Plasmocytes Blood	4,00	0,010	0,082	35,0	10^{-6}
Entropy Splenocytogram	4,23	0,007	0,073	29,1	10^{-6}
Reticulocytes Spleen	2,76	0,046	0,067	24,8	10^{-6}
AMo as Sympathotone	2,72	0,049	0,062	21,9	10^{-6}
Lymphocytes Thymus	1,80	0,151	0,059	19,4	10^{-6}
Microbial Count Neutrophils	1,84	0,144	0,056	17,6	10^{-6}
$(Cap•Pu/Cau•Pp)^{0,25}$ as PTA	1,62	0,191	0,053	16,0	10^{-6}
Microphages Spleen	1,50	0,219	0,051	14,7	10^{-6}
Testosterone	1,32	0,272	0,049	13,6	10^{-6}
Spleen Mass	1,27	0,289	0,047	12,7	10^{-6}
Basophiles Blood	1,10	0,353	0,045	11,9	10^{-6}
Blasttransformation T-Lymph	1,19	0,318	0,043	11,2	10^{-6}

The separating information contained in 15 variables is condensed into three canonical discriminant roots (Table 12). The first root contains 43,5% of discriminant possibilities(r^*=0,844; Wilks' Λ=0,043; $\chi^2_{(45)}$=306; $p<10^{-6}$), the second 35,8% (r^*=0,819; Wilks' Λ=0,151; $\chi^2_{(28)}$=184; $p<10^{-6}$), the third 20,7% (r^*=0,736; Wilks' Λ=0,459; $\chi^2_{(13)}$=76; $p<10^{-6}$).

Table 12. Standardized, Structural and Raw Coefficients and Constants for Variables of Entropy and Neuroendocrine-Immune Complex

Coefficients	Standardized			Structural			Raw		
Variables	Root 1	Root 2	Root 3	Root 1	Root 2	Root 3	Root 1	Root 2	Root 3
Entropy Thymocytogram	-1,118	-0,001	0,464	**-0,621**	0,197	0,236	-1,424	-0,002	0,590
Reticulocytes Spleen	-0,255	0,045	0,102	**-0,297**	-0,025	0,149	-0,273	0,048	0,109
Entropy Splenocytogram	-0,373	0,043	-0,080	**-0,167**	-0,079	0,137	-0,394	0,046	-0,084
AMo as Sympathotone	0,253	-0,180	-0,053	**-0,073**	-0,005	0,073	0,213	-0,152	-0,044
Lymphocytes Thymus	-0,401	-0,016	0,142	**0,203**	-0,018	-0,103	-0,346	-0,014	0,123
$(Cap•Pu/Cau•Pp)^{0,25}$ as PTA	0,162	-0,243	0,125	**0,155**	-0,138	0,080	0,147	-0,221	0,114
Spleen Mass	0,212	0,176	0,064	**0,135**	-0,054	0,027	0,300	0,248	0,090
Blasttransformation T-Lym	-0,237	-0,119	0,214	**0,072**	-0,067	-0,031	-0,233	-0,117	0,210
Entropy Immunocytogram	-0,015	0,795	-0,739	-0,136	**0,815**	-0,390	-0,018	1,007	-0,936
Plasmocytes Blood	-0,129	0,263	0,379	-0,154	**0,475**	0,108	-0,120	0,245	0,352
Basophiles Blood	-0,019	-0,017	0,384	-0,079	**0,365**	0,257	-0,021	-0,018	0,412
Microphages Spleen	-0,043	0,035	0,257	-0,018	**0,030**	0,055	-0,030	0,025	0,180
Entropy Leukocytogram	0,588	0,464	0,598	0,353	0,415	**0,614**	0,683	0,539	0,694
Testosterone	0,126	-0,164	-0,231	-0,080	0,017	**-0,198**	0,082	-0,106	-0,150
Microbial Count Neutroph	0,302	0,132	-0,259	0,032	0,105	**-0,173**	0,186	0,081	-0,159
						Constants	0,571	-0,102	-0,016
						Eigenvalues	2,482	2,042	1,180
						Cum.Propopt.	0,435	0,793	1,000

According to the already involved algorithm, using raw coefficients and constants (Table 12), members of all clusters were visualized in the information field of discriminant roots.

The localization of **T-SnInLn** cluster members in the positive zone of the first root axis (Fig. 4) reflects, first of all, their reduced Entropy level of Thymocytogram and reduced content of reticulocytes in the Splenocytogram, while in animals of other clusters these parameters are more or less increased. This is combined with normal levels of T-lymphocytes in the thymus and their ability to blast transformation, as well as the mass of the spleen, while in other clusters, these immune parameters are more or less reduced. Such an immune constellation is accompanied by normal levels of sympathetic tone and parathyroid activity, whereas in members of other clusters they are increased and decreased, respectively (Table 10).

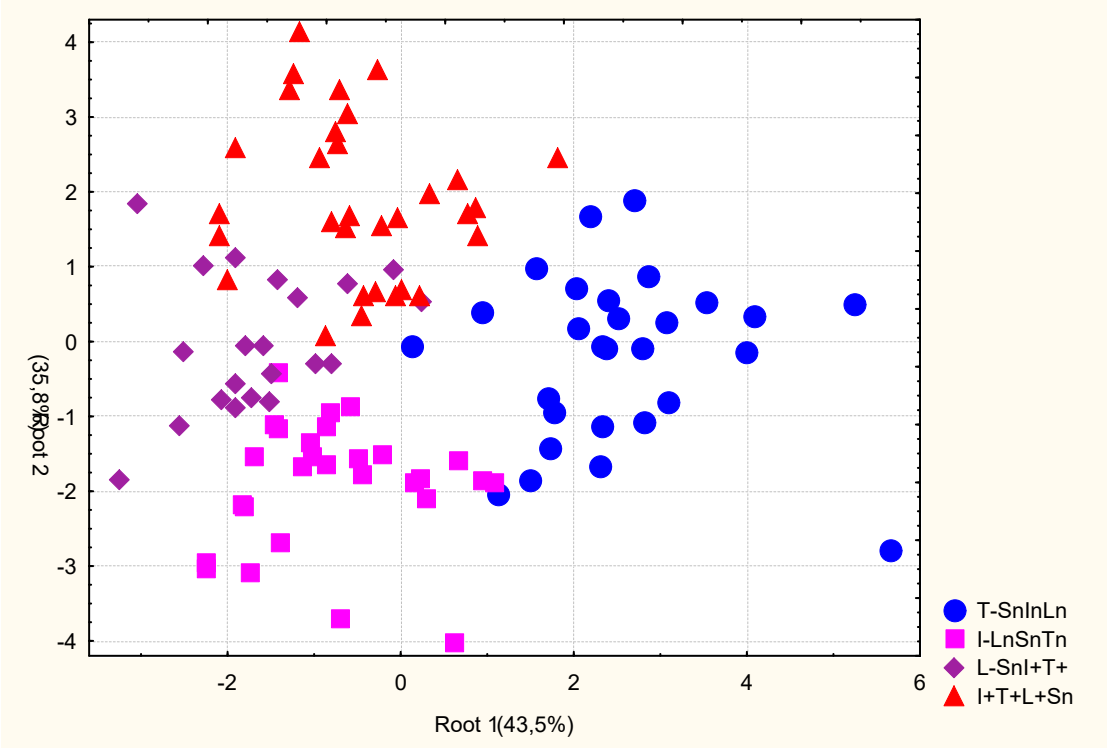

Fig. 4. Individual values of the first and second roots of the parameters of Entropy and Neuroendocrine-Immune Complex in rats of different clusters

The members of the **I+T+L+Sn** cluster are separated from the others along the axis of the second root, occupying a top position that reflects their maximum for the sample level of Entropy Immunocytogram. This is accompanied by maximum sampling levels of plasma cells and basophils in the blood, as well as the normal content of microphages in the spleen, while in other clusters it is slightly reduced.

The opposite position is occupied by members of the **I-LnSnTn** cluster with minimal or reduced levels of these immune parameters (Fig. 4 and Table 10).

The last cluster **L-SnI+T+** is separated from the others along the axis of the third root (Fig. 5), occupying the lower zone, which reflects their minimum level of Leukocytogram Entropy for sampling. This is accompanied by a normal level of phagocytosis intensity of blood microphages, while in other clusters it is reduced, as well as increased plasma testosterone level against the background of its normal levels in members of other clusters.

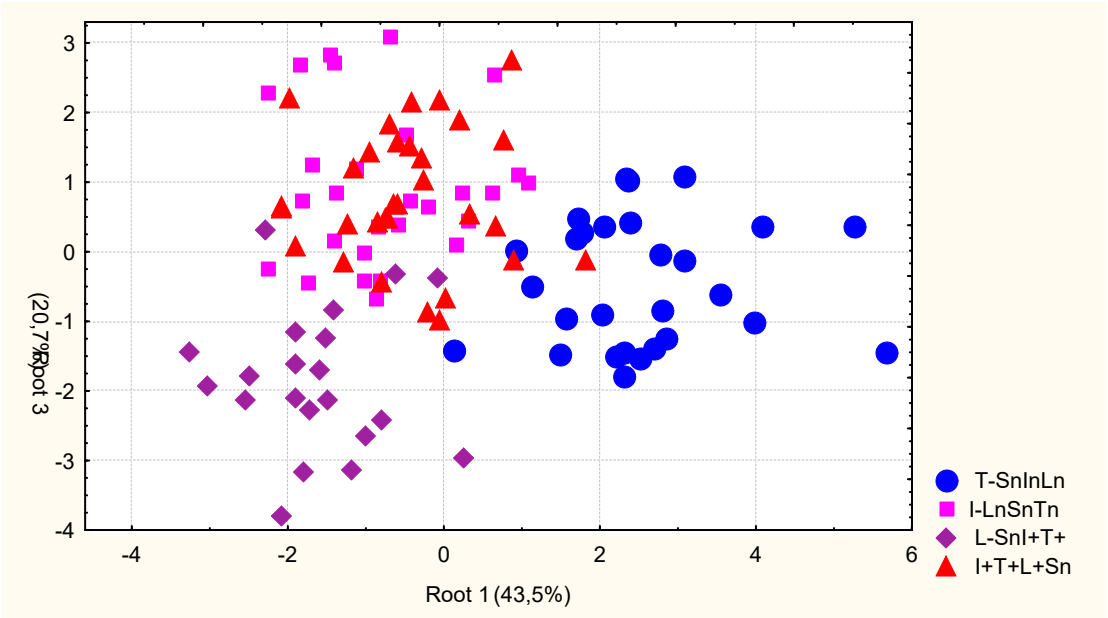

Fig. 5. Individual values of the first and third roots of the parameters of Entropy and Neuroendocrine-Immune Complex in rats of different clusters

Despite some mutual penetrations, in the information field of the three discriminant roots, all four clusters are quite clearly delineated, which is documented by the distances of Mahalanobis between them (Table 13).

Table 13. Squared Mahalanobis Distances between Cluters (over diagonal) and F-values (under diagonal); for all p-level<10^{-6}

Cluters (n)	III (28)	IV (28)	II (21)	I (31)
T-SnInLn	**0,0**	16,3	20,1	15,5
I-LnSnTn	12,7	**0,0**	12,6	15,0
L-SnI+T+	13,3	8,3	**0,0**	12,5
I+T+L+Sn	12,7	12,3	8,7	**0,0**

Selected discriminant parameters can be used to identify the affiliation of a rat to a particular cluster. This goal of discriminant analysis is realized with the help of classifying (discriminant) functions (Table 14).

Table 14. Coefficients and Constants for Classification Functions

Variables	III (28)	IV (28)	II (21)	I (31)
Entropy Immunocytogram	0,092	-2,857	1,667	1,083
Entropy Leukocytogram	1,070	-1,125	-2,649	1,021
Entropy Thymocytogram	-3,391	2,128	1,703	1,604
Plasmocytes Blood	-0,008	0,465	0,042	1,304
Entropy Splenocytogram	-0,664	0,441	1,104	0,507
Reticulocytes Spleen	-0,665	0,303	0,325	0,387
AMo as Sympathotone	0,674	0,166	-0,178	-0,336
Lymphocytes Thymus	-0,908	0,428	0,356	0,251
Microbial Count Neutrophils	0,284	-0,688	-0,250	-0,302
(Cap•Pu/Cau•Pp)^{0,25}	-0,279	-0,231	-1,092	-1,039
Microphages Spleen	-0,183	0,124	-0,306	0,183
Testosterone	0,288	-0,010	0,139	-0,366
Spleen Mass	0,492	-0,793	-0,839	0,225
Basophiles Blood	-0,653	0,021	-1,149	-0,114
Blasttransformation T-Lymph	-0,917	0,342	-0,265	-0,202
Constants	-3,708	-4,673	-6,126	-4,475

These functions are special linear combinations that maximize differences between groups and minimize variance within groups. The coefficients of classification functions are not standardized, so they are not interpreted. The object belongs to the group with the maximum value of the function, calculated by summing the products of the values of variables by the coefficients of classifying functions plus a constant.

The accuracy of retrospective classification for different clusters ranges from 85,7% to 100%, and in general is 95,4% (Table 15).

Table 15. Classification Matrix
Rows: Observed classifications
Columns: Predicted classifications

Cluters (n)	Percent	III (28)	IV (28)	II (21)	I (31)
T-SnInLn	96,4	**27**	0	**1**	0
I-LnSnTn	100	0	**28**	0	0
L-SnI+T+	85,7	0	0	**18**	**3**
I+T+L+Sn	96,8	0	0	**1**	**30**
Total	95,4	27	28	20	33

Therefore, each constellation of independent Entropies of the Thymocytogram, Splenocytogram, Immunocytogram and Leukocytogram is accompanied by a specific constellation of immune and neuroendocrine parameters. We consider this evidence that entropy has a real life force (***vis vitalis***), which is quantified by the coefficient of canonical correlation of entropy levels of morpho-functional immune subsystems with the parameters of immunity of other subsystems [5]. That is, Entropy is the subject (factor) of influence. On the other hand, Entropy is object to the regulatory influence of the autonomic nervous and endocrine systems, in particular, it is subject to the modulating effects of the sympathetic

nerves, parathyroid hormone and testosterone. This is consistent with the literature [9,11,13,15,20-23,25-29].

CONFORMITY TO ETHICAL STANDARDS

Experiments on animals have been carried out in accordance with the provisions of the Helsinki Declaration of 1975, revised and supplemented in 2002 by the Directives of the National Committees for Ethics in Scientific Research.

The carrying out of experiments was approved by the Ethics Committee of the Horbachevskyi Ternopil' State Medical University. The modern rules for the maintenance and use of laboratory animals complying with the principles of the European Convention for the Protection of Vertebrate Animals used for scientific experiments and needs are observed (Strasbourg, 1985).

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