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), 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 (tΘ 40-42°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:

$$h_{LCG} = - [L \log_2 L + M \log_2 M + Eo \log_2 Eo + Bas \log_2 Bas + RN \log_2 RN + SN \log_2 SN] / \log_2 6.$$

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 (MxDm) as markers of the humoral channel of regulation (circulating catecholamines, steroids, 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 flaming spectrophotometer “CФ-47”.

According to the parameters of electrolyte exchange, hormonal activity was evaluated: parathyroid by coefficients \((\text{Cap}/\text{Pp})^{0.5}, (\text{Pu}/\text{Cau})^{0.5}\) and \((\text{Cap} \cdot \text{Pu}/\text{Pp} \cdot \text{Cau})^{0.25}\), calcitomin by coefficients \((1/\text{Cap} \cdot \text{Pp})^{0.5}, (\text{Cau} \cdot \text{Pu})^{0.5}\) and \((\text{Cau} \cdot \text{Pu}/\text{Cap} \cdot \text{Pp})^{0.25}\) as well as mineralocorticoid by coefficients \((\text{Nap}/\text{Kp})^{0.5}, (\text{Ku}/\text{Nau})^{0.5}\) and \((\text{Nap} \cdot \text{Ku} / \text{Kp} \cdot \text{Nau})^{0.25}\), 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 (Thelper) 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:

\[
\text{hiCG} = - [\text{Th} \cdot \log_2 \text{Th} + \text{Tc} \cdot \log_2 \text{Tc} + \text{B} \cdot \log_2 \text{B} + \text{Pla} \cdot \log_2 \text{Pla} + \text{NK} \cdot \log_2 \text{NK} + 0 \text{L} \cdot \log_2 0 \text{L}]/\log_2 6.
\]

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:

\[
\text{hTCG} = - [\text{Lc} \cdot \log_2 \text{Lc} + \text{Lb} \cdot \log_2 \text{Lb} + \text{Ret} \cdot \log_2 \text{Ret} + \text{Mac} \cdot \log_2 \text{Mac} + \text{En} \cdot \log_2 \text{En} + \text{Ep} \cdot \log_2 \text{Ep} + \text{H} \cdot \log_2 \text{H}]/\log_2 7
\]

\[
\text{hSCG} = - [\text{Lc} \cdot \log_2 \text{Lc} + \text{Lb} \cdot \log_2 \text{Lb} + \text{P} \cdot \log_2 \text{P} + \text{R} \cdot \log_2 \text{R} + \text{Ma} \cdot \log_2 \text{Ma} + \text{F} \cdot \log_2 \text{F} + \text{Mi} \cdot \log_2 \text{Mi} + \text{E} \cdot \log_2 \text{E}]/\log_2 8.
\]

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

**RESULTS AND DISCUSSION**

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

| Cluster Number | Cases |
|----------------|-------|
| 3              | 28    |

| Case | Cluster Number 3 |
|------|------------------|
| 1    | 13, 14, 17, 21, 22, 25, 26, 27, 30, 41, 52 | 61, 63, 64, 65, 66 |

| Distances | 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

| Cluster Number | Cases |
|----------------|-------|
| 4              | 28    |

| Case | Cluster Number 4 |
|------|------------------|
| 2    | 4, 6, 11, 12, 15, 19, 20, 23, 28, 29, 39, 40, 45, 46, 48, 51 |

| Distances | 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

| Cluster Number | Cases |
|----------------|-------|
| 2              | 21    |

| Case | Cluster Number 2 |
|------|------------------|
| 9    | 16, 31, 32, 34, 36, 42, 44, 50, 53, 54, 55, 58, 70, 74, 75, 76 |

| Distances | 95, 107, 109, 110 |
|-----------|-------------------|
|          | .71, .97, .94, .52 |

Cluster Number 1 contains 31 cases

| Cluster Number | Cases |
|----------------|-------|
| 1              | 31    |

| Case | Cluster Number 1 |
|------|------------------|
| 3    | 5, 7, 8, 10, 18, 24, 33, 35, 37, 38, 43, 47, 49, 56, 60, 67 |

| Distances | 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 | η² | 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-Sn1Ln, 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' Lambda | Partial Lambda | F-reject | F-enter | Lambda | F-value | p-level |
|------------|---------------|----------------|----------|---------|---------|---------|---------|
| Immunocytogram | 0.194 | 0.425 | 45.5 | 10^-6 | 0.937 | 54.8 | 10^-6 |
| Leukocytogram | 0.180 | 0.459 | 39.6 | 10^-6 | 0.926 | 36.6 | 10^-6 |
| Thymocytogram | 0.162 | 0.509 | 32.5 | 10^-6 | 0.950 | 35.1 | 10^-6 |
| Splenocytogram | 0.092 | 0.895 | 4.0 | 0.010 | 0.909 | 4.0 | 0.010 |

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

| Variables (Entropy) | Correlations | Variables-Canonical Roots | T-SnInLnIII (28) | I+T+L+SnI (31) | I-LnSnTnIV (28) | L-SnI+TII (21) |
|---------------------|--------------|---------------------------|-----------------|---------------|----------------|---------------|
| Root 1(43.4%)       | R1           | R2                        | R3              | -1.68         | -0.64          | 1.23          |
| Thymocytogram       | 0.509        | -0.493                    | 0.631           | -1.01±0.17    | 1.00±0.14      | 0.60±0.15     |
| Splenocytogram      | 0.196        | 0.012                     | 0.235           | -0.33±0.23    | 0.14±0.16      | 0.49±0.15     |
| Root 2(40.5%)       | R1           | R2                        | R3              | 0.94          | -1.44          | 1.45          |
| Immunocytogram      | -0.231       | -0.935                    | -0.262          | -0.19±0.14    | 1.12±0.16      | -1.36±0.14    |
| Root 3(16.1%)       | R1           | R2                        | R3              | -0.64         | 0.77           | 0.64          |
| Leukocytogram       | -0.649       | -0.097                    | 0.727           | 0.35±0.20     | 0.88±0.12      | -0.52±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 Clusters (over diagonal) and F-values (under diagonal); for all p-level<10^{-6}

| Clusters (n)       | III (28) | IV (28) | II (21) | I (31) |
|--------------------|----------|---------|---------|--------|
| T-SnLnInLn        | 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    | IV    | II    | I     |
|--------------------|--------|-------|-------|-------|
| Entropies         | (.259) | (.259)| (.195)| (.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

| Clusters (n)   | Percent (,259) | III (,259) | IV (,195) | II (,287) |
|----------------|----------------|------------|-----------|-----------|
| T-SnLnInLn     | 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-Sn+T+ (21) | I-LnSnTn (28) | I+T+L+Sn (31) | T-SnlnLn (28) | Parameters of Wilk's Statistics |
|---------------------------------|-------------|----------------|----------------|----------------|-----------------------------|
| Root 1 (43.5%)                  | -1.64       | -0.77          | -0.47          | 2.52           |
| Entropy Thymocytes              | 0.81±0.15   | 0.60±0.15      | 1.00±0.14      | -1.01±0.17     |
| Entropy Splenocytes             | 0.13±0.16   | 0.49±0.15      | 0.14±0.16      | -0.33±0.23     |
| Reticulocytes Spleen            | 0.27±0.18   | 0.46±0.19      | 0.27±0.16      | -0.68±0.18     |
| AMo as Sympathotone             | 0.35±0.29   | 0.53±0.20      | 0.47±0.22      | 0.12±0.22      |
| Lymphocytes Thymus              | -0.59±0.24  | -0.68±0.22     | -0.66±0.19     | 0.24±0.25      |
| (Cap•Pu/Cau•Pp)^0.25            | -1.05±0.28  | -0.38±0.22     | -0.91±0.21     | -0.19±0.14     |
| Spleen Mass                     | -0.28±0.10  | -0.07±0.14     | -0.19±0.09     | 0.15±0.19      |
| Blastransform T-Lym             | -0.29±0.23  | -0.18±0.19     | -0.41±0.20     | -0.01±0.18     |
| Root 2 (35.8%)                  | -1.89       | -1.89          | -1.89          | -2.0           |
| Entropy Immunocytogor           | 0.80±0.17   | -1.36±0.14     | 1.12±0.16      | -0.19±0.14     |
| Plasmocytes Blood               | 0.47±0.20   | -0.31±0.19     | 1.60±0.25      | -0.15±0.16     |
| Basophiles Blood                | -0.29±0.16  | -0.32±0.13     | 0.94±0.24      | -0.32±0.14     |
| Microphages Spleen              | -0.33±0.28  | -0.22±0.24     | -0.07±0.32     | -0.34±0.23     |
| Root 3 (20.7%)                  | -1.85       | 0.95           | 0.80           | -0.45          |
| Entropy Leukocytogor            | -1.59±0.16  | -0.52±0.16     | 0.88±0.12      | 0.35±0.20      |
| Testosterone                    | 1.28±0.46   | 0.21±0.29      | 0.34±0.27      | 0.29±0.21      |
| Microb Count Neutroph           | -0.18±0.20  | -1.25±0.45     | -0.54±0.25     | -0.39±0.26     |
| Variables currently not in the model | L-Sn+T+ (21) | I-LnSnTn (28) | I+T+L+Sn (31) | T-SnlnLn (28) | Wilks' Λ | Partial Λ | F-remove | p-level | Tolerance |
| Endotheliocytes Thymus          | 0.23±0.18   | -0.39±0.22     | 0.08±0.21      | -0.94±0.19     |
| Hassal corpuscles Thym           | 0.57±0.17   | 0.54±0.15      | 0.77±0.15      | -0.48±0.15     |
| Epitheliocytes Thymus           | 0.27±0.32   | 0.43±0.20      | 0.33±0.17      | -0.09±0.16     |
| Killing Index Neutroph          | 0.59±0.28   | 0.15±0.21      | 0.18±0.21      | 0.17±0.16      |
| Macrophages Thymus              | 0.74±0.19   | 1.46±0.42      | 1.13±0.41      | 0.33±0.32      |
| Sex Index                       | 0.43±0.20   | -0.06±0.19     | 0.36±0.17      | -0.21±0.19     |
| Reticulocytes Thymus            | -0.07±0.11  | 0.01±0.14      | -0.18±0.10     | 0.56±0.21      |
| Monocytes Blood                 | -0.65±0.17  | -0.14±0.16     | 0.16±0.13      | 0.36±0.18      |
| Mode as Humoral chan            | -0.83±0.31  | -0.81±0.18     | -0.33±0.24     | -0.21±0.19     |
| (Cau•Pu/Cap•Pp)^0.25            | -2.39±0.60  | -0.85±0.37     | -1.62±0.47     | -0.84±0.39     |
| (Nap•Ku/Kp•Nau)^0.25            | 0.16±0.29   | -0.11±0.17     | 0.42±0.24      | 0.15±0.18      |
| Rodnucleary Neutroph B          | -1.14±0.31  | -0.48±0.26     | 0.52±0.22      | -0.17±0.24     |
| T-helper Lymphocytes            | -0.83±0.23  | 0.14±0.31      | -0.74±0.21     | -0.25±0.23     |
| Eosinophils Neutr B             | -0.69±0.11  | -0.36±0.15     | 0.29±0.20      | 0.16±0.19      |
| Segmentonuc Neutr B             | -0.49±0.15  | -0.08±0.14     | 0.38±0.15      | 0.03±0.20      |
| Lymphocytes Blood               | 1.13±0.18   | 0.34±0.14      | -0.70±0.15     | -0.22±0.23     |
| T-cytolytic Lymphocyt           | 0.59±0.19   | -0.59±0.22     | 0.34±0.16      | 0.04±0.21      |
| Phagocytosis Ind Neutr          | 0.06±0.26   | -0.52±0.36     | -0.01±0.24     | -0.17±0.22     |

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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) 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} |
| Blastransformation 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 ($r^*=0.844; \chi^2_{(45)}=306; p<10^{-6}$), the second 35.8% ($r^*=0.819; \chi^2_{(28)}=184; p<10^{-6}$), the third 20.7% ($r^*=0.736; \chi^2_{(13)}=76; p<10^{-6}$).

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

| Variables                           | Coefficients | Standardized | Structural | Raw |
|-------------------------------------|--------------|--------------|------------|-----|
|                                    | 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) 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 |
| Blastransformation T-Lymph         | -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. Prop opt: 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 Thymocytes in and reduced content of reticulocytes in the Splenocytes, 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](image)

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 Leukocytes 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.
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 Clusters (over diagonal) and F-values (under diagonal); for all p-level<10^{-6}

| Clusters (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-Sn+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  |
| Plasmaocytes 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

| Clusters (n) | Percent | III (28) | IV (28) | II (21) | I (31) |
|--------------|---------|----------|---------|---------|--------|
| T-SnLnLn     | 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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