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Original Article

The role of innate muscular endurance and resistance to hypoxia in reactions to acute stress of immunity in rats

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Abstract:
Background. It is known about aerobic fitness variability between individuals explained by genetics factors. On the other hand, it is also known about a wide variety of individual reactions to stress. From the above it follows the hypothesis that inter-individual differences in normal conditions determine the characteristics of the body's response to acute stress. We have been shown that innate muscular endurance and resistance to hypoxia significantly determines the post-stress neuro-endocrine and metabolic parameters as well as injuries of myocardium and gastric mucosa in rats. The purpose of this study is to test the hypothesis conformably to Immunity. **Material and methods.** The experiment is at 58 rats (28 males) Wistar line. Animals were first tested for resistance to hypoxic hypoxia. One week later, aerobic muscular performance was determined by swimming test. On the basis of the received data two qualitatively equivalent groups in a ratio 10/48 were formed. After a week of recovery over the next 10 days, one animal remained intact and 5 other rats were exposed to water-immersion and restraint stress. The next day after stress, the immune testes carried out. **Results**. Wide variability of hypoxic and swimming tests was revealed. 4 clusters were created retrospectively. Each cluster is characterized by specific (correctness of classification 96%) post-stress changes of 18 Immune parameters (6 from the Thymus, 5 from the Spleen, 7 from the Blood). The swimming test determines the post-stress state of the registered parameters of the Immunity by 46,7%, the hypoxic test - by 25,0%, and taken together - by 50,1%. **Conclusion**. Innate muscular endurance and resistance to hypoxia significantly determines the Immune parameters after acute stress in rats.
Keywords: **swimming and hypoxic tests, acute stress, immunity, rats.**

Introduction

It is known about aerobic fitness variability between individuals explained by genetics factors (Alvarez-Romero et al., 2021; Daskalakis et al., 2013; Herold et al., 2021; Overstreet & Wegener, 2013; Savignac et al., 2011; Zannas & West, 2014).

On the other hand, it is important to note that significant individual differences in stress perception, processing, and coping have been observed (Dhabhar & McEwen, 2007; Gunnar & Quevedo, 2007). Individual differences become particularly relevant while studying human subjects because stress perception, processing, and coping mechanisms can have significant effects on the kinetics and peak levels of circulating stress hormones and on the duration for which these hormone levels are elevated. Animal studies showing significant strain differences in stress reactivity and peak hormone levels (Dhabhar et al., 1993), adaptation to stress (Dhabhar et al., 1997), and in distribution and activation of adrenal steroid receptors and corticosteroid-binding globulin levels (Dhabhar et al., 1995], suggest that genetic as well as environmental factors play a role in establishing individual differences (Gomez-Serrano et al., 2001; de Carvalho et al. 2022; Kovács et al., 2022; Beschasnyi et all., 2021).

From the above it follows the hypothesis that inter-individual differences in normal conditions determine the characteristics of the body's response to acute stress. The purpose of our study is to test the hypothesis. The first report provides data on wide variability of hypoxic and swimming tests in Wistar rats of both sexes. Four clusters were created retrospectively. Each cluster is characterized by specific (correctness of classification 100%) post-stress changes of 7 neuro-endocrine, 15 metabolic and 4 ECGs parameters, as well as the length of ulcers and the index of damage to the gastric mucosa. The swimming test determines the post-stress state of the registered parameters of the body by 64,4%, the hypoxic test - by 58,6%, and taken together - by 73,5%. Thus,

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innate muscular endurance and resistance to hypoxia significantly determines the post-stress neuro-endocrine and metabolic parameters as well as injuries of myocardium and gastric mucosa in rats (Fil et al., 2021).

It is known about the close relationships between the nervous, endocrine and immune systems within the framework of the triune neuro-endocrine-immune complex (Korneva, 2020; Kozyavkina et al., 2015; Kul'chyns'kyi et al., 2017; 2017a; 2017b; Nance & Sanders, 2007; Pavlov et al., 2018; Popovych, 2011; Popovych et al., 2017; 2018; 2020; Sternberg, 2006; Sydoruk et al., 2018; Thayer & Sternberg, 2010; Tracey, 2010; Shvets et al., 2020; Goncharenko et al., 2020).

The purpose of this study is to test the hypothesis conformably to Immunity.

Material and methods

Participants. The experiment is at 58 rats Wistar line weighing 170-280 g: 28 males (Mean=216 g; SD=22 g) and 30 females (Mean=196 g; SD=19 g).

Procedure / Test protocol / Skill test trial / Measure / Instruments. At the preparatory stage, all animals were first tested for resistance to hypoxic hypoxia by the classical method of Berezovskyi (1975). To do this, each rat was placed in a pressure chamber with a transparent lid, in which the pump created a vacuum of air equivalent to a rise to a height of 12 km (20 kPa) and recorded the time of the second agonal breath or seizure.

One week later, aerobic muscular performance was determined by the duration of swimming (t^0 water 26^0 C) with a load (5% of body weight) to exhaustion (falling to the bottom of the bath) (Brekhman, 1968).

On the basis of the received data two qualitatively equivalent groups (equally females and males, practically identical average sizes and variances of swimming and hypoxic tests) in a ratio 10/48 were formed. Over the next 10 days, one animal remained intact and 5 other rats were exposed to water-immersion and restraint stress according to the method of Nakamura et al. (1977) in the modification of Popovych (2007), which is to reduce the duration of stay of the rat in a fixed standing position in cold water (t^0 20-21^0 C) to the level of the xiphoid process from 8 to 4 hours.

Data collection and analysis / Statistical analysis.

The day after stressing, 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 Popovych (2007; 2020) on the basis of the classical Shannon's (1948) formula:

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

The experiment was consummated by decapitation of the animals in order to remove the Thymus and Spleen and collect the maximum possible amount of blood in which was determined some Immune parameters.

The parameters of immunity were determined, as described in the manual (Perederiy et al., 1995): the relative content of the population of T-lymphocytes in a test of spontaneous rosette formation with erythrocytes of sheep by Jondal et al. (1972), their theophylline-resistant (TR) and theophylline-susceptible (TS) subpopulations (by the test of sensitivity of rosette formation to theophylline by Limatibul et al. (1978); the population of B-lymphocytes by the test of complementary rosette formation with erythrocytes of sheep by Bianco (1970). Natural killers were identified as large granules contain lymphocytes. The content of zero-lymphocytes (0L) was calculated by the balance method. For these components, as well as plasma cells (Pla), the Entropy of the Immunocytogram (hICG) was calculated by formula:

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

About the condition of the phagocytic function of neutrophils (microphages) and monocytes (macrophages) were judged by the phagocytosis index (percentage of cells, in which found microbes), the microbial count (number of microbes absorbed by one phagocyte) and the killing index (percentage of dead microbes) for Staphylococcus aureus (ATCC N25423 F49). According to these parameters and the content of microphages and macrophages in the blood calculated their Bactericidal Capacity (Bilas & Popovych, 2009; Bilas et al., 2020):

BCC N or M, 10^9 Bacteria/L = Leukocytes, 10^9/L•(Neutrophils or Monocytes, %)•PhI,%•MC, Bac/Phag•KI%.

After decapitation, the spleen and thymus were removed from the animals. Immune organs weighed and made smears-imprints for counting Thymocytogram and Splenocytogram (Horizontov et al., 1983; Bilas & Popovych, 2009; Bilas et al., 2020). The components of the Thymocytogram (TCG) are lymphocytes (Lc), lymphoblastes (Lb), reticulocytes (Ret), macrophages (Mac), basophiles (B), endotheliocytes (En), epitheliocytes (Ep) and Hassal's corpuscles (H). The Splenocytogram (SCG) includes lymphocytes (Lc), lymphoblastes (Lb), plasma cells (Pla), reticulocytes (R), macrophages (Ma), fibroblasts (F), microphages (Mi) and eosinophils (Eo).

For them Shannon's entropy was calculated too:

$hTCG = - (Lc \bullet \log_2 Lc + Lb \bullet \log_2 Lb + Ret \bullet \log_2 Ret + Mac \bullet \log_2 Mac + B \bullet \log_2 B + En \bullet \log_2 En + Ep \bullet \log_2 Ep + H \bullet \log_2 H)/\log_2 8$

$hSCG = - (Lc \bullet \log_2 Lc + Lb \bullet \log_2 Lb + Pla \bullet \log_2 Pla + R \bullet \log_2 R + Ma \bullet \log_2 Ma + F \bullet \log_2 F + Mi \bullet \log_2 Mi + Eo \bullet \log_2 Eo)/\log_2 8$

Statistical processing was performed using a softwarepackage "Microsoft Excell" and "Statistica 64 StatSoft Inc".

Results

The formation of four clusters of animals according to the indicators of swimming and hypoxic tests is described in detail in the previous article (Fil et al., 2021).

In order to identify exactly those post-stress parameters (variables) whose constellation is characteristic for each cluster, the available informational field was subjected to discriminant analysis by the method of forward stepwise (Klecka, 1989). To include in the model (Table 1), the program has selected 18 variables (6 from the **Thymus**, 5 from the **Spleen, 7** from the **Blood**). The rest of the registered immune variables were left out of the model, although some of them carry discriminant (recognizable) information.

Table 1. Discriminant Function Analysis Summary. Immune Variables currently in the model
Step 18, N of vars in model: 18; Grouping: 4 grps; Wilks' Lambda: 0,0305; approx. $F_{(55)}=3,3$; $p<10^{-6}$

	Clusters (n)				Parameters of Wilks' Statistics					
Variables	**II (3)**	**I (11)**	**III (25)**	**IV (9)**	Wilks' Λ	Partial Λ	F-remove	p-value	Tolerancy	**Norm (10)**
Thymus Mass, mg	1368	15412	1338	11113	0,036	0,838	1,74	0,182	0,014	**14410**
Hassal's corpuscles of Thymus, %	3,000,58	1,730,14	1,360,10	1,330,17	0,065	0,472	10,07	10^{-4}	0,432	**1,000,13**
Reticulocytes of Thymus, %	5,821,33	3,410,40	3,870,29	5,540,33	0,038	0,798	2,27	0,103	0,131	**4,160,74**
Macrophages of Thymus, %	6,330,33	5,910,44	6,880,40	7,000,37	0,036	0,853	1,55	0,225	0,349	**5,390,50**
Fibroblastes of Thymus, %	6,001,15	5,360,43	5,680,31	5,670,62	0,038	0,798	2,28	0,102	0,371	**5,330,65**
Entropy of Thymocytogram	0,6550,035	0,5930,019	0,6120,010	0,6220,017	0,046	0,662	4,59	0,010	0,091	**0,5960,015**
Spleen Mass, mg	80780	66841	66329	75335	0,037	0,826	1,90	0,153	0,037	**77358**
Lymphoblastes of Spleen, %	6,72,2	6,40,8	9,70,6	8,30,7	0,041	0,741	3,14	0,042	0,394	**8,61,0**
Plasmocytes of Spleen, %	3,000,58	3,000,54	1,800,22	1,750,17	0,038	0,804	2,20	0,112	0,484	**1,670,22**
Macrophages of Spleen, %	2,330,33	1,730,30	2,760,19	3,830,39	0,041	0,752	2,97	0,050	0,456	**2,560,32**
Microphages of Spleen, %	11,31,8	10,91,3	12,00,6	12,00,7	0,041	0,744	3,10	0,043	0,289	**12,30,9**
Leukocytes of Blood, 10^9/L	11,701,18	14,931,42	15,600,97	14,570,98	0,036	0,848	1,61	0,210	0,591	**13,812,09**
Entropy of Leukocytogram	0,6690,014	0,6690,013	0,6630,008	0,6600,012	0,035	0,862	1,44	0,253	0,337	**0,6820,003**
Killing Index of Neutrophils, %	41,30,9	34,42,3	40,62,0	52,12,1	0,065	0,472	10,05	10^{-4}	0,391	**47,52,9**
Microbial Count of Neutrophils, Bac/Phag	7,20,2	5,80,2	6,10,2	6,50,3	0,046	0,669	4,46	0,011	0,367	**5,50,3**
Bactericidal Capacity Neutrophils, 10^9 B/L	8,230,96	7,401,06	9,441,02	12,01,66	0,037	0,825	1,91	0,152	0,009	**7,541,39**
Phagocytic Index of Monocytes, %	5,30,7	6,60,7	6,00,3	4,40,7	0,038	0,802	2,22	0,108	0,385	**5,90,5**
Bactericidal Capacity of Monocytes, 10^6 B/L	12133	350121	23031	18275	0,038	0,794	2,34	0,096	0,028	**20837**

Notes. In each column, the top row is the average, the bottom is the standard error.

Next, the 18-dimensional space of discriminant variables transforms into 3-dimensional space of a **canonical** discriminant functions (canonical roots), which are a linear combination of discriminant variables. The discriminating (differentiating) ability of the root characterizes the canonical correlation coefficient (r*) as a measure of connection, the degree of dependence between groups (clusters) and a discriminant function. It is for Root 1 0,917 (Wilks' Λ=0,0305; $\chi^2_{(64)}=126$; $p<10^{-6}$), for Root 2 0,820 (Wilks' Λ=0,193; $\chi^2_{(34)}=59$; p=0,005), for Root 3 0,641 (Wilks' Λ=0,589; $\chi^2_{(16)}=19$; p=0,266). The first root contains 65,9% of discriminative opportunities, the second is 25,5% and the third only 8,6%.

The calculation of the discriminant root values for each animal as the sum of the products of raw coefficients to the individual values of discriminant variables together with the constant enables the visualization of each rat in the information space of the roots (Fig. 1).

Variables obtained after acute stress expressed as Z-scores. This approach allows us to compare the variables expressed in different units (%, mg, Bac/Phag etc) in one scale.

Table 2. Correlations Variables-Canonical Roots, Means of Roots and Z-scores of Variables

Variables	Correlations Variables-Canonical Roots			II (3)	I (11)	III (25)	IV (9)
Root 1 (65,9%)	**Root 1**	**Root 2**	**Root 3**	-3,9	-3,1	+0,9	**+2,7**
Macrophages of Spleen	**0,248**	0,150	0,021	-0,22	-0,82	+0,20	**+1,26**
Macrophages of Thymus	**0,101**	0,019	0,127	+0,60	+0,33	+0,95	**+1,02**
Bactericidal Capacity of Neutrophils	**0,060**	0,061	-0,062	+0,16	-0,03	+0,43	**+1,01**
Basophiles of Thymus	Currently not in the model			-0,36	-0,12	+0,02	**+1,27**
Killing Index of Neutrophils	**0,235**	0,269	-0,232	-0,66	-1,40	-0,74	**+0,50**
Lymphoblastes of Spleen	**0,195**	-0,083	0,401	-0,57	-0,69	+0,35	**-0,07**
Microphages of Spleen	**0,056**	0,007	0,078	-0,35	-0,50	-0,13	**-0,12**
Hassal's corpuscles of Thymus	**-0,257**	0,394	0,115	+4,60	+1,67	+0,83	**+0,77**
Plasmocytes of Spleen	**-0,132**	0,091	-0,315	+1,89	+1,89	+0,19	**+0,12**
Phagocytic Index of Neutrophils	Currently not in the model			+0,90	+0,36	+0,22	**+0,20**
Entropy of Leukocytogram	**-0,036**	-0,006	0,003	-0,25	-0,26	-0,36	**-0,43**
Phagocytic Index of Monocytes	**-0,122**	-0,208	0,154	-0,30	+0,45	+0,09	**-0,84**
Thymus Mass	**-0,129**	-0,112	0,060	-0,25	+0,23	-0,37	**-1,08**
Root 2 (25,5%)	**Root 1**	**Root 2**	**Root 3**	**+4,3**	**-0,9**	-0,6	+1,3
Reticulocytes of Thymus	0,064	**0,346**	-0,040	**+0,71**	**-0,32**	-0,12	+0,59
Entropy of Thymocytogram	0,078	**0,245**	-0,037	**+1,19**	**-0,06**	+0,32	+1,35
Microbial Count of Neutrophils	0,037	**0,243**	0,084	**+1,55**	**+0,25**	+0,58	+0,93
Fibroblastes of Thymus	0,018	**0,051**	0,085	**+0,33**	**+0,02**	+0,17	+0,16
Spleen Mass	0,009	**0,233**	-0,115	**+0,19**	**-0,57**	-0,60	-0,11
Pan Lymphocytes of Blood	Currently not in the model			**-0,10**	**-0,89**	-0,86	-0,41
Bactericidal Capacity of Monocytes	-0,068	**-0,186**	0,117	**-0,74**	**+1,20**	+0,18	-0,22
Polymorphonucleary Neutrophils of Blood	Currently not in the model			**+0,68**	**+1,62**	+1,65	+1,23
Microbial Count of Monocytes	Currently not in the model			**-1,03**	**+0,48**	+0,14	+0,28
Leukocytes of Blood	0,042	**-0,140**	0,024	**-0,32**	**+0,17**	+0,27	+0,11

Localization in the extreme right zone of the axis of the first root of the members of the **fourth** cluster (Fig. 1) reflects the maximum for the sample levels of immune variables that represent the root **directly**, as well as the minimum for the sample levels of variables **inversely** related to the root. The polar opposite position is occupied by the members of the **first** and **second** clusters, which are characterized by the minimum/maximum levels of these immunity parameters, mixing among themselves.

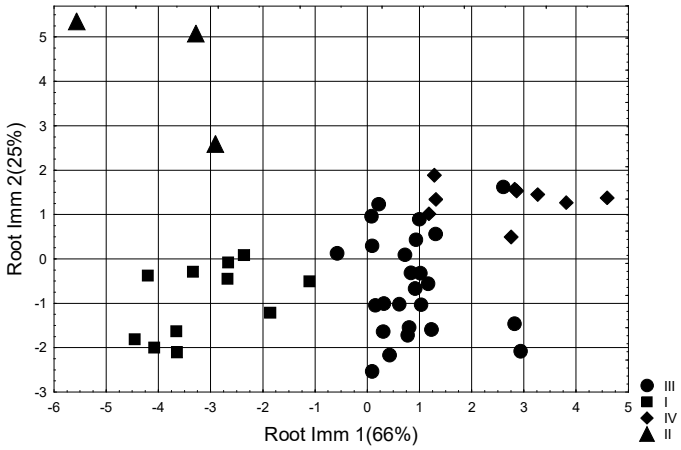


Fig. 1. Diagram of scattering of individual values of first and second immune Roots of rats of different clusters

Instead, along the axis of the second root, both clusters are clearly demarcated. The top position of the three male rats reflects their, as a rule, maximum/minimum levels of the variables representing the direct/inverse root, respectively. The polar opposite position is occupied by the members of the **second** cluster with minimum/ maximum levels of variables.

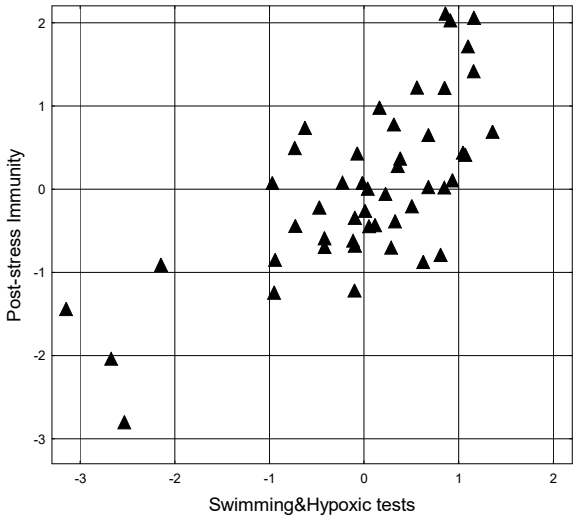
In general, in the information space of the two discriminant roots, all four clusters are clearly separated from each other, that is, they differ from each other in terms of constellation of the 18 parameters of immunity. This distinction is documented by the calculation of the Mahalanobis distances (MD) between clusters (MD^2: 9,8÷57,2; F: 2,2÷6,5; p: 0,03÷10^{-5}). The accuracy of retrospective recognition (classification) is 95,8%.

At the final stage of the analysis, the role of muscular endurance and resistance to hypoxia in determining the post-stress state of immunity was clarified. For this, a correlation matrix was first created, and then regression models were built by stepwise elimination until the maximum Adjusted R^2 levels were reached.

Innate muscular endurance has been found to upregulate post-stress content of Hassal's corpuscles in the Thymus (r=0,42), Reticulocytes in the Spleen (r=0,23) as well as Microbial count of neutrophils in the Blood (r=0,39) while downregulate post-stress content of Rod-shaped neutrophils in the Spleen (r=-0,37) and Phagocytic index of monocytes in the Blood (r=-0,27). In total, the swimming test determines the post-stress state of the registered parameters of the Immunity by 46,7%. The conditioning effect of innate resistance to hypoxia is significantly inferior to such innate muscular endurance accounting for only 25,1% and refers to the post-stress state of other immune parameters: upregulate Killing index of Neutrophils (r=0,34) and content of Plasmocytes in the Spleen (r=0,24) while downregulate content of Plasmocytes in the Blood (r=-0,31) and Reticulocytes in the Spleen (r=-0,21). Taken together, both innate factors of cardiorespiratory fitness determine the acute stress-induced changes in Immunity of rats by 50,1% (Table 3 and Fig. 2).

Table 3. Factor load on first pair of canonical roots

Left set	**Root 1**
Swimming test	**-0,935**
Hypoxic test	**-0,309**
Right set	**Root 1**
Microbial Count of Neutrophils	**-0,588**
Hassal's corpuscles of Thymus	**-0,522**
Plasmocytes of Spleen	**-0,330**
Killing Index of Neutrophils	**-0,265**
Reticulocytes of Spleen	**-0,199**
Rod-shaped Neutrophils of Spleen	**0,579**
Phagocytic Index of Monocytes	**0,495**
Plasmocytes of Blood	**0,477**



R=0,708; R^2=0,501; $\chi^2_{(16)}$=41,6; p=0,0005; Λ Prime=0,367

Fig. 2. Scatterplot of canonical correlation between Swimming and Hypoxic tests (X-line) and Post-Stress Immune parameters (Y-line) in rats

Discussion

There is considerable evidence highlighting the prominent influence of the *genotype* on the responsiveness of a single individual in physical performance parameters, brain structure and function etc. However, the exact influence of genetic factors on inter-individual response variability, at least for physical performance, is not yet exactly known and is currently under debate (Herold et al., 2021).

Previously, we found inter-individual variability, on the one hand, between innate two parameters of cardiorespiratory fitness, and responses of neuro-endocrine, metabolic and ECGs parameters as well as markers of gastric mucosa damage - on the other hand. In addition, we have shown that both aerobic muscular endurance (to a greater extent) and resistance to hypoxia (to a lesser extent) determine not only the severity but also the direction of stress-induced reactions of the autonomic nervous and endocrine systems, which in turn cause

damage to the myocardium and gastric mucosa, the severity of which differs significantly in rats of different clusters (Fil et al., 2021).

Stress is known to suppress immune function and increase susceptibility to infections and cancer. Paradoxically, stress is also known to exacerbate asthma, and allergic, autoimmune and inflammatory diseases, although such diseases should be ameliorated by immunosuppression. Moreover, the short-term fight-or-flight stress response is one of nature's fundamental defense mechanisms that enables the cardiovascular and musculoskeletal systems to promote survival, and it is unlikely that this response would suppress immune function at a time when it is most required for survival (e.g. in response to wounding and infection by a predator or aggressor). These observations suggest that stress may suppress immune function under some conditions while enhancing it under others. Dhabhar (2009; 2018) 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 authors 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.

In this study, as part of the discussion about the role of ***causes and conditions*** in pathogenesis/sanogenesis (Gozhenko, 2010), we showed that acute stress causes both adverse and favorable effects on the parameters of immunity, which are conditioned by innate two parameters of cardiorespiratory fitness. However, their conditioning effect is ambiguous.

In particular, significantly **increased** resistance to hypoxia in rats of the fourth cluster conditions a significant stress-induced increase in the content of neutrophils both in the leukocytogram (+1,23 Z) and in the blood (the content of leukocytes does not change) in combination with increased intensity (+0,93 Z) and improvement of completeness (+0,50 Z) of the phagocytic function of neutrophils, which ultimately gives an increase in their bactericidal ability at least against gram-positive bacteria (+1,01 Z). At the same time, the bactericidal ability of blood monocytes shows a tendency even to decrease (-0,22 Z) due to the predominance of a decrease in the activity of phagocytosis (-0,80 Z) over an increase in its intensity (+0,28 Z). However, it should be borne in mind that the lion's share of monocytes/macrophages is localized in tissues, in particular in the spleen, the mass of which does not change, but there is an increase in the content of macrophages in the splenocytogram (+1,26 Z). Therefore, in rats of this phenotype (at least 1/5 of our sample), increased resistance to hypoxia with normal muscular endurance is associated with their ability to respond to acute stress by increasing the bactericidal ability of blood neutrophils and tissue macrophages. At the same time, in such animals, acute stress reduces the mass of the thymus (-1,08 Z), but the level of macrophages in the thymocytogram increases significantly (+1,02 Z), as well as basophils (+1,27 Z), Hassal's corpuscles (+0,77 Z) and reticulocytes (+0,59 Z), which apparently reflects the activation of immunogenesis.

Instead, moderately **reduced** resistance to hypoxia with normal muscular endurance in rats of the third cluster (half of the sample) conditions a stress-induced decrease in the killing index of neutrophils (-0,74 Z), which, despite an increase in their content in the blood and an increase in the intensity of phagocytosis, leads to only a moderate increase their bactericidal ability (+0,43 Z). The bactericidal capacity of blood monocytes, as in the previous cluster, does not change (+0,18 Z). Instead, the mass of the spleen (-0,60 Z) and the absolute (but not relative) content of macrophages decrease moderately. On the other hand, this phenotype prevents a post-stress reduction in the mass of the thymus, while the content of macrophages and Hassal's corpuscles, but not reticulocytes and basophils, increases in the thymocytogram, as in the previous cluster.

Rats of the second cluster are characterized by a **drastic** (+2,71 Z) duration of swimming to exhaustion, which is associated with an even more drastic (+4,60 Z) post-stress increase in the thymocytogram of the level of Hassal's corpuscles, as well as, to a lesser extent, reticulocytes (+0,71 Z) and macrophages (+0,60 Z) in the absence of a significant change in thymus mass. The mass of the spleen also does not change significantly, but in the splenocytogram, the content of plasma cells increases (+1,89 Z), instead, the content of lymphoblasts decreases (-0,57 Z) and there is a tendency to decrease the content of microphages (-0,35 Z) and macrophages (-0,22 Z). The described post-stressor changes in the cytoarchitecture of the thymus and spleen are accompanied by a decrease in the intensity (-1,03 Z) and activity (-0,30 Z) of the phagocytic function and bactericidal capacity (-0,74 Z) of blood monocytes. At the same time, the bactericidal ability of blood neutrophils does not change, because the increase in the content of neutrophils in it and the intensity of phagocytosis (+1,55 Z) compensates for the deterioration of its completion (-0,66 Z). Regarding the validity of the conclusions, one should bear in mind the small number of members of this cluster (and on the other hand, there are also very few endurance champions).

Finally, in rats of the first cluster with normal, both resistance to hypoxia and muscular endurance, in response to acute stress, the bactericidal ability of blood neutrophils does not change, because the increase in the content of neutrophils in it is leveled by the weakening of the killing function (-1,40 Z). Instead, the bactericidal ability of blood monocytes/macrophages increases (+1,20 Z) due to an increase in both activity (+0,45 Z) and intensity (+0,48 Z) of phagocytosis without increasing their total content. At the same time, the content of macrophages in the splenocytogram decreases (-0,82 Z), as well as microphages (-0,50 Z), and taking into

account the decrease in the mass of the spleen (-0,57 Z), the total number of both types of phagocytes in it decreases even more. The content of lymphoblasts in the spleen also decreases, instead, the content of plasma cells increases. At the same time, the mass of the thymus does not change, while the content of Hassal's corpuscles in the thymocytogram increases, but other form elements do not change significantly.

Conclusion

So, the features of the emergency response of the immune system to acute stress are determined by the features of the innate state of muscular endurance and resistance to hypoxia. A similar polyvariant was previously discovered by us regarding the immune response to chronic stress in rats (Polovynko et al., 2016; 2016a; 2016b; Zajats et al., 2017; 2017a; Popovych et al., 2020) and humans (Lukyanchenko et al., 2019).

Regarding ***entropy***, which is a special subject of research in our laboratory (Flyunt et al., 2008; Kostyuk et al., 2007; Gozhenko et al., 2021; Popovych et al., 2020), it is interesting to note the significant post-stress increase in entropy of the thymocytogram in rats of the second and fourth clusters, that is, with extreme levels of muscular endurance or resistance to hypoxia.

Given the well-documented neuro-endocrine-immune interrelationships (Gozhenko et al., 2021; Khaitov, 2005; Korneva, 2020), the features of the immune response to acute stress revealed in this study are undoubtedly related to the features of autonomic and endocrine reactions revealed in the previous study (Fil et al., 2021). The analysis of neuro-endocrine-immune relationships in this sample of rats will be the subject of the next publication.

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 carry out of experiments was approved by the Ethics Committee of the 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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