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Sexual Dimorphism of the Neuroendocrine-Immune Complex and its Reactions to Chronic Stress in Rats

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In our previous publications [11-18], the effect of chronic stress on immunity parameters and related changes in its neuroendocrine regulation in male and female rats were analyzed. Even a superficial cursory analysis of the data presented gives the impression of the presence of sexual differences between the parameters of the neuroendocrine-immune complex in both intact and stressed rats.

The purpose of this article is a detailed analysis of sexual dimorphism. The research is conducted in line with the concept of the neuroendocrine-immune complex [19-24].

Material and methods. The experiment was carried out on 60 female and 50 male white rats of Wistar line weighing 230-300 grams. 10 animals of each sex were not subjected to any influences while others within 7 days were under moderate stress by daily 30-minute immobilization. The day after being stressed samples of peripheral blood (through a cut tail) were taken to analyze leukocytogram in intact and stressed rats. An hour under light ether anesthesia for 15-20 sec ECG was recorded in standard lead II (introducing needle electrodes subcutaneously) to determine parameters of heart rate variability (HRV) [1]. Then the rats were placed in individual chambers with perforated bottom to collect daily urine, in which the concentration of 17-ketosteroids was determined (by method of colorimetric reaction with m-dinitrobenzene), as well as calcium (by reaction with arsenase III), phosphates (phosphate-molybdate method), sodium and potassium (by flaming photometry).

The next day the animals were decapitated for the purpose of collecting blood. The plasma levels of the hormones of adaptation were determined: corticosterone, triiodothyronine, thyroxine and testosterone (according to ELISA) as well as electrolytes: calcium, phosphates, sodium and potassium.

According to the parameters of electrolyte exchange, hormonal activity was evaluated: parathyrin by coefficients $(\text{Cap/Pp})^{0.5}$ and $(\text{Cap} \cdot \text{Pu/Pp} \cdot \text{Cau})^{0.25}$, calcitonin by coefficients $(1/\text{Cap} \cdot \text{Pp})^{0.25}$ and $(\text{Cau} \cdot \text{Pu/Cap} \cdot \text{Pp})^{0.25}$ as well as mineralocorticoid by coefficients $(\text{Nap/Kp})^{0.5}$ and $(\text{Nap} \cdot \text{Ku/Kp} \cdot \text{Nau})^{0.25}$, based on their classical effects and recommendations by I.L. Popovych [8,19].

The analysis was carried out according to the instructions described in the manual. The analyzers "Tecan" (Oesterreich), "Pointe-180" ("Scientific", USA) and "Reflotron" (Boehringer Mannheim, BRD) were used with appropriate sets and a fiery spectrophotometer "SF-47".

Immunological parameters were determined by tests as described in the handbook [9] and the previously developed algorithm [8]. The state of the

phagocytic function of neutrophils (microphages) and monocytes (macrophages) is judged by the phagocytic index, microbial (phagocytic) number and the index of killing regarding the museum culture *Staphylococcus aureus* (ATCC N 25423 F49) [4,5], with the calculation of bactericidal capacity (the number of microbes that are able to neutralize neutrophils or monocytes contained in 1 litre of blood) [4].

Among the parameters of the immunogram the relative amount of blood population of T-cells was determined by M. Jondal's spontaneous rosette test with sheep erythrocytes [6], their theophylline resistant (T-helper) and theophylline sensitive (T-cytotoxic) subpopulations (by S. Limatibul's test of sensitivity rosette to theophylline [10]), the population of B-lymphocytes by C. Bianco's test of complementary rosette of sheep erythrocytes [3]. Natural killers were identified as big containing granules lymphocytes.

After the blood sample, spleen, thymus and adrenal glands were removed and weighed since spleen and thymus did smears for counting splenocytogram and thymocytogram [2,4]. For the latter, as well as for leukocytogram and immunocytogram we calculated entropy [25]. The thickness of glomerular, fascicular, reticular and medullar zones were measured under a microscope in sections of the adrenal glands.

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

Results and discussion. In order to compare different parameters adequately and their ranking, they were listed according to two indices. In this case, the average values of the male indices (M) were used as the base, for which each individual indexes were calculated for each female (F): F/M and $(F/M - 1)/Cv$, where Cv is the coefficient of variation of the parameter, common for both sexes. Neuroendocrine parameters, which are significantly different between sexes, are given in Table 1.

Table 1

Neuroendocrine indices of intact males/females and their interrelation

Variables	Actual values		Females/ Males	(Females/ Males - 1)/Cv
	Males (n = 10)	Females (n = 10)		
Reticular Zone Adrenal Cortex, μM	20,0 $\pm$ 1,8	40,8 $\pm$ 2,5	2,04 $\pm$ 0,13 ^c	+4,27 $\pm$ 0,52 ^c
Fascicular Zone Adr Cortex, μM	218 $\pm$ 11	370 $\pm$ 20	1,70 $\pm$ 0,09 ^c	+4,15 $\pm$ 0,56 ^c
Adrenals Mass, mg	50 $\pm$ 2	69 $\pm$ 3	1,39 $\pm$ 0,06 ^c	+3,01 $\pm$ 0,49 ^c
Moda HRV, msec	189 $\pm$ 11	124 $\pm$ 5	0,66 $\pm$ 0,0 ^c	+2,21 $\pm$ 0,16 ^c
Glomerular Zone Adrenal Cortex, μM	129 $\pm$ 11	189 $\pm$ 8	1,47 $\pm$ 0,07 ^c	+2,20 $\pm$ 0,31 ^c
Calcitonin Activity as (Cap • Pp) ^{-0,5}	0,48 $\pm$ 0,02	0,65 $\pm$ 0,05	1,36 $\pm$ 0,11 ^b	+1,73 $\pm$ 0,55 ^b
Parathyrin Activity as (Cap • Pu/ Cau • Pp) ^{0,25}	2,97 $\pm$ 0,05	3,40 $\pm$ 0,19	1,15 $\pm$ 0,06 ^a	+1,27 $\pm$ 0,55 ^a
Parathyrin Activity as (Cap • Pu) ^{0,5}	1,67 $\pm$ 0,03	2,11 $\pm$ 0,16	1,27 $\pm$ 0,10 ^a	+1,48 $\pm$ 0,54 ^a
Mineralocortic Activ as (Nap • Ku/ Kp • Nau) ^{0,25}	2,08 $\pm$ 0,12	2,73 $\pm$ 0,25	1,31 $\pm$ 0,12 ^a	+1,33 $\pm$ 0,51 ^a
17-KS urine, nM/100 g • day	26 $\pm$ 6	45 $\pm$ 7	1,75 $\pm$ 0,27 ^a	+1,24 $\pm$ 0,45 ^a
Calcitonin Activity as (Cau • Pu/ Cap • Pp) ^{0,25}	2,45 $\pm$ 0,04	2,71 $\pm$ 0,12	1,11 $\pm$ 0,05 ^a	+1,09 $\pm$ 0,52 ^a
Corticosterone, nM/L	333 $\pm$ 43	466 $\pm$ 57	1,40 $\pm$ 0,17 ^a	+1,02 $\pm$ 0,43 ^a
Mineralocorticoid Activity as (Nap/Kp) ^{0,5}	6,10 $\pm$ 0,37	5,57 $\pm$ 0,17	0,91 $\pm$ 0,03 ^b	-0,60 $\pm$ 0,20 ^b
Triiodo-thyronine, nM/L	2,50 $\pm$ 0,17	2,12 $\pm$ 0,18	0,85 $\pm$ 0,07 ^a	-0,63 $\pm$ 0,30 ^a
Testosterone, nM/L	35,1 $\pm$ 4,6	3,93 $\pm$ 0,34	0,11 $\pm$ 0,01 ^c	-2,57 $\pm$ 0,03 ^c

Note: p < 0,05^a; < 0,01^b; < 0,001^c.

It was found that the most significant sexual differences were observed in the morpho-functional parameters of the adrenal glands. In particular, the females had the highest androgenic, glucocorticoid and mineralocorticoid activity which was estimated for the thickness of the reticular cortex of the adrenal glands and daily excretion of 17-ketosteroids with urine, the thickness of the fascicular corticoadrenal zone and the plasma corticosterone as well as glomerular zone of the adrenal cortex and levels in plasma and daily urine of sodium and potassium. However, there was higher parathyroid and calcitonin activity in females, measured by levels in plasma and daily urine calcium and phosphate. In addition, females had a lower level of Moda of HRV as an inverse rate of heart rate, which was subjected to the influence of the so-called humoral regulation channel (circulating catecholamines, glucocorticoids, electrolytes, etc.), while the HRV-markers of sympathetic and vagal tonus did not significantly differ, making $53 \pm 7 \%$, $56 \pm 5 \%$, 45 ± 8 and 53 ± 13 msec respectively. Instead, the plasma level of triiodothyronine in females was $85 \pm 15 \%$ of males, and an order of magnitude lower testosterone level does not need to be commented.

Among the registered immune indices, 11 of them are significantly higher in females (Table 2). First of all, it is the content in the thymocytoqram of lymphocytes and lymphoblasts, in the immunocytoqram of blood of natural killers and B-lymphocytes, as well as fibroblasts, macrophages and microphages in splenocytoqram. In addition, females had higher levels of phagocytosis by monocytes of blood Staph aureus and leukocytosis as well as the entropy of splenocytoqram and immunocytoqram.

Table 2

Significantly higher immune parameters of intact female rats

Variables	Actual values		Females/ Males	(Females/ Males - 1)/Cv
	Males (n = 10)	Females (n = 10)		
Lymphocytes of Thymus, %	55,6 ± 1,0	70,3 ± 0,8	1,26 ± 0,01 ^c	+5,75 ± 0,24 ^c
NK-Lymphocytes of Blood, %	10,4 ± 0,6	15,0 ± 0,4	1,45 ± 0,03 ^c	+3,53 ± 0,27 ^c
Lymphoblastes of Thymus, %	5,50 ± 0,17	7,40 ± 0,27	1,35 ± 0,05 ^c	+3,29 ± 0,46 ^c
Microbial Count of Monocytes, Bact/Phagoc	2,8 ± 0,1	4,4 ± 0,5	1,56 ± 0,16 ^b	+2,48 ± 0,73 ^c
Fibroblastes of Spleen, %	5,90 ± 0,38	8,20 ± 0,66	1,39 ± 0,11 ^b	+1,69 ± 0,49 ^b
Macrophages of Spleen, %	5,50 ± 0,65	7,90 ± 0,50	1,44 ± 0,09 ^c	+1,51 ± 0,32 ^c
B-Lymphocytes of Blood, %	12,7 ± 0,7	15,4 ± 0,8	1,21 ± 0,06 ^b	+1,27 ± 0,36 ^b
Leukocytes of Blood, 10 ⁹ /L	9,57 ± 0,54	13,10 ± 1,76	1,37 ± 0,17 ^c	+1,22 ± 0,58 ^a
Entropy of Splenocytoqramm (•10 ³)	721 ± 9	753 ± 9	1,04 ± 0,01 ^c	+1,17 ± 0,33 ^b
Entropy of Immunocytoqramm (•10 ³)	849 ± 9	874 ± 6	1,03 ± 0,01 ^c	+1,07 ± 0,23 ^c
Neutrophils of Spleen, %	11,5 ± 0,5	13,0 ± 0,4	1,13 ± 0,04 ^b	+1,06 ± 0,32 ^b

Instead, 10 indicators of immunity in females were significantly lower (Table 3). This is, first and foremost, the activity of phagocytosis of neutrophils/microphages and, to a lesser extent, of monocytes/macrophages, entropy of thymocytoqrams and the proportion of epithelial cells, endothelial cells and macrophages in it, the proportion of lymphocytes and lymphoblasts in splenocytoqram, 0-lymphocytes in immunocytoqram as well as the completeness of phagocytosis of neutrophils blood.

Table 3

Significantly lower immune parameters of intact female rats

Variables	Actual values		Females/ Males	(Females/ Males – 1)/Cv
	Males (n = 10)	Females (n = 10)		
Killing Index of Neutrophils, %	54,7 ± 2,0	50,4 ± 1,6	0,92 ± 0,03 ^a	-0,72 ± 0,27 ^a
Lymphoblastes of Spleen, %	5,10 ± 0,38	3,90 ± 0,38	0,76 ± 0,07 ^b	-0,87 ± 0,27 ^b
0-Lymphocytes of Blood, %	29,8 ± 1,5	22,2 ± 1,9	0,75 ± 0,07 ^b	-1,17 ± 0,30 ^b
Macrophages of Thymus, %	4,70 ± 0,21	2,70 ± 0,42	0,57 ± 0,09 ^c	-1,33 ± 0,28 ^c
Lymphocytes of Spleen, %	53,1 ± 0,9	48,7 ± 0,9	0,92 ± 0,02 ^c	-1,51 ± 0,30 ^c
Phagocytose Index of Monocytes, %	7,8 ± 1,1	2,7 ± 0,2	0,35 ± 0,03 ^c	-1,86 ± 0,08 ^c
Endotheliocytes of Thymus, %	7,4 ± 0,4	2,6 ± 0,3	0,35 ± 0,04 ^c	-2,34 ± 0,15 ^c
Epitheliocytes of Thymus, %	19,9 ± 0,7	8,8 ± 0,6	0,44 ± 0,03 ^c	-3,26 ± 0,18 ^c
Entropy of Thymocytoqramm (•10 ³)	567 ± 9	416 ± 10	0,73 ± 0,02 ^c	-4,08 ± 0,28 ^c
Phagocytose Index of Neutrophils, %	82,3 ± 0,7	70,1 ± 1,0	0,85 ± 0,01 ^c	-4,12 ± 0,35 ^c

The described results of the analysis of sexual dimorphism of the neuroendocrine-immune complex of intact rats are visualized in Figures 1 and 2.

Fig. 1. Significantly larger ranking of Z-scores of neuroendocrine and immune parameters of intact female rats

Fig. 2. Ranking of Z-scores of neuroendocrine and immune parameters of intact rats, significantly lower in females

The purpose of the next stage of the analysis was to identify those parameters of the neuroendocrine-immune complex that characterized sexual dimorphism both in normal and after chronic stress. 38 indicators were selected by the method of discriminatory analysis (forward stepwise [7]), including 6 of thymus, 6 of spleen, 13 of blood and 13 of neuroendocrine ones (Table 4).

The distinguishing information is condensed in three canonical roots. At the same time, the major roots contain its lion's share (97,9 %), while in the second and third only 1,2 and 0,9 % respectively.

Table 4

Distinguishing Function Analysis Summary
Step 38, N of variables in model: 38; Grouping: 4 groups
Wilks' Lambda: 0,002; approx. $F_{(114)} = 13$; $p < 10^{-6}$

Current Variables in the model	Wilks' Δ	Partial Δ	F-remove	p-level	Tolerance	F to enter	p-level	Δ	F value	p-level
Thymus Mass, mg	,002	,883	3,1	,03	,08	1,3	,27	,003	15,8	10^{-6}
Thymus Mass Index, %	,002	,837	4,5	,01	,07	1,9	,13	,004	18,3	10^{-6}
Epitheliocytes of Thymus, %	,002	,815	5,2	10^{-2}	,54	190	10^{-6}	,157	190	10^{-6}
Hassal corpuscles of Thymus, %	,002	,872	3,4	,02	,34	1,7	,17	,004	17,2	10^{-6}
Macrophages of Thymus, %	,003	,742	8,0	10^{-4}	,34	6,2	10^{-3}	,021	33,6	10^{-6}
Endotheliocytes of Thymus, %	,003	,637	13	10^{-6}	,41	19	10^{-6}	,061	60,8	10^{-6}
Spleen Mass, mg	,002	,928	1,8	,16	,49	1,5	,22	,002	13,4	10^{-6}
Reticulocytes of Spleen, %	,002	,938	1,5	,21	,45	1,1	,35	,002	14,0	10^{-6}
Fibroblastes of Spleen, %	,002	,974	0,6	,61	,44	1,1	,37	,003	15,4	10^{-6}
Neutrophils of Spleen, %	,002	,949	1,2	,30	,49	1,1	,37	,002	13,1	10^{-6}
Eosinophils of Spleen, %	,003	,738	8,2	10^{-4}	,44	2,4	,07	,009	21,8	10^{-6}
Plasmocytes of Spleen, %	,002	,809	5,4	10^{-2}	,23	3,6	,02	,008	21,3	10^{-6}
Phagocytose Ind of Monocyte, %	,003	,615	14	10^{-6}	,23	8,5	10^{-4}	,038	42,8	10^{-6}
Microbial Count Mon, Bac/Phag	,002	,950	1,2	,31	,24	8,6	10^{-4}	,025	36,0	10^{-6}
Bactericidal Capac Mon, 10^6 B/L	,002	,862	3,7	,02	,36	3,3	,02	,012	25,1	10^{-6}
Phagocytose Ind of Neutroph, %	,003	,681	11	10^{-5}	,28	7,1	10^{-3}	,031	38,3	10^{-6}
Microbial Count Neut, Bac/Phag	,002	,798	5,8	10^{-3}	,37	4,0	,01	,014	27,8	10^{-6}
Segmented Neutroph Blood, %	,002	,848	4,1	,01	,33	3,1	,03	,005	19,7	10^{-6}
Eosinophils of Blood, %	,002	,866	3,6	,02	,37	2,7	,05	,010	22,8	10^{-6}
Basophils of Blood, %	,002	,860	3,7	,01	,45	1,6	,20	,004	17,7	10^{-6}
B-Lymphocytes of Blood, %	,002	,891	2,8	,04	,19	1,7	,18	,003	14,4	10^{-6}
T-cytotoxic Lymphocyt Blood, %	,002	,880	3,1	,03	,15	1,6	,19	,003	14,6	10^{-6}
NK-Lymphocytes of Blood, %	,002	,900	2,5	,06	,38	9,9	10^{-5}	,047	49,4	10^{-6}
O-Lymphocytes of Blood, %	,002	,854	3,9	10^{-2}	,09	2,8	,05	,006	20,1	10^{-6}
Entropy Immunocytogramm	,002	,858	3,8	,01	,26	1,2	,32	,003	14,9	10^{-6}
Adrenals Mass, mg	,002	,835	4,6	10^{-2}	,56	5,6	10^{-3}	,018	31,6	10^{-6}
Glomerulary Zone Adr Cortex, μ M	,002	,966	0,8	,49	,44	2,5	,06	,011	23,8	10^{-6}
MCA as $(\text{Nap} \cdot \text{Ku/Kp} \cdot \text{Nau})^{0,25}$	,002	,889	2,8	,07	,44	2,4	,09	,005	18,6	10^{-6}
Fascicularary Zone Adr Cortex, μ M	,003	,776	6,7	10^{-3}	,33	24	10^{-6}	,094	79,4	10^{-6}
Corticosterone, nM/L	,002	,899	2,6	,06	,46	2,3	,08	,004	18,8	10^{-6}
Reticulary Zone Adr. Cortex, μ M	,002	,872	3,4	,02	,56	2,5	,07	,005	19,2	10^{-6}
Testosterone, nM/L	,002	,929	1,8	,16	,67	1,5	,23	,003	16,7	10^{-6}
Thyroxine, nM/L	,002	,892	2,8	,05	,29	1,6	,20	,003	16,3	10^{-6}
Triiodo-thyronine, nM/L	,002	,787	6,2	10^{-3}	,23	3,6	,02	,006	20,7	10^{-6}
Parathyrine activity as $(\text{Cap/Pp})^{0,5}$	,002	,945	1,3	,27	,10	3,6	,02	,013	26,4	10^{-6}
PTA as $(\text{Cap} \cdot \text{Pu/Cau} \cdot \text{Pp})^{0,25}$	,002	,915	2,1	,10	,09	1,2	,33	,002	13,6	10^{-6}
Moda HRV, msec	,003	,759	7,3	10^{-3}	,12	3,7	,01	,016	29,4	10^{-6}
Sympathetic tone as AMo HRV, %	,003	,754	7,5	10^{-3}	,13	4,5	,01	,007	21,0	10^{-6}

The calculation of individual values of discriminant roots was based on the individual values of discriminant variables as well as their raw coefficients and constants, given in Table 5, makes it possible to visualize the localization of each of the 110 rats in the information box of discriminant roots (Fig. 3).

Table 5

Standardized and Raw Coefficients and Constants for Canonical Variables

Current Variables in the model	Standardized Coefficients			Raw Coefficients		
	Root 1	Root 2	Root 3	Root 1	Root 2	Root 3
Thymus Mass, mg	-,818	,238	-1,205	-,042	,012	-,062
Thymus Mass Index, ‰	,991	-,697	1,348	12,13	-8,54	16,49
Epitheliocytes of Thymus, ‰	-,542	,281	,101	-,225	,117	,042
Hassal corpuscles of Thymus, ‰	,296	-,537	,482	,438	-,796	,714
Macrophages of Thymus, ‰	-,003	-1,139	,014	-,002	-,823	,010
Endotheliocytes of Thymus, ‰	-,764	,720	-,040	-,601	,567	-,031
Spleen Mass, mg	,034	-,054	,537	,000	-,000	,004
Reticulocytes of Spleen, ‰	,032	,187	-,480	,018	,103	-,265
Fibroblastes of Spleen, ‰	,095	,148	,273	,056	,087	,161
Neutrophils of Spleen, ‰	,296	,107	,144	,138	,050	,067
Eosinophils of Spleen, ‰	-,754	,125	,216	-,836	,138	,239
Plasmocytes of Spleen, ‰	-,671	,674	,477	-,483	,485	,343
Phagocytose Index of Monocytes, ‰	-1,057	,974	-,138	-,609	,561	-,079
Microbial Count Monocytes, Bacter/Phagocyte	,011	-,585	,128	,006	-,337	,074
Bactericidal Capacity of Monocytes, 10 ⁶ Bact/L	-,386	,419	,515	-,005	,006	,007
Phagocytose Index of Neutrophils, ‰	-,907	-,723	-,121	-,239	-,190	-,032
Microbial Count of Neutrophils, Bacter/Phagoc	,515	,216	,701	,500	,210	,681
Segmented Neutroph of Blood, ‰	-,028	-,809	-,374	-,005	-,142	-,066
Eosinophils of Blood, ‰	-,355	,185	,661	-,181	,094	,336
Basophils of Blood, ‰	-,472	,357	-,176	-1,163	,879	-,433
B-Lymphocytes of Blood, ‰	-,548	,217	-,708	-,194	,077	-,252
T-cytotoxic Lymphocytes of Blood, ‰	-,800	-,324	-,432	-,257	-,104	-,139
NK-Lymphocytes of Blood, ‰	,278	,293	-,515	,146	,154	-,271
O-Lymphocytes of Blood, ‰	-1,131	,493	-,466	-,172	,075	-,071
Entropy of Immunocytoqramm (•10 ³)	,616	,474	,242	,022	,017	,009
Adrenals Mass, mg	,520	,205	,042	,047	,018	,004
Glomerulary Zone of Adrenal Cortex, µM	,053	,353	,055	,002	,011	,002
Mineralocorticoid Activity as (Nap•Ku/Kp•Nau) ^{0,25}	,170	,900	-,145	,355	1,850	-,300
Fascicular Zone of Adrenal Cortex, µM	,446	-,884	-,233	,007	-,013	-,003
Corticosterone, nM/L	-,392	-,272	,219	-,002	-,001	,001
Reticulary Zone of Adrenal Cortex, µM	,451	-,213	,018	,049	-,023	,002
Testosterone, nM/L	-,317	-,067	-,078	-,027	-,006	-,007
Thyroxine, nM/L	-,587	-,114	-,192	-,037	-,007	-,012
Triiodo-thyronine, nM/L	-,866	-,250	-,528	-1,738	-,502	-1,060
Parathyrine Activity as (Cap/Pp) ^{0,5}	,173	,904	-,147	,354	1,852	-,301
Parathyrine Activity as (Cap•Pu/Cau•Pp) ^{0,25}	,172	-,789	1,048	,386	-1,777	2,358
Moda HRV, msec	-1,202	-,556	-,768	-,042	-,019	-,027
Sympathetic tone as AMo HRV, ‰	-1,363	,077	-,178	-,059	,003	-,008
	Constants			22,16	4,04	-1,92

Fig. 3. Individual sizes of canonical discriminant roots of intact (I) and stressed (S) males (M) and females (F) rats

After calculating the average values (centroids) of discriminant roots (Table 6), it is possible to visualize the localization of four groups of animals on their planes (Fig. 4).

Table 6
Means of Canonical Variables and Chi-Square Tests with Successive Roots
Removed

	Root 1	Root 2	Root 3
Intact Males (<i>n</i> = 10)	-11,6	3,2	-1,0
Stressed Males (<i>n</i> = 40)	-11,0	-0,8	0,2
Intact Females (<i>n</i> = 10)	9,2	1,2	2,8
Stressed Females (<i>n</i> = 50)	9,3	-0,2	-0,6
Eigenvalues	107,5	1,4	1,0
Canonical R	0,995	0,760	0,705
Wilks' Lambda	0,002	0,212	0,503
Chi-Square	549	136	61
Degree freedom	114	74	36
p-level	10-6	10-4	,006

Fig. 4. Average values ($M \pm m$) of canonical discriminant roots of intact (I) and stressed (S) males (M) and females (F) rats

The drastic delineation along the axis of the first root of males and females reflects the values of the parameters of the neuroendocrine-immune complex significantly more in males, which are correlated with the root negatively, as well as significantly smaller than females, are correlated with the root of the parameters positively. At the same time, for each sex, these parameters do not differ significantly in intact and stressed animals, that is, they are stress-resistant (Table 7).

Table 7

Factor Structure Matrix. Correlations of Variables-Canonical Roots
(within-groups correlations)

Variables currently in the model	Root 1	Root 2	Root 3	Intact Males (10)	Stressed Males (40)	Intact Females (10)	Stressed Females (50)
Epitheliocytes of Thymus, %	-,223	-,105	-,047	19,9±0,7	20,6±0,4	8,8±0,6	9,4±0,3
Phagocytose Ind of Neutroph., %	-,161	,016	,046	82,3±0,7	81,9±0,6	70,1±1,0	69,4±0,5
Endotheliocytes of Thymus, %	-,143	,201	-,109	7,40±0,43	6,15±0,25	2,60±0,31	2,65±0,13
Testosterone, nM/L	-,137	-,124	-,007	35,1±4,6	40,0±2,7	3,93±0,34	4,87±0,32
Macrophages of Thymus, %	-,097	-,257	,018	4,70±0,21	6,13±0,28	2,70±0,42	3,08±0,16
Moda HRV, msec	-,096	,172	,022	189±11	168±6	124±5	114±3
0-Lymphocytes of Blood, %	-,086	-,096	,118	29,8±1,5	33,0±0,9	22,2±1,9	20,4±1,1
Triiodo-thyronine, nM/L	-,037	-,099	-,056	2,50±0,17	2,65±0,10	2,12±0,18	2,28±0,05
Plasmocytes of Spleen, %	-,036	-,003	,138	2,80±0,47	3,03±0,24	2,50±0,50	1,86±0,17
Eosinophils of Spleen, %	-,033	-,093	,049	1,80±0,25	2,18±0,16	1,52±0,33	1,49±0,11
NK-Lymphocytes of Blood, %	,160	,098	-,183	10,4±0,6	9,4±0,2	15,0±0,4	16,0±0,3
Fascicular Zone Adr Cortex, µM	,118	-,130	-,119	217±11	245±8	370±20	417±
Glomerular Zone Adr Cort, µM	,104	,125	-,017	129±11	113±3	189±8	188±6
MCA as (Nap•Ku/Kp•Nau) ^{0.25}	,100	,120	-,015	2,08±0,12	2,18±0,14	2,73±	3,05±0,14
Reticular Zone Adr. Cortex, µM	,104	-,096	-,024	20,0±1,8	23,5±1,1	40,7±2,5	43,6±1,6
Adrenals Mass, mg	,092	,015	-,040	49,6±1,8	49,0±1,6	69,0±3,2	70,5±1,8
Entropy Immunocytogram (•10 ³)	,070	,078	-,101	849±9	838±5	874±6	881±3
B-Lymphocytes of Blood, %	,056	-,002	-,054	12,7±0,7	12,6±0,4	15,4±0,8	16,0±0,4
Fibroblastes of Spleen, %	,055	-,006	,050	5,90±0,38	6,10±0,28	8,20±0,66	7,94±0,23
Neutrophils of Spleen, %	,050	,096	-,039	11,5±0,5	10,6±0,4	13,0±0,4	13,0±0,3
T-cytotoxic Lymphocyte Blood, %	,038	,033	-,105	14,1±0,9	13,3±0,5	15,1±0,9	16,1±0,4
Microbial Count Mon, Bact/Phag	,034	-,121	-,012	2,8±0,1	3,6±0,3	4,3±0,5	4,7±0,3
Reticulocytes of Spleen, %	,034	,057	-,156	14,3±0,6	13,6±0,3	14,3±0,6	15,1±0,3
Phagocytose Ind of Monocytes, %	-,090	,254	-,149	7,8±1,1	5,6±0,3	2,7±0,2	2,9±0,1
Basophils of Blood, %	,018	,062	-,123	0,37±0,14	0,21±0,05	0,28±0,12	0,41±0,07
Segment Neutrophils of Blood, %	-,002	,021	-,036	28,1±1,7	27,4±0,7	26,9±2,2	27,4±0,9
Sympathet tone as AMo HRV, %	-,003	-,144	-,028	53±7	65±4	56±5	63±3
Thymus Mass, mg	-,024	-,145	,031	75±5	86±3	73±7	75±2
Thymus Mass Index, %	-,026	-,130	,044	0,29±0,02	0,34±0,01	0,28±0,04	0,29±0,01
Hassal corpuscles of Thymus, %	,005	-,102	-,080	1,70±0,27	1,91±0,14	1,70±0,17	1,98±0,06
Corticosterone, nM/L	,011	-,077	,081	333±43	411±34	466±57	433±27
PTA as (Cap•Pu/Cau•Pp) ^{0.25}	,024	,130	,316	2,97±0,05	2,92±0,03	3,61±0,22	3,05±0,08
Parathyrine activity as (Cap/Pp) ^{0.5}	,027	,131	,309	1,67±0,06	1,60±0,03	2,38±0,25	1,78±0,09
Microbial Count Neutr, Bact/Phag	-,007	,115	,196	8,1±0,1	7,9±0,1	8,5±0,5	7,7±0,2
Bactericidal Capac Mon, 10 ⁶ B/L	-,017	-,024	,191	81±14	103±10	110±52	67±7
Eosinophils of Blood, %	-,002	-,010	,115	3,50±0,62	3,85±0,32	4,30±0,68	3,57±0,27
Spleen Mass, mg	,011	,031	,108	730±50	732±19	816±83	756±20
Thyroxine, nM/L	-,007	-,014	,097	60±6	62±3	64±5	59±2
Discriminant Properties, %	97,9	1,2	0,9				

Instead, intact and stressed males are clearly delimited along the axis of the second root, which reflects the post-stress decrease in the phagocytic activity of monocytes in the blood and the content of basophils and segmental neutrophils in it, on the one hand, and the increase in the mass of thymus and the content of Hassall's cells in it, and the increase of the sympathetic tone and corticosterone - on the other hand. Post-stress changes of the listed parameters in females in general are insignificant. Significant differences between intact

and stressed females are reflected along the third root axis, which contains the information on stress induced lowering levels of parathyrin and thyroxin, the intensity of *Staph aureus* phagocytosis by microphages and bactericidal capacity of blood macrophages, its content of eosinophils as well as the mass of the spleen. Instead, post-stress changes of the listed parameters in males are generally insignificant.

The obvious differences between the four groups of rats are documented by calculating the squares of Mahalanobis distances (Table 8).

Table 8

Squared Mahalanobis Distances, F-values (df = 39) and p-levels

Groups	IM	SM	IF	SF
Intact Male (n = 10)	0	19	468	465
Stressed Male (n = 40)	2,3 10 ⁻³	0	436	430
Intact Female (n = 10)	36 10 ⁻⁶	55 10 ⁻⁶	0	14
Stressed Female (n = 50)	61 10 ⁻⁶	160 10 ⁻⁶	1,8 10 ⁻²	0

Consequently, the discrepancies between neuroendocrine-immune complexes of intact and stressed rats of both sexes are explained exhaustively by 38 parameters. The information contained in these metrics can be condensed in three recognition roots. Selected parameters can be used to identify each of the four animal groups by means of calculating the classifying functions by their coefficients and constants (Table 9) and the individual values of discriminant parameters.

Table 9

Coefficients and Constants for Classifying Functions

Current Variables in the model	Intact Male	Stressed Male	Intact Female	Stressed Female
1	2	3	4	5
Thymus Mass, mg	1,41	1,26	,27	,46
Thymus Mass Index, %	-435	-374	-104	-146
Epitheliocytes of Thymus, %	9,86	9,32	5,10	4,78
Hassal corpuscles of Thymus, %	-25,09	-20,78	-11,69	-12,91
Macrophages of Thymus, %	3,37	6,68	5,01	6,13
Endotheliocytes of Thymus, %	10,99	8,34	-2,78	-3,52
Spleen Mass, mg	,10	,11	,12	,11
Reticulocytes of Spleen, %	15,76	15,04	14,94	15,68
Fibroblastes of Spleen, %	10,18	10,06	11,77	11,12
Neutrophils of Spleen, %	3,99	3,95	7,00	6,72
Eosinophils of Spleen, %	41,94	41,21	25,17	24,11
Plasmocytes of Spleen, %	35,26	33,47	25,54	23,67
Phagocytose Index of Monocytes, %	19,93	17,25	5,85	5,27
Microbial Count Monocytes, Bacter/Phagocyte	35,04	36,49	36,12	36,35
Bactericidal Capacity of Monocytes, 10 ⁶ Bact/L	-,028	-,046	-,127	-,160
Phagocytose Index of Neutrophils, %	21,37	21,97	16,67	17,03
Microbial Count of Neutrophils, Bacter/Phagoc	-6,59	-6,33	5,94	3,42
Segmented Neutrophils of Blood, %	-1,26	-,77	-1,33	-,91
Eosinophils of Blood, %	-8,21	-8,28	-10,90	-12,17
Basophils of Blood, %	-22,83	-27,53	-50,40	-50,29

Continuation of table 9

1	2	3	4	5
B-Lymphocytes of Blood, %	10,75	10,03	5,60	6,32
T-cytotoxic Lymphocytes of Blood, %	3,02	3,13	-2,64	-2,05
NK-Lymphocytes of Blood, %	-3,75	-4,61	-2,03	-1,33
0-Lymphocytes of Blood, %	6,97	6,48	2,97	3,08
Entropy of Immunocytoqramm ($\cdot 10^3$)	2,140	2,094	2,602	2,550
Adrenals Mass, mg	-,39	-,43	,56	,53
Glomerulary Zone of Adrenal Cortex, μ M	,55	,51	,57	,55
Mineralocorticoid Activity as (Nap $\cdot$ Ku/ Kp $\cdot$ Nau) ^{0,25}	123	133	143	138
Fasciculary Zone of Adrenal Cortex, μ M	,14	,19	,29	,32
Corticosterone, nM/L	,13	,14	,10	,09
Reticulary Zone of Adrenal Cortex, μ M	-1,78	-1,66	-,70	-,67
Testosterone, nM/L	-,24	-,24	-,81	-,79
Thyroxine, nM/L	2,51	2,51	1,72	1,77
Triiodo-thyronine, nM/L	170,4	170,1	131,2	135,3
Parathyrine Activity as (Cap/Pp) ^{0,5}	-100,8	-108,4	-98,3	-99,9
Parathyrine Activity as (Cap $\cdot$ Pu/Cau $\cdot$ Pp) ^{0,25}	122,9	133,1	143,3	138,0
Moda HRV, msec	3,53	3,55	2,60	2,71
Sympathetic tone as AMo HRV, %	4,01	3,95	2,74	2,75
Constants	-3293	-3287	-2822	-2814

In this case, the accuracy of the classification is 100 %.

Conclusion. The data obtained by us indicate the need to take into account the sexual differences in the reactions of the neuroendocrine-immune complex to stress when testing the stress limiting means.

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RESEARCH ARTICLE

Sexual Dimorphism of the Neuroendocrine-Immune Complex and its Reactions to Chronic Stress in Rats

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Introduction. If the sexual differences in endocrine parameters are well understood, then research of immune parameters in this aspect remains relevant.

Materials and methods. The experiment was performed on 50 males and 60 females of the Wistar line rats. Parameters of HRV, blood and daily urine levels of hormones and electrolytes as well as parameters of leukocytogram, immunocytogram, thymocytogram and splenocytogram were determined.

Results. It was found that the most significant sexual differences were observed in the morpho-functional parameters of the adrenal glands. Intact females had the highest androgenic, glucocorticoid and mineralocorticoid activity in particular, as well as parathyrin and calcitonin activity. The HRV-markers of sympathetic and vagal tones were not significantly different. Instead, the plasma level of triiodothyronine in females was 85 % of males. Among the recorded immune indices, 11 of them were significantly higher in females. First of all, it was the content in the thymocytogram of lymphocytes and lymphoblasts, in the immunocytogram of blood natural killers and B-lymphocytes as well as fibroblasts, macrophages and microphages in splenocytogram. Instead, the 10 indicators of immunity in females were significantly lower. This was, first of all, the activity of phagocytosis of microphages and macrophages. The discrepancies between neuroendocrine-immune complexes of intact and stressed rats of both sexes were exhaustively explained by 38 parameters including 6 of thymus, 6 of spleen, 13 of blood and 13 of neuroendocrine ones. The information contained in these metrics can be condensed in three recognition roots. Intact and stressed males were clearly delimited along the axis of the second root which reflected the post-stress decrease in the phagocytic activity of monocytes in the blood and the content of basophils and segmental neutrophils in it, on the one hand and the increase in the

mass of thymus and the content of Hassall's cells in it, and the increase of the sympathetic tone and corticosterone - on the other hand. Post-stress changes of the listed parameters in general were insignificant in females. Significant differences between intact and stressed females were reflected along the third root axis which contains the information on stress induced lowering levels of parathyrin and thyroxin, the intensity of *Staph aureus* phagocytosis by microphages and bactericidal capacity of blood macrophages, its content of eosinophils as well as the mass of the spleen. Instead, post-stress changes in the listed parameters were generally insignificant in males.

Conclusions. The obtained data indicate the need to take into account the sexual differences in the reactions of the neuroendocrine-immune complex to stress when testing the stress limiting means.

Key words: sexual difference, neuroendocrine-immune complex, stress, immunological parameters, immunogram, neuroendocrine indices.