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The monograph presents the literature data and the results of its own study of interindividual differences in the immune responses of rats of both sexes to chronic restraint stress and their neuro-endocrine accompaniment. The neuroendocrine-immune interrelations and sexual dimorphism are analyzed in detail.

For physiologists, endocrinologists, immunologists.

Key words: Immune Responses; Chronic Stress; Neuro-Endocrine Accompaniment.

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CONTENT

INTRODUCTION

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 FS et coworkers [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.

It is important to note that significant individual differences in stress perception, processing, and coping have been observed [Dhabhar FS, McEwen BS, 2007; Gunnar M, Quevedo K, 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 FS, McEwen BS, Spencer RL, 1993], adaptation to stress [Dhabhar FS, McEwen BS, Spencer RL, 1997], and in distribution and activation of adrenal steroid receptors and corticosteroid-binding globulin levels [Dhabhar FS, Miller AH, McEwen BS, 1995], suggest that genetic as well as environmental factors play a role in establishing individual differences [Gomez-Serrano M, Tonelli L, Listwak S, 2001].

Inspired by the ideas of Dhabhar FS laboratory, in 2012, we conducted an experimental study in rats to identify the diversity of immune responses to chronic stress and to compare changes in immunity parameters with changes in neuro-endocrine parameters. In our studies, the traditional “Big Three” was supplemented with sympathetic and vagal tone, androgenic, mineralocorticoid, parathyroid and calcitonin activities, as well as sexual dimorphism of the neuroendocrine-immune complex in intact and stressed animals was analyzed.

Key words: Chronic stress, Autonomic nervous system, Stressory hormones, Immune cell distribution, Immune function, Interindividual differences, Sexual dimorphism, Neuroendocrine-Imune relationships.

SECTION 1

STRESS AND IMMUNITY (review of literature)

Since the landmark discovery of stress by Hans Selye, numerous studies by his laboratory and followers [Selye H, 1936-1987; Meerson FZ, 1986,1993; McEwen BS, 1998; Stress of Life, 1998; Garkavi LKh et al, 1990,1998; Neuroimmunomodulation, 2000; Radchenko OM, 2004; Reznikov OG et al, 2004; Ader R, 2007; Baraboy VA, Reznikov OG, 2013]. These studies show that chronic or long-term stressors can have health-aversive effects, some of which are mediated through immune mechanisms [Dhabhar FS, 2009; Dhabhar FS, Malarkey WB, Neri E, 2012; Padro CJ, Sanders VM, 2014]. However, it is also important to appreciate that a psychophysiological stress response is one of nature's fundamental survival mechanisms. Without a fight-or-flight stress response, a lion has no chance of catching a gazelle, just as the gazelle has no chance of escape. During such short-term stress responses observed in nature, physiological systems act in synchrony to enable survival. Therefore, authors hypothesized that just as the stress response prepares the cardiovascular, musculoskeletal and neuroendocrine systems for fight or flight, under certain conditions, stress may also prepare the immune system for challenges (e.g. wounding or infection) that may be imposed by a stressor (e.g. predator or surgical procedure) [Dhabhar FS, Miller AH, McEwen BS, 1995; Dhabhar FS, McEwen BS, 2001; Dhabhar FS, 2014]. Studies have shown that short duration stressors induce a redistribution of immune cells within the body and that immune function is significantly enhanced in organs like the skin to which leukocytes traffic during acute stress. Studies have also identified mechanisms involving dendritic cell, neutrophil, macrophage, and lymphocyte trafficking, maturation, and function through which acute stressors may enhance innate as well as adaptive immunity. We suggest that the acute stress response may serve as an endogenous psychophysiological adjuvant that enhances immune responses and may have evolved by virtue of the fact that many stressful situations (aggression, accident) result in immune activation (wounding, infection) and vice versa. Interestingly, in modern times, many situations involving immune activation (vaccination, surgery, injury) also induce a stress response. It is also important to recognize that while acute stress-induced immunoenhancement may serve to increase immunoprotection during exposure to infectious agents or wounding, it may also exacerbate immunopathology if the enhanced immune response is directed against innocuous or self-antigens, or dysregulated following prolonged activation as seen during chronic stress. In contrast to acute stress, chronic stress has been shown to dysregulate immune responses [Glaser R, Kiecolt-Glaser JK, 2005; Chrousos GP, Kino T, 2007] by altering the cytokine balance from type-1 to type-2 cytokine-driven responses [Glaser R, MacCallum RC, Laskowski BF, 2001] and accelerating immunosenescence [Epel E, Blackburn EH, Lin J, 2004], and to suppress immunity by decreasing numbers, trafficking [Dhabhar FS, McEwen BS, 1997] and function of protective immune cells while increasing regulatory/suppressor T cells [Saul AN, Oberyszyn TM, Daugherty C, 2005].

Stress: Definition, Mediators, and Individual Differences. Although the word ‘stress' generally has negative connotations, stress is a familiar and ubiquitous aspect of life, being a stimulant for some, but a burden for many others. Numerous definitions have been proposed for the concept of stress. Each definition focuses on aspects of an internal or external challenge, disturbance, or stimulus, on perception of a stimulus by an organism, or on a physiological response of the organism to the stimulus [Goldstein DS, McEwen B, 2002; Sapolsky RM, 2005]. Physical stressors have been defined as external challenges to

homeostasis and psychological stressors as the ‘anticipation justified or not, that a challenge to homeostasis looms' [Sapolsky RM, 2005]. An integrated definition states that stress is a constellation of events, consisting of a stimulus (stressor), that precipitates a reaction in the brain (stress perception), that activates physiological fight-or-flight systems in the body (stress response) [Dhabhar FS, McEwen BS, 1997]. It is important to understand that the only way that a stressor can affect the brain or body is by inducing biological changes in the organism. Therefore, the physiological stress response is critical for mediating the effects of stress on health. This response results in the release of neurotransmitters, hormones, peptides and other factors into the circulation or locally within tissues. Even cytokines, factors that were traditionally thought to be the domain of the immune system, have relatively recently been shown to be released in the systemic circulation during psychological stress [Altemus M, Rao B, Dhabhar FS, 2001; Steptoe A, Hamer M, Chida Y, 2007; Puterman E, Epel ES, O’Donovan A, 2014; Aschbacher K, Epel E, Wolkowitz OM, 2012]. The major mediators of stress effects are norepinephrine and epinephrine that are released by the sympathetic nervous system, and corticotropin-releasing hormone, adrenocorticotropin (ACTH), and cortisol, that make up the hypothalamic-pituitary-adrenal (HPA) axis. Since virtually every cell in the body expresses receptors for one or more of these factors, they can induce changes in almost all cells and tissues and inform them about the presence of a stressor [Foley P, Kirschbaum C, 2010].

Although stress can be harmful when it is chronic or long-lasting [Glaser R, Kiecolt-Glaser JK, 2005; Chrousos GP, Kino T, 2007], it is often overlooked that a stress response has salubrious adaptive effects in the short run [Dhabhar FS, Viswanathan K, 2005; Dhabhar FS, McEwen BS, 2007; Dhabhar FS, 2009]. Therefore, major distinguishing characteristics of stress are duration and intensity. Acute stress has been defined as stress that lasts for a period of minutes to hours, and chronic stress that persists for several hours per day for weeks or months [Dhabhar FS, McEwen BS, 1997].

However, in the Reznikov OG laboratory in studies of the effects of chronic prenatal stress on the offspring of rats, chronic stress is modeled by rigid hourly immobilization of females during the last week (from the 15th to the 21st day) of pregnancy. The authors have shown for the first time that a certain level of reactivity, ie sensitivity to physiological stimuli, physiologically active substances is programmed in early ontogeny. The consequences of these imprinting processes are mainly functional manifestations. In particular, prenatal stress alters the stress reactivity of the HPA axis. Although in the state of physiological rest, the level of corticosterone in the plasma of prenatal stressed males did not differ from that intact (0,70±0,02 versus 0,80±0,02 μM/L), increasing it after acute immobilization stress (1-hour immobilization) was three times smaller than that of intact rats (54% vs 162% or 1,1 μM/L vs 2,0 μM/L). This was associated with delayed releasing of ACTH secretory granules from corticotropocytes of the adenohypophysis and the absence of a stressor decrease in the concentration of norepinephrine in the hypothalamus. On the other hand, the adrenergic sensitivity of the hypothalamus to the introduction of norepinephrine into the III ventricle was even slightly increased in comparison with intact tissues. That is, there is damage to the higher centers of regulation of adrenocortical stress reactivity. According to the authors, it is likely that there is an increase in the number of glucocorticoid receptors of the second type in the hippocampus and frontal cortex, which increases the inhibitory effect of circulating glucocorticoids on stress-induced activation of corticosteroid secretion. Instead, adult females (3-4 months) born from prenatal stressed females respond to acute stress similar to intact ones, namely, a decrease in norepinephrine in the hypothalamus (from 8,4 to 6,0 versus from 8,1 to 6,0 nM/g).) and increased corticosterone levels in the blood (from 1,9 to 3,3 vs 1,4 to 2,4 μM/L) [Reznikov OG et al, 1998-2004].

Dysregulation of the circadian cortisol rhythm is one marker that appears to coincide with

the deleterious effects of chronic stress [Sephton S, Spiegel D, 2003]. The intensity of stress may be gauged by the peak levels of stress hormones, neurotransmitters, and other physiological changes such as increases in heart rate and blood pressure, and by the amount of time for which these changes persist during stress and following the cessation of stress.

The ability of humans to generate and experience internal psychological stressors in the absence of external stressors can result in long-term activation of the physiological stress response that often has deleterious effects. The magnitude and duration of stress-induced elevations in catecholamine and glucocorticoid hormones can have significant effects on immune cell distribution and function [Schwab CL, Fan R, Zheng Q, 2005; Benschop RJ, Rodriguez-Feuerhahn M, Schedlowski M, 1996].

Immune Responses Defined in Terms of Their End Effects: Immunoprotective, Immunopathological, and Immunoregulatory/Inhibitory. While discussing immune responses, it is useful to categorize them in terms of their principal cellular and molecular components [Dhabhar FS; 2009; 2018]. For example, innate, adaptive, type-1 and type-2 cytokine-driven immune responses are all defined in terms of their cellular and cytokine components. In addition to these categorizations, it is also useful to define immune responses in terms of their end-effects. Therefore, we suggest that immune responses can be categorized as being immunoprotective, immunopathological, and immunoregulatory/inhibitory. It is important to bear in mind that while all these categories provide useful constructs with which to organize ideas, concepts and models, an overall in vivo immune response is likely to consist of several types of responses with varying amounts of dominance from each category. Each of the proposed end-effect-based categories is defined below.

Immunoprotective responses are defined as responses that promote efficient wound healing, eliminate viral infections and cancer, and mediate vaccine-induced immunological memory. Key characteristics of immunoprotection involve active immune surveillance, a rapid and robust response upon immune activation, efficient clearance of the activating agent or pathogen, followed by rapid resolution of inflammation. Immunoprotective responses are critical for completion of the proliferative and remodeling phases of wound healing. Wound healing is important not only for frank wounds where the initiating event is tissue damage itself, but also for tissue-intrinsic ‘wounds' where the initiating event is an immune response precipitated by intracellular infection during which there can be collateral tissue damage. Innate and/or adaptive type-1 or type-2 immune responses can all confer immunoprotection depending on the type of the pathogen (viral, bacterial, protozoan, fungal, helminthic), on whether it is intra- or extracellular, and on the accompanying wounding conditions (e.g. sterile, infected, external or internal).

Immunopathological responses are defined as those that are directed against self- (autoimmune disease like multiple sclerosis, arthritis, lupus) or innocuous antigens (asthma, allergies) and responses that involve chronic, non-resolving inflammation. Immunopathology is also involved during low-level, long-term elevations in local and/or systemic inflammatory mediators that are thought to contribute to disorders like cardiovascular disease, obesity, and depression [Van Gaal LF, Mertens IL, De Block CE, 2006; Dantzer R, O'Connor JC, Freund GG, 2008].

Immunoregulatory/inhibitory responses are defined as those that involve immune cells and factors that inhibit the function of other immune cells. Although the previous concept of suppressor T cells became mired in controversy, recent studies suggest that there is an arm of the immune system that functions to inhibit immune responses [Simpson E, 2008; Wing K, Sakaguchi S, 2010]. Regulatory $CD4^{+}CD25^{+}FoxP3^{+}$ T cells, interleukin (IL)-10, and TGF-β have been shown to have immunoregulatory/inhibitory functions. The physiological function of these factors is to keep proinflammatory, allergic, and autoimmune responses in check [Bluestone JA, Tang Q, 2005; Wing K, Sakaguchi S, 2010]. However, it has also been

suggested that immunoregulatory/inhibitory factors may suppress antitumor immunity and be indicative of negative prognosis for cancer [Finn OJ, 2008; Olson BM, McNeel DG, 2013; Whiteside TL, 2014].

Factors That May Determine Whether Stress Will Enhance or Suppress Immune Function and the Potential Health Consequences of These Effects of Stress. Several critical factors are likely to influence the direction (enhancing vs. suppressive) of the effects of stress or stress hormones, and the nature of the immune response (immunoprotective, immunoregulatory/inhibitory, or immunopathological) that is affected [Dhabhar FS, 2009]. These include: the effects of stress on leukocyte distribution in the body; the duration (short-term/acute vs. long-term/chronic) of stress; the differential effects of physiologic versus pharmacologic concentrations of glucocorticoids, and the differential effects of endogenous (e.g. cortisol, corticosterone) versus synthetic (e.g. dexamethasone) glucocorticoids, and the timing of stressor or stress hormone exposure relative to the time of activation and ensuing time course of the immune response. It is important to recognize that factors such as gender, genetics, age, the route of administration and nature of the immunizing antigen, and time of day, may additionally affect the relationship between stress and immune function.

It is also important to bear in mind that whether a stressor enhances or suppresses immune function, it is the end-effect of the affected immune response that affects the health of the organism or individual. Given the definitions in the preceding section, stress-induced enhancement of immunoprotection is likely to have beneficial effects while stress-induced suppression of immunoprotection is likely to be harmful. Similarly, stress-induced enhancement of immunopathology or long-term proinflammation is also likely to be harmful. Finally, stress-induced enhancement of active immunoregulation/inhibition is likely to be beneficial in case of autoimmune and proinflammatory disorders and harmful in case of infections and cancer.

Stress-Induced Changes in Immune Cell Distribution. Effective immunoprotection requires rapid recruitment of leukocytes into sites of surgery, wounding, infection, or vaccination. Immune cells circulate continuously on surveillance pathways that take them from the blood, through various organs, and back into the blood. This circulation is essential for the maintenance of an effective immune defense network [Sprent J, Tough DF, 1994]. The numbers and proportions of leukocytes in the blood provide an important representation of the state of distribution of leukocytes in the body and of the state of activation of the immune system. The ability of acute stress to induce changes in leukocyte distribution within different body compartments is perhaps one of the most underappreciated effects of stress and stress hormones on the immune system [Dhabhar FS, Miller AH, McEwen BS, 1995].

Numerous studies have shown that stress and stress hormones induce significant changes in absolute numbers and relative proportions of leukocytes in the blood. In fact, changes in blood leukocyte numbers were used as a measure of stress before methods were available to directly assay the hormone [Hoagland H, Elmadjian F, Pincus G, 1946]. Studies have also shown that glucocorticoid [Dhabhar FS, Miller AH, McEwen BS, 1996] and catecholamine hormones [Benschop RJ, Rodriguez-Feuerhahn M, Schedlowski M, 1996] induce rapid and significant changes in leukocyte distribution and that these hormones are the major mediators of the effects of stress. Stress-induced changes in blood leukocyte numbers have been reported in numerous species including humans [Bilbo SD, Dhabhar FS, Viswanathan K, 2002; Redwine L, Mills PJ, Sada M, 2004; Rosenberger PH, Ickovics JR, Epel E, 2009]. This suggests that the phenomenon of stress-induced leukocyte redistribution has a long evolutionary lineage, and that perhaps it has important functional significance.

Studies have shown that stress-induced changes in blood leukocyte numbers are characterized by a significant decrease in numbers and percentages of lymphocytes and monocytes, and by an increase in numbers and percentages of neutrophils [Dhabhar FS,

Miller AH, Stein M, 1994]. Flow cytometric analyses revealed that absolute numbers of peripheral blood T cells, B cells, NK cells, and monocytes all show a rapid and significant decrease (40–70% lower than baseline) during stress [Dhabhar FS, Miller AH, McEwen BS, 1995]. Moreover, it has been shown that stress-induced changes in leukocyte numbers are rapidly reversed upon the cessation of stress [Dhabhar FS, Miller AH, McEwen BS, 1995]. In apparent contrast to animal studies, human studies have shown that stress increases rather than decreases blood leukocyte numbers [Mills PJ, Berry CC, Dimsdale JE, 1995; Schedlowski M, Jacobs R, Stratman G, Richter S, 1993]. This apparent contradiction may be resolved by taking the following factors into consideration: First, stress-induced increases in blood leukocyte numbers in humans have been studied using stress conditions which result in the activation of primarily the sympathetic nervous system. These stressors are often of a short duration (few minutes) or relatively mild (e.g. public speaking) [Mills PJ, Berry CC, Dimsdale JE, 1995; Schedlowski M, Jacobs R, Stratman G, Richter S, 1993]. Second, the increase in total leukocyte numbers may be accounted for mainly by stress- or catecholamine-induced increases in granulocytes and NK cells [Benschop RJ, Rodriguez-Feuerhahn M, Schedlowski M, 1996; Schedlowski M, Jacobs R, Stratman G, 1993; Naliboff BD, Benton D, Solomon GF, 1991; Mills PJ, Berry CC, Dimsdale JE, 1995]. Third, stress or pharmacologically induced increases in glucocorticoid hormones induce a significant decrease in blood lymphocyte and monocyte numbers [Dhabhar FS, Miller AH, McEwen BS, 1996; Schedlowski M, Jacobs R, Stratman G, 1993]. Thus, stress conditions that result in a significant and sustained activation of the HPA axis result in a decrease in blood leukocyte numbers.

It has been proposed that acute stress induces an initial increase followed by a decrease in blood leukocyte numbers [Dhabhar FS, McEwen BS, 2001]. Stress conditions that result in activation of the sympathetic nervous system, especially conditions that induce high levels of norepinephrine, may induce an increase in circulating leukocyte numbers. These conditions may occur during the beginning of a stress response, very-short-duration stress (order of minutes), mild psychological stress, or during exercise. In contrast, stress conditions that result in the activation of the HPA axis induce a decrease in circulating leukocyte numbers. These conditions often occur during the later stages of a stress response, long-duration acute stressors (order of hours), or during severe psychological, physical or physiological stress. An elegant and interesting example in support of this hypothesis comes from Schedlowski et al. [1993] who measured changes in blood T-cell and NK cell numbers as well as plasma catecholamine and cortisol levels in parachutists. Measurements were made 2 h before, immediately after, and 1 h after the jump. Results showed a significant increase in T-cell and NK cell numbers immediately (minutes) after the jump that was followed by a significant decrease 1 h after the jump. An early increase in plasma catecholamines preceded early increases in lymphocyte numbers, whereas the more delayed rise in plasma cortisol preceded the late decrease in lymphocyte numbers [Schedlowski M, Jacobs R, Stratman G, 1993]. Importantly, changes in NK cell activity and antibody-dependent cell-mediated cytotoxicity closely paralleled changes in blood NK cell numbers, thus suggesting that changes in leukocyte numbers may be an important mediator of apparent changes in leukocyte ‘activity’. Similarly, Rinner et al. [Rinner I, Schauenstein K, Mangge H, 1992] have shown that a short stressor (1 min handling) induced an increase in mitogen-induced proliferation of T and B cells obtained from peripheral blood, while a longer stressor (2 h immobilization) induced a decrease in the same proliferative responses. In another example, SB Manuck et al. [Manuck SB, Cohen S, Rabin BS, 1991] showed that acute psychological stress induced a significant increase in blood CTL numbers only in those subjects who showed heightened catecholamine and cardiovascular reactions to stress.

Thus, an acute stress response may induce biphasic changes in blood leukocyte numbers.

Soon after the beginning of stress (order of minutes) or during mild acute stress, or exercise, catecholamine hormones and neurotransmitters induce the body's ‘soldiers' (leukocytes), to exit their ‘barracks' (spleen, lung, marginated pool and other organs) and enter the ‘boulevards' (blood vessels and lymphatics). This results in an increase in blood leukocyte numbers, the effect being most prominent for NK cells and granulocytes. As the stress response continues, activation of the HPA axis results in the release of glucocorticoid hormones which induce leukocytes to exit the blood and take position at potential ‘battle stations' (such as the skin, lung, gastrointestinal and urinary-genital tracts, mucosal surfaces, and lymph nodes) in preparation for immune challenges which may be imposed by the actions of the stressor [Dhabhar FS, McEwen BS, 1996]. Such a redistribution of leukocytes results in a decrease in blood leukocyte numbers. Thus, acute stress may result in a redistribution of leukocytes from the barracks, through the boulevards, and to potential battle stations within the body.

Since the blood is the most accessible and commonly used compartment for human studies, it is important to carefully evaluate how changes in blood immune parameters might reflect in vivo immune function in the context of the specific experiments or study at hand. Moreover, since most blood collection procedures involve a certain amount of stress, since all patients or subjects will have experienced acute and chronic stress, and since many studies of psychophysiological effects on immune function focus on stress, the effects of stress on blood leukocyte distribution become a factor of considerable importance.

Dhabhar FS et McEwen BS [1999] were the first to propose that stress-induced changes in blood leukocyte distribution may represent an adaptive response. They suggested that acute stress-induced changes in blood leukocyte numbers represent a redistribution of leukocytes from the blood to organs such as the skin, draining sentinel lymph nodes, and other compartments [Dhabhar FS, McEwen BS, 1996; 2001]. They hypothesized that such a leukocyte redistribution may enhance immune function in compartments to which immune cells traffic during stress. In agreement with this hypothesis, it was demonstrated that a stress-induced redistribution of leukocytes from the blood to the skin is accompanied by a significant enhancement of skin immunity [Dhabhar FS, Satoskar AR, Bluethmann H, 2000].

Functional Consequences of Stress-Induced Changes in Immune Cell Distribution. When interpreting data showing stress-induced changes in functional assays such as lymphocyte proliferation or NK activity, it may be important to bear in mind the effects of stress on the leukocyte composition of the compartment in which an immune parameter is being measured. For example, it has been shown that acute stress induces a redistribution of leukocytes from the blood to the skin and that this redistribution is accompanied by a significant enhancement of skin cell-mediated immunity (CMI) [Dhabhar FS, Viswanathan K, 2005]. In what might at first glance appear to be contradicting results, acute stress has been shown to suppress splenic and peripheral blood responses to T-cell mitogens [Cunnick JE, Lysle DT, Kucinski BJ, 1990] and splenic IgM production [Zalcman S, Anisman H, 1993]. However, it is important to note that in contrast to the skin that is enriched in leukocytes during acute stress, peripheral blood and spleen are relatively depleted of leukocytes during acute stress [Dhabhar FS, 1998]. This stress-induced decrease in blood and spleen leukocyte numbers may contribute to the acute stress-induced suppression of immune function in these compartments.

Moreover, in contrast to acute stress, chronic stress has been shown to suppress skin CMI and a chronic stress-induced suppression of blood leukocyte redistribution is thought to be one of the factors mediating the immunosuppressive effect of chronic stress [Dhabhar FS, McEwen BS, 1997]. Again, in what might appear to be contradicting results, chronic stress has been shown to enhance mitogen-induced proliferation of splenocytes [Monjan AA, Collector MI, 1977] and splenic IgM production [Zalcman S, Anisman H, 1993]. However,

the spleen is relatively enriched in T cells during chronic glucocorticoid administration, suggesting that it may also be relatively enriched in T cells during chronic stress [Miller AH, Spencer RL, Hasset J, 1994], and this increase in spleen leukocyte numbers may contribute to the chronic stress-induced enhancement of immune parameters measured in the spleen.

It is also important to bear in mind that the heterogeneity of the stress-induced changes in leukocyte distribution [Dhabhar FS, Miller AH, McEwen BS, 1995] suggests that using equal numbers of leukocytes in a functional assay may not account for stress-induced changes in relative percentages of different leukocyte subpopulations in the cell suspension being assayed. For example, samples that have been equalized for absolute numbers of total blood leukocytes from control versus stressed animals may still contain different numbers of specific leukocyte subpopulations (e.g. T cells, B cells or NK cells). Such changes in leukocyte composition may contribute to the effects of stress even in functional assays using equalized numbers of leukocytes from different treatment groups. Therefore, stress may affect immune function at a cellular level (e.g. phagocytosis, antigen presentation, killing, antibody production) and/or through leukocyte redistribution that could increase or decrease the number of cells with a specific functional capacity in the compartment being studied.

Effects of Acute Stress on Leukocyte Trafficking to a Site of Surgery or Immune Activation. Viswanathan K. and Dhabhar FS [2005] used a subcutaneously implanted surgical sponge model to elucidate the effects of stress on the kinetics, magnitude, subpopulation, and chemoattractant specificity of leukocyte trafficking to a site of immune activation or surgery. Mice that were acutely stressed before subcutaneous implantation or the surgical sponge showed a two- to threefold higher neutrophil, macrophage, NK cell and T-cell infiltration than non-stressed animals. Leukocyte infiltration was evident as early as 6 h and peaked between 24 and 48 h. Importantly, at 72 h, sponges from non-stressed and acutely stressed mice had comparable and significantly lower leukocyte numbers indicating effective resolution of inflammation in both groups. These authors also examined the effects of stress on early (6 h) leukocyte infiltration in response to a predominantly proinflammatory cytokine, tumor necrosis factor-α (TNF-α), and lymphocyte-specific chemokine, lymphotactin (LTN). Acute stress significantly increased infiltration of macrophages, in response to saline, LTN or TNF-α; neutrophils, only in response to TNF-α, and NK and T cells only in response to LTN. These results showed that acute stress significantly enhances the kinetics and magnitude of leukocyte infiltration into a site of immune activation or surgery in a subpopulation and chemoattractant-specific manner, with tissue damage, antigen-, or pathogen-driven chemoattractants synergizing with acute stress to further determine the specific subpopulations that are recruited [Viswanathan K, Dhabhar FS, 2005]. Thus, depending on the primary chemoattractants driving an immune response, acute stress may selectively mobilize specific leukocyte subpopulations into sites of surgery, wounding, or inflammation. Such a stress-induced increase in leukocyte trafficking may be an important mechanism by which acute stressors alter the course of different (innate vs. adaptive, early vs. late, acute vs. chronic) protective or pathological immune responses.

Acute Stress-Induced Enhancement of Innate/Primary Immune Responses. In view of the skin being one of the target organs to which leukocytes traffic during stress, studies were conducted to examine whether skin immunity is enhanced when immune activation/antigen exposure occurs following a stressful experience. Studies showed that acute stress experienced at the time of novel or primary antigen exposure results in a significant enhancement of the ensuing skin immune response [Dhabhar FS, Viswanathan K, 2005]. Compared to controls, mice restrained for 2.5 h before primary immunization with keyhole limpet hemocyanin (KLH) showed a significantly enhanced immune response when re-exposed to KLH 9 months later. This immunoenhancement was mediated by an increase in numbers of memory and effector helper T cells in sentinel lymph nodes at the time of primary

immunization. Further analyses showed that the early stress-induced increase in T-cell memory may have stimulated the robust increase in infiltrating lymphocyte and macrophage numbers observed months later at a novel site of antigen re-exposure. Enhanced leukocyte infiltration was driven by increased levels of the type-1 cytokines, IL-2 and γ-interferon (IFN-γ), and TNF-α, observed at the site of antigen re-exposure in animals that had been stressed at the time of primary immunization. Given the importance of inducing long-lasting increases in immunological memory during vaccination, it has been suggested that the neuroendocrine stress response is nature's adjuvant that could be psychologically and/or pharmacologically manipulated to safely increase vaccine efficacy.

In a series of elegant experiments, Saint-Mezard P et al. [2003] similarly showed that acute stress experienced at the time of sensitization resulted in a significant increase in the contact hypersensitivity (CHS) response. These investigators showed that acute stress experienced during sensitization enhanced dendritic cell migration from skin to sentinel lymph nodes and also enhanced priming of lymph node CD8+ T cells. These CD8+ T cells responded in greater numbers at the site of antigen re-exposure during the recall phase of the CHS response. These studies also suggested that the effects of acute stress in this case were mediated primarily by norepinephrine [Saint-Mezard P, Chavagnac C, Bosset S,, 2003]. Other investigators have similarly reported stress-induced enhancement of type-1 cytokine-driven CMI [Wood PG, Karol MH, Kusnecov AW, 1993] and type-2 cytokine-driven humoral immunity [Cocke R, Moynihan JA, Cohen N, 1993].

Viswanathan K et al. [2005] further elucidated the molecular and cellular mediators of the immunoenhancing effects of acute stress. They showed that compared to non-stressed mice, acutely stressed animals showed significantly greater pinna swelling, leukocyte infiltration, and upregulated macrophage chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-3α (MIP-3α), IL-1α, IL-1β, IL-6, TNF-α, and IFN-γ gene expression at the site of primary antigen exposure. Stressed animals also showed enhanced maturation and trafficking of dendritic cells from skin to lymph nodes, higher numbers of activated macrophages in skin and lymph nodes, increased T-cell activation in lymph nodes, and enhanced recruitment of surveillance T cells to skin. These findings showed that important interactive components of innate (dendritic cells and macrophages) and adaptive (surveillance T cells) immunity are mediators of the stress-induced enhancement of a primary immune response. Such immunoenhancement during primary immunization may induce a long-term increase immunologic memory resulting in subsequent augmentation of the immune response during secondary antigen exposure.

In addition to elucidating mechanisms that could be targeted to reduce stress-induced exacerbation of allergic, autoimmune, and proinflammatory reactions, the above-mentioned studies provide further support for the idea that a psychophysiological stress response is nature's fundamental survival mechanism that could be therapeutically harnessed to augment immune function during vaccination, wound healing or infection.

Acute Stress-Induced Enhancement of Adaptive/Secondary Immune Responses. Studies have shown that in addition to enhancing primary cutaneous immune responses, acute stress experienced at the time of antigen re-exposure can also enhance secondary or recall responses in skin [Dhabhar FS, McEwen BS, 1996]. Compared to non-stressed controls, mice that were acutely stressed at the time of antigen re-exposure showed a significantly larger number of infiltrating leukocytes at the site of the immune reaction. These results demonstrated that a relatively mild behavioral manipulation can enhance an important class of immune responses that mediate harmful (allergic dermatitis) as well as beneficial (resistance to certain viruses, bacteria, and tumors) aspects of immune function.

Blecha F et al. [1982] reported a similar stress-induced enhancement of CHS reactions in mice, and Flint MS et al. [2000] showed that acute stress enhanced CHS responses in both

male and female mice and that immunoenhancement was partially dependent on glucocorticoid hormones, and a stress-induced enhancement of the elicitation phase of skin CMI has also been reported in hamsters [Bilbo SD, Dhabhar FS, Viswanathan K, 2002]. Taken together, studies show that acute stress can significantly enhance the immunization/sensitization/induction as well as the re-exposure/elicitation/recall phases of skin CMI.

Short-term Stress Induced Enhancement of Immune Function in the context of Cancer. Given the importance of cutaneous cell-mediated immunity in elimination of immuno-responsive tumors such as squamous cell carcinoma (SCC) [Kripke ML, 1994; Granstein RD, Matsui MS, 2004], and given the immuno-enhancing effects of short-term stress, studies have examined the effects of short-term stress administered at the time of ultraviolet light (UV) exposure (minimum erythemal dose, 3-times/week) on gene expression of chemokines and cytokines, infiltration of helper and cytolytic T cells that are critical for controlling and/or eliminating SCC and on tumor incidence, number and size [Dhabhar FS, Saul AN, Daugherty C, 2010]. Compared to controls, the short-term stress group showed greater cutaneous T-cell attracting chemokine (CTACK)/CCL27, RANTES, IL-12, and IFN-γ gene expression, higher infiltrating T cell numbers, lower tumor incidence, and fewer tumors early, but not later during tumor development. These results suggest that activation of short-term stress physiology increased chemokine expression and T cell trafficking and/or function during/following UV exposure, and enhanced Type 1 cytokine-driven cell-mediated immunity that is crucial for resistance to SCC.

Although much work remains to be done, these findings show that short-term stress enhances anti-tumor immunity just as it enhances other aspects of innate and adaptive immunity. These findings raise the tantalizing possibility that the physiological fight-or-flight stress response, and its adjuvant-like immuno-enhancing effects may provide a novel and important mechanism for enhancing immune system mediated tumor-detection/elimination that merits further investigation. These findings also suggest that the beneficial effects of exercise/physical activity in the context of cancer [Schmitz KH, 2011; Speck RM, Courneya KS, Masse LC, 2010; Betof AS, Dewhirst MW, Jones LW, 2013], may be at least partially mediated by activation of short-term stress physiology and it's adjuvant-like effects.

Hormone and Cytokine Mediators of Stress-Induced Enhancement of Immune Function. Although much work remains to be done, to identify molecular, cellular, and physiological mechanisms mediating the adjuvant-like, immunoenhancing effects of acute stress, several studies have begun to identify endocrine and immune mediators of these effects. Studies have shown that corticosterone and epinephrine are important mediators of an acute stress-induced immunoenhancement [Dhabhar FS, McEwen BS, 1999]. Adrenalectomy, which eliminates the glucocorticoid and epinephrine stress response, eliminated the stress-induced enhancement of skin CMI. Low-dose corticosterone or epinephrine administration significantly enhanced skin CMI [Dhabhar FS, McEwen BS, 1999]. In contrast, high-dose corticosterone, chronic corticosterone, or low-dose dexamethasone administration significantly suppressed skin CMI. These results suggested a novel role for adrenal stress hormones as endogenous immunoenhancing agents. They also showed that stress hormones released during a circumscribed or acute stress response may help prepare the immune system for potential challenges (e.g. wounding or infection) for which stress perception by the brain may serve as an early warning signal. Studies by Flint MS et al. [2000] have also suggested that corticosterone is a mediator of the stress-induced enhancement of skin CHS, while Saint-Mezard P et al. [2003] have suggested that the adjuvant-like effects of stress on dendritic cell and $CD8^+$ T-cell migration and function are mediated by norepinephrine.

Nance DM and Sanders VM [2007; 2012] have elucidated the role of the beta-adrenergic receptor in regulating lymphocyte function, and have shown that the level of activation is

influenced by the time of receptor engagement relative to the state of activation and/or differentiation of the lymphocyte and by the cytokine milieu. Taken together, these studies suggest that endogenous stress hormones in physiological concentrations can have immuno-enhancing effects, while endogenous hormones at pharmacologic concentrations, and synthetic hormones, are immuno-suppressive.

Studies have also examined the immunological mediators of an acute stress-induced enhancement of skin immunity. Since IFN-γ is a critical cytokine mediator of CMI and delayed as well as CHS, studies were conducted to examine its role as a local mediator of the stress-induced enhancement of skin CMI [Dhabhar FS, Satoskar AR, Bluethmann H, 2000]. The effect of acute stress on skin CMI was examined in wild-type and IFN-γ receptor gene knockout mice (IFN-γR–/–) that had been sensitized with 2,4-dinitro-1-fluorobenzene (DNFB). Acutely stressed wild-type mice showed a significantly larger CMI response than non-stressed mice. In contrast, IFN-γR–/– mice failed to show a stress-induced enhancement of skin CMI. Immunoneutralization of IFN-γ in wild-type mice significantly reduced the stress-induced enhancement of skin CMI. In addition, an inflammatory response to direct IFN-γ administration was significantly enhanced by acute stress. These results showed that IFN-γ is an important local mediator of a stress-induced enhancement of skin CMI [Dhabhar FS, Satoskar AR, Bluethmann H, 2000].

Another important immunological effect of short-term stress is to induce a significant increase in concentrations of circulating cytokines such as IL-6 and IL-1β [Steptoe A, Hamer M, Chida Y, 2007; Puterman E, Epel ES, O'Donovan A, 2014; Aschbacher K, Epel E, Wolkowitz OM, 2012]. Importantly, this increase is observed in response to psychological stressors such as the Trier Social Stress Test (TSST) and in the absence of immune activating events such as a wound, or antigen/pathogen inoculation. Dhabhar FS [2018] suggest that such short-term stress-induced increases in circulating cytokines may be an additional systemic mechanism mediating stress-induced enhancement of immune function. Interestingly, short-term stress-induced increases in circulating cytokines are related to changes in emotional states experienced during stress. For example, IL-1β reactivity during stress is a significant mediator of the relationship between a decline in positive affect and cognitions during stress, and an increase in depressive symptoms one year later [Aschbacher K, Epel E, Wolkowitz OM, 2012]. Such mediation is particularly salient given the known role of proinflammatory cytokines in inducing sickness behavior, depressive states, and depression [Capuron L, Miller AH, 2011; Miller AH, Haroon E, Raison CL, 2013] and in important reciprocal immune-to-neural signaling [Quan N, 2014].

In another interesting example, anger experienced during a stressor is related to a stress-induced increase in circulating IL-6, however, perceived social support mitigates the effects of anger on IL-6 stress reactivity such that the greater the amount of social support, the lower the stress reactivity of IL-6. In light of these findings, it has been suggested that short-term stress-induced increases in IL-6 and other pro-inflammatory cytokines may confer a survival advantage by facilitating immuno-enhancement during/following short-term stress [Puterman E, Epel ES, O'Donovan A, 2014]. Dhabhar FS [2018] have speculated that individuals with low social support may be more likely to be "out on their own," and have to fend for themselves, and as a result be more susceptible to attack and/or injury. Therefore, such individuals may mount a more robust immunological stress response. Furthermore, an angry individual may be more likely to engage in an aggressive encounter, i.e., choose to fight rather than flee, and as a result may be more likely to need enhanced immune defenses to heal wounds (incurred during the fight) and to defend against accompanying pathogen entry. Such evolutionary underpinnings may partially explain the association among emotional states and stress-reactivity of proinflammatory cytokines. As with most psychological and biological processes, activating this response too frequently or for too long (especially in the absence of

a wound or infection), may result in greater long-term exposure to proinflammatory factors resulting in their deleterious health consequences. Such chronic effects may underlie the proinflammatory milieu that is often observed during various disorders [Schedlowski M, Engler H, Grigoleit JS, 2014] like major depression, alcohol addiction and posttraumatic stress disorder [Pace TW, Miller AH, 2009; Pace TW, Heim CM, 2011; Wieck A, Grassi-Oliveira R, 2014], and in some cases may be facilitated by the genetic makeup of an individual [Fredericks CA, Drabant EM, Edge MD, 2009].

Acute Stress Psychophysiology as an Endogenous Adjuvant. We initially suggested that just as the acute stress response prepares the cardiovascular, musculoskeletal, and neuroendocrine systems for flight or flight, it may also prepare the immune system for challenges such as wounding and infection that are likely to result due to the actions of the stressor (predator, or process of undergoing surgery) [Dhabhar FS, McEwen BS, 1996; Dhabhar FS, 1998]. Upon seeing the evidence in support of the above hypothesis, we put forth the novel hypothesis that a psychophysiological stress response is nature's fundamental survival mechanism that could be therapeutically harnessed to augment immune function during vaccination, wound healing or infection [Dhabhar FS, Viswanathan K, 2005]. In keeping with this hypothesis, studies conducted by our group have shown that patients undergoing knee surgery, who show a robust and adaptive immune cell redistribution profile during the acute stress of surgery, also show significantly enhanced recovery. Similarly, studies conducted by Edwards et al. [Edwards KM, Burns VE, 2006, 2008] have shown that acute stressors can enhance vaccine-induced humoral and CMI in human subjects. Further research is required to test the hypothesis that behavioral and/or pharmacological induction of the acute psychophysiological stress response can be used therapeutically to enhance protective immunity during wound healing, infection, and cancer and to enhance vaccine efficacy. Such intervention is likely to allow for safe and effective enhancement of protective immunity because it would tap into the body's natural, endogenous adjuvant mechanisms to enhance immune function.

Chronic Stress Can Suppress Immunoprotection, While Enhancing Immunopathological and Immunoregulatory/Suppressive Responses. In contrast to acute stressors, chronic stress has been shown to suppress type-1 cytokine-driven protective immune responses while enhancing proinflammatory and type-2 cytokine-driven immune responses. Briefly, chronic stress has been shown to suppress wound healing [Mercado AM, Padgett DA, Sheridan JF, 2002; Christian LM, Graham JE, Padgett DA, 2006] and vaccine [Kiecolt-Glaser JK, Glaser R, 1999] related immune responses. Chronic stress has also been shown to increase susceptibility to viral [Fagundes CP, Glaser R, Johnson SL, 2012; Cohen S, Hamrick N, Rodriguez MS, 2002] and bacterial [Rojas IG, Padgett DA, Sheridan JF, 2002] infection. Chronic stress also appears to mobilize immunoregulatory/inhibitory mechanisms. Therefore, chronic stress is likely to exacerbate proinflammatory diseases and increase susceptibility to cancer. This topic has been the subject of many excellent reviews (such as: [Sanders VM, Straub RH, 2002; Glaser R, Kiecolt-Glaser JK, 2005; Irwin MR, 2008; Webster Marketon JI, Glaser R, 2008; Straub RH, Bijlsma JW, Masi A, 2013; Fagundes CP, Glaser R, Kiecolt-Glaser JK, 2013; Padro CJ, Sanders VM, 2014; Vitlic A, Lord JM, Phillips AC, 2014]).

Dhabhar FS and McEwen BS [1997] conducted studies designed to examine the effects of increasing the intensity and duration of acute stress as well as the transition from acute to chronic stress on skin immune function. These studies showed that acute stress administered for 2 h prior to antigenic challenge significantly enhanced skin CMI [Dhabhar FS, McEwen BS, 1997]. Increasing the duration of stress from 2 to 5 h produced the same magnitude immunoenhancement. Interestingly, increasing the intensity of acute stress produced a significantly larger enhancement of the CMI response that was accompanied by increasing magnitudes of leukocyte redeployment. In contrast, these studies found suppression of the

skin immune response when chronic stress exposure was begun 3 weeks before sensitization and either discontinued upon sensitization, or continued an additional week until challenge, or extended for 1 week after challenge [Dhabhar FS, McEwen BS, 1997]. Interestingly, acute stress-induced redistribution of peripheral blood lymphocytes was attenuated with increasing duration of stressor exposure and correlated with attenuated glucocorticoid responsivity. These results suggested that stress-induced alterations in lymphocyte redeployment may play an important role in mediating the bidirectional effects of stress on cutaneous CMI [Dhabhar FS, McEwen BS, 1997]. An association between chronic stress and reduced skin CMI has also been reported in human subjects [Smith A, Vollmer-Conna U, Bennett B, 2004; Sephton SE, Dhabhar FS, Keuroghlian AS, 2009].

A chronic stress-induced decrease in baseline leukocyte numbers and leukocyte mobilization and trafficking from the blood to other body compartments is an important mediator of stress induced suppression of immune function [Dhabhar FS, McEwen BS, 1997]. In human and animal studies, chronic stress has also been shown to suppress different immune parameters examples of which include: CMI, antibody production, NK activity, leukocyte proliferation, skin homograft rejection, virus-specific T cell and NK cell activity, and anti-mycobacterial activity of macrophages from susceptible mouse strains [review: Dhabhar FS, 2018].

Accelerated biological aging is another important mechanism through which chronic stress suppresses/dysregulates immune function. In a seminal study, Epel E, Blackburn EH, Lin J [2004]. showed that blood lymphocytes and monocytes from women reporting high chronic stress levels have significantly shorter telomeres compared to leukocytes from women reporting low stress. Immune cell telomerase activity was also lower in the high stress women indicating a chronic stress induced decrease in their ability to rebuild shortened telomeres. The study concluded that “women with the highest levels of perceived stress had telomeres that were shorter on average by the equivalent of at least one decade of additional aging compared to low stress women”. Epel ES, Merkin SS, Cawthon R [2009] have also shown that the rate of telomere shortening predicts death from cardiovascular disease, and has significant deleterious effects [Blackburn EH, Epel ES, 2012]. Thus, chronic stress induced telomere attrition can have significant deleterious effects on immune function because it could lead to DNA replication errors and is also likely to result in suppression of immuno-protection and exacerbation of immune dysregulation and immuno-pathology.

Numerous studies have investigated the effects of chronic stress in the context of cancer [Chida Y, Hamer M, Wardle J, 2008]. In light of the immuno-suppressive effects of long-term stress, and given the importance of cell-mediated immunity in elimination of immuno-responsive tumors like squamous cell carcinoma [Kripke ML, 1994; Granstein RD, Matsui MS, 2004], studies have also investigated the effects of chronic stress on cancer emergence and progression [Saul AN, Oberyszyn TM, Daugherty C, 2005; Levi B, Benish M, Goldfarb Y, 2011]. Chronic stress significantly accelerated the emergence and progression of squamous cell carcinoma (SCC). Compared to non-stressed controls, chronically stressed mice had lower IFN-γ, CCL27/CTACK, and CD3ε gene expression and lower $CD4^+$ and $CD8^+$ T cells infiltrating within and around tumors. Chronically stressed mice also showed a shorter median time to first tumor and reached 50% incidence six weeks earlier than controls. Interestingly, stressed mice had higher numbers of tumor infiltrating and circulating regulatory/suppressor T cells than non-stressed mice. These studies showed that chronic stress increased susceptibility to UV-induced squamous cell carcinoma by suppressing skin immunity, Type 1 cytokines, and protective T cells, and increasing active immuno-suppressive mechanisms mediated by regulatory/suppressor T cells [Saul AN, Oberyszyn TM, Daugherty C, 2005]. Similarly, studies have shown that a high-anxious behavioral phenotype, that is likely to be associated with increased susceptibility to chronic stress, is associated with suppressed anti-

tumor immunity, and increased susceptibility to the emergence and progression of squamous cell carcinoma [Dhabhar FS, Saul AN, Holmes TH, 2012].

Given the immuno-suppressive effects of chronic stress, it may be hypothesized that under certain conditions, chronic stress could ameliorate autoimmune diseases. In an elegant series of experiments, Stefanski V, Hemschemeier SK, Schunke K [2013] showed that severe (but not moderate) social stress significantly reduced susceptibility to collagen-induced arthritis in Wistar rats, and that this effect was mediated by decreases in CD4, CD8 T cell numbers and macrophage infiltration at the site of collagen injection.

One would not recommend chronically stressing anyone, leave alone patients with autoimmune disease. However, there may be lessons to be learned from the above-mentioned studies. Important questions for future studies include: **1)** What are the physiological conditions and mechanisms under which chronic stress can exert immuno-suppressive effects in the absence of inducing proinflammatory effects? **2)** Does a chronic stress-induced increase in regulatory/suppressor T (Tregs) [Saul AN, Oberyszyn TM, Daugherty C, 2005], regulatory B cells [Dhabhar FS, Saul AN, Holmes TH, 2012], NK cells, dendritic cells or monocytes/macrophages mediate suppression of autoimmune responses? **3)** Is chronic stress induced amelioration of autoimmune disease observed in human subjects? **4)** If so, could some of the biological mechanisms mediating chronic stress-induced amelioration of autoimmune reactions be safely and selectively harnessed to treat autoimmune diseases without administering chronic stress? Clearly, more research is warranted into investigating whether chronic stress ameliorates autoimmune reactions in humans, delineating the conditions under which such amelioration is observed, and elucidating mechanisms with the goal of identifying targets for pharmacological or biobehavioral interventions [Dhabhar FS, 2018].

Importantly, it has also been suggested that chronic stress-induced exacerbation of inflammatory diseases such as rheumatoid arthritis may be mediated by a loss of immuno-suppression that is normally driven by sympathetic nerves that innervate the inflamed tissue, and by systemic secretion of cortisol through cytokine-induced activation of the hypothalamic-pituitary-adrenal axis [Straub RH, Kalden JR, 2009; Del Rey A, Wolff C, Wildmann J, 2010].

These studies showed that chronic stress increased susceptibility to UV-induced SCC by suppressing skin immunity, type-1 cytokines, and protective T cells, and increasing active immunosuppression through regulatory/suppressor T cells [Saul AN, Oberyszyn TM, Daugherty C, 2005]. In addition, chronic stress has also been shown to suppress several other indices of immunoprotection [Irwin M, Patterson T, Smith TL,, 1990; Bonneau RH, Sheridan JF, Feng N, 1991] and to enhance proinflammatory and type-2 cytokine-driven conditions and disorders [Elenkov IJ et al, 1996,1999,2000,2004; Glaser R, MacCallum RC, Laskowski BF, 2001].

Bidirectional Effects of Glucocorticoid Hormones on Immune Function. In contrast to the well-known immunosuppressive effects of glucocorticoids, several studies have revealed that glucocorticoid hormones also exert immunomodulating [Wilckens T, DeRijk R, 1997] and immunoenhancing effects [Dhabhar FS, McEwen BS, 2007; Dhabhar FS, 2008]. In general, pharmacological concentrations of glucocorticoids exert immunosuppressive effects, whereas under different conditions, physiologic concentrations may exert immunomodulatory, immunoenhancing, or immunosuppressive effects. It is important to recognize that the source (natural vs. synthetic) and concentration (physiologic vs. pharmacologic) of glucocorticoid hormones, the effects of other physiologic factors (hormones, cytokines, and neurotransmitters), and the state of activation of an immune parameter (naïve vs. activated leukocyte, early vs. late activation, etc.) are all important factors which ultimately determine the nature of the effects of glucocorticoids on a given

immune response. Immunoenhancing effects of glucocorticoids are discussed below, whereas immunosuppressive effects are discussed in the following section.

Acute stress-induced enhancement of skin CMI is mediated by adrenal stress hormones [Dhabhar FS, McEwen BS, 1999]. Adrenalectomy, which eliminates the glucocorticoid and epinephrine stress response, eliminated the stress-induced enhancement of skin CMI. Low-dose corticosterone or epinephrine administration significantly enhanced skin CMI and produced a significant increase in T-cell numbers in lymph nodes draining the site of the CMI reaction [Dhabhar FS, McEwen BS, 1999]. Moreover, simultaneous administration of these two stress hormones produced an additive increase in the skin CMI response. These results showed that hormones released during an acute stress response may help prepare the immune system for potential challenges (e.g. wounding or infection) for which stress perception by the brain may serve as an early warning signal [Dhabhar FS, McEwen BS, 1999].

A permissive role for glucocorticoids in antibody production was described over 30 years ago. Several investigators reported that low levels of cortisol were a necessary factor in cell culture media in order to obtain in vitro antibody production [Halliday WJ, Garvey JS , 1964]. Moreover, glucocorticoids were determined to be the critical permissive component present in serum supplements of culture media, and it was suggested that variability in antibody production assays may be the result of variability of glucocorticoid content in different batches of serum [Halliday WJ, Garvey JS , 1964]. Under some conditions, glucocorticoids have been shown to shift the balance of an immune response towards humoral immunity [Mosmann TR, Coffman RL, 1989]. Physiological doses of glucocorticoids have been shown to enhance immunoglobulin production by mitogen-stimulated human lymphocytes in culture [Grayson J, Dooley NJ, Koski IR, 1981], and glucocorticoids have been shown to stimulate B-cell number and antibody production in vitro and in vivo [Plaut M , 1987].

Studies examining the effects of corticosterone on T-lymphocyte proliferation in vitro demonstrate an important mechanism by which corticosterone may mediate the enhancement of immune function [Wiegers GJ, Labeur MS, Stec IE, 1995]. These studies have shown that during the early stages of T-cell activation, low levels of corticosterone potently enhance anti-T-cell receptor (TCR)-induced lymphocyte proliferation. Furthermore, they showed that corticosterone had to be present during the process of TCR activation in order to enhance the proliferative response. Other studies have suggested that low concentration corticosterone-induced enhancement of concanavalin A-stimulated mitogenesis of splenocytes from ADX animals may be mediated by the type-1 or mineralocorticoid receptor [Wiegers GJ, Reul JMHM, 1994]. Finally, it was shown that corticosterone increases T-cell responsiveness to IL-2 and proliferation under conditions of high cell densities in vitro, that mimic conditions that are likely to be found in lymph nodes in vivo [Wiegers GJ, Stec IE, Klinkert WE, 2001]. Thus, these in vitro studies indicate a possible mechanism by which stress and stress hormones may enhance immune function in vivo.

Several lines of evidence indicate that glucocorticoid stress hormones may act synergistically with cytokines to enhance specific immune reactions. Thus, while glucocorticoids inhibit the synthesis of cytokines under some conditions, they have also been shown to induce the release and potentiate the actions of cytokines under other conditions. For example, acute psychological stress has been shown to increase circulating TNF-α, IL-1β and IL-10 levels in human subjects [Altemus M, Rao B, Dhabhar FS, 2001] and acute stress increases circulating IL-1β and IL-6 in rodents [Takaki A, Huang QH, Somogyvari-Vigh A, 1994]. Glucocorticoids have been shown to induce the production of migration inhibitory factor [Calandra T, Bernhagen J, Metz CN, 1995]. Moreover, glucocorticoids synergistically enhance the induction of acute-phase proteins by IL-1 and IL-6 [Baumann H, Gauldie J, 1994]. Glucocorticoids similarly enhance the biological responses of other cytokines such as IL-2, IFN-γ, granulocyte colony-stimulating factor, granulocyte macrophage colony-

stimulating factor, and oncostatin M [Wiegers GJ, Reul JMHM, 1998].

Synergistic interactions between glucocorticoids and cytokines may be mediated by glucocorticoid-induced upregulation of cytokine receptors on target cells as determined by increased cytokine binding or cytokine receptor mRNA expression. For example, glucocorticoid-induced TNF receptor has been shown to promote survival and serve as a costimulatory receptor for T cells [Esparza EM, Arch RH, 2005], and glucocorticoids increase IL-1 binding to human peripheral blood B cells [Akahoshi T, Oppenheim JJ, 1988]. Glucocorticoids also act synergistically with IFN-γ to induce high-affinity Fcγ receptors on human monocytic cell lines [Girard MT, Hjaltadottir S, 1987] and stress-induced increases in endogenous glucocorticoids also appear to facilitate the expression of low-affinity Fcγ receptors on peritoneal macrophages [Kizaki T, Oh-Ishi S, 1996].

Several books and articles have extensively reviewed the anti-inflammatory or immunosuppressive effects of glucocorticoid hormones [Marx J, 1995]. It is apparent from these reviews that under specific conditions, glucocorticoids have been shown to suppress immunoglobulin, prostaglandin, leukotriene, histamine, and cytokine production, neutrophil superoxide production, macrophage function, mitogen- and antigen-induced lymphocyte proliferation, lymphocyte differentiation, NK cell activity and leukocyte migration and activation. Due to their potently immunosuppressive actions, glucorticoids are widely used in the clinic as anti-inflammatory agents [Schleimer RP, Claman HN, 1989].

Immunomodulatory Effects of Timing of Stress or Stress Hormone Administration Relative to the Timing of Immune Activation and the Time Course of the Ensuing Immune Response. Under certain conditions, physiological levels of endogenous glucocorticoids have immunoenhancing effects while under other conditions similar hormone levels suppress autoimmune and inflammatory reactions. Dhabhar FS [2009] hypothesize that these differential effects are achieved by differences in overall glucocorticoid sensitivity or receptivity of the immune response being affected. At the very beginning of an immune response, certain components such as leukocyte trafficking, antigen presentation, helper T-cell function, leukocyte proliferation, cytokine and chemokine function, and effector cell function may all be receptive to glucocorticoid-mediated immunoenhancement. In contrast, at a later, more advanced stage of an immune response these components may be more receptive to glucocorticoid-mediated immunosuppression. While this hypothesis needs to be tested through further experiments, examples from studies showing temporal differences in the sensitivity of immune reactions to the effects of physiologic concentrations of glucocorticoid hormones are presented below.

Studies examining the effects of corticosterone on T-lymphocyte proliferation in vitro support the hypothesis that there may be temporal differences in the receptivity of an immune response to the enhancing versus suppressive effects of endogenous glucocorticoid hormones [Wiegers GJ, Labeur MS, Stec IE, 1995]. These studies have shown that during the early stages of T-cell activation, low levels of corticosterone potently enhance anti-TCR-induced lymphocyte proliferation. However, during later stages of culture, the same levels of corticosterone suppress T-lymphocyte proliferation. Furthermore, Wiegers GI et al. [1995] showed that corticosterone had to be present during the process of TCR activation in order to enhance the proliferative response. If corticosterone was added to the culture system more than 2 h after the initiation of TCR activation, the enhancement of lymphoproliferation was not observed.

Nance DM and Sanders VM [2007; 2012] have elegantly elucidated the role of the beta-adrenergic receptor in regulating lymphocyte function, showing that the level of activation is influenced by the time of receptor activation relative to the state of activation and/or differentiation of the lymphocyte and by the cytokine milieu. Similar bimodal effects of catecholamines dependent on the state (early versus late) of progression of rheumatic disease

have also been shown [Straub RH, Bijlsma JW, Masi A, 2013]. It has been proposed that energy and volume regulation may be one important aspect of interactions between stress (and other) hormones and the immune system and that these factors may take on additional significance during chronic inflammatory conditions [Straub RH, 2012; 2014].

Interestingly, Wiegers GJ and Reul JMHM [1998] have shown that these bidirectional effects of corticosterone on different stages of T-lymphocyte proliferation are mediated by opposing effects of corticosterone on IL-2R versus the cytokine itself. Thus, during the early stages of lymphocyte proliferation, corticosterone induces an increase in IL-2Rα expression. This increases the IL-2 receptivity of lymphocytes and is reflected by an increase in lymphocyte proliferation [Wiegers GJ, Labeur MS, Stec IE, 1995]. Although corticosterone reduces the production of IL-2 under these conditions, this decrease is not rate-limiting at this stage since exogenously added IL-2 fails to increase proliferation. However, if corticosterone is administered at later stages, the enhancement in IL-2R expression is absent, while the suppression of IL-2 production is still present. Under these conditions, the availability of IL-2 does become rate-limiting and hence corticosterone suppresses the lymphoproliferative response. Thus, these studies indicate an important mechanism mediating an endogenous glucocorticoid-induced immunoenhancement during the early stages, and an endogenous glucocorticoid-induced immunosuppression during the later stages of an immune response.

In a series of seminal studies, Sternberg EM et al. [1992-2006] showed that decreased HPA axis reactivity to inflammatory stimuli results in increased susceptibility to experimental autoimmune deseases.

Glucocorticoid hormones may exert their protective immunosuppressive effects by inhibiting the production or actions of proinflammatory molecules as discussed previously. In addition, it has been hypothesized that glucocorticoids may suppress certain autoimmune reactions by inducing a shift towards a Th2 or humoral immune response [Chrousos GP, 1998, 2000; Elenkov IJ, 2004]. For example, stimulation of the HPA axis by inflammatory mediators released during the initiation of an autoimmune response results in increased plasma corticosterone. Increased corticosterone levels may shift the balance of the ongoing immune reaction from a Th1-directed (cell-mediated) response towards a Th2-directed (antibody-mediated) response, by promoting the production of IL-4 and suppressing the production of IL-2 [Danes RA, Araneo BA, 1989].

The Stress-Immune Spectrum. It is often overlooked that a stress response has salubrious adaptive effects in the short run [Dhabhar FS, Miller AH, 1995; Dhabhar FS, 2008], although stress can be harmful when it is long-lasting [Dhabhar FS, McEwen BS, 1997; Glaser R, Kiecolt-Glaser JK, 2005; McEwen BS, 1998]. In order to reconcile these seemingly contradictory effects of stress, we proposed that a stress response and its effects on immune function be viewed in the context of a stress spectrum [Dhabhar FS, McEwen BS, 1997]. One region of this spectrum is characterized by acute stress *or* eustress, i.e., conditions of short-duration stress that may result in immunopreparatory or immunoenhancing physiological conditions. An important characteristic of acute stress is a rapid physiological stress response mounted in the presence of the stressor, followed by a rapid shutdown of the response upon cessation of the stressor. The other region of the stress spectrum is characterized by chronic stress *or* distress, i.e., repeated or prolonged stress which may result in dysregulation or suppression of immune function. An important characteristic of chronic stress is that the physiological response either persists long after the stressor has ceased, or is activated repeatedly to result in an overall integrated increase in exposure of the organism to stress hormones. The concept of ‘allostatic load’ has been proposed to define the “psychophysiological wear and tear” that takes place while different biological systems work to stay within a range of equilibrium (allostasis) in response to demands placed by internal or external chronic stressors [McEwen BS, 1998; Juster RP, McEwen BS, Lupien SJ, 2010].

Dhabhar FS and McEwen BS [1997] suggest that conditions of high allostatic load would result in dysregulation or suppression of immune function. Importantly, a disruption of the circadian corticosterone/cortisol rhythm may be an indicator and/or mediator of distress or high allostatic load. The stress spectrum also proposes that acute or chronic stress is generally superimposed on a psychophysiological health maintenance equilibrium. The extent and efficiency with which an organism returns to its health maintenance equilibrium after stress depends on resilience, which we define as reserve capacity of psychophysiological systems to recover from challenging conditions. Factors such as coping mechanisms, sense of control, optimism, social support, early life experiences, learning, genetics, and sleep may be important mediators of psychological resilience. Factors such as neuroendocrine reactivity, genetics, environment, nutrition, and sleep may be important mediators of physiological resilience. The psychophysiological basis of resilience and reserve capacity are underinvestigated and provide an important opportunity for future research.

The stress spectrum, taken together with the preceding discussion, shows that the duration, intensity/concentration, and timing of exposure to stressor-induced physiological activation (neurotransmitters, hormones, and their molecular, cellular, organ-level and systemic effects) are critical for determining whether stress will enhance or suppress/dysregulate immune function. The model shows that the stressor itself can be acute or chronic. Stress perception and processing by the brain and mechanisms mediating psychological and physiological resilience are critical for determining the duration and magnitude of the physiological stress response. Psychological resilience mechanisms are especially important in humans because they can limit the duration and magnitude of chronic stress responses. Psychogenic stressors are also very important in human subjects because they can generate stress responses long after stressor exposure (e.g. posttraumatic stress disorder following a severe traumatic experience, or in a milder form, lingering anger/mood disturbance following a social altercation) or even in the absence of a physical stressor or salient threat (e.g. worrying about whether one's romantic feelings will be reciprocated). Therefore, following stressor exposure and its processing by the brain, there ensues a physiological stress response. This response may consist of acute or chronic physiological activation (neurotransmitters, hormones, and their molecular, cellular, organ-level and systemic effects) which results in psychophysiological states that have different effects on overall health and immune function. While there is significant evidence from animal studies to support this model, it needs to be further examined in studies involving human subjects.

Conclusion. Stress has long been suspected to play a role in the etiology of many diseases, and numerous studies have shown that stress can be immunosuppressive and hence may be detrimental to health. Moreover, glucocorticoid stress hormones are widely regarded as being immunosuppressive, and are used clinically as anti-inflammatory agents. However, studies have shown that the acute stress response may play a critical adaptive and protective role, with stress hormones and neurotransmitters preparing the immune system for potential challenges (e.g. wounding or infection) that are perceived by the brain (e.g. the detection of predator or attacker) [Dhabhar FS, McEwen BS, 1997; Dhabhar FS, Viswanathan K, 2005; Dhabhar FS, Miller AH, Stein M, 1994; Dhabhar FS, McEwen BS, 1996; Viswanathan K, Dhabhar FS, 2005]. It may be useful to further study, and clinically harness, the acute stress response that evolution has finely sculpted to be one of nature's crucial survival mechanisms, at least as much as we study its maladaptive ramifications (chronic stress) that evolution yet has to catch up with.

This paper illustrates the complex role that stress and stress hormones play as modulators and regulators of an immune response. Stress and glucocorticoid hormones can either enhance or suppress immune function depending on the following factors: (1) The duration (acute vs. chronic) of stress. (2) Changes in leukocyte distribution within the body, and the

compartments in which the immune response occurs. (3) The concentration (physiologic vs. pharmacologic), duration (acute vs. chronic) and nature (endogenous vs. synthetic) of glucocorticoid hormone exposure. (4) The timing of stress or stress hormone exposure relative to the stage (early vs. late) of an immune response. Further elucidation of the interactions among the above-mentioned factors and other nervous, endocrine, and genetic factors in mediating the effects of stress on immune function is necessary. Importantly, whether a stressor enhances or suppresses immune function, it is the end-effect of the affected immune response that affects the health of the organism or individual. For example, stress-induced enhancement of immunoprotective responses is likely to be beneficial while stress-induced enhancement of immunopathology is harmful. These findings need to be explored and investigated further and translated from bench to bedside.

It is important to recognize that humans as well as animals experience stress as an intrinsic part of life, and in conjunction with many standard diagnostic, clinical, and experimental manipulations. Unintended stressors may significantly affect these measures and overall health outcomes. Thus, when conducting clinical, diagnostic, or experimental procedures, it may be important to account for the effects of stress on the specific physiologic parameter or health outcome being measured. For example, it is critical to elucidate and account for the effects of stress and/or stress hormones on changes in leukocyte distribution within different body compartments. Where possible, redistribution needs to be monitored in terms of changes in absolute numbers of specific subpopulations of leukocytes. Stress-induced changes in immune cell numbers and/or function could significantly affect results (in case of experiments), diagnosis (in case of medical tests), or outcome (in case of treatment procedures and surgery).

Due to a host of psycho-sociopolitical factors, stress has unfortunately become an increasing and inevitable part of people's lives. Stress is also a major factor during the diagnosis, treatment, and follow-up for most diseases. Chronic stress has been shown to dysregulate immune function and is thought to play a role in the etiology of many diseases. In contrast, it has been shown that activation of acute stress physiology may enhance protective immune responses [Dhabhar FS, Miller AH, McEwen BS, 1995; Dhabhar FS, Viswanathan K, 2005; Dhabhar FS, McEwen BS, 1996; Viswanathan K, Daugherty C, Dhabhar FS, 2005]. Dhabhar FS et al propose that it is important to further study, and clinically harness the immunological effects of the acute stress response, that evolution has finely sculpted as a survival mechanism, just as we study its maladaptive ramifications (chronic stress) that evolution has yet to resolve. A determination of the physiologic mechanisms through which stress and stress hormones enhance or suppress immune responses is critically important for elucidating risk, developing preventative and therapeutic interventions, and optimizing a patient's response to treatment. The elucidation of such mechanisms would facilitate development of biomedical treatments designed to harness an individual's physiology to selectively enhance (during vaccination, wounding, infections, or cancer) or suppress (during autoimmune or inflammatory disorders) the immune response depending on the outcome most beneficial for the patient.

SECTION 2

MATERIAL AND METHODS

The studies were performed in the form of two experiments.

The first experiment is at 50 white male rats Wistar line weighing 240-280 g. Of these 10 animals not subjected to any influences (intact), accounting for the control group, and the remaining 40 within 7 days subjected to moderate stress by daily 30-minute immobilization [Reznikov AG et al, 2004; Popovych IL, 2008]. The day after the completion of stressing in rats of both groups took samples of peripheral blood (through a cut tail) to analyze leukocytogram. An hour under light ether anesthesia for 15-20 s recorded ECG in standard lead II (introducing needle electrodes subcutaneously) to determine parameters of heart rate variability (HRV) [Baevskiy RM et al, 1984; 2001]. Then the rats were placed in individual chambers with perforated bottom to collect daily urine, in which determined the concentration of 17-ketosteroids (by method colorimetric reaction with m-dinitrobenzene) as well as electrolytes: calcium (by reaction with arsenase III), phosphates (phosphate-molybdate method), sodium and potassium (by flamming photometry) [Goryachkovskiy AM, 1998].

The next day, the animals were decapitated, for the purpose of collecting blood plasma, in which was determined concentration of adaptive hormones: corticosterone, testosterone, thyroxine and triiodothyronine (by ELISA [Instructions …, 2000]) as well as electrolytes: calcium, phosphates, sodium and potassium.

According to the plasma and urine levels of electrolytes hormonal activity was evaluated: parathyroid by coefficients $(Cap/Pp)^{0,5}$ and $(Cap•Pu/Pp•Cau)^{0,25}$, calcitonin by coefficients $(Cap•Pp)^{-0,5}$ and $(Cau•Pu/Pp•Cap)^{0,25}$, mineralocorticoid by coefficients $(Nap/Kp)^{0,5}$ and $(Nap•Ku/Kp•Nau)^{0,25}$ based on their classical effects and recommendations by Popovych IL [2007-2011].

The analyzes were 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 flamming spectrophotometer “СФ-47”.

In the same portion of the blood immunological parameters were determined by tests I and II levels of WHO as described in the handbook [Perederiy VG et al, 1995; Lapovets’ LY, Lutsyk BD, 2002] and the previously developed algorithm [Popovych IL, 2007-2011]. On the state of the phagocytic function of neutrophils (microphages) and monocytes (macrophages) judged by phagocytic index, microbial (phagocytic) number and index of killing regarding museum culture Staphylococcus aureus (ATCC N 25423 F49), with the calculation of derivative indices: microbial capacity (number of microbes that are able to absorb phagocytes contained in 1 L of blood) and bactericidal capacity (number of microbes that are able to neutralize neutrophils or monocytes contained in 1 L of blood) [Bilas VR, Popovych IL, 2009].

Among the parameters immunogram determined the relative amount of blood population of T-cells by spontaneous rosette test with sheep erythrocytes by Jondal M et al [1972], their theophylline resistant (T-helpers) and theophylline sensitive (T-cytotoxic) subpopulations (by test sensitivity rosette to theophylline by Limatibul S et al [1978]), the population of B-

lymphocytes by test complementary rosette of sheep erythrocytes by Bianco C [1970]. Natural killers identified as big containing granules lymphocytes. We set also induced by phytohemagglutinin blast transformation reaction of T-Lymphocytes, as described in the handbook [Perederiy VG et al, 1995].

After a blood sample was removed spleen, thymus and adrenal glands and weighed them. Since the spleen and thymus did smears for counting splenocytogram and thymocytogram [BelousovaOI, Fedotova MI, 1968; Bazarnova MA, 1988; Bilas VR, Popovych IL, 2009]. For the latter, as well as to leukocytogram and immunocytogram we calculated entropy [Shannon CE, 1963; Yushkovs'ka OG, 2001; Popovych IL, 2007]. In sections of the adrenal glands was measured under a microscope the thickness of glomerular, fascicular, reticular and medullar zones[Bilas VR, Popovych IL, 2009].

The second experiment was performed on 60 healthy Wistar female rats weighing 240-290 g. 10 animals remained intact using tap water from ad libitum drinking bowls, and the other rats underwent moderate chronic stress for 6 days according to the procedure described above. All tests were performed using a similar algorithm.

Digital material was statistically processed on a computer using the Statistica 5.5 software package for factor, cluster, variational, correlation, canonical and discriminant analyzes and the algorithm of Truskavets' Scientific School of Balneology [Popovych IL, 2007; 2011].

SECTION 3

VARIANTS OF IMMUNE REACTIONS TO CHRONIC STRESS IN MALS

3.1. Factor analysis of the parameters of the neuro-endocrine-immune complex

Factor analysis was applied to reduce the number of variables (data reduction) and to determine the structure of relationships between variables, that is, their classification. From a number of factor analysis methods, principal component analysis (PC) is involved. It is believed that to study the factor structure of the studied field can be limited to considering the number of PC, the total contribution of which to the total variance of the original data exceeds 2/3. Another approach for determining the number of PCs is to apply the Kaiser ($\lambda>1$) and Cattell criteria (with the maximum deceleration of the eigenvalue λ visualized graphically) [Kim JO, Mueller ChW, 1986].

In the first stage of factor analysis (principal component method) it is found that the dispersion of the information field of 58 parameters of the neuro-endocrine-immune complex and electrolyte exchange is absorbed by 20 factors. Using the Cattel method, we limit the number of factors analyzed to twelve, the total contribution of which to the total variance of the original data is 70,1%, which is significantly higher than the required critical level (2/3).

It is revealed (Table 3.1) that the first PC absorbs 13,8% of the dispersion and includes parathyrine and calcitonin activity on the one hand, and the relative content in the thymus of Hassal's corpuscles as well as in the spleen and in the blood of plasmocytes on the other. The association of endocrine and immune indicators also occurs in the structure of the second PC (9,3% of variability): the first constellation consists of thyroid hormones, their associated body mass and relative adrenal mass, and the second constellation consists the relative masses of the thymus and spleen, content in spleen fibroblasts and lymphoblasts, as well as the total level of blood leukocytes, the content of natural killer cells and the bactericidal capacity of its neutrophils. The third PC explains 8,7% of the variance as regards only the thymocytogram parameters. The urinary excretion of metabolites, mainly of androgens and, in part, of glucocorticoids is reflected in the fourth PC (7,9% scattering). The fifth PC absorbs 5,9% of the variance with respect to the major elements of the leukocytogram. The sixth PC (5,3% of variability) combines three parameters of autonomic regulation, with which one immune - the content in the spleen of macrophages, are associated. The seventh PC includes indicators of peripheral blood immunocytograms together with the thickness of the medullary zone of the adrenal cortex as characteristics of the hormonal element of the sympatho-adrenal system, absorbing 4,7% of the variance. Eight PC explains 4,0% of variability and includes only three immune parameters related to the major element of the splenocytogram: lymphocytes, its entropy, as well as the activity of blood macrophages. Instead, the ninth PC (3,8% of the variance) contains only four indicators pertaining to steroid hormones. The tenth PC (3,4% of variability) combines immune indices of peripheral blood and spleen. It is noteworthy that the thickness of the fascicular area of the adrenal cortex and its product corticosterone have a fairly heavy load on the first and tenth PC, which includes the parameters of immunity. The eleventh PC, absorbing 3,1% of the dispersion, refers, on the one hand, to mineralocorticoids and, on the other, to monocytes/macrophages. It is impossible to overlook the very significant factor load from the endothelial cells of the thymus, which formally belong to the third PC. This suggests that they are related to the bactericidity of blood macrophages. Twelfth PC (2,7% of variability) combines indicators of phagocytosis of macro- and microphages, RBTL on PhHA and thymus epitheliocytes as the source of its hormones. By the way, the macrophages of the thymus could be attributed to the third PC, which includes most elements

of the thymocytogram.

Therefore, there is a general pattern - the combination of **neuro-endocrine** and **immune** indices in individual PCs, which, in our material, illustrates the correctness of the concept of a triple neuroendocrine-immune complex, the elements of which carry out bilateral interaction.

Table 3.1. Factor loadings (Varimax normalized). Clusters of loadings, those determine the oblique factors for hierarchical analysis of parameters of male rat

Variable	Code	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 7	PC 8	PC 9	PC 10	PC 11	PC1 2
Parathyrine activity	Cap/Pp	**0,94**											
Hassal corpuscles of Thymus	GasT	**0,89**											
Calcitonine activity	1/Cap▪Pp	**0,87**											
Plasmocytes of Spleen	Pla S	**0,49**				-0,34			0,36				
Plasmocytes of Blood	Pla	**0,38**			0,35		0,33		0,34				
Triiodethyronine	T_3		**0,78**										
Thyroxine	T_4		**0,63**										
Thymus Mass Index	Thym %		**0,59**		*-0,29*								
Fibroblastes of Spleen	Fib S		**0,55**					*-0,29*					
Body Mass	Massa	-0,39	**0,51**										
Leukocytes of Blood	Leu		**0,50**			-0,36	**-0,34**						
Bacterocidity of Neutrophils	BCCN		**0,50**			0,46					*-0,29*		
Lymphoblastes of Spleen	Lb S		**0,46**								*0,28*		-0,33
Adrenals Mass Index	Adr %		**0,46**				-0,31			0,39			
NK-Lymphocytes Blood	NK		**0,45**			*-0,28*			0,39		0,35		
Spleen Mass Index	Spl %		**0,44**				**-0,43**					-0,42	
Entropy of thymocytogram	hT			**0,84**									
Lymphocytes of Thymus	Lc T			**0,77**									0,47
Reticulocytes of Thymus	Ret T			**0,63**									
Endotheliocytes of Tymus	End T			**0,55**								0,50	
Lymphoblastes of Thymus	Lb T	-0,33		**0,36**					*0,29*				
17-KS urinary excretion	$17\text{-}KS_U$				**0,88**								
Lymphocytes of Blood	L					**0,88**							
Segmented Neutrophils Blood	S					**0,87**							
Stub Neutrophils of Blood	Pal					**0,79**							
Entropy Leukocytogram Blood	hL					**0,66**					0,46		
Vagal tone	ΔX						**0,93**						

Sympathetic tone	AMo						**0,89**						
Humoral channal	Mo						**0,87**						
Macrophages of Spleen	Mac S	0,31					**0,79**						
Entropy Immunocytogram Blood	hI	-0,34						**0,80**					
0-Lymphocytes of Blood	0							**0,79**			-0,40		*0,28*
Tc-Lymphocytes of Blood	Ts							**0,71**					
B-Lymphocytes of Blood	B		-0,32					**0,59**					
Medullary Zone Adrenal Cortex	Medul							**0,45**					
Entropy of Splenocytogram	hS								**0,86**				
Lymphocytes of Spleen	Lc S								**0,83**				
Phagocytose Ind Monoc Blood	FIM						*-0,29*		**0,57**				
Reticulocytes of Spleen	Ret S								**0,42**				
Reticulary Zone Adrenal Cortex	Retic	0,35								**0,62**			
Testosterone	Test					*0,29*				**0,62**			
Fasciculary Zone Adrenal Cortex	Fasc	0,42							-0,30	**0,55**	0,33		
Corticosterone,	Cor			-0,30						**0,48**	0,31		
Th-Lymphocytes of Blood	Th										**0,69**		
Eosinophils of Spleen	E S			-0,39		0,41					**0,62**		
Eosinophils of Blood	E										**0,56**		
Basophils of Blood	Bas										**0,51**		
Neutrophils of Spleen	Neu S				0,33					-0,38	**0,49**		
Killing Ind Neut. Blood	IK		-0,29				0,30				**0,41**		*0,28*
Monocytes of Blood	M											**0,64**	
Bactericidity of Monocytes	BCCM								-0,43			**0,54**	-0,30
Glomerulary Zone Adr Cortex	Glom						-0,39					**0,49**	
Phagocyt Ind Neutr Blood	FIN												**0,77**
Endotheliocytes of Thymus	Epy T								-0,31				**0,74**
Microb Count Neutr Blood	FNN		-0,32					0,40					**0,58**
Microb Count Monoc Blood	FNM		0,43					0,47					**0,58**
Blasttransform of T-Lymphoc	RBTL												**0,56**
Macrophages of Thymus	Mac T			0,50									**0,51**

In the second step, we obtained a correlation matrix for oblique factors, which was subjected to further analysis to distinguish a plurality of orthogonal factors that divide the variability into variables related to total variance (secondary factors) and to individual variations or cluster-related variables (primary factors).

It is revealed (Table 3.2) that there are 4 directly unmeasured hypothetical common

factors. In this case, the first common factor (S1) combines, on the one hand, the immune parameters, first of all, the entropy of leukocytogram as mirrors of neuro-hormonal adaptive systems, then the major elements of leukocytograms are lymphocytes and the segment nuclear neutrophils and its minor elements, stub neutrophils, eosinophils, basophils and leukocytosis, as well as eosinophils of splenocytogram, the relative mass of the thymus with the content of reticulocytes and T-lymphocytes, the blood content of T-helper cells and natural killers; and on the other hand, there are elements of the main adaptive system such as corticosteronemia and the thickness of the fascicular area of the adrenal cortex; it also naturally includes testosterone as an element of the hypothalamic-pituitary-gonadal adaptive system.

Table 3.2. Secondary (S) and Primary (P) Factors Loadings at male rats

Фактор	S1	S2	S3	S4	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12
hL	**-0,73**								-0,39							
L	**0,70**								0,62							
Pal	**-0,53**								-0,58							
S	**-0,53**								-0,68							
E S	**0,48**													0,49		
Cor	**-0,46**												-0,42			
E	**-0,45**		0,30											-0,39		
Ret T	**0,42**		-0,32				-0,47									
Thym%	**0,36**					0,51				*-0,29*						
Fasc	**0,35**			0,33	*-0,29*								0,48			
Leu	**0,35**					0,45										
Th	**-0,35**	-0,30												-0,55		
NK	**-0,33**					-0,39						0,34				
Lc T	**-0,32**		0,31	0,32			0,60									-0,36
Bas	**-0,32**													-0,44		
Test	**0,29**												0,51			
FNM		**0,58**				-0,37					-0,34					0,39
FNN		**-0,58**														-0,38
0		**0,55**									-0,67			0,32		
FIN		**-0,54**														-0,58
hI		**-0,50**									0,69					
PTA		**0,45**			**-0,75**											
Ts		**-0,40**									0,61					
Epy T		**0,38**														0,52
CTA		**-0,37**		*-0,28*	**0,71**											
Fib S		**0,33**				-0,45										

Lb S		**-0,33**				0,41										
RBTL		**-0,31**														-0,45
B		**-0,30**									0,51					
BCCM		**0,31**	-0,49												-0,42	
17-KS_U			**0,46**					**-0,77**								
Pla S			**0,44**		-0,38				-0,33							
M			**-0,42**												-0,56	
hT	0,33		**-0,40**	-0,31			-0,66									
Pla			**-0,34**													
Mac S				**-0,65**						-0,53						
AMo				**-0,61**						-0,64						
ΔX				**0,61**						0,68						
Lc S			-0,31	**0,60**								-0,54				
Mo				**0,55**						0,66						
hS			0,42	**-0,54**								0,55				
Glom				**0,40**											0,44	
IK				**0,39**										0,31		
FIM			-0,37	**0,39**								-0,33				
Ret S				**-0,39**								0,33				
Gas T		-0,36		**-0,37**	**0,71**											
Mac T			-0,33	**-0,34**			-0,39									0,39
BCCN				**0,28**		0,45			-0,46					0,30		
T_3						**0,75**										
T_4						-0,54										
Massa						-0,48										
Adr%						0,43							-0,39			
Lb T					*-0,29*		-0,33									
Medul											-0,44					
Retic													0,56			
Neu S								-0,31					-0,40	0,45		
End T							-0,47								0,48	
Splen%						0,40				0,32					-0,42	

In the structure of the second common factor, the immunity parameters also are at the forefront, especially its phagocytic link, such as the intensity of macrophage phagocytosis and the intensity and activity of blood microphages, as well as T- and B-links, in particular

entropy of immunocytogram and its elements, thymus epitheliocytes as well as spleen fibroblasts and lymphoblasts. On the other hand, there are parathyrin and calcitonin activities.

The third common factor heads up the bactericidal ability of blood microphages, which, in addition, gives a significant load on the second factor. The company of this indicator consists of plasmocytes of the spleen and blood, blood monocytes, as well as entropy of the thymocytogram as a measure of its structural reserve and mobilization of the reserve protective sanogenetic mechanisms. By the way, for the high informativeness of the entropy of the thymocytogram, it is additionally evidenced by its rather heavy load on the first and fourth common factors. Significantly, urinary excretion of 17-ketosteroids, that is, metabolites of hormones that have regulatory effects on both immune and electrolyte metabolism, in this case potassium and sodium, takes second place.

The hierarchy of the fourth common factor is again led by the immune index such as the macrophages of splenocytogram; it is also represented by its reticulocytes, lymphocytes and entropy. The latter give a significant load on the third factor. The bactericidity of microphages and the phagocytic activity of blood macrophages occupy mediocre positions, while Hassal's calves and thymus macrophages close the list of indicators of this factor, but they give almost similar loads to the related common factors.

These immune parameters are accompanied by indicators of autonomic neuro-humoral regulation and mineralocorticoid function of the adrenal cortex, represented by the thickness of their glomerular zone. By the way, the thickness of the fascicular zone gives a considerable weight load to this factor, besides the first one.

Therefore, due to factor analysis, 4 independent clusters of parameters of the neuro-endocrine-immune complex, related to cause/effect functional relationships, were identified.

3.2. Characterization of neuro-endocrine manifestations of stress in male rats

When compared to traditional neuroendocrine parameters in rats subjected to chronic stress, with intact animals, taken as 100%, revealed an increase Corticosterone plasma level by 20,8±10,0% ($p<0,05$), Sympathetic tone (evaluated by AMo HRV) by 20,1±6,5% ($p<0,01$), Heart Rate by 9,6±3,2% ($p<0,01$), thickness of reticular zone of adrenals by 17,1±5,6% ($p<0,01$), Testosterone plasma level by 16,9±8,7% ($p>0,05$), thickness of fascicular zone of adrenals by 12,6±3,6% ($p<0,01$) as well as Triiodethyronine plasma level by 6,2±3,8% ($p>0,10$) while Vagal Tone (evaluated by ΔX HRV), thickness of glomerular zone of adrenals, Mineralocorticoide Activity (evaluated by plasma $(Na/K)^{0,5}$ ratio) as well as Parathyrin Activity (evaluated by plasma $(Ca/P)^{0,5}$ ratio) decreased by 27,5±8,6% ($p<0,01$), 12,5±2,6% ($p<0,001$), 5,6±1,8% ($p<0,01$) and 3,9±1,9% ($p<0,05$) respectively.

According recommendation by Cook IA et al [1998] and Popovych IL [2001] variables obtained after Chronic Stress (SV) expressed as Z-score calculated by formula:

$Z=(SV/IV – 1)/Cv$, where

IV is Initial (Control) Variable, Cv is Coefficient its variation at intact rats.

This approach allows us to estimate variables expressed in various units (μM, %, nM/L, msec etc) not just in one scale, and taking into account their variability as physiological cost 1% change stable setting higher than this parameter fluctuation whose normally wider. As shown in Fig. 3.1, ranking caused by chronic stress changes in neuroendocrine parameters by Z-scores differs of ranking by %.

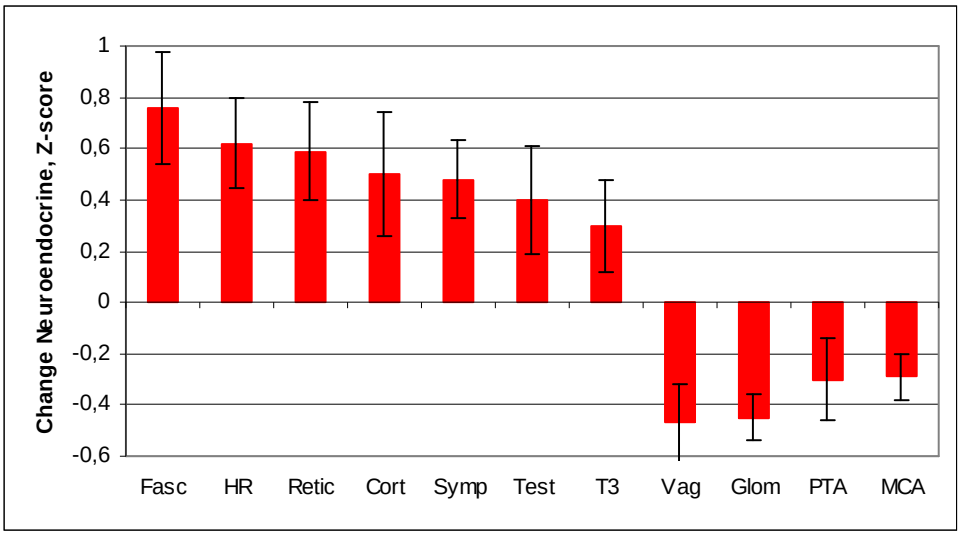


Fig. 3.1. Ranking caused by chronic stress changes in neuroendocrine parameters

Overall, our findings are consistent with a classic conception about the leading role in neuro-endocrine manifestations of Chronic Stress Corticoadrenal and Autonomic Nervous Systems and about functional antagonism between Glucocrticoide and Mineralocorticoide as well as between Sympathetic and Vagal Activities. However, our data support the discussion about the nature of changes by Chronic Stress in other endocrine glands.

If we take as an integrated quantitative measure of neuro-endocrine manifestations of Chronic Stress mean of modules of Z-Scores, it will be 0,47±0,04 σ (or Euklidian units).

Another approach to identify the parameters (variables, options) set of which neuro-endocrine status intact and stressed rats significantly different is discriminant (recognizing) analysis. In applying method forward stepwise [Klecka WR, 1989] variables currently in the model turned out three only (Tables 3.3 and 3.4), while other earlier marked variables currently not in the discriminant model.

Table 3.3. Discriminant Function Analysis Summary

Step 3, N of variables in model: 3; Grouping: 2 groups; Wilks' Λ: 0,795; approx. $F_{(3,5)}$=3,95; p=0,014.

Variables currently in the model	Initial level (Control)	After Chronic Stress	Stressory change as Z-score	Wilks' Λ	Partial Λ	F-remove	p-level	Tolerancy
Fasciculary ZAC, μM	218±11	245±8	+0,76±0,22	,925	,859	7,5	,009	,748
Glomerulary ZAC, μM	129±11	113±3	-0,45±0,09	,899	,884	6,0	,018	,885
$(Cap/Pp)^{0,5}$ as PTA, units	1,66±0,06	1,59±0,03	-0,30±0,15	,838	,949	2,5	,124	,836
Variables currently not in the model	Initial level (Control)	After Chronic Stress	Stressory change as Z-score	Wilks' Λ	Partial Λ	F to enter	p-level	Tolerancy
Heart Rate, beats/min	336±20	375±11	+0,62±0,18	,789	,992	,346	,56	,86
Reticulary ZAC, μM	20,0±1,8	23,5±1,1	+0,59±0,20	,788	,991	,415	,52	,69
Corticosterone, nM/L	340±44	411±34	+0,50±0,24	,778	,979	,966	,33	,86
Sympathotone (AMo), %	55,6±7,4	66,8±3,6	+0,48±0,15	,782	,983	,764	,39	,83
Testosterone, nM/L	34,6±4,6	40,4±3,0	+0,40±0,20	,793	,997	,143	,71	,94
Triiodethyronine, nM/l	2,50±0,17	2,66±0,10	+0,30±0,18	,795	1,00	,015	,90	,94
Vagal Tone (ΔX), msec	42±8	30±4	-0,47±0,15	,786	,988	,532	,47	,84
$(Nap/Kp)^{0,5}$ as MCA, un	6,10±0,37	5,76±0,11	-0,29±0,09	,787	,989	,480	,49	,93

Table 3.4. Summary of Stepwise Analysis and Chi-Square Tests with Successive Root Removed

Variables currently in the model	F to enter	p-level	Lambda	F-value	p-level
Fasciculary Zone of Adrenal Cortex	5,35	,025	,838	4,56	,015
Glomerulary Zone of Adrenal Cortex	3,45	,069	,933	3,45	,069
$(Cap/Pp)^{0,5}$ as Parathyrine Activity	2,45	,124	,795	3,95	,014
Canonical R=0,453; Wilks' Λ=0,795; $\chi^2_{(3)}$=10,7; p=0,014					

Next, a 3-dimensional space of discriminant variables is transformed into a one-dimensional space of a canonical discriminant function (canonical root), which is a linear combination of discriminant variables.

The discriminative ability of the root characterizes the coefficient of canonical correlation (r*) as a measure of relationship, the degree of dependence between groups (intact and stressed rats) and discriminant function. It is 0,453 (Wilks' Λ=0,795; $\chi^2_{(3)}$=10,7; p=0,014).

Table 3.5 summarizes the raw (actual) and standardized (normalized) coefficients of the discriminant variables.

Table 3.5. Standardized, Structural and Raw Coefficients and Constant for Canonical Variables

	Coefficients		
Variables currently in the model	Standardized	Structural	Raw
Fasciculary Zone of Adrenal Cortex	-0,958	-0,47	-0,021
Glomerulary Zone of Adrenal Cortex	0,799	0,53	0,033
$(Cap/Pp)^{0,5}$ as Parathyrine Activity	0,544	0,23	2,818
Eigenvalue	0,258	**Constant**	-3,396

The coefficient in a raw (non-standard) form gives information about the absolute contribution of a given variable to the value of the discriminant function; instead, the standardized coefficients reflect the relative contribution of the variable, independent of the unit of measure. They make it possible to identify the variables that contribute most to the value of the discriminatory function.

There are also complete structural coefficients, that is, correlation coefficients between the discriminant root and the variables. The structural coefficient indicates how closely related variables and discriminant functions are, that is, what proportion of information about the discriminant function (root) is embedded in this variable. As you can see, the root reflects in an inverse way information about the thickness of the fascicular zone of the adrenal cortex and in the direct way the glomerular zone, as well as parathyroid activity.

The sum of the products of the raw coefficients for the values of the discriminant variables together with the constant give the value of the discriminant function (root) for each animal and allow its visualization (Fig. 3.2)

Canonical Neuroendocrine Roots for Intact and Stressed Males Rats averages +0,99±0,40 and -0,25±0,15 respectively (Squared Mahalanobis Distance=1,61; F=3,76; p=0,017)

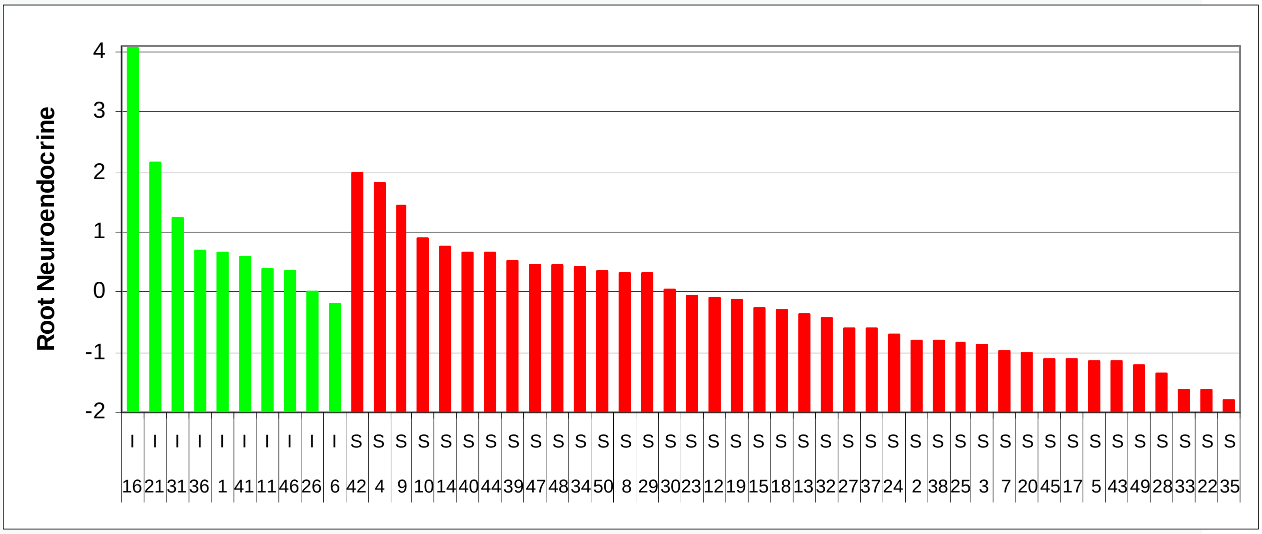


Fig. 3.2. Individual values of the neuro-endocrine root of intact (I) and stressed (S) male rats (animal numbers below)

Despite the wide range of individual root values, the discrepancy between the root mean values of the intact (+0,99±0,40) and stressed (-0,25±0.15) groups is statistically significant (Fig. 3.3), as documented by the Mahalanobis distance calculation (D^2_M=1,61; F=3,76; p=0,017).

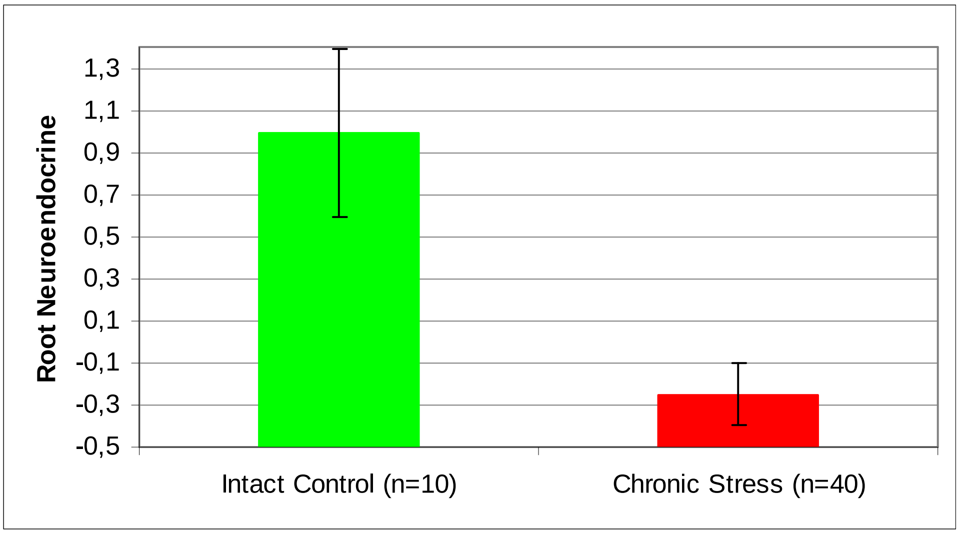


Fig. 3.3. Mean values of the neuro-endocrine root of intact and stressed male rats

3.3. Characterization of the immune manifestations of stress in male rats

Neuroendocrine perturbations caused by chronic stress are accompanied by deviations from the norm of a number of indicators of immunity, in the form of both suppression and activation. The most noticeable decrease was the intensity of phagocytosis by blood neutrophils of Staphylococcus aureus (Fig. 3.4).

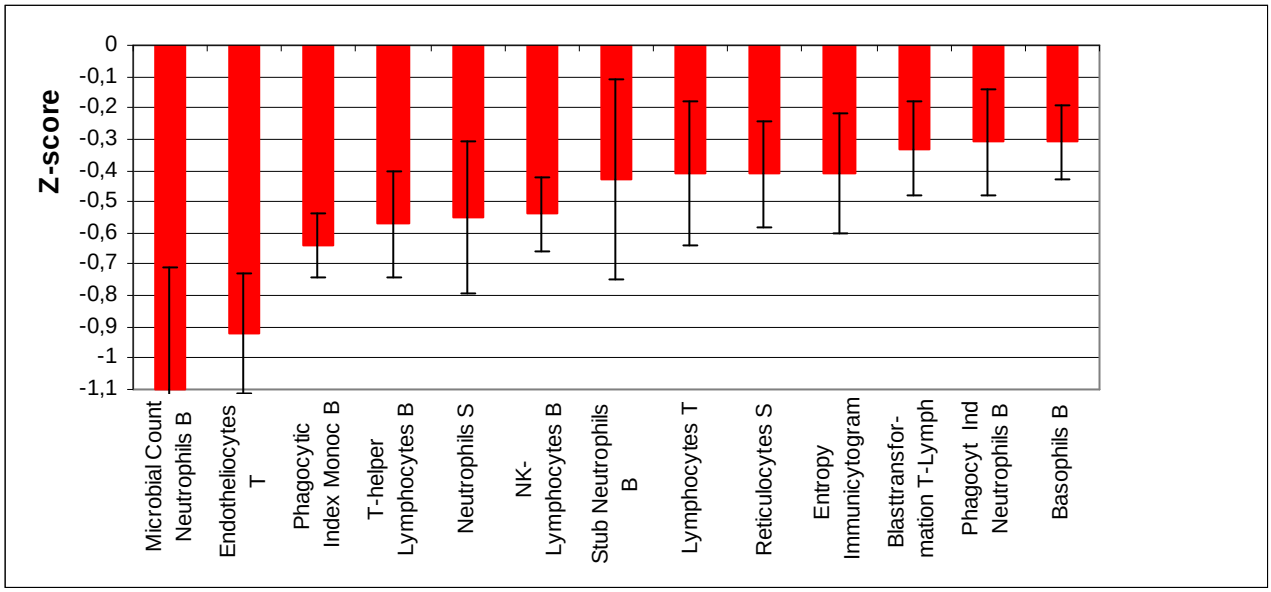


Fig. 3.4. Ranking of chronic stress-induced suppressive abnormalities in immune parameters in male rats

Significant in terms of suppression were also identified: the proportion of endothelial cells and lymphocytes in thymocytogram, the activity of phagocytosis of monocytes/macrophages and neutrophils/microphages, the proportion of Th-lymphocytes and NK-lymphocytes in blood immunocytogram, neutrophiles and reticulocytes in splenocytogram, stub neutrophils and basophils in leukocytogram as well as the entropy of leukocytograms and the blastransformation response of T-lymphocytes to the mitogen phytohemagglutinin.

Instead, another constellation of immune parameters became more active (Fig. 3.5). This is, first and foremost, a microbial count of blood monocytes/macrophages, which, combined with a slight increase in their number, gives (despite a decrease in phagocytic index) a marked increase in bactericidal capacity against Staphylococcus aureus. At the same time, the proportion of macrophages in the splenocytogram and thymocytogram, as well as the mass of the thymus, increases. Other activation manifestations of stress are the increase in entropy of the splenocytogram and the eosinophils share in it. Instead, the increase in the proportion of blood 0-lymphocytes in the immunocytogram, ie immature and/or lost receptors, should be interpreted as a manifestation of immunosuppression.

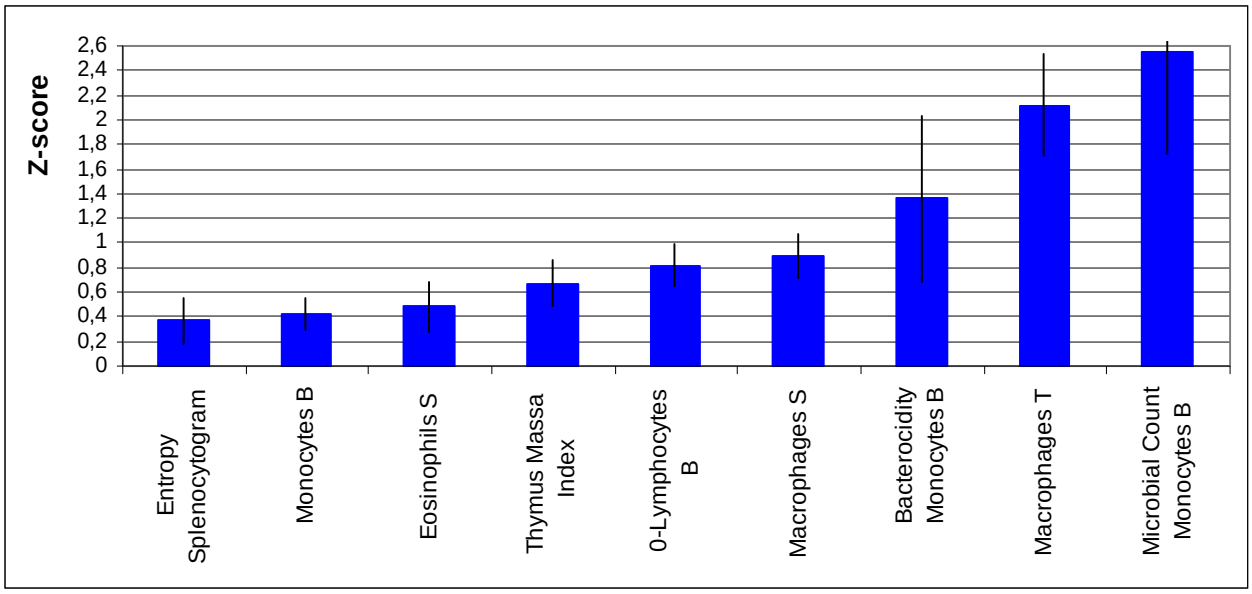


Fig. 3.5. Ranking of chronic stress-induced enhancing deviations from the norm of immune rates in male rats

As a result of the discriminant analysis of the immune parameters included in the model, that is characteristic of the immunotropic effect of chronic stress, only 17 were considered among them, while recognition information from other immune indicators was evaluated by the program as excessive (Table 3.6).

Information on discriminant variables is condensed in the canonical root, which is poorly structured and correlates poorly positively with the content of macrophages in the spleen and thymus, while negatively with the content of endothelial cells in the thymus and NK lymphocytes in the blood (Fig. 3.7).

Table 3.6. Discriminant Function Analysis Summary of immune parameters of male rats

Step 17, N of vars in model: 17; Grouping: 2 grps
Wilks' Lambda: 0,248; approx. $F_{(17)}=5,7$; $p<10^{-5}$

Variables currently in the model	Initial level (Control)	After Chronic Stress	Stressory change as Z-score	Wilks Λ	Par-tial Λ	F re-mo-ve	p-le-vel	Tole ran-cy
Macrophages Thymus	4,70±0,21	6,13±0,28	+2,12±0,42	,402	,617	19,9	,000	,352
Macrophages Spleen	5,50±0,65	7,35±0,31	+0,90±0,15	,356	,696	14,0	,001	,254
Thymus Mass Ind, ‰	0,295±0,022	0,340±0,013	+0,67±0,19	,278	,892	3,9	,058	,512
Eosinophils Spleen	1,80±0,25	2,18±0,16	+0,48±0,21	,325	,763	10,0	,003	,353
Monocytes Blood	4,20±0,73	5,18±0,29	+0,42±0,13	,256	,967	1,1	,304	,426
Entropy Splenocytogram	0,588±0,007	0,597±0,004	+0,37±0,19	,269	,921	2,7	,108	,358

Entropy LCG Blood	0,350±0,007	0,356±0,005	+0,26±0,24	,346	,716	12,7	,001	,050
Reticulocytes Thymus	5,20±0,63	5,51±0,29	+0,16±0,14	,307	,806	7,7	,009	,355
Lymphocytes Blood	60,4±1,4	60,2±1,0	-0,05±0,21	,314	,789	8,6	,006	,075
Killing Ind Neut. Blood	54,7±2,0	54,3±0,7	-0,06±0,11	,347	,715	12,7	,001	,283
Phagocyt Ind Neutr Blood	82,3±0,7	81,9±0,6	-0,31±0,17	,274	,906	3,3	,077	,286
Basophils Blood	0,30±0,15	0,15±0,06	-0,31±0,12	,302	,821	7,0	,013	,380
Stub Neutroph. Blood	3,50±0,17	3,27±0,17	-0,43±0,32	,264	,937	2,1	,153	,290
NK-Lymphocyt Blood	10,4±0,6	9,4±0,2	-0,54±0,12	,261	,949	1,7	,198	,592
Neutrophils Spleen	11,5±0,5	10,6±0,4	-0,55±0,24	,257	,965	1,2	,287	,532
Endotheliocytes Thym	7,40±0,43	6,15±0,25	-0,92±0,19	,268	,925	2,6	,117	,620
Microb Count Neutr Bloo	8,14±0,07	7,91±0,08	-1,10±0,39	,316	,785	8,7	,006	,295
Variables currently not in the model Df for all F-tests: 1,31	Initial level (Control)	After Chronic Stress	Stressory change as Z-score	Wilks Λ	Par-tial Λ	F to en-ter	p-le-vel	Tole ran-cy
Microb Count Monoc Bloo	2,8±0,1	3,6±0,3	+2,55±0,82	,245	,989	,349	,56	,346
Bacterocid. Mon., 10^6 M/l	81±14	141±30	+1,36±0,67	,245	,989	,340	,56	,340
0-Lymphocytes of Blood	29,9±1,5	33,8±0,8	+0,82±0,17	,245	,989	,336	,57	,507
Leukocytes of Blood	9,57±0,54	10,20±0,34	+0,37±0,20	,244	,985	,466	,50	,475
Epitheliocytes of Tymus	19,9±0,7	20,6±0,4	+0,33±0,18	,247	,998	,056	,81	,616
Blasttransform T-Lym Blo	65,8±3,7	62,0±1,7	-0,33±0,15	,247	,998	,058	,81	,390
Lymphocytes of Thymus	55,6±1,0	54,3±0,7	-0,41±0,23	,246	,992	,240	,63	,219
Reticulocytes of Spleen	14,3±0,6	13,6±0,3	-0,41±0,17	,248	1,00	,004	,95	,675
Entropy ICG of Blood	0,522±0,004	0,517±0,003	-0,41±0,19	,247	,996	,126	,72	,570
Th-Lymphocytes of Blood	32,3±0,8	30,9±0,4	-0,57±0,17	,247	,998	,071	,79	,454
Phagoc Ind Monoc Blood	7,8±1,1	5,6±0,3	-0,64±0,10	,248	,999	,046	,83	,289

The calculation of the individual values of the immune root by the raw coefficients and constant given in table 3.7, allows to visualize the immune status of each rat (Fig. 3.4).

Table 3.7. Summary of Stepwise Analysis as well as Standardized, Structural and Raw Coefficients and Constant for Canonical Variables

Variables currently in the model	F to enter	p-level	Λ	F-value	p-level	Coefficients		
						Standardized	Structural	Raw
Macrophages of Spleen	6,93	,011	,874	6,93	,012	1,260	,218	,634
Macrophages of Thymus	4,16	,047	,617	7,00	10^{-3}	1,204	,207	,747
Thymus Massa Index	3,24	,081	,273	6,04	10^{-5}	,530	,133	65,50
Monocytes of Blood	1,09	,304	,248	5,71	10^{-4}	-,321	,118	-,165
Eosinophils of Spleen	2,40	,129	,407	5,67	10^{-4}	,945	,088	,951
Entropy of Splenocytogram	2,15	,152	,256	5,98	10^{-5}	-,541	,075	-20,77
Entropy of LCG of Blood	2,96	,093	,479	5,58	10^{-4}	2,746	,043	87,69
Reticulocytes of Thymus	3,48	,070	,342	5,93	10^{-4}	-,852	,040	-,462
Endotheliocytes of Thymus	7,11	,010	,759	7,46	,002	-,402	-,189	-,261
NK-Lymphocytes of Blood	5,83	,020	,674	7,43	10^{-3}	-,339	-,155	-,224
Microbes Count of Neutr of Blood	1,66	,206	,327	5,70	10^{-4}	-,983	-,115	-2,075
Neutrophils of Spleen	3,39	,073	,374	5,78	10^{-4}	-,297	-,091	-,132
Basophils of Blood	2,01	,164	,513	5,69	10^{-4}	-,792	-,091	-2,044
Stub Neutrophils of Blood	3,73	,060	,568	6,68	10^{-4}	-,537	-,052	-,534
Phagocytose Ind of Neutr of Blood	3,27	,079	,299	5,86	10^{-5}	,663	-,023	,173
Killing Ind of Neutroph. of Blood	2,45	,125	,538	6,16	10^{-4}	1,157	-,017	,236
Lymphocytes of Blood	4,26	,046	,433	5,83	10^{-4}	1,940	-,009	,331
Canonical R=0,867; Wilks' Λ=0,248; $\chi^2_{(17)}$=55; p<10^{-5}							**Constant**	-51,41

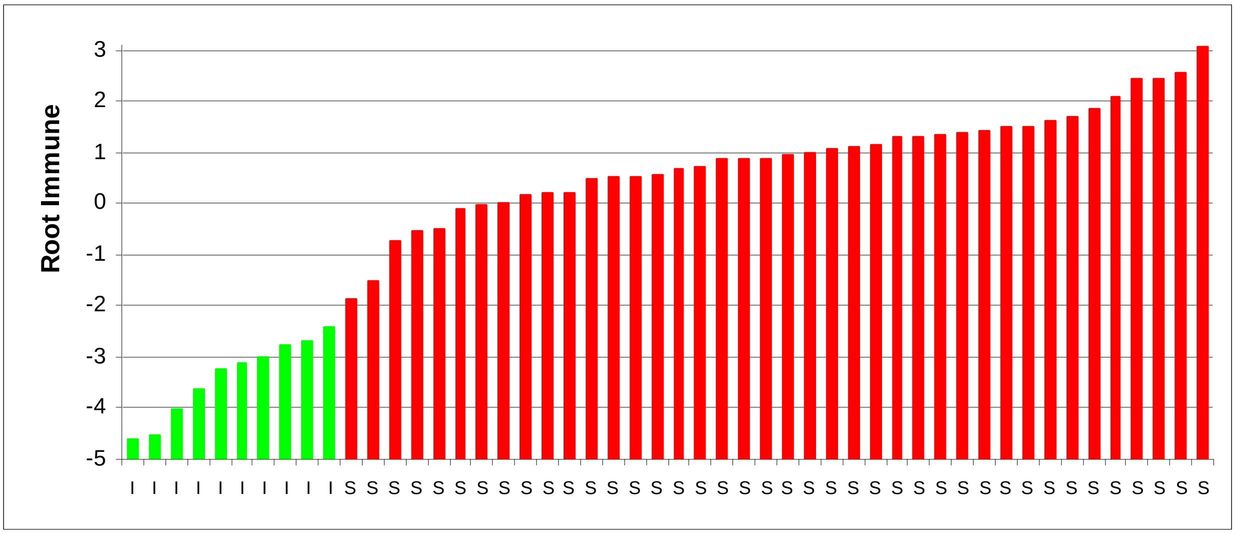


Fig. 3.4. Individual values of the immune root of intact (I) and stressed (S) male rats

Even at first glance, there is a clear distinction between the immune statuses of intact and stressed rats. The visual impression is documented by calculating the mean values of the immune roots: -3,41±0,40 and +0,85±0,15, respectively (D^2_M=19,0; F=5,44; $p<10^{-4}$).

Selected 17 parameters can be used to identify (classify) the affiliation of a rat to a group of intact or stressed. This purpose of discriminant analysis is realized by means of classification (discriminant) functions (Table 3.8).

Table 3.8. Coefficients and Constant for Classification Functions for male rats

Variables currently in the model	Intact Male Rats	Stressed Male Rats
Macrophages of Spleen	22,56	25,27
Endotheliocytes of Thymus	1,32	0,20
NK-Lymphocytes of Blood	-8,28	-9,23
Macrophages of Thymus	54,14	57,33
Stub Neutrophils of Blood	-1,44	-3,72
Killing Index of Neutrophils of Blood	22,31	23,32
Basophils of Blood	-166,8	-175,5
Entropy of Leukocytogram of Blood	11272	11646
Lymphocytes of Blood	52,36	53,78
Eosinophils of Spleen	61,29	65,35
Neutrophils of Spleen	-19,47	-20,04
Reticulocytes of Thymus	-22,04	-24,01
Microbes Count of Neutr of Blood	4,38	-4,48
Phagocytose Index of Neutrophils of Blood	7,20	7,94
Thymus Massa Index	1087	1367
Entropy of Splenocytogram	874,3	785,6
Monocytes of Blood	-33,30	-34,01
Constant	-4692	-4905

These function are special linear combinations that maximize group differences and minimize intra-group variance. The coefficients of the classification functions are not standardized and therefore not interpreted. The object belongs to the group with the maximum value of the function, calculated by summing the product of the quantities of variables by the coefficients of the classification functions plus a constant. In this case, we can retrospectively recognize intact rats unmistakably, and subjected to stress with an accuracy of 97,5% (one error per 40 animals).

3.4. Variants of the post-stress state of immunity (according to cluster analysis)

Even a visual analysis of the individual magnitudes of the canonically discriminatory roots of male rats subjected to chronic stress shows that they differ not only from the intact animals (by definition) but also among themselves. Therefore, we set ourselves the goal of dividing stressed rats into three homogeneous groups. To achieve this goal, a cluster analysis

was applied.

While a routine methodological approach only allows one to analyze one or another feature of a statistical sample, the use of cluster analysis makes it possible to take into account all the features simultaneously. Taking into account the totality of features of persons taken in their relationship and the predisposition of one of them (derivatives) to others (basic, determining) allows to make a natural classification that reflects the nature of things, their essence. It is believed that knowledge of the essence of an object is to identify those qualitative properties that, in fact, and determine the object, distinguish it from others [Aldenderfer MS, Blashfield RK, 1985; Mandel ID, 1988].

Clustering by the parameters of immunity is implemented by the iterative k-means method. In this method, the object is assigned to the class whose Euclidean distance is minimal. The main principle of the structural approach to the selection of homogeneous groups is that the objects of one class are close and different objects are distant. In other words, a cluster is an accumulation of points in an n-dimensional geometric space in which the average inter-point distance is less than the average distance from these points to the rest.

The maximum contribution to cluster distribution, judging by the criterion η^2, which reflects the proportion of intergroup variance in total dispersion, contributes to the ability of T lymphocytes to transform into blasts under the influence of the phytohemagglutinin mitogen. Significantly smaller but significant ($p \leq 0.05$) values of η^2 were found with respect to 7 parameters of lymphocytes, 5 parameters of neutrophils and 4 parameters of monocytes/macrophages, as well as the total blood content of leukocytes (Table 3.9).

Table 3.9. Results of ANOVA. Variables that contribute significantly to the distribution of stressed animals in immune clusters

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

Note. The variance analysis parameters are calculated by the following formulas:

$\eta^2=Sb^2/(Sb^2+Sw^2)$,
$R=\eta$,
$F=[Sb^2 \bullet (n-k)]/[Sw^2 \bullet (k-1)]$, where
Sb^2 is the between (intergroup) variance;
Sw^2 is within (intragroup) variance;
n is the number of animals (40);
k is the number of cluster/groups (3).

Contributions of other parameters of immunity to the division of animals into immune clusters are insignificant or scanty (Table 3.9). Noteworthy are the content of neutrophils and eosinophils in the spleen, epitheliocytes in the thymus, neutrophils in the blood, and the degree of completion of phagocytosis by Staph. aureus.

Table 3.9. Results of ANOVA. Variables that make little or scanty contribution to the distribution of stressed animals in immune clusters

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

Clusters are clearly demarcated, as evidenced by the Euclidean distances between them, far exceeding the distances between members within each cluster (Table 3.10).

Table 3.10. Euclidean distances between immune clusters

Кластер	No. 1	No. 2	No. 3
No. 1	0,00		
No. 2	3,19	0,00	
No. 3	2,61	3,79	0,00

When calculating the deviation from the norm of cluster-forming immune parameters in Z-units revealed the following (Table. 3.11).

Table 3.11. Features of deviation from the norm of cluster-forming immune parameters in individual clusters

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

In cluster III rats, 16 parameters did not deviate from normal (±0,5σ) and only 2 (content of macrophages in the spleen and lymphocytes in the thymus) moderately increased.

Cluster II is characterized by a moderate decrease in 8 parameters (content of lymphoblasts in the spleen, lymphocytes in the thymus, segmented neutrophils and NK as

well as Th-lymphocytes in the blood, RBTL on PhHA, the intensity and activity of phagocytosis by neutrophils) in combination with a marked increase in 5 other parameters (the content of macrophages in the thymus and the intensity of Staph. aureus phagocytosis by macrophages of blood, as well as the blood content of leukocytes, pan- and 0-lymphocytes),. while within range ±0,5σ only 5 parameters remained

In cluster I rats, only 3 parameters did not deviate from the norm, 5 parameters increased significantly (intensity of phagocytosis Staph. aureus by blood macrophages, content macrophages in the thymus and spleen, fibroblasts in the spleen, 0-lymphocytes in the blood), instead 10 parameters decreased significantly (content leukocytes, pan-lymphocytes and Th-lymphocytes in the blood, lymphocytes in the thymus and spleen, lymphoblasts in the spleen, as well as activity and intensity of phagocytosis of blood neutrophil, their microbial capacity and bactericidal ability).

When calculating the average values separately for the parameters that under the influence of stress increase or decrease, it was found that in the III cluster the deviations of 16 parameters on both sides from the average norm are almost identical and are in the acceptable range ±0,5σ (Table 3.11 and Fig. 3.13). Therefore, the immune status of 40% of male rats subjected to chronic moderate stress was resistant to its factors.

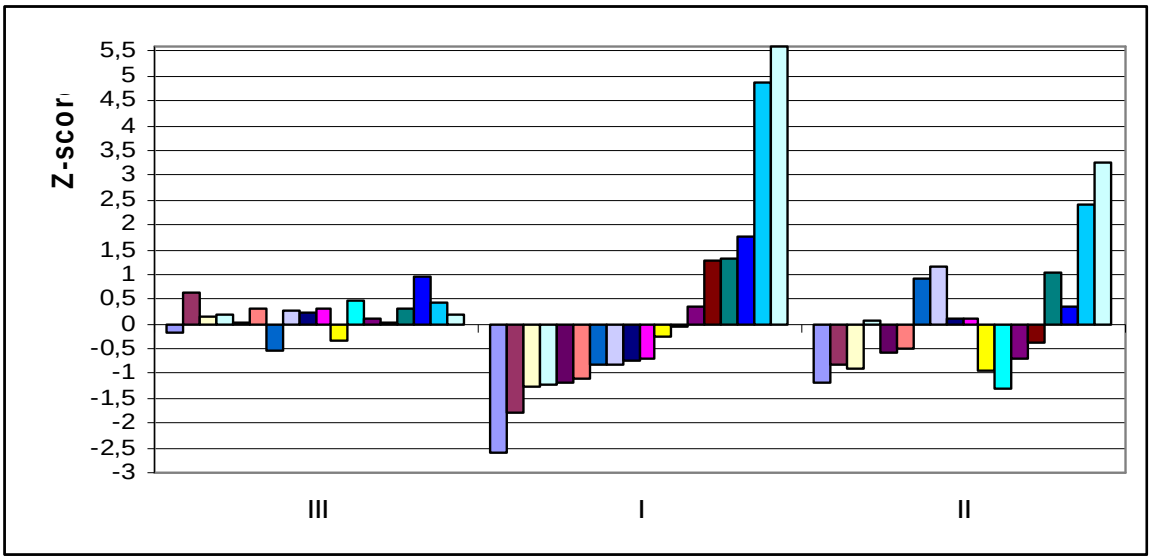


Fig. 3.13. Post-stress immune profiles of male rats of III, I and II clusters (the order of the parameters is the same as in Table 3.11)

The immune status of rats in the cluster II is characterized by moderate inhibition of microphagal, killer and T-links (an increase in the blood content of 0-lymphocytes indicates loss of Th-lymphocytes of $CD4^+$ receptors) in combination with pronounced activation of the macrophagal link.

Post-stress changes in immunity in cluster I differ from those in cluster II both qualitatively and quantitatively. In particular, the rats of this cluster showed no deviation from the norm, neither the response of T-lymphocyte blastransformation nor the level of NK-lymphocytes.

However, other parameters of the T-link, as well as the microphagal link were suppressed to a greater extent, and the parameters of the macrophagal link were activated very noticeably, with the activation areal, additiomal to the thymus and blood, extended to the

spleen.

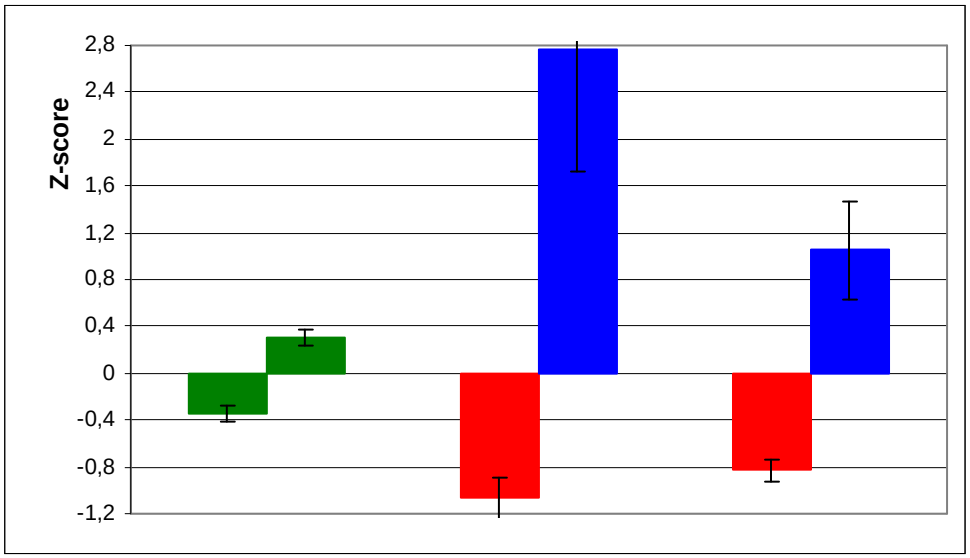


Fig. 3.14. Post-stress abnormalities in immunity of male rats of III, I and II clusters

The **modulus** of deviation from the norm of immune parameters as a measure of stress-induced immunodysfunction is 1,54±0,35σ for the I cluster, 0,93±0,19σ for the II cluster, whereas for the III cluster it is only 0,32±0,05σ.

Another methodical approach to elucidating the characteristic features of post-stress immune clusters was discriminant analysis (the forward stepwise method). Only 12 parameters were selected for inclusion in the model (Table 3.12).

For completeness of the picture in Tables 3.13-3.17 provide data on immune parameters not included in the discriminant model.

Table 3.12. Summary of discriminant analysis of post-stress immune indices of male rats

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

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

%	0	+0,65±0,34	**-1,77±0,30**	**-0,82±0,23**					
Lymphocytes of Spleen, %	53,1±0,9 1 0	53,7±0,8 1,01±0,02 +0,20±0,29	49,6±0,8 0,93±0,02 **-1,21±0,29**	53,3±0,8 1,00±0,01 +0,08±0,26	,023	,857	2,16	,13	,063
Microb Capac. Neutro Blood, 10^9 Bact/L	20,3±1,7 1 0	22,0±1,1 1,22±0,06 +0,32±0,22	16,5±1,0 0,81±0,05 **-0,71±0,18**	20,9±1,6 1,03±0,08 +0,12±0,30	,028	,692	5,79	,01	,401

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

Significant **suppressive** and **enhancing** effects are highlighted

Table 3.13. Leukocytogram indices not included in the discriminant model

Variables	Intact Male Rats (n=10)	Chronic Stressed Male Rats			Parameters of Wilks' Statistics				
		Cluster III (n=16)	Cluster I (n=9)	Cluster II (n=15)	Wilks Λ	Partial Λ	F to enter	p-level	Tolerancy
Stub Neutrophiles of Blood, %	3,50±0,17 1 0	3,50±0,22 1,00±0,06 0,00±0,42	3,67±0,41 1,05±0,12 +0,32±0,77	2,80±0,28 0,80±0,08 **-1,33±0,53**	,020	,992	,098	,91	,423
Eosinophiles of Blood, %	3,50±0,62 1 0	4,63±0,48 1,32±0,14 **+0,58±0,25**	3,44±0,47 0,98±0,14 -0,03±0,24	3,27±0,56 0,93±0,16 -0,12±0,29	,019	,979	,265	,77	,682
Basophiles of Blood, %	0,30±0,15 1 0	0,06±0,06 0,21±0,21 **-0,49±0,13**	0,22±0,15 0,74±0,49 -0,16±0,30	0,20±0,11 0,67±0,36 -0,21±0,22	,019	,946	,719	,50	,738
Lymphocytes of Blood, %	60,4±1,4 1 0	58,1±1,5 0,96±0,02 -0,52±0,32	56,7±1,3 0,94±0,02 **-0,83±0,30**	64,5±1,3 1,07±0,02 **+0,92±0,28**	,019	,984	,208	,81	,139
Leukocytes of Blood, 10^9/L	9,57±0,54 1 0	10,06±0,36 1,05±0,04 +0,29±0,21	8,20±0,29 0,86±0,03 **-0,80±0,17**	11,55±0,62 1,21±0,06 **+1,16±0,36**	,020	,997	,036	,96	,245
Entropy of Leukocytogram (•10^3)	350±7 1 0	367±7 1,05±0,02 **+0,75±0,31**	373±7 1,07±0,02 **+1,03±0,30**	334±9 0,95±0,03 -0,74±0,40	,019	,987	,161	,85	,456

Table 3.14. Indicators of phagocytosis not included in the discriminant model

Variables	Intact Male Rats (n=10)	Chronic Stressed Male Rats			Parameters of Wilks' Statistics				
		Cluster III (n=16)	Cluster I (n=9)	Cluster II (n=15)	Wilks Λ	Partial Λ	F to enter	p-level	Tolerancy
Phagocytic Index Monocytes of Blood, %	7,8±1,1 1 0	6,3±0,6 0,81±0,07 **-0,45±0,17**	4,6±0,7 0,59±0,09 **-0,94±0,21**	5,5±0,4 0,71±0,06 **-0,67±0,13**	,019	,956	,576	,57	,351
Microbial Count Monocytes of Blood, Bact/Phag	2,8±0,1 1 0	2,9±0,1 1,02±0,04 +0,19±0,31	4,6±0,9 1,66±0,32 **+5,59±2,67**	3,9±0,4 1,39±0,15 **+3,26±1,26**	,020	,993	,089	,92	,677
Bactericid Capac Monocytes of Blood, 10^6 Bact/L	81±14 1 0	89±11 1,10±0,14 +0,18±0,26	118±31 1,46±0,38 +0,84±0,70	152±34 1,87±0,42 **+1,61±0,77**	,019	,987	,164	,85	,439
Bactericidal Capacity Neutroph. of Blood, 10^9 Bact/L	11,2±1,15 1 0	12,07±0,77 1,08±0,07 +0,25±0,21	8,55±0,61 0,77±0,05 **-0,72±0,17**	11,60±0,81 +1,04±0,07 +0,12±0,22	,019	,980	,259	,77	,109
Phagocytic Index Neutrophiles of Blood, %	82,3±0,7 1 0	84,3±0,7 1,02±0,01 +0,32±0,17	78,9±1,1 0,95±0,01 **-1,10±0,29**	81,2±1,2 0,98±0,01 -0,50±0,30	,019	,989	,134	,87	,802
Microbial Count Neutrophiles of Blood, Bact/Phag	8,1±0,1 1 0	8,1±0,1 1,00±0,01 -0,16±0,43	7,6±0,2 0,93±0,02 **-2,60±0,92**	7,9±0,1 0,97±0,02 -1,20±0,66	,020	,996	,053	,95	,687
Killing Index of Neutrophiles of Blood, %	54,7±2,0 1 0	54,5±1,2 1,00±0,02 -0,03±0,20	51,7±1,2 0,94±0,02 **-0,48±0,19**	55,8±1,1 1,02±0,02 +0,18±0,17	,019	,991	,118	,89	,744

Table 3.15. Indicators of blood immunogram not included in the discriminant model

Variables	Intact Male Rats (n=10)	Chronic Stressed Male Rats			Parameters of Wilks' Statistics				
		Cluster III (n=16)	Cluster I (n=9)	Cluster II (n=15)	Wilks Λ	Par-tial Λ	F to en-ter	p-le-vel	To-lera-ncy
Th-Lymphocytes of Blood, %	32,3±0,81 0	32,6±0,6 1,01±0,02 +0,13±0,25	29,1±1,0 0,90±0,03 **-1,28±0,40**	30,1±0,4 0,93±0,01 **-0,90±0,16**	,019	,951	,649	,53	,819
Tc-Lymphocytes of Blood, %	14,1±0,91 0	13,6±0,9 0,97±0,06 -0,16±0,30	12,1±0,7 0,86±0,05 **-0,68±0,25**	13,8±0,8 0,98±0,06 -0,10±0,29	,020	,992	,097	,91	,511
B-Lymphocytes of Blood, %	12,7±0,71 0	12,6±0,5 0,99±0,04 -0,06±0,20	12,6±1,2 0,99±0,09 -0,06±0,52	12,7±0,6 1,00±0,05 +0,01±0,26	,019	,978	,280	,76	,677
NK-Lymphocytes of Blood, %	10,4±0,61 0	9,7±0,3 0,94±0,03 -0,34±0,19	9,9±0,5 0,96±0,04 -0,25±0,25	8,6±0,3 0,83±0,03 **-0,93±0,17**	,020	,993	,093	,91	,757
0-Lymphocytes of Blood, %	29,9±1,51 0	31,5±1,1 1,05±0,04 +0,33±0,22	36,3±1,8 1,22±0,06 **+1,34±0,37**	34,8±1,3 1,16±0,04 **+1,02±0,28**	,019	,985	,190	,83	,853
Entropy of Immunocytogram ($\bullet 10^3$)	522±4 1 0	520±4 1,00±0,01 -0,20±0,27	516±6 0,99±0,01 -0,49±0,43	514±5 0,98±0,01 -0,60±0,35	,020	,992	,102	,90	,520

Table 3.16. Thymocytogram indices not included in the discriminant model

Variables	Intact Male Rats (n=10)	Chronic Stressed Male Rats			Parameters of Wilks' Statistics				
		Cluster III (n=16)	Cluster I (n=9)	Cluster II (n=15)	Wilks Λ	Partial Λ	F to enter	p-level	Tolerancy
Thymus Mass Index, ‰ Body Mass	2,95±0,22 1 0	3,47±0,22 1,18±0,07 **+0,78±0,32**	3,06±0,23 1,04±0,08 +0,16±0,36	3,53±0,22 1,20±0,07 **+0,86±0,31**	,019	,959	,529	,60	,482
Epitheliocytes of Tymus, %	19,9±0,7 1 0	19,5±0,5 0,98±0,02 -0,18±0,23	21,8±0,6 1,09±0,03 **+0,81±0,24**	21,3±0,8 1,07±0,04 +0,60±0,35	,019	,986	,174	,84	,327
Endotheliocytes of Thymus, %	7,40±0,43 1 0	5,62±0,31 0,76±0,04 **-1,32±0,23**	6,88±0,49 0,93±0,07 -0,39±0,36	6,33±0,48 0,86±0,07 **-0,79±0,36**	,020	,994	,070	,93	,498
Reticulocytes of Thymus, %	5,20±0,63 1 0	4,81±0,46 0,93±0,09 -0,20±0,23	6,13±0,52 1,18±0,10 +0,47±0,26	5,93±0,47 1,14±0,09 +0,37±0,24	,020	,995	,066	,94	,425
Entropy of Thymocytogram ($\bullet 10^3$)	498±9 1 0	485±9 0,97±0,02 -0,50±0,32	536±7 1,08±0,01 **+1,38±0,27**	513±6 1,03±0,01 **+0,54±0,24**	,019	,982	,225	,80	,057

Table 3.17. Splenocytogram indices not included in the discriminant model

Variables	Intact Male Rats (n=10)	Chronic Stressed Male Rats			Parameters of Wilks' Statistics				
		Cluster III (n=16)	Cluster I (n=9)	Cluster II (n=15)	Wilks Λ	Par-tial Λ	F to en-ter	p-le-vel	To-lera-ncy
Spleen Mass Index, ‰ Body Mass	28,4±1,2 1 0	28,5±0,9 1,00±0,03 +0,02±0,23	27,6±1,7 0,97±0,06 -0,21±0,43	29,6±0,8 1,04±0,03 +0,32±0,21	,020	,991	,115	,89	,573
Lymphoblastes of Spleen, %	5,10±0,39 1 0	5,12±0,27 1,00±0,05 +0,02±0,23	3,67±0,24 0,72±0,05 **-1,20±0,20**	4,40±0,21 0,86±0,04 **-0,58±0,18**	,019	,949	,672	,52	,812
Plasmocytes of Spleen, %	2,80±0,47 1 0	3,00±0,39 1,07±0,14 +0,14±0,26	3,33±0,69 1,19±0,24 +0,36±0,47	2,87±0,32 1,02±0,11 +0,04±0,22	,020	,996	,044	,96	,360
Reticulocytes of Spleen, %	14,3±0,6 1 0	13,5±0,5 0,94±0,03 -0,45±0,27	13,8±0,6 0,96±0,04 -0,30±0,35	13,5±0,5 0,94±0,03 -0,43±0,28	,020	,996	,055	,95	,500
Macrophages of Spleen, %	5,50±0,65 1 0	7,4±0,3 1,35±0,06 **+0,94±0,17**	9,1±0,6 1,66±0,12 **+1,75±0,32**	6,2±0,4 1,13±0,08 +0,34±0,22	,019	,979	,268	,77	,516
Neutrophiles of Spleen, %	11,5±0,5 1 0	9,6±0,4 0,83±0,03 **-1,23±0,23**	10,9±1,0 0,95±0,09 -0,39±0,63	11,6±0,6 1,01±0,06 +0,06±0,40	,020	,991	,114	,89	,597
Eosinophiles of Spleen, %	1,80±0,25 1 0	1,75±0,19 0,97±0,11 -0,06±0,24	2,22±0,36 1,24±0,20 +0,54±0,46	2,60±0,29 1,44±0,16 **+1,02±0,37**	,019	,966	,441	,65	,572

In the next stage of discriminant analysis, the 12-dimensional space of discriminant variables was transformed into a two-dimensional space of canonical discriminant roots (Table 3.18). It was found that the first root contained 67,8% of the discriminatory potential and the second root the remaining 32,2%. Given the structural coefficients, the first root characterizes the inverse image of RBTL on PhHA (ie, the cardinal clustering immune parameter), the relative content of neutrophils in the blood and the Hassal's corpuscles in the thymus, while directly the content in the thymus of the lymphoblastes.

The second root reflects directly the macrophagal links of the thymus, spleen and blood, instead the inverse microphagal link of the blood, as well as the content of lymphocytes in the thymus and spleen.

Table 3.18. Correlations of discriminant variables with canonical roots and post-stress deviations from the norm of discriminant variables in different clusters of male rats

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

Further, according to the Table 3.19 a points in the information space of two roots for each animal was identified (Fig. 3.15).

Table 3.19. Summary step-by-step analysis as well as standardized and raw coefficients and constants for immune discriminant variables

Coefficients	Standardized		Raw		Parameters of Wilks' Statistics				
Variables currently in the model	Root 1	Root 2	Root 1	Root 2	F to enter	p-level	Λ	F-value	p-level
Lymphocytes of Thymus	,211	,015	,060	,004	10,0	10^{-3}	,176	24,9	10^{-6}
Lymphocytes of Spleen	-1,529	,470	-,499	,153	3,83	,032	,048	19,0	10^{-6}
Microbial Capacity Neutroph Blood	-,022	-,976	-,006	-,251	3,90	,030	,059	20,5	10^{-6}
Lymphoblastes of Thymus	,266	,599	,326	,735	1,76	,191	,022	14,0	10^{-6}
Blasttransformation T-Lymphocytes	-1,342	,046	-,229	,008	49,0	10^{-6}	,274	49,0	10^{-6}
Hassal corpuscles of Thymus	-,387	-,159	-,436	-,180	2,62	,089	,033	17,0	10^{-6}
Segmented Neutrophiles of Blood	-,831	,442	-,200	,106	9,52	10^{-3}	,114	22,9	10^{-6}
Plasmocytes of Blood	-,186	,484	-,159	,413	1,69	,204	,020	13,3	10^{-6}
Monocytes of Blood	-,219	,341	-,120	,187	3,78	,034	,038	18,1	10^{-6}
Fibroblastes of Spleen	-,689	,262	-,417	,158	9,42	10^{-3}	,073	22,9	10^{-6}
Entropy of Splenocytogram	-1,084	1,506	-,044	,061	2,12	,138	,029	15,8	10^{-6}
Macrophages of Thymus	,424	,920	,299	,648	1,91	,167	,025	14,9	10^{-6}
	Constants		69,47	-52,84					
Discriminant Properties, %			67,8	32,2					

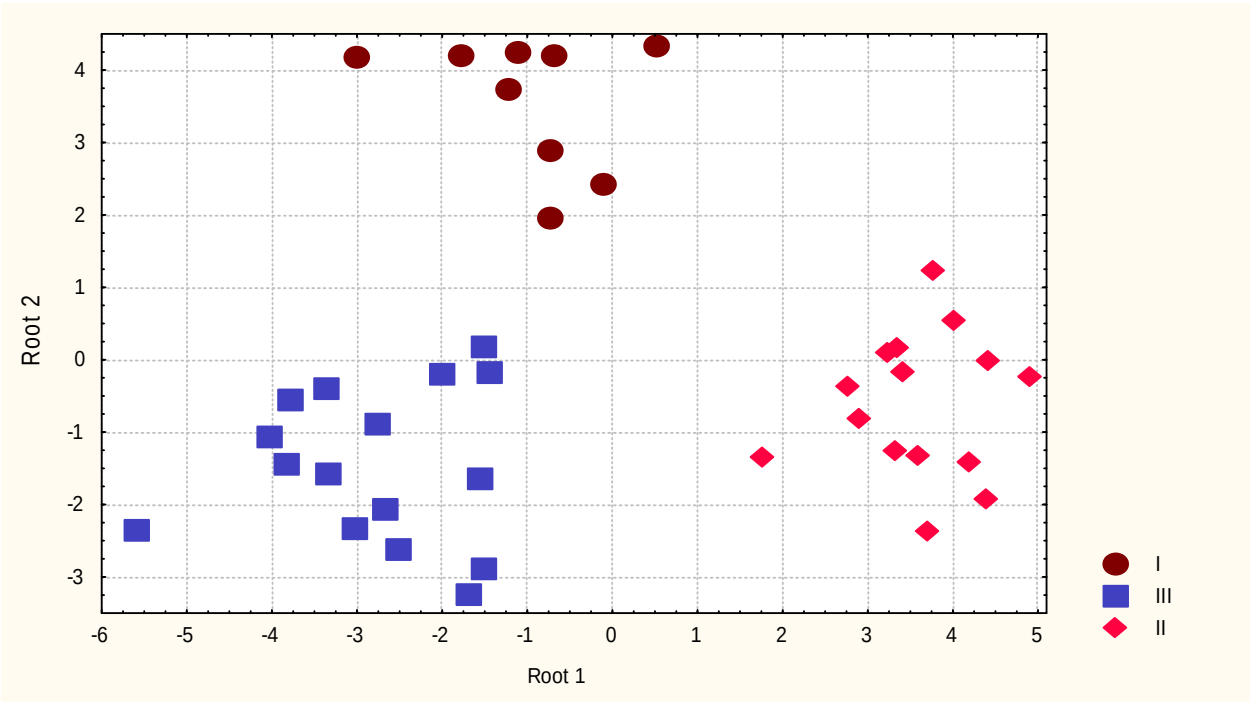


Fig. 3.15. Individual levels of discriminant immune roots of male rats of different clusters of immune response to stress

The figure shows that the set of selected variables post-stress immune status of rats of the three clusters are significantly different from each other. The visual impression is confirmed by the calculation of the squares Mahalanobis distance which are between I and III clusters 30,9 ($F=9.4$; $p<10^{-6}$); between I and II clusters 41,6 ($F=12,4$; $p<10-6$); between II and III clusters 44,7 ($F=18.9$; $p<10^{-6}$).

Extreme localization of members of the second cluster along the axis of the first root reflects (see Table 3.18) their minimum among the stressed cohort of the level of mitogenic blastransformation of T-lymphocytes and the proportion within leukocytes of segmental nuclear neutrophils, as well as Hassal's corpuscles in thymocytogram, on the one hand, and the maximum proportion of lymphoblasts therein - on the other, whereas the listed indices of cluster members I and III are significantly larger/smaller than those of cluster II and are not significantly different, as illustrated by the mixing of points along the axis of the first root.

Instead, the extreme localization of members of the first cluster along the axis of the second root reflects a dramatic increase in the content of **macrophages in the thymus**, **fibroblasts in the spleen, and plasmocytes in the blood**, as well as entropy of splenocytogram in combination with a decrease in the content of **thymus and spleen of lymphocytes and the microbial capacity of blood neutrophils** (Fig. 3.16). The listed immune parameters in the members of the other two clusters are much smaller/larger and, as a rule, differ little, as illustrated by the mixing of points along the axis of the second root.

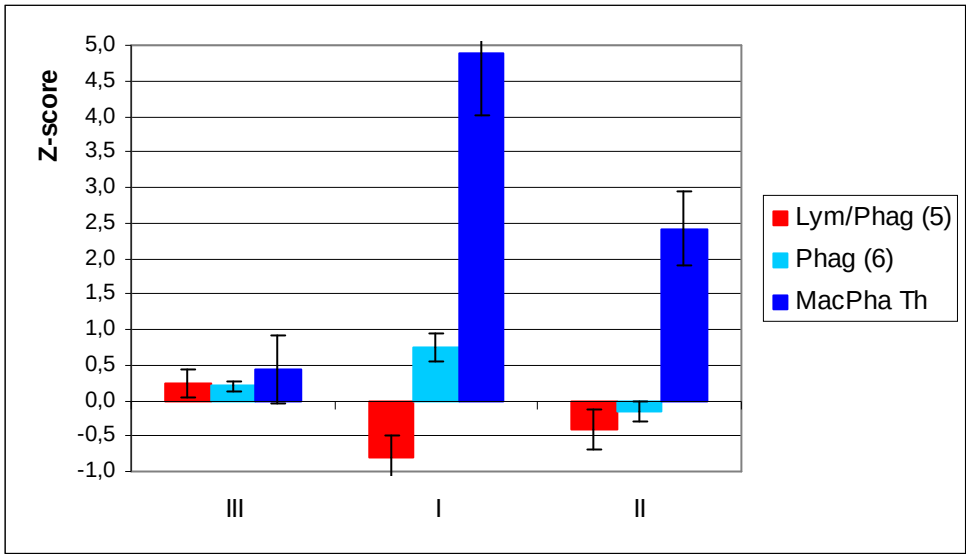


Fig. 3.16. Post-stress condition of three immune sets of male rats of different clusters

Therefore, the differences between post-stress immune statuses are comprehensively explained by 12 parameters that reflect different levels of immunity. The information contained in these parameters can be condensed into two roots (recognition functions). In other words, the selected parameters can be used to identify a particular cluster-type immune response to chronic stress. This purpose of discriminant analysis is realized by means of the classification functions (Table 3.20).

Table 3.20. Coefficients and constants for classification functions of immune parameters

Variables currently in the model	Cl I	Cl III	Cl II
Blasttransformation T-Lymphocytes	15,40	15,78	14,33
Segmented Neutrophiles of Blood	9,71	9,53	8,35
Hassal's corpuscles of Thymus	115,1	116,8	113,9
Lymphoblastes of Thymus	183,5	179,2	181,9
Macrophages of Thymus	98,81	95,01	97,45
Entropy of Splenocytogram	32,69	32,46	32,24
Fibroblastes of Spleen	38,79	38,75	36,22
Monocytes of Blood	3,93	3,20	2,60
Plasmocytes of Blood	73,30	71,51	70,84
Lymphocytes of Thymus	27,25	27,12	27,50
Lymphocytes of Spleen	235,6	235,7	232,6
Microbial Capacity Neutroph of Blood	-25,37	-24,09	-24,34
Constants	**-18246**	**-18103**	**-17706**

In this case, the unmistakable correctness of the classification of objects is achieved.

3.5. Features of neuroendocrine regulation in male rats of different immune clusters (according to discriminant analysis)

From the standpoint of the concept of the triune neuro-endocrine-immune complex, the components of which interact closely, it follows that the revealed features of immune responses to chronic stress are related to the peculiarities of reactions to the stress of the autonomic nervous system and classical adaptive glands: adrenals, gonads and thyroid as well as parathyroid.

It is a surprise that almost all (except for adrenals mass) reported neuro-endocrine parameters (Table 3.21) were included into the model by the discriminant analysis program, that is, they all in one way or another determine the revealed features of post-stress immunity in rats of three clusters.

Table 3.21. Summary of discriminant analysis of post-stress neuroendocrine indices of male rats

Step 15, N of vars in model: 15; Grouping: 3 grps
Wilks' Lambda: 0,120; approx. $F_{(30)}$=2,89; $p<10^{-3}$

Variables currently in the model	Control group (10)	Cluster I (9)	Cluster II (15)	Cluster III (16)	Wilks Λ	Par-tial Λ	F-re-move (2,2)	p-le vel	Tole ran-cy
Triiodo-thyronine, nM/l	2,50±0,17 1 0	2,17±0,21 0,87±0,08 **-0,62±0,40**	2,70±0,14 1,08±0,06 +0,39±0,27	2,88±0,13 1,15±0,05 **+0,72±0,25**	,177	,679	5,44	,012	,295
Fasciculary Zone Adrenals Cortex, µM	218±11 1 0	214±16 0,99±0,07 -0,09±0,43	254±13 1,17±0,06 **+1,00±0,36**	252±12 1,16±0,05 **+0,96±0,32**	,155	,776	3,33	,054	,312
Glomerulary Zone Adrenals Cortex, µM	129±11 1 0	102±10 0,79±0,08 **-0,75±0,27**	116±5 0,90±0,04 **-0,36±0,14**	115±5 0,89±0,03 **-0,38±0,13**	,133	,900	1,28	,296	,371
Reticulary Zone Adrenals Cortex, µM	20±2 1 0	20±3 0,97±0,13 -0,10±0,46	25±2 1,24±0,08 **+0,84±0,28**	24±2 1,20±0,09 **+0,70±0,31**	,140	,857	1,92	,169	,290
Medullary Zone of Adrenals, µM	87±7 1 0	84±13 0,97±0,15 -0,11±0,56	87±7 1,00±0,09 +0,02±0,32	87±6 1,00±0,07 +0,02±0,26	,138	,871	1,70	,205	,558
Thyroxine, nM/L	60±6 1 0	85±10 1,42±0,16 **+1,32±0,51**	58±3 0,97±0,05 -0,11±0,16	59±4 0,99±0,06 -0,04±0,19	,148	,811	2,69	,089	,311
$(Ku/Nau)^{0,5}$ as Mineralocorti-coide Activity	0,73±0,07 1 0	0,93±0,10 1,27±0,14 +0,93±0,49	0,70±0,07 0,96±0,09 -0,13±0,32	0,74±0,05 1,02±0,07 +0,06±0,23	,227	,529	10,2	,001	,277
AMo HRV as Sympathotone, %	56±7 1 0	85±7 1,53±0,12 **+1,25±0,28**	55±5 0,98±0,09 -0,05±0,21	69±5 1,25±0,10 **+0,59±0,23**	,143	,842	2,16	,139	,009
$(1/Cap•Pp)^{0,5}$ as Calcitonine	0,49±0,02 1	0,49±0,02 1,01±0,03	0,48±0,01 0,99±0,03	0,55±0,03 1,12±0,05	,168	,714	4,60	,021	,032

Activity	0	+0,07±0,23	-0,06±0,18	**+0,80±0,36**					
Corticosterone, nM/L	340±45 1 0	417±70 1,23±0,20 +0,55±0,49	342±30 1,01±0,09 +0,02±0,21	472±69 1,39±0,20 +0,94±0,48	,194	,618	7,11	,004	,465
Testosterone, nM/L	34,6±4,6 1 0	33,4±2,6 0,97±0,07 -0,08±0,18	36,5±5,5 1,06±0,16 +0,13±0,38	48,4±4,8 1,40±0,14 **+0,96±0,33**	,133	,900	1,27	,299	,730
$(Cap/Pp)^{0,5}$ as Parathyrine Activity	1,68±0,06 1 0	1,64±0,05 0,99±0,03 -0,07±0,27	1,66±0,04 1,00±0,02 +0,03±0,21	1,50±0,06 0,90±0,03 **-0,79±0,28**	,185	,648	6,26	,007	,029
MxDMn HRV as Vagotone, msec	42±8 1 0	16±5 0,39±0,13 **-1,04±0,22**	42±6 1,00±0,15 -0,01±0,26	26±5 0,63±0,12 **-0,63±0,20**	,135	,892	1,39	,269	,004
Moda HRV, msec	184±11 1 0	154±8 0,84±0,04 **-0,88±0,22**	181±13 0,98±0,07 -0,10±0,36	159±6 0,87±0,03 **-0,72±0,17**	,142	,847	2,08	,148	,017
17-Ketosteroides urine, nM/100 g•day	26±6 1 0	24±7 0,93±0,28 -0,10±0,39	33±7 1,27±0,29 +0,37±0,41	25±4 0,98±0,15 -0,03±0,21	,150	,802	2,85	,079	,432

Identification information is condensed into two canonical roots, the first of which contains 87,1% discriminatory opportunities, while the second contains only 12,9%. After calculating the individual values of both roots using the raw coefficients for neuro-endocrine variables and constants given in Table 3.22, the position of each animal was visualized in the two-dimensional information space of these roots (Fig. 3.17).

Table 3.22. Summary of step-by-step analysis aa well as standardized and raw coefficients and constants for neuro-endocrine discriminant variables in male rats

Coefficients	Standardized		Raw		Parameters of Wilks' Statistics				
Variables currently in the model	Root 1	Root 2	Root 1	Root 2	F to enter	p-le-vel	Λ	F-va lue	p-le-vel
Triiodothyronine	1,103	-,533	1,982	-,957	4,71	,015	,797	4,71	,015
Fasciculary Zone Adren Cortex	,860	-,567	,0180	-,0119	3,07	,061	,297	3,70	10^{-3}
Glomerulary Zone Adren Cortex	,257	-,755	,0123	-,0362	1,27	,298	,147	3,09	10^{-3}
Reticulary Zone Adrenal Cortex	-,754	,302	-,1088	,0436	1,82	,181	,203	3,41	10^{-3}
Medullary Zone of Adrenals	-,528	,128	-,0178	,0043	1,42	,259	,162	3,22	10^{-3}
Thyroxine	-,671	-,804	-,0396	-,0473	2,19	,129	,259	3,62	10^{-3}
$(Ku/Nau)^{0,5}$ as Mineralocort Act	-1,441	-,263	-6,001	-1,097	2,74	,079	,529	4,37	10^{-3}
AMo HRV as Sympathicotone	-4,207	-3,198	-,193	-,147	5,44	,009	,612	5,01	,001
$(1/Cap•Pp)^{0,5}$ as Calcitonine Act	-3,023	-2,010	-39,83	-26,48	2,40	,106	,401	3,82	10^{-3}
Corticosterone	,926	-,586	,0044	-,0028	2,05	,146	,355	3,61	10^{-3}
Testosterone	-,099	-,580	-,0058	-,0340	1,27	,299	,120	2,89	10^{-3}
$(Cap/Pp)^{0,5}$ as Parathyrine Activ	-3,736	-1,463	-20,25	-7,929	2,59	,089	,459	4,04	10^{-3}
MxDMn HRV as Vagotone	-5,646	-3,390	-,2545	-,1528	1,24	,307	,133	2,98	10^{-3}
Moda HRV	3,333	,784	,0913	,0215	1,76	,191	,180	3,33	10^{-3}
17-Ketosteroides urine	-,749	,142	-,0335	,0063	1,85	,176	,230	3,50	10^{-3}
	Constants		57,33	51,20					
Discriminant Properties, %			87,1	12,9					

Table 3.23. Correlations of neuro-endocrine discriminant variables with canonical roots and post-stress abnormalities in different clusters of male rats

Variables currently in the model	Root 1	Root 2	**Z-scores for Variables**		
			Clu I	**Clu II**	**Clu III**
Triiodothyronine	**,246**	-,079	**-0,62**	+0,39	+0,72
Fasciculary Zone Adrenal Cortex	**,153**	,086	**-0,09**	+1,00	+0,96
Glomerulary Zone Adrenal Cortex	**,125**	,075	**-0,75**	-0,36	-0,38
Reticulary Zone Adrenal Cortex	**,118**	,118	**-0,10**	+0,84	+0,70
Medullary Zone of Adrenals	**,021**	,012	**-0,11**	+0,02	+0,02
Thyroxine	**-,221**	-,142	**+1,32**	-0,11	-0,04
$(Ku/Nau)^{0,5}$ as Mineralocorticoid Activ	**-,155**	-,148	**+0,43**	-0,47	-0,09
AMo HRV as Sympathetic tone	-,142	**-,435**	+1,25	**-0,05**	+0,59
$(1/Cap•Pp)^{0,5}$ as Calcitonine Activity	,089	**-,412**	+0,07	**-0,06**	+0,80
Corticosterone	,015	**-,354**	+0,55	**+0,02**	+0,94
Testosterone	,109	**-,305**	-0,08	**+0,13**	+0,95
$(Cap/Pp)^{0,5}$ as Parathyrine Activity	-,100	**,431**	-0,07	**+0,03**	-0,79
MxDMn HRV as Vagal tone	,100	**,407**	-1,04	**-0,01**	-0,63
Moda HRV	,054	**,319**	-0,88	**-0,10**	-0,72
17-Ketosteroides urine	,032	**,204**	-0,10	**+0,37**	-0,03
Tests with Successive Roots Removed			**Means of Variables**		
Canonical R	,898	,618	Root 1		
Wilks' Lambda	,120	,618	-3,59	+0,66	+1,40
Chi-Sqr.	63,6	14,4	Root 2		
Degree freedom	30	14	-0,23	+0,94	-0,75
p-level	,0003	,419			

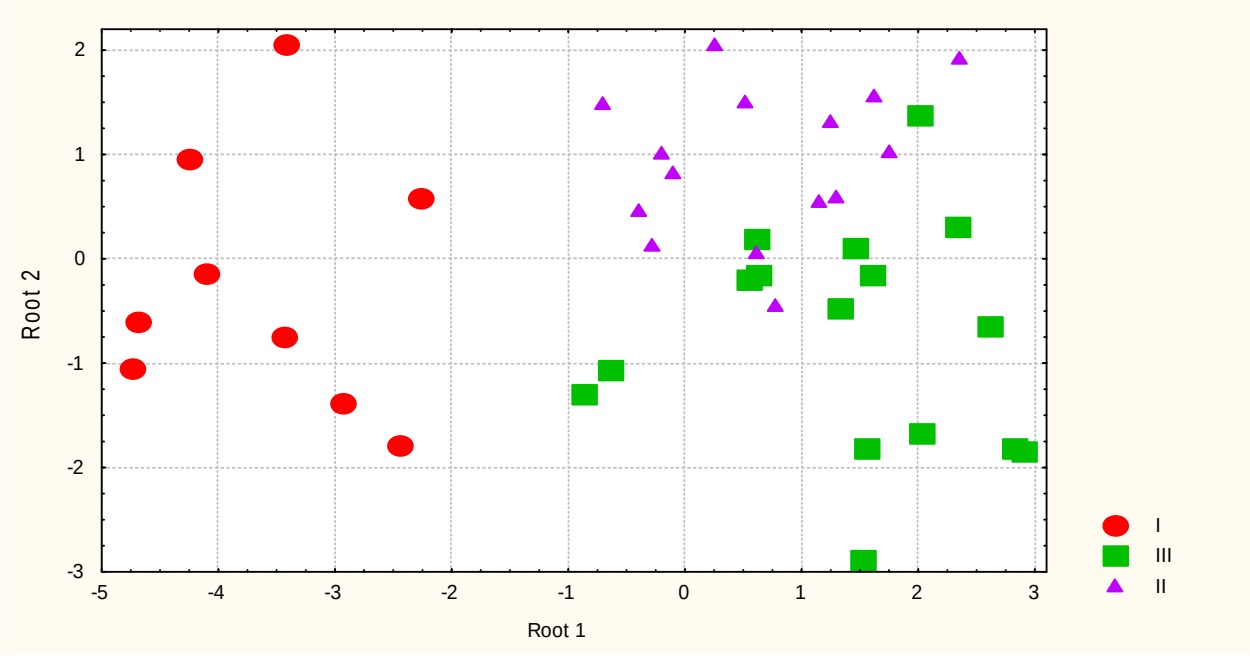


Fig. 3.17. Individual values of the discriminant neuro-endocrine roots of male rats of different immunity response clusters

The calculation of the mean values of neuro-endocrine roots makes it possible to visualize the localization in the two-dimensional information space of the centroids of individual clusters of the immune response to chronic stress (Fig. 3.18).

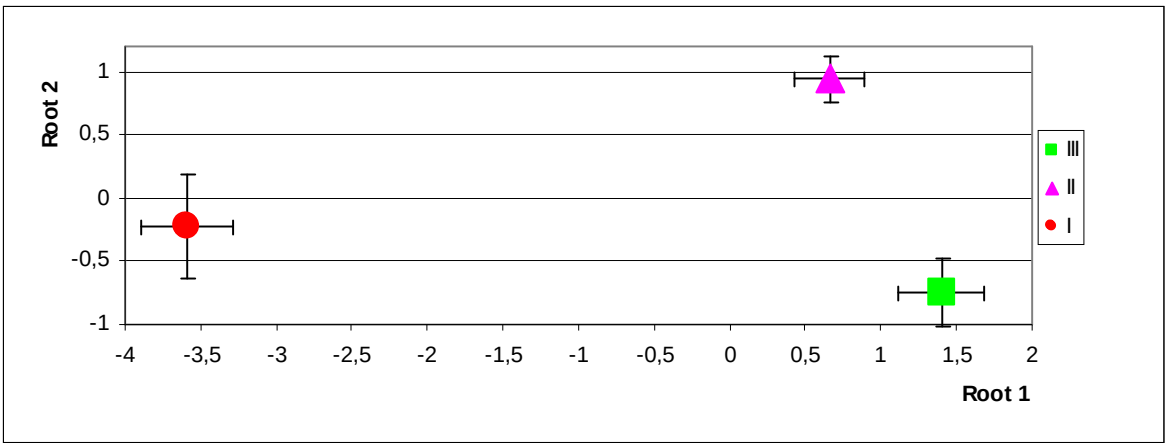


Fig. 3.18. Mean values of discriminant neuro-endocrine roots of male rats of different clusters of the immune response to stress

Figures 3.17 and 3.18 show that the set of selected variables poststress neuro-endocrine status of the three clusters of rats differ to a different extent. The visual impression is confirmed by the calculation of the Mahalanobis distance squares,that make up between clusters I and III 27 ($F=5,9$; $p<10^{-4}$); between I and II clusters 21 ($F=4,4$; $p<10^{-3}$), while between II and III clusters only 4 ($F=1,11$; $p=0,40$).

Extreme localization of members of the cluster I along the axis of the first root reflects their minimum values in the stressed cohort of magnitude of positively correlating with the root level in the plasma triiodothyronine and thickness of all four adrenal zones, as well as the maximum magnitudes of negatively correlating with the root level in the plasma thyroxine and mineralocorticoid activity. In animals II and III clusters the magnitudes (points) of these endocrine indicators are interspersed.

Instead, along the axis of the second root, the values of other endocrine parameters and the parameters of the autonomic nervous system in rats I and III clusters are interspersed, whereas the animals of the cluster II occupy the upper extreme zone of the axis. This reflects their minimum in the cohort of sympathetic tone, calcitonin activity, as well as corticosterone and testosterone levels, which are inversely related to the root, whereas maximum values are directly related to the root of parathyroid activity, markers of vagal tone, and daily excretion 17-ketosteroids.

Retrospective classification of rats by neuro-endocrine parameters (Table 3.24) is not as accurate as immune.

Table 3.24. Coefficients and constants of classification functions for neuro-endocrine parameters of male rats

Variables currently in the model	I	III	II
Triiodothyronine	11,58	21,97	18,87
Fasciculary Zone Adrenal Cortex	-0,444	-0,348	-0,382
Glomerulary Zone Adrenal Cortex	3,013	3,093	3,023
Reticulary Zone Adrenal Cortex	6,416	5,851	6,006
Medullary Zone of Adrenals	1,373	1,282	1,303
Thyroxine	6,797	6,624	6,573
$(Ku/Nau)^{0,5}$ as Mineralocorticoide Activ	514,5	485,1	487,7
AMo HRV as Sympathicotone	25,41	24,52	24,41
$(1/Cap•Pp)^{0,5}$ as Calcitonine Activity	7677	7492	7477
Corticosterone	-0,112	-0,089	-0,097
Testosterone	2,338	2,327	2,274
$(Cap/Pp)^{0,5}$ as Parathyrine Activity	3283	3186	3187
ΔX HRV as Vagotone	30,21	29,02	28,95
Moda HRV	-7,074	-6,630	-6,661
17-Ketosteroides urine	1,803	1,632	1,668
Constants	**-6121**	**-5856**	**-5811**

In particular, only rats of the first cluster are correctly identified, whereas the classification accuracy of the members of the second cluster is 93,3% (one error per 15

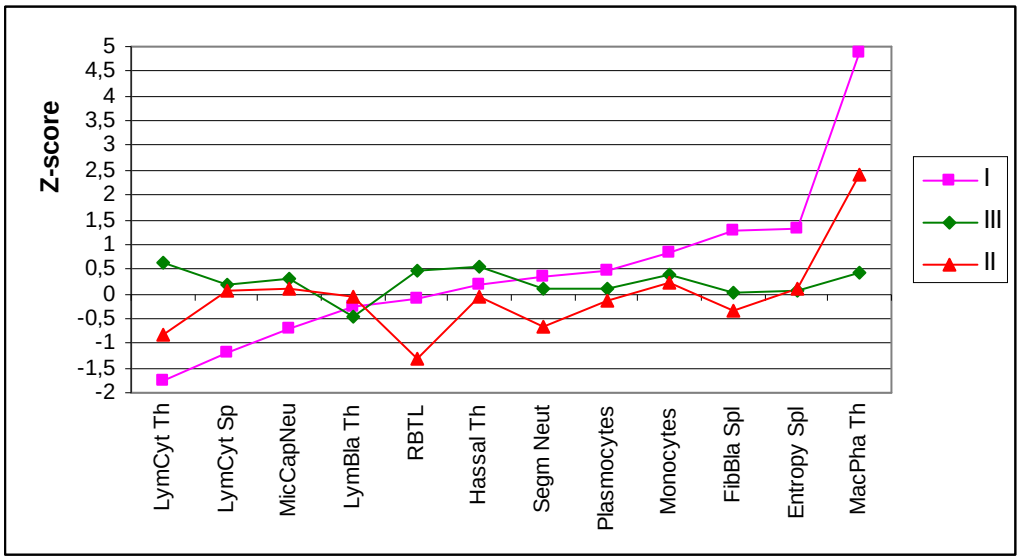
animals) and the third one is only 87,5 (two errors per 16 animals), so that the overall classification accuracy is 92,5%.

3.6. Relationships between neuro-endocrine and immune parameters

The purpose of this subsection is to evaluate the nature and strength of the links between neuro-endocrine and immune parameters. Two methodological approaches are used to achieve this goal. The first approach is to compare immune and neuro-endocrine profiles.

Fig. 3.19 illustrates that the absence of post-stress deviations from the norm of immune status indicators in rats of the cluster III is associated with normal post-stress levels of mineralocorticoid activity, plasma thyroxine and daily excretion of 17-ketosteroids, in contrast to reduced parathyroid activity, vagal tone and thickness of the glomerula zone of adrenal cortex, on the one hand, and increased indicators of sympathetic tone, heart rate, calcitonin activity, thickness of the reticular and fascicular zones of adrenal cortex as well as plasma levels of testosterone, corticosterone, and triiodothyronine. It seems that such a post-stress constellation of neuro-endocrine factors, due to individual reactivity, contributes to the resistance of the immune status to stress-induced abnormalities.

Moderate inhibition of microphagal, killer and T-cell links in combination with pronounced activation of the macrophagal link in cluster rats II is associated with a similar increase in the thickness of the reticular and fascicular zones of adrenal cortex, but without increasing levels in plasma testosterone and corticosterone in combination with a decrease in glomerular zone thickness and mineralocorticoid activity.



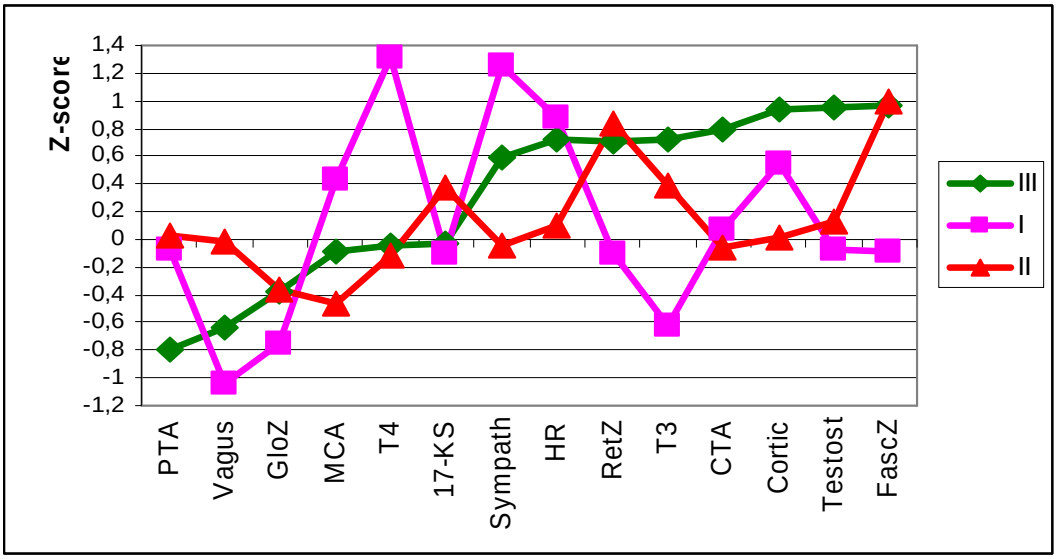


Fig. 3.19. Immune (top) and neuro-endocrine (bottom) profiles of male rats of different clusters of the immune response to stress

More pronounced post-stress changes of immunity in rats of the first cluster, first of all activation of a macrophage link in combination with supression of microphage and T-links, is also associated with the most raised sympathetic tone and a reciprocally reduced level of a vagus tone, in combination with moderate decreased level of triiodethyronine, due to the inhibition of its formation from thyroxine, whose level in plasma is significantly increased.

In addition, there is a dissociation between a decrease in the thickness of the glomerular zone of the adrenal cortex and an increase in mineralocorticoid activity, estimated by the K/Na coefficient of daily urine.

When comparing the neuro-endocrine and immune modules of Z-values as a measure of stress-induced deviations from the norm, it is seen (Fig. 3.20) that minimal immunodysfunction in rats of the cluster III is accompanied by maximal neuro-endocrine perturbation, and a slight attenuation of the latter in rats of the cluster I is accompanied by significant exacerbation of immunodysfunction.

This first suggests the immunoprotective effect of neuro-endocrine factors of chronic stress. However, such a hypothesis is rejected by the fact that further attenuation of neuro-endocrine perturbation in rats of the cluster II leads not to a burden but to a weakening of immunodysfunction.

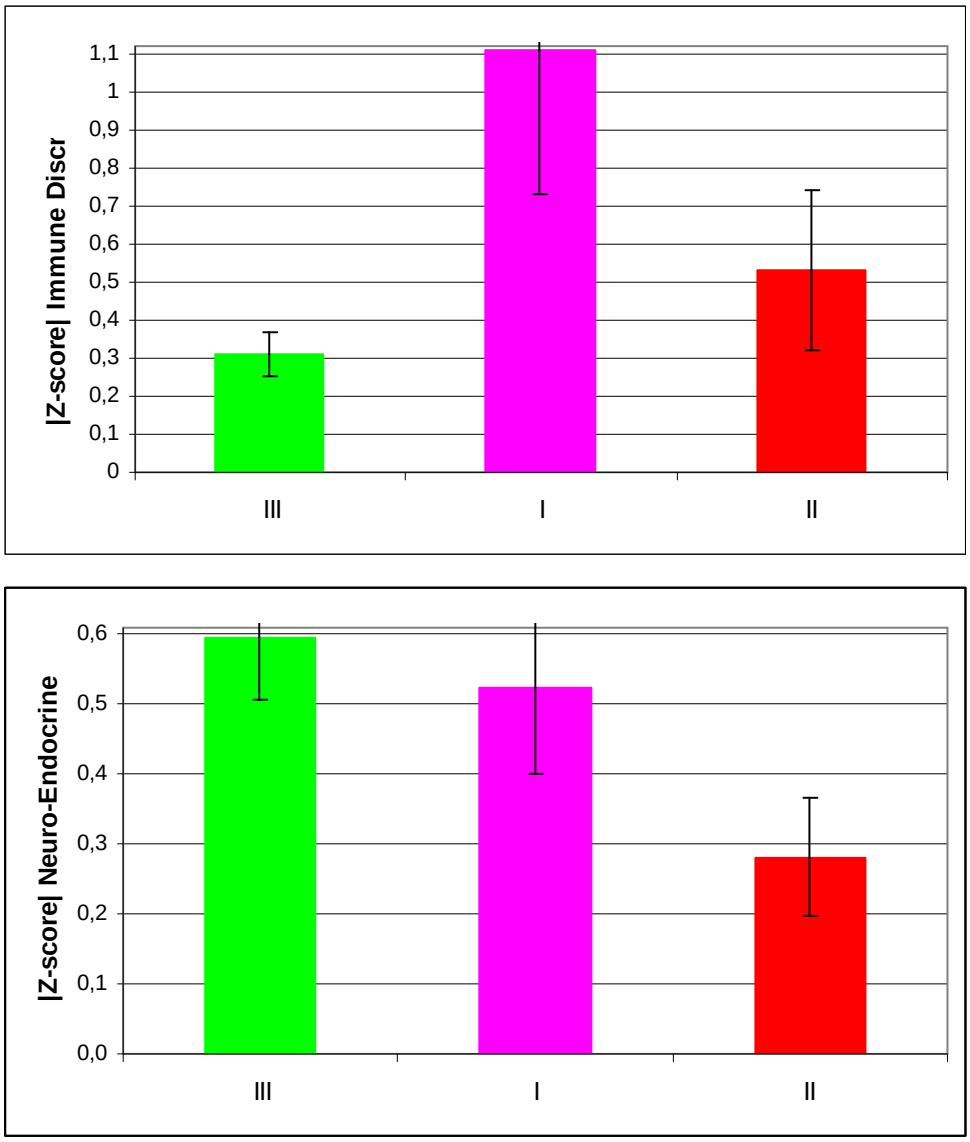


Fig. 3.20. Immune (top) and neuro-endocrine (bottom) modules of Z-values of male rats of different clusters of immunity response to stress

The **nonlinear** character of neuroendocrine-immune relationships is visualized in Fig. 3.21.

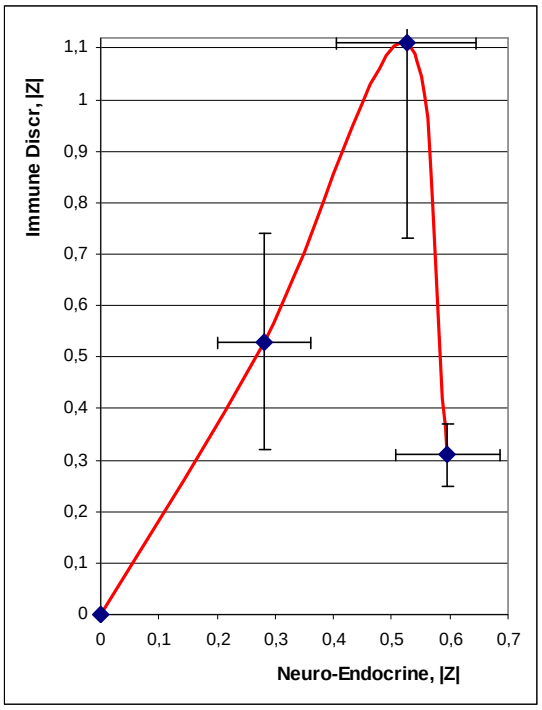


Fig. 3.21. Pattern of compatible post-stress abnormalities (in the form of Z-values) of neuro-endocrine (X-axis) and immune (Y-axis) discriminant indicators of male rats of different immune response clusters

Therefore, the analysis of neuroendocrine-immune relationships required a different approach. For this purpose, a correlation matrix was first created (Table 3.25). For a sample with n=50, the critical value |r| is at $p<0,05$ ($t>2,01$): 0,28; at $p<0,01$ ($t>2,68$): 0,37; at $p<0.001$ ($t>3,50$): 0,46.

In the next step, regression models with stepwise exclusion were constructed for each neuro-endocrine factor, and in the final stage, a canonical correlation analysis of the links between neuro-endocrine and immune constellations was performed.

Table 3.25. Correlation coefficients between neuro-endocrine and immune parameters

Variables	AMo	ΔX	Mo	Fasc	Cort	Glom	MCA	Retic	KS	Test	PTA	T_3	CTA
Stub Neutrophils of Blood	,27	-,24				-,23				-,20	**-,28**		**0,33**
Basophils of Blood	**,30**			**-,30**									
Monocytes of Blood									-0,26			**-,27**	
Lymphocytes of Blood	-,26			,27	-,24					,27	,23		-0,25
Leukocytes of Blood	**-,34**	,27										**,31**	
Phagocytose Ind of Monocytes	**-,28**	,23				,23				-,18			
Microbial Count of Monocytes											,24	**-,29**	-0,23
Killing Index of Neutrophils	**-,35**	**,33**	,25	**,41**		**,29**	**-,28**	**,30**					
Blasttransformat T-Lymphocyt								-,21		,18			
Th-Lymphocytes of Blood						,25	**,35**						
NK-Lymphocytes of Blood				**-,31**	,23			-,22				**-,33**	
0-Lymphocytes of Blood							**-,42**			-,19			
Plasmocytes of Blood	**0,31**	**-0,28**				-0,25			**-0,34**		**-0,34**		0,27
Thymus Mass Index			-,27	**,32**				,26	0,27			**,38**	
Lymphocytes of Thymus				,23	,22								
Hassal corpuscles of Thymus	**0,32**	**-0,32**	**-0,31**	**-0,45**			**-0,34**	**-0,38**	-0,27		**-0,80**		**0,75**
Lymphoblastes of Thymus	-0,26	0,27	**0,28**	0,27							**0,37**	-0,27	**-0,33**
Macrophages of Thymus	,27	**-,28**	**-,31**	**-,33**			**-,25**						
Endotheliocytes of Thymus	,28	**-,28**	-,24		**-,30**	,23							
Lymphoblastes of Spleen						**0,33**					**-0,38**	**0,29**	**0,35**
Plasmocytes of Spleen								**0,29**	0,27		0,27		-0,25
Reticulocytes of Spleen				**-,33**					**-0,30**				
Macrophages of Spleen	**,85**	**-,83**	**-,72**	-,25	,23	**-,44**		-,22			**-,39**		**0,36**
Neutrophils of Spleen	**-,34**	,24					**-,29**	-,22	**0,41**				
Eosinophils of Spleen	**-,29**												
Entropy of Leukocytogram	,23			**-,34**	,22		,24	-,24		-,22			
Entropy of Immunocytogram	**,28**	-,26		**-,31**						,20	**-,37**		**0,33**
Entropy of Splenocytogram	,23	-,25	-,27						0,27				

First of all, a strong correlation was found between sympathetic tone and content in the spleen of macrophages (Fig. 3.22).

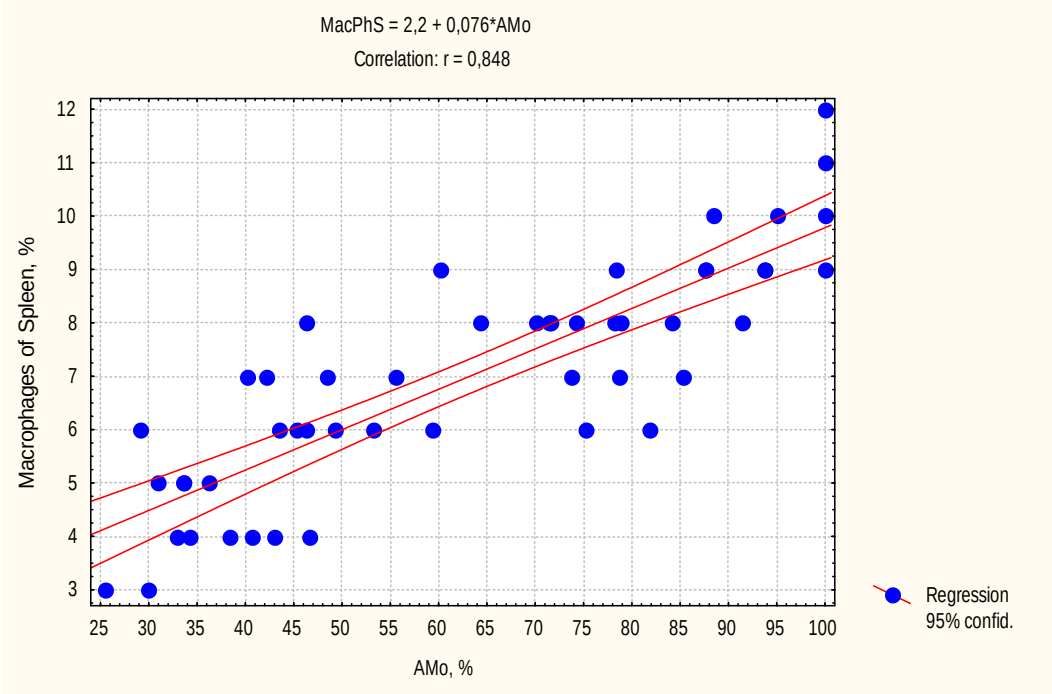


Fig. 3.22. Relationship between sympathetic tone (X-axis) and macrophage spleen content (Y-axis)

In addition, sympathetic tone also significantly affects the content of eosinophils in the spleen and endothelial cells in the thymus, so that the determination of this constellation of immunocytes reaches 77% (Table. 3.26).

Table 3.26. Regression model of multiple correlation of sympathetic tone (AMo) with indicators of immunity

R=0,885; R^2=0,783; Adjusted R^2=0,769; $F_{(3,5)}$=55; $p<10^{-5}$

		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(46)}$	p-level
	r		Intercpt	-16,0	9,3	-1,73	,091
Macrophages of Spleen	0,85	,803	,071	9,01	,80	11,3	10^{-6}
Endotheliocytes of Thymus	0,28	,236	,069	3,47	1,02	3,40	,001
Eosinophils of Spleen	-0,29	-,122	,071	-2,91	1,68	-1,73	,091

Vagal tone affects the content of macrophages and endothelial cells in the same extent, but in the opposite way, and also reduces the entropy of the splenocytogram, determining these immune parameters by 73% (Fig. 3.23, Table 3.27).

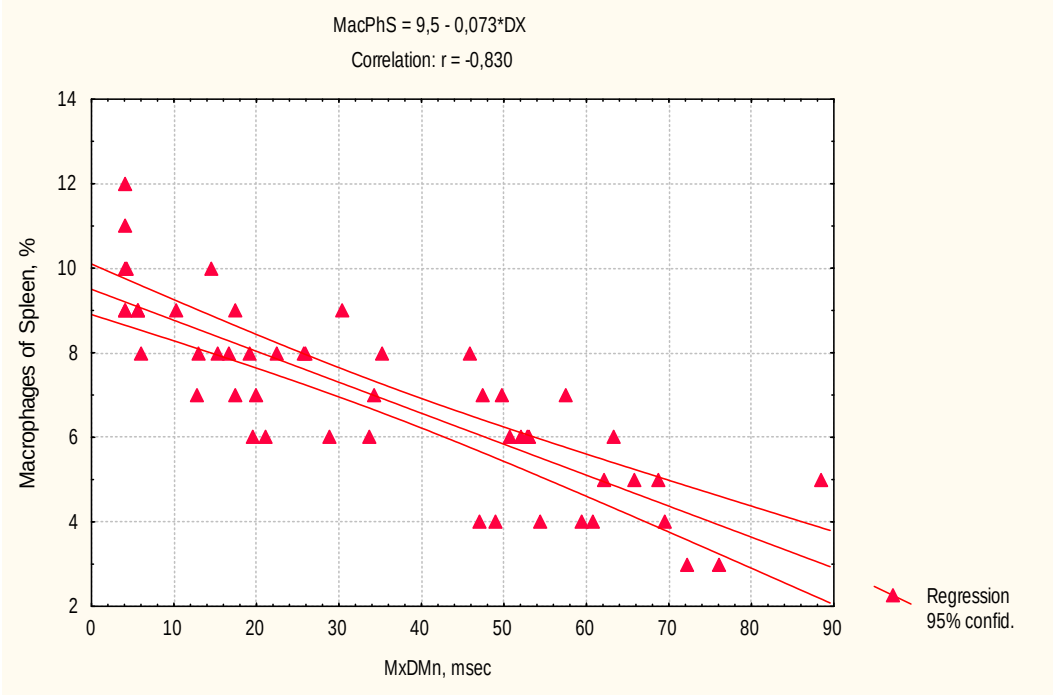


Fig. 3.23. Relationship between vagal tone (X-axis) and spleen content in macrophages (Y-axis)

Table 3.27. Regression model of multiple correlation of parasympathetic tone (MxDMn) with indicators of immunity

R=0,865; R^2=0,748; Adjusted R^2=0,732; $F_{(3,5)}$=45,5; $p<10^{-5}$

		Beta	St.Err.of Beta	B	St. Err.of B	$t_{(46)}$	p-level
	r		Intercpt	58,9	43,6	1,35	,183
Macrophages of Spleen	-0,83	-,866	,082	-9,80	,93	-10,5	10^{-6}
Endotheliocytes of Thymus	-0,28	-,210	,074	-3,13	1,11	-2,82	,007
Entropy of Splenocytogram	-0,25	,117	,082	107,6	75,4	1,43	,161

HRV Mode is considered a marker of a humoral regulation channel. According to our data, it correlates with the level of triiodothyronine (r=-0,41) and parathyroid activity (r=0,30). Mode is somewhat weaker than vagal tone, negatively affecting the content of macrophages and endothelial cells, as well as the relative mass of the thymus, determining these immune parameters by 59% (Table 3.28).

Table 3.28. A regression model of multiple correlation of the HRV Mode with immunity indices

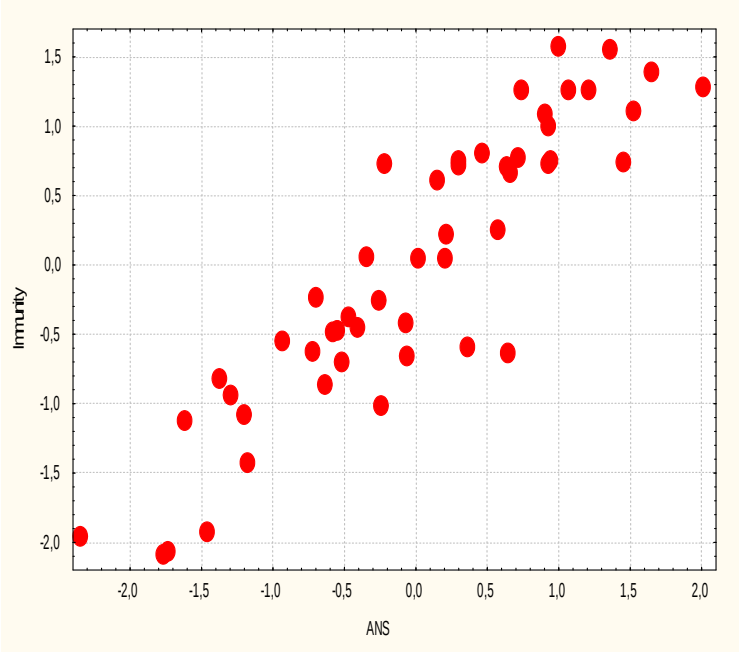
R=0,786; R^2=0,618; Adjusted R^2=0,593; $F_{(3,5)}$=24,8; $p<10^{-5}$

		Beta	St. Err. of Beta	B	St. Err.of B	$t_{(46)}$	p-level
	r		Intercpt	324	22	14,8	10^{-6}
Macrophages of Spleen	-0,72	-,699	,091	-12,4	1,6	-7,64	10^{-6}
Thymus Mass Index	-0,27	-,247	,091	-1119	414	-2,70	,009
Endotheliocytes of Thymus	-0,24	-,191	,091	-4,4	2,1	-2,09	,042

The canonical correlation analysis shows that the three parameters of autonomic regulation determine the mentioned 5 immune parameters by 82% (Table 3.29, Fig. 3.24).

Table 3.29. Factor structure of neuro-immune connections in male rats

Right set	R
Amplitude of Moda	**-,98**
MxDMn	**,96**
Moda	**,84**
Left set	R
Macrophages of Spleen	**-,95**
Endotheliocytes of Thymus	**-,31**
Entropy of Splenocytogram	**-,30**
Thymus Mass Index	**-,19**
Eosinophils of Spleen	**,30**



$R=0,906$; $R^2=0,821$; $\chi^2_{(15)}=93$; $p<10^{-6}$

Fig. 3.24. Canonical correlation between parameters of autonomic nervous system (X-axis) and immunity (Y-axis)

The thickness of the fascicular area of the adrenal cortex as a marker of their permanent glucocorticoid activity is moderately negatively correlated with the content of Hassal's bodies and macrophages in the thymus and reticulocytes in the spleen, as well as entropy of leukocytogram, but positively with thymus mass index and completion of phagocytosis of Staphylococus aureus by neutrophils. The measure of determination of this immune constellation is 39% (Table 3.30).

Table 3.30. Regression model of multiple correlation of the thickness of the fascicular zone of the adrenal cortex with indicators of immunity

$R=0,679$; $R^2=0,461$; Adjusted $R^2=0,385$; $F_{(6,4)}=6,1$; $p<10^{-4}$

		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(43)}$	p-level
	r		Intercpt	367	122	3,00	,004
Hassal corpuscles	-0,45	-,262	,128	-14,15	6,92	-2,05	,047
Entropy of Leukocytogram	-0,34	-,218	,123	-333	188	-1,77	,083
Macrophages of Thymus	-0,33	-,248	,117	-6,967	3,275	-2,13	,039
Reticulocytes of Spleen	-0,33	-,192	,124	-4,922	3,184	-1,55	,129
Killing Index of Neutrophils	0,41	,177	,123	1,734	1,198	1,45	,155

Thymus Massa Index	0,32	,158	,122	916	707	1,30	,202

Corticosterone plasma levels reflect situational glucocorticoid activity, ie at the time of blood collection. Corticosteronemia is poorly correlated with the thickness of the fascicular area of the adrenal cortex (r=-0,28), so it is not surprising that it is associated with another constellation of immune parameters, but very weak, so that their measure of corticosterone determination is only 15% (Table. 3.31).

Table 3.31. Regression model of multiple correlation of corticosterone with immunity parameters

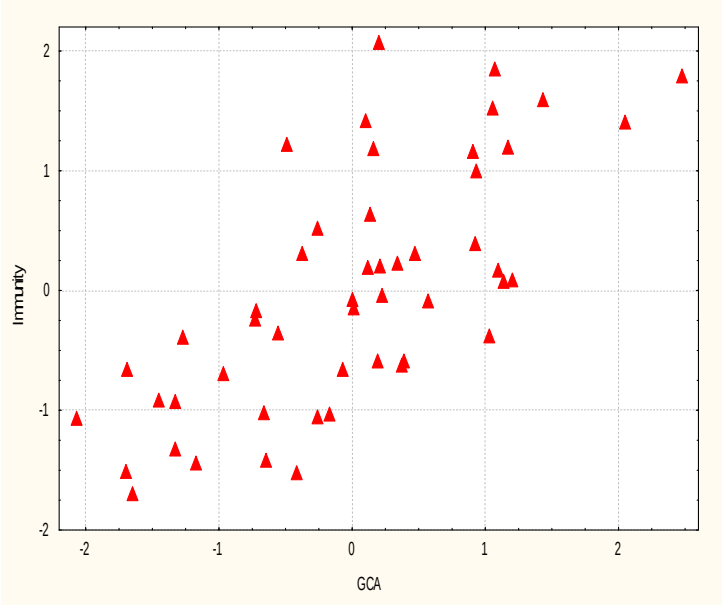
R=0,469; R^2=0,220; Adjusted R^2=0,151; $F_{(4,45)}$=3,2; p=0,022

		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(45)}$	p-level
	r		Intercpt	-264	476	-0,55	,583
Endotheliocytes of Thymus	-0,30	-,258	,143	-32,5	18,0	-1,80	,078
NK-Lymphocytes of Blood	0,23	,208	,133	27,0	17,2	1,56	,125
Macrophages of Spleen	0,23	,259	,136	24,8	13,0	1,90	,064
Lymphocytes of Thymus	0,22	,171	,147	8,0	6,9	1,17	,250

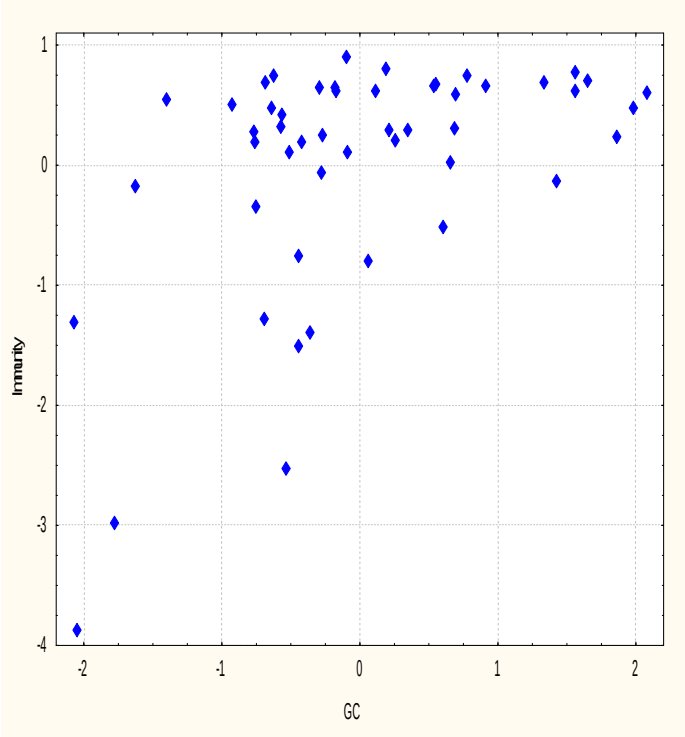
The canonical correlation analysis reveals two pairs of roots (Table 3.32).

Table 3.32. Factor structure of glucocorticoid-immune linkages

Right set	Root 1	Root 2
Fasciculary Zone Adrenal Cortex	**-,95**	,30
Corticosterone of Plasma	-,02	**-0,995**
Left set	Root 1	Root 2
Killing Index of Neutrophils	**-,56**	,16
Lymphocytes of Thymus	**-,43**	**-,41**
Thymus Mass Index	**-,39**	**,40**
Macrophages of Thymus	**,52**	,12
Reticulocytes of Spleen	**,49**	,03
Entropy of Leukocytogram	**,40**	**-,44**
NK-Lymphocytes of Blood	**,36**	**-,45**
Endotheliocytes of Thymus	,14	**,57**
Macrophages of Spleen	,27	**-,44**



R=0,713; R^2=0,508; $\chi^2_{(20)}$=43; p=0,002



R=0,516; R^2=0,267; $\chi^2_{(9)}$=13,2; p=0,155

Fig. 3.25. Canonical correlation between parameters of glucocorticoid activity (X-axis) and immunity (Y-axis)

The glucocorticoid canonical root of the first pair receives the lion's share of the factor load from the fascicular area of the adrenal cortex at a small fraction of corticosterone and determines the constellation of the immune indices by 51% (Fig. 3.25 above). Instead, the

glucocorticoid root of the second pair represents both corticosterone and, in the opposite way, the fascicular area, determining the same constellation of immune parameters by 27% (Fig. 3.25 below).

The thickness of the glomerular zone of the adrenal cortex as a marker of their permanent mineralocorticoid activity is moderately negatively correlated with the content of macrophages in the spleen and plasmocytes in the blood, but positively with the content of lymphoblasts in the spleen, T-helper cells in the blood and endotheliocytes in the thymus, determining the constellation of the immune indices by 32% (Table 3.33).

Table 3.33. Regression model of multiple correlation of the thickness of the glomerular zone of adrenal cortex with indicators of immunity

R=0,622; R^2=0,386; Adjusted R^2=0,317; $F_{(5,4)}$=5,5; $p<10^{-3}$

		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(44)}$	p-level
	r		Intercpt	46,4	39,1	1,19	,241
Macrophages of Spleen	-0,44	-,346	,127	-4,135	1,510	-2,74	,009
Plasmocytes of Blood	-0,25	-,213	,130	-4,862	2,962	-1,64	,108
Lymphoblastes of Spleen	0,33	,211	,124	4,818	2,823	1,71	,095
Th-Lymphocytes of Blood	0,25	,169	,122	1,569	1,134	1,38	,174
Endotheliocytes of Thymus	0,23	,308	,123	4,824	1,931	2,50	,016

Situational mineralocorticoid activity, estimated by the Na/K ratio of the plasma, first, is completely unrelated to permanent (r=0,03), and secondly, moderately negatively correlated with another constellation of immune parameters, determining it by 45 % (Table 3.34).

Table 3.34. Regression model of multiple correlation of mineralocorticoid activity as $(Nap/Kp)^{0.5}$ with indicators of immunity

R=0,706; R^2=0,499; Adjusted R^2=0,454; $F_{(4,4)}$=11; $p<10^{-6}$

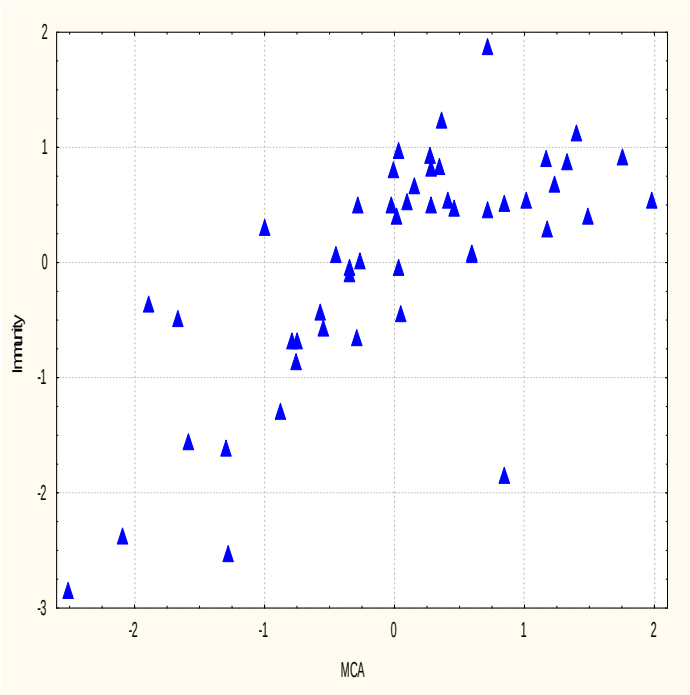
		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(45)}$	p-level
	r		Intercpt	12,1	1,1	10,7	10^{-6}
0-Lymphocytes of Blood	-0,42	-,396	,109	-,059	,016	-3,62	10^{-3}
Hassal corpuscles	-0,34	-,526	,111	-,475	,100	-4,74	10^{-4}
Neutrophils of Spleen	-0,29	-,289	,111	-,102	,039	-2,62	,012
Killing Index of Neutrophils	-0,28	-,262	,111	-,043	,018	-2,36	,023

According to the canonical analysis of mineralocorticoid-immune connections, two pairs of roots were isolated, as well as for glucocorticoid-immune connections (Table 3.35).

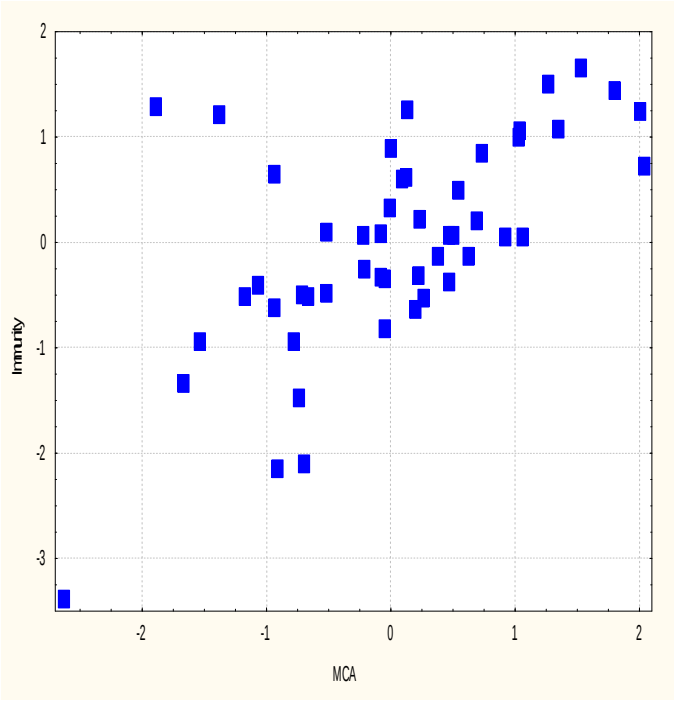
Table 3.35. Factor structure of mineralocorticoid-immune linkages

Right set	**R 1**	**R 2**
MCA as $(Nap/Kp)^{0,5}$	**-,995**	-,10
Glomerulary Zone of AC	,07	**-,998**
Left set	**R 1**	**R 2**
0-Lymphocytes of Blood	**,56**	**,25**
Hassal corpuscles	**,45**	**,29**
Neutrophils of Spleen	**,42**	-,12
Killing Index of Neutrophils	**,43**	**-,43**
Th-Lymphocytes of Blood	**-,46**	**-,44**
Endotheliocytes of Thymus	,03	**-,36**
Lymphoblastes of Spleen	,01	**-,52**
Macrophages of Spleen	**-,25**	**,69**
Plasmocytes of Blood	-,03	**,40**

The pairs were almost equal in magnitude of the canonical correlation coefficients. At the same time, situational mineralocorticoid activity determines the immune constellation by 52% (Fig. 3.26 from above) and permanent by 39% (Fig. 3.26 from below).



$R=0,719$; $R^2=0,517$; $\chi^2_{(18)}=53$; $p<10^{-4}$



R=0,625; R^2=0,391; $\chi^2_{(8)}$=21,3; p=0,006

Fig. 3.26. Canonical correlation between parameters of mineralocorticoid activity (X-axis) and immunity (Y-axis)

The thickness of the reticular zone of the adrenal cortex as a marker of their permanent androgenic activity is moderately negatively correlated with the content of Hassal's bodies in the thymus, macrophages and microphages in the spleen and mitogenic blastransformation of T-lymphocytes, while positively with bactericidity of macrophages of the blood and thymus mass index, determining their by 29,5% (Table 3.36).

Table 3.36. Regressive model of multiple correlation of the thickness of the reticular zone of the adrenal cortex with indicators of immunity

R=0,617; R^2=0,381; Adjusted R^2=0,295; $F_{(6,4)}$=4,4; p=0,0014

		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(43)}$	p-level
	r		Intercpt	32,3	14,3	2,25	,030
Hassal corpuscles	-0,38	-,327	,132	-2,542	1,030	-2,47	,018
Macrophages of Spleen	-0,22	-,137	,149	-,444	,486	-,91	,365
Neutrophils of Spleen	-0,22	-,412	,129	-1,247	,392	-3,19	,003
Blasttransformation T-Lym	-0,21	-,171	,124	-,106	,077	-1,38	,174
Killing Index of Neutrophils	0,30	,174	,141	,244	,199	1,23	,226
Thymus Massa Index	0,26	,188	,126	157	105	1,49	,143

The urinary excretion of 17-ketosteroids as metabolites of androgens positively correlates with entropy of splenocytogram and the share of microphages in it, while negatively with the share of reticulocytes in it, as well as the relative content of plasmocytes and monocytes, determining their by 29,6% (Table 3.37).

Table 3.37. Regression model of multiple correlation of 17-KS with immunity indicators

R=0,618; R^2=0,383; Adjusted R^2=0,296; $F_{(6,4)}$=4,4; p=0,0014

		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(43)}$	p-level
	r		Intercpt	-43,5	65,3	-,67	,509
Neutrophils of Spleen	0,41	,259	,132	2,433	1,238	1,97	,056
Entropy of Splenocytogram	0,27	,169	,133	138,6	108,7	1,27	,209
Thymus Mass	0,27	,164	,126	,170	,131	1,30	,200
Plasmocytes of Blood	-0,34	-,177	,127	-3,428	2,461	-1,39	,171
Reticulocytes of Spleen	-0,30	-,244	,133	-2,799	1,524	-1,84	,073
Monocytes of Blood	-0,26	-,208	,124	-2,252	1,346	-1,67	,102

Testosterone plasma level, correlating with the reticular zone (r=0,36), are poorly correlated with immune parameters, determining their constellation by only 11%, but statistically significant (Table 3.38).

Table 3.38. Regression model of multiple correlation of the testosterone with immunity parameters

R=0,432; R^2=0,187; Adjusted R^2=0,114; $F_{(4,45)}$=2,6; p=0,050

		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(45)}$	p-level
	r		Intercpt	-154	83	-1,84	,072
Lymphocytes of Blood	0,27	,311	,139	,909	,406	2,24	,030
Entropy of Immunocytogram	0,20	,228	,139	245	149	1,64	,107
Blasttransformation T-Lymphocytes	0,18	,184	,138	,283	,212	1,33	,189
Phagocytose Index of Monocytes	-0,18	-,149	,136	-,998	,914	-1,09	,281

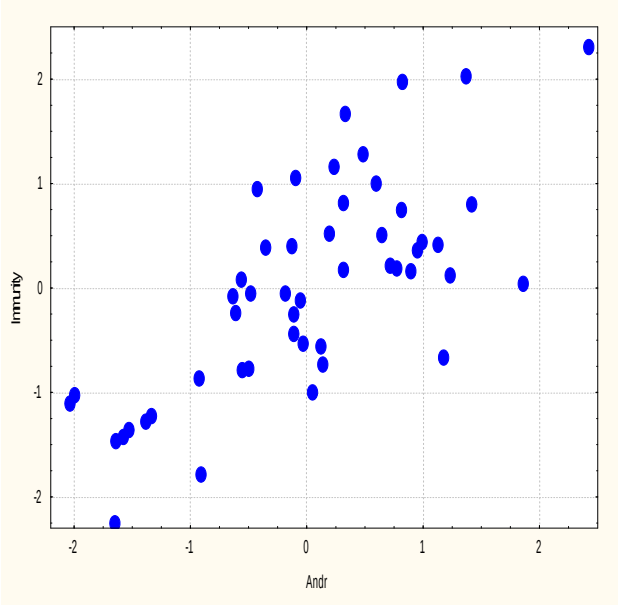
As a result of the canonical correlation analysis of androgen-immune connections, two pairs of roots were identified (Table 3.39). The androgenic root of the first pair receives the lion's share of the factor load from the thickness of the reticular zone of the adrenal cortex at a small fraction from 17-KS and, opposite in sign, from testosteronemia. This constellation of markers of androgen function has a **suppressive** effect on the content of Hassal's bodies in the thymus, macrophages in the spleen and mitogenic blastransformation of T-lymphocytes, and

also reduces the entropy of immunocytogram of the blood, and in turn **activates** bactericidity of microphages in the blood, increases blood level of lymphocytes as well as thymus mass, determinimg these immune parameters by 53% (Fig. 3.27 top).

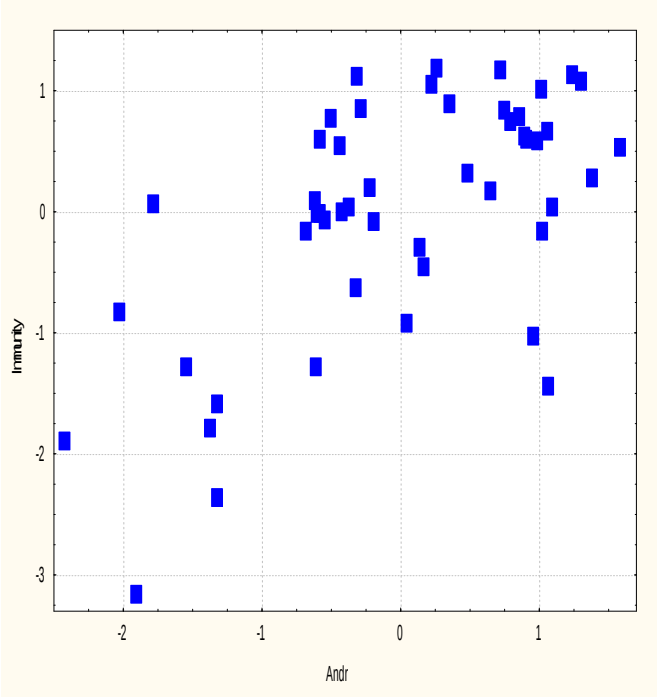
Instead, the androgenic root of the second pair receives the lion's share of the factor load from the 17-KS at a small and opposite in sign from the thickness of the the reticular zone of the adrenal cortex and a negligible fraction from testosteronemia. Such constellation of markers of androgenic function exerts a **suppressive** effect on the content of plasmocytes and monocytes in the blood and phagocytic activity of the latter, as well as the content in the spleen of reticulocytes; instead, it **increases** the proportion of neutrophils and entropy of the splenocytogram, as well as the mass of the thymus, determining these immune parameters by 39% (Fig. 3.27 below).

Table 3.39. Factorial structure of androgen-immune connections in male rats

Right set	R 1	R 2
Reticulary Zone Adrenal Cortex	**,81**	,22
17-Ketosteroides Urine	,23	**-,97**
Testosterone Plasma	**-,20**	-,08
Left set	R 1	R 2
Hassal corpuscles	**-,73**	,24
Blasttransformation T-Lymphocytes	**-,46**	-,04
Macrophages of Spleen	**-,38**	-,00
Entropy of Immunocytogram	**-,35**	,20
Killing Index of Neutrophils	**,48**	,11
Thymus Mass Index	**,37**	**-,23**
Lymphocytes of Blood	**,10**	-,13
Neutrophils of Spleen	-,06	**-,69**
Entropy of Splenocytogram	-,23	**-,50**
Plasmocytes of Blood	-,23	**,49**
Reticulocytes of Spleen	-,22	**,44**
Monocytes of Blood	-,14	**,40**
Phagocytose Index of Monocytes	,00	**,20**



R=0,727; R^2=0,529; $\chi^2_{(42)}$=66; p=0,009



R=0,624; R^2=0,389; $\chi^2_{(26)}$=36; p=0,085

Fig. 3.27. Canonical correlation between parameters of androgen function (X axis) and immunity (Y axis)

In the final stage, we analyzed the canonical correlation between all registered markers of steroid hormones on the one hand, and the constellation of their associated immune parameters on the other.

Two pairs of roots are distinguished, which are worth considering in terms of the canonical correlation coefficients.

The steroid root of the first pair, judging by factor loadings (Table 3.40), represents

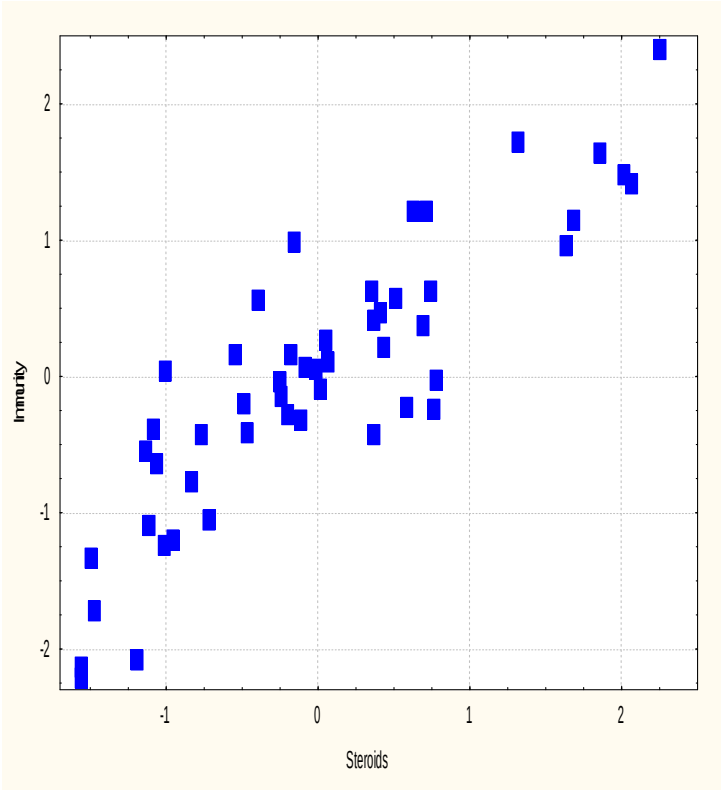
directly the thickness of the glomerular zone of the adrenal cortex and inversely the mineralocorticoid activity as well as plasma testosterone and corticosterone levels. Factor loads from the other three steroid markers are negligible.

Table 3.40. Factor structure of steroid-immune relationships in male rats

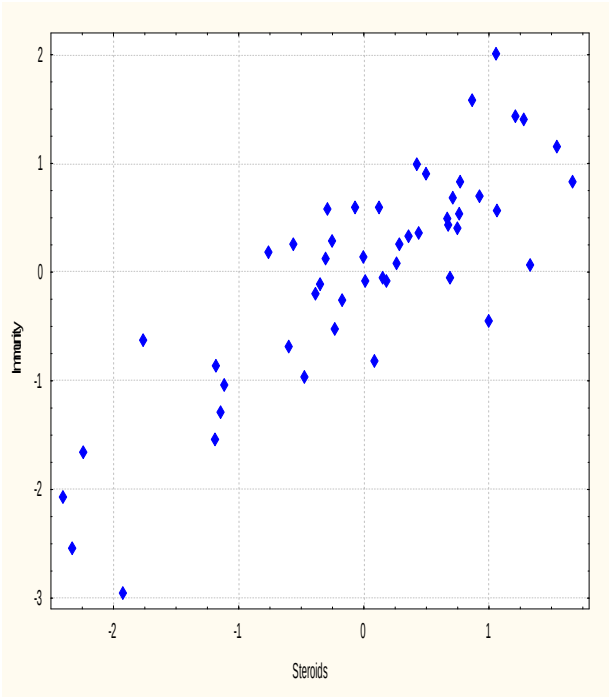
Right set	R 1	R 2
Glomerulary ZAC	**,64**	**-,44**
Testosterone Plasma	**-,50**	,07
MCA as $(Nap/Kp)^{0,5}$	**-,48**	**-,77**
Corticosterone Plasma	**-,43**	**,35**
Fasciculary ZAC	,17	,03
17-Ketosteroide Urine	,13	-,16
Reticulary ZAC	-,06	-,11
Left set	R 1	R 2
Macrophages of Spleen	**-,49**	,12
Entropy of Immunocytogram	**-,36**	-,14
Plasmocytes of Blood	**-,27**	,16
Blasttransformation T-Lymphocytes	**-,26**	,03
NK-Lymphocytes of Blood	**-,25**	-,09
Entropy of Splenocytogram	**-,23**	,00
Killing Index of Neutrophils	**,44**	,20
Phagocytose Index of Monocytes	**,37**	,10
Neutrophils of Spleen	**,34**	,17
Endotheliocytes of Thymus	**,23**	-,17
0-Lymphocytes of Blood	,29	**,46**
Hassal corpuscles	-,06	**,44**
Th-Lymphocytes of Blood	-,07	**-,37**

On the other hand, the immune root represents 6 parameters that are **suppressed** by steroids: the content of macrophages in the spleen, plasmocytes and natural killer cells, mitogenic blastransformation of T-lymphocytes, as well as entropy of immunocytogram and splenocytogram.

Instead, the other 5 parameters under the influence of steroids are **increasing**, namely: the completion of neutrophil/microphage phagocytosis, the activity of monocyte/macrophage phagocytosis, the content of neutrophils in the spleen and endothelial cells in the thymus. The rate of steroid determination of such immune constellation is 76% (Fig. 3.28 above).



R=0,873; R^2=0,763; $\chi^2_{(154)}$=195; p=0,015



R=0,844; R^2=0,712; $\chi^2_{(126)}$=146; p=0,112

Fig. 3.28. The canonical correlation between steroid hormones (X-axis) and immunity (Y-axis)

The factor structure of the steroid root of the second pair is both common and different

from the first root. In particular, it also receives a negative load from the mineralocorticoid activity, but is much more noticeable, and the nature of the loads from the thickness of the glomerular zone of the adrenal cortex and corticosteronemia is inverted; the list of steroid markers with negligible loads is supplemented with testosteronemia.

The immune root of the second pair receives significant load from only three parameters. In particular, the negative from the blood level of T-helper lymphocytes, reflecting the **enhancing** effect of both mineralocorticoid markers, and the positive load from the content of Hassal's calves in the thymus, reflecting the **suppressive** effect on them both mineralocorticoids (major), and androgens (minor) (Table 3.25). A positive sign of factor loading also applies to 0-lymphocytes, which reflects a **decrease** in their proportion in the immunocytogram of blood under the influence of mineralocorticoids, apparently due to an increase in the proportion of T-lymphocytes. The rate of steroid determination of such immune constellation is 76% (Fig. 3.28 above).

Parathyroid activity strongly correlates with the content of Hassal's calves in the thymus (Fig. 3.29). In addition, a moderate negative correlation was found with the content of macrophages and lymphoblasts in the spleen and segmented neutrophils in the blood. This immune constellation is determined by parathyroid activity by 75% (Table 3.41 and Fig. 3.30).

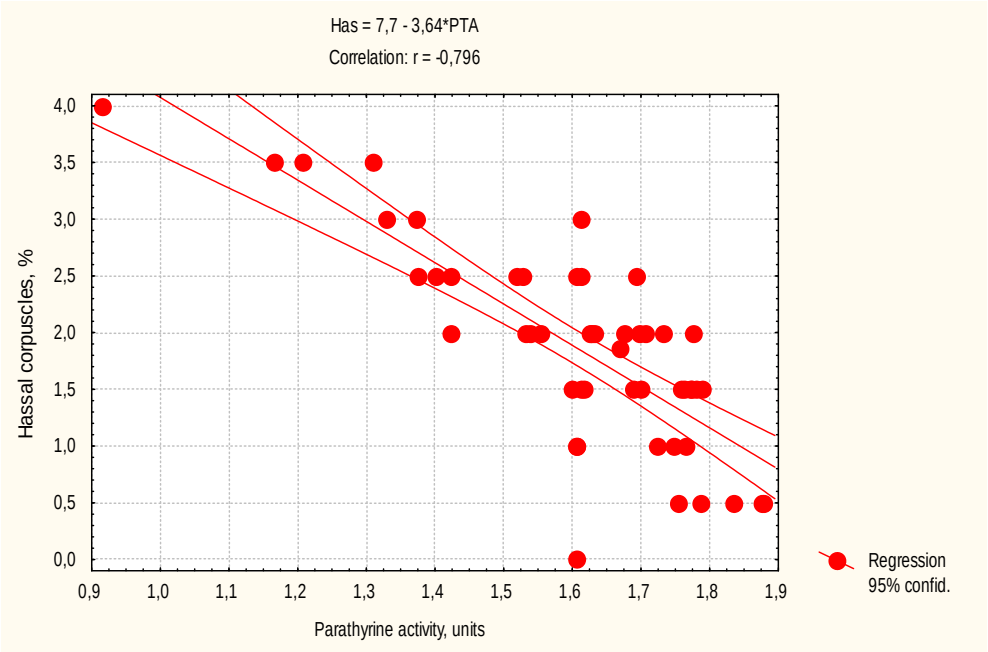


Fig. 3.29. Relationship between parathyroid activity (X-axis) and Hassal' corpuscles of thymus content (Y-axis)

Table 3.41. Regression model of multiple correlation of $(Cap/Pp)^{0,5}$ ratio as parathyroid activity with indicators of immunity

$R=0,879$; $R^2=0,773$; Adjusted $R^2=0,753$; $F_{(4,4)}=38$; $\chi^2_{(4)}=68$; $p<10^{-6}$

		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(45)}$	p-level
	r		Intercpt	2,42	,10	23,6	10^{-6}
Hassal corpuscles	-0,80	-,702	,078	-,1536	,0171	-8,98	10^{-6}
Macrophages of Spleen	-0,39	-,137	,084	-,0125	,0077	-1,63	,110
Lymphoblastes of Spleen	-0,38	-,314	,074	-,0548	,0129	-4,24	10^{-3}
Segmented Neutrophils of Blood	-0,26	-,165	,076	-,0066	,0031	-2,17	,035

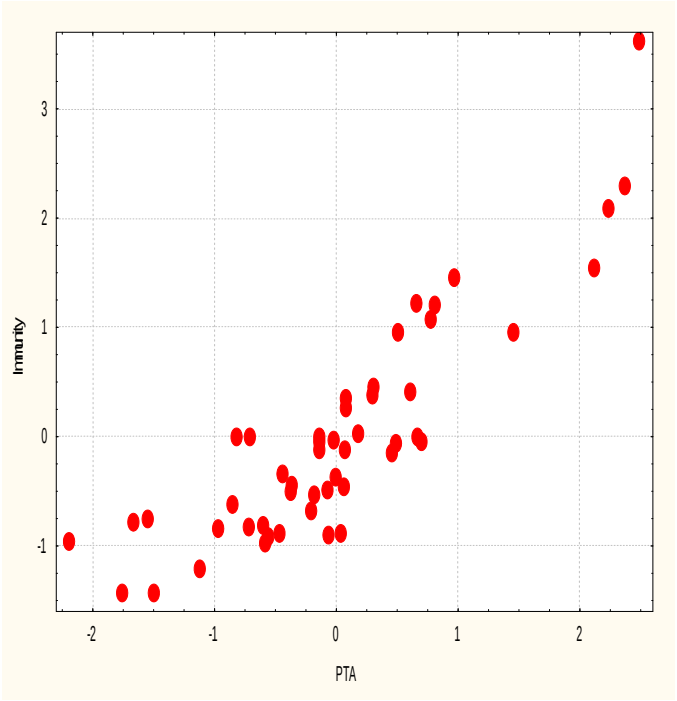


Fig. 3.30. Canonical correlation between parathyroid activity (X-axis) and immunity parameters (Y-axis)

Calcitonin activity is related to the content of Hassal's calves in the thymus to the same extent, but positively (Fig. 3.31), which is consistent with the status of calcitonin as a functional antagonist of parathyrin for calcium-phosphorus metabolism, gastric secretion etc.

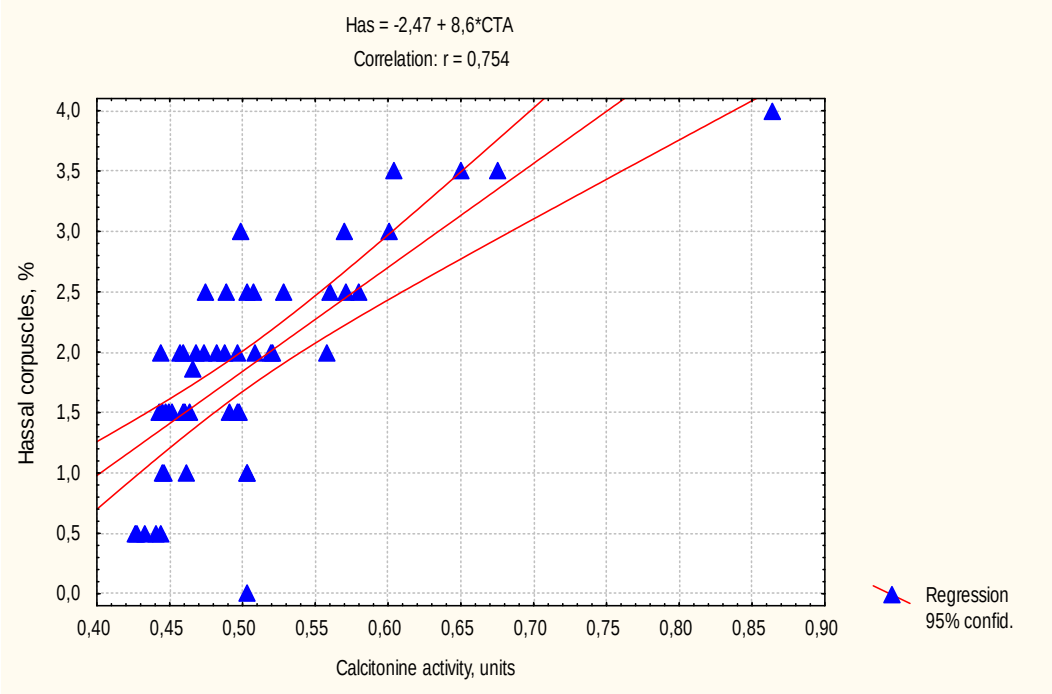


Fig. 3.31. Relationship between calcitonin activity (X-axis) and Hassal calf thymus content (Y-axis)

In addition, calcitonin activity correlates moderately positively with the content of lymphoblasts in the spleen and plasmocytes in the blood, but negatively with the content of lymphoblasts in the thymus, lymphocytes in the blood and plasmocytes in the spleen. This immune constellation is determined by calcitonin activity by 67% (Table 3.42 and Fig. 3.32).

Table 3.42. Regression model of multiple correlation of $(Cap \bullet Pp)^{-0,5}$ as calcitonin activity with indicators of immunity

$R=0,842$; $R^2=0,708$; Adjusted $R^2=0,668$; $F_{(6,4)}=17$; $\chi^2_{(6)}=55$; $p<10^{-6}$

		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(43)}$	p-level
	r		Intercpt	,6126	,1074	5,70	10^{-6}
Hassal corpuscles	0,75	,694	,095	,0607	,0084	7,27	10^{-6}
Lymphoblastes of Spleen	0,35	,234	,085	,0164	,0059	2,76	,008
Plasmocytes of Blood	0,27	-,104	,100	-,0073	,0070	-1,04	,305
Lymphoblastes of Thymus	-0,33	-,118	,089	-,0121	,0091	-1,33	,191
Lymphocytes of Blood	-0,25	-,266	,090	-,0035	,0012	-2,95	,005
Plasmocytes of Spleen	-0,25	-,105	,103	-,0053	,0052	-1,02	,313

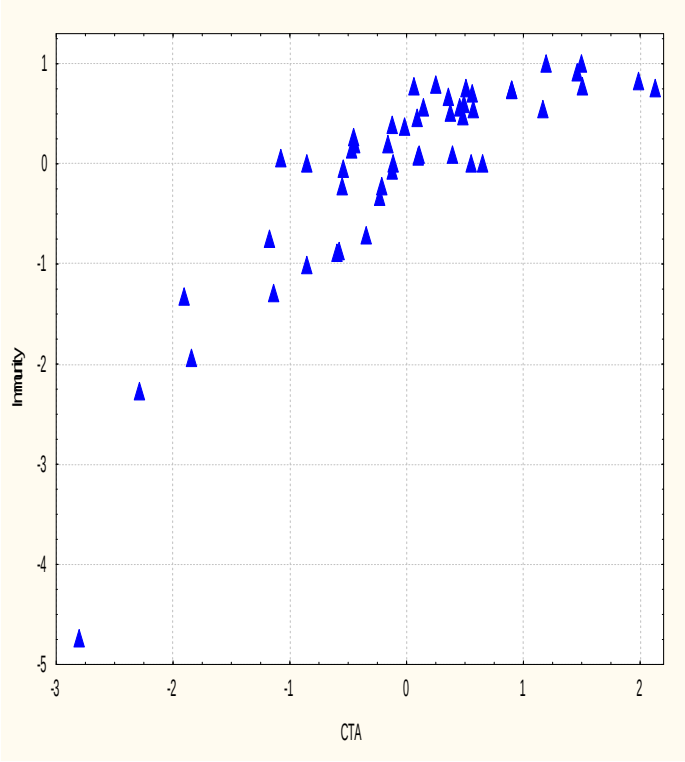


Fig. 3.32. Canonical correlation between calcitonin activity (X-axis) and immunity parameters (Y-axis)

Blood levels of triiodothyronine are moderately positively correlated with thymus mass and total leukocyte content in the blood, but negatively with the natural killer cells and monocytes blood level as well as the intensity of Staphylococcus aureus phagocytosis, lymphoblastes content in the thymus. The above constellation of immune parameters is determined by triiodothyronine by 33% (Table 3.43 and Fig. 3.33).

Table 3.43. Regression model of multiple correlation of triiodothyronine with immunity rates

R=0,640; R^2=0,409; Adjusted R^2=0,327; $F_{(6,4)}$=5,0; $\chi^2_{(6)}$=24; $p<10^{-3}$

		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(43)}$	p-level
	r		Intercpt	3,98	,99	4,01	,0002
Thymus Mass Index	0,38	,186	,136	13,4	9,8	1,37	,178
Leukocytes of Blood	0,31	,220	,121	,0625	,0343	1,82	,075
NK-Lymphocytes of Blood	-0,33	-,221	,134	-,0838	,0511	-1,64	,108
Microbial Count for Monocytes	-0,29	-,293	,120	-,1093	,0447	-2,45	,019
Monocytes of Blood	-0,27	-,171	,124	-,0515	,0373	-1,38	,175
Lymphoblastes of Thymus	-0,27	-,234	,123	-,1838	,0965	-1,90	,064

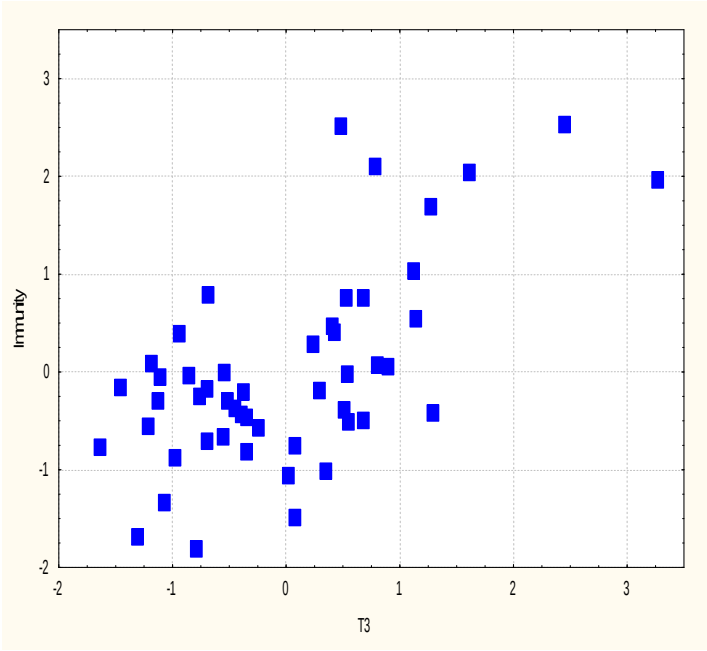


Fig. 3.33. Canonical correlation between triiodothyronine (X axis) and immunity parameters (Y axis)

Resume

The method of factor analysis revealed 4 independent clusters of parameters of the neuroendocrine-immune complex, linked by cause and effect functional relationships. Each of the reported neuro-endocrine factors more or less closely correlates positively or negatively with these or other immune parameters of the thymus, spleen and blood, which testifies to their interaction within the triune neuroendocrine-immune complex.

SECTION 4

VARIANTS OF IMMUNE RESPONSES TO CHRONIC STRESS AND THEIR NEUROENDOCRINE ACCOMPANIMENT IN THE FEMALE

4.1. Factor analysis of the parameters of the neuro-endocrine-immune complex

In the first stage of factor analysis (principal component method) it is found that the dispersion of the information field of 60 actual and calculated parameters of the neuro-endocrine-immune complex and electrolyte exchange is absorbed by 20 factors. Applying the Cattel technique (with the maximum deceleration of the eigenvalue λ, shown graphically in Fig. 4.1), the number of analyzed factors is limited to ten, the total contribution of which to the total variance of the original data is 60.9% (Table 4.1).

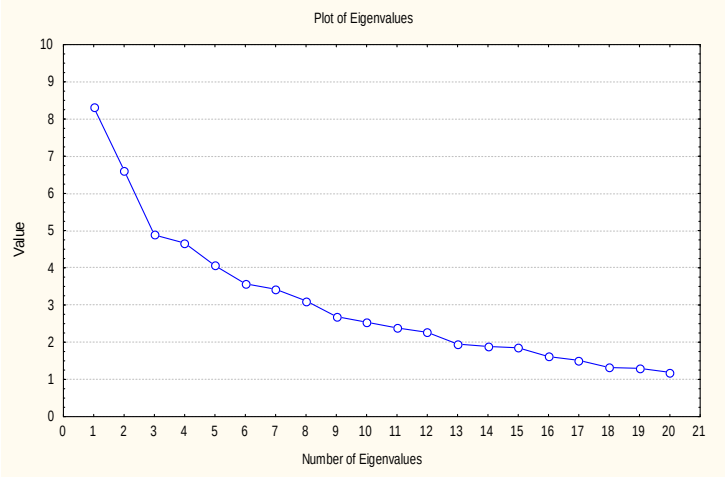


Fig. 4.1. The magnitudes of the eigenvalues of the principal components

Table 4.1. Eigenvalues of the principal components of the information field of the parameters of the neuro-endocrine-immune complex and the electrolyte exchange of rats-females

No	Eigen-value	% total Variance	Cumul. Eigenval	Cumul. %	No	Eigen-value	% total Variance	Cumul. Eigenval	Cumul. %
1	8,32	11,6	8,32	11,6	6	3,57	5,0	32,12	44,6
2	6,62	9,2	14,94	20,7	7	3,43	4,8	35,54	49,4
3	4,88	6,8	19,82	27,5	8	3,11	4,3	38,65	53,7
4	4,66	6,5	24,49	34,0	9	2,69	3,7	41,34	57,4
5	4,06	5,6	28,54	39,6	10	2,54	3,5	43,88	60,9

It has already been mentioned that in one principal component (PC), indicators (variables) are combined, maximally correlated and minimally related to other indicators. It is revealed (Table 4.2) that the first PC contains information about, first of all, the functional state of the autonomic nervous system, estimated by the parameters of HRV: Mode as the

inverse of the rhythm frequency, amplitude of mode as the HRV-marker of sympathetic tone and variational pitch as a HRV-marker of vagal tone. The thickness of the fascicular and glomerular zones of the adrenal cortex producing glucocorticoids and mineralocorticoids, respectively, is shown here.

Among the immune parameters, the first PC receives maximal factor loadings from the relative content in the spleen of macrophages (negative, ie, single-target with sympathetic tone and corticoadrenal activity) and lymphoblasts (positive, ie, single-axis with vagal tone). In addition, the negative load gives the total blood content of leukocytes.

The second PC combines parathyroid and calcitonin activities, on the one hand, and the relative and absolute mass of the spleen as well as the content in the splenocytogram of fibroblasts (a variety of macrophages), on the other.

The third PC contains information about major elements of the blood leukocytogram and its entropy, bactericidal ability of blood neutrophils/microphages and monocytes/macrophages, as well as the content of reticulocytes (a variety of macrophages) in the thymus in combination with blood levels of triiodothyronin.

The fourth PC combines the three elements of the thymocytogram and its entropy with the thickness of the reticular zone of the adrenal cortex as the source of androgens.

The fifth PC contains information about the partial parameters of phagocytosis of blood microphages and macrophages, content in the blood of eosinophils, and in the thymus lymphoblasts in combination with blood levels of testosterone and corticosterone. Note the opposite of signs of factor loadings of microbial count of neutrophils and monocytes.

The sixth PC combines information on the absolute and relative mass of the adrenal glands with the content of eosinophils in the splenocytogram.

Instead, the seventh PC contains information about the share in the splenocytogram of its major element lymphocytes and its entropy, as well as the content of natural killer cells in the blood and endothelial cells in the thymus.

Eight PC combines two endocrine factors - mineralocorticoid activity and thyroxinemia - with 6 immune factors: the relative and absolute mass of the thymus and the its content of epitheliocytes, the content of plasmocytes and neutrophils in the spleen and basophils in the blood.

The ninth PC receives negative factor load from the proportion of 0-lymphocytes in the blood immunocytogram, ie immature and/or devoid receptors, while T-killer lymphocytes and B-lymphocytes give a positive factor load, as does the killing index of neutrophils and content of plasmocytes in the thymus. Another element of the PC - the reticulocytes of the spleen - give it a negative factor load.

The tenth PC carries information about the proportion of T-helper lymphocytes in the blood immunocytogram and its entropy, as well as the share of monocytes in the blood leukocytogram. There was also the body mass of the animal.

Table 4.2. Factor loadings (Varimax normalized). Clusters of loadings, those determine the oblique factors for hierarchical analysis of parameters of female rat

Variable	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 7	PC 8	PC 9	PC 10
Humoral channal	**,886**	,025	,151	,024	,259	,045	-,041	-,016	-,046	,068
Sympathetic tone	**-,874**	-,070	,001	,146	,013	-,091	,091	-,027	,033	,165
Vagal tone	**,786**	,147	,105	-,049	,303	,034	,026	,156	-,061	,259
Macrophages of Spleen	**-,686**	,412	,039	,113	,003	,025	,209	,115	-,122	,069
Lymphoblastes of Spleen	**,473**	-,459	-,176	-,023	-,129	,025	,181	-,200	,312	,263
Fasciculary Zone Adr Cortex	**-,448**	,431	,007	,083	,000	-,018	,081	,367	,010	,185
Glomerul Zone Adr Cortex	**-,446**	-,052	,330	,054	,312	,097	-,227	,121	,136	-,094
Leukocytes of Blood	**-,422**	,114	,278	-,160	,093	,401	,087	-,052	,041	-,053
Parathyrine activity	,016	**-,814**	,078	-,064	,032	,060	-,159	-,036	,017	,095
Calcitonine activity	,067	**-,753**	-,092	,096	,058	,053	,262	,098	,195	-,231
Spleen Mass Index	-,182	**,470**	,087	-,348	-,123	,384	,299	,205	-,129	-,030
Spleen Mass	-,139	**,439**	,075	-,255	-,087	,358	,312	,106	-,153	-,239
Fibroblastes of Spleen	,084	**,297**	,250	-,017	-,065	,215	,265	-,025	-,284	-,304
Lymphocytes of Blood	-,055	,028	**-,896**	-,004	-,046	,147	-,116	-,105	,019	-,050
Stub Neutrophils of Blood	,011	,059	**,819**	,015	,104	,062	-,001	-,154	,085	,104
Segmented Neutroph Blood	-,032	-,168	**,789**	-,140	-,122	-,071	,188	,013	,036	,059
Entropy of Leukocytogram	,047	,098	**,785**	,102	,080	-,192	-,057	,243	-,009	-,092
Bactericidity of Neutrophils	-,298	-,018	**,585**	-,154	,346	,304	,136	-,103	,121	-,046
Bactericidity of Monocytes	,051	,062	**,496**	-,061	-,407	,217	-,342	,156	-,067	-,251
Triiodothyronine	,020	-,213	**-,452**	-,041	,114	,191	-,068	,323	-,281	-,133
Phagocytose Ind Monocytes	,292	-,221	**,442**	-,214	-,436	,174	-,155	,235	-,064	-,030
Reticulocytes of Thymus	-,204	-,117	**,422**	,157	-,211	-,059	-,302	,096	-,061	-,043
Entropy of thymocytogram	-,290	-,053	,096	**,722**	-,081	-,071	,078	,369	,111	-,036
Macrophages of Thymus	,120	-,135	-,252	**,702**	-,222	,263	-,081	-,127	-,126	,073
Lymphocytes of Thymus	,340	-,033	-,106	**-,662**	,059	,134	,041	-,384	-,075	,071
Hassal corpuscles of Thymus	-,095	,096	-,212	**,561**	,107	,124	,463	,126	-,189	-,300
Reticulary Zone Adr Cortex	-,171	,274	,062	**,384**	-,006	-,233	-,004	-,160	-,196	,129
Phagocyt Ind Neutr Blood	,004	-,095	-,058	-,033	**,773**	-,059	-,049	,133	,116	-,078
Microb Count Neutr Blood	,134	-,114	,151	-,105	**,757**	,070	-,002	-,095	-,047	,016

Microb Count Monoc Blood	,163	,217	,247	,072	**-,721**	,233	,002	-,209	,099	-,015
Blasttransform of T-Lymphoc	-,058	-,033	,004	-,095	**-,662**	,059	,134	,041	-,384	-,075
Eosinophils of Blood	,256	,213	,189	,300	**,496**	-,051	,116	-,026	-,128	,314
Testosterone	-,194	,271	-,248	,372	**-,490**	-,185	,009	,005	-,153	,167
Lymphoblastes of Thymus	,164	,010	-,102	-,129	**,439**	-,036	,078	-,248	,396	-,076
Corticosterone,	-,077	-,099	-,160	-,172	**-,296**	,040	,183	-,222	-,135	-,135
Adrenals Mass Index	,123	,168	-,213	,165	-,081	**,375**	-,157	,095	,201	-,014
Adrenals Mass	,117	,236	-,268	,230	,049	**,364**	-,138	-,088	,156	**-,373**
Eosinophils of Spleen	-,055	,172	,020	,207	,042	**-,256**	,072	,070	-,026	-,088
Lymphocytes of Spleen	,175	-,060	-,084	-,008	,034	-,020	**-,876**	,014	,127	,107
Entropy of Splenocytogram	,039	,025	,019	,047	-,071	-,080	**,814**	-,221	,099	-,102
NK-Lymphocytes Blood	,021	-,182	,339	,006	,096	-,034	**,430**	,121	-,074	,241
Endotheliocytes of Thymus	-,034	,046	,167	,054	,139	,112	**,418**	,389	,257	,056
Mineralocorticoid activity	,059	,052	-,082	,057	-,090	-,159	-,001	**,709**	,067	,059
Plasmocytes of Spleen	,283	-,130	-,158	-,157	-,153	-,098	,125	**-,548**	,444	,083
Thymus Mass Index	-,238	-,076	,137	-,262	,096	-,058	-,194	**,544**	,287	,267
Thymus Mass	-,224	-,105	,156	-,170	,145	-,103	-,217	**,520**	,301	,117
Neutrophils of Spleen	,034	-,271	,190	-,242	,038	-,202	,136	**,490**	,072	,159
Basophils of Blood	,102	,297	,039	,225	,173	,075	,108	**,431**	-,016	,129
Epitheliocytes of Tymus	-,391	,252	,009	,169	-,024	-,260	-,223	**,403**	-,202	-,090
Thyroxine	,187	,038	,180	-,017	-,252	-,132	,219	**-,364**	,032	,005
0-Lymphocytes of Blood	,052	,288	-,114	,093	-,116	-,110	-,085	-,087	**-,774**	,298
Tc-Lymphocytes of Blood	,079	-,152	-,002	-,022	,040	-,012	-,085	,110	**,728**	,192
B-Lymphocytes of Blood	-,125	-,254	,091	,010	,018	,172	-,098	,134	**,722**	-,148
Reticulocytes of Spleen	-,199	,047	-,147	,224	,154	,201	,341	-,165	**-,399**	-,321
Killing Ind Neutr Blood	-,052	-,052	,039	,295	,008	,079	,019	-,350	**,377**	,084
Plasmocytes of Thymus	-,049	-,296	,089	,251	-,257	-,185	,166	,006	**,342**	,158
Medullary Zone Adr Cortex	,039	,295	,008	,089	,251	-,257	-,185	,166	**-0,45**	-,100
Th-Lymphocytes of Blood	-,073	-,111	-,051	-,174	,121	,099	,067	-,108	,326	**-,753**
Entropy Immunocytogram	,162	,055	,120	,109	,113	,093	-,071	,194	,065	**,675**
Body Mass	,101	-,102	-,052	,301	,112	-,100	,004	-,252	-,032	**-,499**
Monocytes of Blood	,035	,091	,182	,100	-,024	-,292	-,278	,292	-,100	**-,337**

In the second step, we obtained a correlation matrix for oblique factors, which was subjected to further analysis to distinguish a secondary and primary factors.

It is revealed (Table 4.3) that there are two directly unmeasured hypothetical common factors. In this case, the first common factor (S1) combines, on the one hand, the **neuroendocrine** parameters: gluco- and mineralocorticoids and sympathetic tone, and on the other hand - the **immune**: the mass of the thymus and the relative content of lymphocytes, epitheliocytes and endothelium cells and entropy of thymocytogram and leukocytograms, as well as blood basophils, plasmocytes, neutrophils and spleen macrophages. This suggests that these immune parameters are regulated by gluco- and mineralocorticoids and sympathetic tone.

Spleen macrophages are part of the structure of the second common factor, but with the opposite sign, together with the mass of the spleen and the content of reticulocytes, as well as lymphoblasts in the thymus, 0-, B- and T-cytolytic lymphocytes in the blood and the parameters of neutrophil phagocytosis. This immune constellation is apparently regulated by the endocrine constellation of calcitonin and paratyrin, on the one hand, and testosterone and androgens, on the other.

Table 4.3. Secondary (S) and Primary (P) Factors Loadings at female rats

Variable	S1	S2	P1	P2	P3	P4	P5	P6	P7	P8	P9
Lymphocytes of Thymus	**-,513**	,149	,19	,03	-,06	**-,58**	-,06	,04	,08	,23	-,10
Entropy of thymocytogram	**,491**	-,096	-,16	-,10	,04	**,65**	,09	,01	,04	-,22	,12
Fasciculary Zone Adrenal Cortex	**,445**	-,292	-,29	**,32**	-,02	-,01	-,02	,06	,05	-,24	,08
Epitheliocytes of Tymus	**,438**	-,310	-,24	,14	-,02	,08	,00	-,19	-,26	-,27	-,13
AMo as Sympathetic tone	**,421**	-,165	**-,74**	-,14	-,04	,07	-,02	-,02	,06	,15	,07
Plasmocytes of Spleen	**-,402**	,268	,14	-,03	-,13	-,07	,17	-,17	,16	**,43**	,38
Thymus Mass	**,380**	,241	-,16	-,05	,09	-,20	-,09	-,03	-,25	**-,40**	,23
Thymus Mass Index	**,377**	,227	-,17	-,03	,07	-,29	-,04	,02	-,23	**-,42**	,22
Mineralocorticoid activity	**,378**	,018	,15	,04	-,13	,01	,11	-,09	-,03	**-,59**	,05
Entropy of Leukocytogram	**,323**	,086	,11	,11	**,74**	,07	-,05	-,14	-,08	-,15	-,04
Mode HRV as Humoral channal	**-,322**	,257	**,76**	,11	,17	,10	-,24	-,01	-,02	-,08	-,10
Endotheliocytes of Thymus	**,306**	,150	,02	,07	,12	,03	-,10	,17	**,39**	-,29	,21
Neutrophils of Spleen	**,298**	,285	,07	-,20	,13	-,25	,02	-,14	,11	**-,39**	-,01
Glomerulary Zone Adrenal Cortex	**,281**	,084	**-,39**	-,04	,29	,02	-,29	,15	-,25	-,03	,11
Basophils of Blood	**,267**	-,088	,18	,26	,01	,18	-,17	,12	,09	**-,35**	,00
Macrophages of Spleen	**,411**	**-,412**	**-,52**	,27	,03	,01	-,04	,09	,18	,00	-,02
0-Lymphocytes of Blood	-,047	**-,497**	,11	,15	-,06	,05	,04	-,13	-,08	,06	**-,65**
Calcitonine activity	-,011	**,440**	-,00	**-,62**	-,13	,14	,01	,06	,26	-,09	,08
Testosterone	,146	**-,434**	-,10	,14	-,23	,31	**,43**	-,17	,00	,03	-,05
Parathyrine activity	-,096	**,433**	-,07	**-,68**	,05	-,01	,03	,05	-,15	,02	-,09
Tc-Lymphocytes of Blood	,059	**,429**	,03	-,03	-,05	,01	,03	,01	-,09	-,08	**,62**
B-Lymphocytes of Blood	,083	**,404**	-,16	-,14	,04	,04	,05	,20	-,11	-,10	**,62**
Reticulary Zone Adrenal Cortex	,163	**-,300**	-,09	,18	,07	**,33**	-,03	-,21	-,01	,20	-,12
Lymphoblastes of Thymus	-,167	**,298**	,08	,10	-,11	-,08	**-,41**	-,06	,09	,20	,33
Spleen Mass	-,001	**-,297**	-,09	**,35**	,10	-,29	,04	**,35**	,31	-,11	-,08
Spleen Mass Index	,061	**-,288**	-,12	,38	,10	**-,39**	,08	**,39**	,30	-,19	-,05
Reticulocytes of Spleen	-,032	**-,288**	-,17	-,03	-,12	,20	-,20	,19	**,35**	,15	-,32
Phagocytosis Ind of Neutrophils of Blood	,118	**,279**	-,01	-,02	-,10	-,02	**-,73**	-,03	-,06	-,09	,04
Microbial Count of Neutrophils of Blood	-,057	**,277**	,08	-,03	,14	-,07	**-,72**	,06	,00	,08	-,11

Therefore, due to factor analysis, two independent clusters were identified to identify the

cause-effect functional relationships of the parameters of the neuro-endocrine-immune complex.

4.2. Characterization of neuro-endocrine manifestations of stress in female rats

Applying the previously described methodological approach with the calculation of Z-values, it was found that on the second day after a week of stressing, mineralocorticoid activity, estimated by the Na/K ratio of plasma, maximally increases (Fig. 4.2). Then, in descending order, follow: testosteroneemia, thickness of the fascicular area of the adrenal cortex, heart rhythm (as the inverse of the Mode of HRV), sympathetic tone (estimated for AMo HRV), thickness of the reticular ZAC and plasma level of triiodothyronine. Hormonal constellation consisting of calcitonin activity, adrenals mass, thickness of the glomerular ZAC, corticosteroneemia and vagal tone is not significantly different from that of the control. In contrast, thyroxinemia and paratyrin activity were significantly lowered.

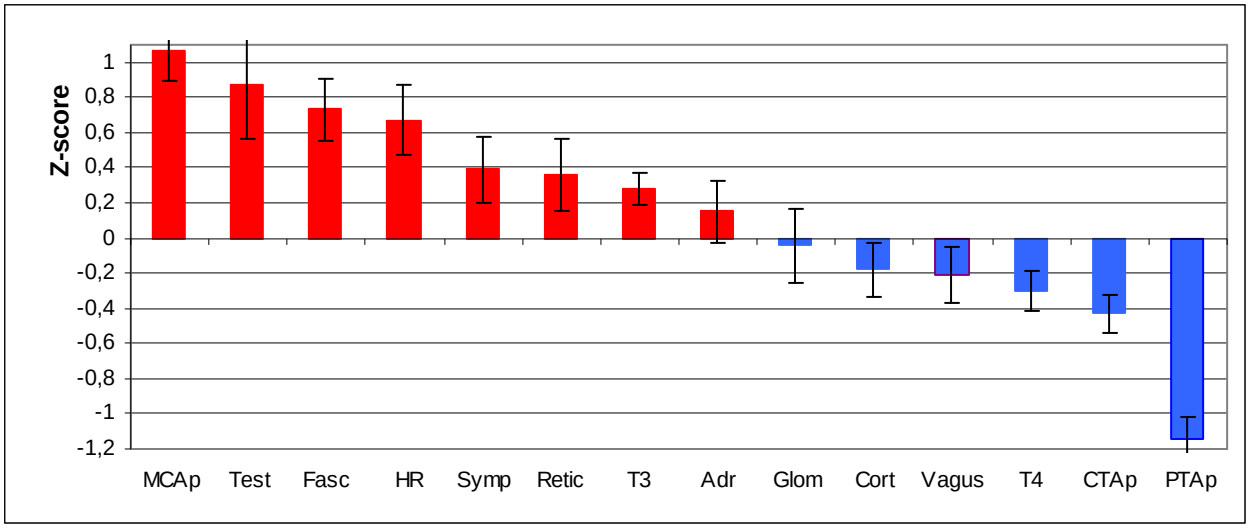


Fig. 4.2. Ranking caused by chronic stress changes in neuroendocrine parameters at female rats

The integral quantitative measure of neuro-endocrine response to chronic stress factors as the average of significant Z-unit modules is 0,61±0,10 σ, that is, not significantly different from that of males.

As a result of the discriminant analysis of neuro-endocrine indicators characteristic of the stress reaction were only four indicators, while others, even though significant deviations from the norm, were out of the discriminatory model, that is, the identifiable information they carry is redundant (Tables 4.4 and 4.5).

Table 4.4. Discriminant Function Analysis Summary for Neuro-endocrine Variables of female rats

Step 4, N of vars in model: 4; Grouping: 2 grps. Wilks' Λ 0,756; approx. $F_{(4,55)}$=4,44; p=0,004

Variables currently in the model	Initial level (Control) (10)	After Chronic Stress (50)	Stressory change as Z-score	Wilks Λ	Partial Λ	F re-move	p-level	Tolerancy
$(Nap/Kp)^{0,5}$ as MCA	5,57±0,17	6,16±0,10	**+1,07±0,18**	,833	,907	5,62	,021	,993
Moda HRV, msec	124±5	114±3	**-0,67±0,20**	,775	,976	1,38	,245	,997
Ttiiodo-thyronine, nM/L	2,12±0,18	2,28±0,05	**+0,28±0,09**	,773	,978	1,26	,267	,977
$(Cap/Pp)^{0,5}$ as PTA	2,38±0,25	1,48±0,12	**-1,14±0,12**	,860	,879	7,57	,008	,980

Variables currently not in the model Df for all F-tests: 1,54	Initial level (Control) (10)	After Chronic Stress (50)	Stressory change as Z-score	Wilks Λ	Partial Λ	F to enter	p-level	Tolerancy
Testosterone, nM/L	3,93±0,34	4,86±0,32	**+0,87±0,30**	,753	1,00	,19	,666	,912
Fasciculary ZAC, µM	370±20	417±12	**+0,73±0,18**	,754	1,00	,17	,684	,803
AMo as Sympathetic tone, %	55,8±5,4	62,5±3,3	**+0,39±0,19**	,756	1,00	,00	,975	,280
Reticulary ZAC, µM	41±2	44±2	**+0,36±0,20**	,753	1,00	,24	,627	,945
Adrenals Mass, mg	69±3	71±2	+0,15±0,18	,756	1,00	,02	,880	,899
Glomerulary ZAC, µM	189±8	188±6	-0,04±0,21	,755	1,00	,04	,850	,908
Corticosterone, nM/L	466±57	433±27	-0,18±0,15	,754	1,00	,16	,689	,936
MxDMn as Vagal tone, msec	53±14	44±6	-0,21±0,16	,756	1,00	,03	,864	,250
Thyroxine, nM/L	63,5±5,3	58,6±1,9	**-0,30±0,11**	,753	1,00	,19	,668	,501
$(Pp•Cap)^{-0,5}$ as CTA	0,86±0,15	0,66±0,05	**-0,43±0,11**	,754	1,00	,13	,721	,730
$(Cau•Pu/Pp•Cap)^{0,25}$ as CTA	1,72±0,15	1,53±0,08	**-0,41±0,17**	,753	1,00	,18	,669	,505
$(Cap•Pu/Pp•Cau)^{0,25}$ as PTA	2,03±0,12	1,54±0,07	**-1,26±0,17**	,753	1,00	,24	,627	,945

Table 4.5. Summary of step-by-step analysis of neuro-endocrine indices of female rats

Variables currently in the model	F to enter	p-level	Λ	F-value	p-level
$(Cap/Pp)^{0,5}$ as Parathytine Activity (PTA)	7,43	,008	,886	7,43	,008
$(Nap/Kp)^{0,5}$ as Mineralocorticoid Activity (MCA)	6,52	,013	,795	7,33	,001
Moda HRV	1,61	,210	,773	5,47	,002
Ttiiodo-thyronine	1,26	,267	,756	4,44	,004

The coefficient of canonical correlation between selected indicators of intact and stressed female rats and discriminant root is 0,494 (Wilks' Λ=0,756; $\chi^2_{(4)}$=15,7; p=0.004). Table 4.6 lists the standardized and nonstandardized coefficients of the discriminant variables.

Table 4.6. Coefficients and constant for neuroendocrine discriminant variables female rats

Variables currently in the model	Coefficients for Canonical Variables		
	Standardized	Structural	Raw
$(Cap/Pp)^{0,5}$ as Parathytine Activity (PTA)	,71	**,63**	1,224
Moda HRV	,32	**,33**	,016
$(Nap/Kp)^{0,5}$ as Mineralocorticoid Activity (MCA)	-,62	**-,59**	-,925
Ttiiodothyronine	-,31	**-,27**	-,759
Chi-Square Tests with Successive Roots Removed		**Constant**	1,609
Eigenvalue	,323		
Canonical R	,494		
Wilks' Λ	,756		
χ^2	15,7		
Degree freedom	4		
p-level	,004		

Calculating the value of the discriminant function for each animal by summing the product of the non-standardized coefficients by the value of the discriminant variables together with the constant make it possible to visualize its position in the root information

field (Fig. 4.3).

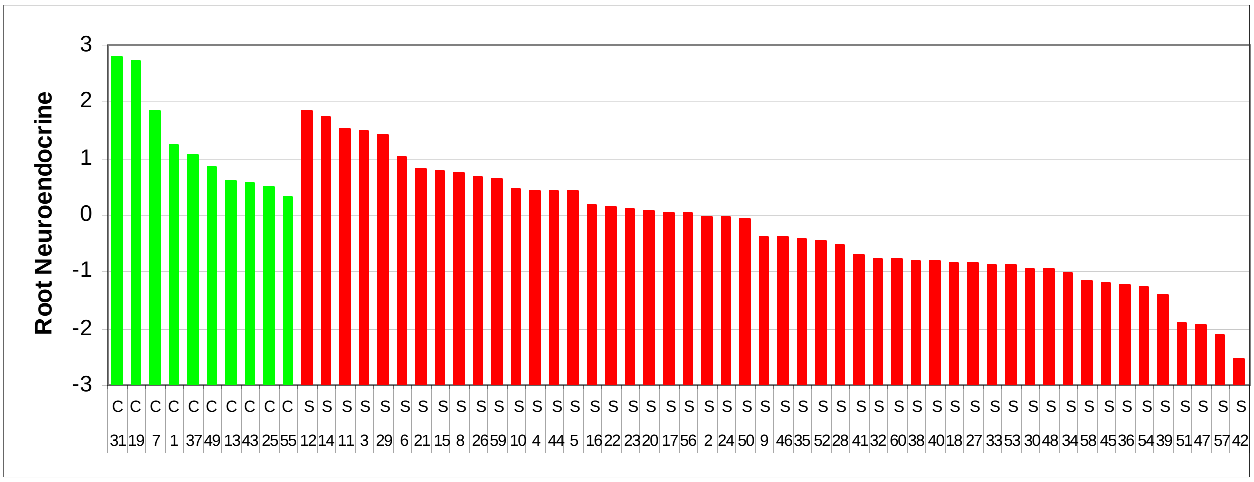


Fig. 4.3. Individual values of the neuro-endocrine root of intact (I) and stressed (S) female rats (animal numbers below)

Calculation of root mean values shows a significant difference between the neuroendocrine status of intact (+1,25±0,29) and stressed (-0,25±0,14) groups (Fig. 4.4), which is documented by the Mahalanobis distance calculation (D^2_M=2,32; F=4,2; p=0,005).

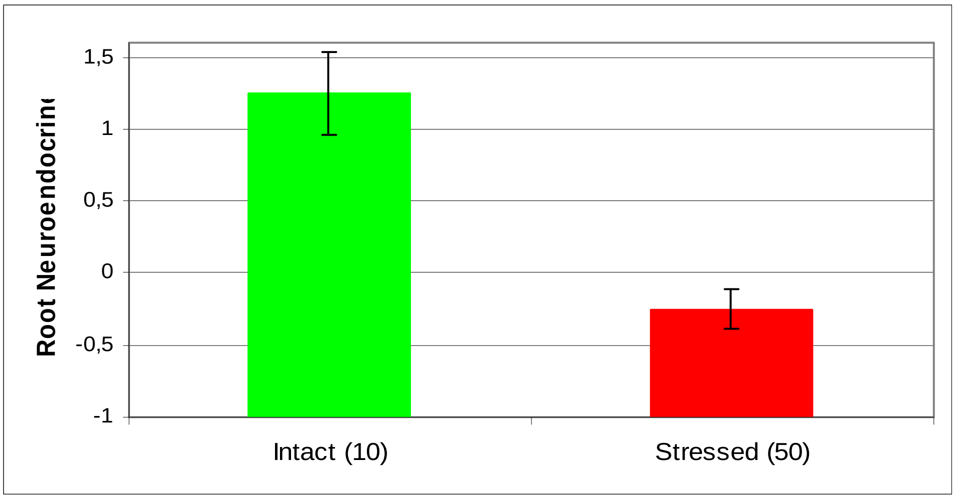


Fig. 4.4. Mean values of the neuro-endocrine root of intact and stressed female rats

Judging by the full structural coefficients given in Table. 4.6, the root directly displays information about the parathyroid activity and duration of the cardiac cycle while in the reverse way about mineralocorticoid activity and plasma level of triiodothyronine. So, Figs. 4.3 and 4.4 illustrate the chronic stress-induced decrease in parathyroid and increased mineralocorticoid activity, as well as the activation of thyroid function and sympathotonic shift of autonomic tone.

4.3. Characterization of the immune manifestations of stress in female rats

The described neuro-endocrine perturbations induced by chronic stress are accompanied by significant deviations from the norm of a number of parameters of immunity, and multidirectional ones. The maximum **suppression** was found in relation to the relative content in the thymus of lymphocytes (Fig. 4.5). Other parameters reflect a decrease in

bactericidal ability against Staph. aureus of neutrophils/microphages of the blood by reducing, first of all, the activity of phagocytosis and, to a lesser extent, its intensity and their content in the blood, as well as the bactericidal capacity of monocytes/macrophages of the blood by reducing their content.

The next pool of suppressed blood parameters are T-helper lymphocytes, 0-lymphocytes and eosinophils, as well as blasttransformation reaction of T-lymphocytes (RBTL). With respect to the spleen, a decrease in its mass was detected, with both absolute and relative body mass, as well as share in the splenocytogram of lymphocytes and plasmocytes. However, a decrease in the proportion of lymphoblastes in the thymocytogram was detected.

On the other hand, another constellation of immune parameters has undergone an **enhancing** influence of chronic stress (Fig. 4.6).

First of all, it is the content of natural killer in the blood, as well as T-killer cells and the microphage killing index, combined with a tendency to increase the activity and intensity of blood macrophage phagocytosis and significantly increase their content in the thymus and spleen. In addition, an increase was found in the content of reticulocytes in the spleen, which have the capacity for phagocytosis, and content in the thymus of epitheliocytes and Hassal's corpuscles.

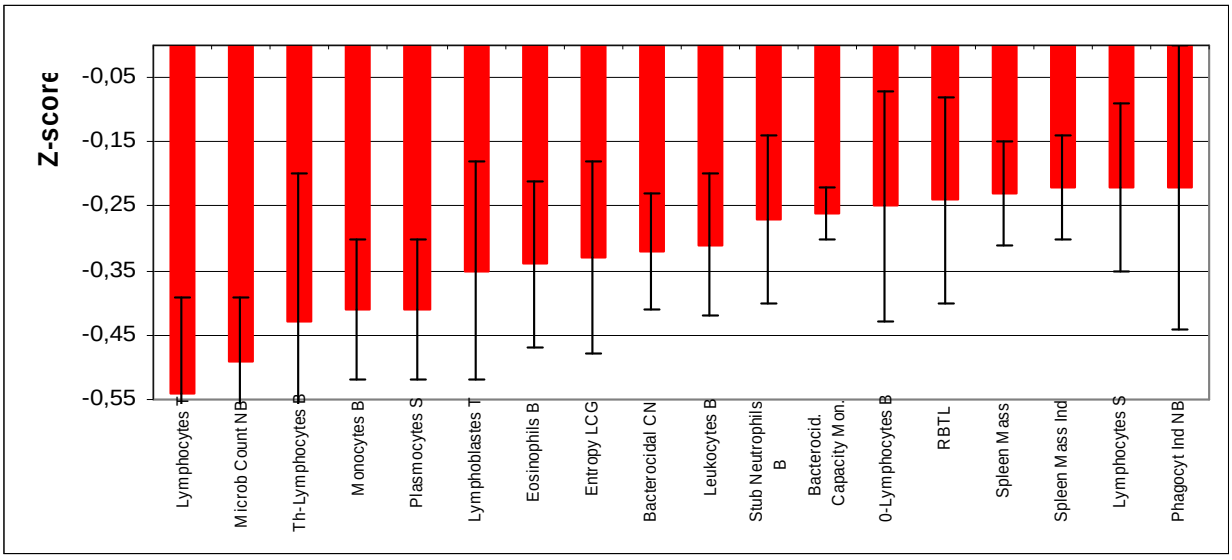


Fig. 4.5. Ranking of chronic stress-induced suppressive abnormalities in immune parameters in female rats

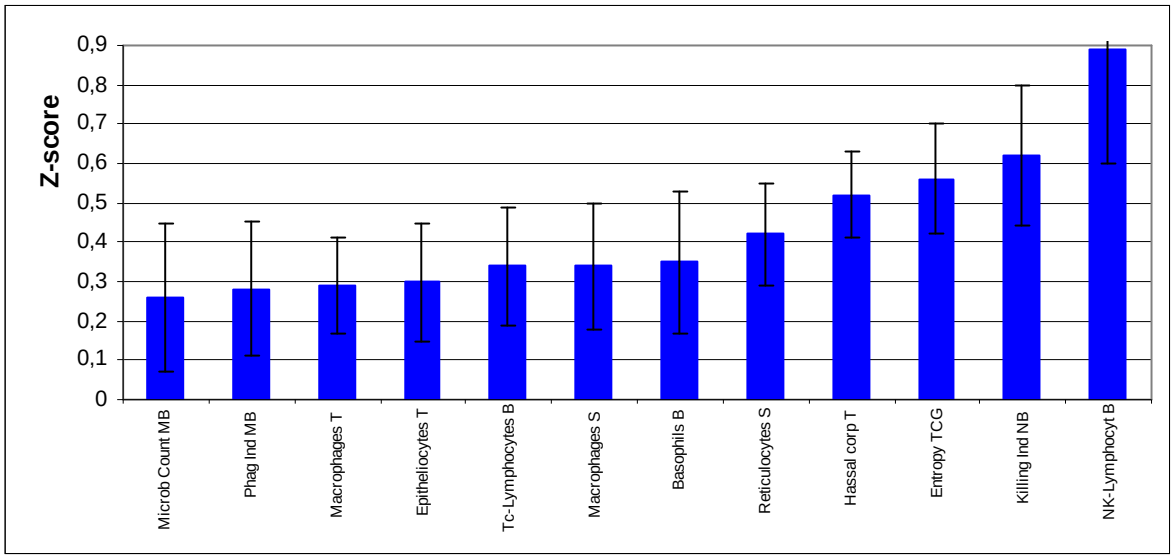


Fig. 4.6. Ranking of chronic stress-induced enhancing deviations from the norm of immune parameters in female rats

Particular attention should be paid to entropy, which for leukocytogram decreases, for thymocytograms increases, and for immunocytograms and splenocytograms does not change.

As a result of the discriminant analysis of the immune indices of female rats, as a characteristic of post-stress changes, only 13 were included in the model (Tables 4.7 and 4.8). Instead, other indicators, including those significantly different from controls, appeared outside the discriminatory model as carriers of excess discriminatory information (Table 4.9).

Table 4.7. Summary of discriminant analysis of the immune indices of female rats

Step 13, N of vars in model: 13; Grouping: 2 grps
Wilks' Lambda: 0,528; approx. $F_{(13)}$=3,16; p=0,002

Variables currently in the model	Initial level (Control) (10)	After Chronic Stress (50)	Stressory change as Z-score	Wilks Λ	Partial Λ	F remove	p-level	Tolerancy
Microb Count Neutr of Blood	8,5±0,5	7,7±0,2	**-0,49±0,10**	,557	,948	2,5	,119	,485
Plasmocytes of Spleen, %	2,50±0,50	1,86±0,17	**-0,41±0,11**	,552	,957	2,0	,159	,472
Lymphoblastes of Thymus, %	7,40±0,27	7,10±0,15	**-0,35±0,17**	,591	,894	5,5	,024	,561
Eosinophils of Blood, %	4,30±0,68	3,57±0,27	**-0,34±0,13**	,572	,923	3,8	,056	,627
Bactericid Capacity of Monoc	110±52	67±7	**-0,26±0,04**	,722	,731	16,9	,000	,443
Phagocyt Ind Neutr Blood, %	70,1±1,0	69,4±0,5	**-0,22±0,17**	,611	,865	7,2	,010	,245
Entropy Splenocytogram, 10^3	613±8	613±18	+0,01±0,10	,550	,961	1,9	,179	,512
Microb Count Monoc Blood	4,3±0,5	4,7±0,3	**+0,26±0,19**	,589	,896	5,3	,026	,193
Phagoc Ind Monoc Blood, %	2,7±0,2	2,9±0,1	**+0,28±0,17**	,539	,980	1,0	,333	,440
Tc-Lymphocytes of Blood, %	15,1±0,9	16,1±0,4	**+0,34±0,15**	,570	,926	3,7	,062	,790
Hassal corpuscles Thymus, %	1,70±0,17	1,98±0,06	**+0,52±0,11**	,579	,912	4,4	,040	,605
Killing Ind Neut. Blood, %	50,4±1,6	53,6±0,9	**+0,62±0,18**	,548	,965	1,7	,201	,585
NK-Lymphocyt of Blood, %	15,0±0,4	16,0±0,3	**+0,89±0,29**	,567	,932	3,4	,073	,666

Table 4.8. Summary of step-by-step analysis of immune indices of female rats

Variables currently in the model	F to enter	p-level	Λ	F-value	p-level
Hassal's corpuscles of Thymus, %	3,4	,071	,945	3,39	,071
Microbial Count Neutrophils Blood, Bact/Phag	3,1	,085	,897	3,29	,044
Bactericidal Capacity of Monocytes, 10^6 Bact/l	4,1	,048	,836	3,67	,017
Phagocytic Index Monocytes of Blood, %	3,6	,062	,784	3,79	,009
Killing Index Neutrophils of Blood, %	2,4	,125	,750	3,60	,007
Plasmocytes of Spleen, %	2,5	,120	,716	3,50	,005
Phagocytic Index Neutrophils of Blood	1,8	,188	,693	3,30	,006
Lymphoblastes of Thymus, %	3,3	,073	,650	3,43	,003
Tc-Lymphocytes of Blood, %	2,5	,121	,619	3,42	,002
Microbial Count Monocytes Blood, Bact/Phag	1,9	,173	,596	3,32	,002
Eosinophils of Blood, %	2,0	,165	,572	3,26	,002
NK-Lymphocytes of Blood, %	1,9	,172	,550	3,21	,002
Entropy of Splenocytogram, 10^3	1,9	,179	,528	3,16	,002

Table 4.9. Immune indicators of female rats not included in the discriminant model

Variables currently not in the model Df for all F-tests: 1,45	Initial level (Control)	After Chronic Stress	Stressory change as Z-score	Wilks Λ	Par-tial Λ	F to en-ter	p-le-vel	Tole ran-cy
Lymphocytes of Thymus, %	70,3±0,8	69,0±0,4	**-0,54±0,15**	,520	,984	,744	,39	,600
Th-Lymphocytes of Blood, %	31,8±0,8	30,8±0,5	**-0,43±0,23**	,527	,997	,117	,73	,803
Monocytes of Blood, %	5,70±0,92	4,51±0,31	**-0,41±0,11**	,523	,991	,422	,51	,486
Entropy of LCG of Blood, 10^3	318±10	308±5	**-0,33±0,15**	,527	,999	,065	,79	,469
Bactericidal Capacity Neutrop	12,5±2,4	10,1±0,7	**-0,32±0,09**	,525	,994	,270	,60	,614
Leukocytes of Blood, 10^9/l	13,1±1,8	11,4±0,6	**-0,31±0,11**	,527	,998	,112	,74	,694
Stub Neutrophils of Blood, %	3,50±0,40	3,16±0,16	**-0,27±0,13**	,528	1,00	,022	,88	,496
0-Lymphocytes of Blood, %	22,7±1,8	21,2±1,1	**-0,25±0,18**	,523	,991	,422	,51	,446
Blasttransfrmation T-Lym, %	78,8±2,3	77,1±1,1	**-0,24±0,16**	,522	,993	,420	,52	,440
Spleen Mass, mg	816±83	756±20	**-0,23±0,08**	,526	,996	,181	,67	,723
Spleen Mass Ind, ‰	3,12±0,32	2,90±0,10	**-0,22±0,08**	,524	,993	,322	,57	,783
Lymphocytes of Spleen, %	48,7±0,9	48,1±0,3	**-0,22±0,13**	,528	1,00	,018	,89	,236
Fibroblases of Spleen, %	8,20±0,66	7,94±0,23	-0,12±0,11	,528	1,00	,003	,96	,680
Eosinophils of Spleen, %	1,50±0,34	1,48±0,11	-0,02±0,10	,526	,996	,190	,66	,606
Neutrophils of Spleen, %	13,0±0,4	13,0±0,3	0,00±0,22	,527	,997	,134	,71	,576
Thymus Mass Index, ‰	0,29±0,04	0,30±0,04	+0,02±0,09	,523	,991	,411	,52	,652
Entropy of ICG of Blood, 10^3	469±2	470±1	+0,03±0,22	,528	1,00	,001	,97	,786
Reticulocytes of Thymus, %	4,70±0,54	4,78±0,16	+0,04±0,09	,527	,997	,147	,70	,606
Endotheliocytes of Thymus, %	2,60±0,31	2,65±0,13	+0,05±0,14	,522	,987	,580	,45	,630
Segmented Neutr of Blood, %	26,9±2,2	27,4±0,9	+0,07±0,13	,527	,998	,100	,75	,395
Thymus Mass, mg	73±7	75±2	+0,08±0,11	,527	,998	,081	,77	,650
Lymphoblases of Spleen, %	3,90±0,38	4,08±0,19	+0,15±0,16	,526	,995	,229	,63	,423
Lymphocytes of Blood, %	59,4±2,5	61,0±1,1	+0,20±0,14	,528	1,00	,000	,99	,414
B-Lymphocytes of Blood, %	15,4±0,8	16,0±0,4	+0,23±0,19	,520	,985	,692	,41	,577

Plasmocytes of Thymus, %	1,80±0,25	2,00±0,12	+0,25±0,15	,528	1,00	,008	,93	,745
Macrophages of Thymus, %	2,70±0,42	3,08±0,16	**+0,29±0,12**	,528	1,00	,004	,95	,667
Epitheliocytes of Thymus, %	8,80±0,63	9,39±0,30	**+0,30±0,15**	,524	,993	,339	,56	,546
Macrophages of Spleen, %	7,90±0,50	8,44±0,26	**+0,34±0,16**	,523	,990	,452	,50	,410
Basophils of Blood, %	0,20±0,13	0,35±0,07	**+0,35±0,18**	,526	,996	,173	,67	,742
Reticulocytes of Spleen, %	14,3±0,6	15,1±0,2	**+0,42±0,13**	,525	,994	,285	,59	,387
Entropy Thymocytogram, 10^3	439±9	455±4	**+0,56±0,14**	,521	,987	,609	,43	,574

Information on discriminant variables is condensed in the canonical root, which is poorly structured and directly reflects the content in the thymus of Hassal's corpuscles, in the blood of natural killers and T-killers, as well as parameters of phagocytosis Staph. aureus: killing activity of neutrophils as well as activity and intensity of phagocytosis of monocytes. Instead, the intensity and activity of neutrophil phagocytosis and the bactericidal ability of monocytes against Staph. aureus are reversed reflected, as well as the content of plasmocytes in the spleen, eosinophils in the blood and lymphoblasts in the thymus.

Table 4.10. Coefficients and constant for immune discriminant variables in female rats

Variables currently in the model	Coefficients		
	Standar-dized	Structural	Raw
Hassal corpuscles of Thymus, %	,556	**,256**	1,272
Killing Index of Neutrophils of Blood, %	,357	**,200**	,056
NK-Lymphocytes of Blood, %	,467	**,185**	,219
Tc-Lymphocytes of Blood, %	,445	**,127**	,144
Phagocytic Index of Monocytes of Blood, %	,313	**,093**	,368
Microbial Count of Monocytes Blood, Bact/Phag	1,067	**,081**	,571
Entropy of Splenocytogram, 10^3	-,401	**,004**	-20,86
Microbial Count Neutrophils Blood, Bact/Phag	-,476	**-,254**	-,360
Microbial Capacity of Monocytes, 10^6 Bact /l	-1,134	**-,220**	-14,53
Plasmocytes of Spleen, %	-,438	**-,203**	-,346
Eosinophils of Blood, %	-,510	**-,150**	-,262
Lymphoblastes of Thymus, %	-,633	**-,120**	-,640
Phagocytic Index of Neutrophils of Blood	1,082	**-,076**	,287
Chi-Square Tests with Successive Roots Removed		**Constant**	**-11,93**
Eigenvalue	0,893		
Canonical R	0,687		
Wilks' Λ	0,528		
χ^2	32,9		
Degree freedom	13		
p-level	0,002		

The calculation of the individual values of the immune root by the low coefficients and constant given in table. 4.10, allows to visualize the immune status of each female rat (Fig. 4.7).

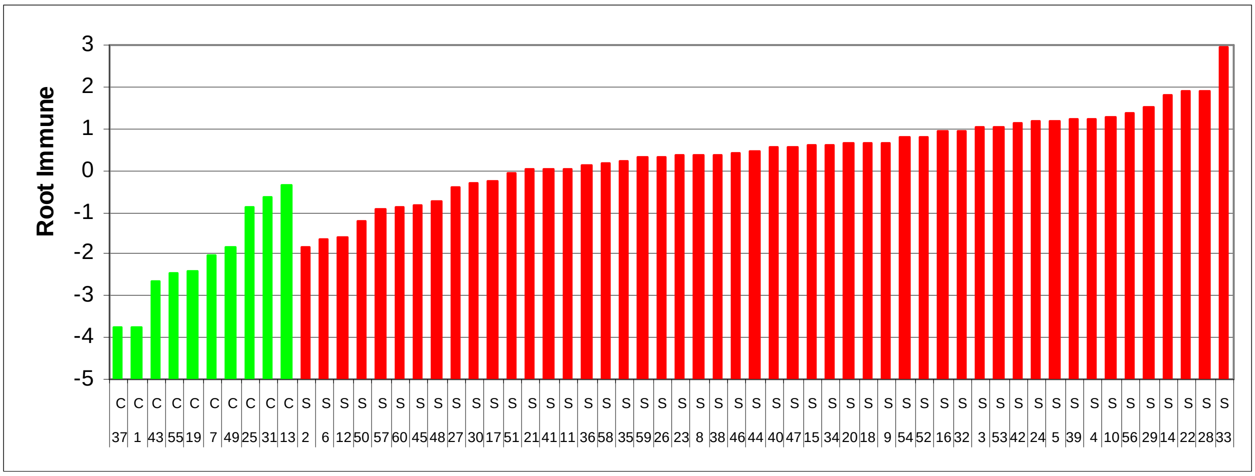


Fig. 4.7. Individual values of the immune root of intact (C) and stressed (S) female rats. The numbers indicate the animal's number

The apparent clear difference between the immune statuses of intact and stressed female rats is documented by calculating the mean values of the immune roots (Fig. 4.8) and the Mahalanobis distance between them (D^2_M=6,4; F=2,98; p=0.003).

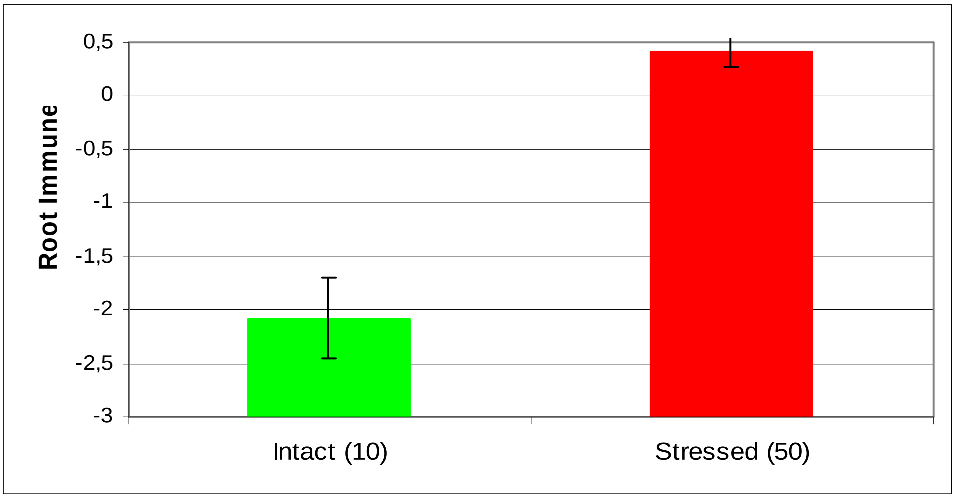


Fig. 4.8. Average values of the immune root of intact and stressed female rats

Selected 13 parameters can be used to identify the belonging of a female rat to a group of intact or stressed. This purpose of discriminant analysis is realized by means of the classification functions (Table 4.11).

Table 4.11. Coefficients and constants for the classification functions of female rats

Variables currently in the model	Intact	Stressed
Hassal corpuscles of Thymus, %	-23,95	-20,78
Microbial Count Neutrophils Blood, Bact/Phag	-7,12	-8,02
Bactericidal Capacity Monocytes, 10^6 M/l	22,87	-13,36
Phagocytic Index Monocytes of Blood, %	20,21	21,12
Killing Index Neutrophils of Blood, %	2,98	3,12
Plasmocytes of Spleen, %	-23,70	-24,57
Phagocytic Index Neutrophils of Blood	16,55	17,26
Lymphoblastes of Thymus, %	-10,07	-11,66
Tc-Lymphocytes of Blood, %	4,02	4,38
Microbial Count Monocytes Blood, Bact/Phag	16,68	18,11
Eosinophils of Blood, %	,52	-,13
NK-Lymphocytes of Blood, %	-3,98	-3,44
Entropy of Splenocytogram, 10^3	2700	2648
Constants	-1433	-1459

In this case, we can retrospectively recognize intact female rats with an accuracy of 70% (3 errors per 10 animals), and subjected to stress with an accuracy of 94% (3 errors per 50 animals). The overall classification accuracy is 90%.

4.4. Variantes of post-stress immunity in female rats

Analysis of the individual values of the canonical discriminant root of female rats subjected to chronic stress, again, as among male rats, revealed their wide variability. Therefore, stressed female rats were also divided into three homogeneous clusters.

The first cluster included 24 rats (48%), the second 12 (24%), and the third 14 (28%). The Euclid's distance between I and II clusters is 23, between I and III 29, between II and III 52,5 units, which is significantly greater than the distances between members within each of the clusters.

The analysis of variance revealed that the maximum contribution to the distribution into clusters, judging by the criterion η^2, is made by the mass of the spleen, more absolute, somewhat less relative to body weight.

Smaller, but **significant** ($p \leq 0.05$) values of η^2 were found relative to the proportion in the splenocytogram of lymphoblasts, macrophages and fibroblasts, as well as the total blood content of leukocytes.

At the **limit of significance** were revealed: the content in the thymocytogram of endothelial cells, Hassal's bodies and plasmocytes, in the splenocytogram of reticulocytes and microphages, in the immunocytogram of T-helper cells and in the leukocytogram of basophils

as well as phagocytosis completeness of microphages and phagocytosis activity of macrophages (Table 4.12).

Table 4.12. Summary of the ANOVA of immune parameters of female rats

Variables	Between SS	Within SS	η^2	R	F	p
Spleen Mass, mg	778143	189275	0,804	0,897	96,6	**$<10^{-6}$**
Spleen Mass Index, ‰	0,050	0,034	0,591	0,769	41,3	**$<10^{-6}$**
Lymphoblastes of Spleen, %	19,8	67,9	0,226	0,475	6,85	**,002**
Macrophages of Spleen, %	24,8	137,5	0,153	0,391	4,24	**,020**
Leukocytes of Blood, 10^9/L	127	781	0,140	0,374	3,81	**,029**
Fibroblastes of Spleen, %	17,8	113	0,136	0,369	3,70	**,032**
Endotheliocytes of Thymus, %	5,0	38,1	0,116	0,341	3,09	**,054**
Th-Lymphocytes of Blood, %	80,3	657	0,109	0,330	2,87	**,066**
Hassal corpuscles of Thymus, %	0,9	7,6	0,106	0,325	2,78	**,07**
Reticulocytes of Spleen, %	15,1	135	0,101	0,317	2,62	**,08**
Killing Index Neutrophils of Blood, %	193	1937	0,091	0,301	2,34	**,11**
Basophiles of Blood, %	1,1	12,0	0,084	0,290	2,15	**,13**
Plasmocytes of Thymus, %	2,8	31,2	0,082	0,287	2,13	**,13**
Neutrophiles of Spleen, %	17,6	210	0,077	0,278	1,97	**,15**
Phagocytose Index of Monocytes, %	2,4	35,1	0,064	0,253	1,61	***,21***
NK-Lymphocytes of Blood, %	14,7	238	0,058	0,241	1,45	*,24*
Blasttransformation of T-Lymph, %	10,0	190	0,050	0,224	1,40	*,27*
Eosinophiles of Blood, %	7,8	170	0,044	0,209	1,08	*,34*
Lymphoblastes of Thymus, %	1,9	48,6	0,038	0,194	0,90	*,41*
Lymphocytes of Thymus, %	6,6	320	0,020	0,142	0,49	*,62*
Entropy of Thymocytogram ($\bullet 10^3$)	0,001	0,071	0,014	0,118	0,40	*,67*

In the Table. 4.12 also included those immune parameters that, despite the meager η^2, were further included in the discriminatory model.

When calculating the deviation (in Z-units) from the norm of cluster-forming immune parameters, revealed the following (Table 4.13 and Fig. 4.9). In cluster I rats, only 2 parameters barely exceeded the limit (±0,5σ). Cluster III rats are characterized by a moderate decrease in the content of lymphocytes in the thymus and lymphoblasts in the thymus and spleen, combined with a moderate increase in the content in the spleen of macrophages and

reticulocytes, in the blood basophils and natural killer cells, and in the thymus Hassal's corpuscles as well as entropy of thymocytogram.

Thus, in 28% of female rats, chronic stress causes moderate suppression of lymphoid elements of the thymus and spleen in combination with moderate activation of phagocytic and cytotoxic blood immunocytes.

In cluster II rats, only 6 of the 20 parameters did not deviate from the norm. Seven parameters were significantly reduced, reflecting a decrease in spleen mass and fibroblastes content, a decrease in the proportion of lymphocytes in the thymocytogram and Th-lymphocytes in the blood immunocytogram, and eosinophils in the leukocytogram in the background, while decrease in total leukocyte content.

Instead, the other 7 parameters increased significantly. First of all, it is the content of natural killer in the blood and killer activity of blood microphages and their content in the spleen, phagocytic activity of blood macrophages against the background of an increase in the proportion of lymphoblasts in the splenocytogram and plasmocytes in the thymocytogram and increase in its entropy.

Thus, in 24% of female rats, stress causes moderate suppression of lymphoid and myeloid cells of blood and thymus and macrophages of spleen in combination with moderate activation of cytotoxic, phagocytic and lymphoid cells of blood, thymus and spleen.

Table 4.13. Features of deviation from the norm of immune parameters in individual clusters

Variables	I	III	II
Th-Lymphocytes of Blood, %	-0,11	-0,15	**-1,35**
Spleen Mass, mg	-0,29	+0,43	**-0,88**
Spleen Mass Index, ‰	-0,35	+0,47	**-0,77**
Lymphocytes of Thymus, %	-0,38	**-0,72**	**-0,65**
Fibroblastes of Spleen, %	-0,04	+0,14	**-0,61**
Eosinophiles of Blood, %	-0,14	-0,50	**-0,52**
Leukocytes of Blood, 10^9/L	-0,50	+0,15	**-0,49**
Endotheliocytes of Thymus, %	+0,07	+0,49	-0,45
Macrophages of Spleen, %	+0,38	**+0,87**	-0,36
Basophiles of Blood, %	**+0,56**	**+0,54**	-0,28
Lymphoblastes of Thymus, %	-0,23	**-0,75**	-0,18
Reticulocytes of Spleen, %	+0,50	**+0,71**	-0,07
Blasttransformation of T-Lymphocytes, %	-0,47	-0,05	+0,03
Hassal's corpuscles of Thymus, %	**+0,60**	**+0,77**	+0,09
Neutrophiles of Spleen, %	-0,41	+0,20	**+0,59**
Entropy of Thymocytogram (•10^3)	+0,43	**+0,70**	**+0,67**
Phagocytose Index of Monocytes, %	-0,01	+0,32	**+0,77**
Lymphoblastes of Spleen, %	+0,29	**-0,63**	**+0,78**
Plasmocytes of Thymus, %	+0,04	+0,16	**+0,78**
Killing Index of Neutrophils of Blood, %	+0,51	+0,24	**+1,27**
NK-Lymphocytes of Blood, %	+0,41	**+1,06**	**+1,62**
Inhibiting effects, Z	**-0,27**	**-0,47**	**-0,55**
SE	0,05	0,11	0,10
n	11	6	12
Enhancing effects, Z	**+0,38**	**+0,48**	**+0,73**
SE	0,06	0,07	0,16
n	10	15	9
Modulating effects, Z	**0,32**	**0,48**	**0,63**
SE	0,04	0,06	0,09
n	21	21	21

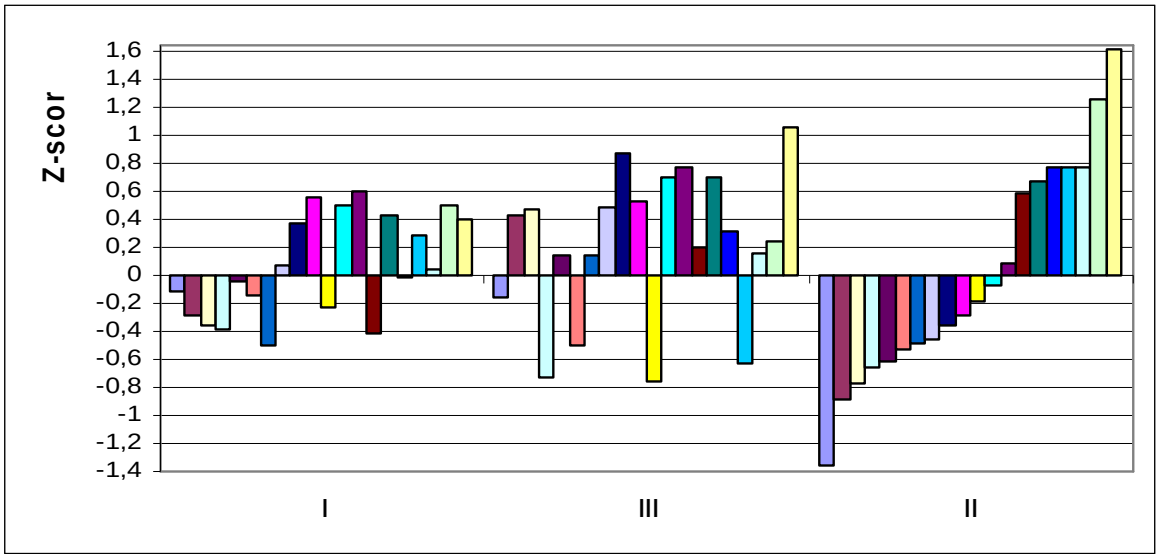


Fig. 4.9. Post-stress immune profiles of female rats of I, III and II clusters (the order of the parameters is the same as in Table 4.13)

When calculating the averages, it is stated that in the first cluster, the deviations of the parameters on both sides of the average norm are almost identical and are within the permissible range ±0,5σ (Fig. 4.10).

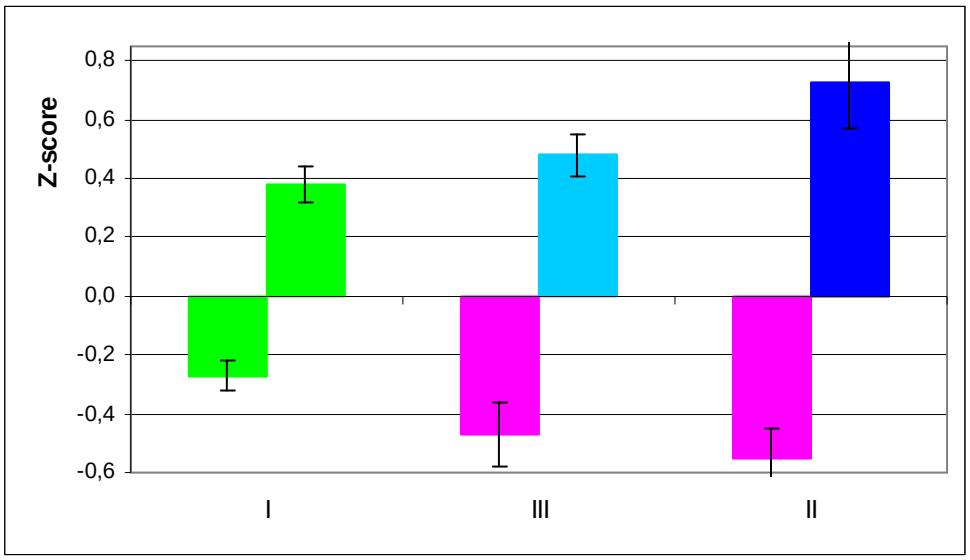


Fig. 4.10. Post-stress deviations from the norm of immunityparameters in female rats I, III and II clusters

Therefore, the immune status of 48% of female rats subjected to chronic moderate stress was resistant to its factors. The **immunosuppressive** effect of stress in the other two clusters turned out to be almost the same, whereas the **enhancing** of immune parameters in cluster III was more prevalent than that in II.

The **modulus** of deviation from the norm of immune parameters as a measure of stress-

induced immunodysfunction is 0,63±0,09σ for the II cluster, 0,48±0,06σ for the III cluster, whereas only 0,32±0,04σ for the cluster I (Fig. 4.11).

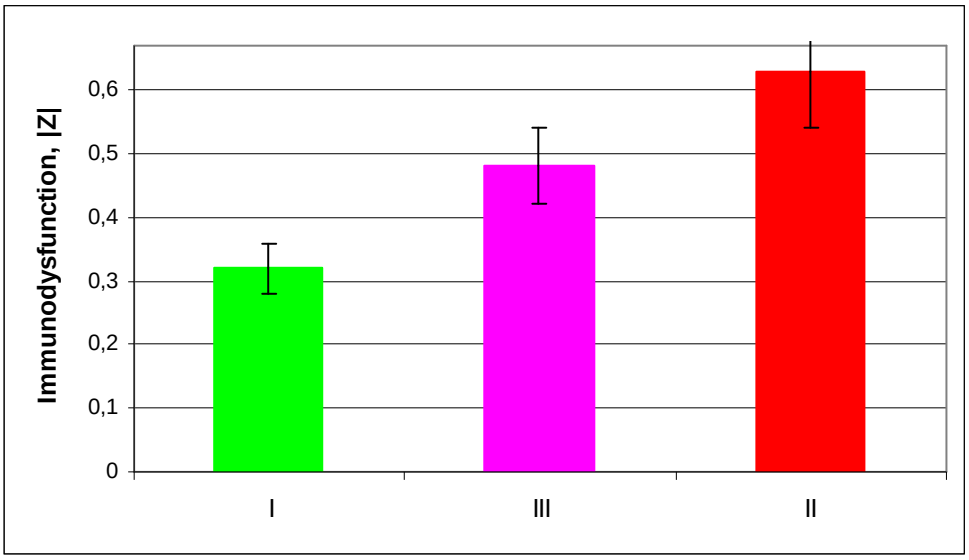


Fig. 4.11. Modules of post-stress deviations from the norm of immunity parameters in female rats I, III and II clusters

Following the adopted algorithm, discriminant analysis was applied to elucidate the characteristics of post-stress immune clusters of female rats. Only 13 parameters were selected for inclusion in the model (Table 4.14).

The data on immune parameters not included in the discriminant model are given in Table. 4.15-4.19.

Table 4.14. Summary of discriminant analysis of post-stress immune indices of female rats

Step 13, N of vars in model: 13; Grouping: 3 grps
Wilks' Lambda: 0,050; approx. $F_{(27)}$=9,3; $p<10^{-6}$

Variables currently in the model	Intact Female Rats (n=10)	Chronic Stressed Female Rats			Parameters of Wilks' Statistics				
		Cluster I (n=24)	Cluster III (n=14)	Cluster II (n=12)	Wilks Λ	Par-tial Λ	F-re-move (2,3)	p-le-vel	To-lera-ncy
Spleen Mass, mg	816±83 1 0	739±9 0,91±0,01 -0,29±0,03	930±25 1,14±0,03 +0,43±0,10	586±14 0,72±0,02 -0,88±0,05	,178	,283	44,4	10^{-6}	,218
Spleen Mass Index, ‰	3,12±0,32 1 0	2,77±0,05 0,89±0,02 -0,35±0,05	3,59±0,14 1,15±0,05 +0,47±0,14	2,35±0,09 0,75±0,03 -0,77±0,09	,071	,710	7,16	,002	,286
Endotheliocy-tes of Thymus, %	2,60±0,31 1 0	2,67±0,14 1,03±0,06 +0,07±0,15	3,08±0,28 1,18±0,11 +0,49±0,29	2,17±0,32 0,83±0,12 -0,45±0,33	,062	,806	4,21	,023	,391
Leukocytes of Blood, 10^9/L	13,1±1,76 1 0	10,32±0,77 0,79±0,06 -0,50±0,14	13,91±1,36 1,06±0,10 +0,15±0,25	10,36±0,98 0,79±0,07 -0,49±0,18	,067	,748	5,89	,006	,569
Basophiles of Blood, %	0,20±0,13 1 0	0,43±0,12 2,17±0,60 +0,56±0,29	0,43±0,14 2,14±0,69 +0,54±0,33	0,08±0,08 0,42±0,42 -0,28±0,20	,069	,728	6,54	,004	,465
Lymphoblaste s of Spleen, %	3,90±0,38 1 0	4,25±0,26 1,09±0,07 +0,29±0,21	3,14±0,21 0,81±0,05 -0,63±0,17	4,83±0,42 1,24±0,11 +0,78±0,35	,059	,848	3,14	,056	,624
Lymphoblaste s of Thymus, %	7,40±0,27 1 0	7,21±0,22 0,97±0,03 -0,23±0,26	6,77±0,22 0,91±0,03 -0,75±0,26	7,25±0,33 0,98±0,04 -0,18±0,39	,053	,939	1,13	,334	,765
Neutrophiles of Spleen, %	13,0±0,4 1 0	12,4±0,4 0,96±0,03 -0,41±0,31	13,3±0,4 1,02±0,03 +0,20±0,31	13,8±0,7 1,06±0,06 +0,59±0,51	,053	,951	,91	,413	,677
NK-Lympho-cytes of Blood, %	15,0±0,3 1 0	15,4±0,5 1,03±0,03 +0,41±0,44	16,2±0,6 1,08±0,04 +1,06±0,55	16,8±0,6 1,12±0,04 +1,62±0,51	,056	,903	1,87	,169	,673
Microb Capac. Monoc Blood,	110±52	57±9	83±12	66±16	,056	,901	1,91	,163	,626

10^6 Bact/L	1 0	0,52±0,08 -0,32±0,06	0,76±0,11 -0,16±0,07	0,60±0,15 -0,27±0,10					
Entropy of Thymocyto-gram ($•10^3$)	439±9 1 0	451±7 1,03±0,02 +0,43±0,24	459±7 1,05±0,01 +0,70±0,23	458±6 1,04±0,01 +0,67±0,22	,055	,913	1,67	,203	,041
Eosinophiles of Blood, %	4,30±0,68 1 0	4,00±0,46 0,93±0,11 -0,14±0,21	3,21±0,41 0,75±0,10 -0,50±0,19	3,17±0,44 0,74±0,10 -0,52±0,20	,055	,909	1,76	,187	,666
Lymphocytes of Thymus, %	70,3±0,8 1 0	69,4±0,6 0,99±0,01 -0,38±0,27	68,6±0,6 0,98±0,01 -0,72±0,24	68,7±0,5 0,98±0,01 -0,65±0,23	,051	,975	,46	,638	,043

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

Table 4.15. Thymocytogram indices not included in the discriminant model

Variables	Intact Female Rats (n=10)	Chronic Stressed Female Rats			Parameters of Wilks' Statistics				
		Cluster I (n=24)	Cluster III (n=14)	Cluster II (n=12)	Wilks Λ	Partial Λ	F to enter	p-level	Tolerancy
Thymus Mass, mg	73±7 1 0	74±4 1,02±0,05 +0,06±0,16	76±4 1,04±0,06 +0,14±0,18	74±5 1,02±0,07 +0,05±0,23	,049	,971	,514	,603	,663
Thymus Mass Index, ‰	0,28±0,04 1 0	0,28±0,02 0,98±0,06 -0,04±0,14	0,29±0,02 1,03±0,06 +0,08±0,16	0,30±0,02 1,04±0,07 +0,09±0,18	,049	,971	,501	,610	,577
Reticulocytes of Thymus, %	4,70±0,54 1 0	4,58±0,27 0,98±0,06 -0,07±0,16	4,62±0,17 0,98±0,04 -0,05±0,10	5,33±0,26 1,13±0,05 **+0,37±0,15**	,050	,993	,119	,888	,577
Epitheliocytes of Thymus, %	8,8±0,6 1 0	9,1±0,5 1,03±0,05 +0,14±0,24	10,2±0,5 1,16±0,06 **+0,72±0,26**	9,1±0,5 1,03±0,06 +0,14±0,26	,050	,996	,068	,934	,122
Plasmocytes of Thymus, %	1,80±0,25 1 0	1,83±0,18 1,02±0,10 +0,04±0,22	1,92±0,20 1,07±0,11 +0,16±0,26	2,42±0,23 1,34±0,13 **+0,78±0,29**	,049	,969	,547	,584	,500
Macrophages of Thymus, %	2,70±0,42 1 0	3,21±0,22 1,19±0,08 **+0,38±0,16**	2,69±0,30 1,00±0,11 -0,01±0,22	3,25±0,33 1,20±0,12 +0,41±0,25	,050	,990	,167	,847	,417
Hassal corpuscles of Thymus, %	1,70±0,17 1 0	2,02±0,09 1,19±0,05 **+0,60±0,16**	2,12±0,08 1,24±0,05 **+0,77±0,15**	1,75±0,13 1,03±0,08 +0,09±0,24	,050	,992	,138	,871	,533

Table 4.16. Splenocytogram indices not included in the discriminant model

Variables	Intact Female Rats (n=10)	Chronic Stressed Female Rats			Parameters of Wilks' Statistics				
		Cluster I (n=24)	Cluster III (n=14)	Cluster II (n=12)	Wilks Λ	Partial Λ	F to enter	p-level	Tolerancy
Lymphocytes of Spleen, %	48,7±0,91 0	48,1±0,5 0,99±0,01 -0,21±0,17	47,1±0,7 0,97±0,01 **-0,60±0,25**	49,3±0,6 1,01±0,01 +0,20±0,23	,049	,980	,343	,712	,667
Plasmocytes of Spleen, %	2,50±0,501 0	1,88±0,27 0,75±0,11 **-0,40±0,17**	1,57±0,17 0,63±0,07 **-0,59±0,11**	2,17±0,41 0,87±0,16 -0,21±0,26	,049	,969	,553	,581	,558
Reticulocytes of Spleen, %	14,3±0,61 0	15,3±0,4 1,07±0,03 **+0,50±0,21**	15,6±0,4 1,09±0,03 **+0,71±0,21**	14,2±0,4 0,99±0,03 -0,07±0,19	,050	,992	,132	,877	,755
Fibroblastes of Spleen, %	8,2±0,71 0	8,1±0,3 0,99±0,03 -0,04±0,12	8,5±0,5 1,04±0,06 +0,14±0,23	6,9±0,5 0,84±0,06 **-0,61±0,25**	,049	,984	,273	,762	,665
Macrophages of Spleen, %	7,9±0,51 0	8,5±0,4 1,08±0,05 +0,38±0,26	9,3±0,3 1,18±0,04 **+0,87±0,22**	7,3±0,4 0,93±0,05 -0,36±0,26	,049	,980	,347	,709	,641
Eosinophils of Spleen, %	1,50±0,341 0	1,46±0,17 0,97±0,11 -0,04±0,16	1,50±0,17 1,00±0,12 0,00±0,16	1,50±0,26 1,00±0,17 0,00±0,24	,049	,980	,356	,703	,785
Entropy of Splenocytogram ($\bullet 10^3$)	613±81 0	614±3 1,00±0,01 +0,04±0,12	616±6 1,01±0,01 +0,12±0,22	608±6 0,99±0,01 -0,19±0,23	,049	,981	,333	,719	,612

Table 4.17. Immunocytogram indices not included in the discriminant model

Variables	Intact Female Rats (n=10)	Chronic Stressed Female Rats			Parameters of Wilks' Statistics				
		Cluster I (n=24)	Cluster III (n=14)	Cluster II (n=12)	Wilks Λ	Par-tial Λ	F to en-ter	p-le-vel	To-lera-ncy
Th-Lymphocytes of Blood, %	31,8±0,81 0	31,5±0,9 0,99±0,03 -0,11±0,35	31,4±0,9 0,99±0,03 -0,15±0,39	28,5±0,9 0,90±0,03 **-1,35±0,35**	,049	,981	,323	,726	,720
Tc-Lymphocytes of Blood, %	15,1±0,91 0	16,4±0,6 1,08±0,04 **+0,43±0,20**	15,2±0,8 1,01±0,05 +0,04±0,28	16,6±1,1 1,10±0,08 +0,51±0,40	,049	,978	,388	,681	,648
B-Lymphocytes of Blood, %	15,4±0,81 0	16,3±0,7 1,06±0,05 +0,37±0,29	15,2±0,8 0,99±0,05 -0,08±0,31	16,2±1,0 1,05±0,07 +0,32±0,43	,048	,964	,637	,535	,589
0-Lymphocytes of Blood, %	22,7±1,81 0	20,4±1,6 0,90±0,07 -0,40±0,28	22,0±1,8 0,97±0,08 -0,12±0,30	22,0±2,3 0,97±0,10 -0,13±0,40	,048	,965	,616	,546	,529
Entropy of Immunocytogram ($\bullet 10^3$)	469±2 1 0	468±2 1,00±0,01 -0,20±0,36	470±2 1,00±0,00 +0,02±0,28	473±3 1,01±0,01 +0,48±0,43	,047	,947	,959	,394	,719
Blasttransformati-on of T-Lymphocytes, %	78,8±2,31 0	75,4±1,9 0,96±0,02 -0,47±0,27	78,5±1,9 1,00±0,02 -0,05±0,26	79,0±1,5 1,00±0,02 +0,03±0,21	,048	,945	,957	,390	,711

Table 4.18. Leukocytogram indices not included in the discriminant model

Variables	Intact Female Rats (n=10)	Chronic Stressed Female Rats			Parameters of Wilks' Statistics				
		Cluster I (n=24)	Cluster III (n=14)	Cluster II (n=12)	Wilks Λ	Par-tial Λ	F to en-ter	p-le-vel	To-lera-ncy
Limphocytes of Blood, %	59,4±2,5 1 0	62,4±1,4 1,05±0,02 **+0,38±0,18**	60,6±2,0 1,02±0,03 +0,15±0,26	58,7±2,6 0,99±0,04 -0,09±0,33	,049	,969	,545	,585	,494
Stub Neutrophiles of Blood, %	3,50±0,40 1 0	3,04±0,19 0,87±0,05 **-0,36±0,15**	3,00±0,36 0,86±0,10 -0,39±0,29	3,58±0,34 1,02±0,10 +0,07±0,26	,050	,999	,026	,975	,576
Segmented Neutrophiles of Blood, %	26,9±2,2 1 0	25,6±1,0 0,95±0,04 -0,19±0,15	27,9±1,6 1,04±0,06 +0,15±0,23	30,3±2,2 1,13±0,08 +0,49±0,32	,048	,960	,703	,502	,666
Monocytes of Blood, %	5,70±0,92 1 0	4,57±0,50 0,80±0,09 **-0,39±0,17**	4,71±0,49 0,83±0,09 -0,34±0,17	4,17±0,67 0,73±0,12 **-0,53±0,23**	,050	,992	,138	,872	,560
Entropy of Leukocytogram ($\bullet 10^3$)	318±10 1 0	305±7 0,96±0,02 **-0,43±0,21**	311±9 0,98±0,03 -0,24±0,28	311±10 0,98±0,03 -0,24±0,31	,050	1,00	,002	,998	,412

Table 4.19. Indicators of phagocytosis not included in the discriminant model

Variables	Intact Female Rats (n=10)	Chronic Stressed Female Rats			Parameters of Wilks' Statistics				
		Cluster I (n=24)	Cluster III (n=14)	Cluster II (n=12)	Wilks Λ	Partial Λ	F to enter	p-level	Tolerancy
Killing Index of Neutrophils of Blood, %	50,4±1,6 1 0	53,0±1,4 1,05±0,03 +0,51±0,27	51,6±1,8 1,02±0,04 +0,24±0,35	56,9±1,5 1,13±0,03 **+1,27±0,29**	,050	,989	,192	,826	,675
Phagocytose Index of Neutrophils, %	70,1±1,0 1 0	70,0±0,6 1,00±0,01 -0,04±0,20	68,9±1,3 0,98±0,02 -0,39±0,40	68,8±1,2 0,98±0,02 -0,39±0,36	,050	,990	,179	,837	,662
Microbial Count of Neutrophils of Blood, Bact/Phag	8,5±0,5 1 0	8,0±0,2 0,94±0,03 **-0,32±0,14**	7,4±0,4 0,87±0,05 **-0,67±0,23**	7,4±0,3 0,87±0,03 **-0,63±0,17**	,049	,983	,298	,744	,772
Bactericid Capacity Neutrophils, 10^9 Bact/L	12,5±2,4 1 0	8,8±0,8 0,71±0,07 **-0,48±0,11**	11,7±1,7 0,94±0,14 -0,10±0,22	10,5±1,4 0,84±0,11 -0,26±0,19	,049	,977	,392	,679	,264
Phagocytose Index of Monocytes, %	2,7±0,2 1 0	2,7±0,2 1,00±0,06 -0,01±0,22	2,9±0,2 1,08±0,08 +0,32±0,32	3,3±0,3 1,20±0,12 +0,77±0,43	,050	,987	,221	,803	,513
Microbial Count of Monocytes of Blood, Bact/Phag	4,35±0,5 1 0	4,6±0,4 1,06±0,09 +0,18±0,26	4,9±0,6 1,13±0,14 +0,38±0,42	4,75±0,5 1,09±0,13 +0,28±0,38	,050	,996	,063	,939	,439

In the next stage of discriminant analysis, the 13-dimensional space of discriminant variables was transformed into a two-dimensional space of canonical discriminant roots (Table 4.20). It is revealed that the lion's share of discriminatory opportunities is found in the first root – 96,2%, while the second root accounts for only 3,8%. Considering the structural coefficients, the first root characterizes in reverse absolute and relative spleen masses (ie, cardinal cluster-forming immune parameters), as well as the relative content of endothelial cells in the thymus, basophils in the blood and its leukocytosis, and in the direct way reflects the content of lymphoblasts in the spleen and thymus.

The second root directly reflects the content of microphages in the spleen, natural killer cells in the blood and bactericidal ability of blood macrophages, as well as the entropy of the thymocytogram, while inversely reflects the content of eosinophils in the blood and lymphocytes in the thymus.

Table 4.20. Correlations of discriminant variables with canonical roots and post-stress deviations from the norm of discriminant variables in different clusters

Variables currently in the model	Root 1	Root 2	**Z-scores for Variables**		
			Clu **III**	Clu **I**	Clu **II**
Spleen Mass, mg	**-,570**	,398	+0,43	-0,29	-0,88
Spleen Mass Index, ‰	**-,361**	,534	+0,47	-0,35	-0,77
Endotheliocytes of Thymus, %	**-,103**	-,017	+0,49	+0,07	-0,45
Leukocytes of Blood, 10^9/L	**-,091**	,349	+0,15	-0,50	-0,49
Basophiles of Blood, %	**-,072**	-,233	+0,54	+0,56	-0,28
Lymphoblastes of Spleen, %	**,148**	-,208	-0,63	+0,29	+0,78
Lymphoblastes of Thymus, %	**,047**	-,151	-0,75	-0,23	-0,18
Neutrophiles of Spleen, %	,028	**,391**	+0,20	**-0,41**	+0,59
NK-Lymphocytes of Blood, %	,030	**,323**	+1,06	**+0,41**	+1,62
Bactericid Capacity Mon Bl, 10^6 Bac/L	-,038	**,273**	-0,16	**-0,32**	-0,27
Entropy of Thymocytogram (•10^3)	-,002	**,186**	+0,70	**+0,43**	+0,67
Eosinophiles of Blood, %	-,003	**-,307**	-0,50	**-0,14**	-0,52
Lymphocytes of Thymus, %	,005	**-,205**	-0,72	**-0,38**	-0,65
Tests with Successive Roots Removed			**Means of Roots**		
Canonical r*	0,962	0,572	Root 1		
Wilks' Lambda	,050	,673	-4,36	-0,03	5,15
Chi-Sqr.	123	16	Root 2		
Degree freedom	26	12	+0,66	-0,70	+0,64
p-level	10^{-6}	,180			

Table 4.21. Summary step-by-step analysis and standardized and raw coefficients and constants for immune discriminant variables

Coefficients	Standardized		Raw		Parameters of Wilks' Statistics				
Variables currently in the model	Root 1	Root 2	Root 1	Root 2	F to en-ter	p-le-vel	Λ	F-va-lue	p-le-vel
Spleen Mass, mg	-1,879	-,300	-,030	-,005	96,6	10^{-6}	,196	96,6	10^{-6}
Spleen Mass Index, ‰	,967	,680	2,692	1,893	4,1	,023	,139	25,3	10^{-6}
Endotheliocytes of Thymus, %	-,700	-,359	-,777	-,398	3,1	,054	,080	14,9	10^{-6}
Leukocytes of Blood, 10^9/L	-,691	-,017	-,170	-,004	5,2	,009	,112	21,8	10^{-6}
Basophiles of Blood, %	-,750	-,446	-1,483	-,883	4,4	,017	,164	33,8	10^{-6}
Lymphoblastes of Spleen, %	,512	-,058	,426	-,048	2,2	,122	,102	18,4	10^{-6}
Lymphoblastes of Thymus, %	,289	-,085	,284	-,083	1,1	,334	,050	9,3	10^{-6}
Neutrophiles of Spleen, %	,140	,408	,066	,193	2,2	,128	,072	13,6	10^{-6}
NK-Lymphocytes of Blood, %	,241	,524	,107	,233	1,4	,266	,058	10,6	10^{-6}
Bactericid Capac Mon Bl, 10^6 Bac/L	,370	,308	,0078	,0065	1,4	,248	,053	10,0	10^{-6}
Entropy of Thymocytogram ($\bullet 10^3$)	1,483	,557	,052	,019	1,4	,270	,067	12,4	10^{-6}
Eosinophiles of Blood, %	-,296	-,414	-,156	-,217	1,7	,205	,062	11,5	10^{-6}
Lymphocytes of Thymus, %	,797	,077	,306	,029	2,2	,121	,092	16,1	10^{-6}
	Constants		-31,94	-16,51					
Discriminant Properties, %			**96,2**	**3,8**					

Further on the raw coefficients and constants given in Table. 4.21, and the individual values of the discriminant immune variables given in Table. 4.14, a point in the information space of two roots for each animal was determined (Fig. 4.12).

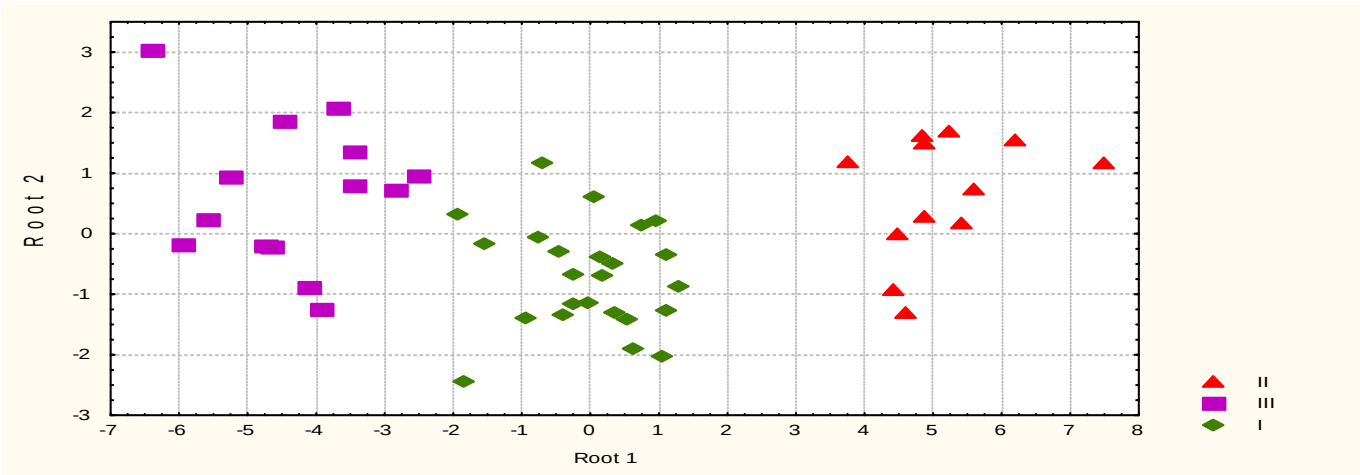


Fig. 4.12. Individual values of discriminant immune roots of female rats of different clusters of immunity response to stress

Figure 4.12 shows that in the set of selected variables, the post-stress immune statuses of female rats in the three clusters differ significantly. The visual impression is confirmed by calculating the Mahalanobis distance squares, which form between clusters II and III 96 ($F=33$; $p<10^{-6}$); between II and I clusters 30 ($F=13,0$; $p<10^{-6}$); between III and I clusters 22 ($F=10,4$; $p<10^{-6}$).

Extreme localization of members of the cluster II along the axis of the first root reflects their minimum values among the stressed cohort of the values negatively correlating with the root of the immune indices and the maximum magnitudes of the immune indices associated with the root positively (see Table 4.20). Similar indicators of members I and III clusters are much larger/smaller than those of II clusters and are less different from each other, which is illustrated by the location of points along the axis of the first root.

Instead, along the axis of the second root, the clusters are interspersed, illustrating its meager discernment. However, the centroid of the cluster I is localized in the negative zone of the axis (-0,70), whereas III and II - in the positive, with almost the same magnitude (+0,66 and +0,64, respectively). This illustrates the post-stress reduction in spleen content of microphages in rats of cluster I, whereas in clusters III and II, their content increases relative to control. However, the immune status of rats in the cluster I is characterized by maximal for the cohort inhibition of bactericidal ability of blood macrophages in combination with a minimal increase in the level of natural killer cells and entropy of thymocytogram as well as a decrease in the level of eosinophils in the blood and lymphocytes in the thymus.

Therefore, the differences between post-stress immune statuses of female rats are exhaustively explained by 13 parameters that reflect different levels of immunity. The parameters selected can be used to identify a particular cluster-type immune response to chronic stress. This is implemented using the classification functions (Table 4.21). In this case, the unmistakable correctness of the classification of objects is achieved.

Table 4.21 Coefficients and constants of the classification functions of the immune indices of female rats

Variables currently in the model	II	III	I
Spleen Mass, mg	-0,68	-0,40	-0,52
Basophiles of Blood, %	21,67	35,74	30,53
Spleen Mass Index, ‰	28,22	2,674	11,74
Leukocytes of Blood, 10^9/L	-9,22	-7,61	-8,33
Lymphoblastes of Spleen, %	-44,70	-48,75	-46,84
Lymphocytes of Thymus, %	367,3	364,4	365,7
Endotheliocytes of Thymus, %	-181,2	-173,8	-176,6
Neutrophiles of Spleen, %	32,27	31,64	31,67
Entropy of Thymocytogram (•10^3)	34,16	33,66	33,86
Eosinophiles of Blood, %	-19,41	-17,94	-18,31
NK-Lymphocytes of Blood, %	3,75	2,74	2,89
Bacterocidal Capacity Monocytes of Blood, 10^6 Bact/L	,218	,144	,168
Lymphoblastes of Thymus, %	-12,00	-14,70	-13,36
Constants	**-20121**	**-19814**	**-19920**

4.5. Features of neuro-endocrine regulation in female rats of different immune clusters (according to discriminant analysis)

Seven neuro-endocrine parameters were identified by regular use of discriminant analysis, the constellation of which the three immune clusters differ from each other. Other reported parameters were not included in the discriminant model (Table 4.22 and 4.23).

Table 4.22. Summary of discriminant analysis of post-stress neuroendocrine indicators of female rats

Step 7, N of vars in model: 7; Grouping: 3 grps; Wilks' Λ: 0,42; approx. $F_{(14)}=3,2$; $p<10^{-3}$

Variables currently in the model	Intact Female Rats (n=10)	Chronic Stressed Female Rats			Parameters of Wilks' Statistics				
		Cluster I (n=24)	Cluster III (n=14)	Cluster II (n=12)	Wilks Λ	Par-tial Λ	F-re-move (2,3)	p-le-vel	To-lera-ncy
Parathytin Activity $(Cap/Pp)^{0,5}$	2,38±0,25 1 0	1,78±0,12 0,75±0,05 **-0,76±0,15**	1,48±0,12 0,62±0,05 **-1,14±0,15**	2,14±0,19 0,90±0,08 -0,30±0,24	,534	,787	5,6	,007	,793
Calcitonin Activity $(Cap•Pp)^{-0,5}$	0,86±0,15 1 0	0,76±0,05 0,89±0,05 **-0,21±0,10**	0,66±0,05 0,77±0,06 **-0,43±0,11**	0,82±0,09 0,96±0,11 -0,07±0,20	,484	,869	3,1	,056	,749
Fasciculary ZAC, µM	370±20 1 0	427±19 1,15±0,05 **+0,89±0,29**	451±17 1,22±0,05 **+1,26±0,26**	361±17 0,98±0,05 -0,14±0,26	,607	,693	9,1	,001	,617
Adrenals Mass, mg	69±3 1 0	74±3 1,07±0,04 +0,51±0,27	70±3 1,01±0,05 +0,08±0,33	64±3 0,93±0,04 -0,48±0,31	,528	,796	5,3	,009	,190
Adrenals Mass Index, ‰	0,26±0,01 1 0	0,28±0,01 1,05±0,04 +0,33±0,25	0,27±0,01 1,01±0,04 +0,08±0,30	0,25±0,01 0,97±0,04 -0,20±0,29	,498	,844	3,8	,031	,188
AMo as Sympathetic tone, %	55,8±5,5 1 0	60,8±5,2 1,09±0,09 +0,29±0,30	63,5±5,9 1,14±0,11 +0,48±0,34	64,9±6,7 1,16±0,12 +0,53±0,39	,465	,904	2,2	,128	,225
Moda HRV, msec	124±5 1 0	117±4 0,94±0,04 -0,48±0,29	109±5 0,88±0,04 **-1,02±0,37**	114±6 0,92±0,05 -0,63±0,39	,457	,919	1,8	,179	,247
Variables currently not in the model	Intact Female Rats (n=10)	Chronic Stressed Female Rats			Parameters of Wilks' Statistics				
		Cluster I (n=24)	Cluster III (n=14)	Cluster II (n=12)	Wilk s Λ	Par-tial Λ	F to en-ter	p-le-vel	To-lera-ncy
Corticosterone nM/L	466±57 1 0	437±39 0,94±0,08 -0,16±0,22	424±60 0,91±0,13 -0,23±0,34	438±44 0,94±0,09 -0,16±0,24	,412	,98	,38	,68	,875
Testosterone, nM/L	3,93±0,34 1	4,34±0,40 1,11±0,10	5,65±0,69 1,44±0,18	4,91±0,67 1,25±0,17	,405	,96	,78	,47	,883

	0	+0,39±0,37	**+1,61±0,65**	+0,92±0,62					
MxDMn as Vagal tone, msec	53±13 1 0	47±10 0,89±0,18 -0,14±0,24	38±11 0,73±0,20 -0,34±0,26	46±15 0,87±0,28 -0,17±0,36	,412	,98	,41	,67	,213
Triiodo-thyronine, nM/L	2,12±0,18 1 0	2,28±0,08 1,08±0,04 +0,29±0,14	2,27±0,06 1,07±0,03 +0,27±0,11	2,27±0,12 1,07±0,06 +0,27±0,20	,417	,99	,16	,85	,849
Thyroxine, nM/L	63,5±5,3 1 0	59,5±2,9 0,94±0,04 -0,24±0,17	58.8±2,8 0,93±0,04 -0,29±0,17	56,7±4,3 0,89±0,07 -0,41±0,26	,415	,99	,28	,76	,942
Glomerulary ZAC, µM	189±8 1 0	184±8 0,97±0,04 -0,19±0,32	190±9 1,00±0,05 +0,02±0,35	194±12 1,03±0,07 +0,18±0,47	,405	,96	,77	,47	,721
Reticulary ZAC, µM	40,8±2,5 1 0	43,1±1,7 1,06±0,04 +0,29±0,22	43,5±3,1 1,07±0,08 +0,34±0,39	44,6±4,4 1,09±0,11 +0,48±0,55	,411	,98	,46	,64	,859
$(Nap/Kp)^{0,5}$ as Mineralocorti-coid activity	5,57±0,17 1 0	6,04±0,13 1,08±0,02 **+0,86±0,24**	6,34±0,22 1,14±0,04 **+1,40±0,39**	6,19±0,17 1,11±0,03 **+1,13±0,31**	,409	,97	,54	,59	,870

Table 4.23. Summary step-by-step analysis as well as standardized and raw coefficients and constants for neuro-endocrine discriminant variables

Coefficients	**Standardized**		**Raw**		**Parameters of Wilks' Statistics**				
Variables currently in the model	Root 1	Root 2	Root 1	Root 2	F to en-ter	p-le-vel	Λ	F-va-lue	p-le-vel
Fasciculary Zone Adren Cortex, µM	-,996	-,114	-,014	-,002	3,6	,034	,702	4,4	,003
Adrenals Mass, mg	-1,345	-1,041	-,109	-,084	2,2	,118	,565	3,6	,001
Adrenals Mass Index, ‰	1,260	,470	28,5	10,6	3,4	,043	,488	3,7	$<10^{-3}$
Parathytin Activity (PTA)	,693	-,421	1,344	-,816	5,4	,008	,813	5,4	,008
AMo as Sympathetic tone, %	,704	-1,054	,030	-,044	1,4	,254	,457	3,4	$<10^{-3}$
Calcitonin Activity (CTA)	-,567	-,301	-,554	-,294	2,9	,065	,622	4,0	,001
Moda HRV, msec	,341	-1,298	,016	-,063	1,8	,179	,420	3,2	$<10^{-3}$
	Constants		-0,106	17,2					
Discriminant Properties, %			**84**	**16**					

Identification information is condensed into two roots. The first root has 84% discriminatory potential and the second root the remaining 16%. Judging by the structural coefficients (Table 4.24), the first root reflects in the reverse way, first of all, the thickness of the fascicular zone of the adrenal cortex, as well as their mass, more absolute than relative to the body weight, and in the directly way characterizes the parathyroid and calcitonin activities.

Table 4.24. Correlations of discriminant variables with canonical roots and post-stress abnormalities of neuro-endocrine discriminant variables in different clusters

Variables currently in the model	Root 1	Root 2	**Z-scores for Variables**		
			Clu **III**	Clu **I**	Clu **II**
Fasciculary Zone Adrenal Cortex, µM	**-,452**	,170	+1,26	+0,89	**-0,14**
Adrenals Mass, mg	**-,275**	-,433	+0,08	+0,51	**-0,48**
Adrenals Mass Index, ‰	**-,156**	-,270	+0,08	+0,33	**-0,20**
Parathytin Activity (PTA)	**,436**	-,456	-1,14	-0,76	**-0,30**
Calcitonin Activity (CTA)	**,352**	-,296	-0,43	-0,21	**-0,07**
Moda HRV, msec	,037	**-,377**	-1,02	**-0,48**	-0,63
AMo as Sympathetic tone, %	,046	**,128**	+0,45	**+0,29**	+0,53
Tests with Successive Roots Removed			**Means of Roots**		
Canonical r*	0,707	0,400	Root 1		
Wilks' Lambda	0,420	0,840	-0,73	-0,43	1,71
Chi-Sqr.	38,1	7,7	Root 2		
Degree freedom	14	6	0,60	-0,40	0,10
p-level	$<10^{-3}$	,265			

The second root correlates negatively with the HRV mode, that is positively with the heart rate, and positively with the amplitude of the mode as a marker of sympathetic tone.

Based on the individual values of selected neuro-endocrine parameters (Table 4.22) and their raw coefficients and constants (Table 4.23), the localization of each rat in the information field of discriminant roots was determined (Figs. 4.13 and 4.14).

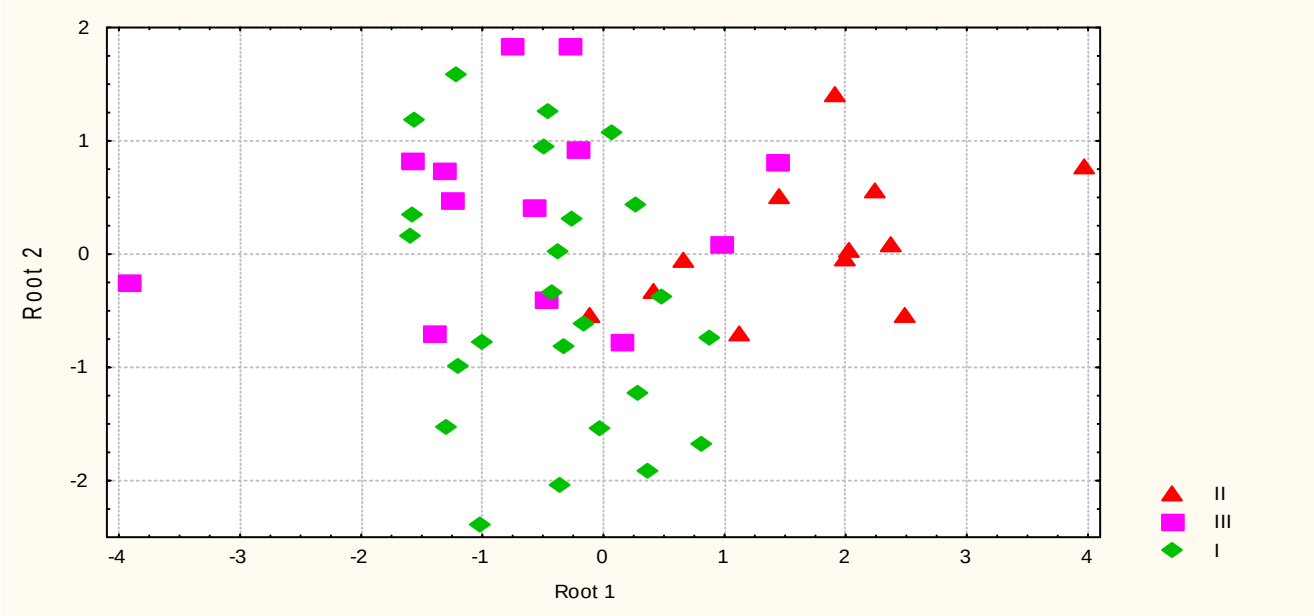


Fig. 4.13. Individual magnitudes of discriminant neuro-endocrine roots of female rats of different immunity response clusters

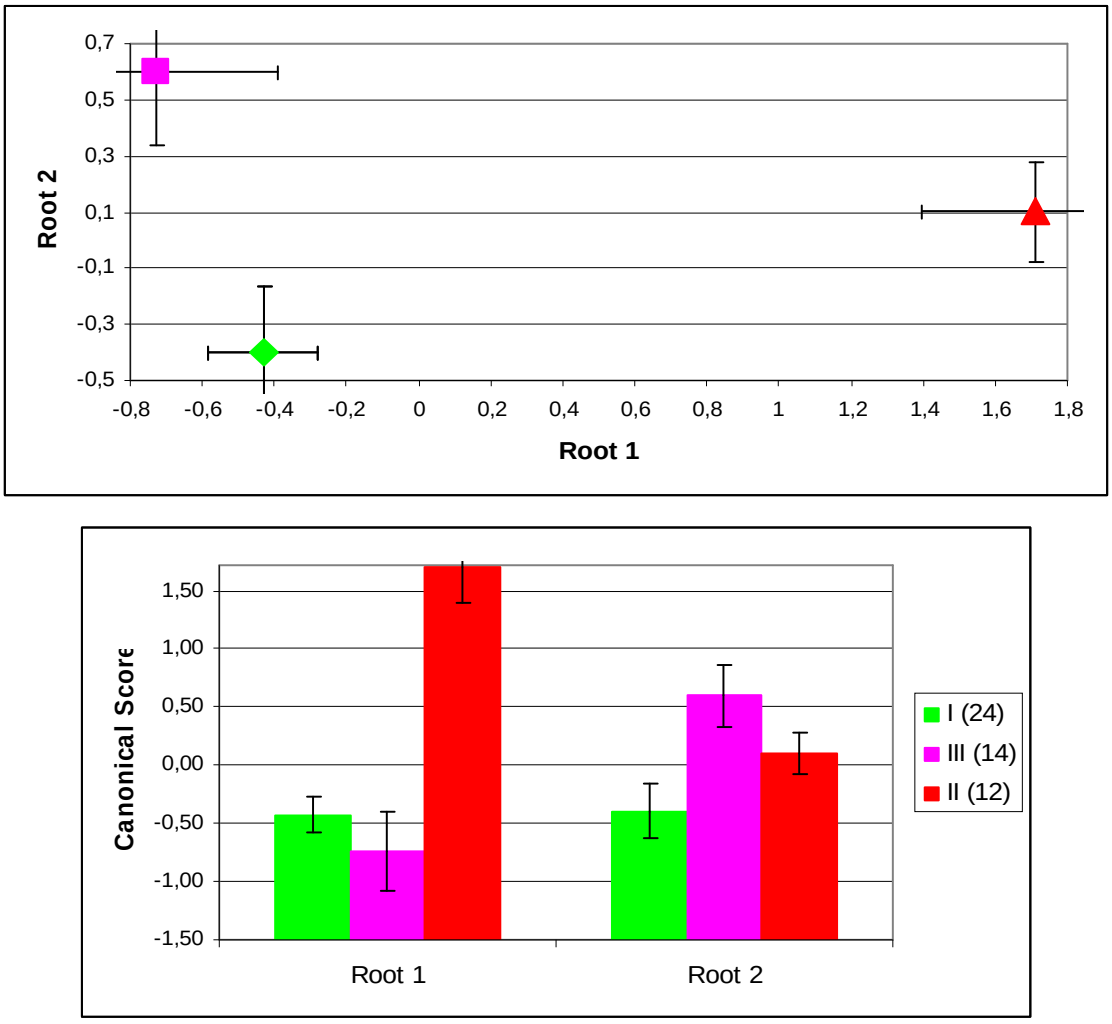


Fig. 4.14. Mean values of discriminant neuro-endocrine roots of female rats of different clusters of immune response to stress

Extreme localization of the members of the cluster II along the axis of the first root reflects their minimal (among the stressed cohort) values of negatively correlating with the root the thickness of the fascicular zone of the adrenal cortex and its mass, as well as the maximum (more precisely minimally reduced) parathyrin and calcitonin activities. In animals I and III clusters, the points of these endocrine indicators are interspersed, yet the centroid of the cluster I takes an intermediate position.

Along the axis of the second root, the lowest position is occupied by the centroid of the cluster I, although more than half of its members are mixed with members of other clusters, whereas the mixing of rats II and III clusters is complete. This reflects the minimally increased values of heart rate and sympathetic tone in cluster I, with approximately the same magnitudes of the listed parameters of rats II and III clusters.

The figures show that the set of selected variables post-stress neuro-endocrine status of the three clusters of rats differ to a different extent. The visual impression is confirmed by the calculation of the Mahalanobis distance squares that are between II and III clusters 6,61 ($F=4,91$; $p<10^{-3}$); between II and I clusters 5,12 ($F=4,75$; $p<10^{-3}$); instead, there are only 1,16 between III and I clusters ($F=1,20$; $p=0.32$).

The retrospective classification of rats by neuro-endocrine parameters (Table 4.25) is not as accurate as that of immune parameters, accounting for a total of 70% (Table 4.26).

Table 4.25. Ratios and constants of classification functions of neuro-endocrine indicators of female rats

Variables currently in the model	II	III	I
Parathytin Activity (PTA)	18,69	15,00	16,22
Fasciculary Zone Adrenal Cortex, μM	0,071	0,104	0,101
Calcitonin Activity (CTA)	-0,023	1,183	1,307
Adrenals Mass, mg	0,649	0,872	0,922
Adrenals Mass Index, ‰	85,9	21,6	19,75
AMo as Sympathetic tone, %	1,348	1,254	1,307
Moda HRV, msec	1,694	1,623	1,690
Constants	-218,5	-208,5	-224,9

Table 4.26. Classification matrix for female rats.
Rows are the observed classification, columns are the predicted classification

Кластер	Точність, %	II	III	I
II	75,0	**9**	0	3
III	57,1	2	**8**	4
I	75,0	0	6	**18**
Total	70,0	11	14	25

4.6. Relationships between neuro-endocrine and immune parameters of female rats

Two approaches were used to assess the nature and strength of the links between neuro-endocrine and immune parameters, as in the previous section.

In the first approach, neuro-endocrine and immune profiles of rats of three clusters were created (Fig. 4.15).

The figure illustrates that the absence of post-stress abnormalities (±0,5σ) of immune status in cluster I rats is associated with normal post-stress levels of the vast majority of neuro-endocrine parameters, however, with a modest decrease in parathyroid activity combined with an increase in mineralocorticoid activity and thickness of the fascicular area of the adrenal cortex as a marker of their glucocorticoid function. This gives the impression that such endocrine responses to chronic stress counteract immunodysfunction.

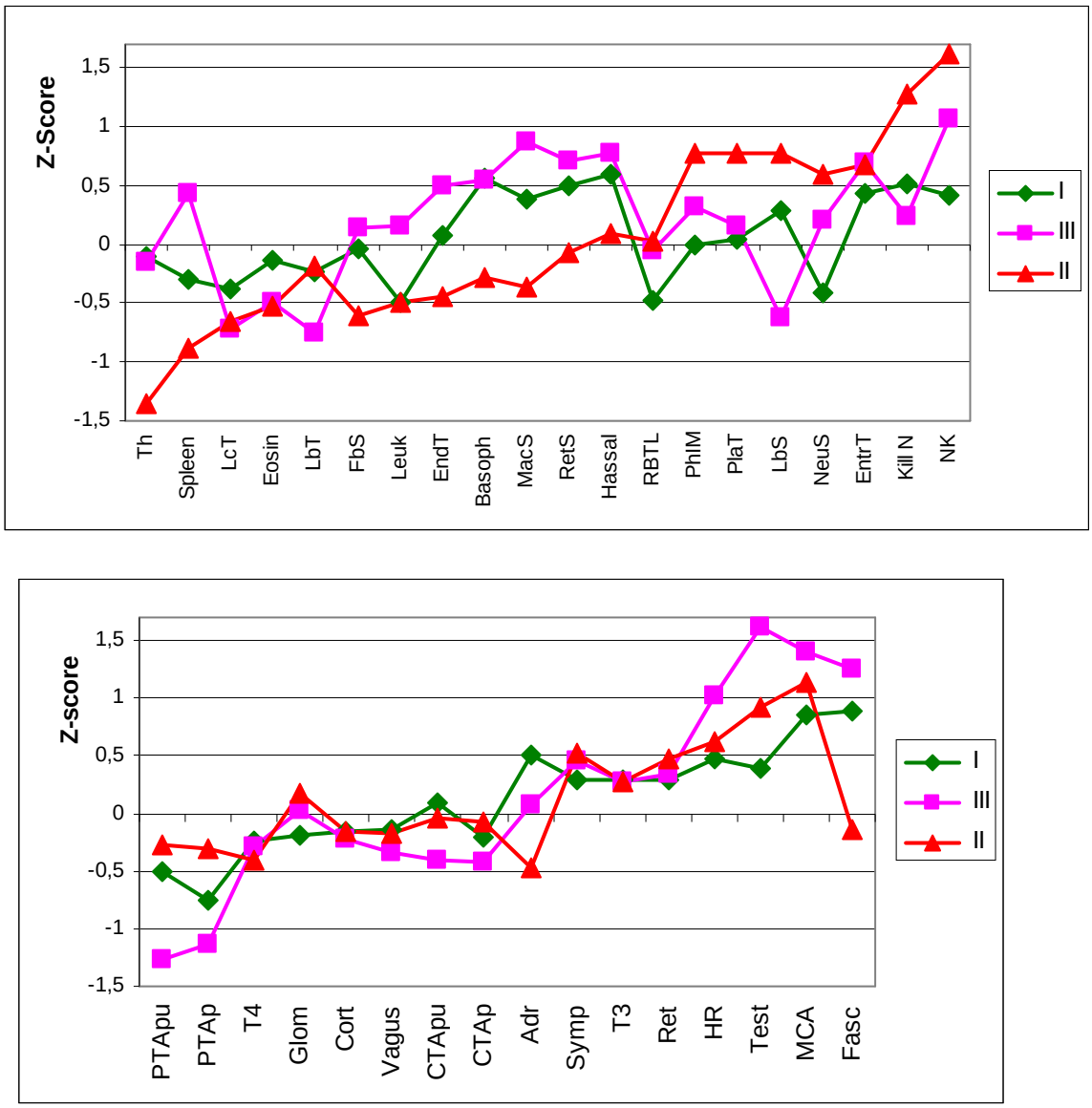


Fig. 4.15. Immune (top) and neuro-endocrine (bottom) profiles of female rats of different immune response clusters

Discovered in rats of the III cluster post-stress moderate suppression of lymphoid elements of the thymus and spleen in combination with moderate activation of phagocytic and cytotoxic blood immunocytes, which is manifested in a moderate decrease in the content of lymphocytes in the thymus and lymphoblasts in the thymus and spleen, in combination with moderate increase in the content in the spleen of macrophages and reticulocytes, in the blood of basophils and natural killer cells, and in the thymus of Hassal's lobules, as well as the entropy of the thymocytogram, is accompanied by another neuro-endocrine pattern. Namely, more pronounced decrease in parathyroid activity and increase of mineralocorticoid and glucocorticoid function are supplemented with hypertestosteronemia and tachycardia as a marker of sympathotonic shift of autonomic balance. So the first impression of the immunoprotective effect of the previous endocrine constellation is dispelled.

Stress-induced moderate suppression in rats of II cluster of lymphoid and myeloid elements of blood and thymus and spleen macrophages (in the form of reduction of spleen mass and content of fibroblasts, reduction of lymphocyte share in thymocytograms as well as Th-lymphocytes in immunocytogram and eosinophils in leukocytogram against the backdrop of diminishing total content of leukocytes) in combination with moderate activation of cytotoxic, phagocytic and lymphoid elements of the blood, thymus and spleen (in the form of an increase in the content of natural killer cells and killer activity of blood microphages and their content in the spleen, phagocytic activity of blood macrophages against the background of increasing the proportion of lymphoblasts in the splenocytogram and plasmocytes in the thymocytogram as well as increasing its entropy), ie more pronounced immunodysfunction, accompanied by normal levels of both parathyroid and glucocorticoid activity and less pronounced, compared with the cluster II, increases in heart rate, plasma testosterone and mineralocorticoid activity.

Therefore, neuroendocrine-immune relationships are non-linear.

With another analytical approach, a correlation matrix was first created (Table 4.27). For a sample with n=60, the critical value |r| is at $p<0,05$ ($t>2,00$): 0,25; at $p<0,01$ ($t>2,66$): 0,33; at $p<0,001$ ($t>3,46$): 0,42.

Table 4.27. Correlation coefficients between neuro-endocrine and immune indices of female rats

Variables	DX	AMo	Mo	Cort	Fasc	MCA	Glom	Test	Ret	Adr	T3	T4	PTA	CTA
Spleen Mass					,22					,23			-,31	
Spleen Mass Ind					,28								-,34	
Lymphoblastes S	,24	-,27	,30		-,46								,37	,31
Plasmocytes S		-,25			-,41	-,30	-,32					,25		
Reticulocytes S						-,22								
Fibroblastes S												,21	-,28	
Macrophages S	-,41	,68	-,64		,55			,32	,30				-,35	-,26
Eosinophils S												-,20		
Thymus Mass				-,23	,22		,30							
Thymus Mass Ind					,24		,24							
Lymphocytes T	,25	-,31	,27		-,32			-,20						
Reticulocytes T										-,25				
Epitheliocytes T		,27	-,29		,52							-,21		-,30
Endotheliocytes T					,27									
Plasmocytes T														,31
Macrophages T								,31		,27				
EntropyThymoCG	-,23	,29	-,25		,25			,23						
Lymphocytes B							-,28				,31			
Stub Neutrophil B								-,28			-,38			
Segment Neutr B											-,26			
Eosinophils Blood	,48		,51	-,32										
Basophils Blood	,24				,28	,22								
Monocytes Blood						,26								
Entropy LeukoCG							,27				-,20			
Killing Ind Neutrs				,21										
Phagoc Ind Neutr				-,23				-,37				-,21		,19
Microbas Count N	,33		,31					-,59						
Bacterocid Cap N							,42	-,40						

Phagoc Ind Mon		-,29							-,24					
Microb Count M								,32			-,24			
Th-Lymphocytes								-,30	-,25					,28
Tc-Lymphocytes				-,23										
B-Lymphocytes							,33		-,26		-,23		,31	,27
0-Lymphocytes								,37	,34				-,25	-,40
NK-Lymphocytes				,24										
Entropy ImmuCG	,31			-,22						-,25				

In the next step, regression models with stepwise exclusion were constructed for each neuro-endocrine factor, and in the final stage, a canonical correlation analysis of the links between neuro-endocrine and immune constellations was performed.

The closest relationship is found between sympathetic tone and macrophage content in the spleen (Fig. 4.16). In addition, sympathetic tone also significantly affects the content in the spleen of lymphoblasts and endothelial cells in the thymus, so that the determination of this constellation of immunocytes reaches 47% (Table 4.28 and Fig. 4.17).

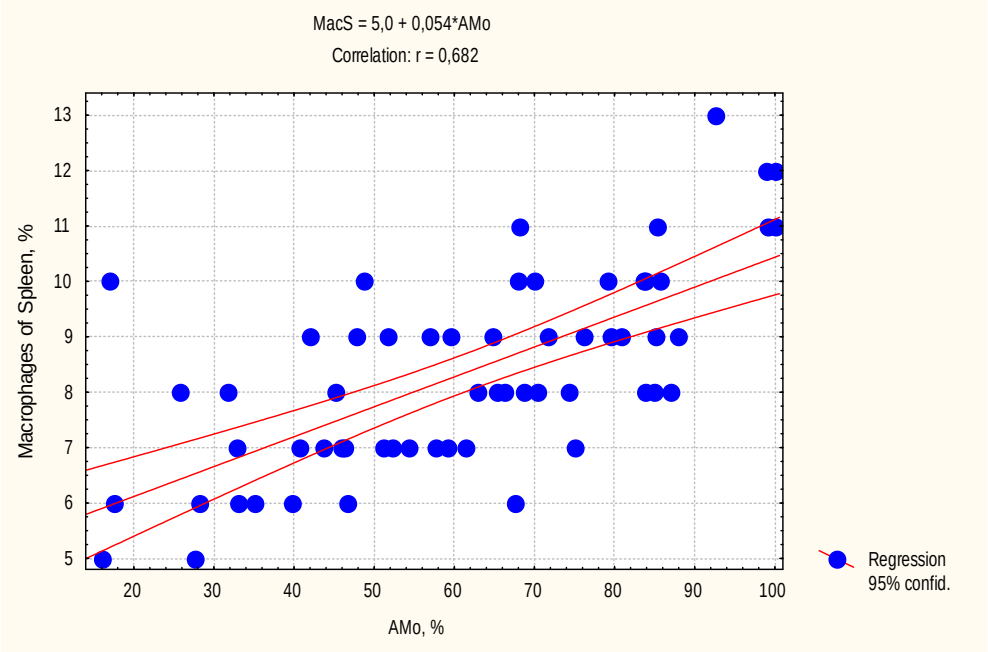


Fig. 4.16. Relationship between sympathetic tone (X-axis) and macrophage spleen content (Y-axis) of female rats

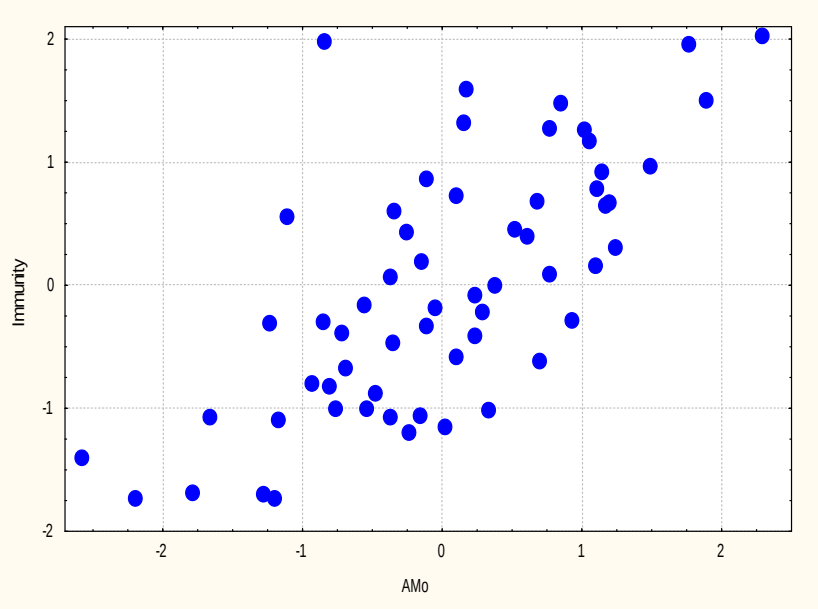


Fig. 4.17. Canonical correlation between sympathetic tone (X-axis) and immunity parameters of female rats (Y-axis)

Table 4.28. Regression model of multiple correlation of sympathetic tone (AMo) with indicators of immunity of female rats

$R=0,702$; $R^2=0,493$; Adjusted $R^2=0,466$; $F_{(3,6)}=18$; $\chi^2_{(3)}=38$; $p<10^{-7}$

		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(56)}$	p-level
	r		Intercpt	77,2	62,9	1,23	,225
Macrophages S	0,68	,696	,110	8,79	1,39	6,33	10^{-6}
Lymphocytes T	-0,31	-,161	,100	-1,40	,87	-1,61	,114
Lymphoblastes S	-0,27	,113	,111	1,94	1,91	1,02	,313

Vagal tone as a sympathetic antagonist is associated with the listed immunocytes in the opposite way and weaker, instead it is significantly correlated with the blood content of eosinophils and basophils, as well as with the entropy of the immunocytogram, determining this immune constellation by 53% (Table 4.29 and Fig. 4.18).

Table 4.29. Regression model of multiple correlation of parasympathetic tone (MxDMn) with indicators of immunity of female rats

$R=0,756$; $R^2=0,571$; Adjusted $R^2=0,531$; $F_{(5,5)}=14$; $\chi^2_{(5)}=47$; $p<10^{-6}$

		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(54)}$	p-level
	r		Intercpt	-438	235	-1,86	,068
Macrophages S	-0,41	-,481	,095	-11,94	2,36	-5,05	10^{-5}
Eosinophils Blood	0,48	,470	,096	10,66	2,17	4,92	10^{-5}
Lymphocytes T	0,25	,188	,094	3,22	1,60	2,01	,050
Basophils Blood	0,24	,213	,098	18,80	8,66	2,17	,034
Entropy ImmunoCG	0,31	,148	,093	,672	,424	1,58	,119

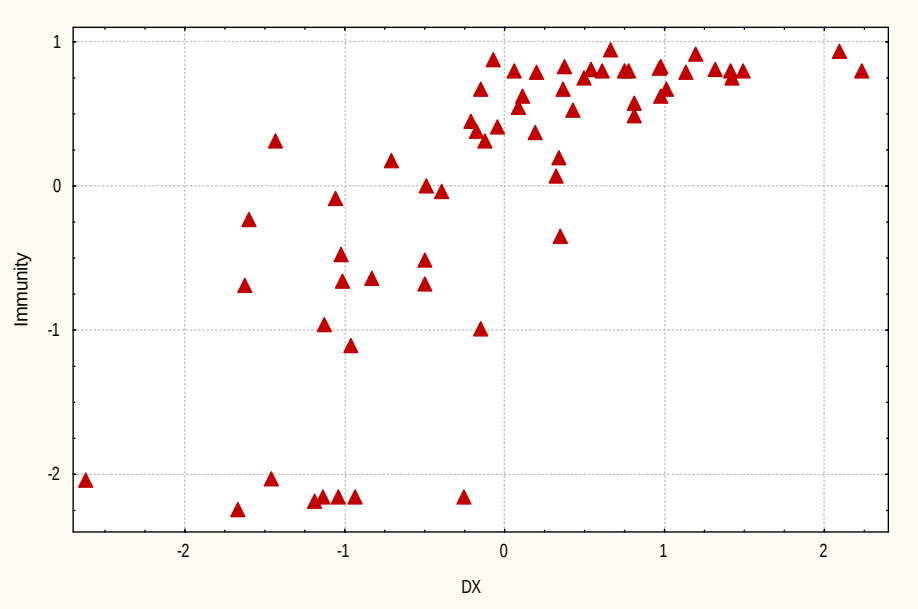


Fig. 4.18. Canonical correlation between parasympathetic tone (X axis) and immunity parameters (Y axis) of female rats

HRV mode, on the one hand, is closely correlated with vagus (r=0,84) and sympathetic (r=-0,84) tone, and on the other hand, is inversely associated with spleen macrophages and directly with blood eosinophils and thymus lymphocytes , determining them by 79% (Table 4.30 and Fig. 4.19).

Table 4.30. Regression model of multiple correlation of HRV mode with indicators of immunity of female rats

R=0,896; R^2=0,803; Adjusted R^2=0,792; $F_{(3,6)}$=76; $\chi^2_{(3)}$=92; $p<10^{-6}$

		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(56)}$	p-level
	r		Intercpt	106,1	34,8	3,05	,003
Macrophages S	-0,64	-,718	,062	-8,04	,696	-11,5	10^{-6}
Eosinophils B	0,51	,626	,060	6,41	,6162	10,4	10^{-6}
Lymphocytes T	0,27	,099	,061	,76	,475	1,61	,113

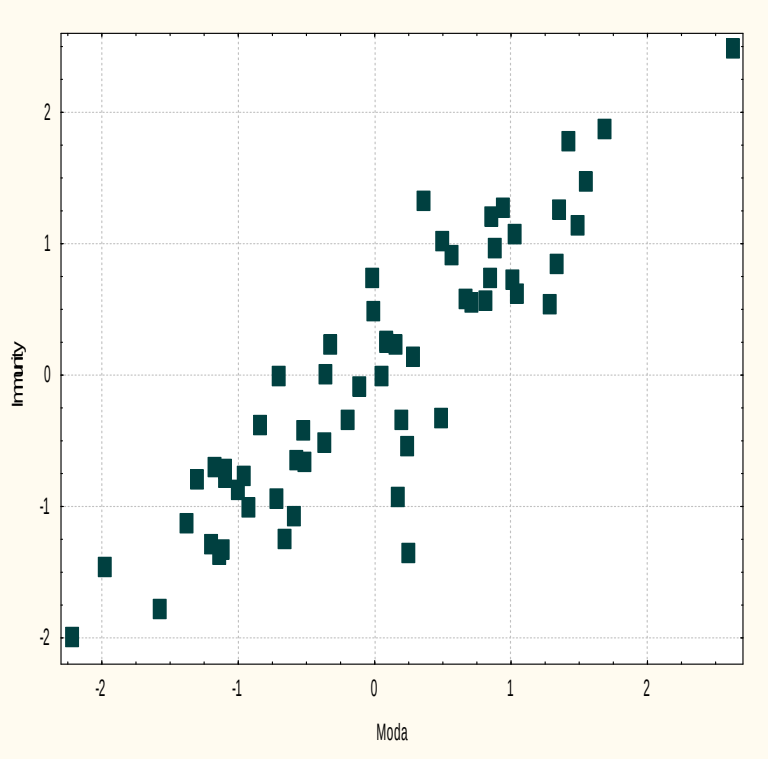
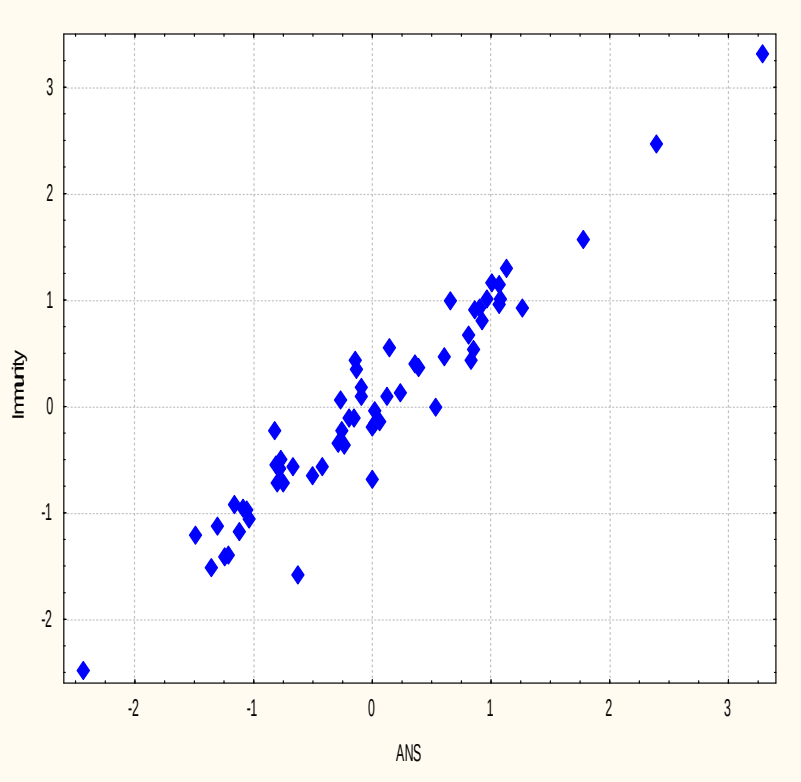


Fig. 4.19. Canonical correlation between HRV mode (X-axis) and immunity parameters (Y-axis) of female rats

The canonical correlation analysis shows that the three parameters of vegetative regulation determine the mentioned 6 immune parameters by 93% (Table 4.31, Fig. 4.20).

Table 4.31. Factorial structure of neuro-immune connections in female rats

Right set	R
Moda HRV	**,89**
MxDMn	**,74**
Amplitude of Moda	**-,48**
Left set	
Eosinophils of Blood	**,79**
Entropy Immunocytogram	**,28**
Lymphoblastes of Spleen	**,25**
Basophils of Blood	,21
Lymphocytes of Thymus	,18
Macrophages of Spleen	**-,46**



$R=0,964$; $R^2=0,929$; $\chi^2_{(18)}=189$; $p<10^{-6}$

Fig. 4.20. Canonical correlation between the parameters of the autonomic nervous system (X-axis) and immunity (Y-axis) of female rats

The thickness of the fascicular area of the adrenal cortex as a marker of their permanent

glucocorticoid activity correlates positively with the mass of the spleen and the content of macrophages in it as well as the mass of the thymus and the content of epitheliocytes and endothelial cells in it, while negatively with the content of lymph in the thymus. The measure of determination of this immune constellation is 45,5% (Table 4.32 and Figure 4.21).

Table 4.32. Regressive model of multiple correlation of the fascicular area of the adrenal cortex with indicators of immunity of female rats

R=0,727; R^2=0,529; Adjusted R^2=0,455; $F_{(8,5)}$=7,2; $\chi^2_{(8)}$=41; $p<10^{-5}$

		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(51)}$	p-level
	r		Intercpt	-523	350	-1,49	,142
Macrophages S	0,55	,383	,112	16,8	4,9	3,42	,001
Epitheliocytes T	0,52	,532	,144	19,9	5,4	3,71	,001
Spleen Mass Ind	0,28	1,673	,847	196	99	1,97	,054
Spleen Mass	0,22	-1,638	,834	-,8	,4	-1,97	,055
Endotheliocytes T	0,27	,256	,114	21,4	9,6	2,24	,029
Thymus Mass Ind	0,24	-1,803	,982	-1723	939	-1,84	,072
Thymus Mass	0,22	1,683	,887	7,1	3,7	1,90	,063
Lymphocytes T	-0,32	,253	,148	7,7	4,5	1,72	,092

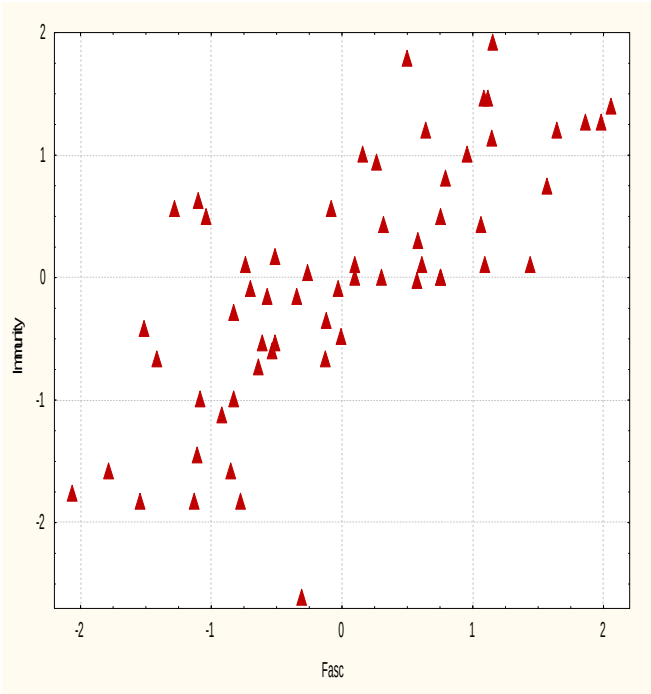


Fig. 4.21. The canonical correlation between the thickness of the fascicular area of the adrenal cortex (X-axis) and the parameters of immunity (Y-axis) of female rats

Plasma corticosterone levels as a marker of situational glucocorticoid activity correlate

very poorly with the adrenal fascicular area (r=-0,19), and are associated with another constellation of immune parameters, negatively with eosinophils and T-killer content in the blood, phagocytosis activity of its microphages, as well as entropy of the immunocytogram and mass of the thymus, while positive - with the content of natural killer in the blood and the completion of phagocytosis of its microphages. The measure of corticosterone determination of the listed constellation of immune parameters is 27,5% (Table 4.33 and Fig. 4.22).

Table 4.33. Regression model of multiple correlation of corticosterone with indicators of immunity of female rats

R=0,601; R^2=0,361; Adjusted R^2=0,275; $F_{(7,5)}$=4,2; $\chi^2_{(7)}$=24; $p<10^{-3}$

		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(52)}$	p-level
	r		Intercpt	1621	1032	1,57	,122
Thymus Mass	-0,23	-,134	,123	-1,27	1,17	-1,09	,282
Eosinophils Blood	-0,32	-,354	,118	-31,8	10,6	-2,99	,004
Killing Ind Neutrophils	0,21	,170	,118	4,62	3,22	1,44	,157
Phagocytose Ind Neutroph	-0,23	-,123	,117	-5,72	5,46	-1,05	,299
T-Cytotoxic Lymphocytes	-0,23	-,216	,123	-12,3	7,0	-1,75	,086
NK-Lymphocytes	0,24	,318	,117	26,0	9,54	2,72	,009
Entropy Immunocytogram	-0,22	-,122	,121	-220	218	-1,01	,317

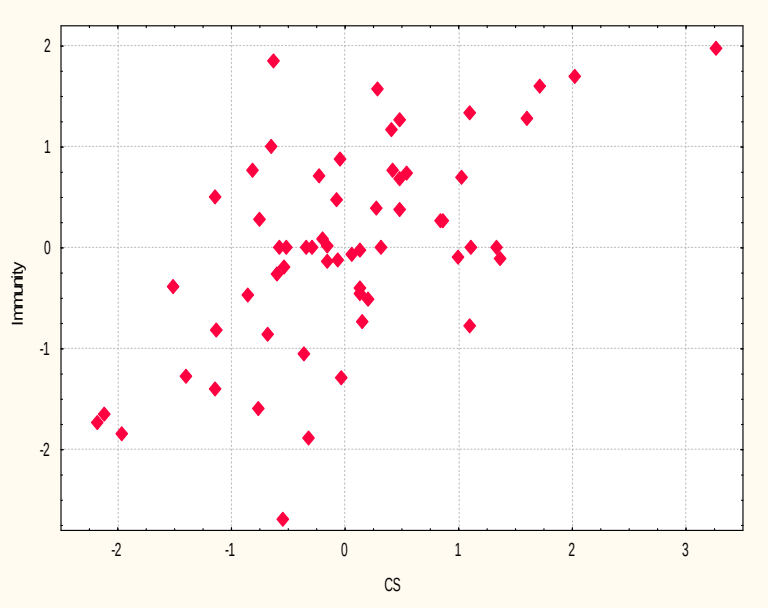
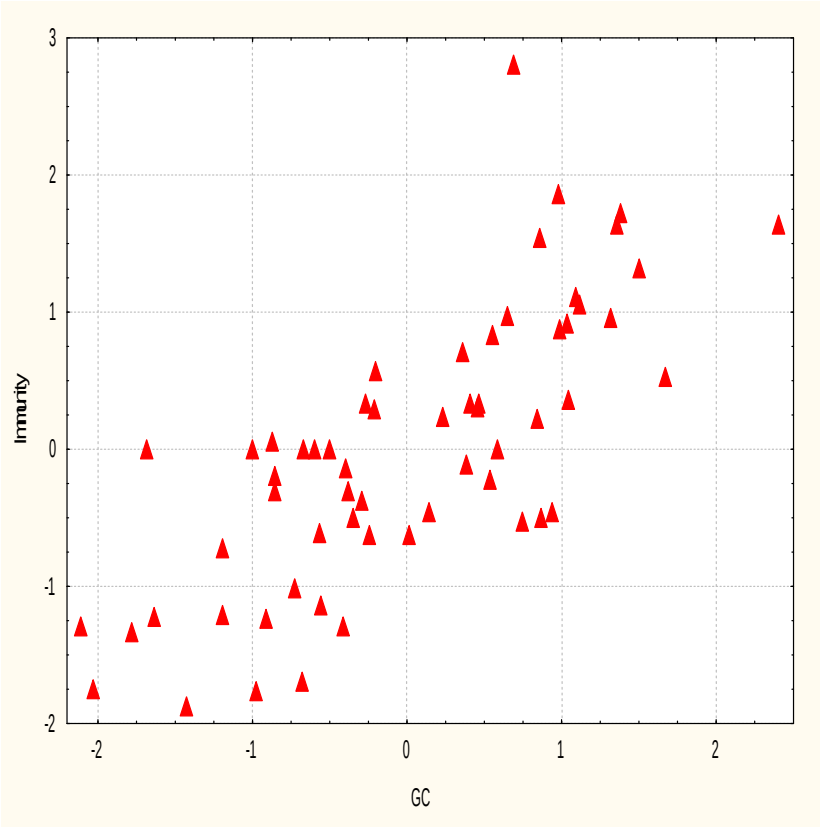


Fig. 4.22. Canonical correlation between corticosteronemia (X axis) and immunity parameters (Y axis) of female rats

Both parameters of glucocorticoid activity, taken together, determine the same constellation of immune parameters stronger - by 57,5% (Table 4.34 and Fig. 4.23).

Table 4.34. Factor structure of glucocorticoid-immune relationships in female rats

Right set	R
Fascicular Zone of Adrenal Cortex	**,98**
Corticosterone of Plasma	**-,39**
Left set	R
Macrophages of Spleen	**,71**
Epitheliocytes of Thymus	**,68**
Endotheliocytes of Thymus	**,37**
Thymus Mass	**,34**
Thymus Mass Index	**,34**
Spleen Mass Index	**,31**
Spleen Mass	**,25**
Eosinophils of Blood	**,25**
Entropy of Immunocytogram	**,25**
Lymphocytes of Thymus	**-,43**
Killing Index of Neutrophils of Blood	**-,24**
T-cytotoxic Lymphocytes of Blood	,04
Phagocytose Index Neutrophils of Blood	,03
NK Lymphocytes of Blood	-,02



$R=0,758$; $R^2=0,575$; $\chi^2_{(28)}=66$; $p<10^{-4}$

Fig. 4.23. Canonical correlation between parameters of glucocorticoid activity (X-axis) and immunity (Y-axis) of female rats

The thickness of the glomerular zone of the adrenal cortex as a marker of its permanent mineralocorticoid activity correlates positively with the bactericidal ability of blood neutrophils and the content of B lymphocytes in the blood as well as the thymus mass and the content of macrophages in the spleen, while negatively with the content in its of plasmocytes determining this immune constellation by 36% (Table 4.35).

Table 4.35. Regression model of multiple correlation of glomerular zone of adrenal cortex with indicators of immunity of female rats

R=0,650; R^2=0,422; Adjusted R^2=0,357; $F_{(6,5)}$=6,5; $p<10^{-4}$

		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(53)}$	p-level
	r		Intercpt	65,8	33,4	1,97	,054
Bacterocidal Capacity N	0,42	,342	,108	2,23	,71	3,17	,003
B-Lymphocytes	0,33	,244	,111	2,86	1,30	2,20	,032
Macrophages of Spleen	0,31	,200	,117	4,07	2,37	1,72	,092
Thymus Mass	0,30	,606	,315	1,19	,62	1,92	,060
Thymus Mass Index	0,24	-,449	,311	-197	138	-1,44	,155
Plasmocytes of Spleen	-0,32	-,204	,117	-5,80	3,32	-1,75	,087

Situational mineralocorticoid activity, estimated by Na/K ratio of plasma, firstly, is not at all related to its permanent activity (r=0,06), and secondly, weakly positively correlated with the content of monocytes and basophiles in the blood while negatively with the content in the spleen of plasmocytes and reticulocytes, so that the measure of determination of these immune parameters reaches only 17% (Table 4.36).

Table 4.36. Regression model of multiple correlation of mineralocorticoid activity as $(Nap/Kp)^{0,5}$ with indicators of immunity of female rats

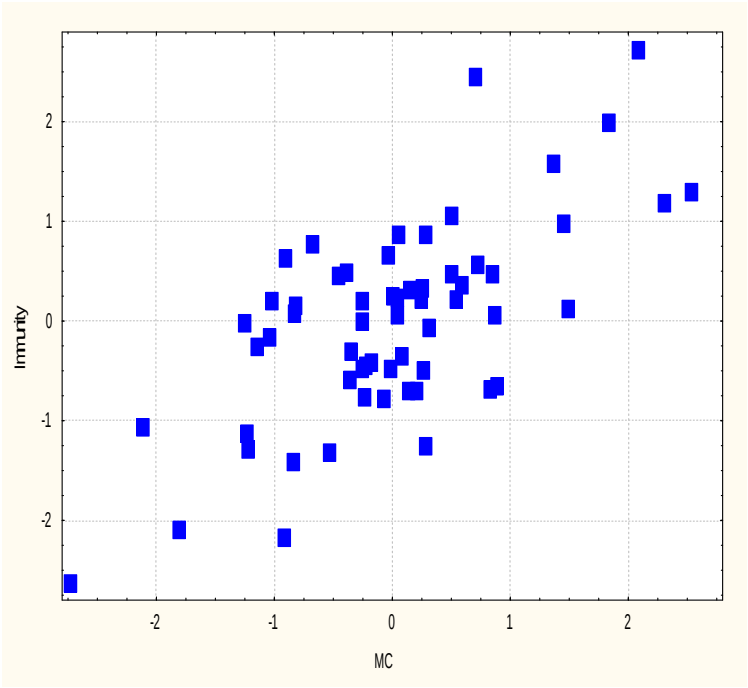
R=0,474; R^2=0,225; Adjusted R^2=0,168; $F_{(4,6)}$=4,0; p=0,007

		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(55)}$	p-level
	r		Intercpt	7,8	0,8	9,27	10^{-6}
Plasmocytes S	-0,30	-,316	,129	-,173	,071	-2,44	,018
Reticulocytes S	-0,22	-,285	,125	-,112	,049	-2,28	,027
Monocytes Blood	0,26	,145	,124	,044	,037	1,18	,244
Basophils Blood	0,22	,132	,125	,184	,174	1,06	,294

Taken together, both parameters of mineralocorticoid activity determine the same constellation of immune parameters more strongly - by 46% (Table 4.37 and Fig. 4.24).

Table 4.37. Factor structure of the mineralocorticoid-immune relationships of female rats

Right set	R
Glomerular Zone of Adrenal Cortex	**-,96**
Mineralocorticoid Activity as $(Nap/Kp)^{0,5}$	**-,33**
Left set	
Bactericidal Capacity of Neutrophils	**-,51**
B-Lymphocytes of Blood	**-,50**
Thymus Mass	**-,50**
Thymus Mass Index	**-,43**
Macrophages of Spleen	**-,45**
Monocytes of Blood	**-,24**
Basophils of Blood	-,07
Plasmocytes of Spleen	**,56**
Reticulocytes of Spleen	,18



$R=0,680$; $R^2=0,462$; $\chi^2_{(18)}=48$; $p<10^{-3}$

Fig. 4.24. Canonical correlation between parameters of mineralocorticoid activity (X

axis) and immunity (Y axis) of female rats

Testosterone plasma level correlates negatively with the intensity of phagocytosis of blood microphages and the content of rod-core neutrophils in the blood as well as lymphocytes in the thymocytogram, while positively with its entropy, the content of macrophages in the spleen and 0-lymphocytes in the blood determining this immune constellation by 52% (Table 4.38).

Table 4.38. Regression model of multiple correlation of testosterone with indicators of immunity of female rats

R=0,753; R^2=0,567; Adjusted R^2=0,518; $F_{(6,5)}$=11,6; $p<10^{-5}$

		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(53)}$	p-level
	r		Intercpt	-44,2	28,9	-1,53	,132
Microbas Count Neutrop	-0,59	-,504	,094	-,76	,14	-5,38	10^{-5}
Stub Neutrophils Blood	-0,28	-,157	,093	-,28	,17	-1,70	,095
Lymphocytes of Thymus	-0,20	,542	,335	,43	,26	1,62	,111
0-Lymphocytes of Blood	0,37	,314	,093	,09	,03	3,38	,001
Macrophages of Spleen	0,32	,170	,097	,19	,11	1,76	,084
Entropy Thymocytogram	0,23	,703	,332	,050	,024	2,12	,039

The thickness of the reticular zone of the adrenal cortex as a marker of their permanent androgenic activity, correlating with testosteroneemia (r=0,61), correlates with only two relevant immune parameters (spleen macrophages and blood 0-lymphocytes), as well as phagocytosis activity of macrophages. As a result, the determination rate of this immune constellation is only 15% (Table 3.36).

Table 4.39. Regression model of multiple correlation of the reticular zone of the adrenal cortex with indicators of immunity of female rats

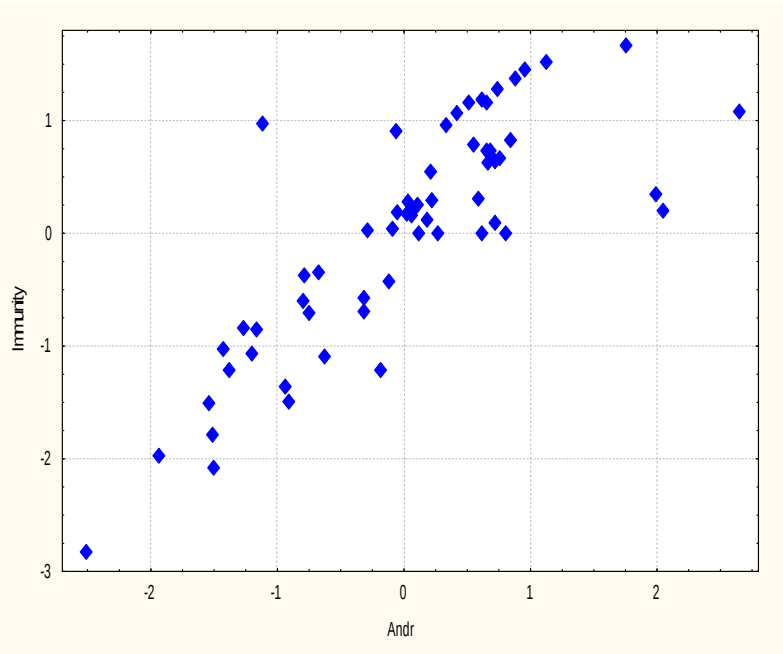
R=0,442; R^2=0,196; Adjusted R^2=0,152; $F_{(3,6)}$=4,5; p=0,006

		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(56)}$	p-level
	r		Intercpt	29,3	9,3	3,15	,003
0-Lymphocytes	0,34	,299	,122	,43	,18	2,46	,017
Macrophages S	0,30	,198	,129	1,15	,75	1,54	,130
Phagoc Ind Mon	0,24	-,146	,128	-1,78	1,55	-1,14	,257

Taken together, both parameters of androgen activity determine the same constellation of immune parameters more strongly - by 66% (Table 4.40 and Fig. 4.25).

Table 4.40. Factorial structure of androgen-immune connections in female rats

Right set	R
Testosterone of Plasma	**-,89**
Reticular Zone of Adrenal Cortex	-,18
Left set	R
Microbial Count of Neutrophils	**,86**
Stub Neutrophils of Blood	**,52**
T-helper Lymphocytes of Blood	**,28**
Lymphocytes of Thymus	**,22**
Phagocytose Index of Monocytes	,04
0-Lymphocytes of Blood	**-,32**
Macrophages of Spleen	**-,27**
Entropy of Thymocytogram	**-,26**



$R=0{,}815$; $R^2=0{,}664$; $\chi^2_{(16)}=74$; $p<10^{-6}$

Fig. 4.25. The canonical correlation between the parameters of androgen function (X-axis) and immunity (Y-axis) of female rats

The total adrenal mass, unlike the thickness of its morpho-functional compartments, is poorly correlated with immune parameters, but the canonical correlation with their

constellation is statistically significant (Table 4.41).

Table 4.41. Regression model of multiple correlation of adrenal mass with indicators of immunity of female rats

R=0,535; R^2=0,286; Adjusted R^2=0,234; $F_{(4,6)}$=5,5; $p<10^{-3}$

		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(55)}$	p-level
	r		Intercpt	223	69,6	3,20	,002
Macrophages of Thymus	0,27	,357	,116	3,90	1,27	3,07	,003
Spleen Mass	0,23	,248	,116	,02	,01	2,13	,037
Reticulocytes of Thymus	-0,25	-,255	,114	-2,63	1,17	-2,23	,030
Entropy Immunocytogram	-0,25	-,279	,115	-35,4	14,6	-2,42	,019

Calcitonin activity (CTA), calculated by the formula: $CTA=(Cau•Pu)/(Cap•Pp)^{0,25}$, negatively correlates with the content of 0-lymphocytes in the blood and epitheliocytes in the thymus, while positively with the content in its of plasmocytes as well as of lymphoblasts in the spleen and T-killers in the blood. This immune constellation is determined by calcitonin activity by 25% (Table 4.42 and Fig. 4.26).

Table 4.42. Regression model of multiple correlation of calcitonin activity with indicators of immunity of female rats

R=0,580; R^2=0,336; Adjusted R^2=0,246; $F_{(7,5)}$=3,8; $\chi^2_{(7)}$=22; p=0,002

		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(52)}$	p-level
	r		Intercpt	2,47	1,57	1,57	,124
0-Lymphocytes of Blood	-0,40	-,434	,154	-,036	,013	-2,81	,007
Epitheliocytes of Thymus	-0,30	-,188	,140	-,053	,040	-1,34	,186
Plasmocytes of Thymus	0,31	,272	,121	,195	,087	2,24	,029
Lymphoblastes of Spleen	0,31	,195	,145	,088	,065	1,35	,183
T-cytotoxic Lymphocytes	0,20	-,195	,159	-,037	,030	-1,22	,227
Phagoc Ind of Neutrophils	0,19	,158	,115	,025	,018	1,37	,176
Plasmocytes of Spleen	0,19	-,153	,147	-,071	,068	-1,04	,302

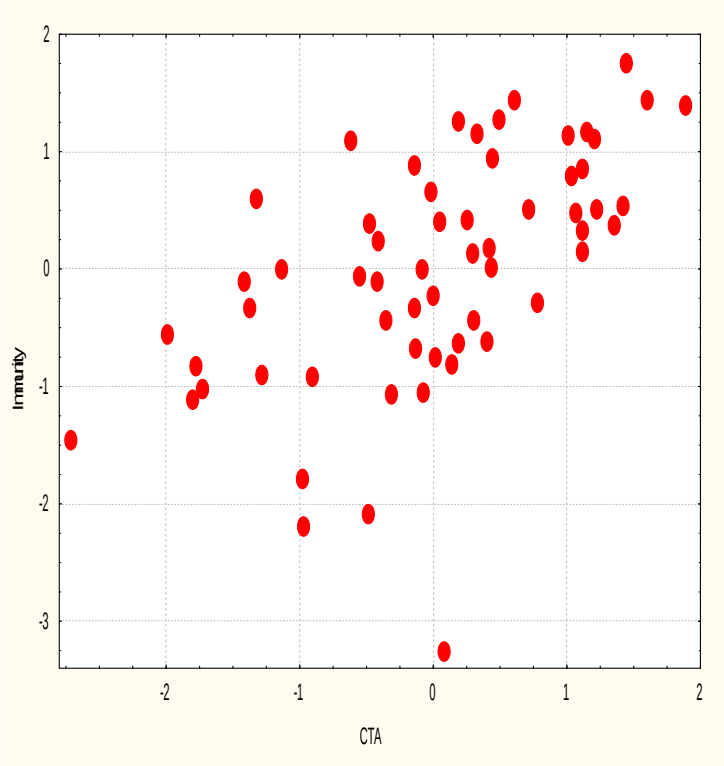


Fig. 4.26. Canonical correlation between calcitonin activity (X axis) and immunity parameters (Y axis) of female rats

Parathyroid activity (PTA), calculated by the formula: $PTA=(Cap \bullet Pu)/(Cau \bullet Pp)^{0,25}$, correlates negatively with the mass of the spleen and the content in it of macrophages, fibroblasts and eosinophils, but positively with the blood content of B-lymphocytes. This immune constellation is determined by 24% (Table 4.43 and Fig. 4.27).

Table 4.43. Regression model of multiple correlation of parathyroid activity with indicators of immunity of female rats

$R=0,551$; $R^2=0,304$; Adjusted $R^2=0,239$; $F_{(5,5)}=4,7$; $\chi^2_{(5)}=20$; $p<10^{-3}$

		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(54)}$	p-level
	r		Intercpt	4,34	,67	6,45	10^{-6}
Spleen Mass Index	-0,34	-,141	,127	-,130	,117	-1,11	,272
Macrophages of Spleen	-0,35	-,247	,123	-,085	,042	-2,01	,049
Fibroblastes of Spleen	-0,28	-,190	,119	-,068	,043	-1,59	,117
Eosinophils of Spleen	-0,23	-,205	,115	-,151	,085	-1,78	,081
B-Lymphocytes of Blood	0,31	,214	,117	,042	,023	1,82	,074

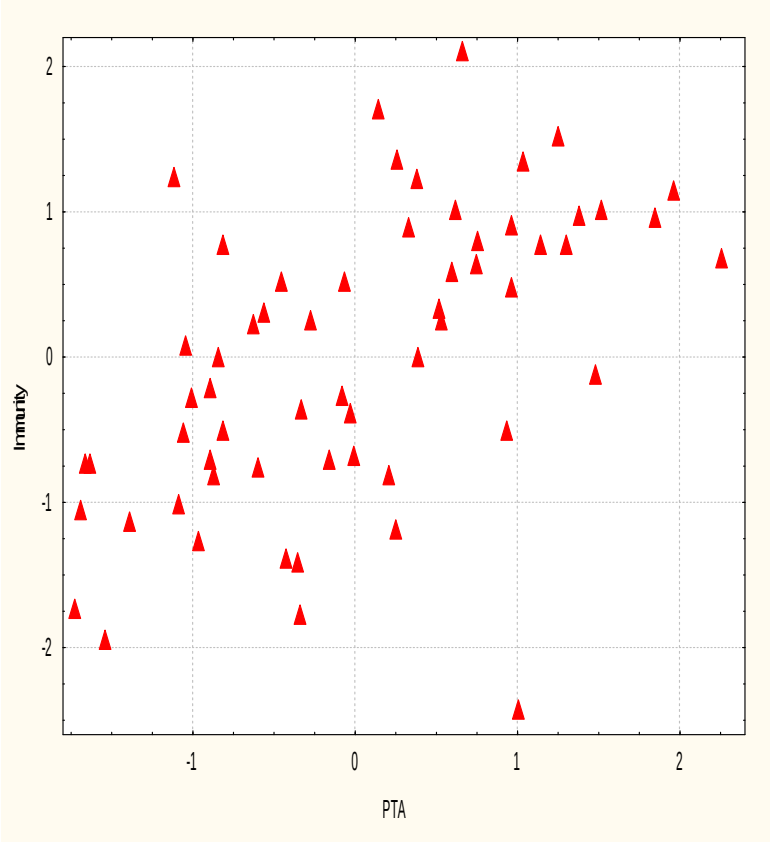


Fig. 4.27. Canonical correlation between parathyroid activity (X-axis) and immunity parameters (Y-axis) of female rats

Blood level of triiodothyronine correlate positively with content of total lymphocytes in the blood, while negatively with population of B-lymphocytes, as well as rod and segmented neutrophils. Together with the intensity of phagocytosis by monocytes Staph. aureus such constellation of immune parameters is determined by triiodothyronine by 22% (Table 4.44).

Table 4.44. Regression model of multiple correlation of triiodothyronine with indicators of immunity of female rats

R=0,544; R^2=0,296; Adjusted R^2=0,216; $F_{(6,5)}$=3,7; p=0,004

		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(53)}$	p-level
	r		Intercpt	-5,82	3,61	-1,62	,112
Stub Neutrophiles	-0,38	-,356	,171	-,127	,061	-2,09	,042
Segmented Neutrophiles	-0,26	,847	,396	,054	,025	2,14	,037
Microbial Count Monoc	-0,24	-,189	,121	-,041	,026	-1,56	,126
B-Lymphocytes of Blood	-0,23	-,256	,119	-,033	,015	-2,16	,035
Entropy Leukocytogram	-0,20	,792	,319	1,001	,404	2,48	,016

Pan-Lymphocytes Blood	0,31	1,412	,579	,076	,031	2,44	,018

The level of thyroxine plasma, firstly, is inversely related to the level of triiodothyronine (r=-0,68), and secondly, it correlates with another constellation of immune parameters, determining them by only 13,5% (Table 4.45) .

Table 4.45. Regression model of multiple correlation of thyroxine with indicators of immunity of female rats

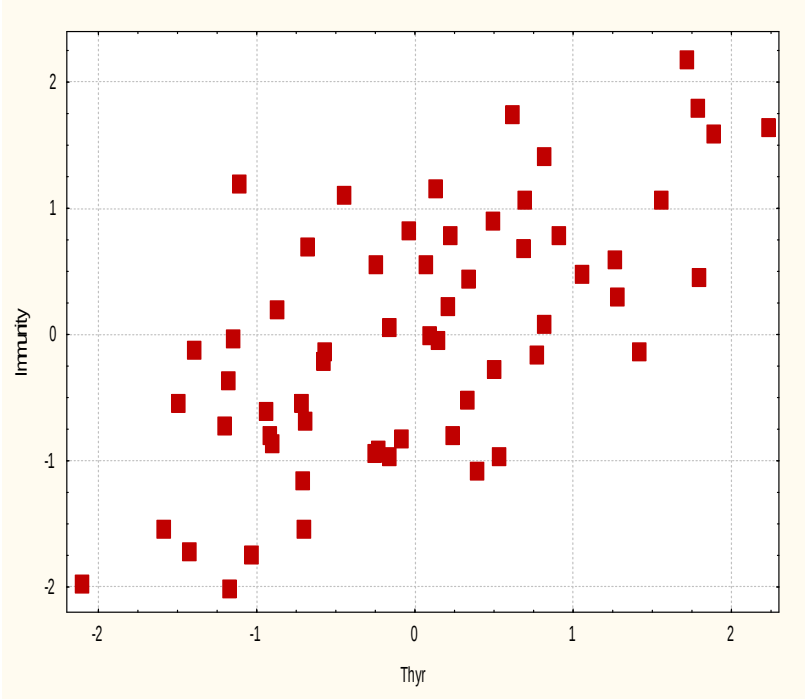
R=0,441; R^2=0,194; Adjusted R^2=0,135; $F_{(4,6)}$=3,3; p=0,017

		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(55)}$	p-level
	r		Intercpt	92,8	33,4	2,78	,007
Plasmocytes of Spleen	0,25	,290	,126	3,13	1,36	2,30	,025
Fibroblastes of Spleen	0,21	,244	,125	1,98	1,01	1,95	,056
Eosinophils of Spleen	-0,20	-,139	,124	-2,30	2,04	-1,13	,265
Phagocytose Ind Neutr	-0,21	-,203	,122	-,75	,45	-1,67	,100

The canonical correlation between the two parameters of thyroid function, on the one hand, and the parameters of immunity, on the other, was much stronger than in relation to individual thyroid hormones. Interestingly, the factor load on the thyroid canonical root by triiodothyronine is four times that of thyroxine, which coincides with the ratio of their physiological activities (4:1). Accordingly, the immune canonical root receives a major load from indicators related specifically to triiodothyronine (Table 4.46). In general, thyroid activity determines the immune parameters by 44% (Fig. 4.28).

Table 4.46. Factor structure of thyroid-immune connections in female rats

Right set	R
Triiodo-thyronine	**-,63**
Thyroxine	**-,14**
Left set	R
Stub Neutrophiles of Blood	**,63**
B-Lymphocytes of Blood	**,63**
Segmented Neutrophiles of Blood	**,46**
Entropy of Leukocytogram	**,51**
Eosinophils of Spleen	**,28**
Microbial Count of Monocytes	,14
Phagocytose Index of Neutrophils	,10
Pan-Lymphocytes of Blood	**-,57**
Plasmocytes of Spleen	-,14
Fibroblastes of Spleen	-,10



$R=0,664$; $R^2=0,440$; $\chi^2_{(20)}=45$; $p=0,001$

Fig. 4.28. Canonical correlation between thyroid function (X-axis) and immunity parameters (Y-axis) of female rats

Thus, as in the experiment with males, in relation to females, it was found that each of neuro-endocrine factors, which we registered, more or less closely correlates positively or negatively with certain immune parameters of the thymus, spleen and blood, which testifies to their interaction within the triune neuro-endocrine-immune complex. However, a number of features have been identified that will become the topic of the next section.

SECTION 5

SEXUAL DIMORPHISM OF THE NEUROENDOCRINE-IMMUNE COMPLEX AND ITS REACTIONS TO CHRONIC STRESS

Even a cursory analysis of the data presented in Chapters 3 and 4 gives the impression that there are sexual differences between the parameters of the neuroendocrine-immune complex of both intact and stressed rats. The purpose of this section is a detailed analysis of sexual dimorphism.

5.1. Sexual dimorphism of the neuroendocrine-immune complex of intact rats

In order to adequately compare the different indicators and rank them, they were listed in two indices. In this case, the average values of males (M) were used as baseline values, relative to which individual indices were calculated for each female: female/M and (female/M-1)/Cv, where Cv is the coefficient of variation of the variable common to both sexes. Neuroendocrine variables, significantly different between the sexes, are given in Table. 5.1.

Table 5.1. Neuroendocrine indicators of intact males and females and their relationships

Variables	Actual values		Females/ Males	(Females/ Males – 1)/Cv
	Males (n=10)	Females (n=10)		
Reticular Zone of Adrenal Cortex, μM	20,0±1,8	40,8±2,5	2,04±0,13 ^{c}	+4,27±0,52 ^{c}
Fascicular Zone of Adrenal Cortex, μM	218±11	370±20	1,70±0,09 ^{c}	+4,15±0,56 ^{c}
Adrenals Mass, mg	50±2	69±3	1,39±0,06 ^{c}	+3,01±0,49 ^{c}
Moda HRV, msec	189±11	124±5	0,66±0,02 ^{c}	+2,21±0,16 ^{c}
Glomerular Zone of Adrenal Cortex, μM	129±11	189±8	1,47±0,07 ^{c}	+2,20±0,31 ^{c}
Parathyrine Activity as $(Cap/Pp)^{0,5}$	1,68±0,06	2,38±0,25	1,42±0,15 ^{a}	+1,90±0,67 ^{a}
Calcitonine Activity as $(Cap•Pp)^{-0,5}$	0,48±0,02	0,86±0,15	1,79±0,19 ^{b}	+1,73±0,55 ^{b}
Mineralocorticoid Ac as $(Nap•Ku/Kp•Nau)^{0,25}$	2,08±0,12	2,73±0,25	1,31±0,12 ^{a}	+1,33±0,51 ^{a}
17-Ketosteroids (KS) urine, nM/100 g•day	26±6	45±7	1,75±0,27 ^{a}	+1,24±0,45 ^{a}
Parathyroid Activity as $(Cap•Pu/Cau•Pp)^{0,25}$	1,72±0,03	2,03±0,12	1,18±0,07 ^{a}	+0,96±0,37 ^{a}
Calcitonin Activity as $(Cau•Pu/Cap•Pp)^{0,25}$	1,41±0,04	1,72±0,15	1,21±0,10 ^{a}	+0,80±0,38 ^{a}
Corticosterone, nM/L	333±43	466±57	1,40±0,17 ^{a}	+1,02±0,43 ^{a}
Mineralocorticoid Activity as $(Nap/Kp)^{0,5}$	6,10±0,37	5,57±0,17	0,91±0,03 ^{b}	-0,60±0,20 ^{b}
Triiodo-thyronine, nM/L	2,50±0,17	2,12±0,18	0,85±0,07 ^{a}	-0,63±0,30 ^{a}
Testosterone, nM/L	35,1±4,6	3,93±0,34	0,11±0,01 ^{c}	-2,57±0,03 ^{c}

Note: $p<0,05^{a}$; $<0,01^{b}$; $<0,001^{c}$

The most significant sex differences were found to be related to the morpho-functional parameters of the adrenal glands. In particular, females have higher androgenic, glucocorticoid, and mineralocorticoid activity, estimated according to the thickness of the reticular zone of the adrenal cortex and daily excretion in the urine of 17-ketosteroids, the thickness of the fascicular corticoadrenal zone, and the plasma level of corticosterone as well as of the thickness of glomerular zone of the adrenal cortex and plasma and urine sodium and potassium. However, females have higher parathyroid and calcitonin activities, as measured by plasma and urinary levels of calcium and phosphate. In addition, females have a smaller amount of HRV mode as an inverse measure of heart rhythm, which is subject to the so-called humoral regulation channel (circulating catecholamines, glucocorticoids, electrolytes, etc.), whereas HRV-markers of sympathetic and vagal tone are not significant, respectively 53±7% and 56±5% and 45±8 and 53±13 ms. Instead, the plasma triiodothyronine level is 85±15% of the male level, and the lower plasma testosterone level requires no comment.

Among the reported immune rates, 12 were significantly higher in females (Table 5.2). First of all, this is the proportion in the thymocytogram of lymphocytes and lymphoblastes, the natural killer cells and B-lymphocytes in the blood immunocytogram, as well as of fibroblasts, macrophages and microphages in the splenocytogram. In addition, females have a higher intensity of phagocytosis by monocytes of Staph. aureus, blood leukocytosis, RBTL on PhHA, as well as entropy of splenocytogram and immunocytogram.

Table 5.2. Immune indicators of intact rats, which are significantly higher in females

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 [a]	+1,22±0,58 [a]
Entropy of Splenocytogramm (•10^3)	721±9	753±9	1,04±0,01 [c]	+1,17±0,33 [b]
Blasttransformation of T-Lymphocytes, %	65,6±3,7	78,8±2,3	1,20±0,03 [c]	+1,11±0,20 [b]
Entropy of Immunocytogramm (•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 are significantly lower (Table 5.3). This

is, first of all, the phagocytosis activity of neutrophils/microphages and, to a lesser extent, monocytes/macrophages, as well as thymocytogram entropy and its proportion of epitheliocytes, endothelial cells and macrophages, content of lymphocytes and lymphoblastes in the splenocytogram and 0-lymphocytes in immunocytogram as well as the completeness of phagocytosis by neutrophils of blood.

Table 5.3. Immune indicators of intact rats, which are significantly lower in females

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 Thymocytogramm ($\bullet 10^3$)	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 results of the analysis of sexual dimorphism of the neuroendocrine-immune complex of intact rats are shown in Fig. 5.1 and 5.2.

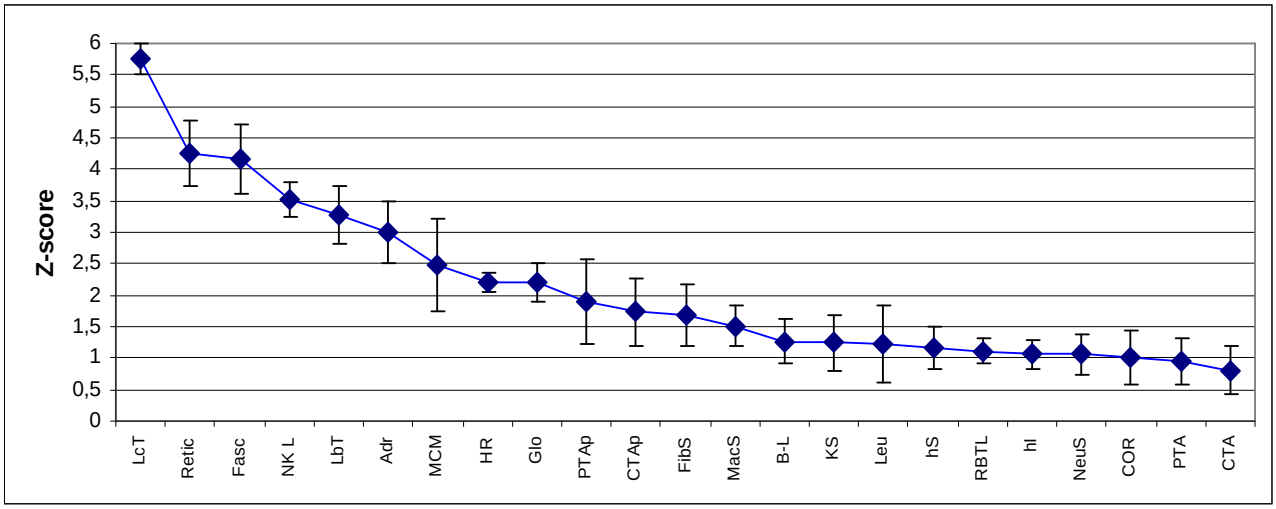


Fig. 5.1. Z-values of neuroendocrine and immune parameters of intact rats significantly larger in females

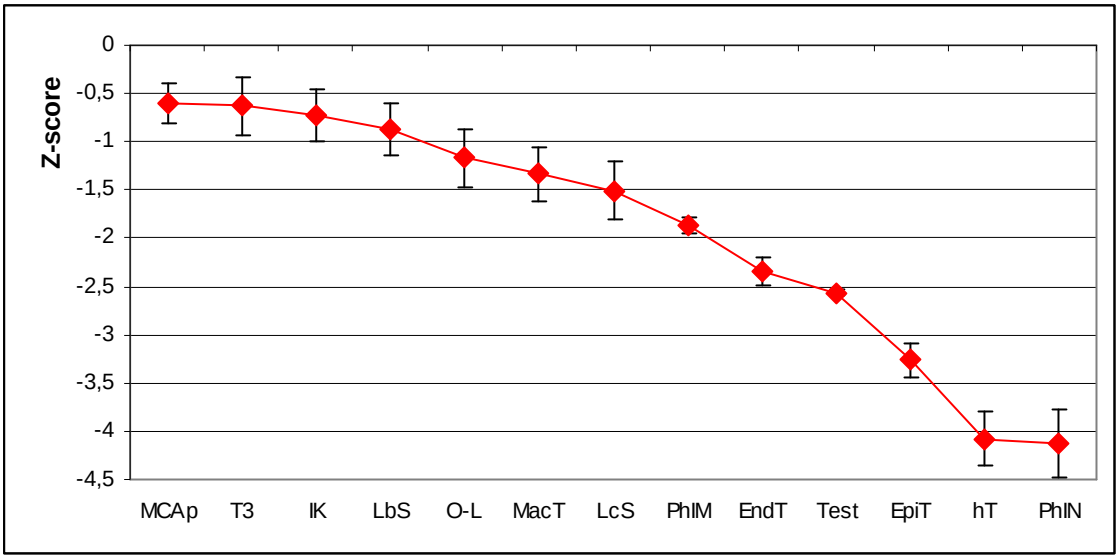


Fig. 5.2. Z-values of neuroendocrine and immune parameters of intact rats significantly smaller in females

5.2. Sexual differences of the neuroendocrine-immune complex in normal and after chronic stress

The purpose of the next stage of analysis was to identify those indicators of the neuroendocrine-immune complex that characterize sexual dimorphism both in normal and after chronic stress. Forward discriminatory analysis selected 38 indicators, including 6 of **thymus**, 6 of **spleen**, 13 of **blood**, and 13 neuroendocrine (Table 5.4).

Discriminatory information is condensed into three canonical roots. The major root contains its lion's share – 97,9%, while in the second and third only 1,2% and 0,9%, respectively.

Calculation of individual values of discriminant roots on the basis of individual values of discriminant variables, as well as their raw coefficients and constants, are given in Table. 5.5, makes it possible to visualize the localization of each of the 110 rats in the information field of discriminant roots (Fig. 5.3).

Table 5.4. Summary of discriminant analysis of indicators of neuroendocrine-immune complex that characterize sexual dimorphism

Step 38, N of vars in model: 38; Grouping: 4 grps. Wilks' Λ: 0,002; approx. $F_{(114)}=13$; $p<10^{-6}$

Variables currently in the model	Wilks' Λ	Partial Λ	F-remove	p-level	Tolerancy	F to enter	p-level	Λ	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}
Bacterocidal 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}
0-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}

Glomerular Zone Adr Cortex, µM	,002	,966	0,8	,49	,44	2,5	,06	,011	23,8	10^{-6}
MCA as $(Nap•Ku/Kp•Nau)^{0,25}$	,002	,889	2,8	,07	,44	2,4	,09	,005	18,6	10^{-6}
Fascicular Zone Adr Cortex, µM	,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}
Reticular Zone Adr. Cortex, µM	,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}
Parathyroid activity as $(Cap/Pp)^{0,5}$	,002	,945	1,3	,27	,10	3,6	,02	,013	26,4	10^{-6}
PTA as $(Cap•Pu/Cau•Pp)^{0,25}$	,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}

Table 5.5. Standardized and raw coefficients and constants for discriminant variables

Variables currently 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's 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
Bacterocidal 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
0-Lymphocytes of Blood, %	-1,131	,493	-,466	-,172	,075	-,071
Entropy of Immunocytogramm ($\bullet 10^3$)	,616	,474	,242	,022	,017	,009
Adrenals Mass, mg	,520	,205	,042	,047	,018	,004
Glomerular 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
Reticular 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
Parathyroid Activity as $(Cap/Pp)^{0,5}$	,173	,904	-,147	,354	1,852	-,301
Parathyroid 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**

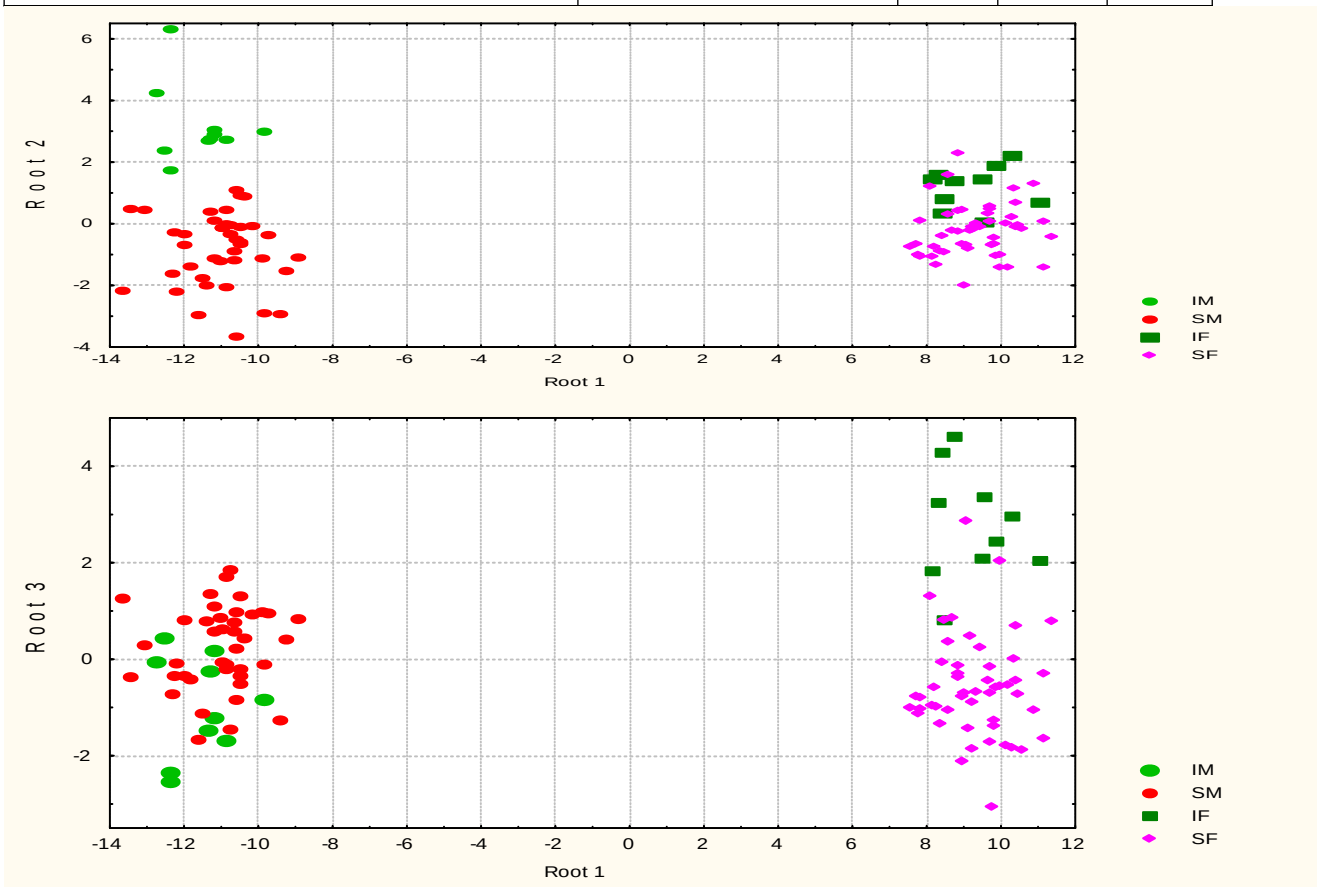


Fig. 5.3. Individual magnitudes of canonical discriminant roots of intact (I) and stressed (S) males (M) and females (F) rats

After calculating the mean values (centroids) of the discriminant roots (Table 5.6), it becomes possible to visualize the localization on their planes of four groups of animals (Figs.

5.4 and 5.5).

Table 5.6. Centroids of the discriminant roots of intact and stressed groups of rats of both sexes

Groups	Root 1	Root 2	Root 3
Intact Males (n=10)	-11,6	3,2	-1,0
Stressed Males (n=40)	-11,0	-0,8	0,2
Intact Females (n=10)	9,2	1,2	2,8
Stressed Females (n=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

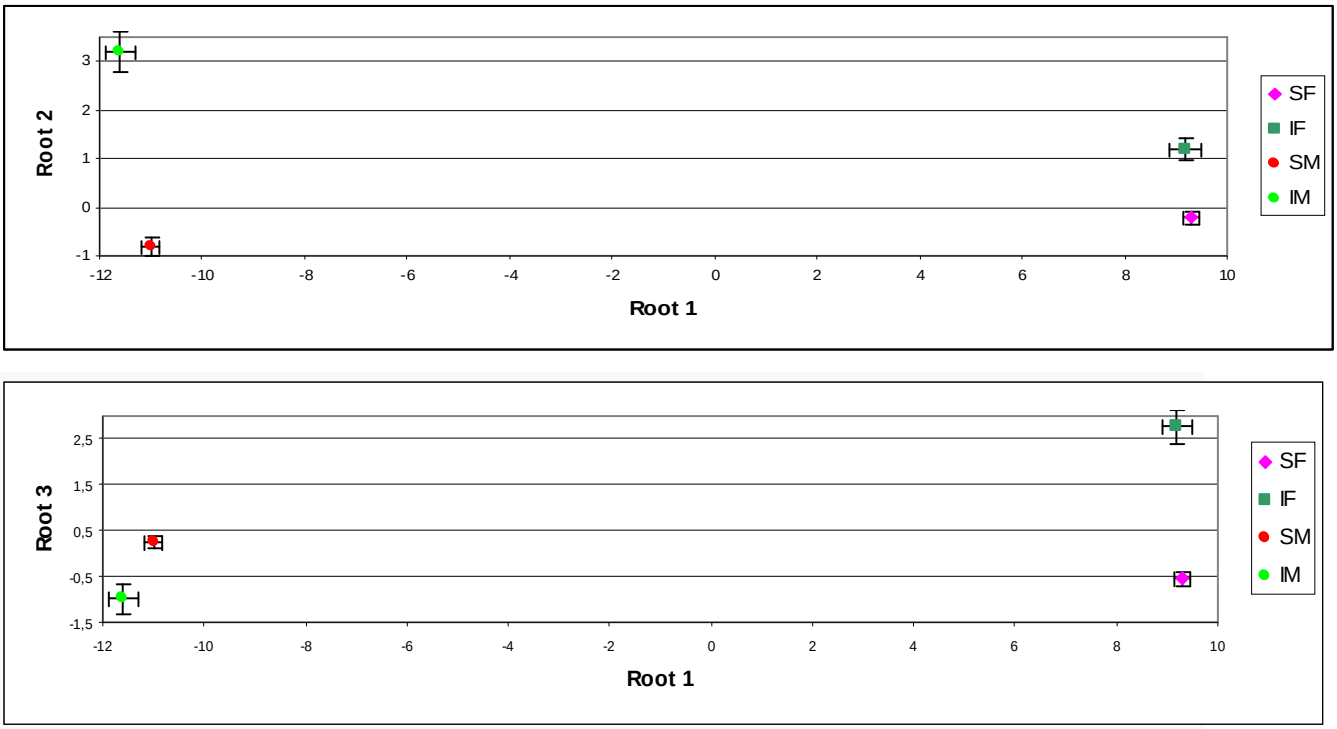


Fig. 5.4. Localization in the information field of centroids (M±SE) of canonical discriminant roots of intact (I) and stressed (S) males (M) and females (F) rats

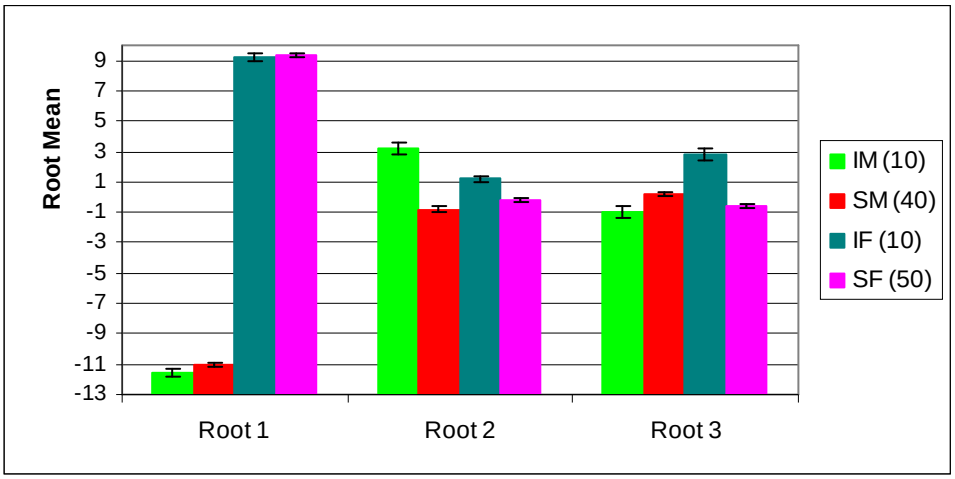


Fig. 5.5. Mean (M±SE) canonical discriminant roots of intact (I) and stressed (S) males (M) and females (F) rats

Drastic delineation along the axis of the first root of males and females reflects significantly greater in males the values of neuroendocrine-immune complex, which correlate with the root **negatively**, as well as significantly smaller than in females, the values of **positively** correlating with the root indices. However, for each sex, these indicators do not differ significantly in intact and stressed animals, ie they are stress-resistant (Table 5.7).

Table 5.7. Correlation coefficients between discriminant variables and roots as well as mean values of discriminant variables intact and stressed rats of both sexes

Variables currently in the model	Root 1	Root 2	Root 3	Intact Male (10)	Stressed Male (40)	Intact Female (10)	Stressed Female (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±12
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's 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**	1,72±0,05	1,69±0,03	**2,03±0,12**	**1,77±0,06**
Parathyroid activity as $(Cap/Pp)^{0,5}$	,027	,131	**,309**	1,68±0,06	1,59±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 clearly delineate along the axis of the second root, which reflects the post-stress reduction of phagocytic activity of blood monocytes and the content of basophils and segmented neutrophils, on the one hand, and the increase in thymus mass and content in it of Hassal's corpuscles, on the other hand. In females, post-stress changes in these indicators are generally insignificant.

Significant differences between intact and stressed females are reflected along the axis of the third root, which contains information about the stress-induced decrease in levels of parathyroid activity and thyroxine, the phagocytosis intensity of Staph. aureus by microphages and bactericidal ability of blood macrophages, the blood content of eosinophils, as well as the mass of the spleen. On the other hand, the post-stress changes in these indicators are insignificant in the males.

The apparent differences between the four groups of rats are documented by the calculation of the Mahalanobis distance squares (Table 5.8).

Table 5.8. Mahalanobis distance squares as well as values of F (df=39) and p

Groups	IM	SM	IF	SF
Intact Males (n=10)	0	**19**	**468**	**465**
Stressed Males (n=40)	**2,3 10^{-3}**	0	**436**	**430**
Intact Females (n=10)	**36 10^{-6}**	**55 10^{-6}**	0	**14**
Stressed Females (n=50)	**61 10^{-6}**	**160 10^{-6}**	**1,8 10^{-2}**	0

Therefore, the differences between the neuroendocrine-immune complexes of intact and stressed rats in both sexes are explained exhaustively by 38 indicators. The information contained in these indicators can be condensed into three distinctive radical functions. The selected indicators can be used to identify each of the four groups of animals by calculating the classification functions by their coefficients and constants (Table 5.9) and the individual values of the discriminant indicators.

Table 5.9. Coefficients and constants for classification discriminant functions

Variables currently in the model	Intact Male	Stressed Male	Intact Female	Stressed Female
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's 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
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 Immunocytogramm (•10^3)	2,140	2,094	2,602	2,550
Adrenals Mass, mg	-,39	-,43	,56	,53
Glomerular Zone of Adrenal Cortex, µM	,55	,51	,57	,55
Mineralocorticoid Activity as $(Nap•Ku/Kp•Nau)^{0,25}$	123	133	143	138
Fascicular Zone of Adrenal Cortex, µM	,14	,19	,29	,32
Corticosterone, nM/L	,13	,14	,10	,09
Reticular Zone of Adrenal Cortex, µM	-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
Parathyroid Activity as $(Cap/Pp)^{0,5}$	-100,8	-108,4	-98,3	-99,9
Parathyroid Activity as $(Cap•Pu/Cau•Pp)^{0,25}$	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, 100% classification accuracy is achieved.

CONCLUSION

The method of factor analysis revealed 4 independent clusters of parameters of the neuroendocrine-immune complex of male rats, which are linked by cause and effect functional relationships. Each of the reported neuro-endocrine factors more or less closely correlates positively or negatively with these or other immune parameters of the thymus, spleen and blood, which testifies to their interaction within the triune neuroendocrine-immune complex.

It was found that daily half-hour immobilization during the week of male rats increases plasma corticosterone level by 20,8%, increases the thickness of the fascicular zone of the adrenal cortex by 12,6%, sympathetic tone by 20,6%, heart rate by 9,6%, the thickness of the reticular zone of the adrenal cortex by 17,1%, the level of plasma testosterone by 16,9%, triiodothyronine by 6,2%. Instead, the vagal tone, thickness of glomerular zone of the adrenal cortex, mineralocorticoid and parathyroid activities decrease by 27,5%, 12,5%, 5,6%, and 4,0%, respectively. Other recorded endocrine parameters do not change significantly.

The integral quantitative measure (mean of Z-units) of the neuroendocrine response to chronic stress factors is 0,47±0.04 Euclid units, which is very moderate, that is consistent with the very mild conditions of stress modeling in our experiment.

Neuroendocrine perturbations caused by chronic stress are accompanied by deviations from the norm of a number of indicators of immunity, in the form of both suppression and enhancing.

As a result of the discriminant analysis, 17 indicators were characteristic of the immunotropic effect of chronic stress. The most significant was the decrease in the intensity of phagocytosis of Staphylococcus aureus by neutrophils, as well as the proportion of endothelial cells and lymphocytes in the thymocytogram, the activity of phagocytosis of monocytes/macrophages and neutrophils/microphages, the share of Th-lymphocytes and NK-lymphocytes in the thymocytogram, of stub neutrophils and basophils in the leukocytogram as well as blastransformation of T-lymphocytes by mitogen PhHA.

Instead, another constellation of immune parameters was increasing, namely, the microbial count of blood macrophages and their bactericidal capacity against Staphylococcus aureus, thymus mass, the proportion of macrophages in the thymocytogram and splenocytogram as well as of eosinophils in the splenocytogram.

The method of cluster analysis identifies three homogeneous groups, different from each other. The immune status of 40% of male rats appeared to be resistant to stressors. The immune status of 37,5% of rats of the II cluster is characterized by moderate inhibition of microphagal, killer and T-cellular links in combination with the expressed activation of the macrophagal link. Post-stress changes in immunity in 22,5% of rats in cluster I differ from those in cluster II both qualitatively and quantitatively. In particular, no deviations from the norm were detected, neither the T-lymphocyte blastransformation reaction nor the level of NK lymphocytes. At the same time, other parameters of the T-link, as well as the microphagal link were suppressed to a greater extent, and the parameters of the macrophagal link were activated very noticeably.

The revealed features of immune responses to chronic stress are related to the stress responses of the autonomic nervous system and the classic adaptive glands - adrenal, gonadal and thyroid, as well as parathyroid. In particular, the absence of post-stress deviations from the norm of immune status indicators in rats of the cluster III is associated with normal post-stress levels of mineralocorticoid activity, plasma thyroxine and daily excretion of 17-

ketosteroids, in contrast with reduced parathyroid activity, vagal tone, thickness of the glomerular zone of the adrenal cortex, while elevated sympathetic tone, heart rate, calcitonin activity, thickness of the reticular and fascicular zones of the adrenal cortex, plasma levels of testosterone, corticosterone and triiodothyronine.

Moderate supression of microphagal, killer and T-cellular links in combination with pronounced enhancing of the macrophagal link in cluster rats II is associated with a similar increase in the thickness of the reticular and fascicular zones of adrenal cortex, but without increasing in plasma levels of testosterone and corticosterone in combination with reduction in thickness of the glomerular zone of the adrenal cortex and mineralocorticoid activity.

More pronounced post-stress changes of immunity in rats of the cluster I, first of all activation of a macrophagal link in combination with suppression of microphagal and T-cellular links, is also associated with the most raised sympathetic tone and reciprocally reduced vagal tone as well as moderately elevated corticosterone level in combination with moderately decreased triiodothyronine level.

In females under conditions of stress, mineralocorticoid activity increases to the maximum extent, followed by ranking: testosteroneemia, thickness of the fascicular area of the adrenal cortex, heart rhythm, sympathetic tone, thickness of the reticular zone of the adrenal cortex, plasma level of triiodothyronine. Instead, thyroxinemia and parathyroid activity were significantly reduced. The integral quantitative measure of neuroendocrine response to chronic stress factors is 0,61±0,10 σ, that is, it is not significantly different from that in males.

The method of discriminant analysis selected 38 indicators (6 of hymus, 6 of spleen, 13 of blood and 13 neuroendocrine), which characterize sexual dimorphism both in normal and after chronic stress. Normally, the most significant sex differences were found to relate to the morpho-functional parameters of the adrenal glands. In particular, females have higher androgenic, glucocorticoid, mineralocorticoid as well as parathyroid and calcitonin activities.

In addition, females have a higher heart rhythm, which is subject to the so-called humoral regulation channel (circulating catecholamines, glucocorticoids, electrolytes, etc.), whereas markers of sympathetic and vagal tone are not significantly different. Instead, the plasma triiodothyronine level is 85% of the male level, and the order of magnitude lower testosterone requires no comment.

Among the reported immune parameters, 11 are significantly higher in females. First of all, this is the share in the thymocytogram of lymphocytes and lymphoblastes, in the blood immunocytogram of the natural killer cells and B-lymphocytes, as well as in the splenocytogram of fibroblasts, macrophages and microphages. In addition, females have a higher leukocytosis and intensity of phagocytosis of Staph. aureus by blood monocytes.

Instead, 10 indicators of immunity in females are significantly lower. This is, first and foremost, the activity of phagocytosis of microphages and, to a lesser extent, macrophages of the blood, the proportion in the thymocytogram of epitheliocytes, endothelial cells and macrophages, the proportion in the splenocytogram of lymphocytes and lymphoblasts, in the immunocytogram of 0-lymphocytes as well as the phagocytosis completion of blood neutrophils.

The post-stress reduction of phagocytic activity of blood monocytes and the content of basophils and segmented neutrophils, on the one hand, and increase of thymus mass and content in it of Hassal's corpuscles as well as increase of sympathetic tone and corticosterone, on the other hand, were found in males, in females was not significant. Instead, females were found to have decreased levels of parathyrin and thyroxine, as well as the intensity of phagocytosis of Staph. aureus by microphages and bactericidal ability of blood macrophages, blood content of eosinophils and mass of the spleen, while in males post-stressor changes of these indicators are generally insignificant.

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For physiologists, endocrinologists, immunologists.

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