Integrated Quantitative Estimation of Neuro-Endocrine Manifestations of Chronic Stress in Female Rats

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Previously in line with the concept of a triune neuro-endocrine-immune complex [3, 5, 7, 9] we have been carried out integrated quantitative estimation of neuroendocrine and immune responses to chronic restraint stress at male rats. The method of discriminant analysis has found that distinctive endocrine signs of chronic stress are increasing thickness of Fascicular Zone whereas decreasing thickness of Glomerulary Zone of Adrenal Cortex as well as plasma (Ca/P)^0.5 ratio as Parathyrine Activity. Other signs of chronic stress such as increasing plasma levels Corticosterone, Testosterone and Triiodo-thyronine, Sympathetic tone, Heart Rate and thickness of Reticular Zone of Adrenals as well as decreasing Vagal Tone and plasma (Na/K)^0.5 ratio as Mineralocorticoide Activity currently not in the discriminant model. Canonical Neuroendocrine Roots for Intact and Stressed Males Rats averages +0.99 ± 0.40 and -0.25 ± 0.15 respectively (Squared Mahalanobis Distance \( D^2_M = 1.61; F = 3.76; p = 0.017 \)).

Among the parameters of Immunity characteristic of chronic stress appeared to increase Thymus Massa Index, level in Thymocytogram of Macrophages and Reticulocytes, in Splenocytogram of Macrophages and Eosinophils, Monocytes in Leukocytogram of Blood have increased as well as Entropy of Leukocytogram and Splenocytogram whereas both Intensity and Activity of Phagocytose by Neutrophils, levels of Endotheliocytes in Thymocytogram, Neutrophils in Splenocytogram, NK-Lymphocytes, Stub Neutrophils and Basophils in Leukocytogram have decreased. Canonical Immune Roots for Intact and Stressed males rats averages -3.41 ± 0.40 and +0.85 ± 0.15 respectively (\( D^2_M = 19.0; F = 5.44; p < 10^{-4} \)). Canonical correlation between Neuroendocrine and Immune parameters is very strong: \( R = 0.976; \chi^2(297) = 432; p < 10^{-6} \) [8, 10, 11].

At the next stage, research has been conducted on female rats. In this article we give the results of quantitative evaluation of neuroendocrine responses to chronic restraint stress

Material and methods. The experiment is at 60 white female rats Wistar line weighing 230-300 g. Of these 10 animals not subjected to any influences and 50 within 7 days subjected to moderate stress by daily 30-minute immobilization. The day after the completion of stressing in rats of both groups under light ether anesthesia for 15-20 sec recorded ECG in standard lead II (introducing needle electrodes subcutaneously) has been recorded and parameters of heart rate variability (HRV) have been determined [1]. Then the rats have been placed in individual chambers with perforated bottom to collect daily
urine, in which a concentration of calcium (by reaction with arsenazo III) and phosphate (by phosphate-molibdate method) has been defined. The next day, the animals have been decapitated, for the purpose of collecting blood, in plasma that has determined concentration of adaptive hormones such as corticosterone, testosterone, thyroxine and triiodothyronine (by ELISA) as well as of calcium, phosphate, sodium and potassium (by flaming photometry). After a blood sample has been taken adrenal glands has been removed and weighed. The thickness of glomerular, fascicular and reticular zones in sections of the adrenal glands has been measured under a microscope [2, 7, 9].

Digital material has been treated using the package of softwares “Statistica 5.5”.

Results and discussion. According to a recommendation of I.L. Popovych [2, 9] variables obtained after a Chronic Stress (SV) can be expressed as Z-score calculated by this formula:

\[ Z = \frac{SV/IV - 1}{Cv} \]

where

IV is Initial (Control) Variable, Cv is Coefficient of its variation at the 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 because a physiological cost 1 % changes a stable figure that is higher than fluctuation parameter that is normally wider.

It has been found that on the second day after weekly stress the mineralocorticoid activity, which is estimated by the Na/K ratio of plasma (Fig. 1), increases to the maximum. Further, in the descending order such features have been appeared as: testosteroneemia, thickness of the fascicular zone of the adrenal cortex (ZAC), heart rate (as the inverse of the Moda of HRV), sympathetic tone (estimated for AMo HRV), thickness of the reticular ZAC and plasma level of triiodothyronine. Hormonal constellation consisting of calcitomin activity (calculated by the formula [7, 9]: \((Cau \cdot Pu/Pp \cdot Cap)^{0.25}\)), adrenals mass, thickness of the glomerular ZAC, corticosteroneemia and vagal tone (estimated for MxDMn HRV) does not significantly differ from that of the control. In contrast, thyroxinemia and paratyrin activity (calculated by the formula: \((Cap \cdot Pu/Pp \cdot Cau)^{0.25}\)) have been significantly decreased.

![Fig. 1. Ranking caused by chronic stress changes in neuroendocrine parameters at female rats.](image)

the integral quantitative measure of the neuroendocrine response to the factors of chronic stress as the mean value of the significant modules of Z-units is 0.61 ± 0.10, that does not significantly differ from revealed ones earlier in the males: 0.47 ± 0.04 [8].
However, there are sexual differences between the post stressed neuro-endocrine profiles not only in the severity of reactions, but also in their sign (Fig. 2). It could be confirmed according to the known data about the gender differences in the responses of neuroendocrine-immune complex on adaptogens [6, 7, 9].

Another approach to identifying the parameters (variables), that significantly differ from a neuro-endocrine status of the intact and stressed rats, is a discriminant (recognizing) analysis. While applying this method only four forward stepwise variables that have been currently in the model have turned out (Tables 1 and 2), while other variables earlier marked are not in the discriminant model, consequently, recognizable information that they depict is redundant.

Information about these four variables has been condensed in canonical root that positively correlates with Parathyrine activity and Moda HRV instead negative correlation with Mineralocorticoid activity and plasma level of Ttiiodo-thyronine (Table 3). The calculation of individual Root values based on Raw Coefficients for discriminant variables and Constant (Table 3) allows to visualize the status of each rat (Fig. 3).

The calculation of the mean values of the roots shows a significant difference between the neuroendocrine status of intact (+1.25 ± 0.29) and stressed (-0.25 ± 0.14) groups (Fig. 4), which is documented by calculating the square of the distance Mahalanobis \(D^2_M = 2.32; F = 4.2; p = 0.005\).
Table 1

Discriminant function analysis summary for neuro-endocrine variables
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) | After Chronic Stress (50) | Stressory change as Z-score | Wilks Λ | Partial Λ | F remove | p-level | Tolerance |
|----------------------------------|-------------------------|---------------------------|-----------------------------|---------|-----------|----------|---------|-----------|
| (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      |
| Ttiodothyronine, nM/L            | 2.12 ± 0.18             | 2.28 ± 0.05               | +0.28 ± 0.09                | ,773    | ,978      | 1.26     | ,267    | ,977      |
| (Cap•Pu/Pp•Cau)⁰.²⁵ as PTA       | 3.61 ± 0.22             | 3.05 ± 0.08               | -0.81 ± 0.12                | ,860    | ,879      | 7.57     | ,008    | ,980      |

| Variables currently not in the model | Initial level (Control) | After Chronic Stress (50) | Stressory change as Z-score | Wilks Λ | Partial Λ | F remove | p-level | Tolerance |
|--------------------------------------|-------------------------|---------------------------|-----------------------------|---------|-----------|----------|---------|-----------|
| 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      |
| Reticularly ZAC, µM                  | 41 ± 2                  | 44 ± 2                    | +0.36 ± 0.20                | ,753    | 1.00      | ,24      | ,627    | ,945      |
| (Cau•Pu/Pp•Cap)⁰.²⁵ as CTA           | 3.52 ± 0.36             | 3.71 ± 0.15               | +0.17 ± 0.13                | ,754    | 1.00      | ,13      | ,721    | ,730      |
| 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, ms              | 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      |

Table 2

Summary of stepwise analysis and chi-square tests with successive root removed for neuro-endocrine variables

| Variables currently in the model | F to enter | p-level | λ      | F-value | p-level |
|----------------------------------|------------|---------|--------|---------|---------|
| (Cap•Pu/Pp•Cau)⁰.²⁵ as PTA       | 7.43       | .008    | .886   | 7.43    | .008    |
| (Nap/Kp)⁰.⁵ as MCA               | 6.52       | .013    | .795   | 7.33    | .001    |
| Moda HRV                         | 1.61       | .210    | .773   | 5.47    | .002    |
| Ttiodothyronine                  | 1.26       | .267    | .756   | 4.44    | .004    |

Canonical R = 0.494; Wilks’ Λ = 0.756; χ²(4) = 10.7; p = 0.004
Table 3 shows program-calculated coefficients for discriminant variables.

### Table 3

| Variables currently in the model                                      | Coefficients for Canonical Variables |
|---------------------------------------------------------------------|--------------------------------------|
|                                                                     | Standardized | Structural | Raw       |
| (Cap•Pu/Pp•Cau)\(^{0.25}\) as Parathyline Activity (PTA)          | ,71          | ,63        | 1,224     |
| Moda HRV                                                            | ,32          | ,33        | ,016      |
| (Nap/Kp)\(^{0.5}\) as Mineralocorticoid Activity (MCA)            | -,62         | -,59       | -,925     |
| Ttiiodo-thyronine                                                  | -,31         | -,27       | -,759     |
| Constant                                                            | 1,609        |            |           |

Fig. 4. Average values of the neuro-endocrine root of intact and stressed females rats.

It is worth saying that neuroendocrine roots for intact and stressed **Males** rats average between +0.99 ± 0.40 and -0.25 ± 0.15 respectively \((D^2_m = 1.61; F = 3.76; p = 0.017)\) [8].

According to the full structural coefficients given in Table. 4, the discriminant root reflects mineralocorticoid activity caused by the chronic stress reduction of parathyrin and increased, as well as the activation of the thyroid gland and sympathetic shift of vegetative tone.

**Conclusion.** Overall, our findings accord to 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 Mineralocorticoid 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 as well as about differences related to sex [3-7].

**CONFORMITY TO ETHICAL STANDARDS.** Experiments on animals have been carried out in accordance with the provisions of the Helsinki Declaration of 1975, revised and supplemented in 2002 by the Directives of the National Committees for Ethics in Scientific Research. The conduct of experiments has been approved by the Ethics Committee of the National Medical University. The modern rules for the maintenance and use of laboratory animals accord to the principles of the European Convention for the Protection of Vertebrate Animals used for scientific experiments and needs are observed (Strasbourg, 1985).
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RESEARCH ARTICLES

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Material and methods. The experiment has been conducted on 60 white female rats Wistar line weighing 230-300 g. 10 of these animals have not subjected to any influences and 50 of them subjected to moderated stress by daily 30-minute immobilization during 7 days. The day after the completion of stressing in rats of both groups the parameters of HRV, blood levels of hormones as well as blood and urine levels Ca, P, Na and K have been registered. The thickness of glomerular, fascicular and reticular zones in sections of the adrenal glands has been measured under a microscope.

Results. It has been found that the mineralocorticoid activity, which is estimated by the Na/K ratio of plasma, increases to the maximum. Further, in the descending order, follow: testosterone level, thickness of the fascicular zone of the adrenal cortex (ZAC), heart rate, sympathetic tone, thickness of the reticular ZAC and plasma level of triiodothyronine. Hormonal constellation consisting of calcitonin activity (calculated by the formula: \((Cau \cdot Pu/Pp \cdot Cap)^{2.29}\)), 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 (calculated by the formula: \((Cap \cdot Pu/Pp \cdot Cau)^{2.26}\)) have been significantly decreased. The integral quantitative measure of the neuroendocrine response to the factors of chronic stress as the mean value of the significant modules of Z-units is 0.61 ± 0.10, that is, it is not significantly different from revealed earlier by males: 0.47 ± 0.04. However, there are sexual differences between the post stressed neuro-endocrine profiles not only in the severity of reactions, but also in their sign.

Key words: Chronic Stress, HRV, adaptation hormones, adrenals, female rats.