KOZYAVKINA, Nataliya, POPOVYCH, Igor, POPOVYCH, Dariya, ZUKOW, Walery & BOMBUSHKAR, Igor. Sexual dimorphism in some psycho-neuro-endocrine parameters at humana. Journal of Education, Health and Sport. 2021;11(5):370-391. eISSN 2391-8306. DOI http://dx.doi.org/10.12775/JEHS.2021.11.05.039
https://apcz.umk.pl/JEHS/article/view/43195
https://zenodo.org/record/7749226

The journal has had 5 points in Ministry of Science and Higher Education parametric evaluation. § 8. 2) and § 12. 1. 2) 22.02.2019.

Received: 15.04.2021. Revised: 22.05.2021. Accepted: 30.05.2021.

Sexual dimorphism in some psycho-neuro-endocrine parameters at human

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Abstract

Background. Despite the well-documented relationships between HRV, EEG and anxiety parameters, studies of their sexual dimorphism, judging by the results of the meta-analysis, have so far been conducted separately. The purpose of this study is to clarify sexual dimorphism in ***almost synchronously*** registered psycho-neuro-endocrine parameters. **Materials and Methods**. The object of observation were practically healthy volunteers: 30 females (30÷76 yr) and 31 males (24÷69 yr). In basal conditions we recorded the ongoing HRV and EEG, determined serum levels of testosterone, calcitonin, cortisol, aldosterone, and triiodothyronine, estimated the severity of the trait and reactive anxiety. After 4 or 7 days, repeated testing was performed. **Results**. Regardless of age, females differ significantly from males, except for drastically lower levels of testosterone and calcitonin by definition (but not cortisol, aldosterone and triiodothyronine), lower levels of HRV-markers of sympathetic tone (but not heart rate), reactive anxiety, and beta-rhythm asymmetry. On the other hand, trait anxiety, levels of HRV-markers of vagal tone, variability and amplitude of the beta-rhythm, and its power spectral density (PSD) in 12 loci (maximum differences in T6, F3, and T3 loci), amplitude of the theta-rhythm and its PSD in 16 loci (maximum differences in F3, C3, and T3 loci), PSD of the alpha-rhythm in T3, T6, F7, and T4 loci as well as entropy of PSD in F7 and F8 loci are significantly higher in females than in males. The listed parameters are determined by testosterone for 31,7%, and calcitonin for 26,3%. The method of discriminant analysis revealed 20 EEG and 6 HRV parameters as well as trait and reactive anxiety, based on the totality of which males can be retrospectively recognized (without testosterone and calcitonin) with an accuracy of 90,3%, and females with an accuracy of 88,3%. **Conclusion.** The

revealed differences between the sexes were not influenced by at least two bioactive factors, that is, they are robust sex markers.

Keywords: EEG, HRV, anxiety, testosterone, calcitonin, cortisol, aldosterone, triiodothyronine, males, females, sexual dimorphism.

INTRODUCTION

Following Gillies GE & McArthur S [22], in this article, the term *sex* will be used to distinguish male or female subjects according to the reproductive organs and functions that derive from the chromosomal complement (individual organisms bearing the male XY or female XX sex chromosomes seen in most mammals). This is distinct from the term *gender*, used to refer to a human subject's self-representation as male or female.

The last decade has seen an exponential increase in evidence for structural, cellular, and molecular sex differences in the brain that can be described as true *dimorphisms*, defined as the occurrence of two forms in the same species. These include regions of human and animal brains that are important for cognition, memory, and affect, such as the hippocampus, amygdala, and cortex, and for regions controlling sensorimotor and reward systems. Indeed, post mortem studies, as well as evidence from new technologies for in vivo imaging, are adding rapidly to the view that sex differences in the human brain may be the norm rather than the exception [review: 22].

Recently Cave AE & Barry RJ [13] stated: surprisingly, differences in resting EEG between females and males have not been investigated systematically in previous literature.

Only Clarke AR et al [15] investigated sex differences (and age-related changes) in the EEGs of normal children (40 boys and 40 girls, between the ages of 8 and 12 years). Sex differences were found, with males having less theta and more alpha than females. Females were also found to have a developmental lag in the EEG compared with males.

Cave AE & Barry RJ [13] in the present study utilised the four traditional EEG bands (delta: 0.5-3.5 Hz, theta: 4.0-7.5 Hz, alpha: 8.0-13.0 Hz, and beta: 13.5-29.5 Hz) to clarify topographical differences between sexes. Participants were 80 healthy young adults (40 female), with a mean age of 20,4 (range 18-26) yr. Continuous resting EEG was recorded from 30 scalp sites during 2-minute condition. Females had greater overall amplitudes in delta, alpha and beta, enhanced midline activity in theta, and parietal and midline activity in the alpha and beta bands. These findings indicate significant differences in neuronal activity between young adult females and males.

Proverbio AM [46] has just published a meta-analysis of sexual dimorphism in hemispheric processing of faces in humans. Thirty-four articles met the inclusion criteria of a sufficiently large and balanced sample size with strictly right-handed and healthy participants aged 18-35 yr and N170 measurements in response to neutral front view faces at left and right occipito/temporal sites. The data of 817 male (n = 414) and female (n = 403) healthy adults were subjected to repeated-measures analyses of variance. The results of statistical analyses from the data of 17 independent studies (from Asia, Europe and America) seem to robustly indicate the presence of a sex difference in the way the two cerebral hemispheres process facial information in humans, with a marked right-sided asymmetry of the bioelectrical activity in males and a bilateral or left-sided activity in females.

Sexual differences in HRV are much better studied.

Abhishekh HA et al [1] assessed effect of sex (and age) on autonomic regulation of heart in healthy volunteers (114 males and 75 females). On multiple regression analysis to control for effect of age and heart rate while comparing males and females, LFnu showed significant reduction suggesting lower sympathetic tone in females and HFnu showed increase at trend level. In conclusion, females showed greater vagal tone than male.

Park SB et al [38] registered HRV in healthy 366 men and 271 women. The mean age of these subjects was 45,1±10,7 yr. The men showed that their TP, LF, and LF/HF values were significantly higher than women whereas SDNN, RMSSD, HF showed no significant differences between sexes. With increasing age, there was no significant decrease in HR. TP, SDNN, LF, and HF were significantly decreased when getting older. On the contrary, LF/HF had no significant difference related to age. In conclusion, middle-aged men had more pronounced sympathetic influence than women in cardiac regulation, and HRV declined linearly with age.

Kuo TB et al [31] determined the various parameters of HRV in a large population of normal humans between 40 and 79 yr of age, from which the effects of sex and aging on cardiac sympathetic and parasympathetic controls were evaluated. ANOVA detected significant effects of age on all the absolute measurements (TP, VLF, LF, HF) and LF/HF and LF% and significant effects of sex on all the relative measurements (LF/HF, LF%, HF%, and HF. For women, significant changes from the age stratum of 40 yr were not detected until the age strata of 50 yr for variance, 55 yr for VLF, 50 yr for LF, 50 yr for HF, and 65 yr for LF%. For men, significant changes in variance, VLF, LF, HF, LF/HF, and LF% were not detected until the age stratum of 60 yr. When the effect of gender was analyzed for each age stratum, it was noted that women exhibited a greater absolute HF at ages 40–49 yr. Dramatic disparities between genders were detected in LF/HF, LF%, and HF% at ages 40–59 yr, when men had higher LF/HF and LF% and lower HF%. All differences disappeared in the age strata ≥60 yr.

Huikuri HK et al [26] studied HRV in randomly selected, age-matched populations of middle-aged women (n = 186; 50±6 yr) and men (n = 188; 50±6 yr) without hypertension, diabetes, or clinical or echocardiographic evidence of heart disease. Authors shown that the LF band was lower in women, whereas the HF band was higher in women than in men. The LF/HF ratio also was lower in the women. The increase of HR and decrease of HF band in response to an upright posture were smaller in magnitude in women than in men. After adjustment for differences in the baseline-variables, such as blood pressure, HR, smoking, alcohol consumption, and psychosocial score, the sex-related differences in HRV variability still remained significant.

Koenig J & Thayer JF [29] carried out a meta-analysis of sex differences in healthy human heart rate variability. Data from 63,612 participants (31,970 females) were available for analysis. Females showed a significantly lower mean RR interval and SDNN. The power spectral density of HRV in females is characterized by significantly less total power that contains significantly greater HF and less LF power. This is further reflected by a lower LF/HF ratio. Although women showed greater mean heart rate, they showed greater vagal activity indexed by HF power of HRV.

Sztajzel J et al [57] studied temporal and spectral HRV indices obtained from 24-hour Holter recordings in 32 healthy volunteers (14 men and 18 women, mean age 29±3 yr) during 2 days of their usual all-day activity. Time-domain measures and the spectral LF and HF components as well as the LF/HF ratio were comparable on both test days. Significantly higher values on test day 2 were observed only for the spectral VLF component and for the resulting total power. Compared to men, women had higher day- and nighttime vagus-associated HRV indices, including RMSSD, pNN_{50}, and HF power, and lower day- and nighttime VLF and LF power with lower LF/HF ratio and total power.

Significant relationships between EEG and HRV parameters are known. Tang YY et al [58] analysed the correlation between the changes in frontal midline θ power (related to generators in the anterior cingulate cortex [10]) and HFnu HRV. After 5 days of integrative body-mind training correlations between HFnu and Fz-θ, FCz-θ and Cz-θ were significantly positive. We [42,43] also found in healthy male correlations between HFnu and F4-θ as well as P4-θ, between HF% and Fp1-θ as well as P4-θ also between RMSSD and P4-θ. Prinsloo

GE et al [44] found that less pronounced changes in HRV, due to work-related stress, accompanied by higher relative PSD Fz-θ, Pz-θ and Cz-θ, lower fronto-central relative β power and higher θ/β ratio. It is also perfectly consistent with our [42,43] data on a negative correlation of LFnu, LF% and LF/HF, on the one hand, with F4-θ, P4-θ, F7-θ, F8-θ while positive correlation with F7-β and F8-β - on the other hand, as well as a positive correlation of HF% with Fp1-θ and P4-θ while negative with P4-β.

The described connections are made within the framework of central autonomic network [8,37] that include following cortical, subcortical, and medullary structures: the anterior cingulate, insular, orbitofrontal, and ventromedial cortices; the central nucleus of the amygdala; the paraventricular and related nuclei of the hypothalamus; the periaqueductal gray matter; the nucleus of the solitary tract; the nucleus ambiguous; the ventrolateral medulla; the ventromedial medulla and the medullary tegmental field.

Therefore, the simultaneous registration of EEG and HRV parameters can be more informative regarding the detection of sexual dimorphism. However, we were unable to find any publication on this issue. The closest is a publication on the sexual dimorphism in HRV and the activity of certain brain regions.

Nugent AC et al [35] by electrophysiology and neuroimaging studies have revealed sex-related differences in autonomic cardiac control, as reflected in measurements of HRV. In this study, young male and female healthy subjects underwent $H_2{}^{15}O$-positron emission tomography and ECG recording while performing a handgrip motor task and an n-back task. Indices of HRV were calculated and correlated with regional cerebral blood flow (rCBF). Authors hypothesized that sex differences would be evident in brain regions known to participate in autonomic regulation: the anterior insula, the anterior cingulate cortex, the orbitofrontal cortex, and the amygdala. The study found that associations between rCBF and parasympathetic indices differed significantly between female and male subjects in the amygdala. Females showed a positive correlation between rCBF and parasympathetic indices while males exhibited negative correlations. This differential correlation of amygdala rCBF and parasympathetic activity between males and females may reflect differences in parasympathetic/sympathetic balance between sexes, consistent with known sexual dimorphism in the amygdala and closely related structures such as the hypothalamus.

Chalmers JA et al [11] carried up meta-analyses based on 36 articles, including 2086 patients with an anxiety disorder and 2294 controls. HF HRV was reduced in participants with any anxiety disorder, regardless of specific diagnosis, relative to healthy controls. LF HRV did not differ between participants with any anxiety disorder and controls. Overall, anxiety disorders were characterized by lower HF HRV than controls. Panic disorder ($n = 447$), post-traumatic stress disorder ($n = 192$), generalized anxiety disorder ($n = 68$), and social anxiety disorder ($n = 90$), but not obsessive-compulsive disorder ($n = 40$), displayed reductions in HF HRV relative to controls. Authors concluded that anxiety disorders are associated with reduced HRV, findings associated with a small-to-moderate effect size. Findings have important implications for future physical health and well-being of patients, highlighting a need for comprehensive cardiovascular risk reduction. A key feature of the neurovisceral integration model is the central autonomic network, a network of brain regions that coordinates autonomic, endocrine, and behavioral responses in goal directed action and in adaptation to environmental challenges. The integrity of this network is compromised in anxiety; sympathoexcitatory responses are unable to be effectively inhibited, leading to behavioral inflexibility. The neurovisceral integration model also links hypervigilance and worry – features observed in all the anxiety disorders – to reductions in HRV.

Based on the above the purpose of this study is to clarify sexual dimorphism in ***almost synchronously*** registered psycho-neuro-endocrine parameters.

MATERIAL AND METHODS

Volunteers-employees of the clinical sanatorium "Moldova" and PrJSC “Truskavets’ Spa” were under our observation: 30 females (30÷76; 49±13 y) and 31 males (24÷69; 47±12 y). The volunteers were considered practically healthy (without a clinical diagnosis), but the initial testing revealed deviations from the norm in a number of parameters of the neuro-endocrine-immune complex as a manifestation of maladaptation. Testing was performed twice with an interval of 4 ("Moldova") or 7 (“Truskavets’ Spa”) days.

We determined the plasma levels of hormones as Testosterone, Calcitonin, Cortisol, Aldosterone, and Triiodothyronine (by the ELISA with the use of corresponding sets of reagents from “Алкор Био”, XEMA Co Ltd, and DRG International Inc). The analyzes were carried out according to the instructions. The analyzers “RT-2100C” (PRCh) used.

To assess the parameters of heart rate variability (HRV) we recorded during 7 min electrocardiogram in II lead (software-hardware complex "CardioLab+HRV", KhAI-MEDICA, Kharkiv). For further analysis the following parameters HRV were selected [6,9,24,49]. Temporal parameters (Time Domain Methods): the standard deviation of all NN intervals (SDNN), the square root of the mean of the sum of the squares of differences between adjacent NN intervals (RMSSD), the percent of interval differences of successive NN intervals greater than 50 msec (pNN_{50}). Spectral parameters (Frequency Domain Methods): absolute ($msec^2$) and relative (% of total) power spectrum density (PSD) bands of HRV: high-frequency (HF, range 0,4÷0,15 Hz), low-frequency (LF, range 0,15÷0,04 Hz), very low-frequency (VLF, range 0,04÷0,015 Hz) and ultralow-frequency (ULF, range 0,015÷0,003 Hz). Derived indices were calculated: (VLF+LF)/HF as Centralization Index, LF/HF as Sympatho-vagal Balance Index, HFnu as Sympathetic Tone Index.

Simultaneosly EEG recorded a hardware-software complex “NeuroCom Standard” (KhAI MEDICA, Kharkiv) monopolar in 16 loci (Fp1, Fp2, F3, F4, F7, F8, C3, C4, T3, T4, P3, P4, T5, T6, O1, O2) by 10-20 international system, with the reference electrodes A and Ref on tassels the ears. Two minutes after the eyes had been closed, 25 sec of artifact free EEG data were collected by computer. Among the options considered the average EEG amplitude (μV), average frequency (Hz), frequency deviation (Hz) as well as absolute ($\mu V^2/Hz$) and relative (%) PSD of basic rhythms: β (35÷13 Hz), α (13÷8 Hz), θ (8÷4 Hz) and δ (4÷0,5 Hz) in all loci, according to the instructions of the device.

In addition, we calculated coefficient of Asymmetry (As) and Laterality Index (LI) for PSD each Rhythm using equations [34]:

As, % = 100•(Max – Min)/Min; LI, % = Σ [200•(Right – Left)/(Right + Left)]/8.

We calculated for HRV and each locus of EEG the Entropy (h) of normalized PSD using Popovych’s IL [40] equations based on classic Shannon’s CE [48] equation:

$hEEG=-[PSD\alpha \bullet \log_2 PSD\alpha + PSD\beta \bullet \log_2 PSD\beta + PSD\theta \bullet \log_2 PSD\theta + PSD\delta \bullet \log_2 PSD\delta]/\log_2 4;$

$hHRV=-[PSHF \bullet \log_2 PSHF + PSLF \bullet \log_2 PSLF + PSVLF \bullet \log_2 PSVLF + PSULF \bullet \log_2 PSULF]/\log_2 4$

At last volunteers filled a questionnaire with the purpose of estimation of level of the trait and reactive anxiety by STAI of Spielberg ChD [53] in modification of Khanin YL [45].

Results processed by using the software package "Statistica 6.4".

RESULTS AND DISCUSSION

According Instruction for use sets of reagents for the determination of Testosterone in human serum or plasma (“Testosterone EIA”, XEMA Co., Ltd, RF), the following normal range (nM/L) is recommended: females 0,15÷4,6; males 20-39 yrs 9,0÷38; 40-55 yrs 6,9÷21; >55 yrs 5,9÷18,1. According Instruction for the determination of Calcitonin (DRG® Calcitonin ELISA) the values obtained on the normal females ranged from 0,1 to 10,0 ng/L while on males from 0,2 to 27,7 ng/L. This sample is characterized (Mean±SE) by

testosteronemia 3,5±0,4 nM/L vs 13,5±0,8 nM/L and by calcitoninemia 5,7±0,4 ng/L vs 10,5±0,9 ng/L respectively.

It is interesting that in comparative studies conducted in the period 1977-2021, the average values were very different, which is due to the peculiarities of the analysis methods, but sexual dimorphism was always found: 31 (range, <25÷51) vs 49 (range, <25÷73) [25]; 63,4±34,7 vs 92,6±35,4 [32]; 1,9 vs 2,7 [52] ng/L in female and male respectively.

Therefore, the cohort observed by us as a whole meets the criteria of normality and sexual dimorphism.

On the other hand, there is no sexual dimorphism in the levels of other determined hormones: Cortisol 304±14 and 298±16 nM/L, Aldosterone 226±5 and 226±4 pM/L, Triiodothyronine 2,19±0,12 and 2,01±0,11 nM/L in females and males respectively.

In order to compare the parameters expressed in nM/L, ng/L, μV, Hz, μV^2/Hz, msec, $msec^2$, %, and points they were recalculated in Z-score according to the equation:

Z = (V/M -1)/Cv, where

V is the current value, M is the average for the sample, Cv is the coefficient of variation.

It was found that the Z-score (Mean±SE) for testosterone is -0,72±0,06 in females vs +0,72±0,11 in males, that is, sexual dimorphism is 1,44. The expression of sexual dimorphism of calcitoninemia is almost half as low: 0,83.

Screening of recorded parameters revealed that regardless of age, females differ significantly from males, except for drastically lower levels of testosterone and calcitonin by definition, lower levels of HRV-markers of sympathetic tone (but not heart rate), reactive anxiety, and beta-rhythm asymmetry. On the other hand, trait anxiety, levels of HRV-markers of vagal tone, variability and amplitude of the beta-rhythm, and its PSD in 12 loci (maximum differences in T6, F3, and T3 loci), amplitude of the theta-rhythm and its PSD in 16 loci (maximum differences in F3, C3, and T3 loci), PSD of the alpha-rhythm in T3, T6, F7, and T4 loci as well as entropy of PSD in F7 and F8 loci are significantly higher in females than in males. It is also worth noting the much greater variability (SE) of neuro-endocrine (but not anxiety) parameters in women compared to men (Figs 1 and 2).

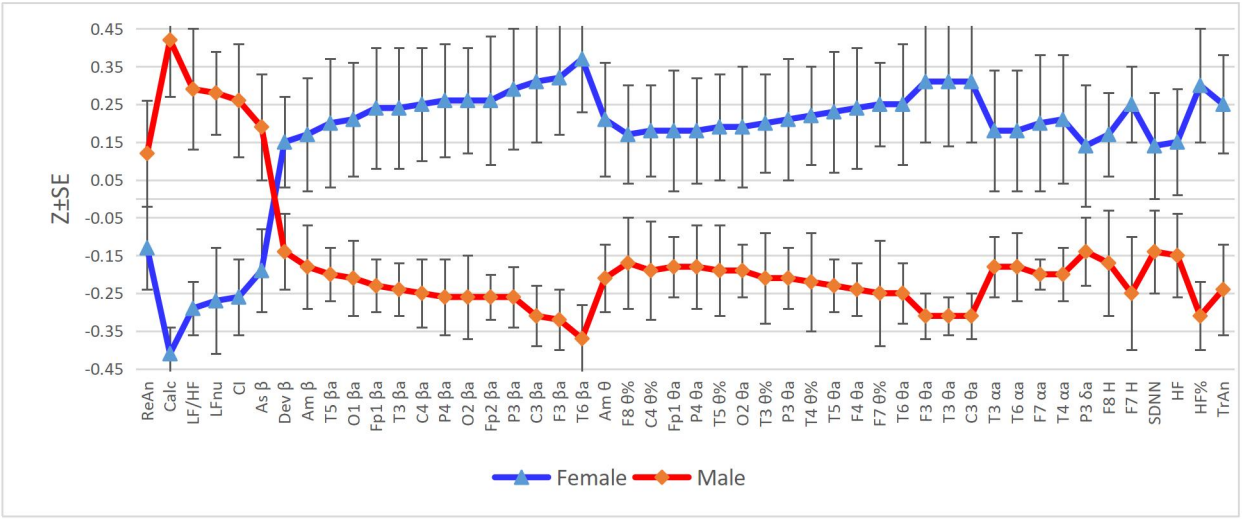


Fig. 1. Profiles of psycho-neuro-endocrine parameters (Z±SE) that differ in female and male. Testosterone (-0,72±0,06 vs +0,72±0,11) is not shown, so as not to coarsen the scale

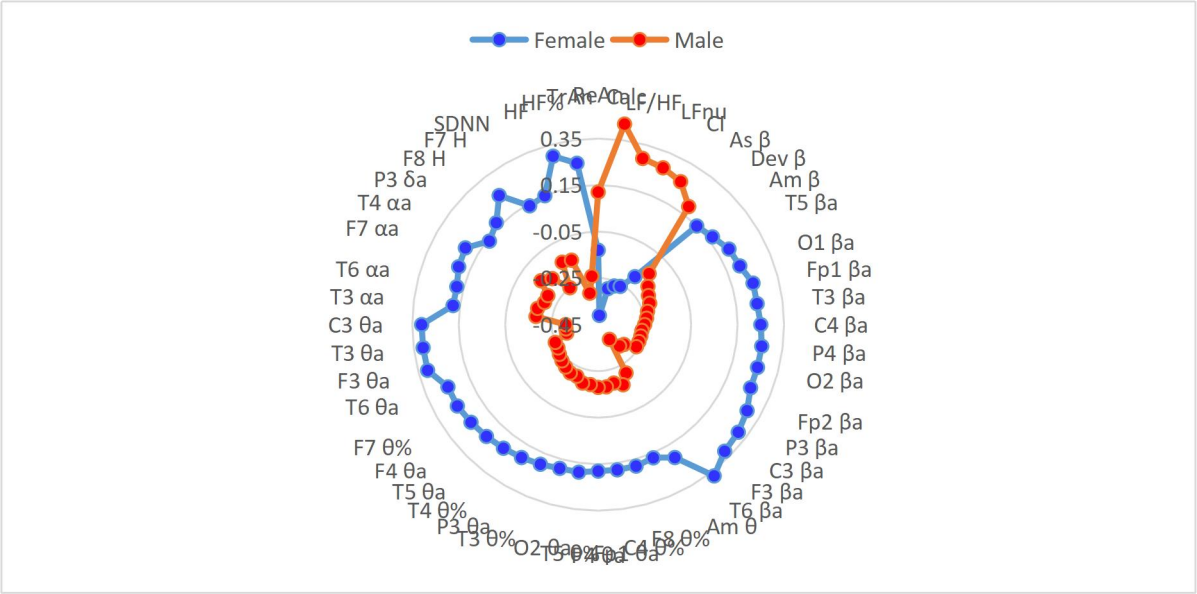


Fig. 2. Petal diagram of sexual dimorphism in psycho-neuro-endocrine parameters

The obviousness of the relationship between testosteroneemia and EEG/HRV parameters is documented by a regression model with stepwise exclusion until the maximum Adjusted R^2 is reached (Table 1).

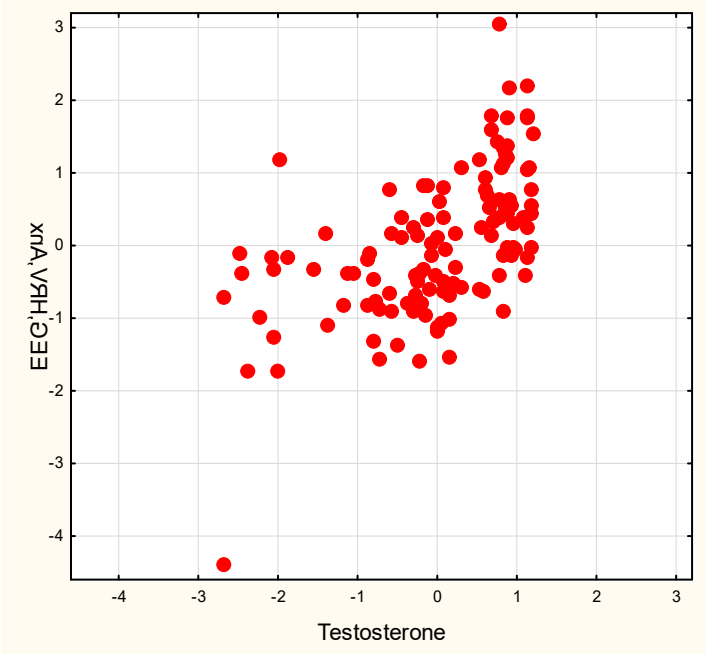
Table 1. Regression Summary for serum Testosterone

$R=0,563$; $R^2=0,317$; Adjusted $R^2=0,242$; $F_{(12,1)}=4,2$; $p<10^{-4}$

N=122		Beta	St. Err. of Beta	B	SE of B	$t_{(109)}$	p-level
Variables	r		Intercpt	24,36	9,52	2,56	0,012
F3-θ PSD, μV²/Hz	**-0,29**	-0,425	0,157	-0,059	0,022	-2,71	0,008
C3-θ PSD, μV²/Hz	**-0,23**	-0,261	0,193	-0,036	0,027	-1,35	0,181
T3-β PSD, μV²/Hz	**-0,23**	-0,300	0,114	-0,028	0,011	-2,63	0,010
Fp1-β PSD, μV²/Hz	**-0,20**	-0,163	0,150	-0,023	0,021	-1,08	0,282
T5-θ PSD, %	**-0,17**	0,331	0,170	0,051	0,026	1,95	0,053
F4-θ PSD, μV²/Hz	**-0,16**	0,208	0,163	0,035	0,027	1,28	0,203
C4-β PSD, μV²/Hz	**-0,13**	0,283	0,147	0,027	0,014	1,92	0,057
HF PSD ,%	**-0,22**	-0,320	0,149	-0,269	0,125	-2,15	0,034
Trait Anxiety, point	**-0,15**	-0,184	0,085	-0,159	0,074	-2,16	0,033
Asymmetry-β, %	**0,25**	0,273	0,083	0,121	0,037	3,31	0,001
LFnu, %	**0,21**	-0,188	0,186	-0,106	0,105	-1,01	0,315
LF/HF	**0,19**	0,180	0,122	0,228	0,155	1,47	0,145

Note. For a sample of 122 observations critical value of correlation coefficient module at $p<0,05$ ($t>1,98$) is 0,17.

It was established that testosteroneemia downregulates the activity of theta- and beta-rhythm generating neurons, which are projected, as a rule, on the left loci of the scalp. This is accompanied by a rightward shift in the symmetry of the beta-rhythm and a decrease in vagal tone as well as trait anxiety in combination with an increase in sympathetic tone. The listed parameters are determined by testosterone for 31,7% (Fig. 3).



R=0,563; R^2=0,317; $\chi^2_{(12)}$=43; $p<10^{-4}$; Λ Prime=0,683

Fig. 3. Scatterplot of canonical correlation between serum Testosterone (X-line) and Psycho-Neural parameters (Y-line) in both sexes

It is obvious that the revealed neurotropic effects of testosterone are realized through androgen receptors (AR) which are widely expressed in the brain [review: 22]. Surprisingly, we were unable to find any publications on the effect of testosterone on EEG. Moreover, only one study was found on the neurotropic effect of testosterone assessed by blood oxygen level dependent (BOLD) signal. Nine healthy estrogen-treated postmenopausal women (55,4±3,8 yr) completed the study. Twenty-six weeks of testosterone therapy was associated with significant **decreases** in BOLD intensity during the mental rotation task in the right superior parietal, left inferior parietal, and left precuneus regions, and during the verbal fluency task in the left inferior frontal gyrus, left lingual gyrus, and medial frontal gyrus, with no change in task performance, accuracy, or speed [17].

Testosterone, the primary circulating androgen, is made by the Leydig cells of the testicles and acts unmodified or following conversion to the more potent dihydrotestosterone. Testosterone can also be converted to estradiol by the aromatase enzyme. In women, estradiol is made primarily in the granulosa and theca cells of the ovarian follicles. In men, ~15% of estradiol is secreted directly from the testes, and the remaining ~85% is derived from peripheral aromatization. Contrary to the abrupt decline of estradiol during menopause, older men do not experience a true “andropause,” and total estradiol concentrations remain above a level sufficient to maintain skeletal homeostasis [14]. From the evolutionary perspective testosterone is a precursor of estradiol, dihydrotestoterone and other metabolites rather than a hormone *per se.* Testosterone does not only act per se, but also *via* the products of its metabolism. Reduction to dihydrotestosterone by 5-alpha reductase increases the androgen activity, conversion to estradiol by aromatase converts the androgen to estrogen activity [review: 14]. Estrogen receptors Eα and Eβ are widely expressed in the brain along with androgen receptors. Thus, circulating testosterone and estradiol levels are intrinsically related [review: 2].

Back in 1994 it has been reported that estrogen has a facilitating effect on cardiac vagal function in rat [19]. So SY & Savidge TC [50] in the recent excellent review note that not surprisingly, the sexual dimorphic findings in autonomic neuronal function relate to sex hormones modulating this system. For instance, sympathetic activity is often increased in the

luteal phase of the menstrual cycle or during menopause when estrogen levels are reduced. Surgical-induced menopause reduced parasympathetic nervous system activity and shifted this towards sympathetic hyperactivity. Although some studies found no effect, others reported that estrogens in hormone replacement therapy facilitate parasympathetic activity and suppress sympathetic signaling in postmenopausal women. Estrogen may reduce sympathetic fiber density directly through affecting Eα receptor expressed in sympathetic neurons, or indirectly through affecting target tissue or specific molecules. However, another study reported that estrogen is positively correlated to sympathetic activity in men. Nevertheless, estrogens are generally reported to inhibit sympathetic activity while sex could possibly influence the effect. In contrast to estrogens, androgens are associated with sympathetic hyperactivity in females. The postmenopausal ovary continues to produce androgens, serum testosterone or other androgen levels do not change significantly across menopause . In polycystic ovary syndrome patients, who often suffer from hyperandrogenism, sympathetic activity was enhanced whereas parasympathetic signaling was suppressed. Furthermore, excess neonatal androgen in female mice increases sympathetic tone in cardiometabolic tissues. However, several studies reported that androgens are positively correlated with parasympathetic activity in males. Also, a study found that males with low testosterone levels were unable to maintain cardiosympathetic and cardiovagal responses. These inconsistent findings suggest that autonomic control mediated by sex steroids could be sex-dependent, as well as modulated by health and hormonal status of the individual.

In addition to a direct effect on brain stem and peripheral neurons of the autonomic nervous system, sex hormones can modulate vagal and sympathetic tones, influencing cortical neurons within the framework of the central autonomic network (Fig. 4).

The prefrontal, cingulate, and insular cortex form an interconnected network with bi-directional communication with the amygdala. The amygdala is under tonic inhibitory control via prefrontal vagal pathways to intercalated cells in the amygdala. The activation of the central amygdala nucleus (CeA) inhibits the nucleus of the solitary tract (NTS) which in turn inhibits inhibitory caudal ventrolateral medullary (CVLM) inputs to the rostral ventrolateral medullary (RVLM) sympathoexcitatory neurons, and simultaneously inhibits vagal motor neurons in the nucleus ambiguus (NA) and the dorsal vagal motor nucleus (DVN). In addition, the CeA can directly activate the sympathoexcitatory neurons in the RVLM. The enhancing of prefrontal activity (particular by electrostimulation) leads to the vagotonic shift in sympatho-vagal balance. While inhibition of prefrontal activity (*particular by testosterone – our note*) leads to disinhibition of sympathoexcitatory circuits [12,37,47,59,61,62]. The above explains the possible mechanism of testosterone-induced higher level of sympathetic tone and lower level of vagal tone in males compared to females.

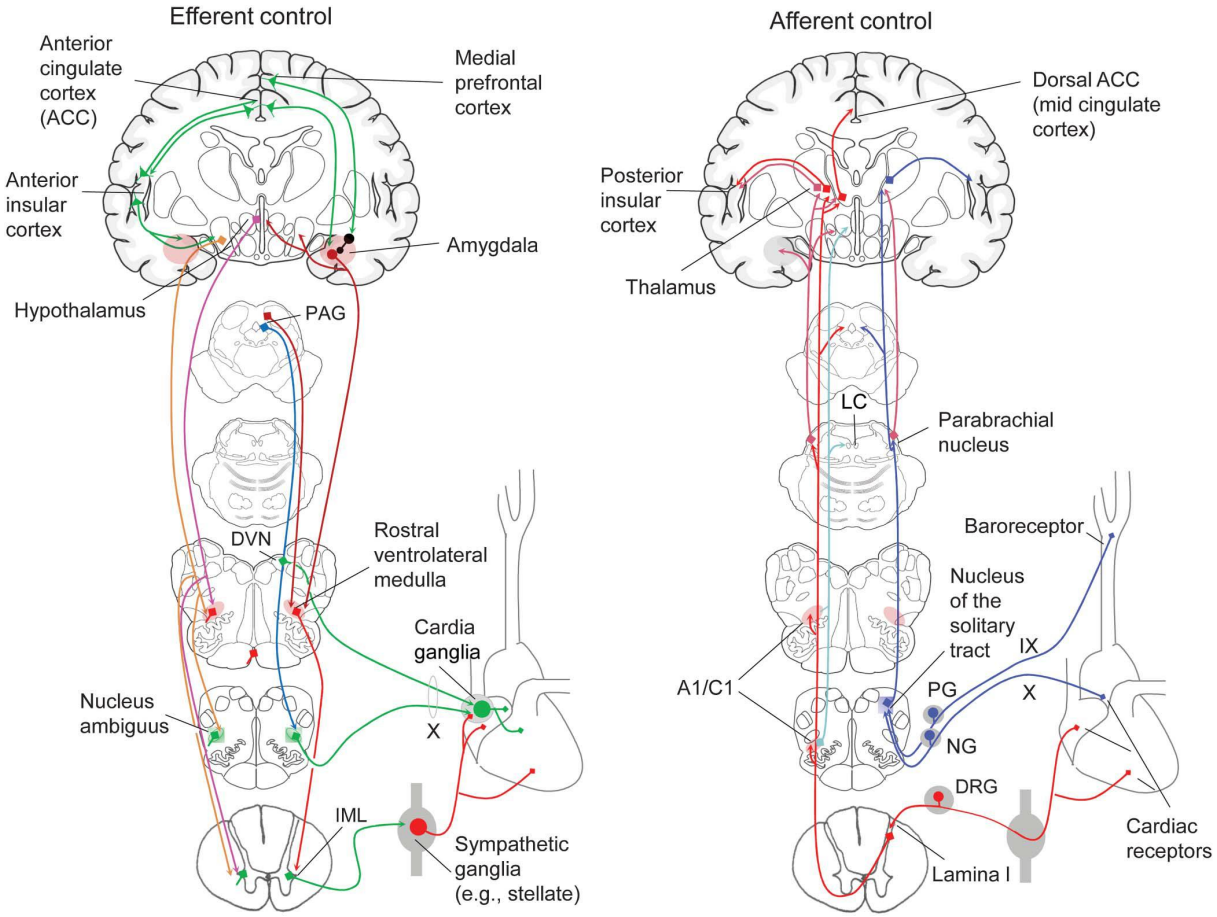


Fig. 4. Efferent and afferent control of cardiac function [37]

Normal or moderate amounts of anxiety naturally occur in a healthy individual. It has been previously reported that people can use anxiety for self-motivation (so-called anxiety motivation) [39,51]. Women are twice as likely to suffer from anxiety disorders when compared to men. The higher prevalence may be related to fluctuations of sex steroide hormones during different life periods, such as puberty, pre-menstruum, pregnancy, postpartum, and menopause [7,33,51]. The presence among objects of testosterone's influence of anxiety is naturally connected with its effect on the amygdala. There is a wealth of evidence for the involvement of amygdala in anxiety disorders. Furthermore, the magnitude of amygdala activation is correlated with symptom severity, such that hyperactivation actually decreases or even normalizes following successful treatment of anxiety disorders [54,55,60].

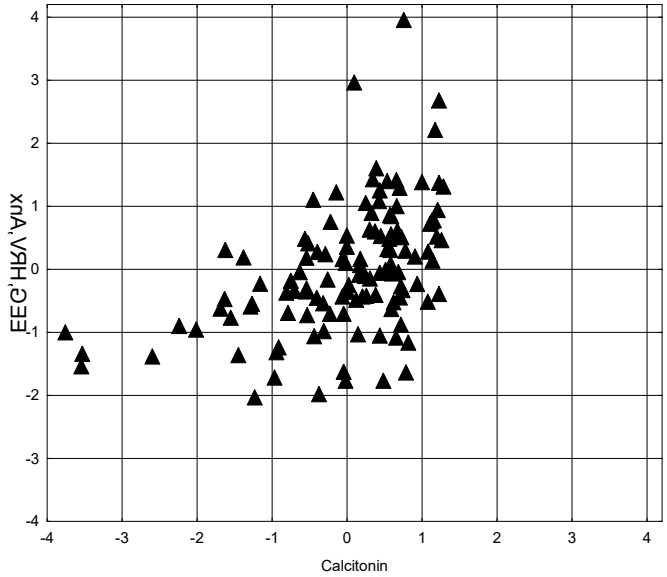
Stefanaki Ch et al [56] detected that in the euglycemic group, anxiety score negatively correlated with LF/HF (rho = -0.781); LF % (rho = - 0.863); and positively correlated with HF % (rho = 0.863). While in the prediabetic group, the anxiety score was negatively correlated with SDNN (rho = -0.585); HF% (rho = -0.609); and positively correlated with LF/HF (rho = 0.602); LF % (rho = 0.63); and HF% (rho = -0.63).

The above explains why a higher level of testosterone in males is accompanied by a lower level of trait, but not reactive, anxiety.

Now let's consider the psycho-neural connections of another endocrine marker of sexual dimorphism. Calcitoninemia exerts a regulatory influence, shared with such testosteronenemia, only on 5 parameters out of 13 included in the regression model (Table 2), determining them by 26,3% (Fig. 5).

Table 2. Regression Summary for serum Calcitonin
R=0,513; R^2=0,263; Adjusted R^2=0,174; $F_{(13,1)}$=3,0; $p<10^{-3}$

N=122		Beta	St. Err. of Beta	B	SE of B	$t_{(108)}$	p-level
Variables	r		Intercpt	-10,21	7,67	2,56	0,186
C3-θ PSD, μV²/Hz	**-0,21**	-0,723	0,275	-0,083	0,032	-2,63	0,010
T3-θ PSD, μV²/Hz	**-0,20**	-0,405	0,177	-0,050	0,022	-2,28	0,024
C4-θ PSD, μV²/Hz	**-0,20**	0,592	0,254	0,058	0,025	2,33	0,022
P4-β PSD, μV²/Hz	**-0,20**	-0,944	0,260	-0,083	0,023	-3,64	10^{-3}
C3-β PSD, μV²/Hz	**-0,16**	0,567	0,235	0,043	0,018	2,42	0,017
F3-β PSD, μV²/Hz	**-0,16**	-0,286	0,182	-0,029	0,018	-1,57	0,120
T3-β PSD, μV²/Hz	**-0,16**	-0,190	0,155	-0,015	0,012	-1,23	0,223
Amplitude-β, μV	**-0,18**	0,566	0,232	0,857	0,351	2,44	0,016
Asymmetry-β, %	**-0,12**	-0,282	0,123	-0,103	0,045	-2,30	0,023
F7-α PSD, μV²/Hz	**-0,12**	0,680	0,200	0,029	0,008	3,40	0,001
Trait Anxiety, point	**-0,13**	-0,170	0,089	-0,121	0,063	-1,92	0,057
HF PSD ,%	**-0,12**	0,293	0,162	0,204	0,113	1,81	0,073
LFnu, %	**0,23**	0,581	0,166	0,270	0,077	3,49	0,001



R=0,513; R^2=0,263; $\chi^2_{(12)}$=29; $p<10^{-3}$; Λ Prime=0,772

Fig. 5. Scatterplot of canonical correlation between serum Calcitonin (X-line) and Psycho-Neural parameters (Y-line) in both sexes

Significant differences regarding the topography of the regulatory neurotropic effects of both hormones are confirmed by the absence of correlations between their levels in the serum of both men (Fig. 6) and women of two age groups (Figs 7 and 8). It should be noted that the levels of testosteroneemia in 1/5 women and calcitoninemia in 1/10 women exceeded the upper limit of normal, while in men, both hormones, with a few exceptions, were within the normal range.

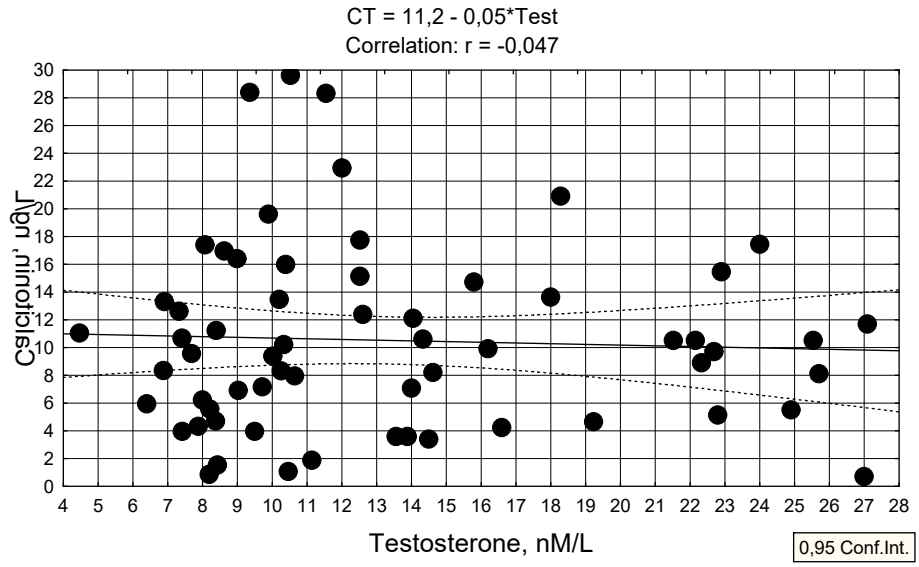


Fig. 6. Scatterplot of correlation between serum Testosterone (X-line) and Calcitonin (Y-line) in Males

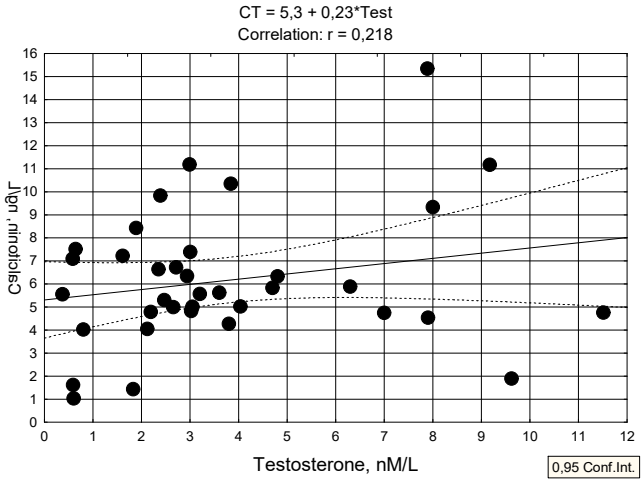


Fig. 7. Scatterplot of correlation between serum Testosterone (X-line) and Calcitonin (Y-line) in postmenopausal Females

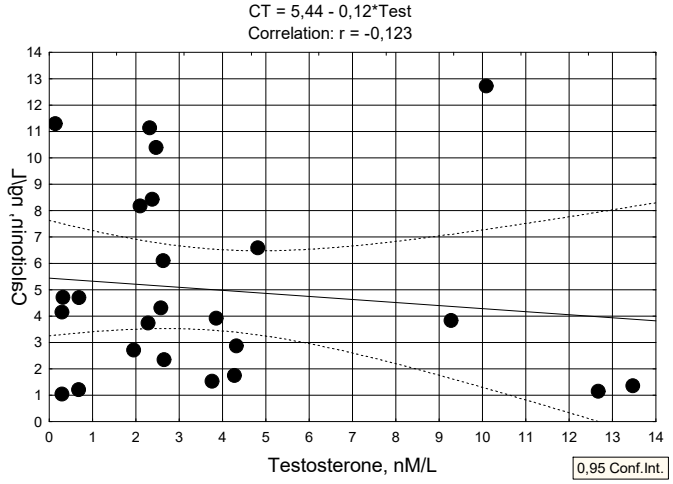
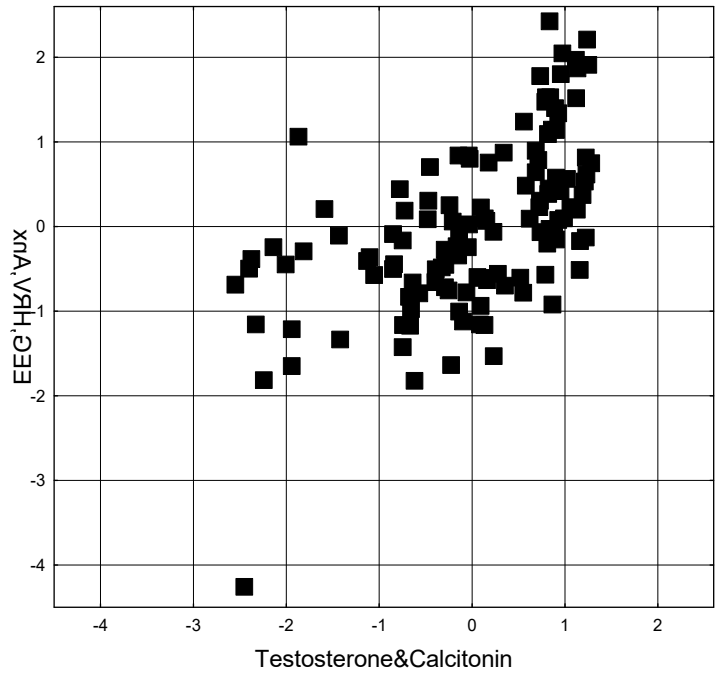


Fig. 8. Scatterplot of correlation between serum Testosterone (X-line) and Calcitonin (Y-line) in reproductine age Females

The canonical correlation analysis revealed that the additional inclusion of calcitonin in the factor structure of the hormonal root has almost no effect on the measure of endocrine determination of psycho-neural parameters: 33,1% vs 31,7% (Fig. 9).

Table 3. Factor structure of the canonical roots, which represent the endocrine and psychoneral parameters

Hormones	Root
Testosterone	-0,995
Calcitonin	-0,338
Psycho-neural	Root
F3-θ PSD	0,524
F3-β PSD	0,398
F4-θ PSD	0,311
T3-β PSD	0,412
C3-β PSD	0,441
C3-θ PSD	0,424
C4-θ PSD	0,400
C4-β PSD	0,259
T5-θ PSD	0,316
P4-β PSD	0,247
Fp1-β PSD	0,367
F7-α PSD	0,323
Amplitude-β	0,187
HF PSD%	0,397
Trait Anxiety	0,278
Asymmetry-β	-0,401
LFnu	-0,389
LF/HF	-0,366



R=0,575; R^2=0,331; $\chi^2_{(36)}$=75; $p<10^{-3}$; Λ Prime=0,508

Fig. 9. Scatterplot of canonical correlation between Endocrine (X-line) and Psycho-Neural parameters (Y-line) in both sexes

It seems that the revealed neurotropic effects of calcitonin are "masked" by the effects of testosterone, although in view of the complete lack of correlation between the serum levels of

both hormones, their combined effect, according to the laws of multiple correlation, should be much more pronounced than the partial effects. We cannot yet explain such a discrepancy.

However, testosterone and calcitonin are somehow related. It is known that androgens (and estrogens) together with calcitonin (as well as parathyroid hormone, calcitriol, and glucocorticoids) are the systemic factors which are important to the maintenance of bone homeostasis. Herewith, androgens, estrogens, and calcitonin act synergistically: suppress bone resorption in trabecular and endocortical bone surfaces by decreasing osteoclast numbers [20]. It is found the enhanced calcitonin response to calcium administration in normal men compared to normal women. Garcia-Ameijeiras A et al [21] conclude that this phenomen is at least partially determined by the higher testosterone levels found among normal men.

Among the detected markers of sexual dimorphism, as a result of the discriminant analysis [28], only 28 are formally considered characteristic, that is, included in the model by the forward stepwise program (Tables 4 and 5). It is interesting that for the 8 variables currently in the model, sexual differences according to the Student's t test are **insignificant** (t<1,98).

Table 4. Discriminant Function Analysis Summary
Step 28, N of vars in model: 28; Grouping: 2 grps; Wilks' Λ: 0,363; approx. $F_{(29)}$=5,8; $p<10^{-6}$

Variables currently in the model	Mean±SE		Parameters of Wilks' Statistics					Student's St	
	Females (60)	Males (62)	Wilks' Λ	Partial Λ	F-remove (1,93)	p-level	Tolerancy	t	p
F3-β PSD, μV²/Hz	100±9	64±5	0,369	0,985	1,44	0,233	0,141	3,67	$<10^{-3}$
HF PSD ,%	13,1±1,2	8,0±0,8	0,402	0,902	10,1	0,002	0,142	3,54	$<10^{-3}$
F7-θ PSD, %	10,7±0,6	8,3±0,6	0,378	0,960	3,91	0,051	0,373	2,89	<0,01
Trait Anxiety, point	43,6±1,0	39,6±1,0	0,461	0,788	25,1	10^{-5}	0,527	2,81	<0,01
SDNN HRV, msec	51,1±3,5	44,2±2,8	0,390	0,932	6,83	0,010	0,323	1,58	>0,05
F3-θ PSD, μV²/Hz	67±8	35±3	0,368	0,986	1,34	0,250	0,301	3,57	$<10^{-3}$
P4-θ PSD, μV²/Hz	61±8	40±6	0,367	0,991	0,85	0,360	0,144	1,97	>0,05
C3-θ PSD, μV²/Hz	68±8	37±3	0,386	0,940	5,91	0,017	0,111	3,52	$<10^{-3}$
O2-β PSD, μV²/Hz	109±8	77±7	0,390	0,932	6,81	0,011	0,395	2,94	<0,01
O1-β PSD, μV²/Hz	119±10	91±7	0,374	0,970	2,86	0,094	0,277	2,35	<0,05
Deviation-β, Hz	1,5±0,1	1,2±0,1	0,386	0,940	5,96	0,017	0,742	1,58	>0,05
P3-δ PSD, μV²/Hz	295±59	188±34	0,384	0,945	5,38	0,023	0,500	1,57	>0,05
C4-θ PSD, %	11,3±0,5	9,7±0,6	0,395	0,920	8,06	0,006	0,310	2,12	<0,05
Reactive Anxiety, p	23,1±0,9	25,1±1,2	0,398	0,913	8,82	0,004	0,521	1,39	>0,10
Asymmetry-β, %	22,0±1,8	28,0±2,2	0,401	0,906	9,64	0,003	0,667	2,14	<0,05
Fp1-θ PSD, μV²/Hz	41±6	28±3	0,365	0,996	0,36	0,549	0,185	1,99	<0,05
C3-β PSD, μV²/Hz	122±12	75±6	0,368	0,986	1,29	0,258	0,160	3,60	$<10^{-3}$
HF PSD, msec²	384±66	249±51	0,380	0,956	4,25	0,042	0,201	1,62	>0,05
T3-β PSD, μV²/Hz	101±12	65±5	0,383	0,948	5,05	0,027	0,342	2,73	<0,01
F8-θ PSD, %	10,8±0,7	8,9±0,6	0,379	0,958	4,09	0,046	0,377	1,95	>0,05
(VLF+LF)/HF	11,3±1,3	18,3±1,9	0,372	0,976	2,32	0,131	0,108	3,00	<0,01
P3-θ PSD, μV²/Hz	64,5±10	39,5±5	0,372	0,976	2,30	0,132	0,390	2,33	<0,05
Fp2-β PSD, μV²/Hz	85±10	53±3	0,370	0,982	1,67	0,199	0,151	2,96	<0,01
Fp1-β PSD, μV²/Hz	78±8	55±4	0,372	0,975	2,36	0,128	0,225	2,64	<0,01
LF/HF	4,3±0,4	7,5±0,9	0,369	0,985	1,42	0,236	0,142	3,35	<0,01
LFnu, %	74,9±1,7	81,8±1,4	0,371	0,979	2,00	0,161	0,481	3,14	<0,01
T5-θ PSD, %	10,4±0,6	8,7±0,5	0,368	0,987	1,25	0,266	0,316	2,11	<0,05
T6-θ PSD, %	9,2±0,5	7,85±0,5	0,369	0,985	1,44	0,233	0,141	1,80	>0,05

Table 5. Summary of stepwise analysis of discriminant variables ranked by criterion Λ

Variables currently in the model	F to enter	p-level	Λ	F-value	p-level
F3-β PSD, μV²/Hz	13,4	10^{-3}	0,900	13,4	10^{-3}
HF PSD ,%	17,6	10^{-4}	0,784	16,4	10^{-6}
F7-θ PSD, %	9,17	0,003	0,728	14,7	10^{-6}
Trait Anxiety, points	5,83	0,017	0,693	13,0	10^{-6}
SDNN HRV, msec	6,79	0,010	0,655	12,2	10^{-6}
F3-θ PSD, μV²/Hz	5,45	0,021	0,625	11,5	10^{-6}
P4-θ PSD, μV²/Hz	5,14	0,025	0,598	10,9	10^{-6}
C3-θ PSD, μV²/Hz	4,34	0,039	0,576	10,4	10^{-6}
O2-β PSD, μV²/Hz	2,50	0,117	0,563	9,64	10^{-6}
O1-β PSD, μV²/Hz	5,11	0,026	0,539	9,51	10^{-6}
Deviation-β, Hz	3,42	0,067	0,522	9,14	10^{-6}
P3-δ PSD, μV²/Hz	2,99	0,087	0,508	8,78	10^{-6}
C4-θ PSD, %	3,47	0,065	0,493	8,56	10^{-6}
Reactive Anxiety, points	5,24	0,024	0,470	8,63	10^{-6}
Asymmetry-β, %	3,51	0,064	0,455	8,48	10^{-6}
Fp1-θ PSD, μV²/Hz	3,38	0,069	0,440	8,34	10^{-6}
C3-β PSD, μV²/Hz	2,40	0,124	0,430	8,10	10^{-6}
HF PSD, msec²	2,31	0,132	0,421	7,87	10^{-6}
T3-β PSD, μV²/Hz	2,16	0,145	0,412	7,65	10^{-6}
F8-θ PSD, %	1,21	0,275	0,407	7,35	10^{-6}
(VLF+LF)/HF	1,80	0,183	0,400	7,14	10^{-6}
P3-θ PSD, μV²/Hz	1,47	0,228	0,394	6,91	10^{-6}
Fp2-β PSD, μV²/Hz	1,39	0,241	0,389	6,70	10^{-6}
Fp1-β PSD, μV²/Hz	1,18	0,280	0,384	6,48	10^{-6}
LF/HF	1,16	0,285	0,380	6,28	10^{-6}
LFnu, %	1,61	0,208	0,373	6,14	10^{-6}
T5-θ PSD, %	1,31	0,255	0,368	5,98	10^{-6}
T6-θ PSD, %	1,25	0,266	0,363	5,82	10^{-6}

Instead, variables with significant sexual differences were outside the discriminant model (Table 6). Obviously, this is due to the duplication/redundancy of the distinguishing information carried by these variables. Testosterone and calcitonin, by default, were not subject to discriminant analysis.

Table 6. Variables currently not in the discriminant model

Variables currently not in the model	Mean±SE		Parameters of Wilks' Statistics					Student's St	
	Females (60)	Males (62)	Wilks 'Λ	Partial Λ	F to enter	p-level	Tolerancy	t	p
Amplitude θ, μV	10,0±0,7	8,2±0,4	0,363	1,000	0,01	0,934	0,168	2.39	<0,02
F4-θ PSD, μV²/Hz	55±7	35±3	0,363	1,000	0,01	0,914	0,144	2,75	<0,01
F7 PSD Entropy	0,82±0,02	0,71±0,03	0,360	0,991	0,87	0,353	0,307	2,90	<0,01
F7-α PSD, μV²/Hz	101±24	44±5	0,363	0,999	0,08	0,775	0,107	2,28	<0,05
T3-θ PSD, μV²/Hz	55±8	26±2	0,362	0,997	0,32	0,575	0,408	3,51	$<10^{-3}$
T4-θ PSD, %	10,4±0,6	8,45±0,6	0,359	0,989	0,99	0,323	0,171	2,47	<0,02
T4-α PSD, μV²/Hz	109±18	64±7	0,363	1,000	0,03	0,859	0,180	2,27	<0,05
C4-β PSD, μV²/Hz	118±12	81±7	0,363	0,999	0,05	0,828	0,339	2,78	<0,01
C4-θ PSD, μV²/Hz	73±10	38±3	0,362	0,997	0,30	0,582	0,153	3,39	$<10^{-3}$
T5-θ PSD, μV²/Hz	52±8	31±3	0,363	0,999	0,05	0,820	0,128	2,59	<0,02
T6-α PSD, μV²/Hz	171±31	99±18	0,363	1,000	0,01	0,904	0,134	2,03	<0,05
T6-θ PSD, μV²/Hz	51±8	26±4	0,363	1,000	0,01	0,933	0,106	2,79	<0,01
P3-β PSD, μV²/Hz	124±12	78±6	0,363	0,999	0,06	0,803	0,334	3,33	<0,01
P4-β PSD, μV²/Hz	109±10	74±6	0,363	0,998	0,18	0,677	0,325	2,95	<0,01
O2-θ PSD, μV²/Hz	51±8	33±3	0,362	0,997	0,28	0,599	0,092	2,11	<0,05

T3-θ PSD, %	10,4±0,6	8,6±0,5	0,363	0,998	0,17	0,678	0,356	2,32	<0,02
T3-α PSD, μV²/Hz	122±21	75±10	0,363	0,999	0,05	0,820	0,128	2,02	<0,05
T5-β PSD, μV²/Hz	97±13	65±5	0,363	1,000	0,01	0,914	0,144	2,25	<0,05
T6-β PSD, μV²/Hz	89±7	55±4	0,363	1,000	0,01	0,933	0,106	4,36	$<10^{-3}$
Amplitude-β, μV	13,0±0,6	11,7±0,4	0,363	1,000	0,01	0,917	0,110	1,95	**>0,05**
F8 PSD Entropy	0,80±0,03	0,72±0,03	0,363	0,998	0,17	0,678	0,356	1,92	**>0,05**
RMSSD HRV, ms	27,7±2,3	22,9±2,0	0,363	1,000	0,01	0,917	0,110	1,57	**>0,05**
Calcitonin, ng/l	5,7±0,4	10,5±0,9						5,00	$<10^{-4}$
Testosterone, nM/l	3,5±0,4	13,5±0,8						11,7	$<10^{-5}$

Calculating the value of the discriminant root for each participant as the sum of the products of non-standardized (raw) coefficients on the individual values of discriminant variables together with the constant (Table 7) allows visualization of each participant (not shown due to redundancy) as well as their separate groups in the information space of the root (Fig. 10).

Table 7. Coefficients and constant for discriminant variables

	Coefficients			Winkelmann T et al [63]	R thickness/ HF PSD
Variables	**Standar-dized**	**Raw**	**Struc-tural**	**Cortical region**	
F3-β PSD, μV²/Hz	0,412	0,008	**0,252**	Caudal anterior cingulate	**0,47**
F3-θ PSD, μV²/Hz	0,272	0,006	**0,246**		
F4-θ PSD, μV²/Hz					**0,55**
T3-β PSD, μV²/Hz	0,486	0,007	**0,188**	Inferior temporal gyrus	**0,53**
P3-θ PSD, μV²/Hz	-0,594	-0,010	**0,160**	Supramarginal gyrus	**0,43**
P3-δ PSD, μV²/Hz	0,415	0,001	**0,108**		
Fp2-β PSD, μV²/Hz	0,312	0,005	**0,204**	Pars triangularis	**0,47**
O2-β PSD, μV²/Hz	0,521	0,009	**0,202**	Lingual gyrus	**0,47**
C4-θ PSD, %	0,636	0,152	**0,145**	Precentral gyrus	**0,44**
F8-θ PSD, %	-0,419	-0,081	**0,133**	Superior frontal gyrus	**0,39**
T6-θ PSD, %	-0,257	-0,063	**0,123**	Transverse temporal cortex	**0,51**
C3-β PSD, μV²/Hz	-0,367	-0,005	**0,247**		
C3-θ PSD, μV²/Hz	0,918	0,019	**0,242**		
F7-θ PSD, %	0,412	0,090	**0,197**		
Fp1-β PSD, μV²/Hz	-0,429	-0,009	**0,182**		
O1-β PSD, μV²/Hz	-0,411	-0,006	**0,161**		
T5-θ PSD, %	0,262	0,059	**0,145**		
Fp1-θ PSD, μV²/Hz	-0,182	-0,005	**0,137**		
P4-θ PSD, μV²/Hz	-0,314	-0,006	**0,135**		
Deviation-β, Hz	0,357	0,459	**0,108**		
Asymmetry-β, %	-0,470	-0,030	**-0,146**		
HF PSD ,%	1,037	0,131	**0,243**		
HF PSD, msec²	-0,585	-0,001	**0,111**		
SDNN HRV, msec	0,577	0,024	**0,109**		
LF/HF	-0,416	-0,079	**-0,228**		
LFnu, %	0,408	0,034	**-0,215**		
(VLF+LF)/HF	-0,043	-0,003	**-0,205**		
Trait Anxiety, points	0,795	0,101	**0,194**		
Reactive Anxiety, p	-0,511	-0,060	**-0,095**		
	Constant	-9,784			
Squared Mahalanobis Distance=6,9; $F_{(29)}$=5,8; $p<10^{-6}$					
Canonical R=0,798; Wilks' Λ=0,363; $\chi^2_{(28)}$=107; $p<10^{-6}$					

Judging by the structural coefficients, the discriminant root reflects directly the electrical activity (more precisely PSD) of theta- and beta-rhythm generating neurons, which are projected to different loci of the scalp, as well as the variability of the beta-rhythm - on the

first side, vagal (directly) and sympathetic (inversely) tone - from the second side, trait (direct) and reactive (inversely) reactivity - from the third side.

If we return to the scheme of central autonomic network (Fig. 4), and also compare our Fig. 10 and Table 7 with the Table 1 and Fig. 2 of Winkelmann's T et al [63] article, which shows the correlation coefficients between cortical thickness of Desikan parcellations and HF PSD in 30 healthy subjects (8 female and 22 male), an interesting picture emerges. As we can see, women differ from men in significantly higher ongoing electrical activity, as well as, probably, a greater number of neurons in precisely those areas of the cortex that are responsible for maintaining a higher vagal and lower sympathetic tone [12,27,63], as well as higher trait and lower reactive anxiety.

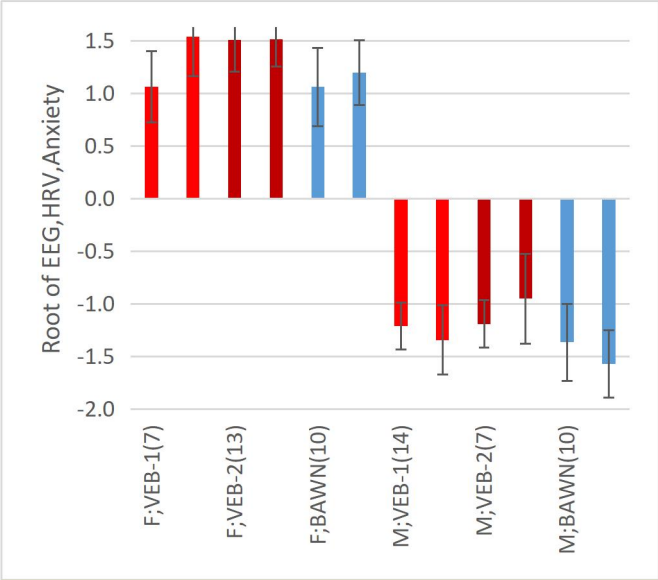


Fig. 10. Average values (Mean±SD) of the discriminant roots before and after interventions

It is especially important to note that such differences are reproduced when the parameters are re-registered after 4- or 7-day interventions in the form of transcutaneous electrical stimulation with VEB-1 VEB-2 devices [4,5,40] or Naftussya bioactive water (BAWN) [30,40] respectively, i.e. the listed parameters can be considered robust psycho-neural markers of sexual dimorphism.

Another result of discriminant analysis is the ability to retrospectively recognize a person's sex based on the constellation of discriminant parameters by calculating classification functions based on coefficients and constants (Table 8).

Table 8. Coefficients and constants of classification functions

	Males	Females
Variables	p=,508	p=,492
F3-β PSD, μV²/Hz	-0,082	-0,062
HF PSD ,%	5,464	5,807
F7-θ PSD, %	0,493	0,729
Trait Anxiety, points	1,228	1,494
SDNN HRV, msec	0,203	0,265
F3-θ PSD, μV²/Hz	0,023	0,038
P4-θ PSD, μV²/Hz	-0,116	-0,131
C3-θ PSD, μV²/Hz	0,101	0,151
O2-β PSD, μV²/Hz	0,043	0,066
O1-β PSD, μV²/Hz	-0,061	-0,077
Deviation-β, Hz	6,550	7,755

P3-δ PSD, μV²/Hz	0,019	0,022
C4-θ PSD, %	-0,448	-0,048
Reactive Anxiety, p	-0,119	-0,277
Asymmetry-β, %	0,008	-0,071
Fp1-θ PSD, μV²/Hz	0,166	0,152
C3-β PSD, μV²/Hz	0,030	0,016
HF PSD, msec²	-0,004	-0,007
T3-β PSD, μV²/Hz	0,045	0,062
F8-θ PSD, %	-0,848	-1,060
(VLF+LF)/HF	0,938	0,929
P3-θ PSD, μV²/Hz	-0,006	-0,033
Fp2-β PSD, μV²/Hz	0,036	0,050
Fp1-β PSD, μV²/Hz	-0,026	-0,049
LF/HF	-4,353	-4,560
LFnu, %	4,268	4,358
T5-θ PSD, %	1,128	1,283
T6-θ PSD, %	1,710	1,543
Constants	-231,0	-256,8

It turns out that by only 28 psycho-neural parameters, male sex can be recognized with 90,3% accuracy, and female sex with 88,3% accuracy (Table 9).

Table 9. Classification matrix

	Rows: Observed classifications Columns: Predicted classifications		
Group	Percent Correct	Men p=,508	Women p=,492
Men	90,3	56	6
Women	88,3	7	53
Total	89,3	63	59

The additional use of calcitoninemia increases the accuracy of recognition of women to 92,6%, but does not affect that of men. Looking ahead, we note that taking into account the serum levels of long-known metabolic sex markers such as cholesterol, creatinine, uric acid and direct bilirubin registered in this cohort brings the accuracy of gender recognition to 93,5 and 96,7%, respectively. Interestingly, the inclusion of testosterone in the discriminant function predictably allows identifying all 60 women without error, while there are 2 errors for men (accuracy 96,8%).

In conclusion, let's return to the study of Proverbio AM [46] about of sexual dimorphism in hemispheric processing of faces in humans.

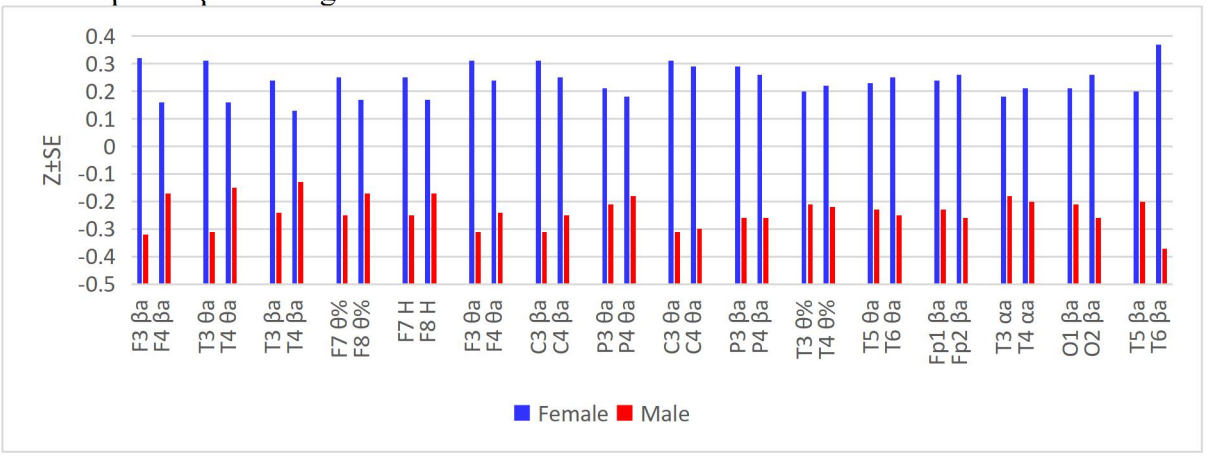


Fig. 11. Z-scores of PSD of EEG rhythms in left (odd) and right (even) loci

At the first stage, we will compare the normalized PSD values in the left and right loci (Fig. 11), and then we will compare the measures of sexual dimorphism (as FemaleDMale) in PSD laterality (Fig. 12).

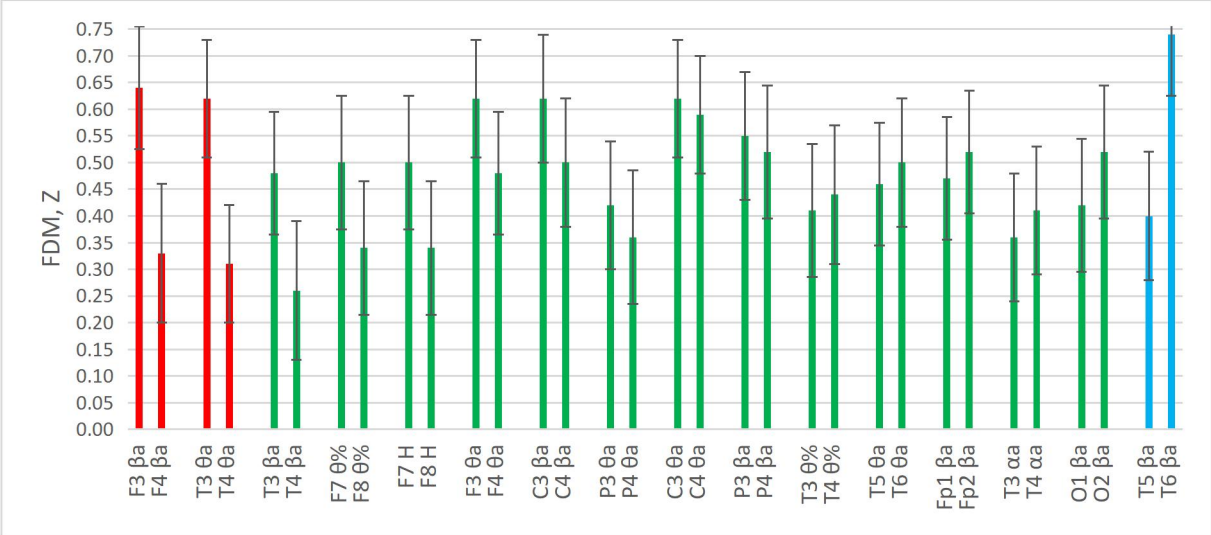


Fig. 12. Measures of sexual dimorphism (FemaleDMale) in Laterality of PSD of EEG rhythms

As we can see, in most loci the asymmetry is insignificant. At the same time, there is a **left-sided** asymmetry in the medial frontal and anterior temporal loci, and a **right-sided** asymmetry in the posterior temporal loci.

This study has a number of both limitations and advantages. We consider the biggest drawback to be the lack of determination of the levels of female sex hormones, at least estradiol, which also has neurotropic and psychotropic activity [16]. It would be desirable to estimate the content of free fractions of sex hormones, because the bioavailability of either androgens or estrogens is restricted by high-affinity binding to circulating sex hormone-binding globulin [23]. On the other hand, the study has several strengths, including almost synchronous registration of EEG, HRV, anxiety and endocrine parameters. Moreover, these same participants have registered parameters of metabolism as well as acupuncture and gas discharge visualization, which are closely related to neuro-endocrine parameters [3,41], and are also characterized by sexual dimorphism, which will be reported in subsequent publications.

ACKNOWLEDGMENT

We express sincere gratitude to administration of clinical sanatorium “Moldova” and PrJSC “Truskavets’ Spa” as well as TA Korolyshyn and VV Kikhtan for help in carrying out this investigation.

ACCORDANCE TO ETHICS STANDARDS

Tests in patients are carried out in accordance with positions of Helsinki Declaration 1975 and directive of National Committee on ethics of scientific researches. During realization of tests from all participants the informed consent is got and used all measures for providing of anonymity of participants.

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