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Relationships between electrical conductivity of acupuncture points and adaptation hormones

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Abstract

Background. Modern scientific research has shown that the inherent regulatory system of the body is a neuro-endocrine-immune (NEI) network. A researcher proposed a hypothesis, that the bidirectional positive regulatory role of acupuncture was achieved by NEI network. The purpose of this study is to find out relationships between electrical conductivity (EC) of acupuncture points (AP) and adaptation hormones one of the elements of NEI network. **Materials and methods.** The object of observation were 10 women (32-76 years) and 10 men (37-67 years) examined twice with a weekly interval. The volunteers were considered practically healthy, but with maladaptation. We recorded EC in AP Pg(ND), TR(X) and MC(AVL) (by complex "Medissa") as well as serum Cortisol, Aldosterone, Testosterone, Triiodothyronine, Calcitonin and PTH (by the ELISA). For statistical analysis used the software package "Statistica 6.4".
Results. The multiple linear regression analysis revealed the closest relationship with AP Testosterone raw level (R=0,485). Testosterone level, normalized by sex and age, was associated with AP to the same extent (R=0,439), but positive correlations were transformed into negative ones. Calcitonin level was also moderately positively correlated with AP (R=0,441). PTH was significantly correlated only with TR(X) lateralization (r=-0,363;

p=0,022) and Triiodothyronine only with MC(AVL) lateralization (r=0,315; p=0,048). The connections of Aldosterone with AP lateralization turned out to be insignificant, and from the side of Cortisol, they were negligible at all. Despite moderate pairwise correlations, the canonical correlation between the levels of adaptation Hormones, on the one hand, and EC of AP and their lateralization, on the other hand, turned out to be quite strong (R=0,856). **Conclusion**. Electrical conductivity of AP and their lateralization is 73% determined by the constellation of adaptation hormones, which are an important component of the NEI network. In the next message, we will demonstrate the existence of connections between AP and parameters of EEG and heart rate variability as well as Immunity.
***Keywords*:** acupuncture points, electrical conductivity, adaptation hormones, relationships.

INTRODUCTION

Modern scientific research has shown that the inherent regulatory system of the body is a neuro-endocrine-immune (NEI) network [5]. A researcher proposed a hypothesis, that “*the bidirectional positive regulatory role of acupuncture was achieved by NEI network”* [9,10,22].

Because of the hypothalamus–pituitary–adrenal (HPA) axis, hypothalamus–pituitary–gonadal (HPG) axis and hypothalamus–pituitary–thyroid (HPT) axis playing an important role in the endocrine activities, recent researches about the effects of acupuncture on the endocrine system more focused on these axes, with related hormones as observation indexes. For example, acupuncture could obviously reduce hormones such as adreno corticotropic hormone, corticosterone related to HPA axis in chronic stress-induced rats [11]. Electroacupuncture could regulate the level of uterus estrogen, pituitary follicule-stimulating hormone and luteinizing hormone and hypothalamic gonadotropin releasing hormone in ovariectomized rats, to restore the disorder of hypothalamus–pituitary–ovary axis [8]. Acupuncture could increase the level of thyrotropin releasing hormone, thyroid stimulating hormone and total three typical thyroid original axis in chronic fatigue rats, to restore the inhibition of HPT axis [21]. All these studies suggest that acupuncture can modulate the function of HPA, HPG and HPT axes.

We started the project "Functional relationships between the parameters of acupuncture points and the neuro-endocrine-immune network", thereby joining the construction of a bridge between the Western and Eastern paradigms of medicine [12]. The purpose of this study is to find out relationships between electrical conductivity of acupuncture points (ECAP) and adaptation hormones.

MATERIAL AND RESEARCH METHODS

The object of observation were 10 women (32-76 years) and 10 men (37-67 years) examined twice with a weekly interval. 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 NEI network (details follow) as a manifestation of maladaptation, which actually prompted them to participate in the study with the hope of recovery.

In the morning in basal condition we recorded electrical conductivity in follow acupuncture points: Pg(ND), TR(X) and MC(AVL) at Right and Left side, which represents the nervous, endocrine and immune systems respectively. Used complex “Medissa”. For each pair, the Laterality Index (LI) was calculated using formula [18]:

LI, % = 200•(Right – Left)/(Right + Left).

In portion of the venous blood the serum levels of major hormones of adaptation: Cortisol, Testosterone, Aldosterone, Triiodothyronine, PTH and Calcitonin assayed with ELISA kits according to the SOP provided by the manufacturer (“Алкор Био”, XEMA Co Ltd, DRG International Inc, “Vector-Best”) with the use of analyzer “RT-2100C”.

Normal (reference) values of variables are taken from the instructions and/or database of the UkrSR Institute of Medicine of Transport [1,20].

For statistical analysis used the software package "Statistica 6.4".

Statistical methods used.

1. Descriptive Statistics. The authors calculated means and standard errors for all measured variables, including electrical conductivity of acupuncture points, hormone levels, and laterality indices. This provides a basic overview of the central tendency and variability in the data.

2. Data Normalization. Raw data was normalized using Z-score transformation. The formula used was: $Z = 4*(V - N)/(Max - Min) = (V - N)/SD = (V/N - 1)/Cv$, where V is the actual value, N is the normal (reference) value, SD is standard deviation, and Cv is coefficient of variation. This normalization allows for comparison between variables measured on different scales.

3. Correlation Analysis. Pairwise correlations were calculated between acupuncture point parameters and hormone levels. This provides an initial look at bivariate relationships between variables. The strength and direction of correlations are reported, with statistical significance noted (e.g., p-values).

4. Multiple Linear Regression. Multiple regression models were constructed to examine relationships between sets of predictor variables (e.g., acupuncture point measures) and individual outcome variables (e.g., specific hormone levels). The authors report multiple correlation coefficients (R), coefficients of determination (R^2), adjusted R^2, F-statistics, p-values, and standard errors of estimate for each model. Beta coefficients, their standard errors, and p-values are reported for individual predictors in each model.

5. Canonical Correlation Analysis. This technique was used to examine relationships between the entire set of acupuncture point measures and the set of hormone parameters simultaneously. The authors report the overall canonical correlation coefficient (R), R^2, chi-square statistic, degrees of freedom, p-value, and Wilks' Lambda. Factor loadings (correlations) between individual variables and the canonical variates are presented in a table.

6. Factor Analysis. Applied to the canonical roots to examine the underlying structure of relationships between variables. Factor loadings are reported, showing how strongly each variable relates to the identified factors.

7. Visualization Techniques. Bar graphs are used to display profiles of multiple correlation coefficients. A scatterplot is presented to visualize the canonical correlation between acupuncture point conductance/symmetry and adaptation hormones.

8. Statistical Significance. The authors consistently report p-values to indicate the statistical significance of their findings. A significance level of $p < 0.05$ appears to be used as the threshold for statistical significance.

This comprehensive statistical approach allows the researchers to examine complex multivariate relationships between acupuncture point parameters and hormone levels, while also looking at specific relationships between individual variables. The combination of techniques provides both broad overviews of relationships and detailed examinations of specific associations.

RESULTS

In order to correctly compare variables expressed in different units and with different variability, the actual/raw parameters were normalized by recalculation by the equations:

$Z = 4•(V – N)/(Max – Min) = (V – N)/SD = (V/N – 1)/Cv$, where

V is the actual value; N is the normal (reference) value; SD and Cv are the standard deviation and coefficient of variation respectively [19].

It was found (Table 1) that the electrical conductivity of acupuncture points exceeds the upper limit of the classical norm (+2σ). In addition, for the Pg(ND) AP pair, a rightward shift of symmetry was noted, which is less pronounced for the TR(X) pair, while the state of both MC(AVL) APs is the same.

Table 1. Raw and normalized values of acupuncture points and adaptation hormones

Variables	Means ± Standard Errors (n)		
	Raw (40)	Reference (40)	Z-score (40)
Pg(ND) Right, units	63,3±0,9	57,5±0,4	2,34±0,38
MC(AVL) Left, units	63,3±0,8	57,5±0,4	2,34±0,31
TR(X) Right, units	63,2±0,9	57,5±0,4	2,32±0,34
MC(AVL) Right, units	63,2±0,9	57,5±0,4	2,30±0,34
TR(X) Left, units	63,1±0,8	57,5±0,4	2,24±0,34
Pg(ND) Left, units	62,9±0,9	57,5±0,4	2,16±0,35
Pg(ND) Laterality, %	0,64±0,50	-0,51±0,39	0,46±0,20
TR(X) Laterality, %	0,27±0,49	-0,31±0,51	0,32±0,27
MC(AVL) Laterality, %	-0,23±0,53	-0,17±0,30	-0,03±0,28
Cortisol, nM/L	397±24	370±18	0,24±0,21
Triiodothyronine, nM/L	2,23±0,14	2,20±0,08	0,06±0,29
Testosterone normalized, Z		0±0,32	-0,11±0,20
Aldosterone, pM/L	226±3	238±7	-0,28±0,06
Calcitonin normalized, Z		0±0,32	-0,40±0,17
Parathyroid hormone, pM/L	3,07±0,11	3,75±0,14	-0,79±0,13

Since the reference serum levels of testosterone in men are approximately 5.5 times higher than in women, and the level of calcitonin is 2.8 times higher [17], in this context we operated with levels normalized by sex, as well as age (in men only). A slight, but statistically probable decrease in the level of aldosterone as well as a more pronounced decrease in calcitonin and parathyroid hormone was noted (Fig. 1).

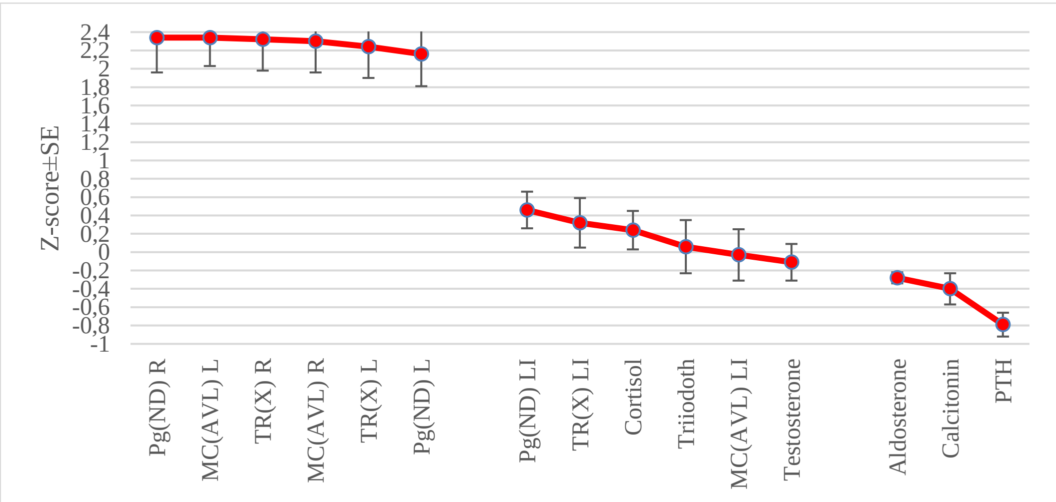


Fig. 1. Profile of normalized values of acupuncture points and adaptation hormones

At the next stage, a correlation matrix was created between AP parameters, on the one hand, and adaptation hormones, on the other hand (Table 2).

Table 2. Matrix of correlations between the parameters of APs and adaptation hormones

Variable	Correlations Pg(ND) R	Pg(ND) L	Pg(ND) LI	MC(AVL) R	MC(AVL) L	MC(AVL)LI	TR(X) R	TR(X) L	TR(X) LI
PTH	-0,09	-0,12	0,03	-0,15	-0,14	-0,07	-0,18	-0,05	-0,36
Aldosterone	-0,09	-0,04	-0,20	0,01	-0,07	0,21	-0,11	-0,19	0,21
T3	-0,14	-0,16	0,02	-0,03	-0,17	0,32	-0,14	-0,17	0,08
Cortisol	-0,16	-0,13	-0,14	0,04	0,02	0,06	-0,15	-0,10	-0,16
Calcitonin	0,26	0,32	-0,12	0,39	0,30	0,29	0,36	0,32	0,13
Testosterone	0,24	0,22	0,10	0,41	0,41	0,09	0,35	0,32	0,09
Test normal	-0,30	-0,30	-0,06	-0,25	-0,35	0,17	-0,37	-0,35	-0,06

Then, on the basis of the matrix, the multiple correlation coefficients between the parameters of adaptation hormones and acupuncture points were first calculated (Fig. 2 and Tables 3-10).

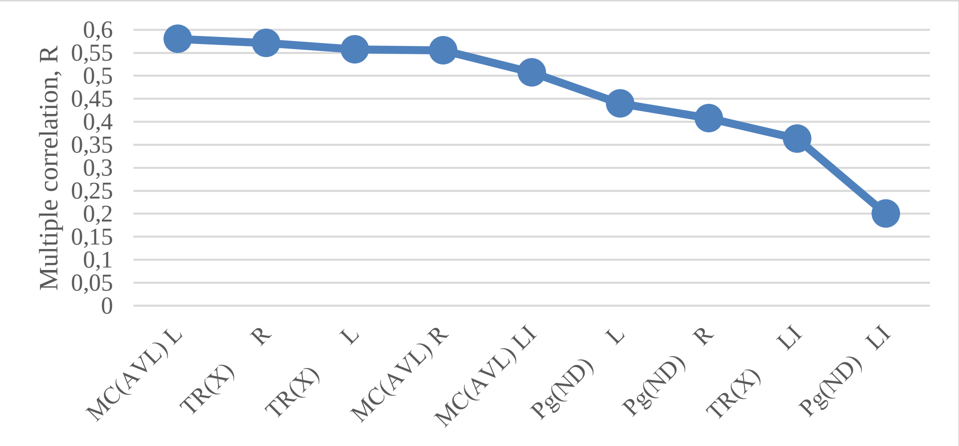


Fig. 2. Profile of multiple correlation coefficients between parameters of adaptation hormones and acupuncture points

Table 3. Regression Summary for Dependent Variable: MC(AVL) L
$R=0.580$; $R^2=0.337$; Adjusted $R^2=0.282$; $F_{(3.4)}=6.1$; $p=0.002$; SE: 4.2 units

N=40		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(36)}$	p-level
Variables	r		Intercpt	59,3	1,6	38,1	10^{-6}
Testosterone, nM/L	**0,41**	0,419	0,140	0,414	0,138	3,00	0,005
Testosterone, Z	**-0,35**	-0,360	0,142	-2,021	0,796	-2,54	0,016
Calcitonin, ng/L	0,30	0,133	0,144	0,188	0,203	0,92	0,362

Table 4. Regression Summary for Dependent Variable: TR(X) R
$R=0.571$; $R^2=0.326$; Adjusted $R^2=0.270$; $F_{(3.4)}=5.8$; $p=0.002$; SE: 4.6 units

N=40		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(36)}$	p-level
Variables	r		Intercpt	58,7	1,7	34,1	10^{-6}
Testosterone, Z	**-0,37**	-0,353	0,143	-2,169	0,877	-2,47	0,018
Calcitonin, ng/L	**0,36**	0,208	0,145	0,322	0,224	1,44	0,159
Testosterone, nM/L	**0,35**	0,345	0,141	0,372	0,152	2,45	0,020

Table 5. Regression Summary for Dependent Variable: TR(X) L

$R=0.557$; $R^2=0.310$; Adjusted $R^2=0.231$; $F_{(4.4)}=3.9$; $p=0.010$; SE: 4.6 units

N=40		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(35)}$	p-level
Variables	r		Intercpt	72,1	10,6	6,80	10^{-6}
Testosterone, Z	**-0,35**	-0,313	0,148	-1,878	0,891	-2,11	0,042
Testosterone, nM/L	**0,32**	0,340	0,145	0,359	0,153	2,34	0,025
Calcitonin, ng/L	**0,32**	0,177	0,149	0,267	0,225	1,19	0,242
Aldosterone, pM/L	-0,19	-0,178	0,143	-0,058	0,047	-1,24	0,222

Table 6. Regression Summary for Dependent Variable: MC(AVL) R
$R=0.555$; $R^2=0.308$; Adjusted $R^2=0.250$; $F_{(3.4)}=5.3$; $p=0.004$; SE: 4.7 units

N=40		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(36)}$	p-level
Variables	r		Intercpt	57,9	1,7	33,3	10^{-6}
Testosterone, nM/L	**0,41**	0,378	0,143	0,409	0,154	2,65	0,012
Calcitonin, ng/L	**0,39**	0,259	0,147	0,401	0,227	1,77	0,086
Testosterone, Z	-0,25	-0,220	0,145	-1,353	0,890	-1,52	0,137

Table 7. Regression Summary for Dependent Variable: MC(AVL) LI
$R=0.507$; $R^2=0.257$; Adjusted $R^2=0.195$; $F_{(3.4)}=4.2$; $p=0.013$; SE: 3.0 %

N=40		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(36)}$	p-level
Variables	r		Intercpt	-17,2	7,2	-2,39	0,022
Triiodothyronine, nM/L	**0,32**	0,360	0,145	1,342	0,539	2,49	0,018
Calcitonin, ng/L	0,29	0,307	0,144	0,299	0,140	2,14	0,039
Aldosterone, pM/L	0,21	0,253	0,145	0,054	0,031	1,75	0,088

Table 8. Regression Summary for Dependent Variable: Pg(ND) L
$R=0.440$; $R^2=0.193$; Adjusted $R^2=0.126$; $F_{(3.4)}=2.9$; $p=0.049$; SE: 5.2 units

N=40		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(36)}$	p-level
Variables	r		Intercpt	59,1	1,9	30,4	10^{-6}
Calcitonin, ng/L	**0,32**	0,211	0,158	0,338	0,253	1,34	0,190
Testosterone, Z	-0,30	-0,267	0,156	-1,694	0,993	-1,71	0,097
Testosterone, nM/L	0,22	0,210	0,154	0,235	0,172	1,36	0,182

Table 9. Regression Summary for Dependent Variable: Pg(ND) R
$R=0.408$; $R^2=0.167$; Adjusted $R^2=0.122$; $F_{(2.4)}=3.7$; $p=0.034$; SE: 5.6 units

N=40		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(37)}$	p-level
Variables	r		Intercpt	61,0	1,5	40,5	10^{-6}
Testosterone, Z	-0,30	-0,329	0,151	-2,263	1,037	-2,18	0,036
Testosterone, nM/L	0,24	0,277	0,151	0,335	0,182	1,84	0,074

Table 10. Regression Summary for Dependent Variable: TR(X) LI
$R=0.363$; $R^2=0.131$; Adjusted $R^2=0.109$; $F_{(1.4)}=5.7$; $p=0.022$; SE: 2.9 %

N=40		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(38)}$	p-level
Variables	r		Intercpt	5,25	2,13	2,47	0,018
Parathyroid hormone, pM/L	**-0,36**	-0,363	0,151	-1,626	0,678	-2,40	0,022

Next, R between AP and hormones parameters were calculated (Tables 11-15 and Fig. 3).

Table 11. Regression Summary for Dependent Variable: Testosterone raw

$R=0.485$; $R^2=0.235$; Adjusted $R^2=0.171$; $F_{(3.4)}=3.7$; $p=0.021$; SE: 4.5 nM/L

N=40		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(36)}$	p-level
Variables	r		Intercpt	-18,6	9,4	-1,98	0,056
MC(AVL) Left, units	**0,41**	0,779	0,272	0,789	0,275	2,87	0,007
Pg(ND) Right, units	0,24	0,417	0,447	0,345	0,370	0,93	0,358
Pg(ND) Left, units	0,22	-0,827	0,518	-0,741	0,464	-1,60	0,119

Table 12. Regression Summary for Dependent Variable: Testosterone normalized
$R=0.439$; $R^2=0.193$; Adjusted $R^2=0.126$; $F_{(3.4)}=2.9$; $p=0.050$; SE: 0.82 Z

N=40		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(36)}$	p-level
Variables	r		Intercpt	4,21	1,70	2,48	0,018
MC(AVL) Left, units	**-0,35**	-0,620	0,442	-0,110	0,079	-1,40	0,169
TR(X) Right, units	-0,30	-0,339	0,301	-0,055	0,049	-1,13	0,268
MC(AVL) Right, units	-0,25	0,609	0,398	0,099	0,065	1,53	0,135

Table 13. Regression Summary for Dependent Variable: Calcitonin
$R=0.441$; $R^2=0.195$; Adjusted $R^2=0.127$; $F_{(3.4)}=2.9$; $p=0.048$; SE: 3.2 ng/L

N=40		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(36)}$	p-level
Variables	r		Intercpt	-7,42	6,75	1,10	0,279
MC(AVL) Right, units	**0,39**	0,673	0,398	0,435	0,257	1,69	0,099
MC(AVL) Left, units	0,30	-0,596	0,442	-0,421	0,312	-1,35	0,186
TR(X) Right, units	0,26	0,317	0,301	0,205	0,195	1,05	0,299

Table 14. Regression Summary for Dependent Variable: Triiodothyronine
$R=0.315$; $R^2=0.099$; Adjusted $R^2=0.076$; $F_{(1.4)}=4.2$; $p=0.048$; SE: 0.87 nM/L

N=40		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(36)}$	p-level
Variables	r		Intercpt	-7,42	6,75	1,10	0,279
MC(AVL) LI, %	**0,32**	0,315	0,154	0,085	0,041	2,05	0,048

Table 15. Regression Summary for Dependent Variable: Aldosterone
$R=0.359$; $R^2=0.129$; Adjusted $R^2=0.056$; $F_{(3.4)}=1.8$; $p=0.169$; SE: 15 pM/L

N=40		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(36)}$	p-level
Variables	r		Intercpt	226	2,5	89,9	10^{-6}
Pg(ND) LI, %	-0,22	-0,220	0,158	-1,109	0,796	-1,39	0,172
TR(X) LI, %	0,21	0,223	0,158	1,165	0,823	1,41	0,166
MC(AVL) LI, %	0,21	0,175	0,157	0,829	0,743	1,12	0,272

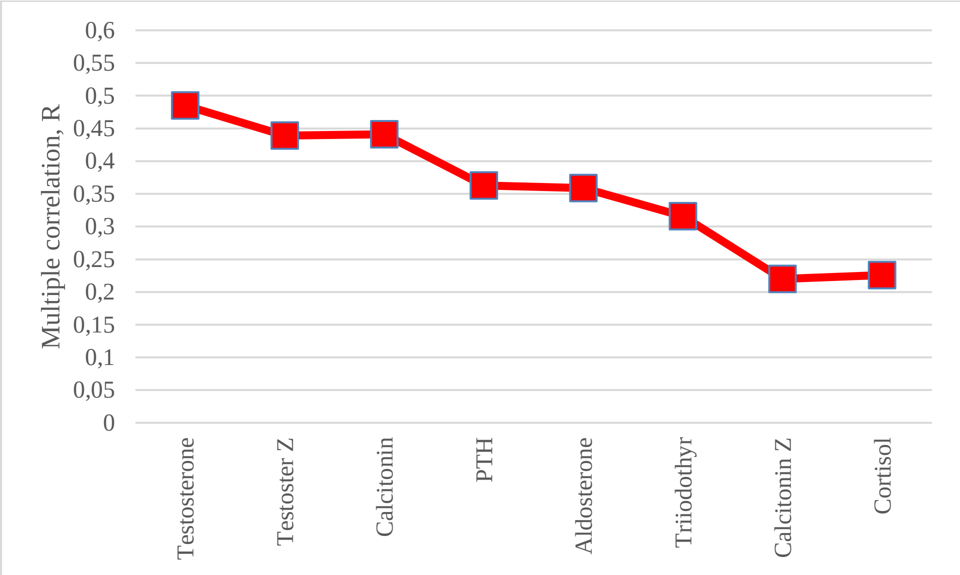


Fig. 3. Profile of multiple correlation coefficients between the parameters of acupuncture points and adaptation hormones

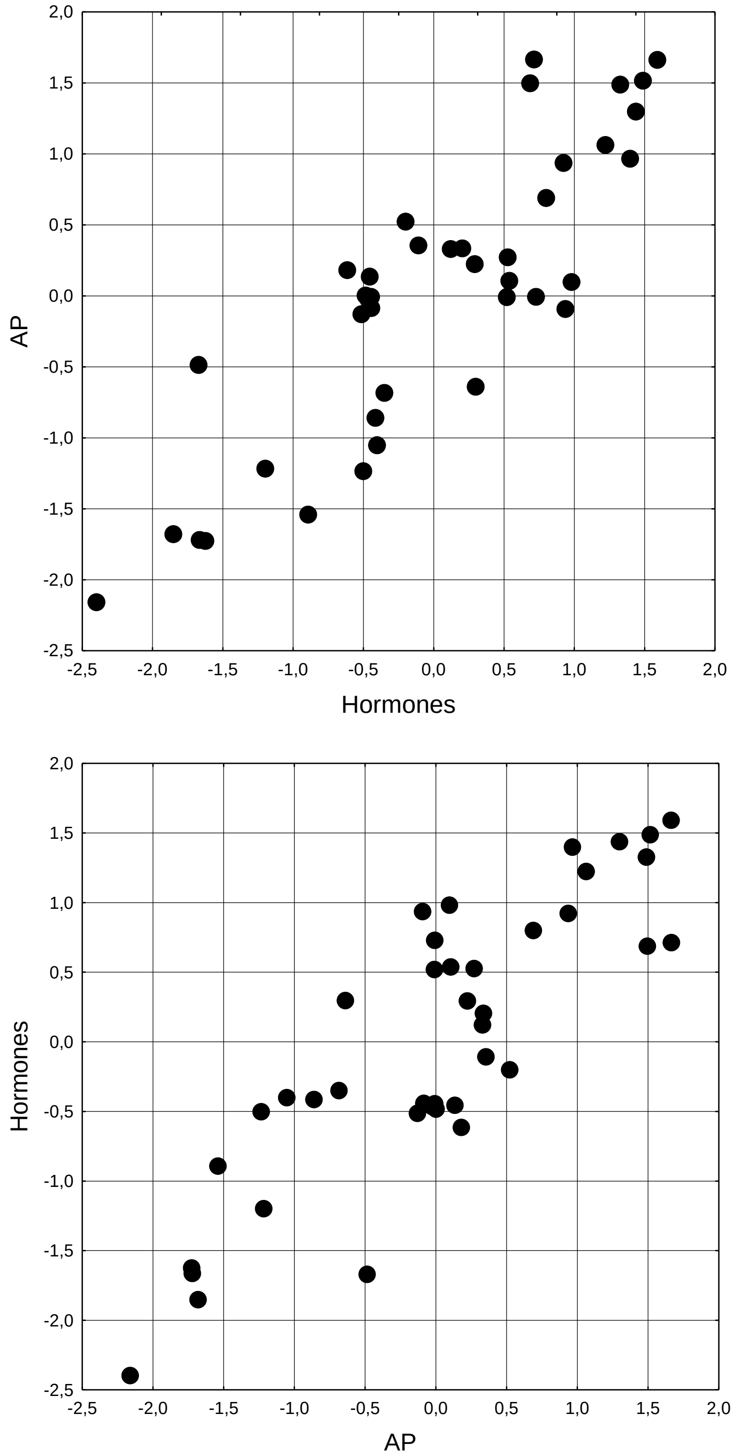
Such a two-way approach to the calculation of multiple correlation was applied by us in order to evaluate the revealed connections from the standpoints of the paradigms of both Western and Eastern medicine.

According to the paradigm of Western medicine the electroacupuncture according to Voll (EAV) belongs to an area of research known as electrodermal activity, galvanic skin response, or sympathetic skin response (SSR). The SSR represents an electrical potential generated in skin sweat glands. It originates by activation of the SSR reflex arch evoked by a variety of internal or externally stimuli. The effectors of the reflex arch activate eccrine sweat glands with cholinergic mediation [13]. Therefore, AP parameters are considered to be an effective sign depending on the regulatory effects of at least sympathetic nerves as well as, as shown in our study, adaptation hormones. According to the paradigm of Eastern medicine AP through meridians are connected with Chakras, which exert a regulatory influence on endocrine glands and nerve plexuses [7]. Therefore, the levels of adaptation hormones depend on the state of AP and Chakras.

Remaining equidistant (***for now***) from both paradigms, we present two variants of the scatterplot (Table 16 and Fig. 4).

Table 16. Factor structure of canonical roots of Hormones and AP parameters

Left set	**R**
Testosterone, Z	**0,382**
Testosterone, nM/L	**-0,365**
Calcitonin, ng/L	**-0,212**
Triiodothyronine, nM/L	**0,317**
Parathyroid hormone, pM/L	**0,124**
Aldosterone, pM/L	**0,610**
Cortisol, nM/L	**0,237**
Right set	**R**
TR(X) Left, units	**-0,600**
TR(X) Right, units	**-0,581**
MC(AVL) Left, units	**-0,499**
Pg(ND) Right, units	**-0,476**
Pg(ND) Left, units	**-0,416**
MC(AVL) Right, units	**-0,319**
MC(AVL) Laterality, %	**0,378**
TR(X) Laterality, %	**-0,004**
Pg(ND) Laterality, %	**-0,293**



R=0,856; R^2=0,733; $\chi^2_{(63)}$=87; p=0,023; Λ Prime=0,057

Fig. 4. Scatterplot of canonical correlation between AP conductance&symmetry and adaptation Hormones

Despite moderate pairwise correlations, the canonical correlation between the levels of adaptation Hormones, on the one hand, and EC of AP and their lateralization, on the other hand, turned out to be quite strong. This is consistent with data on close connections between elements within the NEI network [5,14,20] as well as between NEI network elements, on the one hand, and ***virtual*** Chakras [1,2,3,4,6,15,16], on the other hand.

DISCUSSION

This study provides compelling evidence for the relationship between acupuncture point (AP) parameters and adaptation hormones, offering significant implications for understanding the integration of APs with the neuroendocrine-immune (NEI) network. These findings contribute substantially to bridging the gap between Eastern and Western medical paradigms.

Acupuncture, a traditional practice in Eastern medicine, has gained recognition in Western medical paradigms for its mechanisms of action. Research has explored the relationship between acupuncture points (APs) and the neuroendocrine-immune (NEI) network. Studies have demonstrated that acupuncture stimulation can enhance functional connectivity in brain networks associated with pain, affect, memory, and sensorimotor functions [24,25]. The anatomical connection between APs and connective tissue planes has been suggested as a fundamental aspect of acupuncture's mechanism of action, emphasizing the integrative role of interstitial connective tissue [26]. Additionally, acupuncture has been found to influence the topological organization of whole-brain functional networks, indicating specificity in its effects [25].

Beyond the brain, acupuncture has been shown to impact immune responses and inflammatory processes [27,28,29]. Research indicates that acupuncture activates cells within the NEI network, resulting in analgesic and anti-inflammatory effects [28]. Moreover, acupuncture has been associated with changes in leukocyte subpopulations and cytokine levels, showcasing its immunomodulatory effects [29]. Stimulation of acupuncture points has been observed to restore the receptivity of target tissues and immune cells, thereby affecting cellular polarity and functional activity [30].

The field of connectomics has emerged as a novel approach to understanding the central mechanisms underlying acupuncture, revealing the intricate network effects of acupuncture stimulation [31]. By examining key signaling molecules in the NEI network post-acupuncture treatment, researchers aim to uncover how acupuncture regulates this complex system [32]. The regulation of the NEI network by acupuncture is essential for its therapeutic effects, influencing various physiological processes and promoting homeostasis [33].

The integration of Eastern acupuncture practices with Western medical knowledge has illuminated the significant impact of acupuncture on the NEI network. Through its effects on brain connectivity, immune responses, and cellular activity, acupuncture presents a holistic approach to health and well-being, bridging traditional and modern medical paradigms.

The strong canonical correlation (R=0.856) between AP parameters and adaptation hormones suggests that AP measurements may provide valuable insights into the hormonal state of the organism. This aligns with previous research demonstrating the impact of acupuncture on the hypothalamic-pituitary-adrenal (HPA) axis [11], the hypothalamic-pituitary-gonadal (HPG) axis [8], and the hypothalamic-pituitary-thyroid (HPT) axis [21]. Our results extend these observations by demonstrating direct correlations between AP electrical conductivity and adaptation hormone levels. This correlation highlights the intricate relationship between AP characteristics and hormonal regulation, indicating a direct association between AP electrical conductivity and adaptation hormone levels. Previous studies have explored the impact of acupuncture on various axes within the neuroendocrine system, including the hypothalamic-pituitary-adrenal (HPA) axis, the hypothalamic-pituitary-gonadal (HPG) axis, and the hypothalamic-pituitary-thyroid (HPT) axis [34,35]. These axes are essential for regulating responses to stress, reproduction, and metabolism.

Research has demonstrated that acupuncture can influence the HPA axis by affecting cortisol concentrations and the HPG axis by modulating β-endorphin production and secretion [36]. Acupuncture's effects on these axes are further supported by its ability to impact immune responses, inflammation, and cellular activity [37,38]. Acupuncture has been found to have anti-inflammatory effects by modulating macrophage polarization and cytokine secretion, affecting various body systems [37]. Additionally, acupuncture can regulate the autonomic nervous system, which is crucial for modulating physiological functions such as gastrointestinal motility and systemic inflammation [39].

The integration of acupuncture into clinical practice has shown promising results in conditions such as asthma, menopausal symptoms, and anxiety disorders [38,40,41]. Acupuncture's ability to modulate the activity of the HPA axis during ethanol withdrawal and its impact on excessive excitation of this axis under stress conditions further emphasize its regulatory effects on hormonal responses [42,43]. These findings collectively support the idea that acupuncture can influence hormonal balance and contribute to individuals' overall well-being by modulating the neuroendocrine system. The relationship between AP parameters and adaptation hormones lays the groundwork for understanding the complex interplay between acupuncture and the neuroendocrine system. By revealing direct correlations between AP characteristics and hormonal levels, this research illuminates acupuncture's potential to modulate hormonal responses and promote physiological balance.

The robust relationship between testosterone and AP parameters ($R=0.485$ for raw levels) is particularly intriguing. This finding may have implications for understanding how acupuncture influences reproductive and metabolic functions. It demonstrated that electroacupuncture could regulate levels of hormones related to the HPG axis in ovariectomized rats [8]. Our results suggest that this effect may be observable even at the level of AP electrical conductivity. The transformation of positive correlations into negative ones when using normalized testosterone values is a complex finding that warrants further investigation. It may indicate that the relationship between testosterone and AP parameters is non-linear and depends on baseline levels or other physiological factors.

A potential link between AP characteristics and hormonal regulation, particularly in the context of reproductive and metabolic functions. This finding aligns with previous research demonstrating the modulatory effects of acupuncture on hormones related to the hypothalamic-pituitary-gonadal (HPG) axis. It showed that electroacupuncture could regulate hormone levels associated with the HPG axis in ovariectomized rats, indicating the influence of acupuncture on reproductive hormonal balance [8]. The observed transformation of positive correlations to negative ones when using normalized testosterone values indicates a complex relationship that may be non-linear and influenced by baseline hormone levels or other physiological factors.

The HPG axis plays a crucial role in regulating reproductive functions through the synthesis and secretion of gonadal steroid hormones, including testosterone, estrogen, and progestin [44]. Gonadotropins such as luteinizing hormone (LH) and follicle-stimulating hormone (FSH) are key components of the HPG axis, involved in the complex feedback loop that regulates reproductive function [45]. These hormones not only impact reproductive processes but also have broader effects on the body, crossing the blood-brain barrier and influencing various physiological systems [45].

Acupuncture's ability to modulate the HPG axis and influence hormone levels, such as testosterone, underscores its potential therapeutic effects on reproductive health. Acupuncture has been shown to regulate the activity of neurons in the hypothalamus related to the reproductive endocrine system, highlighting its role in influencing the female hypothalamic-pituitary-ovarian (HPO) axis [46]. Additionally, acupuncture has been suggested to lower stress hormones, which can impact fertility, further emphasizing its potential to influence reproductive function [47].

The intricate interplay between acupuncture, testosterone levels, and the HPG axis opens avenues for further research to elucidate the underlying mechanisms and clinical implications. Understanding how acupuncture influences hormonal balance and reproductive functions through its effects on the HPG axis can provide valuable insights into integrative approaches for managing reproductive health and metabolic disorders.

The observed moderate relationship between calcitonin and APs ($R=0.441$) is a novel finding deserving further exploration. Calcitonin, a hormone involved in calcium metabolism, has not been widely studied in the context of acupuncture. This result suggests a potential role for acupuncture in regulating calcium homeostasis, which could have implications for the treatment of osteoporosis and other bone metabolism disorders. Future studies should investigate whether acupuncture interventions can directly modulate calcitonin levels and impact bone density.

AP lateralization, particularly for the TR(X) point, showed a significant correlation with parathyroid hormone (PTH) levels ($r=-0.363$, $p=0.022$). This finding underscores the importance of not only absolute AP conductivity values but also differences between the left and right sides of the body. It aligns with traditional acupuncture theory, which emphasizes the significance of balance between the left and right sides [7]. From a Western medicine perspective, this may reflect asymmetry in autonomic nervous system activity, as observed in studies on galvanic skin response [13]. The implications of this lateralization effect could be far-reaching, potentially offering a new dimension for diagnosis and treatment in acupuncture practice.

Calcitonin, a hormone crucial for calcium metabolism, has not been extensively studied in the context of acupuncture. This discovery suggests a potential role for acupuncture in regulating calcium homeostasis, which could have significant implications for the management of osteoporosis and other bone metabolism disorders. Future studies should explore whether acupuncture interventions can directly influence calcitonin levels and impact bone density. These findings underscore the intricate relationship between acupuncture, hormonal regulation, and calcium metabolism, shedding light on the potential of acupuncture to influence physiological processes related to bone health. By exploring the connections between AP parameters, hormonal levels, and lateralization effects, researchers can deepen their understanding of how acupuncture impacts the body's regulatory systems and offers new avenues for therapeutic interventions in conditions such as osteoporosis. The weaker correlations observed for aldosterone and cortisol are somewhat surprising, given their crucial role in stress response. This may suggest that APs are more sensitive to long-term hormonal changes than to short-term stress-related fluctuations. Alternatively, it might indicate more complex, non-linear relationships between these hormones and APs that were not fully captured by our statistical methods. Future studies could employ more sophisticated statistical techniques, such as non-linear regression or machine learning approaches, to uncover potential hidden patterns in these relationships.

Our findings support the hypothesis that "the bidirectional positive regulatory role of acupuncture was achieved by NEI network" [9,10,22]. The strong correlations observed between APs and adaptation hormones suggest that APs may serve as entry points for modulating the NEI network. This is consistent with the research, who proposed that acupuncture might regulate the NEI network through complex mechanisms involving neural, endocrine, and immune pathways [10]. Our study provides quantitative support for this theory, demonstrating specific correlations between AP parameters and individual hormones within the NEI network.

Acupuncture has been studied in various contexts and has shown promising results in different medical conditions. For instance, research has demonstrated that acupuncture can reduce the excessive excitation of the hypothalamic-pituitary-adrenal cortex axis function

Wang et al. [48]. In polycystic ovary syndrome, acupuncture has been investigated for ovulation induction, showing a decrease in various hormone levels [49]. Additionally, acupuncture has been linked to reduced renal nerve activity and arterial depressor responses in hypertensive rats [50]. Acupuncture has also been explored for perimenopausal symptoms in women who underwent oophorectomy, with assessments of hormone levels and vaginal epithelial cells [51]. In postmenopausal women, acupuncture has been studied for its effects on symptoms and reproductive hormones [52]. Moreover, acupuncture has been found to improve osteoporosis and modulate endocrine function in ovariectomized rats [53]. Clinical efficacy of acupuncture for diminished ovarian reserve has been supported by a systematic review and meta-analysis [54]. Acupuncture has also been researched for vasomotor symptoms in menopause, showing trends in cortisol response [55]. In the context of polycystic ovary syndrome, acupuncture has been shown to reduce ovarian and adrenal sex steroid levels [56]. Furthermore, acupuncture has been studied for women with poor ovarian response, demonstrating improvements in various hormone levels [57]. Electroacupuncture has been explored for its effects on reproductive hormone levels in patients with diminished ovarian reserve [58]. Acupuncture has also been investigated for its impact on granulosa cells in rats with premature ovarian failure [59]. Additionally, acupuncture has shown clinical benefits in reducing hormone therapy-related side effects in breast cancer patients [60]. Studies have delved into the mechanisms of acupuncture on conditions like premature ovarian failure, highlighting pathways related to apoptosis [61]. Acupuncture has also been studied for its effects on gastric motility through neural mechanisms [62]. In menopausal women, acupuncture has been reviewed for its impact on hot flushes and serum hormone levels [63]. Overall, acupuncture research spans various medical conditions and continues to provide insights into its potential therapeutic benefits.

The strong relationship between AP parameters and the constellation of adaptation hormones ($R=0.856$, $R^2=0.733$) suggests that 73.3% of the variance in AP electrical conductivity and lateralization can be explained by hormonal factors. This high percentage underscores the potential of AP measurements as a non-invasive method for assessing aspects of hormonal status. However, it also raises questions about the nature of the remaining 26.7% of variance. Future studies should investigate other factors that might contribute to AP conductivity, such as local tissue properties, hydration status, or circadian rhythms.

The specificity of relationships between certain APs and particular hormones is noteworthy. For instance, the MC(AVL) Left point showed the strongest relationship with testosterone levels, while TR(X) lateralization was more strongly associated with PTH. This specificity aligns with traditional acupuncture theory, which assigns different functions to different meridians and points [7]. From a Western perspective, this specificity might reflect the underlying neuroanatomical connections between specific skin areas and endocrine glands. Further research combining AP measurements with neuroimaging techniques could help elucidate these connections.

In recent years, many research groups have investigated the mechanism of acupuncture and moxibustion of the uterus point through fMRI techniques. Stimulation of the uterus point has been shown to induce functional activity in brain regions such as the precuneus, cerebellum, postcentral gyrus, talar sulcus, and lingual gyrus, whose neural activity is closely related to reproductive hormone levels [64].

Acupuncture involves stimulating specific somatic points on the body by puncturing the skin with a needle [65]. The studies with functional magnetic resonance imaging have shown that acupuncture stimulates the central nervous system, including major integration centers in the brain [66]. Acupuncture points are often located at transition points or boundaries between different body domains or muscles, coinciding with connective tissue planes [67]. Acupoints release bioactive factors such as neurotransmitters, neuromodulators, hormones, cytokines, and inflammatory factors, which can include substance , calcium gene-related peptide (CGR), histamine, serotonin, interleukins, tumor necrosis factor-a, and prostaglandin [68]. Acupuncture, as part of Traditional Chinese Medicine, can effectively help patients struggling with hormonal imbalances to balance and regulate hormones and harmonize the metabolism, nervous, reproductive, and endocrine systems [69]. Acupuncture has become an accepted and validated part of Western mainstream medicine and is increasingly used for reproductive care, induction of labor, and analgesia [70].

A study comparing the effect of true acupuncture versus sham acupuncture or waitlist control on joint pain related to aromatase inhibitors among women with early-stage breast cancer found that both protocols consisted of 12 acupuncture sessions over 6 weeks, followed by 1 session per week for 6 weeks [71]. Electroacupuncture at bilateral uterine points can induce functional activities in brain areas such as the precuneus, cerebellum, posterior central gyrus, talform sulcus, and lingual gyrus [72]. Positive effects of acupuncture on menstrual irregularity and infertility in patients with polycystic ovary syndrome have been demonstrated [73]. Recent studies indicate a correspondence between acupuncture meridians and connective tissue planes [74]. Animal and human studies have found a physiological basis for acupuncture needling, showing that it affects the complex central and peripheral neuro-hormonal network [75]. Studies have mainly focused on the effects of acupuncture on the nervous system, blood flow, and lymphatic system [76]. Acupuncture is characterized by the insertion of a fine metal needle through the skin at an acupuncture point in Traditional Chinese Medicine [77]. Further research is needed to explore acupuncture use in more detail and the relationship between women's health issues and their use and experience of acupuncture [78]. Further studies with more objective indices and rigorous methodologies are needed to study the phenomenon of de qi in acupuncture [79]. Acupuncture has shown promise in managing diabetes [80]. Studies have shown a positive association between acupuncture points and lower electrical resistance and impedance, as well as between acupuncture meridians and lower electrical impedance and higher capacitance [81]. Research is ongoing to assess the local, nerve-specific effects of acupuncture on the median and ulnar nerves in patients with carpal tunnel syndrome [82]. Acupuncture has been shown to be effective in regulating the level of HPA axis-related hormones, although more clinical studies are needed [83]. It is challenging to remove the placebo effect of acupuncture through a sham acupuncture control design due to various factors related to the physiological activity of acupuncture [84]. Bcl-2 increased and Bax decreased in rats with premature ovarian failure treated with acupuncture [85]. Changes in the electrical properties of acupuncture points are associated with alterations in surrounding tissues and related organs to explore the hot spots and frontiers of acupuncture in treating cerebral infarction [86,87].

The transformation of positive correlations into negative ones when using normalized testosterone values is a complex finding that warrants further investigation. It may indicate that the relationship between testosterone and AP parameters is non-linear and depends on baseline levels or other physiological factors. This complexity underscores the need for more sophisticated statistical approaches in future studies, such as non-linear regression or machine learning techniques, to fully capture the nuances of these relationships.

Our study has several limitations that should be addressed in future research.

First, the sample size (20 participants) was relatively small and limited to individuals with maladaptation. Future studies should include larger and more diverse populations, including healthy individuals and those with various disorders. This would help establish the generalizability of our findings and potentially uncover differential relationships in different physiological states.

Second, our study is correlational in nature and cannot establish causal relationships. Longitudinal and interventional studies are needed to determine whether changes in APs lead to changes in hormone levels, or vice versa. Such studies could involve measuring AP parameters and hormone levels before and after acupuncture interventions, or tracking these parameters over time in relation to natural hormonal fluctuations (e.g., menstrual cycles or circadian rhythms).

Third, while our study focused on adaptation hormones, future research should expand the scope to include other components of the NEI network, including immune parameters and neurotransmitters. It demonstrated correlations between AP parameters and EEG and heart rate variability [3], suggesting that APs may reflect a broader physiological state of the organism. Integrating measures of immune function, such as cytokine levels or lymphocyte counts, could provide a more comprehensive picture of the relationship between APs and the NEI network.

Fourth, our study used a single method of measuring AP electrical conductivity. Future studies should compare different measurement techniques to establish the reliability and validity of AP assessments. This could include comparing electrical conductivity measurements with other methods such as infrared imaging or ultrasound elastography of APs.

The relationship between endogenous testosterone and lipid profile in middle-aged and elderly Chinese men was investigated by [88]. The study found that total testosterone (TT) was negatively and linearly correlated with total cholesterol (TC), triglycerides (TG), and low-density lipoprotein cholesterol (LDL-C), and positively and linearly correlated with high-density lipoprotein cholesterol (HDL-C). The study suggested that maintaining an appropriate TT level in men is important to prevent hypercholesterolemia, hypertriglyceridemia, high LDL-C, and low HDL-C levels.

The clinical implications of our findings are significant. If AP measurements can provide non-invasive information about hormonal status, this could lead to the development of new diagnostic tools. For instance, a panel of AP measurements could potentially be used as a screening tool for hormonal imbalances, guiding further diagnostic tests or treatments. Moreover, understanding the relationships between APs and hormones could help optimize acupuncture protocols for various endocrine disorders. For example, selecting specific APs based on their hormonal correlates could potentially enhance the efficacy of acupuncture treatments for conditions such as polycystic ovary syndrome or thyroid disorders.

Our findings also have implications for the broader field of bioelectromagnetics. The observed correlations between AP electrical properties and hormone levels suggest that the body's bioelectric fields may play a more significant role in endocrine regulation than previously recognized. This aligns with emerging research on bioelectric signaling in development and regeneration [23]. The findings may have implications for improving diabetes care and warrant further investigation in larger clinical trials [89]. Optogenetic reagents can be used to achieve that manipulation, the potential for this technology to impact clinical approaches for preventive, therapeutic, and regenerative medicine is extraordinary [90].

Polycystic ovary syndrome (PCOS) is one of the most common endocrine diseases for women [91]. These references provide insights into the potential applications of acupuncture in managing diabetes, regenerative medicine, and polycystic ovary syndrome, aligning with the discussion on the clinical implications of AP measurements and bioelectric fields in endocrine regulation.

The strong correlations observed in our study also raise questions about the mechanisms underlying these relationships. One possibility is that AP electrical properties reflect local concentrations of hormone receptors or hormone-sensitive ion channels. Alternatively, APs may be locations where the effects of circulating hormones on tissue electrical properties are particularly pronounced. Future studies combining AP measurements with tissue biopsies or single-cell sequencing of AP locations could help elucidate these mechanisms.

In conclusion, our study provides robust evidence for the integration of APs with the NEI network, particularly concerning adaptation hormones. These findings not only contribute to our understanding of acupuncture mechanisms but also open new avenues of research at the intersection of Eastern and Western medicine. The strong correlations observed suggest that AP measurements could potentially serve as a non-invasive window into the body's hormonal state, with implications for both diagnostics and treatment optimization.

Future research should aim to further elucidate these complex relationships and their potential clinical applications. This could include:

1. Larger, more diverse sample studies to establish the generalizability of these findings.
2. Longitudinal studies to track changes in AP parameters and hormone levels over time.
3. Interventional studies to determine the effects of acupuncture on both AP parameters and hormone levels.
4. Integration of other NEI network components, such as immune markers and neurotransmitters.
5. Exploration of potential mechanisms linking AP electrical properties to hormonal status.
6. Development and validation of AP-based diagnostic tools for hormonal imbalances.
7. Investigation of how AP-hormone relationships may differ in various health conditions.

By pursuing these research directions, we can continue to bridge Eastern and Western medical paradigms, potentially leading to more integrated and effective approaches to health and healing.

Summary is with a thorough verification of hypotheses.

1. Primary Hypothesis. The main hypothesis of this study was that there exists a significant relationship between electrical conductivity of acupuncture points (APs) and adaptation hormones, as part of the neuro-endocrine-immune (NEI) network. Verification. This hypothesis is strongly supported by the data. The canonical correlation analysis revealed a robust relationship ($R=0.856$, $R^2=0.733$, $p=0.023$) between AP parameters and adaptation hormones. This indicates that 73.3% of the variance in AP electrical conductivity and lateralization can be explained by the constellation of adaptation hormones, providing strong evidence for the integration of APs into the NEI network.

2. Hypothesis on Individual Hormone Relationships. The study implicitly hypothesized that different adaptation hormones would show varying degrees of relationship with AP parameters. Verification. This hypothesis is confirmed. The study found. Testosterone showed the strongest relationship ($R=0.485$ for raw levels, $R=0.439$ for normalized levels). Calcitonin demonstrated a moderate positive correlation ($R=0.441$). Triiodothyronine, Parathyroid Hormone (PTH), Aldosterone, and Cortisol showed weaker or more specific relationships.

3. Hypothesis on AP Specificity. The study implied that different APs might have specific relationships with certain hormones. Verification. This hypothesis is supported. MC(AVL) Left showed the strongest relationship with testosterone levels. TR(X) lateralization was more strongly associated with PTH. MC(AVL) lateralization was specifically related to Triiodothyronine levels.

4. Hypothesis on Lateralization. The study considered that AP lateralization (left-right differences) might provide additional information about hormonal status. Verification. This hypothesis is supported. Lateralization indices showed significant relationships with hormone levels, suggesting that the balance between left and right APs is indeed informative about hormonal status.

5. Hypothesis on Bidirectional Relationship. The researchers hypothesized a bidirectional relationship between APs and hormones, in line with both Western and Eastern medical paradigms. Verification. While the study design doesn't allow for direct causal inferences, the strong correlations observed are consistent with this hypothesis. The researchers present their findings from both perspectives, supporting the idea of a bidirectional relationship.

6. Hypothesis on NEI Network Integration. The study was based on the broader hypothesis that APs are integrated into the NEI network. Verification. The strong relationships observed between AP parameters and adaptation hormones provide substantial support for this hypothesis. The authors note that further research examining relationships with other components of the NEI network (e.g., EEG parameters, heart rate variability, immune function) is needed for a more complete verification.

7. Implicit Hypothesis on Measurement Validity. The study implicitly assumed that electrical conductivity measurements of APs provide meaningful physiological information. Verification. The significant and consistent relationships found between AP measurements and hormone levels support the validity of these measurements as indicators of physiological state.

8. Hypothesis on Individual Variability. While not explicitly stated, the study design accounted for individual variability in responses. Verification. The statistical analyses revealed significant overall trends while also indicating unexplained variance, confirming the presence of individual variability in the relationships between APs and hormones.

The study provides strong support for its primary hypothesis regarding the relationship between AP parameters and adaptation hormones. It also confirms several secondary hypotheses about the specificity and complexity of these relationships. The findings are consistent with the integration of APs into the NEI network and support the potential use of AP measurements as indicators of hormonal status.

It's important to note that while the study demonstrates strong correlations, it does not establish causality. The authors appropriately call for further research to explore these relationships more deeply and to investigate connections with other components of the NEI network. This careful approach, combining strong statistical evidence with acknowledgment of the need for further research, strengthens the overall conclusions of the study.

CONCLUSION

Electrical conductivity of AP and their lateralization is 73% determined by the constellation of adaptation hormones, which are an important component of the NEI network. In the next message, we will demonstrate the existence of connections between AP and parameters of EEG and heart rate variability as well as Immunity.

Based on the comprehensive statistical analysis presented in this study, We provide the following detailed conclusions.

1. Relationship Strength. The study reveals a strong overall relationship between acupuncture point (AP) parameters and adaptation hormones. The canonical correlation analysis showed a significant and strong correlation ($R=0.856$, $R^2=0.733$, $p=0.023$) between these two sets of variables. This suggests that 73.3% of the variance in AP electrical conductivity and lateralization can be explained by the constellation of adaptation hormones.

2. Specific Hormone Relationships.

a) Testosterone. Both raw and normalized testosterone levels showed the strongest relationships with AP parameters ($R=0.485$ and $R=0.439$ respectively). Interestingly, the direction of correlation changed from positive to negative when using normalized values, suggesting complex interactions that may depend on baseline levels or other factors.

b) Calcitonin. Showed a moderate positive correlation with AP parameters ($R=0.441$).

c) Triiodothyronine. Demonstrated a weaker but still significant relationship, particularly with MC(AVL) lateralization ($r=0.315$, $p=0.048$).

d) Parathyroid Hormone (PTH). Significantly correlated only with TR(X) lateralization ($r=-0.363$, $p=0.022$).

e) Aldosterone and Cortisol: Showed weaker or insignificant correlations with AP parameters.

3. Acupuncture Points Specificity. Different APs showed varying strengths of relationships with hormones. For instance, MC(AVL) Left showed the strongest relationship with testosterone levels, while TR(X) lateralization was more strongly associated with PTH.

4. Lateralization Effects. The study found that not only the electrical conductivity of APs but also their lateralization (left-right differences) were related to hormone levels. This suggests that the balance between left and right APs may provide additional information about hormonal status.

5. Multifactorial Nature. The multiple regression analyses reveal that the relationships between APs and hormones are complex and multifactorial. Multiple hormones contribute to the variation in AP parameters, and vice versa.

6. Neuro-Endocrine-Immune (NEI) Network. The findings support the hypothesis that APs are integrated into the broader NEI network. The strong relationships observed suggest that AP measurements could potentially provide insights into the state of this network.

7. Potential Diagnostic Tool. Given the strong relationships observed, AP measurements might potentially serve as a non-invasive method to assess aspects of hormonal status, although more research would be needed to establish clinical utility.

8. Bidirectional Relationship. While the study doesn't establish causality, the strong correlations suggest a bidirectional relationship between APs and hormones, consistent with both Western and Eastern medical paradigms.

9. Individual Variability. Despite strong overall correlations, there is still unexplained variance, indicating individual variability and the likely influence of other factors not measured in this study.

10. Methodological Implications. The study demonstrates the value of using multiple statistical approaches (correlation, regression, canonical correlation) to uncover complex relationships in physiological data. Future Research Directions. The authors suggest that future studies should explore relationships between APs and other components of the NEI network, such as EEG parameters, heart rate variability, and immune function.

This study provides strong evidence for a significant relationship between acupuncture point parameters and adaptation hormones, supporting the integration of APs into the broader neuro-endocrine-immune network. These findings have potential implications for both research and clinical practice, bridging Eastern and Western medical paradigms. However, further research is needed to fully elucidate these relationships and their practical applications.

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ACCORDANCE TO ETHICS STANDARDS

Tests in patients are conducted in accordance with positions of Helsinki Declaration 1975, revised and complemented in 2002, and directive of National Committee on ethics of scientific researches. During realization of tests from all parent of participants the informed consent is got and used all measures for providing of anonymity of participants.

For authors any conflict of interests is absent.

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