Psychological and cortisol human reactions in experimental stress

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Summary

   The manuscript provides the description of the methodological and instrumental base for experimental stress research. The main results of the research aimed to identify endocrine and psychological characteristics of stress reactivity in both gender young adults in response to social stressors. The algorithm and components of complex stress reactivity assessment was developed. Two types of social daily hassles (microstressors) were modeled in experimental conditions (public speaking and interview with opposite sex interviewer). Stress reactivity was assessed using endocrine (cortisol level in saliva) and psychological (observation and psychometric measurements). Different types of stress reactivity were identified significantly associated with gender, coping strategies, emotional experience, personality traits and specific of stress situation.

social stress / experimental stress / assessment / stress reactivity / coping strategies / cortisol

INTRODUCTION

   Stress and its negative consequences and disorders are considered one of the major problems for different sciences and applied areas in medicine, psychology, sociology. The empirical data shows that the most dangerous consequences associated with the experience of stress are cardiovascular disorders and mental disorders such as depression, anxiety, learned helplessness, health risk behaviors [1,2]. The actual trends in international and national stress research are associated with the investigation of the mechanisms responsible for appearing stress reactions and identification of the hierarchy of their determinants. This type of knowledge could lead to finding new effective medical and psychological treatments.

   The theoretical and methodological base of our experimental method was built on cognitive-phenomenological approach of R. Lazarus and his understanding of stress as transactional process. Lazarus and Folkman [3] definition of stress includes psychological and physiological reactions. Everyday life events are considered as stressors which could be systematized by the level of negative effect and time for post adaptation. The following classification includes everyday stressors or daily hassles (microstressors), traumatic life events (macrostressors) and chronic stressors [4]. In the experimental research daily hassles were modeled by two types of experimental situations.

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OBJECTIVE AND AIMS

The study objective was to identify psychological and endocrine human reactions in response to different everyday stresses in healthy males and females. The following study aims were defined: to identify emotional, cognitive, behavioral and gender specific reactions in response to experimental stressors; to investigate stress reactivity of hypothalamic-pituitary-adrenal axis in two modeled stress situations assessing the cortisol level in saliva.

MATERIAL AND METHODS

Two experimental stress inducing situations were developed in order to address study objective and aims.

Public speaking stress. To model this type of stress situation we used the version of classic Trier Social Stress Test in Halpern's modification [5].

Stress in response to communication with an opposite sex unknown interviewer (sexual behavior). To model this type of stress we used modified experimental situation based on dating interview [6].

The modification and tailoring of Halpern's experimental procedures were important to address cultural, ethical and economic specificity of the population and the site. The study protocol, informed consent forms and procedures were reviewed and approved by the Institutional Review Board of St. Petersburg State University. The inclusion criteria involved the willingness to participate in two-day experiments, age range 18 - 35 y.o. (young adults), no medical condition requiring hormonal therapy, no psychiatric or psychological conditions identified as barriers for understanding informed consent. The total sample size was 151 participants, men and women, age range from 19 to 34 y.o. The recruitment was conducted using two approaches - face-to-face recruiting in educational setting and recruiting in on-line social networks.

Each participant took part in two-day experimental procedures with no longer than one week interval in between. The scheduled times of the experiments were the same to avoid the influence of daily cortisol level variations.

First study visit – modeling experiment "Public speaking stress"

Upon signing the informed consent, participants were asked to provide pre-test saliva test, fill out pre-test psychometric surveys and conduct pre-test psycho-physiological express-tests. Prior to the experiment, the participants received the following instruction: "Today you will participate in the experimental procedure to evaluate your abilities and achievement level in public speaking. You will receive a task to prepare a speech on a proposed topic which has discussing issues. You will have 10 minutes to prepare the speech and a list of facts which should be used to support your arguments. During the speech you need to present the opposite arguments and position on the proposed topic. Your speech will last 5 minutes and it will be videotaped for future scientific analysis". To model public speaking stress situation we used a stressful and unfamiliar topic, that is, a fictional project of "law banning the export of specific species of Conus family mollusk to Russia".

After 10 minutes of preparation the participant was taken to another room with video camera and soffits in front of the place for public speaking and videotaping. The participant had to start his/her presentation after a beep and finish it in 5 minutes after the second beep. In case of early finishing the participant was instructed to keep standing until the final time.

The saliva collection for cortisol level detection from all study participants was conducted using the following algorithm:

a) the saliva samples were collected between 2 PM – 8 PM;

b) 1 hour prior to the saliva collection time (scheduled experiment time) the participants were not allowed to eat, smoke or drink other beverages except water;

c) three samples of saliva were collected from each participant at each of two days of experiments – pre-test before the experimental procedure, 1st post-test right after the experiment and 2nd post-test 10–20 minutes after the experimental procedure.

For quantitative detection of the free cortisol concentration level in saliva (as a marker of stress reactivity) we have used rapid test kits

"Cortisol in Saliva – Immune-Enzyme Assay ("XEMA" production, Russia). Testing of the saliva samples was conducted according to the required algorithm for those test kits. 0.2 – 1.0 ml of saliva samples were collected using sterile disposable SaliCap supplies (IBL production, Germany). Minimally detected free cortisol concentration in saliva for this test was 0.3 ng/ml, standard cortisol concentration as stress marker was 0.5 — 3.0 mg/ml (7.5 — 8.5 hours after morning awakening); 2.8 — 7.7 mg/ml (3.5 — 4.5 hours after morning awakening).

After stress modeling experimental procedures and short debriefing, the participants were asked to fill out post-test psychometric surveys and conduct post-test psycho-physiological express-tests (detailed description will be provided below). At the end of the first study visit the second study visit was scheduled and short description of second study experiment was provided.

Second study visit – modeling experiment "Stress in communication with an opposite sex unknown interviewer"

After filling out pre-test surveys, first saliva test and pre-test psycho-physiological tests, the following instruction was provided to each participant prior to the experiment: "The experimental situation will consist of two parts today. The first part will last 5 minutes. During this time 10 photos of people of the opposite sex will be demonstrated to you on the computer monitor (the duration of exposition for each photo – 30 sec). You will need to evaluate each person by answering two questions: 1) How attractive does this person seem to you? 2) How attractive do you seem to be to this person? Choosing your answers, please, use the cursor under the photo on the computer screen".

The second part of the experimental procedure, an interview with an opposite sex interviewer, was focused on questions related to participants' choices and sexual behavior. The following instruction was provided: "After the first part of the experiment you will meet the interviewer who will talk to you for approximately 15 minutes, asking questions about your impressions and evaluations of presented people and

associations with your actual life experience. The interviewer will assess your ability to speak out and provide arguments freely and assertively". During the interview, 2 – 3 sensitive sexually oriented questions were asked by the interviewer to provoke a stress reaction in participants.

After stress modeling experimental procedures, last saliva sample collection and short debriefing, the participants were asked to fill out post-test psychometric surveys again and conduct post-test psycho-physiological express-tests (the same as after the first experiment).

The following psychometric and physiological measures and instruments were used for pre- and post-experiment assessment and evaluating stress reactivity:

Survey for Stress Reactions Self-assessment. The short version was developed based on the family stress survey [7] and self-assessment survey of emotional states [8] and includes the list of stressors, the dynamics of self-evaluation of emotional states, feelings, casual attributions, the level of self-control, used coping strategies in stress.

Stait-Trait Anxiety Inventory (STAI) [9]

Health Risk Behaviors Survey developed by A. Shaboltas with colleagues from St. Petersburg State University at the Department of Behavioral Psychology and Prevention of Behavioral Deviations, which includes questions on demographic and different health risk behaviors (substance use, sexual behaviors, deviant behaviors, suicidal tendencies, violence experience, etc.) [10].

Short version of Adverse Childhood Experiences Questionnaires (ACE) developed as a part of long-term research study on adverse childhood experiences by Center of Disease Control. Original version of the questionnaire included 200 items. In current study, the short version eligible for international settings was used [11].

Russian version of Dissociation Experiences Scale (DES) developed and adapted by N. Tarabrina [12].

Short version of Personality Traits Inventory Big Five developed by J. Gerris with colleagues. This instrument allows to evaluate the level of 5 basis personality factors: extraversion, agree-

ableness, conscientiousness, emotional stability and openness/resourcefulness which are considered transcultural and universal [13].

Sensation Seeking Scale. The most popular version was developed by Zukerman as an instrument to diagnose the need in sensation seeking in 1964 and consists of 16 pairs of statements [14]. In our study we used a modern version of the instrument with 40 questions which is considered a part (scale) of five-factor personality traits inventory developed by Zukerman [15].

Achievement Motivation Survey. The modified version of the survey was developed by A. Mehrabian and adapted by M. Mahomed-Eminov for Russia. Survey was presented in two forms for male participants (form A) and female participants (form B) [16].

Brief Symptom Inventory (BSI) – short version of SCL-R-90 developed by L. R. Derogatis with the main purpose to identify clinical symptoms. BSI consists of 53 items related to 9 factors: somatization, obsessive-compulsive disorder, interpersonal anxiety, depression, general anxiety, hostility, anxious-phobic thoughts, paranoiac thoughts, psychotism [17].

Observation of Emotional Arousal. Technical and observational algorithms were developed by A.T. Puni in 1977 in order to observe and evaluate the dynamics of visual emotional arousal symptoms [18]. The algorithm includes the eval-

MAIN RESULTS

The types of stress reactivity in response to experimental stressors

Developed experimental model of human stress allowed to identify a significant association between the types of stress reactivity based on the level of cortisol and the self-esteem specific in subjective evaluation of emotional states and coping strategies used in modeled stress situations. The objective significant stress reactivity in cortisol level in saliva after the experimental stress was considered as 30% increasing of cortisol level in one post-test sample or 30% summarized increasing in two post-test samples. The following considerations were based on the analysis of the literature on cortisol stress reactivity research in human [6]. Positive cortisol stress reactivity after two experiments is presented in Table 1.

In the first experiment (public speaking stress) significant positive stress reactivity was found in 53.1% female participants and 61.1% males ($\chi2 = 1.680$, $p > 0.05$). In the second experiment 67.2% of females demonstrated significant cortisol reaction as well as 55.6% of males ($\chi2 = 0.767$, $p > 0.05$). The gender differences were not statistically significant. Four types of cortisol stress reactivity were identified (Table 2).

Table 1. Cortisol reactivity in response to different experimental stress.

Experimental situation	Gender	Significant reactivity	No significant reactivity
Public speaking	Males	61.1%	38.9%
	Females	53.1%	46.9%
Interview	Males	55.6%	44.4%
	Females	67.2%	32.8%

uation of dynamics of the following behavioral parameters: attention, mimic, pantomimic, physical movements, static poses, speech, vegetative characteristics [19].
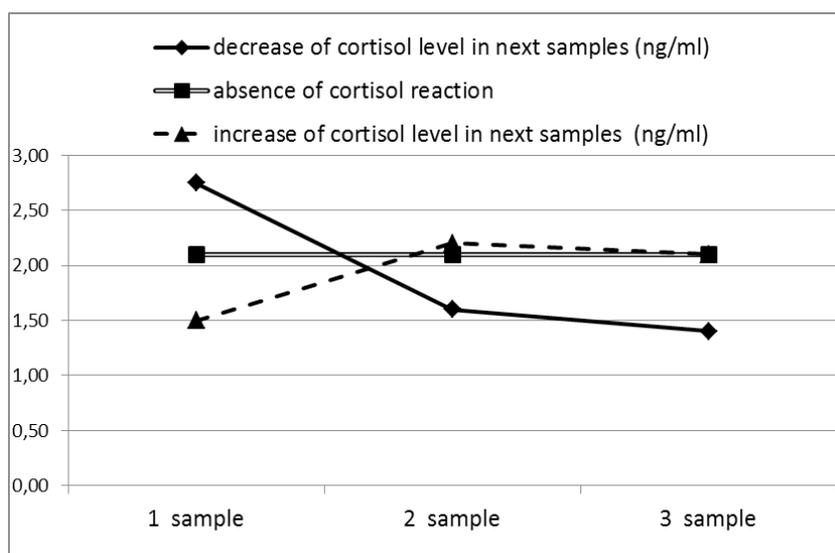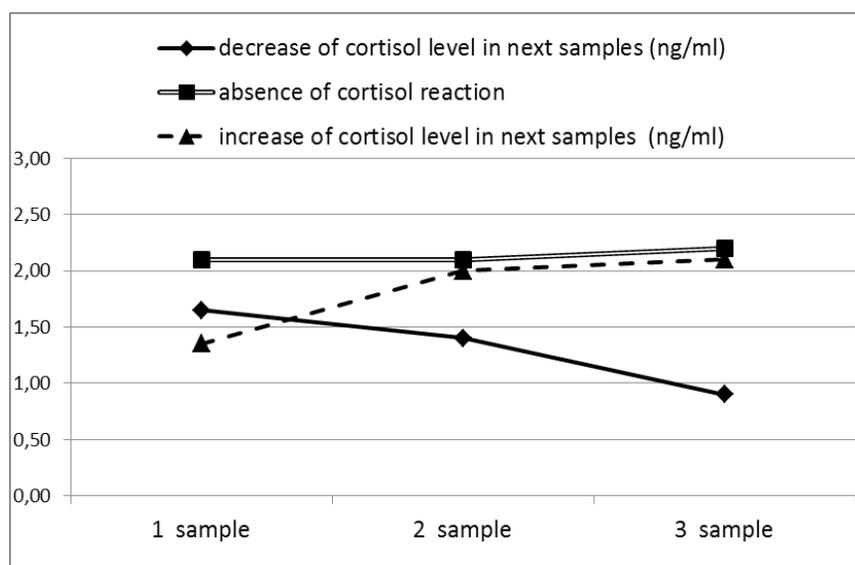
The psycho-physiological express-tests are not discussed in this article.

The following statistical procedures and methods were used to analyse the data: descriptive statistics, dispersion and regression analysis (by SPSS, version 18).

Significant stress reactivity in cortisol level after the first and second experiments was found in 37.0 % males and 37.5 % females, after the first experiment only and after the second experiment only, the figures were 24.1% and 15.6%, 18% and 29.7%, correspondingly. 20.4% males and 17.2% females didn't demonstrate any significant increasing in cortisol level after both experiments. Significant correlation between cortisol level and previous experience of public speaking was not found (Fig. 1 – 2).

Table 2. Cortisol stress reactivity types

Gender	Significant reactivity in both experiments	Significant reactivity after the first experiment	Significant reactivity after the second experiment	No reactivity in both experiments
Males	37.0%	24.1%	18.5%	20.4%
Females	37.5%	15.6%	29.7%	17.2%

Fig. 1. The dynamics of cortizol level in 1st experiment.

Fig. 2. The dynamics of cortizol level in 2nd experiment.

In terms of subjective evaluation, the data shows that after both experiments the participants in general felt more tense which was not always associated with an increase in cortisol level. In general, the coping strategies were complex and included several coping actions. The participants more often used active adaptive actions ("I was thinking how to resolve the experimental tasks"). Passive less adaptive actions were also used by the participants, but not so often. It is concluded that subjective perception of everyday stress could differ significant-

ly from its physiological correlates. The correlations between psychological and biological determinants in social stress situations.

The research data on endocrine, physiological and psychological reactions in response to modeled leads to the following statistically significant conclusions:

1. Modeled situations of everyday stressors don't induce significant stress reactions in all registered parameters. It was found that automatic (involuntary) characteristics of mental states are the most sensitive parameters that demonstrated significant dynamics in experimental situations.

2. Registering the levels of subjective feelings such as active, fresh, tension could be considered as informative indicators of psychological stress and could be utilized in diagnostic and correction. Those subjective feelings as a complex accompanying the feelings of calmness, bravery, satisfaction could be considered func-

ments and personality traits: extraversion, agreeableness, openness to experience. A significant correlation was found between gender and cortisol stress reactivity in the second experiment and with conscientiousness (p=.050). Cortisol stress reactivity in response to communication with a person of the opposite sex was more significant in males with low and females with high level of conscientiousness. A significant correlation was also found between gender and emotional stability (p=.006 in the first experiment and p=.004 in the second experiment). Emotional stability in female participants was lower than in males.

The influence of extraversion on the frequency of using coping strategy was also found: "I was trying to hold my emotions" in both experiments. High extraversion level reduces the frequency of using such strategy.

A negative influence of conscientiousness on the frequency of using coping strategy "I was hoping a miracle would happen and help me resolve the tasks" was detected in the first exper-
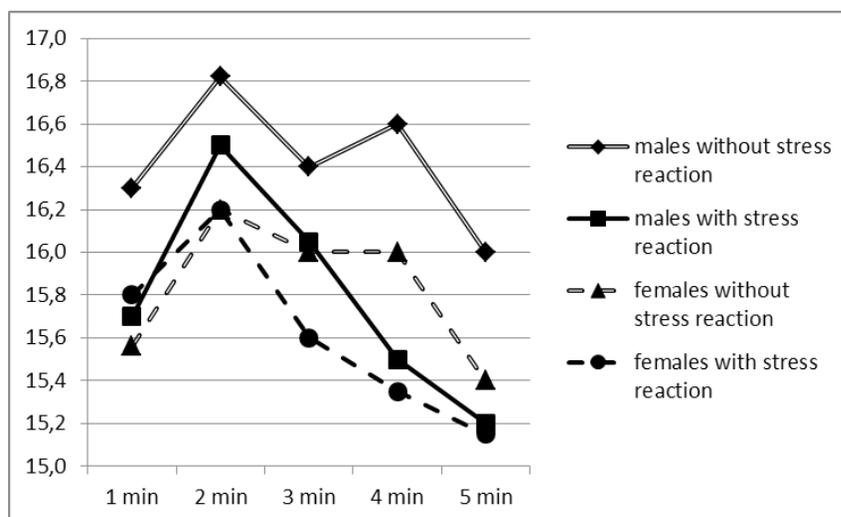

Fig 3. The dynamics of emotional arousal (in balls).

tional mobilizing manifestation in stress situations. Fig. 3 represents the general dynamics of emotional arousal.

The personality factors, coping strategies and cortisol reactivity in experimental stresses

The results of two-factor dispersion analysis didn't discover significant correlations between gender, cortisol stress reactivity in both experi-

iment: a high level of conscientiousness reduces the frequency of using this strategy, while in the second experiment, a high level of conscientiousness reduces the frequency of using coping strategy "I was criticizing the investigator in my mind".

A positive correlation was found for benevolence and the frequency of using strategy "I reduced the significance of the experiment" in the

first experiment: a high level of benevolence reduces the frequency of using this strategy.

A negative correlation was found for emotional stability and the frequency of using coping strategy "I was criticizing myself" in the second experiment: a high level of this personality trait reduces the use of this coping strategy.

In the second experiment it was found that the openness to new experience reduces the frequency of using coping strategy "I have reduced the significance of the experiment".

The combined correlation was detected in the first experiment for conscientiousness and emotional stability and the frequency of using cop-

ing strategy "I was trying to see the positive aspects for myself in the experimental situations": a high level of conscientiousness increases the frequency of using this coping strategy, while high level of emotional stability reduces it.

The cortisol level, adverse childhood experience, dissociations, sensation seeking and achievement motivation

Negative significant correlations were found between the levels of dissociation and achievement motivation in participants who demonstrated significant cortisol level increasing after

Table 3. The correlations of personality factors and coping strategies

Coping	1st experiment	2nd experiment		1st experiment					2nd experiment				
	% of participants			EXT	CONS	AGR	EmSt	RES	EXT	CONS	AGR	EmSt	RES
COP01	61.0%	50.0%	r=	-.162	-.060	-.066	-.002	-.046	-.100	-.014	.066	-.082	-.096
			p=	.040	.259	.239	.494	.310	.141	.440	.240	.188	.150
COP02	48.3)	49.2%	r=	-.073	-.070	.121	-.047	-.005	.052	-.010	-.001	-.136	-.038
			p=	.216	.226	.095	.305	.478	.288	.459	.497	.071	.341
COP03	53.4	52.5%	r=	-.227	.112	-.030	-.044	-.019	-.198	.000	-.148	-.187	-.159
			p=	.007	.114	.372	.319	.417	.016	.500	.055	.021	.043
COP04	24.6	6.8%	r=	-.156	-.208	-.100	-.174	.040	.043	-.039	-.018	-.078	.015
			p=	.046	.012	.140	.030	.333	.323	.336	.423	.200	.436
COP05	5.1%	1.7%	r=	-.141	.143	.031	.005	.138	.100	-.022	-.061	-.055	.048
			p=	.063	.062	.369	.476	.068	.141	.405	.256	.276	.304
COP06	11.0%	13.6%	r=	-.149	.112	-.111	-.070	.037	-.011	-.197	-.170	-.173	-.084
			p=	.054	.114	.115	.227	.347	.453	.016	.033	.031	.184
COP07	37.3%	14.4%	r=	-.169	-.077	.045	-.127	-.111	-.040	-.162	-.077	-.278	-.103
			p=	.033	.205	.316	.085	.116	.333	.040	.203	.001	.133
COP08	51.7%	33.3%	r=	-.106	-.132	-.279	-.090	-.150	-.155	-.143	-.193	-.070	-.260
			p=	.126	.077	.001	.165	.052	.047	.061	.018	.226	.002
COP09	8.5%	1.7%	r=	-.112	.020	.015	-.052	.128	.088	-.109	-.033	-.040	.020
			p=	.113	.414	.435	.289	.084	.172	.120	.362	.333	.415
COP10	15.3	11.0%	r=	-.141	.021	-.139	.051	.060	-.003	-.139	-.057	-.088	.067
			p=	.064	.410	.067	.292	.260	.488	.066	.271	.172	.235
COP11	73.7)	72.0%	r=	.016	-.008	.213	-.152	.130	.031	-.033	-.002	.057	-.093
			p=	.431	.467	.010	.050	.081	.371	.363	.490	.269	.158
COP12	(94.9%)	81.4%	r=	.032	.181	.165	.011	.142	.011	.079	.160	.004	.080
			p=	.366	.025	.037	.453	.063	.452	.198	.042	.481	.193

the first experiment (p=.014) and after the second experiment (p=.002).

Positive correlations were found between sensation seeking and achievement motivation (p=.013) and between dissociation and sensation seeking (p=.009) in participants with significant cortisol reaction after the second experiment.

Gender differences were found in adverse childhood experience. Female participants told about the experience of sexual violence much more often (p=.007), male participants more often told about the alcohol or drug abuse of adults living with them (p =.035). The participants who had experience of living with adults with substance abuse more often demonstrated significant stress reactivity in response to public speaking task (p =.008).

Health risk behaviors

A relatively high prevalence of health risk behaviors was detected in the study sample without significant gender differences: active smoking (27.78% males and 21.88% females); alcohol use (72.22% males and 90.63% females); experience of drug use (66.67% males and 56.25% females); experience of sexually transmitted diseases (14.81% males and 18.75% females). The only statistically significant difference was found in the frequency of alcohol use. Unexpectedly, female participants drink alcohol more often than males (p =.021).

Non-verbal stress reactions in public speaking stress experiment

The technique and algorithm developed by Puni to observe emotional states were used for observational data collection. Statistically significant increase in breathing frequency and sweating appeared during the second minute of speaking which was combined with tension in face muscles (eyes squeezing, winking etc.). The facial expression was becoming tenser during the fourth minute. At the end of the experiment all participants demonstrated a decrease of stress expressions in the voice (p=.014).

Participants without cortisol stress reactivity much more often looked to the right side from the observer in comparison with the participants with cortisol stress reactivity (p=.012). Participants with cortisol stress reactivity more often fixed the gaze direction ("look in one direction") during public speaking. Female participants more often changed the gaze direction in comparison with male participants. The majority of the participants directed their head movements towards the video camera during speaking. Head movements towards the opposite direction were observed only in males. Arms movements towards the opposite direction were observed only in males. Female participants more often moved their arms away from their bodies. Male participants in general and specifically those with stress reactivity were characterized by abrupt change of poses (p=.042). Arms movements were more monotonous in females, especially in those with stress reactions (p=.028).

Male participants without cortisol stress reactivity opened their eyes wide (p=.031) and wrinkled their noses less frequently. Females with stress reactivity more often raised their eyebrows and less often drew them together. The sighs were more common in participants with stress reactivity (p=.016).

The correlation between gender, stress reactivity and voice key was detected. Females without stress reactivity demonstrated higher voice key during pubic speaking (p=.042). Results of two-factor dispersion analysis discovered gender influence on the level of speech characteristics expression. Statistically significant gender differences were found in all registered parameters except general behavioral characteristic and the frequency of changing in static poses. Females were speaking louder, with more variety in intonations, with clearer articulation and higher voice. Statistically significant gender differences were also discovered in the parameters of the frequency of pose changing during the third minute of speaking: males were changing poses more often than females (p=.008). Males with stress reactivity demonstrated more expressive dynamics in the intensity of nonverbal reactions. Females without stress reactivity were demonstrating a smooth increase in characteristics of mimic and pantomimic during public speaking: the muscles were becoming tenser (p=.022). The expressions of the emotional arousal in the voice at the same time were decreasing (p=.007). Fe-

males with stress reactivity were coming back to a comfortable pose by the 3rd minute (p=.014), the speed and the volume of the voice were smoothly going down and the clearness was increasing from the 2nd to the 5th minute (p=.004).

DISCUSSION

Our research supports the conception of Richard Lazarus on stress as transactional process. As well as macrostress, experimental microstress contains behavior, cognitive, emotional and physiological reactions. A significance of this work concerns the relationships between the measurements of different psychological factors but not individual indexes of specific methods. Cortisol as one of "hormones of stress" manifests his role in microstress not always and has different dynamics. We couldn't evaluate the dinamics of cortisol because it's level depends on many other hormones which we didn't control. The level of cortisol could depends on individual level of arousal and coping resources.

CONCLUSION

The proposed model of experimental stress is based on complex investigation of cortisol level, psychophysiological and mental parameters. Research data supports the consideration that stress reactivity is a stable enough individual characteristic which is constantly manifested in different stressful situations. Complex correlations between the types of stress reactivity and self-assessment of mental state and used coping strategies were detected. Important data was discovered about the correlation between adverse childhood experience, health risk behaviors and the level of stress reactivity. The development of experimental model of human micro stress and piloted methods for its assessment were the main result of the conducted study. Assessment of cortisol level is considered as objective parameter of stress level evaluation. Psychological and mental parameters are much more subjective and mediated comprehensively. The choice of psychometric tests and methods should be based on the specificity of evaluation related to the experimental situation.

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