Psychoneuroendocrinology (2007) 32, 451–463

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journal homepage: www.elsevier.com/locate/psyneuen

Acute psychosocial challenge and cardiac autonomic response in women: The role of estrogens, corticosteroids, and behavioral coping styles

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Received 13 November 2006; received in revised form 20 February 2007; accepted 26 February 2007

KEYWORDS
Psychosocial stress;
Menstrual cycle;
Estrogens;
HPA axis;
Heart rate;
Autonomic nervous system;
Submission;
Displacement

Summary
Theoretical statements, as well as clinical and experimental data, suggest that the amplitude of cardiovascular reactivity to acute stressors can be a good predictor of preclinical and clinical cardiovascular states. The aim of the present study is to investigate the role of estrogens, the hypothalamic–pituitary–adrenocortical activity, and the behavioral profile in individual cardiac autonomic reactivity to brief laboratory stressors in women.

Thirty-six adult, healthy women were exposed to a stress interview and a mental task test, each lasting 5 min. They were assigned to two experimental groups: D4, i.e. 4 days after menses beginning (follicular phase, $n = 18$), and D14, i.e. 14 days after menses beginning (ovulatory phase, $n = 18$). The cardiac measurements in the baseline, stress and recovery periods consisted in heart rate (average R–R interval) and parasympathetic tone (r-MSSD) quantification, while the HPA axis activity and stress reactivity were assessed via plasma cortisol and dehydroepiandrosterone concentrations. The ethological profile during the interview was drawn by means of non-verbal behavior analysis. The cardiac, adrenocortical and behavioral responses to the two stressors were similar in groups D4 and D14, despite significantly higher estradiol levels in the latter. Subjects with higher pre-stress cortisol levels had higher heart rate and lower vagal activity in the baseline, stress and recovery phases. Women showing higher level of submission were characterized by higher heart rate acceleration and vagal withdrawal during both the interview and the recovery phase. In addition, the subjects that exhibited greater displacement during the interview

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0306-4530/$ - see front matter
doi:10.1016/j.psyneuen.2007.02.009

were also characterized by lower heart rate increments and less pronounced vagal suppression during post-stress recovery. In conclusion, the present results do not support a clear buffering role of estrogens in cardiovascular response to acute stressors. However, they confirm that baseline HPA axis activity can be predictive of cardiac autonomic activity and stress responsiveness. They also highlight the modulating role of the individual style of behavioral coping in cardiac sympathovagal stress reactivity. Therefore, the objective assessment of the individual behavioral profile via the analysis of non-verbal communication patterns might represent a powerful tool for identifying subjects with higher risk of cardiac events.

1. Introduction

Psychosocial challenges induce robust neuroendocrine responses mainly involving the activation of the sympathetic-adrenomedullary (SAM) system and the hypothalamic–pituitary–adrenocortical (HPA) axis (Kirschbaum et al., 1995; Sgoifo et al., 1996; Gerra et al., 2001; Schommer et al., 2003). In the short term, the HPA axis and the SAM system effectively manage the adaptive response to these stressful events through dynamic mutual interactions. However, as postulated by McEwen in his allostatic theory of stress adaptation and pathology, the same physiologic mediators that maintain homeostasis are also involved in allostatic overload when they are not turned off efficiently after the challenge or not turned on in adequate amounts when needed (McEwen, 1998; Goldstein and McEwen, 2002).

Human and animal data indicate that psychosocial stress can bring about various cardiocirculatory and pathophysiological complications, including atherosclerosis, hypertension, myocardial stunning, acute coronary syndrome, reduced heart rate variability, cardiac arrhythmias and myocardial structural damage (Kaplan and Manuck, 1999; Sgoifo et al., 1999; al'Absi and Arnett, 2000; Knardahl, 2000; Lucini et al., 2002; Costoli et al., 2004; Wittstein et al., 2005; Rozanski et al., 2005, Strike et al., 2006).

There is also evidence suggesting that the amplitude of cardiovascular reactivity to acute stressors can predict the development of preclinical and clinical cardiovascular states (Treiber et al., 2003). The reactivity hypothesis of cardiovascular risk postulates that individuals with exaggerated cardiovascular response to stressors are at higher risk of developing cardiovascular morbidity (Krantz and Manuck, 1984; Burleson et al., 2003). Given that it takes years for such a condition to develop, the tendency towards hyperresponsivity must be stable and reproducible over time (Manuck et al., 1993). Indeed, behaviorally evoked cardiovascular response appears to be a relatively stable individual trait, showing reasonable consistency across time and stressors (Sherwood and Turner, 1995; Sgoifo et al., 2003). For these reasons, it is clinically relevant to investigate individual differences in acute cardiovascular stress responsivity and to try to figure out the role of different modulating variables accounting for such individual variability. In this regard, the HPA axis function, sex steroid levels, and the individual strategy of behavioral coping with psychosocial challenges represent three important modulators.

Clinical and basic science studies underline the role of glucocorticoids in cardiovascular stress responsivity (Litchfield et al., 1998; Sapolsky et al., 2000; Larson et al., 2001; Roy et al., 2001; Schommer et al., 2003). It is generally accepted that appropriate cortisol levels are crucial for catecholamines to exert basic effects on the cardiovascular system, such as vasoconstriction and sinus tachycardia (Dickerson and Kemeny, 2004). Moreover, baseline cortisol levels can be predictive of heart rate reactivity induced by a public speech task (Larson et al., 2001), and individual differences in cardiovascular reactivity appear to predict cortisol changes due to a mental arithmetic challenge (Uchino et al., 1995). Indeed, baseline glucocorticoid levels rather than stress-induced responses are likely to be more essential as modulators of cardiovascular reactivity (Sapolsky et al., 2000).

Besides glucocorticoids, differences in sexual steroid concentrations (due to gender, menstrual cycle phase, menopausal status, and pregnancy) account for different cardiac autonomic and HPA axis stress responses (Kudielka and Kirschbaum, 2005; Kajantie and Phillips, 2006). Earlier studies in women using mild to moderate psychosocial stressors reported similar cardiovascular and HPA axis responses, regardless of the menstrual cycle phase (Abplanalp et al., 1977; Stoney et al., 1990). However, the use of standardized stress protocols has highlighted considerable variations. In particular, the luteal phase (i.e. high estrogen and progestin concentrations) was shown to be characterized by greater adrenocortical response and sensitivity, and buffered cardiovascular responsivity (Tersman et al., 1991; Sita and Miller, 1996; Kirschbaum et al., 1999). As for glucocorticoids, the role of sex hormones in modifying the incidence of cardiovascular disease is largely acknowledged (Kalin and Zumoff, 1990). A substantial body of biological data supports the important role of estrogens in reducing the risk of cardiac and vascular morbidity (Grodstein et al., 1996; Pepine et al., 2006; Bolego et al., 2006; Turgeon et al., 2006). Such protective action appears to be exerted via different mechanisms, involving lipoprotein metabolism, direct effect on the vessel wall, and modulation of sympatho-vagal balance (Farhat et al., 1996; Pare et al., 2002; Saleh and Connell, 2003).

Another important issue about cardiovascular stress reactivity and morbidity concerns the role of individual differences in behavioral response to stressors (Rozanski et al., 1999; Lerner et al., 2005). For instance, when facing stressful tasks, some participants may respond with a proactive coping style, whereas others may adopt a reactive

coping strategy (Ursin and Olff, 1993). These different modes of behavioral coping with a stressor may bring about different autonomic/neuroendocrine responses in ways that have important clinical implications (Korte et al., 2005). The ethological approach to the analysis of human behavior represents a powerful tool for highlighting individual differences in behavioral coping and allows to compare physiological parameters with objectively quantified behavioral scores (Troisi, 1999). Via this approach, a previous study by our research group found a close relationship between the degree of autonomic/neuroendocrine arousal and the style of behavioral adaptation to psychosocial stressors (Sgoifo et al., 2003).

The present study performed on young women aims to shed further light on the role of natural fluctuations of estrogen levels (associated with different phases of the menstrual cycle) on cardiac and HPA axis activity, and stress responsivity. The cardiovascular measurements consisted in indirect evaluation of the sympathovagal balance at the sinoatrial node (heart rate and heart rate variability parameters), while the HPA axis function was assessed by determining plasma cortisol and dehydroepiandrosterone (DHEA) concentrations. We also investigated whether individual differences in the style of behavioral coping, quantified by means of a detailed analysis of non-verbal behavior, were differentially associated with HPA axis and cardiac autonomic stress responsivity.

2. Methods

2.1. Participants

Data were collected from 36 healthy female university students. Their mean age was 23.4 years ($\pm$0.4, SEM), body weight 58.3 kg ($\pm$1.4), height 1.66 m ($\pm$0.01), and body mass index 21.1 kg/m^2 ($\pm$0.04). The participants were requested to confirm that: (i) they had had regular menstrual cycles of 26–30 days for the 6 months prior to participation in the study, (ii) they had not used oral contraceptives, (iii) they were not pregnant, (iv) they were not under any kind of chronic pharmacological treatment, (v) they were not smokers, and (vi) they had no history of cardiovascular disease. In addition, we also instructed participants not to have alcoholic drinks and not to perform sustained physical activity in the 24 h preceding the recording session. The experimental subjects were allowed to have a light breakfast, but at least 1 h before testing. They were also asked to wake up not later than 07:00 h on the morning of the test, in order to avoid blood sampling when cortisol levels are too close to diurnal maximum peak (Steptoe et al., 2007).

The women were assigned to two groups of $n = 18$ subjects each: D4—measurements taken on day 4, after the beginning of the menstrual cycle; D14—measurements taken on day 14, after the beginning of the cycle. All recordings were performed between 9:00 and 13:00 h, in a quiet room at a comfortable temperature (22 $\pm$ 2 °C). Participants received a verbal explanation describing the experiment, but not the purpose of the study, and signed a written informed consent. The experimental protocol was approved by our Institutional Review Board.

2.2. Outline of the experimental set-up

Each subject individually underwent a four-phase experimental procedure, overall lasting around 40 min, in the presence of four experimenters.

- *Adaptation phase* (10 min): electrode positioning for electrocardiographic (ECG) recording, with the subject comfortably seated on a chair in the presence of three experimenters.
- *Baseline phase* (10 min): baseline ECG recording at rest, immediately followed by blood sample collection, in the presence of the same experimenters.
- *Stress phase* (10 min): stress interview with ECG and videotape recording, followed by mental task with ECG recording only, in the presence of four experimenters.
- *Recovery phase* (10 min): recovery ECG recording, immediately followed by blood sample collection, in the presence of three experimenters.

2.3. Radiotelemetry system for ECG recordings

The radiotelemetry system employed in this study for the ECG recordings consisted of a flat transmitter (TA11CTA-F40, Data Sciences International, St. Paul, MN, USA), and a platform receiver (RPC-1, Data Sciences International). The two steel wires protruding from the body of the trasmitter were wrapped to commercial electrodes terminating with a disc-shaped lead (Battaglia-Rangoni, Bologna, Italy). The two electrodes were fixed with paper tape to the right and left parasternal regions, and the transmitter was left lying on the receiver, on a table just in front of the subject.

2.4. Electrocardiographic data acquisition and processing

Continuous ECG recordings (sampling frequency: 1 kHz) were performed in four recording periods, each lasting 5 min: baseline (Bas), stress interview (Test1), mental task (Test2), and recovery (Rec). The ECG waves were acquired on PC with ART-Silver 1.10 data acquisition system (Data Sciences International). The ECG analysis was performed by means of a software package developed in our laboratory (Sgoifo et al., 2001) for quantification of time-domain indexes of heart rate variability (HRV; Stein et al., 1994). The following two parameters were quantified: (i) the mean R–R interval duration (RR, ms) and (ii) the root mean square of successive R–R differences (r-MSSD, ms). Basically, RR corresponds to the average inter-beat-interval in a given period of time. The r-MSSD focuses on high-frequency, short-term variations of the R–R interval, which are due to the activity of the parasympathetic nervous system (Stein et al., 1994; De Meersman and Stein, 2007). Generally speaking, increased sympathetic and/or decreased parasympathetic activity (i.e. shift of sympathovagal balance towards sympathetic dominance) are reflected in decreased values of RR and r-MSSD, while decreased sympathetic and/or increased vagal nervous system activity (i.e. shift of sympathovagal balance towards parasympathetic prevalence) are reflected

in increased values of these parameters. The RR and r-MSSD quantification was performed after removal of the intervals surrounding the arrhythmic events (extremely rare) or recording artifacts.

2.5. Blood collection and endocrine determinations

Blood samples (9 ml each) were collected in sterile test tubes at the end of baseline and recovery phases (the time span between the two samples was approximately 20 min). They were immediately centrifuged at 3500 rpm for 15 min at 4 °C, and the plasma was stored at −80 °C until assayed. The plasma concentrations of cortisol, DHEA, and estradiol were determined. Each hormonal determination was performed in duplicate. Intra- and inter-assay coefficients of variation were less than 10% for each hormonal evaluation.

The cortisol levels were quantified using a radio immuno assay (RIA) kit. The rabbit antihuman cortisol antiserum had 100% cross-reactivity with cortisol, 35.8% with prednisolone, 4.6% with 11-deoxycortisol, 1.2% with cortisone and corticosterone, 0.6% with 17-hydroxyprogesterone, 0.4% with prednisone, 0.3% with deoxycorticosterone and less than 0.1% with progesterone. The DHEA levels were quantified using a RIA, too. The hormone had 100% cross-reactivity with DHEA, 0.45% with isoandrosterone, 0.19% with androstenedione, 0.04 with 5α-androstane-3,17-dione, 0.03% with 5-androstene-3β, 17β-diol, 0.03% with androsterone and less than 0.001% with progesterone, dexamethasone, testosterone, aldosterone, corticosterone, cortisol, estradiol, estrone, and norethindrone. Finally, estradiol levels were also quantified via RIA. The estradiol had 100% cross-reactivity with estradiol, 2.4% with estrone, 0.2% with estrone-β-D-glucuronide, 0.01% with estrone-3-sulfate, 0.34% with equilin, 3.40% with D-equilenin, 0.21% with 17α-estradiol, 0.21% with 16-cheto-estradiol, 2.56% with 17β-estradiol-3-glucuronide, 0.17% with estradiol-3-SO_4, 0.64% with estriol, and less than 0.001% testosterone and DHEA.

2.6. Stress interview and mental task

A psychosocial challenge was performed, consisting of a brief (5 min) stress interview during which the subject was seated in front of an interviewer (middle aged man) and in the presence of three additional experimenters (who remained silent all throughout the test). Differently from the Trier Social Stress Test (TSST) (Kirschbaum et al., 1993), the challenge did not involve a free speech in a simulated job interview, but consisted in a number of questions by the interviewer. In particular, each woman was asked to describe her distinctive personality features in a social context (for instance: "Are you a sociable person?", "What characteristics of your social attitude would you like to change?", "What social contexts do you find most stressful?", etc.) (Troisi, 1999; Sgoifo et al., 2003). In order to render the challenge more stressful, the interviewer often interrupted the participant's answers. The interview was immediately followed by a mental task (5 min) consisting of three exercises of increasing difficulty, in which the subject had to choose the missing element that correctly completed a series of images. For the 1st exercise, the subject had 1 min to find the correct solution, while for exercises 2 and 3, she was given 2 min each. The subject was not allowed to speak during the mental task.

2.7. Behavioral measurements

The subject's non-verbal behavior during the stress interview was quantified by means of the Ethological Coding System for Interviews (ECSI). The ECSI used in the present study is a revised version of Grant's ethogram, specifically designed for clinical interviews (Grant, 1968; Troisi, 1999). Non-verbal behavioral patterns act as social signals and reflect mood and intentions to behave in certain ways (Dixon and Fisch, 1998). This version of ECSI includes 37 different patterns, mostly facial expressions and hand movements. The ECSI was specifically designed for measuring non-verbal behavior during stress interviews by combining behavior patterns described in published human ethograms.

The interview was videotaped with a camera adjusted so that the subject's face and trunk were in full view. Subsequently, a trained observer examined the videotape and scored the subject's behavior according to a one-zero sampling method (Troisi, 1999). Each video recording (lasting 300 s) was divided into 20 successive 15-s sample intervals and the observer recorded whether a certain behavioral pattern had occurred or not, in each sample interval. The score for each pattern was expressed as the proportion of sample intervals (%) during which that pattern occurred as to the total number of sample intervals.

The 37 behavioral patterns were then grouped in eight behavioral categories, each reflecting a different aspect of the subject's emotional and social attitude (see Troisi, 1999 for details on the 37 behavioral patterns and grouping criteria): (1) *eye contact*, which expresses attention and involvement and is considered an essential component in a dyadic interaction; (2) *affiliation*, i.e. a set of patterns which consists of facial expressions and head movements, which invite social interaction and reflect interpersonal attitude, tending to reassure and increase attachment; (3) *submission*, i.e. patterns used to appease the interviewer and prevent or inhibit hostile responses; (4) *flight*, which includes non-verbal behaviors aimed at cutting off sensory systems from incoming social stimuli perceived as stressful or adverse; (5) *assertion*, which includes facial expressions and head movements that signal low-level aggression and hostility; (6) *gesture*, which includes hand movements that accompany, illustrate and, accentuate the verbal content of utterances—this behavioral category reflects the subject's communicative efforts in social interactions and is a reliable index of global psychomotor activity; (7) *displacement*, which includes behavioral patterns that consist of movements which are focused on one's own body or addressed to manipulation of external objects; and (8) *relaxation*, which consists of behavioral patterns indicative of a low level of emotional arousal.

The score of a given behavioral category was expressed as the sum of the percentages of all the behavioral patterns belonging to it. Before the beginning of the study, the observer was trained in order to reach an adequate level of inter-observer reliability (i.e. a *k* coefficient of at least 0.70 for each behavioral pattern). The assessment of

inter-observer reliability was based on a sample of 30 interviews, which did not include the ones with the subjects of this study.

2.8. Data analysis and statistics

All parameters (electrocardiographic, endocrine, and behavioral) were expressed as mean ± SEM. The R–R interval and r-MSSD were quantified as: (i) mean of each 5-min recording period (baseline, stress interview, mental task, and recovery); (ii) Delta value (difference between stress interview and baseline values). The blood cortisol and DHEA concentrations were expressed as Pre (value during baseline phase), Post (value at the end of recovery phase), and Delta (Post minus Pre), while the blood estradiol concentrations were expressed only as Pre. The values of cardiac autonomic (RR and r-MSSD) and adrenocortical (cortisol and DHEA) parameters were statistically analyzed by means of two-way ANOVA, with "menstrual cycle phase" as between-subject factor (two levels: days 4 and 14 after the beginning of the cycle) and "time" as within-subject factor (two levels for adrenocortical measures: Pre and Post; four levels for heart rate measures: Bas, Test1, Test2, Rec). After ANOVAs, posthoc analyses were applied when appropriate using Student's "*t*"-test. Between-group comparison for estradiol concentrations and behavioral category scores were also performed by means of Student's "*t*"-test. The relationships between behavioral, endocrine, and cardiac autonomic measures were statistically explored by hierarchical, multiple regression. Hierarchical multiple regression analyses were carried out with RR and r-MSSD as dependent variables. The variables were entered in the following order: step 1, behavioral categories (eye contact, flight, submission, affiliation, gesture, displacement, assertion, and relaxation); step 2, DHEA and cortisol pre-stress levels. Subsequently, Pearson's correlation coefficient was used to assess relationships between pairs of cardiovascular, endocrine, and behavioral parameters. Statistical significance for all tests was set at $p<0.05$. All statistics were performed using SPSS 11.5 software package (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Cardiac autonomic responses

Two-way ANOVA applied to 5-min mean values, revealed a significant effect of the "time" factor for RR ($F = 8.5$, $p<0.01$) and r-MSSD ($F = 4.2$, $p<0.01$; Table 1).

The within-group post hoc analysis revealed a clear reduction of RR and r-MSSD values during Test1 as compared to baseline, though statistical significance was reached only for RR (RR—D4: $t = 3.09$, $p<0.01$; D14: $t = 2.24$, $p<0.05$; r-MSSD—D4: $t = 1.93$, $p = 0.07$; D14: $t = 1.57$, $p = 0.08$). On the contrary, Test2 did not induce significant RR changes as compared to baseline conditions, for neither group D4 nor D14. In other words, only the stress interview induced robust heart rate accelerations, and such changes were not statistically different between the two experimental groups. D4 and D14 women showed similar changes in RR and r-MSSD even when Delta values (Test1-Bas) were compared (Table 1).

3.2. Hormone levels

In order to quantify possible differences in circulating estradiol between D4 and D14 women, the plasma concentrations were determined at the end of the baseline phase. As expected, D14 women had significantly higher estradiol levels as compared to their D4 counterparts (D4: 102.7 ± 10.8 pM/l; D14: 416.5 ± 62.0 pM/l; $t = -4.9$, $p<0.01$).

Fig. 1 reports values of plasma cortisol and DHEA concentrations before (Pre) and after (Post) the stress phase

Table 1 Values of heart rate parameters (RR and r-MSSD, ms; mean ± SEM) in the different recording periods, in women belonging to group D4 (4 days after the beginning of the menstrual cycle) and group D14 (14 days after the beginning of the cycle).

Group	Period	Heart rate parameter	
		RR	r-MSSD
D_4 ($n = 18$)	Bas	788.1 ± 29.1	45.2 ± 5.0
	Test1	664.9 ± 27[a]	34.5 ± 4.2
	Test2	727.4 ± 28.5	38.9 ± 4.1
	Rec	811.6 ± 29.3	52.0 ± 5.7
	Delta	−123.2 ± 15.2	−10.7 ± 2.3
D_{14} ($n = 18$)	Bas	742.5 ± 24.6	36.8 ± 4.3
	Test1	667.7 ± 22[a]	30.4 ± 2.2
	Test2	729.2 ± 22.5	34.4 ± 3.9
	Rec	764.5 ± 21.8	43.0 ± 5.0
	Delta	−74.8 ± 20.2	−6.4 ± 3.8

Bas, baseline; Test1, stress interview; Test2, mental task; Rec, recovery; Delta, Test1−Bas; RR, average R–R interval; r-MSSD, root mean square of successive R–R differences.

[a]Significantly different from corresponding baseline ($p<0.05$, Student "*t*"-test).

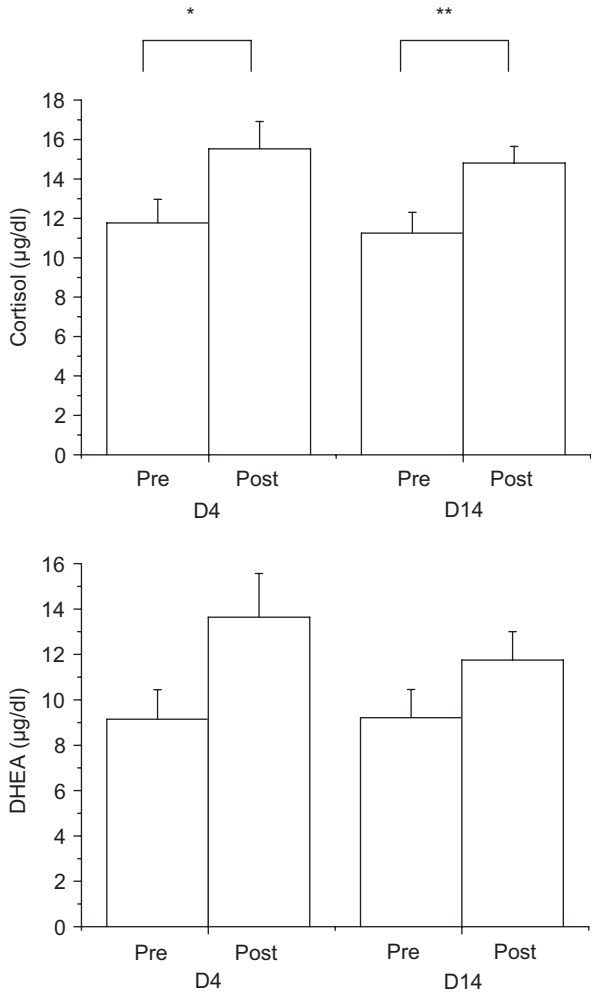

Figure 1 Plasma cortisol and DHEA concentrations (mean ± SEM, µg/dl) before (Pre) and after (Post) the stress phase, in women belonging to group D4 (4 days after the beginning of the menstrual cycle) and group D14 (14 days after the beginning of the cycle). $^{*}p<0.05$, $^{**}p<0.01$.

in both groups of women. Two-way ANOVA on Pre and Post cortisol concentrations revealed a significant effect of "time" ($F = 10.4$, $p<0.01$). The stress phase produced robust increases in cortisol levels in both groups (D4: $t = -2.03$, $p<0.05$; D14: $t = -2.65$, $p<0.01$), but no differences between groups could be detected, for neither Pre nor Post and Delta values. Two-way ANOVA applied to plasma DHEA levels revealed a significant effect of time ($F = 5.9$, $p<0.05$), indicating that plasma concentrations of this hormone increased due to stress exposure, although within-group increase in DHEA concentration did not reach statistical significance, in neither D4 nor D14 women. As for cortisol, Pre, Post and Delta values for D4 women were not statistically different from D14 corresponding values.

3.3. Ethological data

No significant differences between groups were found in the expression of non-verbal behavior, for any of the categories considered (Table 2). In other words, behavioral responses to stress interview did not appear to be influenced by the levels of circulating estrogens.

3.4. Relationships among autonomic, endocrine, and behavioral variables

In light of the lack of any significant difference between the two groups of women, correlation analyses between the various autonomic, endocrine, and behavioral variables were performed considering all the subjects as a single experimental group.

3.4.1. Hierarchical multiple regression analysis

3.4.1.1. Prediction of cardiac response to stress interview (Test1). The baseline hormonal levels predicted the variance of RR during Test1 (RR: $F = 3.64$, $\Delta R^2 = 0.17$, $p<0.05$). In particular, the pre-stress cortisol concentration had a significant independent effect on RR (RR: $t = -2.63$, $p<0.05$). The variance of r-MSSD during Test1 was not predicted by any other variable.

3.4.1.2. Prediction of cardiac response to mental task (Test2). The block of hormonal variables predicted the variance of RR during Test2 ($F = 4.09$, $\Delta R^2 = 0.20$, $p<0.05$). In particular, the cortisol Pre values had a significant independent effect on RR variance ($t = -2.83$, $p = 0.01$), as well as on the variance of r-MSSD during Test2 ($t = -2.54$, $p<0.05$).

3.4.1.3. Prediction of cardiac activity during the recovery phase (Rec). The pre-stress hormonal variables, as well as the non-verbal behavior during the stress interview predicted the variance of RR during the recovery phase ($F = 3.77$, $\Delta R^2 = 0.13$, $p<0.05$; $F = 2.42$, $\Delta R^2 = 0.47$, $p<0.05$). In particular, pre-stress cortisol levels had an independent effect on RR ($t = -2.70$, $p<0.05$). The block of behavioral categories predicted the variance of r-MSSD in the recovery phase ($F = 3.72$, $\Delta R^2 = 0.57$, $p<0.01$); in particular, displacement had an independent effect ($t = 2.62$, $p<0.05$).

3.4.2. Pearson's correlation analysis

3.4.2.1. Correlations between endocrine parameters. There was a positive correlation between DHEA and cortisol levels, both before and after the stressor ($R = 0.52$, $p<0.01$; $R = 0.51$, $p<0.01$). Similarly, the Delta values of the two hormones (Post and Pre) were positively correlated too ($R = 0.54$, $p<0.01$), i.e. the subjects with substantial increments in plasma cortisol also showed marked increments in DHEA.

3.4.2.2. Correlations between cardiac and endocrine parameters. The pre-stress cortisol levels were negatively correlated with the heart rate parameters (RR and r-MSSD) in all the recording periods (Bas, Test1, Test2, and Rec) (Table 3). Overall, these correlations indicate that subjects with higher pre-stress plasma cortisol levels were prone to greater heart rate acceleration and stronger vagal suppression not only in baseline conditions, but also during the stress and recovery phases. On the contrary, pre-stress, post-stress, and Delta values of DHEA did not correlate with cardiac parameters, neither in baseline conditions nor during and after the stress phase.

Table 2 Values of behavioural categories (%, mean ± SEM) exhibited during stress interview by women belonging to group D4 (4 days after the beginning of the menstrual cycle) and group D14 (14 days after the beginning of the cycle).

Behavioral category	D_4 ($n = 18$)	D_{14} ($n = 18$)
Eye contact	98.1 ± 1.2	97.8 ± 0.8
Flight	150.0 ± 7.6	144.4 ± 8.6
Submission	60.0 ± 6.0	59.2 ± 8.1
Affiliation	140.0 ± 12.2	150.8 ± 9.6
Gesture	43.9 ± 5.5	51.9 ± 4.6
Displacement	106.4 ± 14.0	123.1 ± 15.2
Assertion	71.1 ± 12.0	89.2 ± 14.3
Relaxation	44.2 ± 6.6	43.3 ± 5.3

Student's "*t*" test—*t* and *p* values for each behavioral category—eye contact: $t = 0.19$, $p = 0.85$; flight: $t = 0.49$, $p = 0.63$; submission: $t = 0.08$, $p = 0.94$; affiliation: $t = 0.70$, $p = 0.49$; gesture: $t = 1.13$, $p = 0.27$; displacement: $t = 0.81$, $p = 0.43$; assertion: $t = 0.97$, $p = 0.34$; relaxation: $t = 0.09$, $p = 0.92$.

Table 3 Pearson's correlation coefficients and corresponding *p* values between plasma cortisol levels (μg/dl) in baseline conditions and heart rate (HR) parameters (RR and r-MSSD, ms) in the different recording periods.

Period	HR Parameter	*R*	*p*
Bas	RR	−0.42	0.012
	r-MSSD	−0.34	0.043
Test1	RR	−0.35	0.037
	r-MSSD	−0.31	0.068
Test2	RR	−0.38	0.026
	r-MSSD	−0.43	0.011
Rec	RR	−0.44	0.008
	r-MSSD	−0.38	0.025

Bas, baseline; Test1, stress interview; Test2, mental task; Rec, recovery; RR, average R–R interval; r-MSSD, root mean square of successive R–R differences.

3.4.2.3. Correlations between cardiac and behavioral parameters. The RR mean values during Test1 correlated negatively with submission ($R = -0.34$, $p < 0.05$), and so did the values of r-MSSD ($R = -0.44$, $p < 0.01$). In other words, women showing high scores of submission were characterized by low values of RR and r-MSSD, i.e. the more submissive subjects showed a higher heart rate and larger vagal suppression during the stress interview (Fig. 2). Similarly, the values of RR and r-MSSD during Rec correlated negatively with the submission scores ($R = -0.34$, $p < 0.05$; $R = -0.56$, $p < 0.01$), i.e. the higher the level of submission during the interview, the greater the heart rate acceleration as well as the degree of vagal withdrawal during post-stress recovery phase (Fig. 2).

Significant correlations with heart rate and vagal activity also involved displacement behavior. In particular, the values of RR and r-MSSD during Rec correlated positively with the displacement scores ($R = 0.41$, $p < 0.05$; $R = 0.51$, $p < 0.01$). In other terms, the subjects exhibiting a higher degree of displacement during the interview were also characterized by lower heart rate increments and less pronounced vagal suppression during the post-stress recovery (Fig. 3).

4. Discussion

The present study examined short-term cardiac and adrenocortical responses to a laboratory stressor consisting in two brief, consecutive challenges, namely a stress interview and a mental task. The first objective was to shed additional light on the influence of spontaneous sex steroid fluctuations due to menstrual cycling on autonomic and neuroendocrine stress activations, with particular emphasis on the role of estrogens. For this purpose, we studied young women with regular menstrual cycle length (26–30 days) and we focussed on two specific time points, namely days 4 and 14 after menses initiation. Indeed, early follicular and ovulatory phases differ markedly as regards the circulating levels of estradiol, with low concentrations in the former and a peak shortly before or during the latter (Carr and Wilson, 1987). As expected, the group of women tested during (or very close to) the ovulatory phase had much higher (about four-fold) concentrations of estradiol than women screened at the beginning of the follicular phase. Despite this clear hormonal difference, no sign of differentiation was observed in terms of autonomic/neuroendocrine activity and stress reactivity. Apart from slight differences in cardiac sympathovagal balance at rest (women in the ovulatory phase had somewhat lower baseline values of RR and r-MSSD), higher estradiol concentrations did not involve differences in cardiac autonomic and adrenocortical response to the stressors. In other words, heart rate, vagal tone, and cortisol and DHEA concentrations were fully similar in D4 and D14 women before, during, and after the exposure to psychosocial and mental challenges.

So far, human studies have provided inconsistent results with respect to cardiac autonomic and adrenocortical stress reactivity across the menstrual cycle. This inconsistency is likely due to differences in the laboratory challenge choice and cycle phases considered (Kajantie and Phillips, 2006). There is evidence suggesting that estrogens may be protecting women from heart disease via modulation of

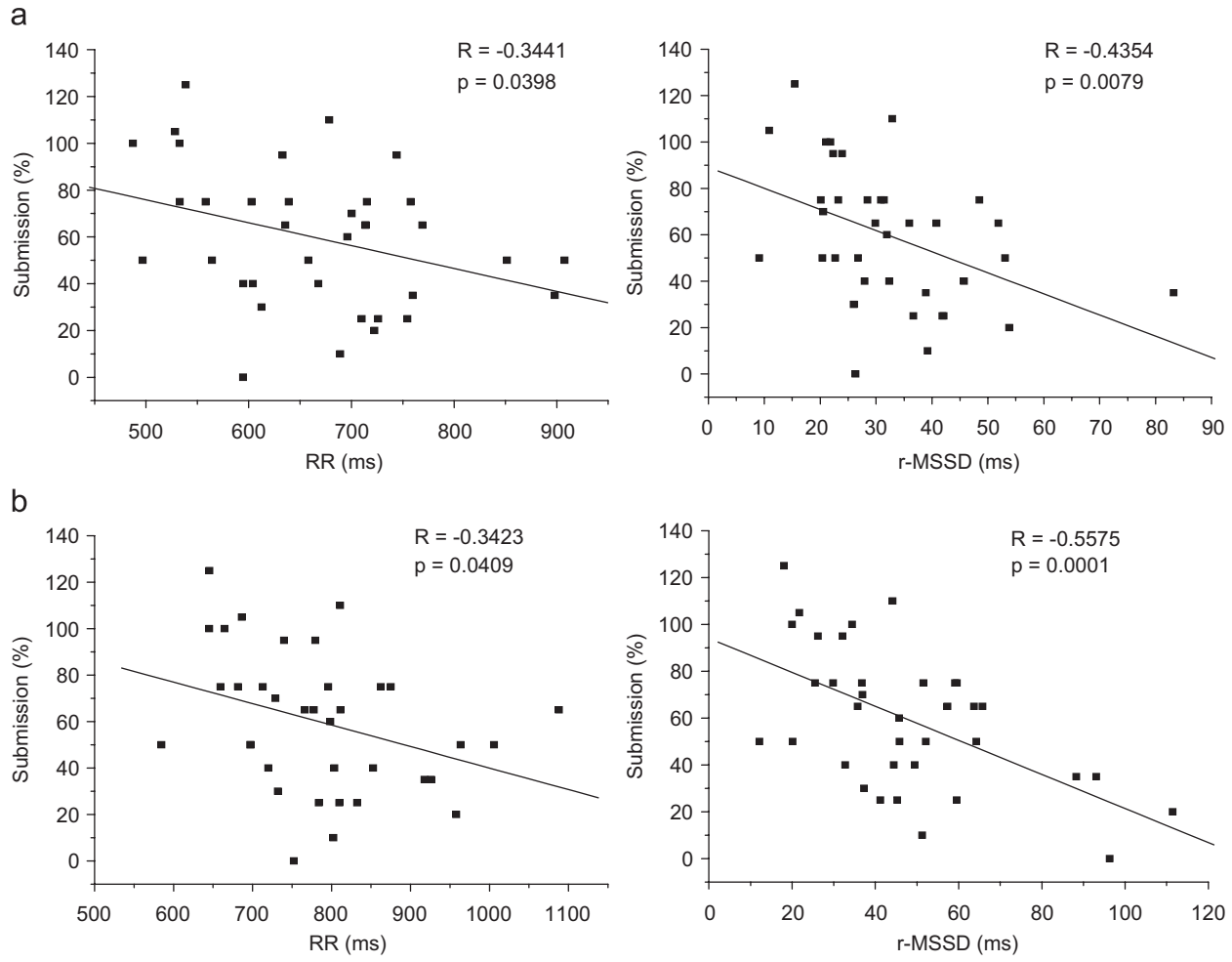

Figure 2 Correlations (Pearson's correlation coefficient) between the score of submissive behavior exhibited during psychosocial challenge and the values of RR and r-MSSD during stress interview (panel A) and recovery (panel B).

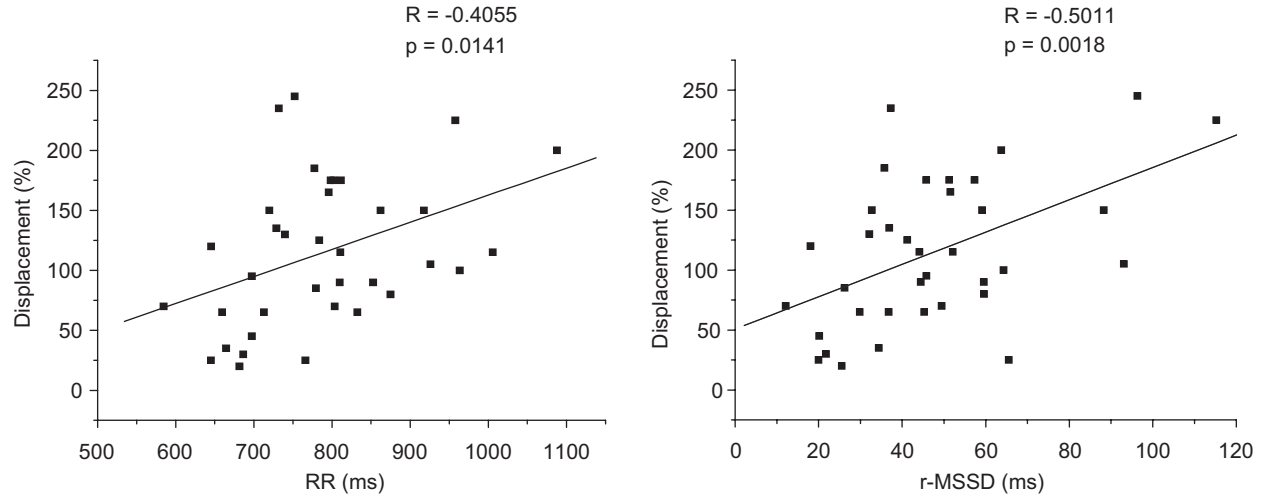

Figure 3 Correlations (Pearson's correlation coefficient) between the score of displacement exhibited during stress interview and the values of RR and r-MSSD during recovery.

cardiovascular physiology. During the luteal phase, higher levels of estradiol were shown to be associated with lower levels of cardiac output responses to combined video game and speech tasks, and lower levels of heart rate and systolic blood pressure responses to cold pressor test (Sita and Miller, 1996). Tersman and colleagues documented a

significant phase-related difference in heart rate and blood pressure reactivity, with women in the luteal phase reacting significantly more than those in the follicular phase to a cold pressor but not to a mental arithmetic test (Tersman et al., 1991). However, a carefully conducted study on women each studied in the menstrual, follicular, and luteal phases, with speech, arithmetic, and isometric exercise tasks as stressors, failed to show any relationship between menstrual phase and cardiovascular response or urinary or plasma catecholamines (Stoney et al., 1990).

Data regarding HPA axis response to acute challenges in relation to menstrual cycle phases are also rather inconsistent. Thirty years ago, Marinari et al. (1976) found that subjects tested premenstrually responded to the psychological test of self-evaluation with a significantly sharper increase in plasma cortisol levels than those tested in the midcycle phase. On the other hand, Abplanalp et al. (1977) found that cortisol response to a stress interview challenge was not related to the menstrual cycle phase. More recent studies, however, indicated that the luteal phase is associated with greater adrenocortical response and sensitivity (Kudielka and Kirschbaum, 2005; Kajantie and Phillips, 2006).

Given that the present paper compared two menstrual cycle phases largely differing for estradiol levels only and not for progesterone, and given that no differences in adrenocortical and cardiac autonomic activity and stress reactivity were found, an obvious inference is that, among female sexual steroids, progestins could have a significant role in modulating cardiovascular and HPA axis responses to stressors. Indeed, acute treatment with progesterone decreases arterial pressure, baroreflex mediated responses and corticosteroid feedback effects on ACTH (Keller-Wood, 1998). In addition, dexamethasone suppression of plasma cortisol levels has been shown to be reduced during the luteal phase of the menstrual cycle, when progesterone levels are high (Altemus et al., 1997). Moreover, progesterone has also been reported to exert rapid, non-genomic inhibiting actions on catecholamine secretion from chromaffin cells, thus potentially contributing to the short-term regulation (buffering) of cardiac stress response (Zinder and Dar, 1999). These are obviously rather indirect explanations of the role of progestins on autonomic/neuroendocrine stress response, and additional studies with targeted hormonal manipulations are expected to provide more conclusive evidence.

The behavioral response during the stress interview also failed to be influenced by the levels of circulating estrogens. The women tested at the ovulatory phase exhibited an ethological profile that was fully similar to that found in women at the early follicular stage. This consistency between different system responses—i.e. the lack of differences between D4 and D14 women regarding endocrine, autonomic, and behavioral stress responses—allowed us to study the sample as a unique group and to explore the role of the style of behavioral coping with a psychosocial challenge on cardiac autonomic stress responsivity. Our data confirm the accepted view that the individual behavioral strategy of coping with a stressor plays an important modulating role in cardiovascular reactivity (Lerner et al., 2005). In the present study, the behavioral response to a social challenge (stress interview in the presence of a small audience) was assessed via a detailed analysis of non-verbal behavior (Troisi, 1999). The amount of submissive behavior exhibited during the stress interview was clearly associated with cardiac sympathovagal balance in baseline conditions, during the psychosocial challenge, during the mental task, and in the post-stress recovery period. As an earlier study from our group pointed out (Sgoifo et al., 2003), the subjects that more frequently showed submissive behavioral patterns also exhibited higher heart rate (low RR values) in baseline, stress and post-stress phases. Coherently, vagal withdrawal (low values of r-MSSD) was larger in more submissive subjects, again in all recording periods. In other terms, individuals prone to adopt a submissive strategy when coping with a social stressor were characterized by a larger sympathetic prevalence at the heart level, which might render them more vulnerable to cardiovascular morbidity in the long term.

This experimental evidence supports previous studies linking chronically impaired autonomic function, social position, and cardiovascular disease risk (Hemingway et al., 2005). In fact, among healthy humans subjected to acute laboratory stressors, those with low social position demonstrated impaired recovery of heart rate variability (Steptoe and Marmot, 2002). These indications have clear clinical relevance, given that high heart rate and low heart rate variability were shown to predict the incidence of coronary disease (Bigger et al., 1993). Interesting data on the relationship between female social attitude and cardiovascular function were obtained in animal models too. For instance, premenopausal social subordination in female monkeys was shown to be a good predictor of postmenopausal atherosclerosis, irrespective of postmenopausal treatment (Kaplan et al., 2002). In the study quoted, such an effect was attributed to estrogen deficiency in the premenopausal phase due to the chronic subordination state, and was prevented by premenopausal oral contraceptive exposure.

Results of ethological analysis also indicated that some behavioral patterns exhibited during the stress interview and grouped together in the category "displacement" were associated with a faster re-establishment of resting cardiovascular physiology. According to Troisi, this behavioral category, largely described in animal models, gathers non-verbal behavioral patterns that appear in situations characterized by social tension and are likely to reflect increased autonomic arousal (Troisi, 2002). Indeed, ethological studies of healthy persons and psychiatric patients during interviews have found that increased displacement behavior does not only correlate with the subjective feeling of a state of anxiety and negative affect, but it also provides more veridical information about the subject's emotional state than verbal statements and facial expressions (Troisi, 2002).

In the literature on human non-verbal communication, displacement activities have been referred to with many labels, including synkinetic movements, self-manipulations, body-focused movements, self-adaptors, and body manipulators. There is a large body of evidence (reviewed in Troisi, 2002) showing that, both in non-human primates and human subjects, the occurrence of these behaviors is associated with social situations that are quite heterogeneous but which have uncertainty and anxiety in common. In addition, displacement activities in human beings correlate

with an emotional condition of negative affect (i.e. anxiety and/or depression). According to Troisi, rather than being just a behavioral epiphenomenon of the psychological and physiological changes associated with stress, displacement activities are likely to be behavioral components of the adaptive stress response, probably causing anxiolytic effects.

Animal data support such a hypothesis. In non-primate species, behavioral "stereotypes" attenuate physiological measures of stress (Dantzer and Mormede, 1985; Dantzer, 1986), and stimulate endorphin production (Cronin et al., 1986) in pigs. Displacement chewing and gnawing reduce the stress-related activation of the hypothalamo–pituitary–adrenal axis in mice exposed to a novel environment (Hennessy and Foy, 1987), and in rats exposed to electric shock (Levine et al., 1989). In rats and mice exposed to a brightly lit novel environment, the animals that engaged in displacement chewing of inedible objects displayed significantly lower activation of the prefrontal cortical (PFC) dopaminergic system, which is generally observed in stressful situations (Berridge et al., 1999). Since the attenuation of the stressor-induced increase in dopaminergic transmission within PFC is similar to that observed following administration of anxiolytics and opposite to that observed following administration of anxiogenics, Berridge et al. (1999) have suggested that displacement chewing may serve an anxiolytic function. In a prosimian species, the animals that performed more displacement activities (foot and chest rubbing) in a novel environment also exhibited lower cortisol responses to restraint stress (Watson et al., 1999).

In the present study, displacement scores during the psychosocial challenge were not associated with cardiac reactivity during the interview or mental task, rather they were significantly correlated with values of cardiac parameters measured just after the stress episode: women exhibiting higher displacement scores had lower heart rate and higher parasympathetic tone during recovery. This behavioral-autonomic association confirms the view on the dearousing properties of displacement behaviors (Spruijt et al., 1992) and suggests that they likely represent a successful strategy for promoting prompter return to baseline, homeostatic conditions.

Plasma cortisol and DHEA levels were positively correlated both before and after the stressors. This relationship can be interpreted in the light of the antiglucocorticoid properties of DHEA in several tissues, e.g. its capacity to antagonize glucocorticoid negative feedback at the brain and pituitary level, thereby facilitating CRF and/or ACTH and cortisol release (Rasmusson et al., 2004). Indeed, ACTH responses to psychological stress have been shown to increase after DHEA treatment in postmenopausal women (Kudielka et al., 1998). Moreover, there is evidence that DHEA-S level is increased by acute stressors and that DHEA-S/cortisol ratio may index the degree to which an individual is buffered against the negative effects of stress (Morgan et al., 2004).

Another interesting indication coming from this study is concerned with the robust correlation between baseline HPA axis activity (plasma cortisol levels) and cardiac autonomic activity and stress responsivity. Subjects with higher plasma cortisol concentrations at rest exhibited higher heart rate and lower vagal activity before, during, and after the challenge. This result is in accordance with a study by Larson et al. (2001) where a similar laboratory challenge (public speaking task) was used in adult men and women. The authors showed that heart rate reactivity was positively correlated with baseline levels of circulating cortisol (Larson et al., 2001). These and our data suggest that short-term cardiovascular stress responses may be directly related to longer-term neuroendocrine modulation and that the degree of resting HPA axis activity can be highly predictive of cardiac autonomic stress reactivity at the individual level. Indeed, as suggested by Sapolsky, glucocorticoids seem to help mediate permissively, rather than suppress, the cardiovascular stress response, over the entire glucocorticoid dose range (Sapolsky et al., 2000).

The delayed recovery of cardiovascular function in subjects with higher baseline cortisol concentrations deserves an additional comment. In fact, this finding is intriguing as it points to the idea that a sluggish return to basal state might be a risk factor for cardiovascular morbidity (Linden et al., 1997) and that chronic elevations in HPA outflow represent a mechanism through which prolonged stress exerts its pathogenic effects on the cardiovascular system (Watt et al., 1992; Litchfield et al., 1998; Goldstein and McEwen, 2002). Returning to the role of the behavioral style of coping, it is worth underlining that such delayed recovery of cardiac autonomic balance was associated with the degree of submission exhibited and that the adoption of dearousing behavioral patterns favored prompter resumption of baseline activity.

In conclusion, the results of this study are in line with the reports that did not support a clear buffering role of estrogens in cardiovascular response to acute psychosocial stressors. Rather, they indirectly suggest that progestins might be crucial in female reduced cardiac reactivity to laboratory tasks. On the other hand, our data confirm that HPA axis activity (baseline plasma cortisol levels) can be highly predictive of cardiac autonomic activity and stress responsivity. Finally, the analysis of non-verbal behavior highlighted the modulating role of the individual style of behavioral coping on cardiac sympathovagal stress reactivity. In particular, submission appears to bring about higher cardiac acceleration and vagal withdrawal, whereas displacement seems to favor a prompter recovery of baseline sympathovagal balance. On this regard, the objective assessment of individual behavioral coping style via the ethological analysis of non-verbal behavior might represent a powerful tool for identifying subjects with higher proneness to cardiac events, and cognitive-behavioral stress management could be successfully used for prevention and therapy of detrimental stress effects (Hammerfald et al., 2006).

Role of the funding source

The funding source was a grant from the the Italian Ministry for Education, University and Research and from the University of Parma. The study reported in the manuscript was one of the experiments detailed in the grants. The funding bodies played no actual role in the collection

analysis or interpretation of the data or in the writing of the report or in the decision to submit the paper for publication.

Conflict of interest

There are no conflicts of interest to report the current manuscript.

Acknowledgments

This study was supported by grants from the Italian Ministry for Education, University and Research and from the University of Parma.

References

Abplanalp, J.M., Livingston, L., Rose, R.M., Sandwisch, D., 1977. Cortisol and growth hormone responses to psychological stress during the menstrual cycle. Psychosom. Med. 39, 158–177.

al'Absi, M., Arnett, D.K., 2000. Adrenocortical responses to psychological stress and risk for hypertension. Biomed. Pharmacother. 54, 234–244.

Altemus, M., Redwine, L., Leong, Y.M., Yoshikawa, T., Yehuda, R., Detera-Wadleigh, S., Murphy, D.L., 1997. Reduced sensitivity to glucocorticoid feedback and reduced glucocorticoid receptor mRNA expression in the luteal phase of the menstrual cycle. Neuropsychopharmacology 17, 100–109.

Berridge, C.W., Mitton, E., Clark, W., Roth, R.H., 1999. Engagement in a non-escape (displacement) behavior elicits a selective and lateralized suppression of frontal cortical dopaminergic utilization in stress. Synapse 32, 187–197.

Bigger, J.T., Fleiss, J.L., Rolnitzky, L.M., Steinman, R.C., 1993. The ability of several short-term measures of RR variability to predict mortality after myocardial infarction. Circulation 88, 927–934.

Bolego, C., Vegeto, E., Pinna, C., Maggi, A., Cignarella, A., 2006. Selective agonists of estrogen receptor isoforms: new perspectives for cardiovascular disease. Arterioscler. Thromb. Vasc. Biol. 26, 2192–2199.

Burleson, M.H., Poehlmann, K.M., Hawkley, L.C., Ernst, J.M., Berntson, G.G., Malarkey, W.B., Kiecolt-Glaser, J.K., Glaser, R., Cacioppo, J.T., 2003. Neuroendocrine and cardiovascular reactivity to stress in mid-aged and older women: long-term temporal consistency of individual differences. Psychophysiology 40, 358–369.

Carr, B.R., Wilson, J.D., 1987. Disorders of the ovary and female reproductive tract. In: Braunwald, E., Isselbacher, K.J., Petersdorf, R.G., et al. (Eds.), Harrison's Principles of Internal Medicine. McGraw-Hill, New York, pp. 1818–1837.

Costoli, T., Bartolomucci, A., Graiani, G., Stilli, D., Laviola, G., Sgoifo, A., 2004. Effects of chronic psychosocial stress on cardiac autonomic responsiveness and myocardial structure in mice. Am. J. Physiol. Heart. Circ. Physiol. 286, H2133–H2140.

Cronin, G.M., Wiepkema, P.R., van Ree, J.M., 1986. Endorphines implicated in stereotypies of tethered sows. Experientia 42, 198–199.

Dantzer, R., 1986. Behavioral, physiological and functional aspects of stereotyped behavior: a review and re-interpretation. J. Anim. Sci. 62, 1776–1786.

Dantzer, R., Mormede, P., 1985. Stress in domestic animals: a psychoneuroendocrine approach. In: Moberg, G. (Ed.), Animal Stress. Waverly Press, Baltimore, pp. 81–95.

De Meersman, R.E., Stein, P.K., 2007. Vagal modulation and aging. Biol. Psychol. 74, 165–173.

Dickerson, S.S., Kemeny, M.E., 2004. Acute stressors and cortisol responses: a theoretical integration and synthesis of laboratory research. Psychol. Bull. 130, 355–391.

Dixon, A.K., Fisch, H.U., 1998. Animal models and ethological strategies for early drug-testing in humans. Neurosci. Biobehav. Rev. 23, 345–358.

Farhat, M.Y., Lavigne, M.C., Ramwell, P.W., 1996. The vascular protective effects of estrogen. FASEB J. 10, 615–624.

Gerra, G., Zaimovic, A., Mascetti, G.G., Gardini, S., Zambelli, U., Timpano, M., Raggi, M.A., Brambilla, F., 2001. Neuroendocrine responses to experimentally-induced psychological stress in healthy humans. Psychoneuroendocrinology 26, 91–107.

Goldstein, D.S., McEwen, B.S., 2002. Allostasis, homeostats, and the nature of stress. Stress 5, 55–58.

Grant, E.C., 1968. An ethological description of non-verbal behavior during interview. Br. J. Med. Psychol. 41, 177–184.

Grodstein, F., Stampfer, M.J., Manson, J.E., Colditz, G.A., Willett, W.C., Rosner, B., Speizer, F.E., Hennekens, C.H., 1996. Postmenopausal estrogen and progestin use and the risk of cardiovascular disease. N. Engl. J. Med. 335, 453–461.

Hammerfald, K., Eberle, C., Grau, M., Kinsperger, A., Zimmermann, A., Ehlert, U., Gaab, J., 2006. Persistent effects of cognitive-behavioral stress management on cortisol responses to acute stress in healthy subjects—a randomized controlled trial. Psychoneuroendocrinology 31, 333–339.

Hemingway, H., Shipley, M., Brunner, E., Britton, A., Malik, M., Marmot, M., 2005. Does autonomic function link social position to coronary risk? The Whiteall II study. Circulation 111, 3071–3077.

Hennessy, M.B., Foy, T., 1987. Nonedible material elicits chewing and reduces the plasma corticosterone response during novelty exposure in mice. Behav. Neurosci. 101, 237–245.

Kajantie, E., Phillips, D.I., 2006. The effects of sex and hormonal status on the physiological response to acute psychosocial stress. Psychoneuroendocrinology 31, 151–178.

Kalin, M.F., Zumoff, B., 1990. Sex hormones and coronary disease: a review of the clinical studies. Steroids 55, 330–352.

Kaplan, J.R., Manuck, S.B., 1999. Status, stress, and atherosclerosis: the role of environment and individual behavior. Ann. NY Acad. Sci. 896, 145–161.

Kaplan, J.R., Manuck, S.B., Anthony, M.S., Clarkson, T.B., 2002. Premenopausal social status and hormone exposure predict postmenopausal atherosclerosis in female monkeys. Obstet. Gynecol. 99, 381–388.

Keller-Wood, M., 1998. ACTH responses to hypotension and feedback inhibition of ACTH increased by chronic progesterone treatment. Am. J. Physiol. (Heart Circ. Physiol.) 274, 81–87.

Kirschbaum, C., Pirke, K.M., Hellhammer, D.H., 1993. The trier social stress test—a tool for investigating psychobiological stress responses in a laboratory setting. Neuropsychobiology 28, 76–81.

Kirschbaum, C., Prussner, J.C., Stone, A.A., Federenko, I., Gaab, J., Lintz, D., Schommer, N., Hellhammer, D.H., 1995. Persistent high cortisol responses to repeated psychological stress in a subpopulation of healthy men. Psychosom. Med. 57, 468–474.

Kirschbaum, C., Kudielka, B.M., Gaab, J., Schommer, N.C., Hellhammer, D.H., 1999. Impact of gender, menstrual cycle phase, and oral contraceptives on the activity of the hypothalamus–pituitary–adrenal axis. Psychosom. Med. 61, 154–162.

Knardahl, S., 2000. Cardiovascular psychophysiology. Ann. Med. 32, 329–335.

Korte, S.M., Koolhaas, J.M., Wingfield, J.C., McEwen, B.S., 2005. The Darwinian concept of stress: benefits of allostasis and costs of allostatic load and the trade-offs in health and disease. Neurosci. Biobehav. Rev. 29, 3–38.

Krantz, D.S., Manuck, S.B., 1984. Acute psychophysiologic reactivity and risk of cardiovascular disease: a review and methodologic critique. Psychol. Bull. 96, 435–464.

Kudielka, B.M., Kirschbaum, C., 2005. Sex differences in HPA axis responses to stress: a review. Biol. Psychol. 69, 113–132.

Kudielka, B.M., Hellhammer, J., Hellhammer, D.H., Wolf, O.T., Pirke, K.M., Varadi, E., Pilz, J., Kirschbaum, C., 1998. Sex differences in endocrine and psychological responses to psychosocial stress in healthy elderly subjects and the impact of a 2-week dehydroepiandrosterone treatment. J. Clin. Endocrinol. Metab. 83, 1756–1761.

Larson, M.R., Ader, R., Moynihan, J.A., 2001. Heart rate, neuroendocrine, and immunological reactivity in response to an acute laboratory stressor. Psychosom. Med. 63, 493–501.

Lerner, J.S., Gonzalez, R.M., Dahl, R.E., Hariri, A.R., Taylor, S.E., 2005. Facial expressions of emotion reveal neuroendocrine and cardiovascular responses. Biol. Psych. 58, 743–750.

Levine, S., Coe, C., Wiener, S., 1989. The psychoneuroendocrinology of stress: a psychobiological perspective. In: Levine, S., Bursh, R. (Eds.), Psychoendocrinology. Academic Press, New York, pp. 181–207.

Linden, W., Earle, T.L., Gerin, W., Christenfeld, N., 1997. Physiological stress reactivity and recovery: conceptual siblings separated at birth? J. Psychosom. Res. 42, 117–135.

Litchfield, W.R., Hunt, S.C., Jeunemaitre, X., Fisher, N.D., Hopkins, P.N., Williams, R.R., Corvol, P., Williams, G.H., 1998. Increased urinary free cortisol: a potential intermediate phenotype of essential hypertension. Hypertension 31, 569–574.

Lucini, D., Norbiato, G., Clerici, M., Pagani, M., 2002. Hemodynamic and autonomic adjustments to real life stress conditions in humans. Hypertension 39, 184–188.

Manuck, S.B., Kamarck, T.W., Kasprowicz, A.S., Waldstein, S.R., 1993. Stability and patterning of behaviorally evoked cardiovascular reactivity. In: Blascovich, J.J., Katkin, E.S. (Eds.), Cardiovascular Reactivity to Psychological Stress and Disease. American Psychological Association, Washington, pp. 111–134.

Marinari, K.T., Leshner, A.I., Doyle, M.P., 1976. Menstrual cycle status and adrenocortical reactivity to psychological stress. Psychoneuroendocrinology 1, 213–218.

McEwen, B.S., 1998. Protective and damaging effects of stress mediators. N. Engl. J. Med. 338, 171–179.

Morgan 3rd., C.A., Southwick, S., Hazlett, G., Rasmusson, A., Hoyt, G., Zimolo, Z., Charney, D., 2004. Relationships among plasma dehydroepiandrosterone sulfate and cortisol levels, symptoms of dissociation, and objective performance in humans exposed to acute stress. Arch. Gen. Psychiatry 61, 819–825.

Pare, G., Krust, A., Karas, R.H., Dupont, S., Aronovitz, M., Chambon, P., Mendelsohn, M.E., 2002. Estrogen receptor-alpha mediates the protective effects of estrogen against vascular injury. Circ. Res. 90, 1087–1092.

Pepine, C.J., Nichols, W.W., Pauly, D.F., 2006. Estrogen and different aspects of vascular disease in women and men. Circ. Res. 99, 459–461.

Rasmusson, A.M., Vasek, J., Lipschitz, D.S., Vojvoda, D., Mustone, M.E., Shi, Q., Gudmundsen, G., Morgan, C.A., Wolfe, J., Charney, D.S., 2004. An increased capacity for adrenal DHEA release is associated with decreased avoidance and negative mood symptoms in women with PTSD. Neuropsychopharmacology 29, 1546–1557.

Roy, M.P., Kirschbaum, C., Steptoe, A., 2001. Psychological, cardiovascular, and metabolic correlates of individual differences in cortisol stress recovery in young men. Psychoneuroendocrinology 26, 375–391.

Rozanski, A., Blumenthal, J.A., Kaplan, J., 1999. Impact of psychological factors on the pathogenesis of cardiovascular disease and implications for therapy. Circulation 99, 2192–2217.

Rozanski, A., Blumenthal, J.A., Davidson, K.W., Saab, P.G., Kuzbansky, L., 2005. The epidemiology, pathophysiology, and management of psychosocial risk factors in cardiac practice: the emerging field of behavioral cardiology. JACC 45, 637–651.

Saleh, T.M., Connell, B.J., 2003. Estrogen-induced autonomic effects are mediated by NMDA and GABAA receptors in the parabrachial nucleus. Brain Res. 973, 161–170.

Sapolsky, R.M., Romero, L.M., Munck, A.U., 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocr. Rev. 21, 55–89.

Schommer, N.C., Hellhammer, D.H., Kirschbaum, C., 2003. Dissociation between reactivity of the hypothalamus–pituitary–adrenal axis and the sympathetic-adrenal-medullary system to repeated psychosocial stress. Psychosom. Med. 65, 450–460.

Sgoifo, A., De Boer, S.F., Haller, J., Koolhaas, J.M., 1996. Individual differences in plasma catecholamine and corticosterone stress responses of wild-type rats: relationship with aggression. Physiol. Behav. 60, 1403–1407.

Sgoifo, A., De Boer, S., Musso, E., Stilli, D., Buwalda, B., Meerlo, P., 1999. Social stress, autonomic neural activation, and cardiac activity in rats. Neurosci. Biobehav. Rev. 23, 915–923.

Sgoifo, A., Pozzato, C., Costoli, T., Manghi, M., Stilli, D., Ferrari, P.F., Ceresini, G., Musso, E., 2001. Cardiac autonomic responses to intermittent social conflict in rats. Physiol. Behav. 73, 343–349.

Sgoifo, A., Braglia, F., Costoli, T., Musso, E., Meerlo, P., Ceresini, G., Troisi, A., 2003. Cardiac autonomic reactivity and salivary cortisol in men and women exposed to social stressors: relationship with individual ethological profile. Neurosci. Biobehav. Rev. 27, 179–188.

Sherwood, A., Turner, J.R., 1995. Hemodynamic responses during psychological stress: implications for studying disease processes. Int. J. Behav. Med. 2, 193–218.

Sita, A., Miller, S.B., 1996. Estradiol, progesterone and cardiovascular response to stress. Psychoneuroendocrinology 21, 339–346.

Spruijt, B., van Hooff, J.A., Gispen, W.H., 1992. Ethology and neurobiology of grooming behavior. Physiol. Rev. 72, 825–852.

Stein, P.K., Bosner, M.S., Kleiger, R.E., Conger, B.M., 1994. Heart rate variability: a measure of cardiac autonomic tone. Am. Heart J. 127, 1376–1381.

Steptoe, A., Marmot, M., 2002. The role of psychobiological pathways in socio-economic inequalities in cardiovascular disease risk. Eur. Heart J. 23, 13–25.

Steptoe, A., Gibson, E.L., Hamer, M., Wardle, J., 2007. Neuroendocrine and cardiovascular correlates of positive affect measured by ecological momentary asessment and by questionnaire. Psychoneuroendocrinology 32, 56–64.

Stoney, C.M., Owens, J.F., Matthews, K.A., Davis, M.C., Caggiula, A., 1990. Influences of the normal menstrual cycle on physiologic functions during behavioral stress. Psychophysiology 27, 125–135.

Strike, P.C., Magid, K., Whitehead, D.L., Brydon, L., Bhattacharyya, M.R., Steptoe, A., 2006. Pathophysiological processes underlying emotional triggering of acute cardiac events. Proc. Natl. Acad. Sci. USA 103, 4322–4327.

Tersman, Z., Collins, A., Eneroth, P., 1991. Cardiovascular responses to psychological and physiological stressors during the menstrual cycle. Psychosom. Med. 53, 185–197.

Treiber, F.A., Kamarck, T., Schneiderman, N., Sheffield, D., Kapuku, G., Taylor, T., 2003. Cardiovascular reactivity and development of preclinical and clinical disease states. Psychosom. Med. 65, 46–62.

Troisi, A., 1999. Ethological research in clinical psychiatry: the study of non-verbal behaviour during interviews. Neurosci. Biobehav. Rev. 23, 905–913.

Troisi, A., 2002. Displacement activities as a behavioral measure of stress in nonhuman primates and human subjects. Stress 5, 47–54.

Turgeon, J.L., Carr, M.C., Maki, P.M., Mendelsohn, M.E., Wise, P.M., 2006. Complex actions of sex steroids in adipose tissue, the cardiovascular system, and brain: insights from basic science and clinical studies. Endocr. Rev. 27, 575–605.

Uchino, B.N., Cacioppo, J.T., Malarkey, W., Glaser, R., 1995. Individual differences in cardiac sympathetic control predict endocrine and immune responses to acute psychological stress. J. Pers. Soc. Psychol. 69, 736–743.

Ursin, H., Olff, M., 1993. Psychobiology of coping and defense strategies. Neuropsychobiology 28, 66–71.

Watson, S.L., Ward, J.P., Davis, K.B., Stavisky, R.C., 1999. Scent-marking and cortisol response in the small-eared bushbaby (*Otolemur garnettii*). Physiol. Behav. 66, 695–699.

Watt, G.C., Harrap, S.B., Foy, C.J., Holton, D.W., Edwards, H.V., Davidson, H.R., Connor, J.M., Lever, A.F., Fraser, R., 1992. Abnormalities of glucocorticoid metabolism and the renin-angiotensin system: a four-corners approach to the identification of genetic determinants of blood pressure. J. Hypertens. 10, 473–482.

Wittstein, I.S., Thiemann, D.R., Lima, J.A., Baughman, K.L., Schulman, S.P., Gerstenblith, G., Wu, K.C., Rade, J.J., Bivalacqua, T.J., Champion, H.C., 2005. Neurohumoral features of myocardial stunning due to sudden emotional stress. N. Engl. J. Med. 352, 539–548.

Zinder, O., Dar, D.E., 1999. Neuroactive steroids: their mechanism of action and their function in the stress response. Acta Physiol. Scand. 167, 181–188.