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Neuroscience and Biobehavioral Reviews 27 (2003) 179–188

NEUROSCIENCE AND BIOBEHAVIORAL REVIEWS

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Cardiac autonomic reactivity and salivary cortisol in men and women exposed to social stressors: relationship with individual ethological profile

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Accepted 29 January 2003

Abstract

The degree of cardiovascular stress responsivity and its possible implications for the onset and progression of cardiovascular pathologies seem to be linked to the individual strategy of behavioral coping with stressors. This study was designed to investigate the relationship among cardiac autonomic, endocrine and behavioral responses to real-life stress episodes. Thirty university students were exposed to two brief social challenges (stress interviews), during which the state of sympathovagal balance (time-domain indexes of heart rate variability) and a number of non-verbal behaviors were quantified. Psychometric measurements were also obtained via SPRAS questionnaire, administered just after each stress interview. Samples of saliva were collected for cortisol determination immediately prior and after the experimental session. Subjects showing higher levels of sympathetic dominance were characterized by higher scores of submissive behavior, larger cortisol increments, and higher perception of psychophysiological arousal. A clear consistency in the individual response to the two stress interviews was found, at the behavioral, physiological and psychophysiological level. Finally, the gender of the subjects did not clearly influence their stress responsivity. These results support the hypothesis of a close relationship between the degree of physiological arousal and the style of behavioral adaptation to social stressors.

Keywords: Social stress; Autonomic nervous system; Heart rate variability; Sympathetic dominance; Salivary cortisol; Nonverbal behavior; Anxiety; Submission; Trait characteristics

1. Introduction

Psychosocial stress factors play a significant role in the onset and progression of stress-related disorders, including psychopathologies and psychosomatic diosorders [1–4]. The influence of such environmental factors on the pathogenesis of cardiovascular disease has received remarkable attention in the last decade, resulting in a number of studies in both humans and animal models [4–8].

In particular, there is evidence indicating that people with relatively large cardiovascular responses to behavioral challenge may be at increased risk for the development of essential hypertension and coronary heart disease [9–11].

Along this line of research, an important determinant of cardiovascular reactivity seems to be the individual behavioral strategy of coping with stressors. This issue appears particularly relevant when one considers that, rather than the quantitative (physical) aspects of the stressor, it is the cognitive appraisal and the style of coping which determine the consequences of a social challenge [12].

The study of the relationship between individual behavioral characterization and cardiovascular stress responsivity gained inspiration and impulse from the work of Henry and coworkers on humans and rodents [5,13,14]. Schematically, they proposed that there are two innate response patterns, which might explain the differential sensitivity in developing hypertension. The pattern related to dominance behavior, characterized by behavioral arousal, high levels of aggression and territorial control, i.e.

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0149-7634/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0149-7634(03)00019-8

the active coping response pattern. It is associated with increased cardiac output and redistribution of blood flow to the brain and skeletal muscles, mediated by a robust activation of the sympathetic-adrenomedullary system. The other pattern, characterizing subordination (i.e. defeat, or perception of a threat of loss of control), consists of a generalized behavioral inhibition and a prominent activation of the hypothalamic–pituitary–adrenocortical axis [5].

These concepts relate back to old studies by Rosenman and Friedman, who defined the type A behavioral pattern as associated to a higher proness to develop coronary disease. This behavioral category includes subjects who are competitive, achievement oriented and hostile. The relative absence of type A characteristics defines the type B non-coronary-prone behavioral pattern [15–17].

Glass studied the relationship between type A behavioral pattern and cardiovascular/catecholaminergic responses to experimental conditions designed to induce hostility and competitiveness [18]. Type A subjects showed significantly larger increments of sistolic pressure, heart rate and plasma adrenaline as compared to type B counterparts, thus supporting the idea that the style of behavioral coping influences in a significant manner the patterns of physiological responsiveness.

Recently, Newton and Bane examined the cardiocirculatory correlates of the ‘expression of’ and the ‘exposure to’ dominance and hostility during dydadic social interactions. Such challenges produced significant effects on arterial pressure in females and on heart rate in males. In both cases, cardiovascular reactivity was positively correlated with the level of dominance/hostility shown by the opponent in the dyadic interaction. Moreover, but in women only, the level of dominance exhibited during the test by the experimental subject was positively correlated with heart rate response [19].

Also on the basis of the studies by Contrada and colleagues, certain behavioral and trait characteristics are well-established correlates of cardiovascular and neuroendocrine activation in response to stressors [20,21]. For example, individuals who are hostile, cynical, with propensity to develop anger and aggression, exhibit high cardiovascular reactivity to laboratory stressors [22].

These and other studies investigating the relationship between behavioral profiles and the level of cardiovascular/neuroendocrine stress response have generally identified psychological–behavioral traits via interviews and questionnaires. This approach provides a partial, scarcely objective picture of the subject’s psychological characteristics and, most of all, does not make use of direct observations of behavior. Therefore, whereas physiological correlates are accurately and objectively measured, behavioral stress responses are assessed almost exclusively via the evaluation of the psychic experiences as reported by the subject [23]. As a consequence, it is difficult to evaluate how much this incongruence between accurate physiological measurements and simplistic behavioral assessments can affect the interpretation of results.

From this point of view, ethology can fill the gap, contributing to the development of objective methods for reliable quantification of human behavior.

In the present paper, we applied an ethological method for the study of non-verbal behavior in subjects undergoing stress interviews. This method, often used in the study of subjects with psychopathologies such as schizofrenia and depression, implies the quantification of up to 37 different patterns of non-verbal behavior. The values of all these patterns, once grouped within behavioral categories, allow to depict a complex and quantitative picture of the behavioral response to psychosocial stress in humans [24].

The rationale of the present study can be summarized with the following questions:

1. Is there any correlation, at the individual level, among cardiac autonomic, endocrine and behavioral responses to acute social stressors?
2. How much does the conscious peception of one’s own psychophysiological state reflect the actual physiological activation?
3. Is there any consistency in the individual stress responsivity ? In other words, how repetitive is a subject in his own level of physiological activation across different episodes of social stress?
4. Are there any gender differences in physiological/behavioral responses to social stressors?

2. Methods

2.1. Subjects

Thirty healthy University students (15 males and 15 females), a well-established model of real-life stress [25], were included in the study. Their mean ± SD age was respectively 25.5 ± 1.5 years and 25.2 ± 2.7 years, their mean ± SD body weight 72.1 ± 8.5 and 52.4 ± 5.9 kg, and their mean ± SD height 179.5 ± 5.4 and 162.5 ± 3.8 cm. They all signed a written informed consent and the experimental protocol was approved by our Institutional Review Board. The subjects were tested during the light phase (between 08:30 and 14:30 h), in quiet rooms at a comfortable temperature (22 ± 2 °C).

2.2. Stress protocol and recording schedule

Each subject underwent individually the following experimental procedure, lasting around 1 h:

Phase 1 (room A): saliva collection (see Section 2.5 for details)

Phase 2 (room B): electrocardiographic and videotape recording during Interview 1 (see Section 2.4 and

'Behavioral measurements' for details), followed by completion of the Sheehan Patient Rated Anxiety Scale (SPRAS) (see 'Psychometric measurements' for details). During interview 1, the subject was asked to describe his/her own distinctive personality features.

Phase 3 (room B): electrocardiographic and videotape recording during Interview 2, again followed by completion of SPRAS. During interview 2, the subject was asked to talk about his/her own university experience.

Phase 4 (room A): saliva collection.

During phase 1 and 4 the subject was assisted by one experimenter, only. In phase 2 and 3 there were four persons assisting the subject. Because of the content of the two interviews, interview 1 was assumed to be more stressful than interview 2. In addition, the effect of familiarity with the interviewer was a further factor reducing potential arousing effects of the second interview.

2.3. Radiotelemetry system for electrocardiographic recordings

The ECG radiotelemetry system employed in this study 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 transmitter were wrapped to commercial electrodes terminating with a disc-shaped lead (Battaglia-Rangoni, Bologna, Italy). The two electrodes were fixed with paper tape to right and left parasternal regions and the transmitter was left lying on the platform receiver, on a table just in front of the subject. All recordings were performed with the subject comfortably seated on a chair.

2.4. Electrocardiographic data acquisition and processing

Continuous ECG recordings were performed at first and second interview, in three recording periods, namely baseline (pre-interview), test (interview) and recovery (post-interview), each lasting 5 min. ECG waves were acquired on PC with ART-Silver 1.10 data acquisition system (Data Sciences Int., St Paul, MN, USA). ECG analysis was performed by means of a software package developed in our lab [26] for quantification of time-domain indexes of heart rate variability [27]. The following parameters were quantified: (i) the mean R–R interval duration (RR, ms), (ii) the standard deviation of RR (SD_{RR}, ms), and (iii) the root mean square of successive R–R interval differences (*r*-MSSD, ms). Basically, RR is an 'instantaneous' measurement of heart rate. SD_{RR} estimates overall heart rate variability and therefore includes the contribution of both branches of the autonomic nervous system to heart rate variations: it measures the state of the balance between the activities of the sympathetic component (low-frequency variations) and the parasympathetic branch (high-frequency variations). The *r*-MSSD focuses on high-frequency, short-term variations of R–R interval, which are mainly due to the activity of the parasympathetic nervous system [27–29]. Generally speaking, increased sympathetic and/or decreased parasympathetic tone (i.e. shift of sympathovagal balance towards sympathetic dominance) are reflected in decreased values of variability indexes, while decreased sympathetic and/or increased vagal nervous system activity (i.e. shift of the balance towards parasympathetic prevalence) are reflected in increased values of heart rate variability parameters [27]. R–R interval variability measurements were performed after removal of the intervals surrounding arrhythmic events (extremely rare) or recording artifacts.

2.5. Saliva collection and cortisol determination

Saliva samples (1 ml) were collected in a plastic tube by direct spitting during a 5 min period. The oral cavity was previously cleaned with tap water. The saliva samples were immediately centrifuged at 3000 rpm and the transparent supernatant was stored at −20 °C until assayed. Salivary cortisol measurements were made in duplicate using a RIA method (commercially available kits from Radium, Rome, Italy) with a sensitivity of 0.9 μg/l. 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, but no cross-reactivity with testosterone, estrone, estradiol, estriol and androstenedione (data from Radium). The intraassay coefficient of variation was 3.9%. In order to avoid interassay variability, all samples were run in a single assay.

2.6. Psychometric and behavioral assessment

At the end of each of the two stress interviews, the subjects were asked to fill the SPRAS [30]. This questionnaire, made of 11 items, enabled to evaluate how well the subject could perceive his/her own psychophysiological state during each interview (for instance: 'heart beating hard and quick', 'hand sweating', etc.). Each item was scored by the subject from 0 to 4.

The non-verbal behavior of the subjects during each 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 an ethogram specifically designed for clinical interviews [24]. The current version of the ECSI includes 37 different behavioral patterns, mostly facial expressions and hand movements. Both interviews were videotaped with a camera adjusted so that the subject's face and trunk were in full view, whereas the audio was not recorded. Subsequently, a trained observer (unaware of the subject's verbal reports), examined the videotape and scored the subject behavior according to a one-zero sampling. The video recordings (lasting 300 s) were divided into successive 15 s sample intervals and the observer recorded whether or not a certain behavior pattern had occurred during each sample interval. The score for each behavioral pattern was expressed as the proportion of all sample intervals during which that behavioral pattern occurred. The 37 behavioral patterns were grouped within nine behavioral categories, reflecting different aspects of the subject's emotional and social attitude, as thoroughly described by Troisi [24]. Briefly, these categories were: (1) eye contact, which serves to express attention and involvement, to monitor the interactant's behavior, and to regulate conversational sequencing; (2) *affiliation*, set of patterns (facial expressions and head movements) displayed in order to express friendliness, which invite social interaction and reflect a positive attitude, tending to reassure and to increase attachment; (3) *submission*, patterns used to appease the interviewer and to prevent or inhibit hostile responses, denoting a submissive attitude; (4) affiliation and submission, which serve a common function in the regulation of social interactions—that is, allowing the actor to establish and maintain non-hostile social contact—can be grouped in the category *prosocial*; (5) *flight*, patterns which serve to cut off the sensory receptors from incoming social stimuli perceived as stressful; as social pressures tie the subject to the interview chair and running away is unlikely, social contact is temporarily broken off by disengaging from any interaction; (6) *assertion*, that includes facial expressions and head movements that signal low-level aggression and hostility; (7) *gesture*: includes hand movements that accompany, illustrate and accentuate the verbal content of utterances; it is also an index of global psychomotor activity; (8) *displacement*, a set of behavioral patterns that consist of movements which are focused on one's own body or which iteratively handle objects; in humans, increased displacement behavior correlates with a subjective feeling state of anxiety and negative affect [31,32]; (9) *relaxation*, that consists of behavior 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 the behavioral patterns belonging to it.

2.7. Statistical analysis

R–R interval parameters (mean R–R interval, SD_{RR}, and r-MSSD) were quantified as: (i) means of each 5 min recording phase (baseline, test, and recovery); (ii) delta values (difference between test and baseline values); (iii) means of 1 min periods within each recording phase (the mean value of the five 1 min time points during baseline was used as basal reference value); (iv) area under the curve, i.e. the area comprised between the response time curve and the baseline (AUC; including all time points—minutes—of the test and recovery phase).

Salivary cortisol concentrations were expressed as pre (beginning of the recording session), post (end of the recording session), and delta (post minus pre) values.

Values of autonomic, endocrine, behavioral, and psychometric parameters were analyzed by means of two-way ANOVA for repeated measures, with Time as within-subject factor (two levels: first and second interview, or pre and post), Gender as between—subject factor (two levels: males and females) and Time × Gender interaction. Scheffè test was used when post-hoc analysis was required.

Response consistency within each parameter and the relationships among different parameters were explored by means of Pearson correlation analysis.

All parameters were expressed as mean ± SEM. Statistical significance was set at $p < 0.05$. All statistics were performed using SPSS 10.0 software package (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Psychometric and ethological responses

Analyzing the data for the two stress interviews with a two-way ANOVA with repeated measures on the Time factor, no gender effect emerged for either SPRAS scores and ECSI behavioral categories. In contrast, we found significant Time effects for SPRAS score ($F = 20.18$, $p < 0.01$), submission ($F = 11.65$, $p < 0.05$), prosocial ($F = 8.07$, $p < 0.01$), and relaxation ($F = 5.54$, $p < 0.05$). Thus, during interview 1, the subjects reported higher levels of anxiety (as measured by the SPRAS), showed fewer prosocial and submissive behaviors, and scored higher on the relaxation category. No significant interaction effect (Time × Gender) was found for psychometric and behavioral measurements.

A positive correlation was found for the overall scores obtained by the subjects in the first and second SPRAS questionnaire ($r = 0.72$; $p < 0.05$), indicating an individual consistency in the evaluation of one's own psychophysiological activation in the two stress contexts.

The subjects exhibited a clear consistency also in the behavioral response to the two social challenges (positive correlations for all nine categories; $0.85 \geq r \geq 0.49$, $p < 0.05$).

3.2. Electrocardiographic responses

ECGraphic responses to stress interviews are shown in Fig. 1. Two-way ANOVA for baseline values of the average R–R interval (RR) revealed a significant effect of Time ($F = 5.74$, $p < 0.05$), a tendency toward a Gender effect ($F = 3.60$, $p = 0.068$), and a significant effect of the interaction Time × Gender ($F = 4.94$, $p < 0.05$). Thus,

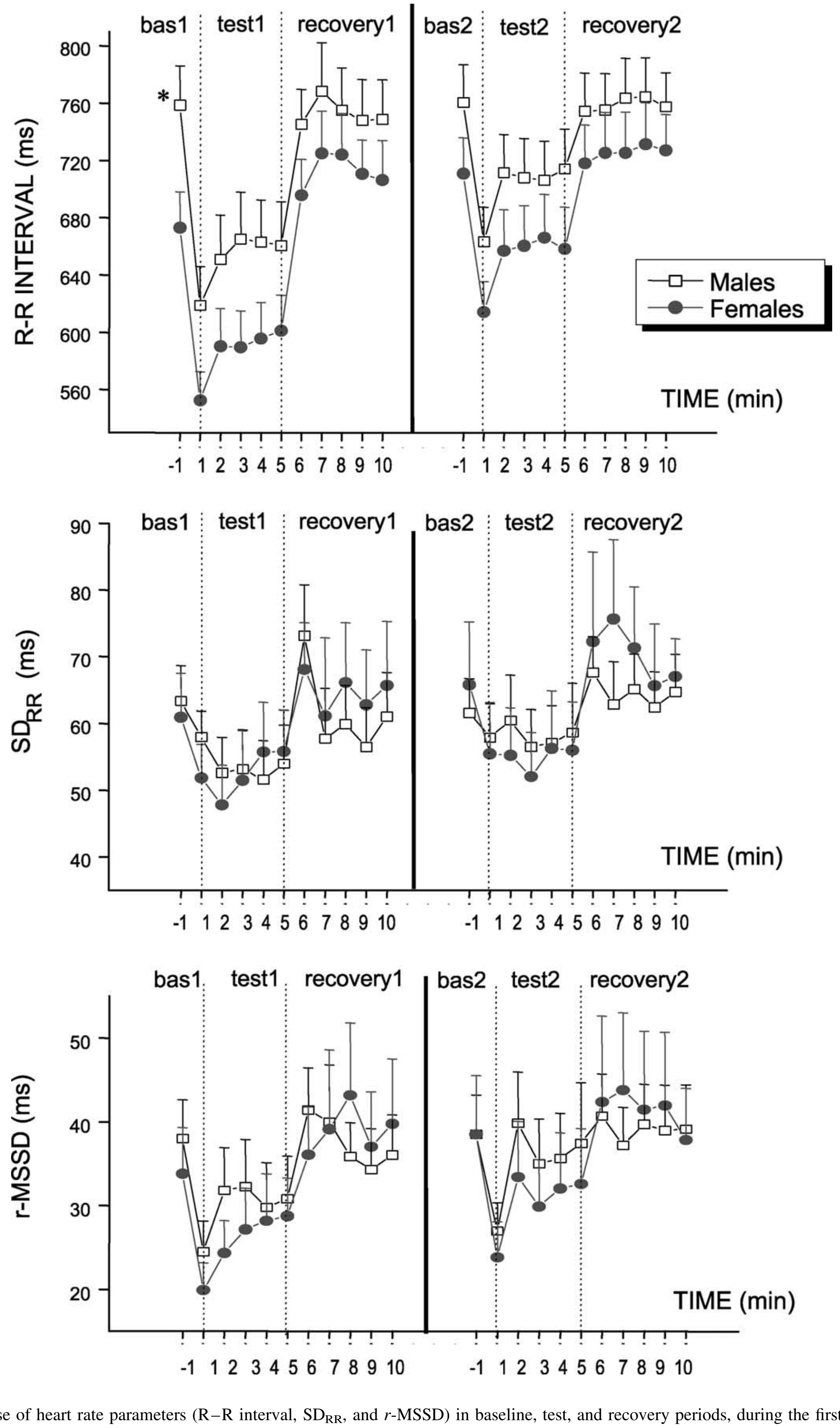

Fig. 1. Time course of heart rate parameters (R–R interval, SD_{RR}, and *r*-MSSD) in baseline, test, and recovery periods, during the first and second stress interview, in males (□, $n = 15$) and females (■, $n = 15$). Each time point represents 1-min mean ± SEM. *Significantly different ($p < 0.05$, Scheffe's Test) from male corresponding value.

when gender is not considered, heart rate was significantly higher at first as compared to second baseline measurement. Post-hoc analysis showed that baseline RR values were significantly lower in females as compared to males at first interview ($t = 2.30$, $p = 0.029$). Two-way ANOVA for test and recovery values of RR revealed significant effects of Time ($F = 42.11$ and $p < 0.01$ for the test; $F = 4.86$ and $p < 0.05$ for recovery), that is to say that heart rate activation during and after the test was much larger at first as compared to second stress interview. No Gender and Gender × Time effects were found. When delta values of RR were considered (test minus baseline), two-way ANOVA showed significant effects only for Time ($F = 15.56$, $p < 0.01$), indicating again that the first interview was more physiologically arousing than the second. Two-way ANOVA for the AUC of RR (area comprised between the response time curve and the baseline) revealed a significant effect of Gender ($F = 3.47$, $p < 0.05$), due to significantly more negative values for males as compared to females at first stress episode ($AUC_{MM} = -257 \pm 176$ vs. $AUC_{FF} = -158 \pm 124$ ms min; $t = -2.07$; $p = 0.048$). In other words, male subjects showed a higher overall heart rate activation at first interview, as compared to females. However, this difference appeared to be determined by the higher resting value of RR (lower heart rate) exhibited by male subjects (Fig. 1). No significant effects were found for Time, Gender, and Time × Gender interaction when baseline, test, recovery, delta and AUC values of SD_{RR} and r-MSSD were considered.

Within each ECGraphic parameter (RR, SD_{RR} and r-MSSD), quantified as baseline, test, recovery, delta and AUC value, many significant (positive, $p < 0.05$) correlations were found between the first and the second stress episode (Table 1). This suggests that individual autonomic stress responsivity was rather consistent across the two stress episodes.

3.3. Salivary cortisol

Fig. 2 reports values of salivary cortisol concentrations in males and females, at the beginning (pre) and the end (post) of the experimental session. Two-way ANOVA on pre and post concentrations (with repeated measures on the Time factor) revealed neither Gender nor Time effects.

3.4. Correlations among psychometric, behavioral, endocrine and cardiac autonomic parameters

Psychometric vs. ECGraphic parameters. During the first interview, significant (negative) correlations were found between SPRAS and ECGraphic parameters (RR, SD_{RR}, r-MSSD) (Table 2). These data indicate that the higher the self-reported anxiety and perceived physiological arousal during the first challenge, the larger the actual autonomic stress activation.

Table 1
Pearson Correlation analyses (r values) within each ECGraphic parameter, measured during first and second stress interview

First interview	Second interview				
	Baseline	Test	Rec	AUC	Delta
RR					
Baseline	0.9[a]				
Test		0.89[a]			
Recovery			0.97[a]		
AUC				0.25	
Delta					0.39[a]
SD_{RR}					
Baseline	0.75[a]				
Test		0.91[a]			
Recovery			0.89[a]		
AUC				−0.05	
Delta					0.27
r-MSSD					
Baseline	0.96[a]				
Test		0.78[a]			
Recovery			0.97[a]		
AUC				0.24	
Delta					0.59[a]

[a] $p < 0.05$.

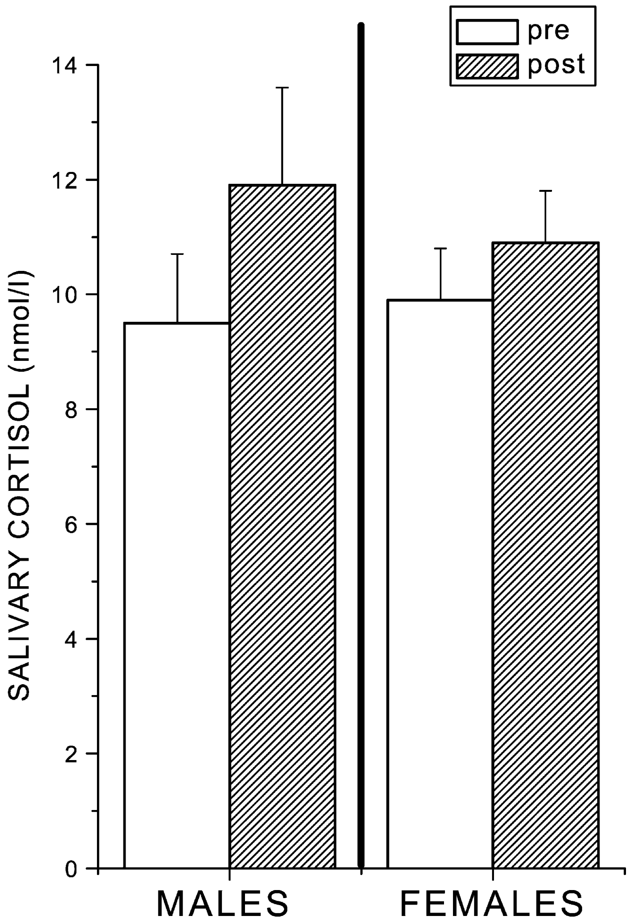

Fig. 2. Salivary cortisol concentrations (nmol/l) at the beginning (pre) and the end (post) of the recording session, in males and females.

Table 2
Pearson correlation analyses (r values) between SPRAS scores and ECGraphic parameter values, during the first stress interview

	RR			SD_{RR}			r-MSSD		
	Baseline	Test	Recovery	Baseline	Test	Recovery	Baseline	Test	Recovery
SPRAS	−0.28	−0.36[a]	−0.40[a]	−0.42[a]	−0.40[a]	−0.34[b]	−0.46[a]	−0.52[a]	−0.41[a]

[a] $p < 0.05$.
[b] $p = 0.07$.

Behavioral vs. ECGraphic parameters. A significant experimental evidence concerning the hypothesis of a correlation between behavioral and cardiac autonomic parameters regarded the set of behavioral patterns classified within the category submission. ECGraphic parameters measured in all the recording phases (baseline, test, recovery) of the first stress episode correlated significantly (negatively) with the scores of this behavioral category (Table 3). These correlations indicate that the subjects exhibiting more frequently submissive behavioral patterns were also characterized by lower values of RR and R–R interval variability—i.e. higher levels of cardiac autonomic activation.

Behavioral vs. endocrine parameters. The scores of the behavioral category eye contact, measured during the first stress interview, correlated positively with delta cortisol, i.e. the higher the level of eye contact, the larger the increment of salivary cortisol ($r = 0.42$, $p < 0.05$). Moreover, we found a negative correlation between the scores of the behavioral category flight during the first stress episode and delta cortisol, i.e. the lower the level of flight behavior the higher the increase of salivary cortisol ($r = -0.38$, $p < 0.05$).

ECGraphic vs. endocrine parameters. The increase in salivary cortisol correlated negatively with the values of delta and AUC of RR during the first social challenge ($r = -0.56$ and $r = -0.57$, respectively; $p < 0.05$). In other words, the higher the increase in salivary cortisol the lower the values of ECGraphic parameters, i.e. the higher the levels of cardiac autonomic activation during interview 1.

4. Discussion

The results of the present study support the hypothesis of a correlation between the level of expression of a number of behavioral patterns and the degree of cardiac autonomic stress responsivity. In particular, this correlation regards those patterns of non-verbal behavior classified within the category *submission*. The subjects which more frequently exhibited submissive behavioral patterns during the first stress interview were also characterized by higher heart rate in baseline, test and recovery periods. Coherently, heart rate variability (expressed as SD and r-MSSD of R–R interval) was lower in the more submissive individuals, again in all recording phases (baseline, test, and recovery). Generally speaking, increased sympathetic and/or decreased parasympathetic activity (i.e. a shift of sympathovagal balance towards sympathetic dominance) are reflected in reduced values of heart rate variability indexes, whereas decreased sympathetic and/or increased vagal activity (i.e. a shift of sympathovagal balance towards parasympathetic dominance) are reflected in increased values of variability indexes. Therefore, the correlations we found suggest that the subjects more prone to exhibit submission in a context of social interaction have a more pronounced sympathetic dominance before, during, and just after the stress episode.

A significant association with cardiac autonomic responsivity was found also for psychometric assessment. During the first social interaction, the level of state anxiety perceived by the subjects (as measured by the SPRAS total score) was negatively correlated with average R–R interval and R–R interval variability indexes in baseline, test, and recovery periods. In other words, the subjects which defined themselves more anxious at the end of the first challenge, also exhibited a higher degree of sympathetic dominance before, during, and after the stressor. These correlations suggest that, when one considers cardiac autonomic arousal as a marker of stress activation, the subjective perception of the level of anxiety experienced during a stressful interview can be accurate. However, we believe that self-reports about one's own anxiety state are reliable (i.e. they reflect an actual stress activation) only

Table 3
Pearson correlation analyses (r values) between submission scores and ECGraphic parameter values, during the first stress interview

	RR			SD_{RR}			r-MSSD		
	Baseline	Test	Recovery	Baseline	Test	Recovery	Baseline	Test	Recovery
Submission	−0.39[a]	−0.42[a]	−0.42[a]	−0.59[a]	−0.42[a]	−0.49[a]	−0.53[a]	−0.34[b]	−0.50[a]

[a] $p < 0.05$.
[b] $p = 0.065$.

when the psychometric evaluation is temporally associated with the stress episode, and not when it is collected away from (e.g. prior to) a specific stressful challenge.

The association between neuroendocrine data (variation of salivary cortisol levels between the beginning and the end of the experimental session, i.e. delta cortisol) and behavioral stress response (quantified via ECSI) was based on two significant correlations. The positive correlation between delta cortisol and frequency of eye contact during the first interview seems to indicate that maintaining higher levels of social communication during a stressful situation determines higher levels of neuroendocrine activation. As a matter of fact, in our species as well as in other primate species, eye contact might represent a stressful stimulus for two different—though strictly linked—reasons. First, eye contact enables the input of social signals via the visual sensory channel which, in our species, represent the main source of information from the surrounding environment. Second, staring at a conspecific represents a threat behavior in most non-human primates [24].

The other significant association between behavioral and neuroendocrine profiles was represented by the negative correlation between delta cortisol and flight behavior: high hormonal changes were accompanied by low frequencies of flight during the first social challenge. In fact, flight behaviors allow a subject to remain physically in a context of social interaction, while providing him/her with the chance to temporary cut off the exchange of social signals [24]. According to the present result, flight behaviors might have the beneficial role of limiting neuroendocrine stress responses during social interactions.

Another interesting result of this study regards the correlation between delta cortisol and average R–R interval response in the first stress interview. The subjects with higher increments of cortisol were also characterized by larger increments of heart rate. This evidence allows to associate these classical stress parameters when estimating the individual degree of physiological stress responsivity.

Noteworthy, all behavioral–physiological, psychometric–physiological, and physiological–physiological correlations so far described were significant only when the first stress interview was examined. This apparent incongruence can be explained when one considers that autonomic (namely average R–R interval) and psychometric (SPRAS score) activations were significantly larger during the first as compared to the second stress episode. This might imply that an aversive social stimulus producing a too moderate stress arousal (the second interview) does not allow any of these associations to emerge.

This study also suggests that there is a clear consistency in the individual responsivity to the two social stress episodes, in terms of cardiac electrical activity (heart rate and heart rate variability indexes), as well as behavior (ethological parameters) and psychophysiological perception of one's own anxiety state (SPRAS questionnaire). The subjects which were high responders during the first challenge were again high responders also in the second social interaction, both from the physiological and the ethological–psychometric point of view. This allows us to consider the autonomic, behavioral and psychometric parameters used in this study as trait characteristics, that is to say, individual characteristics that identify a certain personality, a certain style of coping with a stressor that is substantially stable within each individual [5,12].

Another interesting issue concerns the role of gender in the behavioral and physiological response to social challenge. In the present study, we did not find significant differences between males and females. At the level of cardiac autonomic modulation, the only (apparent) difference concerned the ECGraphic response to the first stress interview: males exhibited higher overall increments of heart rate as compared to females, measured as test, recovery and AUC values of RR. This experimental evidence contrasts with results obtained by other authors, where females showed a higher heart rate responsivity due to a reduced vagal antagonism to sympathetic activation [33]. However, the sexual difference we found seems to depend largely on two elements. First, in the baseline conditions preceding the first challenge, females exhibited significantly higher heart rates as compared to males. Presumably, this was due to a higher level of anxiety (and associated activation of the sympathetic–adrenomedullary system) in anticipation of the upcoming test. Second, in the post-test phase immediately following the first interview, female heart rate not only recovered but reached values which were somewhat lower than baseline. This suggests that the reference RR value we measured in females before the first stressor took place was not corresponding to their actual baseline heart rate. Altogether, these considerations suggest that the gender difference we documented should be taken with caution.

In agreement with this reasoning, Carrillo and colleagues did not find any gender difference in the ECGraphic response to public speaking [34].

Also in terms of neuroendocrine activation (salivary cortisol levels) we did not find differences between genders. This result seems to contrast with data provided by other studies, where males confronted with psychosocial stimuli exhibited higher responses of the hypothalamic–pituitary–adrenocortical axis [35–37]. A significant role in this sexual difference has been ascribed to the effects of estrogens, which are thought to attenuate female glucocorticoid response to psychological stressors [38]. The lack of gender differences in our study might be due to the fact that we found relatively mild cortisol increments at the end of our recording session, likely because the peak of activation was antecedent to our final measurement. Usually, the peak of cortisol response is estimated between the 20th and 30th min after the onset of the stressor [39], whereas our second saliva sample was obtained approximately 45 min after the beginning of the first (and more robust) social challenge.

Moreover, it cannot be disregarded that, within the female group, nine individuals out of 15 were taking oral contraceptive medication at the time of the experiment, which might of course have affected their HPA axis stress responsivity. The lack of control for this variable is clearly a limitation of the present study. As a partial extenuating circumstance for this shortcoming, it is worth to say that, a part from slightly higher pre values of salivary cortisol in the subjects taking oral contraceptive treatment, we did not find significant differences in post and delta values between the two subgroups of females (personal observations).

To summarize the results of the present study, the following conclusions can be drawn:

(i) when facing a social stressor of a certain intensity (corresponding to stress interview 1 in this study), the subjects exhibiting higher neuroendocrine activations (salivary cortisol concentrations) are also characterized by larger shifts of cardiac sympathovagal balance towards sympathetic dominance;
(ii) the individuals showing higher scores of submissive behavior during social challenge are prone to larger sympathetic dominance at the level of the heart, thus are potentially more at risk to develop tachyarrhythmias;
(iii) a higher degree of sympathetic dominance appears to be associated also to the perception (and self-report) of higher levels of anxiety during a social stress test;
(iv) the maintenance of a certain degree of social communication during a psychosocial challenge (higher scores of eye contact with the interviewer) is associated with a higher increment of salivary cortisol;
(v) on the other hand, the intermittent use of behavioral patterns which allow the subject to temporarily cut off social stimuli (flight behavior) seems to dampen such neuroendocrine stress response;
(vi) there is a clear consistency in the individual responsivity to the two stress interviews, at the physiological and behavioral level, as well as at the level of psychophysiological perception. In other words, the parameters used in this study could be considered as trait characteristics, i.e. individual strategies of coping with a stressor that are substantially stable within each individual;
(vii) the behavioral/physiological activation produced by acute social challenges such as stress interviews does not seem to be gender dependent.

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