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Stressed out? Associations between perceived and physiological stress responses in adolescents: The TRAILS study

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Abstract

Studies regarding the interrelation of perceived and physiological stress indices have shown diverging results. Using a population sample of adolescents ($N = 715$, 50.9% girls, mean age 16.11 years, $SD = 0.59$), we tested three hypotheses: (1) perceived responses during social stress covary with concurrent physiological stress responses; (2) high pretest levels of perceived stress predict large physiological responses; and (3) large physiological responses to social stress predict low posttest perceived stress levels. Perceived arousal, unpleasantness, and dominance were related to heart rate, respiratory sinus arrhythmia, and cortisol responses to a laboratory social stress test. Although effect sizes were small, the results suggest covariation of perceived stress and concurrent physiological stress responses in both the ANS and the HPA axis, as well as inverse associations between heart rate responsiveness and the subsequent appraisal of stress.

Descriptors: Stress-reactivity, Heart rate, Cortisol, Self-report

Stress is an umbrella term which designates divergent symptoms such as rapid heartbeat, dizziness, pains, nervousness, agitation, irritability, worrying, concentration problems, and moodiness.

This research is part of the TRacking Adolescents' Individual Lives Survey (TRAILS). Participating centers of TRAILS include various departments of the University Medical Center and University of Groningen, the Erasmus University Medical Center Rotterdam, the University of Utrecht, the Radboud Medical Center Nijmegen, and the Parnassia Bavo group, all in The Netherlands. TRAILS has been financially supported by various grants from the Netherlands Organization for Scientific Research NWO (Medical Research Council program grant GB-MW 940-38-011; ZonMW Brainpower grant 100-001-004; ZonMw Risk Behavior and Dependence grants 60-60600-98-018 and 60-60600-97-118; ZonMw Culture and Health grant 261-98-710; Social Sciences Council medium-sized investment grants GB-MaGW 480-01-006 and GB-MaGW 480-07-001; Social Sciences Council project grants GB-MaGW 457-03-018, GB-MaGW 452-04-314, and GB-MaGW 452-06-004; NWO large-sized investment grant 175.010.2003.005; the Sophia Foundation for Medical Research (projects 301 and 393), the Dutch Ministry of Justice (WODC), the European Science Foundation (EuroSTRESS project FP-006), and the participating universities. We are grateful to all adolescents, their parents, and teachers who participated in this research and to everyone who worked on this project and made it possible.

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That all of these symptoms are referred to as stress suggests that they reflect a single underlying mechanism. The extent to which various stress indicators are actually related to each other determines the generalizability of a single stress measure to stress in a broader sense. Because physiological stress indices are harder to assess than psychological ones, perceived stress is often the initial or even only measure of states of stress, both in research and in clinical practice. It is therefore important to assess whether and how various psychological and physiological stress indices are interrelated. This study explores these interrelationships in a large sample of adolescents.

In the first half of the twentieth century, Selye, often considered the father of stress research, discovered that a variety of different physical stimuli (e.g., cold, pain, toxic agents, extracts of organs) led to similar physical consequences, that is, degeneration of lymphatic structures, gastric ulceration, and increased activity of the adrenal cortex. He postulated these responses to be universal and non-specific, and called them the general adaptation syndrome or GAS (e.g., Selye, 1936). Selye's notion of a universal stress response has been criticized for being an oversimplification of the reality. Mason (1968, 1971) and others after him (e.g., Dickerson & Kemeny, 2004; McCarty & Gold, 1996) noted that not all stress phenomena are nonspecific: some are only triggered if the stimulus requires specific demands to be met. In other words, stress systems may respond to variable degrees and in variable combinations to stressors, depending on

the nature of the stressor (Ulrich-Lai & Herman, 2009). In fact, there is increasing evidence that the two major stress systems of the body, the autonomic nervous system (ANS) and the hypothalamic-pituitary-adrenal (HPA) axis, are more dissociated than is often assumed: high ANS reactivity does not necessarily imply high HPA-axis reactivity (e.g., Gerra et al., 2001; Schommer, Hellhammer, & Kirschbaum, 2003), and vice versa.

Selye was also criticized because he had excluded psychosocial stressors from his research, and ignored that a stressor may also evoke emotional arousal. Mason (1971) and Mikhail (1981) proposed that, rather than the stressor itself, the emotional response to the stressor generates stress phenomena. Lazarus and Folkman (Lazarus, 1966; Lazarus & Folkman, 1984) also focused on the psychological dimension of the stress response. They emphasized the importance of the appraisal of the situation and stated that physiological stress phenomena appear only if the situation is perceived as potentially damaging and hard to manage. Today, the psychological processes provoked by a (psychosocial) stressor are still believed to constitute the bridge between stressor and stress response (Van Praag, De Kloet, & Van Os, 2004).

If the appraisal of the potentially stressful stimulus is the major determinant of the stress response, one might expect a strong positive association between the perceived stressfulness of a situation and the strength of the physiological stress responses. This hypothesis is consistent with the linkage of the ANS and HPA-axis with cortical and limbic structures, important mediators of subjectively experienced stress (e.g., Buijs & Van Eden, 2000; Schlotz et al., 2008). However, despite the intuitive and neurological plausibility of a close link between perceived stress and physiological stress responses, associations reported between the two are generally weak and divergent (Cohen et al., 2000; Hjortskov, Garde, Ørbæk, & Hansen, 2004; Lackschewitz, Hüther, & Kröner-Herwig, 2008; Schlotz et al., 2008). Schommer and colleagues noted that “this dissociation between subjective and biological indices of stress is most interesting from a psychosomatic point of view. Unfortunately, few experimental data are available to help explain why outflow from these different response levels hardly ever converges consistently” (Schommer et al., 2003, p. 458). Indeed, interrelationships between psychological and physiological stress indices have rarely been examined systematically, with a few notable exceptions. Al’ Absi et al. (1997) evaluated cardiovascular, HPA-axis, and psychological responses to public speaking and mental arithmetic, and found substantial correlations between psychological and HPA-axis responses, particularly during public speaking. By contrast, Gaab, Rohleder, Nater, and Ehlert (2005) reported that cortisol responses to social stress were particularly associated with anticipatory stress appraisal (perceived threat), not with (retrospective) ratings of perceived stress during the test. Schlotz et al. (2008) reported positive correlations between psychological stress measures and cortisol levels when psychological stress was assessed before cortisol, and negative correlations when the order was reversed. Though intriguing, these findings need replication and extension, not only because of the partly contradictory reports regarding temporal processes, but also because of methodological limitations of the studies. Al’ Absi et al.’s and Gaab et al.’s results were based on small ($N = 52$ and $N = 81$, respectively) samples of male volunteers, while all females (58%) in Schlotz’ study (total $N = 219$) used oral contraceptives, which are known to affect cortisol responses (e.g., Bouma, Riese, Ormel, Verhulst, & Oldehinkel, 2009). Furthermore, Gaab et al.

and Schlotz et al. examined only the HPA-axis and no cardiac autonomic responses, and Gaab et al. used different measures for anticipatory versus retrospective stress appraisal. In other words, prior studies suggest interesting patterns of associations, but still with many gaps to be filled.

The aim of the present study is to fill part of these gaps and so better understand how perceived stress relates to physiological stress. Associations between various perceived and physiological stress indices were investigated in 715 adolescents (351 boys, 364 girls, age 15–17) from the general population. Adolescents are a valuable population to study (psycho)physiological stress responses, because the prevalence of potentially confounding somatic disorders and medication use is relatively low at this age. Despite the fact that stress reactivity is affected by exposure to stressors earlier in life (e.g., Lupien, McEwen, Gunnar, & Heim, 2009), both perceived stress and physiological stress responses to psychosocial stress have been reported to be fairly invariant across age (e.g., Kudielka, Buske-Kirschbaum, Hellhammer, & Kirschbaum 2004; McManis, Bradley, Berg, Cuthbert, & Lang, 2001; Wood, Maraj, Lee, & Reyes, 2002), although it should be noted that the magnitude of heart rate responses tends to decrease with age (Carroll et al., 2000; Steptoe, Fieldman, Evans, & Perry, 1996).

The adolescents included in this study participated in a series of behavioral tests including a social stress test (public speaking and mental arithmetic), which is considered a useful experimental paradigm to observe integrated psychological and physiological responses (Al’ Absi et al., 1997). The psychological stress indices used reflect bodily, affective, and cognitive dimensions of perceived stress; that is, subjective arousal, unpleasantness, and dominance (sense of being in control). The physiological measures, heart rate, respiratory sinus arrhythmia, and salivary cortisol, reflect (re)activity of two major physiological stress systems, the ANS and HPA-axis.

The value of this study is not only its exceptionally large sample of adolescents, but also the fact that it examines various temporal patterns in the association between perceived and physiological stress. This is important, because Schlotz and colleagues (2008) showed that the direction of associations between psychological and physiological stress response may depend on the time lag between the measures. Based on associations found in the before-mentioned prior studies, three hypotheses were tested with regard to interrelations between perceived stress measures and physiological stress responses:

1. Perceived stress during a social stress test covaries with concurrent physiological stress responses;
2. High pretest levels of perceived stress predict large physiological responses to a social stress test; and
3. Large physiological responses to a social stress test predict low posttest perceived stress levels.

The first hypothesis assumes an association between psychological and physiological stress during the social stress test, as compared to pretest levels, and is hence the most direct test of linkage between the various stress systems. Previous findings in favor of this hypothesis were reported by, among others, Al’ Absi et al. (1997), Roy (2004), and Thayer (1970). By comparing difference scores (that is, stress levels during exposure to a social stressor minus resting levels), it is possible to account for differences in response style, which can weaken estimated associations (e.g., Hjortskov et al., 2004). Response style refers to answer tendencies

that are unrelated to the content of the items, such as acquiescence. Because differences in on- and offsets of the stress responses may obscure covariations (Schlotz et al., 2008), the maximum stress response during the social stress test was used in this study, regardless of its timing.

Justification for the second hypothesis is found in several studies suggesting that anticipatory appraisal processes predict physiological stress responses (e.g., Gaab et al., 2005; Rohrmann, Hennig, & Netter, 1999; Wirtz et al., 2006). Through various neural pathways, appraisal processes, such as perceived threat, provide input for the hypothalamic paraventricular nucleus, which plays a central role in the regulation of autonomic and endocrine stress responses (Gaab et al., 2005). It has been suggested that anticipation of stress, especially when the situation is perceived to be unpredictable and uncontrollable, may result in a state of vigilance toward events that are to occur and, consequently, in exaggerated stress responses (Schulkin, McEwen, & Gold, 1994).

The third hypothesis, in a way, mirrors the second one. It was based on the intriguing phenomenon, observed in multiple studies, that high cortisol levels during stress may reduce post-stress anxiety, arousal, or fatigue (Het & Wolf, 2007; Reuter, 2002; Schlotz et al., 2008; Soravia et al., 2006; Tops, Van Peer, Wijers, & Korf, 2006). This suggests that, apart from normalizing the physiological stress systems, cortisol also regulates stress-induced negative emotions and perceived stress. Based on these findings, high cortisol levels during the social stress test were expected to predict low perceived stress levels afterwards in our study. As opposed to cortisol, autonomic stress responses have, to the best of our knowledge, not been investigated with regard to posttest perceived stress before, hence the analyses regarding heart rate and respiratory sinus arrhythmia were mostly exploratory in this respect.

In sum, considering the wealth of data on psychological and physiological responses to stressful experiences, the relative scarcity of studies on the interrelation between the two is surprising and regrettable. The present study offers the opportunity to shed more light on this issue, because it involves cross-sectional and temporal associations between multiple perceived and physiological stress indices in a large general population sample of adolescents who were submitted to a social stress test. More knowledge about whether and how perceived stress predicts, follows, or covaries with cardiac and cortisol responses not only benefits theoretical stress models, but may also clarify the validity of perceived stress measures with respect to more general notions of stress.

Methods

Participants

The data were collected in a focus sample of TRAILS (TRacking Adolescents' Individual Lives Survey), a large prospective population study of Dutch adolescents with bi- or triennial measurements from age 11 to at least age 25. Thus far, three assessment waves have been completed, running from March 2001 to July 2002 (T1), September 2003 to December 2004 (T2), and September 2005 to December 2007 (T3). During T1, 2230 children were enrolled in the study (response rate 76.0%, De Winter et al., 2005), of whom 1816 (81.4%) participated in T3. During T3, 744 adolescents were invited to perform a series of laboratory tasks (hereafter referred to as the experimental session) on top of the usual assessments, of whom 715 (96.1%) agreed to do so. The costly and labor-intensive nature of the

laboratory tasks precluded assessing the whole sample. Adolescents with a high risk of mental health problems had a greater chance of being selected for the experimental session. High risk was defined based on temperament (high frustration and fearfulness, low effortful control), lifetime parental psychopathology, and living in a single-parent family. In total, 66.0% of the focus sample had at least one of the above-described risk factors; the remaining 34.0% were selected randomly from the low-risk TRAILS participants. Please note that the focus sample still represented the whole range of problems seen in a normal population of adolescents, which made it possible to reproduce the distribution in the total TRAILS sample by means of sampling weights. Descriptive statistics of the focus sample (weighted estimates) are presented in Table 1.

Procedure

Experimental session. The experimental session consisted of a number of different challenges, listed here in chronological order: a spatial orienting task, a gambling task, a startle reflex task, and a social stress test. The session was preceded and followed by a 40-min period of rest. The participants filled out a number of questionnaires at the start and end of the session. Before, during, and after the experimental session, extensively trained test assistants assessed cardiovascular measures, cortisol, and perceived stress. Measures that were used in the present study are described more extensively below. The experimental sessions took place in sound-proof rooms with blinded windows at selected locations in the participants' towns of residence. The total session lasted about 3 1/2 h, and started between 8:00 and 9:30 am (morning sessions, 50%) or between 1:00 and 2:30 pm (afternoon sessions, 50%). The protocol was approved by the Central Committee on Research Involving Human Subjects (CCMO).

The social stress test. The social stress test was the last challenge of the experimental session. It involved a standardized protocol, inspired by (but not identical to) the Trier Social Stress Task (Kirschbaum, Pirke, & Hellhammer, 1993), for the induction of mild performance-related social stress. Socio-evaluative threats are highly salient challenges for adolescents and are known to be effective activators of various physiological stress systems, particularly in combination with uncontrollability; that is, in situations when negative consequences cannot be avoided (Dickerson & Kemeny, 2004). The participants were instructed to prepare a 6-min speech about themselves and their lives and deliver this speech in front of a video camera. They were told that their videotaped performance would be judged on content of speech as well as on use of voice and posture, and ranked by a panel of peers after the experiment. The participants had to speak continuously for the whole period of 6 min. The test assistant

Table 1. Sample Characteristics ($N = 715$)

Variable	Mean (<i>SD</i>) or percentage
Female gender	50.7%
Age	16.11 (0.60)
Smoking (habitual)	28.0%
Physical exercise ^a	3.26 (2.06)
Body mass index	21.45 (3.29)
Use of oral contraceptives (% among girls)	34.4%

Note: Sampling weights were used to represent the distribution in the general population.

^aNumber of days per week with at least 1 hr of physical exercise.

watched the performance critically, and showed no empathy or encouragement. The speech was followed by a 3-min interlude in which the participants were not allowed to speak. During this interval, which was included to assess cardiac autonomic measures that were not affected by speech, the participants were told that they had to wait for a moment because of computer problems, but that the task would continue as soon as these problems were solved. Subsequently, they were asked to perform mental arithmetic. The participants were instructed to repeatedly subtract the number 17 from a larger sum, starting with 13,278. A sense of uncontrollability was induced by repeated negative feedback from the test assistant (e.g., “No, wrong again, begin at 13,278”; “Stop wiggling your hands”; “You are too slow, we are running behind schedule”). The mental arithmetic challenge lasted for 6 min, again followed by a 3-min period of silence, after which the participants were debriefed about the experiment.

Measures

Heart rate (HR). Cardiac autonomic function was assessed at the start of the experimental session (after 40 min of rest), as well as during and after the social stress test, in seven blocks: pretest (300 s), speech preparation (420 s), speech (360 s), silent interlude after speech (180 s), mental arithmetic (360 s), silent interlude after mental arithmetic (180 s), and posttest (300 s). A three-lead electrocardiogram was registered using 3M/RedDot Ag/AgCl electrodes (type 2255, 3M Health Care, Neuss, Germany), while the participant was sitting and breathing spontaneously. With a BIOPAC Amplifier-System (MP100, Goleta, CA), the signals were amplified and filtered before digitization at 250 samples/second. Dedicated software (PreCARSPAN, previously used in, e.g., Dietrich et al., 2007) was used to check signal stationarity, to correct for artifacts, to detect R-peaks, and to calculate the interbeat-interval (IBI) between two heartbeats. Blocks were considered invalid if they contained artifacts with a duration of more than 5 s, if the total artifact duration was more than 10% of the registration, or if the block length was less than 100 s (invalid blocks pretest: $n = 15$, preparation: $n = 28$, speech: $n = 27$, interlude after speech: $n = 35$, mental arithmetic: $n = 29$, interlude after mental arithmetic: $n = 31$, posttest: $n = 32$). HR is inversely related to IBI by the equation $HR = 60000/IBI$. HR was defined as the number of beats per minute (bpm).

Respiratory sinus arrhythmia (RSA). Calculation of RSA was performed by power spectral analysis in the CARSPAN software program (Mulder, 1988) using estimation techniques based on Fourier transformations of IBI series (Robbe et al., 1987). RSA was defined as the power in the high-frequency (0.15–0.40 Hz) band, which is associated with the respiratory cycle, and expressed in ms^2 . RSA mainly results from centrally mediated cardiac vagal activity (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). Because the social stress test involved speech, which is known to interfere with analysis of RSA (e.g., Bernardi et al., 2000; Sloan, Korten, & Myers, 1991), the calculation of RSA was based on HR recordings during the 3-min interludes directly following the speech and mental arithmetic tasks, when the participants were not allowed to speak. The stress level remained relatively high during these interludes, because the participants expected that they had to continue any moment. Nevertheless, it was probably lower than during speech and mental arithmetic tasks and might not reflect the maximum response.

Cortisol. Cortisol levels were assessed just before the start of the social stress test (C1), directly after the end of the test (C2), 20 min after the test (C3), and 40 min after the test (C4). Considering the normal delay (20–25 min) in peak cortisol responses to experimental stressors (Kirschbaum, Read, & Hellhammer, 1992), all samples reflect stress reactions about 20 min earlier. Therefore, the samples were labeled as C1 = pretest, C2 = during test, C3 = end of test (immediately after the test), and C4 = post-test (20 min after the test).

Cortisol was assessed from saliva by the Salivette sampling device (Sarstedt, Numbrecht, Germany). After the experimental session, the samples were placed in a refrigerator at 4°C, and within a few days stored at –20°C until analysis. All samples were analyzed with the same reagent, and all samples from a participant were assayed in the same batch. Cortisol was measured directly in duplicate in 100 μ l saliva using an in-house radioimmunoassay (RIA) applying a polyclonal rabbit cortisol antibody and 1,2,6,7-³H Cortisol (Amersham International Ltd., Amersham, UK) as tracer. After incubation for 30 min at 60°C, the bound and free fractions were separated using activated charcoal. The intra-assay coefficient of variation was 8.2% for concentrations of 1.5 nM, 4.1% for concentrations of 15 nM, and 5.4% for concentrations of 30 nM. The inter-assay coefficients of variation were, respectively, 12.6%, 5.6%, and 6.0%. The detection border was 0.9 nM. Missing samples (C1: $n = 12$, C2: $n = 8$, C3: $n = 10$, C4: $n = 12$) were due to detection failures in the lab (60%) or insufficient saliva in the tubes (40%). Cortisol levels above 5 standard deviations of the mean (C1: $n = 3$, C2: $n = 6$, C3: $n = 3$, C4: $n = 4$) were considered outliers and recoded into missing values.

Perceived stress. Perceived stress was assessed by means of the Self-Assessment Manikin (SAM), a non-verbal pictorial assessment technique to measure the arousal, pleasure, and dominance (i.e., control) associated with a person's affective reaction to a stimulus (Bradley & Lang, 1994). For each of the feelings assessed (i.e., arousal, unpleasantness, dominance), the subjective intensity could be indicated by choosing one out of nine ordered pictures. The pictures were translated into a nine-point scale (range 1–9) in such a way that high scores represented high levels of arousal, unpleasantness, and dominance. Perceived stress during the social stress test was assessed directly after the test, with a reference to the test (“How did you feel during this test?”) Pre- and posttest experiences were measured at the start (after 40 min of rest) and at the end of the experimental session (40 min after the social stress test), respectively. SAM ratings for arousal and unpleasantness have been shown to correlate almost perfectly ($r \geq .95$) with corresponding scales of the Semantic Differential Scale (Mehrabian & Russel, 1974), while the correlation was moderately high ($r = .79$) for dominance (Bradley & Lang, 1994).

Other variables. Smoking, physical activity, and body mass index (BMI) were included as potential confounders of the associations under study. Smoking and physical exercise were assessed as part of the regular T3 questionnaire, which was filled out at school, on average 3.07 months ($SD = 5.12$) before the experimental session. We distinguished between non-smokers and habitual smokers (i.e., at least one cigarette a day). Physical activity was operationalized as the number of days the respondent was physically active for at least 1 h. During the school assessments, length and weight were measured by trained test assistants. BMI is defined as the weight in kilograms divided by

the length in meters squared. Use of oral contraceptives (OC) was assessed by means of a checklist on current medication use administered at the start of the experimental session. In total, OC were used by 125 girls (34.4%).

Analysis

Adolescents with a high risk of mental health problems were overrepresented in the study sample. Therefore, sampling weights were used to reproduce the distribution in the total TRAILS sample in all analyses. Sampling weights denote the inverse probability that a subject is included in a sample. Missing data on any of the variables were handled by multiple imputation, using the ICE (Imputation by Chained Equations) approach available in the statistical package Stata (StataCorp, 2007). Five datasets with imputed missing values were created, given other variables in the dataset. Analyses were performed on each imputation, and subsequently combined into a single result using the Stata program MIM (Royston, 2005). The percentage of missing values was generally low and did not exceed 4.5% for any of the variables included in the analyses. Perceived and physiological stress responses were defined as the maximum level during (or immediately after) the test minus the minimum level before or after the test; for RSA and dominance, this equation was reversed in order to construct response measures that were positively associated with the strength of the response for all variables. Stress responses were defined in relation to either pre- or posttest levels instead of only pretest levels because prior research suggests that posttest stress levels make up better resting measures than pretest levels because posttest levels are not confounded by anticipation effects (Hansen, Johnsen, & Thayer, 2003). A two-sided p -value smaller than .05 was considered statistically significant.

The first step was to calculate descriptive statistics of the (untransformed) variables used in this study, and to test differences between multiple assessments of the same variable by means of repeated measures analysis of variance. In case of significant within-subject changes, pairwise *post hoc* tests were performed to explore the nature of the differences, with Bonferroni correction for multiple testing. The analyses of variance were based on a single imputation dataset, because Stata's multiple imputation procedures do not support repeated measures analysis of variance. The HR, RSA, and cortisol variables were log-transformed before analysis to obtain a more normal distribution. Before transformation, the skewness ranged from 0.53 to 0.97 for the HR variables, from 3.32 to 5.76 for the RSA variables, and from 1.68 to 2.70 for the cortisol variables. After transformation, the skewnesses were between -0.73 and 0.14 , -0.17 and 0.07 , and -0.17 and 0.96 , respectively. Means and standard deviations were based on untransformed (raw) variables.

Subsequently, the three hypotheses outlined in the introduction were tested by a series of linear regression analyses. The hypothesis that perceived stress covaried with concurrent physiological responses during the social stress test (hypothesis 1) was tested by analyses with HR, RSA, and cortisol responses as outcomes, and perceived stress responses (i.e., the difference between test and resting levels of arousal, unpleasantness, and dominance) as predictor variables. The hypothesis that high perceived stress levels at pretest predicted large physiological responses to the social stress test (hypothesis 2) was tested by using the pretest levels of arousal, unpleasantness, and dominance as predictor variables, and HR, RSA, and cortisol responses as outcomes. Finally, the hypothesis that large physiological stress responses

predicted low posttest perceived stress levels (hypothesis 3) was tested by regressing the difference between post- and pretest perceived stress levels on HR, RSA, and cortisol responses. All continuous variables were standardized to mean 0 and standard deviation 1 to obtain internally comparable regression coefficients. Partial η^2 was used as a measure of effect size.

Gender, smoking, and physical exercise were included in all regression analyses as possible confounders. Furthermore, because there is ample evidence for gender differences in psychophysiological responses to stressful situations, both in previous studies (Biondi & Picardi, 1999; Kudielka, Hellhammer, & Wüst, 2009) and in the present dataset (Bouma et al., 2009), all effects under study were tested on gender differences. This was done by including interaction terms in the model, which were maintained if significant. A previous study by Bouma et al. (2009) on the effects of gender, menstrual phase, and use of oral contraceptives in the same sample had indicated that oral contraceptive users (34.4% of the girls) showed no cortisol response to the social stress test. Therefore, in the present study, oral contraceptive users were excluded from all analyses involving cortisol. This exclusion led to an overrepresentation of boys in the cortisol analyses, but not to a dramatic extent (59.6% boys versus 40.4% girls). Moreover, gender was included as covariate in all analyses, which prevented possible bias.

Results

Descriptive Statistics

All stress measures changed significantly during the social stress test, with both psychological and physiological measures indicating that stress levels were higher during the social stress test than preceding or following it (Table 2). Please note that the pretest values of the perceived stress measures and HR and RSA reflect stress levels at the start of the laboratory session (after 40 min of rest), about 1 1/2 h before the start of the social stress test. Pretest RSA was exceptionally low, compared to RSA levels during and after the stress test. This is remarkable since pretest HR correlated $-.67$ with pretest RSA, but was not exceptionally high. RSA levels after speech and mental arithmetic were relatively high compared to RSA during the preparation phase, probably because the speech and mental arithmetic values of RSA were assessed during silent interludes (directly) after the performance rather during the task itself. RSA levels during speech and mental arithmetic were lower indeed (speech: 1872 , $SD = 2380$; mental arithmetic: 1901 , $SD = 2349$), but may have been influenced by the respondents' speaking at that time and are hence less trustworthy. Although RSA levels after speech and mental arithmetic were higher than during these stressors, they were still both significantly lower than posttest RSA. The cortisol statistics presented concern the pooled estimates across morning and afternoon sessions. Cortisol levels were higher in the morning (mean level morning 4.54 nM/L, $SD = 2.16$; afternoon 3.62 nM/L, $SD = 1.98$; $t(588) = 5.45$, $p < .001$), but the response patterns were comparable (Bouma et al., 2009), with significant within-changes in both mornings ($F(3,288) = 41.4$, $p < .001$) and afternoons ($F(3,295) = 41.9$, $p < .001$).

Correlations between subsequent assessments of stress measures were generally moderate to high (arousal: $r = .32$ to $.47$; unpleasantness: $r = .18$ to $.32$; dominance: $r = .44$ to $.59$; HR: $r = .61$ to $.87$; RSA: $r = .69$ to $.87$; cortisol: $r = .47$ to $.87$). Correlations between arousal, unpleasantness, and dominance were higher during stress ($|r| = .41$ to $.54$) than during rest ($|r| = .17$ to

Table 2. Stress Measures Used in this Study, and Tests of Within-Subjects Changes

Variable	Mean (SD)	Within-subject change	Significant differences
A. Arousal pretest	2.68 (1.50)	$F(2,713) = 325.3, p < .001$	C < A < B
B. Arousal during test	4.19 (1.88)		
C. Arousal posttest	2.37 (1.45)		
A. Unpleasantness pretest	2.85 (1.23)	$F(2,713) = 367.1, p < .001$	A < B C < B
B. Unpleasantness during test	4.74 (1.89)		
C. Unpleasantness posttest	2.88 (1.76)		
A. Dominance pretest	6.46 (1.47)	$F(2,713) = 288.8, p < .001$	B < A < C
B. Dominance during test	5.39 (1.85)		
C. Dominance posttest	6.97 (1.44)		
A. HR pretest (bpm)	75.68 (11.13)	$F(4,711) = 457.2, p < .001$	E < A < B < C E < A < D < C
B. HR preparation (bpm)	77.96 (11.12)		
C. HR speech (bpm)	82.05 (13.20)		
D. HR mental arithmetic (bpm)	78.08 (11.49)		
E. HR posttest (bpm)	69.47 (9.96)		
A. RSA pretest (ms ²)	1732 (2820)	$F(4,711) = 72.04, p < .001$	A < B < C < E A < B < D < E
B. RSA preparation (ms ²)	2178 (3209)		
C. RSA after speech (ms ²)	2462 (3447)		
D. RSA after mental arithmetic (ms ²)	2363 (3338)		
E. RSA posttest (ms ²)	2653 (3561)		
A. Cortisol pretest (nM/L)	3.43 (2.04) ^a	$F(3,586) = 76.0, p < .001$	A < D < C < B
B. Cortisol during test (nM/L)	4.59 (2.85) ^a		
C. Cortisol end of test ^b (nM/L)	4.46 (2.98) ^a		
D. Cortisol posttest ^c (nM/L)	3.71 (2.12) ^a		

Note: Sampling weights were used to represent the distribution in the general population. Descriptives for HR, RSA, and cortisol data reflect untransformed data, while log-transformed data were used in the analyses. Analyses were based on single imputation data. Pairwise differences were adjusted for multiple testing (Bonferroni method). HR: heart rate, RSA: respiratory sinus arrhythmia.

^aExclusive of girls using oral contraceptives.

^bImmediately after the social stress test.

^c20 min after the social stress test.

.35). Similarly, HR and cortisol levels were significantly correlated ($r = .08$ to $.17$) during and immediately after the social stress test, but not before the test or 20 min afterwards ($r = -.06$ to $-.05$). Interestingly, pretest cortisol levels were inversely related to HR during and after the test. RSA was negatively associated with HR ($r = -.37$ to $-.67$), but not with cortisol. For an overview of all correlations, see the Appendix. Associations between psychological and physiological stress measures will be discussed in more detail below.

Associations Between Perceived and Physiological Stress Measures

Interrelations between perceived and physiological stress measures (adjusted for gender, smoking, BMI, and physical exercise) are shown in Tables 3–5. None of the effects were significantly different for boys and girls.

The first hypothesis was that perceived responses during the social stress test would covary with concomitant physiological responses. As expected, changes in perceived arousal and unpleasantness responses were associated with changes in HR, RSA, and cortisol (Table 3). Changes in perceived dominance did not covary significantly with any of the physiological stress responses. Effect sizes (partial η^2) for arousal and unpleasantness ranged between .006 and .017, which correspond to Cohen's d -values between 0.15 and 0.25 and thus signify small effects.

To further illustrate the size of the effects, the sample was divided into three groups based on the perceived stress responses: low responders (limited change in perceived arousal and perceived unpleasantness, i.e., 0 or 1, 20.8%), high responders (large changes in perceived arousal or perceived unpleasantness of 5 or

more, 20.1%), and intermediate responders (all other adolescents, 59.1%). HR, RSA, and cortisol responses were plotted for each of these groups (Figures 1–3). Relative to the size of the stress response itself, the differences among the three perceived stress groups were considerably smaller for HR than for RSA and cortisol; Figure 1 suggests hardly any effect of perceived stressfulness on HR responses. This may seem inconsistent with the fact that both the effect sizes and the (standardized) regression coefficients were largely comparable for the three outcome measures. This seeming inconsistency can be explained by the small standard deviation of HR responses, compared to the size of the response.

The graphs in Figures 1 and 3 show larger differences between high and intermediate responders than between low and intermediate responders, which could point to nonlinear effects. To test this (*post hoc*) hypothesis, we added quadratic effects of arousal and unpleasantness responses to the regression models predicting HR and cortisol, but none of these effects were statistically significant (all p -values $> .11$). The patterns shown in Figures 1 and 3 may be due to the definition of the perceived stress response groups: perhaps the group of intermediate responders were on average more comparable to the low responders than to the high responders.

The second hypothesis was that pretest perceived stress levels would be associated with physiological responses to the social stress test. No pretest levels of the perceived stress measures (arousal, unpleasantness, and dominance) predicted any subsequent HR, RSA, or cortisol responses (Table 4).

With regard to the third hypothesis, that physiological responses would predict posttest perceived stress levels, we found that greater HR responses predicted less posttest unpleasantness

Table 3. Perceived Concurrent Stress Responses as Predictors of Physiological Responses to the Social Stress Test

Predictors	Outcomes		
	HR response (ln) <i>B (p)</i>	RSA response (ln) <i>B (p)</i>	Cortisol response (ln) ^a <i>B (p)</i>
Concurrent Arousal	0.12 (.001)	0.08 (.02)	0.09 (.05)
Concurrent Unpleasantness	0.09 (.03)	0.09 (.03)	0.12 (.008)
Concurrent Dominance	0.04 (.25)	-0.00 (.97)	0.01 (.88)

Note: Sampling weights were used to represent the distribution in the general population. HR, RSA, and cortisol variables were log-transformed before analysis. Continuous variables were standardized to mean 0 and *SD* 1. All effects are adjusted for gender, smoking, BMI, and physical exercise. *N* = 715. HR: heart rate, RSA: respiratory sinus arrhythmia, Response: difference between state during the test and pre- or posttest state.

Bold: *p* < .05.

^aAnalyses exclusive of girls using oral contraceptives (*N* = 589).

and more posttest dominance, as compared to pretest levels (Table 5), which lends partial support for the hypothesis that physiological stress responses predict posttest perceived stress levels. Large cortisol responses tended to be associated with low posttest unpleasantness as well (two-sided *p* = .06). RSA responses were not associated with any of the posttest perceived stress measures. Effect sizes were small, with partial η^2 values of around .006 for the (marginally) significant effects, corresponding to a Cohen's *d*-value of 0.15.

Discussion

In this study, we explored the interrelation of perceived and physiological responses to a social stress test in a large sample of adolescents from the general population. The results suggest temporal covariation of psychological and physiological stress systems as well as limited associations between physiological stress responses and subsequent psychological measures. More specifically, perceived arousal and unpleasantness during the stress test covaried with all concurrent physiological stress responses (hypothesis 1), and large HR responses to social stress predicted low posttest unpleasantness and dominance, while a trend was found for an effect of cortisol responses on posttest

Table 4. Perceived Pretest Stress Responses as Predictors of Physiological Responses to the Social Stress Test

Predictors	Outcomes		
	HR response (ln) <i>B (p)</i>	RSA response (ln) <i>B (p)</i>	Cortisol response (ln) ^a <i>B (p)</i>
Pretest Arousal	-0.02 (.55)	-0.02 (.62)	-0.02 (.62)
Pretest Unpleasantness	0.06 (.10)	0.03 (.34)	-0.04 (.32)
Pretest Dominance	-0.06 (.13)	-0.08 (.09)	-0.01 (.82)

Note: Sampling weights were used to represent the distribution in the general population. HR, RSA, and cortisol variables were log-transformed before analysis. Continuous variables were standardized to mean 0 and *SD* 1. All effects are adjusted for gender, smoking, BMI, and physical exercise. *N* = 715. HR: heart rate, RSA: respiratory sinus arrhythmia, Response: difference between state during the test and pre- or posttest state.

^aAnalyses exclusive of girls using oral contraceptives (*N* = 589).

Table 5. Physiological Stress Responses as Predictors of Posttest Perceived Stress

Predictors	Posttest perceived stress ^a		
	Arousal <i>B (p)</i>	Unpleasantness <i>B (p)</i>	Dominance <i>B (p)</i>
HR response	-0.06 (.13)	- 0.09 (.05)	0.09 (.04)
RSA response	-0.03 (.35)	-0.03 (.44)	0.06 (.14)
Cortisol response ^b	-0.02 (.65)	-0.07 (.06)	0.01 (.84)

Note: Sampling weights were used to represent the distribution in the general population. All effects are adjusted for gender, smoking, BMI, and physical exercise. *N* = 715. HR: heart rate, RSA: respiratory sinus arrhythmia, Response: difference between state during the test and pre- or posttest state.

Bold: *p* < .05.

^aAs compared to pretest perceived stress (difference scores).

^bAnalyses exclusive of girls using oral contraceptives (*N* = 589).

unpleasantness (hypothesis 3). There was no support for hypothesis 2, that high pretest perceived stress levels predict physiological responses to social stress.

Hypothesis 1

Our results support the notion of covariation between perceived and physiological stress responses. Despite only weak correlations between HR and cortisol and no significant correlations between RSA and cortisol, most associations with perceived stress levels were largely comparable among the three physiological stress measures. The significant associations of perceived arousal and unpleasantness with both cardiac measures and cortisol suggest that perceived stress reflects, to a certain extent, activity of the HPA-axis as well as the autonomic nervous system. Our data do not allow conclusions about whether the perception of the stressfulness steered physiological responses or vice versa, but we assume bidirectional influences. On the one hand, it is obvious that psychosocial stressors need to be perceived and evaluated as such in order to trigger a stress response (e.g., Ulrich-Lai & Herman, 2009), on the other hand, physiological reactions (e.g., heart pounding) may be interpreted as signs of the apparent stressfulness of the situation and hence inflate perceived stress scores.

Effect sizes were small according to Cohen's conventions (1988), but still considerable compared to the size of the stress responses, specifically for RSA and cortisol (see Figures 2 and 3). Taking into account that this study involved a normal-population sample of adolescents and a mild brief stressor, and that both psychological and physiological stress responses are influenced by a multitude of only partially overlapping factors, we feel that high effect sizes could not be expected. Furthermore, as shown in several meta analyses (e.g., Ioannidis, Trikalinos, Ntzani, & Contopoulos-Ioannidis, 2003), published effect sizes based on large samples are, on average, considerably smaller than those based on small samples. This is probably due to publication bias: in studies with a limited sample size, small effects are usually not statistically significant and therefore less likely to be submitted and accepted for publication (Easterbrook, Berlin, Gopalan, & Matthews, 1991).

Changes in perceived dominance were not significantly related to physiological stress responses. This seems inconsistent with previous reports of uncontrollability as a predictor of the cortisol response (Dickerson & Kemeny, 2004). The Dominance scale of the Self-Assessment Manikin (Bradley & Lang, 1994) depicts a series of schematic figures, ranging from very small

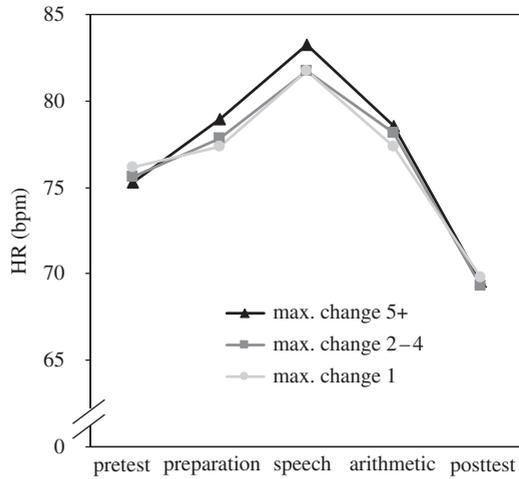


Figure 1. HR responses to the social stress test, by responsiveness level. Responsiveness level is based on a composite index of perceived arousal and perceived competence.

(being controlled, submissive) to very large (being in control, powerful). This measure may not be specific enough to measure feelings of uncontrollability. It is also possible that the assumed effects of uncontrollability on cortisol responses relate to objective task characteristics rather than individual differences in perceived controllability.

Apart from the above-described methodological issues, there may also be a more substantive reason why arousal and unpleasantness, but not dominance, covary with physiological stress responses. Unpleasantness and arousal reflect the desire to change the situation, and the intensity of this desire, respectively. These are primitive motivational parameters integrated in subcortical areas (e.g., Lang, Bradley, & Cuthbert, 1992), which have been associated with various physiological responses (e.g., Lang, Greenwald, Bradley, & Hamm, 1993). Dominance reflects the

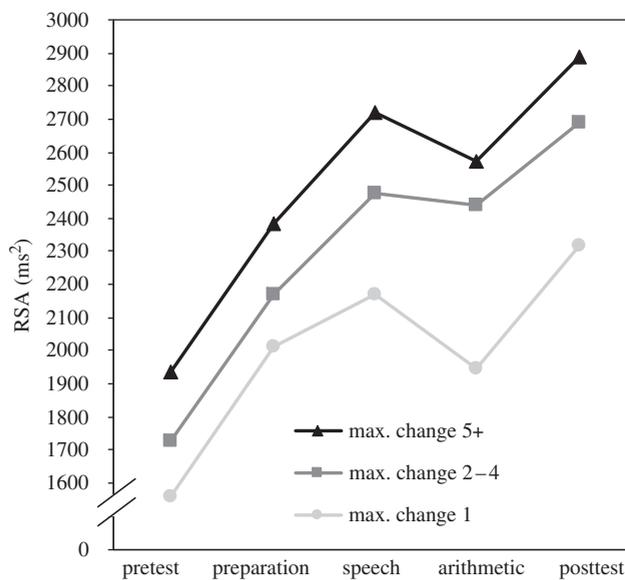


Figure 2. RSA responses to the social stress test, by responsiveness level. Responsiveness level is based on a composite index of perceived arousal and perceived competence.

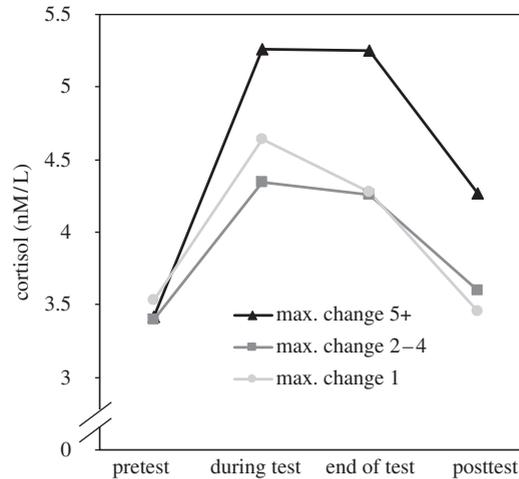


Figure 3. Cortisol responses to the social stress test, by responsiveness level. Responsiveness level is based on a composite index of perceived arousal and perceived competence.

perceived possibilities to change the situation, rather than the actual desire to do so. Consistent with this, dominance has been found to account for less variance in emotional judgments than arousal and pleasure (e.g., Bradley & Lang, 1994), and may hence also be more loosely linked to physiological stress responses.

Hypothesis 2

Contrary to expectations, pretest perceived stress did not predict physiological responses to a social stress test. This could be due to the fact that the pretest perceived stress levels did not reflect how stressful the adolescents expected the social stress test to be, but rather how they felt in general at the start of the laboratory session. This general stress perception is probably not a very accurate measure of anticipatory appraisal, which has been found to predict physiological stress responses in previous studies (Gaab et al., 2005; Rohrmann et al., 1999; Wirtz et al., 2006). In addition, stress responses may have been affected by the considerable time lag between the pretest measure and the social stress test, as well as the various other tasks performed in between. It would thus be inappropriate to conclude that the anticipated stressfulness of a particular task is unrelated to physiological responses to that task.

Although the pretest perceived stress measures used in this study may not assess anticipatory appraisal well, they have a validity of their own, as pretest resting levels. Hence, what can be concluded from the results is that resting levels of perceived arousal, unpleasantness, and dominance are not very informative with regard to subsequent physiological stress responses. In general, there does not seem to be much meaningful variance in stress measures assessed during rest, as is also illustrated by finding that correlations between various stress measures were higher during stress than pre- or posttest. This suggests that individual differences in stress responsiveness can best be ascertained under stressful conditions.

Hypothesis 3

The hypothesis that physiological stress responses predict posttest perceived stress levels was based on prior studies suggesting that high cortisol levels might prevent stressful experiences from inducing negative affect (Het & Wolf, 2007; Reuter, 2002; Sch-

lotz et al., 2008; Soravia et al., 2006; Tops et al., 2006). The marginally significant effect of cortisol responses on posttest perceived unpleasantness lends tentative support to this postulation. It seems contradictory that high cortisol *levels* reflect distress and high cortisol *responses* prevent it. Distinguishing between tonic and phasic cortisol levels might be relevant in this respect: high tonic cortisol levels have adverse effects on mood (e.g., Schmidt, Fox, Goldberg, Smith, & Schulkin, 1999; Wolowitz et al., 1990), while high phasic cortisol levels (i.e., large responses) seem quite adaptive when measured in healthy adolescents. The functional effects of cortisol for regulating emotions are still unknown. Cortisol binds to (glucocorticoid and mineralocorticoid) receptors, which can be found in several brain areas, including prefrontal cortex and limbic areas (e.g., De Kloet, Vreugdenhil, Oitzl, & Joels, 1998), and can influence several catecholaminergic neurotransmitter systems (Joels, 2000). It has been proposed that cortisol modulates pathways of a neural network involving, among other things, the prefrontal cortex, amygdala, and HPA-axis. These networks play an important role in emotional processing (e.g., Davidson & Irwin, 1999; Dolan, 2002), in that the effects of cortisol on the prefrontal cortex reduce emotional responses to stress (Het & Wolf, 2007).

HR responses, which have been suggested to reflect effort rather than distress (e.g., Arnetz & Fjellner, 1986; Peters et al., 1998), were more strongly associated with posttest perceived stress measures than cortisol responses. High HR responses predicted low posttest unpleasantness and high posttest dominance. A possible explanation for the association between HR responses and posttest perceived stress is that a high HR response is an adaptive mechanism to adequately cope with stressors. Assuming a positive association between the strength of the HR response and the amount of effort invested in the task (Arnetz & Fjellner, 1986; Peters et al., 1998), we could speculate that adolescents who invested a lot of effort performed better and hence felt more satisfied and in control afterwards. Otherwise stated, blunted stress responses may signal dysfunctional coping strategies, which in turn may increase feelings of discomfort and lack of control following the stressful experience. In fact, another study in the same sample indicated that adolescents with high effortful control (i.e., high self-regulation skills) had stronger HR responses to the social stress test (Oldehinkel, Hartman, Nederhof, Riese, & Ormel, submitted), which supports the idea that blunted stress responses may reflect poor coping with stress. Analogous to effects of physical exercise on emotional well-being (e.g., Sher, 1998; Yeung, 1996), a direct impact of physiological activity on subsequent subjective emotions is conceivable as well, such as through altered neurotransmitter release (Meeusen & Piacentini, 2001). Alternatively, high HR responses may not actually predict subsequent feelings, but rather mark adolescents who are still energetic and do not feel worn out and therefore report low levels of unpleasantness and uncontrollability at the end of the laboratory session. Why the effect of HR responses on posttest perceived stress was stronger for unpleasantness and low dominance than for arousal might be related to the fact that unpleasantness and uncontrollability are usually rated as negative emotions, while high arousal can be conceived of as either negative or positive. If high HR responses mark a satisfactory performance, as suggested above, this is likely to influence positive affect, but not necessarily relaxation. Hence, HR responses are possibly associated with posttest negative affect rather than (hyper)arousal. However, all these suggestions are highly tentative, and replication in an independent sample is

warranted before firm conclusions can be drawn regarding this association.

RSA responses did not predict any of the posttest perceived stress measures. This could indicate that the effects of HR were mainly accounted for by sympathetic, and not vagal, activation. Prudence is called for, however, because HR and RSA measures during speech and mental arithmetic were not based on the same time periods.

Practical Implications

Given that our sample was large and representative of a normal population of adolescents, this study is particularly suitable to answer the practical question of whether, in clinical or research settings, self-reports of perceived arousal and unpleasantness during a stressful situation provide useful information about the magnitude of HR, RSA, or cortisol responses. Based on our findings, the answer to this question would have to be no. Due to substantial unexplained variance, measures of perceived stress provide only partial knowledge about the responsiveness of the autonomic system and HPA-axis. As suggested by Fahrenberg and Foerster (1982), a set of marker variables seems to be preferable to a single measure to assess individual differences in stress responsiveness, and we propose these marker variables should include both perceived and physiological stress indices.

Strengths and Limitations

The findings should be considered in light of a number of noteworthy strengths and limitations. A significant strength of the study is its very large sample size, compared to most other studies involving laboratory stress tests. This reduces the influence of single outliers and the probability of false-negative or false-positive results. The subjects were adolescents selected from the general population, whose perceived and physiological stress responses are less likely to be disturbed by medical conditions than those of older subjects or clinical patients. An additional strength is the repeated examination of stress indices across the testing session, a procedure which yields more clues about the direction of effects than single assessments.

There are also limitations to this study. First, the social stress test was preceded by a spatial orienting task, a startle-response test, and a gambling task. We did not account for the perceived stressfulness of these challenges. The stress measures assessed during the social stress test could represent the cumulative effect of the prior experimental tasks rather than responses to the social stress test. A large systematic bias due to the experimental design is unlikely, however, because the order of the tasks was the same for all subjects. Hence, not only the exposure to social stress was standardized, but also the activities preceding the social stressor. Furthermore, the social stress test was by far the most stressful element of the session, both conceptually and in terms of subjectively experienced stress as measured by the Self-Assessment Manikin (data available upon request). Still, one cannot rule out effects of the preceding tasks on responses to the social stress test. Moreover, as mentioned before, pretest HR, RSA, and perceived stress measures reflect levels at the beginning of the laboratory session (after 40 min of rest) rather than levels immediately preceding the social stress task, which may have deflated the effects. A second limitation is that RSA was assessed during silent interludes following the periods wherein the participants were actively engaged in public speaking and mental arithmetic, to avoid interference with speech. Although the stress level during these silent interludes was relatively high because the participants anticipated near continuation of the test, it was still likely to be lower

than during the performance. In most participants, the RSA responses reflected the difference between posttest RSA and RSA during the preparation phase, which may not be the maximum response. Third, respiration rate was not recorded in this study and hence could not be controlled for while analyzing RSA, as recommended by, for instance, Berntson et al. (1997). Because RSA was based on periods without speech in which the participants were sitting quietly, the task effects upon respiration rate were probably limited, which reduces the need for respiratory control (e.g., Grossman & Taylor, 2007; Houtveen, Rietveld, & De Geus, 2002), yet some confounding cannot be excluded. Finally, responses to social stress tests as used in laboratory experiments may not reflect responses to potentially pathogenic stressful experiences in real life. The social stress test used in our study lasted for less than half an hour, after which the adolescents were debriefed and could relax again. Real-life stressors and their aftermaths usually persist considerably longer than half an hour and are therefore likely to trigger more pervasive stress reactions.

Conclusions

Our findings suggest that perceived, autonomic, and HPA-axis responses to social stressors covary to some extent in adolescents.

Particularly on-task perceived arousal and unpleasantness may predict concurrent changes in HR, RSA, and cortisol levels. Dominance seems to have a specific, more cognitive role in adolescents' stress appraisals, and to be less associated with physiological stress measures. Pretest resting perceived stress measures are not very informative with regard to physiological responses to stress. Furthermore, large physiological stress responses, notably HR responses, seem to reflect healthy, adaptive mechanisms, which might prevent post-stress negative affect.

In sum, adolescents' reported feelings of arousal and unpleasantness, but not dominance, to some degree reflect concurrent autonomic and HPA-axis activity. This could indicate that—specific—emotional responses to stressors generate physiological stress responses, as postulated in the introduction (Mason, 1971; Mikhail, 1981), be it to a limited extent. However, perceived stress levels do not seem to predict how adolescents will respond to later stressors, and should therefore be considered correlates rather than risk factors of physiological stress responses (Kraemer et al., 1997). This study also suggest that strong physiological stress responses, although perceived as arousing and unpleasant at the time being, can still be adaptive, in that they may increase feelings of pleasantness and dominance afterwards.

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