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An inactive control of the 'Trier Social Stress Test' for Youth 10–17 years: Neuroendocrine, cardiac, and subjective responses

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ABSTRACT

The Trier Social Stress Test for children (TSST-C) adapted from TSST is one of the most commonly used laboratory paradigms for investigating the effects of stress on cognitive, affective and physiological responses in children and adolescents. Considering that laboratory procedures generate a significant amount of stress to children and adolescents, even in the absence of a stress paradigm, it is important to validate TSST-C against an inactive control condition in which the stress components were absent. Using a randomized design, we tested an inactive control condition, which replaced the TSST-C with a benign video clip (nature scenes viewed while standing), thus removing the stress associated components of the TSST-C. Eighty-eight youth between the ages of 10 and 17 years were randomly assigned to complete the TSST-C or the Inactive Control (IC). Subjective anxiety rating, salivary cortisol, systolic and diastolic blood pressure, and heart rate were collected at eight time points. Subjects in the Inactive Control condition showed no significant changes in blood pressure and heart rate, and decreased anxiety rating and salivary cortisol level throughout the study. Subjects in the stress condition (TSST-C) showed increased anxiety ratings, salivary cortisol, systolic and diastolic blood pressure, and heart rate immediately following TSST-C stress induction. Our findings validated that the TSST-C induced a systemic stress response, and that the Inactive Control can be a promising standardized control condition for the TSST-C and a tool for future psychobiological research. Our results also showed that anxiety reactivity decreased with age while HR reactivity increased with age. Cortisol reactivity did not fall in a linear relationship with age but rather via a quadratic curve, suggesting the mid-age adolescents had the highest cortisol responses to stress compared to their younger and older peers, potentially due to a dual factor of pubertal development and self-control and emotion regulation capacity.

1. Introduction

1.1. Background

As the transitional period between childhood and adulthood, adolescence is a time of remarkable physical and psychosocial growth (Chulani and Gordon, 2014). The adolescent developmental period encompasses pubertal development, when sex specific hormones guide the maturation of sex organs and overall body growth and maturation. Psychosocially, adolescents experience significant development in cognitive capacity, moral reasoning, self-identity and relationship building (Chulani and Gordon, 2014). These maturational changes can create a significant amount of stress, such that adolescence is often termed a "period of storm and stress" (Hall 1904).

Interest in psychosocial stress and its impacts on mental and physical well-being has led to the identification of stress biomarkers. Stress facilitates the release of a series of hormones in the Hypothalamic-pituitary-adrenal (HPA) axis (Foley and Kirschbaum, 2010; Frodl and O'Keane, 2013; Kemeny, 2003). This stress response cascade initiates upon the perception of stress, which causes the hypothalamus to release

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corticotrophin releasing hormone (CRH). CRH then triggers the pituitary glands to release adrenocorticotropic hormone, which in turn triggers the adrenal glands to release cortisol (Frodl and O'Keane, 2013; Kemeny, 2003). As the end product of the HPA axis, cortisol directly reflects the stress level in both actual and perceived stress (Allen et al., 2014; Dedovic et al., 2009; Dickerson and Kemeny, 2004; Foley and Kirschbaum, 2010; Miller et al., 2017).

Given the broad range of emergent developmental factors occurring in youth across psychological, maturational, hormonal, and neural domains that bear on well-being (Gunnar et al., 2009; Stroud et al., 2009; Van den Bos et al., 2014; Walker et al., 2001), studying stress responses in adolescence is of particular importance. Indeed, several studies reported that baseline cortisol levels increase with age through childhood to adolescence (Gunnar et al., 2009; Walker et al., 2001), as do stress-induced cortisol levels (Gunnar et al., 2009; Stroud et al., 2009). Prolonged activation of the HPA axis can result in negative physical and psychological consequences (Frodl and O'Keane, 2013). In particular, dysregulated HPA activity to stressors can make adolescents more prone to psychiatric illnesses (Spear, 2000; Stroud et al., 2009; Walker et al., 2001).

A large body of work shows that exposure to relatively short-duration laboratory challenge tasks activate the stress system. The "Trier Social Stress Test" (TSST) is one of the most widely used psychological stress tasks for assessing acute stress responses in the laboratory (Kirschbaum et al., 1993, 1992). The TSST induces stress by exposing participants to a review panel as they perform challenging language and arithmetic tasks. Several factors influence the psychological and physiological responses to a stressor, including uncontrollability, duration, ambiguity, level of cognitive demand, and the presence of social evaluation (Kemeny, 2003). Previous work finds that laboratory performance tasks characterized by both social evaluative threat and uncontrollability yield the largest elevations in cortisol level (Dickerson and Kemeny, 2004). These elements are similarly important for eliciting a significant stress response in children and adolescents (Buske-Kirschbaum et al., 1997; Gunnar et al., 2009). Buske-Kirschbaum et al. developed the TSST for children (TSST-C) (Buske-Kirschbaum et al., 1997), which includes components of performing public speaking and a mental arithmetic task before an audience. The TSST-C has been widely used in youth samples (Buske-Kirschbaum et al., 1997, 2003; Dorn et al., 2003; Gunnar et al., 2009; Stroud et al., 2009; Sumter et al., 2010; Van den Bos et al., 2014; Walker et al., 2001; Yim et al., 2010) and has been adopted for use with children as young as 7 years old (Buske-Kirschbaum et al., 2003; Stroud et al., 2009).

The TSST induces a stress response as evidenced by multiple domains across subjective, neuroendocrine and physiological measurements. Immediately following the TSST manipulation, increases are observed in anxiety (Childs et al., 2006; Hellhammer and Schubert, 2012; Het et al., 2009; Jezova et al., 2004; von Dawans et al., 2011), salivary cortisol (see review of Allen et al (Allen et al., 2014)), systolic blood pressure (Campisi et al., 2012; Gerra et al., 2001; Jezova et al., 2004; Wright et al., 2014), diastolic blood pressure (Jezova et al., 2004), and heart rate (Campisi et al., 2012; Childs et al., 2006; Gerra et al., 2001; Hellhammer and Schubert, 2012; Jezova et al., 2004; Kirschbaum et al., 1992; von Dawans et al., 2011; Wright et al., 2014). Given that children and adolescents experience higher distress than adults in the same stressful environment (Yim et al., 2010), it is important to consider findings in youth regarding stress responses. Similar to adults, salivary cortisol increases after acute stress in children and adolescents (Buske-Kirschbaum et al., 1997, 2003; Dorn et al., 2003; Gunnar et al., 2009; Sumter et al., 2010; Yim et al., 2010). In addition, blood pressure (Dorn et al., 2003) and heart rate rise to the stressor (Buske-Kirschbaum et al., 1997, 2003; Gunnar et al., 2009).

Even though stress responses occur in a laboratory setting with a stress manipulation, the causal relationship between the stress responses and the stressor can only be established through validation against a control condition. Previous work suggests that a significant amount of stress can be generated by the mere experience of going to a laboratory facility to participate in a study, without the induction of stress (Gunnar et al., 2009; Walker et al., 2001). Thus, further qualification of the validity of the TSST-C and its impact on stress reactivity requires consideration of a non-stress control condition in order to rule out the possibility that the stress response is generated due to concurrent events. Moreover, the TSST and TSST-C have been used as a stressor to study the impact of stress in various psychological and cognitive domains in combination of neurocognitive and neurophysiological measures (Andersen et al., 2018; Quesada et al., 2012; Tiferet-Dweck et al., 2016). Given the general effect stress induced by a laboratory visit, it is also relevant to consider when the psychological stressor is or is not combined with other equipment and procedures (i.e., EKG, EEG, BP). Thus, the current study compares two procedures. Procedure 1 (the TSST-C) has a traditional TSST-C in combination with concurrent EEG measurement and other neurocognitive tests. Procedure 2, (the inactive control) shared the same concurrent EEG measurement as procedure 1, but replaced the TSST-C with a benign video clip (nature scenes viewed while standing). The contrast of TSST-C and the inactive control aims to verify the origin of stress responses of the TSST-C manipulation.

In terms of contrasting TSST-C with a control or low stress condition, Het et al. (Het et al., 2009) implemented a similar approach in a sample of young adults by developing a placebo TSST where uncontrollability and social evaluative threats were excluded. Their findings suggested that the placebo TSST is an adequate control condition for young adults as it did not activate the HPA axis compared to the standard TSST (Het et al., 2009). In the youth literature, Yim and colleagues (Yim et al., 2015) examined standard and low stress conditions in children (7–8 years old) and adolescents (12–15 years old). The two conditions carried the same mental and arithmetic tasks, but the low stress condition involved more friendliness and less pressure compared to the standard stress condition. They found that the standard stress condition elicited higher levels of salivary cortisol and subjective stress than the low stress condition. Quesada and colleagues (Quesada et al., 2012) examined children (8–10 years old) in TSST-C and an active non-stress condition. In their study, children did mental and arithmetic tasks but in absence of a review panel. They found that the cortisol level was higher in the TSST-C group than in the control condition. Importantly, no previous studies have compared the TSST-C stress responses against an inactive control condition, in which the TSST-C stressor was completely removed, allowing for demonstration that an inactive control does not activate the HPA axis or other facets of a provoked stress response.

In terms of age trends, cortisol reactivity to an acute stressor generally increases with development. Cortisol reactivity was higher in adults than in children (Strahler et al., 2010), and higher in adolescents than in children (Stroud et al., 2009; Yim et al., 2015). Findings for cortisol reactivity in the adolescent period though, are possibly inconsistent across previous studies. Gunnar and others found that stress-induced cortisol level marginally increased with age in 9, 11, 13, 15 years old (Gunnar et al., 2009), whereas Sumter and others found that the 13–14 years old had the largest cortisol reactivity compared to 9–10, 11–12, and 15–17 years old (Sumter et al., 2010). However, the difference across these studies (linear vs. quadratic effect), could reflect the lack of an older age group in the Gunnar et al. (2009) study. Given the interaction between cortisol and sexual hormones, the puberty-HPA stress hypothesis proposes that increased stress response accompanies sexual maturation (Gunnar et al., 2009). However, sex differences in cortisol reactivity are inconclusive (Hollanders et al., 2017). Some studies reported sex differences in adults, but not in adolescents or children (Strahler et al., 2010; Yim et al., 2010). However, this is inconsistent with Gunnar and colleagues (Gunnar et al., 2009), who found that 13-year-olds exhibited large sex difference in terms of cortisol reactivity due to the delayed puberty development in 13 years old boys compared to girls of the same age. The current study considers a

relatively large age range of adolescence (10–17 years old) dimensionally along with sex.

1.2. The present study

The novelty of the current study centers on three aspects. First, children and adolescents show a significant amount of stress when they had the mere experience of coming to a laboratory, without experimental stress induction (Gunnar et al., 2009; Walker et al., 2001). We designed a procedure that measured both the initial stress response when the participants first arrived the laboratory, and a baseline stress response, ~40 min after the initial arrival, before administration of stress manipulation or our inactive control. Thus, we obtained a baseline to contrast with stress responses after the later stress manipulation. Second, the current study is the first study in youth to compare the TSST-C against an inactive control condition, allowing for evaluation of the TSST-C in the absence of a stressful probe. Third, we provide an important replication of a stress assessment across the 10–17-year-old time period. The large sample and wide age range is able to provide valuable information regarding the sex and age-related stress response in a typically developing adolescent sample.

This study evaluates an Inactive Control (IC) procedure against the TSST-C in an EEG laboratory setting. In a randomized trial, we compared TSST-C and IC on measures of subjective ratings of anxiety, salivary cortisol, systolic and diastolic blood pressure and heart rate. We also considered variables of sex (male vs. female) and age (10–17 years). Based on previous work (Het et al., 2009; Quesada et al., 2012; Yim et al., 2010), we predicted that, the IC condition would produce a stable response of the subjective and biological measures in the IC condition. On the other hand, we expected the TSST-C would lead to greater subjective anxiety and stress responses as reflected by increases in salivary cortisol, systolic, diastolic blood pressure and heart rate. In the TSST-C group alone, we also examine the stress responses on the subjective, neuroendocrine and physiological measures in relation to sex and age. Based on previous study (Gunnar et al., 2009; Sumter et al., 2010) we predict that the stress-induced salivary cortisol level would increase with age in general but the mid-puberty point (13–14 years old) might out perform their younger and older peers.

2. Material and methods

2.1. Participants

Subjects were recruited via a mass mailing list provided by Experian that targeted towns surrounding New Haven, CT. A telephone screening was done with parents to determine that subjects were fluent in English and had no history of serious mental illness. In order to participate, subjects needed to be between 10 and 17 years of age. Children were screened by phone based on parent/caregiver response. Children were accepted for participation if they were fluent in English with no evidence of serious developmental disability or mental illness (e.g., psychosis autism, or bipolar disorder), nor did they have any previous head injury or seizure. This was part of a larger study with an embedded randomized trial. As such, we randomly assigned 88 participants to either the TSST-C or the IC group that participated in the visit. Three participants were excluded because the medicine they were taking would affect heart rate. Our final sample included 85 participants with 44 males and 41 females, age M = 13.81 years, SD = 1.95 years. The participants were 72.9% White not of Hispanic Origin, 7.1% African American, 5.9% Hispanic or Latino, 4.7% Asian, 4.7% American Indian or Alaskan, 4.7% others. Table 1 lists the demographic information of the sample.

2.2. Procedure

This study was part of a larger study on the impact of stress on hedonic capacity, risk taking, and interpersonal interaction (Crowley et al., 2013). The protocol for this study was approved by the Yale University School of Medicine Institutional Review Board. The study was composed of two visits. During visit 1, informed consent and assent were obtained from parents and children. Then parents provided demographic information. Both parents and children completed questionnaires regarding puberty development, emotion, behavior, risk taking, and children performed risk taking tasks.

Within approximately 2 weeks of Visit 1, participants were seen again for a laboratory visit (Visit 2), which is the focus of the current study. Unknown to the participants, they were randomly assigned to either the TSST-C or the IC group. Participants' mean age and sex were tracked such that participants could be preferentially assigned to a particular group in order to maintain comparable age and gender balance (Table 1). Participants were screened for alcohol and drug use by urine and breathalyzer tests prior to the lab procedures.

Subjects arrived at the lab between 4:00 pm to 4:15 pm. Throughout the study, subject ratings of anxiety, salivary samples, blood pressure and heart rate were taken at eight time points (T1 to T8). T1 measurements were taken at the beginning of the visit (T1, + min 0) upon subject's arrival. Then each subject was fitted with an EEG cap (~20 min). Next, the subject was asked to rest for 7 min. After the rest, a progressive muscle relaxation tape was played for 5 min, followed by instructed relaxation exercises for 10 min. Next, T2 measurements were taken (T2, + min 40), followed by an EEG food cue viewing task was administered (7 min) (Wu et al., 2017), and a computer-based reward task was administered the first time (8-min) (Crowley et al., 2013). Then either the TSST-C or IC procedure was conducted (15 min). T3 measurements were taken immediately after (T3, + min 70).

Subsequent to T3 measurements, the impedance of the EEG net was checked and adjusted. Then participants completed the computer-based reward task a second time after which T4 measurements were taken (T4, + min 90). Following T4, a delay discounting task was administered (7 min), followed by a stress booster (5 min, details in session 2.3.1) for the TSST-C group, or a video clip watching for the IC group. Then a Balloon Analog Risk Task was administered (8 min) before the fifth measurement was taken (T5, + min 110). After moving the EEG net, the following three assessments were conducted, designed to be a recovery period, spaced 15 min apart, (T6, + 125 min.), (T7, + 140 min.), (T8, + 155 min.). At the end of the study, participants were congratulated by the experimenters and debriefed (details in session 2.3.1).

Table 1
Demographics of the Inactive Control (IC) and the TSST-C groups on number of participants, number of females, number of participants in each age group (youngest 10–12 yrs, middle 13–14 yrs, oldest 15–17 yrs), age (Mean and SD), puberty (Mean and SD), Body Mass Index (Mean and SD), Body Mass Index Standardized by age and sex (BMI-SD, Mean and SD) and p values for the group comparisons.

	Total	IC	TSST-C	Group comparison
n	85	42	43	
n of female	41	20	21	p = .912
n in each age group	27/33/25	14/18/10	13/15/15	
Age	13.81(1.95)	13.73 (1.98)	13.87 (1.93)	p = .736
Puberty-male	2.38(0.77)	2.34(0.77)	2.43(0.79)	p = .708
Puberty-female	2.62(0.39)	2.61(0.42)	2.62(0.36)	p = .882
Puberty	2.50(0.62)	2.47 (0.64)	2.52 (0.62)	p = .674
BMI	22.62(5.67)	22.93(5.91)	22.33(5.49)	p = .477
BMI-SD	65.26(31.72)	66.78(29.50)	63.77(34.04)	p = .665

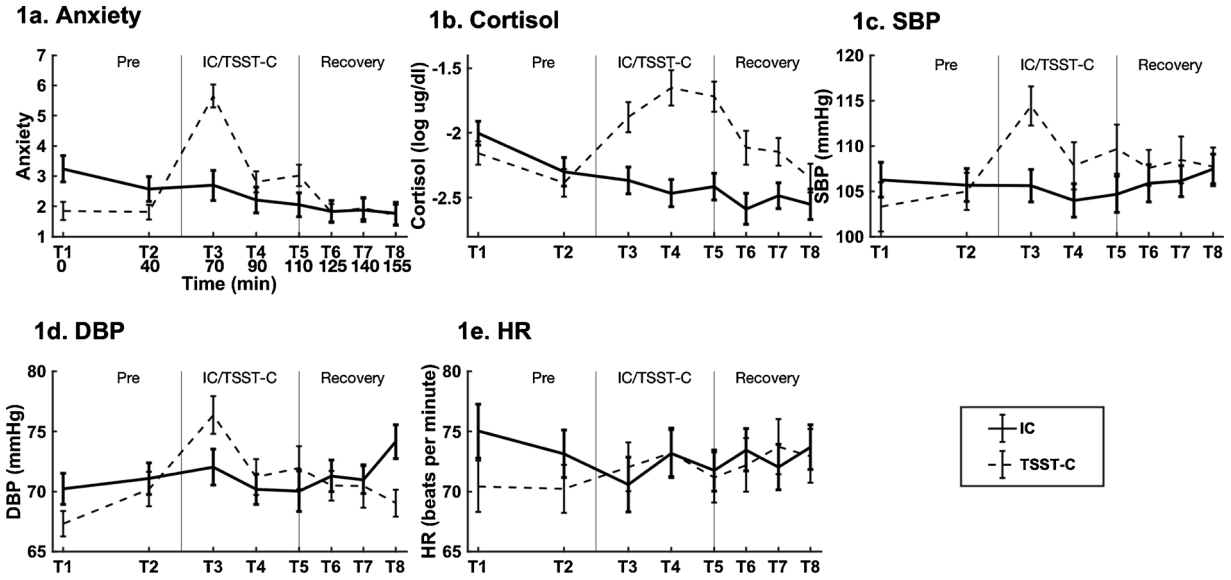

Fig. 1. This figure illustrated the time course of the subjective anxiety rating (1a), salivary cortisol (1b), Systolic Blood Pressure (1c), Diastolic Blood Pressure (1d), and heart rate (1e) in the TSST-C (dashed line) and the Inactive Control (IC) (solid line) group across T1 to T8 time points.

2.3. TSST-C and inactive control

2.3.1. Trier social stress test for children (TSST-C) and stress booster

Stress reactivity was assessed using an adapted version (details see below) of the Trier Social Stress Test for Children (TSST-C). The TSST-C is a widely used social stress task that is effective in eliciting HPA axis activation in children as reflected by increased salivary cortisol and other physiological indicators of stress (Buske-Kirschbaum et al., 1997, 2003; Gunnar et al., 2009; Kudielka et al., 2004).

The TSST-C was performed according to instructions provided by Buske-Kirschbaum and colleagues (Buske-Kirschbaum et al., 1997), except that in this study the adolescent prepared and delivered the speech in the same room (rather than a separate "preparation room"). In our study, two unfamiliar adults (the "judges") entered the laboratory room and told the participants that they will have to finish writing a story and to "make the story as exciting as possible" because they will be "competing against other teenagers". The judges gave the participants a story stem (Buske-Kirschbaum et al., 1997) and then left the room. The research assistant collected self-reported anxiety, salivary cortisol, blood pressure and heart rate levels and then told the participants to prepare the story for 5 min. The judges then re-entered the room. One judge placed an audio-recorder in front of the participant and asked him/her to stand up and recite the story back for 5 min while he/she was audio-taped. If the participant ended their story before 5 min, he/she were told to continue. After the 5 min were finished, the second judge asked the participant to remain standing and complete a math task ("subtract the number 13 from 1023 over and over as quickly and accurately as possible") for 5 min. In the event of an error, the participant heard a buzzer and was told to start from the beginning. The judges were trained research assistants. They were instructed to maintain a neutral facial expression and not to assist the adolescent during the tasks.

As part of a larger study, participants were "re-stressed" with a stress booster procedure ~30 min after the TSST-C. Following procedures similar to the TSST-C numeric subtraction, participants performed a booster TSST-C at 30 min after the end of the TSST-C task. This booster lasted 5 min and participants were audio recorded as in the previous task and observed by a panel of judges and were told that they were competing against other subjects who were also in the same study. Participants were instructed to spell specific words forwards and backwards and were asked to perform to the best of their ability. The initial words used in this booster consisted of 4 letters. If participants made two successive correct answers, they moved on to spelling 5 letter words, progressing in this pattern to 6 letter words, 7 letter words, 8 letter words etc. However, if participants made an error, they heard a buzzer and did not advance to a longer word group.

At the end of the study, after final anxiety rating, cortisol, blood pressure, and heart rate were made, the participants were debriefed. The participants were told that they had done a good job on the story telling and arithmetic components, and also that they were not competing against any other participants in the study. The experimenter also informed the participants that the audience members had been instructed to act in a non-reactive manner as part of the study.

2.3.2. Inactive control (IC)

Participants assigned to the control group performed an IC procedure specifically for this study. In an effort to maintain consistency between the TSST-C and the IC, both tests lasted the same amount of time and were performed standing up. While standing (for comparability to the TSST-C), participants in the IC group watched a 15-minute benign video of nature scenes depicting waterfalls and underwater flora and fauna. During TSST-C booster, the IC group watched the same video for 5 min. The volume was kept at 80-db during these videos. The video replaced the story generation, public speaking and arithmetic tasks in the TSST-C.

2.4. Measures

2.4.1. Subjective ratings of anxiety

Subjects were asked to rate levels of anxiety at time points T1 to T8 during the laboratory procedure (Fig. 1a). Participants rated on the question "How tense, anxious and/or jittery do you feel now?", on a 10-point scale, where 0 represented "none at all" and 10 represented "more than ever" (Chaplin et al., 2018).

2.4.2. Salivary cortisol

Salivary cortisol was used to measure HPA axis response. Cortisol samples were collected by asking the participant to place a cotton swab between his/her tongue and cheek until this swab was completely saturated (approximately 2 min). Samples were immediately placed in an ice bucket and then stored at −20 °C before being transported to a university laboratory for analysis using Coat-A-Count Cortisol Kit

(Diagnostic Products Corporation, Los Angeles, CA). Salivary cortisol samples were taken at T1 to T8 (see Fig. 1b). Cortisol data was log transformed to reduce the potential skewness (Stroud et al., 2009; Sumter et al., 2010; Yim et al., 2015).

2.4.3. Cardiovascular measures: blood pressure and heart rate

Following standard procedures, an Omron® blood pressure monitor cuff (model BP761 N) was used by trained research assistants to measure systolic, diastolic blood pressure and heart rate at time points T1 to T8 (time point details in session 2.2).

2.4.4. Adolescent pubertal status

Participants completed the Pubertal Development Scale (PDS) Self Report, while the primary caregiver completed the Pubertal Development Scale (PDS) Parent Report (Petersen et al., 1988). Both the PDS self-report and parent-report had 5 items estimating the pubertal status based on the presence or absence of critical developmental changes, including growth spurt, pubic hair growth, skin changes in both boys and girls, as well as gender specific changes, voice change and face hair growth for boys, and breast development and menstrual cycle for girls. Each item had a 1 (not yet started) to 4 (somewhat complete) Likert scale. Self-report and parent-report was highly correlated, r = .845, $p < .001$. We used the mean of the two measures to obtain a more reliable measure of puberty.

2.5. Data analysis

First, the IC and TSST-C group comparisons were examined using two approaches. Approach 1 aimed to test the time trajectories of the responses in the two groups. Repeated measures ANCOVAs were conducted with time (8 levels, T1 to T8) as within-subject variable, group (TSST-C vs. IC) and sex (Male vs. Female) as between-subject variables, and age (10–17 years old, standardized before entering the model) as a continuous covariate. Greenhouse-Geisser $p < .05$ was used to determine significance. Approach 2 tested the stress reactivity of IC and TSST-C procedures. Time point (T2) which was right before the IC/TSST-C procedure, was used as the baseline for all the subjective, neuroendocrine and physiological measurements. Time point (T3) which was immediately after the IC/TSST-C manipulation, was used as the acute response measurement. Stress reactivity was the difference between T2 and T3 measurement (T3-T2). Then stress reactivity was contrasted between the two groups for each subjective, neuroendocrine and physiological measurement using independent samples *t*-tests.

Secondly, sex and age-related effects were tested via linear regressions for baseline measures (T2) and for stress reactivity (T3-T2) of the subjective and biological measurements. The baseline measurements were tested across all the participants, and the stress reactivity was tested in the TSST-C group only. The linear regressions were first modeled using sex and age as the predictors (model 1), and then sex, age and sex by age interaction as the predictors (model 2, sex coded as 0-male, 1-female, age was standardized prior to model entry). Lastly, relationship between cortisol reactivity, age and puberty were examined using curve fitting to evaluate potential quadratic trends. The correlation of baseline measures and stress reactivity measures with age, sex and puberty were listed in Table 2. SPSS 24 (IBM) was used to execute the statistical analysis.

3. Results

3.1. Subjective ratings of anxiety

3.1.1. The Trajectory of subjective ratings of anxiety contrasting TSST-C and IC

Using a repeated measures ANCOVA on the subjective anxiety ratings with time (T1 to T8) as within-subjects variable, group (TSST-C vs. IC) and sex (male vs. female) as between-subjects variables and age as a continuous covariate, we observed a significant main effect of time, $F(4.49, 358.77) = 19.74$, $p < .001$, $\eta_p^2 = .20$, a significant group x time interaction, $F(4.49, 358.77) = 14.47$, $p < .001$, $\eta_p^2 = .15$, group x time x sex interaction, $F(4.49, 358.77) = 3.00$, $p = .02$, $\eta_p^2 = .04$, and a significant age effect, $F(1, 80) = 5.40$, $p = .02$, $\eta_p^2 = .06$.

Table 2
Correlations (Pearson's r values) between sex (male and female coded as 1 and 2 respectively), age, puberty with baseline across all participants and stress reactivity measures only in TSST-C, among subjective ratings of anxiety (anxiety), salivary cortisol (cortisol, log transformed), systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate (HR) († for 0.10 trend association, * for 0.05 significance and ** for 0.01 significance.).

baseline measures	sex	age	puberty	stress reactivity	sex	age	puberty
sex		−.026	.191†	sex		.007	.164
age	−.026		.688**	age	.007		.710**
anxiety	−.157	−.193†	−.148	anxiety	.250	−.355*	−.231
cortisol	−.089	.232*	.143	cortisol	.097	−.035	−.148
SBP	−.093	.523**	.559**	SBP	.176	.286†	.050
DBP	−.002	.334**	.286*	DBP	.131	.083	−.028
HR	.150	−.217†	−.082	HR	−.046	.360*	.092

Following the group x time interaction, independent sampled *t*-tests comparing the TSST and IC groups at time points T1 to T8 (means and comparisons see Table 3) showed significant differences at T1 and T3. It indicated that when first arrived (T1), the IC group was more anxious than the TSST-C group, $t(73.31) = 2.65$, $p = .010$, but similar at T2, $t(83) = 1.58$, $p = .12$, and less anxious at T3, $t(83) = -4.75$, $p < .001$, which was immediately following the TSST-C/IC procedure. By T5, there was a trend level of significance, $t(83) = -1.83$, $p = .071$, indicating the TSST-C group had trend-level greater anxiety than the IC after the booster. There are no other significant groups comparisons.

Measurements at T1, T3 to T8 were compared against the baseline T2 (means and comparisons see Table 3) for both the IC and TSST-C groups. The IC group had a trend level higher anxiety at T1 compared to T2, $t = 1.98$, $p = .055$, and there was no difference of T1 and T2 in the TSST-C group. Then, while the anxiety rating in the IC group declined from T3 to T8, (*t* decreased from 0.38 at T3 to -2.49 at T8, *p* decreased from .709 at T3 to .017 at T8), the anxiety rating in the TSST-C group increased through T3 to T5, (*t* ranged 2.57 to 9.96, *p* ranged < .001 to .014), until it declined to baseline level from T6 to T8 (Fig. 1a).

Following the group x time x sex interaction, repeated measures ANOVAs, for each condition separately, suggested the interaction was driven by the IC condition, $F(4.02, 144.66) = 2.64$, p = .036, $\eta_p^2 = .07$, in which at T3 the boys have a higher anxiety ratings than the girls, $t(32.74) = 2.04$, $p = .049$.

3.1.2. Anxiety reactivity contrasting TSST-C and IC

The anxiety reactivity was compared across the two groups using independent-samples *t*-tests. The TSST-C group showed significantly greater anxiety reactivity (M = 3.82, SE = 0.39) than the IC group (M = -0.21, SE = 0.31), $t(83) = 8.08$, $p < .001$ (Table 3).

3.1.3. Subjective ratings of anxiety in relation to sex and age

Using baseline anxiety as the dependent variable, linear regressions were conducted across all participants with sex and age (model 1) and also their interaction (model 2) as the predictors. Neither model reached significance. It indicated that the baseline anxiety level was not related to sex or age in our adolescent sample (Appendix A, Fig. 2a).

Using anxiety reactivity as the dependent variable, linear regressions were conducted in the TSST-C group with sex and age (model 1), and also their interaction (model 2) as the predictors. Both models showed a significant age effect (Model 1, B = −0.91, $p = .016$, Model 2, B = −1.02, $p = .042$), indicating that anxiety reactivity decreased with age in our sample. There was no significant sex or sex by age interaction effect (Appendix B, Fig. 3a).

Table 3
Mean and SE of anxiety rating (anxiety), salivary cortisol level (cortisol, ug/dl, log transformed,), systolic blood pressure (SBP, mmHg), diastolic blood pressure (DBP, mmHg), heart rate (HR, beats per minute) from T1 to T8, plus the stress reactivity for the IC and TSST-C group, and the group comparison p values (uncorrected). Measurements at T1, and from T3 to T8 were marked with significance of comparisons with baseline T2 († for .10 trend level, * for .05 and ** for .01 significance).

		T1	T2 (baseline)	T3	T4	T5	T6	T7	T8	Stress reactivity
anxiety	IC	3.24†	2.57	2.69	2.21†	2.05*	1.83*	1.88*	1.76*	.12
		(0.44)	(0.41)	(0.49)	(0.43)	(0.40)	(0.36)	(0.39)	(0.38)	(.32)
	TSST-C	1.84	1.81	5.65*	2.81*	3.02*	1.79	1.94	1.70	3.84
		(0.30)	(0.24)	(0.38)	(0.35)	(0.36)	(0.30)	(0.36)	(0.35)	(.39)
	p	.009	.116	< .001	.280	.071	.927	.908	.901	< .001
cortisol	IC	−2.00**	−2.30	−2.37	−2.47*	−2.41†	−2.59*	−2.49*	−2.55*	−.09
		(0.09)	(0.15)	(0.10)	(0.10)	(0.10)	(0.12)	(0.10)	(0.12)	(.05)
	TSST-C	−2.16**	−2.38	−1.88**	−1.64**	−1.72**	−2.11*	−2.14**	−2.33	.54
		(0.09)	(0.11)	(0.12)	(0.14)	(0.12)	(0.14)	(0.11)	(0.12)	(.09)
	p	.232	.581	.003	< .001	< .001	.009	.022	.214	< .001
SBP	IC	106.25	105.67	105.61	103.97	104.64	105.86	106.11	107.44	−.06
		(1.93)	(1.81)	(1.79)	(1.86)	(2.00)	(2.05)	(1.73)	(1.63)	(1.37)
	TSST-C	103.28	104.97	114.41**	107.79†	109.64*	107.56†	108.44†	107.69†	9.44
		(2.71)	(2.05)	(2.17)	(2.62)	(2.72)	(1.98)	(2.58)	(2.12)	(1.40)
	p	.383	.802	.003	.238	.143	.552	.457	.927	< .001
DBP	IC	70.22	71.08	72.06	70.19	70.03	71.31	71.00	74.17*	0.97
		(1.31)	(1.31)	(1.50)	(1.27)	(1.70)	(1.32)	(1.17)	(1.40)	(1.26)
	TSST-C	67.31*	70.21	76.36**	71.23	71.97	70.49	70.46	69.03	6.15
		(1.08)	(1.43)	(1.57)	(1.50)	(1.80)	(1.26)	(1.83)	(1.13)	(1.85)
	p	.088	.654	.052	.603	.435	.655	.808	.005	.026
HR	IC	75.03	73.15	70.59*	73.21	71.76	73.47	72.06	73.71	0.06
		(2.25)	(1.96)	(2.29)	(1.96)	(1.71)	(1.76)	(1.90)	(1.86)	(0.73)
	TSST-C	70.44	70.22	72.06†	73.22*	71.19	72.22†	73.75†	72.97*	3.00
		(2.14)	(2.01)	(2.03)	(2.09)	(2.09)	(2.22)	(2.29)	(2.22)	(1.04)
	p	.144	.302	.633	.995	.834	.663	.573	.800	.007

3.2. Salivary cortisol

3.2.1. The trajectory of salivary cortisol contrasting TSST-C and IC

Salivary cortisol was log-transformed to reduce skewness. Using a repeated measures ANCOVA on the salivary cortisol measure with time (T1 to T8) as within-subjects variable, group (TSST-C vs. IC) and sex (male vs. female) as between-subjects variables and age as a continuous covariate, we observed a significant time effect, $F(3.52, 253.53) = 15.64$, $p < .001$, $\eta_p^2 = .18$, and a significant group effect, $F(1, 72) = 7.07$, $p = .010$, $\eta_p^2 = .09$, both qualified by a time x group interaction $F(3.52, 253.53) = 19.11$, $p < .001$, $\eta_p^2 = .21$. There was also a significant main age effect, $F(1, 72) = 6.38$, $p = .014$, $\eta_p^2 = .08$.

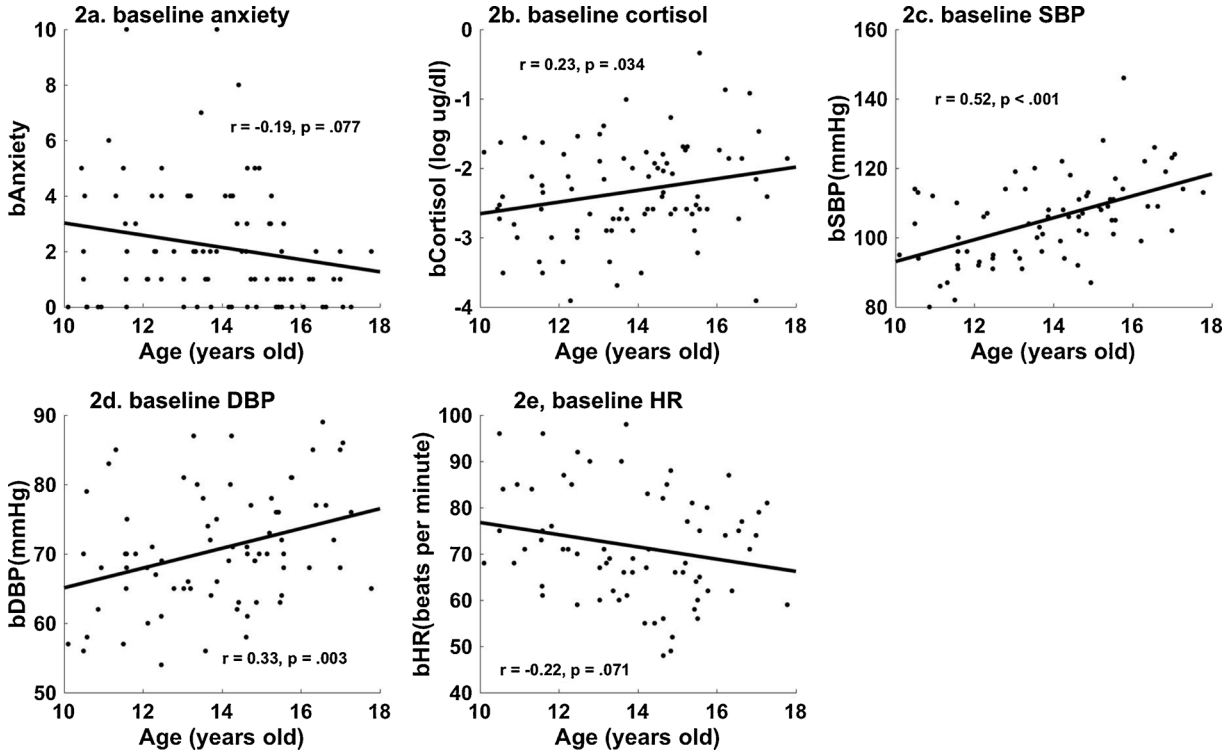

Fig. 2. This figure illustrated the correlational relationship between age and baseline measures: 2a, baseline anxiety, 2b, baseline cortisol, 2c, baseline SBP, 2d, baseline DBP, 2e, baseline HR.

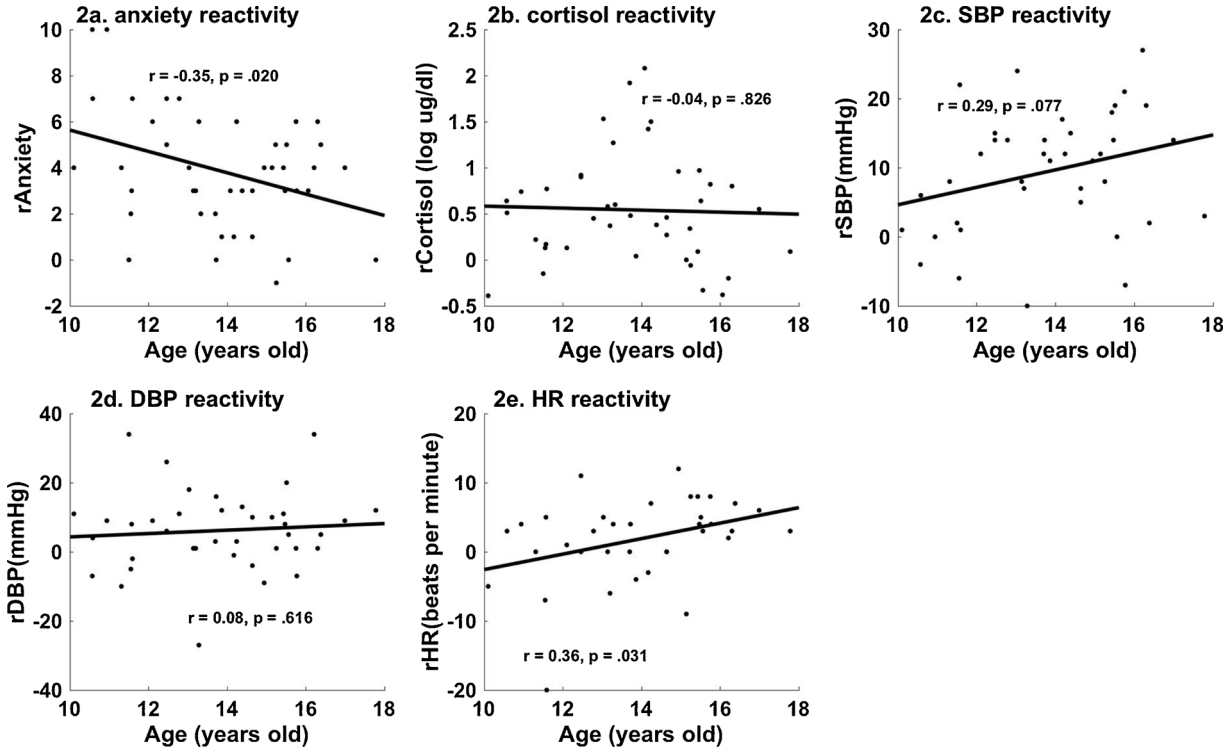

Fig. 3. This figure illustrated the correlational relationship between age and stress reactivity measures: 2a, anxiety reactivity, 2b, cortisol reactivity, 2c, SBP reactivity, 2d, DBP reactivity, 2e, HR reactivity.

Following the group x time interaction contrasting the IC and TSST-C groups (means and comparisons see Table 3), post hoc *t*-tests indicated significant group differences from T3 to T7, with the IC group having a lower salivary cortisol level than the TSST-C group, (*t* values ranged from −4.73 to −2.33 and *p* values ranged from < .001 to .02), until T8 when the groups were comparable (See Fig. 1b).

Measurements at T1, T3 to T8 were compared against the baseline T2 (means and comparisons see Table 3) for both the IC and TSST-C groups. Both groups exhibited a significantly higher cortisol at T1 than T2, *ts* = 5.16, 5.18, *ps* < .001, respectively for IC and TSST-C. It indicated that all participants had a higher stress level when first arrived at the lab at time point T1, and then the stress level decreased at time point T2. Subsequently to T2, the salivary cortisol level in the IC group decreased from T3 to T8, (*t* decreased from −1.67 at T3 to −2.86 at T8, *p* decreased from .103 to .007). On the other hand, the salivary cortisol level in the TSST-C group increased from T3 to T7 (I ranged from 2.39 to 5.88, $p < .02$), until it returned to the baseline at T8, (means and comparisons see Table 3) (Fig. 1b).

3.2.2. Salivary cortisol reactivity contrasting TSST-C and IC

Cortisol reactivity was contrasted in the two groups using independent-samples *t*-tests. The TSST-C group showed a significant higher cortisol reactivity (M = 0.54, SE = 0.09) than the IC group (M = -0.09, SE = 0.33), $t(79) = 5.90$, $p < .001$.

3.2.3. Salivary cortisol in relation to Sex and Age

Using the baseline cortisol as the dependent variable, linear regressions were conducted across all participants using sex and age (model 1) and their interaction (model 2) as predictors. Model 1 showed a significant age effect (B = 0.16, $t = 2.12$, $p = .04$). It indicated that the baseline cortisol level increased with age in our sample. There was no other significant effects for any other predictors in either model (Appendix A, Fig. 2b).

Using cortisol reactivity as the dependent variable, linear regressions were conducted in the TSST-C group only using sex and age (model 1), and their interaction (model 2) as predictors. Neither model showed any significant predictors. It indicated that sex and age were not linearly associated to cortisol reactivity in our sample (Appendix B, Fig. 3b).

3.2.4. Salivary cortisol reactivity in relation to age and puberty

A quadratic curve estimation was conducted on the salivary cortisol reactivity using age as the continuous predictor. The results showed a significant quadratic curve, $F(2,38) = 3.52$, $p = .04$, R Square = 0.16, Constant = −10.22, b1 = 1.62, b2 = −0.06 (Table 4, Fig. 4). It indicated that the mid-age adolescents had a higher stress reactivity than their younger and older peers. The same curve estimation was conducted using puberty as the predictor, but no significant effect emerged, $F(2,38) = 2.05$, $p = .14$. (Table 4).

Gunnar and others found that 13 years old had the largest sex difference in terms of cortisol reactivity due to the sex difference in puberty development (Gunnar et al., 2009). The puberty score for each age was calculated and compared across sex. We observed that boys had a significant lower puberty score than girls at 10, 11, 12 years old,

Table 4
Curve estimate for cortisol reactivity. Salivary cortisol reactivity were estimated by age and puberty separately using a quadratic curve. Both age and puberty were standardized before entering the models. Significant predictors (p < .05) were marked with *.

Independent variable	Equation	R2	F	df1	df2	Sig	Constant	b1	b2
age*	Quadratic	.156	3.52	2	38	.040	−10.22	1.62	−0.06
puberty	Quadratic	.098	2.05	2	38	.142	.673	.023	−0.13

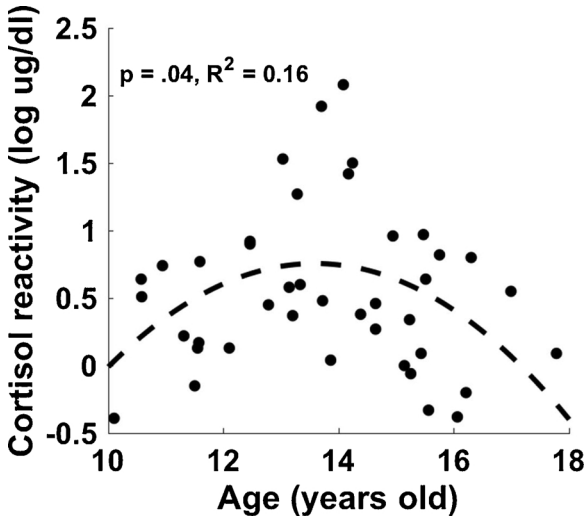

Fig. 4. The quadratic curve estimate of age and cortisol reactivity (ug/dl log transformed).

ts = −3.50, −2.71, −2.51, *p* = .01, .03, .04, respectively. Then by age 13 the puberty levels were comparable across sex, *ts* = −1.76, −.04, 1.25, 1.08 respectively for age 13–16, (age 17 only had 1 male thus not able to conduct statistic testing). For 10–16 years old respectively, boys puberty mean and SE were listed as follows: 1.54(0.18), 1.48(0.19), 1.61(0.19), 2.21(0.21), 2.74(0.13), 2.93(0.10), 3.21(0.25), and girls puberty mean and SE were listed as follows: 2.42(0.15), 2.31(0.20), 2.25(0.15), 2.57(0.08), 2.75(0.10), 2.93(0.10), 3.22(0.25).

3.3. Systolic blood pressure (SBP)

3.3.1. The trajectory of SBP contrasting TSST-C and IC

Using a repeated measures ANCOVA on SBP with time (T1 to T8) as within-subjects variable, group (TSST-C vs. IC) and sex (male vs. female) as between-subjects variables and age as a continuous covariate, we observed a significant time effect, F(5.67, 396.63) = 3.66, *p* = .002, η_p^2 = .05, qualified by a time x group interaction F(5.67, 396.63) = 4.70, *p* < .001, η_p^2 = .06. There was also a significant main age effect, F(1, 70) = 75.07, *p* < .001, η_p^2 = .52.

Post-hoc *t* tests at each time point contrasting the IC and TSST-C group for SBP (means and comparisons see Table 3) showed that the groups were only different at T3 (right after TSST-C), *t*(73) = -3.10, *p* = .003, indicating the IC group had a lower SBP than the TSST-C group at T3 (Fig. 1c).

Contrasting T1, T3 to T8 with the baseline (T2) (means and comparisons see Table 3), the SBP in the IC group did not differ from the baseline at any time point, while the SBP in the TSST-C group was elevated above baseline at T3 and T5, *ts* = 6.73 and 2.24, *p* < .001, = .03 respectively, (Fig. 1c).

3.3.2. SBP reactivity contrasting TSST-C and IC

SBP reactivity was contrasted between the two groups using independent-samples *t* tests. The TSST-C group showed a significant higher SBP reactivity (M = 9.44, SE = 1.40) than the IC group (M = −0.06, SE = 1.37), *t*(73) = 4.83, *p* < .001.

3.3.3. SBP in relation to sex and age

Using baseline SBP as the dependent variable, linear regressions were conducted across all participants with sex and age (model 1) and also their interaction (model 2) as the predictors. Both model 1 and 2 showed a significant age effect (B = 6.15, 8.59, *t* = 5.24, 5.57, *ps* < .001 respectively), indicating that the baseline SBP level increased with age in our 10–17-year-old adolescent sample. Model 2 also showed a significant sex by age interaction (B = −5.35, *t* = −2.35, *p* = 0.02). Correlations of age and baseline SBP, conducted in males and females separately, showed that the positive association of age and SBP was more driven by males, r = .68, *p* < .001, than by females, r = .31, *p* = .07, (two tailed correlation comparison resulted in z = 2.11, *p* = .02). There were no other significant effects (Appendix A, Fig. 2c).

Using SBP reactivity as the dependent variable, linear regressions were conducted in the TSST-C group only using sex and age (model 1), and their interaction (model 2) as the predictors. Neither model revealed any significant predictors, indicating SBP reactivity was not modulated by sex or age in our sample, (Appendix B, Fig. 3c).

3.4. Diastolic blood pressure (DBP)

3.4.1. The trajectory of DBP contrasting TSST-C and IC

Using a repeated measures ANCOVA on DBP with time (T1 to T8) as within-subjects variable, group (TSST-C vs. IC) and sex (male vs. female) as between-subjects variables and age as a continuous covariate, we observed a significant time effect, F(5.67, 396.63) = 3.66, *p* = .002, η_p^2 = .05, qualified by a time x group interaction, F(5.67, 396.63) = 4.70, *p* < .001, η_p^2 = .06. There was also a significant main age effect, F(1, 70) = 75.07, *p* < .001, η_p^2 = .52.

Post-hoc *t*-tests at each time point contrasting the IC and TSST-C group for DBP (means and comparisons see Table 3) showed no group difference except at T8 when IC had a greater DBP than the TSST-C group, *t*(73) = 2.88, *p* = .005. The IC group also had a trend level higher DBP than the TSST-C at T1 in the beginning of the visit, *t* (73) = 1.73, *p* = .09. In addition, the IC group had a trend level lower DBP than the TSST-C at T3 right after the TSST-C administration, *t*(73) = −1.98, *p* = .052, (See Fig. 1d).

Contrasting T1, T3 to T8 with the baseline (T2) (means and comparisons see Table 3), the DBP in the Control group did not differ from the baseline except an increase at T8, *t*(35) = 2.07, *p* = .046. For the TSST-C group, the DBP had a lower level at T1 than T2, *t*(38) = −2.50, *p* = .02, a higher level at T3, *t*(38) = 3.30, *p* = .002, and dropped to the baseline level at T4 to T8 (Fig. 1d).

3.4.2. DBP reactivity contrasting TSST-C and IC

DBP reactivity was contrasted in the two groups using independent-samples *t*-tests. The TSST-C group showed a significant higher DBP reactivity (M = 6.15, SE = 1.85) than the IC group (M = 0.97, SE = 1.26), *t*(73) = 2.28, *p* = .026.

3.4.3. DBP in relation to sex and age

Using baseline DBP as the dependent variable, linear regressions were conducted in the whole sample with sex and age (model 1) and with their interaction (model 2) as the predictors. Model 1 showed a significant age effect (B = 2.77, *t* = 3.00, *p* = .004) indicating that the baseline DBP level increased with age in our 10–17-year-old adolescent sample. There were no other significant predictors for either model (Appendix A, Fig. 2d).

Using DBP reactivity as the dependent variable, linear regressions were conducted in the TSST-C group only using sex and age (model 1), and their interaction (model 2) as the predictors. Neither model revealed any significant predictors, suggesting DBP reactivity was not modulated by sex or age in our sample (Appendix B, Fig. 3d).

3.5. Heart rate (HR)

3.5.1. The trajectory of HR contrasting TSST-C and IC

Using a repeated measures ANCOVA on HR with time (T1 to T8) as within-subjects variable, group (TSST-C vs. IC) and sex (male vs. female) as between-subjects variables and age as a continuous covariate, we did not observe any effect of time, F(4.96, 312.25) = 1.31, *p* = .26, η_p^2 = .02. There was a trend level of significance for the Time x Condition interaction, F(4.96, 312.25) = 1.99, *p* = .08, η_p^2 = .03.

Contrasting T1, T3 to T8 to the baseline (means and comparisons

see Table 3), there was no difference between T1 and T2 measurements in either group. Subsequent to T2, heart rate in the IC group declined at T3, $t(33) = -2.08$, $p = .046$ and remained mostly stable compared to the baseline during the remaining time points. In contrast, heart rate in the TSST-C group had a significant increase at T4 and T8, $t(35) = 2.88$, 2.04, $p = .007$, .049 respectively, and had trend-level significant increases at T3, T6, and T7 (*ts* ranging from 1.81 to 1.97, *ps* ranging from .057 to .079), (See Fig. 1e).

3.5.2. HR reactivity contrasting TSST-C and IC

HR reactivity was contrasted in the two groups using independent-samples *t*-tests. The TSST-C group showed a significant higher HR reactivity (M = 1.83, SE = 1.01) than the IC group (M = −2.56, SE = 1.23), $t(68) = 2.77$, $p = .007$.

3.5.3. HR in relation to sex and age

Using baseline HR as the dependent variable, linear regressions were conducted across the all participants using sex and age (model 1) and their interaction (model 2) as the predictors. Neither model revealed any significant effect for any of the predictors. Model 1 showed a trend level significance of age, B = −2.54, $t = -1.82$, $p = .074$, suggesting that baseline HR at the trend level decreased with age in our 10–17-year-old adolescent sample (Appendix A, Fig. 2e).

Using HR reactivity as the dependent variable, linear regressions were conducted in the TSST-C group with sex and age (model 1), and with their interaction (model 2) as the predictors. Model 1 showed significant age effect (B = 2.17, $t = 2.20$, $p = .04$), indicating HR reactivity increased with age in our 10–17 years old adolescent sample. There were no other significant predictors in any model (Appendix B, Fig. 3e).

4. Discussion

4.1. Inactive control (IC) versus TSST-C

Adolescence is a dynamic period with dramatic biological, psychological and social developments occurring that can be greatly influenced by stress (Gunnar et al., 2009; Stroud et al., 2009; Van den Bos et al., 2014; Walker et al., 2001). As popular laboratory stressors, TSST for adults, and TSST-C for youth have been used in combination of other psychological and neurocognitive measures to study the influence of stress in psychological, cognitive and neurological domains (Andersen et al., 2018; Quesada et al., 2012; Tiferet-Dweck et al., 2016). The current study was designed to answer two questions. First, we asked to what extent might the stress responses (subjective, neuroendocrine, physiological) generated by the TSST-C stressor, reflected concurrent events, such as the novelty of visiting a research facility and being tested using various equipment such as an EEG. Second, we tested whether an inactive control condition, would activate the HPA axis and generate any stress response across subjective, neuroendocrine and physiological measures. Using a randomized control approach in 88 healthy youth aged 10–17, we evaluated our IC procedure against TSST-C on measures of subjective anxiety, salivary cortisol, systolic and diastolic blood pressure and heart rate. Based on previous work, we predicted that all the subjective, neuroendocrine and physiological measures would increase due to the stress induction in the TSST-C group, but not in the IC group.

We found that only those exposed to the TSST-C showed significant changes in subjective, neuroendocrine and physiological measures. Specifically, salivary cortisol in the TSST-C condition showed a significant peak following the TSST-C administration, before returning to baseline levels at the end of the experiment. Our findings are consistent with studies in adults (Allen et al., 2014) and children (Buske-Kirschbaum et al., 1997; Gunnar et al., 2009; Stroud et al., 2009; Sumter et al., 2010; Walker et al., 2001), in which salivary cortisol increased after acute stress. Participants in the IC condition on the other hand, exhibited a decline of salivary cortisol throughout the visit. We confirmed that our TSST-C manipulation was successful, and that our IC condition did not evoke any stress response detectable with our measures. In addition, we found that the initial measurement T1 had a significantly higher level of cortisol than the second measurement T2. This finding confirmed the idea that first arriving at a novel research facility could introduce a significant amount of stress in adolescents (Gunnar et al., 2009; Walker et al., 2001). This finding suggests laboratory researchers to consider a period of adaptation time for the participants to adjust before starting any research procedure.

Consistent with previous studies in adults (Hellhammer and Schubert, 2012; Jezova et al., 2004; von Dawans et al., 2011), and in children and adolescents (Stroud et al., 2009), following a TSST-C manipulation, the subjective anxiety ratings peaked only in the TSST-C group and returned to baseline towards the end of the recovery. Systolic and diastolic blood pressure increased in the TSST-C group, but not in the IC group. The increase of blood pressure after rapid stress is consistent with TSST studies in adults (Jezova et al., 2004; Wright et al., 2014), and a previous study in adolescents (Stroud et al., 2009). Our data on HR did not show an overall trajectory difference between the TSST-C and IC procedures, partially due to the fact that the IC group had a higher HR than the TSST-C group at T1. By T2 the HR levels of both groups were comparable. Then the HR in the IC group remained mostly stable, while the TSST-C group had the trajectory of increased HR right after the stressor. In addition, HR reactivity was significantly higher in the TSST-C than the IC group. Taken together, our results support previous work showing that HR rises after a stressor (Gerra et al., 2001; Hellhammer and Schubert, 2012). However, since the HR increase could happen in a narrow window directly after the stressor (Buske-Kirschbaum et al., 1997; Childs et al., 2006), future work could benefit from monitoring HR during the entire TSST-C procedure.

Contrasting the TSST-C and the IC groups across measures, the IC group showed consistent responses (indicated by systolic and diastolic blood pressure and HR) and more relaxed response (indicated by cortisol level, subjective anxiety rating) throughout the course of experiment. The TSST-C group showed more variability and a significant peak in stress response immediately following the stress paradigm, indicated by subjective anxiety ratings, cortisol levels, systolic and diastolic blood pressure and HR. The contrast between the two groups further validates the causal relationship between stress response and the TSST-C procedure, and that the IC procedure indeed did not activate the HPA axis and generate any stress response.

4.2. Age, sex and puberty

Age accounted for a significant amount of variance for several of our measurements. Regarding the baseline measurements, we found that salivary cortisol level, systolic and diastolic blood pressure increased with age, while heart rate decreased with age (trend level). These findings are consistent with previous research in youth (Gunnar et al., 2009; Stroud et al., 2009; Walker et al., 2001), possibly reflecting different stages of physically maturation (Kotchen et al., 1980). We did not find baseline anxiety level to vary with age, suggesting that all the age groups had a comparable level of self-reported anxiety level at the baseline.

Regarding stress reactivity in the TSST-C group, we found that the anxiety reactivity decreased with age. This finding suggested that although all participants had comparable levels of anxiety before the TSST-C stressor, the older adolescents experienced less of an anxiety increase after the stressor than did the younger adolescents. The same anxiety measure had been used in a previous literature, in which higher anxiety was seen in at-risk adolescent girls with prenatal cocaine exposure after TSST-C compared to those without exposure (Chaplin et al., 2010). The previous study did not find any age-related effect, potentially due to the narrow age range (14.5–16 years old) compared to the current study of 10–17-year-olds. Decreased anxiety response

with development is consistent with model suggesting that self-control and emotion regulation capacity increased with age (Casey et al., 2017; Lawton et al., 1992).

We found that cortisol reactivity did not follow a linear relationship with age as in previous work (Gunnar et al., 2009), but rather followed a quadratic pattern. Specifically, adolescents ~13–15 years old, had a higher cortisol reactivity than the younger (~10–12 years old), and older (~16–17 years old) peers after the TSST-C. Our findings match Sumter and others, who observed that 13–14 years old youth had the highest cortisol reactivity compared to 9–10, 11–12 and 15–17 years old after a speech performance stressor (Sumter et al., 2010). The greater cortisol reactivity for the ~13–15 group could reflect baseline cortisol changes in puberty compared to younger youth, while, the same TSST-C stressor could seem less challenging for older adolescents, who had more life experience, or who have more mature brain circuits for self-control and emotion regulation (Casey et al., 2017; Lawton et al., 1992). The idea that TSST-C may not be as challenging across all ages was supported by other measurements in the study – anxiety reactivity decreased with age, and SBP and DBP reactivity did not vary with age. The contribution of the both the puberty and self-regulation could account for the quadratic shape of the cortisol reactivity and age relationship we observed here, in which the mid-age adolescents showed the largest cortisol increase. It may also suggest that comparing cortisol reaction across different age groups should be dealt with caution, since greater cortisol reactivity does not necessarily mean a more stressful experience. When puberty replaced age in the curve estimate, albeit self/parent report, the quadratic result was no longer significant. This finding was also consistent with Sumter et al. (2010) in that puberty-defined age increase was better at explaining cortisol reactivity than puberty on its own (Sumter et al., 2010).

Sex was related to several baseline measures. First, in the IC group, boys had a higher overall anxiety level than girls. This was consistent with previous work in which the same anxiety scale was used, which reported that normal control boys (14.5–16 years old) experienced higher anxiety than girls of the same age after TSST-C (Chaplin et al., 2010). Secondly, baseline SBP increased with age, and the association was stronger in boys than in girls. This finding was consistent with previous research showing that SBP rises with age, and more steeply during adolescence, especially in boys (Jackson et al., 2007). A sex difference emerged in relation to pubertal maturation in 10, 11, and 12 years old, but not in 13–16 years old. Younger adolescent boys (10–12 years old) were less pubertally mature than girls, but by age 13 the pubertal scores were comparable across sex. It was inconsistent with previous work found the largest pubertal difference at 13 years of age, across sex compared to 9, 11, and 15 years old (Gunnar et al., 2009).

Sex was not found to relate to any stress reactivity measures. This finding is consistent with previous studies reporting cortisol reactivity of sex differences only in older adults, and not in adolescents or children (Kudielka et al., 2004; Strahler et al., 2010). Our findings add to the research that sex effects in cortisol reactivity appear to be absent or inconclusive in children and adolescents (Hollanders et al., 2017). Boys and girls respond to the TSST-C in a similar fashion.

4.3. Limitations

The current study has some limitations. First, our sample was predominantly Caucasian, urban-suburban and low psychosocial risk with regard to socioeconomic status. Children with a different ethnicity/socioeconomic background or with risk factors could potentially have a different stress profile (Buske-Kirschbaum et al., 1997, 2003, Chaplin et al., 2010, 2018; Corbett and Simon, 2014; Dorn et al., 2003). Second, the TSST-C was only validated in the age range of 7–16 years old (Buske-Kirschbaum et al., 1997) and not in 17 years old. Future study might consider adjusting task difficulties for older adolescents (Allen et al., 2017). Third, upon the design of studying risk and reward in a stress environment, the procedure in the study deviated from the standard TSST-C in several ways including having computer-based reward tasks, wearing an EEG cap and having a booster session. Thus, the results should be interpreted with caution if compared to standard TSST-C. Fourth, we did not instruct the participants regarding food and beverage consumption prior to the visit which could contribute to variation in stress level. Fifth, we did not collect the menstrual cycle information which could influence endocrine responses and stress responses. Future study should control these variables to reduce variance not of interest. Finally, we randomized the TSST-C and IC groups based on sex and age, but not on trait anxiety, which could explain why the IC group reported higher subject anxiety ratings at baseline. Future studies would benefit from matching the groups on trait anxiety prior to randomization. Our findings are still robust despite the difference in the baseline anxiety levels between the groups.

5. Conclusions

As the first experimental study to evaluate the stress response of adolescents on TSST-C versus an inactive control condition, the novelty of our study was three-fold. First, we highlight the importance of allowing for lab acclimation in stress research as adolescents generally showed evidence of stress reactivity when they first arrived at the laboratory (inferred from differences between the initial response and the baseline measures after). Second, we further validated the TSST-C manipulation and our control condition, showing that the stress responses (subjective anxiety, salivary cortisol, systolic and diastolic blood pressure and HR) generated from that point at which both groups acclimated, was indeed due to the TSST-C manipulation—the inactive control did not activate a detectable stress response across the visit. Third, our relatively large sample and a wide age range of adolescents (10–17 years old) documents important age-related individual differences in stress measures which vary by context. Baseline cortisol, SBP, DBP increased with age, whereas cortisol response to stress peaked at mid-adolescence (13–14 years).

Conflict of interest

The authors declare no conflicts of interest.

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Appendix A

*Regression Table for baseline measures, significant models and predictors were marked with * indicating $p < .05$*

			B	Std. Error	ß	t	Sig	R	ΔR^2	Model F	Model Sig
Baseline anxiety	Model 1	(Constant)	2.53	0.33		7.74	< .001	.252	.064	2.79	.068
		sex	−0.72	0.47	−.16	−1.52	.133				
		age	−0.44	0.24	−.20	−1.85	.069				
	Model 2	(Constant)	2.53	0.33		7.69	< .001	.253	.064	1.84	.146
		sex	−0.72	0.47	−.16	−1.51	.135				
		age	−0.48	0.34	−.22	−1.40	.164				
		sex by age	0.08	0.48	.03	0.16	.871				
Baseline cortisol	Model 1	(Constant)	−2.28	0.11		21.80	< .001	.246	.061	2.58	.082
		sex	−0.11	0.15	−.08	−0.75	.453				
		age*	0.16	0.08	.23	2.12	.037				
	Model 2	(Constant)	−2.28	0.11		−21.67	< .001	.246	.061	1.70	.173
		sex	−0.11	0.15	−.08	−0.75	.455				
		age	0.17	0.11	.24	1.56	.122				
		sex by age	−0.17	0.15	−.02	−0.11	.912				
Baseline SBP	Model 1*	(Constant)	106.18	1.63		65.19	< .001	.532	.283	14.18	< .001
		sex	−2.19	2.35	−.09	−0.93	.355				
		age*	6.15	1.17	.52	5.24	< .001				
	Model 2*	(Constant)	106.06	1.58		67.12	< .001	.578	.334	11.88	< .001
		sex	−2.00	2.28	−.09	−0.88	.382				
		age*	8.59	1.54	.73	5.57	< .001				
		sex by age*	−5.35	2.28	−.31	−2.35	.022				
Baseline DBP	Model 1*	(Constant)	70.55	1.28		54.96	< .001	.334	.111	4.52	.014
		sex	−0.03	1.85	< .01	−0.02	.988				
		age*	2.78	0.92	.33	3.01	.004				
	Model 2*	(Constant)	70.58	1.28		54.98	< .001	.352	.124	3.35	.024
		sex	−0.09	1.85	< .01	−0.05	.960				
		age	1.92	1.25	.23	1.53	.130				
		sex by age	1.87	1.85	.15	1.01	.316				
Baseline HR	Model 1	(Constant)	70.11	1.92		36.44	< .001	.261	.068	2.45	.094
		sex	3.39	2.76	.15	1.23	.223				
		age	−2.54	1.40	−.21	−1.82	.074				
	Model 2	(Constant)	70.02	1.92		36.44	< .001	.293	.086	2.07	.11
		sex	3.52	2.75	.15	1.28	.205				
		age	−1.12	1.87	−.09	−0.60	.552				
		sex by age	−3.19	2.81	−.18	−1.14	.260				

Appendix B

*Regression Table for stress reactivity measures, significant models and predictors were marked with * indicating $p < .05$*

			B	Std. Error	ß	t	Sig	R	ΔR^2	Model F	Model Sig
anxiety reactivity	Model 1*	(Constant)	3.25	0.50		6.55	< .001	.435	.190	4.68	.015
		sex	1.26	0.71	.25	1.77	.084				
		age*	−0.91	0.36	−.36	−2.50	.016				
	Model 2*	(Constant)	3.26	0.50		6.48	< .001	.438	.192	3.09	.038
		sex	1.25	0.72	.25	1.74	.090				
		age*	−1.02	0.48	−.40	.-2.11	.042				
		sex by age	0.26	0.74	.07	0.35	.730				
cortisol reactivity	Model 1	(Constant)	0.49	0.13		3.70	.001	.104	.011	0.21	.815
		sex	0.11	0.19	.10	0.60	.550				
		age	−0.02	0.10	−.04	−0.22	.829				
	Model 2	(Constant)	0.49	0.13		3.69	.001	.163	.027	0.34	.798
		sex	0.11	0.19	.10	0.58	.563				
		age	0.04	0.13	.07	0.33	.742				
		sex by age	−0.15	−0.20	−.16	−0.78	.441				
SBP reactivity	Model 1	(Constant)	7.85	1.89		4.16	< .001	.343	.118	2.40	.105
		sex	3.28	2.71	.19	1.21	.234				
		age	2.53	1.35	.30	1.88	.068				
	Model 2	(Constant)	7.89	1.89		4.18	< .001	.376	.141	1.92	.144
		sex	3.32	2.71	.19	1.23	.229				
		age	1.46	1.73	.17	0.84	.405				
		sex by age	2.70	2.75	.20	0.98	.334				
DBP reactivity	Model 1	(Constant)	4.66	2.62		1.78	.084	.158	.025	0.46	.634

		sex	3.07	3.75	.14	0.82	.418				
		age	1.00	1.87	.09	0.54	.594				
	Model 2	(Constant)	4.62	2.64		1.75	.089	.193	.037	0.45	.720
		sex	3.03	3.78	.13	0.82	.428				
		age	2.01	2.42	.18	0.83	.411				
		sex by age	−2.55	3.85	−.14	−0.66	.512				
HR reactivity	Model 1	(Constant)	1.85	1.38		1.34	.188	.361	.130	2.47	.100
		sex	−0.32	1.95	−.03	−0.17	.870				
		age	2.17	0.99	.36	2.20	.035				
	Model 2	(Constant)	1.93	1.39		1.39	.176	.381	.146	1.82	.164
		sex	−0.41	1.96	−.03	−0.21	.837				
		age	1.54	1.29	.25	1.20	.240				
		sex by age	1.54	2.02	.16	0.76	.451				

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