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Influence of trauma exposure or early life stress and Serotonin Transporter (5-HTTLPR) Genotype on subjective- and physiological stress reactivity in healthy male subjects

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Abstract

Background: This study investigated whether 5-HTTLPR variation and childhood trauma or early adverse life events independently and interactively predict psychological- and physiological stress responses in healthy adult male subjects. *Methods*: All men were between 18 and 29 years old, mentally and physically healthy and without current or lifetime psychopathology. Of the 79 subjects participating in this study, 40 subjects were allocated to the stress condition, 39 to the control condition. The Trier Social Stress Test for Groups (TSST-G) was used for the induction of psychosocial stress in group format. The polymorphism 5-HTTLPR variation was obtained from blood samples, using two tagging single-nucleotide polymorphisms (SNPs; rs2129785 and rs11867581). The JTV and LSC-R were used to examine childhood trauma and previous life events. Stress reactivity was assessed as (1) subjective assessment with visual analogue scales for stress, anxiety and uncertainty over two minute time points during and before the TSST-G and (2) averaged heart rate over two ten minutes time periods during and before the TSST-G. *Results*: The stress condition showed stronger subjective stress reactivity and heart reactivity than the control condition due to the TSST-G, suggesting a good efficacy of the TSST-G stress protocol. No independent- or interactive effects were found for the 5-HTTLPR polymorphism and childhood trauma or previous stress exposure on psychological- and physiological stress reactivity. *Conclusion*: The 5-HTTLPR S allele and exposure to childhood trauma or previous life events seemed to be unrelated to changes in subjective stress reactivity and heart rate reactivity in healthy men who were free of psychopathology. The current preliminary findings do not confirm the suggested role of a gene- environment interaction to stress reactivity, however further verification with a larger sample size and longitudinal evaluation is required.

1. Introduction

Psychosocial stress is a real or interpreted threat to the physiological or psychological integrity of an individual that results in physiological and / or behavioural responses (Levine, 2005). The two main physiological stress response systems are the hypothalamic-pituitary-adrenal (HPA) axis and the autonomic nervous system (ANS). Activity of both systems is integrated, cross-regulated and centrally controlled by limbic structures of which the amygdala plays a crucial role (Giudice, Ellis, & Shirtcliff, 2011). The HPA- axis provides a delayed and long-term response to environmental challenges through release of cortisol, whereas (the sympathic portion of) the ANS induces a fast physiological 'flight or fight' response through catecholamine secretion. These responses are meant to help the body adapt to and dispose of stressful stimuli (de Kloet, 2009), but long-term, excessive (sympathic) activation can produce neurochemical imbalances that may contribute to the development of psychiatric disorders (Giudice et al., 2011). These physiological responses are often accompanied with changes in psychological state, such as a subjective feeling of anxiety. The physiological and psychological responses to acute stress vary considerably between individuals, with several demographic, physiological, and biological variables moderating the magnitude of the individual stress response (Foley, & Kirschbaum, 2010; Mormede et al., 2011). In this study we will focus on the gene-environment interaction, by studying genetic influence and previous history of stressful experiences in psychosocial stress.

1.1 Genetic factors

Previous studies point to variability in serotonin neurotransmission as a predictor in individual stress response differences. Serotonin (5-HT) regulates avoidance of threat, withdrawal from dangerous or aversive cues and behavioural inhibition (Giudice et al., 2011). The serotonin transporter (5-HTT) regulates 5-HT receptor stimulation and strongly modulates the serotonergic response to stress in humans (Armbruster et al., 2009; Caspi et al., 2003; Karg, Burmeister, Shedden, & Sen, 2011; Munafò, Brown, & Hariri, 2008). The 5-HTTLPR polymorphism in the 5-HTT promoter region affects the transcription rate of the gene (Karg et al., 2011). The 5-HTTLPR is defined by a length variation of a repetitive sequence, with a long (L) allele comprising sixteen copies and a short (S) allele variant comprising fourteen copies of the repeat sequence (Gunthert et al., 2007; Armbruster et al., 2003; Munafò et al., 2008). Previous studies suggest that 5-HTTLPR variations moderate the stress response, with dominance of the S allele over the alternate L allele (Karg et al., 2011; Armbruster et al., 2003; Gunthert et al., 2007; Fredericks et al., 2010). Having one or two S allele copies (either SL or SS genotype) increases amygdala reactivity to threatening stimuli (Caspi et al., 2003; Way & Taylor, 2010). Furthermore, the S allele is associated with neuroanatomical changes in the amygdala, the medial prefrontal cortex and the connecting fibers, and exaggerated HPA- axis response to stress (Caspi, Hariri, Holmes, Uher, & Moffitt, 2010; Pergamin-Hight, Bakermans-Kranenburg, van IJzendoorn, & Bar-Haim, 2012).

Amygdala activation is an important mediator of psychological and physiological stress reactivity of S carriers to threatening social cues (Way & Taylor, 2010). Behaviourally, previous studies show an association between the 5-HTTLPR S allele and increased acquisition of conditioned fear responses; S carriers had enhanced anxiety sensitivity and were associated with biased attention

toward negative outcomes (Gunthert et al., 2007; Caspi et al., 2010; Munafò et al., 2007; Pergamin-Hight et al., 2012). The S allele is associated physiologically with increased heart rate and startle amplitude to aversive or threatening stimuli (Caspi et al., 2010; Fredericks et al., 2010). Fredericks et al (2010) suggest SS carriers have the greatest increase in stress reactivity to laboratory stressors, followed respectively by the SL genotype and homozygous L carriers. Together, these findings demonstrate different 5-HTTLPR variants can affect threat- related amygdala reactivity and predict individual differences in stress sensitivity (Bouma et al., 2011; Caspi et al., 2010; Munafò et al., 2008).

However, others did not find an association (Karg et al., 2011). Gunthert and colleagues (2007), for example, did not find a consistent association between anxiety and variation at 5-HTTLPR; anxiety effects seemed to be independent of 5-HTTLPR-related vulnerability. Another study reported no threat bias regarding to 5-HTTLPR variation (Pergamin-Hight et al., 2012).

1.2. Childhood trauma exposure and previous stressful life events

Besides genetic influences, stressful experiences early in life can permanently change stress response patterns (Elzinga et al., 2008; de Kloet, 2009). Previous findings indicate both behavioural and endocrine stress responses to be influenced by early adverse experiences and inadequate maternal care (Levine, 2005; Elzinga et al., 2008). In animals, prolonged early life stress appears to be associated with increased anxiety and decreased social interaction later in life (Elzinga et al., 2008). Autonomic stress reactivity and experience of early stressful life events are linked as well (Levine, 2005). Several human studies have found a correlation between previous physical and / or sexual abuse and HPA axis reactivity changes upon psychosocial stress exposure (Levine, 2005; Elzinga et al., 2008). Physical- and sexual abuse, but also foster care, institutionalization or the death of parents in childhood are demonstrated to dysregulate the HPA axis and abnormal circadian rhythms of cortisol in human (Levine, 2005). These findings indicate an increase of psychological- and physiological stress reactivity after experiencing more stressful life events early in life.

Nevertheless, conflicting results are present as well in stressful life event studies, where outcome depends on frequency and perceived intensity of the events. Elzinga et al. (2008) found a diminished activation of the HPA- axis and anxiety to novel life stressors after brief early life stress exposure. The experience of previous life stress reversely attenuated sympathetic cardiovascular responses to novel psychosocial stress. When a distinction is made between individuals exposed to none / one adverse event and to relatively many adverse events, no differences were found between the groups with regard to blood pressure, heart rate and subjective distress, either at baseline or in response to the psychosocial stress task (Elzinga et al., 2008).

1.3. Genetic factors and childhood trauma exposure or early stressful life events

Responsiveness to stressful life events differs between individuals and appears to be influenced by genetic makeup. Accumulating evidence contributes this individual variability to 5-HTTLPR variation (Caspi et al., 2003). Caspi and colleagues (2003) found a significant interaction between 5-HTTLPR and stressful life events in the development of depressive symptoms, with the S allele showing greater perceived stress reactivity and a greater risk for developing depressive problems when exposed to high levels of stressful events (Caspi et al., 2003). Several studies have linked the 5-HTTLPR S allele

to psychological problems after early life adversity as well (Obradović & Boyce, 2009; Caspi et al., 2003; Caspi et al., 2010). S carriers with high levels of childhood maltreatment and adversity were more sensitive to anxiety and more biased toward perceiving and expecting negative outcomes. Moreover, children carrying the S allele and raised by unresponsive or nonsupportive mothers showed poor self-regulation of negative affect (Caspi et al., 2010; Obradović & Boyce, 2009). Another study done with rhesus macaques (there is a length polymorphism in the 5-HTT promoter region functionally analogous to the human 5-HTTLPR), have found the S allele interacting with adverse early rearing conditions which results in more distress, less activity, stronger neuroendocrine responses to stress and a lower serotonin turnover in the central nervous system (Uher & McGuffin, 2008). These findings show that the S allele is associated with a vulnerability to early life adverse circumstances, leading to multiple adverse outcomes resembling human psychiatric disorders

Although previous research implicates a gene- environment interaction, this remains controversial. Most research has been conducted in patients suffering from mood and anxiety disorders. Furthermore, the few studies performed in a healthy population are inconsistent and offer equivocal findings. For example, the results of a meta-analysis suggest that the interaction effect of 5-HTTLPR and stressful life events is negligible (Munafò, Durrant, Lewis, & Flint, 2009). Fredericks and colleagues (2010) also found no significant difference in genotypic baseline heart rate variability regarding early life stress (assessed through the Childhood Trauma Questionnaire, CTQ). Another study only found association between 5-HTTLPR and mild stressors, rather than rarer and severe life events (Kendler, Kuhn, Vittum, Prescott, & Riley, 2005).

1.4. Importance of research

Taken together, there are a small number of studies exploring the 5-HTTLPR variation and previous stress experiences on stress reactivity, with inconsistent findings mainly focused on psychiatric disorders. Therefore, the role of 5-HTTLPR and childhood trauma or adverse life events on stress reactivity to psychosocial stress in healthy individuals remains unknown. The present study intends to investigate whether 5-HTTLPR variation and childhood trauma or early life events independently and interactively predict psychological and physiological stress responses in healthy adult male subjects. The Trier Social Stress Test for Groups (TSST-G; Armbruster et al., 2009) is a standardized laboratory stressor, used to evaluate psychosocial stress responses for multiple participants simultaneously. TSST-G has been shown to result in significant endocrine, cardiovascular, immune and subjective responses (Kudielka, Schommer, Hellhammer, & Kirschbaum, 2004). Stress reactivity in the TSST-G is examined physiologically through heart rate frequency and subjectively through Visual Analogue Scales (VAS). The polymorphism 5-HTTLPR variation was obtained from blood samples and can be coupled to stress reactivity in the TSST-G. Childhood trauma was examined using the Dutch version of Childhood Trauma Questionnaire (CTQ; Dutch version JTV) and previous stressful life events are examined using the Life Stressor Checklist- Revised (LSC-R).

The aim is further clarification of stress reactivity in a healthy population, which is an important step to develop future strategies to combat the deleterious impact of stress. This study is split into two separate questions for the effect of 5-HTTLPR on stress and the effect of childhood trauma or stressful

life events, finally the interaction between this genetic and environment effect will be studied (see also model 1. in appendix for a schematic overview of questionnaires and hypotheses).

1. *'What is the effect of 5-HTTLPR on stress reactivity when exposed to stress?'* Upon stress exposure the S allele 5-HTLLPR carriers are expected to have increased autonomic stress reactivity (heart rate frequency) and increased stress perception (VAS stressed, anxious and uncertain) when exposed to the TSST-G
2. *'What is the effect of childhood trauma or previous stressful life events when exposed to stress?'* Higher levels childhood trauma or more previous life events are expected to lead to increased autonomic stress reactivity (heart rate frequency) and increased stress perception (VAS stressed, anxious and uncertain) when exposed to the TSST-G.
3. Question 1 and 2 are integrated in: *'What is the interactive effect of 5-HTTLPR and childhood trauma or previous stressful life events on stress reactivity when exposed to stress?'* It is hypothesized that 5-HTTLPR S allele carriers and higher levels of childhood trauma or more previous life events are associated with increased autonomic stress reactivity (heart rate frequency) and increased stress perception (VAS stressed, anxious and uncertain) upon stress exposure.

2. Methods & materials

2.1. Participants

In total, 80 healthy male subjects participated in this study, with a mean age of 22.8 years (SD = 2.45, with a minimum of 18 years and maximum of 29 years). Half of total sample was randomly allocated to the stress condition, half to the control condition. See also figure 1. Participants were primarily recruited from the healthy individuals who previously participated in the Cannabis study 06-100/E "The influence of cannabis use on symptoms of schizophrenia". Reason of potential inclusion was availability of personal information (blood samples and questionnaires). Participants explicitly gave written informed consent to be approached for further research and were recruited by telephone. Thereby, all participants underwent a semi structured screening. See also table 1. General exclusion criteria were smoking, any psychiatric disorder, current or past drug use, medication use which can possibly influence stress response, less than three grandparents with Dutch nationality, lack of fluency in the Dutch language and / or speech impairments. Participation was restricted to individuals who were psychiatrically and medically healthy, to avoid confounds with psychiatric and medical illness. As the menstrual cycle can influence cortisol levels and 5-HT concentrations (Bouma et al., 2011; Kirschbaum, Kudielka, Gaab, Schommer, & Hellhammer, 1999), only male participants were recruited. Participants were asked to minimize physical exertion within the last two hours, not to smoke cigarettes, to take meals or drinks other than water at least two hours before testing and not to drink alcohol, coffee or other caffeinated drinks at least four hours before testing. These exclusion factors can influence the participants stress reactivity. Further exclusion criteria on arrival were having a cold, acute illness or fever, current use of drug or other medication that might influence the stress response. Based on the exclusion criteria, one participant was excluded from analysis. Participants were

informed about aims of the study, gave written informed consent and received a reimbursement of 40 euros for participation. The study protocol was approved by the Medical Ethical Committee.

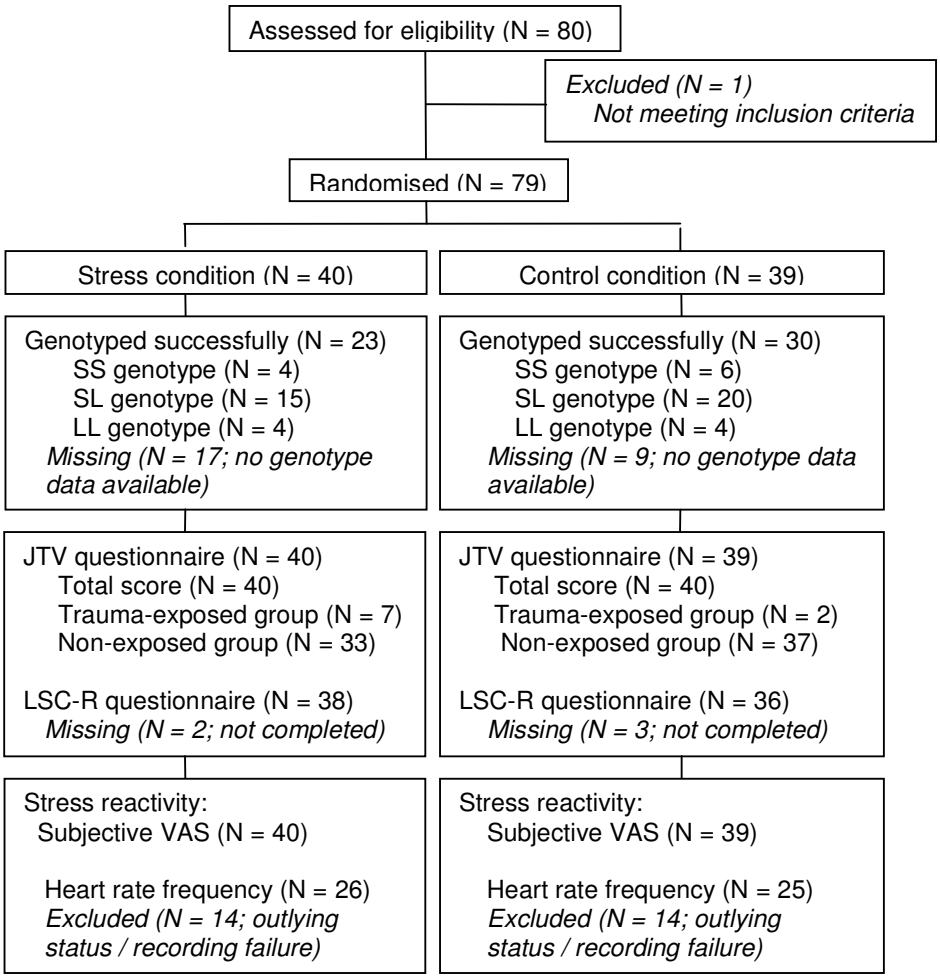

Figure 1. Flow chart of screening, exclusion, and inclusion of healthy male subjects

Table 1. Exclusion criteria for subjects in this study

General exclusion criteria	**Acute exclusion criteria**
Women	Any acute illness or fever
Any psychiatric disorder	Having a severe cold
Smoking (positive urine screen)	Smoke cigarettes
Current or past drug use; amphetamines (including MDMA), barbiturates, cannabinoids, cocaine, benzodiazepines and opiates (positive urine screen)	Use drugs or alcohol 24 hours prior to testing (positive urine screen and breathalyzer with alcohol level above 0%)
Medication use; benzodiazepines, psychotropics, beta blockers, ACE inhibitors and any hormonal treatment excluding any hormonal anticonceptives	Drink other than water or any food within the last two hours; ingestion of coffee or any caffeinated drink within the last four hours
Lack of fluency in the Dutch language	Take meals within the last two hours
Not having three Dutch grandparents	Physical exertion within the last twelve hours
Speech impairments	

2.2. Tasks & materials

2.2.1. Genotyping procedure

The genotype data for participants was already performed in the previous Cannabis study 06-100/E. In this study, three different array platforms were used; Illumina HumanOmniExpress (733,202 SNPs), Illumina Human610-Quad Beadchip (620,901 SNPs) and Illumina HumanHap550 array. For each Single Nucleotide Polymorphism (SNP) platform, quality control procedures were performed separately using the genome association analysis programme 'PLINK'. Participants were excluded based on >5% missing genotypes and gender errors. Linkage disequilibrium (LD) based SNP pruning was used to select the most informative SNPs (R^2<0.2), only for the subsequent quality control step. This resulted in ~78k SNPs for the sets to assess heterozygosity (F<3SD), homozygosity (F>3SD) and relatedness by pairwise IBD values (pihat >0.1). Datasets were merged with Hapmap Phase three to control for ethnicity. Thereafter, a quality check for SNPs was performed; all SNPs were filtered on missingness (>2%) and Hardy Weinberg (*p*>1e-6) before merging the three datasets. The merged dataset is imputed with Hapmap two, release 24 using Beagle. SNPs with an imputation score > 0.8 and SNPs that were present originally in one of the datasets were extracted and 2,504,766 SNPs remained for analysis.

In the present study, SNPs of previous checked datasets were selected on basis of literature review and availability of primer sequences. Based on a study of Vinkhuyzen and colleagues (2011), a preliminary selection of genotypes included the SNPs rs2129785 and rs11867581. Through these two-SNP proxies, a distinction could be made in allelic variations, with the TA haplotype representing the S allele of the 5-HTTLPR genotype (Vinkhuyzen et al., 2011). A subdivision in 5-HTTLPR genotypic variation was made to compare heterozygous or homozygous S carriers and homozygous L carriers. Of the total sample, 53 were successfully genotyped for 5-HTTLPR, with eight homozygous S carriers (four in each condition), 35 LS carriers (fifteen in stress and twenty in control) and ten (four in stress and six in control) having a homozygous L allele.

2.2.2. Previous experienced stressful life events

The 'Jeugd Trauma Vragenlijst' (JTV) and 'Life Symptom Checklist – Revised' (LSC-R) were used to respectively assess frequency and intensity of previous traumatic experiences and previous stress exposure for all participants. Both instruments are attached as appendix.

JTV. This self-administered questionnaire is a Dutch translated short version of the Childhood Trauma Questionnaire (CTQ), developed to provide a reliable and valid screening for child abuse and neglect (Bernstein et al. 2003). The JTV consists of 25 items, divided in the clinical subscales 'physical abuse', 'sexual abuse', 'emotional abuse', 'physical neglect' and 'emotional neglect' (Bernstein et al. 2003). Question 21 is disregarded because of substantive reasons (mistranslation). All items were rated on a five point Likert- type scale, from 'never true' to 'very often true'. This study used the total score of the JTV in order to assess the relation between childhood trauma and subjective- or heart rate reactivity. A dichotomization was also used, with scores 'moderate to severe' on at least one of the variables seen as an inclusion criterion for trauma-exposure, according to norms provided by

Bernstein and Fink (1998; Bernstein et al. 2003). On basis of these criteria, nine subjects were assigned to 'trauma-exposed group' and other 70 to a 'non-exposed group'.

LSC-R. This self-report questionnaire assessed a variety of stressors over the course of lifetime, such as the number of traumatic stressors and negative events previously experienced, age at which the event occurred, and duration and perceived impact of the events. The LSC-R was derived from the LSC, originally developed to screen for life events that meet DSM-IV trauma criteria. It demonstrated a good predictive validity, with the estimated frequency of traumatic events by the LSC corresponding to other standard assessment measures of traumatic exposure (Kimerling, Clum & Wolfe, 2000). The study used a 26- item version, leaving out the questions regarding incidents before the age of sixteen, since the CTQ- SF already covered childhood trauma. The LSC-R consisted of seven items interrogating traumatic exposure: exposure to a natural or human-made disaster, traumatic injury or accident, traumatic death of close other, being mugged or robbed, physical assault, sexual assault and rape. All events were rated for occurrence (with dichotomous outcomes 'yes' and 'no'). Total frequency scores were used to assess the stressful life experiences.

2.2.3. Trier Social Stress Test for Groups

The Trier Social Stress Test for Groups (TSST-G; von Dawan, Kirschbaum & Heinrichs, 2011) was used for the induction of psychosocial stress in groups of three to four participants. The stress protocol is a standardized laboratory psychosocial stress paradigm. It combines uncontrollable and socio-evaluative elements in a standardized manner, by including the presence of an evaluative committee, (which gives no verbal or nonverbal response to the participants performance), microphones, video camera, and presence of the other participants (which possibly introduces competition and performance comparison; Way & Taylor, 2010). Participants were asked to stand in a row in the middle of the room, in front of the committee, microphones and camera. The stress protocol consists of a five minutes standardized public speaking task about a job application and a five minutes arithmetic task. Participants were asked in random order to give a speech to the committee. The members of the committee could return to the participant's performance and asked further questions randomly. The arithmetic task consisted of counting aloud backwards from a large number in seven step sequences. Participants were called to perform in a random order. Miscalculations were indicated by the committee and the participant had to start over again.

For the control condition, a placebo version of TSST-G was used with a comparable cognitive load, but no social evaluative aspects. Participants were instructed to simultaneously talk about a job application for a friend and to not feel stressed as only participation was important. During the tasks no evaluative committee, microphones or camera were present, just one experimenter who did not evaluate their performance or ask any questions. See appendix for precise protocol. Risks were minimal and exposure to the TSST-G did not lead to extreme perceived stress levels. TSST-G leads to significant cardiac and neuroendocrine responses and to increased subjective stress and anxiety (Armbruster et al., 2009). Due to large effect sizes and high reliability, the TSST is a worldwide standard for psychological stress induction under controlled conditions and therefore used in this study (Armbruster et al., 2009).

2.2.4. Physiological and psychological stress reactivity

Stress reactivity was assessed physiologically as well as psychologically. Physiological stress reactivity was measured through heart rate frequency, while stress perception was measured with Visual Analog Scales (VAS). The Dutch translation of the VAS indicates the subjective basal and stress-induced state and has been extensively validated in anxiety research (van der Ploeg, 2000).

Heart rate frequency. Heart rate response variables were computed to analyze if the TSST-G modified autonomic stress reactivity. Heart rate levels were registered continuously, expressed as beats per minute (bpm) and registered using an automatic heart rate monitor (T6, Suunto, Finland), at least one hour before commencing the TSST-G. Dedicated software (Polar Pro Trainer program) was used for data processing. Means and standard deviations were calculated and normal distributions were made using Excel. Heart rate frequency data was considered invalid when outliers or recording failures were present. Two time periods were selected in order to see whether stress affects heart rate frequency; a ten minutes time period prior to the TSST-G as baseline (t -2000 – t-1400 sec) and a ten minutes time period during TSST-G (t180 – t780 sec). The pre-test measure time period was subtracted from the TSST-G measure time period, with a difference larger than zero considered as a meaningful increase.

VAS. This self-report instrument was employed to measure the subjective perception of stress. The VAS contained three items interrogating the presence of stress, anxiety and uncertainty. Participants were asked to judge their perceived stress and mood by rating the presence of the items on a straight line ranging from null ('not at all') to 100 ('very much') before and during the TSST-G, in order to indicate the subjective experienced basal and stress-induced state. The VAS score was measured in centimeters and divided by 8.11 (since the line was 11.8 centimeters instead of 10).

2.3. Procedure

The total experiment was approximately three hours and took place in the afternoon when cortisol levels are more stable. Participants were randomly assigned to stress or control and participation took place in groups of minimal three and maximal four participants. Upon arrival, participants were individually welcomed, informed about the study and asked for cooperation by signing the informed consent form. Subsequently, participants had to pass a written medical screening, urine test and breathalyzer to control for the exclusion criteria. Then, a heart rate belt was strapped on the diaphragm and a digital watch covered with tape was attached to the left wrist. Participants obtained a number (from one to four) and received further instructions including an explicit communication prohibition during the experiment. Participants were placed together in a 'waiting room' and were asked to complete several questionnaires, including JTV and LSC-R. Before receiving written instructions of the TSST-G, participants were asked to evaluate their subjective stress perception with a VAS. After receiving the written instructions there was five minutes preparation time, before participants were guided to the 'stress room'. The details of the stress and procedure are described in 2.2.3. After the speech task, a second VAS was filled out and participants were informed about the second arithmetic task. Altogether, the task lasted approximately fifteen minutes in both conditions. On completion, participants were guided back to the 'waiting room'. At the end of the experiment, a

committee member performed a detailed debriefing and the belt and watch were removed. See appendix for precise study protocols.

2.4. Statistical analysis

The independent variables used in this study are condition, genotypic variability, childhood trauma and previous stressful life events. The study sample was divided into two groups exposed to either a stress- or control condition. Because protocols of both conditions have a comparable cognitive load (only difference is the degree of stress induction during the TSST-G), analyses are done for the total sample to provide a larger sample size and more variability. and for the stress- and control condition separately. Genotypic variability was divided into three categories (SS-, SL- and LL carriers). Childhood trauma was represented by the total- and dichotomized scores of the JTV and previous stressful life experiences were represented as a continuous variable by the total frequency scores of the LSC-R questionnaire. The dependent variable in this study is stress reactivity, which is operationalized psychologically through self-reports of stress, anxiety and uncertainty before and during TSST-G, and physiologically through averaged heart rate reactivity for ten minutes time periods before (t-2000 – t-1400 sec) and during TSST-G (t180 – t780 sec).

From the total of 80 healthy subjects who took part in this study, 79 were included in the statistical analyses after applying the exclusion criteria. Thereof, 40 subjects were placed into the stress condition, 39 subjects in the control condition. VAS- scores of perceived stress, anxiety and uncertainty were available for the total study sample. Averaged heart rate data were only available for 51 subjects due to a malfunction of the cardiac monitor. 53 subjects were successfully genotyped for 5-HTTLPR. There was no missing data for the JTV and 74 subjects completed the LSC- R questionnaire. See also figure 1.

Means and standard deviations of all variables were calculated and analyses were performed using SPSS statistical software package version 20.0. (SPSS Inc., Chicago, Illinois). Because positively skewed distributions were found for self-reports and averaged heart rate differences, they were subsequently logarithmically transformed. A one-way ANOVA for the total sample was used to analyze differences in the log-transformed subjective stress reactivity and averaged heart rate reactivity to the stressor as dependent variables, with 5-HTTLPR variation or childhood trauma as between-subject factors. A one-way ANOVA was done either for the stress condition separately to examine whether 5-HTTLPR variation or childhood trauma show effects on self-reports and averaged heart rate reactivity in the stress condition. The correlations between previous life events and subjective- and averaged heart rate reactivity were analysed using Spearman's correlation coefficients. To give the effect of stress and trauma more power, Z scores were computed for the JTV and LSC-R total scores and the effects were summed. With respect to the stress and trauma effects, Spearman's correlation coefficients of the summed Z scores were run to analyze the correlations with subjective responses to stress, anxiety and uncertainty and heart rate reactivity. With respect to the interaction effect of genotypic variability and trauma, a two-way ANOVA was examined to see if self-reported reactivity and heart rate reactivity show significant sensitivity to a variation of the 5-HTTLPR polymorphism and trauma exposure, with self-report differences to stress, anxiety and uncertainty and

averaged heart rate differences as dependent variables and 5-HTTLPR variability and the dichotomized scores of JTV as between-subjects factor. For readability, original (untransformed) values are used in the tables and figures.

3. Results

3.1. Preliminary analyses; stress induction

Mean scores and standard deviations of the subjective stress responses and heart rate reactivity when being exposed to TSST-G are shown in table 2. A one-way ANOVA revealed condition differences for perceived- and physiological stress reactivity. The stress condition subjects reported significant more stress, anxiety and uncertainty and show significant higher heart rate reactivity than subjects in the control condition.

Table 2: Significant differences in self-reported stress, anxiety and uncertainty and heart rate reactivity due to TSST-G between healthy male subjects in stress- and control condition

	Total		**Stress condition**		**Control condition**			
	Mean (N)	**SD**	**Mean (N)**	**SD**	**Mean (N)**	**SD**	**Test**	**P-value**
VAS stressed; (2-1)*	0.18 (79)	0.23	0.32 (40)	0.23	0.04 (39)	0.13	F (1, 78) = 42.97	<.001
VAS anxious; (2-1)*	0.05 (79)	0.10	0.07 (40)	0.11	0.02 (39)	0.08	F(1, 78) = 4.92	<.001
VAS uncertain; (2-1)*	0.13 (79)	0.19	0.25 (40)	0.18	0.01 (39)	0.12	F (1, 78) = 47.64	0.03
Averaged HR; (2-1)**	14.74 (51)	12.28	21.81 (26)	11.94	7.40 (25)	7.43	F(1, 50) = 26.53	<.001

Note: * (2-1) = difference in subjective reactivity; assessment during TSST-G – pretest assessment.
** (2-1) = difference in averaged heart rate reactivity; assessment during TSST-G time period (t180 - t780 sec) – TSST-G pretest time period (t-2000 – t-1400sec).

3.2. Effect of genetic variability on stress reactivity

As was determined by a one-way ANOVA, no statistically significant effects were found of genotypic variability on subjective reactivity to stress ($F(1, 77) = 4.38$, $p = 0.04$), anxiety ($F(1, 77) = 4.27$, $p = 0.04$), uncertainty ($F(1, 77) = 3.20$, $p = 0.08$) or to heart rate reactivity ($F(1, 49) = 1.04$, $p = 0.31$). These results indicate that 5-HTTLPR S allele carriers do not perceive more stress, anxiety and uncertainty and do not have higher heart rate responses than the L carriers. Since the stress reactivity has been shown to be stronger in the stress condition, a one-way ANOVA was done for the stress condition separately. No effects of 5-HTTLPR genotype on subjective stress reactivity or heart rate reactivity were found for the stress condition either, with $F(2, 20) = 0.90$, $p = 0.42$ for perceived stress, $F(2, 20) = 0.31$, $p = 0.74$ for perceived anxiety, $F(2, 20) = 2.18$, $p = 0.17$ for perceived uncertainty and $F(2, 9) = 2.18$, $p = 0.17$ for heart rate reactivity. These results suggest no evidence for an independent influence of 5-HTTLPR genotypic variation on stress reactivity; S carriers do not to have higher perceived- and physiological stress reactivity than L carriers, even in the most stressful condition. See also figure 2 and 3.

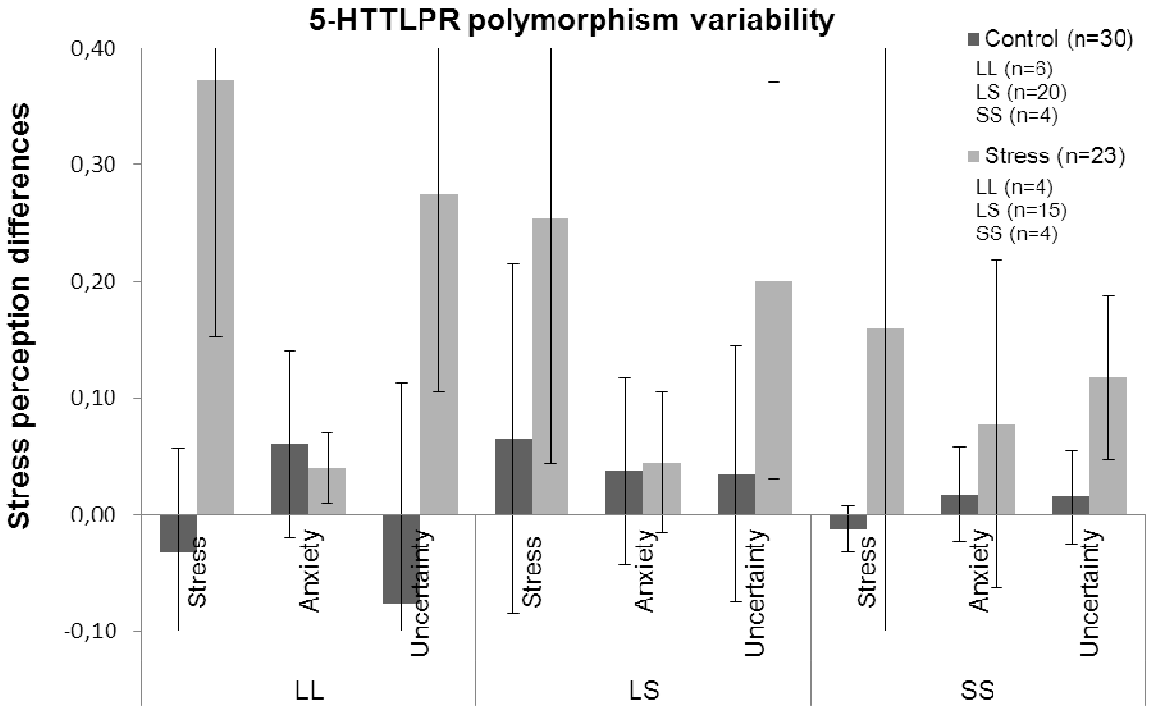

Figure 2: No differences in genotypic variability and self-reporting differences in stress, anxiety and uncertainty to TSST-G for the control- and stress condition.

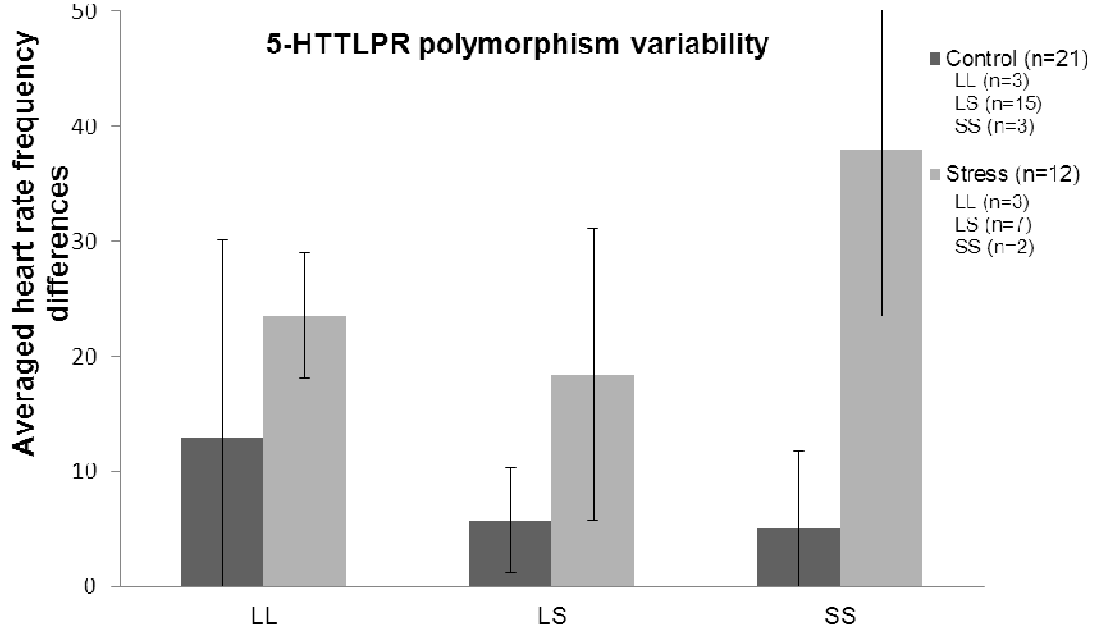

Figure 3. No differences in genotypic variability and averaged heart rate differences to TSST-G for the control- and stress condition.

3.3. Effect of childhood trauma on stress reactivity

Differences for the JTV childhood trauma exposure on subjective assessment and heart rate reactivity to the TSST-G are shown in table 3. A one-way ANOVA showed statistically significant effects for childhood trauma on the perception of stress and anxiety. No significant effect was found for the perception of uncertainty or heart rate reactivity. These results suggest that subjects exposed to childhood trauma feel more stressed and anxious due to the TSST-G than non-exposed subjects. Since stress reactivity was stronger in the stress condition, a one-way ANOVA was done for the stress condition separately showing no significant differences in effects of trauma exposure on perceived reactivity or heart rate reactivity when exposed to the TSST-G. Subjects exposed to trauma during childhood do not have higher stress perceptions and higher heart rate reactivity to the TSST-G than non- exposed subjects.

Table 3: No significant condition differences for childhood trauma and self-reporting differences in stress, anxiety and uncertainty or heart rate reactivity when exposed to TSST-G

	Non-exposed group		**Trauma-exposed group**			
	Mean (N)	**SD**	**Mean (N)**	**SD**	**Test**	**P-value**
Total sample						
VAS stressed; (2-1)*	0.16 (70)	0.23	0.33 (9)	0.16	F (1, 78) = 4.38	0.04
VAS anxious; (2-1)*	0.04 (70)	0.09	0.11 (9)	0.12	F (1, 78) = 4.27	0.04
VAS uncertain; (2-1)*	0.12 (70)	0.18	0.24 , (9)	0.24	F (1, 78) = 3.20	0.08
Averaged HR; (2-1)**	13.99 (13)	12.40	18.82 (8)	11.47	F (1, 50) = 1.04	0.31
Stress condition						
VAS stressed; (2-1)*	0.30 (33)	0.25	0.39 (7)	0.14	F (1, 39) = 7.24	0.40
VAS anxious; (2-1)*	0.06 (33)	0.10	0.12 (7)	0.13	F (1, 39) = 2.10	0.15
VAS uncertain; (2-1)*	0.23 (33)	0.17	0.32 (7)	0.20	F (1, 39) = 1.25	0.27
Averaged HR; (2-1)**	21.44 (20)	12.7	23.07 (6)	9.86	F (1, 25) = 0.08	0.78
Control condition						
VAS stressed; (2-1)*	0.04 (37)	0.13	0.14 (2)	0.11	F (1, 38) = 1.25	0.27
VAS anxious; (2-1)*	0.02 (37)	0.08	0.06 (2)	0.07	F (1, 38) = 0.44	0.52
VAS uncertain; (2-1)*	0.01 (37)	0.12	-0.04 (2)	0.16	F (1, 38) = 0.28	0.60
Averaged HR; (2-1)**	7.51 (23)	7.75	6.06 (2)	1.15	F (1, 24) = 0.07	0.80

Note: * (2-1) = difference in subjective reactivity; assessment during TSST-G – pretest assessment.
** (2-1) = difference in averaged heart rate reactivity; assessment TSST-G time period
(t180 - t780 sec) - TSST-G pretest time period (t-2000 - t-1400sec).

3.4. Effect of previous life events on stress reactivity

No significant correlations were found between the total frequency scores of the LSC-R questionnaire and the subjective stress reactivity or heart rate reactivity for the total sample. Analyzing the stress- and control condition separately, no significant correlations for the LSC-R total frequency scores and perceived responses of stress, anxiety and uncertainty, as well as heart rate reactivity were found either. Experience of more previous stressful life events was not related to higher levels of perceived stress, anxiety and uncertainty or increased heart rate reactivity. See also table 4.

Table 4. No significant correlations between LSC-R total frequency scores and self-reporting differences in stress, anxiety and uncertainty or heart rate reactivity to TSST-G for the total sample and per condition

	Total sample			Stress condition			Control condition		
	N	r_s	P	N	r_s	P	N	r_s	P
VAS stressed; (2-1)*	74	0.11	0.33	74	0.21	0.21	36	0.21	0.23
VAS anxious; (2-1)*	74	0.07	0.54	74	-0.03	0.85	36	0.17	0.32
VAS uncertain; (2-1)*	74	-0.03	0.78	74	0.03	0.86	36	-0.09	0.59
Averaged HR; (2-1)**	47	0.25	0.09	47	0.21	0.32	23	0.29	0.18

Note: *(2-1) = difference in subjective reactivity; assessment during TSST-G – pretest assessment. **(2-1) = difference in averaged heart rate reactivity; assessment TSST-G time period (t180 - t780 sec) – TSST-G pretest time period (t-2000 - t-1400 sec).

3.5. The combined effect of childhood trauma and previous life events on stress reactivity

Although no significant effects were found to stress reactivity for childhood trauma and previous stressful life events separately, complementary analyses were done to see if the interaction of both childhood trauma and previous stressful life events has effect on stress reactivity. Using the sum of both Z scores, a Spearman's correlation coefficient showed no significant correlations between the JTV and LSC-R scores and differences in subjective assessment or heart reactivity due to the TSST-G for the total sample. When taken the stress- and control condition separately, no significant correlations were found either. These results indicate that exposure to trauma in childhood as well as to previous life events were not related to higher subjective assessment and higher heart rate reactivity to the TSST-G. See also table 5.

Table 5. No significant correlations between Z sum scores of the JTV total scores and LSC-R total frequency scores and self-reporting differences in stress, anxiety and uncertainty or heart rate reactivity to TSST-G for the total sample and per condition

	Total sample			Stress condition			Control condition		
	N	r_s	P	N	r_s	P	N	r_s	P
VAS stressed; (2-1)*	79	0.13	0.25	40	0.22	0.18	39	0.18	0.27
VAS anxious; (2-1)*	79	0.11	0.32	40	0.20	0.23	39	-0.08	0.62
VAS uncertain; (2-1)*	79	0.00	0.98	40	0.13	0.44	39	-0.14	0.41
Averaged HR; (2-1)**	51	0.23	0.10	26	0.21	0.30	25	0.38	0.06

Note: * (2-1) = difference in perceived stress reactivity; assessment during TSST-G – pretest assessment. ** (2-1) = difference in averaged heart rate reactivity; assessment during TSST-G time period (t180 - t780 sec) – TSST-G pretest time period (t-2000 – t-1400sec).

3.6. Interaction effect of genetic variability and childhood trauma on stress reactivity

Despite the fact that 5-HTTLPR variability allele and childhood trauma did not show to have any effect on subjective assessment or heart rate reactivity to the TSST-G independently, the interaction effect of 5-HTTLPR variability and trauma exposure was analyzed as well, since previous findings show the 5-

HTTLPR S allele to be associated with a vulnerability to early life adverse circumstances. A two-way ANOVA for the total sample showed no statistically significant effects of trauma exposure and 5-HTTLPR variability on differences in perceived stress ($F(1, 48) = 0.82$, $p = 0.37$), anxiety ($F(1, 48) = 0.863$, $p = 0.36$) and uncertainty ($F(1, 48) = 0.25$, $p = 0.62$) or heart rate frequency ($F(1, 28) = 0.19$, $p = 0.67$) to the TSST-G. When analyzing the stress condition separately, no significant interaction effects were found either (with $F(1, 18) = 0.02$, $p = 0.89$ for perceived stress, $F(1, 18) = 1.17$, $p = 0.29$ for perceived anxiety, $F(1, 18) = 1.18$, $p = 0.29$ for perceived uncertainty and $F(1, 7) = 0.04$, $p = 0.85$ for heart rate reactivity) to the TSST-G. S carriers exposed to trauma during childhood do not show higher subjective assessment and higher heart rate reactivity to the TSST-G. See also figure 3 and 4.

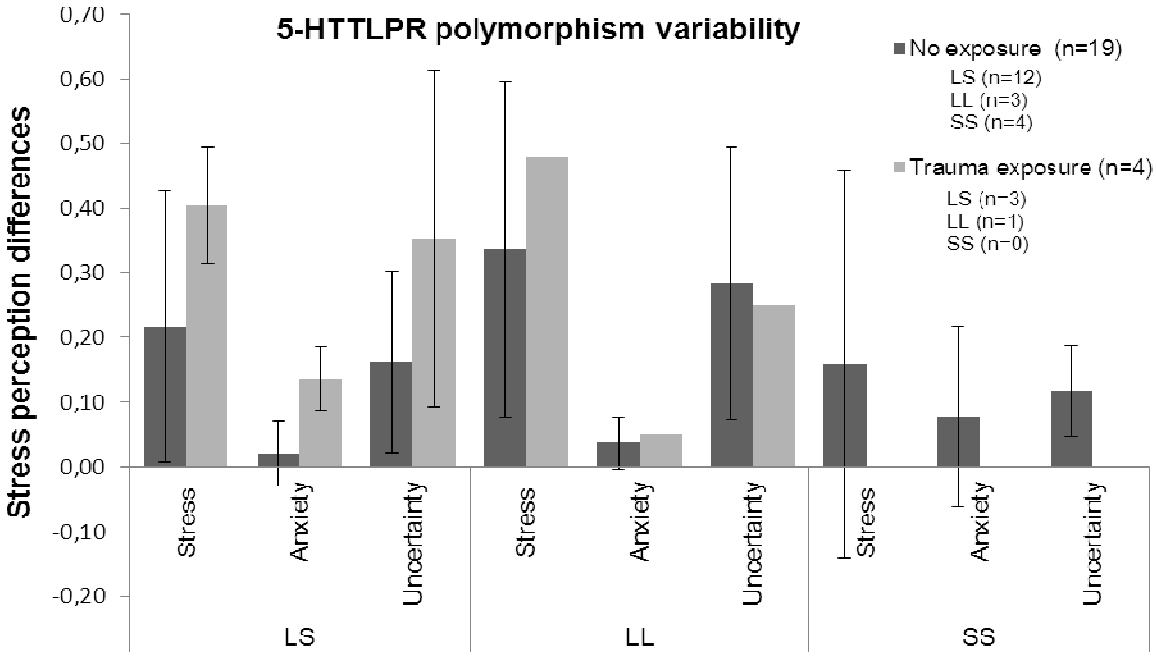

Figure 3. No significant differences in genotypic variability and trauma exposure on self-reporting differences in stress, anxiety and uncertainty to TSST-G in the stress condition

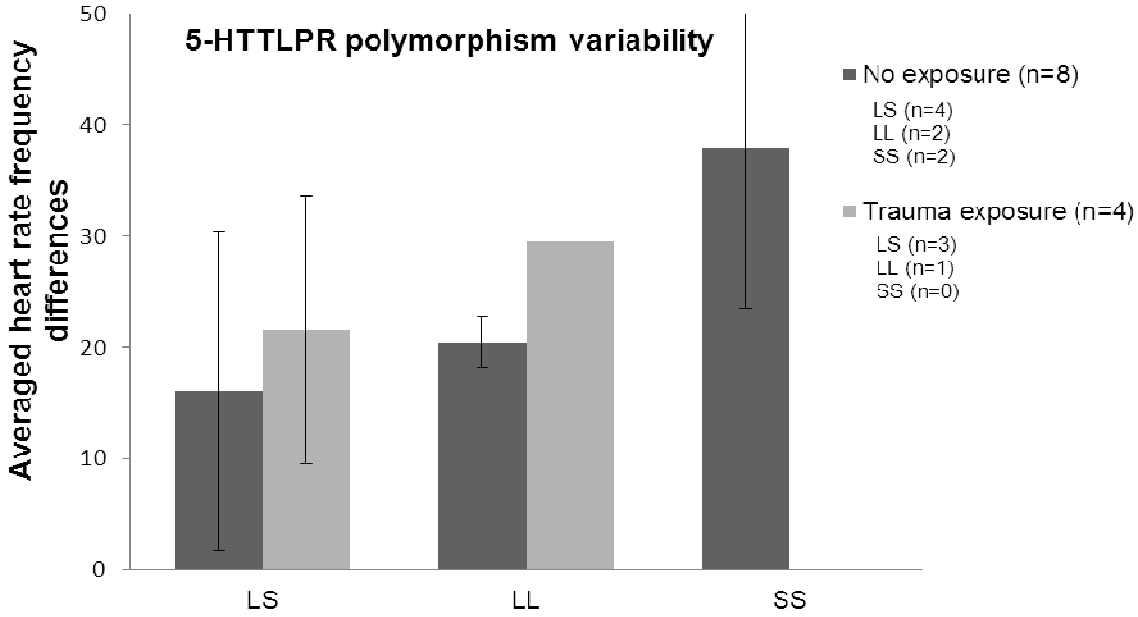

Figure 4. No differences in genotypic variability and trauma exposure on averaged heart rate differences in the stress condition, with time periods during TSST-G (t180 – t780 sec) minus time periods before TSST-G (t-2000 – t-1400 sec).

4. Discussion

The current study investigated whether 5-HTTLPR polymorphism variation, childhood trauma exposure and previous stressful life experiences are independently and interactively related to psychophysiological stress responses following the TSST-G in healthy adult male subjects. It was hypothesized that the presence of a 5-HTTLPR S allele and experience of previous stress exposure (childhood trauma exposure and the amount of early stressful life experiences) are independently and interactively associated with higher subjective responses of stress, anxiety and uncertainty or higher heart rate reactivity upon stress exposure. This study used a TSST-G stress protocol as a validated measure to assess subjective responses and heart rate reactivity in comparison to a TSST-G control protocol. The results show significant condition differences in subjective- and physiological stress reactivity, indicating that the TSST-G stress protocol induced significant elevations of perceived stress, anxiety and uncertainty as well as averaged heart rate reactivity. Contrary to the first hypothesis, the 5-HTTLPR genotypic variability was not associated with subjective stress reactivity and averaged heart rate reactivity. The findings are partly in accordance with the second hypothesis. Interestingly childhood trauma appears to increase stress and anxiety perception when exposed to the TSST-G in the total sample, but not when analyzed separately within the stress condition. This difference could be due to the small sample size for the stress group (n= 40) limiting the power. However, analyzing the entire group sample may confound the results since individuals are subjected to two different interventions, leading to large differences in stress and anxiety unrelated to childhood trauma. Therefore these results should be treated as very preliminary. No effect was found of childhood trauma or previous life experiences on uncertainty perception or heart rate reactivity when exposed to

the TSST-G. When looking at the summed Z scores of both childhood trauma and the amount of previous stressful life events, no relations were found between subjective reactivity and heart rate reactivity either. Finally, the results are not conform to the third hypothesis either, showing no interaction effect on psychological- and physiological stress reactivity for S- carriers of the 5-HTTLPR polymorphism who are exposed to trauma in childhood.

4.1. Possible explanations and inconsistencies with previous research

These results are difficult to reconcile with previous suggestions that 5-HTTLPR genotypic variability and childhood trauma exposure or previous life experiences are related to increased psychological- and physiological stress reactivity (Caspi et al., 2010; Munafò et al., 2007; Fredericks et al., 2010; Bouma et al., 2011). The controversial findings might be due to sample- related factors including sample size, age and the type of assessment. The restricted sample size of the groups limits the conclusions. Moreover, since most of previous research has been conducted in patients suffering from mood and anxiety disorders, these studies can not unravel the stress effects of stressful experiences from the effects of suffering from a psychiatric illness. Contrary to previous studies, the current study used a young, healthy and well-educated sample size, where no severe previous stress experiences (trauma exposure and stressful life events) were expected. Possibly age and / or education level have influenced the impact of trauma experiences in childhood or previous stressful life events (Carpenter, Shattuck, Tyrka, Geracioti & Price, 2011). The results may indicate no relation between previous exposure to relatively mild stressors and stress reactivity. Besides, other contextual factors that may alleviate the stress effects of previous life events were not examined, such as the social support during or after exposure to childhood trauma or previous life events. Furthermore, the fact that no interactions were found between previous stress exposure and genotypic variability to stress reactivity may be due to the self-report instruments not reliably reporting the type of childhood trauma or other life events that interact with the 5-HTTLPR polymorphism. Possibly, a structured interview approach may be needed to elicit previous trauma exposure (Uher, & McGuffin, 2008). The 5-HTTLPR polymorphism may be more robust with childhood trauma than with life events in general. Moreover, previous research indicated that the 5-HTTLPR SS carriers may have effects on stress reactivity more broadly; after stressful life experiences, SS carriers are more likely to attempt suicide, develop a posttraumatic stress disorder or abuse substances (Fredericks et al., 2010). These factors were not taken into account in the current study. Taking these factors into consideration could have potentially leaded to differential findings.

4.2. Limitations

This study has several limitations. First of all, the small sample size is an important flaw that urges us to interpret the findings with caution. The current study lacks statistical power due to small groups unequally distributed over the stress or control conditions and childhood trauma exposure groups. Due to the small sample size, the stress- and control protocols were initially considered as equivalent and analyses were done using data of both protocols at first to provide a larger sample size and more variability. Merging the protocols could have potentially lead to a bias in the results. Likewise, this

study has to do with a relatively large amount of missing values, in particular heart rate assessment and 5-HTTLPR genotypic data. With respect to heart rate assessment, 29 subjects had to be excluded due to malfunctions (recording failures and outlier status) of the cardiac monitor. Thereby, the cardiac monitors used in this study were programmed differently; clock times were not set equally and heart rate was assessed by two and ten second time periods respectively. Other 26 subjects could not be genotyped successfully for the 5-HTTLPR polymorphism, due to missing DNA-data in previous Cannabis study (concerning six subjects) and missing blood samples by subject recruitment through advertisement and face-to-face-approach (partly apart from previous Cannabis study; concerning twenty subjects). And, as the genotyping technology is limited to only two tagging single-nucleotide polymorphisms (SNPs), investigation of this polymorphism in the context of large-scale association studies is precluded and the length of the 5-HTTLPR promoter has a restricted reliability.

Another restriction is the young and healthy male sample used in order to minimize confounding factors and to examine the effects of a genetic risk factor that had not led to psychiatric disorders. Through this homogeneity, results cannot be generalized to women (who tend to react differently to stress, due to the influence of the menstrual cycle phase and the use of contraceptives; Bouma et al., 2011; Kirschbaum et al., 1999), to other ages (since age has shown to influence platelet 5-HT; Muck- Seler, Pivac, Crncevic, Jakovljevic, & Sagud, 2004) or clinical populations.

Moreover, numerous other factors that affect subject's reactivity to social stress were not taken into consideration. Examining these factors (the quality and availability of social support during and after stress exposure for example) may possibly help clarifying the specific mechanisms that can change the psychophysiological stress responses. Analyses were not adjusted for factors such as age, body mass index (BMI) or mood either, which may possibly weaken the associations for subjective- and physiological stress reactivity somewhat. For example, not including mood as a covariate may distort the results in that mood may be associated with both the 5-HTTLPR polymorphism and (subjective and physiological) responses to stress (Bouma et al., 2011).

Another limitation is the varied methodology of assessment used in studies. The JTV and LSC-R are self-report questionnaires that rely on subjects' recall. Thereby, long-term and retrospective reports may be flawed by forgetting and underestimating life events, whereas more recent life events may be biased by mood and overestimation. Some subjects may possibly choose not to disclose their abuse either. As such, even though confidentiality was emphasized, there remains a degree of uncertainty since self-reports cannot be subjected to verification. Another flaw is that the JTV did not characterize the specific timing, duration, or severity of the stressful life events. The misconception may arise that different stressors may be assessed in simultaneous constructs, because they have the same name. For example, some studies count 'death of a spouse' as a stressor, whereas others count 'being the child of a father in an unskilled job' as a stressor. And, some stressors are chronic, others acute (Caspi et al., 2010).

Finally, the TSST-G as a laboratory stressor can only draw near the effect of an actual stressor. The participants knew that there was no real threat and that they could stop at any moment. Moreover, the TSST-G cannot predict the effects of multiple exposure to stressful events. Examining the effects of multiple life events, both in and out the laboratory setting, will likely provide more clarity

about the impact of stressors on following psychiatric disorders (Fredericks et al., 2010). Taking other factors such as social support into account will also be important for defining the efficacy in laboratory stressors in a group as well. Compared to TSST, the laboratory stressor in group format might also cause additional errors when the stress responses of the subjects interact (von Dawans et al., 2011).

4.2. Future research

The current preliminary findings do not confirm the suggested role of a gene- environment interaction to stress reactivity, however further verification is needed. A larger sample size is required to investigate the gene- environment interaction. Increasing the sample size will provide a more adequate statistical power to examine the impact of the 5-HTTLPR polymorphism variability and exposure of childhood trauma or previous life events to stress reactivity in healthy male individuals. Furthermore, to investigate the effects of childhood trauma and previous life events on the regulation of the perceived stress and heart rate reactivity, it is essential to assess numerous other factors such as effects of social support, presence of depressed mood (or other psychopathological problems) and current levels of stress as mediating factors as well. In future, other accurate heart rate test material is preferable and consistent installment is necessary to optimize assessment and avoid possible errors. Moreover, future studies may consider using clinical interviews as a more reliable tool than self-reports to assess childhood trauma and early life events. Furthermore, a longitudinal evaluation of subjective assessment and heart rate reactivity of subjects will clarify how the findings predict future psychological and physical well-being.

5. Conclusion

In summary, the present study found condition differences in subjective reactivity and heart rate reactivity, suggesting a good efficacy of the TSST-G. No independent or interactive effect was found of the 5-HTTLPR polymorphism and experience of childhood trauma or previous stress exposure to psychological- and physiological stress reactivity, indicating no effect of nature and nurture in predicting psychophysiological stress responses. Longitudinal studies with larger sample sizes are needed to further unravel the interplay between genetic influences and the role of trauma exposure or early life events to psychological- and physiological reactivity to stressful situations later in life.

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Appendixes

Appendix 1. JTV questionnaire

JTV

De onderstaande stellingen gaan over ervaringen gedurende uw kinder- en tienerjaren. Omcirkel steeds het antwoord dat het best bij u past. Hoewel sommige vragen persoonlijk zijn, willen we u toch verzoeken om alle vragen zo eerlijk mogelijk te beantwoorden. Uw antwoorden zullen vertrouwelijk worden behandeld.

	Tijdens mijn jeugd….	**Nooit waar**	**Zelden waar**	**Soms waar**	**Vaak waar**	**Zeer vaak waar**
1.	Had ik niet voldoende te eten.	1	2	3	4	5
2.	Wist ik dat er iemand was om voor me te zorgen en me te beschermen.	1	2	3	4	5
3.	Noemden mensen in mijn gezin mij dingen als ‘dom’, ‘lui’ of ‘lelijk’.	1	2	3	4	5
4.	Waren mijn ouders te dronken of stoned (onder invloed van drugs) om voor het gezin te zorgen.	1	2	3	4	5
5.	Was er iemand in mijn gezin die me het gevoel gaf dat ik belangrijk en bijzonder was.	1	2	3	4	5
6.	Moest ik vieze kleren dragen.	1	2	3	4	5
7.	Had ik het gevoel dat er van me gehouden werd.	1	2	3	4	5
8.	Had ik het gevoel dat mijn ouders wensten dat ik nooit geboren was.	1	2	3	4	5
9.	Ben ik door iemand uit mijn gezin zo hard geslagen dat ik naar een dokter of naar het ziekenhuis moest gaan.	1	2	3	4	5
10.	Ben ik zo hard geslagen door mensen in mijn gezin dat ik er blauwe plekken of littekens aan overhield.	1	2	3	4	5
11.	Ben ik gestraft met een riem, een plank, een touw, of een ander hard voorwerp.	1	2	3	4	5
12.	Kwamen mijn gezinsleden voor elkaar op.	1	2	3	4	5
13.	Zeiden mensen in mijn gezin kwetsende of beledigende dingen tegen me.	1	2	3	4	5
14.	Geloof ik lichamelijk mishandeld te zijn geweest.	1	2	3	4	5
15.	Ben ik zo hard geslagen dat het opgemerkt werd door iemand zoals een leraar, een van de buren, of een dokter.	1	2	3	4	5

16.	Had ik het gevoel dat iemand in mijn gezin me haatte.	1	2	3	4	5
17.	Voelden de leden van mijn gezin zich met elkaar verbonden.	1	2	3	4	5
18.	Probeerde iemand mij op een seksuele manier te betasten, of mij ertoe te brengen hem of haar te betasten.	1	2	3	4	5
19.	Dreigde iemand me pijn te doen of leugens over me te vertellen als ik niet iets seksueels met hem of haar deed.	1	2	3	4	5
20.	Wilde iemand mij seksuele dingen laten doen of naar seksuele dingen laten kijken.	1	2	3	4	5
21.	Ben ik door iemand gemolesteerd.	1	2	3	4	5
22.	Geloof ik emotioneel mishandeld te zijn geweest.	1	2	3	4	5
23.	Was er iemand die me naar de dokter bracht als dat nodig was.	1	2	3	4	5
24.	Geloof ik seksueel misbruikt te zijn geweest.	1	2	3	4	5
25.	Was mijn gezin een bron van kracht en ondersteuning.	1	2	3	4	5

Wat is uw leeftijd? …… jaar

Wat is uw geslacht? M/V

Appendix 2. LSC-R questionnaire

Lees dit eerst:

We willen u graag een aantal vragen stellen over gebeurtenissen in uw leven die voor de meeste mensen eng, emotioneel of stressvol zijn. Denkt u alstublieft terug aan uw *hele leven* als u deze vragen beantwoordt. Sommige van deze vragen gaan over emotionele dingen waar u doorgaans niet over praat. Uw antwoorden zijn belangrijk, maar *u hoeft geen vragen te beantwoorden als u dat niet wilt*.

U mag het meest passende antwoord onderstrepen of arceren. Vergeet alstublieft niet uw leeftijd in te vullen (als er sprake is geweest van een dergelijke situatie).

1. Heeft u ooit een ramp meegemaakt (bijvoorbeeld een aardbeving, een overstroming, een brand of explosie)?

Ja - Nee

Indien ja:

1a. Hoe oud was u toen dit gebeurde? *Leeftijd:*

1b. Dacht u toen dat ***u of iemand anders*** gevaar liep ***dood*** te gaan of ernstig ***gewond*** te raken?

Ja - Nee

1c. Voelde u zich toen ***intens*** hulpeloos, angstig of vol van afschuw?

Ja - Nee

1d. In hoeverre heeft dit u het afgelopen jaar beïnvloed?

1	*2*	*3*	*4*	*5*
NIET		**ENIGSZINS**		**HEEL ERG**

2. Heeft u ooit een ernstig ongeluk gezien (bijvoorbeeld een auto-ongeluk of een ongeluk op het werk)?

Ja - Nee

Indien ja:

2a. Hoe oud was u toen dit gebeurde? *Leeftijd:*

2b. Dacht u toen dat ***u of iemand anders*** gevaar liep ***dood*** te gaan of ernstig ***gewond*** te raken?

Ja - Nee

2c. Voelde u zich toen ***intens*** hulpeloos, angstig of vol van afschuw?

Ja - Nee

2d. In hoeverre heeft dit u het afgelopen jaar beïnvloed?

1	*2*	*3*	*4*	*5*
NIET		**ENIGSZINS**		**HEEL ERG**

3. Heeft u ooit een ernstig ongeluk gehad (bijvoorbeeld een auto-ongeluk of een ongeluk op het werk)?

Ja - Nee

Indien ja:

3a. Hoe oud was u toen dit gebeurde? *Leeftijd:*

3b. Dacht u toen dat ***u of iemand anders*** gevaar liep ***dood*** te gaan of ernstig ***gewond*** te raken?

Ja - Nee

3c. Voelde u zich toen ***intens*** hulpeloos, angstig of vol van afschuw?

Ja - Nee

3d. In hoeverre heeft dit u het afgelopen jaar beïnvloed?

1	*2*	*3*	*4*	*5*
NIET		**ENIGSZINS**		**HEEL ERG**

4. Moesten er ooit nabije familieleden in de gevangenis verblijven?

Ja - Nee

Ja - Nee

Indien ja:

4a. Hoe oud was u toen dit gebeurde? *Leeftijd:*

4b En toen het voorbij was? *Leeftijd:*

4c. In hoeverre heeft dit u het afgelopen jaar beïnvloed?

1	*2*	*3*	*4*	*5*
NIET		**ENIGSZINS**		**HEEL ERG**

LSC - R (vervolg)

5. Moest u ooit zelf in de gevangenis verblijven?

Ja - Nee

Ja - Nee

Indien ja:

4a. Hoe oud was u toen dit gebeurde? *Leeftijd:*

4b En toen het voorbij was? *Leeftijd:*

4c. In hoeverre heeft dit u het afgelopen jaar beïnvloed?

1	*2*	*3*	*4*	*5*
NIET		**ENIGSZINS**		**HEEL ERG**

6. Bent u ooit in een pleeggezin geplaatst of geadopteerd?

Ja - Nee

Indien ja:

4a. Hoe oud was u toen dit gebeurde? *Leeftijd:*

4b En toen het voorbij was? *Leeftijd:*

4c. In hoeverre heeft dit u het afgelopen jaar beïnvloed?

1	*2*	*3*	*4*	*5*
NIET		**ENIGSZINS**		**HEEL ERG**

7. Zijn uw ouders ooit uit elkaar gegaan of gescheiden toen u nog bij hen woonde?

Ja - Nee

Indien ja:

4a. Hoe oud was u toen dit gebeurde? *Leeftijd:*

4b En toen het voorbij was? *Leeftijd:*

4c. In hoeverre heeft dit u het afgelopen jaar beïnvloed?

1	*2*	*3*	*4*	*5*
NIET		**ENIGSZINS**		**HEEL ERG**

8. Bent u zelf ooit uit elkaar gegaan of gescheiden?

Ja - Nee

Indien ja:

4a. Hoe oud was u toen dit gebeurde? *Leeftijd:*

4b En toen het voorbij was? *Leeftijd:*

4c. In hoeverre heeft dit u het afgelopen jaar beïnvloed?

1	*2*	*3*	*4*	*5*
NIET		**ENIGSZINS**		**HEEL ERG**

9. Heeft u ooit ernstige geldproblemen gehad (bijvoorbeeld geen geld voor eten of huisvesting)?

Ja - Nee

Indien ja:

4a. Hoe oud was u toen dit gebeurde? *Leeftijd:*

4b En toen het voorbij was? *Leeftijd:*

4c. In hoeverre heeft dit u het afgelopen jaar beïnvloed?

1	*2*	*3*	*4*	*5*
NIET		**ENIGSZINS**		**HEEL ERG**

10. Heeft u ooit een ernstig lichamelijke of geestelijke ziekte gehad (bijvoorbeeld kanker, hartaanval, een operatie, zelfmoordplannen of een psychiatrische opname)?

Ja - Nee

Indien ja:

10a. Hoe oud was u toen dit gebeurde? *Leeftijd:*

En toen het voorbij was? *Leeftijd:*

10b. Dacht u toen dat ***u of iemand anders*** gevaar liep ***dood*** te gaan of ernstig ***gewond*** te raken?

Ja - Nee

10c. Voelde u zich toen ***intens*** hulpeloos, angstig of vol van afschuw?

Ja - Nee

10d. In hoeverre heeft dit u het afgelopen jaar beïnvloed?

1	*2*	*3*	*4*	*5*
NIET		**ENIGSZINS**		**HEEL ERG**

LSC - R (vervolg)

11. Bent u ooit emotioneel verwaarloosd of genegeerd (bijvoorbeeld vernederd of in verlegenheid gebracht, of herhaaldelijk verteld dat u niet deugde)?

Ja - Nee

Indien ja:

11a. Hoe oud was u toen dit gebeurde? *Leeftijd:*
En toen het voorbij was? *Leeftijd:*

11b. Dacht u toen dat ***u of iemand anders*** gevaar liep ***dood*** te gaan of ernstig ***gewond*** te raken?

Ja - Nee

11c. Voelde u zich toen ***intens*** hulpeloos, angstig of vol van afschuw?

Ja - Nee

11d. In hoeverre heeft dit u het afgelopen jaar beïnvloed?

1	*2*	*3*	*4*	*5*
NIET		**ENIGSZINS**		**HEEL ERG**

12. Bent u ooit lichamelijk verwaarloosd (bijvoorbeeld geen eten gekregen, of niet voldoende gekleed of alleen gelaten terwijl u daar te jong voor was)?

Ja - Nee

Indien ja:

12a. Hoe oud was u toen dit gebeurde? *Leeftijd:*
En toen het voorbij was? *Leeftijd:*

12b. Dacht u toen dat ***u of iemand anders*** gevaar liep ***dood*** te gaan of ernstig ***gewond*** te raken?

Ja - Nee

12c. Voelde u zich toen ***intens*** hulpeloos, angstig of vol van afschuw?

Ja - Nee

12d. In hoeverre heeft dit u het afgelopen jaar beïnvloed?

1	*2*	*3*	*4*	*5*
NIET		**ENIGSZINS**		**HEEL ERG**

13. ALLEEN VROUWEN: Heeft u ooit een abortus of miskraam gehad (uw baby verloren)?

Ja - Nee

Indien ja:

13a. Hoe oud was u toen dit gebeurde? *Leeftijd:*
En toen het voorbij was? *Leeftijd:*

13b. Dacht u toen dat ***u of iemand anders*** gevaar liep ***dood*** te gaan of ernstig ***gewond*** te raken?

Ja - Nee

13c. Voelde u zich toen ***intens*** hulpeloos, angstig of vol van afschuw?

Ja - Nee

13d. In hoeverre heeft dit u het afgelopen jaar beïnvloed?

1	*2*	*3*	*4*	*5*
NIET		**ENIGSZINS**		**HEEL ERG**

14. Bent u ooit tegen uw wil gescheiden van uw kind (bijvoorbeeld de voogdij verloren, geen omgangsregeling meer of ontvoering van het kind)?

Ja - Nee

Ja - Nee

Indien ja:

14a. Hoe oud was u toen dit gebeurde? *Leeftijd:*
14b En toen het voorbij was? *Leeftijd:*

14c. In hoeverre heeft dit u het afgelopen jaar beïnvloed?

1	*2*	*3*	*4*	*5*
NIET		**ENIGSZINS**		**HEEL ERG**

LSC - R (vervolg)

15. Heeft een kind van u ooit een ernstige lichamelijke of geestelijk beperking gehad (bijvoorbeeld zwakzinnigheid, aangeboren afwijkingen, doofheid, blindheid)?

Ja - Nee

Ja - Nee

Indien ja:

15a. Hoe oud was u toen dit gebeurde? *Leeftijd:*

15b En toen het voorbij was? *Leeftijd:*

15c. In hoeverre heeft dit u het afgelopen jaar beïnvloed?

1	*2*	*3*	*4*	*5*
NIET		**ENIGSZINS**		**HEEL ERG**

16. Heeft u ooit de verantwoordelijkheid gehad voor iemand in uw omgeving (NIET uw kind) die een ernstige lichamelijke aandoening of geestelijk handicap had (bijvoorbeeld kanker, hersenbloeding, AIDS, psychiatrische aandoening, doofheid, blindheid)?

Ja - Nee

Ja - Nee

Indien ja:

16a. Hoe oud was u toen dit gebeurde? *Leeftijd:*

16b En toen het voorbij was? *Leeftijd:*

16c. In hoeverre heeft dit u het afgelopen jaar beïnvloed?

1	*2*	*3*	*4*	*5*
NIET		**ENIGSZINS**		**HEEL ERG**

17. Is iemand uit uw nabije omgeving ooit onverwacht overleden (bijvoorbeeld door een plotselinge hartaanval, moord of zelfmoord)?

Ja - Nee

Ja - Nee

Indien ja:

17a. Hoe oud was u toen dit gebeurde? *Leeftijd:*

17b. In hoeverre heeft dit u het afgelopen jaar beïnvloed?

1	*2*	*3*	*4*	*5*
NIET		**ENIGSZINS**		**HEEL ERG**

18. Is iemand uit uw nabije omgeving ooit overleden (NIET plotseling of onverwacht)?

Ja - Nee

Ja - Nee

Indien ja:

18a. Hoe oud was u toen dit gebeurde? *Leeftijd:*

18b In hoeverre heeft dit u het afgelopen jaar beïnvloed?

1	*2*	*3*	*4*	*5*
NIET		**ENIGSZINS**		**HEEL ERG**

19. Bent u ooit getuige geweest van een beroving, overval of aanval?

Ja - Nee

Indien ja:

19a. Hoe oud was u toen dit gebeurde? *Leeftijd:*

19b. Dacht u toen dat ***u of iemand anders*** gevaar liep ***dood*** te gaan of ernstig ***gewond*** te raken?

Ja - Nee

19c. Voelde u zich toen ***intens*** hulpeloos, angstig of vol van afschuw?

Ja - Nee

19d. In hoeverre heeft dit u het afgelopen jaar beïnvloed?

1	*2*	*3*	*4*	*5*
NIET		**ENIGSZINS**		**HEEL ERG**

LSC - R (vervolg)

20. Bent u ooit overvallen, beroofd of aangevallen (NIET seksueel) door iemand die u niet kende?
Ja - Nee

Indien ja:
20a. Hoe oud was u toen dit gebeurde? *Leeftijd:*
20b. Dacht u toen dat ***u of iemand anders*** gevaar liep ***dood*** te gaan of ernstig ***gewond*** te raken?
Ja - Nee
20c. Voelde u zich toen ***intens*** hulpeloos, angstig of vol van afschuw?
Ja - Nee
20d. In hoeverre heeft dit u het afgelopen jaar beïnvloed?

1	*2*	*3*	*4*	*5*
NIET		**ENIGSZINS**		**HEEL ERG**

21. *Na uw 16de jaar*, bent u ooit overvallen, beroofd of aangevallen (NIET seksueel) door iemand die u kende (bijvoorbeeld dat uw ouders, vriend of echtgenoot u sloeg, stompte, verstikte of brandde)?
Ja - Nee

Indien ja:
21a. Hoe oud was u toen dit gebeurde? *Leeftijd:*
En toen het voorbij was? *Leeftijd:*
21b. Dacht u toen dat ***u of iemand anders*** gevaar liep ***dood*** te gaan of ernstig ***gewond*** te raken?
Ja - Nee
21c. Voelde u zich toen ***intens*** hulpeloos, angstig of vol van afschuw?
Ja - Nee
21d. In hoeverre heeft dit u het afgelopen jaar beïnvloed?

1	*2*	*3*	*4*	*5*
NIET		**ENIGSZINS**		**HEEL ERG**

22. Bent u ooit lastig gevallen met seksuele opmerkingen, grapjes of verzoeken om seksuele handelingen door iemand van uw werk of school (bijvoorbeeld een collega, uw baas, klant of medeleerling of leraar)?
Ja - Nee

Indien ja:
22a. Hoe oud was u toen dit gebeurde? *Leeftijd:*
En toen het voorbij was? *Leeftijd:*
22b. Dacht u toen dat ***u of iemand anders*** gevaar liep ***dood*** te gaan of ernstig ***gewond*** te raken?
Ja - Nee
22c. Voelde u zich toen ***intens*** hulpeloos, angstig of vol van afschuw?
Ja - Nee
22d. In hoeverre heeft dit u het afgelopen jaar beïnvloed?

1	*2*	*3*	*4*	*5*
NIET		**ENIGSZINS**		**HEEL ERG**

23. *Na uw 16de jaar*, werd u ooit aangeraakt of moest u iemand anders aanraken op een seksuele manier, omdat hij/ zij u dwong of u bedreigde als u dat niet deed?
Ja - Nee

Indien ja:
23a. Hoe oud was u toen dit gebeurde? *Leeftijd:*
En toen het voorbij was? *Leeftijd:*
23b. Dacht u toen dat ***u of iemand anders*** gevaar liep ***dood*** te gaan of ernstig ***gewond*** te raken?
Ja - Nee
23c. Voelde u zich toen ***intens*** hulpeloos, angstig of vol van afschuw?
Ja - Nee
23d. In hoeverre heeft dit u het afgelopen jaar beïnvloed?

1	*2*	*3*	*4*	*5*
NIET		**ENIGSZINS**		**HEEL ERG**

LSC - R (vervolg)

24. *Na uw 16de jaar*, heeft u ooit seks gehad (oraal, anaal, genitaal) tegen uw wil omdat iemand u daartoe dwong of u bedreigde?

Ja - Nee

Indien ja:

24a. Hoe oud was u toen dit gebeurde? *Leeftijd:*
En toen het voorbij was? *Leeftijd:*

24b. Dacht u toen dat ***u of iemand anders*** gevaar liep ***dood*** te gaan of ernstig ***gewond*** te raken?

Ja - Nee

24c. Voelde u zich toen ***intens*** hulpeloos, angstig of vol van afschuw?

Ja - Nee

24d. In hoeverre heeft dit u het afgelopen jaar beïnvloed?

1	*2*	*3*	*4*	*5*
NIET		**ENIGSZINS**		**HEEL ERG**

25. Zijn er gebeurtenissen die we niet gevraagd hebben en die u nog wilt noemen?

Ja - Nee

Indien ja:

25a. Hoe oud was u toen dit gebeurde? *Leeftijd:*
En toen het voorbij was? *Leeftijd:*

25b. Dacht u toen dat ***u of iemand anders*** gevaar liep ***dood*** te gaan of ernstig ***gewond*** te raken?

Ja - Nee

25c. Voelde u zich toen ***intens*** hulpeloos, angstig of vol van afschuw?

Ja - Nee

25d. In hoeverre heeft dit u het afgelopen jaar beïnvloed?

1	*2*	*3*	*4*	*5*
NIET		**ENIGSZINS**		**HEEL ERG**

Welke?

……

26. Is er wel eens iemand anders iets overkomen, die u nabij was, waar u van overstuur raakte terwijl u het zelf niet heeft gezien?

Ja - Nee

Indien ja:

26a. Hoe oud was u toen dit gebeurde? *Leeftijd:*
En toen het voorbij was? *Leeftijd:*

26b. Dacht u toen dat ***u of iemand anders*** gevaar liep ***dood*** te gaan of ernstig ***gewond*** te raken?

Ja - Nee

26c. Voelde u zich toen ***intens*** hulpeloos, angstig of vol van afschuw?

Ja - Nee

26d. In hoeverre heeft dit u het afgelopen jaar beïnvloed?

1	*2*	*3*	*4*	*5*
NIET		**ENIGSZINS**		**HEEL ERG**

Appendix 4. Time schedule Choice study

T-120	Waiting room	Check for inclusion and exclusion criteria, including a urine drug test and breath alcohol test; Informed consent. HR monitor attached and start measurement; I-buttons attached and start measurements Drawing of a number; Questionnaires and task instructions.
T-60	Waiting room	Saliva sample; Blood pressure measurement,.
T-30	Waiting room	Saliva sample
T-10	Waiting room	Saliva sample; Blood pressure measurement; VAS 1; STAI-state 1
T-5	Waiting room	Blood pressure measurement; Written instructions for TSST-G; Preparation period, standing position
T0	Test room	Public speaking task (2min in total pp)
T8	Test room	Saliva Sample; VAS 2 and explanation mental arithmetic task
T10	Test room	Mental arithmetic: serial subtraction (1.30min in total pp)
T16	Test room	Saliva sample; Blood pressure measurement VAS3; STAI-state 2 Cognitive tasks
T20	Waiting room	Saliva sample
T25	Waiting room	Saliva sample; Blood pressure measurement
T35	Waiting room	Saliva sample; Blood pressure measurement
T45	Waiting room	Saliva sample; Blood pressure measurement
T60	Waiting room	Saliva sample; Blood pressure measurement
T90	Waiting room	Saliva sample; Blood pressure measurement
T120	Waiting room	Saliva sample; Blood pressure measurement

Appendix 4. Protocol TSST-G stress condition

Schriftelijke introductie stresstaak aan deelnemer

Stel je voor dat je solliciteert voor een positie als onderzoeker aan de universiteit.
Voor je zal straks een twee koppige **commissie** zitten die zal besluiten of je de baan wel of niet krijgt. Je hebt vanaf nu 5 minuten de tijd om je op dit gesprek voor te bereiden. je kunt tijdens de voorbereidingstijd notities maken. Het is niet toegestaan om deze tijdens het gesprek te gebruiken.
Neem tijdens het gesprek aan dat de commissie je sollicitatiebrief heeft ontvangen. Je zult de commissie dus moeten overtuigen dat je de **goede eigenschappen** hebt om deze baan te krijgen. Hiervoor zal de commissie je meerdere keren naar voor roepen aan de hand van je nummer, waarna er van je verwacht zal worden dat je over jezelf gaat vertellen. Als je niet aan de beurt bent is het belangrijk dat je **achter de streep** blijft staan.
Het is de bedoeling dat je vooral op je persoonlijke kwaliteiten ingaat en dan vooral op deze die jou van andere sollicitanten onderscheiden en je voor deze positie kwalificeren. Het is niet de bedoeling dat je ingaat op je kennis en beroepskwalificaties. Ga hierbij vanuit dat de commissie al je diploma's en certificaten reeds heeft ingezien.
Het gesprek zal op camera en taperecorder opgenomen worden voor **aanvullende analyses van je stem en je gedrag**. De commissie is **getraind om non-verbaal gedrag te observeren**, en ze zullen tijdens het sollicitatiegesprek notities maken. Ze zullen met name letten op **je gebaren en je gezichtsuitdrukking**.
Na het gesprek krijg je een **tweede taak**. De commissie zal je tegen die tijd instructies hierover geven. U wordt altijd per toeval naar voren geroepen en kunt op elk moment aan de beurt komen.
Dit alles zal zo'n 15 minuten duren. Het nummer dat u bij de ontvangst heeft getrokken (1,2,3, of 4) zal dienen als nummer op basis waarvan u naar voor wordt geroepen. Als er vragen zijn, steek dan je hand op.

TSST-G: stress condition group

1 commissielid doet het eerste gedeelte (gesprek) en het tweede gedeelte (rekenkundige taak).

Gesprek instructies
Totale tijd van het gesprek: 8 minuten (2x 1min p.p.) Begroet de kandidaat bij binnenkomst niet of heel formeel, en houd je gezichtsuitdrukking neutraal (dus: niet lachen of contact maken, wel aankijken).
De persoon die de kandidaat binnenleidt heeft al uitleg gegeven over de TSST procedure. De kandidaten wordt verteld dat ze in de hokjes moeten gaan staan op basis van het nummer dat ze getrokken hebben, dit zal de onderzoeksleider doen. Vervolgens wordt verteld dat er een tweede taak geïntroduceerd zal worden na het praatje. De commissie start de test met de woorden: *"Als uw nummer genoemd wordt, begin dan te spreken. U mag tussen de gordijnen blijven staan. We zullen aangeven als het voldoende is. We kunnen op elk moment bij elke deelnemer terugkomen."*
Nu worden de nummers omgeroepen met om de minuut een ander nummer, zodat iedereen 2x aan de beurt komt, voor beurten van 1 minuut per keer zodat elke deelnemer 2x aan de beurt is geweest.

Tijdstip in min.	Proefpersoon
0:00	1
1:00	4
2:00	3
3:00	1
4:00	2
5:00	3
6:00	2
7:00	4
8:00	Eind

De leden van de commissie blijven stil zolang de deelnemer vloeiend blijft spreken. Alleen als de kandidaat langer dan 10 seconden stilvalt in de periode van 2 minuten is het eerste wat de commissie zegt: "*U hebt nog steeds tijd, ga alsjeblieft door*." Als dan na 10 seconden blijkt dat de deelnemer niks meer te zeggen heeft, zullen er voor de rest van de tijd verdere vragen gesteld worden. Voorbeelden van deze vragen staan hieronder en het is geheel aan de commissie om hier vragen uit te selecteren.

- Waaruit blijkt dat u in staat bent anderen te overtuigen?
- Wat zijn uw sterkste punten?
- Wat zijn uw zwakke punten?
- Hoe zou u uzelf omschrijven?
- Welke hobby's hebt u?
- Hebt u wel eens een moeilijke beslissing genomen? Hoe ging dat?
- Kunt u goed samenwerken? Waaruit blijkt dat?
- Hoe maakt u keuzes?
- Waar bent u goed in? Kunt u voorbeelden noemen?
- Hoe zou uw beste vriend u omschrijven?
- Waar krijgt u energie van?
- Waar bent u trots op?
- Waar wordt u ongelukkig van?
- Waar mag men u 's nachts voor wakker maken?
- Heeft u een voorbeeld waarin u iets hebt bereikt wat u onmogelijk leek?
- Waardoor zou u snel geïrriteerd raken?
- Hoe gemakkelijk leert u iets nieuws?
- Hoe zien uw vijanden u?
- Wat onderscheidt u van anderen?

Het doel van deze vragen is niet om de deelnemer te vernederen of om gemeen te zijn. Het doel is om de deelnemer zich te laten presenteren aan een publiek. De vragen dienen dan ook om de presentatie meer diepgang te geven en om informatie te krijgen over specifieke kwaliteiten van de deelnemer.
In sommige gevallen zal de deelnemer in staat zijn om zelf te praten gedurende de twee minuten. Het is dan aan de commissie om te beslissen of het nodig is om in te grijpen tussen de eerste en de tweede minuut. Dit dient afhankelijk te zijn van wat de deelnemer zegt. Het is bijvoorbeeld niet de bedoeling dat de deelnemer heel specifiek over lessen gaat praten die hij gekregen heeft op de universiteit of ergens anders. Sommige deelnemers zullen dit doen om de aandacht af te leiden van hun eigen persoon. In dat geval is het noodzakelijk dat de commissie interrupeert, bijvoorbeeld door te zeggen: “We geloven dat je weet hoe je een markt-analyse moet uitvoeren, maar we zijn meer geïnteresseerd in waarom je zo betrokken bent tot dit gebied.
Na 8 minuten, zeg: *“We beeindigen het gesprek. Pak de salivette die achter u in het mapje hangt en stop deze in uw mond. Ik zal aangeven als u deze uit uw mond kunt halen. (wacht even tot het in de mond zit). Vul dan nu het formulier dat achter u in het mapje hangt in (na 1 minuut:) U mag de salivette weer in het buisje doen en deze in het mapje doen..* (VAS-schaal)) Laat de proefpersonen ook een speekselmonster afnemen. (VAS schaal en salivette op tafeltje achter proefpersoon). Laat de kandidaat dit invullen aan de statafel. Ga over naar de wiskundige taak.

Wiskundige taak
Je zegt: “*Nu vragen we u een tweede rekenkundige taak te doen, die niets te maken heeft met het sollicitatiegesprek. We vragen u om vanaf een getal dat we u geven terug te tellen naar 0 in stappen van 17. Het is de bedoeling dat u dit zo snel en foutloos mogelijk doet. Als u een fout maakt zal de commissie dit aangeven en opnieuw het begingetal zeggen. Het is de bedoeling dat u dan opnieuw begint. De commissie zal iedereen meerdere beurten geven en kan op elk moment bij iedere deelnemer terugkomen. Zijn er vragen?* “
Elke beurt van de arithmetische test zal 45 seconden duren met een totaal van 1,5 minuut per persoon, dit betekent dat elke persoon in willekeurige volgorde twee keer aan de beurt zal komen. Het is de bedoeling dat de commissie bijhoudt hoeveel fouten er zijn gemaakt en het getal dat de deelnemer uiteindelijk bereikt heeft als prestatiemaat. Na een beurt is het de bedoeling dat de commissie de deelnemer bedankt en vraagt om weer op zijn/haar nummer te gaan staan. (Als een proefpersoon een fout maakt, zeg: “Fout, 2023”).

Tijdstip in sec.	proefpersoon	getal
0:00	3	2023
0:45	4	2018
1:30	2	2013
2:15	4	2028
3:00	1	2013
3:45	2	2018
4:30	3	2028
5:15	1	2023
6:00	eind	eind

2023	2028	2018	2013	1394	1399	1389	1384	765	770	760	755	136	141	131	126
2006	2011	2001	1996	1377	1382	1372	1367	748	753	743	738	119	124	114	109
1989	1994	1984	1979	1360	1365	1355	1350	731	736	726	721	102	107	97	92
1972	1977	1967	1962	1343	1348	1338	1333	714	719	709	704	85	90	80	75
1955	1960	1950	1945	1326	1331	1321	1316	697	702	602	687	68	73	63	58
1938	1943	1933	1928	1309	1314	1304	1299	680	685	675	670	51	56	46	41
1921	1926	1916	1911	1292	1297	1287	1282	663	668	658	653	34	39	29	24
1904	1909	1899	1894	1275	1280	1270	1265	646	651	641	636	17	22	12	7
1887	1892	1882	1877	1258	1263	1253	1148	629	634	624	619	0	5	-5	-10
1870	1875	1865	1860	1241	1246	1236	1231	612	617	607	602				
1853	1858	1848	1843	1224	1229	1219	1214	595	600	590	585				
1836	1841	1831	1826	1207	1212	1202	1197	578	583	573	568				
1819	1824	1814	1809	1190	1195	1185	1180	561	566	556	551				
1802	1807	1797	1792	1173	1178	1168	1163	544	549	539	534				
1785	1790	1780	1775	1156	1161	1151	1146	527	532	522	517				
1768	1773	1763	1758	1139	1144	1134	1129	510	515	505	500				
1751	1756	1646	1641	1122	1127	1117	1112	493	498	488	483				
1734	1739	1629	1624	1105	1110	1100	1095	476	481	471	466				
1717	1722	1712	1707	1088	1093	1083	1078	459	464	454	449				
1700	1705	1695	1690	1071	1076	1066	1061	442	447	437	432				
1683	1688	1678	1673	1054	1059	1049	1044	425	430	420	415				
1666	1671	1661	1656	1037	1042	1032	1027	408	413	403	398				
1649	1654	1644	1639	1020	1025	1015	1010	391	396	386	381				
1632	1637	1627	1622	1003	1008	998	993	374	379	369	364				
1615	1620	1610	1605	986	991	981	976	357	362	352	347				
1598	1603	1593	1588	969	974	964	959	340	345	335	330				
1581	1586	1576	1571	952	957	947	942	323	328	318	313				
1564	1569	1559	1554	935	940	930	925	306	311	301	296				
1547	1552	1542	1537	918	923	913	908	289	294	284	279				
1530	1535	1525	1520	901	906	896	891	272	277	267	262				
1513	1518	1508	1503	884	889	879	874	255	260	250	245				
1496	1501	1491	1486	867	872	862	857	238	243	233	228				
1479	1484	1474	1469	850	855	845	840	221	226	216	211				
1462	1467	1457	1452	833	838	828	823	204	209	199	194				
1445	1550	1440	1435	816	821	811	806	187	192	182	177				
1428	1433	1423	1418	799	804	794	789	170	175	165	160				
1411	1416	1406	1401	782	787	777	772	153	158	148	143				

Na 6 minuten: *" Het is tijd. Jullie kunnen de kamer verlaten. Buiten wacht iemand jullie op om jullie naar de wachtkamer te begeleiden."*

Appendix 5. Protocol TSST-G control condition

Schriftelijke introductie controletaak aan deelnemer

Je wordt straks gevraagd namens een vriend of vriendin (niet uw partner) een sollicitatiegesprek te voeren. Het is de bedoeling dat je gaat vertellen waarom juist deze persoon zo geschikt is. Zet daarbij vooral de **positieve eigenschappen** van deze persoon uiteen. Denk daarbij aan zaken als karaktereigenschappen, sociale vaardigheden en capaciteiten.

Je hebt vanaf nu 5 minuten de tijd om een praatje voor te bereiden. Daarna zal je dit praatje 2 minuten lang houden. U doet dit **tegelijk** met de andere deelnemers. Het gaat hierbij **niet** om houding en orginaliteit. Uw praatje wordt niet opgenomen, beoordeeld of bewerkt. Er zal iemand bij zitten, maar verder **niet op jullie letten**. Het is dan ook **niet stressvol**.

Spreek alstublieft zo hard dat men je stem hoort, je hoeft echter niet luid te spreken. Het is vooral belangrijk dat je duidelijk verstaanbaar praat. Tijdens het gesprek ben je vrij om te gaan staan waar je zelf wil.

In vervolg op deze taak zal nog een **tweede taak** worden uitgelegd door de persoon in de kamer. Ook hierbij gaat het **niet om houding**, alleen om een korte niet stressvolle taak.

Alles bij elkaar zal dit zo'n 15 minuten duren. Als er vragen zijn, steek dan je hand op.

TSST-G: control condition group

Controle gesprek

Totale tijd van het gesprek: 2 minuten (2 min tegelijk praten) De onderzoeksleider die de kandidaat binnenleidt heeft al uitleg gegeven over de TSST procedure. De kandidaten wordt verteld dat ze overal mogen gaan staan waar ze willen. Dit zal de onderzoeksleider doen. De toeziend persoon in de stressruimte die het overneemt begint dan met: *"Jullie mogen nu tegelijk beginnen met het sollicitatiegesprek namens een vriend/vriendin met een harde stem, tot we jullie vragen te stoppen. Jullie zullen niet gefilmd worden. Tevens zal er niet naar jullie gekeken of geluisterd worden. Toch is deze taak belangrijk als controle."* De toezichthouder zelf gaat een tijdschrijft of een boek lezen. Na twee minuten zegt de toezichthouder: *"Dank voor jullie medewerking. Jullie kunnen nu verder in het tijdschrift lezen.'' Jullie moeten wel blijven staan. Ik zal zeggen wanneer het tijd is".*

Na 8 minuten, zeg: *"We beeindigen deze taak. Vul nu het volgende formulier in".* (geef de kandidaat een tweede visual-analogue scale formulier, Salivette). Laat de kandidaat dit invullen/afnemen aan de statafel. Ga over naar de wiskundige taak.

Controle wiskundige taak

De toezichthouder zegt: *"We willen jullie nu vragen om een rekenkundige taak uit te voeren. We vragen je om van 2023 terug te tellen naar 0 in stappen van 17. Jullie kunnen dit tegelijk doen met een zachte stem. Het is de bedoeling dat je dit zo snel en foutloos mogelijk doet. Als je een misrekening maakt, zal ik jullie niet verbeteren. Willen jullie nu beginnen. ?"*

Laat de proefpersonen deze taak 1,5 minuut uitvoeren. Waarna de toezichthouder zegt: *"Bedankt voor jullie medewerking. Jullie kunnen nu verder lezen in het tijdschrift. Jullie moeten wel blijven staan. Ik zal zeggen wanneer het tijd is."* Na 6 minuten: *" Het is tijd. Jullie kunnen de kamer verlaten. Buiten wacht iemand om jullie naar de wachtruimte te begeleiden."*