The Effects of Acute Stress on the Neural Correlates of Decision-Making

Jillian L. Toppings, Thomas D. Ferguson, and Olave E. Krigolson^{*}

University of Victoria jill.toppings@sasktel.net

Abstract

Stress has been defined in many ways but is typically a response to a change in the body's current state. Stress affects decision-making, and the effects of stress on processes involved in decision-making can be indirectly measured through EEG. The purpose of this study was to investigate how acute stress affects sub-processes involved in decision-making. We hypothesized that acute stress would affect how individuals respond to rewards and pay attention to environmental changes. Stress was physiologically present in the stress condition group, as seen in a mean increased heart rate compared to the control condition group. The stress condition group reported being more subjectively stressed than the control group, seen in STAI and PANAS questionnaire decreased positive and increased negative affect scores compared to the control group. For neural responses, while insignificant, there was a trend towards being less sensitive to environmental changes (attentional sensitivity; P300 component activity) in the stress condition, but no significant changes for reward sensitivity. Further research is needed to explore the implications for reward sensitivity that utilizes multiple tasks and includes cortisol measurement. Stress is common to everyday life and has been implicated chronically in numerous health conditions. Understanding how stress affects executive function, particularly decision-making, is therefore crucial in both the short- and long-term, as demonstrated by the initial findings of this study.

Keywords: stress; decision-making; ERPs; P300 component; reward positivity component

The Effects of Acute Stress on the Neural Correlates of Decision-Making

Stress occurs frequently in daily life at various levels both internally through expectations, attitudes, and feelings, and externally, through the environment. For example, choosing what to eat for lunch may evoke minimal stress in some individuals but incur a great deal of stress for others. Stress is difficult to define, but generally it occurs in situations that present a physical or mental challenge and is elicited when the demands of the situation threaten homeostasis or resources are perceived to be inadequate to meet the challenge (Pabst, Schoofs, Pawlikowski, Brand, & Wolf, 2013; Starcke & Brand, 2012; Tiferet-Dweck et al., 2016; Wemm & Wulfert, 2017). While under stress, the body enters into what is commonly referred to as "fight or flight" mode, in which our perception of the environment, attention, neural responses, and judgement are adjusted to address the environmental changes (Qi, Gao, & Liu, 2017; Tiferet-Dweck et al., 2016). This short-term response is facilitated by the sympathetic adrenomedullary system (SAM-system), while long-term responses are facilitated by the hypothalamus pituitary adrenal axis (HPA-axis) system. Stress modulates the biological systems through hormonal, neurophysiological, and behavioural adjustments (Godoy, Rossignoli, Delfino-Pereira, Garcia-Cairasco, & de Lima Umeoka, 2018; Lenow, Constantino, Daw, & Phelps, 2017).

^{*}This research was supported by a Jamie Cassels Undergraduate Research Award, University of Victoria.

Effect of Stress in Humans

Stress can be useful in some situations, but detrimental in others. Stress or traumatic events occurring early in life are linked to dysregulation of the HPA-axis and negative impact of glucocorticoids on the development of certain brain areas such as the prefrontal cortex (Lupien, McEwen, Gunnar, & Heim, 2009). Additionally, prolonged or chronic stress has many negative health implications. There is a wealth of literature surrounding the negative impact of acute and chronic stress on health that highlights the importance and impact of stress on people. Stress increases the risk of cardiovascular diseases, psychosomatic diseases, diabetes, hypertension, and psychiatric disorders, particularly depression, anxiety and post-traumatic stress disorder (Juster, McEwen, & Lupien, 2010). Additionally, stress-related disorders have been linked with impaired feedback processing (Banis & Lorist, 2012), chronic HPA-axis dysregulation (Putman, Antypa, Crysovergi, & van der Does, 2010), prolonged cortisol activation (Dickerson & Kemeny, 2004), prolonged catecholamines (Godoy et al., 2018), and blunted behaviour (Banis & Lorist, 2012). Stress has also been implicated in unhealthy lifestyle behaviours such as substance abuse, unhealthy eating, and risk-taking behaviour such as pathological gambling (Putman et al., 2010). Therefore, stress throughout one's life increases the risk of developing many of the listed conditions or impairments.

Acute Stress

The potentially detrimental effects of chronic stress have been well established, but the implications for short-term acute stress have not been as thoroughly investigated. Acute stress can be either beneficial or detrimental depending on the situation and individual (Dickerson & Kemeny, 2004). Acute stress can benefit individuals particularly in tasks that are simple and well-rehearsed or habitual with a low cognitive load as these tasks rely on processing that is more automatic, but stress can impair more complex or novel tasks (Banis & Lorist, 2012; Qi et al., 2017; Shields, Sazma, & Yonelinas, 2016). This concept can be illustrated in the Yerkes-Dodson curve (Yerkes & Dodson, 1908), which depicts the relationship between arousal and performance (see Figure 1). There is an optimal level of arousal or stress to the system that results in optimal performance. Individuals experience higher arousal for tasks that are more complex or novel. Therefore, a high amount of stress would shift the curve to the right and decrease performance.

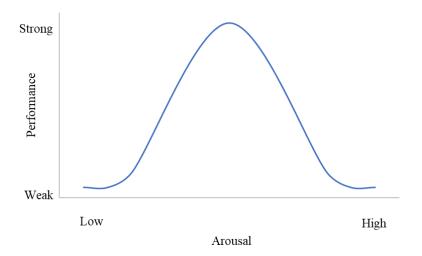


Figure 1: Simplified version of the Yerkes-Dodson curve (1908) depicting the relationship between arousal and performance. (Graph created by Jillian Toppings.)

Physical and Psychological Response to Stress

Physical and psychological stressors result in different neural and cellular responses (Godoy et al., 2018). Physical stressors are defined as stimuli that change physiological status, while psychological stressors are anticipated and affect the current state (Godoy et al., 2018). Physical stressors activate brain regions such as the nucleus of the solitary tract and locus coeruleus, while psychological stressors activate the limbic system and reward systems (Godoy et al., 2018). However, the response to physical and psychological stressors are more similar than different; psychological stressors can evoke similar responses to physical stressors, such as the activation of the HPA axis. For example, the hippocampus is activated from both physical and psychological stressors, which is important for the HPA axis negative feedback system (Godoy et al., 2018).

When a stressor is perceived, brain structures and neuronal networks are recruited and work with the autonomic nervous system to restore homeostasis (McEwen, 2007). The autonomic system is responsible for adapting visceral functions such as heart rate, salivation, respiration, and perspiration (McEwen, 2007). Once the stressor has been detected, the thalamus and frontal lobes integrate the sensory information and evaluate the environmental stimuli (Lovallo, 1997). Additionally, the orbitofrontal cortex and prefrontal cortex process emotional and social responses (Hanson et al., 2010). With the neural networks and brain structures activated, two systems are triggered and work together: the fast-reacting SAM-system and the slow-reacting HPA-axis (McEwen & Sapolsky, 1995). The SAM-system is mediated by catecholamines and is primarily responsible for the short-term and rapid "fight or flight" reaction by increasing alertness and vigilance, allowing for strategic decision-making (McEwen & Sapolsky, 1995). The HPA-axis is a slower system activated and regulated by excitatory and inhibitory loops of limbic and prefrontal structures (McEwen & Sapolsky, 1995). The system is activated by the release of corticotropin releasing hormone (CRH) from the hypothalamus, which in a cascade triggers adrenocorticotropin hormone (ACTH) and then the adrenal cortex, which then releases cortisol that can bind to the limbic and prefrontal structures like the amygdala, hippocampus, and prefrontal cortex (Herman et al., 2003). The role of cortisol includes helping to mobilize energy resources as well as being a natural anti-inflammatory for the body (Sapolsky, Romero, & Munck, 2000; van Oort et al., 2017). Cortisol is elicited particularly when the stressors are a threat to self-preservation, when they are uncontrollable, or when they are social-evaluative stressors (Dickerson & Kemeny, 2004).

Stress and Cognitive Function

There have been many studies that measure cortisol as an indication of increased stress. Many studies have focused on executive function, which includes working memory, cognitive flexibility, attention, and inhibition, which rely on the prefrontal cortex, a brain structure particularly sensitive to stress. Executive function is important in the ability to focus on or switch activities, make decisions about the present and future, resist temptations or impulses, and maintain and update working memory (Dierolf, Fechtner, Bönke, Wolf, & Naumann, 2017). The current literature about acute stress largely surrounds working memory, attention, and response inhibition (Dierolf et al., 2017) using tasks such as the digit span task or n-back task. A common theory to explain the impairments stress exhibits on executive function is that stress reallocates cognitive resources to inhibition to enhance attention on the stressor (Banis & Lorist, 2012; Dierolf et al., 2017; Qi et al., 2017; Shields et al., 2016). Shields et al. (2016) suggest that resources reallocated to inhibition come from working memory and cognitive flexibility.

Stress and Decision-Making

One crucial part of how stress can affect an individual's health is reliant on their decisionmaking abilities. The relationship between decision-making and stress is bidirectional, as decisions are often made under varying levels of stress, and situations that require a decision often induce stress (Kirschbaum, Pirke, & Hellhammer, 1993; Starcke & Brand, 2012; Wemm & Wulfert, 2017). Additionally, brain regions that underlie decision-making can be affected by stress (Ossewaarde et al., 2011). This influence is important because the way in which an individual responds to stress affects their appraisal of the environment or situation, which can lead to suboptimal choices (Lenow et al., 2017) that can lead to unhealthy behaviours and/or negative health conditions over time.

Starcke and Brand's (2012) theory suggests decision-making is a complex process that involves selecting between competing options by comparing their relative values of consequences, while also accounting for highest benefit or social or moral factors. Individuals are capable of calculating the risks and benefits associated with different choices when there are explicit rules for gains and losses by using executive functioning including working memory, planning, and categorization. However, there are situations in which individuals make decisions based on intuition, biases, or heuristics rather than strategic decisions involving risk and benefit calculation. The dual process theory encompasses various decision forms, stating that humans make strategic decisions through the rational-analytical system, and intuitive decisions through the intuitive-experiential system (Pabst et al., 2013; Starcke & Brand, 2012). The rational-analytical system is slower and rule-governed, while the intuitive-experiential system relies on fast and emotional processing (Pabst et al., 2013; Starcke & Brand, 2012).

The dual process theory systems are also similar to the concepts of model-based behaviour and model-free control (Otto, Raio, Chiang, Phelps, & Daw, 2013; Radenbach et al., 2015). Model-based behaviour is driven by an internally built mental model, and planned actions are future-oriented. Model-free control is driven by past rewards and repeats actions that were previously awarded, neglecting a potentially advantageous model (Otto et al., 2013; Radenbach et al., 2015). Radenbach et al. (2015) investigated the impact of acute stress, stress reactivity, and previous exposure to life events on the shift of model-free and model-based control systems during a two-step decision task. They did not find a significant shift in the sample towards model-free behaviour but found that individuals with higher chronic stress displayed a shift towards model-free behaviour.

Decisions often involve exploring new options (exploration) or sticking with a known reward (exploitation) (Wilson, Geana, White, Ludvig, & Cohen, 2014). Stress can bias decision-making towards model-free or habitual decision-making, leading to exploitation rather than exploration, or lead to an increase in high-risk behaviour (Lenow et al., 2017; Putman et al., 2010; Radenbach et al., 2015; van den Bos, Harteveld, & Stoop, 2009; Wilson et al., 2014).

Stress and ERPs

A majority of the literature on stress has used cortisol and behavioural measures only, despite extensive literature on the neurophysiological mechanisms of inhibition (Dierolf et al., 2017). There are only a few event-related potential (ERP) studies that have looked at the effects of stress. EEG studies are an appropriate method to investigate stress as they show temporal changes. Qi et al. (2017) used ERPs when examining the effects of acute stress on response inhibition and found that cognitive control processes were enhanced and early selective attention processes were reduced. Dierolf et al. (2017) looked at the effects of stress on response inhibition and its neural correlates and found enhanced response inhibition, demonstrating support for the theory of cognitive resource reallocation. Two studies were done using ERPs to examine the effects of acute noise stress. Banis et al. (2012) studied the effects of stress on the feedback-related negativity (FRN) component and found impaired cognitive control functioning. Banis et al. (2014) also examined the FRN component, theta, and oscillatory power, and found that acute noise stress impairs both males and females. Stress increases cortisol levels in a linear relationship for men, and an inverted U-shape for women. However, there are currently minimal studies looking at the effects of acute stress on ERPs and decision-making.

Decision-making involves processes such as attending to or adapting to context changes and learning from rewards. Context sensitivity is thought to be related to the neuromodulator norepinephrine, while learning from rewards is thought to be related to the neuromodulator dopamine. Therefore, the effect of stress on these underlying decision-making processes can be indirectly measured through the effects on noradrenergic and dopaminergic activity by using EEG.

Stress impacts the levels of norepinephrine (Tanaka, 1999), which is reflected in the P300 component reduction. The P300 component peaks at approximately 300 ms post-stimulus (Luck, 2005) and is thought to reflect context updating of a mental model (Polich, 2007). This occurs when there is a change in stimulus in the current environment, and this component is reduced with stress (Banis et al., 2014). The P300 is elicited in tasks where participants can continue to exploit an option or choose to explore new options.

In addition, stress activates dopaminergic neurons and dopamine levels (Deutch et al., 1991). The effect on dopamine affects reward sensitivity in explore/exploit tasks. Dopamine is reflected in the reward positivity (RewP) component or the feedback related negativity (FRN) component, which peaks at approximately 250-350 ms after feedback is presented (Proudfit, 2015). The impact of stress on dopamine leads to a reduction in reward positivity (Otto et al., 2013; Sambrook & Goslin, 2015). The reinforcement learning system underlying the RewP component has to do with evaluating the net value of the reward for the available action choices while also computing a reward prediction error (Sambrook & Goslin, 2015; Walsh & Anderson, 2012), which is crucial to decision-making. These two components will be evaluated in this study as they are both impacted by stress.

Present Study

While the wealth of literature on acute and chronic stress is highly informative, there are areas that can be critiqued. First, the type of stressor used in each study varied, which makes it difficult to compare the results. Additionally, the timing, operationalization, intensity, degree of uncertainty, and type of task often differed between studies.

The current literature on acute stress and cognitive processes has been limited to memory and relied mostly on behavioural measures. The current ERP research is narrow and does not address the effects of acute stress on decision-making. The purpose of the current study is to address the gap in the literature by using ERPs as a tool to investigate the effects of acute stress both physiologically and psychologically on decision-making, reward processing, and their neural correlates. We hypothesized that acute stress would impact the sensitivity of reward and attentional processing, seen through both diminished P300 and reward positivity component activity.

Method

Participants

Participants in this experiment were 26 university-aged individuals (14 male, 12 female, four left-handed, one ambidextrous, $M_{age} = 20.38$, age range: 18-35 years) with no known neurological impairments and with normal or corrected-to-normal vision. They were assigned too the control or stress condition such that 13 participants were in each group. The control condition group consisted of five males and eight females, and the stress condition group consisted of nine males and four females.

All participants volunteered for this study and received credit in an undergraduate psychology course for their participation. Informed consent was approved by the Human Research Ethics Board at the University of Victoria, and written consent was provided voluntarily by participants.

Participants were informed of specific exclusion criteria prior to sign-up and then asked a series of questions confirming their eligibility prior to the experiment start. The exclusion criteria were individuals with neuropsychological or chronic illnesses, individuals taking medications, individuals who regularly smoke, or individuals who are taking birth control pills. Additionally, participants were instructed to avoid eating large meals, doing strenuous exercise, consuming acidic beverages, or smoking any substances two hours prior to the scheduled start of the experiment.

Materials

Trier Social Stress Test (TSST) Protocol (Stress Condition)

The TSST protocol is modelled after Kirschbaum et al.'s (1993) protocol (see Appendix). At time 0 min, participants were taken from room A to room B, which had two persons sitting behind a table in white lab coats, as well as a video camera. The subject was instructed to behave as a job applicant at an interview with the panel. Participants were informed that after a three-minute preparation period, they were to introduce themselves and convince the panel in five minutes of free speech that they were the best candidate for a job position of their choice. The participant was made aware that the panel had special training in monitoring nonverbal behavior, and that there would be both voice frequency and video analysis of the performance. Participants had paper and pens to aid their preparation but were not permitted to use any preparatory materials for their speech.

After the preparation period, the panel chair would welcome the participant and instruct them to deliver their speech for five minutes. If the speech concluded prior to five minutes, the panel would remain silent for 20 seconds before asking prepared questions until the five minutes were over. Following the speech period, the panel asked the participant to serially subtract in steps of 17 from 2023 as fast and accurately as they could. If the subject made an error, the panel would intervene and ask them to restart at 2023. This task concluded after five minutes.

Placebo TSST (Control Condition)

The placebo TSST protocol was adopted from Het, Rohleder, Schoofs, Kirschbaum, and Wolf (2009) and designed to be as similar as possible to the TSST without the aspect of stress. The participant was taken into an empty room in the same seated position as in the TSST. The experimenter told the participant they would be given a three-minute preparation period and then asked to speak aloud for five minutes about a recent movie, novel, or holiday. They were informed that no one would be in the room recording or listening. The experimenter came in following the five-minute speech period to instruct the participant to add up the number 15 starting at 0 for five minutes. The placebo TSST was performed in the same room as the stress TSST, but the elements meant to induce stress (committee, video camera, tape recorder) were not present. This removed the social evaluative threat and uncontrollability elements present in the stress condition TSST protocol, as per Dickerson and Kemeny's (2004) theory of stress.

SAM Axis Measurement and Analysis

Heart rate (HR) was measured to evaluate the effectiveness of the TSST to activate the short-term response system, the SAM axis. HR (in beats per minute, bpm) was measured continuously at 250 Hz throughout the experiment using a ZephyrTM heart rate monitor strapped to the chest underneath the sternum.

The average heart rate was calculated for the baseline period during questionnaires, during the TSST, and during cognitive assessment for each participant. Then, the percent change from baseline for the TSST was computed using 95% confidence intervals for the control and stress conditions.

Subjective Stress Measurement (STAI and PANAS)

The State-Trait Anxiety Inventory (STAI) (Spielberger, 1983) questionnaire was used to evaluate the effectiveness of the TSST to induce anxiety, which is indicative of the psychological perception of stress. We used the standard two, 20-item portions for state and trait anxiety. Participants selected responses on a four-point scale from *not at all* to *very much so*. Higher scores for negative affect indicated higher levels of anxiety.

The Positive and Negative Affect Schedule (PANAS) (Watson, Clark, & Tellegen, 1988) questionnaire was also used to evaluate the effectiveness of the TSST. We used the standard two, 20 item portions. Participants selected responses on a five-point scale from *very slightly* or *not at all* to *extremely*. Higher scores for negative affect and lower scores for positive affect indicated higher levels of stress.

The STAI and PANAS surveys' mean positive and negative affect scores were calculated post-TSST using 95% confidence intervals for the control and stress conditions.

Cognitive Assessment

Cognitive Assessment consisted of four tasks. The first task involved focusing on a cross in the centre of the screen for 30 seconds to gain a baseline. The second task consisted of focusing on the cross while mentally counting backwards in steps of 7 from 1000. The third and fourth task, described below, were randomized in order. These two tasks were examined for this study as they pertain to attentional and reward sensitivity, respectively.

Task 3 was the oddball task, in which participants viewed two blocks of 100 trials of either common or rare (oddball) circles flashing on a screen (see Figure 2). The task was to actively respond with key presses to the oddball, meant to elicit the P300 component.

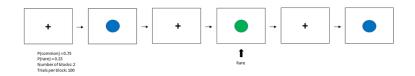


Figure 2: Oddball paradigm

The fourth task was a gambling task, in which participants chose between two coloured squares on a screen by pressing either "f" or "j" on the keyboard (see Figure 3). Each coloured square was assigned a probability of a "win," which changed with each block. The task was to identify and correctly select the coloured block with the highest probability of a "win" over five blocks of 20 trials each. This task elicited the reward positivity component.

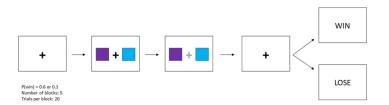


Figure 3: Gambling task paradigm.

P300 and Reward Positivity Components

Independent-samples t-tests were conducted to compare the average peak amplitude and average latency for control and stress groups using 95% confidence intervals for the P300 and reward positivity components.

Procedure

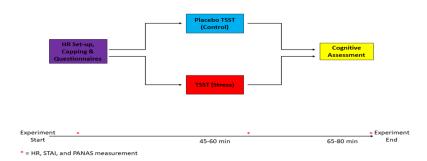


Figure 4: Experimental protocol timeline.

The experimental sessions were carried out between 1 and 5 p.m. in order to account for the fluctuation of cortisol levels the morning and evening (Dickerson & Kemeny, 2004). All testing was conducted in quiet distraction-free rooms.

Overall, the experimental portion took approximately 80 minutes (see Figure 4). Upon their arrival, participants were assigned to the stress or control group such that there were approximately equal numbers of participants in each group. The experimenter was not blind to which group the participant was assigned to in order to prepare the appropriate TSST to match the condition.

Participants gave informed consent, then EEG setup occurred while participants filled out demographic and baseline STAI and PANAS questionnaires in room A. During this period, participants were introduced to and fitted with the HR monitor. Stress condition participants then engaged in the TSST protocol, while the control group engaged in the placebo TSST protocol. Following the TSST, participants carried out the Cognitive Assessment task, which included the key oddball and gambling tasks. The PANAS and STAI surveys were given at baseline, after the TSST and after Cognitive Assessment.

Data Collection

Thirty-two channels of EEG data, referenced to channel AFz, were recorded using Brain Vision Recorder (Version 1.20, Brain Products GmbH, Munich, Germany). Thirty electrodes were placed in a fitted cap according to the International 10-20 system, while an additional two electrodes were affixed to the left and right mastoids. Conductive gel was applied to each electrode to ensure electrode impedances were below 20 k Ω prior to recording, and the EEG data were sampled at 500 Hz and amplified (actiCHamp, Brain Products GmbH, Munich, Germany).

Statistical Analysis

Prior to beginning the study, a power analysis was conducted. The "pwr" package in R (Version 3.3.0, The R Foundation, Vienna, Austria) was used to compute the sample size needed to determine an effect. To determine sample size, a power of 80% and a .05 significance level were assumed. Based on data collected from a pilot sample, it was observed that there was a large effect (0.8; Cohen, 1988) between conditions in the event-related potentials, and thus, a final sample size of 26 participants in each condition was determined. The experimenter was not blind to which group the participant was assigned to in order to prepare the appropriate TSST to match the condition and analyze the data.

MATLAB (Version 8.3, Mathworks, Natick, USA) and the Psychophysics Toolbox Extension (Brainard, 1997; Pelli, 1997) were also used to pre-process the data using custom scripts (www.github.com/Neuro-Tools) that depended on EEGLab (Delorme & Makeig, 2004). Finally, R (R Core Team, Version 3.6.2) and Excel (Microsoft Excel, Build 13127.20408) were utilized to complete the statistical analysis.

It should be noted that given the small sample size, sex differences were not evaluated in this study.

Data Pre-Processing

EEG data were processed using standard methods in the Krigolson Laboratory (http:// www.krigolsonlab.com/data-analysis.html). Data from faulty electrodes or electrodes with excessive noise were removed. The average of the two mastoid channels were used for referencing. Data were filtered through a (0.1 Hz - 30 Hz pass band) phase shift-free Butterworth filter (60 Hz notch). Independent component analysis (Delorme & Makeig, 2004; Luck, 2005) was used to remove blinks, and then inverse ICA was conducted on the removed components. We then used topographic interpolation and the method of spherical splines to add back in any removed channels. This was followed by the construction of 1000 ms epochs of EEG data from the 200 ms prior to the 800 ms following feedback onset. The ERP waveforms were created by averaging the epochs of EEG data for each channel and participant. Segments were common and rare (oddball) stimuli for the P300 component and win or loss feedback for the reward positivity component. Difference waves were created by subtracting the average common stimuli from the average rare (oddball) stimuli and by subtracting the average loss feedback from the average win feedback. All trials were baseline corrected with a 200 ms pre-feedback window, with any trials with a voltage change exceeding 10 μ V per sampling point or voltage greater than 100 μ V discarded.

Results

Heart Rate

Heart rate was calculated for the TSST as the mean percent (%) change from baseline for the control and stress conditions. The control group had a mean % change of -0.52 ± 8.02 %, and the stress group had a mean % change of 8.54 ± 6.16 % (see Figure 5). An independent-samples *t*-test

was conducted to compare the control and stress groups mean % change from baseline, t(21) = 3.07, p = 0.01, d = -1.24.

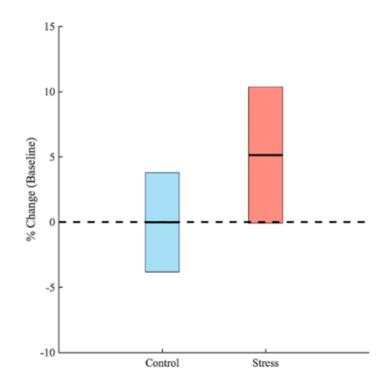


Figure 5: Mean heart rate % change from baseline during the TSST comparing control (n = 13) and stress (n = 13) conditions. Error bars indicate 95% confidence intervals.

STAI

Mean positive and negative affect scores were computed post-TSST (see Figure 6). The mean positive score was 2.92 ± 0.59 for the control group and 2.34 ± 0.76 for the stress group. An independent-sample *t*-test was conducted to compare the control and stress groups' positive affect scores, t(23) = 2.10, p = 0.05, d = 0.86 and it showed a decrease in positive affect scores for the stress group.

The mean negative score was 1.37 ± 0.29 for the control group and 2.08 ± 0.87 for the stress group. An independent-samples *t*-test was conducted to compare the control and stress groups mean negative affect scores, t(23) = 2.51, p = 0.02, d = -1.10, and it showed an increase in negative affect scores for the stress group.

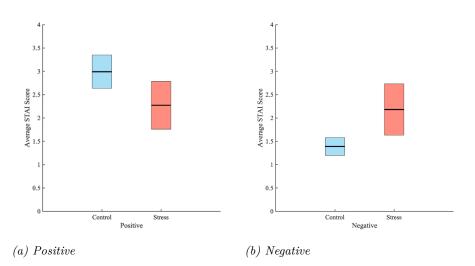


Figure 6: Mean STAI score post-TSST for positive (a) and negative (b) affect scores for control (n = 13) and stress (n = 13) conditions. Error bars indicate 95% confidence intervals.

PANAS

Mean positive and negative affect scores were computed post-TSST (see Figure 7). The mean positive score was 3.11 ± 0.46 for the control group and 2.54 ± 0.66 for the stress group. An independent-samples *t*-test was conducted to compare the control and stress groups mean positive affect scores, t(23) = 2.44, p = 0.02, d = 1.00, and it showed a decrease in positive affect scores for the stress group.

The mean negative score was 1.52 ± 0.92 for the control group and 1.73 ± 0.58 for the stress group. An independent-samples *t*-test was conducted to compare the control and stress groups mean negative affect scores, t(23) = 0.66, p = 0.51, d = -0.26 and did not show statistically significant changes.

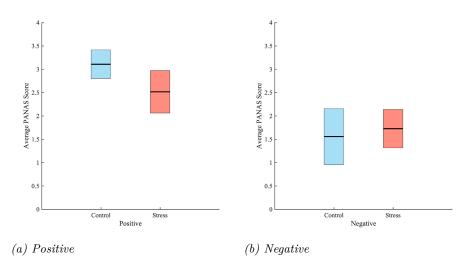


Figure 7: Mean PANAS score post-TSST for positive (a) and negative (b) affect scores for control (n = 13) and stress (n = 13) conditions. Error bars indicate 95% confidence intervals.

P300

(a)

The mean P300 component peak amplitude (μ V) was calculated for the control condition as 9.62 ± 6.01 μ V and for the stress condition as 6.29 ± 3.97 μ V, t(22) = 1.67, p = 0.11, d = 0.65. The mean P300 component latency (ms) was calculated for the control condition as 379.67 ± 40.94 ms and 384.82 ± 37.10 ms for the stress condition, t(22) = 0.28, p = 0.78, d = -0.13.

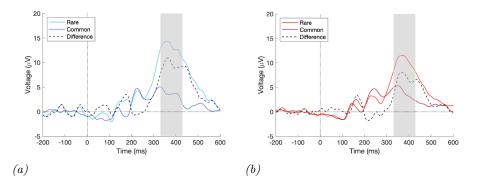


Figure 8: ERP components for the oddball task comparing the mean common and rare (oddball) trials for the control (n = 13) (a) and stress (n = 13) (b) conditions.

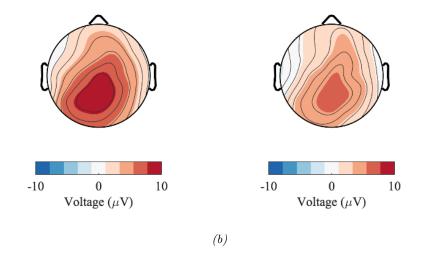


Figure 9: Topoplots for the control (n = 13) (a) and stress (n = 13) (b) conditions for the oddball task.

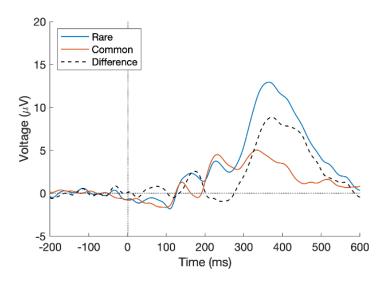


Figure 10: Grand average of the P300 component showing the difference wave for the oddball task between rare and common stimuli.

Reward Positivity (RewP)

The mean RewP component peak amplitude (μ V) was calculated for the control condition as $4.67 \pm 4.81 \ \mu$ V, and for the stress condition as $3.82 \pm 5.61 \ \mu$ V, t(22) = 0.53, p = 0.60, d = 0.16. The mean RewP component latency (ms) was calculated for the control condition as 311.33 ± 27.16 ms and 306.33 ± 42.88 ms for the stress condition, t(22) = 0.34, p = 0.74, d = 0.14.

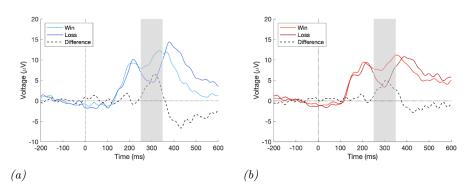


Figure 11: ERP components for the gambling task comparing the mean win and loss feedback for the control (n = 13) (a) and stress (n = 13) (b) conditions.

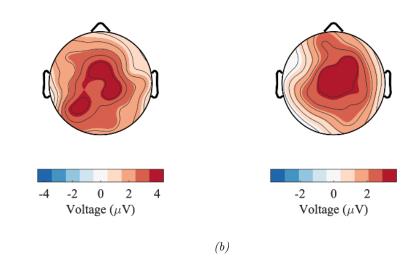


Figure 12: Topoplot for the control (n = 13) (a) and stress (n = 13) (b) conditions.

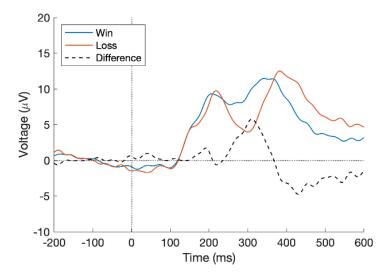


Figure 13: Grand average of the reward positivity component showing the difference wave for the $\hat{a}\check{A}\check{O}$ gambling task between win and loss feedback.

Win Percentage

(a)

Mean win percentages were calculated for the gambling task as 52.42 ± 7.35 % for the control condition and 48.75 ± 7.40 % for the stress condition, t(22) = 1.00, p = 0.33, d = 0.50.

Discussion

The purpose of this study was to address the gap in the literature by using ERPs as a tool to investigate the effects of acute stress both physiologically and psychologically on decision-making, reward processing, and their neural correlates. We hypothesized that acute stress would impact the sensitivity to attentional and reward processing, seen through both diminished P300 and reward

positivity component activity. While the results were not statistically significant to support our hypothesis, there are trends that emerged.

Validation

Heart rate

The induction of stress was successful as seen through the manipulation checks of heart rate and subjective measures of anxiety and affect. The HR % change from baseline comparing control and stress groups was significant for the stressor, providing physiological evidence that stress was induced. HR has been used as a manipulation check in several other tasks that utilized the TSST as the stressor, and those studies also found statistically significant higher heart rates in the stress groups (Starcke, Wiesen, Trotzke, & Brand, 2016; Wemm & Wulfert, 2017).

STAI and **PANAS**

In addition to the statistically significant HR results, there were statistically significant results for the STAI and PANAS questionnaires, providing evidence of subjective or psychological stress. The STAI questionnaire was used to examine the induction of anxiety from the TSST. The stress group had decreased positive affect scores and increased negative affect scores. Studies by Wemm and Wulfert (2017), Villada et al., (2016), and Starcke et al. (2016) also found statistically significant higher anxiety scores in the stress condition compared to the control condition post-TSST.

The PANAS questionnaire was utilized to evaluate the effectiveness of the TSST to impact positive and/or negative affect scores for mood. The stress group had decreased positive affect scores for the PANAS questionnaire. The results for the PANAS negative affect scores were not statistically significant. Other studies have shown statistically significant changes for increased negative affect in the stress condition post-TSST (Capobianco, Morrison, & Wells, 2018; Villada, Hidalgo, Almela, & Salvador, 2016; Wemm & Wulfert, 2017).

However, some literature suggests that the physiological measures of stress and subjective experience may not always be correlated with each other (Campbell & Ehlert, 2012). For example, the peak cortisol response occurs 20-40 minutes post-stressor, and the subjective anxiety and mood results typically return to baseline (Pabst et al., 2013). It is also possible that a significant number of individuals in the stress condition were cortisol nonresponders, which would indicate they are less sensitive to the effects of the TSST (Starcke & Brand, 2012). This effect would be seen with an additional task post Cognitive Assessment.

P300 component

The P300 component is elicited during the oddball task when comparing the averaged trials for the common and rare (oddball) stimuli. The results were not statistically significant for the P300 component between the control and stress conditions (see Figure 8). However, there was a slight decrease in mean peak amplitude for the stress condition. Since the P300 is thought to be involved in context sensitivity or selective attention (Shackman, Maxwell, McMenamin, Greischar, & Davidson, 2011), this decrease may suggest that stress, induced by the TSST, may decrease this context sensitivity which can result in a failure to attend fully to the environment (Wemm & Wulfert, 2017). The P300 component is thought to reflect the activity of the locus coeruleus-norepinephrine system, particularly sensitive to stress (Shackman et al., 2011). The attenuation of the P300 component thus may result in alterations to selective attention, resulting in a disruption of top-down control (Shackman et al., 2011). Stress can reallocate the resources that would typically be used in selective attention or bias the information most relevant to the stressor at that point in time (Shields et al., 2016). This can have negative ramifications for decision-making, as the individual may not consider all available contextual and environmental information.

Reward positivity component

The reward positivity component is elicited during the gambling task when comparing the averaged trials between win and loss feedback. The results show no significant differences between the control and stress groups (See Figure 11). These results are not surprising, as cortisol levels peak 20-40 minutes post-stress onset (Pabst et al., 2013), occurring after Cognitive Assessment, specifically the gambling task. Therefore, significant results may be found for a second task occurring 20-40-minutes post-stressor. There are some expected results for that time frame based on other similar studies. Wemm and Wulfert (2017) found an inverted U relationship between stress and performance, which can be interpreted as stress enhancing performance, but only up to a certain point, consistent with the Yerkes-Dodson Law. Stress can affect sensitivity to reward through the modulation of the dopamine system, reflected in the reward positivity component. Literature suggests that acute stress can enhance learning of positive feedback and inhibit learning of negative feedback (Banis et al., 2014; Lighthall, Gorlick, Schoeke, Frank, & Mather, 2013; Mather & Lighthall, 2012; Petzold, Plessow, Goschke, & Kirschbaum, 2010; Wemm & Wulfert, 2017). This enhancement and impairment of selective feedback due to stress can be beneficial or detrimental, depending on the situation. A similar study by Banis et al. (2014) conducted a gambling task where participants were given feedback indicative of monetary gain or loss following their choice. Probabilities were randomized instead of constant as in the current study, and the results were that even without constant probabilities, the participants' behavioural data suggested that they were using the feedback from the previous trial to bias their decisions. The results of this study were the trend towards reduced reward positivity component activity for the stress group compared to the control group, which are the expected results for a second task in the current study.

To further provide evidence that a second task was needed to potentially see a reward positivity difference, there was no significant difference in win percentage between control and stress conditions. This suggests that either stress did not significantly influence the choices made in the gambling task or that participants in both groups were not learning the tasks, as the win percentages were only 52.42 ± 7.35 % and 48.75 ± 7.40 % for the control and stress conditions, respectively.

Limitations and Future Directions

There were several limitations in this study. The experimenters were aware of which condition each participant was assigned to in order to appropriately prepare for the TSST, which may have resulted in unintentional biasing towards each condition. With a small sample size, it was difficult to analyze gender or age differences. However, there is literature to suggest that males and females respond differently to stress (Banis et al., 2014; Kirschbaum, Kudielka, Gaab, Schommer, & Hellhammer, 1999; Kudielka & Kirschbaum, 2005; Starcke & Brand, 2012; van den Bos, Homberg, & de Visser, 2013), and this could be a future research direction. Also due to sample size, it was difficult to determine statistically significant results. There were also limitations with the tasks themselves, as they may not have been complex enough to reflect real-world stress. Future studies should add further tasks to see the potential effects of stress on reward positivity component activity, along with the measurement of cortisol.

Utilizing EEG, researchers cannot examine subcortical regions. Furthermore, EEG has poor

spatial resolution (Luck, 2005). In addition, the size of scalp ERPs are small in comparison to the size of noise, and scalp ERPs only record when they meet particular conditions. This means that a particular mental or neural process may not be reflected in the recorded voltage patterns unless it meets all conditions, and that particular brain process has a distinct ERP component (Luck, 2005). Therefore, future studies could utilize multiple methods in addition to EEG to achieve optimal spatial and temporal resolution. This study used standard EEG pre-processing (Luck, 2005) for the analysis, but future studies could conduct single trial analysis or examine time-frequency wavelets or fast-Fourier transformations. Last, it is extremely difficult to use ERPs to measure brain activity for tasks that are longer than a few seconds, which limits testing tasks that reflect real-world stress.

Conclusions

In summary, the purpose of this study was to investigate the effects of acute stress on decisionmaking and its neural correlates, as there is limited ERP research regarding stress and its effects on decision-making. We hypothesized that acute stress would affect the underlying processes of decision-making of context sensitivity and learning from rewards. This would be seen through the reduction of P300 and reward positivity ERP component activity. We found no statistically significant results for these components, but there was a trend of reduced P300 component activity for the stress condition. Stress is quite common to everyday life and has been implicated chronically in numerous health conditions. Understanding how stress affects executive function, particularly decision-making, is therefore crucial in both the short- and long-term.

References

- Banis, S., Geerligs, L., & Lorist, M. M. (2014). Acute stress modulates feedback processing in men and women: Differential effects on the feedback-related negativity and theta and beta power. PLoS ONE, 9(4). https://doi.org/10.1371/journal.pone.0095690
- Banis, S., & Lorist, M. M. (2012). Acute noise stress impairs feedback processing. Biological Psychology, 91(2), 163-171. https://doi.org/10.1016/j.biopsycho.2012.06.009
- Brainard, D.H., 1997. The psychophysics toolbox. Spatial Vis. 10, 433-436.
- Campbell, J., & Ehlert, U. (2012). Acute psychosocial stress: Does the emotional stress response correspond with physiological responses? *Psychoneuroendocrinology*, 37(8),1111-1134. Retrieved from http://10.0.3.248/j.psyneuen.2011.12.010
- Capobianco, L., Morrison, A. P., & Wells, A. (2018). The effect of thought importance on stress responses: A test of the metacognitive model. *Stress: The International Journal on the Biology of Stress, 21*(2), 128-135. https://doi.org/10.1080/10253890.2017.1417378
- Delorme, A., & Makeig, S. (2004). EEGLAB: An open source toolbox for analysis of singletrial EEG dynamics including independent component analysis. *Journal of Neuroscience Methods*, 134(1), 9. Retrieved from http://10.0.3.248/j.jeumeth.2003.10.990
- Deutch, A. Y., Lee, M. C., Gillham, M. H., Cameron, D. A., Goldstein, M., & Iadarola, M. J. (1991). Stress selectively increases Fos protein in dopamine neurons innervating the prefrontal cortex. *Cerebral Cortex*, 1(4), 273-292. https://doi.org/10.1093/cercor/1.4.273
- Dickerson, S. S., & Kemeny, M. E. (2004). Acute stressors and cortisol responses: A theoretical integration and synthesis of laboratory research. *Psychological Bulletin*, 130(3), 355-391, https://doi.org/10.1037/0033-2909.130.3.355
- Dierolf, A. M., Fechtner, J., Böhnke, R., Wolf, O. T., & Naumann, E. (2017). Influence of acute stress on response inhibition in healthy men: An ERP study. *Psychophysiology*, 54(5), 684-695. https://doi.org/10.1111/psyp.12826
- Godoy, L. D., Rossignoli, M. T., Delfino-Pereira, P., Garcia-Cairasco, N., & de Lima Umeoka, E. H. (2018). A comprehensive overview on stress neurobiology: Basic concepts and clinical implications. *Frontiers in Behavioral Neuroscience*, 12(July), 1-23. https://doi.org/ 10.3389/fnbeh.2018.00127
- Hanson, J. L., Chung, M. K., Avants, B. B., Shirtcliff, E. A., Gee, J. C., Davidson, R. J., & Pollak, S. D. (2010). Early stress is associated with alterations in the orbitofrontal cortex: A tensor-based morphometry investigation of brain structure and behavioral risk. *Journal* of Neuroscience, 30(22), 7466-7472.
- Herman, J. P., Figueiredo, H., Mueller, N. K., Ulrich-Lai, Y., Ostrander, M. M., Choi, D. C., & Cullinan, W. E. (2003). Central mechanisms of stress integration: Hierarchical circuitry controlling hypothalamo-pituitary-adrenocortical responsiveness. *Frontiers in Neuroendocrinology*, 24(3), 151-180. https://doi.org/https://doi.org/10.1016/j .yfrne.2003.07.001
- Het, S., Rohleder, N., Schoofs, D., Kirschbaum, C., & Wolf, O. T. (2009). Neuroendocrine and psychometric evaluation of a placebo version of the "Trier Social Stress Test." *Psychoneuroendocrinology*, 34 (7), 1075-1086. https://doi.org/10.1016/j.psyneuen.2009.02.008
- Juster, R.-P., McEwen, B. S., & Lupien, S. J. (2010). Allostatic load biomarkers of chronic stress and impact on health and cognition. *Neuroscience & Biobehavioral Reviews*, 35(1), 2-16. Retrieved from http://10.0.3.248/j.neubiorev.2009.10.002
- Kirschbaum, C., Kudielka, B. M., Gaab, J., Schommer, N. C., & Hellhammer, D. H. (1999). Impact of gender, menstrual cycle phase, and oral contraceptives on the activity of the

hypothalamus-pituitary-adrenal axis. *Psychosomatic Medicine*, 61(2), 154-162. https://doi.org/10.1097/00006842-199903000-00006

- Kirschbaum, C., Pirke, K. M., & Hellhammer, D. H. (1993). The "Trier Social Stress Test"–a tool for investigating psychobiological stress responses in a laboratory setting. *Neuropsychobiology*, 28(1-2), 76-81. https://doi.org/10.1159/000119004
- Kudielka, B. M., & Kirschbaum, C. (2005). Sex differences in HPA axis responses to stress: A review. *Biological Psychology*, 69(1), 113-132. https://doi.org/10.1016/j.biopsycho .2004.11.009
- Lenow, J. K., Constantino, S. M., Daw, N. D., & Phelps, E. A. (2017). Chronic and acute stress promote overexploitation in serial decision making. *The Journal of Neuroscience*, 37(23), 5681-5689. https://doi.org/10.1523/JNEUROSCI.3618-16.2017
- Lighthall, N. R., Gorlick, M. A., Schoeke, A., Frank, M. J., & Mather, M. (2013). Stress modulates reinforcement learning in younger and older adults. *Psychology and Aging*, 28(1), 35-46. https://doi.org/10.1037/a0029823
- Lovallo, W. R. (1997). Behavioral medicine and health psychology series, Vol. 1.Stress & health: Biological and psychological interactions. Sage Publications, Inc.
- Luck, S. J. (2005). An introduction to the event-related potential technique. Cambridge, MA: MIT Press.
- Lupien, S. J., McEwen, B. S., Gunnar, M. R., & Heim, C. (2009). Effects of stress throughout the lifespan on the brain, behaviour and cognition. *Nature Reviews Neuroscience* 10(6), 434-445. https://doi.org/10.1038/nrn2638
- Mather, M., & Lighthall, N. R. (2012). Both risk and reward are processed differently in decisions made under stress. Current Directions In Psychological Science, 21(2), 36-41.
- McEwen, B. S. (2007). Physiology and neurobiology of stress and adaptation: Central role of the brain. *Physiological Reviews*, 87(3), 873-904. Retrieved from http://10.0.4.128/ physrev.00041.2006
- McEwen, B. S., & Sapolsky, R. M. (1995). Stress and cognitive function. Current Opinion in Neurobiology, 5(2), 205-216. https://doi.org/10.1016/0959-4388(95)80028-X
- Ossewaarde, L., Qin, S., Van Marle, H. J. F., van Wingen, G. A., Fernández, G., & Hermans, E. J. (2011). Stress-induced reduction in reward-related prefrontal cortex function. *NeuroImage*, 55(1), 345-352. Retrieved from http://10.0.3.248/j.neuroimage.2010.11.068
- Otto, A. R., Raio, C. M., Chiang, A., Phelps, E. A., & Daw, N. D. (2013). Working-memory capacity protects model-based learning from stress. *Proceedings of the National Academy of Sciences*, 110(52), 20941-20946. https://doi.org/10.1073/pnas.1312011110
- Pabst, S., Schoofs, D., Pawlikowski, M., Brand, M., & Wolf, O. T. (2013). Paradoxical effects of stress and an executive task on decisions under risk. *Behavioral Neuroscience*, 127(3), 369-379. https://doi.org/10.1037/a0032334
- Pelli, D.G., 1997. The VideoToolbox software for visual psychophysics: Transforming numbers into movies. *Spatial Vis*, 10(4), 437-442. https://doi.org/10.1163/156856897X00366
- Petzold, A., Plessow, F., Goschke, T., & Kirschbaum, C. (2010). Stress reduces use of negative feedback in a feedback-based learning task. *Behavioral Neuroscience*, 124(2), 248-255. https://doi.org/10.1037/a0018930
- Polich, J. (2007). Updating p300: An integrative theory of P3a and P3b. Clinical Neurophysiology, 118(10), 2128-2148. https://doi.org/10.1016/j.clinph.2007.04.019
- Proudfit, G. H. (2015). The reward positivity: from basic research on reward to a biomarker for depression. *Psychophysiology*, 52(4), 449-459. https://doi.org/10.1111/psyp.12370
- Putman, P., Antypa, N., Crysovergi, P., & van der Does, W. A. (2010). Exogenous cortisol acutely influences motivated decision making in healthy young men. *Psychopharmacology*,

208(2), 257-263.

- Qi, M., Gao, H., & Liu, G. (2017). Effect of acute psychological stress on response inhibition: An event-related potential study. *Behavioural Brain Research*, 323, 32-37. https://doi.org/ 10.1016/j.bbr.2017.01.036
- Radenbach, C., Reiter, A. M. F., Engert, V., Sjoerds, Z., Villringer, A., Heinze, H. J., Schlagenhauf, F. (2015). The interaction of acute and chronic stress impairs model-based behavioral control. *Psychoneuroendocrinology*, 53, 268-280. https://doi.org/10.1016/ j.psyneuen.2014.12.017
- Sambrook, T. D., & Goslin, J. (2015). A neural reward prediction error revealed by a metaanalysis of ERPs using great grand averages. *Psychological Bulletin*, 141(1), 213-235. https://doi.org/10.1037/bul0000006
- Sapolsky, R. M., Romero, L. M., & Munck, A. U. (2000). How do glucocorticoids influence stress responses? Preparative actions. *Endocrine Reviews*, 21(1), 55-89.
- Shackman, A. J., Maxwell, J. S., McMenamin, B. W., Greischar, L. L., & Davidson, R. J. (2011). Stress potentiates early and attenuates late stages of visual processing. *The Journal of Neuroscience*, 31(3), 1156-1161. https://doi.org/10.1523/JNEUROSCI.3384-10.2011
- Shields, G. S., Sazma, M. A., & Yonelinas, A. P. (2016). The effects of acute stress on core executive functions: A meta-analysis and comparison with cortisol. *Neuroscience and Biobehavioral Reviews*, 68, 651-668. https://doi.org/10.1016/j.neubiorev.2016.06 .038
- Spielberger, C. D. (1983). Manual for the State-Trait Anxiety Inventory. In Self-Evaluation Questionnaire. Retrieved from http://ci.nii.ac.jp/naid/10007926257/en/
- Starcke, K., & Brand, M. (2012). Decision making under stress: A selective review. Neuroscience and Biobehavioral Reviews, 36(4), 1228-1248.https://doi.org/10.1016/j.neubiorev .2012.02.003
- Starcke, K., Wiesen, C., Trotzke, P., & Brand, M. (2016). Effects of acute laboratory stress on executive functions. *Frontiers in Psychology*, 7(MAR), 1-8. https://doi.org/10.3389/ fpsyg.2016.00461
- Tanaka, M. (1999). Emotional stress and characteristics of brain noradrenaline release in the rat. Industrial Health, 37(2), 143-156.
- Tiferet-Dweck, C., Hensel, M., Kirschbaum, C., Tzelgov, J., Friedman, A., & Salti, M. (2016). Acute stress and perceptual load consume the same attentional resources: A behavioral-ERP Study. *PLoS ONE*, 11(5), 1-19. Retrieved from http://10.0.5.91/journal.pone .0154622
- van den Bos, R., Harteveld, M., & Stoop, H. (2009). Stress and decision-making in humans: Performance is related to cortisol reactivity, albeit differently in men and women. *Psychoneuroen-docrinology*, 34(10), 1449-1458. https://doi.org/10.1016/J.PSYNEUEN.2009.04.016
- van den Bos, R., Homberg, J., & de Visser, L. (2013). A critical review of sex differences in decision-making tasks: Focus on the Iowa Gambling Task. *Behavioural Brain Research*, 238, 95-108. https://doi.org/10.1016/j.bbr.2012.10.002
- van Oort, J., Tendolkar, I., Hermans, E. J., Mulders, P. C., Beckmann, C. F., Schene, A. H.,... van Eijndhoven, P. F. (2017). How the brain connects in response to acute stress: A review at the human brain systems level. *Neuroscience and Biobehavioral Reviews*, 83(April), 281-297. https://doi.org/10.1016/j.neubiorev.2017.10.015
- Villada, C., Hidalgo, V., Almela, M., & Salvador, A. (2016). Individual differences in the psychobiological response to psychosocial stress (Trier Social Stress Test): The relevance of trait anxiety and coping styles. Stress & Health: Journal of the International Society for the Investigation of Stress, 32(2) 90-99. Retrieved from http://10.0.3.234/smi.2582

- Walsh, M. M., & Anderson, J. R. (2012). Learning from experience: Event-related potential correlates of reward processing, neural adaptation, and behavioral choice. *Neuroscience* and Biobehavioral Reviews, 36(8), 1870-1884. https://doi.org/10.1016/j.neubiorev .2012.05.008
- Watson, D., Clark, L. A., & Tellegen, A. (1988). Development and validation of brief measures of positive and negative affect: The PANAS scales. *Journal Of Personality And Social Psychology*, 54(6), 1063-1070.
- Wemm, S., & Wulfert, E. (2017). Effects of acute stress on decision making. Applied Psychophysiology & Biofeedback, 42(1), 1-12.
- Wilson, R. C., Geana, A., White, J. M., Ludvig, E. A., & Cohen, J. D. (2014). Supplementary Material: Humans use directed and random exploration to solve the explorationexploitation dilemma. *Journal of Experimental Psychology: General*, 143(6), 2074-2081. https://doi.org/10.1037/a0038199.Humans
- Yerkes, R. M., & Dodson, J. D. (1908). The relation of strength of stimulus to rapidity of habit formation. Journal of Comparative Neurology & Psychology, 18, 459-482. https:// doi.org/10.1002/cne.920180503

Appendix

TSST Protocol and Script

General Protocol

1.1. Pre-TSST: Introduction

The research participant is then led to Room 4.

This room is set up in the following way: two people are seated behind a table in front of the wall facing the door. The experimenter explains to the research participants their first task, which is to give an introductory talk in front of the panel present, in which he/she is to imagine having been invited to an interview to apply for a job. (experimenter introductions; see below)

1.2. TSST: Preparation Phase

The experimenter than leaves and the participant stays in Experiment Room 4, where they have three minutes to prepare his/her talk. The participant is allowed to take notes but he/she must not use them during his/her speech in front of the audience. The panel is in the room with them.

1.3. TSST: Task 1 (free speech)

The camera is switched on and the research participant is asked to start the talk. All members of the audience remain quiet, as long as the research participant continues to speak fluently. Only after a pause of more than twenty seconds prior to the end of the five-minute period are questions asked.

1.4. TSST: Task 2 (arithmetic task)

After the five-minute period the research participant is then informed by the chair about the second half of his/her task (instructions: see below). This part of the test should be concluded after a maximum of five minutes, insofar as the participant does not reach a count of "0" before that. It is recommended that prime numbers be used as subtractors for this task, since these make the task more difficult.

1.5. Post-TSST: Further Assessment (saliva samples and questionnaires)

The research participant then goes back to Room 1, where the post-TSST assessment takes place.

The TSST Panel Members Instructions

1.6. Neutral impression

A crucial characteristic of the TSST is the impression that the panel should make. The principal aspect of the TSST is the role play, and for that it is important that everybody involved play their respective roles to the best of their abilities. As for the panel, which has to decide about the acceptance of an applicant for a specific position, the issue is therefore to make an impression that leaves no doubt about the seriousness of this endeavour. Furthermore, the TSST is meant to be a psychological stress situation; for that, it is important to maintain a serious impression.

In any case, talk about the situation as such should be avoided before the TSST. Any role play loses its realism (and with that its stress-inducing effect) if the research participant is made aware of it through discussion. It is clear that a real job interview would never take place like this in real life and that the TSST can only be a compromise - however, that should only, if at all, be discussed at the time of the introduction or post-test assessments and debriefing, but not during the actual task. Therefore, it is recommended that during the introduction by the exerimenter none of the panel members talk or smile; should the research participant address the panel, one should only return the greeting courteously. If necessary, it can be pointed out that any questions of the research participant should be directed to the experimenter, rather than to the panel.

Furthermore, all panel members should seek eye contact with the participant during the talk; the knowledge that all persons present give the research participant their undivided attention further reinforces the seriousness of the situation for the research participant.

The point of these questions is not to embarrass the research participants or to be mean to him/her. This is neither the purpose nor the task of the TSST and would also distort the contents of this role play. The research participant's task is to present him/herself before an audience. The questions should serve to deepen this presentation and to receive information about specific qualities of the applicant.

1.6.1 Taking notes

The actual task of the panel starts three minutes after the research participant has begun taking notes. Furthermore, the panel can note the number of errors and the number that the research participant has eventually reached as a performance measure.

1.7. The TSST Chairperson of the Panel

1.7.1 Opening of the session, starting of taping devices

At this time, the chairperson of the panel should turn on the video camera by hand or remote control (making sure he/she knows the operating instructions beforehand). He/she opens up the session with the words "*Please begin your talk*". The Chair will also have access to a clipboard, piece of paper, and timer. The clipboard and piece of paper are to be used to mark down errors during the interview and cognitive task, while the timer is to make sure the participant is not going over time.

1.7.2 Addressing the research participant for Task 1

Only the chairperson should address the research participant directly, so that coordination problems between the panel members can be avoided. During the interview, the Chair should take notes infrequently. They should be related to the behaviour of the participant (e.g., "Participant stumbled when explain their talk"). We will not actually be analyzing these notes, but they are to enhance the realism of the task. Try to avoid taking too many notes, as we do want to give the appearance that your attention is focused on the participant.

The chair should let the research participant speak for the first three minutes. In most cases, the participant will come to the end of the talk even before three minutes have passed. The chair should then give him/her time to formulate additional elaborations. In any case, there should be a pause. After about a twenty-second pause, the chair can alert the research participant to the remaining time, as with the phrase "You still have time, please continue...". Should it appear after another ten seconds that the participant has nothing further to say, then the chair should ask questions until the end of the time period. The phrasing of these questions is left to the chair's discretion; it may also be solely oriented towards the participants' previous statements.

Only in rare instances, will the research participant be able to talk alone for the full five minutes.

In this case, it is left to the discretion of the chair whether he/she wants to intervene between the third and fifth minute to ask questions of the research participant or whether the participant is allowed to continue. This should also be dependent on what is being said by the participant. For instance, it is not appropriate for the applicant to speak in great detail about specific lessons he/she may have learned in the course of training at university or elsewhere. Some research participants use their school-knowledge to distract from their own person. In this case, the chair should certainly intervene, for example by saying "We believe you that you know how to execute a market analysis, but we would be more interested to find out why you were so involved in or drawn to this area".

1.7.3 Addressing the research participant for Task 2

After the first five minutes, it is the chair's duty to explain the second part of the stress protocol. To avoid the possibility of the research participant becoming annoyed, it is very important to make it clear that this is indeed a second task that has nothing to do with the application talk. In the past, some participants have refused to engage in mental arithmetic because they felt (rightly so) that it had nothing to do with their job application. The chair will mark down errors during the task on the paper provided.

1.7.4 End of the session

At the end of the test period, the chair should thank the research participant for his/her participation and ask him/her to go to the neighbouring room for post-test assessments and debriefing. With that, the panel's role in the TSST is concluded.

Script

Before beginning make sure that the following is in the TSST room:

- 1. Clipboard with script
- 2. Pen (x2)
- 3. Loose piece of paper for the participant
- 4. Loose piece of paper to mark down errors
- 5. Stopwatch

The 'Pre-TSST Experimenters' Introduction

Experimenter: "Your task in this part of the experiment is the following: please imagine that you have applied for your ideal job and have been invited for an interview. You must now convince the panel members why you are the ideal candidate by giving a talk. You will have three minutes to prepare a talk to convince the panel, before having five minutes to present the talk. Please note that you will be recorded by a camera for subsequent voice and behavioural analysis. This is the "selection panel" (introduce panel). This selection panel has been trained to monitor your behaviour and will take notes during your talk. You should try to leave the best possible impression and assume the role of the applicant for the duration of the talk as best as you can. The panel will reserve the right to ask follow-up questions in case of uncertainties to receive all necessary information from you. Following your talk, you will be given a second task by the panel, which will only be explained to you by the panel. You may take some notes now, which you must not use during your talk. Do you have any questions?

You now have three minutes to prepare your speech. There is a pen and pencil on the table for your use."

Opening up the session

Chair begins video recording and hits start on the timer

Chair: "Please begin your talk. You may not use the notes you have made."

(If participant stops before 5 minutes, wait for about 20 seconds and then say)

Chair: "You still have time left. Please continue."

(If participant does not continue, start asking questions after 10 seconds)

Questions to ask the research participant during the "job interview"

- 1. What are your personal strengths?
- 2. What are your major weaknesses?
- 3. Why do you think you are especially well-qualified for this task?
- 4. Why do you think you are better qualified then the other applicants?
- 5. You just mentioned your qualities in respect to..., what do you in particular think about...?
- 6. You just spoke about..., what exactly do you then think about...?
- 7. What kind of leading qualities do you have?
- 8. What do you think about teamwork?
- 9. Where do you see your position in a team?
- 10. What can you constructively add to a team?
- 11. What do your employees appreciate about you most?
- 12. Would you be willing to work on the weekends if this be deemed necessary?
- 13. What kind of qualities to you expect from your co-workers?
- 14. Under what circumstances would you be willing to compensate for the mistakes your co-wworkers make?
- 15. What do your family/friends especially appreciate about you?
- 16. Please complete the following sentence: "I am the best at/in..."

(When either the time is up or if the participant goes over the five minutes, let them finish their sentence, and then say)

Chair: "Stop, the interview is now over"

Cognitive task (5 minutes)

Chair: "We now want you to solve a calculation task. This task is unrelated to the job interview. Please count aloud backwards from 2023 in steps of 17. Please calculate as quickly and correctly as possible. Should you miscalculate, we will point out your mistake and you have to start over again. Do you have any questions?"

(Chair should mark down errors on the paper. If the participant looks for whether they are correct or not, simply nod for correct answers. Should the participant miscalculate say)

Chair: "Stop - mistake. Start over at 2023 please."

2023	1683	1343	1003	663	323
2006	1666	1326	986	646	306
1989	1649	1309	969	629	289
1972	1632	1292	952	612	272
1955	1615	1275	935	595	255
1938	1598	1258	918	578	238
1921	1581	1241	901	561	221
1904	1564	1224	884	544	204
1887	1547	1207	867	527	187
1870	1530	1190	850	510	170
1853	1513	1173	833	493	153
1836	1496	1156	816	476	136
1819	1479	1139	799	459	119
1802	1462	1122	782	442	102
1785	1445	1105	765	425	85
1768	1428	1088	748	408	68
1751	1411	1071	731	391	51
1734	1394	1054	714	374	34
1717	1377	1037	697	357	17
1700	1360	1020	680	340	0

End of Session (5 minutes)

Chair: "Thank you for your time. Please leave the room where the experimenters will be waiting. You will then complete the rest of the experimental session and will be given a full debrief at the end of the experiment."

Placebo TSST Instructions (Het et al., 2009)

(participant is led by the experimenter to an empty room where they are instructed)

Experimenter: "You are now going to have three minutes to think about a talk about a recent movie, novel, or holiday. After the three minutes is up, you will have to talk aloud for 5 minutes about the topic of interest. You will not be giving the talk to anyone and you will not be recorded. Just take the time to think about what you would like to say about the topic of interest."

(The experimenter leaves the room)

(After three minutes has elapsed)

Experimenter: "You will now have five minutes to start your talk. Again, there is no recording, and no one is listening."

(The experimenter leaves the room)

(After five minutes has elapsed)

Experimenter: "Okay please start counting up in steps of 15, starting at 0. This will last for five minutes."

(Experimenter then leaves the room)

(After five minutes has elapsed)

Experimenter: "What number did you get to?"