FACULTY OF PSYCHOLOGY AND EDUCATIONAL SCIENCES

THE EFFECTS OF STRESS ON BRAIN-FUNCTIONALITY: AN EEG-STUDY

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2021 has proven to be an incredibly difficult year, not only on a global level but also on a more personal level. Therefore, I am very proud that I managed to finish this master's thesis. It is ironic how one could write a thesis on stress while writing a thesis in itself is one of the most stressful processes in the Master's programme. Of course, I couldn't have done this without the support of a great load of people, so many that I cannot give everyone the thank you that they deserve in this small section. There are some people I would like to address personally, hoping not to insult anyone I may have forgotten.

To anyone who may read this:

> Enjoy reading this Master dissertation. Take your time reading it, because it for sure took some time writing it.

To everyone who helped me:

> Thank you for all the support. Thank you to my friends and family, whom I could always count on for advice and feedback. Thank you to my supervisor, Gert Vanhollebeke, for the countless revisions and feedback sessions. Thank you to all the participants who let me poke on their skull to get good EEG signals and spent hours in the University Hospital in the middle of a global pandemic.

To my grandmother:

> Thank you for all the support, guidance and wise lessons you thought me throughout your life. I hope you are proud, knowing I gave it my all.

Abstract

Living under stress has an immense impact on one's body. This is also the case for the human brain. A possible indicator for stress has been identified as frontal alpha asymmetry. This study tries to identify this frontal alpha asymmetry using electroencephalography (EEG) and sees which personality characteristics predict the magnitude of this effect. By showing participants a false comparison in relation to a fictional, successful group, participants are brought under psychosocial stress while their brain activity is being monitored. Based on this data, we did not find an increased frontal alpha asymmetry after the experimental manipulation. Instead, individual characteristics such as ruminative behaviour and trait regret were predictive for frontal alpha asymmetry following the stress condition. Frontal alpha asymmetry did not variate with age but we did see a stronger asymmetry shift in women compared to men. These findings highlight the importance of individual characteristics in frontal alpha asymmetry. Instead of focussing on a universal diagnostic feature of stress, the focus should shift towards understanding the impact of these individual differences on one's stress response.

Nederlandse samenvatting

Langdurige stress heeft vele negatieve invloeden op het menselijke lichaam. Dit is ook het geval in de hersenen. Voorgaand onderzoek heeft frontale alfa asymmetrie geïdentificeerd als een mogelijke indicator voor stress. Met deze studie trachten we deze frontale alfa asymmetrie bloot te leggen aan de hand van elektro-encefalografie (EEG) en te zien welke persoonlijkheidskenmerken de grootte van dit effect voorspellen. Door deelnemers een valse vergelijking te tonen ten opzichte van een fictieve, succesvolle groep worden deelnemers onder psychosociale stress gebracht terwijl hun hersenactiviteit wordt gemeten. Een algemene verhoging van frontale alfa asymmetrie is niet teruggevonden tijdens de stress conditie, maar persoonlijkheidskenmerken zoals ruminatief gedrag en spijtgevoelens bleken een verklarende rol te hebben in de frontale alfa asymmetrie variaties na de stress conditie. Het effect varieerde niet met de leeftijd van de participanten, maar we zagen wel een verschil in frontale alfa asymmetrie tussen mannen en vrouwen. Zo hadden vrouwen een meer negatieve alfa asymmetrie, wat wijst op een grotere alfa power in de linkerhersenhelft. Op basis van deze resultaten lijkt frontale alfa asymmetrie geen universele maat voor stress te zijn, en wijst het op het belang van individuele verschillen in de stress respons.

Content

The Effects of Stress on Brain-Functionality: an EEG-Study

Defining Stress

Stress is used in a variety of contexts and has a broad meaning. One definition, proposed by Lazarus and Folkman (1984), defined stressful situations as situations where there is a discrepancy between the demands and expectations of the environment and a person's ability to meet these expectations. This definition is very broad, making stress a widely applicable concept.

Stress is linked to some of the most deadly diseases of the 21st century and has a significant effect on people's mental health. Studies show that stress can be linked to the onset of manic episodes in bipolar disorder (Paykel, 2003) and the onset of depression (Kubo, 2007). In a review by Tennant (2002), twin studies showed that environmental stressors explain as much variance in depression as genetic factors. Stress also has an impact on brain development (Lupien, McEwen, Gunnar & Heim, 2009), where continuous exposure to stress could lead to altered activation in the frontal cortex, amygdala and hippocampus.

When we look at the list of the most deadly diseases in 2016 (WHO.it, 2018), cardiovascular disease is reported as the most deadly disease, causing 15.2 million deaths worldwide. One key factor in the onset and development of these diseases is stress. Chronic exposure to stress increases the risk of coronary heart disease and one of the most common triggers for major cardiac events are stressful events (Steptoe & Kivimäki, 2013). Stress plays a role in various physical diseases. In a meta-analysis by Chida, Hamer, Wardle and Steptoe (2008) the incidence of cancer was higher in populations living in stressful conditions. Not only was the incidence of cancer higher, but the cancer mortality rate was also elevated for this group.

The definition of stress by Lazarus and Folkman (1984) can be applied to a variety of situations. Therefore, these situations should be further specified in research to make valid comparisons. This raises the question for a universal taxonomy of stress.

A Taxonomy for Stress (Epel et al., 2018).

Epel et al. (2018) proposed a taxonomy of stress which aims to classify and describe stress based on its responses, duration and dimensions of exposure. The framework first makes a clear distinction between two properties of stress: the situation that causes stress and the stress response. The situation eliciting stress is referred to as the *stressor* and is characterised by its timeframe, life period and assessment window. The *stress response*, which is caused by this stressor, is defined in terms of its specificity to a certain stressor.

Characteristics of the stressor.

Timeframe.

The first specification of the stressor is the timeframe in which the stressor occurs. Epel et al. (2018) divide this into four categories: acute stressors, daily hassles, life events and chronic stressors. *Acute stressors* are by definition short term, these events pass quickly and can be used as an experimental manipulation. An example of an acute stressor could be giving an important presentation, which is also used as a stressor in the Trier Social Stress Test (Kirschbaum, Pirke & Hellhammer, 1993). *Daily hassles* are minor events that occur in daily life. *Life events*, on the other hand, are more stressful than daily hassles and are elicited by a specific, often major, event. An example of a life event could be the death of a family member, while an argument would be labelled as a daily hassle. The final category, *chronic stressors*, are stressful situations that last six months or longer. This could refer for example to growing up in an abusive family.

Life period.

The second characteristic is the life period in which the stressor occurs. This can either be *in utero*, *during the childhood*, *adulthood* or *across the entire lifespan*.

Assessment window.

The final specification is the assessment window of the stressor. This refers to the proximity of the assessment to the actual stressor and the length of the time

period that is evaluated. The time window can either be the current assessment of stress, the assessment at the end of a day or a retrospective assessment, for example reporting the perceived stress over the past 10 years. The proximity of the assessment is then quantified as the time between the assessment and the stressor.

Characteristics of the stress response.

The stress response can be divided into three main components, depending on the specificity of the stressor. The first one is the *global subjective stress*, referring to a stress response that is not linked to a specific stimulus. If we look at a more specific response, we can research the *stress response regarding a specific life domain* such as relationships or work. The most specific response we can investigate is the *response to a specific stimulus or event*.

Impact of Stress on Physiological Measures

Stress causes several measurable changes in the physiological functioning of the human body. Since there is a difference in mechanisms and effects between short-term and long-term exposure to stress, this division should also be made in the literature.

Short-term effects of stress.

When we look at the short-term effects of stress, we look at the changes in bodily arousal when people are confronted with a stressor. This stress response is only temporary, and the body can slowly return to its previous state when the stressor disappears. This is often referred to as the fight-or-flight response.

One of the key circuits in the stress response is the *hypothalamo-pituitary-adrenal (HPA) axis*. This axis is responsible for an increase in cortisol in the body during acute stress (Harbuz & Lightman, 1992). However, this is not the only circuit involved in the processing of stressful stimuli. Another important circuit is the *sympathetic-adrenal-medullary (SAM) system* which is responsible for a release in adrenaline and noradrenaline (Shields, Sazma & Yonelinas,

2016). This system results in an increase in heart rate, increased respiratory rate, sweat secretion and suppression of the immune system (Padget & Glaser, 2003).

Long-term effects of stress.

While the fight-or-flight response is considered an adaptive strategy, being in a nearly constant state of stress is not adaptive. In normal situations, the body slowly returns to its previous state when the stressor disappears. When the body cannot return to its resting state, we refer to this as chronic stress. This type of stress is responsible for the majority of the negative effects of stress.

One of the most important effects of chronic stress, as mentioned earlier is the one on the cardiovascular system. Chronic activation of the HPA axis also impacts the immune system. This increases the risk for various diseases such as diabetes (Wellen & Hotamisligil, 2005), obesity (Jin & Flavell, 2013) and even cancer (Chida et al., 2008). When we look at psychopathology, a review by Staufenbiel et al., (2012) concluded that stress can be linked to mood disorders such as depression and bipolar disorder but also to various anxiety disorders including generalised anxiety disorder, obsessive-compulsive disorder, specific phobia and post-traumatic stress disorder.

Measuring stress in laboratory environments.

Since stress appears to be involved in many different types of disorders, research that looks into its mechanisms is very relevant. The level of stress can be indirectly measured using various techniques. Depending on the type of research, the most appropriate combination of techniques should be chosen to operationalise the stress level of the participants.

Temperature.

In 1991, Marazziti, Di Muro and Castrogiovanni already discovered the impact of the stress response on body temperature. In their study, students showed an increase in axillary temperature during exams compared to a few weeks post-exams. In later research, the division was made between core temperature and skin temperature. A study by Vinkers et al. (2013) revealed that stress has

differential effects, depending on the type of temperature that was measured. Participants were exposed to an acute stressor, the TSST, and temperature was measured during the exposure to this stressor. Core temperature was measured intestinal and at the temporal artery. Here, only the intestinal temperature decreases in response to a stressor while the temperature at the temporal artery remained stable. Skin temperature was measured at the fingertips and the infraclavicular area. For these areas, we see a decrease in temperature at distal areas such as the fingertips while the temperature remains stable at more proximal areas. Looking at the facial skin temperature, we see sex differences with an increase located at the nose for females, and at the cheeks for males. All these differences were found across all subjects which advocate for the inclusion of temperature as a measurement of stress.

Heart rate.

The fight-or-flight response results in a change in heart rate. When we look at the ability of the heart rate to adapt to this response, we often consider the heart rate variability (HRV) which is used to detect differences in the balance between the sympathetic and parasympathetic nervous system. In a review by Castaldo et al. (2015), including 12 articles, the effect of acute stress on different HRV measurements was considered. Looking at the time domain, various measurements of the time between two consecutive peaks showed a decrease in stressful situations.

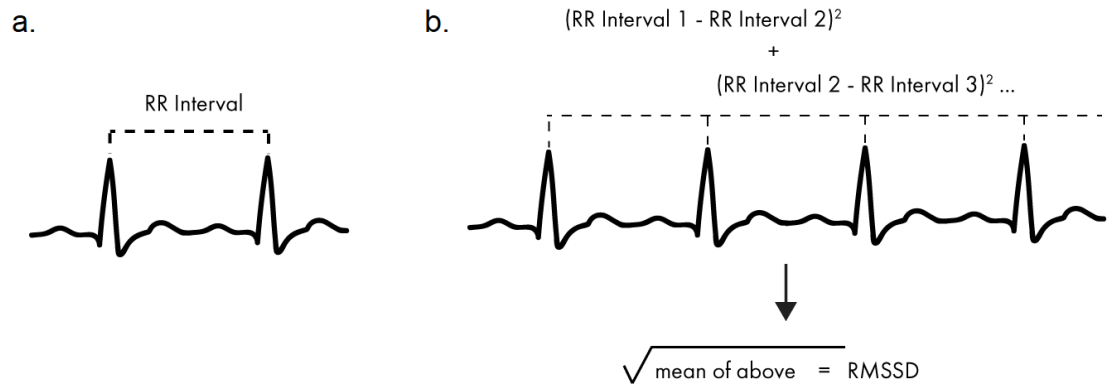


Figure 1. Illustration of (a) the R-R intervals and (b) the RMSSD Illustration retrieved from Farnsworth (2019)

These measurements were retrieved from Malik et al. (1996) and included the mean of R-R intervals (Figure 1a), also referred to as N-N intervals, which reflects the time between two consecutive peaks of the R-wave of the ECG, pNN50 is a proportion calculated by dividing the normal-to-normal intervals (NN) that differ more than 50ms by the total number of NN, and RMSSD (Figure 1b) which is the square root of the mean squared difference between two peaks, giving the absolute value of the difference between the peaks.

Frequencies of HRV can be divided into two bands: high frequency (HF), going from 0.15 to 0.4 Hz and low frequency (LF) from 0.04 to 0.15 Hz. Frequency analysis of HRV includes power in these two frequency bands and the LF/HF ratio. In the review, power in LF showed opposite results with most studies showing an increase in LF power. HF power increased in stressful situations for all papers. Looking at the LF/HF ratio, most papers showed a significant increase during stressor exposure.

Respiratory rate.

During stress, the respiratory rate (RR) increases (Widjaja, Orini & Vlemincx, 2013). But the effect of respiration does not stand alone and seems to interact with the HRV. More specifically, the frequencies of the HF band depend on the RR (Hernando et al., 2016). To perform a correct HRV analysis in the frequency domain, RR should be recorded simultaneously with the heart rate

Saliva samples.

As mentioned earlier, the HPA-axis causes an increase in cortisol. A minimally invasive way to measure cortisol levels is using saliva samples. In the paper by Bozovic, Racic and Ivkovic (2013) saliva cortisol levels increase after a stressful event and reach a peak after 10 to 30 minutes. Another biological marker of stress found in saliva is immunoglobulin A. This component shows an acute increase immediately following stressful events and has a delayed decrease up till days after the stressor (Tsujita & Morimoto, 1999). In the paper by Rohleder et al. (2006), salivary alpha-amylase was proposed as an indirect measure of stress. The levels of salivary alpha-amylase significantly increased during stressful events.

Hair strands.

Cortisol can also be detected in the hair of participants. Because hair has a stable growth rate of 1 cm per month, it provides a retrospective measurement of chronic stress. In the review by Russell, Koren, Rieder and Van Uum (2012) hair cortisol is proposed as a long-term stress measurement.

Skin conductance.

Skin conductance measures the activity of sweat glands which reflect sympathetic activity. In 1963, Lazarus, Speisman and Mordkoff already proposed skin conductance as an autonomic measurement of stress. They used movies to induce stress which is an acute stressor. Their study measured the stress response while watching the movies and they found increased levels of skin conductance while watching a stressful movie. This has later been replicated by multiple studies such as the study by Lin, Lin, Lin and Huang (2011). In this study, participants performed a mental arithmetic task and showed elevated sympathetic activity including an increased skin conductance.

The Impact of Stress on Brain Activity

In the research regarding the impact of stress on brain activity electroencephalography (EEG) is used. EEG is a neuroimaging technique that uses electrodes to pick up voltage changes across the scalp which originate from large groups of pyramidal neurons in the brain (Niedermeyer & da Silva, 2005, p. 23). An EEG cap (see Figure 2a) can consist of anywhere between 1 and 256 electrodes, with 32, 64 and 128 being the most common. These electrodes are divided into different regions such as frontal electrodes, central electrodes, parietal electrodes, temporal electrodes and occipital electrodes, corresponding to the respective brain lobes that lay underneath. The electrodes on a cap are labelled and have odd numbers on the left side and even numbers on the right side (Niedermeyer & da Silva, 2005, p. 145). A schematic representation of an EEG setup can be found in Figure 2b.

Based on these studies, two main effects of stress have been found: *frontal asymmetry* and *frontal midline theta*.

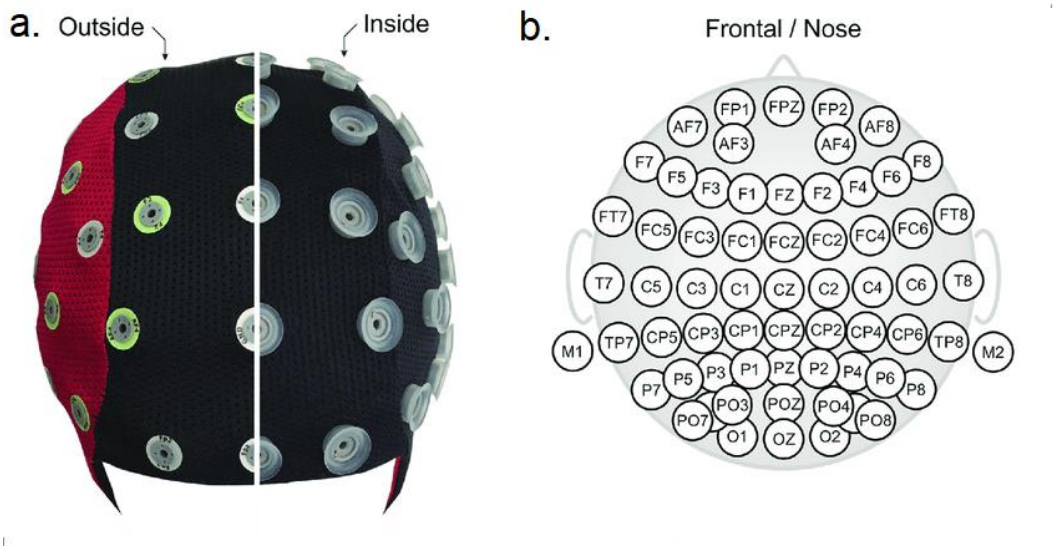


Figure 2. (a) picture of an EEG cap and (b) EEG topography.
Retrieved from di Fronso et al. (2019)

Frontal Midline Theta.

The first effect detectable by EEG is frontal midline theta (FMT). The theta band in EEG usually refers to the frequency band from 4 to 8Hz. FMT is observed during working memory tasks that activate the prefrontal cortex. In stressful situations, the activation in the prefrontal cortex is attenuated, which results in alterations in FMT.

In 1990, Yamamoto and Matsuoka studied the effects of stress on operators of visual display terminals. During acute stressful tasks, theta band increase started on the frontal midline and after one hour the increase was picked up in more parietal regions. Theta activity was also correlated with performance and when the performance decreased, theta activity went down. This effect has also been found in animal models such as in the study of Jacinto et al. (2013). In their study, rats explored novel environments while local-field potentials were recorded. For the control rats, theta power decreased when they got more

familiar with their environment. This was not the case for the rats who were acutely stressed, for whom theta power in the prefrontal cortex and hippocampus increased. Paul et al. (2018) used another acute stressor, a socially evaluated cold pressor test in which the participants then performed a category learning task whilst EEG was recorded. They found FMT increases during stress for atypical trials.

However, in the study by Gärtner, Rohde-Liebenau, Grimm and Bajbouj (2014), the effect was reversed. They looked at the EEG data of 31 healthy male subjects while performing a difficult or easy n-back task. In one block, an acute stressor was presented prior to the task: watching aversive videos. They found that frontal theta power was increased with increasing task difficulty but that stress attenuated this effect and thus resulted in lower theta power. This effect was replicated by Gärtner, Grimm and Bajbouj in 2015. While performing a mental arithmetic task, FMT was increased but for acutely stressed participants FMT decreased.

Tough the direction of the effect of stress on FMT is still unknown, the alteration of FMT during stress is well-established. Future research should look into the causes of these opposite effects and find a common ground that connects both directions into one general framework.

Frontal Asymmetry.

Frontal EEG asymmetry is an index for emotion regulation and is also predictive of the stress response. The frontal lobe is lateralised with the left hemisphere associated to approach motivation while the right hemisphere is involved in avoidance related behaviour (Kelley, Hortensius, Schutter & Harmon-Jones, 2017). To study frontal asymmetry, alpha activity (8-13Hz) is often used as an inverse for brain activity, meaning that more alpha power reflects less cognitive processing in the brain (Jensen & Mazaheri, 2010; Laufs et al., 2003). This has all led to the interest in frontal alpha activity in the field of emotion regulation, anxiety and stress.

In 1992, Fox, Bell and Jones conducted an experiment in which babies between 1 and 2 years old were separated from their caretaker. The response to this acute stressor was assessed during the separation and a resting-state EEG was recorded two months after this separation. From these recordings, the power in the 6 to 9 Hz frequency band was extracted. Babies who cried during the separation showed a relative power increase in the right hemisphere.

Looking at patient studies, a meta-analysis by Meyer et al. (2015) looked into the role of frontal asymmetry in PTSD patients. They found a relative power increase in the right hemisphere when presenting trauma-related images. For depression, participants with a family history of depression and increased stress during their childhood showed elevated right frontal activity during affect-inducing tasks (Lopez-Duran et al, 2012). Since the effects found in patient populations could be attributed to many other factors other than stress, research has also tried to replicate these effects in healthy populations. In the study by Zhang et al. (2018) an acute stressor, the cold pressor test, elicited a stronger right frontal activity during exposure. This effect has also been found when an individual alpha band was used (Quaedflieg, Meyer, Smulders & Smeets, 2015).

Lewis, Weekes & Wang (2007) investigated frontal asymmetry without a stress-evoking experimental paradigm. Instead, they used daily hassles as a stressor and tested 49 students during an examination period. They found that in a week where students had a lot of exams and assignments, they were presented with an increased right alpha activity compared to a week in which they had no exams or assignments.

This frontal asymmetry also has practical applications, it has been used as a classifier using Support Vector Machines (Hou et al., 2016). Arpaia, Moccaldi, Prevete, Sannino and Tedesco (2020) tried to recognise stress on the work floor using a wearable EEG device that consisted of a left and right frontal electrode. Based on these two electrodes and using classifiers, they tried to recognise the response to an acute stressor, namely negative social feedback

during a task. With a latency of 2 seconds, they could correctly identify stress in 97.5% of the cases and an accuracy of 100% when the latency was 4 seconds.

The Present Study

This thesis focuses on the effects of psychosocial stress on frontal alpha activity and its interaction with various measurements including perceived stress, depression, rumination and trait regret. Psychosocial stress is evoked by presenting participants false feedback after they perform Raven's Matrices. This feedback compares their performance to the performance of a fictional reference group, namely people who excelled in their school or work career. We hypothesise that this stressful situation will lead to an increase in frontal alpha asymmetry, meaning a relative increase of alpha power in the right hemisphere compared to the left hemisphere. Furthermore, we explore the influence of individual characteristics such as age, gender, perceived stress, rumination, depression or trait regret that may influence the magnitude of this effect.

Methods

Participants

In total, 83 participants took part in the experiment. 11 of them were excluded due to faulty EEG recording. After exclusion, 72 participants were included in the analysis of which 25 men and 46 women. The mean age was 23.15 (SD = 6.29). Based on a power analysis for a within factors, repeated measures anova (f = 0.15, alpha = 005 and power = 0.8) the required sample size was 73.

The participants were recruited online. They all were born after 1970, natively Dutch-speaking and had no history of cardiac, respiratory, neurological or psychiatric disorders. All participants had normal or corrected eyesight and were right-handed. Psychology students were not allowed to participate, as well as pregnant women. Further exclusion criteria were dreadlocks, facial skin conditions, electronic implants, cochlear implants, metal or magnetic objects in or around the head and recent neurological procedures. Participants could not be on psychotropic medications and were asked not to use caffeine or nicotine two hours before the start of the experiment as this can influence the heart rate and blood pressure (MacDougall, Musante, Castillo & Acevedo, 1988) and the EEG power (Gilbert, Dibb, Plath & Hiyane, 2000). All participants received a reward of €30 for participating.

Materials

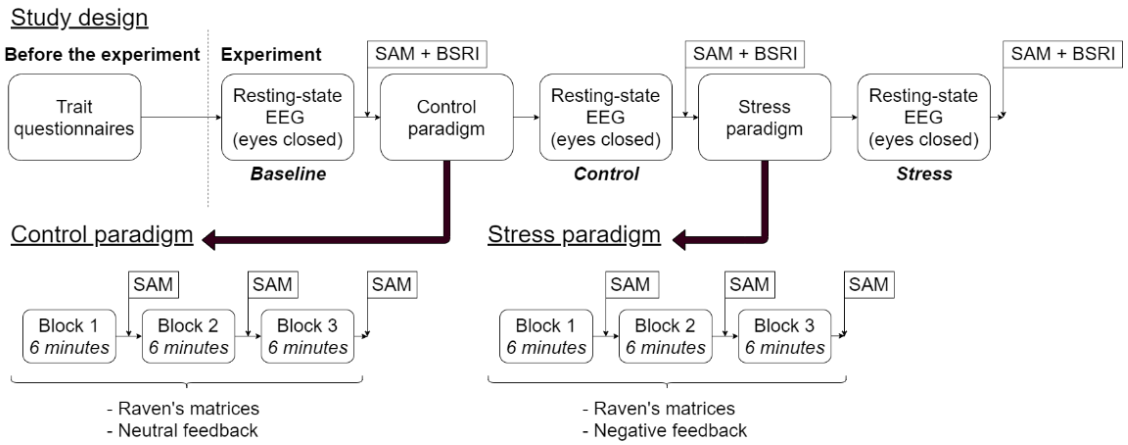


Figure 3. Experimental design

Questionnaires prior to the experiment.

Before the start of the experiment, participants filled in an online survey which consisted of 5 trait questionnaires: the *Perceived Stress Scale* (PSS), *Beck's Depression Inventory* (BDI), the *Ruminative Response Scale* (RRS), the Self-*Critical Rumination Scale* (SCRS) and the *Trait Regret Scale* (TR) (Trait Questionnaires in Figure 3).

The PSS (α = 0.85) was designed by Cohen, Kamarck and Mermelstein in 1983 to measure the extent to which situations are perceived as stressful. The questionnaire consists of 14 items for which the participant should indicate how often this occurred in the last month. Scores for each item ranged from 0 to 4 with 0 representing never and 4 very often. This results in a total score between 0 and 56 with higher scores representing more perceived stress.

The behavioural manifestations of depression were quantified using the BDI (α = 0.93) (Beck, Steer & Brown, 1996). This scale uses 21 categories with 4 or 5 items per category. For each category, a score between 1 and 3 is administered, resulting in a score between 0 and 63. Scores between 0 and 13 are considered minimal range, 14-19 mild depression, 20-28 moderate depression and 29-63 as severe depression.

The RRS (α = 0.90) (Nolen-Hoeksema & Morrow, 1993, Treynor, Gonzalez & Nolen-Hoeksema, 2003) measures the two key components of rumination: brooding and reflection. 22 items are scored on a 4-point Likert scale with a score of 0 indicating almost never and a score of 4 meaning almost always. This results in a total score between 22 and 88.

The SCRS consists of 10 items and was designed to specifically measure self-critical rumination (α = 0.92) (Smart, Peters & Baer, 2015). The participant had to score each item on a 4-point scale, indicating how well each item described them (1 = not at all, 2 = a little, 3 = moderately, 4 = very much). This results in a total score between 10 and 40.

The last questionnaire was the TR (α = 0.67), which was used to measure regret (Schwartz et al., 2002). This scale uses 9 items and a 7-point Likert scale (1= completely disagree, 7 = completely agree) which results in a summed score between 9 and 63.

Measurements during the experiment.

During the experiment, various physiological measurements are taken. The first set of measurements relate to the stress level of the participants and will be used to check if the acute stressor that is used as our experimental manipulation did result in a physiologically measurable stress response. *Skin conductance* (SC) is measured by placing two electrodes on the middle phalanx of the index and middle finger of the left hand (Figure 4a). The *respiratory rate* (RR) is measured by placing a band with resistive sensors around the chest of the participants. The last measurement is the *heart rate* (HR) which is recorded using an electrocardiogram (ECG). This is done by placing two electrodes on the left upper and lower ribcage (Figure 4b). Based on the HR, various measurements of heart rate variability (HRV) can be computed for each participant.

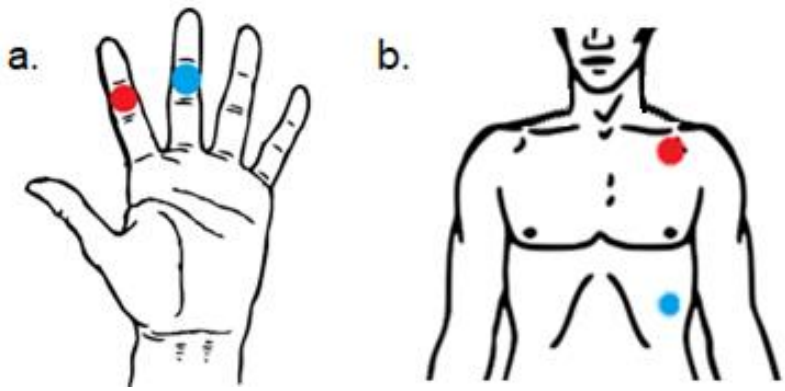


Figure 4. Placement of the electrodes for (a) skin conductance and (b) electrocardiogram.

Additionally, the participants also wore two smartwatches. On the left wrist, the participants wore a Chill+ smartwatch (Imec, Leuven, Belgium). On the right wrist, participants wore an E4 smartwatch (Empatica, Milano, Italy). These smartwatches were both designed to measure galvanic skin response,

photoplethysmography, skin temperature and movement. However, the results of these smartwatches will not be discussed further in this paper since it is beyond the scope of this research.

For the EEG an ant-neuro waveguard medium 64 electrodes (wet) cap (ANT neuro, Hengelo, Netherlands) was used and the signal was recorded using Micromed System PLUS EVOLUTION software (Micromed, Mogliano Veneto, Italy) and amplified with Micromed SD LTM (Micromed, Mogliano Veneto, Italy). The system uses both the ground electrode and Cz as a reference and had a sampling rate of 512 Hz.

The experiment used both a computer and a tablet to record responses. On the computer, the experiment was displayed using Eprime 2.0 professional (Psychology Software Tools, Sharpsburg, USA), the tablet used a custom-made application. During the experiment, the participants filled in the *Self-Assessment Manikin* (SAM) and *Brief State Rumination Inventory* (BSRI) on this tablet. The SAM is used as a non-verbal measure of pleasure, arousal and dominance (Bradley & Lang, 1994). The BSRI ($\alpha = 0.89$) measures state rumination using 8 items on a visual analogue scale ranging from “completely disagree” to “completely agree” (Marchetti, Mor, Chiorri & Koster, 2018).

Another part of the study is stress recognition in speech (See Speech blocks in Figure 5 and 7). For this research, participants were asked to read a text which was retrieved from Van den Broecke (1988). These speech sessions will be discussed in the method section to provide a full overview of the experimental paradigm but will not be included in the results since this is beyond the scope of our research.

Procedure

Baseline measurements.

At the start of the experiment, an EEG baseline recording was collected while the participants sat in front of the screen with their eyes closed for 10 minutes (see Resting-State EEG in Figure 3). When the 10 minutes were finished, participants were informed by a beep (440 Hz sine wave) that they could open

their eyes again. Then they had to switch to the tablet where they had to read a text aloud and fill in the BSRI (see Figure 3).

Control paradigm.

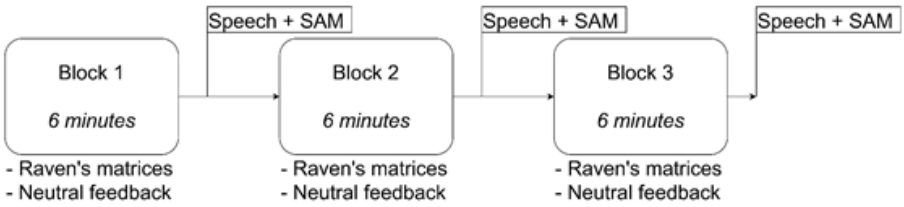


Figure 5. Control Paradigm

The second phase of the study is the control paradigm (see Figure 3 and Figure 5), the participants performed three blocks of Raven's Matrices on the computer. Each block consisted of maximum 11 matrices, the block ended when the participants either solved all matrices or when the block lasted longer than 6 minutes.

After each matrix, the participants received feedback which consisted of three key elements: the correct/false indication, the reaction time and a comparison with other participants. In the control paradigm, participants were told that this comparison was based on previous participants. A visual representation of the feedback is shown in Figure 6. What the participants did not know was that this comparison was randomly generated and always displayed a similar or higher score than the participant. Between each block, the participants had to fill in the SAM on the tablet and read the same text aloud (see Speech and SAM in Figure 3 and Figure 5).

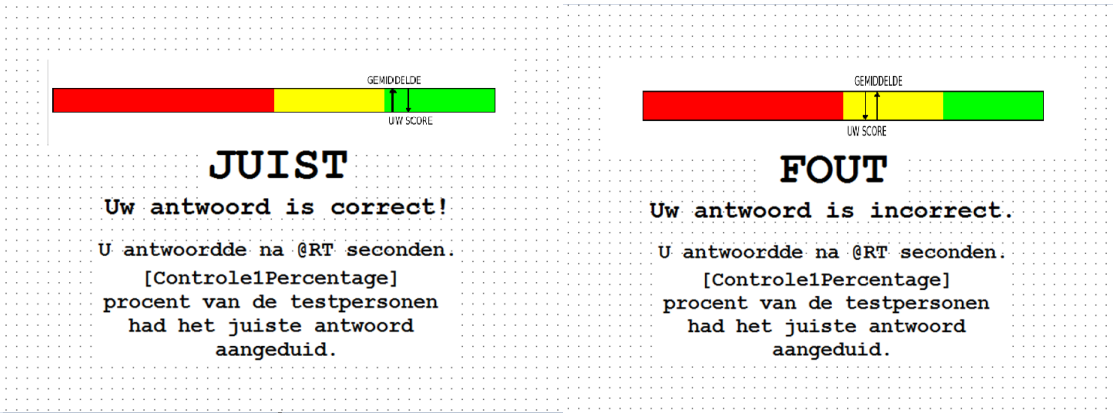


Figure 6. Feedback during the control paradigm

After the three blocks with Raven's Matrices, the participants did another rest block of 10 minutes where we recorded the EEG while the participants had their eyes closed (Resting-state EEG block in Figure 3). This rest block was also followed by a speech session and filling in BSRI and SAM on the tablet (Figure 3).

Stress paradigm.

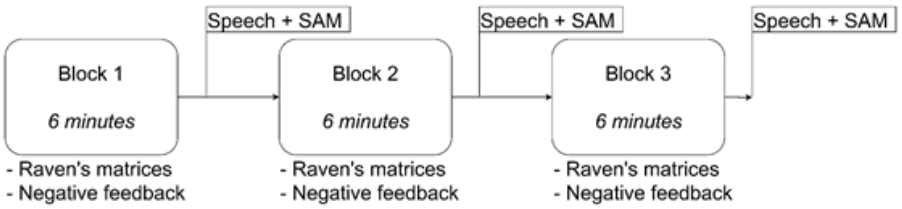


Figure 7. Stress Paradigm

In the second part of the study, the participants performed three more blocks of Raven's matrices, alternated with speech sessions and filling in the SAM (Figure 3 and Figure 7) At the beginning of this phase, participants were informed that their performance would be compared to a group that has successfully completed higher education and performed above average professionally.

For this phase, the feedback was altered as an acute stressor. Now the performance of the participant started at the level of the comparison group but gradually started to decrease until it was in the red zone (Figure 8). For false responses, the feedback was also formulated differently than for the control paradigm (see Figure 8).

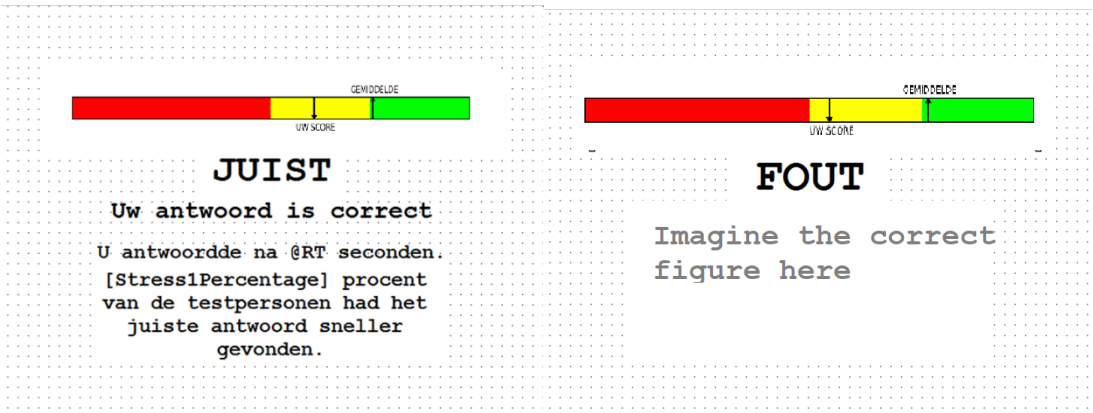


Figure 8. Feedback during the stress paradigm

After the three blocks with Raven's Matrices, the participants performed a final rest block of 10 minutes where the participants had their eyes closed (see Resting-state EEG in Figure 3). This rest block was lastly followed by a speech session and filling in BSRI and SAM on the tablet.

At the end of the experiment, participants were asked what they thought the study was about. Using these reports, we could identify participants who did not believe the feedback. After these reports, participants were informed that the feedback was false and got an explanation about the actual purpose of the experiment.

Results

Data-extraction and analysis

The EEG data was pre-processed using Brainvision Analyzer 2 (Brain Products GmbH, Gilching, Germany). Powerline noise was eliminated using a Notch filter at 50 Hz and the data was subsequently filtered between 0.5 and 40 Hz using a low pass and a high pass filter (8th order, Butterworth). After filtering, bad channels were interpolated using spline interpolation (order 4). Next data from the three segments of interest, namely the baseline, control and stress resting-state blocks, were extracted and for each segment separately artefact correction was performed using independent component analysis (ICA). ICA components containing eye movement or electrical artefacts were identified manually. After ICA correction, data was re-referenced to an average all reference. The remaining artefacts were removed using manual inspection by means of a custom-made tool in MATLAB R2018a (The MathWorks Inc, Natick, USA) as well as the epoching of the data, which was also conducted in Matlab.

All data was then analysed using MATLAB R2018a. After pre-processing, the EEG dataset consisted of 201 epochs of 3 seconds for each condition, resulting in 603 epochs per participant. Frontal alpha asymmetry was calculated using the method of Smith et al. (2017). We extracted the data from F7 and F8, two frontal electrodes located respectively on the left and right side. each 3-second epoch was transformed into a frequency spectrum ranging from 0 Hz to 256 Hz by using a Fast Fourier transformation. From this spectrum, the alpha power (8-13 Hz) were extracted and log-transformed. These transformed alpha powers were summed together and averaged to obtain an average alpha power value for each electrode, per participant, per condition. The asymmetry score was then computed by subtracting the score for the left hemisphere from the score of the right hemisphere (F8-F7).

To examine the differences between each condition a one-way ANOVA was executed and to look at the effect of questionnaire results and demographic variables, univariate linear regression models were constructed.

Questionnaire results

Table 1

Correlations, Means, and Standard Deviations of Questionnaire scores

Questionnaire	1	2	3	4	5	VIF
1. PSS	—					2.40
2. BDI	0.70***	—				2.13
3. RRS	0.65***	0.63***	—			2.24
4. SCRS	0.65***	0.64***	0.72***	—		2.66
5. TR	0.29*	0.36*	0.27*	0.41**	—	1.24
M	17.21	6.65	34.21	20.21	19.35	
SD	5.30	6.82	9.96	6.60	3.68	

Note. PSS = Perceived Stress Scale; BDI = Beck's Depression Inventory; RRS = Ruminative Response Scale; SCRS = Self-Critical Rumination Scale; TR = Trait Regret Scale, VIF = variance inflation factor

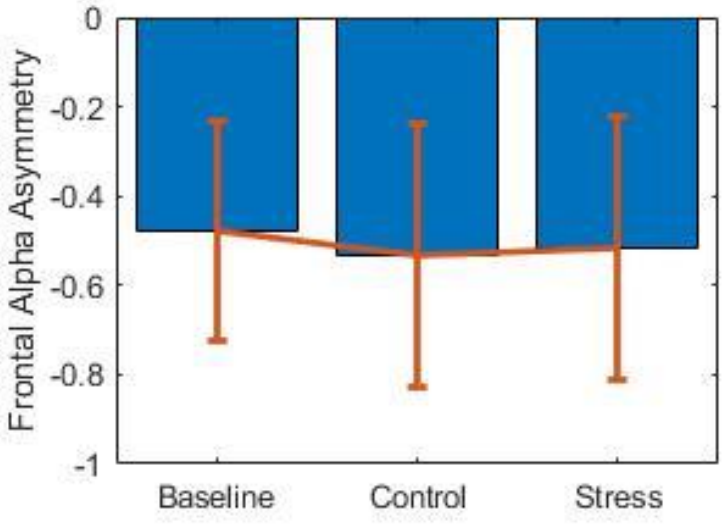
* = p < .05, ** = p < 0.01, *** = p < 0.001

Table 1 contains the means, standard deviations and the correlation between all questionnaires. The PSS scores had a mean of 17.21 out of 56 with a minimum score of 6 and a maximum of 35. The mean BDI score was 6.65 with a minimum score of 0 and a maximum of 44. Based on the interpretation by Beck, Steer, & Brow (1996), the majority (N = 64) of the participants fall within the minimal range. However, 6 participants are considered mild depressed, 1 moderate and 1 severely depressed. For the rumination scales, RRS had a

mean score of 34.21 with a minimum of 22 and a maximum of 66, covering almost the entire possible range of scores between 22 and 88. The mean SCRS score is 20.21 with a minimum of 10 and a maximum score of 36, once again almost completely covering the possible range of 10 to 40. For the final questionnaire, TR, the mean was 19.35 with a minimum score of 10 and a maximum of 29.

Looking at the correlations in Table 1, we see high correlations between all questionnaires with the highest correlations between PSS, BDI, RRS and SCRS and the lowest correlations between TR and all other questionnaires. Furthermore, the variance inflation factors (VIF) are reported in Table 1. Since all factors are below the cut-off of 4, there was no problem of multicollinearity detected.

Frontal Alpha Asymmetry



M	- 0.48	- 0.53	- 0.52
SD	0.24	0.29	0.29

Figure 9. Descriptive statistics of the Frontal Alpha Asymmetry Score for each condition

In general, all three conditions showed a negative frontal alpha asymmetry (Figure 9) indicating a relative larger alpha power at the F7 electrode site, which is on the left hemisphere. Since alpha power is an inverse for brain activity, this means a relative larger right hemispheric activity compared to the left hemisphere.

Table 2

One-way ANOVA for Frontal Alpha Asymmetries of different conditions

Source	SS	df	MS	F	p-value
Condition	0.115	2	0.057	0.74	0.48
Error	16.479	213	0.077		
Total	16.593	215			

Note. Asymmetry based on alpha power on F7 and F8 electrode site.
SS = Sum of squares, df = degrees of freedom, MS = Mean squares (SS/df)

To test our manipulation and our hypotheses, a one-way ANOVA was executed, testing whether the mean of the frontal alpha asymmetry scores differs between the three conditions. If our manipulation worked, we should see no difference in the asymmetry scores following the baseline condition and the control condition since the control condition should not evoke stress. We do expect to find a difference in the frontal alpha asymmetry measured after the stress condition. Unfortunately, the difference between these conditions was relatively small (Figure 9) and not significant ($F(2,213) = 0.74$, $p = 0.48$) (Table 2). This leads to the conclusion that stress did not elicit a higher frontal alpha asymmetry and thus contradicting our hypothesis.

Effect of Individual Differences on Frontal Alpha Asymmetry

Our second hypothesis focussed on the individual differences in the frontal alpha asymmetry. To test which individual differences coincide with differences in frontal alpha asymmetry, multiple linear regressions were executed. In the first model, we looked at the asymmetry scores computed at the start of the experiment, which is considered the baseline frontal alpha asymmetry.

Table 3

Linear Regression for baseline asymmetry with demographic variables and questionnaire results as predictors

Variable	B	SE	t-value	p-value
Sex (male)	0.122	0.064	1.89	0.06
Age	0.006	0.005	1.32	0.19
PSS	-0.002	0.009	-0.20	0.84
BDI	-0.007	0.007	-1.06	0.30
RRS	-0.001	0.005	-0.28	0.78
SCRS	0.010	0.007	1.34	0.18
TR	0.015	0.009	1.70	0.09

Note. PSS = Perceived Stress Scale; BDI = Beck's Depression Inventory; RRS = Ruminative Response Scale; SCRS = Self-Critical Rumination Scale; TR = Trait Regret Scale

* = $p \leq .05$

The results of this linear regression can be found in Table 3. In this table, the estimated B, t-value and p-value is reported. Regarding the frontal alpha asymmetry at baseline, we do not see any significant differences for any of the demographic variables or self-reported measures. The effect of sex is the one closest to the significance level, with a beta estimate indicating a more negative

frontal alpha asymmetry score for women, but this did not reach significance and is thus only up for speculation.

Since there was a high correlation between the questionnaire scores, we computed the VIF to check for multicollinearity between the questionnaire scores. These results are reported in Table 1. Since all VIF are below 4, there seems to be no problem with multicollinearity. Furthermore, the model test-statistic indicated that this model did not predict the frontal alpha asymmetry better than the null model ($F(7,64) = 1.44$, $p = 0.20$).

Table 4

Linear Regression for stress asymmetry with demographic variables and questionnaire results as predictors

Variable	B	SE	t-value	p-value
Sex (male)	0.162	0.070	2.30	0.02*
Age	0.007	0.005	1.30	0.20
PSS	-0.004	0.010	-0.44	0.66
BDI	-0.013	0.007	-1.85	0.07
RRS	-0.008	0.005	-1.62	0.12
SCRS	-0.019	0.008	2.54	0.01*
TR	0.022	0.010	2.33	0.02*

Note. PSS = Perceived Stress Scale; BDI = Beck's Depression Inventory; RRS = Ruminative Response Scale; SCRS = Self-Critical Rumination Scale; TR = Trait Regret Scale

* = $p \leq .05$

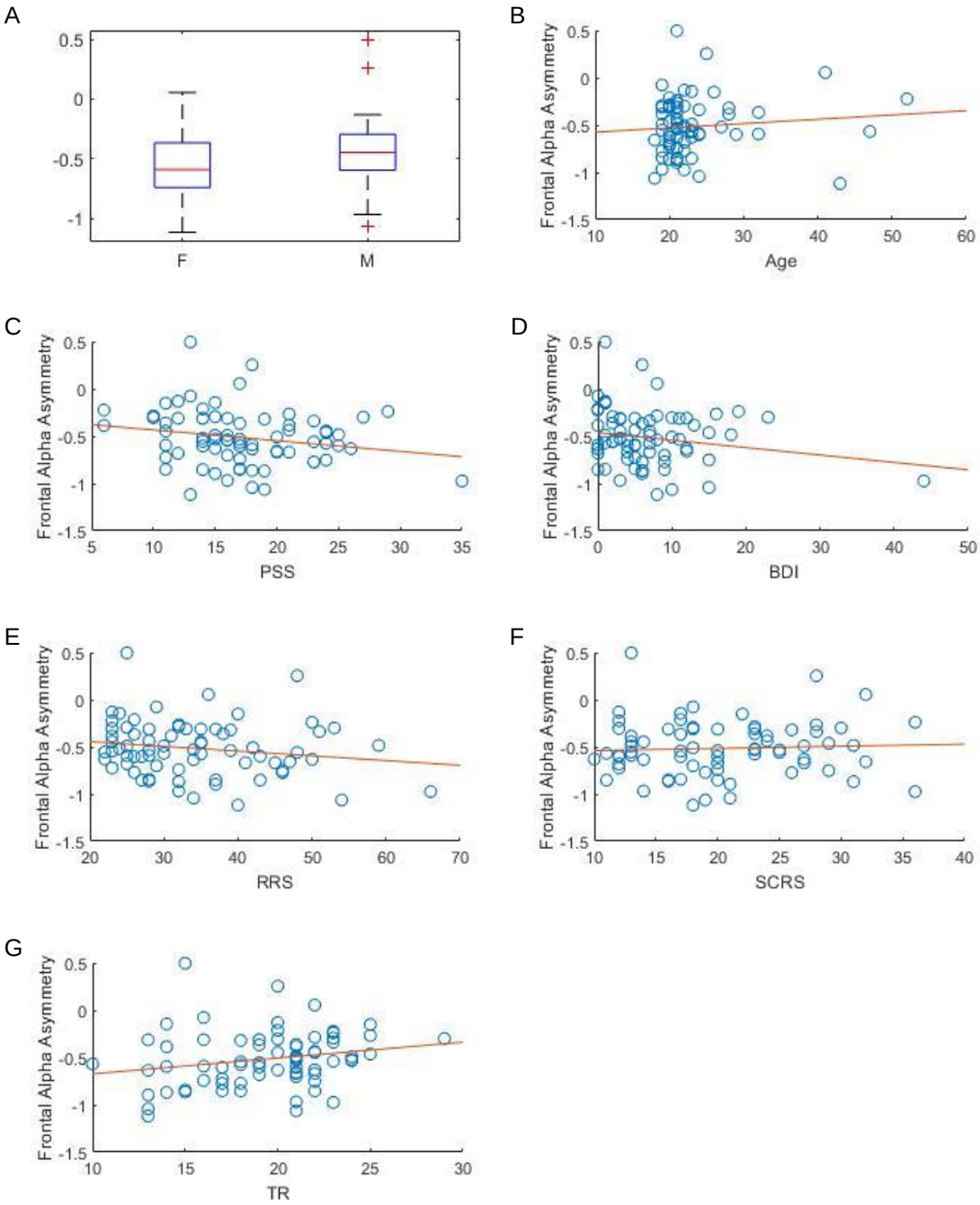


Figure 10. Scatterplot or boxplot for each Variable in the Stress Linear Regression Model

The second variable we looked at was the frontal alpha asymmetry following the stress condition. The results of the linear regression can be found in Table 4 and Figure 10 consists of a boxplot for sex and a scatterplot for each numerical independent variable.

Based on the univariate linear regression analysis (Table 4), we see a significant influence of both sex and two of the self-reported measures. Based on the beta estimate (Table 4) and the scatterplots and boxplot (Figure 10), we see that women show a slightly more negative alpha asymmetry, reflecting a larger left frontal alpha power compared to the right hemisphere. For the questionnaire, we see that for SCRS, the estimated beta is negative, meaning that higher scores on these questionnaires are associated with even more negative alpha asymmetry scores. For TR, this pattern is reversed with lower scores resulting in more negative alpha asymmetry. Once again, the VIF scores indicated no problem of multicollinearity. For this model, the model comparison resulted in a significant p-value ($F(7,64) = 3.35$, $p = 0.004$) indicating that this model predicts frontal alpha asymmetry in the stress condition better than the null model without any regressors.

Table 5

Linear Regression for the difference between control and stress asymmetry with demographic variables and questionnaire results as predictors

Variable	B	SE	t-value	p-value
Sex (male)	0.151	0.075	2.02	0.05
Age	-0.006	0.006	-1.14	0.26
PSS	0.008	0.011	0.77	0.44
BDI	-0.007	0.008	-1.02	0.31
RRS	-0.001	0.005	-0.20	0.83
SCRS	-0.006	0.008	-0.67	0.50
TR	0.010	0.010	1.01	0.32

Note. PSS = Perceived Stress Scale; BDI = Beck's Depression Inventory; RRS = Ruminative Response Scale; SCRS = Self-Critical Rumination Scale; TR = Trait Regret Scale; * = $p \leq .05$

In the final model, the dependant variable is the difference in frontal alpha asymmetry between the stress measurement and the control measurement. In Table 5, the estimated B, t-value and p-value is reported. The results of the final model resemble the results of the baseline model with a nearly significant effect of sex and no significant effects for age or any of the self-reported measures. The model comparison test-statistic also returned a non-significant p-value ($F(7,64) = 1.34$, $p = 0.248$), indicating that the model did not predict the difference between the control condition and the stress condition better than the null model did.

Discussion

Based on our study, we conclude that there is no difference in frontal alpha asymmetry when comparing the resting state after stressful situations with a control condition. Over the entire experiment, we saw negative frontal alpha asymmetry scores which reflects a larger alpha power in the left hemisphere when compared to the right hemisphere. Since alpha activity is considered an inverse for brain activity, this reflects a larger activity in the right hemisphere. Looking at the influence of individual differences on frontal alpha asymmetry, we did not see any significant effects in either the baseline frontal alpha asymmetry or the difference between the control condition and the stress condition. We did see significant effects during stress, with women presenting a more negative and thus more imbalanced frontal alpha asymmetry. Moreover, there was also an effect of rumination as measured by SCRS and trait regret. For rumination, frontal alpha asymmetry was smaller for people who reported higher scores on SCRS, while for trait regret alpha asymmetry scores were larger.

The absence of a stronger frontal alpha asymmetry following a stressful condition is in contradiction with the previous literature cited like Meyer et al. (2015) and Zhang et al. (2018). However, there are also other studies that failed to replicate this effect like the study by Brouwer et al. (2011) where participants played a game in virtual reality. In this game, participants patrolled in a police car in a simulated city. The patrol was conducted in two different neighbourhoods, a stressful environment where they received negative feedback and a bomb explosion occurred that ended the simulation. The neutral environment consisted of positive feedback and no bombs. In this study, both midfrontal (F4-F3) asymmetry was computed, as well as lateral-frontal (F8-F7) asymmetry. Here they also did not find an effect of the condition on any of the alpha asymmetry scores. They did, however, find a correlation between the alpha asymmetry scores and cortisol levels, indicating that alpha asymmetry was indeed correlated with other physiological measurements of stress.

One of the differences between our study and previous studies is the experimental manipulation. Previous studies that did find a difference in alpha asymmetry between their control condition and manipulation used speech tasks like the Trier Social Stress Test (Düsing, Tops, Radtke, Kuhl, & Quirin, 2016; Hofmann et al., 2005; Pérez-Edgar, Kujawa, Nelson, Cole & Zapp, 2013) or a cold pressor test (Quaedflieg et al., 2015; Zhang et al., 2018) or ostracism (Kawamoto, Nittono & Ura, 2013; Peterson, Gravens & Harmon-Jones, 2011).

The current paradigm resembles the paradigms of Subhani et al. (2017) and Al-shargie et al. (2018), in which participants performed a mental arithmetic task where stressors consisted of negative feedback about their performance in the relationship of their peers. However, both studies focussed on machine learning and SVM classification and thus no direct statistics were computed for frontal alpha asymmetry in the stress condition and the control condition. Both studies did, however, reach a classification accuracy above 90%.

Another possible difference is the electrodes that were used. In our study, alpha asymmetry was computed by comparing alpha power in lateral frontal electrodes F7 and F8. This is a formula used in some studies (Kawamoto et al., 2013; Lopez-Duran et al., 2012; Verona, Sadeh & Curtin, 2009), but other studies use midfrontal electrodes F3 and F4 (Crost, Pauls & Wacker, 2008; Hofmann et al., 2005; Papousek et al., 2019; Pérez-Edgar et al., 2013), a combination of F3, F4, F7 and F8 (Brouwer et al., 2011; Quaedflieg et al., 2015, Zhang et al., 2018) or even a larger collection of frontal electrodes including F1 and F2, F5 and F6, frontal-central and frontal-parietal electrodes (Al-Shargie, Tang, Badruddin & Kiguchi, 2018; Düsing et al., 2015; Peterson et al., 2011).

In our study, we found a more negative frontal alpha asymmetry in women in the stress condition. This is an effect that was not found in any of the previously mentioned studies on frontal alpha asymmetry. Moreover, the participant sample in the studies by Crost et al. (2018) and Hofmann et al. (2005) consisted only of men. According to Crost et al. (2018), this was to eliminate the effects of the menstrual cycle and the use of oral contraceptives. There are however some gender differences found in the stress response. In the study by Matud

(2004), who used a sample of 2816 people to examine the gender differences in coping and stress. In this study, they found that women scored significantly higher in terms of chronic stress and minor daily stress. Moreover, women use more emotional and avoidance coping styles. Wang et al. (2007) looked at the differences in neural response between men and women and used fMRI to identify a gender-specific neural activation model for stress. For men, this manifests in asymmetric prefrontal activity and for women in limbic activation.

We saw that self-reported measures of rumination and trait regret are significant predictors of frontal asymmetry during stress. This supports the idea that frontal alpha asymmetry may be a good detector for vulnerability for depression and anxiety since rumination is related to both depression and anxiety (McLaughlin& Nolen-Hoeksema, 2011; Nolen-Hoeksema, 2000). However, this effect is only present in the stress condition and thus cannot be detected at any given point in time. A recent meta-analysis (Van Der Vinne, Vollebregt, Van Putten & Arns, 2017) looked at 16 studies between 1998 and 2016, comparing frontal alpha asymmetry between participants with a diagnosis of major depressive disorder and healthy controls. This analysis revealed differences between patients and healthy controls, but only for people over the age of 53. Moreover, the effect seems to be different, depending on the gender of the participant. Where depressed women showed a more positive frontal alpha asymmetry, indicated relative larger right-hemispheric alpha power, while for men, this alpha asymmetry was left-sided. This study indicates that frontal alpha asymmetry may be more complicated and dependant on individual characteristics than anticipated, making its use as a diagnostic marker fairly complicated. Other possible mechanisms for detection of stress and risk for depression and anxiety that could be explored include frontal midline theta and prefrontal relative gamma power (Minguillon, Lopez-Gordo & Pelayo, 2016).

The absence of an effect of the condition has led to some questions about the limitations of the manipulation. Since this is in contrast to previous literature, one might wonder whether the experimental manipulation failed to elicit stress. One way of testing this is by looking at the skin conductance levels, respiratory

rate and heart rate variability conducted. Based on analyses that were not part of this dissertation, HRV did not seem to differ between the conditions. This may be an indication that our manipulation failed and not all participants responded to the psychosocial stressor. Another possibility is that the task in itself and the cover story we used, which was predicting future success, already placed a lot of stress on the participants and in that way already induced stress at the baseline and the control condition.

Furthermore, some participants indicated that they were sceptic about the cover story and did not believe that the feedback in the stress block was real. These participants were not excluded from the analysis which may have influenced the comparison at the group level. Here, it might also be helpful to see whether there was a difference in frontal alpha asymmetry, skin conductance or heart rate variability when compared to non-sceptic participants.

In our study, the order of conditions was not counterbalanced, mainly because the effects do not immediately subside when the manipulation stops. This might lead to some leakage of the stress condition to the control condition. This does, however, imply that we cannot cancel out sequential effects in the data. It could be, for example, that participants were stressed at the beginning of the experiment because it was their first time experiencing EEG, which may lead to induced stress in the control condition. Regarding the data analysis, all participants were included and there was no control for outliers. This may result in distorted means, which has an impact on the statistical tests that are conducted. Another limitation in the analysis of the data is that there was no control for multiple comparisons and multiple univariate regressions were used instead of one multivariate regression. This does increase the chance of type I errors and therefore, results should be interpreted with caution.

Conclusion

Based on our study we do not see a significant difference in frontal alpha asymmetry between the recovery period after the control condition and the stress condition. There is, however, a relationship between frontal alpha asymmetry during stress and self-reported measures of rumination and trait regret. Moreover, the frontal alpha asymmetry is more lateralised in women compared to men. This suggests that, unlike previous studies, research should focus more on the gender differences in frontal alpha asymmetry and what this could imply. When we combine the finding of gender differences and the relation with self-reported measures of rumination and trait regret, it suggests that frontal alpha asymmetry is highly reliant on individual characteristics. Therefore, the focus of future research on alpha asymmetry should shift to the importance of these individual differences in one's stress response.

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