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AUTOMATIC PROCESSING OF VISUAL INFORMATION: RELATIONS WITH SELF-REPORTED DEPRESSIVENESS AND ANXIETY

Master’s thesis

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Running head: Visual processing: depressiveness and anxiety

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Abstract

The aim of this thesis was to examine how depressiveness and anxiety influence automatic discrimination of emotional and unemotional visual stimuli. The thesis consists of two studies: first, we used schematic faces to elicit visual mismatch negativity (vMMN) to see if the processing of emotional stimuli differs for subjects with high and low scores of depressiveness and anxiety; secondly, we used capital letters as stimuli to see if the emotional content of the stimuli is relevant or is there a more general relationship between automatic processing and depression and/or anxiety. Subjects with no formal psychiatric diagnosis were divided into groups of high and low depression/anxiety based on their scores on a self-report emotional state questionnaire. For schematic faces, there were some relations showing subjects with higher scores of anxiety having smaller vMMN amplitudes for angry and happy deviants. In response to capital letters, subjects with higher scores of depressiveness and anxiety exhibited larger vMMN amplitudes. The emotional central task for those with higher depressiveness was more difficult, leaving the peripheral vMMN stimuli more likely to be ignored. These results indicate that some early differences in the processing of stimuli can be seen on the brain level for people with higher scores of depressiveness and anxiety and these may be affected by difficulty in switching attention away from irrelevant information.

Keywords: automatic processing, vMMN, depression, anxiety, visual processing, affective processing, schematic faces
Visuaalse info automaatne töötlus: seosed eneseraporteeritud depressiivsuse ja ärevusega

Lühikokkuvõte

Käesoleva magistritöö eesmärk oli uurida, kuidas depressiivsus ja ärevus mõjutavad emotionaalsete ja mitteemotsionaalsete visuaalsete stiimulite automaatset eristamist. Töö koosneb kahest uuringust: esmalt kasutasime skemaatilisi nägusid kutsudes esile visuaalset lahknevusnegatiivsust (vMMN), et vaadata, kas emotionaalsete stiimulite töötlus erineb kõrge ja madala skooriga depressiivsete ja ärevate isikute vahel; teiseks, kasutasime stiimulitena trükitähti, vaatamaks, kas stiimuli emotionaalne komponent on oluline või on automaatse töötuse ning depressiivsuse ja/või ärevuse vahel üldisem seos. Ilma ametliku psühhiatriilise diagnoosita katseisikud jagati kõrgesse ja madalasse gruppiga eneseraporteeritud emotionaalsete enesetunde küsimusteku tulemuste põhjal. Skemaatiliste nägude puhul näitasid mõned seosed, et kõrgema ärevuse skooriga isikutel esines väiksema amplituudiga vMMN vastusena kurjale ja rõõmsale deviantstiimulile. Trükitähtedel töötlemisel tekkinud vMMNi amplituud leiti olevat suurem kõrgema skooriga depressiivsuse ja ärevuse puhul. Keskne emotionaalsete sisuga ülesanne kõrgema depressiivsuse ja ärevuse skooriga isikute puhul on aju tasandil mõningaid erinevusi stiimulite töötusel ning need võivad olla seotud raskusega suunata tähelepanu eemale ebavajalikult infolt.

Märksõnad: automaatne töötlus, vMMN, depressioon, ärevus, visuaalne töötlus, afektiivne töötlus, skemaatilised näod
Introduction

Psychiatric disorders are a common problem in today’s society, becoming more and more the reason for poor life quality, major health problems and even death. It has been estimated that the point-prevalence of generalized anxiety disorder, major depressive episodes or the comorbidity of these two among primary care patients is almost 10% (Wittchen, Kessler, Beesdo, Krause, & Hoyer, 2002). Because of this, early diagnosis is essential as it leads to better treatment and less negative outcomes. As for now, the diagnosis is often set by using clinical interviews and self-report questionnaires. Despite working fairly well, this could be argued of being subjective and also insufficient in some cases where the patient purposely tries to conceal the problem.

Brain imaging could be considered as an objective method to accompany clinical interviews and/or self-reported questionnaires, but in order to use it effectively, clear biomarkers need to be set for diagnostics. For now, there still remains limited knowledge about the effects that mood disorders have on the brain level. What kind of changes in emotional processing are happening and how early are they detectable? Studying these changes gives us a practical outcome in two ways – first, the probability of someday diagnosing affective disorders using brain imaging; secondly, understanding the disorders more on a neural level to develop even better treatment. Thus, the current thesis will add to the existing knowledge by investigating the automatic visual processing in subclinical depressiveness and anxiety.

Automatic Processing of Stimuli

Usually, people tend to process emotional stimuli faster, because noticing the correct emotion helps to prevent conflicts and warns us about possible threats, being evolutionary important. One of the most frequent emotional stimuli in the outside world is a facial expression – we are surrounded by faces on a daily basis from where we get a lot of information about the possible emotions and intentions of the owner. Palermo and Rhodes (2007) state in their study that people pay more attention to faces than to other objects. Emotional, especially threatening faces, are processed faster compared to happy faces (Weymar, Löw, Öhman, & Hamm, 2011). Paying quick attention to one angry face in a happy crowd is evolutionary more important than finding one happy face among angry ones. Also, paying greater attention to an emotional face is more relevant than noticing a neutral one.
Although being evolutionary important to all of us, there are some differences in processing emotions between people with various conditions. In order to compare the processing of emotions between different groups, experiments with schematic faces can be used. Schematic faces have been often used to reflect emotions as they are simple and standard, but have just enough features to give off a clear emotion. While they may not be as ecologically valid as real photos, videos or real-time viewing of a person, they are sometimes preferred because the variable facial features (e.g. the mouth or eyebrows) are better to control (Ashwin, Wheelwright, & Baron-Cohen, 2006). Also, we can assume that using schematic faces decreases the possibility of having perceptual biases for attraction or gender due to the absence of specific features (e.g. hair, differences in face shape or size) that could be addressed as being more or less attractive or masculine/feminine. The processing of schematic faces has been compared to real photos and the event related potential (ERP, a direct measured brain response to an event) component related to facial perception, N170, was found to have a similar electroencephalography (EEG) amplitude and latency both for schematic faces and real photos. This suggests that the neural mechanisms underlying the processing are similar if not the same for both stimulus types (Bentin & Sagiv, 2001).

But if emotional stimuli have such an evolutionary advantage in processing speed and drawing attention, one might ask if the processing of stimuli with no direct emotional content is irrelevant? While they may not always be the priority in processing, they do still carry relevant information, depending on the situation. There have been studies that show differences in processing non-emotional stimuli for diagnostic groups compared to controls, suggesting a more overall difference in perception in some cases. For example, patients with unipolar depression have shown impairments in executive control as compared to healthy controls, by having trouble executing a Go/NoGo task where response inhibition is required, while listening to low-pitched and high-pitched auditory stimuli (Ladouceur, Dahl, Williamson, Birmaher, Axelson, Ryan, & Casey, 2006). A typical non-emotional stimulus used in visual experimental studies is a capital letter or a geometric figure. For studying non-emotional processing in people with different disorder levels, capital letters are good to use as they are a very common and simple stimuli and differentiating between them should be no problem to a person, regardless of diagnosis. It has been shown that lexical information can be processed automatically in the visual modality (Shtyrov, Goryainova, Tugin, Ossadchti, & Shestakova, 2013), therefore being useful in studies where automatic processing is relevant.

For a person with a psychiatric disorder, the processing and experiencing of emotions can be somewhat different, with a difficulty in detecting and distinguishing between different
emotions. Depression has been found to accompany excessively intense or lengthy unpleasant emotions (Boden & Thompson, 2015). Depressed young patients have been shown to exhibit biased perceptions of threat, when identifying facial emotions, in comparison to healthy or remitted subjects (Jenness, Hankin, Young, & Gibb, 2015). Also, adults with major depressive disorder are known to have problems with emotional processing (Ritchey, Dolcos, Eddington, Strauman, & Cabeza, 2011). In the case of anxiety, it is suggested that there is a central role of hyper-reactivity and a fear of negative emotional shifts (Newman, Llera, Erickson, Przeworski, & Castonguay, 2013). Compared to healthy controls, children with general anxiety disorder (GAD) have also been shown a bias towards threat relative to neutral faces (Waters, Bradley, & Mogg, 2014). In addition, patients with GAD have shown a strong reactivity to ambiguous or neutral stimuli as if they were negatively valenced, which suggests that their threat-detection system can become over-responsive to any potential danger (Newman et al., 2013). This biased perception of threat in both depression and anxiety could lead to incorrect processing of an emotional situation, for example seeing a friendly dog expose its’ teeth could be understood as a threatening sign and one might be unnecessarily frightened.

The impairment in processing for these conditions is not only seen for negative emotions, but for positive as well. Falkenberg, Kohn, Schoepker and Habel (2012) state in their study that for depression, the enhanced negative affect is accompanied by reduced positive affect, a state called anhedonia, which is the reduced ability to experience pleasure. Anhedonia is one of the main clinical symptoms in the affective domain of the disorder and in their study the authors found that there may even be underlying deficits in depressed patients’ ability to express their felt arousal in response to funny cartoons. Grillo (2016) discusses in his review that as anxiety and depression are often comorbid, in up to 70% of cases, they could be part of the same continuum of problems and anhedonia could be a possible candidate in causing both depression and anxiety, therefore the same reduced positive affect is apparent in anxiety as well.

Due to the apparent deficits in perception for people with depression and anxiety, studying the automatic processing of both emotional and unemotional stimuli for these disorders is crucial, as it leads to a better understanding of the illness. In the current thesis, the focus is set on processing schematic faces as emotional stimuli and capital letters as unemotional, comparing the automatic brain responses of people with higher and lower depressiveness and anxiety to see if there could be an underlying biomarker for future diagnostic purposes.
Means to Study Automatic Processing – Mismatch Negativity

In modern perception theories, it is thought that the processing of the outside world is an ongoing process of predictive coding (see Friston, Stephan, Montague, & Dolan, 2014 for review). When we have a prediction about a situation and this does not match with what really happens, we get a prediction error. After processing these errors, we learn more about the environment and make more precise assumptions in the future. A component which can be used to measure these errors in our brain is called mismatch negativity (MMN). The mismatch negativity is a change-specific component of the event related potential elicited by any discriminable change in stimulation irrespective of the subjects’ or patients’ attention or behavioral task (Näätänen, Gaillard, & Mäntysalo, 1978; Näätänen, Pakarinen, Rinne, & Takegata, 2004). MMN is elicited by presenting a long sequence of identical stimuli, i.e. the standard, and adding some rare unexpected stimuli, deviants, in between. The difference wave for MMN is calculated by subtracting the averaged ERP signal for an often occurring standard stimulus from the response to the rare deviant stimulus. The frequent stimulus is thought to create a memory representation and each stimulus presented next is compared to this. When they coincide, the brain gets positive feedback, but when they differ, an error occurs and this can be seen as a negative peak in the ERP signal (see more on predictive coding and MMN in Winkler & Czigler, 2012).

Earlier, MMN was only studied in auditory experiments, but from the beginning of 1990’s it has been found in several other modalities too – visual (e.g. Kreegipuu, Kuldkepp, Sibolt, Toom, Allik, & Näätänen, 2013; Kuldkepp, Kreegipuu, Raidvee, Näätänen, & Allik, 2013; Susac, Ilmoniemi, Piiko, & Supek, 2004; Astikainen & Hietanen, 2009), olfactory (Krauel, Schott, Sojka, Pause, & Ferstl, 1999) and somatosensory (Strömmer Tarkka, & Astikainen, 2014; Spackman, Towell, & Boyd, 2010). Visual MMN (vMMN) is a good indicator for studying automatic processing and discriminating stimuli. As it does not rely on the subjects’ attention, it is especially favorable in studying clinical groups, because patients could have problems communicating or focusing on a certain task. MMN in vision has been discovered mainly in the occipital area and rarely in the frontal area (Kreegipuu et al., 2013; Stefanics, Csukly, Komlosi, Czobor, & Czigler, 2012). Also, in the review article by Kremláček, Kreegipuu, Tales, Astikainen, Pöldver, Näätänen and Stefanics (2016) it is shown that clinical vMMN studies for mood disorders have mainly found differences in the temporal and occipital regions. For this reason, the current thesis focuses only on the occipital area.
**MMN – a Possible Diagnostic Tool?**

MMN has been found to differ between the diagnostic and control group in various diseases. The amplitude of the difference wave is the most promising measure here, as it seems to be lower or nonexistent in clinical groups, therefore enabling a possible diagnostic value. In the review article by Näätänen, Kujala, Kreegipuu, Carlson, Escera, Baldeweg, & Ponton (2011) the authors give an extensive overview of different disorders which have been studied regarding mostly auditory and seldom visual MMN. These include schizophrenia, bipolar disease, chronic alcoholism, stroke and aphasia, multiple sclerosis, epilepsy, Parkinson’s disease, dementia, Alzheimer’s disease, Huntington’s disease and even normal ageing, resulting in a smaller MMN amplitude and in some cases a prolonged peak latency for the clinical groups. Näätänen et al (2011) state that what all of these conditions have in common is cognitive impairment, which can be objectively measured using MMN and its magnetic equivalent MMNm (magnetic MMN, i.e. measured with magnetic resonance imaging – MEG). Näätänen, Kujala, Escera, Baldeweg, Kreegipuu, Carlson and Ponton (2012) paper adds to this with an even more exhaustive overview of the diseases where MMN provides an objective index of affected auditory discrimination. In addition to the impairment in discrimination, the difference in MMN may also reflect a decreased sensory-memory duration, abnormal perception or attention control. In the recent review by Kremláček and colleagues (2016), the authors shed light on the possible clinical value of visual MMN, indicating its potential as a possible biomarker.

**Depression, Anxiety and MMN**

It has been found that automatic processing of emotional schematic faces as deviants and neutral faces as standards evoke a smaller visual mismatch negativity for depressive participants as compared to controls (Chang, Xu, Shi, Zhang, & Zhao, 2010). This phenomenon has been previously studied more in auditory MMN (aMMN), where depressed patients show a smaller or nonexistent MMN amplitude in response to sad sounds (Pang, Xu, Chang, Tang, Zheng, Liu, & Sun, 2014) and sound duration changes (Chen, Zhang, Wei, Wu, Fu, Xu, Wang, Ye, Ma, Yang, & Zhang, 2015). This also applies for measurements done with MEG, where auditory MMNm amplitude in response to duration and frequency changes of pure-tone stimuli and to a vowel across-category change was smaller for depressed patients in comparison with the control group (Takei, Kuman, Hattori, Uehara, Kawakubo, Kasai, Fukuda, & Mikuni, 2009). These findings have been thought to be the result of occurring depressive episodes which could lead to impaired pre-attentive information processing, even
suggesting that the MMN amplitude could be a stable trait biomarker for the appearance of depression (Chen et al., 2015).

There is some inconsistency regarding the studies relating depression and MMN, still, as He, Chai, Zheng, Yu, Chen, Li, Chen, & Wang (2010) instead found that depressed patients had a larger aMMN amplitude than healthy controls. They suggest this may be a result of difficulty in avoiding irrelevant stimuli or of a higher neuronal activity in the frontal area. However, they used treatment resistant depression patients in their study, so this could also be one of the causes of the difference with the usual findings. Also, in the study of Kähkönen, Yamashita, Rytsälä and Suominen (2007), with frequency changes as deviant tones, they found the patients with major depressive disorder (MDD) to have a larger aMMN amplitude in the frontal area. The authors also suggested this possibly being a result of deficits in involuntary attention shifting for the patient group. Due to the discrepancies in this field, further analysis is anticipated.

For anxiety, there are not as many studies conducted using MMN, so the existing knowledge is rather limited regarding if similar effects could occur as for depression. However, as people with anxiety also experience a bias to threatening stimuli and negative valence, as do those with depression (Jenness, Hankin, Young, & Gibb, 2015; Waters et al., 2014), and experience similar anhedonia (Grillo, 2016), this is worth looking into more. Also, MacNamara and Hajckak (2010) found, using behavioral and ERP measures, that threatening stimuli have a bigger impact on people with generalized anxiety disorder than on healthy controls, showing greater attention towards threat. The knowingly only vMMN study done to date that focuses specifically on anxiety, was done by Jia, Chang, Xu, Shi, Pang, & Tang (2013), who used emotional faces to elicit vMMN for GAD patients and controls. The authors found that the vMMN amplitudes were significantly lower in the GAD group, suggesting an impairment of automatic processing of emotional faces. For panic disorder, again a smaller vMMN amplitude was found in response to schematic emotional faces, implying that the ability to automatically detect facial emotions is impaired in patients with panic disorder (Tang, Xu, Chang, Zheng, Shi, Pang, & Zhang, 2013). However, the results suggested that the vMMN amplitude in that study was not influenced by comorbid anxiety. Based on the latest review article by Kremláček and colleagues (2016), it can also be confirmed that there are surprisingly few studies conducted for investigating the relationship between anxiety and vMMN. In order to understand if and how the automatic discrimination process in terms of vMMN is deficit in anxiety, more studies are again needed for clarification.
In the current study, we used subjects who had not been diagnosed with either depression or anxiety, to see if there are any noticeable changes in the vMMN amplitude for people with higher depression/anxiety scores who would therefore more likely belong to the risk group. The depressiveness and anxiety here was measured using ESQ-2 (Emotional State Questionnaire, Aluoja, Shlik, Vasar, Luuk, & Leinsalu, 1999). ESQ-2 is composed of common symptoms for the disorders and the subjects give answers about their thoughts and experiences for the past month. By looking at subjects with no formal diagnosis but with higher level of symptoms relevant in development of different disorders, it could be possible to indicate the time frame on when these changes in automatic processing start to show. This could be a helpful method in preventing the progression of the disease and the findings will also support the possibility of using MMN as a diagnostic tool for early disease detection and progression monitoring in the future. In addition, as previous studies have focused more on the clinical value of auditory MMN and studies in the visual modality are still at its’ infancy, the current thesis will give additional contribution to the field.

The Current Thesis

The aim of this thesis is to see how people with higher and lower scores of depression and anxiety differ in automatic processing of visual stimuli. Furthermore, to examine how depressiveness and anxiety influence discriminating of emotions and unemotional stimuli. The thesis consists of two studies: first, we looked into processing of emotional schematic faces in relation to depression and anxiety. The aim was to see if the vMMN elicited by emotional facial stimuli would differ between people with high scores and those with low scores. Secondly, we measured vMMN to non-emotional stimuli by using capital letters, to see if the emotional content of the stimuli is relevant here or is there a more general relationship between automatic processing and depression and/or anxiety. We expected the vMMN amplitude to be significantly smaller for people with higher scores for both emotional and unemotional stimuli, as this is mostly found in similar studies and coincides with given theory that both depression and anxiety inhibit the ability to distinguish between different emotions. The phenomenon has been previously studied more in auditory modality, but as frightening and emotional input is often visual, it is an important modality to look into more.

The thesis offers a new insight into vMMN studies on mood disorders and perhaps a larger contribution is made for studying anxiety. The latter seems to be poorly assessed with only a few studies done to date. Also, as most studies have used diagnosed patients in the vulnerable group and compared the results with healthy controls, this thesis adds a new side,
looking at healthy subjects with no formal diagnosis that present different scores for depressiveness and anxiety. This approach would be beneficial in early detection of the disorder and therefore possibly better treatment and a chance of recovery. Overall differences in vMMN regarding stimuli features and experimental design remain undiscussed in the current thesis and can be read further from the work of Saar (2016), Uutma (2015), Juuse (2014) and Kask (2014).

**Study 1 – Schematic Faces**

1.1. Hypotheses

S1H1: vMMN amplitude in the occipital area for schematic faces is smaller for subjects with higher depression scores.

S1H2: vMMN amplitude in the occipital area for schematic faces is smaller for subjects with higher anxiety scores.

S1H3: There is a main effect of depression and anxiety for the amplitudes of the vMMN.

1.2. Method

1.2.1. Subjects. Thirty three subjects took part in the original experiment. Given the purpose of the current study, all subjects who had not filled in the Emotional State Questionnaire were left out from the analysis. Twenty-three (12 female) healthy right-handed volunteer subjects remained, aged 19-39 (mean age 23.26, SD = 4.51) with normal or corrected to normal vision. Subjects did not report any diagnosed psychiatric disorders and no diagnostic interview was conducted. Before participating, the subjects signed a written consent. According to the experimental design, subjects with epilepsy or migraine were not allowed to participate. Psychology students were able to earn credit for their compulsory course. Subjects were introduced to the study being a vision experiment and further content was not specified. The study was approved by the Research Ethics Committee of the University of Tartu (based on The Code of Ethics of the World Medical Association (Declaration of Helsinki)).

1.2.2. Stimuli. Emotional schematic faces \( n = 11 \) with modified gaze direction (looking right, forward, left) and eyebrow angles \( (0, 19 \text{ and } 38\) degrees) showing neutral, angry or happy emotion were used as deviant stimuli (Figure 1c). Two scrambled face-like figures were used as standards. The stimuli were 674 x 789 pixels big and were presented in the middle of a white screen. Experimenters avoided using the word ‘face’ and naming the
emotions represented by the stimuli. The stimuli used were first adapted from the Öhman, Lundqvist, & Esteves (2001) study for the Master’s thesis of Gerly Kukk (2010) and since then has been used several times in studies by students and members of the laboratory of experimental psychology (e.g. Kreegipuu et al., 2013). For the current thesis, only the original stimuli without gaze direction or eyebrow modifications were analyzed: neutral, angry and happy faces as deviants; one scrambled face as the standard (Figure 1).

1.2.3. Procedure. Before coming to the laboratory, subjects filled the ESQ-2 using the Kaemus web-based research portal. The experiments were conducted in January and February 2014 at 12 a.m. - 6 p.m. in the laboratory of experimental psychology at University of Tartu Chemicum building. One testing with one subject was approximately 3 hours long (including pre- and post-procedures and resting pauses). Upon arrival, the subject was first introduced with the upcoming experiment and was then asked to sign a written consent. Participants’ fatigue in the central nervous system was measured using the critical flicker frequency test (CFF; Simonson & Brozek, 1952).

The experiment was held in a dimmed and electrically shielded room. The subject was connected to the EEG device (see subchapter “EEG measurement” p. 14 for details) and advised to sit calmly, avoid excessive blinking and follow the instructions presented on the Mitsubishi Diamond Pro 2080SB 22” screen, which was located 114 cm from the subject. During the experiment, participants evaluated their subjective tiredness by filling an adapted Borg CR10 scale (Borg, 1998). For the EEG, first the resting state of the brains’ electrical activity was measured. The overall experimental design is illustrated in Figures 1a and 1b.

A short trial set, consisting of two blocks, was presented, where the participant could get used to the experimental paradigm. The experiment itself had four 15 minute long sets, which were presented using MATLAB (MathWorks, Natick, Massachusetts, United States). Subjects were instructed to focus on the center of the screen, to avoid them consciously processing the whole faces. The task was to press the left mouse button when the prearranged target was presented on the screen and subjects’ reaction times were recorded. The sets consisted of 30 blocks, every block had 43 stimuli of which there were 14 deviants, 4 targets and 25 standards. An optimum paradigm (Näätänen, Pakarinen, Rinne, & Takegata, 2004) with several features was used to elicit MMN. Stimulus onset time was 252 ms and interstimulus interval 452 ms. Order in which the sets were presented was varied between subjects.
Figure 1. a) Experimental design. The green boxes indicate the sets analyzed in the current thesis, blue boxes show the stimuli analyzed from the sets; b) An example of a single trial in Study 1 set 1; c) all the stimuli in Study 1, black boxes show the stimuli analyzed here.

At the end of the experiment, resting state EEG was measured again and the subject was then detached from the EEG device. The CFF test was repeated and subjects gave a saliva sample. Finally, the subject filled in a post-experiment questionnaire, where the task was to evaluate all the stimuli presented in the study using a 9-point Likert scale (Likert, 1932). In the current thesis, fatigue, genetic samples, reaction times, subjective stimuli evaluations and resting state EEG will not be analyzed.
1.2.4. EEG measurement. 32-channel active electrode kit (Active Two, Biosemi B.V., Amsterdam, The Netherlands) was used for the EEG recordings (online recording frequency 512 Hz, band pass filtered 0.16-100 Hz). The electrodes were placed by the international 10-20 system (Jasper, 1958). In addition, four electrodes were attached to the subjects’ face to detect blinks and eye movement and two reference electrodes were attached behind the linked earlobes.

The raw EEG data were offline post-processed in BrainVision Analyzer 1.05 (Brain Products GmbH, Munich, Germany). The data were referenced to the linked earlobe electrodes, unused channels were removed and names from the 10-20 system were added. Then the data were filtered with Butterworth Zero Phase filter (24 dB/oct, 0.1-30 Hz) and corrected for eyeblinks using the Gratton and Coles algorithm (Gratton, Coles, & Donchin, 1983). Segments starting at -200 ms before and ending at 700 ms after the stimuli were separated for analysis. Baseline correction was made at 100 ms before stimulus onset and the following artefacts were removed: segments that were lower in amplitude than -100 μV or higher than 100 μV; segments that had at least 100 ms of activity lower than 0.5 μV; or segments where the difference between two neighboring values was larger than 50 μV.

For each participant, event-related potentials were calculated by averaging signals from individual stimuli. These were visually inspected, in case some bad channels were left unnoticed by the automatic corrections. As standards were presented more often than deviants, 9% of all standards were randomly selected (55 from the original 609 was left) for comparison. Individual MMN difference waves were calculated for each participant by subtracting the signal to the standards from the signal to the deviants. The data were then exported as individual data points from the BrainVision Analyzer as numerical values and the amplitudes were averaged in 19-20 ms intervals of interest, altogether 80-340 ms post-stimulus. The given intervals (n = 13) were the following: 80-100 ms; 101-120 ms; 121-140 ms; 141-160 ms; 161-180 ms; 181-200 ms; 201-220 ms; 221-240 ms; 241-260 ms; 261-280 ms; 281-300 ms; 301-320 ms; 321-340 ms. Mean amplitudes in these intervals were analyzed in the pooled occipital electrode site comprising of O1, O2 and Oz electrodes.

1.2.5. Emotional State Questionnaire. ESQ-2 consists of 33 statements which are derived from the diagnostic criteria of depression and anxiety in DSM-IV and ICD-10. There are five subscales: anxiety, agoraphobia, depression, asthenia and insomnia. Subjects have to rate how much the stated problem has bothered them in the last month on a 5-point Likert (1932) scale (from “not at all” to “often”). For the current thesis, depression and anxiety
subscale scores are analyzed, cut-off thresholds for low and high groups were selected on the basis of median score – 11/32p for depression and 10/24p for anxiety. Threshold used in the current study for anxiety was slightly different from the one set in the diagnostic guidance (originally 11p) to have approximately the same number of subjects in both high (n = 14 for depression, n = 12 for anxiety) and low (n = 9 for depression and n = 11 for anxiety) groups.

1.2.6. Statistical analysis. ESQ-2 responses were exported from the Kaemus web-environment and combined with the EEG-data in Microsoft Excel 2000 (Microsoft, Redmond, Washington, United States) for further analysis. The data were organized using RStudio (RStudio Inc., Boston, United States) and analyzed with STATISTICA 8 (StatSoft Inc., Tulsa, United States) and IBM SPSS Statistics 20.0 (IBM Corp., Armonk, New York, United States). The normal distribution was tested with Shapiro-Wilk’s Test of Normality. For abnormally distributed values (p < .05), nonparametric tests were used. Differences between standards and deviants were calculated with Paired Samples Test or equivalent Wilcoxon Signed Ranks Test in all intervals for all stimuli to see if there was a prominent vMMN. Only the intervals with significant vMMN were used hereinafter and Pearson correlations were performed between the average vMMN amplitudes for each deviant and scores for depression and anxiety in ESQ-2 to see if the amplitudes were lower for higher scores. To further see if there was a main effect for depression or anxiety regarding vMMN amplitudes, mean vMMN amplitudes were subjected to general linear model repeated-measures analysis of variance (ANOVA) with interval (n = 7/5) and emotion (angry, happy, neutral) as within-subject factors and group (high and low depression/high and low anxiety) as the between-subjects factor. The three-way ANOVA was conducted separately for both sets measured in the experiment and subsequently for depression and anxiety. Degrees of freedom were corrected for nonsphericity, when necessary, using the Greenhouse-Geisser adjustment. Post-hoc comparisons were conducted using the Bonferroni test. The statistical analysis was done separately for both experimental sets in order to separate the responses to the standard stimuli, which could be dependent on the set they were presented in and on which deviants they were accompanied by.

As one could argue the high depressiveness and anxiety in this study are similar or even the result of a single underlying trait, depression and anxiety scores were correlated using Pearson’s correlation and a significant relation (r = 0.70, p < .05) was found. However, by merging the two subscales together and comparing those with both high scores in anxiety and depression to those with both low scores in an analogous Repeated Measures ANOVA
analysis, no additional or contradictory results were found, resulting only in slightly decreased effect sizes and a smaller amount of subjects ($n = 19$, leaving out those with high depressiveness and low anxiety or low depressiveness and high anxiety). Therefore the decision was made to keep the two subscales apart.

1.3. Results

Paired Samples T-test and Wilcoxon Signed Ranks Test indicated the intervals in which the averaged ERP signal for deviant stimulus was significantly different from the response to standard stimulus, i.e. the vMMN occurred (Table 1).

Table 1. Differences between responses to deviant and standard stimuli in 13 time intervals for the two sets.

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Note: * = $p < .05$, ** = $p < .01$; set 1 – eyebrow modifications, set 3 – gaze direction modifications.

1.3.1. Individual differences. The averaged vMMN amplitudes in the selected intervals with prominent vMMN were correlated with ESQ-2 depression and anxiety scores using Pearson’s correlation. In set 1 there was a significant positive correlation 141-160 ms post-stimulus for both angry ($r = .48, p < .05$) and happy ($r = .46, p < .05$) deviant with anxiety. In set 3 there was also a positive correlation with anxiety for angry deviant in 141-160 ms ($r = .46, p < .05$) and in 161-180 ms ($r = .42, p < .05$) post-stimulus intervals (see also Figure 3b). No significant relations were found for depression in either sets. Note that the hypothesized correlation here was positive, because the smaller the vMMN amplitude, the closer the value comes to zero or even turns positive.
1.3.2. Main effects. For further analysis, a three-way general linear model repeated measures analysis of variance (ANOVA) was conducted using only the intervals in which vMMN occurred. In set 1 separate models were conducted for depression and anxiety. The within-subjects factors were interval (\(n = 7\), see Table 1) and emotion (angry, happy, neutral) and the between subjects factor was either the categorical value for depression or anxiety (high and low). For depression, a main effect for interval \([F (3.39, 71.09) = 8.21, \eta^2_p = 0.28, p < .001]\) and emotion \([F (2, 42) = 21.76, \eta^2_p = 0.51, p < .001]\) was found. There was also a significant interaction between interval and emotion \([F (5.05, 106.11) = 3.60, \eta^2_p = 0.15, p < .01]\). Post-hoc Bonferroni test was conducted for the main effect for emotion and a significant difference was found between all of the vMMN amplitudes for the three schematic faces \((p < .01)\). No main effect or interaction was found for depression. For anxiety, a main effect for interval \([F (3.51, 74.78) = 7.68, \eta^2_p = 0.27, p < .001]\) and emotion \([F (2, 42) = 22.52, \eta^2_p = \)
0.52, $p < .001$] was found. Also, similarly, a significant interaction between interval and emotion was found [$F (5.3, 111.24) = 3.81, \eta^2_p = 0.15, p < .01$]. Post-hoc Bonferroni test showed a significant difference between the vMMN amplitudes for all the three deviants ($p < .01$).

Figure 3. a) Grand Averaged ERP waveforms; b) Grand Averaged difference waves (vMMN), shaded area shows the intervals significantly correlated with ESQ-2 scores; c) scalp distributions of vMMN in low and high scored anxiety groups in Study 1 set 3 for the occipital area. Note that the negative amplitude values are presented on the upper part of the y-axes.

In set 3, an analogous analysis was conducted for depression and anxiety. The within-subjects factors were interval ($n = 5$, see Table 1) and emotion (angry, happy, neutral) and the between-subjects factor was depression (high and low) or anxiety (high and low). For depression, a main effect for emotion [$F (2, 42) = 4.59, \eta^2_p = 0.18, p < .05$] was found. A significant interaction for interval and emotion also occurred [$F (3.83, 80.40) = 3.52, \eta^2_p = 0.14, p < .05$]. Post-hoc comparisons using the Bonferroni test were conducted for the main effect for emotion and it showed a significant difference ($p < .05$) between the vMMN
amplitude response to the neutral deviant and the two emotional deviants but not between the angry and happy deviant.

For anxiety in set 3, a main effect for emotion \( [F (2, 42) = 4.34, \eta^2_p = 0.17, p < .05] \) was found. Again, there was a significant interaction between interval and emotion \( [F (4.28, 89.91) = 4.37, \eta^2_p = 0.17, p < .01] \). Bonferroni test showed a similar result – the vMMN amplitude for the neutral stimuli differed significantly from the vMMN amplitude for angry and happy \( (p < .05) \). A marginally significant interaction between interval, emotion and anxiety was found \( [F (4.28, 89.91) = 2.45, \eta^2_p = 0.10, p = 0.047] \). No significant main effect or interaction was found for depression. Although no main effects were found for depression or anxiety in Study 1, looking at the difference waves (vMMN) for the angry deviant, for example, there seem to be noticeable differences in the amplitudes for subjects with high anxiety and for those with low (Figure 3), but no clear differences for depression scores (Figure 2).

1.4. Discussion

The aim of Study 1 was to see if there is a difference in visually processing schematic faces on the brain level for subjects with high and low scores of depression or anxiety. We hypothesized that the vMMN amplitudes in both depressiveness and anxiety will be smaller, suggesting an impairment in emotional processing and furthermore in discriminating between facial expressions.

The first hypothesis remained unconfirmed as there was no significant correlation found between the vMMN amplitude and the ESQ-2 scores for depression in either sets. This indicates there was no difference in automatic processing of faces for different levels of depressiveness. The result does not comply with previous results where the MMN amplitude has been found to be smaller for patients with depression (Chang et al., 2010; Pang et al., 2014; Chen et al., 2015; Takei et al., 2009). However, as we measured subjects with no formal diagnosis, the fact that there is no evident deficit could suggest that the subjects with higher scores for depression do not have specific problems (or they have not occurred yet) with discriminating the facial stimuli. This does not exclude the possibility that the deficit could occur in later stages of the disease for those who might develop it. Furthermore, as mentioned in the introduction, the findings for MMN and mood disorders have resulted in contradictory results, in some cases finding larger amplitudes for depressed patients.
(Kähkönen et al., 2007; He et al., 2010), so no clear correct answer has been found yet in how the processing deficit should affect the MMN amplitude.

In addition, in the current study, there were quite few intervals which had a prominent vMMN present, so there is a possibility that the differences in the deviants may have been too small to be processed and therefore complicating the deviant detection for all subjects, let alone for subjects with higher depression scores, therefore resulting in no difference between the vMMN amplitudes. For studies conducted with mood disorders and vMMN, the differences have been found in latencies around 120-250 ms (Chang et al., 2010; Chang, Xu, Shi, Pang, Zhang, & Cai, 2011) and 220-350 ms (Chang et al., 2010; Chang et al., 2011; Qiu, Yang, Qiao, Wang, Ning, Shi, Zhao, & Yang, 2011; Maekawa, Katsuki, Kishimoto, Onitsuka, Ogata, Yamasaki, Ueno, Tobimatsu, & Kanba, 2013). Despite looking at the same timeframes in the current study, the occurrence of vMMN was modest. As the used experimental design allowed to process the faces entirely, if wanted, the processing was possibly more attentional and this could have overridden the possibility for automatic vMMN to occur.

The second hypothesis was moderately confirmed, as there was a statistically significant correlation found for the anxiety score and vMMN amplitude in both sets for the angry deviant. However, as the relation was prominent in only few of the intervals and stimuli studied, one must remain cautious in making any firm conclusions. The found relations coincided with our assumptions that the more higher the score for anxiety, the smaller the vMMN amplitude. A possible reason to why only anxiety was correlated with the vMMN amplitude could be because it is found that anxious subjects report greater perceived intensity in emotional experiences in comparison to subjects with depression (Newman et al., 2013). This is supported by the case that only the vMMN in response to emotional and especially the angry deviant stimulus was related to anxiety scores. Perhaps subjects with higher anxiety scores perceived the emotions for the deviant stimuli more intense and negative and therefore were less successful in discriminating between them, resulting in a lower vMMN amplitude. A negative bias in perceiving emotional faces for anxious subjects has been found in previous studies (Waters et al., 2014), but as the same goes for depression (Jenness et al., 2015), the difference could be only in the perception of intensity. The difference was only visible in the angry deviant which could be stated as the most intense of the three, adding confirmation to this suggestion.

The third hypothesis remained unconfirmed, as it resulted from the repeated measures analysis, no significant main effect was found for depression nor anxiety in either sets,
therefore confirming the findings that in the current study depressiveness nor anxiety affected the amplitude of the vMMN produced by schematic faces. Although, as previously stated, there were some significant correlations indicating there could be an effect for anxiety. There was also an interaction including anxiety in set 3, therefore indicating some relations with anxiety. In addition, a main effect for emotion was found, which indicated that the vMMN response to the three schematic faces was different, the smallest amplitude belonging to the neutral face and a larger one to happy and angry faces. Therefore some discrimination for these three deviants was present on the brain level, especially between emotional (angry, happy) and non-emotional (neutral) stimuli, respectively.

There also seems to be a tendency towards a difference in vMMN occurrence in case of different emotions in latency, as the vMMN for the angry deviant is only present in earlier intervals and in later intervals only the happy and neutral deviant elicited a vMMN. This could be due to a difference in valence of the stimuli – angry as the negative is processed quicker and happy and neutral as more positive are processed slower. This is especially relevant for depressive or anxious subjects, as they are more vulnerable to possible threat, therefore their processing of the angry face could be even faster. In the review article by Olofsson, Nordin, Sequeira and Polich (2008) the authors give an overview of ERP studies using affective pictures and state that the ERP effects for arousal and valence are temporally separated – valence being usually related to short latencies (100-200 ms) in ERPs and arousal related to longer ERP latencies (200-300 ms). Therefore it could be the case that the angry schematic face processed here was subject to greater valence.

**Study 2 – Capital Letters**

As the question remains if the vMMN amplitude is vulnerable to the state of a subject in case of higher depressiveness or anxiety, Study 2 was conducted. Given that the vMMN in response to facial stimuli was moderate in Study 1, Study 2 looked more into capital letters as stimuli, as they have less details and are more likely easier to discriminate, therefore resulting in a more prominent vMMN. This expectation is supported by previous studies using capital letters (e.g. Sulykos, Kecskés-Kovács, & Czigler, 2015). The main purpose of Study 2 was to examine the processing of unemotional stimuli and to see if there is a deficit in discriminating between them for subjects with more depressiveness or anxiety, indicating that the discrimination deficit has a more overall effect which does not rely on emotional content nor
faces per se. Because anxiety has been closely associated with attentional control theory (Derakshan & Eysenck, 2009; Reinholdt-Dunne, Mogg, & Bradley, 2009) and problems in shifting attention away from irrelevant stimuli in depression (Kähkönen et al., 2007), in Study 2 set 2 we further analyzed if there was a difference in the working memory task performance in the center of the screen for the different levels of depressiveness and anxiety. The task was meant to attract attention away from the periphery, where the vMMN eliciting stimuli were presented, to allow processing of the latter to be as automatic as possible.

However, for subjects with higher scores in depressiveness and anxiety, the emotional content of the central task in Study 2 set 2 could have distracted them more, taking away more resources, and therefore result in worse performance compared to those with low scores. In Study 1, there was a difference in processing emotional and neutral faces, but subjective evaluations were not analyzed, therefore it remained open if this was affected by subjectively perceiving the stimuli different between groups. This question was solved in Study 2. As previous studies have shown biased perceptions of threat in both depression and anxiety (Jenness et al., 2015; MacNamara & Hajckak, 2010), subjective evaluations to the central stimuli were analyzed here in order to see if subjects with higher scores for depressiveness and anxiety evaluated the three basic stimuli – angry, happy and neutral – as more arousing, higher in valence and attracting more attention. The subjective evaluations could affect the vMMN occurrence and also the performance in the central task.

2.1. Hypotheses

S2H1: vMMN amplitude in the occipital area for capital letters is smaller for subjects with higher depression scores.

S2H2: vMMN amplitude in the occipital area for capital letters is smaller for subjects with higher anxiety scores.

S2H3: There is a main effect of depression and anxiety for the amplitudes of the vMMN.

S2H4: Subjective evaluations for arousal, valence and drawing attention to angry, happy and neutral stimuli are higher for subjects with higher scores in depression and anxiety.

S2H5: In set 2, where the central task involves schematic faces, the reaction times are higher and task performance is lower for subjects with higher scores in depression and anxiety.
2.2. Method

The general method of Study 2 was the same as in Study 1 (see the corresponding subchapters of chapter “Method” in pp. 11-15 for details). The differences in Study 2 will be described as follows.

2.2.1. Subjects. Fifty subjects participated in Study 2. Thirty-seven (19 female) healthy right-handed volunteer subjects aged 20-40 (mean age 27.32, \(SD = 5.99\)) with normal or corrected to normal vision remained for analysis in the current thesis. Psychology students were able to earn 4 hours of credit for their compulsory course. Subjects were introduced to the study as a working memory experiment and were not specified about the further content.

2.2.2. Stimuli. Emotional schematic faces (\(n = 5\)) showing angry, sad, scheming happy or neutral emotion and capital letters (\(n = 7\)) H, K, L, R, B, S and T (Figure 4) were used. The schematic faces used here were again adapted from the Öhman et al., (2001) study for the Master’s thesis of Gerly Kukk (2010). In addition, two scrambled images used here were designed by Kertu Saar, using all the same facial features as the schematic faces have. The capital letters were derived from the Tahoma font and edited by Kertu Saar in order to be more consistent (e.g. modifying the width, matching K and R, etc.). All of the stimuli presented were sized 250 x 295 pixels.

All 14 stimuli were used in the central task. In the peripheral vMMN paradigm for faces: the standard was always a neutral face, happy and angry were deviants; for capital letters: B was always the standard, S and T were used as deviants (see Figure 4). As it resulted from the Master’s thesis of Kertu Saar (Saar, 2016), the vMMN for the schematic faces did not occur in the experiment and therefore only the series with capital letters in the periphery will be analyzed in the current thesis.

2.2.3. Procedure. The experiments were conducted in February and March of 2015 at 9 a.m. - 6 p.m. in the laboratory of experimental psychology in University of Tartu Chemicum building. One testing was approximately 3.5 hours long. The outline of the experiment is visualized in Figures 2a and 2b. Before the start of the EEG measurement, the subjects were given an opportunity to see all of the stimuli and it was brought to their attention that there are two different scrambled images, as this was often unnoticed in the pilot trials, but was relevant for the working memory task. The subjects were asked to perform a working memory task in the center of the screen and at the same time 4 other stimuli (which were all the same for one trial) were presented in the corners of the screen with an oddball paradigm to elicit
vMMN. The stimuli in the middle and in the periphery could be from one of two categories: faces or capital letters. The presentation of the stimuli was out of sync for the center and periphery to avoid concurrent reactions: stimulus onset time in the periphery was 450 ms with an inter-stimulus interval of 250 ms; stimulus onset time in the center was 2500 ms with an inter-stimulus interval of 500 ms.

Figure 4. a) Experimental design for Study 2. The green boxes indicate the sets analyzed in the current thesis; b) an example of a single trial in Study 2 set 2; c) all the stimuli in Study 2, the black box shows the stimuli analyzed here regarding vMMN.

The experiment had six 8 minute blocks, consisting of 141 central task stimuli and 600 presentations of the peripheral stimuli, 120 of them being deviants. Stimuli were presented with MATLAB (MathWorks, Natick, Massachusetts, United States). Subjects sat approximately 90 cm from the screen and were instructed to conclude the working memory tasks – 0-back or 2-back. The task was to press the right mouse button when the current
presented stimuli was the same as the one before the previous (for 2-back) or when it was a prearranged stimuli (for 0-back), and the left button in all other cases. The subjects were asked to focus on the middle of the screen, to avoid consciously processing the events in the periphery. The order in which the sets were presented, was varied between subjects and their reaction times and answers were recorded.

2.2.4. EEG measurement. 64-channel active electrode kit (Active Two, Biosemi B.V., Amsterdam, The Netherlands) was used for the EEG recordings (online recording frequency 512 Hz, band pass filtered 0.16-100 Hz). The offline post-processing of the raw EEG data was analogous as done in Study 1. For each participant, event-related potentials were calculated by averaging signals from individual stimuli. As standards were presented more often than deviants, only the responses to the standards which immediately preceded the deviants were included for comparison. The exported data was divided to the same 13 intervals as noted in Study 1 and the mean amplitudes in these intervals were analyzed in the pooled occipital electrode site.

2.2.5. Emotional State Questionnaire. The questionnaire used in Study 2 was the same as in Study 1. Depression and anxiety groups of high and low were again divided by median scores, which in this case set the thresholds to 13/32p for depression (n = 15 for high, n = 22 for low) and 10/24p for anxiety (n = 19 for high, n = 18 for low).

2.2.6. Statistical analysis. The statistical analysis conducted was analogous as in Study 1. In Study 2, mean vMMN amplitudes were subjected to a general linear model repeated-measures analysis of variance (ANOVA) with interval (n = 13/9/9) and deviant (S and T) as within-subject factors and group (high and low depression/high and low anxiety) as the between-subjects factor. The three-way ANOVA was conducted separately for the three sets measured in the experiment and subsequently for depression and anxiety. In addition, as in set 2, the central task involved emotional faces, the reaction times and task performance were compared here using an Independent Samples T-test analysis, reaction times and percentage of correct answers as dependent variables, high/low depressiveness/anxiety as the grouping variable. The post-experiment subjective evaluations to the central stimuli – angry, happy and neutral – were analyzed with regards to arousal, valence and attracting attention between high and low scored groups with Independent Samples T-test.

Depression and anxiety scores were again correlated using Pearson’s correlation and a significant relation (r = 0.58, p < .05) was found. No additional or contradictory results were
found when merging depression and anxiety, resulting only in slightly decreased effect sizes and a smaller amount of subjects \((n = 28)\). Therefore the decision was made to keep the two subscales apart also in Study 2.

2.3. Results

Paired Samples T-test and Wilcoxon Signed Ranks Test indicated the intervals in which the averaged ERP signal for deviant stimulus was significantly different from the response to standard stimulus, i.e. the vMMN occurred (Table 2).

Table 2. Differences between responses to deviant and standard stimuli in 13 time intervals for three sets.

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Note: * = \(p < .05\), ** = \(p < .01\); set 1 – 0-back with letters, set 2 – 2-back with faces, set 3 – 2-back with letters.

2.3.1. Individual differences. The Pearson’s correlation analysis for the vMMN amplitudes in the significant intervals and ESQ-2 scores showed the following results. In set 1, the deviant T correlated with the score for depression in the interval 101-120 ms post stimulus \((r = -.40, p < .05)\). There was no significant relation to anxiety in the sets. In set 2, there was a significant correlation with depression for deviant T in interval 101-120 ms \((r = -.45, p < .05)\), interval 121-140 ms \((r = -.37, p < .05)\), and for deviant S in interval 241-260 ms \((r = -.41, p < .05)\). A significant correlation with anxiety was found for deviant T in 121-140 ms \((r = -.35, p < .05)\), also in interval 141-160 ms \((r = -.33, p < .05)\), 241-260 ms \((r = -.39, p < .05)\) and in interval 261-280 ms \((r = -.41, p < .05)\). The vMMN amplitude for deviant S
correlated with the score for anxiety in interval 241-260 ms ($r = -0.37, p < 0.05$). In set 3, there was a significant correlation with the score of anxiety with deviant T in intervals 141-160 ms ($r = -0.33, p < 0.05$) and 281-300 ms ($r = -0.41, p < 0.05$).

2.3.2. Main effects. The repeated measures ANOVA was conducted in each set to further examine the results. In set 1 model for depression, the results showed a main effect for interval $[F(5.12, 179.07) = 4.55, \eta^2_p = 0.12, p < .001]$. For anxiety, again there was a main effect for interval $[F(5.08, 178) = 4.33, \eta^2_p = 0.11, p < .001]$. No main effect or interaction was found for depression or anxiety in set 1. In set 2, with depression, a main effect for depression $[F(1, 35) = 9.5, \eta^2_p = 0.21, p < .01]$ and an interaction between interval and stimulus $[F(4.06, 142.1) = 2.59, \eta^2_p = 0.07, p < .05]$ was found.

![Figure 5](image-url)

Figure 5. a) Grand Averaged ERP waveforms; b) Grand Averaged difference waves (vMMN), shaded area shows the intervals significantly correlated with ESQ-2 scores; c) scalp distributions of vMMN in low and high scored depressive groups in Study 2 set 2 for the occipital area. Note that the negative amplitude values are presented on the upper part of the y-axes.
For anxiety in set 2, there was a significant interaction between interval and stimulus \( [F(4.43, 155.09) = 2.93, \eta^2_p = 0.08, p < .05] \).

In set 3 for depression, there was an interaction for interval and stimulus \( [F(4.92, 172.12) = 2.38, \eta^2_p = 0.06, p < .05] \). For anxiety, again an interaction for interval and stimulus \( [F(4.94, 172.79) = 2.37, \eta^2_p = 0.06, p < .05] \) was found. No interaction or main effect was found with depression or anxiety in Set 3. The results are visualized for set 2 deviant T in Figure 5 and 6. As it appears from also visually inspecting the difference waves, the response to deviant T is different for both depressive and anxiety groups, therefore resulting in a larger vMMN for those with higher scores.

Figure 6. a) Grand Averaged ERP waveforms; b) Grand Averaged difference waves (vMMN), shaded area shows the intervals significantly correlated with ESQ-2 scores; c) scalp distributions of vMMN in low and high scored anxiety groups in Study 2 set 2 for the occipital area. Note that the negative amplitude values are presented on the upper part of the y-axes.
2.3.3. **Subjective evaluations and task performance.** No significant differences were found between high and low groups in both depressiveness and anxiety for subjective evaluations for valence, arousal or drawing attention ($p > .05$). From the results of the Independent Samples T-test in set 2 between the higher and lower scored groups for depressiveness a significant difference ($t (34) = 2.12, p < 0.05$) was found in case of central memory task performance, showing a higher success rate in the low depression group ($M = .902, SD = 0.63$) compared to the high depression ($M = .856, SD = .69$). The reaction times were also significantly different ($t (34) = -2.91, p < .01$), showing faster reaction times for the group with low scores ($M = 834.55, SD = 152.01$) compared to those with high scores ($M = 1069.13, SD = 256.24$). No differences in task performance or reaction times were found for anxiety groups.

2.4. **Discussion**

The aim of Study 2 was to see if there is a difference on the brain level in visually processing capital letters as unemotional stimuli for subjects with high and low scores of depression or anxiety.

Based on the results, the first hypothesis remained unconfirmed, as although there was a significant relationship between the vMMN amplitude and the score for depression, it was in the opposite direction – the higher the score, the larger the amplitude. The direction of the relation was not the same as expected in the current thesis, however, there have been similar findings in studies where the clinical group has the larger amplitude or there is no difference found in comparison to the control group. An aforementioned study by He and colleagues (2010) had a similar finding, employing an acoustic frequency deviance paradigm, where the treatment resistant depression group had a larger aMMN amplitude. The authors suggested the reason to be a difficulty in avoiding irrelevant stimuli for the patient group. Kähkönen and colleagues (2007) also used frequency changes as deviant tones in their study for patients with MDD compared to healthy controls and found a significant difference in the patient group, resulting in an increased aMMN amplitude for subjects with MDD. They suggested this could be a reflection of reduced inhibition and increased excitability of cortical neurons responsible for the regulation of involuntary attention.

The second hypothesis, similarly to the first in Study 2, remained unconfirmed, as the relationship between anxiety scores and vMMN amplitudes for the subjects was again found in the opposite direction. The direction of the relation was assumed to be the opposite, as the
studies in vMMN for clinical groups done until now, have mostly found a smaller amplitude for the vulnerable group, although there are still inconsistencies (see Kremláček et al., 2016). However, anxiety as a state or as a disease has not been studied as much regarding MMN, so there is no clear theory here for if and how there is a modulation in vMMN amplitudes for more anxious subjects.

In set 2, there was a main effect found for depression, but not for anxiety, so we can say that people with more depressive symptoms differed significantly from those with low scores. This also partly confirmed the third hypothesis. The effect for depressiveness was found in the only set analyzed here which had a remote emotional content – the working memory task executed had emotional schematic faces as stimuli, while the vMMN in the periphery was produced with a sequence of capital letters. There have been studies using non-emotional stimuli, for example bar shapes (Chang et al, 2010) or windmill patterns (Maekawa et al, 2013), which have found a smaller vMMN amplitude for people with major depressive disorder or bipolar disorder. However, the current findings suggest that to some extent the emotional processing could be relevant to observe a clearer difference in subjects with higher depressiveness.

From the previous result, it would seem probable that the stimuli processed in the second set were perceived to be more arousing, higher in valence or drawing more attention, therefore distracting the subjects with higher depressiveness more, leaving the peripheral stimuli unnoticed and resulting in a more apparent vMMN. The fourth hypothesis, however, remained unconfirmed, as there was no difference found between high and low groups of depressive or anxious subjects. They evaluated the stimuli similarly and the possible effect of depressiveness or anxiety did not show here. One must bear in mind that the evaluations for the stimuli were given in the end of the whole experiment, therefore the possible emotional reactions occurring during the experiment may have been worn off by then. However, it seems as the task performance varied between subjects with higher and lower scores of depression, resulting in lower response rates and higher reaction times in the high depressiveness group. Therefore the fifth hypothesis was partly confirmed, because no differences were found for anxiety. This suggests the central task with emotional schematic faces was more difficult for those with higher depressiveness, perhaps affecting the vMMN amplitudes.
General Discussion

The aim of this thesis was to see if subjects with higher level of depressiveness and anxiety differ from those with low scores by producing a smaller vMMN in response to visual stimuli. Two studies were conducted to examine this, using emotional schematic faces and capital letters as stimuli. Results found in the current thesis were quite controversial as the two studies showed opposite directions for the relation tested.

With regards to vMMN produced in the studies, capital letters seemed to result in a more prominent vMMN compared to schematic faces. This also coincided with more relations found in Study 2 with regards to ESQ-2 scores for depressiveness and anxiety. Most of these results occurred from the deviant T in Study 2, which could be because of it being visually the most easily distinguishable from the deviant (B). This could be taken into account when developing a standardized method for clinical studies, where the occurrence of vMMN is crucial in order to conduct comparisons between patients and healthy controls. The differences found here in processing both emotional stimuli and simple shapes as capital letters, indicate deficits in processing both high-level and low-level features. Current findings also suggest a possible importance of emotional content in studying those with mood or anxiety disorders, perhaps a design using simple stimuli with some emotional connotation would be best.

The somewhat controversial results found in this thesis contribute important new information to the field, suggesting the relationship with depressiveness and anxiety is complex and the direction of the relation needs further examining. The more apparent vMMN findings from Study 2 indicated a larger vMMN amplitude for more depressive subjects. The higher anxiety scores were also related to larger amplitudes via correlations. Therefore, the discrimination of capital letters was better achieved in the vulnerable groups, which complies with findings from the studies of Kähkönen et al (2007) and He et al (2010). As the authors of these studies suggested in the light of their results, this may be a case of an inability to shift attention away from irrelevant stimuli, in our case, perhaps the facial stimuli in the demanding working memory task. Such emotional content could lead more depressive and anxious people to focus more on the faces and the negative emotions accompanying these, therefore making it harder for them to move on to processing the next stimulus or paying attention to other events in the situation. This could also explain the opposite results in Study 1, where the vMMN amplitude in some cases was smaller for more anxious subjects. Perhaps the presented schematic faces involved a more relevant meaning to the more anxious, resulting in
processing them longer and more consciously, also having trouble shifting attention away to the next standard stimulus presented. The conscious attentional processing of the faces could have overridden the chance of automatic processing and the occurrence of vMMN, thus resulting in a smaller amplitude. Results from Study 2 offered confirmation to this in case of depressiveness, where the central memory task including emotional schematic faces was more difficult for those with higher scores, suggesting the need to consciously pay more attention to the faces.

Limitations and Future Directions

Although common in EEG studies, the amount of subjects used in this thesis could be argued to be too small for studying individual differences. Because of this, a certain level of caution must be taken into consideration while making generalizations from the results. This could, however, mean that by using a bigger amount of formally diagnosed subjects, the differences may be more prominent. In addition, ESQ-2 is not a strict diagnostic tool for disorders, but rather a good indicator for screening possible symptoms. This means we cannot say for sure that the subjects belonging to the high score group will develop and be diagnosed with a mood or anxiety disorder further along, but they do have a higher risk in doing so, as they experience the common symptoms more often. Also, the thresholds used in the current study were slightly different than the usual 11 p set in the diagnostic criteria, so this could have also affected the results. As we had no formally diagnosed subjects, a median-split was used to divide the groups based on their scores, instead of referring to the actual diagnostic thresholds. However, the used cut-off points were not far from the actual ones.

For further analysis in this field, it would be necessary to use diagnosed patients to say with more confidence if there are differences between a control group and a diagnostic group. Also, when looking at healthy subjects with higher scores, a pre-selection would be best – to have a large number of people fill in the test, then select the highest quartile and the lowest quartile and invite them to an experiment where both emotional and non-emotional stimuli are used in one study and with the same experimental design. As mentioned before, when it comes to studying individual differences with brain imaging, it is necessary to increase the amount of measured subjects, to reduce the possibility of getting randomly significant results.

Furthermore, the two studies used different experimental designs and different paradigms to elicit vMMN. This could be one reason for the different results as in the first study the task of the subject was to only look at the screen and click when the target appeared,
making it easier to purposely look at the entire screen and see the faces. In the second study, the vMMN stimuli were presented in the periphery and a memory task was performed in the center to avoid looking at the periphery and keep the subjects busy. In Study 1, there were 7 deviants used in one set, three of them analyzed here. The large amount of deviant stimuli and the small changes between them (eyebrow angles or gaze directions) could have also affected the occurrence of vMMN as the differences between deviants were perhaps not so evident. In Study 2, only two deviants were used, so the automatic discrimination process was perhaps more simple, resulting in a more prominent vMMN and subsequently more differences between the high and low groups.

As Kremláček and colleagues (2016) discuss in their meta-analysis of clinical vMMN studies, there is a need for more standardized methods and paradigms, so that the results of different studies and of different disorders could be comparable and more consistent conclusions could be drawn from the findings. When looking at the current thesis, we used two different paradigms and experimental designs, which resulted in controversial results, in one case indicating the vMMN amplitude for the higher scored groups is smaller, in the other case indicating the opposite – a larger vMMN amplitude. It is difficult to state based on the current thesis which result is more correct and it is quite impossible to do so in one study, so if we would have studies more similar to each other, more consistent patterns of results could be drawn.

Furthermore, if MMN as a diagnostic tool improved and were more reliable for individual assessment, it would be interesting to see if there is a reverse change when a person has recovered from a disorder e.g. the MMN amplitude becomes gradually more similar to healthy subjects again, using longitudinal studies. A similar effect is noted in coma patients, who are slowly restoring consciousness that is accompanied by their mismatch negativity starting to resemble more that of a healthy conscious human (Daltrozzo, Wioland, Mutschler, & Kotchoubey, 2007). For depression, the brain reactions to emotional stimuli have also been recorded before and after treatment and there have been some noticeable improvements of emotion regulation processes in response to cognitive behavioral therapy (Ritchey, Dolcos, Eddington, Strauman, & Cabeza, 2011).

The proportion of people with generalized anxiety disorder is thought to be comparable to those with mild depressive episodes, but it is even more difficult to diagnose as people with the disorder often do not know their symptoms are indicative of an emotional disorder with a specific name and an established treatment (Wittchen et al., 2002). As
mentioned in the introduction, anxiety has not been studied as much in relation to vMMN yet. Further studies are definitely needed in order to examine how people with anxiety disorders or with no formal diagnosis but with anxiety symptoms automatically process visual information and how the vMMN response changes during the progression of the disease and in case of recovery. This would give us a better understanding of how the process of differentiating visual stimuli is modified in highly anxious people, therefore leading to possible misconceptions and flaws in everyday processing of information, which could in turn be one of the reasons why the anxiousness upholds.

Conclusions

The current thesis examined the relations between depressiveness, anxiety and visual mismatch negativity amplitudes. As seen from the results of Study 1, there were some indications showing a relation of higher anxiety scores and the vMMN amplitude for schematic emotional faces, demonstrating a smaller amplitude for those with higher scores. However, in Study 2, contradictory results were found – an increased vMMN amplitude for those with higher scores of depression or anxiety. The current literature on this topic is still lacking clarity in the direction of the relation and the results of the thesis indicate that the difference in processing, rather than a clear direction in the deficit, could be relevant in differentiating between diagnosed and healthy subjects. From the results of the current thesis it could be suggested that higher depressiveness and anxiety are accompanied by a difficulty in switching attention away from irrelevant stimuli, especially when it has emotional meaning. Further studies regarding anxiety are needed to better understand the accompanied processes on the brain level. In order to use vMMN as a clinical tool in the future, more standardized experimental designs and stimulus materials are essential.
Contribution of the Author

The author was responsible for conducting part of the measurements from Study 1 and 2, preparing the materials and instructions for Study 2, discussing the experimental design and research questions, analyzing the datasets and writing this thesis.

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References


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