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Functional neural differences in emotional regulation between patients with MDD and healthy controls in the context of the vulnerability factors: family history of MDD, BDNF Val66Met polymorphism

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2012
Declaration

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Summary

Emotional regulation is a key mechanism which shows disturbances during major depressive disorder (MDD), and which may be modified by certain factors enhancing vulnerability to MDD. Two vulnerability factors were tested in this study: family history of MDD and Val66Met gene polymorphism (rs6265). In previous research, both factors have been found to have a connection to emotional regulation, and therefore to represent modifiers of vulnerability to MDD. The main aim of this study was therefore to determine to what extent these factors modulate differences between patients with MDD and healthy controls in terms of emotional regulation. The other main aim of this study was to investigate the key abilities of emotional regulation – emotional processing and attention shifting with inhibition of emotional stimuli – as observed in the participant groups, to examine vulnerability and resilience to the disease. An investigation of impairments in MDD episodes with and without the vulnerability, as well as of mechanisms of vulnerability and resilience to MDD, as connected to the two modulating factors, has not been presented in any previous research to date.

Functional magnetic resonance imaging was applied to record functional neuronal activation of subjects. The subjects were compared during performance in an event-related task, probing the emotional regulation processes under scrutiny.

It was found that an MDD episode without family history vulnerability is characterized by an enhanced reaction of limbic areas responsible for visceral aspect of affective display, and also, by a weakened activation in limbic regions involved in informative processing of emotional experience. These alterations were observed during emotional processing of negative stimuli, in particular. An MDD episode with family history vulnerability, in contrast, is characterized by a decreased affective activation in response to positive stimuli, and by an increased visceral neural activation during
attention shifting from negative stimuli. In addition, it is characterized by a weakened reaction in executive control areas during attention shifting from negative information. The vulnerability mechanism connected with family history of MDD is distinguished by a heightened activation in the somatic-affective and the visceral areas of the brain, as well as balanced out by an increased executive control activation. This mechanism is noted especially in relation to negative stimuli inhibition.

An MDD episode with Val homogenous allele is characterized by a decreased activation in the areas responsible for the informative aspect of emotional processing and for executive control. In contrast, an MDD episode with Met allele is marked by an increased activation in the regions involved in the visceral aspect of emotional display.

The findings of this study thus confirm that both family history of MDD and Val66Met gene polymorphism modulate differences between patients with MDD and healthy controls in terms of emotional regulation. Also, mechanisms of vulnerability and resilience to MDD have been shown to play an important role in alterations in emotional regulation.
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List of abbreviations

5-HTT – serotonin transfer protein
AC – accuracy
ACC – anterior cingulate cortex
ATD – acute tryptophan depletion
BDI II – Beck Depression Inventory
BDNF – brain derived neurotrophic factor
BOLD – blood oxygen level dependent
CNS – central nervous system
COMT – catechol-O-methyltransferase
CREB1 – cyclic adenosine monophosphate response element binding protein 1
DLPFC – dorsolateral prefrontal cortex
DSM-IV – Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition
FDR – false discovery rate
FHN – negative family history of major depressive disorder
FHP – positive family history of major depressive disorder
fMRI – functional magnetic resonance imaging
FWE – family wise error
HC-FHN – healthy controls without family history of major depressive disorder
HC-FHP – healthy controls with family history of major depressive disorder
HC-Met – healthy controls with at least one methionine allele
HC-Val – healthy controls with two valine alleles
HDRS – Hamilton Depression Rating Scale
HPA – hypothalamic-pituitary-adrenal
IAPS – International Affective Picture System
IFG – inferior frontal gyrus
IPG – inferior parietal gyrus
MCC – middle cingulate cortex
MDD – major depressive disorder
MDD-FHN – patients with major depressive disorder without family history of major depressive disorder
MDD-FHP – patients with major depressive disorder with family history of major depressive disorder
MDD-Met – patients with major depressive disorder with at least one methionine allele
MDD-Val – patients with major depressive disorder with two valine alleles
MDRS – Montgomery-Asberg Depression Rating Scale
MFG – middle frontal gyrus
MNI – Montreal Neurological Institute
MRI – magnetic resonance imaging
OFC – orbitofrontal cortex
PCC – posterior cingulate cortex
PFC – prefrontal cortex
PPI – psychophysiological interaction
RT – reaction time
SD – standard deviation
SMA – supplementary motor area
SNP – single nucleotide polymorphism
SPM – Statistical Parametric Mapping
SSRI – selective serotonin reuptake inhibitor
STG – superior temporal gyrus
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1 Introduction

1.1 Key research questions

Previous research has been consistent in suggesting that the pathology of major depressive disorder (henceforth MDD) is caused by alterations in the emotional regulation of an individual (Aldao & Nolen-Hoeksema, 2010; Almeida et al., 2011; Beauregard, Paquette, & Levesque, 2006; Campbell-Sills, Barlow, Brown, & Hofmann, 2006; Davidson, Pizzagalli, Nitschke, & Putnam, 2002a; Erk et al., 2010; Etkin & Schatzberg, 2011; Feldman, Harley, Kerrigan, Jacobo, & Fava, 2009; Gotlib & Joormann, 2010; Johnstone, van Reekum, Urry, Kalin, & Davidson, 2007; Joormann & Gotlib, 2010; Kennedy, Koepp, Young, & Zubieta, 2006; Liverant, Brown, Barlow, & Roemer, 2008; Mennin, Holaway, Fresco, Moore, & Heimberg, 2007; Rottenberg, Gross, Wilhelm, Najmi, & Gotlib, 2002a; Taylor & Liberzon, 2007; Wang et al., 2008b; Yap, Allen, & Sheeber, 2007). Patients with MDD have been discovered to be impaired in regulating their emotions (Aldao & Nolen-Hoeksema, 2010; Almeida, et al., 2011; Campbell-Sills, et al., 2006; Davidson, Pizzagalli, Nitschke, & Putnam, 2002b; Erk, et al., 2010; Etkin & Schatzberg, 2011; Johnstone, et al., 2007; Joormann & Gotlib, 2010; Kennedy, et al., 2006; Mennin, et al., 2007; Wang, et al., 2008b). The emotional regulation has been suggested as a valid middle ground between biological underpinnings of MDD and emotional and cognitive symptoms of the disease (Almeida, et al., 2011; Beauregard, et al., 2006; Erk, et al., 2010; Etkin & Schatzberg, 2011; Gotlib & Joormann, 2010; Johnstone, et al., 2007; Kennedy, et al., 2006; Rottenberg, et al., 2002a; Taylor Tavares et al., 2007; Wang, et al., 2008b; Yap, et al., 2007). It has also been suggested that the emotional regulation of a person is affected by a number of biological and psychological factors, thus making an individual more vulnerable or
resilient to MDD (Hariri & Holmes, 2006; Jabbi, Korf, Ormel, Kema, & Den Boer, 2008; Stein, Campbell-Sills, & Gelernter, 2009). These factors influence both behavioural and neural correlates of emotional regulation. Different processes involved in the emotional regulation such as emotional recognition and shifting attention from emotional processing may be disturbed during an MDD episode or by factors influencing it (Beauregard, et al., 2006; Joormann, Siemer, & Gotlib, 2007a).

In this study, two factors associated with vulnerability and resilience to MDD and their influence on changes characteristic for the disease are explored: family history of MDD (Fanous, Gardner, Prescott, Cancro, & Kendler, 2002; Weissman et al., 2006) and polymorphism in the Val66Met gene (Bueller et al., 2006; Hunnerkopf, Strobel, Gutknecht, Brocke, & Lesch, 2007; Koolschijn, van Haren, Lensvelt-Mulders, Hulshoff Pol, & Kahn, 2009; Lonsdorf et al., 2010; Nemoto et al., 2006; Pezawas et al., 2004; Porter, Gallagher, Thompson, & Young, 2003; Schumacher et al., 2005; Szeszko et al., 2005). These factors have both been found to participate in the pathogenesis of the disease (Brunoni, Lopes, & Fregni, 2008; Castren, Voikar, & Rantamaki, 2007; Fanous, et al., 2002; Groves, 2007; Weissman, et al., 2006). No previous study, however, has investigated modulations in emotional regulation caused by these two factors in patients with MDD, as compared to healthy controls. Also, this study is unique in examining the issue at both behavioural and neural levels, and as such it offers a new approach to investigating the mechanisms of vulnerability, resilience and variation in the course of MDD, as related to alterations in the emotional regulation of an individual.

Therefore, the aims of this study are:

1) to investigate the differences between healthy controls and patients with MDD in respect to emotional regulation in its various aspects (emotional recognition,
shifting attention from emotional processing, inhibition of emotional regulation) and at the two different levels (behavioural and neural level);

2) to examine the influence of family history of MDD and polymorphism in the Val66Met gene (rs6265) on the emotional regulation and

3) to establish the repercussions of the modulation in the emotional regulation for vulnerability, resilience to the disease and the difference between healthy controls and patients with MDD.

1.2 Clinical overview of major depressive disorder

1.2.1 General overview

Major depressive disorder is a disease affecting millions of people worldwide. It strongly impacts public health systems all around the world (WHO, 2001) by increasing the number of days spent by patients in clinical care and outside work. Furthermore, it increases additive cost of medical care in cases where MDD is accompanied by yet another disease (Katon, 2003). It alters lives of whole families when one of their members requires help and assistance. Most of all, it influences lives of individuals suffering from the disease, changing many aspects of their functioning and also diminishing their quality of life (Punkanen, Eerola, & Erkkila, 2011). Approximately 800,000 people die worldwide committing suicide and – a significant proportion of those deaths has been found to be connected with MDD (Frodl et al., 2010b).

MDD is a disease with both a biological and a psychological background (Seligman, Walker, & Rosenhan, 2001), and thus both aspects need to be considered in investigations aiming at a full understanding of the mechanism of the disorder. The biological characteristics of the disease include its genetic basis (Levinson, 2006) (described in the chapter 1.2.4), changes in modulation of neurotransmitters such as in
noradrenaline, dopamine, serotonin, glutamine, and in GABA (aan het Rot, Mathew, & Charney, 2009; Nutt, 2008), hypothalamic-pituitary-adrenal (HPA) axis and its related reaction to stress (Praag, Kloet, & Os, 2004), thyroxin abnormalities (Jackson, 1998) and alterations in particular areas of the central nervous system (CNS) (Fitzgerald, Laird, Maller, & Daskalakis, 2008; Koolschijn, et al., 2009) and its circuitries (Frodl et al., 2010a; Lisiecka et al., 2011; Seminowicz et al., 2004). In turn, the psychological facets of MDD involve cognitive negative perception of self, future and external environment (Beck & Alford, 2009), changed emotional regulation with associated cognitive impairments (Bylsma, Morris, & Rottenberg, 2008; Clark, Chamberlain, & Sahakian, 2009), learnt helplessness (Seligman, et al., 2001), personality and temperamental traits promoting the development of the disease (Zinbarg & Lira Yoon, 2008), and less efficient mechanisms of coping with negative and stressful life events (Martin & Dahlen, 2005). The two aspects of the disorder co-occur and cannot therefore be considered as separate or antagonistic: the biological characteristics stimulate the psychological responses and vice versa.

1.2.2 Definition

According to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV-TR), major depressive disorder is a mood disorder defined by 1) all-encompassing lower mood or 2) loss of pleasure or interest (American Psychiatric Association., 2000). For MDD to be diagnosed, five or more depressive symptoms, such as low mood, loss of interest, changes in weight and sleep pattern, feeling of worthlessness and guilt, fatigue, inability to concentrate or death ideations, must be present for the same two-week-long period, and must represent a change from the previous functioning of a person. One of the symptoms must be either the lower mood or the loss of interest and pleasure. Also, the symptoms must not be explained by the
use of substances, such as medication or drugs, by a general clinical condition or by bereavement. The symptoms also need to impair person's functioning in social, occupational or other important aspects of their life. Finally, depressive episodes must not be accompanied by manic episodes, that is episodes of excessively high mood (American Psychiatric Association., 2000). This definition is used as a basis of diagnosis of MDD in clinical and research settings, and will therefore be followed in this study as well.

The DSM-IV-TR definition of MDD describes core symptoms of the disease, pointing to a number of critical features which distinguish the disorder from other related conditions. First of all, the change in a person's mood towards the sad, unpleasant, and low is the main characteristic of MDD and a chief complaint of the majority of patients with the disorder (Beck & Alford, 2009). This symptom can be described as a disturbance in "pleasure principal". In terms of emotional regulation, it signifies a certain counter-intuitive disruption in the homeostasis of an organism, when the sad element is present constantly, and where there is neither interest in seeking anything enjoyable or any gratification nor desire to maximize one's pleasure. That is not to say that sadness is not a natural part of human emotions. However, in MDD it dominates the mood and motivation of a person, whereas pleasure cannot balance it and stops serving its purpose as a motivating/ attracting emotion.

People suffering from MDD may perceive this change in various ways – as sadness, loneliness, hopelessness, helplessness, but also emptiness or apathy. Some patients experience generalized complaints about alterations in their actions and reactions as well as a changed attitude toward life and future (Beck & Alford, 2009). At times, an increased irritability in teenagers (Beck & Alford, 2009) and increased anger and aggression in males (Winkler, Pjrek, & Kasper, 2005) may be the primary symptoms of
an MDD episode. The change in the mood can also manifest itself as a physical symptom, such as pain (Beck & Alford, 2009). For many patients with MDD participating in this study, tiredness was one of the chief complaints during an acute episode of the disease. One of the participants described his experience with MDD as follows:

*It is like being in a deep dark well which you can’t leave. On a better day you are in a bleak grey zone. But you can always slide back to the well. That’s the worst part – there is no hope in the well, nothing exists apart from the well. And you can’t get out of it and you know that you never will.*

In terms of emotional regulation, a lowered ability to control processing of unpleasant and pleasant emotions that is an inability to down-regulate the former and impaired motivation to seek the latter as well as difficulties in shifting one’s attention from affective processing are the main symptoms of MDD.

Second, the symptoms of MDD must be constantly present for at least two weeks before the disorder can be diagnosed (American Psychiatric Association., 2000). In other words, the change in mood needs to be of a persistent nature (Belmaker & Agam, 2008). In contrast, behaviours and actions which represent a transient response to losses present in everyday life and/or a temporary imbalance in psychological homeostasis and in emotional regulation are not classified as a psychiatric disorder. Among these, bereavement represents an exception; it is a reaction to a highly significant loss in an individual’s life, which requires establishing a new homeostasis or a new self-regulatory order of an individual’s emotional states (American Psychiatric Association., 2000). The period of sadness in this case must be longer than two months and must be accompanied by such symptoms as guilt, a morbid preoccupation with worthlessness or suicidal ideations, if it is to be considered an episode of MDD (American Psychiatric
Association., 2000). This means that in the case of a significant loss, a longer period is considered a norm, and a necessity demanded for the self to regulate its emotional states.

Third, to be classified as an MDD episode, depressive symptoms must be caused by neither an action of a chemical substance nor a general medical condition. Substances such as codeine (Halaris, 2011), opium (Mowla, Kianpor, Bahtoe, Sabayan, & Chohedri, 2008) and interferon-α (Wichers et al., 2005) have long been known to induce depressive-like symptoms and to influence emotional regulation via neurotoxicity mechanisms. They impact the emotional regulation in a slightly different manner than an acute episode of MDD does, making the changes in the mood more visceral and less awareness-based (Beck & Alford, 2009; Wichers, et al., 2005). It is important to distinguish between an acute MDD episode and chemically induced depressive symptoms since the course and treatment may differ in these two cases. When an MDD episode coexists along with another disease, such as an infection, a cardiovascular disease, a neurological disorder, diabetes mellitus or cancer, it is diagnosed as a comorbid depression (WHO, 2001).

Fourth, episodes of depression must not be accompanied by a manic episode. In such a case a diagnosis refers to bipolar disorder, where a depressive episode forms a part of a broader symptomatology. Since the distinction between the two types of disorders in a particular patient may not be always definite at the onset of the disease, further study or a repeated examination is needed in order to distinguish all cases accurately (Hantouche & Akiskal, 2005). Also, the results from recent neuroimaging studies indicate that techniques such as electroencephalography and magnetic resonance imaging (MRI) can be used successfully to distinguish between MDD and bipolar disorder on an individual level (Khodayari-Rostamabad, Reilly, Hasey, Debruin, & Maccrimmon, 2010).
Finally, the DSM-IV-TR definition of MDD emphasizes that the disorder affects many aspects of patient’s life. According to the World Health Organization (WHO), MDD constitutes the 4th most severe disease burden in public health systems expressed by the number of healthy years (12.5%) lost to death or disability (McMahon et al., 2006; WHO, 2001). The entire functioning of a patient can be affected by the disease, and personal costs caused by its presence are high. For example, patients with MDD have been shown to experience difficulties in participating in their family and social life as well as in keeping employment (Seligman, et al., 2001). It has also been shown that clusters of symptoms present in the disease affect at least four domains of patient’s functioning: mood, cognitions, motivation and physical well-being (Beck & Alford, 2009; Seligman, et al., 2001). Disturbances of emotional regulation, which form a core feature of MDD, influence a person’s ability to fulfil their needs, further leading to diminished levels of both subjective and objective well-being of an individual (Diener, Oishi, & Lucas, 2003; Parker, Summerfeldt, Hogan, & Majeski, 2004). MDD is an affective disorder which is characterized by persistent low mood or inability to feel pleasure which affect many aspects of person’s life.

1.2.3 Prevalence

According to a number of reports estimating lifetime prevalence of MDD, up to 17% of people in the population are going to suffer from MDD in their lifetime (Blazer, Kessler, McGonagle, & Swartz, 1994; Kessler et al., 2003; Kessler, Zhao, Blazer, & Swartz, 1997). Estimates of the point prevalence show that at any given moment, 4.9% of people in the population experience symptoms of an MDD episode (Blazer, et al., 1994). In fact, and as mentioned previously, MDD constitutes the 4th most severe disease burden when it comes to the number of healthy years (12.5%) lost to death or
disability with only respiratory infections, perinatal conditions and HIV/AIDS causing more difficulties (WHO, 2001).

MDD is more frequent in women than in men with reported ratios from 5:1 to 2:1 (WHO, 2001). When stratified by gender, lifetime prevalence of MDD in men is reported to be 5% to 12%, whereas in women it is 10% to 25% (Beck & Alford, 2009). The point prevalence of MDD is estimated to be 2% to 3% in men, and 5% to 9% in women (Beck & Alford, 2009).

In addition, age is a factor connected with the prevalence of MDD. People aged 30 to 44 appear to be the most vulnerable group as to developing MDD, with the lifetime prevalence at around 20% regardless of gender (Beck & Alford, 2009; Blazer, et al., 1994; WHO, 2001). The last few decades have also seen a dramatic increase in prevalence of MDD in the population of teenagers (Seligman, et al., 2001; WHO, 2001).

When it comes to cultural and ethnical differences in the prevalence of MDD, it has been reported that the percentage of population affected by MDD varies from 5.4% in South Korea to 31.4% in Canada (Waraich, Goldner, Somers, & Hsu, 2004). People of Caucasian origin have been found to experience MDD more frequently than individuals of African origin (Beck & Alford, 2009; Blazer, et al., 1994; Riolo, Nguyen, Greden, & King, 2005). The reports on the prevalence of MDD in the population of Hispanic origin have been contradictory; some state the prevalence to be closer to the Caucasian levels (Beck & Alford, 2009; Blazer, et al., 1994), while other reports note it to be similar to the African prevalence (Kessler, et al., 1997; Riolo, et al., 2005).

1.2.4 Genetics and heritability of MDD

Major depressive disorder is a disease with a significant genetic component influencing its incidence in the population (Sullivan, Neale, & Kendler, 2000). The heritability of
the disease is estimated to be 27% to 39% by family, adoption, and twin studies (Kendler & Prescott, 1999; Sartor et al., 2012; Sullivan, et al., 2000; Wray & Gottesman, 2012). However, some studies estimate the heritability index to reach up to 70% (Lesch, 2004; McGuffin, Katz, Watkins, & Rutherford, 1996). The differences between the reports occur due to varying assumptions about the population risk (McGuffin, et al., 1996). The rest of the variance in aetiology of MDD is explained by environmental influences specific to an individual (McGuffin, et al., 1996; Sullivan, et al., 2000). This indicates that people whose relatives experienced an episode of major depressive disorder are more likely to develop the disease than people without family history of MDD mainly due to shared genetic background (Aukes et al., 2012; Fanous, et al., 2002; Lesch, 2004; Weissman, et al., 2006).

There is no single gene responsible for heritability of MDD (Lopez-Leon et al., 2007; Savitz & Drevets, 2009). However, several genes are implicated by previous studies in either vulnerability to the disease or the psychological traits connected with this vulnerability (Lesch, 2004; Lopez-Leon, et al., 2007; Savitz & Drevets, 2009). Previous genetic studies of major depressive disorder indicated that these include:

- genes connected with the serotonergic system in the CNS that is the serotonin transporter protein (5-HTT) gene with polymorphism allocated in its promoter, the serotonin receptor (5HT1A) gene and tryptophan hydroxylase (TPH1/TPH2) gene (El Hage, Powell, & Surguladze, 2009; Holmes, Bogdan, & Pizzagalli, 2010; Lesch, 2004; Lopez-Leon, et al., 2007; Savitz & Drevets, 2009);

- genes associated with HPA axis that is glucocorticoid receptor (GR, NR3C1, FKBPs) genes and mineralocorticoid receptor (MR1180V) gene (Binder et al., 2004; Claes, 2009; El Hage, et al., 2009; Praag, et al., 2004);
- brain derived neurotrophic factor (BDNF) gene (rs6265) with its allelic variant Val66Met also connected with HPA axis and responsible for the synaptic growth (Lesch, 2004; Lopez-Leon, et al., 2007; Praag, et al., 2004; Savitz & Drevets, 2009);

- catechol-O-methyltransferase (COMT) gene with its allelic variant Val158Met involved in dopaminergic and norepinephrine systems (Lesch, 2004; Lopez-Leon, et al., 2007; Savitz & Drevets, 2009);

- cyclic adenosine monophosphate response element binding protein 1 (CREB1) gene involved in RNA transcription (Lesch, 2004; Lopez-Leon, et al., 2007; Savitz & Drevets, 2009; Zubenko et al., 2002).

Carrying a risk allele of each of these genes increases a person's susceptibility to major depressive disorder. The genes implicated in vulnerability to MDD are responsible for coding of proteins participating in or influencing synaptic transmission (Lesch, 2004; Lopez-Leon, et al., 2007; Savitz & Drevets, 2009). The genes through their involvement in dopamine and serotonin networks as well as in HPA axis interact with the limbic system (amygdala in particular) and influence its ability to withstand stress and regulate emotional responses (El Hage, et al., 2009; Lesch, 2004; Lopez-Leon, et al., 2007; Praag, et al., 2004; Savitz & Drevets, 2009). Carrying a risk allele changes either one of the neurotransmitter pathways mentioned previously or its interaction with the limbic system. Thus, emotional regulation of a person is influenced indirectly by the genes involved in vulnerability to MDD.

A good example of a gene influencing interaction between one of the pathways and the limbic system is the BDNF gene with Val66Met polymorphism (rs6265). Substituting valine to methionine in a single-nucleotide polymorphism in the Val66Met BDNF gene alters activity-dependent secretion of BDNF (Chen et al., 2006). The BDNF
neurotrophin can be found in the entire nervous system, however, its highest concentration is observed in the limbic and para-limbic system regions involved in emotional processing and memory – the hippocampus, frontal lobes and limbic midbrain areas (Binder & Scharfman, 2004; Castren, et al., 2007; Duman & Monteggia, 2006). The secretion of BDNF is altered by a stress reaction of HPA axis and its secretion of glucocorticoids (Duman & Monteggia, 2006; Groves, 2007). Depressive symptoms and episodes of MDD can be evoked by hyperactivity of HPA and prolonged stress reaction, both are associated with altered levels of BDNF (Dawood et al., 2007). Carrying at least one methionine allele in the Val66Met gene alters the levels of BDNF further and therefore may influence the levels of stress tolerable for an organism and may overload emotional regulation system of a person (Chen, et al., 2006).

The polymorphisms and alterations in the candidate genes do not explain the entire variance of major depressive disorder in the population (Kendler & Prescott, 1999; Sartor, et al., 2012; Sullivan, et al., 2000; Wray & Gottesman, 2012). However, genetic affinity can to some extend explicate why MDD is considered a familial disease and why the first degree relatives of people suffering from the disorder are more likely to develop the disease than the people without such a family history (Aukes, et al., 2012; Fanous, et al., 2002; Lesch, 2004; Weissman, et al., 2006). The first degree relatives of patients suffering from MDD share 50% of genetic makeup with people affected by the disease. Therefore, they are more likely to carry genetic alleles associated with susceptibility to MDD than people without family history of the disease.

1.2.5 Symptoms

Major depressive disorder is characterized by a broad and complex symptomatology, affecting a variety of aspects in the functioning of an individual. The symptoms of
MDD are commonly divided into four clusters: mood, cognitive, motivational and physical manifestations (Beck & Alford, 2009; Seligman, et al., 2001).

These symptoms can differ among individuals in their intensity and occurrence, according to demographic and psychological characteristics of patients, as well as in severity of an episode (Beck & Alford, 2009). They may also vary due to the presence of vulnerability factors (Joffe & Regan, 1991; Nierenberg et al., 2007).

### 1.2.5.1 Mood and emotional symptoms

#### 1.2.5.1.1 Depressed mood, dysphoria

Feelings of sadness, unhappiness or guilt are one of the primary characteristics of major depressive disorder (American Psychiatric Association., 2000). Individuals suffering from MDD describe themselves as sad, unhappy, lonely, bored, hopeless, guilty, worried, ashamed, useless, irritated, empty, etc. (Beck & Alford, 2009). All these feelings point to sadness, the extent of which is not proportional in relation to the external circumstances in which the patient found themselves. In terms of the emotional regulation, altered emotional processing of the negative and/or punishing signals are at play.

The depressed mood persists throughout the course of a day and is present almost every day during an MDD episode (American Psychiatric Association., 2000). Diurnal changes are observed since the feelings of sadness are usually strongest in the morning, slowly alleviating towards the end of the day (Beck & Alford, 2009). Depending on the severity of the feelings, it may be possible to ease them through external stimuli; however, the change is seldom lasting (Beck & Alford, 2009).
1.2.5.1.2 Negative feelings toward oneself, self-dislike

Self-dislike can be connected with a general low mood, but because of its direction toward self, it is distinguished from the universal feeling of sadness. The symptom is also discriminable from low self-esteem – a cognitive symptom of MDD. Self-dislike is a feeling of disappointment or disgust with oneself (Beck & Alford, 2009). Patients with an acute MDD episode report having this feeling significantly more often than individuals with chemically induced depressive symptoms (Pasquini et al., 2008).

1.2.5.1.3 Reduction in gratification, anhedonia

Anhedonia is the most common symptom of MDD and is experienced by 92% of patients (Beck & Alford, 2009). Individuals suffering from anhedonia lose the ability to feel pleasure and joy, first in relation to their duties, and later in most areas of their life as well. People with MDD are unable to feel happy or satisfied, and experience indifference toward things they do or toward people they meet. In terms of emotional regulation, anhedonia indicates a diminished reaction to rewarding stimuli. The more pronounced the symptom, the less likely the patient is to recover (Rottenberg, Kasch, Gross, & Gotlib, 2002b).

1.2.5.1.4 Loss of emotional involvement

Patients suffering from MDD start to be indifferent to activities and people in their lives. Also, the emotional attachment to some aspects of oneself diminishes in the process. Patients suffering from the disease may no longer be concerned with their appearance or whether they are fulfilling their basic needs (Beck & Alford, 2009).

1.2.5.1.5 Crying

Patients suffering from MDD cry easier than before the onset of the disease (Beck & Alford, 2009; Rottenberg, et al., 2002b), with desire to cry being more frequent in
women than in men (Beck & Alford, 2009; Carter, Joyce, Mulder, Luty, & McKenzie, 2000; Vredenburg, Krames, & Flett, 1986). Moreover, patients with MDD, unlike non-depressed individuals, do not feel a relief after the act of crying (Beck & Alford, 2009; Rottenberg, et al., 2002a).

1.2.5.1.6 Loss of mirth response

Patients with MDD have a diminished joy reaction to humorous stimuli (Beck & Alford, 2009; Uekermann et al., 2008). They are unable to notice the positive side of situations. This, in turn may be associated with emotional regulation through a diminished ability to react to rewarding stimuli.

1.2.5.2 Cognitive symptoms

1.2.5.2.1 Low self-evaluation

Negative perception of oneself is a common characteristic of individuals with MDD. The patients describe themselves as inferior, inadequate, worthless, failures, having lots of defects, etc. Their reaction to errors is excessive – mistakes are seen in proportions that do not correspond to reality, and a single mistake is seen as a reflection of a general defect. Moreover, a patient can interpret a neutral occurrence as a personal failure (Beck & Alford, 2009).

Patients with MDD have the lowest self-esteem among all psychiatric patients (Silverstone, 1991). Furthermore, individuals with MDD are more sensitive to negative feedback and follow it more readily than healthy controls (Murphy, Michael, Robbins, & Sahakian, 2003). This implies that they selectively attend to negative information and experience difficulties in disengaging their attention from such information.
1.2.5.2.2 Negative view of the future

Individuals suffering from the disease expect negative things to happen to them and believe that they are not going to be able to prevent them (Marquart, Overholser, & Peak, 2009). This may imply an impaired ability to disengage one’s attention from emotional processing to form a plan how to handle everyday events. This symptom has been found to be highly correlated with depression scores (Miranda & Mennin, 2007).

1.2.5.2.3 Self-blame

Patients with MDD blame their mistakes and general misfortunes on their excessive inadequacy. A single mistake on their part is enough to infer a major defect in them. In more severe cases, individuals may blame themselves for events that are clearly outside their power (Beck & Alford, 2009).

1.2.5.2.4 Indecisiveness

Patients with MDD procrastinate and postpone deciding. Moreover, their resolution is not as firm as before the disease – they tend to change their mind more frequently and go back and forth between alternatives. Patients are unable to concentrate long enough to go through the decision-making process. They are also afraid that once a decision is made, they need to act on it although they do not feel the required motivation to do so (Beck & Alford, 2009).

1.2.5.2.5 Perceiving and processing of external emotional information

Altered perception and processing of emotional information from the external environment represents a symptom on the borderline between emotional and cognitive domains. This symptom strongly distinguishes patients with MDD from healthy individuals, and can manifest itself in three different ways. First of all, patients with MDD may not notice external positive stimuli or may perceive them as less positive or
as neutral. This is called positive attenuation (Berenbaum, Raghavan, Le, Vernon, & Gomez, 2003; Bylsma, et al., 2008) and is associated with anhedonia. Secondly, it has been suggested that patients with MDD are quicker to perceive negative information, and tend to see it as more negative than healthy controls. This is called negative potentiation (Bylsma, Taylor-Clift, & Rottenberg, 2011; Gollan, Pane, McCloskey, & Coccaro, 2008; Myin-Germeyser et al., 2003; Pukananen, et al., 2011), and is associated with dysphoria. A negative interpretation of neutral stimuli is also associated with it. Finally, it is observed that patients with MDD may be insensitive to emotional information coming from external environment regardless of its appetitive valence. This is called emotion-context insensitivity, and is associated with a general lack of motivation in patients with MDD (Langenecker et al., 2005; Rottenberg, Gross, & Gotlib, 2005).

1.2.5.2.6 Impairments in cognitive processes

In addition to all the alterations under discussion, individuals suffering from MDD are impaired in the modal aspect of cognitive functioning. Patients with MDD have difficulties with executive function, short-term memory, and attentional and inhibitory control, especially in term of the processing of emotional stimuli (Austin, Mitchell, & Goodwin, 2001; Castaneda, Tuulio-Henriksson, Marttunen, Suvisaari, & Lonqvist, 2008; Kennedy, 2008; Murrough, Iacoviello, Neumeister, Charney, & Iosifescu, 2011; Paelecke-Habermann, Pohl, & Leplow, 2005; Porter, et al., 2003; Ravnkilde et al., 2002; Reppermund, Ising, Lucae, & Zihl, 2009; Stordal et al., 2004; Zhao, Gong, Chen, & Miao, 2010). Some of these deficits are content-dependant: patients with MDD have been shown to have deficient inhibitory control mostly over negative stimuli (Christensen, Kyvik, & Kessing, 2006; Goeleven, De Raedt, Baert, & Koster, 2006; Mathews & MacLeod, 1994), being unable to shift their attention from it. Some of the
cognitive deficits, such as an impaired inhibition of the negative stimuli, are also observed in individuals with remitted MDD (Hammar et al., 2010). This implies that such impairments are connected with vulnerability to MDD.

1.2.5.3 **Motivational symptoms**

1.2.5.3.1 **Loss of internal motivation**

The internal drive to act and plan is reduced in patients with MDD. In milder depression, patients are unable to commit to actions connected with the routine of their lives, such as work or family life, whereas in a more severe state of depression, inertia and inability to self-motivate can affect almost all activities. The inability to act does not mean that patients do not know what to do; rather, they cannot find enough will power to do it (Beck & Alford, 2009).

1.2.5.3.2 **Wishes to withdraw**

Patients suffering from MDD have wishes to withdraw from their pattern of life. Patients wish to escape towards activities that are more relaxing and that offer at least a temporary refuge. Individuals suffering from MDD often do not believe that they are able to fulfil their previous duties or that they will find something interesting in them. Therefore, they have explicit wishes to avoid them (Beck & Alford, 2009).

1.2.5.3.3 **Suicidal wishes**

A wish to commit a suicide is observed among patients with MDD more frequently than in healthy population (Angst, Angst, & Stassen, 1999). The intensity of the symptom is positively correlated with the severity of depression (Beck & Alford, 2009). Actual suicide attempts are most frequently observed in patients whose state changed rapidly, patients with a late onset (Angst, et al., 1999) as well as patients with comorbid anxiety disorder (Bronisch & Wittchen, 1994). Individuals suffering from a more severe
depressive state, and whose will is not entirely paralyzed are more likely to commit a suicide (Beck & Alford, 2009).

1.2.5.3.4 Increased dependency

A wish to be guided or protected by another person is a common characteristic among patients suffering from MDD (Beck & Alford, 2009). Patients with MDD perceive themselves and others more negatively than healthy controls, although they will see others more favourably than themselves (Fexias, Erazp-Caicedo, Lewis Harter, & Bach, 2008). The patients perceive themselves to be inadequately prepared to face the world.

1.2.5.4 Physical symptoms

1.2.5.4.1 Loss of appetite and weight change

One of the first symptoms individuals notice when an MDD episode starts is loss of appetite. Meals lose their taste and do not seem that necessary (Beck & Alford, 2009). The loss of appetite is often followed by loss of weight (Garfinkel, Garner, Kaplan, Rodin, & Kennedy, 1983). In very severe states patients may require force-feeding. However, some patients may gain weight during an MDD episode (Beck & Alford, 2009; Casper et al., 1985). In milder depression eating is one of the last activities evoking a pleasant reaction.

1.2.5.4.2 Sleep disturbances

Patients with MDD suffer from insomnia, wake up early in the morning and have difficulties with falling asleep (Beck & Alford, 2009). The quality of their sleep is lower, which decreases their sleep efficiency and makes them feel less-rested during the day (Gottesmann & Gottesman, 2007; Thase et al., 1997). However, similarly to the case of weight loss, some patients experience hypersomnia instead (Casper, et al., 1985).
1.2.5.4.3 Loss of libido

Some loss of libido is common among both men and women (Williams & Reynolds, 2006). Patients report a diminished arousal and a decreased interest in sexual activity (Kennedy, Dickens, Eisfeld, & Bagby, 1999). This symptom correlates with loss of appetite, anhedonia and a depressed mood. Similarly to appetite, in milder cases of MDD, a slightly heightened libido is sometimes observed due to the fact that sexual behaviours are one of the last remaining gratifications (Beck & Alford, 2009).

1.2.5.4.4 Fatigability

Fatigue is very often one of the main symptoms observed at the onset of MDD (Demyttenaere, De Fruyt, & Stahl, 2005). The symptom is also one of the slowest to respond to therapy and most difficult to treat (Baldwin & Papakostas, 2006). Patients with MDD may experience it as a psychomotor retardation or a general loss of energy (Beck & Alford, 2009).

1.2.6 Burdens of MDD

Given the foregoing, it seems clear that major depressive disorder represents a severe burden for both patients suffering from MDD and for their environment. Morbidity, mortality, prevalence and the enormous impact the disease exerts on person’s life all indicate that prevention and appropriate treatment are fundamental in this regard. Therefore, studies examining vulnerability, resilience and mechanisms of developing MDD are of utmost importance both in terms of individual clinical care and public health.

1.3 Emotional regulation in major depressive disorder

The emotional regulation model is a framework in the context of which the mechanisms of major depressive disorder are to be discussed in this thesis. The underlying
assumption for the choice of this explanatory framework is that the ability to successfully regulate emotional states is crucial to mental health (Gross & Muñoz, 1995; Yap, et al., 2007).

1.3.1 Basic concept of emotion regulation
A healthy person needs to be able to recognize which sections of the environment are rewarding or punishing to them. To be able to do this, the person must perceive rewarding stimuli as pleasant and punishing stimuli as unpleasant, since these are the biological signals to approach or to withdraw. The person must also be able to correctly recognize an emotional reaction in themselves, which encapsulates an informative aspect of emotional processing (Longstaff, 2005). Furthermore, to efficiently regulate an emotional state, the person needs to know how to shift attention from processing the state to perform other tasks. This ability allows the person to perceive the entire environment. It also enables a successful planning of how to approach a rewarding situation or how to withdraw from a punishing one. The capacity to shift attention from an emotional state is then based on the ability to inhibit processing of emotional information. In other words, a successful emotional regulation involves the ability to adjust one’s own emotional states to context, situation, and to one’s own goals and needs (Campos, Frankel, & Camras, 2004; Goldsmith & Davidson, 2004).

1.3.2 Benefits of emotional regulation model for studying MDD
The emotional regulation model postulates that major depressive disorder is a disease of impaired or altered emotional regulation and that this dysregulation is a key feature of MDD (Aldao & Nolen-Hoeckema, 2010; Almeida, et al., 2011; Beauregard, et al., 2006; Campbell-Sills, et al., 2006; Davidson, et al., 2002a; Erk, et al., 2010; Etkin & Schatzberg, 2011; Feldman, et al., 2009; Gotlib & Joormann, 2010; Johnstone, et al., 2007; Joormann & Gotlib, 2010; Kennedy, et al., 2006; Liverant, et al., 2008; Mennin,
et al., 2007; Rottenberg, et al., 2002a; Taylor & Liberzon, 2007; Wang, et al., 2008b; Yap, et al., 2007). In this study, it is further postulated that the abilities described above as fundamental for an efficient emotional regulation, that is emotional recognition and processing, attention shifting and inhibition of emotional processing are altered in individuals with MDD. As a result, patients with MDD are expected to experience changes in both behavioural and neural correlates of emotional regulation.

The model of emotional regulation represents a useful framework for examining the development and course of MDD for several reasons. First of all, the model is capable of combining the biological, psychological and social aspects of major depressive disorder, incorporating inputs and evidence from all three perspectives. Since all these aspects of an individual’s homeostasis are affected in MDD, it is crucial to work within a framework of a representation of the disease which addresses all the aspects.

In addition, there is compelling evidence coming from all the three deregulated domains indicating that the model of emotional regulation describes MDD accurately. From the biological perspective, there is evidence that major depressive disorder can be described as a pattern of functional changes in the regions of CNS involved in the emotional regulation such as the limbic system, the frontal lobes and the inferior parietal cortex (Almeida, et al., 2011; Beauregard, et al., 2006; Clark, et al., 2009; Davidson, et al., 2002a; Erk, et al., 2010; Etkin & Schatzberg, 2011; Green & Malhi, 2006; Heller et al., 2009; Johnstone, et al., 2007; Ritchey, Dolcos, Eddington, Strauman, & Cabeza, 2011; Taylor & Liberzon, 2007; Wang, et al., 2008b). Further evidence suggests that such alterations may be associated with changes in the secretion of and responsiveness to certain inhibitory neurotransmitters, such as serotonin and endorphin, in emotional regulation networks of individuals suffering from MDD (Hariri et al., 2003; Kennedy, et al., 2006; Prossin et al., 2010). In the framework of the model, immunology and
cardiovascular changes accompanying MDD are explained by alterations in the autonomic regulatory system, responsible for a vegetative emotional (visceral) response of the organism to the external environment (O'Connor, Allen, & Kaszniak, 2002).

From the psychological perspective, patients with MDD experience difficulties in affective and cognitive processes involved in the emotional regulation. Individuals suffering from MDD perceive and process emotional stimuli differently from healthy controls (Bylsma, et al., 2008; Bylsma, et al., 2011; Ellis, Beevers, & Wells, 2009; Gollan, McCloskey, Hoxha, & Coccaro, 2010; Heller, et al., 2009; Mah & Pollock, 2010; Mennin, et al., 2007; Punkanen, et al., 2011; Ritchey, et al., 2011; Rottenberg, et al., 2002b). That is, the external environment affects their mood in a way that is not characteristic of the healthy population. Thus, the levels of anhedonia and dysphoria are increased in MDD sufferers. Also, the patients are not as efficient as healthy controls in applying executive control and in shifting attention from emotional processing (Austin, et al., 2001; Castaneda, et al., 2008; Kennedy, 2008; Murrough, et al., 2011; Paelecke-Habermann, et al., 2005; Porter, et al., 2003; Ravnkilde, et al., 2002; Reppermund, et al., 2009; Stordal, et al., 2004; Zhao, et al., 2010). They find it difficult to inhibit processing of negative emotional information (Goeleven, et al., 2006; Gotlib & Joormann, 2010; Green & Malhi, 2006; Joormann & Gotlib, 2010; Mennin, et al., 2007; Wang, et al., 2008b), which suggests that they are less flexible in shifting their attention when emotional regulation is involved.

Evidence from an inter-individual domain also confirms that the emotional regulation model is an appropriate framework to describe MDD. The disorder is more likely to develop when an individual lacks support from the familial environment in the regulation of his or her emotions. For instance, adolescents who do not receive adequate help and support from their parents in regulating their anger and sadness suffer from
MDD more frequently than their supported peers (Calkins, 1994; Feng et al., 2009). However, vulnerability to MDD can also increase when other individuals attempt to completely take over person’s role in his or her emotional regulation (Yap, et al., 2007). Next, the symptoms observed in MDD can be described as a result of alterations in the emotion regulation system of an individual suffering from the disease. The severity of depressive symptoms is correlated with the inability to regulate emotional states in individuals with MDD (Aldao & Nolen-Hoeksema, 2010; Campbell-Sills, et al., 2006; Erk, et al., 2010; Gollan, et al., 2010; Heller, et al., 2009; Joormann & Gotlib, 2010; Phillips, Drevets, Rauch, & Lane, 2003b; Qing et al., 2010). Emotional symptoms of MDD, such as anhedonia, dysphoria, self-dislike as well as cognitive symptoms such as low self-esteem are associated with alterations in the neural regions responsible for emotional regulation (Grimm et al., 2009; Koenigs & Grafman, 2009; Phillips, et al., 2003b). Furthermore, low self-esteem and pessimistic outlook on personal future are based on disproportional and excessive emotional reaction of an individual to negative cues that are often unconnected with him or her (Grimm, et al., 2009). Emotional recognition and control over emotional processing are thus the core abilities of emotional regulation, whereas alterations in these processes are cognitive symptoms of MDD.

Yet another advantage of the model of emotional regulation is that despite its broadness, it is specific enough to generate precise verifiable hypotheses. Additionally, a gradual development of MDD (Feng, et al., 2009; Yap, et al., 2007) as well as vulnerability to it (Ehring, Tuschen-Caffier, Schnulle, Fischer, & Gross, 2010; Gross & Muñoz, 1995; Joormann & Gotlib, 2010; Joormann, Talbot, & Gotlib, 2007b; Phillips, et al., 2003b) can be explicated in the context of the model. Last but not least, the patient’s emotional regulation has been shown to improve when he or she starts
responding to pharmacological or psychological treatment (Anand et al., 2005; Canli et al., 2005; Feldman, et al., 2009; Gross & Muñoz, 1995; Ritchey, et al., 2011; Rottenberg & Gross, 2007).

1.3.3 Emotional regulation and its neural correlates in healthy population

An effective emotional regulation involves abilities to recognize the emotional valence of stimuli as well as to generate and promote emotional arousal congruent with the individual’s goals, and with the context of a situation (Campos, et al., 2004; Goldsmith & Davidson, 2004). Additionally, a successful emotional regulation requires the ability to shift attention from such emotional information which is not congruent with individual’s goals (Gross, 1998). Inhibition of emotional processing is then a part of attention shifting process. An effective emotional regulation is thus critical for mental health (Eippert et al., 2007), which has been demonstrated in healthy population.

1.3.3.1 Emotion generation and recognition of emotional valence

Emotions can be defined as subconscious evaluations made by a person about situations being potentially rewarding or punishing (Longstaff, 2005). Those situations which are potentially rewarding evoke pleasant emotions, whereas those situations which are potentially punishing evoke unpleasant emotions. On the basis of these evaluations a person knows or feels whether they should approach a situation or withdraw from it. Therefore, emotions form a part of a biological signalling system. For this system to be effective, the person needs to correctly recognize situations as potentially harmful or beneficial, to respond to them with an appropriate emotional state, and to be able to correctly interpret one’s own emotional condition. Emotional states are known to consist of three components: a visceral sensory component resulting in autonomic changes in the organism, a motor component involving changes in facial expression and
body posture, and a cognitive/informative/analytical component connected with knowledge about one's own reaction to a particular stimulus (Longstaff, 2005).

Emotions arise as a response to a change in either an external or an internal environment which may have important consequences to the person (Longstaff, 2005). The consequences may be immediate or delayed. Also, emotions can be evoked by association when the person encounters a representation of a punishing or a rewarding object, rather than the object itself (Smith, Cacioppo, Larsen, & Chartrand, 2003). Furthermore, an effective emotional signalling system must be able to distinguish between those situations that lead to potential consequences for a person and those that are irrelevant.

On the basis of the role emotions play in the approach and withdrawal, they may be classified as either pleasant or unpleasant. Similarly, the situations, environments, and stimuli to which the emotional states are the response, can be categorized as positive, negative or neutral (the last category refers to situations which are potentially unimportant). It has been observed that healthy individuals react quicker, with a higher startle effect and with more attentional allocation to stimuli that are positive or negative in valence, in comparison to neutral cues (Amrhein, Mühlberger, Pauli, & Wiedemann, 2004; Rothermund, Wentura, & Bak, 2001; Smith, et al., 2003; Stormark, Nordby, & Hugdahl, 1995). Out of the three, negative stimuli receive the highest attentional allocation and evoke the strongest somatic reaction (Carretié, Mercado, Tapia, & Hinojosa, 2001; De Houwer & Hermans, 1994; Hietanen & Korpela, 2004; Smith, et al., 2003). However, positive stimuli have been shown to be the quickest to be processed and the hardest to ignore at the onset (Hare, Tottenham, Davidson, Glover, & Casey, 2005). These patterns appear to be present across cultures (Elfenbein & Ambady, 2002) and independently of the sensory modality to which the stimuli are
presented (Baumgartner, Esslen, & Jäncke, 2006; De Houwer & Hermans, 1994; Paulmann, Ott, & Kotz, 2011; Wallbott & Scherer, 1986). As for the speed and accuracy of the emotional recognition, they can be influenced by the mood of an individual, especially when different from the emotional valence of the observed stimuli (Murphy & Zajonc, 1993; Rusting, 1998; Wentura, 1999). The mood, in contrast, can be influenced by individual personality traits, such as neuroticism and extraversion, which points at a moderation role of individual differences in emotional recognition (Hamann & Canli, 2004; Rusting, 1998).

Neural foundations of emotion generation and emotional valence evaluation have been demonstrated well in healthy individuals. For instance, a meta-analysis conducted by Kober et al. (Kober et al., 2008), including 162 neuroimaging studies, showed that six functional groups of CNS areas are involved in emotional valence recognition and generation of emotional states in healthy controls. It is noteworthy that these areas are all activated in emotion recognition paradigms regardless of the content of stimuli (Britton, Taylor, Sudheimer, & Liberzon, 2006).

At this point it may also be important to mention that some of the areas consistently activated by emotion recognition paradigms may participate in the inhibition of emotional processing. Some of these regions have a double role, either promoting emotional arousal or inhibiting it. Their involvement in the inhibition domain is described in the next chapter (1.3.3.2). The regions may be consistently activated in emotional paradigms because there is a great need for control processes in emotion recognition tasks requiring a correct answer in a trial (Costafreda, Brammer, David, & Fu, 2008). Such areas are, however, most strongly activated in the tasks requiring attention switching. These areas and their role are briefly mentioned here in view of
being discussed in greater detail in the next chapter (1.3.3.2) dealing with attention shifting.

There are six important groups activated during emotional processing tasks: the lateral occipital/visual association group, the medial posterior group, the cognitive/motor group, the lateral paralimbic group, the medial prefrontal cortex group and the core limbic group (Kober, et al., 2008). The first two groups consist mostly of the visual regions, and have thus been considered as a connected functional entity. The lateral occipital/visual association group includes the right and left lateral occipital lobe, the right occipital-temporal area (superior temporal gyrus), the inferior temporal cortex and the superior cerebellum, whereas the medial posterior group involves the cuneus, precuneus and the posterior cingulate cortex. The role of these regions is mostly visual. In the majority of previous studies, the stimuli have been visual, hence the reported increase of activation in primary and associative visual areas. However, affective stimuli activate the two groups of CNS regions more robustly than neutral stimuli (Hendler, Rotshtein, & Hadar, 2001). Interestingly, the regions receive a direct connection from the amygdala (Freese & Amaral, 2005), one of the main components of the emotional network of the CNS. What is more, damage to the amygdala reduces the difference in response of visual areas to emotional versus neutral faces (Vuilleumier, Richardson, Armony, Driver, & Dolan, 2004), which suggests a top-down control mechanism between the regions. The sensory cortices are a part of the emotional system in the sense that they are specifically attuned to detect emotional stimuli as a category of special importance.

The superior temporal gyrus (STG) together with the left supramarginal gyrus included in the lateral occipital/visual association group is the multimodal associative cortex (Kandel, Schwartz, & Jessell, 2000; Macaluso & Driver, 2001). In healthy controls, the
region is involved in the processing of visual emotional stimuli in three instances: a) when a person imagines how they would feel in a situation requiring an emotional decision (association between somatic/visceral and visual processing) (Burnett, Bird, Moll, Frith, & Blakemore, 2009; Damasio et al., 2000; Ruby & Decety, 2004), b) when an individual is affected by visual stimuli to such a degree that they relate the stimuli to their well-being, and therefore a somatic arousal occurs (Craig, 2002; Hooker, Verosky, Germine, Knight, & D'Esposito, 2010; Northoff et al., 2004; Saxe & Kanwisher, 2003) and c) when a person makes attributions about the other person’s emotional state by unconsciously simulating changes in their expressions (mirror neurons system) (Hooker, et al., 2010; Kober, et al., 2008; Saxe & Kanwisher, 2003; Saxe, Xiao, Kovacs, Perrett, & Kanwisher, 2004). Since the area coproduces somatic reactions to emotional stimuli and is closely linked to semantic regions of the brain (the left angular gyrus, for example), the multimodal associative cortex participates in categorization and naming of individual’s emotional states (Hutcherson et al., 2005). Thus, it forms a part of the network which processes affective meaning (McRae et al., 2010).

Posterior cingulate cortex (PCC), which belongs to the medial posterior group, is a relay area between the two visual groups and the other affective clusters of regions (Kober, et al., 2008). It specifically reacts to emotional stimuli (Maddock & Buonocore, 1997; Maddock, Garrett, & Buonocore, 2003). Together with the middle cingulate cortex (MCC), the region allows an individual to be aware of other’s and one’s own feelings, with more relative activation in the 3rd person perspective (Bargh & Tota, 1988; Jackson, Brunet, Meltzoff, & Decety, 2006; Lamm, Batson, & Decety, 2007; Lou et al., 2004; Maddock, et al., 2003; Moran, Macrae, Heatherton, Wyland, & Kelley, 2006; Northoff, et al., 2004; Singer et al., 2004; Tomlin et al., 2006). It participates in assessing whether a stimulus refers to a person’s self and whether it is important in the
context of self (Moran, et al., 2006). According to the definition presented previously, emotions are generated in response to changes connected with potentially important consequences to self. The more self-relevant a stimulus, the stronger emotional reaction it evokes. Therefore, emotional processing engages the CNS self-referential system of a person, i.e. areas such as STG and PCC. Employment of these areas may be a gating mechanism through which information gathered in sensory cortices, after reaching a certain threshold stimulates affective arousal in the CNS.

Cerebellum, a part of the lateral occipital/visual association group, may also play a role in emotional processing. It has rich connections with other regions belonging to the emotional processing network (Middleton & Strick, 1994, 2000; Schutter & Van Honk, 2005) and reacts to negative stimuli (Wager, Phan, Liberzon, & Taylor, 2003). Lesions in the region result in a fluctuation between flattened affect, depressive symptoms and dysregulation of behaviour (Schmahmann & Sherman, 1998; Wolf, Rapaport, & Schweizer, 2009). A stimulation of the region leads to an increase of activation in the mesolimbic affective areas and may cause rage (Heath, Dempsey, Fontana, & Myers, 1978; Heath, Cox, & Lustick, 1974). Cerebellum is suggested to have a role in stabilizing mood (McGrady, Yonker, Tan, Fine, & Woerner, 1981; Schmahmann, 2000).

The cognitive/motor group consists of the left pre-supplementary motor area (SMA)/the middle frontal gyrus, the right frontal operculum and the bilateral inferior frontal gyri (IFG). It has been consistently activated in the studies of emotional recognition. However, the main role of the group may lie in choosing an appropriate response to a question about the valence of a stimulus, rather than in generating an emotional state or recognizing it per se. The pre-SMA is involved in an action selection and energizes cognitive and motor systems to respond to internal cues (Lau, Rogers, Haggard, &
Passingham, 2004; Stuss & Alexander, 2007). The frontal operculum and the IFG have been found to be most active in studies which require switching between emotions, tasks or attention focus as well as in studies eliciting response inhibition (Aron, Fletcher, Bullmore, Sahakian, & Robbins, 2003; Aron et al., 2004b; Badre, Poldrack, Paré-Blagoev, Insler, & Wagner, 2005; Chong, Williams, Cunnington, & Mathingley, 2008; Coull, Frith, Buchel, & Nobre, 2000; Gabrieli, Poldrack, & Desmond, 1998; Hooker, et al., 2010; Macaluso & Patria, 2007; Martin & Chao, 2001; Moss et al., 2005; Poldrack et al., 1999; Swick, Ashley, & Turken, 2008; Wager, Jonides, Smith, & Nichols, 2005; Wagner, Maril, Bjork, & Schacter, 2001; Zhang, Feng, Fox, Gao, & Tan, 2004). It may be concluded that in comparison to non-emotional stimuli shifting attention from or inhibiting of emotional stimuli is more demanding. Therefore, any task that requires a repeated disengagement of one’s attention from emotional stimuli (for example, to select a proper response) activates these areas.

The lateral paralimbic group consists of the bilateral orbitofrontal cortex (OFC), the bilateral insula, the bilateral striatum and the left hippocampus. In the context of emotional processing, the group is believed to contribute to its motivational aspect, i.e. to the willingness of an individual to approach a situation or to withdraw from it.

The OFC and striatum are areas in the CNS responsible for predicting and discriminating a reward or a punishment value of stimuli (Cardinal, Parkinson, Hall, & Everitt, 2002; Delgado, Nystrom, Fissell, Noll, & Fiez, 2000; Kringelbach & Rolls, 2004; O'Doherty et al., 2004; O'Doherty, Kringelbach, Rolls, Hornak, & Andrews, 2001), and as such participate in the self-motivation of a person. The OFC is involved in the decision-making process. According to the somatic marker hypothesis, the OFC can recreate an approximation of an emotion which a person would feel in a given situation (Bechara, Damasio, & Damasio, 2000) by integrating inputs from numerous
regions of the brain (sensory cortices, prefrontal cortex, anterior cingulate cortex, thalamus, amygdala, hippocampus) (Kringelbach & Rolls, 2004). Therefore, an activated OFC allows a person to feel which situation is rewarding and which punishing, before it takes place. In this manner, the OFC creates willingness in a person to approach the situation or to withdraw from it.

The striatum has been classified to belong to two functional groups, on the basis of its activation, function, location and connections: the lateral paralimbic group and the core limbic group (Kober, et al., 2008). Its role in emotion generation, sustenance and recognition is described in the study in the section presenting the limbic group. Its participation in a behavioural inhibition is delineated in the next chapter (1.3.3.2).

The ventral insula is associated with strong elicited emotional states (Goldin, McRae, Ramel, & Gross, 2008; Northoff, et al., 2004; Wager & Feldman Barrett, 2004). The region is laterally specialized when it comes to emotional processing. The right insula is involved in interoception and representation of bodily responses (Craig, 2002; Critchley, Wiens, Rotshtein, Öhman, & Dolan, 2004), and plays a role in the integration of the internal and external cues into motivational states. Since it is an interoception area, the insula plays a role in recognizing and naming emotional states of an individual (Hutcherson, et al., 2005).

Finally, the hippocampus is responsible for memory processes, such as encoding and retrieval (Vincent et al., 2006). Its activation during emotional processing indicates that memory retrieval plays a role in emotion and in integrating its various components (Kober, et al., 2008).

The medial prefrontal cortex group includes the anterior portion of the anterior cingulate cortex (ACC) and the dorsomedial prefrontal cortex. The group takes part in generation, regulation and recognition of emotional states (Northoff, et al., 2004). A
functional division of the ACC has been suggested by other studies. Its anterior/ventral part is strongly associated with affect generation and visceral reaction, while the posterior/dorsal part of the ACC is involved in the cognitive control of emotions (Bush, Luu, & Posner, 2000; Devinsky, Morrell, & Vogt, 1995; Vogt, Finch, & Olson, 1992). The anterior portion of the ACC takes part in self-awareness processes of an individual and in interpreting exteroceptive and interoceptive emotional cues as rewarding or punishing (Knutson, Fong, Bennett, Adams, & Hommer, 2003; Lane et al., 1998). It is involved in recognizing and naming emotional states of an individual (Hutcherson, et al., 2005). Additionally, the area participates in emotional processing of rewarding and painful stimuli (Knutson, et al., 2003; Posner, Rothbart, Sheese, & Tang, 2007; Rogers et al., 2004b).

The medial prefrontal cortex (PFC) is assumed to take part in making attributions about emotional states of oneself and of others (Adolphs, 2001; Blakemore, Winston, & Frith, 2004; Lane & McRae, 2004; Ochsner et al., 2004b). The area is responsible for the meta-cognitive ability to recognize and name emotions as well as to monitor and to re-evaluate them (Ochsner & Gross, 2005; Ochsner et al., 2004a). It is a part of the network which processes affective meaning (McRae, et al., 2010). The medial PFC is also involved in the regulation and generation of the stress response of the HPA axis (Sullivan & Gratton, 2002).

Finally, the core limbic group consists of the bilateral amygdalae/hippocampi, the thalamus, the hypothalamus and partially the striatum. This group is responsible for basic affect generation and holds a primary role in this among all the CNS regions. It is responsible for producing visceral emotional states.

The thalamus is a general integrative centre of the whole affective system, which projects to the brain stem and the peripheral nervous system (Edelman & Tononi,
2003). Its stimulation produces a strong affective arousal (Hamann, Herman, Nolan, & Wallen, 2004; Lenz et al., 1995). It is also involved in producing pain sensations, which is a biological regulative signal more basic than emotions are (Bushnell & Duncan, 1989; Jeanmonod, Magnin, & Morel, 1993; Tölle et al., 1999; Zubieta et al., 2001).

The hypothalamus is involved in affective vegetative homeostasis of an organism and in stress reaction (Damasio, et al., 2000; Praag, et al., 2004). The hypothalamic activity increases during emotional processing since emotional states change the homeostasis of the body to prepare the organism to quickly and efficiently approach a situation or to withdraw from it. This reaction is triggered even if a situation is important to the person only potentially (Beauregard, Levesque, & Bourgouin, 2001).

The striatum, as a part of mesolimbic dopamine system, participates in generating pleasant or unpleasant emotional states in response to either rewarding or punishing stimuli as well as to stimuli that are novel, intense or unexpected (Horvitz, 2000; Jensen et al., 2003; O’Doherty, et al., 2004; Schultz, Dayan, & Montague, 1997). The reward and punishment may be current or potential. The striatum is also responsible for sustaining an emotional state and for reinforcing continued attention to it (Aouizerate et al., 2004; Cardinal, et al., 2002; Davidson & Irwin, 1999; Drevets et al., 2001). In the case of healthy population, it reinforces sustained attention mostly to positive, pleasant and appetitive emotional states. Moreover, together with the orbitofrontal cortex, anterior cingulate cortex and dorsolateral prefrontal cortex (DLPFC) the striatum takes part in the primary motivation circuit – the first and basic network that is oriented on gratifying an individual’s needs (Chambers, Taylor, & Potenza, 2003; Knutson, Fong, Adams, Varner, & Hommer, 2001).

When it comes to its paralimbic function, the striatum is yet another gating area inhibiting or reinforcing an emotional signal. The other one is the PCC/MCC complex.
described previously. The striatum is a part of the basal ganglia-thalamocortical circuitry consisting of frontal cortex–striatum–globus pallidus and substantia nigra–thalamus–frontal cortex, and is responsible for motivated behaviour and motor activity in humans (Tekin & Cummings, 2002). The system supports five parallel circuits involved in motivation, motor expression of emotions, social responses and cognitive motor planning. The striatum is the area which, by integrating inputs from different parts of frontal cortex (OFC, DLPFC, ACC, SMA), excites (inhibiting of inhibition) or inhibits the whole system with the use of GABA-ergic connections in the direct and indirect rout in the said circuitry (Balleine, Delgado, & Hikosaka, 2007; Wichmann & DeLong, 2006). The outcome is a directed, goal-oriented movement coordinated with person’s motivation and social surrounding. The striatum also participates in recognizing and naming emotional states (Hutcherson, et al., 2005).

The amygdala/hippocampus complex verifies basic affective significance of a stimulus and reacts to it by generating an emotional arousal if the stimulus is either strongly positive or strongly negative (Adolphs, 2002; Beauregard, et al., 2001; Breiter et al., 1996; Costafreda, et al., 2008; Fernandez-Egea et al., 2009; Hamann & Mao, 2002; Paton, Belova, Morrison, & Salzman, 2006; Phelps & Anderson, 1997; Sergerie, Chochol, & Armony, 2008; Whalen et al., 2001). It prepares the CNS for experiencing an emotional state and creates a basic affective tone (Barrett et al., 2007). The amygdala is more prone to be aroused in passive processing of affective stimuli, in comparison to tasks containing active processing instructions (Costafreda, et al., 2008). It is assumed that the amygdala starts a basic affective reaction and if the emotional state needs further processing in cortical areas, the activation in amygdala is down-regulated. The area responds strongly to novelty and to highly arousing stimuli (Hamann, et al., 2004;

A meta-analysis by Phillips et al. (Phillips, Drevets, Rauch, & Lane, 2003a) divided emotional processing into three stages: a) the identification of emotional significance of a stimulus, b) the production of an emotional state in response to it and c) the regulation of the said state. The first two stages are parallel with the emotional processing and recognition described in this chapter. The third stage will be described in the following chapter (1.3.3.2) about attention switching and inhibitory control. The CNS regions with an increased activation during stages a) and b) are called the limbic/ventral compartment. The regions include: the amygdala, the insula, the striatum, the ventral ACC and the OFC.

1.3.3.2 Shifting attention and inhibitory control as processes of emotional regulation

As discussed in the previous chapter (1.3.3.1), an effective emotional regulation requires appropriate emotional responses which are in accordance with an individual's needs and a situational context. The second element of the regulatory system is the ability to inhibit affective processing and shift attention from it (Gross, 1998), balancing the first element. On the one hand, attention and its shifting can moderate and control the expression and duration of emotional states (Beck & Alford, 2009). On the other hand, shifting attention from and inhibition of emotional processing are associated with limiting or halting affective reaction. Inhibition of distracting emotional reaction is then as crucial for mental health as spreading an adaptive emotional arousal in a given context (Eippert, et al., 2007; Koole, 2009).

Shifting attention from emotional processing enables a flexible adjustment of emotional reactions to one's goals and requirements of the external environment. It takes place
when processing of emotional valence is no longer adaptive towards external requirements or internal goals. Therefore, it is a process supporting the homeostasis of an organism. For example, after an initial recognition of rewarding or punishing value of a situation and deciding whether to approach or to withdraw, a person needs to form a plan about how to do this and to follow through on it. In the process, the person must shift more attention to specific informative aspects of the situation and to his or her internal resources. This requires a disengaging of attention from focusing on affective valence of a situation by inhibiting its further emotional processing.

Also, when a change in the external environment or in the internal goal occurs, attention needs to be shifted to a new stimulus and the preceding reaction needs to recede. In other words, attention must be shifted from emotional processing when the processing is no longer central, when it has served its informative purpose, and when it has initiated a motivational reaction. To achieve this result, further emotional processing of the stimulus needs to be limited or inhibited to allow for an attention shift to a different content. This does not mean that the emotional cue is stopped before it enters the attention system, or that it is only the motor reaction to an emotional stimulus which is inhibited (suppression). This suggests instead that an emotional arousal in an individual's CNS is actively inhibited, while an individual's goal or the external environment changes.

Shifting attention from emotional stimuli leads to greater psychological costs – longer reaction times and less accuracy – than shifting attention from non-emotional cues (Hare, et al., 2005; Kim & Hamann, 2007). This applies to both positive (Hare, et al., 2005) and negative information (Compton, 2000; Koster, Crombez, Verschuere, & De Houwer, 2004). However, the cost of attention disengagement is greatest for negative cues (Kim & Hamann, 2007). From an evolutionary point of view, this is
understandable because avoiding an immediate danger represents one of the basic strategies for survival of an organism. Therefore, any negative cue in the environment attracts a considerable share of attention.

Behaviourally, gender is not a significant factor in the ability to shift attention from emotional processing: men and women do not differ in the accuracy of this ability, although they may use different strategies in this (McRae, Ochsner, Mauss, Gabrieli, & Gross, 2008). In contrast, certain personality traits have been found to influence the willingness and ability to shift attention from emotional stimuli; for example, individuals with high scores in neuroticism and extraversion have been shown to experience greater difficulties in shifting attention from emotional processing (Kokkonen & Pulkkinen, 2001b).

Some of the cognitive coping strategies are based on an individual's ability to shift attention from emotional processing (Augustine & Hemenover, 2009; Garnefski & Kraaij, 2007; John & Gross, 2004). Such strategies seem to be healthier than behavioural strategies as cognitive strategies can bring both more immediate and delayed positive results, such as healthier patterns of affect, better social functioning, and well-being (Augustine & Hemenover, 2009; Goldin, et al., 2008; Gross, 2001; John & Gross, 2004). Healthy individuals with a limited ability to shift attention from emotional processing have difficulties with using some of these strategies, and consequently report a lower general mood (Joormann, 2006). Extraversion and neuroticism, which influence the willingness to shift attention from emotional processing, can also affect an individual's general choice in coping strategies (Kokkonen & Pulkkinen, 2001a).

Neural foundations of shifting attention from and inhibiting of emotional processing have been demonstrated in healthy population. In the previous chapter (1.3.3.1), the
regions consistently activated in tasks with emotional processing have been described. Some of these regions are not primarily affective and pose a cognitive inhibitory control over affect generation and processing. They are active in the emotion recognition tasks because responding to these tasks requires some modulation of affective arousal, while planning an answer. However, once shifting attention from emotional processing becomes the main focus of the task, these regions switch from a secondary to a primary role. Moreover, some of the areas described in the previous chapter (1.3.3.1) hold a double role of either promoting or inhibiting further emotional arousal. All these regions as well as other areas not presented in the previous chapter (1.3.3.1) are discussed below.

Shifting attention from emotional processing and inhibitory control of it mostly engages the areas involved in associative and integrative processes. According to the meta-analysis presented by Phillips et al. (Phillips, et al., 2003a) and a model proposed by Mesulam (Mesulam, 1981), the regulation of affective states is dependent on prefrontal and cingulate cortices. Additionally, regions such as the inferior parietal gyrus (IPG) with supramarginal gyrus, the SMA (Wager, Jonides, & Reading, 2004), and the striatum and the cerebellum (Ravizza & Ivry, 2001) have been found to participate in the attention shifting network. The most predominant role in shifting attention from emotional processing or in its inhibition belongs to the prefrontal cortex (Corbetta, 1998; Coull, et al., 2000; Goldin, et al., 2008; Kim & Hamann, 2007; McRae, et al., 2010; McRae, et al., 2008; Phillips, et al., 2003a; Ridderinkhof, Van Den Wildenberg, Segalowitz, & Carter, 2004; Wager, Davidson, Hughes, Lindquist, & Ochsner, 2008).

Several regions in the PFC are of special interest in this domain. The first area engaged in attention shifting and inhibitory control is the inferior frontal gyrus together with the inferior triangular frontal gyrus. They have been mentioned in the previous chapter
(1.3.3.1) as forming a part of the cognitive/motor group activated in emotion processing tasks, mostly when tasks require switching of some sort. The area is responsible for shifting attention from processing irrelevant information, for selection, as well as for response and processing inhibition (Aron, et al., 2003; Aron, et al., 2004b; Badre, et al., 2005; Chong, et al., 2008; Coull, et al., 2000; Dove, Pollmann, Schubert, Wiggins, & Von Cramon, 2000; Gabrieli, et al., 1998; Hooker, et al., 2010; Macaluso & Patria, 2007; Martin & Chao, 2001; Moss, et al., 2005; Poldrack, et al., 1999; Ridderinkhof, et al., 2004; Swick, et al., 2008; Wager, et al., 2005; Wagner, et al., 2001; Zhang, et al., 2004). The role of the area is general – it is involved in shifting attention from both emotional and non-emotional processing, depending on the current goal of an individual (Aron, Robbins, & Poldrack, 2004a; Chikazoe, Konishi, Asari, Jimura, & Miyashita, 2007; Nee, Wager, & Jonides, 2007). However, its activation increases when an individual is using a cognitive strategy to reduce an emotional arousal by means of attention shifting (Ochsner, Bunge, Gross, & Gabrieli, 2002; Ochsner, et al., 2004b).

To some degree, its specialization is lateralized. The right IFG together with the right frontal operculum mostly participates in attention shifting processes (Aron, et al., 2004a; Chikazoe, et al., 2007; Corbetta & Shulman, 2002; Garavan, Ross, & Stein, 1999; Hampshire, Chamberlain, Monti, Duncan, & Owen, 2010; Liddle, Kiehl, & Smith, 2001; Nee, et al., 2007). The left IFG takes part in both attention shifting and selection, especially among compelling alternatives (Moss, et al., 2005; Swick, et al., 2008; Zhang, et al., 2004). It also contributes to controlling behaviour and mental processing through maintenance of inner speech (Morin & Michaud, 2007). The IFG is inversely correlated with amygdala and may exercise an inhibitory control over it (Johnstone, et al., 2007).
The dorsolateral prefrontal cortex also plays a role in attention shifts (Beauregard, et al., 2001; Eippert, et al., 2007). Like it is the case for the IFG, the area is activated when an individual is using a cognitive strategy to down-regulate an emotional state with attention shifting (Banks, Eddy, Angstadt, Nathan, & Phan, 2007; Urry et al., 2006). Its activation predicts attenuation of an affect following the strategy (Banks, et al., 2007).

The area has rich connections with the motor system (the SMA, pre-SMA) (Koski & Paus, 2000), and takes part in the inhibition of motor reactions (Hadland, Rushworth, Passingham, Jahanshahi, & Rothwell, 2001). The DLPFC is involved in especially demanding and difficult attention shifts (Wager, et al., 2005).

The next area of the PFC that takes part in shifting attention from emotional processing is the ventromedial prefrontal cortex (Wager, et al., 2005). The area regulates activation of the amygdala (Urry, et al., 2006), and is functionally connected with both striatum and amygdala (Wager, et al., 2008). The region participates in the control of impulses (Potenza et al., 2003). It is also involved in reducing emotional arousal with cognitive strategies based on attention shifting (Urry, et al., 2006; Wager, et al., 2008). The area is associated with rather low cost attention shifts (Wager, et al., 2005).

The orbitofrontal cortex described previously as an area integrating emotional impulses and information from the entire CNS, and creating a would-be version of a potential emotional state, also participates in the inhibition of emotional processing. The activation in the area increases when a person inhibits a pre-potent response in favour of a less habituated one (Szatkowska, Szymańska, Bojarski, & Grabowska, 2007). The region is also affected when inhibition refers to a strong impulse (Coull, et al., 2000; Horn, Dolan, Elliott, Deakin, & Woodruff, 2003). It means that the area is activated when information is particularly difficult to block. Additionally, the OFC plays a role in
down-regulating an emotional arousal with cognitive strategies involving attention shifting (Banks, et al., 2007; Eippert, et al., 2007).

Apart from the PFC, the cingulate cortex also takes part in inhibition and shifting attention from emotional processing. The area of great importance in attention shifting and inhibitory control is the anterior cingulate cortex, in particular its dorsal cognitive part (Beauregard, et al., 2001; Bush, et al., 2000). The ACC is responsible for emotional conflict resolution as well as motivated selection and maintenance of goals (Bush, et al., 2000; Etkin, Egner, Peraza, Kandel, & Hirsch, 2006; Van Veen & Carter, 2002). It helps a person to focus on a chosen stimulus and inhibits processing of distracting information (Braver, Barch, Gray, Molfese, & Snyder, 2001). Thus this area can block affective and motivational responses to a particular stimulus, depending on whether the stimulus is considered a goal or a distractor (Matthews, Paulus, Simmons, Nelesen, &Dimsdale, 2004). The ACC also participates in conscious down-regulating of both pleasant and unpleasant emotions (Eippert, et al., 2007). The region is generally involved in self-regulation since it ensures that inner goals and pursuits are followed in the context of a given situation (Posner, et al., 2007). The area participating in attention shifts and in inhibition stretches towards the middle cingulate cortex (Kana, Keller, Minshew, & Just, 2007).

The inferior parietal gyrus is a very important part of the inhibition and attention re-orienting network (Corbetta, 1998; Macaluso & Patria, 2007). Its role is connected with attention shifting per se and not limited to a particular type of attention shifts or stimulus (Corbetta, Miezin, Dobmeyer, Shulman, & Petersen, 1991; Coull, et al., 2000; Shapiro, Hillstrom, & Husain, 2002; Wager, et al., 2004). It helps an organism to orient itself in the environment by shifting its attention from irrelevant stimuli (Coull & Nobre, 1998). The IPG takes part in high-cost attention shifts most challenging for an
individual (Wager, et al., 2005). The area stretches to the pre-SMA, the SMA and the precentral gyrus (Rushworth, Krams, & Passingham, 2001) involved in motor control (Coull, et al., 2000; Dove, et al., 2000; Northoff, et al., 2004; Wager, et al., 2004). The activation responsible for attention shifting is observed mostly in the left IPG. In the right hemisphere, the targeted area in collaboration with the right supramarginal gyrus is involved in the selection of the correct response and feeling of agency (Booth et al., 2003; Chaminade & Decety, 2002; Collette, Hogge, Salmon, & Van der Linden, 2006; Garavan, et al., 1999; Menon, Ford, Lim, Glover, & Pfefferbaum, 1997).

A particular role in inhibition and attention shifting is played by striatum which, as mentioned in the previous chapter (1.3.3.1), is involved in the primary motivation circuit (Chambers, et al., 2003; Corbetta, 1998). The area can either promote a certain motivational or emotional reaction or inhibit it, depending on the activation in the entire CNS (Balleine, et al., 2007; Wichmann & DeLong, 2006). The striatum belongs to the network involved in the control of motivation, motor expression of emotions, social responses and cognitive motor planning (Tekin & Cummings, 2002). It integrates motivational, emotional and motor domains (Chambers, et al., 2003; Ravizza & Ivry, 2001). It is distinguished for an optional activation in cognitive copying strategies based on attention shifting, with the result that the stronger the connectivity between the PFC and the striatum, the better an individual’s performance in these strategies (McRae, et al., 2008; Wagner, et al., 2001).

The inhibitory network also reaches the cerebellum. The area is involved in motor inhibition and movement monitoring (Bellebaum & Daum, 2007; Nulsen, Black, & Drake, 1948; Ravizza & Ivry, 2001). This region is further responsible for switching motor reactions (Ravizza & Ivry, 2001).
1.3.4 Emotional regulation and its neural correlates in MDD

The ability to self-regulate one’s emotional states and reactions is altered and impaired in major depressive disorder (Beauregard, et al., 2006; Joormann, et al., 2007a). Generally speaking, people require efficient emotional regulation in modulating their affective reactions and states to accommodate to the requirements of their goals and the external environment conditions. In this way, individuals are able to gratify their needs by recognizing what is rewarding for them and by adjusting their behaviour accordingly. Major depressive disorder changes this process. When the condition is mild, people require help from the external environment to modulate their emotional reactions (Bylsma, et al., 2011). For instance, patients with mild MDD may react to a joke; their mood is slightly increased and negative thoughts are not overbearing when a passive activity such as watching TV attracts their attention (Beck & Alford, 2009). However, the modulation is never long-lasting and is seldom sustained or started as an act of inner will. When MDD becomes severe, patients with the disorder are often unable to gratify their basic needs without an external help. At times they need to be fed or dressed (Beck & Alford, 2009). This does not mean that they have no awareness about what to do or how to cater to their needs. It means instead that their affective regulatory system cannot modulate their emotional and motivational state to allow them to feel compelled or encouraged to gratify their needs. It is therefore crucial to recognize that the consequences of the inability to regulate emotions in patients suffering from MDD can be far-reaching (Koole, 2009).

1.3.4.1 Behavioural correlates of emotional regulation in MDD

The emotional state of patients with MDD occurring in response to both positive and negative stimuli is different from that of healthy controls (Bylsma, et al., 2008; Bylsma, et al., 2011; Punkanen, et al., 2011). The mood of the patients interacts with how they
perceive emotional valence and their reaction to it (Ellis, et al., 2009). First of all, patients with MDD are characterized by positive attenuation (Berenbaum, et al., 2003; Bylsma, et al., 2008). This means that in comparison to healthy controls, they experience a smaller amount of pleasant emotions in response to both positive daily events (Bylsma, et al., 2011) and positive experimental stimuli (Ellis, et al., 2009; Punkanen, et al., 2011). Furthermore, they do not react to positive cues as strongly as healthy subjects do (Bylsma, et al., 2008; Mah & Pollock, 2010; Rottenberg, et al., 2005), and their reactivity to reward is diminished (McFarland & Klein, 2009). Moreover, individuals suffering from the disorder experience attentional bias away from positive emotional cues (Leppänen, 2006). These characteristics of patients with MDD can be explained either by the patients’ reduced ability to experience pleasant emotions or by their inability to sustain them for long (Heller, et al., 2009).

There are two concurrent explanations regarding the question of how patients with MDD react to negative stimuli, and why subsequently they experience unpleasant emotions (Bylsma, et al., 2008). A theory of negative potentiation claims that patients with MDD experience highly unpleasant emotions in response to negative stimuli. In contrast to healthy subjects, the patients indeed report greater unpleasant affect in reaction to negative everyday events (Bylsma, et al., 2011; Myin-Germeys, et al., 2003) as well as to negative experimental stimuli (Gollan, et al., 2008; Punkanen, et al., 2011). Individuals suffering from MDD are also characterized by a greater accuracy in recognizing sad faces (Ritchey, et al., 2011), the accuracy increasing with the severity of symptoms (Gollan, et al., 2010). Finally, they display attentional bias towards processing negative emotional stimuli (Leppänen, 2006). However, a theory of emotion context insensitivity states that patients with MDD are generally less-reactive to emotional stimuli and that their reaction to affective cues is flattened, regardless of the
valence of the signal (Bylsma, et al., 2008; Ellis, et al., 2009; Mah & Pollock, 2010; Rottenberg & Gross, 2007; Rottenberg, et al., 2005).

It seems possible that both theories offer valid points for consideration. On the one hand, it is not surprising that the emotion patients experience in response to a negative cue is lower than that in healthy controls, given their general low mood. On the other hand, the patients may still experience a smaller relative change in affect between their passive state and the time when they process negative information. The mood in patients with MDD is close to one of the ends of emotional valence continuum where possible changes may be limited. In this way the patients experience a greater unpleasant emotion in response to negative stimuli, but the relative change from their basic mood is smaller. This suggests that their emotional processing system is less flexible than in healthy controls, and that it has less capacity to adjust to signals from the external environment.

Finally, patients with MDD also react differently to the stimuli that are considered neutral since they are less accurate in classifying them as neutral (Mah & Pollock, 2010). For example, they tend to interpret neutral faces as negative more often than healthy controls do (Gollan, et al., 2008).

When it comes to behavioural correlates of attention shifting, patients with MDD have more difficulties than healthy controls to disengage from an emotional material (Gotlib & Joormann, 2010). This is particularly true for a material with negative content. The patients are characterized by deficient cognitive inhibition of negative stimuli (Christensen, et al., 2006; Goeleven, et al., 2006; Gotlib & Joormann, 2010; Joormann, Yoon, & Zetsche, 2007c; Langenecker, et al., 2005; Lau, Christensen, Hawley, Gemar, & Segal, 2007; Murphy et al., 1999). They are less accurate and slower in performing an actual task if negative information is a distractor (Langenecker, et al., 2005). Shifting
attention from negative stimuli and down-regulating its processing is costly for the patients with MDD (Beauregard, et al., 2006). This can lead to a prolonged, goal-irrelevant processing of negative cues, which results in a sustained negative mood and in an inability to stay focused on the goal other than the affective processing (Joormann, et al., 2007c). Together with the alterations in the processing of rewarding stimuli, this may result in changes of the motivation characteristic for MDD. It also increases a chance of engaging in negative thinking and rumination of negative thoughts (Gotlib & Joormann, 2010; Joormann, et al., 2007c; Lau, et al., 2007).

1.3.4.2 Neural correlates of emotional regulation in MDD

The changes in emotional reactivity and valence recognition as well as in inhibiting of emotional stimuli observed in major depressive disorder are associated with alterations in the CNS of a depressed person. According to models of neural alterations in emotional regulation in MDD (Davidson, et al., 2002a; Haldane & Frangou, 2006; Mayberg, 1997; Phillips, et al., 2003b; Taylor & Liberzon, 2007), the changes influencing emotional dysregulation in the disease occur in the three compartments of the CNS. The compartments are: the limbic (or the ventral) compartment responsible for generation and recognition of emotions, the cortical (or the dorsal) compartment involved in the cognitive control over emotional processing, and the behaviour and the subcortical compartment mediating between the former two (Mayberg, 1997, 2003; Phillips, et al., 2003a).

According to these models, individuals suffering from the disease generally experience an increase of activation in the limbic compartment of the CNS responsible for emotional generation and processing (Phillips, et al., 2003a). The areas of the limbic compartment characterized by an increase of activation in MDD are suggested to be as follows in the model: the amygdala (Davidson, et al., 2002a; Drevets, 1998; Fu et al.,
2004; Mayberg, 1997; Mayberg et al., 1999; Phillips, et al., 2003b; Taylor & Liberzon, 2007), the hippocampus (Davidson, et al., 2002a; Fu, et al., 2004; Goldapple et al., 2004; Mayberg, 1997; Mayberg, et al., 1999; Ritchey, et al., 2011), the parahippocampal gyrus (Fu, et al., 2004), the hypothalamus (Mayberg, 1997; Mayberg, et al., 1999), the thalamus (Drevets, 1998; Mayberg, 1997; Mayberg, et al., 1999; Phillips, et al., 2003b), the insula (Mayberg, 1997; Phillips, et al., 2003b; Ritchey, et al., 2011; Taylor & Liberzon, 2007), the ventral prefrontal cortex (Beauregard et al., 1998; Davidson, et al., 2002a; Mayberg, 1997; Phillips, et al., 2003b; Ritchey, et al., 2011; Taylor & Liberzon, 2007) and the anterior/subgenual part of the anterior cingulate cortex (Beauregard, et al., 1998; Drevets, 1998; Frodl et al., 2009; Goldapple, et al., 2004; Mayberg, 1997; Mayberg, et al., 1999; Phillips, et al., 2003b; Ritchey, et al., 2011; Taylor & Liberzon, 2007). Abnormalities found in these regions appear to explain the changes in identification of emotional significance and generation of affective states and behaviour observed in MDD (Taylor & Liberzon, 2007).

It is important to mention, however, that an increase of activation in the regions responsible for emotional generation is sometimes connected with processing negative stimuli only, in the case of patients with MDD. The reactivity of the regions to a different type of cues has been observed to lead to a pattern of dissimilarities between patients with MDD and healthy controls, unlike the assumption in the models. There are studies reporting on patients with MDD, as compared to healthy controls, who experienced an increased activation in the amygdala, the striatum, the hippocampus, the thalamus, the insula and the anterior/subgenual part of the ACC in reaction to negative stimuli, but a decreased activation in the same regions in response to positive stimuli (Beauregard, et al., 1998; Fu et al., 2007; Fu, et al., 2004; Joormann, et al., 2007a;
Keedwell, Andrew, Williams, Brammer, & Phillips, 2005; Lawrence et al., 2004; Surguladze et al., 2005; Tremblay et al., 2005).

Regarding, the cortical compartment of the CNS responsible for attention and inhibitory control, it displays a decrease in activation in MDD (Davidson, et al., 2002a; Drevets, 2001; Eugene, Joormann, Cooney, Atlas, & Gotlib, 2010; Gotlib & Joormann, 2010; Haldane & Frangou, 2006; Mayberg, 1997; Phillips, et al., 2003b; Taylor & Liberzon, 2007; Tekin & Cummings, 2002). The areas characterized by the greatest decrease in activity are: the dorsolateral prefrontal cortex (Davidson, et al., 2002a; Goldapple, et al., 2004; Mayberg, 1997; Mayberg, et al., 1999; Phillips, et al., 2003b; Taylor & Liberzon, 2007), the dorsomedial prefrontal cortex (Goldapple, et al., 2004; Mayberg, 1997; Mayberg, et al., 1999; Phillips, et al., 2003b; Taylor & Liberzon, 2007), the orbitofrontal cortex (Drevets, 1998; Drevets, 1999; Drevets & Raichle, 1992; Elliott, Rubinsztein, Sahakian, & Dolan, 2002; Fitzgerald, et al., 2008; Lee et al., 2008; Rogers et al., 2004a), the posterior/dorsal part of the anterior cingulate cortex (Davidson, et al., 2002a; Mayberg, 1997; Mayberg, et al., 1999; Phillips, et al., 2003b; Taylor & Liberzon, 2007), the posterior cingulate cortex (Mayberg, 1997; Mayberg, et al., 1999), and the inferior parietal gyrus (Mayberg, 1997, 2003; Mayberg, et al., 1999). The deactivation of these regions may be responsible for difficulties in disengaging one's attention from emotional material, as characteristic for MDD (Ottowitz, Tondo, Dougherty, & Savage, 2002).

In patients with MDD the compartment is either over- or under-activated, depending on the context (Mayberg, 1997, 2003; Mayberg, et al., 1999).

It is important to note that the changes in all compartments may occur during emotional processing as well as during shifting attention from it. Tasks with an emotional generation require some degree of goal-oriented cognitive control. Therefore, diminished activation in the cortical compartment may be observed in patients with MDD during both emotional recognition and attention shifting. Similarly, increases in the limbic compartment can be present during both processes. In the case of attention shifting, patients may not be able to control emotional arousal sustained by the limbic regions, which can stretch in time into the inhibitory phase (Ottowitz, et al., 2002).

Moreover, changes in the emotional regulation present in MDD do not only depend on altered activation in the three compartments, but also on the connectivity between the compartments. Its disruption characteristic for the disease is of great importance in the regulation of emotional states. As mentioned previously, the connection between the PFC and the striatum as well as the amygdala is the primary motivation circuit (Chambers, et al., 2003; Knutson, et al., 2001). The functional coupling between the frontal areas, such as the OFC, the dorsal ACC and the DLPFC, and the subcortical regions, i.e., the amygdala, the striatum and the thalamus, is decreased in MDD (Almeida, et al., 2011; Anand, et al., 2005; Beauregard, et al., 2006; Dannlowski et al., 2009; Frodl, et al., 2010a; Haldane & Frangou, 2006; Heller, et al., 2009; Matthews, Strigo, Simmons, Yang, & Paulus, 2008). Additionally, a structural connectivity between the two CNS compartments is diminished in patients with MDD, and physical damages of the connection may result in depressive symptoms (Rigucci, Serafini, Pompili, Kotzialidis, & Tatarelli, 2010; Seminowicz, et al., 2004; Shah, Glabus,
Goodwin, & Ebmeier, 2002). This leads to the dysregulation of cognitive and inhibitory
total over emotional states observed in MDD.

Also, patients with MDD experience altered patterns of activation in the visual cortex in
the processing of visual emotional cues. The patients do not filter irrelevant information
in the same way as healthy subjects do, which results in lesser activation in their visual
cortex in response to emotional cues (Desseilles et al., 2009; Fu, et al., 2007).
Furthermore, the patients seem to be generally less reactive to the external emotional
stimuli in the neural areas responsible for external cues processing, such as the
precuneus and the MCC (Fu, et al., 2007). A decreased activation in the MCC during
emotional processing may also be associated with an increased self-focus in MDD
(Frodl, et al., 2009; Grimm et al., 2011; Grimm, et al., 2009). Finally, changes
characteristic for the emotional regulation in MDD manifest themselves in the
alterations in activity of the HPA axis (De Raedt & Koster, 2010; Gotlib & Joormann,
2010).

1.4 Vulnerability, resilience to MDD and types of MDD episodes in the
context of emotional regulation model

Major depressive disorder is a disease with a high prevalence and significant personal
cost for a suffering individual (Blazer, et al., 1994; Hasin, Goodwin, Stinson, & Grant,
2005; Kessler, et al., 2003; Kessler, et al., 1997). Nevertheless, as it was explained in
the chapter 1.2.4, the vulnerability to the disorder is not equally distributed among
healthy population and certain factors predispose particular individuals to developing
the disease (Haeffel & Grigorenko, 2007). Similarly, certain characteristics make
people more resistant to MDD, by promoting a capacity for emotional regulation
(Glicken, 2006). The issue of risk and resilience is not a trivial matter since it sheds
light on the development and onset of the disease. Moreover, it is of high clinical
importance since it suggests efficient methods of prevention and diagnosis of individuals.

There is also a number of vulnerability factors which may change the way the disease manifests itself. Patients who were vulnerable to suffering from MDD prior to the disease onset may experience more severe depressive symptoms or impairments, and have greater difficulties recovering from the disease (Branchi, 2011; Savitz & Drevets, 2009; Tozzi et al., 2008). Thus traits associated with vulnerability may form a foundation for distinguishing different endophenotypes of the disorder, and are of crucial importance in psychiatric research. This is particularly significant from the clinical perspective since patients representing different groups may respond to various types of treatment differently. Furthermore, their premorbid state to which they are to return after an effective therapy may set different goals for the treatment for various endophenotypes. A good understanding of the liability and resilience, as well as different types of types of MDD is thus highly important for to the development of new therapy mechanisms and for the establishment of biological risk markers relevant for an early detection of the disease.

There is a variety of factors influencing the risk and resilience in MDD, originating from all levels of homeostasis disturbed in the disorder. Genetic polymorphisms of nucleotides described in the chapter 1.2.4 that is e.g. the BDNF, CREB1, 5HTT, COMT genes (Hashimoto, 2010; Hashimoto, Shimizu, & Iyo, 2004; Juhasz et al., 2011; Mukherjee et al., 2011; Neumeister et al., 2002; Terracciano et al., 2010), history of MDD in the immediate family (Evers et al., 2006; Fanous, et al., 2002; Focht-Birkerts & Beardslee, 2000; Kendler et al., 1995; Shannon, Beauchaine, Brenner, Neuhaus, & Gatzke-Kopp, 2007; Tozzi, et al., 2008), personality traits, such as neuroticism and low self-esteem (Carbonell et al., 2002; Shannon, et al., 2007), and stressful life events and
major losses (Erdem & Slesnick, 2010; Focht-Birkerts & Beardslee, 2000; Kendler, et al., 1995; Tozzi, et al., 2008) are among such factors. There is some evidence suggesting that vulnerability factors may change the mechanisms of emotional regulation (Hariri & Holmes, 2006; Jabbi, et al., 2008; Stein, et al., 2009). Two such factors – family history of MDD and Val66Met gene polymorphism – are examined in this study.

These two factors were chosen because of their direct association with emotional regulation. Previous research has shown that family history of MDD affects not only vulnerability to the disease, but also reactivity to positive and negative cues, and negative thoughts perseveration (Giles, Etzel, & Biggs, 1990; Le Masurier, Cowen, & Harmer, 2007). The Val66Met gene encodes a protein responsible for flexibility of the CNS regulatory systems and cognitive impairments in MDD (Duman, 2005). The two factors are thus fundamental in organising emotional regulation and in mechanisms of vulnerability to MDD (Fanous, et al., 2002; Koolschijn, et al., 2009; Nierenberg, et al., 2007; Weissman, et al., 2006).

In this study it is hypothesized that family history of MDD and Val66Met gene are significant modulators of changes associated with diagnosis of MDD. Patients with MDD – distinguished by these factors – are expected to show unique ways of developing the disease. An acute episode of MDD associated with family history of the disease and an episode connected with particular alleles of the Val66Met gene are believed to manifest changes characteristic for interplay between MDD and vulnerability to the disease. These patterns may follow the assumptions set by previous models of MDD, but they may also have some original features (Davidson, et al., 2002a; Drevets, 2001; Haldane & Frangou, 2006; Mayberg, 1997; Phillips, et al., 2003b). Groups of patients discriminated by each factor, may demonstrate different
impairments in their behavioural and neural performance in emotional regulation. Groups of healthy controls differentiated by the factors may experience variation in emotional regulation processes. As a result, the two groups of healthy controls may be more likely to develop vulnerability to the disease. Individuals who thus become vulnerable to MDD and still do not develop it represent resilience to the disease. With this approach, a distinction between different types of MDD episodes, characterized by vulnerability or lack thereof, can thus be suggested, and observations, based on behavioural and neural correlates of processes engaged in emotional regulation, made. Vulnerability and resilience to the disease, both of great importance in diagnosis and prevention of MDD, can be also observed within this approach.

Within the framework of emotional regulation, the vulnerability to MDD can be defined as a diminished ability to self-regulate one’s emotional states in the face of the demands of life. This decreased ability to self-regulate may lead to the development of an acute episode of the disease. In turn, resilience can be understood in two ways. Firstly, it can be defined as an ability to regulate one’s emotions after a traumatic event (Stein, et al., 2009). Secondly, it can be understood as resilience to the greater vulnerability to developing MDD, meaning that a person resists the disease through factors promoting emotional regulation in spite of an existing vulnerability (Chiland, 1974; Gr Holt, Ekeberg, Wichstr M, & Haldorsen, 1998; Lengua, 2002). Finally, within the framework of emotional regulation endophenotypes of MDD are understood as different ways of disturbing this regulation. Various disturbances may lead to similar symptoms, although they may require different treatment.

1.5 Functional magnetic resonance imaging in psychiatry

Magnetic resonance imaging has been applied for clinical and research purposes since 1970’s (Damadian, 1971). The original name of the technique is nuclear magnetic
resonance imaging, for being related to properties of atomic nucleus and its behaviour in applied magnetic field.

The MRI technique is based on the fact that the atomic nuclei of at least one isotope of every element in the Periodic Table possess spin or an intrinsic spin angular momentum. As such, spin is an inherent property of the nucleus. The nucleus keeps rotating with constant velocity around an axis, which is perpendicular towards the direction of the rotation. The axis of rotation determines the direction of the spin of the nucleus. The value of the spin depends on the atomic weight and the atomic number. The spin can take three groups of values: zero (no spin), half-integral values, and integral values. Nuclei with a spin value zero do not respond to an applied magnetic field and cannot be examined by MRI. In contrast, nuclei with half-integral and integral values of spin can be inspected with the technique. The most frequently used element in MRI studies is the nucleus of hydrogen (H') with the spin value of $\frac{1}{2}$. Its nucleus consists of a single proton and its response to an applied magnetic field is one of the strongest in nature. Also, human body is constructed of the substances which contain a vast number of hydrogen atoms.

The MRI techniques are based on the fact that the spin produces a magnetic moment. A magnetic moment is a magnetic field oriented parallel to the axis of the rotation of the atomic nucleus. Thanks to this, a nucleus behaves like a tiny dipole. In the absence of an external magnetic field, the spin vectors of all the nuclei in a material or a tissue are randomly arranged. However, when an external magnetic field $B_0$ steps in the spin vectors become oriented along the axis of this field (axis z) and individual magnetic moments adopt a cone-shaped spin around this axis. A radio frequency pulse tips the magnetic moments away from axis z towards plane x-y at different angles. When the pulse is switched off, the nuclei dephase. $T_2$ is the time necessary for the magnetic
moments to lose the net magnetisation brought by the radio pulse, while $T_1$ is the time in which the main magnetic field restores the spin momentums to the axis $z$. During these processes, nuclei emit energy which is measured by the radiotransmitter coil. In an MRI scanner there are three gradient coils generating $B_0$, allowing a spatial localization of energy emitted during $T_2$ and $T_1$.

In functional MRI (fMRI), these magnetic properties are applied to measure activation in the CNS. The measures are based on the blood-oxygen-level-dependent (BOLD) contrast. BOLD refers to the fact that oxygenated haemoglobin is far less magnetic than its deoxygenated version. When a certain area of the CNS becomes active, the blood starts to supply it with glucose and oxygen. The region often uses less oxygen than it is provided with. This means that there is more oxygenated blood near the active areas of the CNS, which increases the ratio of the oxygenated to deoxyginated haemoglobin in these regions. Since the oxygenated blood is less magnetic, the dephasing of the near-by hydrogen atoms is less disturbed. These differences in the signal allow us to measure the level of activation of a particular brain structure.

Being able to observe the activation of the CNS structures in vivo when an individual is performing a particular task, offers unprecedented opportunities for psychiatric research; it helps in discovering the mechanisms behind the most disturbing symptoms of disorders. In fact, understanding of MDD in the context of impaired neural functioning would not be possible without fMRI techniques (FitzGerald, Gruener, & Mtui, 2007; Kandel, et al., 2000) since such changes are impossible to observe in vivo. The technique thus promises to integrate both psychological and biological explanations of psychopathology (Bylsma, et al., 2008; Ebmeier, Rose, & Steele, 2006), and can assist in exploring a wide range of issues important for psychiatry and MDD research. It is also useful in the understanding of basic pathophysiology of disorders (Bylsma, et al.,
2008; Ebmeier, et al., 2006) as well as in verification of treatment efficacy (Aihara et al., 2007; Fu, et al., 2007; Lisiecka, et al., 2011; Narasimhan, Richardson, Hodge, Rickels, & Lohoff, 2011). Finally, the application of fMRI can lead to the discovery of mechanisms behind vulnerability and resilience to disorders, and to a more thorough classification of diseases, on the basis of their action on the CNS (Brunoni, et al., 2008; Castren, et al., 2007; Groves, 2007; Wolfensberger et al., 2008).

1.6 Aims of the study

Following from what has been discussed in the previous section, this study is to examine what changes occur in emotional regulation during an episode of major depressive disorder and whether two vulnerability factors – family history of MDD and Val66Met gene polymorphism – produce different types of episodes of MDD. Also, the study is to investigate whether groups of healthy controls – distinguished by the presence of either one vulnerability factor under scrutiny – differ in their emotional regulation. In this way, the mechanisms of vulnerability to the disease are to be determined. Next, this study aims to explore whether patients with MDD, distinguished by these factors, can demonstrate a unique impairments during an acute episode of the disease. It is to examine whether these changes follow the assumptions set by models of MDD, and whether they carry some original features (Davidson, et al., 2002a; Drevets, 2001; Haldane & Frangou, 2006; Mayberg, 1997; Phillips, et al., 2003b). Finally, it is to be examined whether groups of patients, distinguished by the presence of either vulnerability factor under scrutiny, may also demonstrate different impairments in their behavioural and neural performance. As discussed above, these contrasts can be observed on the basis of behavioural and neural correlates of processes engaged in emotional regulation.
1.7 Hypotheses of the study

In the line with the formulated aims, the following hypotheses have been generated for this study:

**Hypothesis 1 (H1):** Patients with MDD and healthy controls differ when it comes to behavioural and neural correlates of emotional recognition, shifting attention from emotional processing and inhibition of emotional processing. These two groups are dissimilar in processes constituting emotional regulation:

a) in terms of behavioural correlates; patients with MDD are likely to be slower and less accurate in these processes than age-matched and gender-matched healthy individuals;

b) in terms of neural correlates; the two groups are likely to differ in the activation of areas presented in the chapter 1.3. The direction of the differences will be such that patients with MDD will experience a heightened activation in the limbic compartment and a decreased activation in the cortical compartment of the CNS.

**Hypothesis 2 (H2):** Family history of MDD is a significant factor on its own and in relation to the diagnosis of MDD when it comes to emotional regulation.

**Hypothesis 2A (H2A):** Individuals with family history of MDD differ from individuals without family history of the disease in behavioural and neural correlates of emotional regulation.

**Hypothesis 2B (H2B):** Patients with MDD without family history of the disease differ from healthy controls without the family history of the disease in behavioural and neural correlates of emotional regulation. The differences are unique for the comparison between the groups. The differences represent changes characteristic for an episode of MDD without familial vulnerability to it.
**Hypothesis 2C (H2C):** Patients with MDD with family history of the disease differ from healthy controls with family history of the disease in behavioural and neural correlates of emotional regulation. The differences are unique for the comparison between the groups. The differences represent changes characteristic for an episode of MDD with familial vulnerability to it. They also represent resilience to the vulnerability to MDD, which characterises healthy controls, but not patients with MDD.

**Hypothesis 2D (H2D):** Healthy controls with family history of MDD differ from healthy controls without family history of the disorder in behavioural and neural correlates of emotional regulation. The differences represent vulnerability to MDD without the acute symptoms of the disease.

**Hypothesis 2E (H2E):** Patients with MDD with family history of the disease differ from patients with MDD without family history of the disorder in behavioural and neural correlates of emotional regulation. The differences represent vulnerability to MDD associated with the acute symptoms of the disease.

**Hypothesis 3 (H3):** The Val66Met gene polymorphism is also a significant factor on its own and in relation to the diagnosis of MDD when it comes to emotional regulation.

**Hypothesis 3A (H3A):** Individuals with Met allele differ from individuals without it in behavioural and neural correlates of emotional regulation.

**Hypothesis 3B (H3B):** Patients with MDD without Met allele differ from healthy controls without the allele in behavioural and neural correlates of emotional regulation. The differences are unique for this comparison. The differences represent an acute episode of MDD with diminished genetic vulnerability to the disease.

**Hypothesis 3C (H3C):** Patients with MDD with Met allele differ from healthy controls with the allele in behavioural and neural correlates of emotional regulation. The
differences are unique for this comparison. The differences represent an acute episode of MDD with enhanced genetic vulnerability to the disease.

Hypothesis 3D (H3D): Healthy controls with Met allele differ from healthy controls without Met allele in behavioural and neural correlates of emotional regulation. The differences represent genetic vulnerability to MDD without the acute symptoms of the disease.

Hypothesis 3E (H3E): Patients with MDD with Met allele differ from patients with MDD without Met allele in behavioural and neural correlates of emotional regulation. The differences represent genetic vulnerability to MDD associated with the acute symptoms of the disease.
2 General methodology

2.1 Participants

Fifty patients with major depressive disorder (N=50) and forty six healthy controls (N=46) took part in the study. MDD was defined according to DSM-IV-TR standards (American Psychiatric Association., 2000). Patients with MDD attended local psychiatric outpatient clinics in Dublin or psychiatric wards of The Adelaide and Meath Hospital incorporating the National Children's Hospital in Dublin, St. James's Hospital in Dublin and psychiatric services in Dublin South-West. All the patients were outpatients and were informed about a possibility to take part in the study by the psychiatrist in charge of their treatment. Healthy controls were recruited from among the patients' first degree unaffected healthy relatives and via advertisements. Patients with MDD could inform their unaffected healthy relatives about the possibility of taking part in the study. This way the group of healthy controls with family history of MDD was recruited. Healthy controls without family history of MDD were recruited via posters placed in various locations inside and outside the campus (e.g. hair-dressing salons, supermarkets, students' dormitories) of Trinity College Dublin. People between 18 and 65 years of age were eligible to participate in the study.

From the medical point of view, participants' health and eligibility for the study were verified via a psychiatric interview, based on the structured Clinical Interview for DSM-IV (First, Spitzer, Gibbon, & Williams, 1997), Hamilton Depression Rating Scale (HDRS) (Hamilton, 1959), Montgomery-Asberg Depression Rating Scale (MDRS) (Montgomery & Asberg, 1979), and Beck Depression Inventory (BDI II) (Beck, Steer, & Brown, 1996). The MDD diagnosis and lack of comorbidities for all the subjects were confirmed by the consensus of at least two consultant psychiatrists. The medical exclusion criteria of the study were as follows: previous or present head injury, a
current or past psychiatric or neurological disease (apart from MDD in the case of the patients), current medical disease influencing the central nervous system, alcohol, or drug dependency.

From the psychological point of view, participants’ eligibility for the study was confirmed via task training and an associated interview. The psychological exclusion criteria for an fMRI task included an inability to read or understand the presented words, or to see the content displayed on the screen.

Following this procedure, two groups of participants were formed: depressed individuals and healthy controls, who differed significantly in all applied depression ratings. The two groups were balanced in respect to age, gender and handedness (Table 1). The age range for the recruited patients with MDD was 23 to 64 years and for the recruited healthy controls 21 to 65 years.

**Table 1. Demographic and clinical characteristics of patients with major depressive disorder (MDD) and healthy controls**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patients with MDD (N=50)</th>
<th>Healthy controls (N=46)</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (SD)</td>
<td>41.7 (10.8)</td>
<td>37.4 (13)</td>
<td>0.081</td>
</tr>
<tr>
<td>Gender (f/m)</td>
<td>32/18</td>
<td>25/21</td>
<td>0.407</td>
</tr>
<tr>
<td>Handedness (r/l)</td>
<td>50/0</td>
<td>46/0</td>
<td></td>
</tr>
<tr>
<td>Hamilton Depression Rating Scale (SD)</td>
<td>28.5 (6.3)</td>
<td>2.6 (2.7)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Montgomery-Asberg Depression Rating Scale (SD)</td>
<td>30 (6.6)</td>
<td>1.7 (3.2)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Beck Depression Inventory II (SD)</td>
<td>33.3 (11.3)</td>
<td>2.8 (4.3)</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

Statistical significance set to p<0.05; *Statistically significant difference

As for the group of patients, seventeen (N=17) individuals were under treatment with selective serotonin reuptake inhibitors (SSRIs), eighteen (N=18) with dual action substances and fifteen (N=15) were not medicated. The patients treated with different methods differed neither in age, gender, and handedness nor in their scores on the
depression rating scales, except for Montgomery-Asberg Depression Rating Scale. Patients treated with dual action substances had slightly higher Montgomery-Asberg Depression Rating Scale scores than individuals treated with SSRIs (Table 2).

Table 2. Demographic and clinical characteristics of patients treated with SSRIs, patients treated with dual action substances and not medicated patients

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patients treated with SSRIs (N=17)</th>
<th>Patients treated with dual action substances (N=18)</th>
<th>Not medicated patients (N=15)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (SD)</td>
<td>41.1 (11.1)</td>
<td>43.4 (9.9)</td>
<td>40.2 (11.7)</td>
<td>0.671</td>
</tr>
<tr>
<td>Gender (f/m)</td>
<td>11/6</td>
<td>12/6</td>
<td>9/6</td>
<td>0.922</td>
</tr>
<tr>
<td>Handedness (r/l)</td>
<td>17/0</td>
<td>19/0</td>
<td>15/0</td>
<td></td>
</tr>
<tr>
<td>Hamilton Depression Rating Scale (SD)</td>
<td>27.5 (5.2)</td>
<td>30.2 (6)</td>
<td>27.7 (7.7)</td>
<td>0.367</td>
</tr>
<tr>
<td>Montgomery-Asberg Depression Rating Scale (SD)</td>
<td>27.6 (5.5)</td>
<td>33 (6.4)</td>
<td>29.2 (7.1)</td>
<td>0.043*</td>
</tr>
<tr>
<td>Beck Depression Inventory II (SD)</td>
<td>31 (10.9)</td>
<td>34.2 (9.6)</td>
<td>35 (13.7)</td>
<td>0.573</td>
</tr>
</tbody>
</table>

Statistical significance set to p<0.05; *Statistically significant difference

Following an extensive description of the aims of the study to the participants, a written informed consent was obtained from them. The research protocol for the study was prepared in accordance with the ethical standards laid down in the Declaration of Helsinki and approved by the local Ethics Committee of Trinity College Dublin.

2.2 Emotion processing and attention shifting/MRI task

In this study, emotional regulation was operationalized as emotion perception/processing and shifting attention from emotional content with inhibition of emotional processing. The task applied in the study was adapted from Northoff et al. (Northoff, et al., 2004), who established neural correlates of the task in healthy controls. The aim of the task was to probe the processes that represent emotional regulation, i.e. emotional recognition and shifting attention from emotional processing with inhibition.
The task consisted of two conditions and a baseline; the first condition involved an act of emotion recognition, whereas the second one required the participants to shift their attention from emotion processing to processing of non-emotional information. Both conditions involved observing visual stimuli with an established emotional valence, which were resourced from International Affective Picture System (IAPS) (Lang, Bradley, & Cuthbert, 2008) database. Next, the conditions required making judgment about emotional or non-emotional features of the stimuli. Emotional recognition was tested by comparing the emotional recognition condition with a baseline; attention shifting was verified by comparing attention shifting condition with a baseline; and inhibition of emotional processing as a central component of attention shifting and a process absent in emotional recognition was examined by comparing the attention shifting condition to the emotional recognition condition.

The experiment utilised an fMRI event-related design with 180 pseudo-randomized trials, where participants were asked to processes certain features of visual stimuli. Each trial consisted of a viewing stage, during which participants looked at a picture, and a response stage during which they answered a question concerning the picture (Figure 1). Participants answered ‘Yes’ or ‘No’ to all the questions depending on whether, in their opinion, the question stated truth or falsehood, by pressing one of two buttons on a response box from Current Design Inc., USA with their right hand. The trials were alternated with a picture of a fixation cross, which served as a baseline for the analysis. This procedure was validated in previous fMRI studies as well as in the study from which the task was adapted during which it was used to probe non-relative signal variability caused by the group-differences (Newman, Twieg, & Carpenter, 2001; Northoff, et al., 2004; Stark & Squire, 2001). The null-event was jittered and lasted two seconds on average.
Figure 1. The structure of the emotional regulation task. Two trials are presented with alternated null-events. The first trial is an attention shifting trial, the second an emotion processing trial.

Out of the 180 trials, half of them belonged to the emotion processing condition and the other half belonged to the attention shifting condition. Participants started each trial by observing a picture taken from the IAPS database. Subsequently, in trials with the processing of emotions (henceforward emotional trials), after observing the picture, they answered a question referring to its emotional content (Was it positive? Was it negative? Was it neutral?). In trials with the attention shifting (henceforward non-emotional trials), after observing the picture, participants answered a question about the shape of the picture (Was it horizontal? Was it vertical?), and inhibited their processing of emotional information. The two conditions were pseudo-randomly distributed in the experiment. Participants did not know – either before the start of each trial or during the picture viewing – which of the two types of questions would be asked. To provide a correct answer, they had to process both types of information (about the emotional valence and the shape of the picture) until the question was asked after the picture viewing stage. After that, their attention was re-focused onto one of the pieces of information. Therefore, in the emotional trials, emotional information was being processed until the end of the trial. In contrast, the non-emotional trials involved shifting attention away from the processing of emotional information, by inhibiting this
type of processing before the end of the trial. Each trial lasted four seconds. In this way, the emotional trials were indicative of the participants’ emotional processing, whereas the non-emotional trials were indicative of their attention shifting. It was assumed that the latter – attention shifting – was based on the participants’ inhibition of emotional processing, and that this process was absent in the emotional trials. Therefore, inhibition of emotional processing was operationalized in this study as non-emotional trials minus emotional trials.

To address a broad range of emotional values, the pictures used in this experiment were taken from the International Affective Picture System database (Lang, et al., 2008), and were positive, negative or neutral in emotional valence, as well as horizontal or vertical in shape, with 60 unrepeated pictures in each valence category and 30 unrepeated pictures in each valence-shape category (e.g. 30 positive horizontal pictures). In each of the valence-shape categories, the correct answers ‘Yes’ and ‘No’ were equally distributed among the trials (with 15 ‘Yes’ and 15 ‘No’ as a correct answer to the trial). The valence of IAPS pictures is described on a scale from 1 to 9, where 1 represents very negative and 9 very positive; in this study, the pictures in the interval from 1 to 3 were classified as negative, from 4 to 6 as neutral, and from 7 to 9 as positive. The pictures selected for the experiment were as close as possible to 1, 9 and 5 for the negative, positive and neutral category, respectively. To ensure that the chosen pictures have a consistent appraisal in healthy population, the ones with minimal standard deviations (SD) in emotional valence and the ones judged similarly by men and women were eventually selected for the task. Since the examined group consisted of emotionally vulnerable participants, negative pictures presenting a highly disturbing content, such as victims of violent deaths were omitted after a consultation with a psychiatrist. In the end, the respective mean valence values for the negative, positive
and neutral category were 2.54 (SD=0.34), 7.64 (SD=0.34), and 4.97 (SD=0.23), respectively. The selected pictures of different valence and shape were randomly and equally distributed across the two types of trails.

The performance of the task was preceded by a standardized psychological training outside the scanner, which included both a non-computerized and a computerized learning sessions. The two learning sessions were included to account for differences in computer-literacy in the participants of the study. In the first session, the meaning of words appearing on the screen during the task was explained, and pictures representing examples of each category were shown ("positive", "negative", "neutral", "horizontal", "vertical"). Subsequently, each participant was asked to divide a group of paper pictures (N=15) into a positive, a negative or a neutral category. On this basis, a mini-task was performed, in which the participants saw a picture, observed it getting covered, and were asked one of the five following questions: Was it positive? Was it negative? Was it neutral? Was it horizontal? Was it vertical?. A mini-task performed on the computer followed, which was a replica of the main task applied in the scanner. However, the two exceptions were present in this task. There were eighteen trials involved, and different pictures from IAPS database than those used in the main task were presented. Since the goal of this part was to teach the method, yet keep the novelty of the pictures, none of the pictures used in the main task appeared during the training session.

### 2.3 Data acquisition

#### 2.3.1 Image data acquisition

The participants were scanned using a 3T MRI scanner (Philips Achieva, The Netherlands). The MRI protocol consisted of the acquisition of a high resolution 3D T1-weighted structural dataset (SPGR sequence with TR/TE=8.5/3.9 ms and 1mm3=spatial resolution).
resolution), followed by an fMRI experiment with the task presented in the chapter 2.2 (SE-EPI sequence with TR/TE=2000/35 ms, in-place resolution= 3x3 mm², 4.8 mm slice thickness, 550 dynamic scans each with 2 sec duration).

2.3.2 Behavioural data acquisition

Presentation®, a stimulus delivery software manufactured by NeuroBehavioral Systems, was used to program the task and to record participants’ answers and reaction times (RTs). The reaction time was defined as a period of time starting at the question onset and finishing when a participant pressed a button to give an answer. RTs were calculated for each trial of every participant in Excel. The participants’ answers, after their correctness verification, formed an accuracy measure, which was also calculated in Excel. Accuracy was defined as a percentage of correct answers in non-emotional trials and as a percentage of answers in accordance with standardized appraisals of emotional valence in emotional trials.

2.4 Data analysis

2.4.1 Pre-processing

Data was pre-processed with Statistical Parametric Mapping (SPM8) software (http://www.fil.ion.ucl.ac.uk/spm/software/spm8/). The EPI images were realigned to the first volume to correct for motion. Realignment parameters were inspected visually to identify any potential subjects with excessive head movement (4.8 mm, the thickness of one slice was considered an excessive movement). Each participant’s structural image was co-registered to the mean of the motion-corrected functional images, using a 12-parameter affine transformation. Images slice time was corrected to TR/2. The structural images were segmented according to the standard procedure in SPM8 (Ashburner & Friston, 2005). Spatial normalization to standard 3mm×3mm×3mm
Montreal Neurological Institute space was then applied to the functional images to allow for inter-subject analysis. Finally, the images were smoothed using an 8 mm full width, half maximum (FWHM) Gaussian kernel.

2.4.2 First level analysis

The functional data analysis was first performed with SPM8 for each individual subject, where seven types of events were distinguished. The types of events were as follows: null-events (baseline), neutral non-emotional trials, neutral emotional trials, negative non-emotional trials, negative emotional trials, positive non-emotional trials and positive emotional trials. The contrasts for each individual were calculated in each event type.

For each individual, each condition was contrasted separately with null-events. Additionally, in each condition trials were separated into neutral, negative and positive. In consequence, six t-test contrasts were acquired for every participant: a) neutral emotional trials > null-events, b) negative emotional trials > null-events, c) positive emotional trials > null-events, d) neutral non-emotional trials > null-events, e) negative non-emotional trials > null-events and f) positive non-emotional trials > null-events. Motion correction values were taken into account in the model as a covariate of no interest. These tests were used in the analysis of changes in emotional processing and attention shifting characteristic for diagnosis and family history of MDD.

To estimate inhibition of the processing of emotional information, three t-test contrasts were calculated comparing non-emotional trials with emotional trials for each valence separately. In consequence, a set of three subsequent contrasts was acquired for each individual: a) neutral inhibition trial > neutral emotional trial, b) negative inhibition trial > negative emotional trial and c) positive inhibition trial > positive emotional trial.
Also, two t-contrasts were modelled: a) emotional trials $>$ null-events and b) non-emotional trials $>$ null-events. These contrasts were calculated for the analysis of changes in emotional processing and attention shifting characteristic for the Val66Met polymorphism.
3 Family history of MDD in emotional regulation in patients with MDD and healthy controls (study 1 chapter 1)

3.1 Introduction

Family history of MDD plays a crucial role in the vulnerability to the disease as well as in the course and severity of a depressive episode. Individuals with a first degree relative suffering from major depressive disorder are at two-to-threefold greater risk of developing depression than those without family history of MDD (Aukes, et al., 2012; Fanous, et al., 2002; Lesch, 2004; Weissman, et al., 2006). Relatives of depressed patients, when compared to individuals without family history of psychiatric disorders, are characterized by elevated neuroticism and rigidity (Lauer et al., 1997), and by stability of these traits over time (Lauer et al., 1998). They react faster to negative stimuli (especially fear) and slower to positive information, compared to healthy controls (Le Masurier, et al., 2007). Moreover, relatives of patients with MDD tend to have more negative cognitions and beliefs (Giles, et al., 1990; Lauer, et al., 1997). Furthermore, monozygotic twins of patients with MDD have cognitive impairments in selective and sustained attention, in executive function and in working memory, the impairments being similar in quality but not severity to those observed in MDD patients (Christensen, et al., 2006).

Family history of MDD also influences the onset and the course of the disease itself. Patients with MDD who had relatives suffering from an affective disorder display higher neuroticism (Holma, Melartin, Holma, Paunio, & Isometsa, 2011), greater feelings of guilt, anxiety symptoms, functional impairments, and have an earlier age of onset of MDD (Nierenberg, et al., 2007). In contrast, patients without family history of MDD seem to be more dependant and impulsive (Joffe & Regan, 1991).
Apparently, family history of MDD alters susceptibility to depression as well as the course of an acute MDD episode. The factor is clinically important since it takes part in mechanisms of an elevated risk of developing MDD (for relatives of patients with MDD, compared to healthy controls) and resilience to the disease (for relatives of patients with MDD, compared to patients). It points to different groups of healthy controls and patients with MDD. Furthermore, it is suggested that an episode of MDD with family history of the disease may have its specific impairments not observed in MDD without the vulnerability. A good understanding of these mechanisms should not be underestimated if diagnosis, therapy and prevention of the disorder are to be enhanced.

Individuals with family history of MDD have been found to have unique neural characteristics. In two studies of structural changes in the CNS (Amico et al., 2011; Boccardi et al., 2010), individuals with family history of MDD had a reduced volume of the right hippocampus, the DLPFC and the putamen, and an enlarged volume of the left amygdala, when compared to healthy controls without family history of psychiatric disorders. A diffusion tensor imaging study by Frodl et al. (Frodl, et al., 2010b) detected differences between first degree relatives of patients with MDD and healthy controls in the white matter tracts and bundles, such as the posterior body of the corpus callosum, left superior longitudinal fasciculus, left inferior fronto-occipital fasciculus, left external capsule, and left anterior thalamic radiation.

In a functional magnetic resonance imagining study, a small volume-correction procedure revealed that healthy adolescents with family history of MDD, in comparison to healthy controls, have altered activation in the amygdala and the striatum when observing emotional faces (Monk et al., 2008). In another fMRI study, healthy monozygotic twins of patients with MDD were compared to subjects without family
history of the disorder (Wolfensberger, et al., 2008). The twins displayed a higher activation in the left IFG during verbal encoding and retrieval. The neural differences were discovered in spite of the absence of negative bias on a behavioural level. This finding suggests that susceptibility and associated changes can manifest themselves without behavioural signals. Therefore, using the neuroimaging techniques appears a necessity (Kathmann, Hochrein, Uwer, & Bondy, 2003; Savitz & Drevets, 2009). In yet another fMRI study (Evers, et al., 2006) with acute tryptophan depletion (ATD), women with family history of MDD were compared to healthy controls using emotional and cognitive Stroop task. ATD triggered depressive mood in the relatives of patients with MDD, but not in healthy controls. No group effect was found on brain activation during the emotional or cognitive Stroop task. For both groups, severity of depressive mood after ATD was correlated with an activation in the posterior part of the ACC.

Despite remarkable improvements, the neural pathophysiology of this vulnerability and its consequences for MDD are not yet entirely understood. The behavioural data suggest that the key skills impaired in individuals with family history of MDD are: processing of emotions (Hammen & Brennan, 2001; Joormann, et al., 2007b; Le Masurier, et al., 2007) and shifting of attention from emotional content (Christensen, et al., 2006; Hall & Smoller, 2010). These impairments could explain elevated levels of neuroticism in relatives of patients with MDD. Emotional regulation – one of the fundamental abilities disturbed in neuroticism (Bono & Vey, 2007) – requires efficient emotional processing and an effective ability to inhibit it. Interestingly, neural correlates of these abilities have not yet been investigated in individuals with family history of MDD. Furthermore, it has not been verified how these processes change during an acute episode of MDD when family history of the disease is taken into account.
To the best of my knowledge, no previous study has examined whole-brain functional neural correlates of family history of MDD in both healthy controls and patients with MDD. Furthermore, changes in emotional regulation associated with family history vulnerability are yet to be demonstrated. No study compared patients with MDD and healthy controls for correlates of emotional regulation when the family history of the disease was present and when it was not. This study is the first one to do these. It investigates differences in emotion recognition and attention shifting between the groups distinguished by diagnosis of MDD and family history of the disease. Its second part presented in the chapter 4 examines differences in inhibition of emotional processing between these groups.

### 3.2 Aims and hypotheses

The aim of the study is to examine how family history of MDD influences behavioural and neural correlates of emotion processing and attention shifting in patients with MDD and healthy controls. It is also to test what differences there are in the behavioural and neural correlates of the two processes between patients with MDD and healthy controls when family history of the disease is present and when it is not. It is also to examine the changes in the two processes associated with vulnerability and resilience to the vulnerability to the disease.

On the basis of a diagnosis and family history of MDD, participants were divided into 4 groups:

1) patients with MDD with family history of depression (MDD-FHP);
2) patients with MDD without family history of depression (MDD-FHN);
3) healthy controls with family history of depression (HC-FHP – in this case, unaffected healthy first-degree relatives of patients with MDD);
4) healthy controls without family history of MDD (HC-FHN).
The four groups were compared among each other to test the hypotheses 1 and 2 presented in the chapter 1.7.

3.2.1 **Healthy controls vs patients with MDD (H1)**

It was important for this study to test what changes in emotional processing and attention shifting were caused by MDD in general. It was hypothesized that during these two processes patients with MDD may experience a lower activation in the cortical compartment and a higher activation in the limbic compartment of the CNS, when compared to healthy controls (Mayberg, 1997). Furthermore, it was assumed that patients with MDD were slower and less accurate than healthy controls in the two examined processes.

3.2.2 **Subjects with family history of MDD vs subjects without family history of MDD (H2A)**

Secondly, this study sought to test whether subjects with and without family history of MDD differed in neural and behavioural correlates of emotional processing and attention shifting regardless of their diagnosis. It was hypothesized that the neural difference between individuals with and without family history of MDD occurred in the areas named previously as the neural correlates of the examined processes (Northoff, et al., 2004). It was also anticipated that subjects with family history of MDD are slower and less accurate in emotional recognition and attention shifting.

3.2.3 **Healthy controls without family history of MDD vs patients with MDD without family history of MDD (H2B)**

This comparison tested how emotional processing and attention shifting are changed by an acute depressive episode, when the vulnerability caused by family history of MDD is absent. It was hypothesized that the MDD-FHN, compared to the HC-FHN have less
neural activation in the areas connected with the cortical compartment and more activation in the regions of limbic compartment of the CNS (Mayberg, 1997) during the examined processes. It was also anticipated that the MDD-FHN may be slower and less accurate than the HC-FHN.

3.2.4 Healthy controls with family history of MDD vs patients with MDD with family history of MDD (H2C)

When the HC-FHP were compared to the MDD-FHP, both resilience to the risk of developing MDD and an acute MDD episode with vulnerability were observed. It was hypothesized that, in contrast to the MDD-FHP, the HC-FHP experience less activation in the limbic areas and more activation in the cortical compartment of the CNS (Mayberg, 1997) during both processes. It was further assumed that patients may have less activation in other areas identified as those associated with the targeted processes in healthy controls (Northoff, et al., 2004). The HC-FHP may also display a similar or higher activity in the limbic compartment or other affective processing areas, when compared to the MDD-FHP especially during attention shifting. Together with elevated activation in the cortical compartment, this points to a compensation mechanism being at play. It was also assumed that the MDD-FHP are slower and less accurate than the HC-FHP.

3.2.5 Healthy controls with family history of MDD vs healthy controls without family history of MDD (H2D)

The changes in correlates of emotional processing and attention shifting in the HC-FHP, compared to the HC-FHN, show an elevated vulnerability to MDD without acute depressive symptoms. It was hypothesized that the HC-FHP display neural alterations in the areas associated with emotional processing and attention shifting in healthy controls. The regions include frontal and cingulate gyri, parietal cortex, premotor...
cortex, and subcortical areas (Kober, et al., 2008; Northoff, et al., 2004). Differences may also be found in the inferior frontal gyrus, the striatum and the amygdala, named previously as a neural correlates of cerebral changes characteristic for the HC-FHP (Evers, et al., 2006; Wolfensberger, et al., 2008). The HC-FHP could also experience a decrease of activation in the cortical compartment and an increase in the limbic lobe (Mayberg, 1997). It is also likely that in their neural functioning they resemble the patients with MDD. Alternatively, the HC-FHP may demonstrate increase of activation in both cortical and limbic compartments or in the areas associated with emotional processing. This would indicate a mechanism of compensation. Behaviourally, it was anticipated that the HC-FHP may be less accurate in emotion recognition.

3.2.6 Patients with MDD with family history of MDD vs patients with MDD without family history of MDD (H2E)

Finally, the study aimed to test to what extent the neural characteristics of emotional processing and attention shifting change in familial vulnerability to MDD associated with an acute depressive episode of the disease. It was assumed that the MDD-FHP, as carriers of both the vulnerability and the acute symptoms, are less efficient in behavioural performance during emotional processing and attention shifting. Their neural activity may be reduced in the areas involved in the two targeted processes (Northoff, et al., 2004). Alternatively, they can experience heightened in the limbic areas during attention shifting.

3.3 Groups tested

The patients with MDD and healthy controls recruited for the study (chapter 2.1.) were divided into subjects with family history of MDD and subjects without such a history. Family history was assessed by a psychiatrist through a structured interview. In
particular, participants were asked whether any of their first or second degree relatives had been diagnosed with a psychiatric disease, had been in treatment with antidepressant, other psychotropic medications or electroconvulsive therapy or had attended a psychiatric service. Moreover, case notes were evaluated by a psychiatrist in order to collect information about possible exclusion criteria, family history and course of depression. Additionally, unaffected healthy subjects with family history of MDD were recruited only among the first-degree relatives of patients participating in the study. Only one unaffected healthy first-degree relative per patient with MDD was permitted to participate in the study. Ultimately, four groups of participants (N=96) took part in the study: 1) thirty MDD-FHP, 2) twenty MDD-FHN, 3) twenty-one HC-FHP and 4) twenty-five HC-FHN. Depressed individuals and healthy controls differed significantly in all the applied depression ratings (Table 3).

Table 3. Demographic and clinical characteristics of groups distinguished by diagnosis of MDD and family history of the disease (F MANOVA)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>MDD-FHP (N=30)</th>
<th>MDD-FHN (N=20)</th>
<th>HC-FHP (N=21)</th>
<th>HC-FHN (N=25)</th>
<th>p values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (SD)</td>
<td>40.5 (9)</td>
<td>43.4 (13)</td>
<td>38.7 (14.4)</td>
<td>36.3 (11.9)</td>
<td>0.247</td>
</tr>
<tr>
<td>Gender (f/m)</td>
<td>19/11</td>
<td>13/7</td>
<td>11/10</td>
<td>14/11</td>
<td>0.801</td>
</tr>
<tr>
<td>Handedness (r/l)</td>
<td>30/0</td>
<td>20/0</td>
<td>21/0</td>
<td>25/0</td>
<td>N/A</td>
</tr>
<tr>
<td>Hamilton Depression Rating Scale (SD)</td>
<td>29 (6.4)</td>
<td>28 (6.4)</td>
<td>3.7 (3.1)</td>
<td>1.8 (1.9)</td>
<td>&lt;0.001a</td>
</tr>
<tr>
<td>Montgomery-Asberg Depression Rating Scale (SD)</td>
<td>30.7 (6.5)</td>
<td>29 (6.8)</td>
<td>3.1 (4)</td>
<td>0.5 (1.7)</td>
<td>&lt;0.001a</td>
</tr>
<tr>
<td>Beck Depression Inventory II (SD)</td>
<td>34.7 (11.7)</td>
<td>31.3 (10.7)</td>
<td>3.7 (5.7)</td>
<td>2 (2.5)</td>
<td>&lt;0.001a</td>
</tr>
</tbody>
</table>

MDD-FHP – patients with major depressive disorder with family history of the disease; MDD-FHN – patients with major depressive disorder without family history of the disease; HC-FHP – healthy controls with family history of the disease; HC-FHN – healthy controls without family history of the disease

Statistical significance set to p<0.05; a Statistically significant difference
All the healthy participants scored within the norm interval in HDRS, MDRS and BDI II characteristic of healthy individuals, and all the subjects with MDD scored over the threshold characteristic for the disease. There was no significant difference in the ratings between the two groups of healthy controls and between the two groups of patients with MDD (Table 4).

Table 4. Post-hoc comparisons in demographic and clinical characteristics between groups distinguished by diagnosis of MDD and family history of the disease

<table>
<thead>
<tr>
<th>Characteristics (p values)</th>
<th>HC-FHN vs MDD-FHN</th>
<th>HC-FHP vs MDD-FHP</th>
<th>HC-FHN vs HC-FHP</th>
<th>MDD-FHN vs MDD-FHP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.206</td>
<td>0.947</td>
<td>0.906</td>
<td>0.846</td>
</tr>
<tr>
<td>Gender</td>
<td>0.760</td>
<td>0.565</td>
<td>1.000</td>
<td>1.000</td>
</tr>
<tr>
<td>Hamilton Depression Rating Scale (SD)</td>
<td>&lt;0.001(^a)</td>
<td>&lt;0.001(^a)</td>
<td>0.933</td>
<td>0.939</td>
</tr>
<tr>
<td>Montgomery-Asberg Depression Rating Scale</td>
<td>&lt;0.001(^a)</td>
<td>&lt;0.001(^a)</td>
<td>0.977</td>
<td>1.000</td>
</tr>
<tr>
<td>Beck Depression Inventory II</td>
<td>&lt;0.001(^a)</td>
<td>&lt;0.001(^a)</td>
<td>0.879</td>
<td>1.000</td>
</tr>
</tbody>
</table>

MDD-FHP – patients with major depressive disorder with family history of the disease; MDD-FHN – patients with major depressive disorder without family history of the disease; HC-FHP – healthy controls with family history of the disease; HC-FHN – healthy controls without family history of the disease

Statistical significance set to \( p<0.05 \); \(^a\) Statistically significant difference

The four groups were balanced in relation to age, gender and handedness (Tables 3). No significant differences were observed between the examined paired of groups in relation to age, gender and handedness (Table 4).

3.4 Data analysis

3.4.1 Design

The study was a four sample design with MDD-FHP, MDD-FHN, HC-FHP and HC-FHN as comparison groups. On the basis of the selection procedure presented above, participants were assigned to one of the groups. An event-related fMRI experiment measuring processing of emotions and shifting attention from emotional information...
with inhibition of emotional processing was used to record the BOLD signal for each subject.

3.4.2 Behavioural data analysis

All the calculations were performed in SPSS 19.0. The threshold for the significance of all differences was set at \( p < 0.05 \). Patients with MDD and healthy controls were compared in accuracy and reaction times in emotional processing and attention shifting, together and separately for all the valence categories. General linear model was used to calculate the \( P \) values. A similar comparison was performed for subjects with and without family history of MDD. Subsequently, a 4-group MANOVA and a post-hoc analysis were calculated to determine whether the four groups differed in reference to RTs and accuracy measures in the two tested processes. The results are presented in the results sub-section (3.5.1).

3.4.3 Image data second level analysis

In accordance with previous studies (Prata et al., 2012; Rehme, Fink, Von Cramon, & Grefkes, 2011; Sanchez-Carrion et al., 2008) a 2x2x6 factorial analysis was performed with: the diagnosis of MDD (patients with MDD, healthy controls) as the first factor, the family history of MDD (subjects with family history of MDD as the second, subjects without family history of MDD) as the second factor and the type of the trial (neutral emotional processing, negative emotional processing, positive emotional processing, neutral attention shifting, negative attention shifting, positive attention shifting) as the third factor. Participants’ age and gender were added as covariates of no interest.

The differences between patients and healthy controls, as well as between individuals with family history of MDD and without family history of MDD were established for both emotional processing and attention shifting jointly and separately for all valence
categories. The contrasts were calculated to determine the differences between HC-FHP and HC-FHN, between HC-FHP and MDD-FHP, between HC-FHN and MDD-FHN, between MDD-FHP and MDD-FHN. Contrasts between all groups were calculated separately for emotion processing condition and attention shifting condition. All the groups were also contrasted separately for both conditions in each valence category. The regions detected in all the contrasts in emotional processing and attention shifting (surviving the whole-brain family-wise error (FWE) cluster correction with p<0.05) are presented results sub-section. Their localization was verified in Montreal Neurological Institute (MNI) space. The automated anatomical labelling was used to localize the significant results in a standard stereotactic space (template – MNI).

An additional analysis was performed for patients with MDD to determine if individuals treated differently varied in neural activation during the task and if the treatment method could interfere with family history in the group of patients with MDD. A 3x6 factorial analysis was carried out with type of medication as the first factor (SSRIs, dual action and no medication), type of the processing as the second factor and age and gender as covariates of no interest. The methods of verifying of statistical significance and labelling the results were the same as with the previous test.

3.5 Results

3.5.1 Behavioural results

3.5.1.1 General effect of diagnosis (H1)

Significant differences were found between healthy controls and patients with MDD in behavioural correlates of both emotional processing and attention shifting. Healthy controls were characterized by higher accuracy and shorter reaction times in both conditions. The results are presented in Table 5.
Table 5. Differences in accuracy and reaction times between patients with MDD and healthy controls in emotion processing and attention shifting

<table>
<thead>
<tr>
<th>Behavioural characteristics (±SD)</th>
<th>Patients with MDD (N=50)</th>
<th>Healthy controls (N=46)</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Emotion processing</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Accuracy – general (% correct)</td>
<td>74.8 (±12.8)</td>
<td>82.9 (±9.4)</td>
<td>0.001a</td>
</tr>
<tr>
<td>Accuracy – negative stimuli (% correct)</td>
<td>81.3 (±16.1)</td>
<td>87 (±13.6)</td>
<td>0.064</td>
</tr>
<tr>
<td>Accuracy – positive stimuli (% correct)</td>
<td>74.7 (±16.4)</td>
<td>90.1 (±9.1)</td>
<td>&lt;0.001a</td>
</tr>
<tr>
<td>Accuracy – neutral stimuli (% correct)</td>
<td>68.5 (±14.1)</td>
<td>71.5 (±14.4)</td>
<td>0.304</td>
</tr>
<tr>
<td>RTs – general (s)</td>
<td>1.51 (±0.44)</td>
<td>1.33 (±0.31)</td>
<td>0.020a</td>
</tr>
<tr>
<td>RTs – negative stimuli (s)</td>
<td>1.51 (±0.43)</td>
<td>1.32 (±0.31)</td>
<td>0.017a</td>
</tr>
<tr>
<td>RTs – positive stimuli (s)</td>
<td>1.37 (±0.45)</td>
<td>1.17 (±0.28)</td>
<td>0.013a</td>
</tr>
<tr>
<td>RTs – neutral stimuli (s)</td>
<td>1.66 (±0.49)</td>
<td>1.49 (±0.37)</td>
<td>0.059</td>
</tr>
<tr>
<td><strong>Attention shifting</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Accuracy – general (% correct)</td>
<td>77.6 (±16.6)</td>
<td>88.7 (±11.1)</td>
<td>&lt;0.001a</td>
</tr>
<tr>
<td>Accuracy – negative stimuli (% correct)</td>
<td>73.5 (±17.2)</td>
<td>85.3 (±12.9)</td>
<td>&lt;0.001a</td>
</tr>
<tr>
<td>Accuracy – positive stimuli (% correct)</td>
<td>79.9 (±16.6)</td>
<td>88.8 (±11.5)</td>
<td>0.003a</td>
</tr>
<tr>
<td>Accuracy – neutral stimuli (% correct)</td>
<td>79.4 (±18.7)</td>
<td>91.9 (±11.1)</td>
<td>&lt;0.001a</td>
</tr>
<tr>
<td>RTs – general (s)</td>
<td>1.65 (±0.43)</td>
<td>1.3 (±0.27)</td>
<td>&lt;0.001a</td>
</tr>
<tr>
<td>RTs – negative stimuli (s)</td>
<td>1.73 (±0.42)</td>
<td>1.36 (±0.3)</td>
<td>&lt;0.001a</td>
</tr>
<tr>
<td>RTs – positive stimuli (s)</td>
<td>1.64 (±0.47)</td>
<td>1.28 (±0.29)</td>
<td>&lt;0.001a</td>
</tr>
<tr>
<td>RTs – neutral stimuli (s)</td>
<td>1.6 (±0.43)</td>
<td>1.26 (±0.26)</td>
<td>&lt;0.001a</td>
</tr>
</tbody>
</table>

Statistical significance set to p<0.05; *Statistically significant difference

When specific valence categories were considered, patients were less likely to recognize positive stimuli and were slower in reacting to positive and negative images. As for attention shifting, healthy controls were quicker and more accurate in all the valence categories. The results are presented in Table 5.

### 3.5.1.2 General effect of family history of MDD (H2A)

No significant differences were found in behavioural correlates of emotional processing and attention shifting between subjects with and without family history of MDD, when the diagnosis was not taken into account. The results are presented in Table 6.
Table 6. Differences in accuracy and reaction times between individuals with family history of major depressive disorder (FHP) and individuals without family history of the disease (FHN) in emotion processing and attention shifting

<table>
<thead>
<tr>
<th>Behavioural characteristics (±SD)</th>
<th>FHP (N=51)</th>
<th>FHN (N=45)</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Emotion processing</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Accuracy – general (% correct)</td>
<td>78.5 (±12.6)</td>
<td>79.1 (±11.2)</td>
<td>0.818</td>
</tr>
<tr>
<td>Accuracy – negative stimuli (% correct)</td>
<td>83.5 (±16.3)</td>
<td>84.7 (±13.7)</td>
<td>0.704</td>
</tr>
<tr>
<td>Accuracy – positive stimuli (% correct)</td>
<td>81.3 (±15.6)</td>
<td>83.3 (±15.1)</td>
<td>0.527</td>
</tr>
<tr>
<td>Accuracy – neutral stimuli (% correct)</td>
<td>70.7 (±16.3)</td>
<td>69.2 (±11.7)</td>
<td>0.604</td>
</tr>
<tr>
<td>RTs – general (s)</td>
<td>1.41 (±0.44)</td>
<td>1.44 (±0.33)</td>
<td>0.728</td>
</tr>
<tr>
<td>RTs – negative stimuli (s)</td>
<td>1.4 (±0.42)</td>
<td>1.43 (±0.35)</td>
<td>0.691</td>
</tr>
<tr>
<td>RTs – positive stimuli (s)</td>
<td>1.27 (±0.45)</td>
<td>1.28 (±0.31)</td>
<td>0.936</td>
</tr>
<tr>
<td>RTs – neutral stimuli (s)</td>
<td>1.55 (±0.48)</td>
<td>1.6 (±0.39)</td>
<td>0.614</td>
</tr>
<tr>
<td><strong>Attention shifting</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Accuracy – general (% correct)</td>
<td>81.9 (±15.8)</td>
<td>84.3 (±14.4)</td>
<td>0.443</td>
</tr>
<tr>
<td>Accuracy – negative stimuli (% correct)</td>
<td>77.9 (±16.6)</td>
<td>80.9 (±15.9)</td>
<td>0.384</td>
</tr>
<tr>
<td>Accuracy – positive stimuli (% correct)</td>
<td>83.4 (±15.1)</td>
<td>85.3 (±14.9)</td>
<td>0.548</td>
</tr>
<tr>
<td>Accuracy – neutral stimuli (% correct)</td>
<td>84.4 (±18.2)</td>
<td>86.8 (±14.6)</td>
<td>0.481</td>
</tr>
<tr>
<td>RTs – general (s)</td>
<td>1.53 (±0.45)</td>
<td>1.42 (±0.34)</td>
<td>0.185</td>
</tr>
<tr>
<td>RTs – negative stimuli (s)</td>
<td>1.6 (±0.43)</td>
<td>1.49 (±0.37)</td>
<td>0.182</td>
</tr>
<tr>
<td>RTs – positive stimuli (s)</td>
<td>1.52 (±0.5)</td>
<td>1.4 (±0.33)</td>
<td>0.134</td>
</tr>
<tr>
<td>RTs – neutral stimuli (s)</td>
<td>1.47 (±0.44)</td>
<td>1.38 (±0.33)</td>
<td>0.276</td>
</tr>
</tbody>
</table>

Statistical significance set to p<0.05; *Statistically significant difference

3.5.1.3 Four-group analysis and post-hoc tests

MANOVA tests revealed significant differences between the four groups in accuracy of emotion processing and attention shifting and RTs in attention shifting. When each valence category was considered separately, the four groups differed in accuracy and RTs in each valence type in attention shifting. In emotional processing, the four groups varied in the accuracy of recognizing positive emotional stimuli (Table 7).
Table 7. Differences in accuracy and reaction times in emotion processing and attention shifting between groups distinguished by diagnosis of MDD and family history of the disease (F MANOVA)

<table>
<thead>
<tr>
<th>Behavioural characteristics (±SD)</th>
<th>MDD-FHP (N=30)</th>
<th>MDD-FHN (N=20)</th>
<th>HC-FHP (N=21)</th>
<th>HC-FHN (N=25)</th>
<th>p values</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Emotion processing</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Accuracy – general (% correct)</td>
<td>75.4 (±12.6)</td>
<td>73.8 (±13.5)</td>
<td>82.9 (±11.6)</td>
<td>82.9 (±7.3)</td>
<td>0.010</td>
</tr>
<tr>
<td>Accuracy – negative stimuli (% correct)</td>
<td>82.2 (±16)</td>
<td>79.6 (±16.6)</td>
<td>85.4 (±17)</td>
<td>88.4 (±10.1)</td>
<td>0.241</td>
</tr>
<tr>
<td>Accuracy – positive stimuli (% correct)</td>
<td>74.6 (±15.9)</td>
<td>75 (±17.6)</td>
<td>91 (±8.8)</td>
<td>89.3 (±9.5)</td>
<td>&lt;0.001a</td>
</tr>
<tr>
<td>Accuracy – neutral stimuli (% correct)</td>
<td>69.6 (±15.4)</td>
<td>66.7 (±12)</td>
<td>72.2 (±17.7)</td>
<td>71 (±11.4)</td>
<td>0.661</td>
</tr>
<tr>
<td>RTs – general (s)</td>
<td>1.5 (±0.51)</td>
<td>1.53 (±0.29)</td>
<td>1.28 (±0.26)</td>
<td>1.37 (±0.35)</td>
<td>0.111</td>
</tr>
<tr>
<td>RTs – negative stimuli (s)</td>
<td>1.48 (±0.49)</td>
<td>1.55 (±0.32)</td>
<td>1.28 (±0.26)</td>
<td>1.35 (±0.35)</td>
<td>0.096</td>
</tr>
<tr>
<td>RTs – positive stimuli (s)</td>
<td>1.37 (±0.53)</td>
<td>1.37 (±0.29)</td>
<td>1.13 (±0.23)</td>
<td>1.21 (±0.31)</td>
<td>0.086</td>
</tr>
<tr>
<td>RTs – neutral stimuli (s)</td>
<td>1.64 (±0.56)</td>
<td>1.68 (±0.35)</td>
<td>1.42 (±0.31)</td>
<td>1.54 (±0.42)</td>
<td>0.220</td>
</tr>
<tr>
<td><strong>Attention shifting</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Accuracy – general (% correct)</td>
<td>76.3 (±16.1)</td>
<td>79.8 (±17.6)</td>
<td>89.9 (±11.5)</td>
<td>87.6 (±10.8)</td>
<td>0.003a</td>
</tr>
<tr>
<td>Accuracy – negative stimuli (% correct)</td>
<td>72 (±16.4)</td>
<td>75.9 (±18.6)</td>
<td>86.3 (±12.9)</td>
<td>84.4 (±13)</td>
<td>0.003a</td>
</tr>
<tr>
<td>Accuracy – positive stimuli (% correct)</td>
<td>78.7 (±16)</td>
<td>81.9 (±17.9)</td>
<td>90.2 (±10.9)</td>
<td>87.7 (±12.1)</td>
<td>0.023a</td>
</tr>
<tr>
<td>Accuracy – neutral stimuli (% correct)</td>
<td>78.1 (±19.1)</td>
<td>81.5 (±18.5)</td>
<td>93.3 (±12.6)</td>
<td>90.7 (±9.7)</td>
<td>0.002a</td>
</tr>
<tr>
<td>RTs – general (s)</td>
<td>1.71 (±0.48)</td>
<td>1.56 (±0.32)</td>
<td>1.28 (±0.22)</td>
<td>1.32 (±0.31)</td>
<td>&lt;0.001a</td>
</tr>
<tr>
<td>RTs – negative stimuli (s)</td>
<td>1.78 (±0.45)</td>
<td>1.64 (±0.35)</td>
<td>1.34 (±0.22)</td>
<td>1.38 (±0.35)</td>
<td>&lt;0.001a</td>
</tr>
<tr>
<td></td>
<td>MDD-FHP</td>
<td>MDD-FHN</td>
<td>HC-FHP</td>
<td>HC-FHP</td>
<td></td>
</tr>
<tr>
<td>----------------------</td>
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<td>---------</td>
<td>--------</td>
<td>--------</td>
<td></td>
</tr>
<tr>
<td>RTs - positive stimuli (s)</td>
<td>1.71 (±0.55)</td>
<td>1.52 (±0.29)</td>
<td>1.26 (±0.26)</td>
<td>1.3 (±0.32)</td>
<td></td>
</tr>
<tr>
<td>RTs - neutral stimuli (s)</td>
<td>1.64 (±0.48)</td>
<td>1.52 (±0.35)</td>
<td>1.23 (±0.23)</td>
<td>1.28 (±0.28)</td>
<td></td>
</tr>
</tbody>
</table>

MDD-FHP – patients with major depressive disorder with family history of the disease; MDD-FHN – patients with major depressive disorder without family history of the disease; HC-FHP – healthy controls with family history of the disease; HC-FHN – healthy controls without family history of the disease

Statistical significance set to p<0.05; *Statistically significant difference
Subsequently, post-hoc analysis was performed for all the contrast. The results are presented in Table 8.

3.5.1.3.1 Patients with MDD without family history of MDD vs healthy controls without family history of MDD (H2B)

The HC-FHN were more accurate in emotion recognition of positive stimuli than the MDD-FHN. The results are presented in Table 8.

3.5.1.3.2 Patients with MDD with family history of MDD vs healthy controls with family history of MDD (H2C)

The HC-FHP were more accurate and quicker than the MDD-FHP in attention shifting in general and in all the valence types. They were also more accurate in recognition of positive stimuli. The results are presented in Table 8.

3.5.1.3.3 Healthy controls with family history of MDD vs healthy controls without family history of MDD (H2D)

There were no significant differences between the HC-FHP and the HC-FHN in behavioural correlates of emotion processing and attention shifting (Table 8).

3.5.1.3.4 Patients with MDD with family history of MDD vs patients with MDD without family history of MDD (H2E)

There were no significant differences between the MDD-FHP and the MDD-FHN in behavioural correlates of emotion processing and attention shifting (Table 8).
Table 8. Post-hoc comparisons in accuracy and reaction times for emotion processing and attention shifting between groups distinguished by diagnosis of MDD and family history of the disease

<table>
<thead>
<tr>
<th>Behavioural characteristics (p values)</th>
<th>HC-FHN vs MDD-FHN</th>
<th>HC-FHP vs MDD-FHP</th>
<th>HC-FHN vs HC-FHP</th>
<th>MDD-FHN vs MDD-FHP</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Emotion processing</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Accuracy – general</td>
<td>0.054</td>
<td>0.109</td>
<td>1.000</td>
<td>0.960</td>
</tr>
<tr>
<td>Accuracy – negative stimuli</td>
<td>0.240</td>
<td>0.879</td>
<td>0.906</td>
<td>0.938</td>
</tr>
<tr>
<td>Accuracy – positive stimuli</td>
<td>0.005&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;0.001&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.977</td>
<td>0.990</td>
</tr>
<tr>
<td>Accuracy – neutral stimuli</td>
<td>0.773</td>
<td>0.915</td>
<td>0.990</td>
<td>0.907</td>
</tr>
<tr>
<td>RTs – general</td>
<td>0.502</td>
<td>0.187</td>
<td>0.863</td>
<td>0.991</td>
</tr>
<tr>
<td>RTs – negative stimuli</td>
<td>0.328</td>
<td>0.251</td>
<td>0.933</td>
<td>0.939</td>
</tr>
<tr>
<td>RTs – positive stimuli</td>
<td>0.544</td>
<td>0.120</td>
<td>0.879</td>
<td>1.000</td>
</tr>
<tr>
<td>RTs – neutral stimuli</td>
<td>0.720</td>
<td>0.296</td>
<td>0.807</td>
<td>0.991</td>
</tr>
<tr>
<td><strong>Attention shifting</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Accuracy – general</td>
<td>0.287</td>
<td>0.006&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.944</td>
<td>0.843</td>
</tr>
<tr>
<td>Accuracy – negative stimuli</td>
<td>0.284</td>
<td>0.008&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.973</td>
<td>0.825</td>
</tr>
<tr>
<td>Accuracy – positive stimuli</td>
<td>0.554</td>
<td>0.031&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.941</td>
<td>0.880</td>
</tr>
<tr>
<td>Accuracy – neutral stimuli</td>
<td>0.231</td>
<td>0.005&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.938</td>
<td>0.886</td>
</tr>
<tr>
<td>RTs – general</td>
<td>0.147</td>
<td>&lt;0.001&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.977</td>
<td>0.512</td>
</tr>
<tr>
<td>Stimuli Type</td>
<td>RTs (sec)</td>
<td>p-value 1</td>
<td>RTs (sec)</td>
<td>p-value 2</td>
</tr>
<tr>
<td>----------------------</td>
<td>-----------</td>
<td>-----------</td>
<td>-----------</td>
<td>-----------</td>
</tr>
<tr>
<td>Negative stimuli</td>
<td>0.107</td>
<td>&lt;0.001</td>
<td>0.982</td>
<td>0.542</td>
</tr>
<tr>
<td>Positive stimuli</td>
<td>0.265</td>
<td>0.001</td>
<td>0.987</td>
<td>0.405</td>
</tr>
<tr>
<td>Neutral stimuli</td>
<td>0.148</td>
<td>0.001</td>
<td>0.962</td>
<td>0.681</td>
</tr>
</tbody>
</table>

MDD-FHP – patients with major depressive disorder with family history of the disease; MDD-FHN – patients with major depressive disorder without family history of the disease; HC-FHP – healthy controls with family history of the disease; HC-FHN – healthy controls without family history of the disease

Statistical significance set to p<0.05; *Statistically significant difference
3.5.2 Functional MRI results

3.5.2.1 General effect of diagnosis (H1)

3.5.2.1.1 Healthy controls > patients with MDD

3.5.2.1.1.1 Emotional processing condition

During emotional processing healthy controls displayed a higher activation than patients with MDD in the following regions: the right precuneus/ PCC, the right Rolandic operculum, the right insula, the left angular gyrus, the left supramarginal gyrus and the left Rolandic operculum (Figure 2). The results are presented in Table 9.

![Figure 2. Regions of stronger neural activation observed in healthy controls, as compared to patients with MDD, during general emotion processing; FWE cluster corrected; scale represents t values.](image)

When the valence categories were taken into account healthy controls experienced greater activation than patients with MDD did during processing of neutral and positive stimuli. For the positive stimuli, the differences were noted in the following regions: the left calcarine fissure, the left middle occipital gyrus, the left superior occipital gyrus, the left supramarginal gyrus, the left postcentral gyrus and the left Rolandic operculum (Figure 3).
During processing of neutral stimuli, healthy controls showed more activation in the left caudate nucleus, the left insula, the left rolandic operculum, the right insula, the right precuneus and the right rolandic operculum (Figure 4).

When the family history of MDD was not considered, healthy controls noted no increase of activation in the CNS during processing of negative stimuli. The regions are presented in Table 9.

3.5.2.1.2 Attention shifting condition

During attention shifting from emotion processing healthy controls displayed higher activation than patients with MDD in the right superior frontal gyrus, the bilateral SMA/ MCC, the right precuneus, the right rolandic operculum and the left inferior parietal gyrus (Figure 5). The regions are presented in Table 9.
Additionally, when the valence categories were taken into account healthy controls experienced more activation than patients with MDD during shifting attention from neutral stimuli. The differences were noted in: the right precuneus, the left inferior parietal gyrus, the right caudate nucleus, in the right superior frontal gyrus and the bilateral supplementary motor area (Figure 6). When family history of MDD was not considered, healthy controls showed no increase of activation in the CNS when shifting attention from positive and negative stimuli. The regions are presented in Table 9.
3.5.2.1.2 Patients with MDD > healthy controls

3.5.2.1.2.1 Emotional processing condition

In comparison to healthy controls, patients with MDD did not show any increase in the neural activation during emotional processing, when the groups were not divided into subjects with and without family history of MDD. However, when the valence categories were taken into account, the right caudate nucleus approached significance (p=0.066 FWE corrected), when patients with MDD were compared to healthy controls in processing of negative stimuli (Figure 7). When the family history of MDD was not considered, patients with MDD showed no increase of activation in the CNS during processing of positive or neutral stimuli. The regions are presented in the Table 9.

Figure 7. Regions of stronger neural activation observed in patients with MDD, as compared to healthy controls, during emotional processing of negative stimuli; FWE cluster corrected; scale represents t values.

3.5.2.1.2.2 Attention shifting condition

In comparison to healthy controls, patients with MDD did not show any increase in the neural activation during attention shifting when the groups were not divided into subjects with and without family history of MDD.
Table 9. Significant differences in neural activation during emotional processing and attention shifting between patients with MDD and healthy controls

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Process</th>
<th>Cluster size (no. of voxels)</th>
<th>Cluster corrected P values</th>
<th>Region</th>
<th>MNI location</th>
</tr>
</thead>
<tbody>
<tr>
<td>HC &gt; MDD</td>
<td>General emotional processing</td>
<td>500</td>
<td>&lt;0.001</td>
<td>R precuneus/ posterior cingulate cortex</td>
<td>27 -43 25</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>R rolandic operculum</td>
<td>45 -25 25</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>R insula</td>
<td>36 -22 25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>283</td>
<td>&lt;0.001</td>
<td>L angular gyrus</td>
<td>-33 -55 22</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>L supramarginal gyrus</td>
<td>-48 -25 25</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>L rolandic operculum</td>
<td>-33 -43 22</td>
</tr>
<tr>
<td></td>
<td>Emotional processing of positive stimuli</td>
<td>116</td>
<td>0.016</td>
<td>L calcarine fissure</td>
<td>-6 -91 10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>L middle occipital gyrus</td>
<td>-15 -88 10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>L superior occipital gyrus</td>
<td>-18 -85 25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>109</td>
<td>0.020</td>
<td>L supramarginal gyrus</td>
<td>-51 -28 25</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>L postcentral gyrus</td>
<td>-57 -16 25</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>L rolandic operculum</td>
<td>-39 -37 25</td>
</tr>
<tr>
<td></td>
<td>Emotional processing of neutral stimuli</td>
<td>428</td>
<td>&lt;0.001</td>
<td>L caudate nucleus</td>
<td>-21 -13 25</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>L rolandic operculum</td>
<td>-30 -43 25</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>L insula</td>
<td>-33 -34 25</td>
</tr>
<tr>
<td>General attention shifting</td>
<td>213</td>
<td>0.001</td>
<td>R insula</td>
<td>30 -22 28</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>R precuneus</td>
<td>21 -46 19</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>R rolandic operculum</td>
<td>33 -40 22</td>
<td></td>
</tr>
<tr>
<td>L superior frontal gyrus</td>
<td>149</td>
<td>0.006</td>
<td>R superior frontal gyrus</td>
<td>15 17 46</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>L supplementary motor area/ middle cingulate cortex</td>
<td>-9 8 46</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>R supplementary motor area/ middle cingulate cortex</td>
<td>6 5 46</td>
<td></td>
</tr>
<tr>
<td>R precuneus</td>
<td>961</td>
<td>&lt;0.001</td>
<td>R precuneus</td>
<td>27 -46 25</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>R rolandic operculum</td>
<td>42 -40 19</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>L inferior parietal gyrus</td>
<td>-33 -28 34</td>
<td></td>
</tr>
<tr>
<td>L inferior parietal gyrus</td>
<td>1491</td>
<td>&lt;0.001</td>
<td>R precuneus</td>
<td>27 -46 25</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>L inferior parietal gyrus</td>
<td>-33 -28 34</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>R caudate nucleus</td>
<td>24 -19 31</td>
<td></td>
</tr>
<tr>
<td>L superior frontal gyrus</td>
<td>498</td>
<td>&lt;0.001</td>
<td>R superior frontal gyrus</td>
<td>15 17 46</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>L supplementary motor area</td>
<td>-3 5 49</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>R supplementary motor area</td>
<td>6 5 46</td>
<td></td>
</tr>
<tr>
<td>Emotional processing of negative stimuli</td>
<td>MDD &gt; HC</td>
<td>72</td>
<td>0.066</td>
<td>right caudate nucleus</td>
<td>6 5 -2</td>
</tr>
</tbody>
</table>

MDD – patients with major depressive disorder; HC – healthy controls. Results FWE cluster corrected; Statistical significance set to p<0.05
3.5.2.2 General effect of family history of MDD (H2A)

3.5.2.2.1 Subjects without family history of MDD > subjects with family history of MDD

3.5.2.2.1.1 Emotional processing condition

In comparison to subjects with family history of MDD, subjects without family history of MDD did not show any increase in the neural activation during emotional processing when the diagnosis was not taken into account.

3.5.2.2.1.2 Attention shifting condition

In comparison to subjects with family history of MDD, subjects without family history of MDD did not show any increase in the neural activation during attention shifting from emotional processing when the diagnosis was not taken into account.

3.5.2.2.2 Subjects with family history of MDD > subjects without family history of MDD

3.5.2.2.2.1 Emotional processing condition

In comparison to subjects without family history of MDD, subjects with family history of MDD did not demonstrate any increase in the neural activation during emotional processing when the diagnosis was not taken into account.

3.5.2.2.2.2 Attention shifting condition

In comparison to subjects without family history of MDD, subjects with family history of MDD did not demonstrate any increase in the neural activation during attention shifting from emotional processing when the diagnosis was not taken into account.

However, when the valence categories were considered separately, subjects with family history of MDD experienced more neural activation than subjects without such history.
while shifting attention from negative stimuli in the left supramarginal gyrus, the left superior temporal gyrus and the left rolandic operculum (Figure 8). While shifting attention from neutral and positive stimuli the increase of neural activation was not observed in the group with family history of MDD. The regions are presented in Table 10.

Figure 8. Regions of stronger neural activation observed individuals with family history of MDD, as compared to individuals without family history of MDD, during shifting attention from negative stimuli; FWE cluster corrected; scale represents t values.

The negative interaction between the diagnosis of MDD and family history of the disease was observed in the left middle occipital gyrus. The regions are presented in Table 10.

Table 10. Significant differences in neural activation during emotion processing and attention shifting between individuals with family history of MDD and individuals without family history of MDD with interaction between diagnosis and family history of the disease

<table>
<thead>
<tr>
<th>Clustering</th>
<th>Cluster size (no. of voxels)</th>
<th>Cluster corrected P values</th>
<th>Region MNI location</th>
</tr>
</thead>
<tbody>
<tr>
<td>FHP &gt; FHN</td>
<td>Attention shifting from negative stimuli</td>
<td>108</td>
<td>0.021</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>left superior temporal gyrus -57 -31 13</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>left rolandic operculum -48 -10 19</td>
</tr>
<tr>
<td>Negative interaction between diagnosis and family history</td>
<td>80</td>
<td>0.049</td>
<td>left middle occipital gyrus -27 -79 28</td>
</tr>
</tbody>
</table>

FHP – individuals with family history of major depressive disorder; FHN – individuals without family history of major depressive disorder

Results FWE cluster corrected; Statistical significance set to p<0.05
3.5.2.3 Patients with MDD without family history of MDD vs healthy controls without family history of MDD (H2B)

3.5.2.3.1 HC-FHN > MDD-FHN

3.5.2.3.1.1 Emotional processing condition

While processing emotional valence of the stimuli, the HC-FHN showed higher activation than the MDD-FHN in the right insula, the right rolandic operculum and the right precuneus/posterior cingulate gyrus (Figure 9). The regions are presented in Table 11.

![Figure 9. Regions of stronger neural activation observed in the HC-FHN, as compared to the MDD-FHN, during general emotional processing; FWE cluster corrected; scale represents t values.](image)

When the valence categories were considered separately, the HC-FHN noted more neural activation than the MDD-FHN during processing of negative stimuli. The difference was observed in the right postcentral gyrus, the right middle cingulate cortex and the right angular gyrus (Figure 10). The change was not noted in the processing of positive and neutral stimuli. The regions are presented in Table 11.
3.5.2.3.1.2 Attention shifting condition

The increase of neural activation experienced by the MDD-FHN, as compared to the HC-FHN, during emotional processing was observed in the vermis 3 and the left caudate nucleus (Figure 11). The regions are presented in Table 11.

Also, when valence categories were separated, the MDD-FHN in comparison to the HC-FHN experienced an increase of activation while processing negative stimuli. The increase was observed in the bilateral caudate nuclei, the vermis 3, the left parahippocampal gyrus and the bilateral thalami (Figure 12). However, the change was not noted during processing of positive and neutral stimuli. The regions are presented in Table 11.
Figure 12. Regions of stronger neural activation observed in the MDD-FHN, as compared to the HC-FHN, during emotional processing of negative stimuli; FWE cluster corrected; scale represents t values.

3.5.2.3.2 MDD-FHN > HC-FHN

3.5.2.3.2.1 Emotional processing condition

The increase of neural activation experienced by the MDD-FHN, as compared to the HC-FHN, during emotional processing was observed in the vermis 3 and the left caudate nucleus (Figure 13). The regions are presented in Table 11.

Figure 13. Regions of stronger neural activation observed in the MDD-FHN, as compared to the HC-FHN, during general emotional processing; FWE cluster corrected; scale represents t values.

Also, when valence categories were separated, the MDD-FHN in comparison to the HC-FHN experienced an increase of activation while processing negative stimuli. The increase was observed in the bilateral caudate nuclei, the vermis 3, the left parahippocampal gyrus and the bilateral thalami (Figure 14). However, the change was not noted during processing of positive and neutral stimuli. The regions are presented in Table 11.
3.5.2.3.2.2 Attention shifting condition

In comparison to the HC-FHN, the MDD-FHN did not show any increase in the neural activation during shifting attention from emotional processing.

3.5.2.4 Patients with MDD with family history of MDD vs healthy controls with family history of MDD (H2C)

3.5.2.4.1 HC-FHP > MDD-FHP

3.5.2.4.1.1 Emotional processing condition

In emotion processing, the HC-FHP compared to the MDD-FHP displayed a higher activation in the left cuneus, the left supramarginal gyrus and the left superior occipital gyrus (Figure 15). The regions are presented in Table 12.
Table 11. Significant differences in neural activation during emotional processing and attention shifting between patients with MDD without family history of the disease and healthy controls without family history of the disease

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Process</th>
<th>Cluster size (no. of voxels)</th>
<th>Cluster corrected P values</th>
<th>Region</th>
<th>MNI location</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HC-FHN &gt; MDD-FHN</strong></td>
<td>General emotional processing</td>
<td>338</td>
<td>&lt;0.001</td>
<td>right insula</td>
<td>36 -22 25</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>right rolandic operculum</td>
<td>45 -25 25</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>right precuneus/ posterior cingulate cortex</td>
<td>27 -43 25</td>
</tr>
<tr>
<td></td>
<td>Emotional processing of negative stimuli</td>
<td>114</td>
<td>0.017</td>
<td>right postcentral gyrus</td>
<td>30 -40 28</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>right middle cingulate cortex</td>
<td>21 -28 28</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>right angular gyrus</td>
<td>33 -52 28</td>
</tr>
<tr>
<td></td>
<td>General attention shifting</td>
<td>539</td>
<td>&lt;0.001</td>
<td>right supramarginal gyrus</td>
<td>45 -22 25</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>right insula</td>
<td>27 -31 22</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>right rolandic operculum</td>
<td>36 -19 22</td>
</tr>
<tr>
<td></td>
<td>Attention shifting from neutral stimuli</td>
<td>1125</td>
<td>&lt;0.001</td>
<td>right precuneus</td>
<td>27 -46 25</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>right caudate nucleus</td>
<td>21 -25 28</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>right posterior cingulate cortex</td>
<td>15 -40 25</td>
</tr>
<tr>
<td><strong>MDD-FHN &gt; HC-FHN</strong></td>
<td>General emotional processing</td>
<td>108</td>
<td>0.021</td>
<td>vermis 3</td>
<td>-3 -34 -5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>82</td>
<td>0.045</td>
<td>left caudate nucleus</td>
<td>-3 5 10</td>
</tr>
<tr>
<td></td>
<td>Emotional processing</td>
<td>140</td>
<td>0.008</td>
<td>bilateral caudate nucleus</td>
<td>0 5 7</td>
</tr>
</tbody>
</table>
of negative stimuli  |  144  |  0.007  |  vermis 3  |  3  |  34  |  5
|  left thalamus  |  0  |  25  |  1  |
|  left parahippocampal gyrus  |  18  |  25  |  17  |

MDD-FHN – patients with major depressive disorder without family history of the disease; HC-FHN – healthy controls without family history of major depressive disorder
Results FWE cluster corrected; Statistical significance set to p<0.05
Also, when the valence categories were considered separately, the HC-FHP showed an increased activation in comparison to the MDD-FHP during processing of positive stimuli. The increase was observed in the left superior occipital gyrus, the left middle occipital gyrus and the left angular gyrus (Figure 16). The difference was not noted during processing of negative and neutral stimuli. The regions are presented in Table 12.

Figure 16. Regions of stronger neural activation observed in the HC-FHP, as compared to the MDD-FHP, during emotional processing of positive stimuli; FWE cluster corrected; scale represents t values.

3.5.2.4.1.2 Attention shifting condition

While shifting attention from emotional processing, the HC-FHP compared to the MDD-FHP displayed a higher activation in the bilateral superior occipital gyrus and the left inferior parietal gyrus (Figure 17). The regions are presented in Table 12.

Figure 17. Regions of stronger neural activation observed in the HC-FHP, as compared to the MDD-FHP, during general attention shifting; FWE cluster corrected; scale represents t values.

Also, when the valence categories were considered separately, the HC-FHP showed an increased activation in comparison to the MDD-FHP when shifting attention from
negative and neutral stimuli. The increase during attention shifting from negative stimuli was observed in the left middle/anterior cingulate cortex and the left superior occipital gyrus (Figure 18).

Figure 18. Regions of stronger neural activation observed in the HC-FHP, as compared to the MDD-FHP, during shifting attention from negative stimuli; FWE cluster corrected; scale represents t values.

The increase characteristic for attention shifting from neutral stimuli was noted in the left postcentral gyrus, the left inferior parietal gyrus, the bilateral supplementary motor area and the right superior frontal gyrus (Figure 19). The regions are presented in Table 12.

Figure 19. Regions of stronger neural activation observed in the HC-FHP, as compared to the MDD-FHP, during shifting attention from neutral stimuli; FWE cluster corrected; scale represents t values.

3.5.2.4.2 MDD-FHP > HC-FHP

3.5.2.4.2.1 Emotional processing condition

In comparison to the HC-FHP, the MDD-FHP did not show any increase in the neural activation during processing of emotional valence of the stimuli.
3.5.2.4.2.2 Attention shifting condition

In comparison to the HC-FHP, the MDD-FHP did not show any general increase in the neural activation during attention shifting from emotional processing. However, when the valence categories were regarded separately, an effect was discovered for shifting attention from negative stimuli. The MDD-FHP displayed an increased activation in comparison to the HC-FHP in the cluster encompassing the left cerebellum 4_5, the left fusiform/parahippocampal gyrus and the right cerebellum 6 (Figure 20).

![Figure 20](image)

Figure 20. Regions of stronger neural activation observed in the MDD-FHP, as compared to the HC-FHP, during shifting attention from negative stimuli; FWE cluster corrected; scale represents t values.

The effect was not observed for shifting attention from positive and neutral stimuli. The regions are presented in Table 12.
Table 12. Significant differences in neural activation during emotional processing and attention shifting between patients with MDD with family history of the disease and healthy controls with family history of the disease

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Process</th>
<th>Cluster size (no. of voxels)</th>
<th>Cluster corrected P values</th>
<th>Region</th>
<th>MNI location</th>
</tr>
</thead>
<tbody>
<tr>
<td>HC-FHP &gt; MDD-FHP</td>
<td>General emotional processing</td>
<td>828</td>
<td>&lt;0.001</td>
<td>left cuneus</td>
<td>-12 -85 28</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>left supramarginal gyrus</td>
<td>-57 -52 28</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>left superior occipital gyrus</td>
<td>-21 -82 28</td>
</tr>
<tr>
<td></td>
<td>Emotional processing of positive stimuli</td>
<td>765</td>
<td>&lt;0.001</td>
<td>left superior occipital gyrus</td>
<td>-15 -85 25</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>left middle occipital gyrus</td>
<td>-15 -88 13</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>left angular gyrus</td>
<td>-33 -55 22</td>
</tr>
<tr>
<td></td>
<td>General attention shifting</td>
<td>443</td>
<td>&lt;0.001</td>
<td>left superior occipital gyrus</td>
<td>-15 -85 25</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>right superior occipital gyrus</td>
<td>24 -73 25</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>left inferior parietal gyrus</td>
<td>-24 -40 37</td>
</tr>
<tr>
<td></td>
<td>Attention shifting of negative stimuli</td>
<td>798</td>
<td>&lt;0.001</td>
<td>left superior occipital gyrus</td>
<td>-21 -79 25</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>left middle/ anterior cingulate cortex</td>
<td>-3 -16 34</td>
</tr>
<tr>
<td></td>
<td>Attention shifting of neutral stimuli</td>
<td>601</td>
<td>&lt;0.001</td>
<td>left postcentral gyrus</td>
<td>-36 -25 40</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>left inferior parietal gyrus</td>
<td>-30 -37 40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>199</td>
<td>0.001</td>
<td>left supplementary motor area</td>
<td>-3 8 46</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>right superior frontal gyrus</td>
<td>15 17 46</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>-------------------------</td>
<td>--------------------------</td>
<td>--------------------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>MDD-FHP &gt; HC-FHP</strong></td>
<td>Attention shifting of</td>
<td>right supplementary motor</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>negative stimuli</td>
<td>area</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>205</td>
<td>9849</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>left cerebellum 4_5</strong></td>
<td></td>
<td>-9 -58 -20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>**left fusiform/</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>parahippocampal gyrus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>right cerebellum 6</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>-21 -55 -23</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

MDD-FHP – patients with major depressive disorder with family history of the disease; HC-FHP – healthy controls with family history of major depressive disorder

Results FWE cluster corrected; Statistical significance set to p<0.05
3.5.2.5 Healthy controls with family history of MDD vs healthy controls without family history of MDD (H2D)

3.5.2.5.1 HC-FHN > HC-FHP

3.5.2.5.1.1 Emotional processing condition

In comparison to the HC-FHP, the HC-FHN did not show any increase in the neural activation during processing of emotional valence.

3.5.2.5.1.2 Attention shifting condition

In comparison to the HC-FHP, the HC-FHN did not show any increase in the neural activation during shifting attention from emotional processing.

3.5.2.5.2 HC-FHP > HC-FHN

3.5.2.5.2.1 Emotional processing condition

During processing of emotional valence, the HC-FHP displayed a higher neural activation than the HC-FHN did in the following regions: the left supramarginal gyrus, the left inferior parietal gyrus and left postcentral gyrus (Figure 21). The regions are presented in Table 13.

![Figure 21. Regions of stronger neural activation observed in the HC-FHP, as compared to the HC-FHN, during general emotional processing; FWE cluster corrected; scale represents t values.](image)
Also, when the valence categories were considered separately, the HC-FHP compared to the HC-FHN displayed an increased neural activation during emotional processing of negative stimuli in the left inferior parietal gyrus and the left postcentral gyrus (Figure 22). The increase was not noted during processing of positive and neutral stimuli. The regions are presented in Table 13.

Figure 22. Regions of stronger neural activation observed in the HC-FHP, as compared to the HC-FHN, during emotional processing of negative stimuli; FWE cluster corrected; scale represents t values.

3.5.2.5.2.2 Attention shifting condition

While shifting attention from emotional processing, the HC-FHP compared to the HC-FHN displayed a higher neural activation in the following regions: the left superior occipital gyrus, the left inferior parietal gyrus and the left supramarginal gyrus (Figure 23). The regions are presented in Table 13.

Figure 23. Regions of stronger neural activation observed in the HC-FHP, as compared to the HC-FHN, during general attention shifting; FWE cluster corrected; scale represents t values.
Also, when the valence categories were taken into account separately, the HC-FHP displayed a higher neural activation than the HC-FHN did in shifting attention from neutral, negative and positive stimuli. The increase during attention shifting from negative stimuli was observed in the bilateral middle cingulate cortex and the left postcentral gyrus (Figure 24).

![Figure 24](image)

**Figure 24.** Regions of stronger neural activation observed in the HC-FHP, as compared to the HC-FHN, during shifting attention from negative stimuli; FWE cluster corrected; scale represents t values.

The increase representative for attention shifting from positive stimuli was discerned in the left middle occipital gyrus, the left superior occipital gyrus and the left cuneus (Figure 25).

![Figure 25](image)

**Figure 25.** Regions of stronger neural activation observed in the HC-FHP, as compared to the HC-FHN, during shifting attention from positive stimuli; FWE cluster corrected; scale represents t values.

The increase characteristic for attention shifting from neutral stimuli was noted in the left inferior parietal gyrus (Figure 26). The regions are presented in Table 13.
3.5.2.6 Patients with MDD with family history of MDD vs patients with MDD without family history of MDD (H2E)

3.5.2.6.1 MDD-FHN > MDD-FHP

3.5.2.6.1.1 Emotional processing condition

In comparison to the MDD-FHP, the MDD-FHN did not demonstrate any increase in the neural activation during processing of emotional valence of the stimuli.

3.5.2.6.1.2 Attention shifting condition

In comparison to the MDD-FHP, the MDD-FHN did not display any increase in the neural activation during attention shifting from emotional processing.

3.5.2.6.2 MDD-FHP > MDD-FHN

3.5.2.6.2.1 Emotional processing condition

In comparison to the MDD-FHN, the MDD-FHP did not show any increase in the neural activation during processing of emotional valence of the stimuli.
Table 13. Significant differences in neural activation during emotional processing and attention shifting between healthy controls with family history of MDD and healthy controls without family history of MDD

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Process</th>
<th>Cluster size (no. of voxels)</th>
<th>Cluster corrected P values</th>
<th>Region</th>
<th>MNI location</th>
</tr>
</thead>
<tbody>
<tr>
<td>HC-FHP &gt; HC-FHN</td>
<td>General emotional processing</td>
<td>539</td>
<td>&lt;0.001</td>
<td>left supramarginal gyrus</td>
<td>-57 -49 31</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>left inferior parietal gyrus</td>
<td>-51 -49 43</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>left postcentral gyrus</td>
<td>-39 -43 40</td>
</tr>
<tr>
<td></td>
<td>Emotional processing in negative</td>
<td>452</td>
<td>&lt;0.001</td>
<td>left inferior parietal gyrus</td>
<td>-54 -40 40</td>
</tr>
<tr>
<td></td>
<td>stimuli</td>
<td></td>
<td></td>
<td>left postcentral gyrus</td>
<td>-57 -19 43</td>
</tr>
<tr>
<td></td>
<td>General attention shifting</td>
<td>594</td>
<td>&lt;0.001</td>
<td>left superior occipital gyrus</td>
<td>-24 -79 28</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>left inferior parietal gyrus</td>
<td>-45 -46 37</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>left supramarginal gyrus</td>
<td>-57 -22 40</td>
</tr>
<tr>
<td></td>
<td>Attention shifting of negative</td>
<td>313</td>
<td>&lt;0.001</td>
<td>right middle cingulate cortex</td>
<td>3 -19 34</td>
</tr>
<tr>
<td></td>
<td>stimuli</td>
<td></td>
<td></td>
<td>left middle cingulate cortex</td>
<td>-9 -22 37</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>left postcentral gyrus</td>
<td>-36 -16 34</td>
</tr>
<tr>
<td></td>
<td>Attention shifting of positive</td>
<td>146</td>
<td>0.006</td>
<td>left middle occipital gyrus</td>
<td>-33 -73 25</td>
</tr>
<tr>
<td></td>
<td>stimuli</td>
<td></td>
<td></td>
<td>left superior occipital gyrus</td>
<td>-24 -79 28</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>left cuneus</td>
<td>-9 -76 25</td>
</tr>
<tr>
<td></td>
<td>Attention shifting of neutral</td>
<td>141</td>
<td>0.007</td>
<td>left inferior parietal gyrus</td>
<td>-45 -28 40</td>
</tr>
<tr>
<td></td>
<td>stimuli</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

HC-FHP – healthy controls with family history of major depressive disorder; HC-FHN – healthy controls without family history of major depressive disorder; Results FWE cluster corrected; Statistical significance set to p<0.05
3.5.2.6.2.2 Attention shifting condition

In comparison to the MDD-FHN, the MDD-FHP did not experience any increase in the neural activation during attention shifting from emotional processing.

3.5.2.7 Summary of the results

This study verifies hypotheses 1 and 2. The summary of the results is presented in the Table 14.

Table 14. Summary of neural differences between group distinguished by diagnosis and family history of MDD in attention shifting and emotion recognition

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDD vs HC (H1)</td>
<td>- deactivated visual and emotion recognition cortices in patients with MDD during emotion processing (positive, neutral)</td>
</tr>
<tr>
<td></td>
<td>- increased core limbic group activation in patients with MDD during emotion processing (negative)</td>
</tr>
<tr>
<td></td>
<td>- decreased activation in attention shifting areas in patients with MDD during attention shifting</td>
</tr>
<tr>
<td>FHN vs FHP (H2A)</td>
<td>- increased activation in language and mirror neurons area during attention shifting (negative) in subjects with family history of MDD</td>
</tr>
<tr>
<td>MDD-FHN vs HC-FHN (H2B)</td>
<td>- increased activation in the core limbic group during emotional processing (negative) in MDD-FHN</td>
</tr>
<tr>
<td></td>
<td>- decreased activation in the paralimbic group during emotional processing (negative) in MDD-FHN</td>
</tr>
<tr>
<td>MDD-FHP vs HC-FHP (H2C)</td>
<td>- decreased activation in the visual cortex during emotional processing (positive) in MDD-FHP</td>
</tr>
<tr>
<td></td>
<td>- decreased activation in attention controlling areas (negative) and increased activation in core limbic group (negative) during attention shifting in MDD-FHP</td>
</tr>
<tr>
<td>HC-FHN vs HC-FHP (H2D)</td>
<td>- increased activation in language /mirror neurons area during emotional processing (negative) in HC-FHP</td>
</tr>
<tr>
<td></td>
<td>- increased activation in attention controlling and language/mirror neurons areas during attention shifting (negative) in HC-FHP</td>
</tr>
<tr>
<td>MDD-FHN vs MDD-FHP (H2E)</td>
<td>- no differences observed</td>
</tr>
</tbody>
</table>

MDD-FHP – patients with major depressive disorder with family history of the disease; MDD-FHN – patients with major depressive disorder without family history of the disease; HC-FHN – healthy controls with family history of the disease; HC-FHP – healthy controls without family history of the disease
3.5.3 Differences between patients receiving different types of treatment

Patients with MDD treated with different types of medication and untreated individuals with MDD did not differ in behavioural and neural correlates of emotion processing and attention shifting (Table 15).

**Table 15. Differences in accuracy and reaction times between patients treated with SSRIs, patients treated with dual action substances and not medicated patients in emotion processing and attention shifting (F MANOVA)**

<table>
<thead>
<tr>
<th>Behavioural characteristics (±SD)</th>
<th>Patients treated with SSRIs (N=17)</th>
<th>Patients treated with dual-action (N=18)</th>
<th>Not medicated patients (N=15)</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Emotional processing</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AC – general (% cor)</td>
<td>77.8 (±7.7)</td>
<td>73.8 (±11.3)</td>
<td>70.8 (±20.1)</td>
<td>0.409</td>
</tr>
<tr>
<td>AC – negative stimuli (% cor)</td>
<td>87.1 (±10.9)</td>
<td>78.5 (±12.4)</td>
<td>75.8 (±24.9)</td>
<td>0.177</td>
</tr>
<tr>
<td>AC – positive stimuli (% cor)</td>
<td>78.7 (±9.7)</td>
<td>74.8 (±16.2)</td>
<td>68.6 (±24.5)</td>
<td>0.329</td>
</tr>
<tr>
<td>AC – neutral stimuli (% cor)</td>
<td>67.6 (±13.1)</td>
<td>68.1 (±12.4)</td>
<td>68.1 (±19.7)</td>
<td>0.994</td>
</tr>
<tr>
<td>RTs – general (s)</td>
<td>1.44 (±0.43)</td>
<td>1.61 (±0.53)</td>
<td>1.43 (±0.34)</td>
<td>0.465</td>
</tr>
<tr>
<td>RTs – negative stimuli (s)</td>
<td>1.42 (±0.39)</td>
<td>1.6 (±0.51)</td>
<td>1.46 (±0.4)</td>
<td>0.474</td>
</tr>
<tr>
<td>RTs – positive stimuli (s)</td>
<td>1.28 (±0.39)</td>
<td>1.49 (±0.62)</td>
<td>1.33 (±0.31)</td>
<td>0.448</td>
</tr>
<tr>
<td>RTs – neutral stimuli (s)</td>
<td>1.63 (±0.56)</td>
<td>1.75 (±0.47)</td>
<td>1.5 (±0.4)</td>
<td>0.410</td>
</tr>
<tr>
<td><strong>Attention shifting</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AC – general (% correct)</td>
<td>81.9 (±13.8)</td>
<td>75.9 (±17.4)</td>
<td>75.9 (±19.1)</td>
<td>0.538</td>
</tr>
<tr>
<td>AC – negative stimuli (% cor)</td>
<td>79.1 (±14.5)</td>
<td>69.8 (±19.8)</td>
<td>71.4 (±16.9)</td>
<td>0.298</td>
</tr>
<tr>
<td>AC – positive stimuli (% cor)</td>
<td>83.6 (±14.5)</td>
<td>79 (±16.1)</td>
<td>78.6 (±20)</td>
<td>0.678</td>
</tr>
<tr>
<td>AC – neutral stimuli (% cor)</td>
<td>83.1 (±15.6)</td>
<td>79 (±18.7)</td>
<td>77.8 (±22.8)</td>
<td>0.736</td>
</tr>
<tr>
<td>RTs – general (s)</td>
<td>1.69 (±0.42)</td>
<td>1.68 (±0.46)</td>
<td>1.54 (±0.42)</td>
<td>0.621</td>
</tr>
<tr>
<td>RTs – negative stimuli (s)</td>
<td>1.74 (±0.41)</td>
<td>1.76 (±0.45)</td>
<td>1.6 (±0.42)</td>
<td>0.569</td>
</tr>
<tr>
<td>RTs – positive stimuli (s)</td>
<td>1.68 (±0.46)</td>
<td>1.66 (±0.5)</td>
<td>1.49 (±0.44)</td>
<td>0.541</td>
</tr>
<tr>
<td>RTs – neutral stimuli (s)</td>
<td>1.65 (±0.45)</td>
<td>1.61 (±0.46)</td>
<td>1.52 (±0.45)</td>
<td>0.769</td>
</tr>
</tbody>
</table>

AC – accuracy, cor – correct, RTs – reaction times, SSRIs – selective serotonin reuptake inhibitors
Statistical significance set to p<0.05; *Statistically significant difference

MANOVA tests revealed no significant differences between the pairs of groups of patients in accuracy and RTs of emotion processing and attention shifting (Table 16).
Table 16. Post-hoc comparisons in accuracy and reaction times for emotion processing and attention shifting between groups of patients with MDD distinguished by different types of medication

<table>
<thead>
<tr>
<th>Behavioural characteristics (p values)</th>
<th>SSRIs vs dual</th>
<th>SSRIs vs no treatment</th>
<th>dual vs no treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Emotional processing</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Accuracy – general (% correct)</td>
<td>0.692</td>
<td>0.348</td>
<td>0.830</td>
</tr>
<tr>
<td>Accuracy – negative stimuli (% correct)</td>
<td>0.324</td>
<td>0.191</td>
<td>0.902</td>
</tr>
<tr>
<td>Accuracy – positive stimuli (% correct)</td>
<td>0.807</td>
<td>0.298</td>
<td>0.618</td>
</tr>
<tr>
<td>Accuracy – neutral stimuli (% correct)</td>
<td>0.994</td>
<td>0.996</td>
<td>1.000</td>
</tr>
<tr>
<td>RTs – general (s)</td>
<td>0.549</td>
<td>0.996</td>
<td>0.531</td>
</tr>
<tr>
<td>RTs – negative stimuli (s)</td>
<td>0.470</td>
<td>0.963</td>
<td>0.676</td>
</tr>
<tr>
<td>RTs – positive stimuli (s)</td>
<td>0.448</td>
<td>0.967</td>
<td>0.645</td>
</tr>
<tr>
<td>RTs – neutral stimuli (s)</td>
<td>0.792</td>
<td>0.749</td>
<td>0.376</td>
</tr>
<tr>
<td><strong>Attention shifting</strong></td>
<td></td>
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<tr>
<td>Accuracy – general (% correct)</td>
<td>0.580</td>
<td>0.628</td>
<td>1.000</td>
</tr>
<tr>
<td>Accuracy – negative stimuli (% correct)</td>
<td>0.301</td>
<td>0.487</td>
<td>0.968</td>
</tr>
<tr>
<td>Accuracy – positive stimuli (% correct)</td>
<td>0.727</td>
<td>0.728</td>
<td>0.998</td>
</tr>
<tr>
<td>Accuracy – neutral stimuli (% correct)</td>
<td>0.816</td>
<td>0.749</td>
<td>0.985</td>
</tr>
<tr>
<td>RTs – general (s)</td>
<td>0.995</td>
<td>0.642</td>
<td>0.688</td>
</tr>
<tr>
<td>RTs – negative stimuli (s)</td>
<td>0.992</td>
<td>0.658</td>
<td>0.581</td>
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<tr>
<td></td>
<td>RTs – positive stimuli (s)</td>
<td>RTs – neutral stimuli (s)</td>
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<td>0.994</td>
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<td>0.752</td>
<td>0.881</td>
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dual – patients with major depressive disorder treated with dual action substances, no treatment – untreated patients with major depressive disorder, RTs – reaction times, SSRIs – patients with major depressive disorder treated with selective serotonin reuptake inhibitors
Statistical significance set to p<0.05; *Statistically significant difference
3.6 Discussion

This study is the first to explore modulations caused by family history of MDD in emotional regulation in people with and without a diagnosis of MDD. The findings of this study suggest that family history of MDD modulates both behavioural and neural correlates of emotional regulation in both healthy controls and patients with MDD, which is in line with what was formulated in the second general hypothesis. The results of this study further point at the neural mechanisms which are at play in relation to family history of MDD, and which influence emotional regulation. These mechanisms have been shown to play an important role in respect to different diagnoses of MDD. This study thus improves our understanding of certain features associated with family history of MDD and adds to the body of previous research into family history of depression as a crucial factor in diagnosis (Christensen, et al., 2006; Fanous, et al., 2002; Giles, et al., 1990; Hall & Smoller, 2010; Hammen & Brennan, 2001; Holma, et al., 2011; Joffe & Regan, 1991; Joormann, et al., 2007b; Lauer, et al., 1997; Lauer, et al., 1998; Le Masurier, et al., 2007; Nierenberg, et al., 2007; Weissman, et al., 2006).

This study is also the first to investigate what role emotional regulation plays in mechanisms of vulnerability and resilience connected with family history of MDD. Finally, this study is the first to observe different changes in emotional regulation leading to an MDD episode in two distinct groups of patients with and without family history of the disease.

These following sections discuss the results in greater detail. First, inhibition of emotional processing is considered, separately from emotional recognition and attention shifting, and each of the specific points of the two individual analyses is addressed. Second, the findings from both areas under scrutiny are compared, in view of arriving at a deeper understanding of the theme. Finally, general comparisons between patients
with MDD and healthy controls as well as between individuals with and without family history of MDD, are also presented. This is to contextualise the upcoming discussion on risk and resilience in this study.

3.6.1 Family history and diagnosis of MDD

3.6.1.1 General effect of diagnosis

The differences observed between a general group of healthy controls and a general group of patients with MDD confirmed the first main hypothesis of this work, which stated that healthy controls and patients with MDD differ in their emotional processing and attention shifting. As demonstrated in other studies as well, these are the two vital components of emotional regulation (Eippert, et al., 2007). In this study, the patients experienced impairments in both processes, which is in accordance with findings from behavioural studies of MDD (Berenbaum, et al., 2003; Bylsma, et al., 2008; Gotlib & Joormann, 2010). In terms of emotional regulation, the findings suggest that recognition of rewarding or punishing situations is reduced in patients with MDD, and so is their ability to withdraw from emotional processing. This finding thus provides further evidence that patients with MDD are impaired in their ability to self-regulate their emotional states (Beauregard, et al., 2006; Joormann, et al., 2007a).

As for the behavioural changes observed in the patients with MDD, these accorded with the postulates of the positive attenuation theory. The theory claims that during an MDD episode recognition of positive stimuli is impaired (Berenbaum, et al., 2003; Bylsma, et al., 2008), which, in turn, may be linked to the emotional symptom of anhedonia generally observed in the disease (Beck & Alford, 2009).

It was difficult to determine from the behavioural data derived from this study whether positive attenuation is caused by: a) a decrease in pleasant emotions (Bylsma, et al., 2011; Ellis, et al., 2009; Punkanen, et al., 2011), b) lack of behavioural reaction to
positive cues (Bylsma, et al., 2008; Mah & Pollock, 2010; McFarland & Klein, 2009; Rottenberg, et al., 2005) or c) an attention bias (Leppänen, 2006). Yet some insights were gained from the fMRI results. These results show that the patients of this study experienced neither enough emotional arousal during processing of positive stimuli nor did they perceive positive stimuli in a manner characteristic for emotionally loaded information. First, the patients experienced a lower activity in the areas responsible for emotional processing and arousal such as the rolandic operculum and the postcentral gyrus (Kanske & Kotz, 2011; Kensinger & Schacter, 2006; Koelsch, Fritz, Müller, & Friederici, 2006). Second, they showed lesser activity in the visual regions, which is in line with results of previous research in the area (Desseilles, et al., 2009; Freese & Amaral, 2005; Fu, et al., 2007; Hendler, et al., 2001; Kober, et al., 2008; Vuilleumier, et al., 2004). Attention filters which select significant information in healthy controls in response to emotional cues thus do not appear to work for patients with MDD (Desseilles, et al., 2009; Fu, et al., 2007). It is noteworthy that in this study, the decrease of activation was observed mainly for positive cues, suggesting that positive stimuli do not evoke a strong emotional reaction in patients with MDD.

The behavioural observations made in this study were also inconclusive about the selection of the most useful theory of processing of negative stimuli for the model of MDD. The negative potentiation (Bylsma, et al., 2011; Gollan, et al., 2008; Myin-Germeys, et al., 2003; Punkanen, et al., 2011) or the emotion context insensitivity (Bylsma, et al., 2008; Ellis, et al., 2009; Mah & Pollock, 2010; Rottenberg & Gross, 2007; Rottenberg, et al., 2005) represented two competing theories in this regard. No changes in accuracy of emotional processing of neutral and negative cues were observed. The fact that the patients were found to be slow in recognizing negative stimuli might be pointing at both theories. Their neural reaction to the stimuli was either
reduced (emotion context insensitivity theory) or their psychomotor retardation was greater as they were affected by the stimuli to a greater extent (negative potentiation). However, the neural results suggest that the second alternative may be more plausible; there is nevertheless a need for caution with this conclusion since the statistical significance for the observed difference was $p=0.066$. Finally, during the processing of negative stimuli, the patients experienced an increase of activation in the areas responsible for strong emotional arousal (Edelman & Tononi, 2003; Hamann, et al., 2004; Horvitz, 2000; Jensen, et al., 2003; Lenz, et al., 1995; O'Doherty, et al., 2004; Schultz, et al., 1997), which suggests that MDD is associated with an enhanced processing of negative stimuli (Canli et al., 2004).

Regarding impairments in attention shifting observed in patients with MDD, the patients in this study found it difficult to disengage their attention from emotional processing. This issue has been recently raised by Gotlib and Joormann (Gotlib & Joormann, 2010). Their study indicated that patients with MDD are unable to keep focus on a goal different from emotional processing (Joormann, et al., 2007c). This study confirmed these findings, as well as general theories on specific problems of attention control in patients with MDD (Ottowitz, et al., 2002).

The findings of the present study in respect to the neural changes observed in patients with MDD validated the models of the disease only partially. The models under discussion postulate that MDD is characterized by a lower activation in the cortical compartment and a heightened activation in the limbic compartment of the brain (Davidson, et al., 2002a; Drevets, 2001; Haldane & Frangou, 2006; Mayberg, 1997; Phillips, et al., 2003b). In the present study, the patients with MDD did experience an increase of activation in the limbic compartment of the CNS (during emotional processing of negative stimuli). They also displayed a decrease of activation in the
cortical compartment. Both the results support the predictions of the models. However, a drop of activation was also observed in the limbic compartment of the brain. The insula, a limbic region involved in the informative and motivational aspect of emotional experience (Goldin, et al., 2008; Northoff, et al., 2004; Wager & Feldman Barrett, 2004) displayed lesser activation in patients, in comparison to healthy controls. This finding thus does not support the predictions of the models. Instead, it suggests that the limbic compartment from the models of MDD is not as undiversified as the models propose. This notion is actually in accordance with the findings based on a meta-analysis conducted by Kober et al. (Kober, et al., 2008), which classified the striatum as a part of the core limbic group responsible for an integrated emotional arousal. This meta-analysis also placed the insula in the paralimbic group involved in informative and motivational aspect of emotional experience (Longstaff, 2005). It therefore seems possible appears that these two regions can be separated for patients with MDD.

The decrease observed in patients during emotional processing involved a wide range of structures. It occurred in a region previously discovered to be responsible for visual attention and processing, that is in the right precuneus/PCC (Goldin, et al., 2008). This region belonged to the visual attention group involved in reactivity to affective stimuli (Kober, et al., 2008). The decrease was also observed in the areas participating in emotional recognition and arousal, that is in the rolandic operculum and the insula (Goldin, et al., 2008; Kanske & Kotz, 2011; Kensinger & Schacter, 2006; Koelsch, et al., 2006; Northoff, et al., 2004; Wager & Feldman Barrett, 2004). The structures were classified within the paralimbic group responsible for the informative and motivational aspect of emotional experience (Kober, et al., 2008). Moreover, the decrease encompassed areas associating somatosensory information with lexical categories, that
is, the left angular gyrus and the left supramarginal gyrus (Anders, Lotze, Erb, Grodd, & Birbaumer, 2004; Binder et al., 1997).

All these regions have been shown to be involved in emotional processing and recognition (Kober, et al., 2008). Moreover, the function of these areas is connected with gathering and processing information about emotional state (Kober, et al., 2008). The decrease experienced by patients during emotional processing indicates a limited control that conceptual and cognitive processes, such as language or emotional categorization, have over valence recognition in patients with MDD. In healthy population, the language and its concepts can modulate how one’s emotional states are perceived (Anders, et al., 2004; Moseley, Carota, Hauk, Mohr, & Pulvermuller, 2011). Results from the present study suggest that patients with MDD may experience reduction in this control.

The regions in which the patients experienced a diminished neural activity during attention shifting were, to some extent similar to the ones just described, with one significant exception, however, – the areas that in healthy controls were involved in attentional shifts and executive control (Banks, et al., 2007; Beauregard, et al., 2001; Corbetta, 1998; Corbetta, et al., 1991; Coull, et al., 2000; Eippert, et al., 2007; Macaluso & Patria, 2007; Shapiro, et al., 2002; Urry, et al., 2006; Wager, et al., 2004). The right dorsolateral prefrontal cortex, the SMA and the left inferior parietal gyrus are important parts of the cortical compartment (Davidson, et al., 2002a; Drevets, 2001; Haldane & Frangou, 2006; Mayberg, 1997; Phillips, et al., 2003b). A diminished activation in these areas may explain why patients with MDD are less accurate and slower in shifting attention from emotional processing and why it is so difficult for them to stay focused on other goals than emotional processing (Joormann, et al., 2007c).
When the valence of the stimuli was considered separately, the patients experienced an increased activation of the limbic compartment during emotional processing of negative stimuli. It was observed in the regions responsible for strong emotional arousal (Edelman & Tononi, 2003; Hamann, et al., 2004; Horvitz, 2000; Jensen, et al., 2003; Kober, et al., 2008; Lenz, et al., 1995; O'Doherty, et al., 2004; Schultz, et al., 1997), that is the striatum, which form a part of the core limbic group (Kober, et al., 2008). The areas have been shown to integrate emotional inputs from the CNS and to transfer the integrated signal to the peripheral nervous system (Balleine, et al., 2007; Edelman & Tononi, 2003; Wichmann & DeLong, 2006). Their increased involvement implies that during processing of negative cues, patients' experience of emotions is more visceral. This can also explain why physical symptoms of MDD fit within the framework of emotional regulation (Beck & Alford, 2009). Physical symptoms may actually originate from exaggerated and inadequate vegetative reactions to punishing stimuli characteristic for the patients. This increase may be also associated with the emotional symptom of dysphoria (Beck & Alford, 2009).

Regarding the areas displaying an increase in the study, these participate in sustenance of emotional states (Aouizerate, et al., 2004; Cardinal, et al., 2002; Davidson & Irwin, 1999; Drevets, 2001). Such a pattern of activation can affect motivational system of a person. This can result in a diminished will to interact with the experimental stimuli (Gollan, et al., 2008; Punkanen, et al., 2011) or everyday situations (Bylsma, et al., 2011; Myin-Germeys, et al., 2003). This, in turn, may lead to such symptoms as loss of internal motivation or wishes to withdraw, a characteristic of patients with MDD (Beck & Alford, 2009).
3.6.1.2 General effect of family history of MDD

When considered separately from the diagnosis of the disease, family history of MDD in this study modulated neural, rather than behavioural, correlates of emotional regulation. Individuals with family history of MDD displayed more neural activation than individuals without such history during shifting from negative stimuli.

As for shifting attention from negative stimuli, the increase in this connection was observed in the multimodal associative cortex (Kandel, et al., 2000; Macaluso & Driver, 2001). This area is responsible for merging lingual, affective and somatic experiences of an individual (Goldin, et al., 2008; Kanske & Kotz, 2011). The region has also been shown to participate in processing of visual emotional stimuli when a person links their situation or their well-being to the observed stimuli (Craig, 2002; Hooker, et al., 2010; Northoff, et al., 2004; Saxe & Kanwisher, 2003). The fact that such an increase is observed in individuals with family history of MDD when they are to shift their attention from negative images points at certain difficulties; the individuals with family history of the disease may link their negative stimuli excessively to their situation, when it is required to shift attention to something else. This can evolve into a tendency to ruminate. This point can perhaps partially explain the higher levels of neuroticism observed in the group (Holma, et al., 2011; Lauer, et al., 1997; Lauer, et al., 1998).

3.6.1.3 Patients with MDD without family history of MDD (MDD-FHN) versus healthy controls without family history of MDD (HC-FHN)

The comparison between the patients without family history of MDD and healthy controls without family history of MDD indicates an acute episode of MDD without vulnerability caused by family history of the disease. It determines impairments which occur in emotional regulation during an MDD episode when the risk associated with family background is not present.
When one considers behavioural correlates of emotional regulation, the MDD-FHN of this study were particularly impaired in emotional processing of positive valence. The patients were less accurate than the healthy controls. These results point to positive attenuation (Berenbaum, et al., 2003; Bylsma, et al., 2008) in the MDD-FHN. This implies anhedonia (Keedwell, et al., 2005) and the fact that an acute episode of MDD without family history risk is likely to be characterized by a limited recognition of rewarding stimuli.

The findings on neural differences between the MDD-FHN and the HC-FHN partially supported the models of MDD (Davidson, et al., 2002a; Drevets, 2001; Haldane & Frangou, 2006; Mayberg, 1997; Phillips, et al., 2003b). During emotional processing, the MDD-FHN experienced an increase of activation in the core limbic group, which complies with the prediction of the models under discussion. Moreover, during attention shifting, the MDD-FHN displayed a decreased activation in the cortical compartment, which further supports the models.

However, the HC-FHN in comparison to the MDD-FHN also showed higher activation in the paralimbic group of regions during both emotional processing and attention shifting. This finding stands in opposition to the discussed theories. These results indicate that the limbic compartment postulated by the MDD models is not one entity. In the HC-FHN its the two components are activated in a different manner.

During emotional processing, the decrease of activation in the MDD-FHN was observed in the paralimbic and visual areas (Kober, et al., 2008). These structures are crucial for recognition of emotional valence and for motivation (Kober, et al., 2008). The decrease is noted in the right insula responsible for recognizing emotional states and for incorporating them into one’s motivation (Goldin, et al., 2008; Hutcherson, et al., 2005; Northoff, et al., 2004; Wager & Feldman Barrett, 2004). It is displayed in the
right cuneus/PCC – the visual attention group working as a relay area between the visual cortex and affective regions (Kober, et al., 2008). This region is actually involved in awareness of other’s and one’s own feelings (Bargh & Tota, 1988; Jackson, et al., 2006; Lamm, et al., 2007; Lou, et al., 2004; Maddock, et al., 2003; Moran, et al., 2006; Northoff, et al., 2004; Singer, et al., 2004; Tomlin, et al., 2006). Finally, the decrease was observed in the right rolandic operculum, a subcortical region found to be integrating external and internal cues into person’s motivation (Hutcherson, et al., 2005).

These differences indicate that, in comparison to the HC-FHN, the MDD-FHN may be less aware of their own emotional states and emotional states of others. This was also suggested in studies of empathy with MDD patients (Cusi, Macqueen, Spreng, & McKinnon, 2011; Singer, et al., 2004). Furthermore, the MDD-FHN may be less motivated by external emotional cues. The informative recognition of their own emotional response to such cues (Longstaff, 2005) may be disturbed and may not be incorporated into their motivation. This is in accordance with the emotion context insensitivity theory (Bylsma, et al., 2008; Ellis, et al., 2009; Mah & Pollock, 2010; Rottenberg & Gross, 2007; Rottenberg, et al., 2005). It is further argued that the areas mediating between visual and affective regions and filtering crucial affective information may not function fully in the MDD-FHN. This suggestion is in accordance with the theory of the neural visual filters which select most important affective stimuli and their malfunction in general population of patients MDD (Desseilles, et al., 2009; Fu, et al., 2007). In an episode of MDD without family history risk informative aspect of emotional processing may be disturbed, and as such not properly incorporated into a person’s motivation. This can lead to difficulties in catering to one’s needs observed in patients with MDD in connection with an increased dependency (Beck & Alford, 2009).
The neural decrease has been found in this study to be the strongest during emotional processing of negative stimuli. A lower activity in the MCC observed in the MDD-FHN was in previous studies connected to the decrease in focus on external cues in MDD (Frodl, et al., 2009; Grimm, et al., 2011; Grimm, et al., 2009). A lower activation in the right angular gyrus implies lower recognition of complex visual entities (Andersen et al., 2001; Binder et al., 2003). This together indicates that the MDD-FHN do not process the informative aspect of negative stimuli properly. They may also not be aware of the informative value of their own emotional reaction to it (Longstaff, 2005).

Moreover, during emotional processing, the MDD-FHN of this study have been found to experience an increase of activation in the core limbic group of the CNS regions. The overactivated area is known to participate in various stages of producing a strong emotional arousal (Kober, et al., 2008). The striatum is associated with the emotional integration and arousal transferring emotional signals to the peripheral nervous system (Kober, et al., 2008). This indicates that the visceral, rather than the informative, component dominates an emotional display in the MDD-FHN (Longstaff, 2005).

This pattern of activation was observed during processing of negative stimuli, in particular. The MDD-FHN seemed to be processing the external negative stimuli more viscerally and less cognitively. Given the fact that most of the drastic pictures in the IAPS were discarded from the experiment, the strength of the suggested tendency is noteworthy. This point is in accordance with the negative potentiation theory (Bylsma, et al., 2011; Gollan, et al., 2008; Myin-Germeys, et al., 2003; Punkanen, et al., 2011).

Moreover, the observed involvement of memory areas indicates that the MDD-FHN may be more influenced by negative mnemonic associations. This has also been noted in a general group of patients with MDD (Hamilton & Gotlib, 2008). However, this
study is unique in suggesting such a tendency solely in an MDD episode without the family history vulnerability.

During attention shifting, the patients with MDD without family history vulnerability displayed a diminished neural activation. The clusters affected combine regions involved in executive control, such as the right supramarginal gyrus (Booth, et al., 2003; Collette, et al., 2006), and in emotional recognition, that is the right insula (Goldin, et al., 2008; Hutcherson, et al., 2005; Northoff, et al., 2004; Wager & Feldman Barrett, 2004).

The drop of activation in the regions responsible for emotional recognition to some extent resembles the neural patterns in emotional processing. Reductions of activation in the right supramarginal gyrus have in the past been reported in elderly patients with MDD, during focusing on a visual target (Wang et al., 2008a). The findings indicate that attention control may not be as effective in the MDD-FHN as it is in the HC-FHN. The lack of behavioural impairments in attention shifting in the group may signify that the tendency is not particularly strong. However, since emotional signals in the MDD-FHN display a limited informative aspect, they may not pose a strong competition for other material. This notion is implied by a diminished activation in the areas of emotional recognition observed in the MDD-FHN during attention shifting. The HC-FHN who, in contrast to the MDD-FHN, perceived emotional signals as more informative may be actually afflicted by competing of signals. Nevertheless, due to involvement of executive control, the HC-FHN seem to be able to perform other tasks when situation requires it and attention shifting is a necessity.

To conclude, the changes in emotional processing and attention shifting observed in this study suggest that, relative to the HC-FHN, the MDD-FHN are less able to focus on informational aspect. This may refer to external emotional cues as well as their internal
reaction to them. The visceral, rather than informative, aspect of emotional experience is stronger in these patients. This suggests that the MDD-FHN may react strongly to emotional information, yet be unaware of its importance. This point appears to indicate a discord between the core limbic and paralimbic groups of regions in the MDD-FHN. Further, it indicates that the MDD-FHN are not easily motivated by external emotional cues. Memory may play a role in their processing of emotional valence, whereas details of external situation causing the visceral emotional reaction may be unobserved. These tendencies have been noted in processing of negative cues, in particular. In terms of emotional regulation, this point may imply an exaggerated and non-proportional reaction to negative situations and difficulties in coping with them. It further indicates that both the negative potentiation theory and the emotion context insensitivity theory may be true. Last but not least, the MDD-FHN have stronger visceral reaction to negative stimuli, and their understanding of its informative, contextual aspect is limited.

3.6.1.4 Patients with MDD with family history of MDD (MDD-FHP) versus healthy controls with family history of MDD (HC-FHP)

In this study, the healthy controls with family history of MDD were recruited from among the first degree unaffected healthy relatives of patients with MDD. It is important to note that this population shares 50% of genotype with individuals suffering from the disease (Glowinski, Madden, Bucholz, Lynskey, & Heath, 2003; Sullivan, et al., 2000). In contrast to the MDD-FHP, the HC-FHP represent resilience to vulnerability to the disease. This is especially important in respect to prevention of MDD, since the disease features among the 4th most severe health burdens in the world at the moment (WHO, 2001). This level of comparison is also important in respect to determining how MDD is developed in people with family history vulnerability. An examination of such a contrast, for correlates of emotional processing and attention
shifting, promises to explicate the role of emotional regulation in these mechanisms further.

When one considers behavioural correlates of emotional regulation, the MDD-FHP – similarly to the MDD-FHN – are less prone to correctly recognize positive stimuli. This is in accordance with the predictions of the positive attenuation theory (Berenbaum, et al., 2003; Bylsma, et al., 2008). However, the MDD-FHP in this study experienced additional impairments in terms of shifting attention from emotional stimuli, regardless of its emotional valence. This finding, instead, is in accordance with previous studies of the general population of patients with MDD (Gotlib & Joormann, 2010). In addition, the results indicate that these difficulties are more characteristic for a particular endophenotype – the MDD-FHP, suggesting that patients with MDD with family history of the disease have difficulties focusing away from emotional processing. In terms of emotional regulation, this points at problems with planning and acting on the informative value of emotions. This finding goes hand in hand with the motivational symptoms observed in MDD – mainly losses in motivation and inability to plan (Beck & Alford, 2009).

The neural differences between the MDD-FHP and the HC-FHP participants in this study confirmed the predictions of the models of MDD, when it comes to shifting attention from negative stimuli (Davidson, et al., 2002a; Drevets, 2001; Haldane & Frangou, 2006; Mayberg, 1997; Phillips, et al., 2003b). While shifting attention from negative and neutral cues, the MDD-FHP experienced a decreased activation in the cortical compartment. During shifting attention from negative stimuli, the MDD-FHP displayed an increased activation in the limbic areas. Interestingly, the difference was most apparent during shifting attention from negative information, rather than during emotional processing of such cues. Shifting attention from negative stimuli has actually
been found to be a difficult process for patients with MDD (Gotlib & Joormann, 2010; Taylor Tavares, et al., 2007). The MDD-FHP are thus likely to experience great difficulties in performing such a task.

During emotional processing, the HC-FHP, in comparison to the MDD-FHP, displayed a higher activation in the visual attention group (Kober, et al., 2008). In healthy controls, visual cortex and cuneus are known to react with a higher activation to emotional stimuli (Freese & Amaral, 2005; Hendler, et al., 2001; Kober, et al., 2008; Vuilleumier, et al., 2004). As emotional cues are important for the well-being of a person, more information must be gathered about them (Desseilles, et al., 2009; Fu, et al., 2007). This is not the case for MDD-FHP, however, for whom the reaction of visual areas to affective cues is blunted. This pattern of activation is most apparent in the processing of positive stimuli and can be explained by the visual filters theory (Desseilles, et al., 2009; Fu, et al., 2007). The MDD-FHP may not be able to distinguish positive cues as important in their emotional homeostasis. This may indicate that the patients with MDD with family history vulnerability have a limited ability to notice rewarding stimuli. It also indicates that resilience to vulnerability of developing the disease is characteristic of the HC-FHP emotional responsiveness to rewarding cues.

Furthermore, during emotional processing, the HC-FHP, in contrast to the MDD-FHP, displayed a higher activation in the left supramarginal gyrus. This structure is involved in language operations, associating somatosensory information with lexical knowledge (Anders, et al., 2004; Binder, et al., 1997). Such an activation has been observed in previous studies in that language was found to be involved in emotional processing of individuals (Anders, et al., 2004; Moseley, et al., 2011). This may be the case with the HC-FHP, suggesting an additional mechanism used for understanding and modulating of one’s own emotional reactions.
During attention shifting, the MDD-FHP, in comparison to the HC-FHP, displayed a decrease of activation in the visual cortex. This finding may indicate that even during attention shifting the HC-FHP have a higher reaction to emotional stimuli. However, in this case, the area of deactivation stretched to the left inferior parietal gyrus, which participates in particularly demanding attention shifting and in attention re-orienting (Corbetta, 1998; Corbetta, et al., 1991; Coull, et al., 2000; Macaluso & Patria, 2007; Shapiro, et al., 2002; Wager, et al., 2004). This points at an increased involvement of attention control in the task in the case of the HC-FHP. As postulated in the models under scrutiny, the left IPG forms a part of the cortical compartment (Davidson, et al., 2002a; Drevets, 2001; Haldane & Frangou, 2006; Mayberg, 1997; Phillips, et al., 2003b). It has been shown to have a decreased neural activation in general population of patients with MDD (Davidson, et al., 2002a; Mayberg, 1997, 2003; Mayberg, et al., 1999; Phillips, et al., 2003b; Taylor & Liberzon, 2007). Here, the lower activation in the region during attention shifting was found to be particularly characteristic of the MDD-FHP, which may explain behavioural impairments observed in the MDD-FHP. In terms of emotional regulation, this point may be related to the observed inability to step away from emotional stimuli in order to plan how to interact with the demands of a situation.

The neural differences between the HC-FHP and the MDD-FHP were mainly observed in respect to shifting attention from neutral and negative cues. With both types of stimuli, the MDD-FHP displayed a decrease of activation, particularly in the areas responsible for attention shifting and conflict resolution. As to neutral cues, it was the case for the left inferior parietal gyrus, whereas for negative information, the decrease in the MDD-FHP was mainly observed in the ACC/MCC. The posterior ACC (stretching towards the MCC) is responsible for arbitrating between competing signals, and allows focusing on a chosen stimuli (Bush, et al., 2000; Etkin, et al., 2006). It is the
main area responsible for attention modulation in emotional signalling. Its decreased activation indicates that the control system of the MDD-FHP does not respond in an appropriate way to an emotional conflict, which further suggests that shifting attention from negative cues may be more difficult for the MDD-FHP. This finding is in accordance with the observations made in the general population of patients with MDD (Christensen, et al., 2006; Goeleven, et al., 2006; Gotlib & Joormann, 2010; Joormann, et al., 2007c; Langenecker, et al., 2005; Lau, et al., 2007; Murphy, et al., 1999). In this study it was shown, however, that difficulties in shifting attention from negative stimuli are especially true for the MDD-FHP.

There are certain indications in the findings of this study that these difficulties may be even more enhanced. In comparison to the HC-FHP, the MDD-FHP displayed a higher activation in the core limbic group (Kober, et al., 2008) when shifting attention from negative cues. This activation is associated with an increased visceral aspect of emotional experience (Fu, et al., 2004; Kober, et al., 2008). The change may also be associated with greater difficulties in encoding of information in the presence of negative cues (Kandel, et al., 2000). The observed overactivation of the core limbic group may stem from a lowered ability of the ACC/MCC to control this cluster of regions. This may lead to an inability to focus on anything else when negative information is available. Motivational symptoms of MDD, such as indecisiveness and increased dependency (Beck & Alford, 2009), may also be explained in this connection.

In summary, the MDD-FHP are characterised by a lower ability to recognize positive stimuli. They are also distinguished by difficulties in shifting attention from emotional processing, particularly in the case of negative cues. The latter is due to a lower activation of their attention controlling areas as well as a greater involvement of their core limbic group in attention shifting. This may lead to difficulties in recognizing
rewarding stimuli and focusing away from emotional processing when other cues require attention. In contrast, the resilience to MDD vulnerability observed in the HC-FHP is characterized by a strong neural response to emotional stimuli, particularly to rewarding cues. This is nevertheless associated with a greater control of emotional processing with attentional and language operations as well.

3.6.1.5 Healthy controls without family history of MDD (HC-FHN) versus healthy controls with family history of MDD (HC-FHP)

In comparison to the MDD-FHP, the HC-FHP displayed resilience to vulnerability to MDD and an ability to cope with an increased risk of developing the disease. However, when the HC-FHP were contrasted with the HC-FHN, their vulnerability was more apparent. Previous research has indicated that due to this vulnerability, the HC-FHP develop the disease more frequently than the HC-FHN (Fanous, et al., 2002; Weissman, et al., 2006), experience cognitive impairments (Christensen, et al., 2006), and higher levels of neuroticism (Holma, et al., 2011). All together, these impairments may influence emotional regulation. This study was designed to test whether changes in emotional regulation are caused by this type of vulnerability.

The two groups of healthy controls in this study were not found to differ in behavioural correlates of emotional regulation. However, the HC-FHP displayed a higher neural activation than the HC-FHN in numerous CNS structures during both emotional processing and attention shifting.

During emotional processing, the increase of activation characteristic for the HC-FHP was observed in the left postcentral gyrus. This region participates in associating emotional and somatosensory sensations (Kanske & Kotz, 2011; Kensinger & Schacter, 2006; Koelsch, et al., 2006). It is activated when an individual is highly affected by visual emotional stimuli (Craig, 2002; Hooker, et al., 2010). The family history
vulnerability to MDD is characterized by its increased involvement in making decisions about emotional valence.

Furthermore, an increase was observed in the HC-FHP during emotional processing in an area commonly associated with executive control that is the left parietal gyrus (Corbetta, 1998; Corbetta, et al., 1991; Coull, et al., 2000; Shapiro, et al., 2002; Wager, et al., 2004). The increase was also noted in a region involved in language operations, the left supramarginal gyrus (Anders, et al., 2004; Binder, et al., 1997; Moseley, et al., 2011). Both types of regions participate in managing emotional arousal. They do so either through focusing attention on and off the affective cues or through naming emotional states and thus organizing them (Lane, Fink, Chau, & Dolan, 1997; Ochsner & Gross, 2005; Thompson, 1994).

The differences between the HC-FHP and the HC-FHN in activation during emotional processing were most notable in the case of negative stimuli. The changes, in fact, followed the pattern observed in general emotional processing. The HC-FHP showed an increased activation in the areas responsible for affective-somatosensory arousal (Freese & Amaral, 2005; Hendler, et al., 2001; Kanske & Kotz, 2011; Kober, et al., 2008; Maddock & Buonocore, 1997; Maddock, et al., 2003; Vuilleumier, et al., 2004) and for attention control (Anders, et al., 2004; Binder, et al., 1997; Corbetta, 1998; Corbetta, et al., 1991; Coull, et al., 2000; Moseley, et al., 2011; Shapiro, et al., 2002; Wager, et al., 2004).

In the present study, the HC-FHP were distinguished by an increase of activation in the executive regions and in the emotional areas. This may indicate that while the HC-FHP reacted more strongly to emotional stimuli than the HC-FHN, they also applied more cortical control to it. This could explain why there were no behavioural differences between the two groups. In fact, dissimilarities in the neural activation with no
behavioural differences have previously been reported between the HC-FHP and the HC-FHN (Wolfensberger, et al., 2008), which suggests that vulnerability may appear without any observable behavioural symptoms. This also points at the presence of a mechanism of compensation. The differences reported on in this study were most notable during the processing of negative stimuli. This again indicates that family history vulnerability to MDD is associated with an increased emotional response to negative stimuli. It is especially notable in the areas of the CNS responsible for evoking somatosensory sensations. This point can be linked to reported observations of the HC-FHP being quicker to react to fearful faces (Le Masurier, et al., 2007) and having more negative cognitions (Giles, et al., 1990; Lauer, et al., 1997) than the HC-FHN. This increase is nevertheless managed by a heightened activation in attention control areas. During attention shifting, the HC-FHP, in comparison to the HC-FHN, displayed a greater activation in the region responsible for executive control and attention shifts. Like in previous studies, it was observed in the left inferior parietal gyrus (Aron, et al., 2003; Aron, et al., 2004b; Badre, et al., 2005; Chong, et al., 2008; Coull, et al., 2000; Dove, et al., 2000; Gabrieli, et al., 1998; Hooker, et al., 2010; Macaluso & Patria, 2007; Martin & Chao, 2001; Moss, et al., 2005; Ochsner & Gross, 2005; Poldrack, et al., 1999; Ridderinkhof, et al., 2004; Swick, et al., 2008; Wager, et al., 2005; Wagner, et al., 2001; Zhang, et al., 2004). The increase was also noted in the left supramarginal gyrus involved in language processing and emotional modulation through language (Anders, et al., 2004; Moseley, et al., 2011). In the HC-FHP, this region was even more involved in attention shifting than in the healthy controls without family history of MDD. The mean behavioural scores showed that the HC-FHP were indeed scoring highest out of the four participating groups in terms of shifting attention from emotional stimuli. However, the differences between the two groups of healthy controls did not reach a
statistical significance, which leads to an interesting question on the reason for such an increase in use of areas responsible for executive control.

The answer to the question may lie in the neural activation of the areas responsible for visual attention (Freese & Amaral, 2005; Hendler, et al., 2001; Kober, et al., 2008; Vuilleumier, et al., 2004). During attention shifting, the HC-FHP experienced more activation in these regions than the HC-FHN did. This suggests that there is an increased emotional reaction on the part of these subjects, even in situations when their attention is being shifted away. Nevertheless, a greater involvement of the executive areas may control the increase being a compensation mechanism characteristic for the HC-FHP.

The differences between the groups under discussion were observed in shifting attention from all types of the examined valence. While shifting attention from negative stimuli, the HC-FHP displayed a stronger activation in emotional-somatosensory processing regions and in executive areas filtering emotional information. As to neutral stimuli, the increase was observed in the attention shifting region. Finally, with positive stimuli the differences were noted in the areas reacting to important emotional stimuli.

All this suggests that during attention shifting the HC-FHP, on the one hand, react strongly to pictures that are either very low or very high on the valence continuum. On the other hand, their activation in attention control areas is stronger for non-rewarding stimuli. This implies that their compensation mechanism is strongest in shifting attention from negative stimuli.

The HC-FHP are thus characterized by an increased neutral activation in emotional arousal regions and in cognitive control areas during emotional processing and attention shifting. This points at a general mechanism of compensation characteristic for an emotional regulation associated with vulnerability to MDD. This compensation results
in unchanged behavioural correlates of emotional regulation in the HC-FHP. The pattern of activation has been found the most notable for negative stimuli.

It may be implied from the foregoing that emotional regulation is likely to be more difficult for the first-degree unaffected healthy relatives of patients with MDD. Although behavioural changes may not be notable, greater attention resources are necessary to deliver the performance. One can speculate that under adverse circumstances, the system would reach its exhaustion level quicker in HC-FHP than in controls. Thus, HC-FHP may be prone to “crumbling” under stress quicker and more frequently than people without any family history of the disease (Fanous, et al., 2002; Weissman, et al., 2006).

3.6.1.6 Patients with MDD without family history of MDD (MDD-FHN) versus patients with MDD with family history of MDD (MDD-FHP)

Finally, the MDD-FHN and the MDD-FHP did not differ in behavioural and neural correlates of emotional regulation. This suggests that the differences between the two groups may be more subtle. They may be explained by the differences in inhibition of emotional material, which are to be presented in the next section.

3.6.2 Different types of medication

Patients with MDD treated with different types of medication as well as untreated individuals with MDD did not differ in emotion processing and attention shifting. Since there was no difference found between patients treated with different types of antidepressants in terms of neural activation, it was concluded that in the applied task, treatment was a factor of no interest. It did not influence either the interplay between the diagnosis and family history of MDD or its role in emotional regulation. The neural correlates of emotion processing and attention shifting evoked by the task of this study were localized in the areas which are commonly not connected with a response to
antidepressant treatment in MDD (Aihara, et al., 2007; Fu, et al., 2007; Lisiecka, et al., 2011; Narasimhan, et al., 2011). It is possible though that some patients may not have yet responded to their treatment at the time of the study, due to a great variability in the length of treatment reported.
4  Family history of MDD in inhibition of emotional processing in patients with MDD and healthy controls (study 1 chapter 2)

4.1  Introduction

The previous chapter (3) presented differences in emotional processing and attention shifting between groups distinguished by diagnosis of major depressive disorder and family history of MDD. This chapter complements the conclusions drawn in the previous section by presenting differences between the groups in inhibition of emotional processing.

Inhibition of emotional processing is a key skill required in shifting attention from emotional content (Grossberg & Schmajuk, 1987; Rinehart, Bradshaw, Moss, Brereton, & Tonge, 2001) and therefore, can be considered a part of emotional regulation mechanism. To transfer attention to a different task while processing emotional stimuli a person needs first to inhibit the emotional processing (Grossberg & Schmajuk, 1987; Rinehart, Bradshaw, Moss, Brereton, & Tonge, 2001).

The disturbances in inhibiting of emotional processing have been noted in various psychiatric disorders in general (Krause-Utz et al., 2012; Rinehart, et al., 2001; Verona, Sprague, & Sadeh, 2012), and in the mood disorders in particular (Ladouceur et al., 2005; Murphy, et al., 1999; Siegle, Steinhauser, Thase, Stenger, & Carter, 2002). Patients with MDD have been observed to have significantly lower ability to inhibit emotional processing especially of negative stimuli (Ladouceur, et al., 2005; Murphy, et al., 1999; Siegle, et al., 2002). This diminished ability is attributed to a sustained activation of the amygdalar region during the inhibition phase (Siegle, et al., 2002). Understanding this process in relation to family history of MDD would help establishing further correlates of emotional regulation in vulnerability and resilience to the disease.
4.2 Aims and hypotheses

The aim of the study is to examine to what extent family history of MDD influences behavioural and neural correlates of inhibition of emotional processing. It is also to test what differences there are in the behavioural and neural correlates of this process between patients with MDD and healthy controls when family history of the disease is present and when it is not. It is also to examine the changes in the process associated with vulnerability and resilience to the vulnerability to the disease. Similarly to the first part of the study participants were divided into 4 groups. The four groups were compared among each other to test the hypotheses 1 and 2 presented in the chapter 1.7.

4.2.1 Healthy controls vs patients with MDD (H1)

Similarly to the first part of the study it was important to establish what changes in inhibition of emotional processing were caused by MDD in general. It was hypothesized that during the process patients with MDD may experience a higher activation in the limbic compartment of the CNS (especially amygdala), when compared to healthy controls (Siegle, et al., 2002). Furthermore, it was assumed that patients with MDD were slower and less accurate than healthy controls in inhibiting emotional processing. It was hypothesized that the effect may be observable only for negative stimuli (Siegle, et al., 2002).

4.2.2 Subjects with family history of MDD vs subjects without family history of MDD (H2A)

Secondly, this part of the study sought to test whether subjects with and without family history of MDD differed in neural and behavioural correlates of inhibition of emotional processing regardless of their diagnosis. It was hypothesized that the neural difference between individuals with and without family history of MDD occurred in the areas
associated with inhibitory control and gating in the brain (Desseilles, et al., 2009; Fu, et al., 2007; Tekin & Cummings, 2002). It was also anticipated that subjects with family history of MDD are slower and less accurate in inhibition of emotional processing. It was hypothesized that the effect may be observable only for negative stimuli (Siegle, et al., 2002).

4.2.3 Healthy controls without family history of MDD vs patients with MDD without family history of MDD (H2B)

This comparison tested how inhibition of emotional processing is changed by an acute depressive episode, when the vulnerability caused by family history of MDD is absent. It was hypothesized that the MDD-FHN, compared to the HC-FHN have more activation in the limbic regions of the CNS (especially amygdala) during the examined process (Siegle, et al., 2002). It was also anticipated that the MDD-FHN may be slower and less accurate than the HC-FHN. It was hypothesized that the effect may be observable only for negative stimuli (Siegle, et al., 2002).

4.2.4 Healthy controls with family history of MDD vs patients with MDD with family history of MDD (H2C)

When the HC-FHP were compared to the MDD-FHP, both resilience to the risk of developing MDD and an acute MDD episode with vulnerability were observed. It was hypothesized that, in contrast to the MDD-FHP, the HC-FHP experience less activation in the limbic areas of the CNS (especially amygdala) during the inhibition (Siegle, et al., 2002). It was further assumed that patients may have less activation in other areas identified as those associated with inhibitory control and gating in the brain (Desseilles, et al., 2009; Fu, et al., 2007; Tekin & Cummings, 2002). It was also assumed that the MDD-FHP are slower and less accurate than the HC-FHP. It was hypothesized that the effect may be observable only for negative stimuli (Siegle, et al., 2002).
4.2.5 Healthy controls with family history of MDD vs healthy controls without family history of MDD (H2D)

The changes in correlates of inhibition of emotional processing in the HC-FHP, compared to the HC-FHN, show an elevated vulnerability to MDD without acute depressive symptoms. It was hypothesized that the HC-FHP display neural alterations in the areas associated with inhibitory control and gating in healthy controls (Desseilles, et al., 2009; Fu, et al., 2007; Tekin & Cummings, 2002). The HC-FHP could also experience an increase of activation in the limbic lobe (especially amygdala) (Siegle, et al., 2002). Behaviourally, it was anticipated that the HC-FHP may be less accurate in inhibition of emotional processing. It was hypothesized that the effect may be observable only for negative stimuli (Siegle, et al., 2002).

4.2.6 Patients with MDD with family history of MDD vs patients with MDD without family history of MDD (H2E)

Finally, the study aimed to test to what extent the neural characteristics of emotional processing and attention shifting change in familial vulnerability to MDD associated with an acute depressive episode of the disease. It was assumed that the MDD-FHP, as carriers of both the vulnerability and the acute symptoms, are less efficient in behavioural performance during inhibition of emotional processing. Their neural activity may be increased in the limbic compartment (especially amygdala) (Siegle, et al., 2002). It was hypothesized that the effect may be observable only for negative stimuli (Siegle, et al., 2002).

4.3 Groups tested

Groups tested are presented in the chapter 3.3.
4.4 Methods

4.4.1 Design

Design of the study was identical with the one presented in the chapter 3.2.1 with the exception that the targeted processes was inhibition of emotional processing. In the study by Siegel et al. the inhibition of emotional processing was conceptualized as a difference between attention shifting and emotion processing (Siegle, et al., 2002). In this study it is conceptualized similarly. It was reasoned that during trials with emotion processing individuals keep processed emotional content of the picture until the end of a trial. On the other hand, in trials with attention shifting individuals needed to inhibit their processing of emotional content of the picture to consider the shape of the picture.

4.4.2 Behavioural data analysis

All the calculations were performed in SPSS 19.0. The threshold for the significance of all differences was set at \( p<0.05 \). Accuracy in inhibition of emotional processing was calculated as a difference between accuracy in attention shifting and accuracy in emotional processing separately for each subject. Similarly, reaction times in inhibition were evaluated by deducting an average reaction time in emotional processing from an average reaction time in attention shifting for each participant separately.

Patients with MDD and healthy controls were compared in accuracy and reaction times in inhibition of emotional processing, together and separately for all the valence categories. A similar comparison was performed for subjects with and without family history of MDD. Subsequently, a 4-group MANOVA and a post-hoc analysis were calculated to determine whether the four groups differed in reference to RTs and accuracy measures – first for inhibition of emotional stimuli in general, and then separately in each valence category. The results are presented in results sub-section.
4.4.3 Image data second level analysis

In accordance with previous studies (Prata, et al., 2012; Rehme, et al., 2011; Sanchez-Carrion, et al., 2008) a 2x2x3 factorial analysis was performed on the contrasts between non-emotional and emotional trials, where the first factor was a diagnosis (patients with MDD versus healthy controls), the second factor was a family history of MDD (subjects with family history of MDD versus subjects without family history of MDD) and the third factor was emotional valence of inhibited material (neutral versus negative versus positive). Participants' age and gender were added as covariates of no interest.

The differences between patients and healthy controls, as well as between individuals with family history of MDD and without family history of MDD were established. An interaction between the diagnosis, family history of MDD and emotional valence was calculated and a post-hoc analysis performed to determine the differences between the following pairs of groups: HC-FHP and HC-FHN, HC-FHP and MDD-FHP, HC-FHN and MDD-FHN, and MDD-FHP and MDD-FHN.

The regions detected in the contrasts in terms of the inhibition of emotional processing, (surviving the whole brain FWE voxel correction with the p<0.05) are presented in results sub-section. The automated anatomical labeling atlas was used to localize the significant areas in a standard stereotactic space (template from the MNI).

4.5 Results

4.5.1 Behavioural results

4.5.1.1 General effect of diagnosis (H1)

In general terms, healthy controls were quicker in inhibiting emotional processing than patients with MDD. The two groups did not differ when the general accuracy in inhibition was taken into account (Table 17), which also displays costs of inhibition. In
case of RTs, it shows seconds lost to inhibiting of emotional processing (non-emotional trials – emotional trials), that is additional time needed to disengage one’s attention from affective material. As for accuracy, it shows the number of trials that were incorrect because there was a necessity to inhibit emotional stimuli (emotional trials – non-emotional trials). When the valence categories were considered separately, healthy controls and patients with MDD differed in accuracy of inhibition of neutral, negative and positive stimuli. The two groups also varied in the speed of inhibiting negative and positive information. Healthy controls, as compared to patients with MDD, were more accurate in inhibiting negative stimuli processing and were quicker in inhibiting negative and positive information. Patients with MDD were more accurate in inhibiting positive stimuli. In fact, the results suggest that the patients did not need to inhibit processing of positive information to shift their attention to different objectives. Finally, patients with MDD displayed a smaller difference between accuracy in neutral emotional processing trials and accuracy in neutral attention shifting trials than healthy controls did. Table 17 presents means, SDs and significance levels for all the contrasts.

Table 17. Differences in accuracy and reaction times between patients with MDD and healthy controls in costs of inhibiting of emotional processing

<table>
<thead>
<tr>
<th>Behavioural characteristics – inhibition of emotional processing (±SD)</th>
<th>Patients with MDD (N=50)</th>
<th>Healthy controls (N=46)</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accuracy – general (% difference)</td>
<td>-2.8 (±13.3)</td>
<td>-5.8 (±7.8)</td>
<td>0.178</td>
</tr>
<tr>
<td>Accuracy – negative stimuli (% difference)</td>
<td>7.8 (±16.2)</td>
<td>1.7 (±12.1)</td>
<td>0.044*</td>
</tr>
<tr>
<td>Accuracy – positive stimuli (% difference)</td>
<td>-5.1 (±16)</td>
<td>1.2 (±10.3)</td>
<td>0.024*</td>
</tr>
<tr>
<td>Accuracy – neutral stimuli (% difference)</td>
<td>-10.9 (±20.2)</td>
<td>-20.4 (±12.8)</td>
<td>0.008*</td>
</tr>
<tr>
<td>RTs – general (s difference)</td>
<td>0.14 (±0.35)</td>
<td>-0.02 (±0.19)</td>
<td>0.005*</td>
</tr>
<tr>
<td>RTs – negative stimuli (s difference)</td>
<td>0.22 (±0.38)</td>
<td>0.04 (±0.19)</td>
<td>0.005*</td>
</tr>
<tr>
<td>RTs – positive stimuli (s difference)</td>
<td>0.27 (±0.39)</td>
<td>0.11 (±0.21)</td>
<td>0.016*</td>
</tr>
<tr>
<td>RTs – neutral stimuli (s difference)</td>
<td>-0.06 (±0.4)</td>
<td>-0.23 (±0.27)</td>
<td>0.023*</td>
</tr>
</tbody>
</table>

Statistical significance set to p<0.05; *Statistically significant difference
4.5.1.2 General effect of family history of MDD (H2A)

Subjects without family history of MDD were quicker than subjects with family history in inhibiting emotional processing in general. The two groups did not differ in accuracy of general inhibiting (Table 18).

When the valence categories were taken into account separately, subjects without family history of MDD were quicker in inhibiting negative and positive stimuli. The two groups did not differ in respect to the accuracy of inhibiting any type of information (Table 18).

Table 18. Differences in accuracy and reaction times between individuals with family history of MDD (FHP) and individuals without family history of MDD (FHN) in costs of inhibition of emotion processing

<table>
<thead>
<tr>
<th>Behavioural characteristics – inhibition of emotional processing (±SD)</th>
<th>FHP (N=51)</th>
<th>FHN (N=45)</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accuracy – general (% difference)</td>
<td>-3.4 (9.6)</td>
<td>-5.3 (12.5)</td>
<td>0.420</td>
</tr>
<tr>
<td>Accuracy – negative stimuli (% difference)</td>
<td>5.6 (12.8)</td>
<td>3.9 (16.5)</td>
<td>0.566</td>
</tr>
<tr>
<td>Accuracy – positive stimuli (% difference)</td>
<td>-2.1 (12.3)</td>
<td>-1.9 (15.6)</td>
<td>0.958</td>
</tr>
<tr>
<td>Accuracy – neutral stimuli (% difference)</td>
<td>-13.7 (19.9)</td>
<td>-17.7 (14.3)</td>
<td>0.280</td>
</tr>
<tr>
<td>RTs – general (s difference)</td>
<td>0.13 (0.35)</td>
<td>-0.01 (0.2)</td>
<td>0.023*</td>
</tr>
<tr>
<td>RTs – negative stimuli (s difference)</td>
<td>0.2 (0.36)</td>
<td>0.06 (0.23)</td>
<td>0.019*</td>
</tr>
<tr>
<td>RTs – positive stimuli (s difference)</td>
<td>0.25 (0.37)</td>
<td>0.12 (0.24)</td>
<td>0.042*</td>
</tr>
<tr>
<td>RTs – neutral stimuli (s difference)</td>
<td>-0.08 (0.41)</td>
<td>-0.22 (0.25)</td>
<td>0.061</td>
</tr>
</tbody>
</table>

Statistical significance set to p<0.05; *Statistically significant difference

4.5.1.3 Four-group analysis and post-hoc tests

In MANOVA tests, the four groups differed significantly, in general terms, in the speed of inhibition of emotional processing. They did not differ in general accuracy of inhibiting (Table 19).

When the valence categories were taken separately into account, the groups varied in reaction times in inhibiting positive, negative and neutral stimuli. As for accuracy, the groups differed in inhibiting neutral information. Differences in accuracy of inhibiting
negative information approached statistical significance with p=0.052 (Table 19). Subsequently, post-hoc analysis was performed for all the contrast (Table 20).

4.5.1.3.1 Patients with MDD without family history of MDD vs healthy controls without family history of MDD (H2B)

There were no significant differences between the MDD-FHN and the HC-FHN in behavioural correlates of inhibition of emotional processing (Table 20).

4.5.1.3.2 Patients with MDD with family history of MDD vs healthy controls with family history of MDD (H2C)

The HC-FHP were quicker in general inhibition and more accurate and quicker than the MDD-FHP in inhibiting negative information processing. The results are presented in Table 20.

4.5.1.3.3 Healthy controls with family history of MDD vs healthy controls without family history of MDD (H2D)

There were no significant differences between the MDD-FHN and the HC-FHN in behavioural correlates of inhibition of emotional processing (Table 20).

4.5.1.3.4 Patients with MDD with family history of MDD vs patients with MDD without family history of MDD (H2E)

The difference between the MDD-FHP and the MDD-FHN was observed in the speed of inhibition of negative stimuli, and approached the significance levels at p=0.08. The MDD-FHN were quicker than the MDD-FHP. The results are presented in Table 20.
Table 19. Differences in accuracy and reaction times in costs of inhibition of emotion processing between groups distinguished by diagnosis of MDD and family history of the disease (F MANOVA)

<table>
<thead>
<tr>
<th>Behavioural characteristics – inhibition of emotional processing (±SD)</th>
<th>MDD-FHP (N=30)</th>
<th>MDD-FHN (N=20)</th>
<th>HC-FHP (N=21)</th>
<th>HC-FHN (N=25)</th>
<th>p values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accuracy – general (% difference)</td>
<td>-0.8 (11.1)</td>
<td>-6 (16.2)</td>
<td>-7.1 (5.4)</td>
<td>-4.7 (9.3)</td>
<td>0.186</td>
</tr>
<tr>
<td>Accuracy – negative stimuli (% difference)</td>
<td>10.2 (11.6)</td>
<td>3.7 (21.6)</td>
<td>-1 (11.8)</td>
<td>4 (12.1)</td>
<td>0.052</td>
</tr>
<tr>
<td>Accuracy – positive stimuli (% difference)</td>
<td>-4.1 (14.3)</td>
<td>-6.9 (18.9)</td>
<td>0.8 (8.2)</td>
<td>1.6 (11.9)</td>
<td>0.140</td>
</tr>
<tr>
<td>Accuracy – neutral stimuli (% difference)</td>
<td>-8.6 (22)</td>
<td>-14.8 (16.8)</td>
<td>-21.1 (13.7)</td>
<td>-19.7 (12.2)</td>
<td>0.037^a</td>
</tr>
<tr>
<td>RTs – general (s difference)</td>
<td>0.21 (0.4)</td>
<td>0.03 (0.22)</td>
<td>&lt;0.00 (0.22)</td>
<td>-0.04 (0.17)</td>
<td>0.006^a</td>
</tr>
<tr>
<td>RTs – negative stimuli (s difference)</td>
<td>0.3 (0.41)</td>
<td>0.09 (0.28)</td>
<td>0.06 (0.21)</td>
<td>0.03 (0.18)</td>
<td>0.003^a</td>
</tr>
<tr>
<td>RTs – positive stimuli (s difference)</td>
<td>0.34 (0.43)</td>
<td>0.16 (0.29)</td>
<td>0.14 (0.22)</td>
<td>0.09 (0.21)</td>
<td>0.023^a</td>
</tr>
<tr>
<td>RTs – neutral stimuli (s difference)</td>
<td>&gt;-0.01 (0.45)</td>
<td>-0.16 (0.28)</td>
<td>-0.19 (0.31)</td>
<td>-0.26 (0.23)</td>
<td>0.047^a</td>
</tr>
</tbody>
</table>

MDD-FHP – patients with major depressive disorder with family history of the disease; MDD-FHN – patients with major depressive disorder without family history of the disease; HC-FHP – healthy controls with family history of the disease; HC-FHN – healthy controls without family history of the disease

Statistical significance set to p<0.05; ^Statistically significant difference
Table 20. Post-hoc comparisons in accuracy and reaction times between groups distinguished by diagnosis of MDD and family history of the disease in costs of inhibiting of emotional processing

<table>
<thead>
<tr>
<th>Behavioural characteristics – inhibition of emotional processing</th>
<th>HC-FHN vs MDD-FHN (p values)</th>
<th>HC-FHP vs MDD-FHN (p values)</th>
<th>HC-FHN vs HC-FHP (p values)</th>
<th>MDD-FHN vs MDD-FHP (p values)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accuracy – general</td>
<td>0.981</td>
<td>0.187</td>
<td>0.882</td>
<td>0.388</td>
</tr>
<tr>
<td>Accuracy – negative stimuli</td>
<td>1.000</td>
<td>0.034&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.641</td>
<td>0.417</td>
</tr>
<tr>
<td>Accuracy – positive stimuli</td>
<td>0.194</td>
<td>0.588</td>
<td>0.997</td>
<td>0.907</td>
</tr>
<tr>
<td>Accuracy – neutral stimuli</td>
<td>0.787</td>
<td>0.054</td>
<td>0.993</td>
<td>0.609</td>
</tr>
<tr>
<td>RTs – general</td>
<td>0.835</td>
<td>0.048&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.948</td>
<td>0.135</td>
</tr>
<tr>
<td>RTs – negative stimuli</td>
<td>0.916</td>
<td>0.023&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.990</td>
<td>0.082</td>
</tr>
<tr>
<td>RTs – positive stimuli</td>
<td>0.904</td>
<td>0.114</td>
<td>0.962</td>
<td>0.220</td>
</tr>
<tr>
<td>RTs – neutral stimuli</td>
<td>0.806</td>
<td>0.219</td>
<td>0.919</td>
<td>0.410</td>
</tr>
</tbody>
</table>

MDD-FHP – patients with major depressive disorder with family history of the disease; MDD-FHN – patients with major depressive disorder without family history of the disease; HC-FHP – healthy controls with family history of the disease; HC-FHN – healthy controls without family history of the disease

Statistical significance set to p<0.05; <sup>a</sup>Statistically significant difference
4.5.2 Functional MRI results

4.5.2.1 General effect of diagnosis (H1)
There were no significant differences between patients with MDD and healthy controls in inhibiting emotional processing when family history of MDD was not taken into account.

4.5.2.2 General effect of family history of MDD (H2A)
There were no significant differences between subjects with family history of MDD and subjects without family history of MDD in inhibiting emotional processing when diagnosis was not considered.

4.5.2.3 Interaction between diagnosis, family history of MDD and valence of inhibited stimuli
A factorial analysis revealed a significant negative interaction between diagnosis, family history of MDD and valence of the inhibited stimuli in the right middle cingulate cortex and the left caudate nucleus (Figure 27).

![Image](image_url)

Figure 27. Regions in which interaction between diagnosis of MDD, family history of MDD and valence of the inhibited stimuli is observed; FWE voxel corrected; scale represents F values.

The regions are presented in Table 21.
Table 21. Significant differences in neural activation during inhibition of emotional processing between the groups distinguished by diagnosis of MDD and family history of the disease

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Region</th>
<th>MNI location</th>
<th>Voxel corrected p values</th>
<th>Cluster size (no. of voxels)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative interaction between diagnosis and family history</td>
<td>right middle cingulate cortex</td>
<td>3 -19 34</td>
<td>3 -19 34</td>
<td>36</td>
</tr>
<tr>
<td>of MDD</td>
<td>left caudate nucleus</td>
<td>-21 -22 31</td>
<td>-21 -22 31</td>
<td>7</td>
</tr>
<tr>
<td>HC-FHP &gt; MDD-FHP – inhibition of negative stimuli</td>
<td>left middle cingulate cortex</td>
<td>-3 -22 34</td>
<td>0.07</td>
<td>11</td>
</tr>
<tr>
<td>HC-FHP &gt; HC-FHN – inhibition of negative stimuli</td>
<td>right middle cingulate gyrus</td>
<td>3 -19 34</td>
<td>&lt;0.001</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>left caudate nucleus/ thalamus</td>
<td>-21 -16 31</td>
<td>0.005</td>
<td>16</td>
</tr>
<tr>
<td>MDD-FHP &gt; MDD-FHN – inhibition of negative stimuli</td>
<td>left cerebellum 6</td>
<td>-12 -61 -14</td>
<td>0.002</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>vermis 4.5</td>
<td>6 -61 -17</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td></td>
<td>left inferior temporal gyrus</td>
<td>-42 -40 -14</td>
<td>0.008</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>left putamen/ hippocampus</td>
<td>-30 -16 -8</td>
<td>0.019</td>
<td>4</td>
</tr>
</tbody>
</table>

MDD-FHP – patients with major depressive disorder with family history of the disease; MDD-FHN – patients with major depressive disorder without family history of the disease; HC-FHP – healthy controls with family history of the disease; HC-FHN – healthy controls without family history of the disease

Results FWE voxel corrected; Statistical significance set to p<0.05;
4.5.2.4 Healthy controls without family history of MDD vs patients without family history of MDD (H2B)

There were no significant differences between the HC-FHN and the MDD-FHN in terms of inhibiting emotional processing.

4.5.2.5 Healthy controls with family history of MDD vs patients with MDD with family history of MDD (H2C)

There were no significant differences between the HC-FHP and the MDD-FHP in general inhibiting of emotional processing. However, differences were observed in terms of inhibiting negative information. The HC-FHP experienced more neural activation in the left middle cingulate cortex (Figure 28). The regions are presented in Table 21.

![Image](image_url)

*Figure 28. Regions of stronger neural activation observed in the HC-FHP, as compared to the MDD-FHP, during inhibition of negative stimuli; FWE voxel corrected; scale represents t values.*

4.5.2.6 Healthy controls with family history of MDD vs healthy controls without family history of MDD (H2D)

There were no significant differences between the HC-FHP and the HC-FHN in general inhibiting of emotional processing. However, differences were observed in inhibiting negative information. The HC-FHP experienced more neural activation in the right
middle cingulate cortex and the left caudate nucleus/thalamus (Figure 29). The regions are presented in Table 21.

Figure 29. Regions of stronger neural activation observed in the HC-FHP, as compared to the HC-FHN, during inhibition of negative stimuli; FWE voxel corrected; scale represents t values.

4.5.2.7 Patients with MDD with family history of MDD vs patients with MDD without family history of MDD (H2E)

There were no significant differences between the MDD-FHP and the MDD-FHN in general inhibiting of emotional processing. However, differences were observed in inhibiting negative information. The MDD-FHP experienced an increase of neural activation in the left cerebellum, the left inferior temporal gyrus, the left putamen and the left hippocampus (Figure 30). The regions are presented in Table 21.

Figure 30. Regions of stronger neural activation observed in the MDD-FHP, as compared to the MDD-FHN, during inhibition of negative stimuli; FWE voxel corrected; scale represents t values.

4.5.2.8 Summary of the results

The results of this study verify hypotheses 1 and 2 of the thesis. The summary of the results is presented in the Table 22.
Table 22. Summary of neural differences between group distinguished by diagnosis and family history of MDD in inhibition of emotional processing

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDD vs HC (H1)</td>
<td>- no differences observed</td>
</tr>
<tr>
<td>FHN vs FHP (H2A)</td>
<td>- no differences observed</td>
</tr>
<tr>
<td>HC-FHN vs MDD-FHN (H2B)</td>
<td>- no differences observed</td>
</tr>
<tr>
<td>HC-FHP vs MDD-FHP (H2C)</td>
<td>- decreased activation in gating area of MCC during inhibition (negative) in MDD-FHP</td>
</tr>
<tr>
<td>HC-FHN vs HC-FHP (H2D)</td>
<td>- increased activation in the core limbic group and gating area of MCC during inhibition (negative) in HC-FHP</td>
</tr>
<tr>
<td>MDD-FHN vs MDD-FHP (H2E)</td>
<td>- increased activation in the core limbic group during inhibition (negative) in MDD-FHP</td>
</tr>
</tbody>
</table>

MDD-FHP – patients with major depressive disorder with family history of the disease; MDD-FHN – patients with major depressive disorder without family history of the disease; HC-FHP – healthy controls with family history of the disease; HC-FHP – healthy controls without family history of the disease

4.5.3 Differences between patients receiving different types of treatment

Patients with MDD treated in different ways did not differ in behavioural and neural correlates of inhibition of emotional processing (Table 23).

Table 23. Differences in accuracy and reaction times between patients treated with SSRIs, patients treated with dual action substances and not medicated patients in costs of inhibiting emotional processing (F MANOVA)

<table>
<thead>
<tr>
<th>Behavioural characteristics – inhibition of emotional processing (±SD)</th>
<th>Patients treated with SSRIs (N=17)</th>
<th>Patients treated with dual-action (N=18)</th>
<th>Not medicated patients (N=15)</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>AC – general (% dif)</td>
<td>-4.2 (±13.4)</td>
<td>-2.1 (±12.8)</td>
<td>-5.1 (±14)</td>
<td>0.827</td>
</tr>
<tr>
<td>AC – negative stimuli (% dif)</td>
<td>8 (±17.6)</td>
<td>8.8 (±17)</td>
<td>4.5 (±15.5)</td>
<td>0.784</td>
</tr>
<tr>
<td>AC – positive stimuli (% dif)</td>
<td>-4.9 (±17)</td>
<td>-4.2 (±14.4)</td>
<td>-10 (±15.7)</td>
<td>0.590</td>
</tr>
<tr>
<td>AC – neutral stimuli (% dif)</td>
<td>-15.6 (±18.7)</td>
<td>-10.8 (±18)</td>
<td>-9.7 (±26)</td>
<td>0.730</td>
</tr>
<tr>
<td>RTs – general (s dif)</td>
<td>0.25 (±0.4)</td>
<td>0.06 (±0.33)</td>
<td>0.11 (±0.38)</td>
<td>0.367</td>
</tr>
<tr>
<td>RTs – negative stimuli (s dif)</td>
<td>0.33 (±0.4)</td>
<td>0.16 (±0.37)</td>
<td>0.14 (±0.39)</td>
<td>0.356</td>
</tr>
<tr>
<td>RTs – positive stimuli (s dif)</td>
<td>0.4 (±0.42)</td>
<td>0.17 (±0.33)</td>
<td>0.17 (±0.43)</td>
<td>0.204</td>
</tr>
<tr>
<td>RTs – neutral stimuli (s dif)</td>
<td>0.01 (±0.44)</td>
<td>-0.14 (±0.33)</td>
<td>0.03 (±0.47)</td>
<td>0.458</td>
</tr>
</tbody>
</table>

AC – accuracy, dif – difference, RTs – reaction times, SSRIs – selective serotonin reuptake inhibitors
Statistical significance set to p<0.05; *Statistically significant difference
MANOVA tests revealed no significant differences between the pairs of groups of patients in accuracy and RTs of emotion processing and attention shifting (Table 24).

Table 24. Post-hoc comparisons in accuracy and reaction times for costs of inhibiting emotional processing between groups of patients with MDD distinguished by different types of medication

<table>
<thead>
<tr>
<th>Behavioural characteristics – inhibition of emotional processing (p values)</th>
<th>SSRIs vs dual</th>
<th>SSRIs vs no treatment</th>
<th>dual vs no treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>AC – general (% dif)</td>
<td>0.903</td>
<td>0.982</td>
<td>0.825</td>
</tr>
<tr>
<td>AC – negative stimuli (% dif)</td>
<td>0.992</td>
<td>0.849</td>
<td>0.782</td>
</tr>
<tr>
<td>AC – positive stimuli (% dif)</td>
<td>0.991</td>
<td>0.681</td>
<td>0.599</td>
</tr>
<tr>
<td>AC – neutral stimuli (% dif)</td>
<td>0.803</td>
<td>0.750</td>
<td>0.989</td>
</tr>
<tr>
<td>RTs – general (s dif)</td>
<td>0.354</td>
<td>0.605</td>
<td>0.940</td>
</tr>
<tr>
<td>RTs – negative stimuli (s dif)</td>
<td>0.444</td>
<td>0.418</td>
<td>0.990</td>
</tr>
<tr>
<td>RTs – positive stimuli (s dif)</td>
<td>0.259</td>
<td>0.288</td>
<td>0.999</td>
</tr>
<tr>
<td>RTs – neutral stimuli (s dif)</td>
<td>0.546</td>
<td>0.997</td>
<td>0.540</td>
</tr>
</tbody>
</table>

AC – accuracy, dif – difference, dual – patients with major depressive disorder treated with dual action substances, no treatment – untreated patients with major depressive disorder, RTs – reaction times, SSRIs – patients with major depressive disorder treated with selective serotonin reuptake inhibitors

Statistical significance set to p<0.05; *Statistically significant difference

4.6 Discussion

The processes under discussion – emotional processing and attention shifting – were found in this study to be of a general and broad nature, encompassing other smaller subtle processes. When the two broad conditions are compared, a significant amount of the subtle processes is shared between the two. However, some of them may be specific to one condition only. Inhibition of emotional processing is a more specific, discrete process, which is a crucial and distinctive part of attention shifting. In this study, the inhibition was operationalized as the difference between an attention shifting condition and an emotional processing condition (attention shifting condition – emotional processing condition). The inhibition is hypothesized to be most crucial part of this difference, present in attention shifting.
The findings of this study provide evidence that both the diagnosis and family history of MDD are crucial for inhibition of emotional processing, the inhibition being a critical part of emotional regulation. The process has been extensively studied to date, and described as impaired in many psychiatric disorders, such as ADHD (Adams, Milich, & Fillmore, 2010; Alderson, Rapport, Hudec, Sarver, & Kofler, 2010; Boonstra, Kooij, Oosterlaan, Sergeant, & Buitelaar, 2010; Braet et al., 2011; Depue, Burgess, Willcutt, Ruzic, & Banich, 2010; Huizenga, van Bers, Plat, van den Wildenberg, & van der Molen, 2009; Liotti, Pliszka, Higgins, Perez III, & Semrud-Clikeman, 2010; Raiker, Rapport, Kofler, & Sarver, 2012; Schecklmann et al., 2012) and schizophrenia (Beech, Powell, McWilliam, & Claridge, 1989; Volk & Lewis, 2002; Waters, Badcock, Maybery, & Michie, 2003; Williams, 1995, 1996). However, inhibition of emotional processing has been rarely addressed in research on MDD, and has never been studied in connection with the role of family history of the disease.

The present study fills this gap and adds to our understanding of the process by demonstrating how changes in inhibition reflect vulnerability to MDD. Although the role of inhibition of emotional stimuli has been previously noted in the context of vulnerability to MDD and its association with remission of the disease (Hammar, et al., 2010) the issues of family history of MDD and emotional regulation have not been examined in the current context before.

Behaviourally, healthy controls are known to be better at inhibiting negative stimuli than patients with MDD (Christensen, et al., 2006; Goeleven, et al., 2006; Mathews & MacLeod, 1994). However, inhibition of positive stimuli can be easier for patients with MDD than for healthy controls. In the previous analysis, it was demonstrated that patients with MDD do not recognize rewarding cues as readily as healthy controls. They also react to positive stimuli with a weaker neural emotional activation in
comparison to individuals without MDD. Therefore, inhibition of the processing of such stimuli may not be difficult for them.

A similar behavioural pattern occurs when individuals with and without family history of MDD are compared. Individuals without family history of MDD (individuals without vulnerability to depression) are more efficient in inhibiting negative and positive stimuli.

When the two factors (diagnosis and history) are considered separately, there are no observable neural differences between the groups in terms of inhibition of emotional processing. However, when they are considered together, the changes are observed both at a behavioural and at a neural level. This suggests that inhibition plays an important role in our understanding of the vulnerability to MDD. It is also crucial for a distinction between endophenotypes of patients with MDD. This is especially relevant in the context of the burden of the disease (WHO, 2001) and possibilities for its prevention.

The finding that the MDD-FHN do not differ from the HC-FHN in behavioural and neural correlates of emotional inhibition may come as surprising. In studies carried out with a general population of patients with MDD, the patients were observed to perform worse in inhibiting emotional processing, especially if the affective cues were negative (Christensen, et al., 2006; Goeleven, et al., 2006; Mathews & MacLeod, 1994). The MDD-FHN in this study did not experience these difficulties. The present result is, however, in accordance with the findings from the previous part of this study where strongest differences between MDD-FHN and the HC-FHN were observed in emotional processing. This may indicate in that the MDD-FHN represent a particular type of patients with MDD, whose impairments of emotional regulation may actually be driven by changes in the processing of affective negative stimuli.
The HC-FHP, representatives of both vulnerability and resilience to MDD, were quicker than the MDD-FHP in inhibiting negative stimuli. This finding complies with previous studies in the general population of patients with MDD (Christensen, et al., 2006; Goeleven, et al., 2006; Mathews & MacLeod, 1994). In contrast, inhibition of positive and neutral cues was not impaired in the MDD-FHP, suggesting that the pathophysiology of MDD in the MDD-FHP is associated with impairments in the inhibition of negative cues. In contrast, the pathophysiology of the disease in the MDD-FHN is then connected with a changed processing of negative stimuli.

As explained before, this study is unique in that it distinguishes for the first time, between these two mechanisms of developing MDD, as related to the presence or absence of family history vulnerability. MDD with family history of the disease has been associated with an earlier onset of an acute episode and with higher anxiety symptoms (Nierenberg, et al., 2007). This suggests that inhibition may be more complex and fragile than emotional processing, and that it is disturbed more easily. In addition, disturbances of inhibition are a crucial part of anxiety (Wood, Mathews, & Dalgleish, 2001), an observable symptom in the MDD-FHP.

Furthermore, while inhibiting negative cues, the HC-FHP experienced stronger activation than the MDD-FHP did in the left middle/ posterior cingulate cortex. This increase of activation in the inhibition of negative cues is characteristic for resilience to the risk of developing MDD. The area has been shown in previous research as being involved in filtering of emotional information (Desseilles, et al., 2009; Fu, et al., 2007). It also participates in gathering external emotional observations (Bargh & Tota, 1988; Jackson, et al., 2006; Lamm, et al., 2007; Lou, et al., 2004; Maddock & Buonocore, 1997; Maddock, et al., 2003; Moran, et al., 2006; Northoff, et al., 2004; Singer, et al., 2004; Tomlin, et al., 2006). Given these functions, the HC-FHP are likely to focus on
external emotional cues more than the MDD-FHP while also filtering the information better. In emotional regulation terms, this means that the MDD-FHP are easily distracted from following through on their plan by other stimuli or internal cues. This points at the well-known motivational symptoms of the disease (Beck & Alford, 2009). This pattern is only observed in inhibiting negative stimuli, and one may speculate that this is the basis of anxiety observed in the group (Joffe & Regan, 1991).

The HC-FHP, in comparison to the HC-FHN, represent the mechanism of vulnerability to MDD. No observable behavioural differences between the two groups in inhibiting emotional processing can be detected. In a study with monozygotic twins of patients with MDD, these did not differ behaviourally from healthy controls (Wolfensberger, et al., 2008). This may suggest that there are either no differences in inhibition of emotional information connected with vulnerability to MDD or that there is a mechanism of compensation at work.

The neural evidence of this study indicates that a mechanism of compensation is employed during inhibition of negative stimuli. The HC-FHP, in comparison to the HC-FHN, experienced an increased neural activation in the right MCC/PCC and in the left caudate nucleus. An increased activation in the MCC/PCC during the inhibition of negative cues is a neural correlate of resilience, observed in comparison of the HC-FHP and the MDD-FHP groups. The activation in the region is also heightened when the HC-FHP are contrasted with the HC-FHN. This point indicates that the HC-FHP are a group with the strongest activation in the area during inhibition of negative cues. Since the increase is observed for both vulnerability and resilience, a mechanism of compensation is likely at play. Given the functional role of the area (Desseilles, et al., 2009; Fu, et al., 2007), the mechanism seems to be based on the filtering of negative emotional stimuli.
The differences in activation unique for a comparison between the HC-FHP and the HC-FHN and therefore characteristic only for vulnerability to MDD may explain why an increased emotional filtering is needed in the HC-FHP. During inhibition of negative stimuli, the HC-FHP experienced a heightened activation in the core limbic group (Kober, et al., 2008). This region is involved in emotional arousal (Horvitz, 2000; Jensen, et al., 2003; O'Doherty, et al., 2004; Schultz, et al., 1997) and in the visceral aspect of emotional experience (Longstaff, 2005). Most importantly, it is involved in emotional sustenance (Aouizerate, et al., 2004; Cardinal, et al., 2002; Davidson & Irwin, 1999; Drevets, et al., 2001). This suggests an excessive visceral reaction to emotional cues in the HC-FHP during the inhibition of emotional processing, when the observed cues are negative. It also suggests that the HC-FHP sustain their affective arousal even when they are to inhibit emotional processing. This increase can be controlled by enhancement in the functioning of emotional filters, which may constitute the observed mechanism of compensation.

Family history vulnerability to MDD can be also considered within an acute episode of the disease, when the MDD-FHP and the MDD-FHN are contrasted. The MDD-FHP display higher neuroticism (Holma, et al., 2011), excessive guilt, anxiety symptoms, functional impairments and an earlier age of onset of MDD (Nierenberg, et al., 2007). Indeed, the results of the present study suggest that there may be a tendency for the MDD-FHN to be quicker in inhibiting negative cues that the MDD-FHP. The MDD-FHP are as efficient in inhibiting negative information as the MDD-FHN. However, it takes them longer to achieve the same result and they may need to put in more effort.

During the inhibition of negative stimuli, the MDD-FHP displayed a higher activation in cortical and limbic regions, in comparison to the MDD-FHN. The regions have been known to be involved in visceral emotional reactions (Edelman & Tononi, 2003;
Hamann, et al., 2004; Horvitz, 2000; Jensen, et al., 2003; Lenz, et al., 1995; O'Doherty, et al., 2004; Schultz, et al., 1997) and in memory operations (Kober, et al., 2008; Otten, Henson, & Rugg, 2002). Similarly to the activation observed in the HC-FHP, the MDD-FHP may experience an extensively high residual visceral arousal while attempting to inhibit emotional processing. However, unlike the HC-FHP, the MDD-FHP may not inhibit the processing effectively. Behavioural results seem to confirm this notion. The MDD-FHP experienced most difficulties in inhibiting emotional stimuli, when compared to other three participant groups in the study.

Changes in the inhibition of emotional processing were specifically notable in the two groups with family history of MDD. The MDD-FHP displayed an impaired inhibition of negative stimuli, in comparison to both healthy controls and other patients with MDD. The observed neural pattern of activation suggests that the MDD-FHP did not inhibit negative information entirely, and that they experienced an elevated emotional arousal during inhibiting. The first-degree unaffected healthy relatives of patients with MDD also experienced such an increase. However, they seem to have compensated for this with a heightened activation in the area responsible for emotional filtering. In terms of emotional regulation, this indicates that the MDD-FHP may have difficulties in disengaging from the processing of punishing stimuli. They may also be easily distracted by negative information and abandon their goals. This may lead to indecisiveness observed in the disease (Beck & Alford, 2009). It may also result in anxiety characteristic for the group (Nierenberg, et al., 2007). The HC-FHP, in turn, may need to use more attentional and energetic resources while inhibiting negative stimuli, given their use of compensation mechanism. Therefore, under an emotional strain, such as stress, the HC-FHP may “crumble” quicker than the HC-FHN and may develop pathological patterns of coping faster. This is in accordance with the
observation that an acute episode of the disease has usually an earlier onset in the group with family history of MDD (Nierenberg, et al., 2007).

4.6.1 Different types of medication

The absence of behavioural and neural differences between patients treated in distinct ways can be explained in the same way as in the previous part of the study presented in the chapter 3.6.2.

4.6.2 General conclusions of the Study 1

Family history of major depressive disorder increases a risk of developing the disease in healthy subjects and accelerates its onset in patients with MDD (Fanous, et al., 2002; Nierenberg, et al., 2007; Weissman, et al., 2006). This study examines potential neuropsychological mechanism of these alterations. Family history of MDD in this study has been shown to influence the neural correlates of emotion processing, attention shifting and inhibition of the emotional processing in both patients with MDD and healthy controls. These processes have been shown to be responsible for emotional regulation. When altered, they are likely to lead to developing affective and cognitive dysfunctions, or even symptoms of MDD, such as anhedonia, dysphoria, and indecisiveness (Beck & Alford, 2009).

One of the strong points of this study is the high number of participants included within each subgroup. This design allowed for a thorough examination of the differences between individuals with and without family history of MDD in terms of the diagnosis of the disorder. Also, the fact that the whole-brain analysis of processes was possible changes occurring in the entire network involved in emotion processing, attention shifting and inhibition could be explored.

This study is the first to determine risk, resilience, and different types of acute depressive episodes connected with family history of MDD, where neural changes in
the processes of emotional regulation empower the observed alterations. Emotional processing, attention shifting and inhibition of emotional processing are of high importance in emotional regulation. Therefore, these changes may lead to an increase in the risk of developing MDD. According to the models of MDD (Davidson, et al., 2002a; Drevets, 2001; Haldane & Frangou, 2006; Mayberg, 1997; Phillips, et al., 2003b), and in line with the findings of this study, the disease is a disorder of the representation and regulation of emotions and mood.

In this study, both groups of healthy controls experienced a greater neural activation, compared to patients with MDD, in regions responsible for higher cognitive functions. In contrast, patients with MDD were characterized by a decrease of activation in the cortical compartment. These findings are in accordance with the assumptions of the models under scrutiny (Davidson, et al., 2002a; Drevets, 2001; Haldane & Frangou, 2006; Mayberg, 1997; Phillips, et al., 2003b). Furthermore, both groups of patients displayed a higher activation in the core limbic group of the CNS (Kober, et al., 2008), which also confirms the hypothesis of the models emphasizing the role of emotional regulation in MDD (Davidson, et al., 2002a; Drevets, 2001; Haldane & Frangou, 2006; Mayberg, 1997; Phillips, et al., 2003b). In the MDD-FHN, the differences postulated by the models were mostly observed during the emotional processing of negative stimuli. The MDD-FHP experienced these alterations while shifting attention from negative cues and while processing positive information, in particular.

However, and in contrast to the assumptions of the models, both groups of patients with MDD also experienced a decrease of activation in the paralimbic group or in the visual attention group (Kober, et al., 2008). These areas are largely responsible for the recognition of one’s own emotional states and for filtering emotional information (Desseilles, et al., 2009; Fu, et al., 2007; Goldin, et al., 2008; Northoff, et al., 2004;
Wager & Feldman Barrett, 2004). The MDD-FHN, in particular, were characterized by an increase in the visceral aspect of emotional processing and a decrease in the informative aspect (Longstaff, 2005).

The MDD-FHN experienced a reduced activation in the areas responsible for the emotional recognition and for emotional filtering, during their processing of affective stimuli (Desseilles, et al., 2009; Fu, et al., 2007; Goldin, et al., 2008; Northoff, et al., 2004; Wager & Feldman Barrett, 2004). They also experienced an increase of activation in the regions involved in visceral reactions to emotional stimuli (Edelman & Tononi, 2003; Hamann, et al., 2004; Horvitz, 2000; Jensen, et al., 2003; Lenz, et al., 1995; O'Doherty, et al., 2004; Schultz, et al., 1997). This implies that in this group the visceral aspect of emotional states may dominate their perception, whereas their informative aspect is reduced. This may lead to anhedonia, dysphoria and physical symptoms of the disease (Beck & Alford, 2009). In respect to the emotional regulation, this indicates that the MDD-FHN may not be able to absorb enough information from a given situation to take an effective approach. It may also indicate that, although greatly affected by negative stimuli (visceral aspect), the MDD-FHN may experience feelings of emptiness (informative/motivational aspect). Behaviourally, the MDD-FHN were mostly impaired in emotional recognition. All this suggests that an acute episode of MDD without family history vulnerability is characterized by two core features. First, it is a diminished ability to recognize and be motivated by external emotional cues. Second, it is a strong visceral reaction to affective stimuli, especially when negative.

The MDD-FHP displayed a reduced activation in the visual cortex during emotional processing (Freese & Amaral, 2005; Hendler, et al., 2001; Kober, et al., 2008; Vuilleumier, et al., 2004). This suggests a weaker response to emotional stimuli, especially with positive valence. During attention shifting, the MDD-FHP experienced
a reduced activation in the areas involved in attention allocation and in resolving emotional and attentional conflicts (Bush, et al., 2000; Etkin, et al., 2006). During the same process, they also showed a stronger activation in the regions involved in the visceral response to emotions (Edelman & Tononi, 2003; Hamann, et al., 2004; Horvitz, 2000; Jensen, et al., 2003; Lenz, et al., 1995; O'Doherty, et al., 2004; Schultz, et al., 1997).

Behaviourally, the MDD-FHP were most strongly impaired in attention shifting and inhibition. During inhibition, they displayed a reduced activation in the areas filtering an emotional content (Desseilles, et al., 2009; Fu, et al., 2007). When contrasted with the MDD-FHN, the MDD-FHP show more activation in the visceral-emotional areas during the inhibition (Edelman & Tononi, 2003; Hamann, et al., 2004; Horvitz, 2000; Jensen, et al., 2003; Lenz, et al., 1995; O'Doherty, et al., 2004; Schultz, et al., 1997). All this suggests that an acute episode of MDD with family history vulnerability is marked by a disturbed ability to deviate attention from emotional processing. This may lead to difficulties in focusing on planning, which can result in an indecisiveness and increased dependency observed as symptoms in the disease (Beck & Alford, 2009).

The HC-FHP represented both vulnerability to MDD and resilience to increased risk of developing the disease. The group tended to react more strongly to emotional stimuli through a heightened activation in the areas associated with gathering information about external and internal emotional cues (Freese & Amaral, 2005; Hendler, et al., 2001; Kanske & Kotz, 2011; Kober, et al., 2008; Maddock & Buonocore, 1997; Maddock, et al., 2003; Vuilleumier, et al., 2004). The HC-FHP showed such an increase in response to positive stimuli, when compared to the MDD-FHN. When compared to the HC-FHN, however, their response was stronger in relation to negative stimuli. This explains how the HC-FHP may be both vulnerable and resilient to the disease. A similar increased
reaction to emotional stimuli was noted for their attention shifting, suggesting impairments in this process. However, these are not observed in the behavioural correlates of emotional regulation, characteristic for the HC-FHP. This may be because the group displays a stronger activation in the areas associated with executive control and attention shifts (Aron, et al., 2003; Aron, et al., 2004b; Badre, et al., 2005; Chong, et al., 2008; Coull, et al., 2000; Dove, et al., 2000; Gabrieli, et al., 1998; Hooker, et al., 2010; Macaluso & Patria, 2007; Martin & Chao, 2001; Moss, et al., 2005; Ochsner & Gross, 2005; Poldrack, et al., 1999; Ridderinkhof, et al., 2004; Swick, et al., 2008; Wager, et al., 2005; Wagner, et al., 2001; Zhang, et al., 2004), compensating for the increased emotional activation.

This mechanism can also be observed in greater detail during inhibition of emotional processing of negative stimuli. In that case, the HC-FHP show a stronger activation in the areas responsible for emotional arousal (Edelman & Tononi, 2003; Hamann, et al., 2004; Horvitz, 2000; Jensen, et al., 2003; Lenz, et al., 1995; O'Doherty, et al., 2004; Schultz, et al., 1997) than the HC-FHN do. This indicates that at the neural level, the HC-FHP do not inhibit emotional processing as effectively as the HC-FHN do. This, again, has no equivalent in the behavioural performance. This point can be explained by the fact that during the inhibition of negative stimuli the HC-FHP experience an increase of activation in the area involved in emotional filtering and managing (Desseilles, et al., 2009; Fu, et al., 2007). This increase is observed when the HC-FHP are compared to both the HC-FHN and the MDD-FHP groups, indicating a mechanism of compensation at play. This mechanism is probably responsible for the resilience to vulnerability observed in the HC-FHP. This also suggests that keeping such a balance may require more energy, and is therefore more prone to exhaustion.
This study is the first to show that the MDD-FHN and the MDD-FHP develop the disorder differently, and that they suffer from different impairments in their emotional regulation. The study is also the first to show that vulnerability to MDD, as connected with family history of the disease, is based on the mechanism of compensation, when cognitive control faces an excessive emotional arousal.
5 Val66Met polymorphism in emotional regulation in patients with MDD and healthy controls (study 2)

5.1 Introduction

According to the neuroplasticity theory, brain-derived neurotrophic factor (BDNF) plays a significant role in the pathogenesis of major depressive disorder (Brunoni, et al., 2008; Castren, et al., 2007; Groves, 2007). The BDNF is a neurotrophin responsible for neuronal proliferation, migration, survival and synaptic genesis (Binder & Scharfman, 2004), with the highest concentration in the regions of the limbic system involved in emotional processing, memory and attention shifting – the hippocampus, frontal lobes and limbic midbrain areas (Binder & Scharfman, 2004; Castren, et al., 2007; Duman & Monteggia, 2006).

The secretion of the BDNF in the central nervous system is influenced by a stress reaction of the hypothalamic-pituitary-adrenal axis (Duman & Monteggia, 2006; Groves, 2007). Episodes of MDD can actually be evoked by the hyperactivity of the HPA, which brings a prolonged stress reaction (Dawood, et al., 2007). The BDNF plays a role in that process by participating in the negative feedback system for the HPA and by controlling the secretion of hormones participating in the stress response (Dawood, et al., 2007; Praag, et al., 2004). An increase of BDNF in a specific neural area or network can either promote an antidepressant-like reaction or heighten depressive symptoms (Castren, et al., 2007; Groves, 2007).

One mechanism of action of venlafaxine, escitalopram, paroxetine as well as non-medicinal MDD therapies, such as repetitive transcranial magnetic stimulation, involves an increase of concentration of the BDNF in the hippocampus and frontal lobes (for a review, see (Brunoni, et al., 2008)). The change is positively correlated with a reduction in depressive symptoms, in cognitive impairments, in particular (for a meta-analysis...
In post-mortem studies, patients with MDD have a reduced volume of the hippocampus and frontal lobes (Knable, Barci, Webster, Meador-Woodruff, & Torrey, 2004; Rajkowska et al., 1999; Stockmeier et al., 2004), which suggests a limited neuronal growth and survival, two symptoms connected with a reduced protective functionality of the BDNF. A further post-mortem evidence for a reduced concentration of the BDNF in the hippocampus and frontal lobes comes from reports on suicidal individuals with MDD (Dwivedi et al., 2003). Rodent models of depression show that a direct injection of the BDNF into the hippocampus diminishes depressive symptoms (Shirayama, Chen, Nakagawa, Russell, & Duman, 2002). Together, this evidence points to the neurogenesis in the hippocampus and frontal lobes as a protective factor against MDD (Duman, 2005). The neurogenesis in these areas allows for an adaptive reaction to stress and to a changing environment – e.g. paying attention to new information, generating new solutions and memorizing them for upcoming situations which interfere with the organism’s homeostasis.

However, in rodent models it was also observed that a high concentration of the BDNF in midbrain is depressogenic, and that its reduction eliminates a depressive-like behaviour (Berton et al., 2006; Eisch et al., 2003). Considering that GABA-ergic fibres project from midbrain to inhibitory network surrounding the HPA axis, the more active the midbrain areas are, the more prolonged the HPA stress reaction becomes (inhibition of inhibition). This partially explains why a higher concentration of the BDNF in the midbrain leads to depressive symptoms, by extending the stress reaction.

A single-nucleotide polymorphism in the Val66Met BDNF gene (rs6265), where substituting valine by methionine decreases an activity-dependent secretion of the BDNF (Chen et al., 2004), is linked to MDD in complex ways. Firstly, carriers of at least one methionine display increased depressive (Schumacher, et al., 2005) and
anxious behaviours (Chen, et al., 2006) (the latter in rodent models). Furthermore, patients with MDD (Koolschijn, et al., 2009) and Met carriers have a reduced volume of frontal lobes (Nemoto, et al., 2006; Pezawas, et al., 2004) and hippocampus (Bueller, et al., 2006; Pezawas, et al., 2004; Szeszko, et al., 2005). Additionally, the two groups display impairments in working memory (Porter, et al., 2003). Moreover, Met carriers are less susceptible to emotional conditioning, which may be connected with slower emotional learning (Lonsdorf, et al., 2010). These facts suggest that the Met allele carriers have an increased vulnerability to MDD.

Conversely, Val/Val individuals score higher on neuroticism scale in NEO personality inventory (Hunnerkopf, et al., 2007). This may suggest a different pathway of developing MDD in the two genotypes, requiring a good examination of neural differences between patients and healthy controls, separately for both genotypes. This study is the first to address this question with the use of fMRI in adult population.

The fMRI technique has been used previously to explore neural differences associated with the Val66Met polymorphism in healthy controls. The task-dependent functional differences were observed in the hippocampus – compared to Val homozygotes, Met carriers activate the structure less in memory tasks when an increased activation of the region is expected in healthy controls (Hariri, et al., 2003) and more in the N-back task when healthy controls experience a deactivation in the structure (Egan et al., 2003). This suggests a certain lack of flexibility of the hippocampal cortex in the Met individuals. The decrease of neural activation in Met carrying subjects has been noted in frontal lobes (particularly in the left hemisphere) in attentional tasks (Schofield et al., 2009). In contrast, Met carriers experience an increased activation in the cingulate cortex, the insula, the brain stem and the amygdala during facial recognition.
(Mukherjee, et al., 2011; van Wingen et al., 2010) and in the caudate nucleus during spatial navigation (Banner, Bhat, Etchamendy, Joober, & Bohbot, 2011).

There is a paucity of research exploring neural correlates of the connection between the Val66Met polymorphism and the diagnosis of MDD in both healthy controls and patients with MDD (Lau et al., 2010). However, no study to date compared healthy controls and patients with MDD separately for both genotypes. Therefore, drawing conclusions about different patterns of depressive changes for the two genotypes has not been possible. Finally, no study has examined combined influence of the diagnosis and Val66Met polymorphism on the processes engaged in emotional regulation. This study was the first to address these questions.

5.2 Aims and hypotheses

The aim of Study 2 was to examine whether the polymorphism in the Val66Met gene coding BDNF influences behavioural and neural correlates of emotional processing and attention shifting in patients with MDD and in healthy controls.

On the basis of a diagnosis and genotyping, the participants in this study were divided into 4 groups:

1) patients with MDD with two Val alleles (MDD-Val);
2) patients with MDD with at least one Met allele (MDD-Met);
3) healthy controls with two Val alleles (HC-Val);
4) and healthy controls with at least one Met allele (HC-Met).

All these groups were compared among each other in terms of emotional processing and attention shifting. The performed comparisons are described next.
5.2.1 Subjects with two Val alleles vs subjects with at least one Met allele (H3A)

The first hypothesis of Study 2 states that: subjects with two Val alleles and subjects with at least one Met allele vary in neural and behavioural correlates of emotional regulation, regardless of their diagnosis. It is also hypothesized that the neural difference will appear in the areas known as the neural correlates of the examined processes (Northoff, et al., 2004) as well as in areas rich in BDNF secretion (Brunoni, et al., 2008). Finally, it is also anticipated that subjects with at least one Met allele might be slower and less accurate in this connection.

5.2.2 Healthy controls with homogenous Val allele vs patients with MDD with homozygous Val allele (H3B)

This second comparison in Study 2 sets to examine to what extent an acute depressive episode changes emotional processing and attention shifting in carriers of two Val genes. It is hypothesized that patients with MDD with two Val alleles, as compared to the HC-Val, will have less neural activation in the areas connected with cortical compartment and more activation in the regions of limbic compartment (Mayberg, 1997). It is further expected that the patients might show less activation in other areas associated with the targeted processes in healthy controls (Northoff, et al., 2004). Finally, it is also anticipated that the MDD-Val might be slower and less accurate than the HC-Val in this connection.

5.2.3 Healthy controls carrying Met allele vs patients with MDD carrying Met allele (H3C)

When the HC-Met and the MDD-Met groups were compared in this study, changes in emotional regulation in an acute depressive episode, as characteristic for the Met allele carriers, were observed. Also, an increased resilience to the greater risk of MDD characteristic for the HC-Met was confirmed. As a result, the following third hypothesis...
for Study 2 was formulated. The HC-Met, in contrast to the MDD-Met, will experience less activation in the limbic compartment and more activation in the cortical compartment of the CNS (Mayberg, 1997). Differences between the two groups are also likely to occur in the areas with abundant BDNF secretion that is in the hippocampus and the frontal lobes (Brunoni, et al., 2008). Finally, it is expected that the MDD-Met will be slower and less accurate than the HC-Met.

5.2.4 Healthy controls carrying Met allele vs healthy controls with homogenous Val allele (H3D)

Finally, when the HC-Met were compared to the HC-Val in this study, alterations in emotional regulation characteristic for two endophenotypes of healthy controls were found. Therefore, the fifth hypothesis for Study 2 states that the HC-Met will experience less activity in the regions with rich BDNF secretion (Brunoni, et al., 2008). It is further expected that the HC-Met will not be as accurate as the HC-Val.

5.2.5 Patients with MDD carrying Met allele vs patients with MDD with homogenous Val allele (H3E)

In study 2, it is of further interest to test how emotional processing and attention shifting are changed in an acute depressive episode by the presence of at least one Met allele. It is thus hypothesized that patients with at least one Met allele, as carriers of both the vulnerability and the acute symptoms, will be less efficient in behavioural responding to the stimuli, and that their neural activity will be reduced in the areas with high BDNF secretion (Brunoni, et al., 2008).

5.3 Groups tested

Out of the recruited individuals described in the chapter 2.1, thirty-seven (N=37) patients with MDD and thirty-nine (N=39) healthy controls – matched for age, gender
and handedness – participated in Study 2. Depressed individuals and healthy controls differed significantly in all the applied depression ratings.

5.3.1 Genotyping

Both patients with MDD and healthy controls were first genotyped for the variants of the rs6265 single nucleotide polymorphism (SNP). The Val66Met BDNF SNP (rs6265) was genotyped in the sample using a Taqman® SNP Genotyping Assay on a 7900HT Sequence Detection System (Applied Biosystems). The call rate for the Taqman genotyping was >95%, and all samples were in Hardy-Weinberg equilibrium (p>0.05). Along with the test samples, a number of HapMap CEU DNA sample positive controls (www.hapmap.org) and non-template negative controls were genotyped for each SNP for quality control purposes. For positive controls, all genotypes were found to be concordant with the available online HapMap data. All non-template samples returned a negative result.

As a result, participants were divided into three genotype groups: a homozygous group for Met, a homozygous group for Val alleles, and a heterozygous group carrying one Met and one Val allele. Met homozygotes are infrequent (Shimizu, Hashimoto, & Iyo, 2004), and having at least one Met allele is associated with structural changes in the brain (Frodl et al., 2007). Therefore, heterozygous individuals and subjects with two Met alleles were combined in the subsequent analysis into one group of Met-carriers, as has been done in previous functional magnetic resonance studies investigating Met66Val polymorphism (Lau, et al., 2010; Montag, Reuter, Newport, Elger, & Weber, 2008). Overall, 22 subjects were identified as Met-carriers and 54 as Val homozygous individuals. The two groups did not differ in age, gender and handedness (Table 25). The two groups were also comparable in MDD ratings, except for BDII, in which the
Met carriers scored higher. However, the difference disappeared when the two groups were divided into a group of patients with MDD and a group of healthy controls.

Table 25. Demographic and clinical characteristic of subjects with homogenous Val allele and subjects carrying Met allele

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Subjects with homogenous Val allele (N=54)</th>
<th>Subjects carrying Met allele (N=22)</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (SD)</td>
<td>39.6 (12.9)</td>
<td>38.8 (11.2)</td>
<td>0.815</td>
</tr>
<tr>
<td>Gender (f/m)</td>
<td>36/18</td>
<td>13/9</td>
<td>0.601</td>
</tr>
<tr>
<td>Handedness (r/l)</td>
<td>54/0</td>
<td>22/0</td>
<td></td>
</tr>
<tr>
<td>Hamilton Depression Rating Scale (SD)</td>
<td>13.8 (13.9)</td>
<td>18.8 (14)</td>
<td>0.155</td>
</tr>
<tr>
<td>Montgomery-Asberg Depression Rating Scale (SD)</td>
<td>13.5 (15)</td>
<td>19.9 (13.7)</td>
<td>0.085</td>
</tr>
<tr>
<td>Beck Depression Inventory II (SD)</td>
<td>14.2 (16.5)</td>
<td>24.3 (17.8)</td>
<td>0.021*</td>
</tr>
</tbody>
</table>

Statistical significance set to p<0.05; *Statistically significant difference

After a diagnostic and genotyping procedure, four groups of participants were distinguished: Val homozygous patients with MDD (N=23), Met-carrying patients with MDD (N=14), Val homozygous healthy controls (N=31), and healthy Met-carriers (N=8). The groups did not differ in age, gender and handedness (Table 26). They differed in all applied ratings of MDD (Table 26).

Table 26. Demographic and clinical characteristics of groups distinguished by diagnosis of major depressive disorder and Val66Met polymorphism (F MANOVA)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>MDD-Met (N=14)</th>
<th>MDD-Val (N=23)</th>
<th>HC-Met (N=8)</th>
<th>HC-Val (N=31)</th>
<th>p values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (SD)</td>
<td>41.9 (8.1)</td>
<td>41.3 (12.4)</td>
<td>33.5 (14.1)</td>
<td>38.3 (13.3)</td>
<td>0.369</td>
</tr>
<tr>
<td>Gender (f/m)</td>
<td>9/5</td>
<td>17/6</td>
<td>4/4</td>
<td>19/12</td>
<td>0.623</td>
</tr>
<tr>
<td>Handedness (r/l)</td>
<td>14/0</td>
<td>23/0</td>
<td>8/0</td>
<td>31/0</td>
<td>N/A</td>
</tr>
<tr>
<td>Hamilton Depression Rating Scale (SD)</td>
<td>28.3 (6.9)</td>
<td>28.8 (6.5)</td>
<td>2.3 (1.7)</td>
<td>2.6 (2.4)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Montgomery-Asberg Depression Rating Scale (SD)</td>
<td>29.4 (4.9)</td>
<td>29.6 (7.4)</td>
<td>3.3 (4.8)</td>
<td>1.5 (2.6)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Beck Depression</td>
<td>35.7 (10.6)</td>
<td>30.7 (12.2)</td>
<td>4.3 (4.9)</td>
<td>1.9 (2.3)</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

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MDD-Val – patients with major depressive disorder with two Val alleles in Val66Met gene, MDD-Met – patients with major depressive disorder with at least one Met allele in Val66Met gene, HC-Val – healthy controls with two Val alleles in Val66Met gene, HC-Met – healthy controls disorder with at least one Met allele in Val66Met gene

Statistical significance set to p<0.05; *Statistically significant difference

However, when post-hoc analysis was performed, there was no significant difference in the depression ratings between the two groups of healthy controls and between the two groups of patients with MDD (Table 27).

**Table 27. Post-hoc comparisons in demographic and clinical characteristics between groups distinguished by diagnosis of MDD and Val66Met polymorphism**

<table>
<thead>
<tr>
<th>Characteristics (p values)</th>
<th>HC-Val vs MDD-Val</th>
<th>HC-Met vs MDD-Met</th>
<th>HC-Val vs HC-Met</th>
<th>MDD-Val vs MDD-Met</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age – p value</td>
<td>0.806</td>
<td>0.426</td>
<td>0.765</td>
<td>0.999</td>
</tr>
<tr>
<td>Gender – p values</td>
<td>0.392</td>
<td>0.662</td>
<td>0.694</td>
<td>0.713</td>
</tr>
<tr>
<td>Hamilton Depression Rating Scale – p values</td>
<td>&lt;0.001*a</td>
<td>&lt;0.001*a</td>
<td>0.998</td>
<td>0.988</td>
</tr>
<tr>
<td>Montgomery-Asberg Depression Rating Scale – p values</td>
<td>&lt;0.001*a</td>
<td>&lt;0.001*a</td>
<td>0.820</td>
<td>1.000</td>
</tr>
<tr>
<td>Beck Depression Inventory II – p values</td>
<td>&lt;0.001*a</td>
<td>&lt;0.001*a</td>
<td>0.304</td>
<td>0.898</td>
</tr>
</tbody>
</table>

MDD-Val – patients with major depressive disorder with two Val alleles in Val66Met gene, MDD-Met – patients with major depressive disorder with at least one Met allele in Val66Met gene, HC-Val – healthy controls with two Val alleles in Val66Met gene, HC-Met – healthy controls disorder with at least one Met allele in Val66Met gene

Statistical significance set to p<0.05; *Statistically significant difference

### 5.4 Data analysis

#### 5.4.1 Design

Study 2 utilized a four sample design with Met-carrying patients with MDD, Val homozygous patients with MDD, Met-carrying healthy controls, and Val homozygous healthy controls as comparison groups. After the ascertainment procedure presented above, participants were assigned to one of the four groups. An event-related fMRI
experiment measuring processing of emotions and shifting attention from emotional information was used to record BOLD signal for each subject.

5.4.2 Behavioural data analysis

Individuals with homogenous Val allele were compared to individuals carrying Met allele in accuracy and reaction times in emotional processing and attention shifting. A 4-group MANOVA was calculated to determine whether the four groups differed in respect to RTs and accuracy measures for emotional processing and attention shifting. Subsequently, a post-hoc analysis was performed for participants’ RTs and accuracy.

5.4.3 Image data second level analysis

Similarly to previous studies (Prata, et al., 2012; Rehme, et al., 2011; Sanchez-Carrion, et al., 2008), a 2x2x2 factorial analysis was performed with the diagnosis of MDD (patients with MDD versus healthy controls) as the first, the Val66Met gene allele (Val homozygotes versus Met-carriers) as the second, and the type of the trial (emotional processing versus attention shifting from emotional processing) as the third factor. Participants’ age and gender were added as covariates of no interest.

A difference between Val homozygotes and Met-carriers was established. Contrasts were calculated to determine the differences between HC-Val and HC-Met, between HC-Val and MDD-Val, between HC-Met and MDD-Met, and between MDD-Val and MDD-Met. Contrasts between all groups were calculated for the emotion processing condition and the attention shifting condition separately.

The regions detected in the contrasts in general emotional processing and attention shifting, which were the main interest of the study (surviving the whole-brain false error discovery (FDR) cluster correction with p<0.05) are presented in results section. Automated anatomical labelling was used here to localize the significant results in a standard stereotactic space (template MNI).
5.5 Results

5.5.1 Behavioural results

5.5.1.1 General effect of the gene allele (3A)

No significant differences were found in behavioural correlates of emotional processing and attention shifting between homozygous Val subjects and Met carriers when the diagnosis was not taken into account. The results are presented in Table 28.

Table 28. Differences in accuracy and reaction times between subjects with homogenous Val allele (Val) and subjects carrying Met allele (Met) in emotion processing and attention shifting

<table>
<thead>
<tr>
<th>Behavioural characteristics (±SD)</th>
<th>Val (N=54)</th>
<th>Met (N=22)</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accuracy – emotion processing (% correct)</td>
<td>80 (±11.2)</td>
<td>77.6 (±12.6)</td>
<td>0.430</td>
</tr>
<tr>
<td>RTs – emotion processing (s)</td>
<td>1.4 (±0.33)</td>
<td>1.53 (±0.5)</td>
<td>0.184</td>
</tr>
<tr>
<td>Accuracy – attention shifting (% correct)</td>
<td>85.4 (±13.2)</td>
<td>80 (±17.7)</td>
<td>0.149</td>
</tr>
<tr>
<td>RTs – attention shifting (s)</td>
<td>1.42 (±0.33)</td>
<td>1.58 (±0.53)</td>
<td>0.203</td>
</tr>
</tbody>
</table>

Statistical significance set to p<0.05; *Statistically significant difference

5.5.1.2 Four group analysis and post hoc tests

The MANOVA test yielded significant differences for the four groups in terms of accuracy of emotional processing and attention shifting, and RTs in attention shifting. The results are presented in Table 29.

Subsequently the post-hoc analysis was performed. The results are presented in Table 30.
Table 29. Differences in accuracy and reaction times in emotion processing and attention shifting between groups distinguished by diagnosis of MDD and Val66Met polymorphism (F MANOVA)

<table>
<thead>
<tr>
<th>Behavioural characteristics (±SD)</th>
<th>MDD-Met (N=14)</th>
<th>MDD-Val (N=23)</th>
<th>HC-Met (N=8)</th>
<th>HC-Val (N=31)</th>
<th>p values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accuracy – emotion processing (% correct)</td>
<td>76.9 (±10.5)</td>
<td>74.8 (±13)</td>
<td>79.3 (±16.2)</td>
<td>83.7 (±8.1)</td>
<td>0.031*</td>
</tr>
<tr>
<td>RTs – emotion processing (s)</td>
<td>1.64 (±0.58)</td>
<td>1.5 (±0.32)</td>
<td>1.36 (±0.31)</td>
<td>1.33 (±0.32)</td>
<td>0.070</td>
</tr>
<tr>
<td>Accuracy – attention shifting (% correct)</td>
<td>76.2 (±17.7)</td>
<td>79.9 (±15.9)</td>
<td>86.1 (±16.6)</td>
<td>89.4 (±9.3)</td>
<td>0.017*</td>
</tr>
<tr>
<td>RTs – attention shifting (s)</td>
<td>1.78 (±0.56)</td>
<td>1.56 (±0.36)</td>
<td>1.27 (±0.31)</td>
<td>1.32 (±0.26)</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

MDD-Val – patients with major depressive disorder with two Val alleles in Val66Met gene, MDD-Met – patients with major depressive disorder with at least one Met allele in Val66Met gene, HC-Val – healthy controls with two Val alleles in Val66Met gene, HC-Met – healthy controls disorder with at least one Met allele in Val66Met gene. Statistical significance set to p<0.05; *Statistically significant difference.
5.5.1.2.1 Patients with MDD with homogenous Val allele vs healthy controls with homogenous Val allele (H3B)

The HC-Val were more accurate in emotional recognition than the MDD-Val. The results are presented in Table 30.

5.5.1.2.2 Patients with MDD carrying Met allele vs healthy controls carrying Met allele (H3C)

The HC-Met were quicker than the MDD-Met in attention shifting. The results are presented in Table 30.

5.5.1.2.3 Healthy controls with homogenous Val allele vs healthy controls carrying Met allele (H3D)

No significant differences between the HC-Val and the HC-Met in behavioural correlates of emotional processing and attention shifting were found (Table 30).

5.5.1.2.4 Patients with MDD with homogeneous Val allele vs patients with MDD carrying Met allele (H3E)

No significant differences between the MDD-Val and the MDD-Met in behavioural correlates of emotional processing and attention shifting were found (Table 30).
Table 30. Post-hoc comparisons in accuracy and reaction times for emotion processing and attention shifting between groups distinguished by diagnosis of MDD and Val66Met polymorphism

<table>
<thead>
<tr>
<th>Behavioural characteristics (p values)</th>
<th>HC-Val vs MDD-Val</th>
<th>HC-Met vs MDD-Met</th>
<th>HC-Val vs HC-Met</th>
<th>MDD-Val vs MDD-Met</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accuracy – emotion processing</td>
<td>0.025&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.948</td>
<td>0.748</td>
<td>0.965</td>
</tr>
<tr>
<td>RTs – emotion processing</td>
<td>0.368</td>
<td>0.344</td>
<td>0.997</td>
<td>0.707</td>
</tr>
<tr>
<td>Accuracy – attention shifting</td>
<td>0.077</td>
<td>0.389</td>
<td>0.935</td>
<td>0.872</td>
</tr>
<tr>
<td>RTs – attention shifting</td>
<td>0.089</td>
<td>0.014&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.985</td>
<td>0.338</td>
</tr>
</tbody>
</table>

MDD-Val – patients with major depressive disorder with two Val alleles in Val66Met gene, MDD-Met – patients with major depressive disorder with at least one Met allele in Val66Met gene, HC-Val – healthy controls with two Val alleles in Val66Met gene, HC-Met – healthy controls disorder with at least one Met allele in Val66Met gene

Statistical significance set to p<0.05; <sup>a</sup>Statistically significant difference
5.5.2 Functional MRI results

5.5.2.1 General effect of the Val66Met polymorphism (H3A)

5.5.2.1.1 Subjects with homogenous Val allele > subjects carrying Met allele

5.5.2.1.1.1 Emotional processing condition
In comparison to Met carrying subjects, the subjects with homogenous Val allele did not show any increase in the neural activation during emotional processing when the diagnosis was not considered.

5.5.2.1.1.2 Attention shifting condition
In comparison to Met carrying subjects, the subjects with homogenous Val allele did not show any increase in the neural activation during attention shifting when the diagnosis was not considered.

5.5.2.1.2 Subjects carrying Met allele > subjects with homogenous Val allele

5.5.2.1.2.1 Emotional processing condition
In comparison to Val homozygous subjects, the Met carrying subjects displayed a stronger neural activation in the right superior frontal gyrus and in the right middle frontal gyrus (Figure 31). These regions are presented in Table 31.
5.5.2.1.2.2 Attention shifting condition

In comparison to subjects with homogenous Val allele, the Met carrying subjects did not show any increase in the neural activation during attention shifting when the diagnosis was not considered.

The positive interaction between the diagnosis of MDD and the Val66Met polymorphism approached statistical significance, and was observed in the right fusiform gyrus. The regions are presented in Table 31.

**Table 31. Significant differences in neural activation during emotion processing and attention shifting between individuals at least one Met allele in Val66Met gene and individuals with two Val alleles in Val66Met gene with interaction between diagnosis and Val66Met polymorphism**

<table>
<thead>
<tr>
<th>Contrast</th>
<th>Cluster size (no. of voxels)</th>
<th>Cluster corrected P values</th>
<th>Region</th>
<th>MNI location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Met &gt; Val – emotion processing</td>
<td>98</td>
<td>0.048</td>
<td>right middle frontal gyrus</td>
<td>36 17 49</td>
</tr>
<tr>
<td>Positive interaction</td>
<td>28</td>
<td>0.057</td>
<td>right fusiform gyrus</td>
<td>33 -64 -2</td>
</tr>
</tbody>
</table>

Met – individuals with at least one Met allele in Val66Met polymorphism; Val – individuals with two Val alleles in Val66Met polymorphism

Results FDR cluster corrected; Statistical significance set to p<0.05
5.5.2.2 Patients with MDD with homogenous Val allele vs healthy controls with homogenous Val allele (H3B)

5.5.2.2.1 HC-Val > MDD-Val

5.5.2.2.1.1 Emotional processing condition

During emotional processing, the HC-Val, in comparison to the MDD-Val, displayed more neural activation in the left angular gyrus, in the left middle occipital gyrus, in the right insula, in the right precuneus and in the right supramarginal gyrus (Figure 32). The regions are presented in Table 32.

![Figure 32. Regions of stronger neural activation observed in the HC-Val, as compared to the MDD-Val, during emotional processing; FDR cluster corrected; scale represents t values.](image)

5.5.2.2.1.2 Attention shifting condition

While shifting attention from emotional processing, the HC-Val, in comparison to the MDD-Val, showed more neural activation in the right superior frontal gyrus, in the right postcentral gyrus, in right angular gyrus and in the right middle occipital gyrus (Figure 33). The regions are presented in Table 32.
Table 32. Significant differences in neural activation during emotional processing and attention shifting between the groups distinguished by diagnosis of MDD and Val66Met polymorphism

<table>
<thead>
<tr>
<th>Contrast</th>
<th>Process</th>
<th>Cluster size (no. of voxels)</th>
<th>Cluster corrected P values</th>
<th>Region</th>
<th>MNI location</th>
</tr>
</thead>
<tbody>
<tr>
<td>HC-Val &gt; MDD-Val</td>
<td>Emotional processing</td>
<td>97</td>
<td>0.047</td>
<td>left angular gyrus</td>
<td>-33 -58 22</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>left middle occipital gyrus</td>
<td>-27 -64 31</td>
</tr>
<tr>
<td></td>
<td></td>
<td>184</td>
<td>0.005</td>
<td>right insula</td>
<td>36 -34 28</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>right precuneus</td>
<td>24 -43 28</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>right supramarginal gyrus</td>
<td>45 -22 28</td>
</tr>
<tr>
<td></td>
<td>Attention shifting</td>
<td>73</td>
<td>0.049</td>
<td>right superior frontal gyrus</td>
<td>18 14 43</td>
</tr>
<tr>
<td></td>
<td></td>
<td>216</td>
<td>0.001</td>
<td>right postcentral gyrus</td>
<td>33 -40 28</td>
</tr>
<tr>
<td>MDD-Met &gt; HC-Met</td>
<td>Emotional processing</td>
<td>50</td>
<td>0.029</td>
<td>left caudate nucleus</td>
<td>-1 11 13</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>right caudate nucleus</td>
<td>6 2 7</td>
</tr>
<tr>
<td></td>
<td>Attention shifting</td>
<td>76</td>
<td>0.047</td>
<td>left caudate nucleus</td>
<td>-1 2 13</td>
</tr>
<tr>
<td>MDD-Met &gt; MDD-Val</td>
<td>Emotional processing</td>
<td>102</td>
<td>0.017</td>
<td>right middle frontal gyrus</td>
<td>42 17 40</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>right superior frontal gyrus</td>
<td>21 26 49</td>
</tr>
</tbody>
</table>

MDD-Val – patients with major depressive disorder with two Val alleles in Val66Met gene, MDD-Met – patients with major depressive disorder with at least one Met allele in Val66Met gene, HC-Val – healthy controls with two Val alleles in Val66Met gene, HC-Met – healthy controls disorder with at least one Met allele in Val66Met gene

Results FDR cluster corrected; Statistical significance set to p<0.05
5.5.2.2 MDD-Val > HC-Val

5.5.2.2.1 Emotional processing condition
In comparison to the HC-Val, the MDD-Val did not show any increase in the neural activation during emotional processing.

5.5.2.2.2 Attention shifting condition
In comparison to the HC-Val, the MDD-Val did not show any increase in the neural activation during shifting of attention from emotional processing.

5.5.2.3 Patients with MDD carrying Met allele vs healthy controls carrying Met allele (H3C)

5.5.2.3.1 HC-Met > MDD-Met

5.5.2.3.1.1 Emotional processing condition
In comparison to the MDD-Met, the HC-Met did not show any increase in the neural activation during emotional processing.

5.5.2.3.1.2 Attention shifting condition
In comparison to the HC-Val, the MDD-Val did not show any increase in the neural activation during shifting of attention from emotional processing.
5.5.2.3.2 MDD-Met > HC-Met

5.5.2.3.2.1 Emotional processing condition

During processing of emotional content, the MDD-Met displayed a heightened activation in contrast to the HC-Met in the bilateral caudate nucleus (Figure 34). The regions are presented in Table 32.

![Figure 34](image)

**Figure 34.** Regions of stronger neural activation observed in the MDD-Met, as compared to the HC-Met, during emotional processing; FDR cluster corrected; scale represents t values.

5.5.2.3.2.2 Attention shifting condition

During attention shifting, the MDD-Met displayed a heightened activation in the left caudate nucleus, as compared to the HC-Met (Figure 35). The regions are presented in Table 32.

![Figure 35](image)

**Figure 35.** Regions of stronger neural activation observed in the MDD-Met, as compared to the HC-Met, during attention shifting; FDR cluster corrected; scale represents t values.
5.5.2.4 Healthy controls with homogenous Val allele vs healthy controls carrying Met allele (H3D)

5.5.2.4.1 HC-Val > HC-Met

5.5.2.4.1.1 Emotional processing condition
In comparison to the HC-Met, the HC-Val did not show any increase in the neural activation during emotional processing.

5.5.2.4.1.2 Attention shifting condition
In comparison to the HC-Met, the HC-Val did not display any increase in the neural activation during attention shifting from emotional processing.

5.5.2.4.2 HC-Met > HC-Val

5.5.2.4.2.1 Emotional processing condition
In comparison to the HC-Val, the HC-Met did not demonstrate any increase in the neural activation during emotional processing.

5.5.2.4.2.2 Attention shifting condition
In comparison to the HC-Val, the HC-Met did not experience any increase in the neural activation during attention shifting from emotional processing.

5.5.2.5 Patients with MDD with homogeneous Val allele vs patients with MDD carrying Met allele (H3E)

5.5.2.5.1 MDD-Val > MDD-Met

5.5.2.5.1.1 Emotional processing condition
In comparison to the MDD-Met, the MDD-Val did not show any increase in the neural activation during emotional processing.
5.5.2.5.1.2 Attention shifting condition

In comparison to the MDD-Met, the MDD-Val did not show any increase in the neural activation during attention shifting from emotional processing.

5.5.2.5.2 MDD-Met > MDD-Val

5.5.2.5.2.1 Emotional processing condition

During processing of emotional information, the MDD-Met experienced more neural activation than the MDD-Val did in the right middle frontal gyrus and in the right superior frontal gyrus (Figure 36). The regions are presented in Table 32.

![Figure 36. Regions of stronger neural activation observed in the MDD-Met, as compared to the MDD-Val, during emotional processing; FDR cluster corrected; scale represents t values.](image)

5.5.2.5.2.2 Attention shifting condition

In comparison to the MDD-Val, the MDD-Met did not show any increase in the neural activation during attention shifting from emotional processing.

5.5.2.6 Summary of the results

The results of this study verify hypotheses 1 and 2 of the thesis. The summary of the results is presented in the Table 33.
Table 33. Summary of neural differences between groups distinguished by diagnosis of MDD and Val66Met polymorphism in attention shifting and emotion recognition

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Val vs Met (H3A)</td>
<td>- increased activation in the frontal lobe during emotion processing in Met individuals</td>
</tr>
<tr>
<td>HC-Val vs MDD-Met (H3B)</td>
<td>- decreased activation in the paralimbic group during emotion processing and attention shifting in MDD-Val</td>
</tr>
<tr>
<td>HC-Met vs MDD-Met (H3C)</td>
<td>- increased activation in the core limbic group during emotion processing and attention shifting in MDD-Met</td>
</tr>
<tr>
<td>HC-Val vs HC-Met (H3D)</td>
<td>- no differences observed</td>
</tr>
<tr>
<td>MDD-Val vs MDD-Met (H3E)</td>
<td>- increased activation in the frontal lobe during emotion processing in MDD-Met</td>
</tr>
</tbody>
</table>

MDD-Val – patients with major depressive disorder with two Val alleles in Val66Met gene, MDD-Met – patients with major depressive disorder with at least one Met allele in Val66Met gene, HC-Val – healthy controls with two Val alleles in Val66Met gene, HC-Met – healthy controls disorder with at least one Met allele in Val66Met gene

5.6 Discussion

The findings of Study 1 demonstrated that a factor associated with vulnerability to MDD can modulate changes in emotional regulation characteristic for an acute episode of the disease. In Study 2 it was examined whether polymorphism in the Val66Met gene, previously associated with BDNF secretion (Chen, et al., 2004) and vulnerability to the disease (Brunoni, et al., 2008; Castren, et al., 2007; Groves, 2007), modulated correlates of emotional regulation for patients with MDD and healthy controls. No previous study has tested how this modulation influenced mechanism of emotional regulation at behavioural and neural levels in both patients with MDD and healthy controls.

The examination pursued in this study is most important since BDNF coded by the Val66Met gene affects regions of the CNS and their depressive reaction in a most complex way (Brunoni, et al., 2008). Although an increase of BDNF concentration in the hippocampus and in the frontal lobes has been shown to alleviate depressive
symptoms (Brunoni, et al., 2008; Duman, 2005; Dwivedi, et al., 2003; Shirayama, et al., 2002), it can have a reversed effect in the midbrain structures (Berton, et al., 2006; Eisch, et al., 2003). It is therefore, important to understand exactly which brain areas are influenced by the Val66Met polymorphism during emotional regulation.

A difference between the individuals with homogenous Val allele and the individuals carrying a Met allele was indeed observed during emotional regulation in a region associated with high BDNF concentration (Binder & Scharfman, 2004; Castren, et al., 2007; Duman & Monteggia, 2006). The individuals carrying a Met allele experienced a stronger neural activation during emotional processing in the right frontal cortex. This area has been previously shown to have a disturbed functioning during stimulus categorization in non-clinical individuals with homogenous Met allele (Schofield, et al., 2009). However, the region was found to be less active in the healthy individuals with homogenous Met allele. Moreover, it was found that the healthy Met carriers displayed significant reductions in the volume of the area (Nemoto, et al., 2006; Pezawas, et al., 2004). This discrepancy between the results of this study and previous research can be explained by the fact that in this study both patients with MDD and healthy controls were examined, whereas similar studies in this area have tested only non-clinical population. This may indicate that the variation observed between genetically different patients with MDD may not be the same as the difference between the two groups of healthy controls.

The comparison between the HC-Val and the MDD-Val describes an acute episode of MDD when no Met allele is present. The Val/Val genotype is usually associated with a high concentration of BDNF in the CNS (Chen, et al., 2004). In this study, the MDD-Val were slower and less accurate in both emotional processing and attention shifting in comparison to the HC-Val. This finding links the difficulties of the MDD-Val with
impairments noted in a general population of patients with MDD (Berenbaum, et al., 2003; Bylsma, et al., 2008; Gotlib & Joormann, 2010).

Furthermore, and in accordance with the models of MDD under discussion here (Davidson, et al., 2002a; Drevets, 2001; Haldane & Frangou, 2006; Mayberg, 1997; Phillips, et al., 2003b), the MDD-Val experienced a lowered activation in the cortical compartment of the CNS. During emotional processing, the MDD-Val displayed a decrease of activation in the areas associated with visual attention and recognition, and classified as the visual association group (Andersen, et al., 2001; Binder, et al., 2003; Freese & Amaral, 2005; Hendler, et al., 2001; Kober, et al., 2008; Vuilleumier, et al., 2004). These areas are not commonly associated with activity of BDNF (Binder & Scharfman, 2004; Castren, et al., 2007; Duman & Monteggia, 2006). However, they are known to be involved in an emotional response to visual stimuli (Hendler, et al., 2001). In this sense the MDD-Val are similar to the MDD-FHP from Study 1, who experienced a similar decrease during emotional processing. Such a pattern of activation can be shared by many groups of patients with MDD. In reference to emotional regulation, this indicates that the MDD-Val may not perceive external emotional cues as highly important for their well-being (Desseilles, et al., 2009; Fu, et al., 2007).

Moreover, during emotional processing, the MDD-Val experienced a decrease of activation in the paralimbic group. The group is associated with recognition of one's own internal affective states, and is strongly involved in the informative aspect of emotional processing (Kober, et al., 2008). This result, does not actually confirm the predictions of the MDD models and their postulated increase of activation in the limbic compartment in patients with the disease (Davidson, et al., 2002a; Drevets, 2001; Haldane & Frangou, 2006; Mayberg, 1997; Phillips, et al., 2003b). However, the
alteration observed here does resemble the dissimilarity observed in Study 1 in respect to the MDD-FHN and the HC-FHN. The MDD-FHN also demonstrated a lowered activation in the right insula during emotional processing. Val homogenous allele, like negative family history of MDD, is associated with lesser vulnerability to the disease (Bueller, et al., 2006; Hunnerkopf, et al., 2007; Koolschijn, et al., 2009; Lonsdorf, et al., 2010; Nemoto, et al., 2006; Pezawas, et al., 2004; Porter, et al., 2003; Schumacher, et al., 2005; Szeszko, et al., 2005). This indicates that an acute episode of MDD without vulnerability is related to a weaker activation in areas responsible for the informative aspect in emotional processing (Lonsdorf, et al., 2010). In respect to emotional regulation, this seems to suggest that the MDD-Val are not as responsive to external emotional cues as healthy controls are.

During attention shifting, the MDD-Val displayed a decrease of activation in the areas similar to those with an altered functionality in emotional processing. However, in the study, an additional decrease was observed in the right frontal cortex – the area associated with attention shifts and executive control (Aron, et al., 2003; Aron, et al., 2004a; Aron, et al., 2004b; Badre, et al., 2005; Chikazoe, et al., 2007; Chong, et al., 2008; Corbetta & Shulman, 2002; Coull, et al., 2000; Dove, et al., 2000; Gabrieli, et al., 1998; Garavan, et al., 1999; Hampshire, et al., 2010; Hooker, et al., 2010; Liddle, et al., 2001; Macaluso & Patria, 2007; Martin & Chao, 2001; Moss, et al., 2005; Nee, et al., 2007; Poldrack, et al., 1999; Ridderinkhof, et al., 2004; Swick, et al., 2008; Wager, et al., 2005; Wagner, et al., 2001; Zhang, et al., 2004). This decrease suggests that the MDD-Val may be impaired in shifting attention from emotional processing. The alterations found in their performance in this study confirm this assumption. In previous studies, the decrease of volume in the area (Nemoto, et al., 2006; Pezawas, et al., 2004) and its altered functioning (Schofield, et al., 2009) has also been shown to be correlated
with the Val66Met polymorphism. In terms of emotional regulation, the MDD-Val may thus have difficulties with focusing on something else than emotional processing. Together with changes observed in emotional processing, the present results suggest that the MDD-Val will have difficulties focusing on an external environment.

An episode of MDD with the Met allele can be observed in comparisons between the HC-Met and the MDD-Met. In this study, the MDD-Met were slower than the HC-Met in shifting attention from emotional processing. Such a change has been previously noted in a general population of patients with MDD (Gotlib & Joormann, 2010), as well as in the MDD-FHP from Study 1. This alteration suggests that attention shifting is more costly for the MDD-Met than for the HC-Met. In fact, this is one of the characteristics of an MDD episode with Met allele.

An episode of MDD can also be observed in the neural correlates of emotional regulation. During emotional processing, the MDD-Met, in comparison to the HC-Met, experienced a stronger activation in the striatum. This region has been noted to participate in the visceral aspect of emotional experience and display (Edelman & Tononi, 2003; Hamann, et al., 2004; Horvitz, 2000; Jensen, et al., 2003; Kober, et al., 2008; Lenz, et al., 1995; O'Doherty, et al., 2004; Schultz, et al., 1997). The area forms a part of the core limbic group (Kober, et al., 2008). Its increased activation in patients with MDD is predicted by the models of the disease (Davidson, et al., 2002a; Drevets, 2001; Haldane & Frangou, 2006; Mayberg, 1997; Phillips, et al., 2003b). This was also observed in the MDD-FHN in Study 1 during emotional processing. Such a pattern of activation suggests that for the MDD-Met the visceral aspect of emotional experience is stronger than for the HC-Met (Longstaff, 2005).

The increase of activation in the affective arousal area (Edelman & Tononi, 2003; Hamann, et al., 2004; Horvitz, 2000; Jensen, et al., 2003; Lenz, et al., 1995; O'Doherty,
et al., 2004; Schultz, et al., 1997) is also observed in the MDD-Met during attention shifting. The MDD-Met overactivate the core limbic group (Kober, et al., 2008) when their task is to focus away from emotional processing. This may explain why the Met carrying patients display impairments in the process. It may also imply that the MDD-Met do not inhibit emotional reaction to the stimuli even when they are to shift their attention to non-affective cues.

Next, this point indicates that the midbrain structures are overactive in the MDD-Met in many aspects of emotional regulation. This activation seems to characterise the group, and at least partially explains the development of its depressive symptoms. The increase of activation in the region (Banner, et al., 2011) and its decreased volume (Nemoto, et al., 2006) have been associated with vulnerability to MDD linked to Met allele. It has further been noted that an increased level of BDNF observed in the region can produce depressive-like symptoms (Berton, et al., 2006; Eisch, et al., 2003). This suggests that MDD in the Met carriers group may be associated with an incorrect distribution of BDNF in this area. In addition, the activation in the region is known to stimulate the stress response of the HPA axis (Dawood, et al., 2007; Praag, et al., 2004). In other words, if the area is overactive, the stress response does not follow its usual course and does not recede after a time (Dawood, et al., 2007; Praag, et al., 2004). The area is thus systematically overactive in the MDD-Met during emotional regulation. Therefore, emotional regulation - the very process to promote homeostasis of an individual may endanger it with a constant stress reaction.

Finally, it has been found that the Val66Met polymorphism distinguishes two genetically unique groups of patients with MDD. The two groups differed in neural correlates of emotional processing. During emotional processing, and in comparison to the MDD-Val, the MDD-Met experienced an increased activation in the right DLPFC.
The region has been recognized as participating in categorization and inhibition of affective processing (Aron, et al., 2004a; Chikazoe, et al., 2007; Corbetta & Shulman, 2002; Garavan, et al., 1999; Hampshire, et al., 2010; Liddle, et al., 2001; Nee, et al., 2007). Its increased activation suggests a stronger neural top-down control over emotional arousal (Egner & Hirsch, 2005; Lévesque et al., 2003; Ochsner & Gross, 2005; Ochsner, et al., 2004b), and a greater adaptability to changes (Fletcher et al., 2001). The frontal regions, instead, have a strong connection with BDNF activity (Berton, et al., 2006; Eisch, et al., 2003; Nemoto, et al., 2006; Pezawas, et al., 2004). A high concentration of BDNF in the regions then promotes a better stress response and a stronger adaptability to change (Brunoni, et al., 2008; Dwivedi, et al., 2003). The MDD-Val in this study experienced a decrease activation in the right DLPFC, also when they were compared to the HC-Val. The disturbed functioning of the region may thus represent a characteristic change for the group, and be responsible, at least in part, for the MDD symptoms.

The BDNF gene has been shown to be important in describing different sub-types of clinical populations (Bath & Lee, 2006; Rybakowski et al., 2006). The psychiatric patients with Met allele (bipolar disorder and schizophrenia) have been found to be more impaired in tasks connected with working memory than the psychiatric patients with homogenous Val allele have. This study is thus the first to report on neural differences in emotional regulation between patients with MDD with homogenous Val alleles and patients with MDD carrying Met allele. The patients with two different genetic backgrounds differed in neither clinical features nor behavioural performance. However, it was suggested that the two groups may, in fact, follow their own pathways in developing MDD.
6 General conclusions

The findings of the two studies discussed in this research indicate that factors associated with vulnerability to MDD, such as family history of the disease and Val66Met gene polymorphism, do modulate differences between patients with MDD and healthy controls. First of all, such factors help distinguish between individuals with and without increased vulnerability to developing the disease (Duman, 2005; Fanous, et al., 2002; Haeffel & Grigorenko, 2007; Weissman, et al., 2006). The understanding of specific patterns of changes characteristic of people at risk of MDD can help explain such fundamental questions as which mechanisms bring about greater vulnerability to the disease, and under what circumstances this vulnerability triggers the disease (Bueller, et al., 2006; Nemoto, et al., 2006; Pezawas, et al., 2004; Szeszko, et al., 2005). Moreover, such changes point at a protective aspect at play, which allows people to stay resilient to the vulnerability (Duman, 2005; Glicken, 2006). Finally, the modulations associated with the vulnerability factors may help account for the different directions known for major depressive disorder and the various ways of its development (Holma, et al., 2011; Nierenberg, et al., 2007; Schumacher, et al., 2005).

Functional magnetic resonance imagining has been shown to be an extremely effective method of observing such modulations since in some cases the mechanisms of vulnerability may be present, but without behavioural manifestations (Wolfensberger, et al., 2008). Especially in those cases, fMRI enables us to investigate the neural mechanisms of vulnerability, resilience and differentiated development of MDD associated with risk factors (Amico, et al., 2011; Banner, et al., 2011; Boccardi, et al., 2010; Duman, 2005; Egan, et al., 2003; Evers, et al., 2006; Frodl, et al., 2010b; Hariri, et al., 2003; Kathmann, et al., 2003; Lau, et al., 2010; Monk, et al., 2008; Mukherjee, et al., 2011; Savitz & Drevets, 2009; Schofield, et al., 2009; Shirayama, et al., 2002; van
Emotional regulation has been known to represent a crucial ability disturbed during an acute episode of MDD (Eippert, et al., 2007; Gross & Muñoz, 1995; Yap, et al., 2007). There are models of the disease which indicate that neural disturbances in emotional regulation are, in fact, one of the key features of an MDD episode and pathogenesis (Davidson, et al., 2002a; Drevets, 2001; Haldane & Frangou, 2006; Mayberg, 1997; Phillips, et al., 2003b). The results of this study confirm that indeed basic mechanism of emotional regulation such as emotional processing and recognition, attention shifting and inhibition of emotional processing are altered during an acute episode of MDD. The alterations characteristic for the general population of patients with MDD include an accentuated visceral aspect of emotional display, a diminished informative aspect of affective experience and a decreased control over shifting attention from emotional processing.

The present study is unique in that it demonstrates that modulations caused by both family history of the disease and Val66Met gene polymorphism influence mechanisms of emotional regulation within and across the diagnosis of MDD. Therefore, separate patterns of emotional regulation can be observed for vulnerability, resilience and differentiated development of MDD connected with risk factors. In this study such patterns were detected in both behavioural and neural correlates of emotional regulation.

Patients with MDD without family history of the disease, representatives of an acute MDD episode without vulnerability, appear to experience a stronger visceral reaction to emotional states. However, their informative processing of external emotional cues is impaired, in comparison to healthy controls.
Patients with MDD with family history of the disease, who combine an acute episode with greater vulnerability, seem to experience the greatest impairments in attention shifting and inhibition of emotional processing. During such processes they tend to show a reduced activation in the areas responsible for executive control. They also display a stronger reaction in the neural regions involved in visceral aspect of emotional processing.

First-degree unaffected healthy relatives of patients with MDD are vulnerable to the disease, which is connected with their increased neural activation in emotional somatosensory areas during emotional processing and attention shifting. Their vulnerability to the disease is also hinted at a heightened activation in the regions associated with affective arousal during inhibition of emotional processing. However, this group utilizes a compensation mechanism, which aids their resilience to the vulnerability of the disease. The compensation mechanism concerned is associated with an increased activation of the regions responsible for various ways of control over emotional processing.

Patients with MDD with homogenous Val allele appear to experience a decrease in their visual reactivity to emotional stimuli and a lowered activation in the areas responsible for executive function. Patients with MDD carrying Met allele show a higher activation in the areas involved in the visceral aspect of emotional display.

In conclusion, this study is significant in demonstrating that both family history of MDD and Val66Met polymorphism distinguish two separate types of patients with MDD. Each of these groups suffers from its unique set of disturbances in emotional regulation. The patterns of changes characteristic for these endophenotypes of MDD are complex. However, this study has attempted to shed more light on this issue, and has contributed to our growing understanding of the clinical symptoms of the disease. It has
offered explanations for reasons why particular episodes of MDD are often so variable in course and outcomes (Beck & Alford, 2009).

It is also hoped that the findings reported in this study will help selecting appropriate therapies, targeting difficulties experienced by a particular individual with MDD in a more effective manner (Lisiecka, et al., 2011; Salvadore et al., 2010; Sheline et al., 2001). Behavioural and neural correlates of emotional regulation appear a very promising candidate for a biomarker associated with vulnerability to the disease. People who are characterized by susceptibility to MDD have more difficulties in switching their attention from negative stimuli. In the future such difficulties could be considered as a forewarning of the latent susceptibility to the disease. However, this would need further research.

Furthermore, this study – having explicated a number of changes associated with vulnerability to MDD – hopes to motivate further work in respect to improvements in prevention of major depressive disorder. A good understanding of the alterations and impairments associated with vulnerability promises to lead to more effective prevention strategies compensating for the specific difficulties encountered by individuals at risk in terms of emotional regulation. The behavioural and neural changes observed in this study indicate that certain clinical traits, such as a diminished ability to recognize rewarding and punishing situations, are likely to distinguish certain groups of healthy controls and patients with MDD. Patients with MDD with familial susceptibility to the disease display different alterations in emotional regulation than the patients without the increased familial risk of the disorder. This suggests that there may be different subtypes of MDD when the risk factors are taken into account. The next step then would be to verify the claims of this study in clinical settings and in everyday life of individuals. The possibilities of prevention strategies appear more than real, given that resilience to
MDD was found to be clearly associated with mechanism of compensation in this study.

6.1 Limitations

In this thesis it was argued that family history of major depressive disorder as well as Val66Met gene polymorphism are significant factors for the difference between patients with MDD and healthy controls. It was reasoned further that these factors alter the difference between patients with MDD and healthy controls in behavioural and neural correlates of emotional regulation. The findings also indicate that the two factors are associated with vulnerability and resilience to MDD. However, it is essential to consider the limitations of these findings and of the approach applied in the studies. First of all, the matter of statistical power needs to be taken into account. Statistical power in fMRI studies is dependent on several factors: the mean difference in signal observed between experimental and control conditions, intra and inter-subject variability in the observed mean difference of the signal and the sample size (Desmond & Glover, 2002). The observed difference in the BOLD signal is associated with correspondence between experimental and control condition (Desmond & Glover, 2002). Intra and inter-subject variability in the mean difference between the conditions is not directly manipulated by the researcher. However, the size of the examined groups can be adjusted to optimize a specific experimental design (Desmond & Glover, 2002). The differences between experimental and control conditions used in this thesis suggested that the optimal number of participants for testing the hypotheses was between 18 to 30 participants (Desmond & Glover, 2002). It is in accordance with previous literature concerning fMRI and vulnerability to MDD studies (Amico, et al., 2011; Monk, et al., 2008; Zhong et al., 2011), although smaller groups are used in similar studies as well (Boccardi, et al., 2010; Holmes, et al., 2010).
The study testing the importance of family history of MDD in the context of emotional regulation achieves an optimal number of participants in each of the examined groups. However, in the study establishing the role of Val66Met polymorphism in vulnerability and resilience to MDD the examined groups of Met allele carriers were smaller than expected. This reflects the distribution of the polymorphism in the population (Shimizu, et al., 2004), but also under-powers the study. Therefore, the study findings need to be treated with due caution.

Secondly, the possibility of Type I error needs to be taken into account. Functional magnetic resonance imaging due to its multiple statistical testing (multiple voxels) is prone to Type I error, that is the false rejection of the null hypothesis (Lieberman & Cunningham, 2009). In the studies presented in this thesis a number of measures to correct for multiple comparisons were taken. In the first part of the study investigating the role of family history of MDD in vulnerability and resilience to the disease FWE cluster-level correction with $\alpha=0.05$ was used to control Type I error. This method is considered to be one of the most efficient ways of correcting for false positive discoveries in MRI (Bullmore et al., 1999; Rubia et al., 2006). In the second part of this study, FWE voxel-level correction with $\alpha=0.05$, also an approved way of correcting for the Type I error (Lieberman & Cunningham, 2009), was applied. In this part of the study, voxel-level correction was used because less extensive yet more intense changes were the focus of the analysis due to high correspondence of experimental and control conditions. In the second study examining the role of Val66Met gene polymorphism in vulnerability and resilience to MDD, FDR cluster-level correction with $\alpha=0.05$ was used to control the Type I error. The FDR correction is less conservative than FWE correction (Lieberman & Cunningham, 2009). Whereas FWE correcting trends towards elimination of Type I error, FDR correction offers a more balanced approach towards
Type I and Type II errors (Lieberman & Cunningham, 2009). This helps to account for the smaller number of participants in study 2. However, it may also result in an increased probability of Type I error in the study 2. Additionally, it is necessary to consider Type I error in behavioural comparisons presented in the studies. Due to a complex nature of emotional regulation more than one behavioural contrast was explored in every group comparison. This signified increased likelihood of Type I error caused by multiple comparisons. If 95% level of certainty is assumed 5% of all the contrasts in any intergroup comparison may prove significant due to Type I error. However, if the number of significant contrasts in a given comparison is more than 5%, it indicates a significant finding not resulting from the error. The latter is true for most of the comparisons with significant behavioural contrasts presented in the thesis. For the rest it is necessary to bear in mind that their significance may be due to the Type I error.

Next, homogeneity of the control group needs to be taken into consideration. As the studies presented in this thesis indicate, vulnerability to MDD has more than one component. Taking into account all of the vulnerability factors in one analysis would not be practical due to their number. Therefore, when examining one of them, appropriate group sampling is important. When the vulnerability factor of interest is examined, random sampling in regards to other vulnerability factors should lead to their population-like distribution in the examined groups. Such was the case in the first study presented in this thesis (Shimizu, et al., 2004). However, in case of study 2 participants were not chosen randomly in regards to the family history of MDD – although in this study it was a vulnerability factor of no interest, whereas Val66Met gene polymorphism was a vulnerability factor of focus. This way the control group did not resemble the population in regards to distribution of family history of MDD. Unaffected first degree
relatives of patients with MDD differ significantly from healthy controls without family history of the disease. Therefore, their over-representation in the examined group may limit translatability of the results of study 2 to general population. It is, however, important to note that individuals with and without family history of MDD were equally distributed among the groups examined in study 2.

Finally, it needs to be stated that the differences observed between the groups in both studies do not exhaust all possible differences between examined groups in emotional regulation. Attention shifting with inhibition and emotional recognition are two processes selected from a wider and complex phenomenon that is emotional regulation. If different processes constituting emotional regulation were to be examined the results of the presented studies might have been different.
7 References


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8 Appendix

8.1 Study description for participants and a consent form

Title of research study:
Structural and functional integrity of the brain with respect to gene-environmental interactions in patients with major depression and healthy controls

This study and this consent form have been explained to me. My doctor has answered all my questions to my satisfaction. I believe I understand what will happen if I agree to be part of this study.

I have read, or had read to me, this consent form. I have had the opportunity to ask questions and all my questions have been answered to my satisfaction. I freely and voluntarily agree to be part of this research study, though without prejudice to my legal and ethical rights. I have received a copy of this agreement.

PLEASE TICK YOUR RESPONSE IN THE APPROPRIATE BOX

- I have read and understood the attached Participant Information Leaflet
  Yes ☐ No ☐
- I have had the opportunity to ask questions and to discuss the study
  Yes ☐ No ☐
- I have received satisfactory answers to all my questions
  Yes ☐ No ☐
- I have received enough information about this study
  Yes ☐ No ☐
- I understand that I am free to withdraw from the study at any time without giving a reason and without this affecting my future medical care
  Yes ☐ No ☐
- I agree to take part in this study without prejudice to my legal or ethical rights
  Yes ☐ No ☐
- I filled out the MRI screening form
  Yes ☐ No ☐

PARTICIPANT’S NAME:

PARTICIPANT’S SIGNATURE:

Date:
Date on which the participant was first furnished with this form:
Where the participant is incapable of comprehending the nature, significance and scope of the consent required, the form must be signed by a person competent to give consent to his or her participation in the research study (other than a person who applied to undertake or conduct the study). If the subject is a minor (under 18 years old) the signature of parent or guardian must be obtained:

NAME OF CONSENTOR, PARENT or GUARDIAN:  
SIGNATURE:  
RELATION TO PARTICIPANT:  

Where the participant is capable of comprehending the nature, significance and scope of the consent required, but is physically unable to sign written consent, signatures of two witnesses present when consent was given by the participant to a registered medical practitioner treating him or her for the illness.

NAME OF FIRST WITNESS:  
SIGNATURE:  
NAME OF SECOND WITNESS:  
SIGNATURE:  

Statement of investigator's responsibility: I have explained the nature, purpose, procedures, benefits, risks of, or alternatives to, this research study. I have offered to answer any questions and fully answered such questions. I believe that the participant understands my explanation and has freely given informed consent.

Physician's signature:  
Date:  

(Keep the original of this form in the participant's medical record, give one copy to the participant, keep one copy in the investigator's records, and send one copy to the sponsor (if there is a sponsor).)
8.2 Instructions for the task and a consent form

Instructions and consent form

You are going to see some pictures – each one for two seconds. Some of them will be emotionally positive, some emotionally neutral and some emotionally negative. All of them will have rectangular shape. Sometimes the rectangular will be placed vertically (portrait) and sometimes horizontally (landscape).

You are going to answer a question about each picture.

1. First you are going to see a picture. Please look at it.

2. Then the question will appear on the screen to inform you which task to do.

   There are 5 types of questions:

   a) Was it positive?
   b) Was it negative?
   c) Was it neutral?
   d) Was it vertical?
   e) Was it horizontal?

   The question refers to the pictures that is before it. In each trial you are going to answer only one of those questions.

3. Your task is to decide if the correct answer is “Yes” or “No”.

   a) For “Yes” you must press the button under your index finger.
   b) For “No” you must press the button under your middle finger.

The task consists of 180 trials and takes about 18 minutes to complete.

If you have any questions please do not hesitate to ask us.

Thank you.
I confirm that I have read the instructions of the task and that I am familiar with it. I consciously and voluntarily agree to participate in it.

........................................
(Date)

........................................
(Signature)
8.3 Hamilton Depression Rating Scale

The Hamilton Rating Scale for Depression (to be administered by a health care professional)

Patient's Name

Date of Assessment

To rate the severity of depression in patients who are already diagnosed as depressed, administer this questionnaire. The higher the score, the more severe the depression.

For each item, write the correct number on the line next to the item. (Only one response per item)

1. DEPRESSED MOOD (Sadness, hopeless, helpless, worthless)
   0= Absent
   1= These feeling states indicated only on questioning
   2= These feeling states spontaneously reported verbally
   3= Communicates feeling states non-verbally—i.e., through facial expression, posture, voice, and tendency to weep
   4= Patient reports VIRTUALLY ONLY these feeling states in his spontaneous verbal and non-verbal communication

2. FEELINGS OF GUILT
   0= Absent
   1= Self reproach, feels he has let people down
   2= Ideas of guilt or rumination over past errors or sinful deeds
   3= Present illness is a punishment. Delusions of guilt
   4= Hears accusatory or denunciatory voices and/or experiences threatening visual hallucinations

3. SUICIDE
   0= Absent
   1= Feels life is not worth living
   2= Wishes he were dead or any thoughts of possible death to self
   3= Suicidal ideas or gesture
   4= Attempts at suicide (any serious attempt rates 4)

4. INSOMNIA EARLY
   0= No difficulty falling asleep
   1= Complains of occasional difficulty falling asleep—i.e., more than 1/2 hour
   2= Complains of nightly difficulty falling asleep

5. INSOMNIA MIDDLE
   0= No difficulty
   1= Patient complains of being restless and disturbed during the night
   2= Waking during the night—any getting out of bed rates 2 (except for purposes of voiding)

6. **INSOMNIA LATE**
   - 0 = No difficulty
   - 1 = Waking in early hours of the morning but goes back to sleep
   - 2 = Unable to fall asleep again if he gets out of bed

7. **WORK AND ACTIVITIES**
   - 0 = No difficulty
   - 1 = Thoughts and feelings of incapacity, fatigue or weakness related to activities; work or hobbies
   - 2 = Loss of interest in activity; hobbies or work—either directly reported by patient, or indirect in listlessness, indecision and vacillation (feels he has to push self to work or activities)
   - 3 = Decrease in actual time spent in activities or decrease in productivity
   - 4 = Stopped working because of present illness

8. **RETARDATION: PSYCHOMOTOR** (Slowness of thought and speech; impaired ability to concentrate; decreased motor activity)
   - 0 = Normal speech and thought
   - 1 = Slight retardation at interview
   - 2 = Obvious retardation at interview
   - 3 = Interview difficult
   - 4 = Complete stupor

9. **AGITATION**
   - 0 = None
   - 1 = Fidgetiness
   - 2 = Playing with hands, hair, etc.
   - 3 = Moving about, can't sit still
   - 4 = Hand wringing, nail biting, hair-pulling, biting of lips

10. **ANXIETY (PSYCHOLOGICAL)**
    - 0 = No difficulty
    - 1 = Subjective tension and irritability
    - 2 = Worrying about minor matters
    - 3 = Apprehensive attitude apparent in face or speech
    - 4 = Fears expressed without questioning

11. **ANXIETY SOMATIC** Physiological concomitants of anxiety, (i.e., effects of autonomic overactivity, "butterflies," indigestion, stomach cramps, belching, diarrhea, palpitations, hyperventilation, paresthesia, sweating, flushing, tremor, headache, urinary frequency). Avoid asking about possible medication side effects (i.e., dry mouth, constipation)
    - 0 = Absent
    - 1 = Mild
    - 2 = Moderate
    - 3 = Severe
    - 4 = Incapacitating
12. SOMATIC SYMPTOMS (GASTROINTESTINAL)
   0 = None
   1 = Loss of appetite but eating without encouragement from others. Food intake about normal
   2 = Difficulty eating without urging from others. Marked reduction of appetite and food intake

13. SOMATIC SYMPTOMS GENERAL
   0 = None
   1 = Heaviness in limbs, back or head. Backaches, headache, muscle aches. Loss of energy and fatigability
   2 = Any clear-cut symptom rates 2

14. GENITAL SYMPTOMS (Symptoms such as: loss of libido; impaired sexual performance; menstrual disturbances)
   0 = Absent
   1 = Mild
   2 = Severe

15. HYPOCHONDRIASIS
   0 = Not present
   1 = Self-absorption (bodily)
   2 = Preoccupation with health
   3 = Frequent complaints, requests for help, etc.
   4 = Hypochondriacal delusions

16. LOSS OF WEIGHT
   A. When rating by history:
      0 = No weight loss
      1 = Probably weight loss associated with present illness
      2 = Definite (according to patient) weight loss
      3 = Not assessed

17. INSIGHT
   0 = Acknowledges being depressed and ill
   1 = Acknowledges illness but attributes cause to bad food, climate, overwork, virus, need for rest, etc.
   2 = Denies being ill at all

18. DIURNAL VARIATION
   A. Note whether symptoms are worse in morning or evening. If NO diurnal variation, mark none
      0 = No variation
      1 = Worse in A.M.
      2 = Worse in P.M.
   B. When present, mark the severity of the variation. Mark "None" if NO variation
      0 = None
      1 = Mild
      2 = Severe
19. DEPERSONALIZATION AND DEREAUZATION (Such as: Feelings of unreality; Nihilistic ideas)

0 = Absent
1 = Mild
2 = Moderate
3 = Severe
4 = Incapacitating

20. PARANOID SYMPTOMS

0 = None
1 = Suspicious
2 = Ideas of reference
3 = Delusions of reference and persecution

21. OBSESSIONAL AND COMPULSIVE SYMPTOMS

0 = Absent
1 = Mild
2 = Severe

Total Score ___________
8.4 Montgomery-Asberg Depression Rating Scale

Montgomery-Åsberg Depression Rating Scale (MADRS)
Montgomery-Åsberg Depression Rating Scale (MADRS)

1. Apparent sadness
   Representing despondency, gloom and despair (more than just ordinary transient low spirits), reflected in speech, facial expression, and posture. Rate by depth and inability to brighten up.

   | 0. = No sadness. |
   | 2. = Looks dispirited but does brighten up without difficulty. |
   | 4. = Appears sad and unhappy most of the time. |

2. Reported sadness
   Representing reports of depressed mood, regardless of whether it is reflected in appearance or not. Includes low spirits, despondency or the feeling of being beyond help and without hope.

   | 0. = Occasional sadness in keeping with the circumstances. |
   | 2. = Sad or low but brightens up without difficulty. |
   | 4. = Pervasive feelings of sadness or gloominess. The mood is still influenced by external circumstances. |
   | 6. = Continuous or unvarying sadness, misery or despondency. |
Montgomery-Åsberg Depression Rating Scale (MADRS)

3. Inner tension
Representing feelings of inner tension, edginess, inner turmoil, mental tension mounting to either panic, dread or anguish. Rate according to intensity, frequency, duration and the extent of reassurance called for.

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0.</td>
<td>Placid. Only fleeting inner tension.</td>
</tr>
<tr>
<td>2.</td>
<td>Occasional feelings of edginess and ill-defined discomfort.</td>
</tr>
<tr>
<td>4.</td>
<td>Continuous feelings of inner tension or intermittent panic which the patient can only master with some difficulty.</td>
</tr>
<tr>
<td>6.</td>
<td>Unrelenting dread or anguish. Overwhelming panic.</td>
</tr>
</tbody>
</table>

4. Reduced sleep
Representing the experience of reduced duration or depth of sleep compared to the subject's own normal pattern when well.

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
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<tbody>
<tr>
<td>0.</td>
<td>Sleeps as usual.</td>
</tr>
<tr>
<td>2.</td>
<td>Slight difficulty dropping off to sleep or slightly reduced, light or fitful sleep.</td>
</tr>
<tr>
<td>4.</td>
<td>Sleep reduced or broken by at least 2 hours.</td>
</tr>
<tr>
<td>6.</td>
<td>Less than 2 or 3 hours sleep.</td>
</tr>
</tbody>
</table>
Montgomery-Åsberg Depression Rating Scale (HAMRS)

5. Reduced appetite
Representing the feeling of a loss of appetite compared with when well. Rate by loss of
desire for food or the need to force oneself to eat.

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.</td>
<td>Normal or increased appetite.</td>
</tr>
<tr>
<td>2.</td>
<td>Slightly reduced appetite.</td>
</tr>
<tr>
<td>4.</td>
<td>No appetite. Food is tasteless.</td>
</tr>
<tr>
<td>6.</td>
<td>Needs persuasion to eat at all.</td>
</tr>
</tbody>
</table>

6. Concentration difficulties
Representing difficulties in collecting one's thoughts mounting to an incapacitating lack of
concentration. Rate according to intensity, frequency, and degree of incapacity produced.

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.</td>
<td>No difficulties in concentrating.</td>
</tr>
<tr>
<td>2.</td>
<td>Occasional difficulties in collecting one's thoughts.</td>
</tr>
</tbody>
</table>
| 4.    | Difficulties in concentrating and sustaining thought which reduces ability to
       read or hold a conversation.                                              |
| 6.    | Unable to read or converse without great difficulty.                         |
### Montgomery-Åsberg Depression Rating Scale (MADRS)

#### 7. Lassitude
Representing difficulty in getting started or slowness in initiating and performing everyday activities.

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>= Hardly any difficulty in getting started. No sluggishness.</td>
</tr>
<tr>
<td>2</td>
<td>= Difficulties in starting activities.</td>
</tr>
<tr>
<td>4</td>
<td>= Difficulties in starting simple routine activities, which are carried out with effort.</td>
</tr>
<tr>
<td>6</td>
<td>= Complete lassitude. Unable to do anything without help.</td>
</tr>
</tbody>
</table>

#### 8. Inability to feel
Representing the subjective experience of reduced interest in the surroundings, or activities that normally give pleasure. The ability to react with adequate emotion to circumstances or people is reduced.

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>= Normal interest in the surroundings and in other people.</td>
</tr>
<tr>
<td>2</td>
<td>= Reduced ability to enjoy usual interests.</td>
</tr>
<tr>
<td>4</td>
<td>= Loss of interest in the surroundings. Loss of feelings for friends and acquaintances.</td>
</tr>
<tr>
<td>6</td>
<td>= The experience of being emotionally paralysed, inability to feel anger, grief or pleasure and a complete or even painful failure to feel for close relatives and friends.</td>
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[www.cnsforum.com](http://www.cnsforum.com)
9. Pessimistic thoughts
Representing thoughts of guilt, inferiority, self-reproach, sinfulness, remorse and ruin.

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>= No pessimistic thoughts.</td>
</tr>
<tr>
<td>2</td>
<td>= Fluctuating ideas of failure, self-reproach or self-depreciation.</td>
</tr>
<tr>
<td>4</td>
<td>= Persistent self-accusation, or definite but still rational ideas of guilt or sin. Increasingly pessimistic about the future.</td>
</tr>
<tr>
<td>6</td>
<td>= Delusions of ruin, remorse or irredeemable sin. Self-accusations, which are absurd and unshakable.</td>
</tr>
</tbody>
</table>

10. Suicidal thoughts
Representing the feeling that life is not worth living, that a natural death would be welcome, suicidal thoughts, and preparations for suicide. Suicide attempts should not in themselves influence the rating.

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>= Enjoys life or takes it as it comes.</td>
</tr>
<tr>
<td>2</td>
<td>= Weary of life. Only fleeting suicidal thoughts.</td>
</tr>
<tr>
<td>4</td>
<td>= Probably better off dead. Suicidal thoughts are common, and suicide is considered as a possible solution, but without specific plans or intention.</td>
</tr>
<tr>
<td>6</td>
<td>= Explicit plans for suicide when there is an opportunity. Active preparations for suicide.</td>
</tr>
</tbody>
</table>
8.5 Beck Depression Inventory

Name: ______________________________________________ Marital Status: ___________ Age: _______ Sex: ________
Occupation: _________________________________________ Education: ________________________________________

Instructions: This questionnaire consists of 21 groups of statements. Please read each group of statements carefully, and
then pick out the one statement in each group that best describes the way you have been feeling during the past two
weeks, including today. Circle the number beside the statement you have picked. If several statements in the group
seem to apply equally well, circle the highest number for that group. Be sure that you do not choose more than one
statement for any group, including Item 16 (Changes in Sleeping Pattern) or Item 18 (Changes in Appetite).

<table>
<thead>
<tr>
<th>Group</th>
<th>Statement</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Sadness</td>
<td>0 I do not feel sad. 1 I feel sad much of the time. 2 I am sad all the time. 3 I am so sad or unhappy that I can't stand it.</td>
</tr>
<tr>
<td>2. Pessimism</td>
<td>0 I am not discouraged about my future. 1 I feel more discouraged about my future than I used to be. 2 I do not expect things to work out for me. 3 I feel my future is hopeless and will only get worse.</td>
</tr>
<tr>
<td>3. Past Failure</td>
<td>0 I do not feel like a failure. 1 I have failed more than I should have. 2 As I look back, I see a lot of failures. 3 I feel I am a total failure as a person.</td>
</tr>
<tr>
<td>4. Loss of Pleasure</td>
<td>0 I get as much pleasure as I ever did from the things I enjoy. 1 I don't enjoy things as much as I used to. 2 I get very little pleasure from the things I used to enjoy. 3 I can't get any pleasure from the things I used to enjoy.</td>
</tr>
<tr>
<td>5. Guilty Feelings</td>
<td>0 I don't feel particularly guilty. 1 I feel guilty over many things I have done or should have done. 2 I feel quite guilty most of the time. 3 I feel guilty all of the time.</td>
</tr>
<tr>
<td>6. Punishment Feelings</td>
<td>0 I don’t feel I am being punished. 1 I feel I may be punished. 2 I expect to be punished. 3 I feel I am being punished.</td>
</tr>
<tr>
<td>7. Self-Dislike</td>
<td>0 I feel the same about myself as ever. 1 I have lost confidence in myself. 2 I am disappointed in myself. 3 I dislike myself.</td>
</tr>
<tr>
<td>8. Self-Criticalness</td>
<td>0 I don’t criticize or blame myself more than usual. 1 I am more critical of myself than I used to be. 2 I criticize myself for all of my faults. 3 I blame myself for everything bad that happens.</td>
</tr>
<tr>
<td>9. Suicidal Thoughts or Wishes</td>
<td>0 I don’t have any thoughts of killing myself. 1 I have thoughts of killing myself, but I would not carry them out. 2 I would like to kill myself. 3 I would kill myself if I had the chance.</td>
</tr>
<tr>
<td>10. Crying</td>
<td>0 I don’t cry anymore than I used to. 1 I cry more than I used to. 2 I cry over every little thing. 3 I feel like crying, but I can’t.</td>
</tr>
</tbody>
</table>

Subtotal Page 1 Continued on Back
<table>
<thead>
<tr>
<th>11. Agitation</th>
<th>17. Irritability</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 I am no more restless or wound up</td>
<td>0 I am no more irritable than usual.</td>
</tr>
<tr>
<td>than usual.</td>
<td>1 I am more irritable than usual.</td>
</tr>
<tr>
<td>1 I feel more restless or wound up</td>
<td>2 I am much more irritable than</td>
</tr>
<tr>
<td>than usual.</td>
<td>usual.</td>
</tr>
<tr>
<td>2 I am so restless or agitated that</td>
<td>3 I am irritable all the time.</td>
</tr>
<tr>
<td>it's hard to stay still.</td>
<td></td>
</tr>
<tr>
<td>3 I am so restless or agitated that</td>
<td></td>
</tr>
<tr>
<td>I have to keep moving or doing</td>
<td></td>
</tr>
<tr>
<td>something.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>12. Loss of Interest</th>
<th>18. Changes in Appetite</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 I have not lost interest in other</td>
<td>0 I have not experienced any change</td>
</tr>
<tr>
<td>people or activities.</td>
<td>in my appetite.</td>
</tr>
<tr>
<td>1 I am less interested in other</td>
<td>1a My appetite is somewhat less than</td>
</tr>
<tr>
<td>people or things than before.</td>
<td>usual.</td>
</tr>
<tr>
<td>2 I have lost most of my interest in</td>
<td>1b My appetite is somewhat greater</td>
</tr>
<tr>
<td>other people or things.</td>
<td>than usual.</td>
</tr>
<tr>
<td>3 It's hard to get interested in</td>
<td>2a My appetite is much less than</td>
</tr>
<tr>
<td>anything.</td>
<td>before.</td>
</tr>
<tr>
<td></td>
<td>2b My appetite is much greater than</td>
</tr>
<tr>
<td></td>
<td>usual.</td>
</tr>
<tr>
<td></td>
<td>3a I have no appetite at all.</td>
</tr>
<tr>
<td></td>
<td>3b I crave food all the time.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>13. Indecisiveness</th>
<th>19. Concentration Difficulty</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 I make decisions about as well as</td>
<td>0 I can concentrate as well as</td>
</tr>
<tr>
<td>ever.</td>
<td>usual.</td>
</tr>
<tr>
<td>1 I find it more difficult to make</td>
<td>1 I can't concentrate as well as</td>
</tr>
<tr>
<td>decisions than usual.</td>
<td>usual.</td>
</tr>
<tr>
<td>2 I have much greater difficulty in</td>
<td>2 It's hard to keep my mind on</td>
</tr>
<tr>
<td>making decisions than I used to.</td>
<td>anything for very long.</td>
</tr>
<tr>
<td>3 I have trouble making any decisions.</td>
<td>3 I find I can't concentrate on</td>
</tr>
<tr>
<td></td>
<td>anything.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>14. Worthlessness</th>
<th>20. Tiredness or Fatigue</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 I do not feel I am worthless.</td>
<td>0 I am no more tired or fatigued</td>
</tr>
<tr>
<td>1 I don't consider myself as</td>
<td>than usual.</td>
</tr>
<tr>
<td>worthwhile and useful as I used to.</td>
<td>1 I get more tired or fatigued more</td>
</tr>
<tr>
<td>2 I feel more worthless as compared</td>
<td>easily than usual.</td>
</tr>
<tr>
<td>to other people.</td>
<td>2 I am too tired or fatigued to do</td>
</tr>
<tr>
<td>3 I feel utterly worthless.</td>
<td>a lot of the things I used to do.</td>
</tr>
<tr>
<td></td>
<td>3 I am too tired or fatigued to do</td>
</tr>
<tr>
<td></td>
<td>most of the things I used to do.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0 I have as much energy as ever.</td>
<td>0 I have not noticed any recent</td>
</tr>
<tr>
<td>1 I have less energy than I used to</td>
<td>change in my interest in sex.</td>
</tr>
<tr>
<td>have.</td>
<td>1 I am less interested in sex than</td>
</tr>
<tr>
<td>2 I don't have enough energy to do</td>
<td>I used to be.</td>
</tr>
<tr>
<td>very much.</td>
<td>2 I am much less interested in sex</td>
</tr>
<tr>
<td>3 I don't have enough energy to do</td>
<td>now.</td>
</tr>
<tr>
<td>anything.</td>
<td>3 I have lost interest in sex</td>
</tr>
<tr>
<td></td>
<td>completely.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>16. Changes in Sleeping Pattern</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0 I have not experienced any change</td>
<td></td>
</tr>
<tr>
<td>in my sleeping pattern.</td>
<td></td>
</tr>
<tr>
<td>1a I sleep somewhat more than usual.</td>
<td></td>
</tr>
<tr>
<td>1b I sleep somewhat less than usual.</td>
<td></td>
</tr>
<tr>
<td>2a I sleep a lot more than usual.</td>
<td></td>
</tr>
<tr>
<td>2b I sleep a lot less than usual.</td>
<td></td>
</tr>
<tr>
<td>3a I sleep most of the day.</td>
<td></td>
</tr>
<tr>
<td>3b I wake up 1-2 hours early and can't</td>
<td></td>
</tr>
<tr>
<td>get back to sleep.</td>
<td></td>
</tr>
</tbody>
</table>

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8.6 Edinburgh Handedness Inventory

<table>
<thead>
<tr>
<th>Surname</th>
<th>Given Name</th>
<th>Date of Birth</th>
<th>Sex</th>
<th>LEFT</th>
<th>RIGHT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Please indicate your preferences in the use of hands in the following activities by putting + in the appropriate column. Where the preference is so strong that you would never try to use the other hand unless absolutely forced to, put + + . If in any case you are really indifferent put + in both columns.

Some of the activities require both hands. In those cases the part of the task, or object, for which hand preference is wanted is indicated in brackets.

Please try to answer all the questions, and only leave a blank if you have no experience at all of the object or task.

<table>
<thead>
<tr>
<th></th>
<th>LEFT</th>
<th>RIGHT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Writing</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Drawing</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Throwing</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Scissors</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Toothbrush</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Knife (without fork)</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Spoon</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Broom (upper hand)</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Striking Match (match)</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Opening box (lid)</td>
<td></td>
</tr>
</tbody>
</table>

1. Which foot do you prefer to kick with?
2. Which eye do you use when reading singly or in pairs?

L.O. Leave these spaces blank. DECILE

MARCH 1970
8.7 Examples of pictures from IAPS database used in the task

8.7.1 Positive horizontal

![Image of a baby](image1)

![Image of pasta](image2)
8.7.2 Positive vertical
8.7.3 Negative horizontal
8.7.4 Negative vertical
8.7.5 Neutral horizontal
8.7.6 Neutral vertical
Publications

Articles


Posters


biomarker for treatment outcome with mirtazapine and venlafaxine. 10th World Congress of Biological Psychiatry 2011, Prague, Czech Republic


Contributed talks

an fMRI study. Joint Conference of the Czech and Slovak Neuroscience Societies 2011, Slovak Academy of Science, Smolenice, Slovakia