The Serotonin Transporter Polymorphism in Cognition: Intermediate Phenotypes associated with Emotion Regulation and Brain Function

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List of Papers

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List of Abbreviations

5-HT	5-Hydroxytryptamine, Serotonin
5-HTT	5-Hydroxytryptamine Transporter
5-HTTLPR	5-Hydroxytryptamine Transporter Linked Polymorphic Region
DTI	Diffusion Tensor Imaging
MRI	Magnetic Resonance Imaging
fMRI	functional Magnetic Resonance Imaging
MDD	Major Depressive Disorder
DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
mRNA	Messenger ribonucleic acid
SERT	Serotonin transporter
SNP	Single nucleotide polymorphism
SSRI	Selective serotonin reuptake Inhibitors
ADHD	Attention- deficit/Hyperactivity Disorder
VNTR	Variable number of tandem repeats
BDNF	Brain derived neurotrophic factor
GABA	Gamma amino butyric acid
ACC	Anterior Cingulate Cortex
PFC	Prefrontal cortex
EPI	Echo-planar imaging
BOLD	Blood-oxygen-level-dependent
FA	Fractional anisotropy
UF	Uncinate fascicule
VLPFC	Ventrolateral prefrontal cortex
SLF	Superior longitudinal fascicule

Background and Context

The Theme of this Thesis

Symptoms of major depression (MDD) have a serious impact on an individual's capacity to cope with the fundamental demands of modern society. In the year 2000, depression was the fourth leading contributor to the global burden of disease. The World Health Organization predicted major depression will rank second by 2020 (WHO, 2011). Major depression has a severe impact on fundamental human instincts and basic motivation for survival. Self-preservation, the maternal instinct, sexual instinct and pleasure instinct may be distraught or reversed. Some theories have linked the manifestation of depression, such as hopelessness, loss of motivation, self-criticism, and suicidal wishes to a systematic cognitive bias in information processing leading to selective attention to negative aspects of experiences, negative interpretations, and blocking of positive events and memories (A. T. Beck, 2008). The concept of cognitive reactivity has been discussed in mood intervention research (A. T. Beck, 2008). Following these mood interventions, clinically vulnerable subjects have reported more dysfunctional attitudes, negative cognitive biases, and erosion of normal positive biases compared to non vulnerable subjects (Scher, Ingram, & Segal, 2005). Cognitive reactivity may interact with stressful life events and cause depressive symptomatology. The concept of cognitive vulnerability, however, is descriptive and does not address why certain individuals are more reactive to negative environmental stimuli than others.

The search for specific genetic sequence variations that increase one`s risk of developing complex disorders has become one of the most researched fields in cognitive and affective neuroscience. Recently, the combination of multiple methods on a structural-, functional- and system level has contributed substantially to our understanding of the interplay between molecular biology, brain function and behaviour. Symptoms associated with major depression are among the most common targets of this type of research. Individual differences in the human brain's functional and structural connectivity could strongly affect one's ability to adaptively process emotion.

One of the major challenges in the search for potential underlying mechanisms is the distinction between coincidental versus instrumental components. Altered or biased emotion processing may be either a precursor or an artifact of the depressive state. Herein, the studies of healthy individuals at environmental and/or genetic risk, may uncover the underlying instrumental mechanisms that lead to depressive symtpomatology. A second aim is to explore

to which extent these potential intermediate phenotypes mimic cognitive deficits observed in MDD and/or after MDD remission.

We know that multiple genetic and nongenetic factors are involved in these complex interplays and that the searches for a dominant or recessive gene, such as a "blue gene", probably represent an erroneous simplification of the actual processes involved. The conceptualisation of phenotypes refers to measurable, heritable categories and dimensions. Endophenotypes refers to biological markers and may be closer to the level at which genes operate than more complex and heterogeneous behavioural measures or clinical phenotypes (Gottesman & Gould, 2003). Imaging genetics has the potential to integrate neuroimaging and molecular genetics to examine the structural and functional correlates of common genetic variation in the human brain. Neuropsychological measures probably represent an intermediate level and may be combined with neuroimaging data, behavioural data, clinical data, or a combination of these.

The current thesis explores the functional polymorphism located in the promoter region (5-HTTLPR) of the transporter gene (SLC6A4) in intermediate phenotypes. The presence of a variable number of tandem repeats (VNTR) generates either short (S) or long (L) variant alleles, directing low or high transcriptional activity, respectively (K. P. Lesch et al., 1996). Functional and structural magnetic resonance imaging and cognitive measures are used to uncover an apparent depression vulnerability associated with carriers of the short variant (McGuffin, Alsabban, & Uher, 2011).

Four major molecular genetic methodologies are common in the search for individual differences associated with psychological disorders (Levinson, 2009). Genomewide linkage studies, genomewide association studies, large scale resequencing, and candidate gene associations. A linkage study requires analysis of two or more affected relatives to explore whether a particular DNA sequence has been inherited by affected individuals more frequently that would be expected by chance. Linkage studies rely on the size of the genetic effect to estimate the approximate location of a potential susceptible gene. These methods have mainly been applied in studies of disorders with very high heritable estimates, such as the different types of schizophrenia. Major depression, with its lower heritability (40-70%) is thought to have substantially higher etiological heterogeneity. Genome wide associations gather information about common single nucleotide polymorphisms in large portions of the human genome. Resequencing determines the sequence of an individual`s DNA to explore

whether rare variants are common in particular groups. Candidate gene association studies attempt to grasp the underlying mechanisms of these associations.

The selection of candidate genes, such as the serotonin transporter polymorphism (5-HTTLPR), requires an etiological hypothesis (Levinson, 2009). Candidate genes involved in monoamine neurotransmission, particularly those linked to dopamine and serotonin, have dominated studies in this field with the 5-HTTLPR as one of the single most studied genes. The rationale for choosing the serotonin system and the associated polymorphic region (5-HTTLPR) as the most central gene candidate comes mainly from two sources. The first source is from pharmacological studies of, and treatment with, serotonin-linked antidepressants. The other comes from neuroscience studies that show considerable overlap between abnormal brain structure and functioning in major depression and serotonin circuitry (Price & Drevets, 2010).

Serotonin (5-HT) -containing neurons are mainly collected in the nine cores called raphe nuclei. Raphe nuclei are groups of neurons in the whole length of the brainstem and are especially centred around the reticular formation, one on each side of the brainstem's midline. Each nucleus has projections to particular brain areas and structures. Caudally located nuclei in the medulla affect processes associated with the spinal cord where they moderate pain-related sensorial signals. Rostral nuclei in the pons and midbrain affect sweeping areas of the brain in a more diffuse manner. Raphe nuclei cells are most active during the conscious awake condition and in active high physiological conditions. Serotonin plays an important role in cortical development, shaping neuronal circuitry by regulating synaptic plasticity and neuronal activity patterns of serotonergic and non- serotonergic neurons (Gaspar, Cases, & Maroteaux, 2003)

Importantly, serotonin has broad developmental effects, promoting differentiation not only of serotonergic but also of glutamatergic neurons, which transiently express 5-HTT in limbic regions such as the cingulated cortex. A growing amount of evidence indicates 5-HT balance to be crucial for the development, differentiation and maturation of nerve cells and networks in brain areas that control sensorial input, stimulus processing and motor response. Some examples of this are 1) 5-HT modulates projections from the cell bodies of thalamocortical glutaminergic neurons in cultures that involve serotonin receptors; 2) the 5-HT contributes to differentiation of cortical glutaminergic neurons via one of the 5-HT receptor subgroups (Lieske et al., 1999); and 3) the accession of 5-HT increases the likelihood for long-term potential in the visual cortex (Kojic et al., 2000). In mice, chronic treatment

with antidepressants affects the birth of new nerve cells in the hippocampus (Santarelli et al., 2003). This and similar findings accent the importance of a developmental, dynamical perspective when researching 5-HTTLPR variability. Research on how gene-gene and gene-environment interactions affect the development, plasticity and formation of synaptic connections during childhood, adult life and ageing is therefore only in its early beginnings.

The Serotonin Transporter 5-HTT

The brain's serotonin activity is regulated by the serotonin transporter 5-HTT. The 5-HTT is a sodium chlorine-dependent transporter located in the plasma membrane of the cell. When serotonin is released in the synaptic gap the presynaptically located 5-HTT will return serotonin to the cell for recycling and metabolic decomposition under normal physiological circumstances. The 5-HTT's major purpose is the efficient removal of serotonin from extracellular areas. Abnormal or manipulated functioning will alter the duration and intensity of 5-HT communication with its receptors and postsynaptic targets located in limbic structures, mediating emotional processing, or in presynaptic receptors with inhibitory control of the 5-HT neuron itself. Decreased 5-HTT gene function increases serotonin levels and leads to reduced receptor binding to receptors 5-HT1A and 5-HT1B, but increased 5-HT2A, 5-HT2C and 5-HT3 receptor mRNA levels and/or ligand binding. This means that 5-HTT function has both excitatory and inhibitory effect on the postsynaptic cell. Seven distinct families of 5-HT receptors have been identified (5-HT1–5HT7), and subpopulations have been described for several of these (at least 15 subpopulations) (Glennon, Malgorzata, & Westkaemper, 1998). If we imagine that the presynaptic cell is a raphe nucleus efferent and the postsynaptic cell is coupled to limbic structures involved in mood regulation, the net effect is best understood as a spectrum of different variants rather than one functional and one dysfunctional variant.

Genetic Variability in Serotonin Transporter Functioning

In humans, transcriptional activity of the 5-HT transporter gene SLC6A4, located on chromosome 17q11.1-q12, is modulated by the polymorphic repetitive element (5-HTT gene-linked polymorphic region, 5-HTTLPR) located upstream on the transcriptional start site. The majority of alleles consist of 14 or 16 repetitive elements that correspond to the division of short and long variants (S and L) respectively. Repetitions of 15, 18-20 or 22, plus some other variants, occur but are rare. Alleles and genotypic distributions have substantial variations

across different populations (Lesch & Gutknecht, 2005). The different 5-HTTLPR variants form bases for the formation of a second DNA structure that has the potential to regulate transcriptional activity in the associated 5-HTT gene promoter. A promoter is a part of the DNA molecule that contributes to the regulation of gene expression. Expression depends on the kinds of transcriptional factors that are connected to the gene in the particular case.

The transcriptional factor has at least two different effects on the gene product, one connected with DNA binding and another that provides gene activation. The transcriptional factor's main function is to turn on and off other genes. For this purpose, they have to form a complex with other proteins and bind to the start site to turn on the process that copies the gene by the enzyme RNA-polymerase. The 5-HTT gene promoter contains special sequences recognized by proteins called transcriptional factors. In eukaryotes (cells with soma), we know seven such factors. This accents the complicated biochemical connections in the transcriptional process where the described polymorphic region constitutes a restricted component. The genetic sequence we study is a component that modulates the effect of the DNA promoter, thought to determine the quantity and activity of the gene that codes for the reuptake protein 5-HTT/SERT. This means that both the quantity of the 5-HTT protein and the activity in this channel is pivotal to establishing a link between the 5-HTTLPR genotype and 5-HTT function. The hypotheses of a link between variations in 5-HTTLPR length and 5-HTT function have been studied by looking at the 5-HTTLPR genotype, 5-HTT gene transcription and 5-HT reuptake activity in human lymphoblast cell lines. It has been suggested that cells homozygous for the long variant produce higher concentrations of 5-HTT mRNA compared with cells with one or two copies of the short variant (K. P. Lesch et al., 1996). Transcription activity does not necessarily follow protein production or the quality of the proteins produced. Short and long 5-HTTLPR variants are quantitative units and we therefore expect heterozygote variants to subsume between the homozygote variants in an additive manner on the RNA- level. Serotonin promotor activity has been suggested to give rise to variation in serotonin transporter mRNA levels as in heterologous expression systems (Heils et al., 1996). When fused to a luciferase reporter gene and transferred into human 5-HTT expressing cell lines the short and long 5-HTTLPR variants differently modulate transcriptional activity of the 5-HTT gene promoter (K. P. Lesch et al., 1996). Exploring gene transcription activity and uptake activity in human lymphobastoid cell lines revealed higher concentrations of 5-HTT mRNA in cells homozygous for the long variants compared to short variants. Homozygote long lymphoblasts showed higher inhibitory binding than did

homozygote short variants and the rate of specific 5-HT uptake was more than twofold higher in cells homozygous for the long form compared to one or two copies of the short variants.

Decrease in 5-HTT protein expression in the postmortem human brain, and uptake in lymphoblast and platelet are reported to be associated with the short allele (Greenberg et al., 1999; K. P. Lesch et al., 1996; Little et al., 1998). Similar findings are reported in studies investigating other cell lines (Mortensen, Thomassen, Larsen, Whittemore, & Wiborg, 1999) and by using several other procedures (K. P. Lesch & Gutknecht, 2005). Heinz et al. (2000) have found a significantly higher ratio of in vivo 5-HTT raphe availability in homozygous long carriers compared to carriers of short variants. Others have reported no effect of 5-HTTLPR genotype on 5-HTT binding in vivo (Lim, Papp, Pinsonneault, Sadee, & Saffen, 2006; Mann et al., 2000; Patkar et al., 2004; Zalsman et al., 2005). The complexity of findings indicates that we already, at this early level in the space between 5-HTTLPR and 5-HT functioning, have shifted from the level on which genes operate. Further analyses on higher levels will be prone to effects caused by other factors in these processes.

Several algorithms are developed to facilitate uncovering of promoters in genetic sequences. The uncovering of promoters is common in many methods developed for predicting genetic expression. Present biochemical technologies are able to uncover the functional 5-HTTLPR variants and divide the three categories based on the pairing of short and long variants (LL, SS and LS). These two original variants have been studied intensively. Recently, functional variants were identified within the long variant, designated L_A and L_G variants, representing a single nucleotide polymorphism (SNP). Based on such findings, suggestions have been put forward that this is really a three allelic functional polymorphism (Hu et al., 2006). The L_G and S alleles have comparable levels of 5-HTT transporter expression, and both are lower than that of the L_A allele. Martin, Cleak, Willis-Owen, Flint, & Shifman (2007) identified 55 SNPs in or near the gene and measured expression levels of the gene transcript in lymphocytes from carriers of different SNPs in combination with 5-HTTLPR variants. They found two SNPs that predicted expression levels more strongly than did 5-HTTLPR but only about 10% of the population carries one of these SNPs, whereas a majority of the population carries one of the short 5-HTTLPR variants. Furthermore, Wendland, Martin, Kruse, Lesch, & Murphy (2006) have demonstrated that the single nucleotide polymorphism can be associated with either long or short variants. Thus, hesitancy in the division of genetic categories typifies fundamental challenges in present studies of 5-HTTLPR variability. When it comes to the net effect of this complex process, much remains

unclear, but a considerable quantity of studies conclude with variation in emotional processing associated with decreased 5-HTT functioning in carriers of the genetic type denoted “short variants” compared with ”long variants”. The first findings that pointed in this direction were the pharmacological findings that indicated associations between response time and total response to SSRIs and the short 5-HTTLPR variants among patients with depressive disorders. This means that the same amount of drugs have different force depending on one single polymorphic region (K. P. Lesch & Gutknecht, 2005). A growing number of studies conclude with 5-HTTLPR-dependent allelic variation in 5-HTT expression and brain functions in anxiety and depression, but also in conditions associated with aggression and other psychological disorders. The most consistent findings are found within disorders with a considerable affective symptomatology, like depression, bipolar disorder, anxious personality disorders (cluster C), eating disorders, substance-related disorders and neurodegenerative disorders (K.-P. Lesch & Mossner, 1998). Increased 5-HTT function is seen in ADHD, obsessive-compulsive disorder and autism. The latter is based on overrepresentation of the long polymorphic variant in individuals with these diagnoses (K. P. Lesch & Murphy, 2003). Hence, this may also reflect advantages linked to the short variants. These findings indicate that both short and long variants represent diatheses and that operating with a short dysfunctional variant and a long functional variant may be an erroneous simplification. As early in the process as on the synaptic level, a given amount of 5-HT in the synaptic gap leads to both downregulated and upregulated synaptic activity and the net effect will therefore depend on sequences in receptor binding. This means that both less and more 5-HT in the synaptic gap have the potential to facilitate communication with postsynaptic targets.

Phenotypes linked to 5-HTTLPR variability

In psychiatric illness apparent etiological pathways are rare. Interaction effects are more common and reflect our understanding of dynamical interplays between genetics, environmental exposure and development. The interactions between the serotonin transporter gene, stressful life events and risk of depression have been widely published (McGuffin et al., 2011). One of the most cited and replicated studies on these topics concludes that individuals with short 5-HTTLPR variants were more often diagnosed with major depression, had higher subjective ratings of depressive symptomatology, were rated higher on informant reports of depression and had more suicide ideation and attempts (Caspi et al., 2003). Importantly, however, this association was only present or stronger among individuals who had

experienced several traumatic life stressors, assessed as the number of stressful life events at age twenty-one to twenty-six. They also reported interactions between genotype and maltreatment during the first decade of life, assessed as maltreatment between the ages of three and eleven. The effect of life events on depression was stronger both among heterozygote LS carriers and homozygote SS carriers compared to homzygote LL carriers, indicating a dominant genetic pathway. The study provides evidence for a gene-environment interaction, in which an individual's response to environmental insults is moderated by the individual's genetic make-up.

Marcus R. Munafo, Durrant, Lewis, & Flint (2009) systematically reviewed 15 studies to assess the robustness of the claim of a serotonin transporter polymorphism x stressful life event interaction. The authors conclude that the simulation indicates that published studies are underpowered and comparable with chance findings. In a meta-analysis, Risch et al. (2009) included 14 published studies on the association between 5-HTTLPR genotype, number of stressful life events and categorical measures of depression. The results yielded no evidence that the serotonin transporter genotype alone or in interaction with stressful life events is associated with an elevated risk of depression. Several studies have found associations of only one of the genotypes (SS vs LS plus LL or SS plus LS vs LL) corresponding to effects restricted to "recessive" and "dominant" genetic models, (Brummett et al., 2008; Kaufman et al., 2004; Kendler, Kuhn, Vittum, Prescott, & Riley, 2005; Lazary et al., 2008; Sjoberg et al., 2006; Taylor et al., 2006) while others report an interaction between the long allele, stressful life events and major depression (Brummett et al., 2008; Cervilla et al., 2007; Grabe et al., 2005; Laucht et al., 2009; Sjoberg et al., 2006; Zhang et al., 2009).

Recently, a larger meta-analysis based on 34 studies concludes with a real interaction between 5-HTTLPR , stressful life events and depression (McGuffin et al., 2011). Studies using objective evidence or detailed interviews to assess environmental adversity in context consistently found an interaction in the expected direction, while studies relying on brief self-report measures often showed negative results (Uher & McGuffin, 2008, 2010). The indications of a systematic relationship between methods and results calls for conclusions about the causal mechanisms involved.

A number of studies have indicated that biased processing of emotional information is associated with the presence of the short 5-HTTLPR allele. Beevers, Gibb, McGeary, and Miller (2007) have reported 5-HTTLPR-dependent attention bias in a psychiatric inpatient group using a standard dot-probe reaction time task. Participants carrying one or two copies of

the short 5-HTTLPR variants had a stronger attention bias for anxious word stimuli compared with participants homozygous for the long 5-HTTLPR variants. The attention bias was not found when presented with dysphoric word stimuli. The study indicates an emotion specific association between 5-HTTLPR variants and biased attention, not only indicated by amygdala activation, but also when measured with a behavioral reaction time task. Beevers, Wells, Ellis, & McGeary (2009) later found short 5-HTTLPR negative cognitive response to a sad mood induction. A meta- analysis based on 10 published articles on the association between biased attention for emotional information and 5-HTTLPR concludes with selective attention to negative stimuli in low transmission efficacy genotypes (5-HTTLPR + A>G SNP) (Pergamin-Hight, Bakermans-Kranenburg, van Ijzendoorn, & Bar-Haim, 2011).

Cognition and 5-HTTLPR variability

Studies investigating other basic cognitive performance in association to 5-HTTLPR variability are sparse and the findings are somewhat mixed. Paaver et al. (2007) have reported inferior task performance in carriers of the short 5-HTTLPR allele using a visual comparison task indicating increased impulsivity. Walderhaug, Herman, Magnusson, Morgan, and Landro (2010) found that male short 5-HTTLPR carriers exhibited a more impulsive response style than long allele carriers on a continuous performance test. However, another study found no effects of 5-HTTLPR variants on the ability to inhibit a prepotent response applying the Stop Signal Task (Clark et al., 2005). Lage et al. (2011) found no association between genotype and two neuropsychological measures of impulsivity. Strobel et al. (2007) found that carriers of the short 5-HTTLPR allele outperformed the homozygous long 5-HTTLPR carriers on the AX Continuous Performance Task (AX-CPT), which is designed to maximize conflict. Borg et al. (2009) found a highly significant association between 5-HTTLPR genotype and performance in the Wisconsin Card Sorting Test. Carriers of the short allele had a superior performance compared to the homozygote long carriers. Improved cognition in short 5-HTTLPR carriers has also been demonstrated in several tryptophan depletion procedures in the context of potential emotion evoking stimuli, including reward appraisal and incentive motivation (Roiser, Blackwell, et al., 2006; Roiser, Muller, Clark, & Sahakian, 2007). A recent study addressed action monitoring and activation in subdivisions of the anterior cingulated cortex during fMRI and found impaired post-error and post-conflict behaviour adjustment in healthy short 5-HTTLPR carriers (A. J. Holmes, Bogdan, & Pizzagalli, 2010). Others have claimed that single specific dimensions of cognitive function is not moderated by

the 5-HTTLPR (Homberg & Lesch, 2010). Specific cognitive functions in healthy individuals are typically indirectly addressed in relation to 5-HTTLPR as covariates. O'Hara et al. (2007) have reported poorer memory function in healthy older adults carrying the short 5-HTTLPR allele. They also found that inferior memory function in short 5-HTTLPR carries was reflected in hippocampal volume and interacted with cortisol levels within the HPA-axis. There exists preliminary evidence of differences in decision-making, executive function, and episodic memory in healthy control subjects according to polymorphism at the 5-HTTLPR (Roiser, Rogers, Cook, & Sahakian, 2006). Better conceptual predictive relations between emotion processing and cognition may enlighten our understanding of the role of basic cognition and 5-HTTLPR.

Sex Differences Associated with the 5-HTTLPR Genotype

Recently, sex of participants has attracted substantial attention when examining 5-HTTLPR variability. There is a wealth of preclinical and clinical evidence supporting sex differences in serotonin neurotransmission (Fink et al., 1998). Consideration of sex has varied markedly across studies. Some have failed to specifically address sex differences for example by using sex as a covariate, matching groups, or exclusively including male or female participants. Brummett et al. (2007) have demonstrated that, in females, homozygote short variants are associated with susceptibility to depression under stressful life conditions, whereas in males, it is the homozygote long variants that are susceptible, revealing an opposite genotype-environment pattern in males and females. Eley et al. (2004) and Sjöberg (2006) showed an interaction effect in the expected direction for females only and a trend for an opposite effect in males. When positive results have been reported separately for both sexes, the gene-environment effect has shown to be stronger among females (Kendler et al., 2005). The implication of sex could also be age-specific or stronger among specific age cohorts. In studies on adolescent and elderly participants, negative results are more often reported, especially in male adolescents (Uher & McGuffin, 2008). Hence, polymorphism refers to clearly different phenotypes that exist in the same population of a species (more than one morph). Sexual dimorphism is the major systematic difference in form between individuals of different sex in the same species. With this in mind, it does not make sense to study genes and at the same time espouse that gender does not matter, as this can be tested statistically based on phenotypes.

Associated Genes

Common genetic variations linked to the serotonin receptors, as well as genes whose products are involved in the catabolism and metabolism of serotonin, have the potential to mediate or moderate links between 5-HTTLPR and serotonin activity. The serotonin receptors modulate the release of other neurotransmitters, including glutamate, GABA, dopamine, epinephrine/norephinerine and acetylcholine. The influence of various hormones, including oxytocin, prolactin, vasopressin, cortisol, corticotrophin and substance P, represent further complexity in these interplays with the serotonin system representing one factor among many others. A full exhaustive mapping of all relevant genes and their behavioral correlates is therefore an ambitious project. The rationale behind the choice of serotonin system associated genes in the study of depressive symptomatology and emotion regulation is straightforward, as a wealth of data has suggested that serotonin is involved in the pathophysiology of affective disorders (Thase, 2009). Holmes' (2008) review of genetic variation in cortico-amygdala serotonin function illustrates that the serotonin system is by its nature modulatory and extremely plastic. Genetic variance associated with a particular neurobiological component would be expected to lead to compensatory changes. The introduction of epigenetics (meaning "beyond genetics) refers to changes in the genetic expression caused by mechanisms other than changes in the underlying DNA sequence. Both brain-derived neurotropic factor (BDNF), histone and chromatin play pivotal roles in gene regulation (Autry & Monteggia, 2009). Epigenetics have lead to challenges in the question of causality. While it is tempting to assign epigenetic changes as the mechanism of depressive symptomatology, it is crucial to explore how these alterations themselves are coincidental versus instrumental in causing symptoms associated with major depression. Epigenetic changes may remain through cell divisions for the remainder of the cell's lifetime and may also last for multiple generations. It is not completely right to postulate that the environment changes genes (the DNA sequence does not change), but rather that environment has the potential to change the regulation of genetic information due to interactions involving epigenetic factors. Genes have potential and some genes have wide potential while others have more conservative potential. Genetic polymorphims will receive attention only if they are functional, meaning that they represent individual variance at the neurobiological level. So, a functional polymorphism refers to several variants (poly) with the same genetic loci that differ in quality or quantity (morph). There are also several other polymorhisms within the 5-HTTLPR gene and some of them have been shown to be functional (Martin et al., 2007).

Models of emotion processing

One of the major challenges in models of emotion regulation in the context of neuroscience lies in the construction of a system. Many structures and cells have several functions and this makes it difficult to operate with delimited systems. Emotional identification and further processing are dependent on components that are associated with emotional processing, but sometimes also on structures traditionally linked to other cognitive processes. Several neuropsychological subprocesses are involved in many different aspects of behavior and these make concepts based on delimited systems erroneous. Phillips et al. (2003) discuss three processes central to emotional perception. The processes are linked to different brain structures and functional systems. The first process concerns the identification of the stimulus' emotional significance, the second concerns the production of the affective state and automatic regulation, and the last process is the one in which the affective state is further regulated. Others have focused on a twofold process involving emotional appraisal and cognitive strategies to regulate the appraisal of the emotion-eliciting stimulus. Appraisal refers to a continuously unfolding process of assessing the significance of a stimulus to one's current goals, wants and needs. This approach will distinguish the emotional response from mood, where the latter is objectless and more enduring. Emotion regulation amounts to any explicit or implicit process that alters which emotion an individual feels, how long they feel it, and how they express it (Denny, Silvers, & Ochsner, 2010). Gross & Thomson (2007) have focused on the temporal aspects involved in emotion regulation. Antecedent focused strategies refer to those used before the behavioral and physiological responses and response tendencies have been fully activated. Response focused strategies refer to what one does once an emotion is underway. Situation selection, situation modification, attention deployment, cognitive change and response modulation refer to the different strategies involved in emotion regulation and each of these may be used in adaptive or maladaptive ways. Situation selection will often involve avoidance of people and situations that may elicit the emotion. Situation modification involves acting on the situation itself to regulate its emotional impact. Attention deployment is the processes wherein one "chooses" which aspects of the situation to focus on and cognitive change refers to how one may change the way one constructs the meaning of the situation. Maladaptive response-focused strategies (response modulation) are typically attempts to avoid unwanted emotions. Emotion regulation difficulties occur when the strategies are implemented poorly, in inflexible, context-insensitive ways.

Emotion perception in the brain

A ventral system involving the amygdala, insula, ventral striatum and ventral areas of the anterior cingulated gyrus and the prefrontal cortex are particularly important in emotion activation. A dorsal system including the hippocampus, dorsal areas of the anterior cingulated gyrus and prefrontal cortex are central to the regulation of the affective state and the following behavior. The models predict that functions associated with the prefrontal cortex play important roles in emotional perception and processing (Mary L. Phillips, Drevets, Rauch, & Lane, 2003; Price & Drevets, 2010). The prefrontal cortex is the anterior part of the frontal lobes of the brain, lying in front of the motor and premotor areas. It can be divided in several ways, and this represents a major challenge when reviewing different studies to compare and contrast them. It is typical to roughly separate the orbitofrontal and ventromedial areas, the dorsolateral prefrontal cortex and the cingulated cortex. Together, these brain regions have been implicated in planning complex cognitive behaviors, personality expression and moderating social behaviour. These basic activities are the orchestration of thoughts and actions in accordance with internal goals and are often labeled "executive functions" or "cognitive control functions". Functional brain imaging has shown that individuals with a major depressive episode show increased activation in areas central to the identification of emotional stimuli, and for generating emotional behavior during uninstructed resting conditions compared with healthy control participants. The structures include the amygdala, orbitofrontal cortex, anterior cingulated, ventrolateral- and medial prefrontal cortex, insula and striatum (Drevets, 2000). Enhanced striatal and amygdala response to sad faces (Elliott et al., 2004; Surguladze et al., 2005), diminished neural activity to happy faces (Lawrence et al., 2004; Surguladze et al., 2005) and sustained amygdale reactivity to sad emotional words (Siegle, Thompson, Carter, Steinhauer, & Thase, 2007) indicate that negative affective information is preferentially detected and processed over positive information in individuals suffering from MDD. People with MDD show decreased activation in structures central to the regulation of emotional behavior including the medial and lateral prefrontal cortex (Grimm et al., 2008; Matsuo et al., 2007; Walter, Wolf, Spitzer, & Vasic, 2007). The structural and functional abnormalities within these structures could help explain why depression consolidates by shortages in regulating mechanisms. Whether these findings reflect constraints in downregulation mechanisms or biased appraisal of emotional information is still unclear. A review by Price and Drevets (2010) demonstrates dominant findings that involve amygdaloid projections to the medial, caudal, and lateral edges of the orbital cortex, to the

rostroventral insula, and to the temporal pole and inferior temporal cortex, extending caudally to the primary visual cortex. There are also amygdaloid interactions with the entorhinal and perirhinal cortex and the hippocampus and projections from the amygdale to the posterior cingulate cortex. Russchen et al. (1985) found extensive major amygdaloid projections between the striatum and the nucleus accumbens, and adjacent medial caudate nucleus and ventral putamen. These striatal areas in turn project to the ventral and rostral pallidum, which itself sends GABAergic axons to the mediodorsal thalamic nucleus. The perigenual prefrontal cortex is also connected to the same ventromedial part of the striatum and the same region of mediodorsal thalamic nucleus (magnocellular division). This leaves us with overlapping and interconnected medial prefrontal cortico-striato- pallido- thalamic and amygdalo-striato-pallido- thalamic loops.

Functions traditionally linked to the anterior cinglate cortex (ACC) may contribute to our understanding of the interplay between emotional activation and emotion regulation. The ACC may be divided into of four regions. The dorsal-, rostral-, and subgenual ACC, where the latter is often further divided into the anterior and the posterior component. The dorsal ACC has dense connections to subgenual ACC, medial prefrontal cortex, orbito frontal cortex and amygdala. The rostral and subgenual regions are connected to the amygdale and orbito frontal cortex. In healthy subjects there is a relative deactivation in dorsal ACC during emotional paradigms and a relative deactivation in ventral ACC during cognitive tasks (Schlosser et al., 2008). When the rostral ACC is stimulated it causes inhibition in the amygdala (Maren & Quirk, 2004). The perigenual part of the anterior cingulate is the phylogentically older archifortical portion of the cingulated cortex and the region that displays the highest density of 5-HTT terminals within the human cortex. This is also the region that displays the highest target zone of dense projections from the amygdala. The feedback coupling between these regions is implicated in the processing of negative affect. These areas within the anterior cingulate are most prominent in rostral parts, commonly associated with emotional conflict processing as opposed to a more cognitive caudal subdivision. The cognitive subdivision is part of a distributed attention network. It maintains strong reciprocal interconnections with the lateral prefrontal cortex, parietal cortex and premotor and supplementary motor areas. The “emotional” subdivision is connected to the amygdala, periaqueductal grey, nucleus accumbens, hypothalamus, anterior insula, hippocampus and orbitofrontal cortex (Bush, Luu, & Posner, 2000). These studies represent plausible explanations for the aforementioned increased amygdala activity in carriers of the short 5-

HTTLPR variants by decreased downregulation via the anterior cingulated cortex. These findings strongly suggest 5-HTTLPR-dependent variability in the interplay between cognitive and emotional processing. Anatomical studies in the primate brain reveal massive amygdala projections to the rostral anterior cingulate and efferent projections from the caudal anterior cingulate back to the amygdala (Paus, 2001). Convergent evidence suggests that these interactions constitute a functional feedback circuitry that regulates amygdala processing of adverse environmental stimuli. Stimulation of the limbic prefrontal cortex inhibits amygdala function in the macaque monkey (Stefanacci & Amaral, 2002), and medial prefrontal cortex neurons also exert an inhibitory influence on the amygdala (Maren & Quirk, 2004). Given the evidence that the rostral anterior cingulate modulates amygdala activity by inhibition, an uncoupling of the structures can explain the association between these areas under a strong serotonergic influence and 5-HTTLPR variability. Reduced coupling would translate into altered feedback regulation of amygdala activity. The reviewed coupling between the amygdala and the ventromedial prefrontal cortex, which may participate in amygdala activity (Heinz et al., 2005), is most likely based on indirect anatomical interconnections. This assumption is based on the fact that direct connections between those structures are sparse, if they exist at all (Carmichael & Price, 1995; Ghashghaei & Barbas, 2002). Pezawas et al. (2005) speculate that the connection represents a compensatory mechanism for a primary regulatory loop involving the anterior cingulated cortex.

Prefrontal circuitry in emotion regulation

Price and Drevets (2010) recently published a detailed review of the neurocircuitry of mood disorders. An orbital- and a medial prefrontal network have been distinguished based on local cortico-cortical connections. The orbital network appears to be involved in the integration of multi-modal stimuli (such as smell and taste) and the value assessment of those stimuli. The orbital cortex has also been associated with the ability to use reward as a guide to behavior and abstract assessment of reward. The medial prefrontal network consists of areas on the ventromedial surface of the medial edge of the orbital cortex, and a small caudolateral orbital region at the rostral end of the insula. As a system, these structures represent an output system that can modulate visceral function in relation to emotion. The medial prefrontal system is also connected to the rostral part and dorsal bank of the superior temporal sulcus, the anterior and posterior cingulate cortex, and the enthorinal and parahippocampal cortex. Connections to

the anterior cingulate cortex are different from the orbital network, which is mainly connected to the middle portion of the anterior cingulate.

Cortico-striatal-thalamic circuits related to the orbito-medial prefrontal cortex can be divided based on connections to a medial and a lateral segment of the thalamic nucleus. A dorsal prefrontal system, a ventral prefrontal system and a caudal prefrontal system within the lateral prefrontal cortex have been described (Price & Drevets, 2010). The systems are distinguished based on local cortico- cortical connections. Each region is preferentially connected to other local areas in the same region, and each is connected to a specific set of areas in other parts of the cortex. An exception is Area 45a in the caudo-ventral prefrontal cortex, which is connected to areas in all parts of the lateral prefrontal cortex. Miller & Cohen (2008) have outlined a theory of prefrontal functioning based on current reports from cognitive neuroscience. They argue that particular regions in the brain play important roles in the orchestrating of thought and action with internal goals. Cognitive control stems from the active maintenance of patterns of activity in the prefrontal cortex that represent goals and the means to achieve them. The prefrontal cortex sends bias signals to other areas in the brain to facilitate the expression of task- appropriate responses in the face of competition with potentially stronger alternatives. When the strong alternative responses involve affect, the ventromedial prefrontal cortex, in particular, is implicated (Davidson, Pizzagalli & Nitschke, 2009). The reviewed literature demonstrates that the circuitry involved in emotional perception, processing and regulation is widespread, involves several cortical and subcortical areas of the brain, and many of these areas are interconnected (Figure 1.).

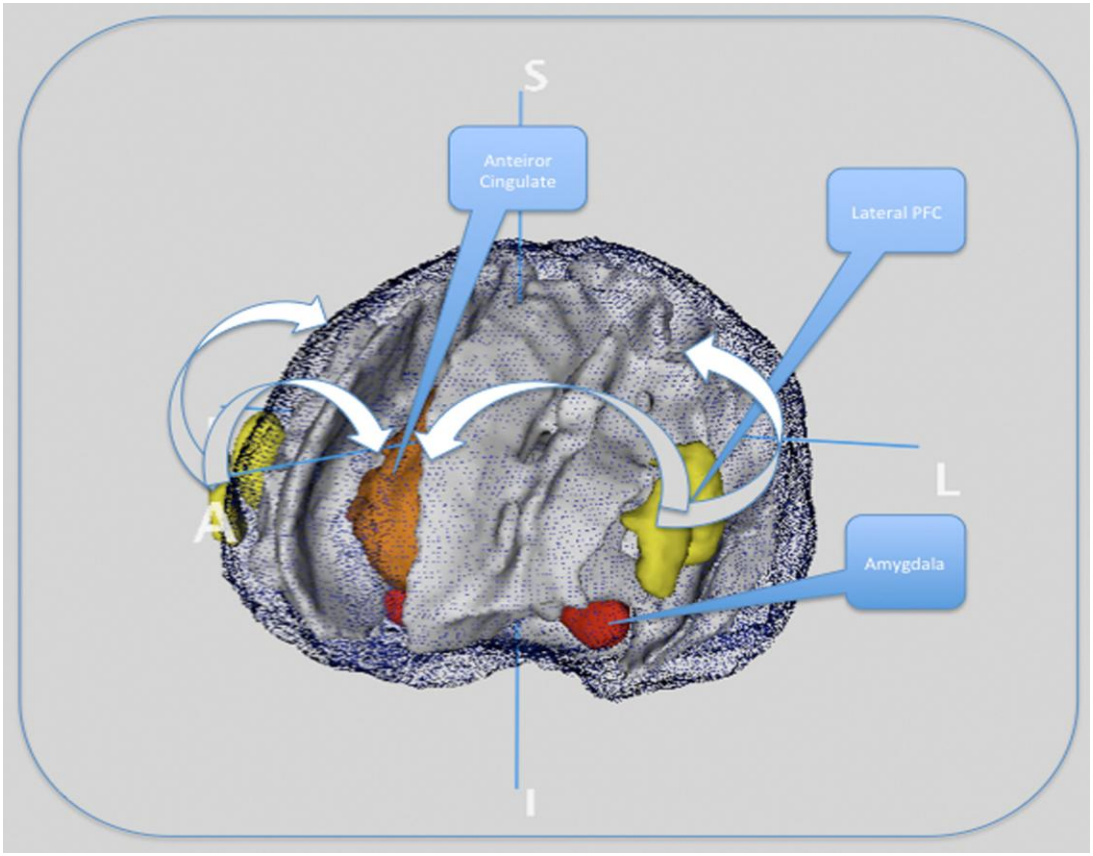

Figure 1. Brain structures involved in emotion processing embedded in the brains white matter. The curved arrows illustrate circuitry associated with cognitive control of emotion. The white matter shows that cortical and subcortical areas are highly interconnected.

Imaging 5-HTTLPR genotype

The 5-HTTLPR endophenotypes are basically found within limbic structures and different subdivisions of the prefrontal cortex, overlapping considerably with the structures known to be altered in major depression. Increased amygdala activation in carriers of short 5-HTTLPR variants reflects a direct or indirect increased sensitivity toward emotionally significant stimuli. The sensitivity will make these individuals emotionally labile. Decreased prefrontal downregulation represents altered function in circuits important for the modulation and inhibition of emotional processing and will have implications for automatic regulation and further regulation of an affective state. Under normal circumstances, emotional activation, produced when one is presented with salient stimuli, will be downregulated. The model posits increased sensitivity to alter this down-regulating mechanism. Short 5-HTTLPR carriers may be sensitive and reactive when identifying negative emotional stimuli. This sensitivity may leave these individuals in danger of enduring functional and structural changes associated with depressive symptomatology. Top- down regulation of amygdale may be diminished in

individuals carrying one or two copies of the short 5-HTTLPR variant. Other neural pathways may be strengthened in these individuals, like functional coupling of the left amygdala and ventromedial prefrontal cortex (Mary L. Phillips et al., 2003).

Heinz et al. (2005) found 5-HTTLPR dependent functional coupling between the amygdala and the ventromedial prefrontal cortex. Both positive and negative emotional pictures elicited amygdala activation but only negative pictures varied with 5-HTTLPR genotype. The data supports the hypothesis that 5-HTT function plays an important role in the development of a negative mood state. Dysfunctional amygdale-prefrontal coupling may be associated with 5-HTTLPR dependent constraints in the capacity to regulate emotional states. Hariri et al. (2002) have shown with fMRI that participants with at least one copy of the short variant have a higher amygdala response, compared with subjects homozygous for the long variant, when exposed to potential fright- evoking stimuli. This report indicates that differences in amygdala excitability contribute to fear- and anxiety-related responses. After these early studies, the topic was elaborated upon considerably and widely published, showing different associations between the polymorphic region and amygdala activation. A meta-analysis by Munafo, Brown, & Hariri (2008) concludes that 5-HTTLPR polymorphism accounts for up to 10% of phenotypic variance. The results indicate that alterations in 5-HT signaling and 5-HTTLPR contribute significantly to amygdala activation in response to a broad range of salient environmental stimuli. Furman, Hamilton, Joormann, and Gotlib (2011) found both stronger and earlier activation in left amygdala as they increased a sad mood state in individuals carrying one or two copies of the short variant compared to the homozygote long variant. Canli et al. (2005) found that allelic variation is associated with differential activation to negative positive and neutral stimuli in limbic, striatal, and cortical regions using a variant of the Emotional Stroop Task in a study of whole brain activation. When the participants were presented with negative, relative to neutral stimuli, increased amygdale activation was observed in carriers of one or two copies of the short 5-HTTLPR variant compared to homozygous long carriers. The observed effects were determined by decreased activation to neutral stimuli, rather than increased activation to negative stimuli. They also found that the homozygote long carriers had significantly greater volume in several subdivisions of the prefrontal cortex and greater gray matter density in areas of the insula, frontal lobe, right temporal lobe, anterior cingulate and cerebellum. The authors suggest that 5-HT transport efficiency has a broader role in modulation controlling affective, cognitive and motor processes. T. Canli et al. (2006) also found interactions between 5-HTTLPR and life

stress on amygdala tonic activation and connectivity, using fMRI and perfusion imaging. The stress depression hypothesis was further supported by 5-HTTLPR-dependent hippocampus activation and connectivity. Frodl et al. (2008) have also reported reduced hippocampal volume associated with long variants in a clinical group suffering from major depression. The latter study indicates homozygote long carriers' vulnerability to hippocampal changes.

Pezawas et al. (2005) analyzed the effect of genotype on functional coupling between amygdala activity, rostral subgenual anterior cingulate (rACC) and caudal anterior cingulate (cACC). The cACC activity was negatively correlated with amygdala activity. The rACC and cACC showed strong positive connectivity with each other. Short 5-HTTLPR carriers showed highly significant reduction of amygdale- ACC connectivity in comparison to homozygote long 5-HTTLPR carries, particularly in the rostral subgenual anterior cingulate. Within the ACC, rostral subgenual- caudal- anterior cingulated connectivity did not differ by genotype. Carriers of the S allele also had more than 25% gray matter volume reduction in the perigenual anterior cingulated and an approximately 15% reduction in the amygdale. Disruption of amygdale- posterior anterior cingulated circuitry could underlie observations of increased amygdale activity in short 5-HTTLPR carriers. Heinz et al. (2005) demonstrated 5-HTTLPR dependent functional coupling between the amygdala and the ventromedial prefrontal cortex in favor of short 5-HTTLPR carries, an opposite pattern. Pezawas et al. (2005) reanalyzed their data with mainly similar results.

A primary affective circuit including the rACC and the amygdale and a more indirect role of the ventromedial prefrontal cortex has been suggested based on the lack of direct connections between the latter and the amygdala (Hamann, 2005). When Beevers, Pacheco, Clasen, McGeary, & Schnyer (2009) found biased attention for emotional stimuli, lateral prefrontal cortex morphology was inversely associated with maintained attention for positive and negative stimuli in carriers of the short 5-HTTLPR allele. No such associations were found in the medial prefrontal cortex or the amygdala. The study suggests that brain structures involved in conscious regulation of emotion varies by 5-HTTLPR genotype. The 5-HTTLPR polymorphism has also been associated with alteration in microstructure of frontal-limbic white matter tracts linked to working memory. Pacheco et al. (2009) found reduction in FA values (fractional anisotropy) in the uncinate fasciculus, a white matter pathway connecting the amygdala to medial and orbital prefrontal cortex, in carriers of the short 5-HTTLPR allele using diffusion MRI (DTI). By combining fMRI and DTI, Wang et al. (2009) also found

significant positive associations between fractional anisotropy in the uncinate fasciculi and functional coupling between the amygdale and the perigenual anterior cingulate.

The reviewed literature strongly supports biased amygdale reactivity and activation in carriers of the short 5-HTTLPR allele. The association between brain structures involved in automatic regulation of emotion and 5-HTTLPR is also well established. Studies on working memory function and brain circuitry involved in conscious regulation of emotion are sparse, despite evidence that demonstrates that variation associated with 5-HTTLPR is not restricted to brain regions and functions involved in emotion identification, reactivity and appraisal (C. G. Beevers, J. Pacheco, et al., 2009; Turhan Canli et al., 2005). Recently, a larger study has reported reduced gray matter density within circuitry involved in conscious cognitive control function in carriers of the short 5-HTTLPR allele, particularly within the right Inferior frontal gyrus (IFG) (Selvaraj et al., 2011).

Research questions and Predictions

The overarching aim is to investigate 5-HTTLPR dependent impairments in basic cognitive control and underlying brain function in healthy subjects. A main thesis constituting a context for the project is that 5-HTTLPR variability is linked to prefrontal modulation in conscious emotion regulation.

Previous work suggests 5-HTTPLR dependent variance in the suppression of response to emotional stimuli , and a 5-HTTLPR dependent hyper vigilance towards certain emotional categories, such as a sad category in healthy female carriers of homozygote short 5-HTTLPR variants (Landrø et al., 2009). We wanted to distend our sample from the preliminary analyzes to explore the specificity linked to emotional category, genotype and gender.

In phase two we introduced a functional MRI modified n-back paradigm. Different n-back procedures will typically reveal activation within the lateral prefrontal cortex (Owen, McMillan, Laird, & Bullmore, 2005). The n-back procedure has the potential to uncover associations between levels of cognitive load and brain activation. Finally, structural correlates are explored by means of white matter integrity measured by fractional anisotropy based on recent reports of unique statistical contribution of 5-HTTLPR in frontal white matter connectivity (Pacheco et al., 2009).

Specific Research Aims

Paper I

Down regulation of serotonin transporter (5-HTT) expression has been associated with brain function and major depression. The polymorphic region (5-HTTLPR) in this gene has repeatedly been linked to automatic biased attention towards negative stimuli, while studies top-down processing of emotion are sparse. The primary aim of this study was to explore the allelic variation (short and long) of the 5-HTTLPR polymorphism in emotion perception associated with cognitive control of emotion.

Paper II

We used fMRI and an n-back task to unmask altered brain function in healthy women who were grouped based upon the 5-HTTLPR genotypes. We tested the hypothesis that short 5-

HTTLPR allele carriers, but not long 5-HTTLPR carriers would show altered brain function in a circuit that has been consistently implicated in MDD (Price & Drevets, 2010) while performing the n-back task. It was also predicted that short 5-HTTLPR carriers would have inferior performance on the n-back task and that task performance would be inversely associated with lateral PFC activation.

Paper III

It has been demonstrated that adult female short 5-HTTLPR carriers have less integrated white matter in the UF compared to long 5-HTTLPR carriers (Pacheco et al., 2009), but this sample consisted of adolescent females and young adults, a restricted age cohort. Herein, we explored the hypotheses of unique effects of age and genotype in healthy women over an expanded adult age span (21-61 years of age). We also wanted to test the specificity of this association by calculating FA values within the Superior longitudinal fascicule (SLF) that connect the lateral prefrontal cortex to the occipital and temporal lobe, a predominantly cognitive pathway.

Materials and Methods

Design

The research design is best described as a natural experiment in a double blind setting. Neither the test leader nor the participant had information about genotypes under test administration. The study focused on a predefined healthy sample of the population.

Participants and Procedures

The participants were recruited by local posters and advertisements in the local newspaper. After providing written informed consent, participants underwent medical and psychiatric evaluations. Criteria for inclusion were individuals between 18 and 65 years, without organic brain disease or head trauma, no actual psychopharmacologic medications, no actual or former psychological disorder according to DSM-IV and no BDI > 13 or BAI > 7. General cognitive functioning was estimated by scaled scores of two subtests from WAIS-III, Picture Completion and Similarities (Wechsler, 2003). Lap top computers were used in different test laboratories with standardized set ups and instructions. Participants received 250 NOK (approximately 32 Euros or 28 British Pounds).

Clinical Evaluations

Diagnostics were done in accordance with the Structural Clinical Interviews for DSM-IV criteria (APA, 1994). Both Axis I and Axis II (SCID-I and II) were utilized by trained professional clinical psychologists. All interviews were audio taped with consent and participants were instructed not to mention names or other identifying information. For participants over whom there was doubt concerning diagnostics, the audiotape was blindly repeated for consensus by an external experienced clinical psychologist. Symptoms associated with depression and anxiety were addressed by the use of Beck Depression Inventory (BDI) and Beck Anxiety Inventory (BAI) (A. Beck & Steer, 2005; A. Beck, Steer, & Brown, 2005), which were filled out by the participants at the time of testing.

Demographics

Education level was classified by means of The International Standard Classifications of Education (UNESCO, 1997). Demographic information was assessed by a modified version of the Diagnostic Interview for Genetic Studies (Nurnberger et al., 1994). For participants with MDD history, recurrent depressive episodes, duration of episodes, hospitalizations,

pharmacotherapy and sick leaves for each episode were registered. A history of only one earlier MDD episode was accepted (n=18) for the Emo 1-back study. Herein, we defined 162 Norwegian individuals as healthy control subjects, females (n=110) and males (n=52), between 19 and 64 years old (M=36). A total subsample of 45 healthy women was included in piloting and fMRI and DTI scanning procedures. Eight participants where excluded in this phase due modifications of the fMRI setup. Thirty seven participants were included after piloting and 33 participants between 21 and 61 years old (M=37) were included in the final analysis (Figure 2). One participant terminated the MR scanning procedure due to scanner-related anxiety. Three participants were excluded based on low quality MR images.

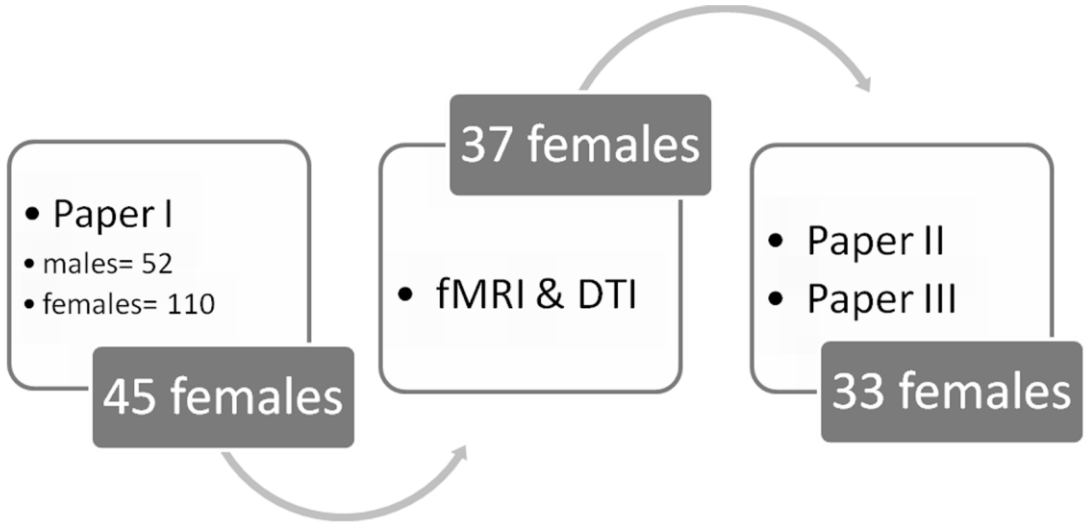

Figure 2. The selection and inclusion of participants over the three studies. Thirty-three healthy women, drawn from the original sample from Paper I, were included in the fMRI and DTI analyzes.

The Emo 1-back Task

We constructed a 1-back paradigm containing emotional expressions in human faces created by Landrø et al. (2009). The paradigm was programmed using E-prime E-studio software. 360 pictures of sad, happy, fearful and neutral faces were presented sequentially for 1000ms. A centered fixation point was presented as an inter-stimulus interval for 1000ms. The stimuli were presented in 3 blocks of 120 trials, each containing 8 targets for each emotional category. Stimuli were selected from validated images of facial expressions from The

Karolinska Directed Emotional Faces (Lundqvist, Flykt, & Õhman, 2008). Images were set in black backgrounds and were blurred in the borders between hair and background. We included a practice procedure with "right", "wrong" and "should have responded" feedback. The participants were instructed to press "1" on the keyboard each time the same facial expression, but not the same face, was presented twice in a row (1-back). Outcome measures were percentage accuracy and reaction time. The emotional faces were contrasted to the neutral faces to explore whether the emotional faces interfered with cognitive performance.

Functional Magnetic Resonance Imaging

Magnetic resonance measures how radio frequency electromagnetic waves act upon dipoles in a magnetic field. In the presence of a strong magnetic field, there is a tendency for the magnetic moment of these nuclei to align parallel or antiparallel to the main static field (B0) of the fMRI scanner. MR signals arise mainly from the hydrogen nuclei in water. Water dipoles are presented in the brain in a density which has the potential to support measurement at a high spatial resolution. Different signals from the transition of these dipoles between different energy states are detected by radio frequency pulses. Radio frequency (rf) pulses are used to flip the angle of the protons. Radio waves have to be the right frequency to excite protons and the frequency is proportional to the strength of the magnetic field, known as the Larmor frequency. The radio pulse contributes precisely the amount of energy needed to cause the nuclei to transition to a higher energy level and realign in the magnetic field. By creating gradients of magnetic fields, different frequencies will affect protons in different parts of space (referred to as K-space). K-space contains information about frequencies in the image. Information about the nearby tissue is derived from the rate at which the hydrogen nuclei return to the low-energy state following the excitation. Coils are placed within the scanner to detect changes in the local magnetic field. Echo-planar imaging (EPI) samples k-space in a linear zig-zag trajectory, spiral imaging (SI) samples k-space in a spiral trajectory. It is also possible to sample k-space with one trajectory or with multiple trajectories. The most common measures are based on how quickly the protons realign with the main magnetic field (T1) or on how quickly the protons give off energy as they recover to equilibrium (T2). In addition to T1 and T2 images there is a third kind called T2* (tee-two-star) In T2* images, artefacts occur near junctions between air and tissue, like sinuses and ear canals, causing a lack of homogeneity in the transverse relaxation in the process where protons are recovering to equilibrium. T2* refers to the decay constant in instances when lack of homogeneities are present (Logothetis & Wandell, 2004).

The BOLD signal

The causal relation between neural activity and the blood-oxygen-level-dependent (BOLD) fMRI signals is linked to the brain's synaptic activity and the associated action potentials. In a process called hemodynamic response, blood releases glucose to neurons and astrocyte neurons to a greater rate than to inactive neurones. When neurons are active they increase their consumption of energy from glucose and switch to less energetically effective, but more rapid aerobic glycolysis. The local response to this energy utilization is to increase blood flow to regions of increased neural activity resulting in a surplus of oxyhemoglobin in the veins of the area and distinguishable change of the local ratio of oxyhemoglobin to deoxyhemoglobin. Hemodynamic response is detected by the MRI scanner and gives us the fMRI BOLD response. The signals are detected on the bases of the magnetic field strength. A typical BOLD signal consists of an initial dip, an overshoot, a positive BOLD response and a post stimulus undershoot. Local field potentials (LFP) reflect post- synaptic potentials similar to what EEG (ERPs) and MEG measure. Multi- Unit Activity (MUA) reflects action potentials similar to what most electrophysiology measures measure. Logothetis et al. (2001) found that BOLD activity is more closely related to LFPs than MUA. As fMRI measures the population activity, the signals depend on how active the neurons are and on how many neurons are activated. Manipulations that change the activity of many neurons a little will show bigger activation differences than manipulations that change the activation of a few neurons a lot. The time course of the human BOLD response to a brief stimulus, the temporal impulse response function, is often called the hemodynamic response function (HRF). There is heterogeneity in HRF across the cortex of a human observer and also between observers. This is why fMRI allowed us to look at relative activation across different structures and loci in the human brain.

Blood- oxygen- level- dependant imaging data were acquired on a 3T Philips Scanner using gradient echo EPI 34 transverse 3mm slices (no gap), parallel to the AC- PC line. Repetition time (TR) =2000 ms, slice echo time (TE) = 30 ms. Flip angle = 80%, field of view (FOV 240 x 240 x 102 mm). Acquisition time = 2000ms per frame. For higher level analysis FMRIB's Local Analysis of Mixed Effects was used for modeling and estimating the inter-session and inter-subject random-effects component of the mixed-effects variance.

The fMRI modified n-back

In a working memory functional MRI paradigm, participants were instructed to monitor a series of stimuli and to respond whenever a stimulus was presented that was the same as the one presented n- trials previously. The increase in cognitive load is based on the parametric increase between the different n-backs. The paradigm was constructed in E-prime 2.0 studio software. The stimuli were a series of 2 times 12 small and large centred letters in 16 randomized blocks. Stimulus duration time was set to 300ms with inter-stimulus interval fixation points of 1650ms. Four types of stimulus procedures were randomized, two types containing 2 n-backs and two types containing 4 n-backs, giving a total of 48 events and 144 non events in each run (64/128 in the 0-back condition). Each of the 16 series had a total duration of 23,4s. An 8000 ms resting condition was presented between series in the form of a centred exclamation point. Behavioural measures for group comparison were accuracy and reaction time. Outcome measures used in the fMRI analysis were onset time and duration in the n-back series compared to the 8000ms resting conditions in a block related design (Figure 3).

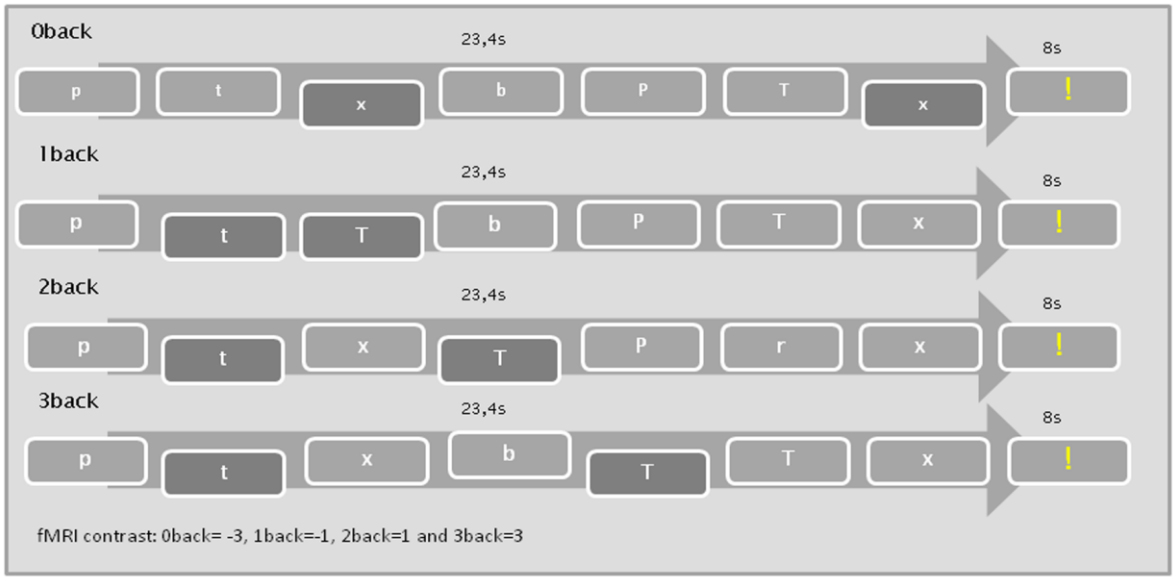

Figure 3. Illustration of the fMRI modified n-back procedure. The four n-back conditions were contrasted in a parametric model.

The BOLD activation across the whole sample resembled activation patterns reported from similar n-back designs (Owen et al., 2005). The strongest activation was found within the lateral prefrontal cortex, the occipital cortex, the anterior cingulate cortex, and the basal

ganglia (Figure 4).

Cluster	Voxels	Z-Max	MNI Coordiate			Probabilistic atlas label
			x	y	z	
1	4068	6.03	136	131	94	41% Precentral Gyrus, 30% Inferior Frontal Gyrus, pars opercularis
2	5390	5.85	127	80	111	32% Supramarginal Gyrus, posterior division, 19% Superior Parietal Lobule
3	400	5.53	73	54	134	53% Lateral Occipital Cortex, superior division
4	749	5.47	102	58	121	19% Precunious Cortex, 16% Lateral Occipital Cortex, superior division
5	1372	5.42	64	120	119	16% Precentral Gyrus, 15% Superior Frontal Gyrus
6	645	5.42	79	141	69	65% Right Caudate, 33% Right Accumbens
7	1762	5.41	107	131	69	53% Left Pallidum, 41% Left Putamen
8	250	5.40	100	145	112	55% Paracingulate Gyrus

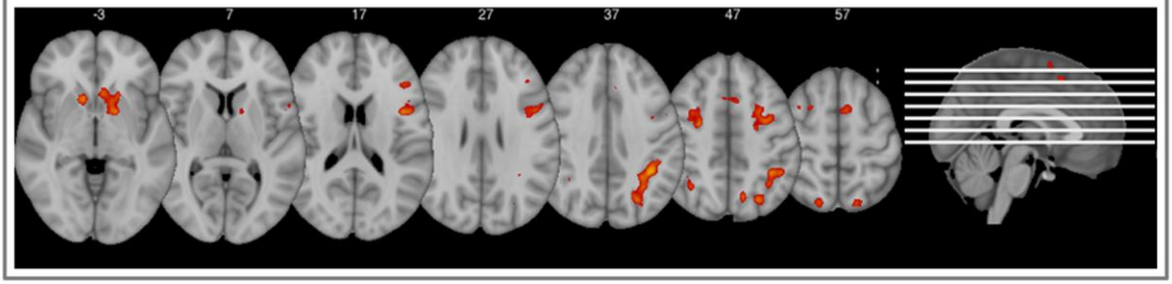

Figure 4. Location BOLD contrast activation across the whole sample (n=33). Z-threshold=2.3 (p=.05).

Diffusion Tensor Imaging

Diffusion tensor imaging is based on the measure of an internal fibrous structure analogous to the anisotropy of water crystals in the brain. Water will diffuse more rapidly in the direction aligned with the internal structure, and more slowly as it moves perpendicular to the preferred direction. Diffusion is an intrinsic physical process that is totally independent of the magnetic field. The MRI does not interfere with the diffusion process itself. Diffusion is encoded in the MRI signal by using magnetic field gradient pulses and only molecular displacements that occur along the direction of the gradient are visible (Le Bihan et al., 2001). By changing the direction gradient pulses we will be able to observe the variation in the diffusion measurement based on the anisotropic quality of water crystals in the brain's white matter. The properties of each voxel of a single DTI image is usually calculated by vector or tensor math from different diffusion weighted acquisitions, each obtained with a different orientation of the diffusion sensitizing gradients. Tractography refers to the process where you use the directional information to select and follow neural tracts through the brain. Diffusion tensor imaging scans derive neural tract directional information from the data using 3D or multidimensional vector algorithms (a vector is a based gradient direction, sufficient to compute the diffusion tensor). The tensor describes molecular mobility along each direction and correlation between these directions. From the diffusion tensor, diffusion anisotropy measures, such as the fractional anisotropy (FA), can be computed. The diffusion tensor is applied based on the fact

that one cannot describe diffusion as a single scalar coefficient when anisotropy is present. By the use of linear regression one can use the diffusion tensor components to calculate indices that reflect the average diffusion or the degree of anisotropy in each voxel. Eigen- vectors and eigen-values are calculated on the basis of the main direction of diffusivities in each voxel (the diffusion coefficient). We then apply a three- dimensional representation (ellipsoid) of the diffusion distance covered in space by molecules in a given diffusion time. The main axis of the ellipsoid gives the main diffusion direction in the voxel corresponding to the main direction of the fibers. The eccentricity of the ellipsoid provides infomation about the degree of anisotropy and its symmetry. The length of the ellipsoid in any direction of space is given by the diffusion distance covered in this direction (Le Bihan et al., 2001). The FA value is the magnitude of the diffusion coefficient that can be ascribed to anisotropic diffusion, or in other words how strongly directonal the local tract structure is. Summarized, you have three measures for DTI analyzes; the mean diffusity, the degree of anisotropy, and the direction of diffusiveness. One of the main challenges have been how to align FA images from multiple subjects based on the subsequent voxelwise analyses. To solve this problem, we project the voxelwise analyses into an alignment-invariant tract representation (the mean FA skeleton). Voxelwise statistical analysis of the FA data was carried out using Tract-Based Spatial Statistics (Smith et al., 2006), part of FSL (Smith et al., 2004). First, FA images were created by fitting a tensor model to the raw diffusion data using FDT, and then brain-extracted using BET (Smith, 2002). All subjects' FA data were then aligned into a common space using the nonlinear registration tool FNIRT (J .L. R Andersson, M. Jenkinson, & S. Smith, 2007; J. L. R. Andersson, M. Jenkinson, & S. Smith, 2007), which uses a b-spline representation of the registration warp field (Rueckert et al., 1999). Next, the mean FA image was created and thinned to create a mean FA skeleton that represents the centres of all tracts common to the group. Each subject's FA data was then projected onto this FA skeleton multiplied by pathways of interest from the JHU white-matter tractography probabilistic atlas (Hua et al., 2008) in binary masks. The FSL tool fslmeants was used to create mean FA values from the predefined POI's.

Genotyping

The genotyping was performed on the Clinical Chemical Unit at Ullevål University Hospital using a polymerase chain reaction (PCR) procedure. Primers (the initial RNA molecule) bind to the denaturized DNA when it's heated and then gradually cooled down. PCR is basically a method used to amplify the amount of DNA linked to a particular genetic segment. The

biallelic 5-HTTLPR polymorphism, located in the regulatory region of the serotonin transporter gene (SLC6A4), was genotyped essentially as described in detail elsewhere (Gelernter, Kranzler, & Cubells, 1997; Stein, Seedat, & Gelernter, 2006). A real-time fluorescence Light Cycler instrument was used to amplify genomic DNA by PCR in a final volume of 20 ul using Light Cycler Faststart DNA SYBR Green kit (Roche cat no 12239264001) with specific primers (0.5 uM) (Gelernter et al., 1997) generating a long (L) 419 base pair (bp) or a short (S) 375 bp PCR product. Differences in product length depend on the variable number of a 22 bp tandem repeat (VNTR) sequence in the promoter region. Cycle conditions were initiated by 10 min denaturation (95 oC) followed by 45 cycles at 95 oC (10 s), 66 oC (10 s) and 72 oC (10 s). Based on the identification of a single nucleotide polymorphism (SNP) within the long variant, suggestions have been put forth that this is a triallelic functional polymorphism (Hu et al., 2006). For the detection of the additional A>G SNP (rs25531), the PCR fragments were digested with 1 U MspI restriction enzyme (New England Biolabs, Beverly, Massachusetts) for 2 hours at 37 oC. The PCR fragments contain two obligatory MspI sites, whereas the A>G substitution creates an additional MspI site. The PCR reaction followed by restriction digestion and gel electrophoreses provides classification of the S, LA and LG alleles. The triallelic classification was then reclassified into a biallelic functional model, based on the 5-HTTLPR-directed level of transcriptional activity of the transporter gene as follows: LgS, LgLg and SS genotypes were classified as SS (low leveled RNA transcription); LaS and LaLg genotypes were classified as LS (intermediate leveled); and LaLa genotype was classified as LL (high leveled) (Neumeister et al., 2006).

Statistical Analyzes

Paper I

Two way ANOVAs with post hoc comparisons using the Tukey HSD test were conducted to explore potential group differences in age, education level, BDI, BAI and the subscales Similarities and Picture Completion from the WAIS III. Multivariate ANOVAs were conducted using analyzes of variance to explore potential statistical effects of sex and genotype on accuracy and reaction time in neutral versus emotional categories. The data were analyzed according to both a biallelic and triallelic genetic model. A multivariate ANOVA was conducted to explore group differences in the neutral category, which was used as the baseline contrast.

Paper II

Two way ANOVAs were conducted to explore potential group differences in age, education level, Beck Depression Inventory, Beck Anxiety Inventory, WAIS III; Similarities and Picture Completion, and the fMRI data processing was carried out using FEAT (FMRI Expert Analysis Tool) version 5.98, a part of FSL (FMRIB's Software Library). Z (Gaussianised T/F) statistics determined by $Z > 2.3$ and (corrected) cluster significance threshold using significant threshold of $p=.05$. Mid level analysis was carried out using a fixed effects model, by forcing the random effects variance to zero. Contrast parameter estimates (COPE's) were contrasted for each mask based on the parametric increase from the four n-back runs. Percentage signal change based on median COPE per non zero voxels was collected for each ROI. Two way ANOVAs were conducted using PASW Statistics 18 to explore potential effects of genotype within the 4 ROI's. Given the large age span, age was added as covariate in all analyzes. The behavioural measure of accuracy from events was reaction time corrected to prevent potential speed accuracy effects and separately analyzed for each n-back condition using two way ANOVAs.

Paper III

The average FA over all voxels in the mask was plotted into two way ANOVAs using PASW Statistics 18 to explore potential effects of genotype within the POI's. Two way ANOVAs were conducted to compare FA values for the 5-HTTLPR genotypes. Next, potential effects associated with white matter microstructure development were explored by adding participant age in two way ANCOVAs.

Ethical Considerations

The participants were recruited from non-clinical populations. Scientists that do studies approved by the Regional Ethics Committee, which adheres to the Helsinki Convention, have, in line with the administrations law §13e, the duty to remain silent about the participants personal information. This principal is also found in The National Committee for Research Ethics in the Social Sciences and the Humanities (NESH) and overlaps with the claim of confidentiality. The claim of informed consensus refers to the importance of giving the participants information about how the data and results of the studies will be stored, used and interpreted.

It is important to note that great psychological interest is linked to endophenotypes of individual differences. Studies of emotional processing on the neurobiological level have the potential to give important contributions relevant for clinical psychology. Both psychotherapeutic aspects and pharmacological aspects may be addressed based on these studies. Cognitive neuroscience has become one of the main sources of support when justifying particular therapeutic approaches. This will raise substantial ethical concerns linked to interpretations of the results from these studies.

Important decisions are linked to details in the methods, some mentioned earlier in this paper and others out of the scope of this paper. The main message is that results from neuroimaging, such as fMRI and DTI, are not just pictures of functional and structural attributes of the brain. The results, instead, rely heavily on the researchers' designs and the nature of the statistics used to create these images. All of this is basically the result of general linear modelling. The ethical concerns emerge when potential results are communicated to the general population. Pictures of brain activation should not be translated into proof of the existence of "blue genes". The dynamics between genes and environment are complex and often misinterpreted, even among scientists. How to fulfil the standard for informed consensus will for these reasons represent a major ethical concern. Studies based on imaging genetics may be interpreted in several ways by different scientists from different disciplines. The participants will typically only be informed about the general framework of how psychologists understand the interaction between genes and environment. Disagreements are often linked to how different scientists weigh the importance of biology and environmental factors. Genes are quantitative units at the DNA level but every aspect associated with genes is in nature dynamical. The introduction of epigenetics has shown that environment has the potential to change genes. General explanations of gene-environment relationships are often based on causality and will not grasp the dynamical nature of genes.

In the end we have to ask ourselves if our studies have any relevance for anything (other than giving the student a Ph.D degree). Genetic polymorphisms are common categories in the general population. Syndromes such as depressive disorders, anxiety disorders, bipolar disorders, schizophrenia, and personality disorders are also categories. These categories are used to explain some common variation within heterogeneous groups of individuals. On a population level, the different psychological disorders are associated with distinguishable (though often overlapping) development, manifestation and outcome. Genetic polymorphisms

have the potential to be used in similar ways and may reflect more homogeneous groups, at least on the biological level. Studies exploring phenotypes and intermediate phenotypes will contribute to a better understanding, better treatment and better predictions of outcome related to psychological disorders. The ethics related to the accomplishments of these studies have to be evaluated in light of these potential benefits.

This study is part of a project that applied to the Regional Ethics Committee of Norway (North) and adheres to the Helsinki Convention. All data was collected and stored according to prescribed procedures fulfilling these standards.

Results

Paper I

Two way ANOVAs revealed no statistically significant group effects of education level, BDI, BAI or the two subscales Similarities and Picture Completion from the WAIS-III for the biallelic or triallelic model. We found no statistically significant effects of age in the biallelic model, but a statistically significant effect of age in the triallelic model [$F(2, 156) = 3.73$, $\eta^2 = .04$, $p = .026$]. Post hoc comparisons using the Tukey HSD test indicated older mean age for the SS variants compared to the LL variants [MD = -7.6, SE = 2.9, $p < .05$]. Predicting reaction time by genotype and sex revealed no unique effect of genotype for the triallelic or biallelic model in the fearful versus neutral, sad versus neutral, happy versus neutral or in the neutral contrast condition. Predicting accuracy from the triallelic gene model and sex did not reveal unique effects of genotype. Applying the biallelic genetic model revealed statistically significant unique effects of genotype [$F(2, 156) = 3.60$, $\eta^2 = .04$, $p = .029$] and sex [$F(1, 156) = 7.68$, $\eta^2 = .04$, $p = .006$] for the sad versus neutral category. There were no interaction between genotype and sex for the sad versus neutral category [$F(2, 156) = .67$, $p = .673$]. There were also main effects of genotype [$F(2, 156) = 4.35$, $\eta^2 = .05$, $p = .014$] and sex [$F(1, 156) = 6.41$, $\eta^2 = .04$, $p = .012$] in the fearful versus neutral category. There were no interaction between genotype and sex in the fearful versus neutral category [$F(2, 156) = .54$, $p = .581$]. The main effect of genotype did not reach statistical significance in the happy versus neutral [$F(2, 156) = .04$, $p = .958$] or in the neutral contrast condition [$F(2, 156) = .54$, $p = .581$]. No effect of sex or interaction between genotype and sex where found in the happy versus neutral category or in the neutral contrast condition. Polynomal contrast tests of linearity revealed statistically linear gene effects for the sad versus neutral category [CE= -.077, $p = .009$] and fearful versus neutral category [CE= -.076, $p = .007$].

Paper II

Applying the 5-HTTLPR genotype revealed statistically significant age corrected main effects of genotype for the right [$F(2, 30) = 4.8$, $\eta^2 = .25$, $p = .026$] and left [$F(2, 30) = 4.1$, $\eta^2 = .22$, $p = .015$] Inferior frontal gyrus, pars triangularis. No statistically significant effects were found within the inferior frontal gyrus pars opercularis by applying the 5-HTTLPR genotype. Age and reaction time corrected two way ANOVAs revealed a statistically significant difference between genotypes on accuracy for the 3-back condition [$F(2, 30) = 4.9$, $\eta^2 = .26$, $p = .014$]. Applying a polynomial contrast revealed a statistically significant linear relationship [CE= -

.123, p=.005] between number of short alleles and accuracy for the 3-back condition. There was also a statistically significant three way interaction (percentage signal change x 3back accuracy x genotype) in both the right [F(2, 30)=5.1, η^2=.26, p=.012] and left [F(2, 30)=4.0, η^2=.22, p=.027 Inferior frontal gyrus, pars triangularis.

Paper III

Two way ANOVAs revealed a statistically significant effect of genotype in the left frontal UF [F(2, 30)=3.3, η^2=.18, p<.047]. There was also a clear trend toward statistically significant effects of genotype in the right SLF [F(2, 30)=3.2, η^2=.17, p=.054]. Polynomial contrast analyses indicated a linear effect for the UF (CE=-.015,p=.022) but not for the SLF (CE=-.007,p=.129). Despite no statistically significant group differences in age, adding age as a covariate explained the statistically significant effect of genotype in the left frontal uncinate fascicule [F(1, 29)=13.3, η^2=.31 p<.001] and the near significant effect of genotype in the right superior longitudinal fascicule [F(1, 29)=8.6, η^2=.22, p=.008]. The remaining statistics for 5-HTTLPR was [F(2, 29)=1.4, η^2=.09, p=.257] for the right SLF and [F(2, 29)=1.6, η^2=.10, p=.208] for the left frontal UF. There was also a main effect of age in the right SLF [F(1, 29)=10.9, η^2=.27, p=.002]. No statistically significant effects of age or genotype were found within temporal parts of the UF or the right frontal UF. Bonferroni corrected alpha levels (.05/6=.008) did however reveal statistically significant local main effects of age in the left frontal UF and right and left SLF.

Summary of Papers

Paper I

Small to moderate statistical 5-HTTLPR effects on accuracy in both negative (sad and fearful) emotion categories, but not in the positive (happy) emotion or neutral baseline contrast conditions, were revealed. The association between 5-HTTLPR variability and cognition seems to be linked to subcomponents that are part of the interplay between emotion perception and cognitive control, which is involved in emotion regulation (Price & Drevets, 2010).

Paper II

Herein we show that in healthy women the 5-HTTLPR polymorphism influences task performance and the VLPFC activation pattern during an n-back procedure. The fMRI modified n-back paradigm revealed a main effect of genotype within both the right and left VLPFC. Short 5-HTTLPR carriers showed higher percentage signal change within these subregions of the LPFC. Short 5-HTTLPR carriers compared to the long 5-HTTLPR carriers showed lesser reaction time corrected accuracy from events. The short 5-HTTLPR carriers showed higher percentage signal change within the lateral prefrontal cortex combined with inferior performance in the n-back procedure. This behavioral and neural pattern resembles the impaired cognitive performance and exaggerated VLPFC activation seen in patients during a depressive episode (Fitzgerald, Laird, Maller, & Daskalakis, 2008).

Paper III

Despite no statistically significant group differences in age, entering age as a covariate explained the finding of an association between 5-HTTLPR and white matter integrity in healthy adult women. Thus, similar to the previously reported findings in adolescence and young adults, these results did not reveal unique effects of age and genotype in the left frontal UF. Calculating FA values for a predominately cognitive pathway, the SLF, did not reveal a unique effect of genotype after age corrections either. The larger age span from the present study may involve both white matter changes associated with the aging process and those associated with growth and development. Herein, the results from Pacheco et al. (2009) and the present study may reflect two different basal processes.

Discussion

Never-depressed, healthy carriers of the short 5-HTTLPR allele showed more interference from negative emotional stimuli in cognition than non carriers. No statistical effect of genotype in neutral or positive stimuli indicated that inferior performance in cognition in short carriers is only pronounced when they are presented with negative emotional stimuli, and therefore cannot be explained by cognition alone. By using a cognitive n-back paradigm in the fMRI scanner and including a subsample it was demonstrated that carriers of short 5-HTTLPR allele have hyperactivity within a circuitry involved in emotion regulation. This activation pattern is similar to what has been shown in individuals during an MDD episode (Grimm et al., 2008; Matsuo et al., 2007; Walter et al., 2007). Inferior performance on the n-back task was also observed. Age seems to be an important factor in the association between 5-HTTLPR and frontal white matter integrity in the same subsample.

The findings from the present studies suggest intermediate phenotypes that resemble biased cognition and altered brain activity similar to that seen in MDD (A. T. Beck, 2008; Fitzgerald et al., 2008). Herein, the serotonin transporter polymorphism may reflect endophenotypes associated with these underlying abnormalities. The amygdala's role in the perception of emotional valence has led to a series of studies to determine the role of the 5-HTTLPR in processing emotionally salient information in MDD (M. R. Munafo et al., 2008). Short 5-HTTLPR carriers have shown highly significant reduction of amygdala-anterior cingulate cortex connectivity in comparison to homozygote long 5-HTTLPR carriers (Pezawas et al., 2005). Short 5-HTTLPR carriers have also shown more functional coupling between the amygdala and the ventromedial prefrontal cortex, compared to long 5-HTTLPR carriers (Heinz et al., 2005). The findings from the present thesis extend this literature by pointing to the importance of cognitive control in the top-down processing of emotion.

Unique statistically significant effects of 5-HTTLPR and age on frontal white matter integrity has been reported (Pacheco et al., 2009). Replicating this study in a sample covering the whole adult lifespan revealed an age mediated effect of 5-HTTLPR. These results may reflect structural changes associated with the aging process and those associated with growth and development. The results from Pacheco et al. (2009) and this study may reflect two different basal processes. Studies on larger samples are needed to explore the nature of age x gene interactions.

Beck (2008) posits that connections among various negatively oriented schemas will become strengthened over time in response to negatively interpreted events. The negatively

biased schemas function as automatic information processors, efficient but maladaptive. In contrast, the role of cognitive control systems is attenuated during depression. A negative mood or mind-set may have been adaptive in the context of stressful life events. Cognitive reactivity may represent diathesis linked to which stimuli an individual will perceive to be emotional salient, and therefore which stimuli will be perceived as stressors for different persons. The trajectories that link brain structure, brain function and biased information processing to symptoms characteristic in MDD are not addressed directly over the three studies included in the present thesis. Despite this notion, these studies of healthy individuals are heavily based on a hypothesis of dynamical links between genes, brain, behaviour, traits, symptoms and syndromes. The results from the present study are mainly in line with the majority of the conclusions from 5-HTTLPR literature that have demonstrated short 5-HTTLPR carriers disadvantage at all these levels.

It is known that serotonin serves as a neurotrophic factor during early development (Buznikov, Lambert, & Lauder, 2001; Whitaker-Azmitia, 2001). Thus, it can be expected that the genetic variation in serotonin transporter function early in life results in an increase of extracellular 5-HT concentration that could affect the development of numerous systems, not restricted to the serotonergic circuitry, such as the hypothalamic-pituitary, adrenal (HPA) axis (Jabbi et al., 2007). These alterations may be associated with habitual reaction- and relations patterns, often linked to the concepts of personality. Some of the structures associated with 5-HTTLPR variability contain mirror neurons involved in goal-directed behavior or the observation of such behavior in others. Others include Von Economo neurons, which are believed to play a role in social bonding (T. Canli & Lesch, 2007; K. P. Lesch, 2010). These findings suggest a broader focus on the interplay between emotion processing and social cognition that also includes a developmental perspective. The pioneer study by Caspi et al. (2003) revealed an association between early life stressors, in the form of maltreatment in the first decade of life, 5-HTTLPR and depressive symptomatology. These hypotheses may overlap with a number of primate studies, which show that the influence of the serotonin transporter polymorphism on behavior is modified by early life experience, affecting both temperament and social competence with peers (Herman, Winslow, & Suomi, 2010). The results from the Emo 1-back study may lead to speculations of how this is related to experience. Attending to negative emotional faces may well have been adaptive in a hostile environment. Following this reasoning, there should be no relation between 5-HTTLPR and biased information processing in healthy control subjects, unless the gene “creates” a hostile environment. This interpretation may sound rather creative at the first glance, but genes are

heritable in their very nature and carriers of short 5-HTTLPR may therefore run in family constellations dominated by negative affect and hostility.

Methodological Considerations

Methodological determinants were highly influenced by the decision to include only healthy subjects. The potential statistical effects of 5-HTTLPR genotype in the background of a wealth of other environmental and genetic factors were predicted to be very small.

Hesitancy when defining the independent variable (genotype) represents a major concern in the 5-HTTLPR literature. Inclusion of the single A>G polymorphism (rs25531) has been the standard for the last decade, but several studies, including one of the studies from this thesis, show that the inclusion of this particular SNP may decrease the sensitivity and power of the measure. The meta-analysis by Clarke, Flint, Attwood, and Munafo (2010) also showed that studies that used the triallelic genotyping of the 5-HTTLPR revealed reduced effect size compared to studies based on the biallelic genotyping. These results are not in line with the assumption that the A version of the SNP has functional qualities similar to the long 5-HTTLPR allele (Hu et al., 2006). This study was also done by comparing subgroups as small as 7 participants and calls for replications. Wendland, Martin, Kruse, Lesch, & Murphy (2006) have demonstrated that the A>G SNP may be linked to both the short and long variants and have for this reason recommended that this SNP should be treated separately from the 5-HTTLPR polymorphism. The divisions based on the triallelic model may have been premature as they are actually based on the interaction between two genes. Following this reasoning, our measures may well be associated with both the biallelic and/or triallelic gene model, without posing that one of them is more or less correct than the other. Further studies should include calculations of unique effects of A>G SNP, 5-HTTLPR, and their potential interactions. The challenge this raises is that this will demand higher sample sizes since some of the gene variants are rare. Finally, other SNP's within the 5-HTTLPR include two with larger contributions to 5-HTT expression variation than the 5-HTTLPR itself (Martin et al., 2007). It is important to note that categorisations based on gene expression are not the same as categorisations based on the DNA structure. Pooling, or dichotomizing, genotypes refer to the situations in which we operate with dominant and recessive models. The heterozygous LS alleles can either be placed with the SS variants (dominant) or the LL variants (recessive). The rationales for pooling genotypes are often not accounted for in the literature.

Variance analysis based on the general linear model (GLM) is prone to several factors when it comes to sensitivity, or statistical power. Power estimates calculate the probability that the test will reject the null hypothesis when the null hypothesis is actually false. These probabilities are highly influenced by the sample sizes used to detect a particular effect, but also by the significance criterion used in the test and the nature of the real effect in the population. Effects of single genes are predicted to be very small. The MR sequences and analyses based on 33 participants probably do not have the power to detect all unique effects of 5-HTTLPR genotype and their interaction with other variables. It can be argued that the lack of unique statistical effects of 5-HTTLPR in the DTI measure after age has been accounted for may be due to a lack of statistical power, while the reported effects of 5-HTTLPR in Emo 1-back performance, fMRI n-back performance, and BOLD activation are detected because these effects are really larger in the population. The power challenge may also lead to biased publications rates of false positives. False replications based on similar sample sizes may be barred in peer reviewing due to lack of statistical power. The DTI study from this thesis indicates that the lack of an age corrected association between 5-HTTLPR and frontal white matter integrity are driven by the older participants. The relatively small sample size does not give us the statistical power to test this assumption. Limited statistical power represents threats towards the sensitivity of the measures (increasing the probability of Type-II errors). Power estimates have to be based on the observed effect sizes (post hoc) as we do not know what effect sizes to predict in the population. The sample size (n=162) in study I is among the largest in this literature ((Beevers et al., 2011) n=140, (Osinsky et al., 2008) n=50, (C. G. Beevers, T. T. Wells, A. J. Ellis, & J. E. McGeary, 2009) n=144, (Perez-Edgar et al., 2010) n=117 and (E. Fox, K. Zougkou, A. Ridgewell, & K. Garner, 2011) n=111). Larger sample sizes still have the potential to unveil statistical effects in other measures from this study where the effect sizes are smaller in the real population.

There are several ways to approach MRI data, and particularly fMRI data. Most of the critical steps are done in an effort to reduce error variance, when we merge the individual functional and structural images, or when we normalize our images to compare different individuals. There are many existing conventions for MR analyses, but there is still considerable variation in the methods sections of articles published using these methods. Positive findings from fMRI analyzes are particularly believable when they are combined with positive findings from behavioral measures and resemble data derived from other MR sequences. Herein, one can argue that the most convincing and transparent fMRI analyzes is that which is simplest, straight forward and manual based. The cost is that these types of

analyzes may not be well suited for the particular research questions in scope. The MR analyzes tools are also rapidly improving and new solutions for familiar problems are reported parallel to the research using these methods. While the fMRI results represent new findings linked to brain regions involved in conscious control of emotion, the results from applying the Emo 1-back are fairly consistent with the present literature.

Clinical Implications

Achievements from the studies on endophenotypes, meaning any hereditary characteristic that is normally associated with some condition but is not a direct symptom of that condition, have been criticized for failing to translate into clinical practice. This mismatch between scientific progress and clinical exploitation likely originated from the limited biological validity of the current behavioral conceptualization of mood disorders (Scharinger, Rabl, Pezawas, & Kasper, 2011). Psychological and psychopharmacological treatment is often based on heterozygous diagnoses sharing some common symptoms. Genetic polymorphisms, like the 5-HTTLPR, are relatively homogeneous categories compared to syndrome diagnoses. Exploring individual variation associated with genetic polymorphism represents a promising contribution to understand dynamical aspects of psychotherapy. Well documented emotion processing circuitry underlines the importance of taking both automatic and conscious processes into account in the treatment of major depression (M. L. Phillips, 2003; Price & Drevets, 2010). Both the literature and results presented in the present thesis demonstrate that there exist endophenotypes, analogous to individual differences. These categories underline the importance of fitting individual therapeutic approaches, not only for different diagnoses, but for different people. Other implications concern psychotherapy based on manuals or therapeutic approaches with clear preferences towards affective or cognitive aspects in the treatment of psychological disorders. The general literature derived from cognitive neuroscience, including the results from the present thesis, clearly indicate that symptoms associated with MDD cannot be understood by focusing on emotion or cognition separately. There may even be situations where the same constellations of symptoms should be treated with different therapies for different individuals. If this is true, general approaches for the treatment of a particular syndrome, such as a national guidance for the treatment of MDD, may be a sidetrack. Attention biased modification (ABM) treatment (MacLeod, Rutherford, Campbell, Ebsworthy, & Holker, 2002) represents a promising therapeutic approach based upon current knowledge of emotion processing from neuroscience. A recent study showed that carriers of the low transmission efficiency genotype are more sensitive to ABM (Elaine

Fox, Konstantina Zougkou, Anna Ridgewell, & Kelly Garner, 2011). This means that the carries of a genotype associated with MDD are also the persons that have the best outcome of psychotherapy. This provides a potential mechanism through which those with the low-expression form of this gene are more susceptible to environmental events for better and for worse. This is in line with the literature that has demonstrated that serotonin is important in shaping neuronal circuitry by regulating synaptic plasticity and neuronal activity patterns of serotonergic and non- serotonergic neurons (Gaspar et al., 2003). The 5-HTTLPR may therefore reflect individual differences in the degrees of synaptic plasticity.

Future perspectives

Studies on 5-HTTLPR related to major depression are well suited for fMRI resting state analyses as models for depression circuitry are well established (Price & Drevets, 2010). Individual differences in resting-state suggest that networks may be disrupted in persons at risk for major depression (Norbury, Mannie, & Cowen, 2011). Resting-state fMRI could also be combined with other functional and structural measures.

Psychotherapy is most often based on clinical evaluations. The accumulated knowledge on how to use information about 5-HTTLPR and other common genetic variance for individual adjusted therapy has not reached the clinics. The studies of intermediate endophenotypes, such as those of healthy control subjects, reveal individual differences that can be measured objectively. This kind of objective information is rare in clinical psychology and may add important information to traditional diagnostic interviews. In the future, the transition from complicated correlations to useful predictions may lead to improved premorbid risk assessment, preventive strategies, and treatment individualization.

Conclusions

Carriers of the short 5-HTTLPR alleles in the serotonin transporter gene (SLC6A4) demonstrated less accuracy in The Emo 1-back Task when presented with successive images of sad or fearful faces, but not for happy or neutral emotional faces. The study suggests an association between 5-HTTLPR variation in the serotonin transporter gene and altered emotion processing. The presentations of emotional stimuli in the context of continuous updating extend the previous literature by shifting the allocation of attention from bottom-up processing to top-down processing of emotion.

Cognitive control of emotion plays an important role in emotion downregulation when emotion activation is no longer adaptive (A. T. Beck, 2008). Study II extends the present literature by demonstrating impaired cognitive control and its neural correlate in healthy women carrying the short 5-HTTLPR allele. In study III, entering age as a covariate explained the hypothesis of an association between 5-HTTLPR and white matter integrity in healthy adult women. Thus, unlike the previously reported findings in adolescence and young adults (Pacheco et al., 2009), these results did not reveal unique effects of both age and genotype in the left frontal UF. Calculating FA values for a predominately cognitive pathway, the SLF, did not reveal a unique effect of genotype after age corrections either. Differences in white matter changes associated with the aging process and those associated with growth and development may explain why the earlier reported unique effects of genotype in frontal white matter integrity do not persist into adulthood as indicated by this study. The study underscore the importance of a developmental perspective when exploring endophenotypes associated with 5-HTTLPR.

Together, the three studies demonstrate intermediate phenotypes related to serotonin function that is associated with emotion regulation. These endophenotypes and behavioural phenotypes may be critical in the trajectories leading to symptomatology characteristic for major depression.

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