The Effect of 5-HTTLPR Variability on Cognitive Control Functioning

Implications of Emotional Processing and Sex

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Background: Although it is widely accepted that serotonin plays a pivotal role in emotional perception and processing, the role of serotonin in cognition is less clear. The present study investigated the implications of introducing emotional faces in a measure of cognitive control functioning. The measure was explored in association with the serotonin transporter polymorphism (5-HTTLPR), linked to serotonin transmission in the brain. An integrative model of emotional processing was used to illustrate cognitive control functioning in emotional processing and potential 5-HTTLPR-dependent diatheses for depressive symptomatology. As previous studies have shown the effect of sex on the relationship between 5-HTTLPR subtypes and cognitive measures, we also included sex as a variable in our analyses.

Methods: Sixty healthy participants were recruited in an experimental design. The participants underwent an extensive screening procedure and gave blood samples for 5-HTTLPR analysis. A new computerized test, labeled the Emo n-back, was constructed to explore the implications of presenting emotional categories of human faces in a paradigm measuring aspects of cognitive control functioning. Emotional categories within the Emo n-back were analyzed to spot 5-HTTLPR-dependent variation in cognitive and emotional perception and processing in males and females.

Results: Exposure to different emotional categories had specific effects on cognitive control functions as measured by the Emo n-back. Participants showed significantly decreased accuracy on the n-back task when presented with successive images from the negative emotional categories, and this effect was most pronounced for sad compared with neutral faces. A three-way interaction effect was found between sex, 5-HTTLPR polymorphism and emotional categories within the Emo n-back. Examination of this interaction revealed a distinct pattern for female short 5-HTTLPR carriers indicating an increased sensitivity to the sad emotional category in this subgroup of participants.

Conclusion: Sex and 5-HTTLPR polymorphism had a significant impact on accuracy in a measure of cognitive control function. The effect is most pronounced when presented with sad human faces, indicating altered sex- 5-HTTLPR-dependent processing linked to specific emotional content. The Emo n-back indicated mechanisms that might represent diatheses for depressive symptomatology by demonstrating the different genotypes to reveal distinct patterns in the way they process adverse environmental stimuli.

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Introduction

An important approach to the study of mood and cognition has been to consider the genetic, neural, neurochemical and neuropsychological substrates of mental illness. Studies in this area have uncovered important aspects of diathesis, development, symptoms and recovery of psychological disease. The studies also contribute to the development of global structures of emotion and normal emotional perception and processing. Integrating global constructs of emotion and specific objective measurable constituents represents a bridging in the literature on both normal emotional processing and disorders of emotion.

The mapping of the human genome has enabled explorations of the specific genetic contributions to neural structures and neurochemistry, as well as the associated emotional and cognitive derivates.

Looking for biological markers associated with specific genes amounts to the study of endophenotypes. Most current studies on endophenotypes have used different neuroimaging procedures, such as measures of neural activation of specific brain regions. In contrast, the strategy of mapping the genes' cognitive architecture is in its infancy and is often even neglected. This is probably based on the assumption that the endophenotypes convey a simpler architecture and may be closer to the level at which genes operates than more remote phenotypes (Meyer-Lindenberg & Weinberger, 2006). This assumption probably holds for measures based on self reports, life stories or clinical symptoms, although this view has also been contested based on reliability analysis (Flint & Munafò, 2007). The use of objective neuropsychological measures represents a promising contribution to the understanding of genes' phenotypic expression. Furthermore, exploring psychological resolution in cognitive processes may contingently help us to delimit 5-HTTLPR-associated phenotypes that otherwise would be overlooked based on neuroimaging studies alone.

The neuropsychological picture is broadly addressed in studies of cognitive deficits associated with the symptomatology of major depression, but is sparse in studies investigating the potential gene candidates that we know are involved in cognitive and emotional processing involved in the same phenomena. This paper will address this view by showing that hypotheses based on neuropsychological data constitute a major contribution to the understanding of genes phenotypic expression. The focus will be on serotonin (5-HT) functioning and a polymorphic genetic region linked to the transcription of a reuptake protein

central to serotonin metabolism (5-HTTLPR within the transcriptional region SLC6A4). The objective of this study was to supplement existing studies by constructing an objective cognitive paradigm able to spot subprocesses in cognitive and emotional processing.

It's important to bear in mind that causes of mental illness are complex, and interact and vary according to the particular disorder and individual. Genetics, early development, disease or injury, neurocognitive and psychological mechanisms, life experiences, society and culture can all contribute to the development or progression of different metal disorders. The serotonin-linked polymorphism will represent a relatively small effect against a background of substantial genetic and environmental variation.

Context

The rationale for choosing the serotonin system and the associated polymorphic region (5-HTTLPR) as the most central gene candidate mainly comes from two sources. The first comes from pharmacological studies of, and treatment with, serotonin-linked antidepressants. The other comes from neuroscience and studies that show considerable overlap between abnormal brain structure and functioning in major depression and serotonergic circuits.

The serotonin system is a common target for antidepressants, anxiolytics and antipsychotic drugs. Serotonin controls neural specificity, differentiation and phenotypic duration of various mental disorders. The formation and integration of neural networks depends on processes tied to several proteins, but a string of studies indicates that variability in the expression of the serotonintransporter (5-HTT) is critical for development and plasticity in delimited neural pathways (Lesch & Gutknecht, 2005).

Serotonin controls a highly complex system of neural communication circuits mediated by several pre- and postsynaptic subgroups of 5-HT receptors. A frequent transport of 5-HT in the presynaptic neuron, that leads to the maintenance of 5-HT and further release in the synaptic gap, is mediated by a unique protein. This protein, the 5-HT transporter, 5-HTT, is the functional site of antidepressants, like prototypical tricyclic clomipramine and, in particular, for the group labelled "selective serotonin reuptake inhibitors" (SSRIs), like fluvoxamine, paroxetine, citalopram and sertaline. The different subgroups of SSRIs don't share identical pharmacokinetic functioning but are all linked to the 5-HT transporter. These drugs are in frequent use for the treatment of depression, anxiety and disorders associated

with impulsivity, like obsessive-compulsive disorder (Lesch & Murphy, 2003), as well as several potential neurotoxin preparates like MDMA (ecstasy).

Until recently, focus has been on kinetic variability (what the body does to the drug) while dynamic variability (what the drug does to the body) has obtained less attention. However, it now seems that pharmacodynamic variability in humans is large, reproducible and usually more pronounced than pharmacokinetic variability. Many drug targets, like receptors, transporters and enzymes, don't only contribute to the regulation of the transmitter system but also, directly or indirectly, modulate the plasticity of neurocircuits. Both variation in structure and variation in expression influence the gene product, availability and function. This will have a substantial influence on further functioning and efficacy in a dynamical manner.

When we know there is a genetic polymorphism that has a major influence on kinetic serotonin functioning, the same polymorphism should have neuroanatomical and cognitive derivates based on dynamical developmental variability. Mood, cognition and motor functioning, as well as circadian and neuroendocrine rhythms, are all modulated by the brainstem serotonin system and distinctively altered in anxiety and affective disorders. This variability should be uncovered and checked in relation to the current gene candidate. Different methods are required to explore variability both within and between the different serotonin-modulated functions.

Contemporary literature on depressive disorders is massive on both functional and structural aspects. Even mild depression is associated with pronounced deficits in cognitive, emotional, motor, perceptual and communication tasks. Wide-ranging neuropsychological deficits in depressed patients are found in areas like executive function (Elliot, 1998; Murphy et al., 2001; Landrø, Stiles & Sletvold, 2001), identification of emotionally significant stimuli (Rubinow & Post, 1992), negative emotional bias (Bradley, Mogg & Millar, 1996; Murphy et al., 1999; Williams, Mathews & Macleod, 1996) and negative, or reduced, positive attention bias while identifying emotional expressions of human faces (David & Cutting, 1990; Gur et al., 1992; Surguladze et al., 2004).

Structural abnormalities are found in several areas associated with the processing of emotional stimuli. The most consistent findings involve the amygdala (Sheline et al., 1998; Bowley et al., 2002), subgenual region of the anterior cingulated gyrus (Drevets et al., 1997;

Cotter et al., 2001; Botteron et al., 2002), prefrontal cortical regions (Coffey et al., 1993; Goodwin et al., 1997; Ongur et al., 1998; Rajkowska et al., 1999, 2001), including the orbitofrontal cortex (Bremner et al., 2000) and the hippocampus (Sheline et al., 1999; Bremner et al., 2000). Others report volumetric reductions involving the putamen (Husain et al., 1991), caudate nucleus (Krishnan et al., 1992) and entorhinal cortex (Bernstein et al., 1998). Discrepant findings of no significant volume reduction in the hippocampus and amygdala (Pantel et al., 1997; Ashtari et al., 1999) and striatal structures (Lenze & Sheline, 1999) are also reported.

Studies based on functional brain imaging often associate major depression with increased blood flow and metabolism within the amygdala (Drevets et al., 1992). Increased blood flow within the ventrolateral prefrontal cortex, cingulated cortex, orbitofrontal cortex, ventrolateral prefrontal cortex, thalamus and insula are also reported (Drevets, 2003). Amygdala activation reveals a positive correlation with depression severity and duration (Drevets et al., 1992; Abercrombie et al., 1998). Others report reduced blood flow within ventral limbic regions and the ventral striatum (Mayberg et al., 1999) and reduced blood flow in the dorsomedial and dorsolateral prefrontal cortex (Baxter et al., 1989; Bench et al., 1993; Buchsbaum et al., 1997; Soares & Mann, 1997).

What make these structural and functional imaging findings particularly interesting are that many of the same structures listed are often traced as endophenotypes in 5-HTTLPR variation and are under considerable serotonergic control.

Importantly, most imaging studies in patients with major depression have either investigated only one sex, most frequently females, or have not analysed sex differences (Yurgelun-Todd, Sava & Dahlgren, 2007). Sex effects are common in genetic, neural, neurochemical and neuropsychological substrates of mental illness (Cosgrove, Mazure & Staley, 2007). Depression is also more common in females than in males (Piccinelli & Wilkinson, 2000).

One of the major concerns in genetic studies is that the expected contribution from one single gene to explain brain activation or measures of behaviour has to be moderate or minimal, given the explained dynamical interaction between genes and environmental factors. These can be additive, nonadditive or synergic gene and environment effects and can be difficult to uncover. An example is that the magnitude of any association between the 5-HTTLPR and 5-

HTT functioning may be affected by other polymorphisms within the same transcriptional region (SLC6A4) as the 5-HTTLPR, which have the potential to moderate the effects of the 5-HTTLPR on gene expression and subsequent biological processes potentially sensitive to alterations in serotonin neurotransmission. This means that factors closely linked to the polymorphic region probably mediate functional variability even before we consider endophenotypes or cognitive phenotypes. Global measures of brain structures, brain activity, personality traits or symptoms run the risk of conveying unsubtle measures by neglecting the impact of sex, actual symptomatology or the quality and specificity of the inventories.

The expected small gene effect against a background of substantial genetic and environmental variation represents serious challenges when modelling neuropsychological functional studies. Modelling requires, more than usual, measures of specific subprocesses to avoid irrelevant factors that complicate the study or extinguish an actual effect. Many studies try to compensate for this problem by considering several gene candidates and behavioural measures to explore interactions between them. As we will see, many of the most cited studies on the field have this objective and conclude with interaction effects. One major concern is that potentially relevant candidate variables and their interaction over time are almost infinite.

Serotonin in the Brain

The brain's serotonin activity is, as we have seen, 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. The other monoamines are mediated by the serotonin transporter and the same functional principle holds for other monoamines, like dopamine, norephineprine, epinephrine, melatonin, histamine and thyronamines and their respective reuptake transporters.

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 presynaptic receptors with inhibitory control of the 5-HT neuron itself (**Fig. 1**).

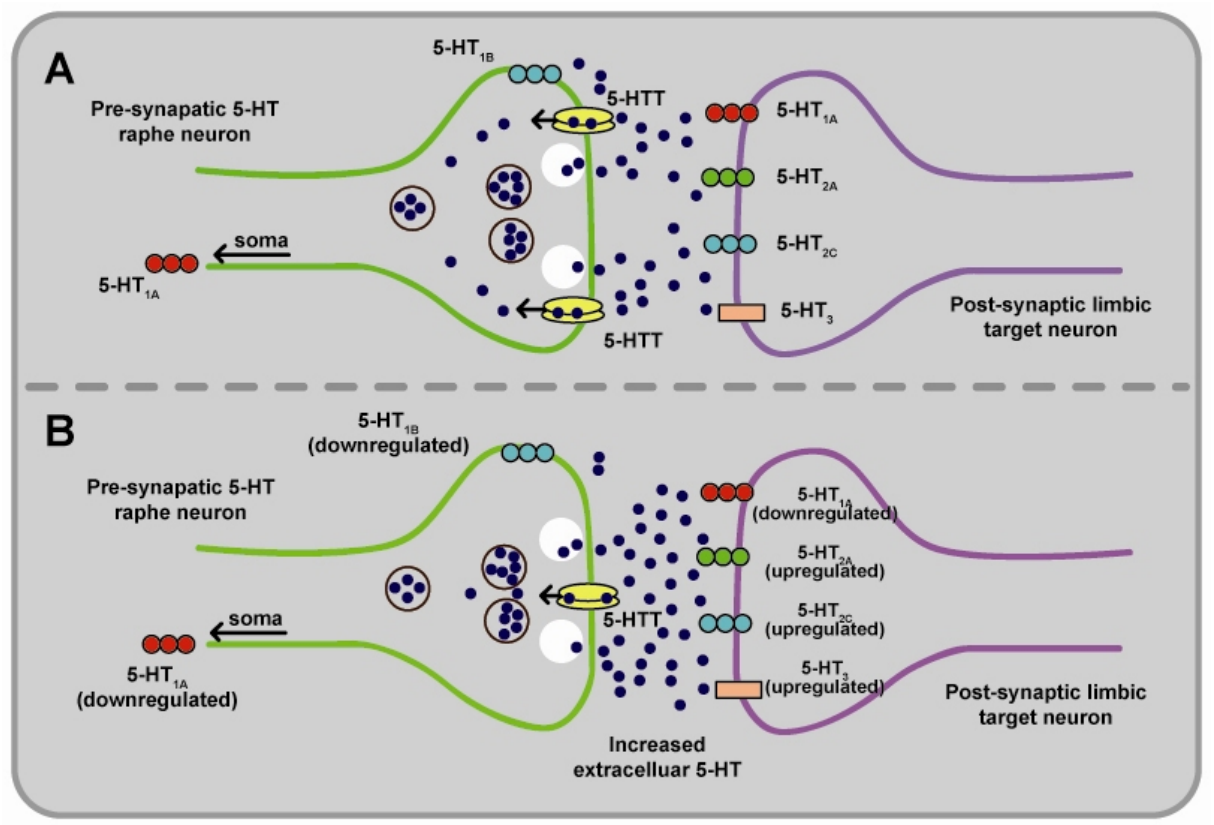

Figure 1 Illustration of the 5-HTT's role in human serotonin metabolism.

A: When 5-HT is released in the synaptic gap, 5-HT returns to the presynaptic neuron through 5-HTT reuptake. This makes the 5-HTT significant for the duration and intensity of 5-HT's communication with postsynaptic targets.

B: The effect of low 5-HTT activity leads to downregulated presynaptic activity, but upregulation of several postsynaptic targets. Presynaptic receptors, on top and towards soma of the presynaptic neuron, represent inhibitory control of the neuron itself.

As we can see from the principal model, 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). 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. This highlights the complex dynamical interaction between gene transcription, neurochemical processes and

neural development and will be of substantial interest in exploring the gene-linked phenotypic variants.

When it comes to the net effect of this complex process, a lot 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 studies that found that unmedicated patients with mood and anxiety disorders had significantly reduced 5-HTT binding compared with healthy controls (Malison et al., 1998; Arango et al., 2001). Subsequently, pharmacological findings 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 (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 (Lesch & Mössner, 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 people with these constellations of symptomatology (Lesch & Murphy, 2003). The findings indicate both short and long variants to represent diatheses and that operating with a short dysfunctional variant and a long functional variant may be an erroneous simplification.

Genetic Variability in Serotonin Transporter Functioning

In humans, transcriptional activity of the 5-HT transporter gene SLC6A4 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, respectively, short and long variants. 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 the 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. Transcriptional activity in the 5-HTT gene promoter is mediated by the presentation of short or long 5-HTTLPR variants (Lesch et al., 1996).

The transcriptional factor has at least two different effects on the gene product, one connected with DNA binding and another that provides gene activation (**Fig. 2**). 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.

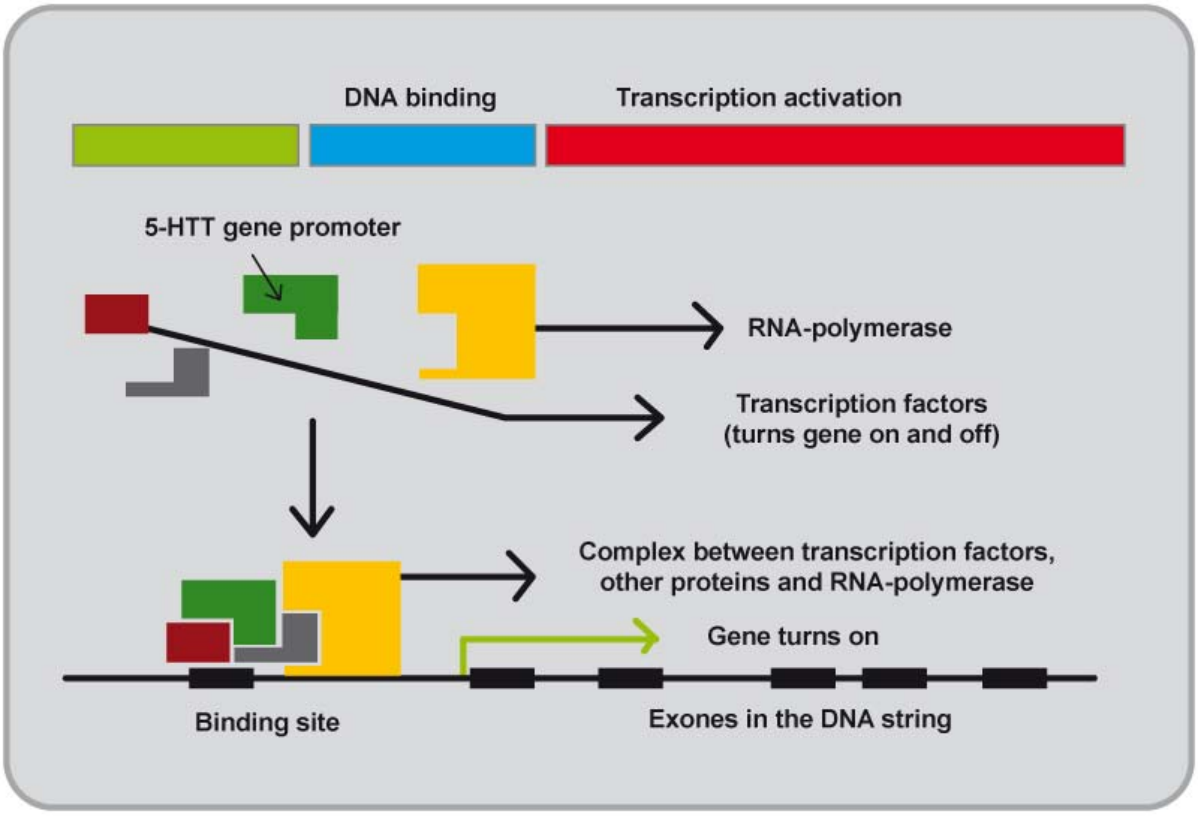

Figure 2 gives a schematic illustration of central transcriptional factors. The line on top shows the two main elements in a transcriptional factor: one element that has the capability to bind to DNA and another element that stands for the transcriptional activation or turning on or off of other genes. The illustration shows the transcriptional factor that, to accomplish its function, binds to other factors to form a complex. This complex usually connects to a binding site upstream of the gene's start site. When the complex is on-site, the gene turns on and is copied by the enzyme RNA-polymerase.

As we have seen, 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 again 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 and that will determine the quantity and activity of the reuptake protein 5-HTT.

The link between variations in 5-HTTLPR length and 5-HTT function is studied by looking at the 5-HTTLPR genotype, 5-HTT gene transcription and 5-HT reuptake activity in human lymphoblast cell lines (lymphoblast cell lines are precursors of lymphocytes that constitute one-quarter of the white blood cells). 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. This means that both the quantity of the 5-HTT protein and the activity in this channel linked to 5-HTTLPR variation can be detected. Similar findings are reported in studies investigating other cell lines (Mortensen et al., 1999) and by using several other procedures (Lesch & Gutknecht, 2005).

When 5-HTTLPR variants melt together with a luciferase reporting gene and are transported into human cell lines, they modulate the transcriptional activity of the 5-HTT gene promoter (Lesch et al., 1996). Luciferase is an enzyme that gives off light by oxidation of the chemical substrate luciferin. Thus, the amount of light indicates transcriptional activity, and in this way the transcriptional activity in living cells can be measured. This means seeing genetic modulation effects in real time, and not only in association with 5-HTT activity or density. This is an important finding consolidating the assumption that there exists an actual causal link between the 5-HTTLPR and serotonin function. It also shows that we, already at this early level in the space between 5-HTTLPR and 5-HT functioning have started the motion from the level on which genes operates, and that further analyses on higher levels will be prone to effects and interactions caused by 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. Current technologies can, by using data technology, mathematics, biochemistry and statistics, uncover the functional 5-HTTLPR variants and divide the three categories

based on the pairing of short and long variants (long/long, short/short and long/short). These two original variants (long and short) 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. 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 (Hu et al., 2006). However, this finding has not been consistently replicated (Martin et al., 2007). Furthermore, Wendland et al. (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.

Serotonin Circuits

Serotonin-containing neurons are mainly collected in the nine cores called raphe nuclei. Raphe nuclei are groups of neurons in the brainstem's whole length 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. In addition to the role of these nuclei in emotional perception and mood regulation, they are involved in sleep and sleep cycles (Bear, Connors & Paradiso, 2001). Present findings suggest that serotonin isn't transported via terminal buttons, as in classic neurotransmission, but by varicosities (swelling) along axons to the extracellular space. In this way, by diffusion, serotonin can reach a relatively wide area and activate dendrites' 5-HTT receptors, cell bodies and presynaptic terminals in adjacent neurons.

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 et al., 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. Examples are that 5-HT modulates projections from the cell bodies of thalamocortical glutaminergic neurons in cultures that involve serotonin receptors, the 5-HT contributes to differentiation of cortical glutaminergic neurons via one of the 5-HT receptor subgroups (Lieske et al., 1999), 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 accomplishing studies on 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 only in its early beginnings.

Sex Differences Associated with the 5-HTTLPR Genotype

Recently, sex 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 address specifically sex differences by using sex as a covariate, matching groups, exclusively including male or female participants or by no consideration of potential sex effects. Brummett et al. (2007) have recently demonstrated that, in females, homozygote short variants are associated with susceptibility to depression under stressful life conditions, whereas the homozygote long variants do so in males, revealing an opposite genotype-environment pattern in males and females. Eley et al. (2004) and Sjöberg (2006) showed an interaction 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 (Uher & McGuffin, 2008). The studies posit the importance of the use of sex as a grouping variable to spot specific sex effects.

Neurocognitive Functions Associated with the 5-HTTLPR Genotype

Genotype-phenotype correlations are seen by using functional neuroimaging procedures like ERP (event-related potentials), fMRI (functional magnetic resonance imaging), perfusion MR and PET (positron emission tomography) and compose the majority of studies on 5-HTTLPR variation. Falgatter et al. (1999, 2004) reported associations between the 5-HTTLPR genotype and prefrontal cortex-limbic excitability by using tasks that involve cognitive response control

(Go-NoGo and error-processing tasks) in healthy volunteers without known symptomatology. Participants with one or two short variants in 5-HTTLPR showed higher prefrontal ERP activity compared with subjects homozygous for the long variant. The findings indicate an increased response in the prefrontal cortex, especially the anterior cingulated cortex (ACC).

Hariri et al. (2002) have shown that participants with at least one copy of the short variant have a higher amygdala response, examined by fMRI, when introduced fo potential fright-evoking stimuli compared with subjects homozygous for the long variant. This indicates that differences in amygdala excitability, when presented with potentially emotionally significant stimuli, contribute to fear- and anxiety-related responses. After these early studies, the topic was considerably elaborated and widely published, showing associations between the polymorphic region and amygdala activation. A meta-analysis by Munafò, Brown and Hariri (In Press) concludes that 5-HTTLPR polymorphism accounts for up to 10% of phenotypic variance when reanalysing data from fourteen studies using fMRI, PET and perfusion MR. They also included unpublished data sets to avoid possible publication bias. The results indicate that alternations in 5-HT signalling associated with the 5-HTTLPR appear to contribute significantly to amygdala activation in response to a broad range of salient environmental stimuli. The studies never compare or separate the different categories of negative emotional stimuli.

Functional neuroimaging has revealed increased blood flow and brain activation in healthy controls within the amygdala when presenting unfamiliar faces (DuBois et al., 1999). Similar findings are seen when participants are shown fearful (Breiter et al., 1996; Morris et al., 1996; Phillips et al., 1997, 2001; Wright et al., 2001), sad (Blair et al., 1999) and happy (Breiter et al., 1996) faces. Amygdala activation is also seen in response to several other potential emotionally significant stimuli linked to different sensorial modalities. Taken together, the findings indicate that the amygdala plays a decisive role in the modulation, vigilance and attention to emotionally significant information (Davis & Whalen, 2001). The findings further suggest human faces as one of the potentially most successful stimuli for amygdala activation. Importantly, the amygdala is not activated only when presented with negative stimuli but also when presented with positive emotional stimuli.

Recently, Beevers et al. (2007) have reported 5-HTTLPR-dependent attention bias in a psychiatric inpatient group using a standard dot-probe reaction time task. Participants

homozygous for the long 5-HTTLPR variants had a stronger attention bias for anxious word stimuli compared with participants carrying one or two copies of the short 5-HTTLPR variants. The attention bias was not found when presented with dysphoric word stimuli. The study indicates an association between 5-HTTLPR variants and biased attention, not only indicated by amygdala activation, but also when measured with a behavioural reaction time task.

The amygdala is a complex of nuclei and afferents to the amygdala come from a large variety of sources, including the neocortex, in all lobes of the brain, and the hippocampal and cingulated gyri. All sensory systems feed into the amygdala and each sensory system has different projection patterns to the amygdala nuclei. Interconnections within the amygdala allow the integration from different sensory systems. As a whole unit, the amygdala's main function is the production of an affective state, but it's still possible that different emotions use different amygdala circuits. Given the interconnection with other areas in the brain, 5-HTTLPR-depended altered amygdala activation probably has implications for different aspects of information processing that are not limited to emotional processing. The reviewed studies demonstrate increased amygdala activation in carriers of one or two short 5-HTTLPR variants compared with homozygote long variants. The comparison indicate that the heterozygote 5-HTTLPR variants do not constitute a qualitatively distinct variant but will have functional qualities lying somewhere between the two homozygote variants. Higher resolution in cognitive measures will contribute to the clarity in functional aspects of heterozygote 5-HTTLPR variants.

Traits and Symptomatology Associated with the 5-HTTLPR Genotype

When they first spotted associations between 5-HTTLPR and its role in the brain's serotonin function, Lesch and his collaborators (1996) reported that individuals carrying short variants show increased trait anxiety, particularly neuroticism and harm avoidance, compared with individuals homozygous for the long variant. Neuroticism is related to anxiety, stress reactivity and depression. The association between the short variants and several measures on fear- and anxiety-related traits is replicated in a substantial number of studies and is also the conclusion from three independent meta-analyses (Schinka et al,. 2004; Sen et al., 2004; Munafò et al., 2005). In contrast to these apparently robust findings, one study with more than one hundred thousand participants found no significant associations between 5-HTTLPR and neuroticism, as measured using the Eysenck Personality Questionnaire (EPQ) or DSM-IV

major depression based on extreme scores of neuroticism (Willis-Owen et al., 2005). The authors suggest that 5-HTT function contributes less to an anxious phenotype in extreme scores. This and other inconsistent findings highlight the pronounced challenges when studying single-gene contributions to polygenetic phenomena interacting with different environmental factors. Small sample sizes, heterogeneous subject populations, differing methods of personality assessment, selection of extreme scores and abstruse division of genotypes may also contribute to the diversity in these findings.

Meta-analytic approaches might have helped in clarifying the data but have also produced ambiguous outcomes. Some studies conclude with an association between 5-HTTLPR and neuroticism but not with harm avoidance, whereas others report the opposite pattern (Canli & Lesch, 2007). One major concern is based on differences in the approach to statistical analysis as different analyses have been shown to reveal opposite conclusions.

Animal and human studies also conclude that relatively decreased 5-HTT function isn't only linked to higher anxiety levels, but also has a negative impact on the capability to cope with stress. One of the most cited 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. 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 (Caspi et al., 2003). 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 study provides evidence for a gene-environment interaction, in which an individual's response to environmental insults is moderated by the person's genetic make-up.

It is important to note that this result does not in itself constitute unambiguous evidence of a gene-environment interaction, because exposure to life events may be influenced by genetic factors like a heritable tendency to enter situations in which they encounter stressful life events in the form of genes not considered. Again, we meet the huge challenges in studies trying to uncover contributions from genes and the environment. As was done in the cited study, it is important to include cause-order analysis in order to decide on causality in human

development. Other gene candidates that share variance with the 5-HTT genotype can also make other explanations - not directly associated with serotonin - plausible.

Eley (2004), Kaufman (2004) and Kendler et al. (2005) broadly confirmed Caspi et al.'s (2003) study, using different measures of stressful life events and different analytic methods, and conclude with a relatively increased risk of depression in the context of environmental adversity. After these studies, an additional fourteen similar studies have been published, with all but three studies concluding with gene-environment interaction effects (Uher & McGuffin, 2008).

The specific contribution of one single gene is difficult to uncover but the studies represent solid evidence indicating that stress increases the risk of emotional disorders, and that this vulnerability is under considerable genetic influence. Several brain structures and functional systems are assumedly involved in complex dynamical interaction and transaction processes.

Studies exploring endophenotypes, such as functional measures of neural activation, represent a promising bridging of the gap between variants with small effects and complex behaviour associated with traits and symptoms. Canli et al. (2006) found interactions between 5-HTTLPR and life stress on amygdala activation and connectivity, using fMRI and perfusion imaging, indicating differences in the amygdala resting state. The stress depression hypothesis was further supported by 5-HTTLPR-dependent hippocampus activation and connectivity. Interconnections between amygdala and hippocampus are important in stress regulation and coping (Bear, Connors & Paradiso, 2001). Frodl et al. (In Press) have recently reported reduced hippocampal volume associated with long variants in patients with major depression using both the traditional diallelic and the triallelic 5-HTTLPR classifications. The study indicates homozygote long carriers' vulnerability to hippocampal changes.

Other studies have shown that when depression is clinically manifested the short variants are associated with more severe symptomatology and a worse prognosis, including a lower effect of treatment with antidepressants (Hariri and Holmes, 2006). The question of causality still remains. The clinical patients could have diatheses that contribute to inferior coping and worse symptomatology and the short variants could make the antidepressants less potent due to pharmacokinetic and pharmacodynamic factors. Nonetheless, it is becoming increasingly clear that even common variations in genetic sequence impact on function and contribute to

measurable variances to this complex phenomena, not only of the transcend phenomenological diagnosis, but may also represent the ultimate mechanisms of disease. The studies demonstrate 5-HTTLPR variation in both endophenotypes and more remote measures of traits and symptomatology.

Conceptual Framework

Traditionally, the circuits and concepts designated "the limbic system" have been central to understanding different aspects of emotion. The limbic system as a concept was suggested by Paul Broca as early as the eighteen-seventies, but was at that time associated with olfactory perception. Later, the limbic system was used to describe a group of medially located brain structures representing a connection between the neocortex and hypothalamus into a functional system. As the American neurologist James Papez presented this system in the nineteen-thirties, the emotions were regulated by the activity in the anterior cingulated cortex and - but in a less direct manner - other cortical areas. Papez postulated that the emotional expression is governed by the hypothalamus. The cingulated cortex has projections to the hippocampus and projections to the hypothalamus via a bundle of neurons called the fornix. Further, the signal reaches the neocortex via the anterior thalamic nucleus. The hypothalamus and neocortex are therefore arranged as a reciprocal influence system. Anatomical studies verify the existence of the system, but the functional connection to different aspects of emotional perception and processing remains speculative. Contemporary neuroscience still includes some of the main aspects of these early concepts by Broca and Papez.

One of the major challenges lies in the construction of a system. Many structures and cells have several functions and make it difficult to operate with delimited systems. Emotional identification and further processing are dependent on components not only associated with emotional processing, and sometimes on structures traditionally linked to other cognitive processes. Several neuropsychological subprocesses are involved in many different aspects of behaviour and make concepts based on delimited systems erroneous. An integrative model will illustrate important structures involved in, but not always delimited to, emotional perception and processing.

An Integrative Model of Emotional Processing

Phillips et al. (2003) operate with 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 is about the production of the affective state and automatic regulation, and the last process is the one where the affective state is further regulated. A ventral system involving the amygdala, insula, ventral striatum and ventral areas of the anterior cingulated gyrus and the prefrontal cortex are particularly important for processes one and two. 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 behaviour. Regulation of the affective state and emotional behaviour probably involves inhibition, or modulation, of the two other processes (**Fig. 3**).

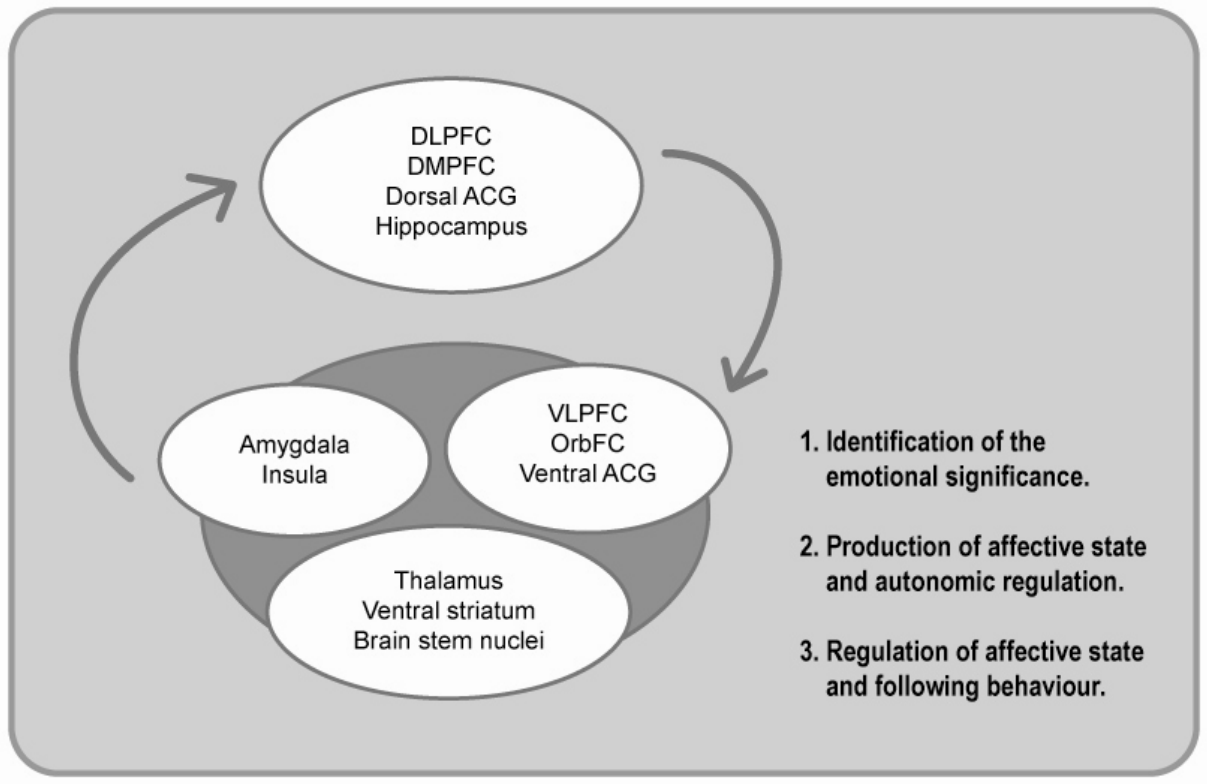

Figure 3 illustrates important functions and structures involved emotional processing. A predominant ventral system is central to the identification of the stimulus' emotional significance, production of the affective state and automatic regulation of responses (listed in the grey circle). A predominant dorsal system is central to non-automatic regulation of the affective state (white circle on top). Regulation of the affective state and emotional behavior probably involves inhibition or modulation of processes 2 and 3 (indicated by curved arrows). VLPFC = ventrolateral prefrontal cortex; DLPFC = dorsolateral prefrontal cortex; DMPFC = dorsomedial prefrontal cortex; OrbFC = orbitofrontal cortex; ACG = anterior cingulated gyrus.

The model shows that the prefrontal cortex plays an important role in emotional perception and processing. 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, which represents a huge challenge when reviewing different studies to compare and contrast them. We usually roughly separate the orbitofrontal and ventromedial areas, the dorsolateral prefrontal cortex and the cingulated cortex. Together, the brain regions have been implicated in planning complex cognitive behaviours, personality expression and moderating social behaviour. The basic activities are the orchestration of thoughts and actions in accordance with internal goals and are often labelled "executive functions" or "cognitive control functions". Miyake et al. (2000) have, by use of factor analysis, presented diversity in executive functioning in three factors labelled "mental shifting" (shifting), "information updating and monitoring" (updating) and "inhibition of prepotent responses" (inhibition).

Functional brain imaging has, as earlier reviewed, shown that individuals with a major depressive episode show increased activation in areas central to the identification of emotional stimuli, and for generating emotional behaviour, compared with healthy control participants. The structures include the amygdala, orbitofrontal cortex, anterior cingulated, ventrolateral prefrontal cortex, anterior insula, ventral striatum and thalamus. The same group shows decreased activation in structures central to the regulation of emotional behaviour including the dorsomedial and dorsolateral prefrontal cortex. For our purpose, it's important to note that this pattern is reversed in full remission, with increased activation in the dorsomedial prefrontal cortex and lower activation in the anterior cingulated, hippocampus, thalamus, ventral striatum and insula (Phillips et al., 2003).

Phillips et al. (2003) hypothesize that these processes result in a limited emotional spectrum, with a bias towards the perception of negative, before positive, stimuli that results in a depressive mood. The structural and functional abnormalities within the dorsolateral and dorsomedial cortex, linked to conscious regulation of the affective state, could explain why the depression consolidates by shortages in downregulating mechanisms. In healthy control participants, we have presented several studies that demonstrate the same structures to be linked to 5-HTTLPR variability and serotonin function. Based on these findings, functional phenotypic studies should reveal 5-HTTLPR-dependent variability in association with negative stimuli.

The basolateral amygdala is densely connected with the ventromedial prefrontal cortex, a limbic area implicated in the emotional expression. Increased activation in these areas is often, but not consistently, observed in major depression (Drevets, 2003). In basolateral amygdala, serotonin is thought to modulate incoming projections from the prefrontal cortex (Pinto & Sesack, 2003). Heinz et al. (2005) demonstrated 5-HTTLPR-dependent functional coupling between the amygdala and the ventromedial prefrontal cortex. The data supports the hypothesis that 5-HTT function plays an important role in the development of a negative mood state. Dysfunctional amygdala-prefrontal coupling may be associated with 5-HTTLPR-dependent constraints in the capacity to regulate emotional states. This will enhance the risk of clinical depression in these individuals.

Pezawas et al. (2005) demonstrated reduced grey matter volume in short 5-HTTLPR carriers in the limbic regions, particularly the perigenual cingulate and amygdala. The perigenual 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, as well as beeing the region that displays the highest target zone of dense projections from the amygdala. The feedback coupling between these regions, stimulated by potentially fearful stimuli, is implicated in the processing of negative affect. Short 5-HTTLPR carriers show relative uncoupling of this circuit. These findings suggest a disruption in the feedback between the amygdala and anterior cingulated cortex. The 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 attentional 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 et al., 2000). The studies represent plausible explanations for the mentioned increased amygdala activity in carriers of the short 5-HTTLPR variants by decreased downregulation via the anterior cingulated cortex. The 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 environmental adverse stimuli. Stimulation of the limbic prefrontal cortex inhibits amygdala function in primates (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. In this case, over activation of the amygdala will lead to compensatory over activity in the ventromedial prefrontal cortex. The studies represent massive evidence which indicates several structures to be involved in the activation, inhibition, modulation and mediation of components involved in emotional perception and processing.

The conceptual framework represents a profitable fundament to understand both structural and functional implications of altered 5-HTT function in emotional processing. 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 functions 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 (**Fig. 4**).

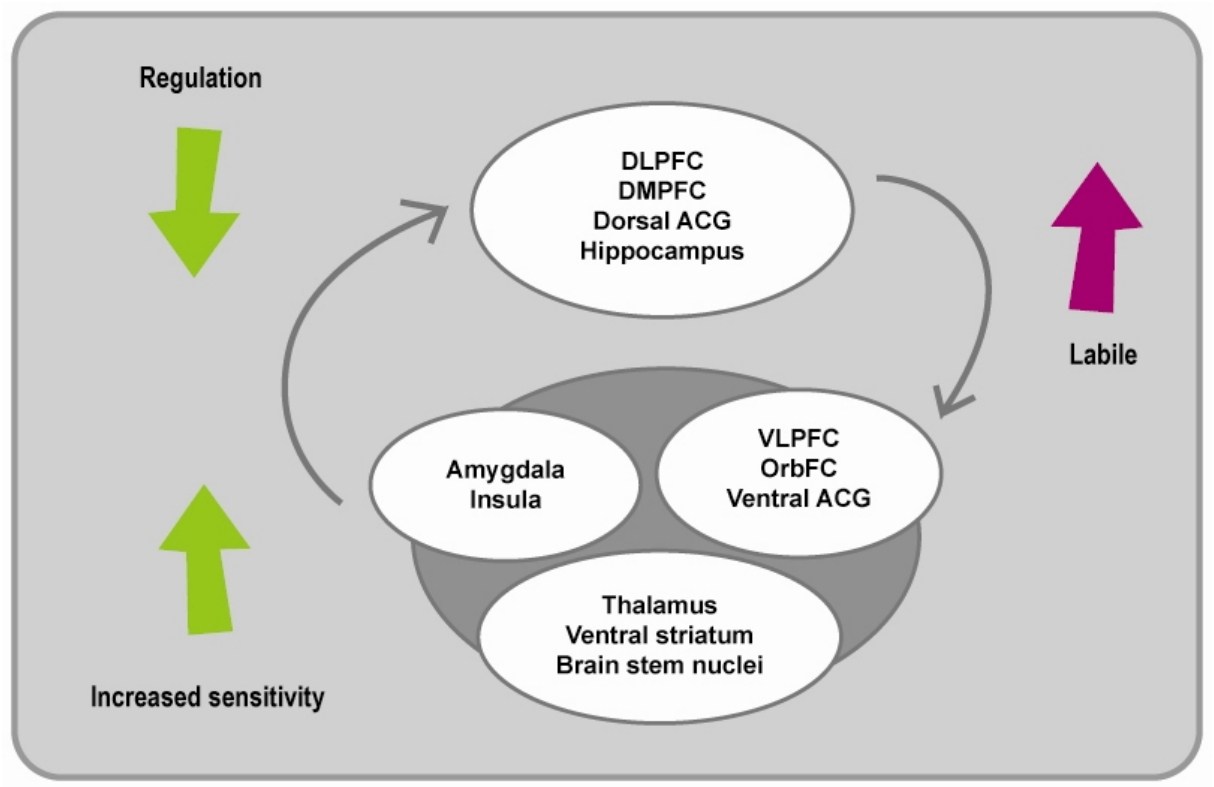

Figure 4 illustrate predictions of increased 5-HTTLPR-dependent amygdala activation, and decreased frontal 5-HTTLPR activation. Altered activation will have implications for all three aspects of emotional processing. Increased sensitivity and altered downregulation (green arrows) make individuals labile in association with emotional activation (red arrow). VLPFC = ventrolateral prefrontal cortex; DLPFC = dorsolateral prefrontal cortex; DMPFC = dorsomedial prefrontal cortex; OrbFC = orbitofrontal cortex; ACG = anterior cingulated gyrus.

Under normal circumstances emotional activation, produced when presented with salient stimuli, will be downregulated. The model posits increased sensitivity in short 5-HTTLPR carriers to alter this down-regulating mechanism. Short 5-HTTLPR carriers are sensitive and labile when identifying negative emotional stimuli. The presented neuroimaging studies indicate that this sensitivity makes these individuals endangered to endurable functional and structural changes associated with depressive symptomatology.

Objective

By constructing a measure of cognitive control functioning containing both neutral and negative emotional categories, we can explore 5-HTTLPR-depended variability in cognitive functioning and emotional processing. The resolution in this measure has the potential to uncover specific 5-HTTLPR-dependent patterns associated with specific emotional categories

and represents an important supplement to contemporary studies which have been dominated by studies based on neuroimaging, reporting of life events, personality traits and symptomatology.

The measure is based on the conceptual model and the reviewed findings which demonstrate that the 5-HTTLPR-dependent variability is linked to frontal- and limbic structures and the corresponding functions. Measures to assess cognitive control functions exist in many forms. Ideally, we want to cover all of Miyake et al.'s (2000) sub-components of executive functioning (updating, inhibition and shifting). The use of an n-back task was chosen based on that the procedure being considerably elaborated and known to activate the cingulated cortex and the frontal lobes (Owen, McMillan, Laird & Bullmore, 2005). The n-back task mainly covers information updating in Miyake et al.'s diversity but also involves aspects of inhibition as participants have to ignore irrelevant categories, and shifting as participants have to attend to more than one emotional category at the same time. The introduction has presented massive evidence that constitutes the rationale for using human emotional faces as stimuli for emotional activation (Breiter et al., 1996; Morris et al., 1996; Blair et al., 1999; DuBois et al., 1999; Phillips et al., 1997, 2001; Wright et al., 2001; Davis & Whalen, 2001).

Hypotheses

The first question we want to answer is whether altering emotional stimuli will affect cognitive control functioning. Our hypothesis is that presenting potentially significant emotional stimuli will affect cognitive control functioning independent of 5-HTTLPR variant or sex.

The second question we want to answer is whether the variation is 5-HTTLPR-dependent. Our hypothesis is that short 5-HTTLPR variants will be associated with increased sensitivity to negative emotional stimuli compared with long 5-HTTLPR carriers.

We also want to investigate potential sex-specific effects as some studies show effects delimited to females, stronger among females and even sometimes the opposite pattern in males and females.

Methods and Materials

Participants

A total of 60 healthy Norwegian individuals, 34 females and 26 males (M age=32,8), screened according to the Structural Clinical Interview for DSM-IV, Axis I and II disorders (SCID I and SCID II). We also included background interviews to assess demographical information relevant to inclusion or as control variables. Pre-criteria for inclusion were individuals between 18 and 60 years, without organic brain disease, psychopharmacologic medication or alcohol/drug addiction, willing to accomplish a 3-4 hour session containing genotyping (blood sample), screening of symptomatology and neuropsychological testing. Participants received a 250 NOK favour. Two participants were excluded from the study based on screening procedures revealing DSM-IV criteria of an Actual Major Depressive Episode.

The study is part of a project which applied to the Regional Ethics Committee and adheres to the Helsinki Convention. All data were collected and stored according to prescribed procedures fulfilling these standards.

Genotyping

The procedure for genotyping the triallelic 5-HTTLPR polymorphism located in the SLC6A4 gene, coding the serotonin transporter protein (HTT), has been performed essentially as described in detail elsewhere (Gelernter, Kranzler & Cubells, 1997; Stein, Seedat & Gelernter, 2006). Briefly, genomic DNA was amplified by polymerase chain reaction (PCR) on a real-time fluorescence LightCycler instrument in a final volume of 20 ul using LightCycler Faststart DNA SYBR Green kit (Roche cat no 12239264001) with specific primers (0.5 uM) (Gelernter, Kranzler & Cubells, 1997) generating a long (L) 419 bp or a short (S) 375 bp PCR product depending on the presence of a 16 bp or a 14 bp sequence repeat, respectively, in the promoter region. Cycle conditions were the following: 10 min denaturation (95 ^{o}C), 45 cycles at 95 ^{o}C (10 s), 66 ^{o}C (10 s) and 72 ^{o}C (0 s). For the detection of an additional A/G single nucleotide polymorphism (SNP) that occurs within the L fragment (L allele), the PCR fragments were digested with 1 U MspI restriction enzyme (New England Biolabs, Beverly, Massachusetts) for 2 hour at 37 ^{o}C. The PCR fragments contain two obligatory MspI sites, whereas the A/G substitution creates an additional MspI site. Thus, a single PCR reaction and restriction digest followed by size fractioning on a gel provides classification of the S, L_A and L_G alleles. The triallelic classification was then reclassified into

a diallelic model, based on the level of 5-HTT gene expression as follows: L_G/S, L_G/L_G and S/S participants were classified as S/S; L_A/S and L_A/L_G participants were classified as L/S; and L_A/L_A participants were classified as L/L (Hu et al., 2006; Neumeister et al., 2006).

A biobank was established at the Psychopharmacological Department at Diakonhjemmet Hospital, where the blood samples were implemented. The Clinical Chemical Department at Ullevål University Hospital conducted genotyping procedures using polymerase chain reaction (PCR). Extracted DNA and classification from Yale University School of Medicine, used in Walderhaug's (2007) doctoral dissertation, were used as control samples.

The Emo n-back

To assess the implications of potentially emotional stimuli in measures of cognitive control functioning, we constructed an n-back paradigm containing emotional expressions in human faces. The paradigm was programmed using E-primes E-studio software. We showed 1000ms of sequential presentations of 360 pictures of sad, happy, fearful and neutral faces. A centred fixation point represented another thousand milliseconds' stimulus interval. The stimuli were presented in 3 blocks of 120 trials, each containing 8 targets for each emotional category. Stimuli were selected from the material from validated images of facial expressions from The Karolinska Directed Emotional Faces (Lundqvist, Flykt & Öhmann, 1998). Images were set in black backgrounds and adjusted for borders and image resolution. We also included a practice procedure with "right", "wrong" and "should have responded" feedback. The participants are instructed to respond each time the same facial expression, hence not the same face, are presented twice in a row (1-back). Outcome measures were percentage accuracy and reaction time in correct responses (**Fig. 5**).

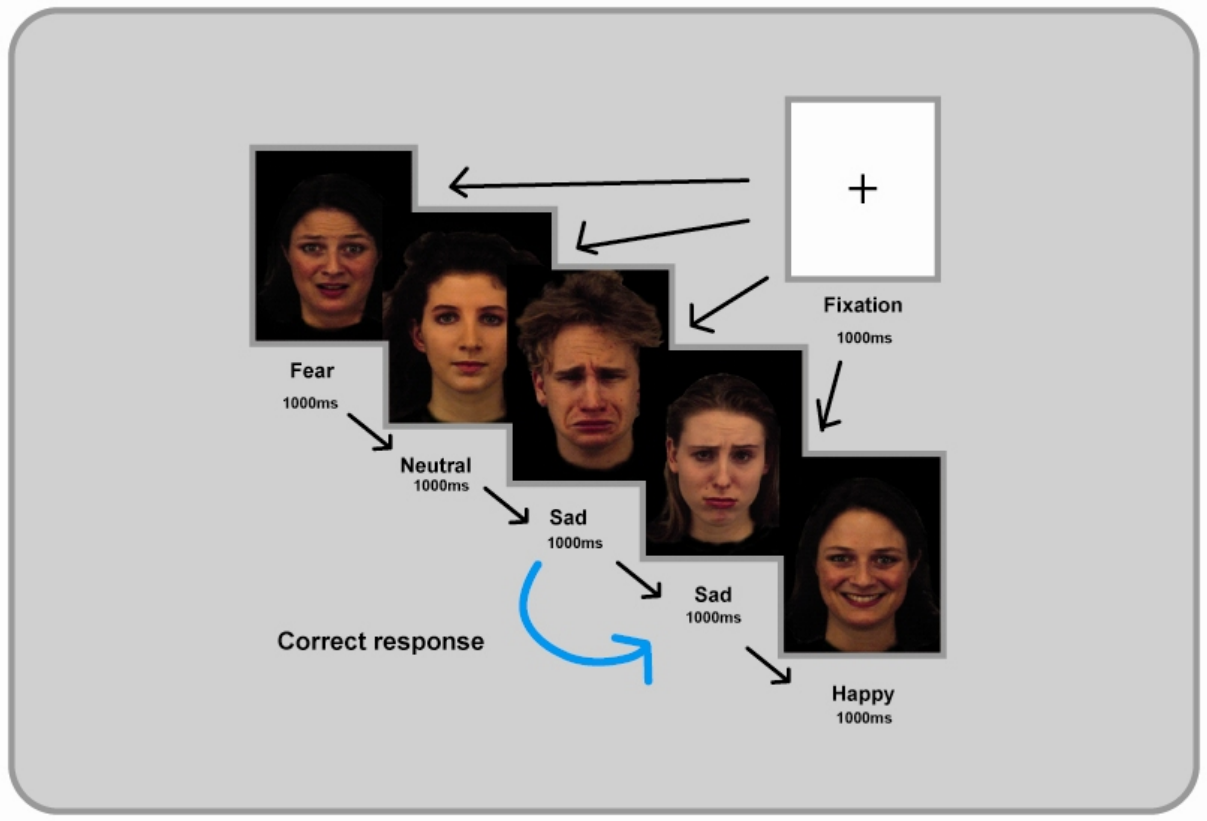

Figure 5 illustrates the Emo n-back. Participants are presented with four emotional categories of facial expressions with a stimulus interval of 1000 ms. Another 1000 ms fixation point is presented between each image. The participants are instructed to respond each time the same facial expression, hence not the same face, is presented twice in a row.

Control Variables

The subscales Similarities (M=24,9) and Picture Completion (M=22,6) from WAIS III were used to detect potential group differences in general intelligence. The Beck Anxiety Inventory (M=3,3) and Beck Depression Inventory (M=4,6) were used to detect potential differences in actual symptomatology.

Statistical Analyses

Multivariate tests were conducted using Wilk's Lamda (λ) as the most commonly reported statistics. The main reason for using λ is that the multivariate approach is less affected by violations of the sphericity assumption than the univariate alternatives (Pallant, 2001). A one-way repeated measures ANOVA was conducted to compare emotional categories within the Emo n-back. Paired sample t-tests were conducted post hoc to evaluate the directions of the potential differences within the Emo n-back. A mixed between–within subject analysis of

variance was conducted to explore the impact of sex and genotype on accuracy in emotional categories as measured by the Emo n-back (emotional category × sex × 5-HTTLPR genotype). A two-way univariate ANOVA was conducted for each emotional category to investigate a potential specificity. A mixed between-within subject analyses of variance was used to compare the sad category to the neutral condition.

Mixed between-within subject analyses was conducted post hoc to explore the implications of reaction time. The p-value to achieve statistical significance was set to the .05 level in all analyses. Effect size was evaluated according to Cohen (1988): Eta^2=.01=small effect, Eta^2= .06= moderate effect and Eta^2=.14=large effect.

Results

Validating the Emo n-back

A one-way repeated measures ANOVA was conducted to compare emotional categories within the Emo n-back. The means and standard deviations are presented in **Table 1**. There was a significant effect for category (Eta^2=.75, λ=.25, F[3,59]=50.07, p<.0005).

Descriptive Statistics for Emotional Categories within the Emo n-back.

Emotional Category	N	Mean	SD
Neutral	60	.8107	.17087
Happy	60	.8963	.13288
Fear	60	.7210	.16426
Sad	60	.6697	.21002

Table 1 shows number of participants, mean and standard deviation for emotional categories based on percentage accuracy in correct responses.

Paired sample t-tests were conducted post hoc to evaluate the directions of the differences within the Emo n-back. All t-tests revealed statistically significant differences between categories. Accuracy was statistically decreased in the sad and fear compared to the neutral condition. There was also significantly increased accuracy when comparing neutral to happy and fear to sad. Neutral-fear (t[59]=5.540, p<.0005), neutral-sad (t[59]=6.038, p<.0005), neutral-happy (t[59]=-5.403, p<.0005) and fear-sad (t[59]=-2.555, p=.013).

There were no correlations between reaction time and accuracy. This suggests an absence of speed-accuracy tradeoff effect, which would predict an association between faster responses and less correct responses. The decreased accuracy in negative compared with neutral stimuli is therefore not indicated by reaction time artifacts within the Emo n-back.

Group Differences

A mixed between-within subject analysis of variance was conducted to explore the impact of sex and genotype on accuracy in emotional categories as measured by the Emo n-back. Subjects were divided into three genotypes according to the triallelic division. In addition to the reported effect of category, multivariate tests revealed a statistically significant sex-category interaction effect, Eta^2=.31, λ=.69, F[3,49]=7,21, p<.0005. There was also a statistically significant three-way interaction between category, sex and genotype, Eta^2=.12, λ=.77, F(6,98)=2,23, p=.047 (**Fig. 6**).

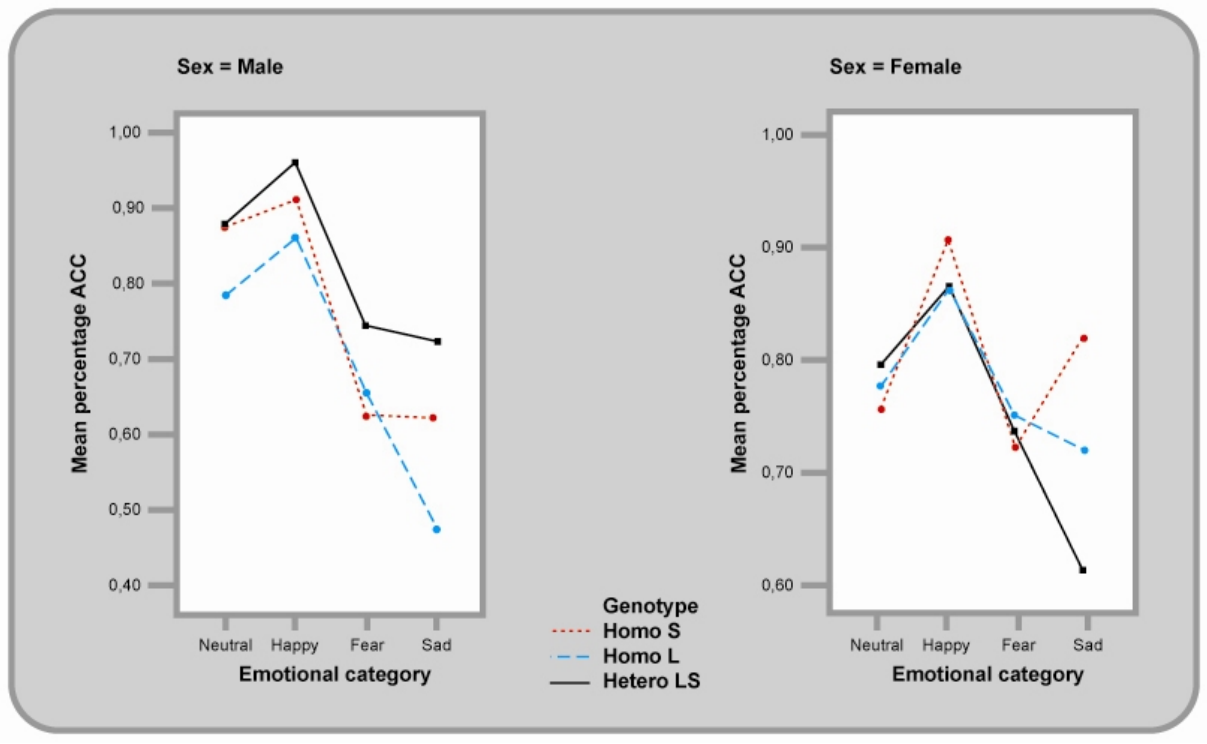

Figure 6 illustrate the results reported from the mixed between-within ANOVA. The figures show the three-way interaction between sex, category and genotype. The figures indicate that the genotype-dependent pattern for males and females are distinctively pronounced in the sad category.

A two-way univariate ANOVA was conducted for each emotional category to investigate the specificity of the three-way interaction indicated by the figures from the multivariate tests. These tests revealed an effect specific and restricted to the sad category $Eta^2=.15$, $F[2,51]=4.59$, $p=.015$ (effect size for the neutral, happy and fear categories was respectively $Eta^2=.01$, $Eta^2=.03$ and $Eta^2=.02$). It's important to note that the original multivariate analyses are based on stricter criteria and represent stronger statistical power than this two-way univariate ANOVA.

We also want to conduct a mixed between-within subject analysis of variance to compare the sad category to the neutral condition. Multivariate tests revealed a statistically significant sex-category interaction effect ($Eta^2=.21$, $\lambda=.79$, $F[1,51]=13,34$, $p=.001$). There was a statistically significant three-way interaction between category, sex and genotype ($Eta^2=.19$, $\lambda=.81$, $F[2,51]=6,11$, $p=.004$) (**Fig. 7**).

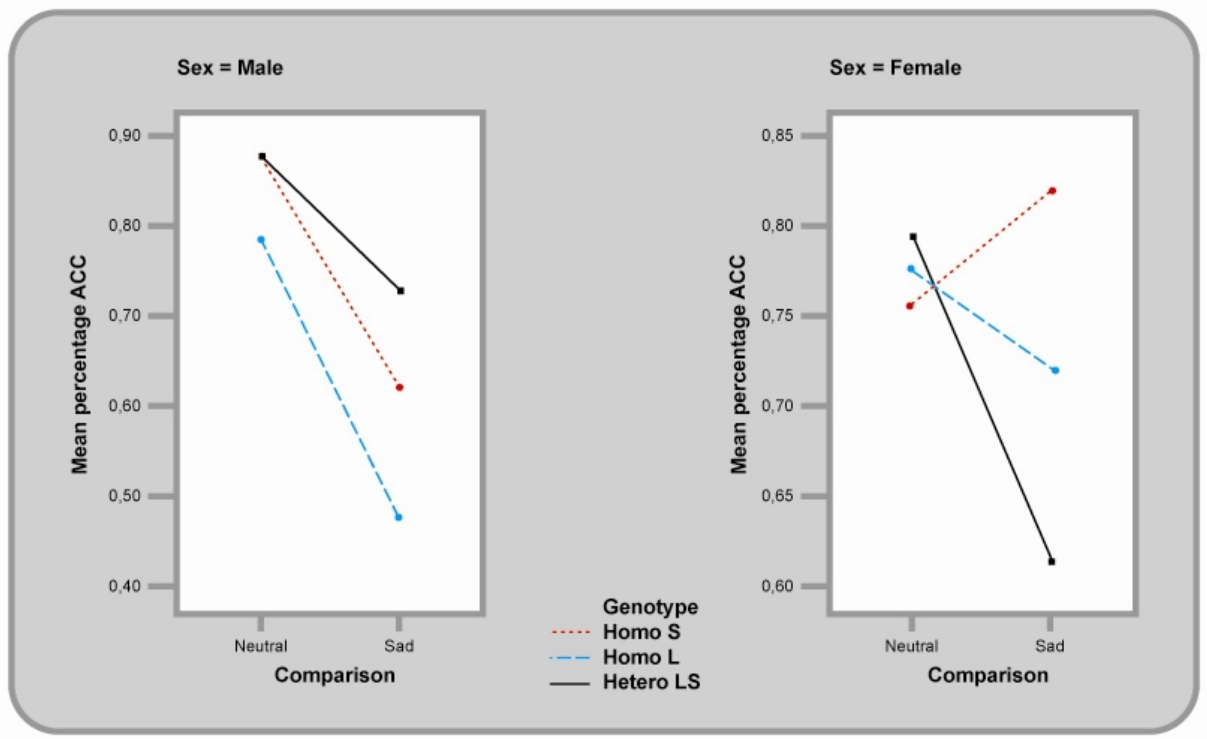

Figure 7 illustrate the results from the comparison between the neutral and sad categories. The mixed between-within ANOVA revealed a three-way interaction between category, sex and genotype. The short 5-HTTLPR variants demonstrate a distinct pattern for females.

There were no statistically significant effects of sex or genotype on BDI or BAI, indicating group differences in actual symptomatology linked to anxiety or depression, and no group differences in age or in general intelligence indicated by the raw scores from the subscales Similarities and Picture Completion from WAIS III.

A post hoc mixed between-within subject analysis of variance was conducted to explore group differences in reaction time. The mixed between-within subject ANOVA revealed no statistically significant tree-way interaction effects between category, sex and genotype, but a statistically significant category-genotype interaction effect (Eta^2=.14, λ=.73, F[6,98]=2,74, p=.017). There was also a marginal sex-genotype interaction effect (Eta^2=.12, λ=.87, F[3,49]=2,25, p=.094). Comparing single emotional categories to the neutral condition revealed no statistically significant effects.

Discussion

Introducing the Emo n-back

The introduction of the Emo n-back supports the hypothesis that the stimulus' emotional content affects cognitive control functioning as assessed by an n-back procedure. Decreased accuracy in categories sad and fear, but increased accuracy in the happy category, compared with the neutral condition, indicate an effect restricted to the presentation of negative emotional categories. The sad emotional faces reveal the lowest accuracy, indicating sad emotional stimuli as the most difficult emotional category, and also significantly more difficult than the fear emotional category.

Of Miyake et al.'s (2000) sub-components of executive functioning, the n-back procedure is probably linked to information updating and monitoring, but also involves aspects of inhibition as participants have to ignore irrelevant categories, and shifting as participants have to attend to more than one emotional category at the same time. The output from the Emo n-back could also represent cognitive components not attended to, like perceptual differences between categories, priming effects or impact of commission errors within the Emo n- back. The indications from the Emo n-back need to be further explored and replicated to spot the nature of the paradigm and its outcome measures. Absent speed-accuracy effects indicate no reaction time-related artifacts within the Emo n-back, but do not outrun potential 5-HTTLPR-dependent or gender specific patterns in reaction time linked to actual differences in cognitive and emotional processing.

Introducing the Emo n-back supports our hypothesis by demonstrating different emotional stimuli to play an important role in a behavioural measure of cognitive control functioning. The anatomical and conceptual correlates are indicated both in the reviewed studies and our conceptual framework, and are consistent with our predictions introducing the Emo n-back. Some of the results observed could conceivably be associated with circuitry which serves to regulate emotion. Negative emotional categories could represent more complex emotional and cognitive processing in the interplay between the identification of emotional significance, the production of an affective state and downregulation of the affective state.

The 5-HTTLPR-Dependent Variability

The analyses from the Emo n-back demonstrate statistically significant three-way interaction between category, sex and genotype and also an interaction between sex and emotional category within the Emo n-back. The findings accent the importance of taking sex into account as several studies indicate sex-specific effects (Brummett et al., 2007; Elley et al., 2004; Kendler et al., 2005; Sjöberg et al., 2006; Uher & McGuffin et al., 2008).

The three-way interaction is clearly linked to, and mostly pronounced when introducing sad human faces. Homozygote short 5-HTTLPR carriers have higher accuracy for both males and females compared with the homozygote long 5-HTTLPR carriers. The effect is not seen when presented with emotional expressions of fearful, neutral or happy faces but also indicates a different pattern for men and women in these categories. It's important to note that our primal analyses take all categorical differences into account and most of all demonstrate different functional patterns. Heterozygote 5-HTTLPR carriers reveal an opposite pattern for men and women within the sad category but are not considered when comparing the homozygote variants.

When we compared the sad category to the neutral category we saw a distinct pattern for short female 5-HTTLPR carries. Our hypotheses that short 5-HTTLPR variants are associated with increased sensitivity to negative emotional stimuli, compared to long 5-HTTLPR carries, seems to be sex specific. Female short 5-HTTLPR carries demonstrate better accuracy in the sad category compared to the neutral category indicating vigilance toward negative emotional stimuli. No distinct 5-HTTLPR dependent patterns are found within the fear category as measured by the Emo n-back.

Importantly, specific effects linked to the sad category are not predicted by the reported imaging studies, but are also found when presented to other negative emotional categories, like the fear emotional category (Munafò, Brown & Hariri, In Press). Beevers et al.'s (2007) behavioural reaction time measure also reported an attention bias restricted to anxious word stimuli, hence in a heterogenic inpatient group, when comparing long 5-HTTLPR carriers to carriers of one or two copies of short 5-HTTLPR variants. Exploring potential effects of reaction time within The Emo n-back did not reveal a specific effect of reaction time but a statistically significant 5-HTTLPR-dependent effect of the total differences between emotional categories.

Operating with three 5-HTTLPR categories (S/S, L/S and L/L, based on the triallelic model), the separation of emotional categories, including accuracy and reaction time, and the consideration of sex may contribute to the more specific effects linked to sad emotional stimuli reported from the Emo n-back.

Depression is more common among females than in males (Piccinelli & Wilkinson, 2000) and the impact of life events, as measured by interviews or self reports are stronger among females than in males (Silberg et al., 1999; Maciejewski, Prigerson & Mazure, 2001). We also know that there exists a considerable sex difference in serotonin neurotransmission (Cosgrove, Mazure & Staley, 2007). Our study indicates increased sensitivity towards negative emotional stimuli in female short 5-HTTLPR carriers and suggests mechanisms that may represent diatheses to depressive symptomatology by sensitivity that makes female short 5-HTTLPR carriers emotionally labile. The results from the Emo n-back support sex specific findings by demonstrating a statistically significant category-sex-genotype interaction effect. The effects of sex should be further explored by matching the genotypes by sex-specific variables like the female menstrual cycle.

Comparing emotional categories does not necessarily uncover 5-HTTLPR dependent differences in emotional activation. Recently, studies have pointed at the importance of considering the implications of phasic and tonic models of 5-HTTLPR-dependent emotional activation. The traditional phasic activation model posits that the presence of short 5-HTTLPR variants represents the same baseline activation but differences linked to the degree of activation when presented with emotionally significant stimuli. Comparing the neutral

category to the sad category will only address phasic differences between these two conditions.

The tonic model explains brain activity in terms of greater baseline activation. Apparent differences between emotional categories may be driven by differences in tonic absolute activation. A combination of tonic and phasic activation represents a third model. Different 5-HTTLPR variants could have higher baseline activation and an additional increased reactivity to specific stimuli. The reviewed endophenotypic studies often rely on relative activation, in the form of differences between two task conditions, rather than on absolute activation values.

Canli et al. (2006) reported, by using perfusion imaging, that short allele carriers have clearly higher amygdala activation at rest. The 5-HTTLPR-dependent activation and connectivity were not restricted to the amygdala but linked to a wide network of other regions. The observations support the assumption that 5-HTTLPR variability are driven by tonic activation and also that the activation modulates extensive domains in brain function, not restricted to emotional processing. Rao et al. (2007) also found 5-HTTLPR variations in resting brain functions in healthy individuals using perfusion fMRI during a resting state. Individuals homozygous for the short variant showed significantly increased cerebral blood flow (CBF) in the amygdala and decreased CBF in the ventromedial prefrontal cortex. The three-way interaction from the Emo n-back demonstrates differences between four emotional categories and can't be explained by the tonic model alone. The observed 5-HTTLPR-dependent amygdala activation could also represent modulation from other brain regions, like inhibitory activity from frontal structures involved in downregulation of an affective state.

"It is possible that the observed amygdala activation patterns reflect influences from other brain systems. We have no evidence for or against it and make no assumptions either way" (Turhan Canli, e-mail, 04.02.08).

Based on our findings, we believe that amygdala-associated activation both affects and reflects activation from many brain regions and that these circuits represent functional systems involved in emotional perception and processing. The 5-HTTLPR-dependent deficits caused by tonic differences should be present independent of the stimulus' emotional content and are not supported by our study. The Emo n-back will, on the other hand, not discover the existence of potential tonic plus phasic 5-HTTLPR-dependent difference between emotional

categories. Further studies should address this problem by including a fifth non-facial category as a neutral baseline. This could represent an indicator of tonic contribution to explain variance within the Emo n-back. Limitations in comparisons lie in the assumption that processing of different emotional categories and a neutral baseline share the same functional and structural properties.

To assume emotional over activation directly to predict accuracy in a measure of cognitive control function is probably a simplification of the actual processes involved. This paper has demonstrated several structures and their interconnections to be involved in complex functional systems. The passage of serotonin in the brain and 5-HTTLPR variation has demonstrated that the net effect of 5-HTTLPR variants is best understood as a spectrum of different variants rather than functional and dysfunctional variants. The distinct pattern in the heterozygote 5-HTTLPR carriers supports this argument. Future studies should therefore treat genetic heterozygotes as potentially distinct functional variants.

The accuracy for different categories within the Emo n-back does neither reveal dysfunctions in cognitive control function per se. Short 5-HTTLPR carriers actually show higher accuracy for both men and women demonstrating better cognitive control functioning when presented with sad human faces compared with homozygote long variants. The 5-HTTLPR variability in cognitive control function seems to reflect subprocesses involved in emotional processing, like sensitivity that increase the likelihood to produce an affective state. More similar designs are needed to explore the reliability and nature of the reported findings.

Some of the functional variants seem to represent diathesis for depressive symptomatology (Uher & McGuffin, 2008). The results from the Emo n-back indicate 5-HTTLPR variability, not better explained by cognitive deficits associated with depressive symptomatology, in basal cognitive and emotional processing. The stress-depression hypotheses linked to short 5-HTTLPR carriers are also consistent with our study indicating 5-HTTLPR-dependent emotional processing. The different genotypes show distinct patterns in the way they process adverse environmental stimuli. Short female 5-HTTLPR carriers show a particular specific pattern suggesting sex-specific sensitivity towards negative stimuli.

Conclusion

Introducing the Emo n-back represents an advantageous contribution in a field that until now have focused on endophenotypes, personality traits and symptomatology

As measured by the Emo n-back, the role of serotonin in cognition seems to be sex-specific and linked to subcomponents involved in emotional processing. The Emo n-back reveal effects that are particularly pronounced when presented with a sad emotional category, indicating altered sex-genotype dependent processing linked to specific emotional content.

In the context of an integrative model of emotional processing, the Emo n-back indicates mechanisms that might represent diatheses for depressive symptomatology by demonstrating different genotypes to reveal distinct patterns in the way they process adverse environmental stimuli.

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