Thesis for doctoral degree (Ph.D.) 2010

Genetic Gating of Human Fear, Fear Learning and Extinction

Tina B. Lonsdorf

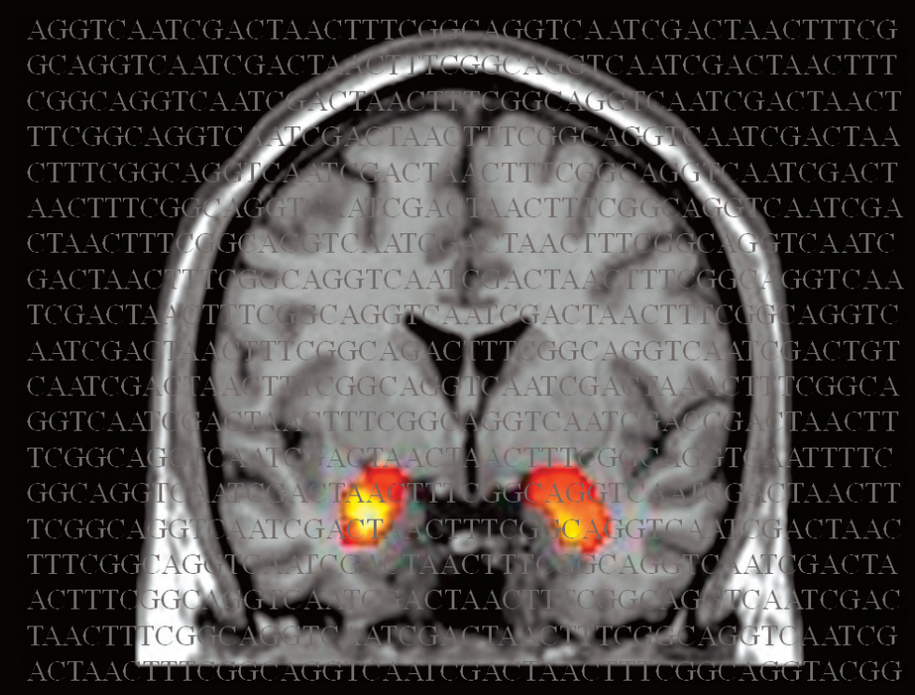
Karolinska Institutet

200 1810 – 2010 Years

Thesis for doctoral degree (Ph.D.)
2010

Genetic Gating of Human Fear, Fear Learning and Extinction

Psychophysiological, brain imaging (fMRI) and clinical studies



Tina B. Lonsdorf

Karolinska Institutet

200 1810 – 2010 Years

From THE DEPARTMENT OF CLINICAL NEUROSCIENCE
Karolinska Institutet, Stockholm, Sweden

GENETIC GATING OF HUMAN FEAR, FEAR LEARNING AND EXTINCTION

Psychophysiological, brain imaging (fMRI) and clinical studies

Tina B. Lonsdorf

Karolinska Institutet

Stockholm 2010

Cover illustration: modified from a download from Wikimedia Commons. No known restrictions on publication.

All previously published papers were reproduced with permission from the publisher.

Published by Karolinska Institutet.

ISBN 978-91-7409-967-6

Printed by
REPROPRINT AB
Stockholm 2010
www.reproprint.se
Gårdsvägen 4, 169 70 Solna

ABSTRACT

Individuals differ in their reaction to the same environmental stressor. After being opposed to the same trauma, some individuals will develop affective pathologies, while others will not. This is assumed to result, at least partly, from a differential genetic vulnerability. Fear conditioning and extinction have been suggested to be mechanisms involved in anxiety disorders and treatment respectively and represent laboratory models to study the reaction of individuals to a stressor in a controlled setting.

In this thesis we investigated the effect of common polymorphisms on fear conditioning and extinction (**Study I** & **II**) and aimed to translate our results into the clinical setting using a sample of patients suffering from panic disorder (**Study III** & **IV**).

As the amygdala is the core region involved in fear learning and extinction as well as highly implicated in anxiety disorders, we also studied the effect of common polymorphisms on amygdala reactivity and habituation to negative emotional stimuli using functional magnetic resonance imaging (**Study V**).

In **Study I** we report facilitated fear conditioning in healthy individuals carrying at least one 5-HTTLPR s-allele (as opposed to non-carriers, l/l), and resistance to extinction in individuals with the COMTval158met met/met genotype (as opposed to val-allele carriers), using fear-potentiated startle (FPS) as an index of conditioned fear. Similarly, in **Study II** we show that also carriers of the BDNFval66met met-allele (as opposed to non-carriers, val/val) display deficits in the acquisition of FPS reactions.

In **Study III** and **IV** we aimed to translate the experimental findings from **Study I** into a clinical setting using a sample of patients suffering from panic disorder. In **Study III,** we report a more severe symptomatic profile (both panic and depressive symptoms) in carriers of the 5-HTTLPR s-allele and in **Study IV,** we report reduced efficacy of exposure-based cognitive behavioural treatment modules in panic patients with the COMTval158met met/met genotype.

Study V investigated amygdala reactivity and habituation during the passive viewing of angry faces in healthy volunteers, selected based on gender, 5-HTTLPR/rs25531 and COMTval158met genotype. We report higher right amygdala reactivity and less habituation in 5-HTTLPR s-carriers as opposed to non-carriers (l/l) as well as enhanced left amygdala reactivity in individuals with the COMT met/met genotype as opposed to those carrying at least one val-allele.

In sum, the results of this thesis support a role for 5-HTTLPR/rs25531, BDNFval66met and COMTval158met in fear learning and extinction respectively which may have important implications for the risk to develop anxiety disorders (in particular after traumatic events) as well as the efficacy of their treatment. In addition, our results may have unraveled a mechanism underlying gene x environment interactions in anxiety disorders. Our finding of slower amygdala habituation may furthermore represent an underlying mechanism of the enhanced amygdala reactivity commonly found in 5-HTTLPR s-carriers in imaging genetic studies.

LIST OF PUBLICATIONS

This thesis is based on the following publications, which are referred to in the text by their roman numerals (Study I-V):

I. **Lonsdorf, T.B.**, Weike, A.I., Nikamo, P., Schalling, M., Hamm, A. & Öhman A. (2009). Genetic gating of human fear learning and extinction: Possible implications for anxiety disorders. *Psychological Science*, 20(2), 198-206.

II. **Lonsdorf, T.B.**, Weike, A.I., Golkar, A., Schalling, M., Hamm, A. & Öhman, A. (2010). Amygdala-dependent fear conditioning in humans is modulated by the BDNFval66met polymorphism. *Behavioral Neuroscience*, 124(1), 9-15.

III. **Lonsdorf, T.B.**, Rück, C., Bergström, J., Andersson, G., Öhman, A., Schalling, M. & Lindefors, N. (2009). The symptomatic profile in panic patients is affected by the 5-HTTLPR polymorphism, *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, 33(8), 1489-1586.

IV. **Lonsdorf, T.B.**, Rück, C., Bergström, J., Andersson, G., Öhman, A., Lindefors, N. & Schalling, M. The COMTval158met polymorphism affects efficacy of exposure based CBT in panic patients. *submitted manuscript*

V. **Lonsdorf, T.B.**, Golkar, A., Lindström, K.M., Fransson, P., Schalling, M., Öhman, A. & Ingvar, M. 5-HTTLPR and COMTval158met genotype independently gate amygdala reactivity and habituation during passive viewing of angry faces. *submitted manuscript*

ADDITIONAL PUBLICATIONS

These additional publications were produced during my PhD time, but are not included in the thesis.

I. Jensen, K.B., **Lonsdorf, T.B.**, Kosek, E., Schalling, M. & Ingvar, M. (2009). Increased Sensitivity to Thermal Pain Following a Single Opiate Dose Is Influenced by the COMT val^{158}met Polymorphism, *PLoSOne, 4(6): e6016.*

II. Kosek, E, Jensen, K.B., **Lonsdorf, T.B.**, Schalling, M. & Ingvar, M. (2009) Genetic variation in the serotonin transporter (5-HTTLPR, rs25531) influences the analgesic response to the short acting opioid Remifentanil in humans. *Molecular Pain, 5:37.*

III. Golkar A., **Lonsdorf T.B**., Olsson, A., Lindström K.M., Fransson P., Schalling M., Ingvar M. & Öhman A. Distinct contributions of the dorsolateral and orbitofrontal cortex during emotion regulation. *submitted manuscript*

IV. Lindström, K.M., **Lonsdorf, T.B.**, Golkar, A., Sankin, L., Britton, J., Fransson, P., Schalling, M., Öhman, A., Pine, D. & Ingvar, M. 5-HTTLPR genotype influence on right amygdala activation during threat orientation. *submitted manuscript*

CONTENTS

1 Introduction 1
1.1 Basic genetics 1
1.1.1 DNA 1
1.1.2 The genetic code 2
1.1.3 Genetic variation 2
1.1.4 Gene expression 4
1.2 Behavioral and psychiatric genetics 5
1.2.1 Heritability 5
1.2.2 Gene-finding approches 6
1.2.3 Genetics of complex traits/disorders 7
1.2.4 Endophenotypes 7
1.2.5 Imaging genetics 8
1.3 Fear, fear conditioning and extinction 9
1.3.1 Brain areas involved in fear, fear learning and extinction 10
1.3.2 Psychophysiological indicators of fear conditioning 13
1.3.3 Clinical relevance of fear conditioning and extinction 15
1.3.4 Genetic factors in fear conditioning and extinction 16
1.4 Panic disorder 17
1.4.1 Diagnostic classification 17
1.4.2 Defining appropriate phenotypes for genetic studies 18
1.4.3 The neurobiology of panic disorder 19
1.4.4 Genetics of panic disorder 20
1.4.5 Treatment of panic disorder 20
1.5 Serotonin 21
1.5.1 The serotonin system 21
1.5.2 Serotonin synthesis and turnover 22
1.5.3 The serotonin transporter and its polymorphisms 23
1.5.4 Associaiton studies of the 5-HTTLPR 25
1.6 Dopamine 27
1.6.1 The dopamine system 27
1.6.2 Dopamine synthesis and turnover 28
1.6.3 The *COMT* gene and its val158met polymorphism 29
1.6.4 Association studies of the COMTval158met 29
1.7 The brain-derived neurotrophic factor (BDNF) 31
1.7.1 BDNF synthesis and turnover 31
1.7.2 The BDNF gene and its val66met polymorphism 31
1.7.3 Associations studies of the BDNFval66met 32
2 Aims 34
3 Methods and Materials 35
3.1 Research participants and patients 35
3.2 Genotyping 37
3.2.1 5HTTLPR/rs25531 37
3.2.2 5’ Nuclease (TaqMan®) assay 38
3.3 Salivary cortisol measurement 38
3.4 Psychophysiological measurements 39

3.4.1 Fear potentiated startle 39
3.4.2 Skin conductance responses 40
3.5 Brain Imaging 40
3.5.1 Functional Magnetic Resonance Imaging 41
3.6 Stimulus material 42
3.7 Questionnaires and symptom ratings 42
4 Results and brief discussion 44
4.1 STUDY I 44
4.2 STUDY II 45
4.3 STUDY III 46
4.4 STUDY IV 47
4.5 STUDY V 48
5 General discussion 50
6 Future perspectives 53
7 Acknowledgements 55
8 References 57

LIST OF ABBREVIATIONS

5-HT	Serotonin
5-HTT	Serotonin transporter
5-HTTLPR	5-HTT linked polymorphic region
ACC	Anterior cingulate cortex
AG	Agoraphobia
APA	American Psychiatric Association
BA	Broadman area
BDNF	Brain derived neurotrophic factor
BOLD	Blood-oxygen-level-dependent
CBT	Cognitive behavioral treatment
COMT	Catechol-O-methyltransferase
CS	Conditioned stimulus
CS+	Conditioned stimulus coupled to the US
CS-	Conditioned stimulus not coupled to the US
CR	Conditioned reaction
CT	Computed tomography
DA	Dopamine
DAT	Dopamine transporter
DZ	Dizygotic twins
EDA	Electrodermal activity
EEG	Electroencephalography
fMRI	Functional Magnetic Resonance Imaging
FPS	Fear potentiated startle
GWAS	Genome-wide association study
HADS	Hospital Anxiety Depression Scale
ICD	International Classification of Diseases
ins/del	Insertion/deletion
NT	Neurotrophic factor
MADRS	Montgomery Åsberg Depression Rating Scale
MAO	Monoamine oxidase
MB-COMT	Membrane-bound COMT
MRI	Magnetic resonance imaging
mRNA	Messenger RNA
MZ	Monozygotic twins
S-COMT	Soluble COMT
SCR	Skin conductance response
SNP	Single-nucleotide polymorphism
SN	Substantia nigra
SSRI	Selective serotonin reuptake inhibitor
OFC	Orbitofrontal Cortex
PFC	Prefrontal Cortex
PCR	Polymerase chain reaction
PD	Panic Disorder
PDSS	Panic Disorder Severity Scale
PTSD	Post traumatic stress disorder
RNA	Ribonucleic acid
STAI	Spielberger Trait-State Anxiety Inventory
US	Unconditioned stimulus
UR	Unconditioned reaction
WHO	World Health Organization
vlPFC	Ventrolateral PFC
vmPFC	Ventromedial PFC
VNTR	Variable number of tandem repeats
VTA	Ventral tegmental area

1 INTRODUCTION

Anxiety disorders are one of the most common categories of psychiatric disorders and much effort has been put into the development of treatments. The most prevailing treatments are pharmacological treatment and psychological treatment (cognitive-behavioral therapy, CBT). There is great interest in possible genetic predictors of disease predisposition, treatment outcome and/or selection of type and intensity of treatment.

Fear conditioning and extinction are experimental paradigms that are used as laboratory analogues for the acquisition and treatment of pathological anxiety respectively. Studying these processes in controlled experimental settings allows studying the impact of specific genetic variants while controlling for confounding factors.

In this thesis, the candidate gene approach was used to study the role of candidate polymorphisms in the acquisition and extinction of experimental fear (**Study I** and **III**), amygdala reactivity as well as habituation to fearful faces in healthy volunteers (**Study V**), symptom severity (**Study III**) and the outcome of CBT in panic patients (**Study IV**).

First, a general introduction into basic genetics is given before the paradigms of fear conditioning and extinction as well as the clinical syndrome panic disorder (PD) are introduced. Last, the genetic polymorphisms studied in this thesis are described in detail, followed by a description of the methods used in the studies, a summary of results and a general discussion as well as future perspectives.

1.1 BASIC GENETICS

1.1.1 DNA

Deoxyribonucleic acid (DNA) [1], contains the information for the development and functioning of all organisms and carries out two main functions: self replication during cell division and direction of protein synthesis.

The molecular units of DNA are nucleotides with backbones of sugars and phosphate groups. Attached to each sugar is one of four types of bases: adenine (A), guanine (G), thymine (T) or cytosine (C). The sequence of these four bases along the backbone encodes information according to the genetic code, which specifies the sequence of the amino acids within proteins. DNA consists of two long polymers of nucleotides, sugars and phosphate groups. Both DNA strands, that coil around each other and thereby form the famous DNA double helix, are connected to each other by base pairing. Due to their structural properties, A always pairs with T via 2 hydrogen bonds and C always pairs with G via 3 hydrogen bonds.

DNA is not distributed randomly within the nucleus but arranged on structures called *chromosomes*. The human genome is organized into 23 pairs of chromosomes with one chromosome of each pair inherited paternally and one maternally. Twenty-two of the chromosome pairs are autosomes (chromosome 1-22) and one is a pair of sex chromosomes (XX for females, XY for males).

The information carried by the DNA to build and maintain cells and pass genetic traits to offspring is contained in the sequence of DNA parts called *genes*. The human genome contains approximately 25.000 genes and these are arranged linearly on the

chromosomes. Each gene has a specific position on the chromosome, the gene *locus*, and typically, a gene is made up of *exons*, *introns* and a *promoter* region.

Exons are nucleic acid sequence that are represented in the mature form of an ribonucleic acid (RNA) molecule and are often referred to as coding sequences, even though non-protein-coding exons exist (5' and 3' untranslated regions).

Introns or non-coding sequences are DNA regions that are not translated into a protein but are removed by splicing during the processing of mature RNA. Introns have for a long time been considered as useless junk-DNA with no biological function. However, it is now known that introns contain important sequences for efficient splicing (donor and acceptor sites) and that introns can be transcribed into microRNA regulating gene expression.

The *promoter region* is the regulatory area upstream to the gene that controls gene expression. It contains motifs which transcription factors bind to; the promoter element is the site where the RNA polymerase will begin to read and transcribe the DNA coding region into messenger RNA (mRNA, see 1.1.4).

1.1.2 The genetic code

The sequence of bases in the DNA encodes 20 different amino acids. The genetic code, that is the same for all living organisms, consists of combinations of three bases which are called *codons*. For example the codon TAC codes for the amino acid methionine while the codon CAC codes for the amino acid valine. Given the four different bases there are 64 (4^3) possible triplet codons that encode for the 20 different amino acids. This implies that some amino acids are encoded by more than one triplet combination, e.g. valine is encoded not only by the codon CAC but also by CAA, CAG and CAT.

1.1.3 Genetic variation

There is no single genome and every individual carries an individual genetic profile. All people share 99,9% of their DNA sequences and thus the 0,1% of the DNA sequences, that vary between individuals are responsible for the biological differences between individuals [2].

The two copies of a gene at corresponding loci on a pair of homologous chromosomes commonly harbor sequence variations. Usually, a rare variation (e.g., present in <1% of the population) is referred to as a mutation while a more common variant (e.g., present in > 1% of the population) has been referred to as *genetic polymorphism*.

The alternative DNA sequences at the same physical gene locus are referred to as *alleles* and the frequency of different alleles can vary extremely between populations. If the two alleles inherited maternally and paternally are identical, an individual is said to be *homozygous* at that locus while the individual is said to be *heterozygous* if they differ. A combination of alleles at several loci on the same chromosome that are transmitted together is called a *haplotype* (for an alternative definition of haplotype see 1.1.3.1)

Genetic polymorphisms may affect the functional or structural features of the protein, the amount of protein that is produced as well as the genes capacity to regulate the expression of other genes. These are the processes through which genetic

variation creates physiological differences that may ultimately affect behavior or diseases. Still, a polymorphism can also be silent and not have any impact on the genes function or structure at all.

But not only variations in coding regions have the potential to affect physiological processes. Intronic polymorphisms may influence mRNA processing, stability of the translation product or regulatory mechanisms such as transcription rate and polymorphisms in the promoter region may affect gene expression.

There are different types of genetic polymorphisms. The most common type are single-nucleotide polymorphisms (SNPs) and other types of polymorphisms include insertion/deletion (ins/del) polymorphisms and repeat polymorphisms, also referred to as variable number of tandem repeats (VNTRs). Copy number variations are deletions or duplications of sequences that are longer than 1000 base pairs.

In this thesis, SNPs and an ins/del polymorphism (located in a VNTR region) were investigated and thus these types of polymorphisms are described in more detail below.

1.1.3.1 Single-nucleotide polymorhpisms

Single-nucleotide polymorphisms (SNPs) are the most common type of genetic variation occurring in about 1 in 1000 bases of the approximately three billion bases in our genome. Hence, there are approximately three million SNPs.

All SNPs are assigned a reference SNP ("rs") number in the Single Nucleotide Polymorphism Database (dbSNP) at the National Center of Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov/SNP).

A SNP is a variation in the DNA sequence caused by a difference in a single nucleotide (A, T, C, G) at a specific locus between the two chromosomes of an individual or between members of the same species (see Figure 1). Usually there are at least two different alleles of a SNP (e.g. A and G) but also more alleles (e.g. A, G and T) are possible.

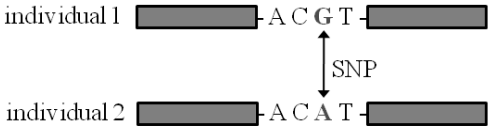


Figure 1. Schematic representation of an A→G SNP with individual 1 carrying the G-allele and individual 2 carrying the A-allele at a specific locus (The figure shows a single strand of a single chromosome).

A SNP that is located in an exon may change the base sequence of a codon. This sort of SNP is called *non-synonymous* and may either result in a different amino acid being incorporated in the protein (missense), or result in a premature stop codon (nonsense).

In contrast, *synonymous* SNPs are situated in coding regions but do not change the amino acid that is encoded by the codon.

It should also be mentioned that the term haplotype (see above) is alternatively also used to refer to a set of statistically associated SNPs on a single chromatid.

1.1.3.2 Insertion/deletion polymorphisms

Single nucleotides may not only be substituted as in the case of SNPs, but can also be removed (deletions) or added (insertion) to a nucleotide sequence (see Figure 2). Occurring in coding regions, an insertion/deletion (ins/del) may lead to a shift in reading if a non-multiple of 3 nucleotide bases is inserted or deleted. This may severely impair the function of the resulting protein, if it is formed at all, and thus ins/dels are rather rare in protein-coding sequences.

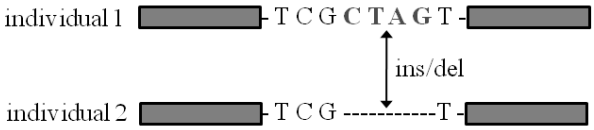


Figure 2. Schematic representation of an 4bp ins/del with individual 1 carrying an insertion and individual 2 carrying a deletion at a specific locus (The figure shows a single strand of a single chromosome).

1.1.3.3 Variable numbers of tandem repeats

Variable numbers of tandem repeats (VNTR) consist of short nucleotide sequence repeat units. The different length variants act as inherited alleles. VNTRs can be sub-classified in microsatellites (repeats of sequences < 5 base pairs) and minisatellites (repeats of sequences > 5 base pairs).

1.1.4 Gene expression

In total, only about 2% of our total genome is expressed and even though almost all cells in our body share the same genome, only 10-20% of the genes are expressed in any given cell type. In brain cells, more genes are expressed than in any other tissue of the body which highlights that the brain is our most complex organ [3].

Which genes are activated in an individual cell depends on complex interactions between molecules in the cell itself, its neighboring cells as well as the organism's external environment. When a gene is active, both coding and non-coding sequences are copied into RNA (where T is substituted for by uracil, U) in a processed called *transcription*. The mature RNA molecule, where introns are spliced off, then directs the synthesis of proteins via the genetic code. In the *translation* process, which takes place outside of the nucleus, the ribosome reads the codons of the messenger RNA (mRNA), and builds the protein from the amino acids that these codons encode. The molecules resulting from gene expression, whether protein or RNA, are referred to as gene products, and are responsible for the development and functioning of the organism.

Here it becomes clear, that genes do not only serve the function of transmitting heritable information from one generation to the next. Another function is to direct the production of, e.g., specific proteins (gene expression).

Gene expression is highly responsive to environmental factors. Internal (e.g., hormones, developmental stages) as well as external factors (e.g., stress, learning) can alter the binding of transcriptional regulators to the enhancer element of the promoter region. This aspect of gene regulation, which refers to changes in gene expression

caused by mechanisms other than changes in the underlying DNA sequence, is termed *epigenetics* but will not be addressed here further.

1.2 BEHAVIORAL AND PSYCHIATRIC GENETICS

The relatively new field of human behavioral genetics aims at elucidating the genetic underpinnings of individual variation in human behavior. Psychiatric genetics in turn aims at understanding the genetic contribution to psychiatric disorders.

There is good evidence supporting that both behavior and psychiatric disorders have a strong biological basis and are heritable. First, brain injury can lead to dramatic changes in the affected individual's personality and behavior. Second, specific mouse behaviors can be created or extinguished by inserting or disabling specific genes. Third, we routinely modify the behavioral manifestation of common psychiatric disorders, that have clearly demonstrated to "run in families", with drugs that alter brain chemistry.

Nearly all behavioral domains and psychiatric disorders studied to date show moderate to high heritability usually to a somewhat greater degree than common medical illnesses [4].

It is noteworthy, that genes do not code for behavior or psychiatric disorders *directly* and that it is not the behavior itself which is inherited. Genes code for proteins and these proteins may be involved in the generation, functioning or maintenance of neurons and neural networks which ultimately give rise to behavior. Approximately one third of our protein coding genes are expressed only in the brain and DNA variations in these genes may create differences in physiological systems that have the potential to affect behavior. Human behavior and psychiatric diseases and the neural networks producing them are the product of hundreds of thousands of genes acting in concert with multiple environmental events and thus they are called *complex traits*.

A major problem in the field of behavioral and psychiatric genetics is the difficulty in defining appropriate phenotypes (for a more detailed discussion see 1.4.2) as well as the quantification or measurement of a particular phenotype.

1.2.1 Heritability

Heritability defines whether a specific trait or disorder is influenced by genetics and what proportion of the phenotypic variation is due to variation in our genetic make-up. The variance not explained by heritability is attributed to environmental influences.

Heritability has been shown to be rather high for behavioral traits as well as psychiatric disorders with heritability estimates up to 50%, which is slightly higher than for other common medical diseases [4].

The three key methods used in quantitative behavioral and psychiatric genetics to estimate heritability have been family studies, twin studies and adoption studies.

Family studies compare the prevalence of a phenotype, e.g. a disease, among relatives of affected and unaffected individuals in order to examine whether a phenotype aggregates in families. While family studies cannot disentangle the contribution of genetics and environmental effects, *twin studies* and *adoption studies* in turn can be used to solve the question whether the clustering of a trait or a disease in a family is due to environmental or genetic factors. Twin studies compare concordance rates between genetically monozygotic (MZ) twins and dizygotic twins (DZ). While MZ twins share

100% of their genome [but see 5], DZ twins share, like normal siblings, approximately 50% of their genes. Thus MZ twins are genetically twice as similar as DZ. A third method, adoption studies, compares the similarity between twins reared apart and reared together as well as between offspring and their biological parents and adoption parents.

1.2.2 Gene-finding approches

Several different approaches can be used for finding genes that play a role in a certain behavior or a disease. The two main types of studies are linkage and association studies.

Linkage studies are used to map the relevant loci for a disease/trait in question in pedigrees, once heritability has been established. Linkage analysis has only limited power to identify loci influencing traits that are complex and inherited in a non-mendelian fashion. While linkage studies test whether a disease and an allele show correlated transmission within a pedigree, association studies test whether they show correlated occurrence in populations.

Because in this thesis, the association study approach using candidate genes has been employed, this approach will be described in more detail below.

Association studies allow for the detection of genetic factors that have a modest effect and this approach is less sensitive to the existence of phenocopies and non-penetrant individuals (see below). However, association studies are sensitive to population stratification and a major disadvantage is that a significant association between a specific behavior or disease and a polymorphism can either be due to a real effect of this specific variant or to a variant that is in close linkage disequilibrium.

Three partly overlapping approaches of association studies exist: Candidate gene studies, case-control association studies and genome-wide association studies (GWAS).

Candidate gene studies investigate genetic variants in genes that are *a priori* hypothesized to be causally related to the trait or disease studied. This hypothetical causal relationship is based on prior biological or genomic evidence (e.g. from linkage studies). Hence, this approach depends on knowledge about the pathophysiology underlying the disease/trait studied. Because of the limited pathophysiological knowledge for most psychiatric disorders and psychological traits, candidate genes are selected based on pharmacological, neurochemical and clinical evidence. Single-locus allele, genotype or haplotype frequencies in or around candidate genes are compared between unrelated healthy individuals and those suffering from a specific disease (*case-control association studies*). Alternatively the results of experimental tests are being compared between individuals carrying different alleles, genotypes or haplotypes at a specific locus.

In this thesis, all studies used the candidate gene approach to study (functional) polymorphisms in neurotransmitter systems that have a high a priori biological likelihood of being causally related to traits of relevance for anxiety disorders.

Despite their popularity, there is considerable concern about the robustness of findings from association studies. Many positive associations obtained using this approach, have proven difficult to replicate. This may be due to false positives or false negatives. The latter may be due to too small sample sizes to reliably detect an effect. Thus, replications are needed until association data can finally be accepted as facts.

In *GWAS*, a large number of genetic markers (e.g., >500.000) that capture genetic variation across the entire genome are examined in their relationship to a specific disease or trait. GWAS's have been used lately and require very large samples. However, despite of high expectations, GWAS have not proven to yield as much information as it was originally hoped for [6].

1.2.3 Genetics of complex traits/disorders

The inheritance of complex traits or diseases does not follow simple mendelian monogenetic patterns and there is no simple correspondence between genotype and phenotype. The same gene can affect different phenotypes (*pleiotropy*) and the same phenotype can result from different genotypes e.g. due to genetic variations in any of several genes that affect a final common biochemical pathway (*locus heterogeneity*). Thus, individuals will suffer from the same disease for different genetic reasons. This hampers genetic mapping because in different families, different genomic regions will co-segregate with the disease.

The heritability of complex traits and diseases likely involves the effects of multiple genetic loci as well as their interaction which is referred to as *epistasis* (the terms gene-gene interaction and epistasis are in most cases used interchangeable). Epistasis takes place when the effects of one gene are modified by one or several other genes, which are sometimes called *modifier genes*. This may explain why a genetic variant acts as a risk factor in one individual while it does not in another individual. Thus gene-gene interactions can explain a large amount of why there is variation in complex diseases as well as between individuals with different ethnic backgrounds.

Gene-gene effects can be additive (in case the effects are independent), s*ynergistic* (the effect of one variant is potentiated by the genotype on another locus) or *antagonistic* (the combined effect is smaller than the sum of the individual effects).

For complex traits, the whole is greater than the sum of its parts and in fact may be different from the sum of its parts. In addition, detection of disease-causing genes is hindered because some individuals that have inherited a genetic predisposition will not manifest the phenotype (*incomplete penetrance*) and some who have not inherited the genetic predisposition will manifest the phenotype e.g. as a result of environmental effects (*phenocopies*).

It also needs to be considered that genes may not only act as risk factors or protective factors for the development of the disease, but that they also may modify phenotypic variation of a trait or disease, e.g. symptom severity (see **Study III**). Furthermore different individuals faced with the same stressful environmental event react differently which can partly be attributed to the individual's genetic make-up (*gene x environment interaction*).

A promising approach to facilitate genetic research on complex traits or disorders and to bridge the gap between gene variants with small effects and complex behavior is the use of so called endophenotypes (see 1.2.4).

1.2.4 Endophenotypes

The term endophenotype was introduced into psychiatry nearly four decades ago [7] and has been adopted from insect biology. An endophenotype describes an *intermediate*

phenotype that lies on the pathways between genes and a disease and is assumed to be closer to the action of the gene than the disease itself [8, 9]. Consequently, endophenotypes are thought to have a simpler genetic architecture than their associated phenotype and thus may be more readily linked to a specific genetic locus [9].

For a marker to be considered an endophenotype, it must be shown to (1) be highly heritable, (2) be associated with the phenotype (e.g. a formal clinical diagnosis) due to shared genes, (3) be independent of clinical state but may only manifest at a certain age or after a certain challenge, (4) must cosegregate with the phenotype within a family, with nonaffected family members displaying it more frequently relative to the general population, and (5) should be reliable and validly measured through various methods e.g. endocrinology, biochemistry, neuropsychological or cognitive measurements as well as neuroimaging [8-10].

However, it needs to be kept in mind that not all of these criteria may be easy to demonstrate and they may need to be indirectly inferred. Furthermore, even an endophenotype that meets all these criteria may still not lie on the actual disease pathway but may represent a pleiotropic epiphenomenon [8].

Even though the endophenotype concept is a promising approach for psychiatric and behavioral genetics it still remains to be seen if the effect sizes found in studies employing endophenotypes indeed are greater than those found for the respective phenotypes [10].

1.2.5 Imaging genetics

In the causal chain from genes via proteins to behavior and disease, brain activity is considered an endophenotype that could help to bridge the gap between genes and behavior [11].

Imaging genetics is a form of genetic association study in which the phenotype is not a disease or a behavior but a measure of brain function (e.g., physiological response of the brain during specific information processing), chemistry (e.g., receptor density) or structure [12]. It is assumed that brain function, chemistry or structure is closer to the gene's functions than the behavior or the disease itself and thus can be considered an endophenotype for a specific behavior or disease. Thus, it is expected that genetic polymorphisms have a more robust impact at the level of the brain than at the level of disease or behavior even though this view has recently been challenged [11].

It has been demonstrated that brain structure is substantially heritable [13, 14] and evidence supporting the heritability of functional magnetic resonance imaging (fMRI) measurements is emerging [15, 16], providing the rationale for studying the specific genetic underpinnings of brain activation during information processing.

The strongest candidates for imaging genetics studies are well characterized polymorphisms in coding regions of genes involved in neurotransmission or regions affecting gene-expression or splicing. Still, the expected effects of any single polymorphism on brain activation is likely to be rather small and thus the success of any imaging genetics study also critically depends on the validity of the selected task. Most suited are well-characterized tasks that effectively engage a neural network and produce robust signals while also displaying variance between individuals [12]. Defining

an appropriate phenotype is thus most critical for the success of imaging genetic studies.

As imaging genetics employs the association study approach, which is known to be susceptible to population stratification, it is also critical to carefully match and select participants in a way that control for other potentially confounding factors (e.g. ethnicity, age, gender etc).

Still, the young field of imaging genetics has been paved by failures to replicate and thus, another much discussed issue is the problem of how to account for multiple testing in thousands of voxels. There is however evidence suggesting that commonly used methods (e.g., Familiy-wise-error correction or False Discovery Rate correction) may reduce false positive associations to an accepted level (e.g., <5%) [17].

1.3 FEAR, FEAR CONDITIONING AND EXTINCTION

Fear is a strong aversive emotional state that can be elicited by internal or external events. These activate the defensive fear system and are caused by either the awareness or the anticipation of danger. In this sense, fear is an adaptive emotion as it prepares the organism for escape or avoidance and thereby helps to adjust to environmental demands and enables effective coping with potentially harmful stimuli. It is less costly to activate these defensive responses to an innocuous stimulus than failures to do so to real threats and in fact our perceptual system seems to be biased towards efficient identification of threat [18].

Fear, as an active coping emotion that is elicited by a distinct stimulus must be distinguished from anxiety, which is more diffuse and occurs when passively avoiding a dangerous situation or when coping strategies fail [18].

Even though fear is in principle an adaptive emotion, it can become pathological when it is too persistent and/or unreasonable intense. It is assumed that many symptoms of pathological anxiety are acquired through learning processes and the acquisition and extinction of fear can be examined in the laboratory using classical conditioning and extinction procedures which are described in more detail below.

Fear conditioning and extinction

Learning to predict danger from previous experience is critical to an organism's survival. It is of high importance to be able to anticipate a threatening event in order to be able to activate the defensive system early. Potentially threatening events may be announced by cues (e.g. noises or smells) which may, via learning processes, become warning signals for the imminent threat [19]. The associative learning taking place is referred to as (aversive) classical conditioning. Thereby, associations between two previously unrelated stimuli are learned: a naturally aversive, fear inducing *unconditioned stimuli* (US) that reflexively activates unconditioned fear responses (UR) and an originally neutral stimulus (the to-be *conditioned stimulus*, CS). This previously neutral stimulus becomes intrinsically aversive and fear-eliciting itself via the temporal pairing with the US and gains the ability to elicit conditioned responses (CR) that share characteristics similar to the UR. These responses are not learned but fear conditioning rather allows new threats to automatically activate the fear system.

In *differential* fear conditioning paradigms, there are two initially neutral stimuli whereof one (CS+) is paired with the US and thus becomes fear eliciting while the second neutral stimulus remains unpaired (CS-) and thus does not gain fear-eliciting capacities.

In experimental settings, two different types of conditioning paradigms have been widely used and can be distinguished based on the timing between CS+ and US: *delay* and *trace* conditioning. During delay conditioning, as used in **Study I** and **II**, the CS+ is immediately followed by the US (or even overlaps with it), while in trace conditioning, the CS+ and the US are separated by a time-interval of 500ms to 10s [20]. Delay conditioning leads to a more rapid learning of the CS-US association and also to faster extinction of theses associations as compared to trace conditioning [20].

In addition to the classification in trace and delay conditioning, experiments vary with respect to the type of stimuli used as CS (e.g. emotional pictures, faces, geometric figures, olfactory cues), US (e.g. a tactile electrotactile stimulation, visual pictures, auditory tones, olfactory cues), CS-US contingency (the rate of pairing between the CS+ and the US) and thus predictability as well as the instructions provided to the participant (explicit information about the CS-US contingency vs. no explicit information) [20].

After conditioning has occurred, the repeated presentation of the CS+ without pairing with the US (exposure) leads to a gradual weakening of the fear reaction, a process that is referred to as *extinction*. Extinction represents not simply forgetting the US-CS+ association but is an active learning process resulting in structural and chemical changes in the brain [21]. Phenomena like spontaneous recovery, renewal and reinstatement demonstrate clearly that extinction rather occurs via new inhibitory associative learning processes than simple erasure [21, 22].

Thus, extinction also is an adaptive process as a previously appropriate, but now unnecessary behavior is inhibited. Despite this long held notion, recent advances in animal work have suggested that it may be critical to distinguish between immediate and delayed extinction, as only the latter may involve inhibition processes while immediate extinction may lead to an erasure of the learned responses [23]. Mixed evidence has emerged lately from human research [24, 25]. This is of critical experimental interest as human studies, for practical reasons, mostly apply immediate extinction while in animal studies extinction does commonly not follow immediaty after the acquisition phase. Therefore we used a 24-hour delayed extinction in **Studies I** and **II.**

Both animal studies and human brain imaging studies have investigated the neural processes associated with fear conditioning and extinction and the crucial neural network underlying these processes has been identified [20, 26] and will be described in more detail in the next section 1.3.1.

1.3.1 Brain areas involved in fear, fear learning and extinction

Both fear conditioning and extinction have been shown to induce robust and specific neural activation patterns. The core neural network involved in fear conditioning and extinction includes the amygdala, the (anterior) insula and prefrontal areas but also striatal areas and the hippocampus are involved [20, 26]. Still, the neural network involved in fear extinction is less understood than that involved in fear conditioning. Because the

amygdala and prefronal areas are of particular interest and importance for this thesis, these structures as well as their interactions will be described below.

Amygdala

Evidence from pharmacological, neurophysiological and lesion studies in both animals and humans have identified the amygdala (see Figure 3) as the most central brain region in both normal and pathological emotional behavior and particularly in fear [27]. The amygdala is thought to rapidly appraise the environment for threat and stimuli of biological significance [28] and direct attention to affectively salient stimuli such as novel, surprising or ambiguous stimuli [29]. In addition the amygdala seems to be especially sensitive to uncertainty [30].

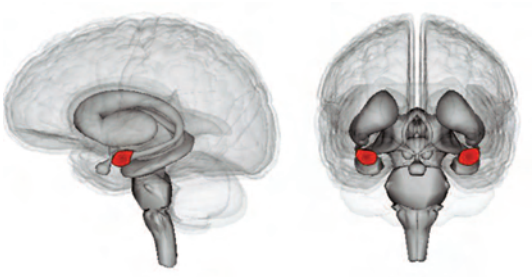


Figure 3. Schematic representation of the location of the amygdala (highlighted in red colour) in the human brain (modified from a download from Wikimedia Commons. No known restrictions on publication).

External sensory information signaling potential danger can reach the amygdala from the thalamus via two distinct routes: A direct pathway from the thalamus (*thalamo-amygdala pathway*) can rapidly and reflexively activate the amygdala even at an unconscious level [27, 31, 32] while information that reaches the amygdala via the indirect pathway (*thalamo-cortico-amygdala pathway*) is slower but more processed and reappraised. In the latter pathway, sensory input from the thalamus reaches the amygdala first after processing in sensory cortices in conjunction with prefrontal areas and the hippocampus [27].

The basal and lateral nuclei of the amygdala, where CS-US associations are thought to be formed, receive sensory input from diverse brain areas (e.g. thalamus, cortical areas, hippocampus) and send information to the central nucleus of the amygdala. From there, projections are sent to autonomic and somatomotor structures that mediate specific and measureable fear responses [26, 33].

A plethora of data across species and methods have demonstrated the importance of the amygdala in both the acquisition (particular the early phases) and expression of learned fear [26, 30] as well as the extinction of conditioned fear [34]. Lesions of the amygdala are known to block several measures of innate fear in different species.

Damage of the lateral nucleus of the amygdala interferes with fear conditioning in animal studies while damage of the central nucleus interferes with expression of the conditioned fear resonse [33]. The prominent Kluver-Bucy syndrome, which involves removal of the monkeys bilateral temporal lobes leads to marked changes in emotional behavior, including reduced fearfulness [35].

Also in human studies, deficits in fear conditioning, as measured by skin conductance responses (see 1.3.2), are seen after demage of the amygdala [36] and the temporal lobe including the amygdala [37]. Removal of the amygdala is furthermore also associated with deficits in the recognition of emotion in people's faces - in particular fear [30]. Lesions restricted to the bilateral amygdalae impair the acquisition of conditioned autonomic responses but leave declarative knowledge about the CS-US contingencies intact, while the opposite pattern has been seen in patients with specific hippocampal lesions [36].

Furthermore fMRI studies have shown increased amygdala activity during fear conditioning [38] which seems to be independent of the CS-US contingency rate (rate of pairing between CS+ and US) and the CS and US modality [20]. In addition, accumulating evidence suggests that amygdala activity may be a traitlike marker associated with inhibited behaviour [39-41].

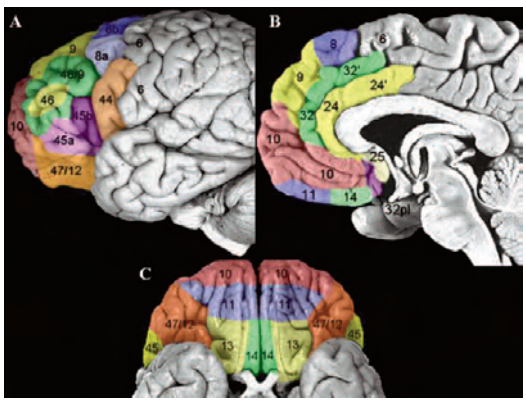


Figure 4. Broadman areas (BA) in the prefrontal cortex (PFC). Modified after Riddarinkhoff K.R. et al., 2004, Brain and Cognition [42] and published with kind permission from the publisher.

The prefrontal frontal cortex

The prefrontal cortex (PFC, see Figure 4) is particularly implicated in the regulation of emotions and in extinction of conditioned fear [22].

The PFC is thought to regulate fear expression by top-down inhibition of the amygdala and thereby inhibition of conditioned reactions (both aversive and appetitive). In particular the involvement of medial and ventromedial (vm) PFC regions have been highlighted in extinction in both animal [22, 43] and human studies [20 , 44, 45].

The terms medial and ventromedial PFC are not always used in the same way by different researches. Generally, the *medial PFC* (mPFC) refers to regions in the frontal lobe from the medial wall of the hemispheres to the base of the frontal lobe and includes e.g. the anterior cingulate, infralimbic, prelimbic, and the medial orbiofrontal cortex [22].

The term *ventromedial PFC* (vmPFC) can refer to the infralimbic cortex, the prelimbic cortex, the subgenual PFC and medial orbitofrontal areas. The *vmPFC* has specifically been shown to be involved in emotion regulation and in particular the ability to interpret emotional stimuli and change behavior accordingly [46] as well as recall of extinction memory in animal studies [43] and humans [44]. Animal studies have demonstrated, that the vmPFC and the amygdala are reciprocally connected [43].

Some sub-regions are also worth mentioning: The *orbitofrontal cortex* (OFC), has bidirectional anatomical and functional connections to the amygdala and plays a key role in the processing of emotional salience as well as updating and integrating affective information to guide (social) behavior [47]. Animals with OFC lesions are unable to inhibit a prepotent affective response [48] and humans with lesions in this area have been described as inflexible and emotionally disinhibited [49].

The *anterior cingulate* (ACC), sometimes referred to as a part of the medial prefrontal cortex, has been suggested to be a bridge between emotion and cognition [50]. It has generally been implicated in initiation, motivation, and goal-directed behaviors [51]. Specifically the ACC is involved in error monitoring and detection of competing responses [22] as well as the reappraisal of negative emotional stimuli [52] and plays an important role in approach and avoidance learning as well as fear learning [53]. The dorsal part of the ACC (Broadman area [BA] 32) projects to the basolateral amygdala and is implicated in conditioned fear, particularly the expression of fear [54] and has been shown to be involved in the generation of autonomic fear responses [55].

Amygdala-prefrontal interactions

Recent excitement and interest has emerged concerning a possible role of amygdala-PFC (in particular the vmPFC) interactions in extinction and emotion regulation. Specifically, it has been suggested that the vmPFC inhibits amygdala neurons and thereby promotes fear inhibition [56, 57].

By now, there is substantial evidence suggesting that anxiety disorders like post traumatic stress disorder (PTSD), PD and phobias are characterized by deficits in prefrontal-amygdala interactions and connectivity subserving fear learning, extinction and emotion regulation [43] which provides a possible mechanism for the development and maintenance of anxiety disorders.

1.3.2 Psychophysiological indicators of fear conditioning

Emotional or salient events, e.g. the presentation of the CS+ in conditioning experiments, result in arousal of the sympathetic nerve system. Two commonly used psychophysiological measurements in research on classical conditioning and extinction are conductance responses (SCR) and fear potentiated startle responses (FPS). Both SCR and FPS have been employed in the experimental studies included in this thesis (**Study I, II** and **V**) and will therefore be described in more detail below.

Fear potentiated Startle

The startle reaction (see also 3.4.1) is a fast defensive response to a sudden, unexpected and intense stimulus, such as a loud noise (acoustic startle reflex), a flash of light, or a quick movement close to the face. The startle reflex is a cross species reaction [58, 59] and in humans, the reaction includes physical movement away from the stimulus and often blinking but also changes in blood pressure, respiration, and breathing. While the latter responses take somewhat longer, the muscle reactions resolve themselves within seconds. In humans the contraction of the orbicularis oculi muscle, which is the first and most reliable component of the human startle reflex to abrupt sensory events, is measured.

The basis for FPS is that the startle blink response, triggered by a sudden burst of noise (startle probe), is larger when the individual is in an aversive or fearful state. Startle potentiation has been proved to be a specific and reliable index of fear learning in both animals [26] and humans [60, 61]. Human research has convincingly demonstrated that the acoustic startle response is augmented when startle probes are administered in the presence of the CS+ in fear conditioning experiments [62] as well as during passive viewing of unpleasant pictures [58] . Furthermore, the magnitude of the startle response has been shown to be directly related to affective valence [63].

The neurobiology of the startle reflex pathway as well as the anatomical basis for the PFS reflex is well known. The afferent and efferent fibers of the startle reflex converge in the nucleus reticularis pontis caudalis, which receives direct and indirect projections from the (central) nucleus of the amygdala. Activation of the central nucleus of the amygdala increases startle via direct and indirect connections between the amygdala and the nucleus reticularis pontis caudalis in the acoustic startle pathway [61]. Lesions of the amygdala block occurrence of FPS [64] and the startle blink reflex is thus particularly appropriate for the study of amygdala-dependent learning. In addition, a major advantage of using FPS as an index of acquired fear state is that the human startle response is comparable to the whole body startle response employed in animal studies which facilitates the translation of the results from one field to the other because. In both animals and humans a similar neural pathway seems to be involved in startle reflex potentiation to the CS+ [61].

Skin conductance responses

Electrodermal activity (EDA, see also 3.4.2), also known as Galvanic skin response, has been one of the most widely used response system in the history of psychophysiology. In general, it has to be discriminated between the tonic skin conductance level in the absence of a phasic response and skin conductance responses (SCR) that are phasic increases in conductance superimposed on the tonic level of conductance.

Phasic SCR have been associated with the psychophysiological concepts of emotion, arousal, orienting and attention and are sensitive to stimulus novelty, and intensity [65].

EDA is primarily under sympathetic control and based on the activity of eccrine sweat glands which are most dense on the palms and soles of the feet. Generally their primary function is thermoregulation, but those located on the palm have been suggested to be more responsive to emotional than to thermal stimuli, which is why they have been of primary interest for psychophysiological research [65]. Depending on the degree of sympathetic activation, sweat rises in the sweat duct which leads to a more conductive path through the relatively resistant corneum. The higher the sweat rises, the lower the resistance and the higher the conductance which is measured as changes in EDA.

Multiple sites in the human brain are involved in the control and generation of SCRs specifically the hypothalamus, the amygdala, the hippocampus, motor areas, prefrontal areas as well as the reticular formation in the brainstem [65] and each of these areas has a distinct functional role. While e.g. SCRs associated with the amygdala most likely reflect affective processes, SCRs associated with the hypothalamus serve

thermoregulation. However, in the living human being all these central influences will act in concert at any point of time.

In human fear conditioning experiments, SCR have been widely used as an indicator of successful fear learning and have been shown to reflect a cognitive level of contingency learning [58, 66]. SCRs can be dissociated from amygdala activations in imaging studies [67].

1.3.3 Clinical relevance of fear conditioning and extinction

Fear conditioning and extinction are basic forms of associative learning with considerable clinical relevance and have been implicated in the pathogenesis of anxiety disorders since a long time [68, 69]. The "conditioning model of anxiety disorders" has however changed during the years and has suggested simple classical conditioned fear as a drive for and reinforcement of avoidance, evolutionary prepared associations, stimulus generalization, associative learning deficits and enhanced conditionability in the pathogenesis of anxiety disorders [70]. By now, research has accumulated supportive evidence for the conditioning model of anxiety disorders and a recent metaanalysis [70] has demonstrated that patients suffering from anxiety show enhanced fear conditioning and deficits in extinction. This metaanalysis further suggestes greater excitatory conditioning to danger cues and impaired inhibitory conditioning to saftety cues as possible underlying mechanisms. Understanding the neurobiological underpinning of fear learning and extinction may enhance our understanding of anxiety disorders and ultimately facilitate their treatment. This section summarizes the general clinical relevance of fear conditioning and extinction focusing on PD, given the relevance for this thesis.

Fear conditioning

Conditioning theory has long been suggested as a theory for the etiology of PD [71, 72] and fear conditioning has been proposed as a central mechanism for the acquisition of symptoms of phobic avoidance and anticipatory anxiety [73].

Early theories have focused on the role of conditioning in the onset of AG and situation-bound panic attacks (PA's) as well as interoceptive conditioning [69, 74].

Modern conceptualizations of the role of learning theory in PD assume that an initial "conditioning episode" is critically involved in the etiology of PD. This conditioning episode often involves a PA. Through associative learning processes, the PA itself or cues that triggered the PA become associated with initially neutral cues (e.g. a specific situation, interoceptive or exteroceptive stimuli). Consequenly, these cues gain the potential to elicit anxiety, which becomes a precursor and intensifier of panic.

Importantly, the conditioning processes described above do not necessarily occur with conscious awareness [69] and it deserves also to be mentioned that other theories about the origins of PD exist theories (i.e., Cognitive theory and Anxiety sensitivity theory)[75], that are not discussed here as only conditioning theory is of theoretical importance to **Study III** and **IV**.

Extinction

It is of considerable clinical relevance to understand how fear memories are diminished *after* they have been acquired. Deficits in the extinction of learned fear associations have been observed in patients suffering from anxiety disorders like PTSD, phobias and PD [20, 75].

Fear extinction has inspired the clinical use of exposure [76] and is experimentally used as a laboratory analogue for the main therapeutic ingredient of exposure-based cognitive behavioral therapy (CBT) which is effectively used to treat anxiety disorders like PD, phobias and PTSD [77].

The underlying assumption is that the fear response has been classically conditioned (see above) and that avoidance behavior is subsequently negatively reinforced by the reduction in fear. Through repeated and gradual *in vivo* and/or *in sensu* exposure to the feared or trauma-relevant stimuli this vicious circle can be interrupted and leads to extinction and desensitization to the anxiety-provoking stimuli.

Research has clearly shown that the changes in affect, behavior and cognition seen after CBT treatment have biological underpinnings and are accompanied by significant and disorder specific changes in brain activity and metabolism [78-80]. Furthermore, both pharmacological and CBT treatment seem to act through final common pathways as indicated by similar patterns of changes in neural activity after both types of treatment not only in PD but also in depression, phobias, as well as obsessive-compulsive disorder [78-80].

PA's are thought to originate form an abnormally sensitive fear network involving the amygdala [75] which is thought to be inhibited during treatment. This inhibition can be either direct by the use of medications, such as SSRIs, or indirectly mediated by CBT via a kind of cognitive control over the amygdala by strengthening the ability of prefrontal areas to inhibit the amygdala [20, 73]. Augmenting prefrontal activity pharmacologically, physiologically or psychologically may restore emotion regulation mechanisms and lead to symptom relief. In animal studies, first attempts have been made to strengthen extinction learning pharmacologically [81, 82] and to strengthen prefrontal functioning in order to enhance extinction learning [43]. In human research first attempts to pharmacologically enhance the extinction process have occurred as well [82, 83].

Given the clinical relevance of both fear conditioning and extinction, studies on the genetic architecture of fear conditioning and extinction may provide important insights into the genetic underpinnings of anxiety disorders and my ultimately lead to advances in terms of personalized medicine that may help to adjust treatment to the individual and thereby accelerate CBT and/or make its effects longer lasting.

1.3.4 Genetic factors in fear conditioning and extinction

Genetic association studies optimally study simple behavioral paradigms with a well-defined underlying neural circuitry that elicite robust behavioral responses which are easy to measure and quantify. Fear conditioning is a prototype of a behavioral paradigm fulfilling all prerequisites for promising studies of its genetic underpinnings.

First, both human [84, 85] and animal studies [86] support that genetic factors represent a significant source of individual variation in the habituation, acquisition, and extinction

of fear. Specifically, about one third of the variance in human fear conditioning [85] and the risk to develop anxiety disorders [87] can be attributed to genetic factors. Established heritability of the trait to be studied is of course a major prerequisite for even considering studying its specific genetic underpinnings.

Second, the neural network underlying fear conditioning and extinction has been studied intensively in both animals and humans. A well delineated neural network is advantageous for imaging genetic studies (see 1.2.5) of a trait.

Third, fear responses can be easily and reliably measured using e.g. SCR and/or FPS (see 1.3.2) and importantly, twin studies have proven the reliability of both SCRs [85] and FPS [84] for the study of the heritability of conditionability. Furthermore, animal research can be rather easily translated into human research because similar measurements (e.g. FPS) can be used in both animal and human work.

Promising for human research, animal work has identified some positional candidates for fear conditioning [88, 89] as well as heightened emotionality [90] which confer susceptibility for anxiety. In human research, the study of specific genetic markers contributing to these processes has only just emerged (see **Study I** and **II)** [91, 92].

1.4 PANIC DISORDER

Panic disorder (PD) is a psychiatric condition characterized by recurring PAs that seem to occur unexpectedly. PAs are however not exclusive to PD. The diagnostic critera for a PA and PD are summarized below (see 1.4.1).

PD also has a strong cognitive component of worry and anticipatory anxiety that leads to avoidance behavior, which often develops into Agoraphobia (AG) [75]. Comorbidity of PD with AG is common and associated with more severity and impairment [93].

Traditionally, AG is the fear of public places and open spaces and is often triggered by the fear of having a PA in a situation from which there is no easy escape. Consequently, agoraphobics avoid public and/or unfamiliar places, especially large open, spaces and in severe cases even become confined to their home as a safe place.

Usually PD emerges in the early 20s and is a rather common disease affecting 3.5-5.3% of the population [94] with females being affected twice as often as males across nationalities [95]. PD is quite common and therefore places a heavy economical burden upon society as it represents a potentially disabling disease when it is not treated successfully.

1.4.1 Diagnostic classification

The classification of mental disorders, also referred to as psychiatric nosology, is mainly based on two widely established classification systems that both list categories of mental disorders in the form of codes: the “Diagnostic and Statistical Manual of Mental Disorders (DSM)” and the “International Classification of Diseases” (ICD).

The DSM is published by the American Psychiatric Association's (APA) and is the primary diagnostic system for psychiatric and psychological disorders within the US as well as other countries and is also widely used in research. Currently the DSM is published in the 4th revision (DSM-IV) since it was first published in 1948 and a 5th revision is expected in 2012.

The ICD also classifies diseases, including mental and behavioral disorders (chapter V), and is published by the World Health Organization (WHO). The ICD is currently in its tenth edition (ICD-10), that was developed in 1992 and a new revision, ICD-11, is planned for 2015.

As in **Study IV** and **V**, DSM-IV criteria were used during the diagnostic procedure for PD patients, only these are listed in detail here (see Figure 5).

Panic attack

A discrete period of intense fear or discomfort, in which four (or more) of the following symptoms developed abruptly and reached a peak within 10 minutes:

- Palpitations, or accelerated heart rate
- Sweating
- Trembling or shaking
- Sensations of shortness of breath or smothering
- Feeling of choking
- Chest pain or discomfort
- Nausea or abdominal distress
- Feeling dizzy, unsteady, lightheaded, or faint
- Derealization (feelings of unreality) or depersonalization (being detached from oneself)
- Fear of losing control or going insane
- Fear of dying
- Paresthesias (numbness or tingling sensations)
- Chills or hot flashes
- Weaknees in the knees
- Confusion
- Blank mind
- Sensing time going by very slowly
- Feeling the need to escape

Panic Disorder

A. Recurrent unexpected panic attacks and at least one of the attacks has been followed by 1month (or more) of one (or more) of the following:
- Persistent concern about having additional attacks
- Worry about the implications of the attack or its consequences (e.g. losing control, having a heart attack, "going crazy")
- Significant change in behavior related to the attacks

B. The presence (or absence of Agoraphobia)

C. The panic attacks are not due to direct physiological effects of a substance (e.g., a drug of abuse, a medication) or a general medical condition (e.g., hyperthyroidism)

D. The panic attacks are not better accounted for by another mental disorder, such as social phobia (e.g., occurring on exposure to feared social situation), specific phobia, obsessive-compulsive disorder or separation anxiety disorder.

Figure 5: Diagnostic Critera for a PA and PD in DSM-IV [96].

Since the 1990s, both APA and WHO have aimed at bringing DSM and ICD codes into concordance. Today, both are broadly comparable but still do not always match and some differences remain. With respect to PD and AG it is important to note that DSM-IV and ICD-10 do not agree on the relationship between PD and AG. While DSM-IV reflects the view that AG is almost always a complication of PD and not a discrete disorder, ICD-10 classifies AG as a distinct disorder that may or may not co-occur with PD. In fact, the diagnosis of PD with AG explicitly excludes AG as a primary diagnosis. It is noteworthy, that research has shown that agoraphobia is best viewed as a separate diagnosis [97, 98] and in the upcoming DSM-V, AG is proposed to become a discrete, independent diagnosis [99]. For a review on the discussion whether AG represents a more severe form of PD or a distinct disorder see Smoller et al. [100].

1.4.2 Defining appropriate phenotypes for genetic studies

The accurate and appropriate definition of a heritable phenotype is critical to the success of molecular genetics studies. Traditionally, phenotypes in psychiatric genetics research are defined as clinical diagnoses based on classification systems like DSM-IV and ICD-10.

Even though categorization into distinct disorders is useful from a clinical point of view and has lead to major advances in epidemiological studies, the categorical

nosology based on DSM-IV and ICD-10 may have severe draw-backs when it comes to clinical genetic studies.

Molecular genetic studies as well as quantitative genetics suggest that the genetic influence on anxiety disorders transcends the boundaries of diagnostic categories [100]. In fact there is accumulating evidence that several psychiatric disorders share genetic risk factors (e.g., PD and Bipolar disorder, anxiety disorders and major depression). Furthermore, DSM-IV and its successors have explicitly aimed at being atheoretical and descriptive and have not based their diagnostic categories on psychopathological factors. Consequently, what has proven useful from a clinical point of view may not be optimally suited for clinical genetic studies as psychiatric diagnoses do not seem to correspond to distinct genetic entities [100]. Additionally, diseases are typically considered to be dichotomous traits (affected vs. non-affected) which represents a simplification of the phenotype for diagnostic and clinical purposes. However, complex traits like anxiety and also different symptoms within a syndrome usually are normally distributed in the population and can be measured on a continuum (e.g., from very severe to mild).

One approach to minimize the arbitrariness of categorical diagnoses and the resulting loss of information is to view anxiety phenotypes as dimensional or continuous intermediate phenotypes (e.g. symptom severity, personality traits etc.). This approach allows for more variance in the phenotype and considers clinical heterogeneity that is not captured by the diagnosis itself. Hence it may also more accurately capture the complex underlying genetic architecture with the different symptoms within a syndrome/clinical diagnosis possibly being under the control of distinct genetic loci.

However most studies in PD to date have applied a simple case/control design, comparing allele frequencies between affected and non-affected individuals while studies on intermediate phenotypes and clinical subgroups have only just begun to emerge [101, 102].

Another approach to improve genetic validity may be to consider specific aspects of clinical variation e.g. age of onset, number of affected family members or clinical as well as sub-clinical comorbidity with other psychiatric or medical disorders.

In conclusion, it seems that the success of psychiatric genetics is critically dependent on the definition of appropriate and heritable phenotypes that correspond to genetic entities. This may in fact be the "rate-limiting step" for the success of psychiatric genetics research [cf. 100]. Elucidating the neurobiological underpinnings of PD may help to find such promising heritable endophenotypes.

1.4.3 The neurobiology of panic disorder

The neurobiological mechanisms underlying PD and panic symptoms are largely unknown, but alterations in neural networks subserving emotion, attention and executive functions have been suggested to be critically involved. Specifically the "Neuroanatomic Hypothesis of Panic Disorder" by Gorman et al. [73] suggests, that PD may involve pathways that subserve conditioned fear centering around the amygdala.
In fact, the similarities between autonomic changes during a PA and the consequences of stimulation of brainstem sites by the amygdala that have been seen in animal studies are compelling [30]. In addition, human brain imaging studies have shown enhanced

amygdala activity during a PA and enhanced reactivity to fearful and phobic stimuli as well as after a pharmacological challenge with panicogenic agents [103].

1.4.4 Genetics of panic disorder

Twin and family studies have supported a hereditary component of anxiety disorders in general and PD with and without AG specifically [101, 104, 105]. Of the anxiety disorders, PD has the strongest evidence of a genetic component with a heritability of approximately 48% [105]. Despite of the relatively strong evidence of a genetic component of PD, molecular genetics approaches including linkage and association studies have not yet consistently identified specific genetic risk factors. The failure to identify these may be attributed to complex genetics underlying PD as well the existence of phenocopies, incomplete penetrance and genetic as well as clinical heterogeneity (see 1.2.3). Furthermore an important question is how to define appropriate phenotypes for genetic studies (see 1.4.2).

To date, most genetic association studies have focused on biologically plausible candidate genes or on positional candidates, located within chromosomal regions previously implicated in linkage studies. Genes encoding receptors, transporters, and enzymes involved in neurotransmitter systems that are the target of therapeutic agents used to treat PD (e.g. serotonin, norepinephrine, glutamate, dopamine) or that have been implicated in animal models of anxiety have been the focus of research so far [106]. Today, there is some support for the involvement of genetic polymorphisms in the genes encoding for monamine oxidase A (MAO-A), cholecystokinin, catechol-O-methyltransferase (COMT) and the serotonin transporter (5-HTT) [101].

Given a heritability of approximately 48%, it is clear that environmental influences are also substantial. In fact many anxiety disorders can be linked to environmental or critical life events, in particular PTSD, phobias and PD. These disorders are clearly related to experience and they are likely to involve changes in gene transcription that may be restored by proper treatment. However, some individuals may have inherited a genetic profile rendering them more vulnerable to environmental stressors than others (gene x environment interactions) possibly via the processes of enhanced fear conditioning and deficient extinction (see 1.3). Studies investigating this are emerging lately for PTSD and PD **Study V,** [107, 108].

1.4.5 Treatment of panic disorder

PD is commonly treated using psychopharmaceuticals and/or cognitive behavioral therapy (CBT) and relaxation techniques have also proven beneficial.

The *pharmacological treatment* of choice for PD are selective serotonin reuptake inhibitors, SSRIs e.g. sertraline, paroxetine and fluoxetine, [109, 110] but also benzodiazepines [111], MAO inhibitors [112] and tricyclic antidepressants [113] are effective in the treatment of PD and PA's.

CBT employes techniques stemming from both behavioural (e.g. exposure) as well as cognitve therapy (e.g. cognitive restructuring) and has been proven to be effective in PD with/without AG [114].

Behavior therapy is based on the principles of classical (see 1.3.) and operant conditioning (which analyzes and changes behavior in terms of its rewarding or

punishing consequences) and assumes that dysfunctional behavior is aquired via normal learning processes. Consequently new learning experiences (e.g. aquired via therapy) may change them again, e.g. if the patient would be exposed to the feared situation without escaping (avoidance), the fear response would weaken and disappear (extinction). Thus, behavioral treatment involves in-vivo as well as interoceptive exposure to break this vicious circle.

The theory underlying cognitive therapy in turn assumes that a panic attack arises from misinterpreted physiological sensations and aims at modifying these fear inducing interpretations into realistic cognitive appraisal.

A recent metanalysis found no evidence for differences between drug treatment and CBT in PD and a combination of both seems to be most beneficial [115]. However, while pharmacological treatments are widely accessible to patients, access to CBT is often limited [116, 117], which is probably mainly due to the lack of trained therapists. In clinical settings, group CBT is commonly used instead of individual therapy to increase the number of patients that get access to evidence-based psychological treatment. During the last decade, internet CBT (see **Study IV**), which is an internet-based guided-self help, has developed and has proven to be efficient in the treatment of PD with/without AG in a number of controlled trials [118-122]. Furthermore, it has been shown that internet-guided CBT and traditional face-to-face CBT are equally efficient in PD [123-125].

1.5 SEROTONIN

Serotonin (5-hydroxytryptamine, 5-HT) was originally described in blood serum as a vasoconstrictor substance, hence its name serotonin: a serum agent affecting vascular tone. Since the 1950s, 5-HT has also been known as a monoamine neurotransmitter in the human brain [126].

5-HT has been demonstrated to play a role in diverse mammalian behaviors such as sleep, appetite, sexuality, thermoregulation, aggression, mood, anxiety as well as memory and learning [127]. 5-HT is also an important regulator in (early) brain development (particularly of limbic areas) and is implicated in neuroplasticity in the adult brain [128]. 5-HT is also known to be a key modulator in the generation, modulation and regulation of emotion [127, 129] and drugs targeting the 5-HT system (e.g. SSRI's) are effectively used to treat affective disorders (e.g. anxiety and depression). The serotonergic system has furthermore been suggested to play a crucial role in fear conditioning and stress responses [130] and manipulation of the 5-HT system has been shown to affect fear conditioning in animals and humans [27, 131]. The acute administration of citalopram, an SSRI, before or after fear acquisition enhances learning and expression of conditioned fear in rats [132, 133].

A recent focus of interest has been the effect of genetic variations on 5-HT metabolism and action and its association with 5-HT related behavior and disorders.

1.5.1 The serotonin system

As 5-HT cannot pass the blood-brain barrier, all 5-HT in the brain is synthesized by neurons. Serotonin producing neurons in the adult human brain are located in the

brainstem nuclei raphe [134]. The efferent axons from the raphe nuclei form a neurotransmitter system that reaches large areas of the brain (see Figure 6).

1.5.2 Serotonin synthesis and turnover

5-HT is synthesized from the essential amino acid L-tryptophan, which is taken up via diet. Tryptophan is converted by the enzyme tryptophan hydroxylase (TPH) to 5-hydroxytryptophan (5-HTP). 5-HTP is then converted by L-amino acid decarboxylase (AADC) to 5-HT. In the synthesis pathway, the enzyme TPH represents the rate-limiting step for the production of 5-HT and it is found in two isoforms: TPH1 which is found in several tissues and TPH2 which is a brain specific isoform [135].

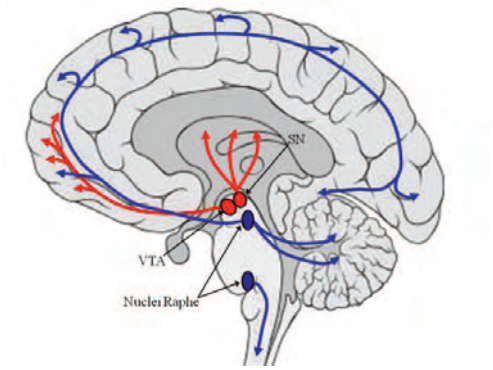


Figure 6: Schematic representation of the brains serotonin system (displayed in blue) and dopamine system (displayed in red) [modified from a download from Wikimedia Commons. No known restrictions on publication).

5-HT is released from serotonergic varicosities into the extra neuronal space and diffuses over a relatively wide gap to activate 5-HT receptors on postsynaptic terminals, dendrites and cell bodies. To date, seven different 5-HT receptor types (5-HT1R – 5-HT7R) and 14 different subtypes are known [136].

The major mechanism of synaptic signal termination is reuptake into the presynaptic intracellular space through the serotonin transporter (5-HTT, see below). If not restored into new vesicles there, 5-HT is degraded by MAO-A and -B and further converted by aldehyde dehydrogenase to 5-hydroxyindolacetic acid (5-HIAA). Given the relevance of the 5-HTT for this thesis it is described in more detail below.

1.5.2.1 The serotonin transporter

The serotonin transporter (5-HTT), driven by the ionic and electrical gradient over the cell membrane, regulates the active presynaptic reuptake of 5-HT from the synaptic cleft. Thereby it is important for the homeostatic regulation of the magnitude, duration and spatial distribution of signals reaching 5-HT receptors.

The 5-HTT is the main target of antidepressant drugs (i.e. SSRI's and tricyclic antidepressants) but is also a target of drugs of abuse like cocaine or ecstasy [137] .

The *5-HTT* gene (5-HTT, SLC*6A4*, *SERT*) spans 37.8 kB, is located on chromosome 17q11.2 and codes for a 630 amino acid long protein with 15 exons [138]. 5-HTT expression is regulated by alternate promoters in combination with differential splicing

in different tissues as well as functional polymorphisms in its promoter region (see 1.5.3).

1.5.3 The serotonin transporter and its polymorphisms

5-HT is a major modulator in anxiety, stress and mood as well as emotion regulation and thus, these processes may be affectd by polymorphisms in genes coding for critical bottlenecks of the 5-HT system e.g. the 5-HTT.

The 5-HTT is a critical regulator of the 5-HT concentration in the synaptic cleft and subsequently of the effect of 5-HT on the receiving neuron. Hence any genetic variation affecting the availability or functionality of the 5-HTT has the potential to have a major impact on behavior and diseases associated with the 5-HT system.

In **Study I, III** and **V**, polymorphisms in the *5-HTT promoter* have been studied and these will be described in the following sections.

5-HTTLPR

Probably the most studied polymorphism in the *5-HTT* gene is the *5-HTT* linked polymorphic region (5-HTTLPR), a 43bp ins/del in a C/G-rich VNTR sequence in its promoter region, located upstream of the transcription start site. The 5-HTTLPR is comprised of a short (s) and a long (l) variant. The short (s) allele comprises 14 copies of a 20-23bp imperfect repeated unit and the long (l) variant comprises 16 imperfect copies. The role of heterozygotes is still under discussion with most studies indicating a dominant effect of the s-allele [139]. Thus, carriers of one or two s-alleles are often *a priori* combined to an s-carrier group even though the role of heterozygotes is still unclear [139].

In caucasians, the s-allele is, with a frequency of 43%, less common than the l-allele and there is considerable frequency variation between ethnic groups [140]. While the s-allele is by far the major allele in Asia it is the minor allele in Caucasians.

The 5-HTTLPR modulates transcriptional activity of the 5-HTT promoter, yielding differences in 5-HT mRNA levels and thus 5-HT uptake activity in human lymphoblastoid cells, platelets and brain [141]. The s-allele is thereby associated with a ~50% reduction of transcriptional activity [142]. This should ultimately lead to less 5-HTT and a higher level of synaptic 5-HT. However, human studies failed to reveal a consistent functional effect of 5-HTTLPR on 5-HTT availability with some studies showing reduced 5-HTT binding in s-carriers in vivo [143] and post mortem [144], while others report contradictory findings [145, 146]. However, more complex mechanisms like differences in methylation patterns [146], receptor up- and down regulation as well as developmental effects may underlie the functional effects of the 5-HTTLPR.

rs25531

Recently, a functional A→G SNP (db number rs25531) has been identified just upstream of the 5-HTTLPR promoter variant but still within the greater repeat structure [147]. The minor G-allele has a frequency of approximately 9-15% in Caucasians, is almost always in phase with the 5-HTTLPR l-allele and only very rarely observed on the s-allele background [147, 148]. The G-allele is as the 5-HTTLPR s-allele associated with a reduced *5-HTT* transcriptional efficacy due to the creation of an activator protein

2 (AP-2) transcription factor binding site [147, 149]. However, this has not been replicated consistently [150]. Furthermore, the impact of the rs25531 on the function of the 5-HTTTLPR s-allele is still unclear.

"triallelic 5-HTTLPR"

Because 5-HTTLPR and rs25531 are located in close proximity and also because it was initially speculated that rs25531 may be located *within* the 43bp insertion of the l-allele [149, 151], both polymorphisms are often combined as a functional mini-haplotype, which is thought to better capture the functional genetic variance in the *5-HTT* gene.

Grouping of individuals based on this mini-haplotype in the literature is often referred to as the "triallelic 5-HTTLPR" (as opposed to s- and l-allele of the "biallelic" 5-HTTLPR). The l-allele of the 5-HTTLPR is thereby further subdivided into L_A and L_G depending on the rs25531 allele present on the same chromosome. In fact, the combination of 5-HTTLPR and rs25531 results in four possible allelic combinations (L_A, L_G, S_A and S_G). However, as the frequency of the rs25531 G-allele on the 5-HTTLPR s-allele background (S_G) is extremely low, the existence of the S_G-allele has mostly been neglected. Thus, in the literature the 5HTTLPR/rs255431 minihaplotype is referred to as "triallelic" when in reality it is "fourallelic". Another reason for the common use of the somewhat misleading term "triallelic" is, that it has been initially postulated that rs25531 is located *within* the 43bp insertion of the 5-HTTLPR l-allele (see above).

Functionally, it has been demonstrated, that the L_G allele has effects that are functionally equivalent to the low expressiong s-allele [149] and grouping of individuals based on the 5-HTTLPR/rs25531 mini-haplotype (see Figure 7) is thus based on putative 5-HTT expression levels [149].

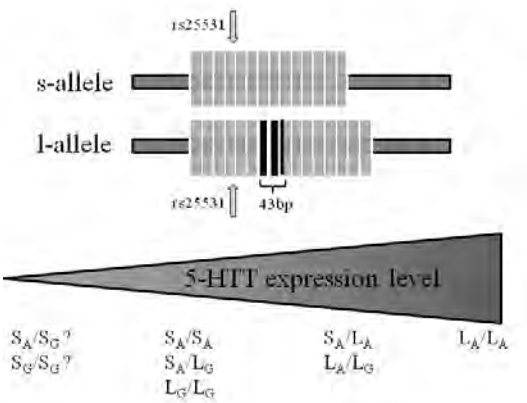


Figure 7: Graphical representation of the 43bp ins/del comprising the 5-HTTLPR and location of the rs25531 as well as putative 5-HTT expression level of the different genotypes based on the 5-HTTLPR/rs25531 mini-haplotype.

Even though there is accumulating evidence from in vivo imaging, that the mini-haplotype explains more variance in the traits studied than the 5-HTTLPR alone [143, 152] also contradictory findings are reported [153]. It needs to be kept in mind, that using this mini-haplotype as a proxy for 5-HTT expression levels still is a simplification as

multiple (rare) sub-variants of the short and long alleles are known to exist [154] as well as other polymorphisms that also have been shown to affect 5-HTT expression e.g. rs25532 [155].

1.5.4 Associaiton studies of the 5-HTTLPR

Animal studies as well as human studies within the fields of behavioural, psychiatric and imaging genetics have shown associations of the bi- and triallelic 5-HTTLPR with anxiety-related traits and disorders as well as altered structure, function and connectivity of brain areas critically involved in emotional behaviour. In the following sections, the relevant literature on behavioral, imaging and psychiatric genetics will be summarized, focusing on human research.

Behavioral Genetic Studies

The 5-HTTLPR s-allele has been confirmed to be associated with anxiety related *personality traits*, in particular neuroticism, by independent metaanalyses [156-159].

In line with anxiety-proneness, several studies using the dot-probe paradigm have demonstrated *attentional bias* in 5-HTTLPR s-carriers to various (negative) emotional stimuli (e.g. anxiety related words, negative IAPS pictures, pictures of spiders and emotional faces [160-163]). In contrast, non-carriers (l/l) have shown an attentional bias away from, but towards to positive IAPS pictures and towards to happy faces [162, 163].

Furthermore, the 5-HTTLPR has been associated with alterations in the *humoral stress resonse*. S-allele carriers or homozyogous s-carriers have shown to display higher morning cortisol levels [164, 165] as well as an elevated and prolonged cortisol reactivity to various stress tasks in general but see [165, 166, 167] or in combination with a history of stressfull life envents [168]. Cross-species studies also show elevated *HPA axis reactivity* to aversive or threatening stimuli in 5-HTTLPR s-carriers or analogues in the animal world e.g. SERT knockout mice, macaques carrying a similar polymorphism while typically baseline levels are unaffected [141, 169].

Of relevance for this thesis, 5-HTTLPR s-allele carriers have been shown to acquire *conditioned fear* responses more readily in both classical conditioning [**Study I**, 91] as well as observational conditioning tasks [92].

Imaging Genetic Studies

Imaging genetics studies have demonstrated significantly higher *amygdala reactivity* in 5-HTTLPR s-carriers as compared to non-carriers (l/l) during various experimental paradigms e.g. matching of angry and fearful faces [170-172], passive viewing of negative pictures [173], faces displaying different emotional expressions [174, 175, 176, Study V, 177, 178] as well as during a public speaking task [179]. This has been found in healthy volunteers as well as different patient populations e.g. depressive patients [174, 175], social phobics [177, 179] and PD patients [180]. A recent metaanalysis has provided formal support for the robustness of an association between enhanced amygdala reactivity and the 5-HTTLPR s-allele [181]. Still, it needs to be mentioned that the 5-HTTLPR genotype has also been shown to affect reactivity in other brain areas than the amygdala e.g. in the hippocampus, the ACC, the putamen or the fusiform gryrus [173, 176, 178].

Noteworthy, most of the studies to date analyzed only the biallelic 5-HTTLPR and studies appreciating also the rs25531 are just emerging [173, 175].

Despite the rather consistent picture, the interpretation of increased amygdala reactivity in 5-HTTLPR s-carriers is controversial. Increased amygdala reactivity to negative stimuli in this group has been proposed to to be driven by decreased activation during neutral control conditions [182] but was subsequently shown to be driven by increased activity during the fixation rest condition [183]. A model of tonic and phasic amygdala activation was proposed suggesting higher tonic activation rather than an increased phasic amygdala response in 5-HTTLPR s-carriers [184]. Indeed it could be demonstrated that s-carriers exhibit higher absolute blood flow in the amygdala at rest than non-carriers as well as reduced blood flow in the vmPFC [185].

Additionally, altered functional and structural connectivity between the amygdala and prefrontal cortical regions has been associated with the 5-HTTLPR. More precisely, increased functional coupling between the amygdala and the vmPFC [186, 187] as well as reduced functional coupling between the amygdala and parts of the ACC, specifically the rostral ACC (BA32/24), has been described in 5-HTTLPR s-carriers [186]. This region has been suggested to directly regulate the amygdala via inhibitory connections. When this pathway is impaired (as indicated by reduced connectivity between the regions), amygdala reactivity to emotional stimuli is stronger and the vmPFC (BA10, BA11) becomes activated as a compensatory mechanism to support rACC function [188].

Interestingly, a recent diffusion tensor imaging study demonstrated impaired white matter integrity between the amygdala/anterior temporal lobe and the medial and orbital PFC as a function of the number of s-alleles/L_G-alleles [189] which may provide a possible mechanism behind the results of the aforementioned connectivity studies.

In addition to altered functional brain activity and connectivity, voxel based morphometry and post mortem studies have shown volume differences and differences in gray matter density depending on the 5-HTTLPR genotype in various brain areas e.g. prefrontal areas, amygdala, cerebellum, and the pulvinar [182, 186, 187, 190].

Psychiatric Genetics focusing on PD

The 5-HTTLPR has achieved attention in a broad range of psychiatric disorders e.g. depression in combination with stressfull life-events [191,] however see [192], anxiety disorders [193] obsessive-compulsive disorder [194], suicidality [195] and irritable bowel syndrome [196]. However, due to the relevance for this thesis, this section focuses mainly on its associations with PD.

Most studies investigating an association between the 5-HTTLPR and PD have used a case-control design. No differences in allele frequencies between patients and controls were found in german [197], korean [198], japanese [199, 200] and scandinavian [201] samples. A recent metaanalysis [202] also concluded that there is no association between the 5-HTTLPR and the diagnosis of PD, while some studies suggested that the l-allele or the l/l genotype may be overrepresented among PD patients [203, 204]. Noteworthy, the aforementioned studies investigated only the biallelic 5-HTTLPR and thus studies appreciating the additional role of rs25531 may reveal different results.

Furthermore, as discussed in chapter 1.4.2, studies based on dichotomous clinical diagnosis may not be well suited for genetic association studies and appreciating

symptoms as continuous variables may increase the power to detect associations with genetic polymorphisms. However, few studies have so far investigated an association of the 5-HTTLPR with the symptomatic profile and symptom severity of PD patients in terms of continuous variables. While no association was found in korean panic patients [198], we report a linear relationship with the 5-HTTLPR s-allele and symptoms of panic and depression in a sample of swedish PD patients (see **Study III**).

Additionally, the 5-HTTLPR has been associated with the outcome of pharmacological treatment in various psychiatric populations. Pharmacogenetic studies have linked the 5-HTTLPR l/l genotype to better efficacy of SSRI treatment in depression [205] as well as in different cohorts of unipolar, bipolar and psychotic depression patients [139]. Very recently, a study in PTSD patients provided preliminary evidence that 5-HTTLPR s-carriers may not only profil less from pharmacological treatment but also may profit less from CBT [108].

Few studies have investigated the role of 5-HTTLPR in the outcome of pharmacological treatment in PD. While no association was found in a korean sample [198], the l-allele was associated with better treatment response to paroxetine females italians [206].

1.6 DOPAMINE

Dopamine (DA) as a neurotransmitter was discovered in 1958 by Arvid Carlsson and Nils-Åke Hillarp in Sweden [207]. DA is a catecholamine neurotransmitter in the human brain and has pleiotrophic effects on the brain and human behavior including critical roles in movement, motivation, learning, sleep, attention and mood. DA is also known for its pivotal role in the mediation of stimulus salience such as sources of reward or danger [208]. Furthermore, DA has been implicated in a wide rage of disorders and diseases [209].

Animal models demonstrate that DA is involved in fear conditioning for a review see [210] and may bias affective responses by augmenting excitatory sensory input and attenuating inhibitory prefrontal input to the amygdala [211]. Thus, DA has the potential to alter the functional interplay between the core structures of the neural network underlying emotion generation and regulation [33] as well as fear conditioning and extinction.

A recent focus of interest has been the effect of genetic variations on DA metabolism and action and its association with DA related behaviour and disorders.

1.6.1 The dopamine system

As DA cannot pass the blood-brain barrier, all DA in the brain is synthesized by neurons. In the brain, DA is produced mainly in the substantia nigra (SN) pars compacta and the ventral tegmental area (VTA) in the mesencephalon as well as in some hypothalamic nuclei. Dopaminergic neurons located in these areas send their axons to large areas of the brain (see Figure 6, page 22) through four major pathways that serve distinct functions: the nigrostriatal (movement regulation), the mesolimbic (emotion and motivation), the mesocortical (learning and memory) and the tuberoinfundibular system (inhibition of prolactin).

1.6.2 Dopamine synthesis and turnover

DA is a member of the catecholamine family and is a precursor to norepinephrine (noradrenaline) and epinephrine (adrenaline) in the biosynthetic pathway. In DAergic neurons, DA is synthesized from the amino acid tyrosine, which is able to pass the blood-brain barrier. In catecholamine neurons, tyrosine is converted into L-3,4-dihydroxyphenylalanine (L-DOPA) by the enzyme thyrosine hydroxylase (TH), which is the rate limiting step in DA synthesis. L-DOPA is then converted by L-amino acid decarboxylase (AADC) to DA.

After synthesis, DA is packed into vesicles which are then released into the synaptic cleft in response to a presynaptic action potential. Subsequently, DA binds to its receptors on the postsynaptic or the presynaptic neuron. DA receptors belong to five different receptor subtypes, labeled D1-D5, which can be classified into the D1-subtype (D1-R, D5-R) or the D2-subtype (D2-R, D3-R, D5R) [212].

The termination of DA signaling is achieved by two major mechanisms: reuptake into the presynapse by the dopamine transporter (DAT) or by enzymatic breakdown by the enzymes catechol-O-methyltransferase (COMT) and MAO-A.

Because a genetic polymorphism in the gene coding for the enzyme COMT was studied in **Study I, IV**, and **V**, the next chapter describes the functions of COMT and its val158met polymorphism in more detail.

1.6.2.1 Catechol-O-methyltransferase

Catechol-O-methyltransferase (COMT) was discovered in 1957 by Julius Axelrod [213] and is an one out of several enzymes that degrade catecholamines such as DA, norepinephrine and epinephrine. COMT introduces a methyl group to the catecholamine, which leads to inactivation of catecholamine neurotransmitters [214], and converts dopamine to inactive 3-methoxytyramine (3-MT). Two COMT protein isoforms are known: The soluble cytoplasmic isoform (S-COMT) is the predominant form in most tissues [215] while a longer, membrane-bound form (MB-COMT) is the major form expressed in the brain [216].

Despite the fact, that reuptake by the DAT is the primary mechanism for removing extracellular DA, enzymatic degradation by COMT is particularly important in brain areas devoid of DAT e.g. the prefrontal cortex [216], as opposed to DAT rich areas like the striatum. This is supported by studies in COMT knockout mice that provided evidence for increased DA levels in frontal areas while no effect was observed on DA levels in the striatum. Furthermore, in the frontal cortex, COMT seems to have a major impact selectively on DA levels but not on other catecholamine levels which may be due to the presence of noradrenalin transporters in this brain region [214].

In the brain, the hippocampus and the PFC show the most abundant expression of COMT mRNA while it is basically absent in the amygdala [217].

In general, females express lower levels of COMT [218, 219] due to an inhibitory effect of estrogen on *COMT* gene transcription [220]. Human studies often, but not always, report female specific genotype effects [221], probably due to significantly lower COMT activity and possibly because COMT activity may have to fall below a specific threshold to yield associations.

1.6.3 The *COMT* gene and its val158met polymorphism

The *COMT* gene, located on 22q11.1 – q11.2, harbors an interesting and widely studied functional A→G SNP that leads to the substitution of the amino acid valine by methionine at codon 158 of MB-COMT (COMTval158met, rs4680 previously rs165688), and codon 108 of S-COMT. The COMT val158met SNP is human specific and evolutionary recent [222].

Both the valine (val) and the methionine (met) allele have a frequency of about 50% in caucasian populations but frequencies differ widely between geographical regions and ethnical groups [222].

The COMTval158met SNP affects the stability of the COMT protein at body temperature. The more stable val-variant has been shown to have a four times higher enzymatic activity as compared to the met-variant and thus lower extracellular DA levels [223]. Therefore, the val-allele is sometimes also referred to as the high activity (H) allele while the met-allele is referred to as the low activity (L) allele. Both alleles seem to act in a co-dominant way, leading to a trimodal distribution of COMT activity in human populations [224]. The COMTval158met SNP affects the effectiveness of DA degradation by COMT (primarily in the PFC, see 1.6.2.1) and thereby the availability of synaptic DA after neurotransmitter release and the stimulation of post-synaptic DA receptors.

1.6.4 Association studies of the COMTval158met

As enzymatic brake-down by COMT is the major way of dopamine degradation in prefrontal areas, the functional COMTval158met polymorphism has the potential to affect functions relying heavily on frontal cortex involvement such as the top-down regulation of emotion and executive control functions. There is also considerable evidence for gender specific effects of the COMTval158met polymorphism [225].

In the following sections, the relevant literature on behavioral, imaging and psychiatric genetics will be summarized, focusing on human data.

Behavioral Genetics

The association of the COMTval158met met-allele with better *cognitive functions* (in particular executive functions and working memory) is the best known association of the COMTval158met polymorphism with behavioral phenotypes [226, 227], even though a recent meta-analysis did not confirm this association [228].

There is also evidence for an effect of COMTval158met on affect modulation and *emotional processing*. Individuals with the met/met genotype have been shown to display increased affective startle modulation to aversive pictures (as opposed to startle probe alone) [229].

Additionally, the met-allele has been associated with increased maintenance and impaired flexibility [230], which may ultimately lead to emotional preservation and behavioural rigidity (as seen in anxiety and depression). The val-allele in turn has been associated with increased proneness to overcome a dominant response but impaired cognitive stability, which may lead to distractibility and impulsiveness (as seen e.g. in schizophrenia) [231].

Imaging Genetics

Like behavioral genetic studies have associations of the COMTval158met polymorphism with executive functions and emotional processing been found in imaging genetics studies.

The COMTval158met polymorphism has consistently been associated with human *cognition*, *executive functions* and frontal lobe functioning [214]. Specifically, the met-allele is associated with better performance and a more focused physiological response in the ventrolateral (vl) PFC as measured by fMRI [214, 232].

Furthermore, met-carriers demonstrate increased *activation in limbic areas* (e.g. amygdala, hippocampus and thalamus) and connected prefrontal brain regions (e.g. vlPFC) during the processing of unpleasant stimuli (e.g. aversive pictures, faces displaying negative emotions) using fMRI [233, 234]. Individuals with the met/met genotype also responded more sensitively to unpleasant stimuli using EEG [235].

Is has been suggested, that the signaling of emotional salience may be enhanced in individuals with the COMT met-allele [221]. The impact of the COMTval18met polymorphism on amygdala activation (see above) may, as shown in animal studies, be explained by its modulatory effect on prefrontal DA levels. DA has been shown to potentiate the response of the amygdala by attenuating the effect of inhibitory input from the prefrontal cortex and augmenting the effect of excitatory input from sensory cortices [236].

Psychiatric Genetics focusing on PD and pharmacogenetics

Most studies investigating an association between the COMTval158met polymorphism and PD have used a case-control design and yielded contradictory findings.

The val-allele has been shown to be more frequent in female german [237], canadian [238] as well as mixed caucasian PD patients [239], while the met-allele has been shown to be overrepresented in korean PD patients [240, 241] and no association was found in japanese patients [242]. A recent metaanalysis [243] thus concluded, that the val-allele may be associated with an increased risk of PD in caucasians while in asian populations the met-allele may be the risk allele.

Little research has been done on the association of the COMTval158met polymorphism and panic related endophenotypes like treatment outcome or the symptomatic profile. Two studies in korean PD patients associate the met/met genotype with poor improvement after pharmacological treatment with paroxetine [240, 241] but no studies in caucasians have been reported so far.

Furthermore, treatment outcome to various other types of treatment in different psychiatric populations has also been associated to the COMTval158met polymorphism. In depressive patients, the COMT val/val genotype was associated with a positive response to electroconvulsive therapy [244] and a better response to pharmacological treatment with mitrazapine but not paroxetine [245] while patients with the met/met genotype had an increased risk of nonremission after treatment with SSRI [246]. Similarly, schizophrenic patients with the met/met genotype required higher doses of antipsychotic medication to achieve a significant reduction in psychotic symptoms [247]. Additionally, in **Study IV**, we report preliminary evidence that PD patients with the

met/met genoptye seem to profit less from exposure-based CBT treatment than val-carriers.

It is possible, that patients with the met/met genotype may be generally less responsive to treatment as compared to non-carriers irrespective of the underlying disease.

1.7 THE BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF)

Neurotrophins (NT), including brain-derived neurotrophic factor (BDNF), nerve-growth factor, neurotrophin 3 and neurotrophin 4 , are regulatory factors that mediate the differentiation and survival of neurons and are modulators of activity-dependent synaptic plasticity [248, 249]. NTs arise from precursors (pro-NTs) which are proteolytically cleaved to produce the mature NT-protein. Pro-NTs are also physiologically active, serve as signaling molecules and seem to promote cell death while the mature NTs promote cell survival , [249].

BDNF is the most abundant and widely distributed NT in the central nervous system and has been implicated in synaptic plasticity as well as survival, differentiation and growth of neurons during both development and adulthood. Due to the relevance for this thesis the following sections will focus on BDNF.

1.7.1 BDNF synthesis and turnover

Pro-BDNF is synthesized and folded in the endoplasmatic reticulum and the mature domain of the protein is folded properly in the Golgi by binding to sortilin. Subsequently, BDNF is sorted into the *regulated* or the *constitutive* secretory pathway, depending on the presence or absence of a specific motif in the mature domain. The secretory granules containing BDNF/pro-BDNF are then transported to dendrites or axons where they are secreted postsynaptically. BDNF and pro-BDNF bind to different receptors (tyrosine receptor kinase family and p^{75} neurophine receptor respectively).
Neurons secrete BDNF and pro-BDNF also in an activity-dependent (regulated) way, while many cell types only secrete NTs constitutively [249]. Regulated secretion is a cellular process in which the fusion of vesicles and the secretion of their content are triggered by extracellular signals.

Pro-BDNF is predominantly secreted directly by neurons and the pro-domain is subsequently cleaved extracellularly but can also be cleaved intracellularly to yield the mature BDNF protein. The pro-domain of NTs is highly preserved between different NTs and species and is crucial for intracellular trafficking and secretion [249]. Thus, genetic variation in the pro-domain has the potential to significantly affect the function of the neurotrophin.

1.7.2 The BDNF gene and its val66met polymorphism

The *BDNF* gene is located on chromosome 11p13, has several alternative splicing variants and harbors a common functional G→A SNP in codon 66 of its pro-domain. This SNP, which was studied in **Study II,** leads to a valine (val) to methionine (met) substitution (BDNFval66met, rs6265).

The met-allele of the BDNFval66met polymorphim has been shown to lead to impairments in intracellular trafficking and activity-dependent secretion of BDNF,

while no differences in constitutive secretion have been observed [250, 251]. The region of the pro-domain containing the BDNFval66met SNP seems to be of importance for the regulated secretion of BDNF (see also 1.7.1) and the met-variant likely leads to inefficient trafficking of BDNF to secretory granules for a review see e.g., [249]. It has been suggested that the met-variant leads to impaired binding to sortilin (see also 1.7.1) which may be the underlying mechanism [250].

The met allele is the minor allele with a frequency of approximately 20-30% in caucasians, with pronounced differences between ethnic groups [252]. Homozygous met-carriers comprise as little as 4% of the caucasian population and therefore most studies so far have *a priori* combined carriers of one or two met-alleles to a met-carrier group. As this group commonly contains only very few homozygous individuals, little data exist on a possible allele load or heterosis effect. Even though the literature may suggest a specific effect in met-carriers, only conclusions about a difference between heterozygous and those homozygous for the val-allele can be drawn.

In the following sections, the relevant literature on behavioral, imaging and psychiatric genetics will be summarized, focusing on human data.

1.7.3 Associations studies of the BDNFval66met

Alterations in brain maturation and development, in concert with plastic changes induced by environmental events may contribute to altered processing of emotional information and learning. BNDF is a regulatory factor that affects these neuronal processes in an activity-dependent manner and thus, genetic variation in the BDNF gene including its pro-domain has the potential to significantly affect these processes.

Behavioral Genetics

The involvement of BDNF in hippocampus-dependent cognitive learning and memory has been known from studies in both rodents [253] and humans [251, 254]. More recently, a similar role for BDNF in amygdala-dependent learning processes like Pavlovian fear conditioning has been shown in rodents using pharmacological and genetic approaches [255-257]. Furthermore, it has been shown that prelimbic cortical BDNF is required for the memory of *learned fear* but not innate fear or extinction [258].

Due to its critical involvement in the molecular processes underlying amygdala-dependent learning, BDNF has been suggested to e a critical modulator of emotional memory formation.

In humans, an association has been reported, between the met-allele of the BDNFval66met and deficient as FPS in a conditioned fear generalization paradigm [259]. Furthermore, we report deficient fear conditioning as measured by FPS but not SCR (**Study II**). However, a third study found deficient fear extinction in animals and humans to be associated with the met-allele but did not find an effect on the acquisition of fear as measured by SCR and fMRI [260].

Furthermore, several studies have been published on an association of the BDNFval66met polymorphism with *anxiety-related personality traits* (e.g. neuroticism or harm avoidance). In sum, findings in personality genetics are inconclusive and contradictory. Still, a recent metaanalysis supports an association of the BDNF met-allele with lower neuroticism scores [261].

Psychiatric Genetics

The human *BDNF* gene has been suggested to be a candidate gene for a variety of neuropsychiatric disorders including mood disorders [262, 263], schizophrenia [264] and eating disorders [265]. A recent metaanalyis yields support from case-control studies for an association of the BDNFval66met polymorphism with substance-related disorders (met-carriers), eating disorders (val/val) and schizophrenia (met/met) [266] while no association was found with anxiety disorders [261].

Furthermore, evidence from both human and animal studies has suggested a critical involvement of BNDF in the therapeutic mechanisms of antidepressive treatments for a review see e.g., [267], but a recent study did not find supporting evidence for this in depressive patients [268].

Imaging Genetics

Few imaging genetics studies have investigated the BDNFval66met polymorphism as compared to the wealth of data available for the 5-HTTLPR or the COMTval158met polymorphism.

One of the most reliable effects observed in morphometric studies is smaller hippocampal volumes in carriers of the BDNF met-allele [269-271], extending into the parahippocampal gyrus and the amygdala [272]. In line with this, functional imaging studies have shown that carriers of the BDNF met-allele perform more poorly and display less hippocampus activation than homozygous val/val individuals in memory tasks relying on the hippocampus [251, 254]. Recently also differential amygdala reactivity to affective pictures has been shown with met-carriers displaying enhanced amygdala reactivity [273].

Very recently, an fMRI study on the role of the BDNFval66met polymorphism in fear conditioning and extinction was published [260]. This study resports deficits in fear extinction on the psychophysiological level as well as the neural level (lower vmPFC activation during extinction in met-carriers as well as atypical frontal-amygdala activity).

2 AIMS

The work presented in this thesis focused on translational studies and tried to bring together insights from molecular genetics, psychophysiology as well as functional neuroimaging. The ultimate aim of the studies presented was to help unravelling the genetic underpinnings of anxiety related traits (e.g. fear conditioning and extinction, amygdala reactivity to angry faces) and anxiety disorders (e.g. panic disorder, PD).

Both healthy research volunteers and patients suffering from PD were studied for this purpose.

The specific objectives were to investigate:

- if and which impact the 5-HTTLPR and COMTval158met polymorphisms have on human fear conditioning and extinction using psychophysiological methods (**Study I**).

- if and which impact the BDNFval66met polymorphism has on human fear conditioning and extinction using psychophysiological methods (**Study II**).

- if the 5-HTTLPR/rs25531 mini-haplotype affects the symptomatic profile in PD patients (**Study III**) and thereby trying to translate the experimental findings from **Study I** into the clinical setting.

- if the COMTval158met polymorphism affects outcome of (exposure-based) CBT treatment in PD patients (**Study IV**) and thereby trying to translate the experimental findings from **Study I** into the clinical setting.

- if and which impact the 5-HTTLPR and COMTval158met polymorphisms have on amygdala reactivity and habituation during the passive viewing of angry faces using fMRI (**Study V**).

3 METHODS AND MATERIALS

3.1 RESEARCH PARTICIPANTS AND PATIENTS

Studies I and II were performed at the Ernst-Moritz-Arndt University of Greifswald in Germany. For **Study I**, in total, eighty-one Caucasian volunteers were screened and genotyped for participation in the experiment (see Figure 8). Out of these, 63 Caucasian volunteers ultimately participated in the experiment (blind to genotype). Thirty-four participants completed the experiment before being genotyped (Sample I) and 29 out of 47 previously genotyped participants (Sample II) were selectively invited in order to balance out the allele frequencies for the 5-HTTLPR and COMTval158met polymorphisms as well as the sex distribution in the experimental sample. From the 63 participants, 15 participants were excluded from the primary analysis (6 due to technical problems, 9 because of failure to correctly report the conditioning contingencies) leaving a final sample of 48 participants for analyses.

Research participants from **Study I** were also genotyped for the BDNFval66met polymorphism (**Study II**) at a later date.

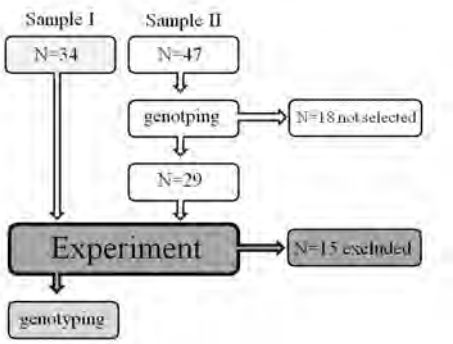


Figure 8. Flow chart for the participant recruitment in **Study I**.

Study III and IV were based on sub-samples of patients diagnosed with PD with/without AG. These patients were recruited from a clinical equivalence trial of regular CBT group therapy (gCBT) vs. internet-based CBT (iCBT) [125]. Figure 9 gives a graphical display about the recruitment and inclusion of patients for the study. All patients provided written informed consent and were diagnosed by a psychiatrist or a resident in psychiatry under supervision of a psychiatrist using the Mini-International Neuropsychiatric Interview (M.I.N.I) [274].

At assignment to the study (e.g. the clinical equivalence trial), patients were allowed to be pharmacologically treated (SSRI, other antidepressants, Benzodiazepine), but only with a stable dosage for at least 2 month. Comorbidity with depression was allowed if PD and not depression was the primary diagnosis. Patients were genotyped for 5-HTTLPR/rs25531 (**Study III**) and COMTval158met (**Study IV**). All patients included for **Study III** and **IV** were Caucasians.

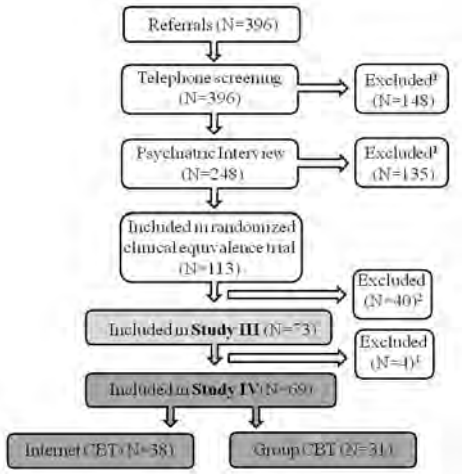


Figure 9: Flow chart displaying the patient recruitment for **Study III** and **IV** (Dr. Jan Bergström contributed to the design of this figure).

[1] Reasons for exclusion were e.g. the exclusion criteria mentioned in the text (see 3.1), that the patient did not accept to be randomly assigned to any of the two treatment-types, was not reachable via phone or did not show up for the interview.

[2] Patients that were not willing to donate DNA samples or patients that are excluded for other reasons e.g. questionable clinical relevance

[3] In **Study IV** one patient with a south-american origin was excluded as well as three patients that only provided pre-treatment symptom scores for **Study III**.

For the fMRI study (**Study V**), participants were selected from a pool of 597 volunteers recruited between May 2007 and April 2008 as well as in November/December 2008 in the Stockholm area. All volunteers donated 20ml of blood (May 2007 - April 2008) or a saliva sample using the Oragene®-DNA Self-Collection Kit (DNA Genotek Inc., Kanata, Kanada; November/December 2008) for DNA extraction and genotyping. All volunteers provided informed consent to participate in the current study and consented to be contacted and invited to behavioral or fMRI studies within the project "känslors biologiska grund" (see Figure 10 for a graphical display of the participant recruitment).

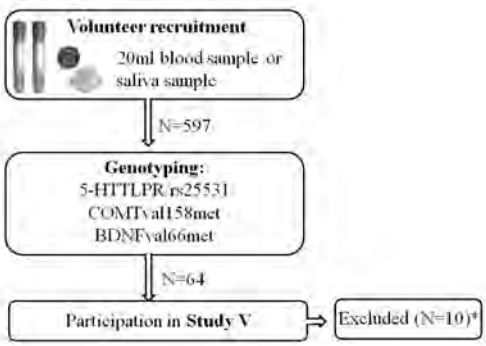


Figure 10. Flow chart for participant recruitment for Study V.

* Ten participants were excluded from data analyses due to technical problems, left handedness, excessive head movement or pathological anatomy.

For **Study V**, we recruited 64 Caucasian participants from this pool of volunteers (see above) based on their 5-HTTLPR genotype (on a constant rs25531 A-allele background) and COMTval158met genotype. Participants within each genotype group

were matched for gender. The experiment was performed with both experimenter and participant being blind to the genotype of the latter. Ten of the participants had to be excluded due to technical problems, pathological anatomy, left handedness or excessive head motion leaving a final sample of 54 participants for the analyses.

3.2 GENOTYPING

Genotyping was based on the Polymerase Chain Reaction (PCR), which is used to amplify specific copies of DNA sequences. A PCR reaction consists of three major steps: denaturation, annealing and elongation, which are cycled for 20-50 times. During the denaturation step, usually at 95°C, the DNA doublestrand is melted to yield two DNA single stands. During annealing, which is performed at varying temperatures depending on the template and primer sequence, ionic bonds between the DNA single strand and the primers are formed. During elongation, usually at 72°C, the DNA polymerase synthesizes a new DNA strand complementary to the template strand by adding dNTPs to the template in 5' to 3' direction.

3.2.1 5HTTLPR/rs25531

Two different protocols were used for genotyping of 5-HTTLPR in **Study I** and **Study III** / **V**. In **Study I** only the 5-HTTLPR was genotyped while for **Study III** and **V** also rs25531 was determined. The PCR protocol employed in **Study I** used the modified nucleotide 7-deaza-dGTP. We were unable to obtain a satisfying digestion of the PCR product with either MSPI or HPAII enzymes to determine the "triallelic" 5-HTTLPR when attempting this for **Study III** and **V**. This problem with -deaza-dGTP has been reported earlier [275] and therefore a different protocol and subsequent digestion of the fragments by the enzyme MSPI was used to determine the 5-HTTLPR and the rs25531 genotypes in **Study III** and **V** (see Figure 11).

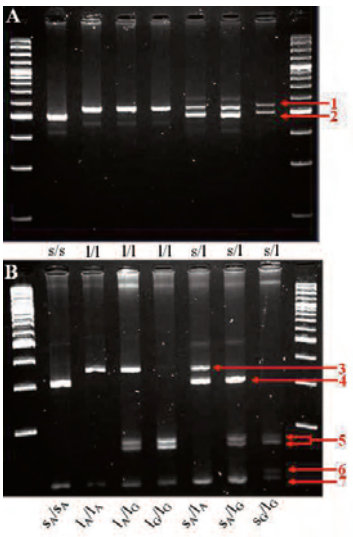


Figure 11: Representative genotyping results for 5-HTTLPR (**A**) and 5-HTTLPR/rs25531 genotypes (**B**) determined by the protocol described in **Study III** and **V**.
1: 5-HTTLPR l-allele, 2: 5-HTTLPR s-allele, 3: undigested 5-HTTLPR l-allele (L_A), 4: undigested 5-HTTLPR s-allele (S_A), 5: digested 5-HTTLPR l-allele (L_G), 6: digested 5-HTTLPR s-allele (S_G), 7: non-allele specific band

3.2.2 5' Nuclease (TaqMan®) assay

Genotyping for COMTval158met (rs4680) and BDNFval66met (rs6265) was performed using the 5' Nuclease (TaqMan®) assay [276]. The TaqMan® assays were ordered from Applied Biosystems and the kits contain two allele specific probes, complementary to the two alleles of the respective SNP. Each probe is labelled at the 5' end with different fluorescent reporter dyes (FAM and VIC respectively), in addition to a fluorescent quencher molecule (TAMRA) at the 3' end. As long as the fluorophore and the quencher are in proximity, quenching inhibits any fluorescence signal.

The labelled TaqMan® probes are hybridized to the template DNA and amplified by a specific set of primers during a PCR reaction. Hybridization conditions are designed to discriminate between the matched and the mismatched probes so that only the allele-specific probe will be able to hybridize. As the Taq polymerase extends the primer and synthesizes the nascent strand, its 5' to 3' exonuclease activity degrades the probe that has annealed to the template. The degradation of the probe releases the fluorophore and thereby breaks the proximity to the quencher. This relieves the quenching effect and allows the fluorescence of the fluorophore. Hence, fluorescence detected is directly proportional to the fluorophore released. If only one allele is present (in case of a homozygote genotype, in Figure 12 "Allele X" or "Allele Y") only a FAM or a VIC-specific fluorescence signal is detected while detection of both signals indicating heterozygositiy ("Both", see Figure 12).

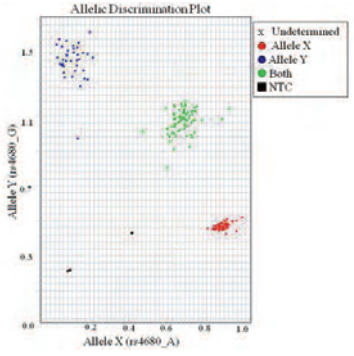


Figure 12: Representative TaqMan® results for a COMTval158met genotyping run.

3.3 SALIVARY CORTISOL MEASUREMENT

For **Study V**, saliva samples were collected using Salivette cotton pads (Salivette, Sarstedt Inc., Rommelsdorf, Germany). Saliva samples were provided approximately 20 min before the experiment started. Samples were stored (for max. 4 days) at +4°C until centrifugation and at -20°C until analyzed. Salivary cortisol concentrations were determined by the RIA method (Coated Tube Radioimmunoassay, Orion Diagnostica Oy, Espoo, Finland) detecting cortisol concentrations in saliva samples between 1.0-100nmol/L and an analytical detection limit of 0.8nmol/L.

3.4 PSYCHOPHYSIOLOGICAL MEASUREMENTS

3.4.1 Fear potentiated startle

Startle responses were recorded in **Study I** and **II**. They were measured by recording electromyographic (EMG) activity to a startle probe of 95dB[A] over the orbicularis oculi muscle beneath the left eye using miniature Ag/AgCl surface electrodes. The raw EMG signal (see Figure 13A) was amplified and filtered through a 30-Hz high-pass filter (Coulbourn S75-01) and a 400-Hz low-pass filter (Kemo KEM-VBF8-03; Beckenham, Kent, United Kingdom), rectified (see Figure 13B), and integrated with a time constant of 10ms.

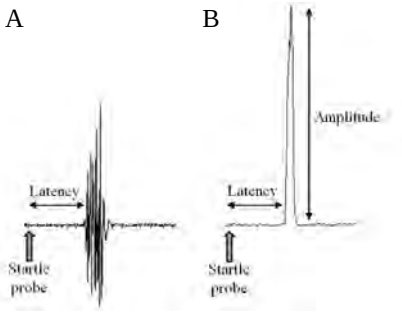


Figure 13. **(A)** Representative raw startle reaction and **(B)** Representative rectified startle reaction.

Startle responses were counted as valid when the onset of the response occurred 20-100ms after stimulus onset. The magnitude of the startle eyeblink (in microvolt, μV) was scored onset to peak (see Figure 13B). Startle blink magnitudes were normalized using z-standardization and converted to T-scores. Three different indices of fear learning (fear potentiation) can be calculated (see Figure 14): CS+ Potentiation ([Startle amplitude to the CS+] - [Startle amplitude to the ITI]), CS- Potentiation ([Startle amplitude to the CS-] - [Startle amplitude to the ITI]) as well as Discriminiation ([Startle amplitude to the CS+] - [Startle amplitude to the CS-]). While CS+ Potentiation (and also CS- Potentiation) requires only excitatory learning, both excitatory and inhibitory learning processes are necessary for CS+/CS- Discrimination [70].

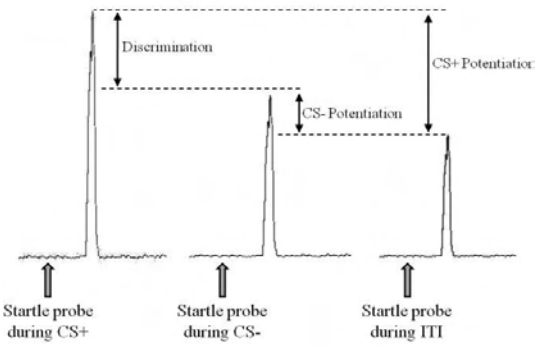


Figure 14. Graphical respresentation of different measurements (CS+ Potention, CS- Potentiation, Discrimination) that are calculated from T-transformed startle amplitudes to startle probes presented during the CS+, CS- and the ITI.

3.4.2 Skin conductance responses

SCR's were recorded in **Study I, II** and **V** using standard electrodes placed adjacently on the hypothenar eminence of the palmar surface of the right hand (**Study I** and **II**) or the left hand (**Study V**).

In **Study I** and **II** a Coulbourn system was used to record SCRs while in **Study V**, SCRs was acquired using fMRI compatible equipment (Biopac Systems, Goleta, CA).

Despite of the two different recording equipments used in **Study I/II** and **V** respectively, SCR magnitudes (in μSiemens, μS) were scored as the largest response from onset to peak occurring 0.9-4.0s after stimulus onset (latency, see Figure 15A) for both CR to the CS+ and CS- and UR to the US (see Figure 15B). The minimum amplitude accepted in order to count as a valid reaction, was 0.049μS in **Study I** and **II** while a more liberal minmum amplitude of 0.029μS was accepted in **Study V**. Logarithms of all values were computed, which normalizes the distribution, and these log values were range corrected (individual score/individual max response) to reduce interindividual variability.

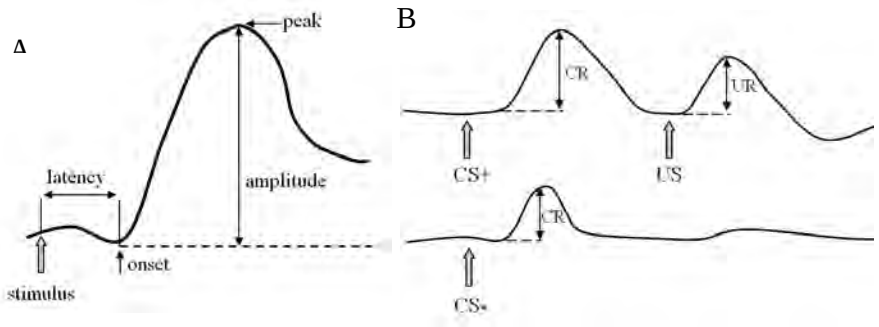


Figure 15. Graphical represenation of (**A**) SCR latency and amplitude and (**B**) conditioned response (CR) to the CS+ and the CS- as well as unconditioned response (UR) to the unconditioned stimulus (US, electrotactile stimulation).

3.5 BRAIN IMAGING

Brain Imaging involves structural as well as functional imaging methods. There are different methods available to image brain structure e.g. computed tomography (CT) and magnetic resonance imaging (MRI). Similarly, different methods can be used to measure neural activity during information processing indirectly though associated changes in brain metabolism or blood flow like electrophysiological methods (e.g., electroencephalogy [EEG], magnetoencephalogy [MEG]) or hemodynamic techniques (e.g. positron emission tomography [PET], single-photon emission computerized tomography [SPECT] and functional magnetic resonance imaging [fMRI]).

Different methods have different strengths and weaknesses and may offer a high temporal but lower spatial (e.g. EEG, MEG) or a high spatial but lower temporal resolution (e.g. fMR, PET, SPECT).

Functional magnetic resonance (fMRI) imaging is the most commonly used method to investigate information processing in the human brain. fMRI is a non-invasive technique with high spatial resolution (1-10mm) and satisfying temporal resolution (hundreds of ms). The temporal resolution of fMRI is worse than that offered by

electrophysiological methods. However, signals from brain structures deep inside the brain (e.g. the amygdala) can be recorded, which is rather difficult with EEG or MEG.

As fMRI was applied in **Study V**, the following sections will describe this method as tools for human brain imaging. Even though structural MRI images are usually acquired for fMRI studies as well, the physics behind this method will not be discussed in detail here. Briefly, both MRI and fMRI are non-invasive techniques that use strong magnetic fields (usually 1.5 - 3 Tesla, which is approximately 25000 times the strength of the Earth's magnetic field) and radio waves to generate images. Structural MRI allows imaging of both the brain surface and deep brain structures with a high degree of anatomical detail. The gray-scale pictures obtained are based on differences in densities of hydrogen protons between tissues.

3.5.1 Functional Magnetic Resonance Imaging

Functional Magnetic Resonance Imaging (fMRI) uses changes in regional cerebral blood flow (rCBF) as an indirect measurement of neural activation [277] to draw inferences about brain areas involved in specific information processing.

Increased activity in a specific brain area leads to a concomitant increase in energy requirements which is followed by an increase in blood flow in order to supply the neurons with glucose and oxygen. This increased supply of oxygenated blood however exceeds oxygen utilization [278]. In fMRI, the so called blood-oxygen-level-dependent (BOLD) effect [279] is used as an indirect measurement of neural activity. The BOLD effect is based on differences between magnetic properties of oxygenated and deoxygenated hemoglobin in the blood. While oxygenated hemoglobin is not magnetic (diamagnetic) deoxygenated hemoglobin is paramagnetic and disturbs the magnetic field more than oxyhemoglobin. In activated brain areas, the MR signal increases due to decreases of deoxyhemoglobin and unproportional increases in blood flow and oxyhemoglobin.

Images sensitive to the BOLD contrast are collected via high speed echo-planar imaging, whereby one whole slice image is acquired for every excitation radiofrequency pulse. This very fast image acquisition leads to loss of image quality in terms of spatial resolution, higher sensitivity to inhomogenity and susceptibility artefacts.

Functional MRI data for **Study V** were obtained using a GE Signa Echo Speed 1.5T scanner at the MR-Center/Stockholm Brain Institute in Stockholm and an 8-channel head-coil (see **Study V** for details on image acquisition).

Methodological considerations

In fMRI, differences between neural activities recoded during two conditions (e.g. an experimental condition and a control or a fixation-rest condition) are calculated because the brain is always highly active, even at rest. Thus, it is important to keep in mind that there is no true baseline assessable with fMRI [280]. Therefore, the results of a study always dependend on the type of baseline that is used.

Furthermore, fMRI images are prone to artefacts, are rather noisy and include head movement artefacts. These problems are accounted for by preprocessing the images before the statistical analyses using *realignment* (motion correction via spatial matching

of a voxels acquired at different time points), spatial *normalization* (normalizes the brain volume into standard brain shape and size allowing data to be averaged across scans and subjects) as well as spatial *smoothing* (low-pass filtering whereby each voxel is replaced with an average of itself and the surrounding voxels). These preprocessing steps increase the signal-to-noise ratio of the hemodynamic effects and prepare the data for statistical analyses.

During statistical analyses of the data, the BOLD signal changes occurring during the experiment are divided into components corresponding to the experimental conditions using the General Linear Model (GLM) [281]. The GLM expresses the observed data as a linear combination of the expected data and a residual error. For every voxel in the brain, a statistical model is estimated to assess differences in BOLD contrast for the different conditions in the model.

Analyses can be performed as a *whole-brain* analysis or as a *region-of-interest* (ROI) analysis. While the former is often more explorative, the ROI-approach limits the analyses to a-priori specified regions and is thus more specific and hypothesis-driven.

The output from the statistical analyses is an image indicating brain activation in response to a specific stimuli or a specific task (see Figure 20A and D for an example). This image is then further segmented into active and inactive areas by statistical thresholding. Ultimately the results can be depicted graphically by superimposing the statistical maps onto high resolution anatomical images or by listing peak activations numerically in form of three-dimensional coordinates in standard stereotaxic spaces (e.g. the atlas from the Montreal Neurological Institute, MNI).

3.6 STIMULUS MATERIAL

The stimulus material for **Study I, II and V** was taken from the "Karolinska Directed Emotional Faces" [282]. For **Study I and II**, four different color pictures depicting male faces (AM09, NES09, AM28, NES28) were selected to serve as CSs (two pictures each depicting neutral and angry facial expressions). For **Study V**, two different faces showing angry facial expression (ANS06, ANS09) were selected as stimuli.

3.7 QUESTIONNAIRES AND SYMPTOM RATINGS

For **Study III**, the symptom severity of PD patients was rated using the Panic Disorder Severity Scale (PDSS) [283] and the Montgomery-Åsberg Depression Rating Scale (MADRS) [284] and in **Study IV** the Hospital Anxiety Depresssion Scale (HADS) [285] was used.

The PDSS is a 7-item observer-rated instrument which assesses panic symptom severity, rated on a 4-point Likert scale (0: no symptoms, 4: extremely severe symptoms).

The MADRS is a 10-item observer-rated scale used to assess severity of depressive symptoms and is rated on a 7-point Likert scale (0: symptom is not present, 6: extremely severe symptoms).

The HADS is a 14-item self-rating scale, which is subdivided into an anxiety subscale (7 items), and a depression subscale (7 items). All items are rated on a 4-point Likert scale (0: never, 3: always).

For **Study V,** the state version of the Spielberger Trait-State Anxiety Inventory STAI, [286], which is a 20-item self-rating scale rated on a 4-point Likert scale (1: disagree completey, 4: agree completely) was used to assess state anxiety before the experiment.

4 RESULTS AND BRIEF DISCUSSION

4.1 STUDY I

Genetic Gating of Human Fear Learning and Extinction – Possible Implications for Gene-Environment Interaction in Anxiety Disorder

In this study, we investigated the ability to acquire and extinguish conditioned fear responses in relationship to the 5-HTTLPR and the COMTval158met polymorphism respectively. Research participants were partly selected by genotype and completed a differential delay conditioning task and a 24-hour delayed extinction task during which SCR and PFS reactions were recorded as psychophysiological indicators of fear learning. Angry male faces were used as CS and an aversive but individually adjusted electrotactile stimulation to the wrist was used as the US. Participants unaware of the conditioning contingencies were excluded from the analysis leaving a final sample of 48 healthy volunteers.

We found facilitated acquisition, as measured by CS+ Potentation of PFS reactions, in carriers of the 5-HTTLPR s-allele (see Figure 16a) and remainders of this facilitated acquisition were seen in early extinction 24-hours later which measures retention of conditioning. In addition individuals with the 5-HTTLPR l/l genotype demonstrated significantly more CS- inhibition during extinction than s-carriers (see Figure 16b).

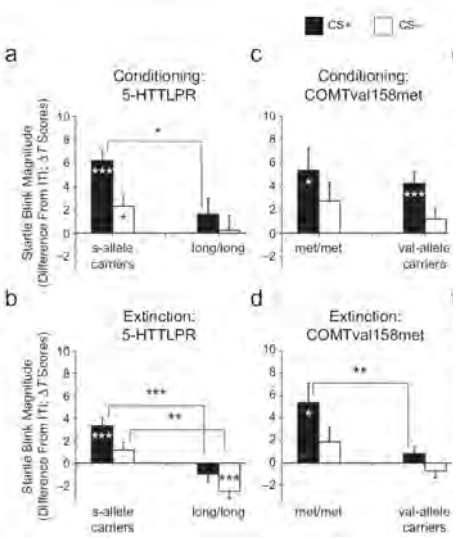


Figure 16. Potentiation of the startle-response magnitude as a function of genotype and stimulus. Black bars show the difference between magnitude of the startle responses elicited during the presentation of the CS+ and the magnitude of the resonse elicited during the ITI and white bars show the difference between magnitude of the startle response elicited during the CS- and the magnitude of the resonse elicited during the ITI. * $p < .05$, ** $p < .005$,*** $p < .001$.

As facilitated acquisition of fear memory has been reported in anxiety patients, we propose that facilitated fear memory acquisition may be an underlying mechanism for a higher vulnerability to affective disorders seen in carriers of the 5-HTTLPR s-allele in particularly after negative life events.

In contrast, the COMTval158met genotype did not have an impact on the acquisition of conditioned responses (see Figure 16c) but resistance to extinction was observed in volunteers with the COMT met/met genotype as measured by CS+ Potentation of PFS reactions (see Figure 16d).

SCRs showed reliable CS discrimination in the absence of genotype effects for either 5-HTTLPR or COMTval158met.

We propose that s-carriers may be particularly vulnerable to develop affective pathologies after negative life events if they also carry the COMT met/met genotype. In particular, the combination of facilitated fear memory acquisition (associated with the 5-HTTLPR s-allele) and resistance to fear memory extinction (associated with the COMT met/met genotype) may be seen as a vulnerability factor for the development of affective and in particular anxiety symptoms.

In support of our findings, a very recent publication by Kolassa et al. [107] found that the risk to develop PTSD after trauma exposure is influenced by the COMTval158met genotype with individuals with the met/met genotype being at higher risk even with less severe trauma exposure.

4.2 STUDY II

Amygdala-dependent fear conditioning in humans is modulated by the BDNFval66met polymorphism

In this study, the ability to acquire and extinguish conditioned fear responses was investigated in relationship to the BDNFval66met polymorphism. Research participants and study design was identical to **Study I** but all participants were genotyped after completing the experiment.

We found that aware BDNF met-carriers show a deficit in amygdala-dependent fear conditioning as indicated by an absence of a fear potentiation of startle responses in the last acquisition block (see Figure 17A). This deficit was maintained in the first block of extinction most likely reflecting retention of (existing differences in) fear memory (see Figure 17B). No genotype differences were found in conditioned SCR discrimination during acquisition and extionction, despite of significant overall CS discrimination (see Figure 17C and D).

Furthermore, we found that participants carrying the BDNF met-allele more often fail to report the correct conditioning (CS-US) contingencies.

These data support findings from animal studies showing an involvement of BDNF in fear conditioning and provide evidence for the importance of BDNF signaling also in human amygdala-dependent learning processes.

We suggest that the minor BDNF met-allele may have a protective effect for the development of affective pathologies. This protective effect may be mediated via reduced synaptic plasticity in the amygala induced by negative experience.

However, at the same time as **Study II** was published (Feb. 2010), a study by Soliman et al. [260] reported deficits in extinction as assessed by SCR and fMRI in carriers of the BDNF met-allele despite of no differences in fear conditioning. This finding stands in contrast to our report and it is possible that differences in task design may account for these inconsistencies.

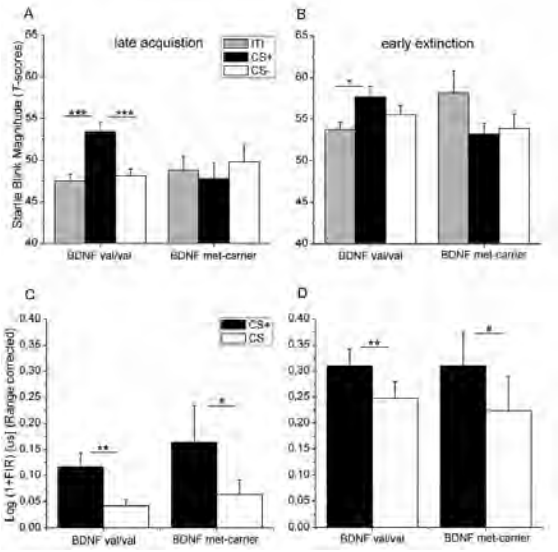


Figure 17. Fear potentiated startle reactions elicited during the conditioned stimuli (CS+, CS-) and the intertrial interval (ITI) for the BDNFval66met genotype groups (val/val vs. met-carriers) during (**A**) late acquisition and (**B**) early extinction, as well as skin conductance responses to the CS+ and CS- for the BDNFval66met genotype groups during (**C**) late acquisition and (**D**) early extinction. # $p < .10$, * $p < .05$, ** $p < .005$,***$p < .001$.

4.3 STUDY III

The symptomatic profile in panic patients is affected by the 5-HTTLPR polymorphism

Study III was an attempt to translate parts of our experimental findings from **Study I** into a clinical setting. Specifically, in **Study I** we demonstreated facilitated fear conditioning in healthy 5-HTTLPR s-allele carriers using psychophysiological methods (FPS). Facilitated fear conditioning has been implicated in anxiety disorders and has been proposed as a mechanism for the acquisition of symptons in PD. Consequently 5-HTTLPR s-carriers were hypothesized to display more severe symptoms than non-carriers.

In this study, 73 patients suffering from PD participated. The symptomatic profile was assessed by clinician-rated PDSS and MADRS scales. These ratings were acquired when the patient was admitted to a randomized clinical equivalence trial of regular cognitive behavioral therapy (CBT) vs. internet-based CBT (see **Study IV**).

Patients were genotyped for the 5-HTTLPR and the rs25531 polymorphism and the symptomatic profile was compared based on the "biallelic" 5-HTTLPR genotypes (s-carriers vs. non-carriers [l/l]) and the "triallelic" 5-HTTLPR genotypes. For the "triallelic" 5-HTTLPR, patients are grouped based on putative 5-HTT expression levels, which are inferred by 5-HTTLPR and rs25531 genotypes.

We found a significant allele-load dependent effect of the the biallelic 5-HTTLPR genotype on the symptomatic profile of PD patients as measured by both panic symptom (PDSS) and depressive symptom (MADRS) severity (see Figure 18a and c). Patients with the s/s genotype reported most symptoms and non-carriers (l/l) reported the least symptoms, while heterozygotes always scored intermediate. An item-wise

analysis revealed significant effects for agoraphobic anxiety, anticipatory anxiety and social impairment (PDSS) and for pessimistic thoughts, concentration problems and melancoly (MADRS).

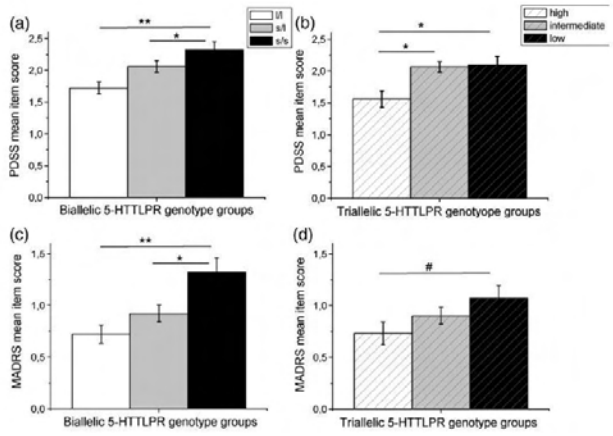


Figure 18. Mean item sum scores and S.E.M. for PDSS (**a,b**) and MADRS (**c,d**) for bi-allelic 5-HTTLPR (**a,c**) and triallelic 5-HTTLPR (**b,d**). ***p<0.001, **p<0.01, *p<0.05, #p<0.1.

Similar results were found for the triallelic 5-HTTLPR for PDSS scores (see Figure 18b). For MADRS scores, a non-significant trend for an allele-load dependent pattern was observed in the same direction as for the biallelic 5-HTTLPR (see Figure 18d).

In sum, our results show an association of the 5-HTTLPR s-allele and the low expressing mini-haplotype (triallelic 5-HTTLPR) with observer-rated panic and depressive symptoms in PD patients. Thus, we provide first evidence that our experimental findings from **Study I,** which were obtained using healthy volunteers, can be translated into a clinical setting.

Given that case-control studies do not find evidence for an involvement of the 5-HTTLPR in PD, we suggest that the s-allele of the 5-HTTLPR may not be associated with the clinical diagnosis of PD, but rather modify the severity of PD and possibly other anxiety disorders.

4.4 STUDY IV

The COMTval158met polymorphism affects efficacy of exposure-based CBT in panic patients.

Study IV was an attempt to translate parts of our experimental findings from **Study I** into a clinical setting. Specifically, in **Study I** we demonstrated resistance to extinction in healthy individuals with the COMTval158met met/met genotype. CBT used to treat patients with anxiety disorders (e.g. PD) is based on the principle of extinction and thus patients with the COMT met/met genotype were expected to profit less from exposure-based treatment modules as compared to val-carriers.

In this study, we investigated the efficacy of CBT in a sample of 69 panic patients in relationship to the COMTval158met polymorphism. Patients were part of a randomized clinical equivalence trial, showing equivalence of regular cognitive behavioral therapy (CBT) and internet-based CBT. Treatment lasted for 10 weeks and consisted of 10

different modules that contained both cognitive and exposure-based methods. After completion of each of the 10 modules/weeks, patients filled in the anxiety and depression scales of the Hospital Anxiety and Depression Scale (HADS).

For statistical analyses, the 10 treatment modules were grouped based on their content in two blocks of cognitive vs exposure-based methods.

No differences in efficacy of the cognitive modules was observed between the COMTval158met genotype groups, while patients with the met/met genotype seemed to profited significantly less from exposure-based modules than patients carrying at least one COMTval-allele (see Figure 19).

Furthermore, while patients carrying a COMTval-allele reported tendentially more severe sypmtoms prior to CBT, no differences in symptom severity were found between the two COMTval158met genotype groups after CBT. This further strengthens the interpretation of the results described above as differential profiting from CBT based on the COMTval158met genotype.

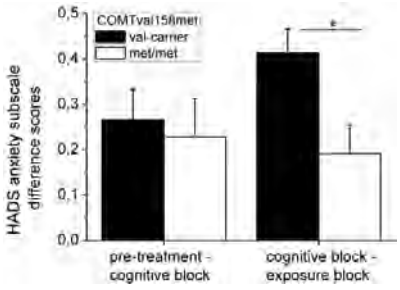


Figure 19. Difference scores between the HADS mean scores pre-treatment and during the cognitive block as well as during the cognitive vs. the exposure block for COMTval-carriers (black bars) and patients with the met/met genotype (white bars). *p<0.05.

In sum, we report suggestive evidence that the findings on the effect of the COMTval158met polymorphism from an experimental extinction study obtained using healthy volunteers (see **Study I**) may be translated into a clinical setting. This finding may have important clinical implications in terms of personalize medicine.

4.5 STUDY V

5-HTTLPR and COMTval158met genotype independently gate amygdala reactivity and habituation during passive viewing of angry faces

For **Study V**, 64 healthy volunteers were selected based on their 5-HTTLPR/rs25531 and COMTval158met genotypes as well as gender from a large pool of genotyped participants (N=597). We tested the impact of these polymorphisms and a possible interaction on amygdala reactivity and habituation to the passive viewing of angry faces using fMRI. The analyses were basd on 54 volunteers.

When viewing angry faces (as opposed to a fixation cross), a significant effect of 5-HTTLPR on right amygdala reactivity (s-carrier>l/l, see Figure 20A and B) and COMTval158met on left amygdala reactivity (met/met>val-carrier, see Figure 20D and E) was observed in absence of an interaction or additive effect. Additionally, we

provide preliminary evidence that different habituation curves of the amygdala may partly underlie the differences in amygdala activity between the 5-HTTLPR genotype groups. Furthermore, the validity of *a priori* pooling carriers of one or two 5-HTTLPR s-alleles and COMT val-alleles was empirically confirmed (see Figure 20C and F respectively).

SCRs to pictures of angry faces revealed a significant interaction between the 5-HTTLPR and COMTval158met genotypes, in absence of main effects. Participants carrying the 5-HTTLPR s-allele that also carry the COMT met/met genotype showed the highest SCR reactions. No differences in salivary cortisol concentrations prior to the experiment were found between the genotype groups.

Our results support that 5-HTTLPR s-carriers and individuals with the COMT met/met genotype may be more sensitive to the detection of biologically and socially relevant information as indicated by enhanced amygdala reactivity. We suggest further that different habituation slopes of the amygdala may partly underly the differences

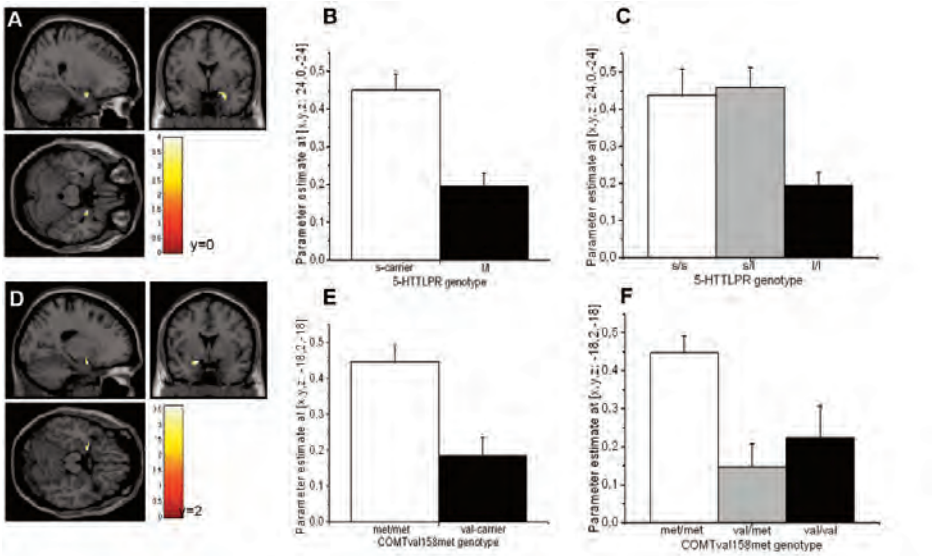


Figure 20. (**A**) Mean signal in the right amygdala for the 5-HTTLPR genotype groups (s-carrier vs. l/l). Activations shown are thresholded at p<0.01 uncorrected for illustrative purposes and based on the ROI (**B**) Parameter estimates (and S.E.M) for 5-HTTLPR s-carrier and l/l at peak activation coordinates (**C**) Parameter estimates (and S.E.M.) for 5-HTTLPR s/s, s/l and l/l genotype groups at peak activation coordinates. (**D**) Mean signal in the right amygdala as a function of COMTval158met genotype. Activations shown are thresholded at p<0.01 uncorrected for illustrative purposes and based on the ROI (**E**) Parameter estimates (and S.E.M.) for COMTval158met met/met and val-carrier at peak activation F coordinates (**F**) Parameter estimates (and S.E.M.) for COMTval158me met/met, val/met, val/val genotype groups at peak activation coordinates.

seen between the 5-HTTLPR genotype groups.

5 GENERAL DISCUSSION

The general aim of this thesis was to investigate the involvement of candidate polymorphisms (5-HTTLPR/rs25531, COMTval158met and BDNFval66met) in fear- and anxiety related processes.

Fear conditioning and extinction was used as a laboratory model to experimentally study gene-environment interactions in anxiety disorders and their treatment respectively. Furthermore, the symptomatic profile as well as the efficacy of (exposure-based) CBT was studied in a sample of PD patients as well as amygdala reactivity and habituation during the viewing of angry faces in healthy individuals. A broad range of state-of the art neuroscience methods was applied to answer these research questions including psychophysiology, molecular genetics and functional brain imaging (fMRI).

In fear conditioning an environmental stimulus becomes a fear elicitor through associative learning processes, and via extinction it may loose this potential. Individual differences depending on their genetic profile may exist in the ability to learn and retain these associations. This would possibly render individuals at different risks to develop anxiety disorders (e.g. PD, phobias and PTSD), in particular after a traumatic or critical life event (gene x environment interaction) and possibly render them differentially susceptible to exposure-based treatments.

In **Study I** and **II** we describe an association of the 5-HTTLPR s-allele and the BDNF val-allele with facilitated fear conditioning and the COMT met/met genotype with resistance to extinction. We propose that facilitated fear conditioning may be an underlying mechanism for the vulnerability of 5-HTTLPR s-allele carriers to develop affective psychophathologies (in particular after adverse life events) as well as of individuals with the BDNF val/val genotype and 5-HTTLPR s-carriers to display higher neuroticism scores.

Still, the *ecologic validity* of laboratory conditioning and extinction may be questioned. Out of obvious ethical reasons, all individuals participated voluntarily, were aware that they will receive electrotactile stimulation during the course of the experiment and were also fully aware that they may cancel the experiment at any time. This stands in contrast to unpredictable traumatic events that happen unescapable. Thus, it needs to be tested if our experimental results could be translated into a clinical setting. In **Study III** and **IV** we attempted this translation of our experimental results using a sample of well characterized PD patients and provide preliminary evidence in favor of our results.

Classical conditioning has long been proposed as a mechanism through which anxiety symptoms are acquired. Consequently, any genetic variant that is associated with facilitated conditioning should also be associated with a more severe symptomatic profile, as shown in **Study III** for the 5-HTTLPR s-allele/low-expressing mini-haplotype.

Psychotherapy can be regarded as a form of learning from the environment, and the learning process occurring during CBT most likely produce alterations of gene expression and synaptic connections in the brain. The efficacy of this process is thus critically dependent on the potential of the individual brain to adapt to these changing

(environmental) contingencies during therapy. By demonstrating that a common genetic polymorphism (COMTval158met) differentially affects efficacy of exposure-based treatment in PD patients (**Study IV**), we provide a potentially interesting new avenue of gene x environment interaction in psychiatric genetics that has so far been unexplored.

In **Study V** we again demonstrate higher reactivity to negative stimuli in 5-HTTLPR s-carriers. During the passive viewing of negative faces (vs. fixation cross), s-carriers demonstrated higher amygdala reactivity than non-carriers. Our data further suggest that this may, at least partly, be due to a slower habituation slope of the amygdala in this group. Furthermore our data also show higher amygdala reactivity to negative faces (vs. fixation cross) in individuals with the COMT met/met genotype as compared to val-carriers. Still, we have used a fixation cross as our only control condition. Using neutral faces as an (additional) control condition may yield different results as in particular the 5-HTTLPR genotype has been associated with the neural response to ambiguous or undefined stimuli [182, 287].

This thesis reports associations of common polymorphisms (5-HTTLPR/rs25531, BDNFval66met, COMTval158met) with human fear learning and extinction, amygdala reactivity and habituation in healthy individuals as well as the symptomatic severity and efficacy of exposure-based CBT in PD patients. Though, these associations may be summarized under the umbrella of fear-relevant processes, all polymorphisms studied in this thesis have been intensively investigated during the last years and have been associated with a wide range of other (psychiatric) disorders and behaviours. Consequently, the *specificity of the findings* reported in this thesis to fear-and anxiety-relevant processes remains to be adressed.

In general, it cannot be expected that the association between a common polymorphism and a complex behaviour/disease is very specific. Behavior is the product of our most complex organ: the brain, and it needs to be kept in mind that genetic variation does not exert a direct effect upon behaviour and disease. Genetic variation induced leads to subtle molecular and cellular changes in systems that promote the development and maintenance of brain structure and function. Genes do not code for behaviour or diseases but encode for gene-products (e.g. proteins) which are usually involved in multiple biological processes (pleiotropy). Thus, genetic variation in a gene encoding a specific gene product will also affect these multiple processes rather than a very specific function.

Even though the polymorphisms that we have studied are all functional, they still only affect single components within a neutotransmitter system. The alteration of one of the bottlenecks within a system may have profound effects. In general however this has to be expected to be rather subtle, given the number of different bottlenecks involved in a well functioning system. Furthermore there is probably no behavior or complex disease that is solely modulated by a single transmitter system. Neurotransmitters are known to have broad effects on behaviour and disease and thus the subtle changes induced by functional polymorphisms in these broad systems cannot be expected to be more specific than the systems general function. Additionally it is important to keep in mind, that the effect of any genetic variation on behaviour and disease will be probabilistic rather than deterministic and also depends on environmental factors.

Another issue arising when questioning the specificity of the findings is the question of *vulnerability vs. plasticity* [288] , as **Study I, II** and **IV** investigated individual differences in associative learning processes. Individuals acquiring and extinguishing fear responses more readily may have a more plastic neural system rather than a more vulnerable one, which would be the most self-evident interpretation. This would imply, that these individuals may also be more sensitive to positive events and in fact the literature provides suggestive evidence for this [288].

In Closing,

the work included in this thesis provides evidence for differential responsivity to adverse environmental events, operationalized as fear conditioning and amygdala reactivity, depening on the 5-HTTLPR and BDNF val66met polymorphisms. Furthermore, we provide a possible explanation for the association of the 5-HTTLPR s-allele with enhanced amygdala reactivity as we found less habituation of amygdala activity in this group.

We also find preliminary evidence for different capacity to extinguish learned fear responses depening on the COMTval158met polymorphism. Furthermore we were able to demonstrate that our experimental findings linking 5-HTTLPR to facilitated fear acquisition and the COMTval158met polymorphism to resistance to extinction may be translated into the clinical setting, using a sample of well-characterized PD patients. However, it also needs to be considered that all studies included in this thesis employ the association study approach and thus a causual link can not yet be claimed. Once an association is established future research needs to focus on elucidating the (molecular) mechanisms underlying these associations.

6 FUTURE PERSPECTIVES

Understanding the neurobiological pathways of fear, fear learning and extinction may enable us to identify high risk individuals and develop suitable therapies (both pharmacological and psychological) to help people suffering from anxiety disorders. Thus, the identification of individuals at high (e.g. genetic) risk for a specific disease may permit interventions even before the disease occurs. In the future, the genetic profile of the individual patient may be taken into account when selecting type and intensity of treatment (*personalized medicine*), e.g. it may be possible that individuals with the COMT met/met genotype need a prolonged or more intense treatment as compared to their val-counterparts to achieve the same symptom relief.

Despite of possible advances in the field of personalized medicine, genetic studies may help to classify currently existing diagnoses into subtypes which may have implications for treatment and may ultimately lead to changes in the *classification systems*.

Condidering the methods used in this thesis, future studies need to go beyond simply establishing associations between genetic polymorphisms and behavior or disease and need to focus on unraveling the *mechanisms* through which genes and genetic variation exert their effects. Association studies are needed and accumulate knowledge about the effects of specific polymorphisms but in order to really profit from this knowledge we need to deepen our understanding of the underlying physiological and molecular processes. Still, the success of association studies is dependent on the *definition of the phenotype* and its reliable and valid measurement. Thus, the tasks used should be carefully evaluated.

Methodological developments within the specific research area will need to lift research to another level.

In the *optimal genetic association study of the future*, more research participants/patients, matched for age, gender, sex, ethnicity and critical environmental factors/life events will be enrolled and a gene-based approach studying haplotypes instead of single polymorphic markers will be employed to better capture the genetic variation in a gene. Furthermore, genetic variants in different bottlenecks of the same neurotransmitter system (e.g. in the 5-HT system: TPH, 5-HTTLPR, MAO-A) and epigenetic analyses will be applied to better capture the variation in the transmitter system of interest.

The *optimal Imaging study of the future* is looking for brain networks rather than single brain regions and using advanced techniques from computational modeling for connectivity analyses. Furthermore the field will profit from emplying methods going beyond "simple" fMRI experiments e.g. resting state fMRI, diffusion tensor imaging and morphometric measurements. Additionally, the use of appropriate and possibly multiple control conditions should be considered in order to be able to rule out alternative explanations and nonspecific effects.

Despite of methodological advances within the separate research areas, *translational studies* bringing together insights from molecular genetics, psychophysiological and behavioral studies as well as neuroimaging and pharmacological challenge studies may

ultimately help to unravel the neural underpinnings of anxiety related traits and anxiety disorders and help to optimize treatment or even prevent their occurrence.

7 ACKNOWLEDGEMENTS

First, I want to thank all the research participants and patients in Stockholm and Greifswald that took part in my studies. Special thanks also to all my friends that patiently volunteered as pilots and research participants in particular for the fMRI experiments!

I have been engaged in close work with five research groups during my PhD. Thus, there are enormous amounts of co-workers at the Psychology section, the Neurogenetics section, the (former) MR-center and the Psychiatry Section at the Karolinska Institute as well as the Section for Clinical and Biological Psychology at the University of Greifswald that deserve to be mentioned here. Especially, I would like to thank…

… my main supervisor **Arne Öhman.** You allowed and helped me to build up my "perfect" PhD project when I started as your student and turned your interest into new avenues. Thank you for giving me as much scientific freedom as I needed, for supporting me in everything as well as letting me travel to every conference I wanted to. Thank you for taking your time to discuss, read, comment and teach. Last, but not least, thank you for honestly caring about how your students are doing and feeling.

…my co-supervisor **MartinI Ingvar** for keeping spirits high, for amazing personal commitment, in particular when it came to home-made solutions for the MRI environment (I will always remember your self-made "mechanical mouse"), brazing cables, saturday morning sessions at the scanner and for just being convinced that everything will be fine.

…my co-supervisor **Martin Schalling** for introducing me to the Neurogenetics lab. Thank you for introducing me to Nils and Christian and providing the chance to participate in the clinical genetics studies included in this thesis. Thank you also for always seeing and providing opportunities for me.

…my co-supervisor **Alfons Hamm** for giving me a flying start at your lab and with my PhD work. I am really thankful, that I had the opportunity to meet you and join your research group for a couple of month.

…my inofficial co-supervisor **Almut Weike** for much more support, involvement and help than one could expect and for always having time for discussions and sharing your knowledge. Thank you for great company in Greifswald and on many conferences. Special thanks also for being the absolutely best proof-reader that just finds every tiny mistake.

…my closest co-worker and office-mate **Armita Golkar**. Everything became so much more fun after you and your spirit moved into "my" office and projects. It was great to have someone to exchange ideas with and fantazise about what could and should be done. Thank you for that fMRI project of ours – I could not have done it without you. Thanks for taking care of the "technical stuff", intense literature reading weeks, late night scans, weekend scans, sharing up's and down's, for laughter and for the Iceland trip ("perform").

…**Kara Lindström** for sharing the up's and down's of our fMRI project and for proof-reading.

…**Andreas Olsson** for inspiring discussions, an open ear and for spreading this unstressed spirit around you.

…my co-authors and collaborators **Christian Rueck, Jan Bergström** and **Nils Lindefors** for providing me with the opportunity to work with your patient material and for a fruitful collaboration.

…**Karin Jensen** and **Eva Kosek** for introducing me into the work on pain and the opioid system.

…**Jonathan Berrebi** for help with the Matlab scripts (again and again and again…), for making work life much easier and for great company.

…**Peter Fransson** for expert help with all kinds of fMRI related issues.

…**Marie "Bloody Mary" Lundberg** and **Carmen Hamm** for taking seemingly endless amounts of bloodsamples.

…**Heino Mohrmann** for very good technical support and hands-on help when getting started with Presentation and VPM.

…**Pernilla Nikamo** for getting me started with genotyping and sequencing 5-HTTLPR and rs25531.

…**Agneta Gunnar** for help with digestion (of the 5-HTTLPR) and for letting me use the NanoDrop.

…**Fred Lindstedt** for the work on pain genetics and for your enthusiasm.

…**Gerhard Andersson** for helpful comments on the psychiatric genetics manuscripts.

…**Christian Garheden** for IT-support.

…KMP**, Karl-Magnus Petterson**, for help with fMRI design and Matlab scripts.

… **Erik Schäfer, Andrew Ketterer, Christin Rhode, Anders Görling, Katarina Hodges** and **Markus Ronnheden,** the students that helped me with my projects during the years.

..my present and former colleagues at the **Psychology Section,** in particular Floor 4: Anna, Aila, Andreas, Annika, Bianka, Bo, John, Lisa, Lotta, Malin, Pernilla, Peter, Susanna and Tina. Thanks for so many chats during fika brakes and lunches!

…my present and former colleagues at the **Neurogenetics Section**: Ana, Anna, Annika, Björn, Cattis, Charlotte, Dzana, Dalila, Elin, Ida, Jeanette, Karin, Lollo, Malin, Maria, Philippe, Rifat, Santi, and Selim. Special thanks to Anna-Lee for helping out with the formamide work while I was pregnant!

…my present and former colleagues at the (former) **MR-centrum**: Deepak, Fiona, Jeremy, Julia, Kattis, Mimmi, Sissela, Stina and Tracy.

… the **SBI juniors**, in particular Anke, Linda, Nathalie, Örjan and Valeria. It was a pleasure! Thanks for great ski conferences, retreats and lots of fun.

… my colleagues at the **University of Greifswald**: Anke, Christiane, Jan, Julia, Katharina, Kathrin, Mattias v.R., Matthias W., Sylvie and Thomas. Thanks for "adopting" me during my visits in Greifswald as well as at various conferences, courses and meetings!

…the **Nordic Center of Excellence in Cognitive Control** for support, courses and meetings.

…the **Stockholm Brain Institute (SBI) research school** for mobility support and courses.

…the **German Academic Exchange Service** for partial financial support.

… my friends outside work: Kristina, Christian, Florian, Ursina, Hanna, Hervé, Therese, Stefan, Ilaria, Matteo, Laura, Jens, Alison, Anne-Marie, Maria, Sven, Julia, Eva, Yvonne, Jolanta, Anders and Sara. Thanks for many rememberable moments in Stockholm and in particular the summer of '05.

…meinen Eltern Inge und Manfred und meine Schwestern Heike und Anke sowie Eike und Charlotte. Danke für groβe Unterstützung und die vielen Besuche in Stockholm.

…my little family: **Sönke** and **Nils**. Thank you for keeping my mind off brains and genes.

8 REFERENCES

1. Watson JD, Crick FHC. Molecular Structure of Nucleic Acids - a Structure for Deoxyribose Nucleic Acid. *Nature* 1953; **171**(4356)**:** 737-738.
2. Plomin R, Crabbe J. DNA. *Psychological Bulletin* 2000; **126**(6)**:** 806-828.
3. Kandel E, Schwartz J, Jessel T. *Principles of Neural Science*. 3rd edn. Conn, Appelton & Lange: East Norwalk, 1991.
4. Plomin R, Owen MJ, McGuffin P. THE GENETIC-BASIS OF COMPLEX HUMAN BEHAVIORS. *Science* 1994; **264**(5166)**:** 1733-1739.
5. Bruder CEG, Piotrowski A, Gijsbers A, Andersson R, Erickson S, de Stahl TD *et al.* Phenotypically concordant and discordant monozygotic twins display different DNA copy-number-variation profiles. *Am J Hum Genet* 2008; **82**(3)**:** 763-771.
6. Maher B. Personal genomes: The case of the missing heritability. *Nature* 2008; **456**(7218)**:** 18-21.
7. Gottesmann II, Shields J. *Schizophrenia and Genetics: a Twin Study Vantage Point.* Academic Press: New York, 1972.
8. Walters JTR, Owen MJ. Endophenotypes in psychiatric genetics. *Molecular Psychiatry* 2007; **12**(10)**:** 886-890.
9. Gottesman, II, Gould TD. The endophenotype concept in psychiatry: Etymology and strategic intentions. *American Journal of Psychiatry* 2003; **160**(4)**:** 636-645.
10. Flint J, Munafo MR. The endophenotype concept in psychiatric genetics. *Psychological Medicine* 2007; **37**(2)**:** 163-180.
11. Green AE, Munafo MR, DeYoung CG, Fossella JA, Fan J, Gray JR. Using genetic data in cognitive neuroscience: from growing pains to genuine insights. *Nature Reviews Neuroscience* 2008; **9**(9)**:** 710-720.
12. Hariri AR, Weinberger DR. Imaging genomics. *British Medical Bulletin* 2003; **65:** 259-270.
13. Glahn DC, Thompson PM, Blangero J. Neuroimaging endophenotypes: Strategies for finding genes influencing brain structure and function. *Human Brain Mapping* 2007; **28**(6)**:** 488-501.
14. Peper JS, Brouwer RM, Boomsma DI, Kahn RS, Poll HEH. Genetic influences on human brain structure: A review of brain imaging studies in twins. *Human Brain Mapping* 2007; **28**(6)**:** 464-473.
15. Koten JW, Wood G, Hagoort P, Goebel R, Propping P, Willmes K *et al.* Genetic Contribution to Variation in Cognitive Function: An fMRI Study in Twins. *Science* 2009; **323**(5922)**:** 1737-1740.
16. Blokland GAM, McMahon KL, Hoffman J, Zhu G, Meredith M, Martin NG *et al.* Quantifying the heritability of task-related brain activation and performance during the N-back working memory task: A twin fMRI study. *Biological Psychology* 2008; **79**(1)**:** 70-79.
17. Meyer-Lindenberg A, Nicodemus KK, Egan MF, Callicott JH, Mattay V, Weinberger DR. False positives in imaging genetics. *Neuroimage* 2008; **40**(2)**:** 655-661.
18. Öhman A. Fear and anxiety: Overlaps and dissociations. In: Lewis M, J.M. H-J, L.F. B (eds). *Handbook of Emotions, 3rd Edition.* Guilford Press: NY, 2008, pp 709-729.
19. Öhman A, Mineka S. Fears, phobias, and preparedness: Toward an evolved module of fear and fear learning. *Psychological Review* 2001; **108**(3)**:** 483-522.
20. Sehlmeyer C, SchÃ¶ning S, Zwitserlood P, Pfleiderer B, Kircher T, Arolt V *et al.* Human Fear Conditioning and Extinction in Neuroimaging: A Systematic Review. *PLoS ONE* 2009; **4**(6)**:** e5865.

21. Myers KM, Davis M. Mechanisms of fear extinction. *Molecular Psychiatry* 2007; **12**(2)**:** 120-150.

22. Sotres-Bayon F, Cain CK, LeDoux JE. Brain mechanisms of fear extinction: Historical perspectives on the contribution of prefrontal cortex. *Biological Psychiatry* 2006; **60**(4)**:** 329-336.

23. Myers KM, Ressler KJ, Davis M. Different mechanisms of fear extinction dependent on length of time since fear acquisition. *Learn Mem* 2006; **13**(2)**:** 216-223.

24. Huff NC, Hernandez JA, Blanding NQ, LaBar KS. Delayed Extinction Attenuates Conditioned Fear Renewal and Spontaneous Recovery in Humans. *Behav Neurosci* 2009; **123**(4)**:** 834-843.

25. Norrholm SD, Vervliet B, Jovanovic T, Boshoven W, Myers KM, Davis M *et al.* Timing of Extinction Relative to Acquisition: A Parametric Analysis of Fear Extinction in Humans. *Behav Neurosci* 2008; **122**(5)**:** 1016-1030.

26. Kim JJ, Jung MW. Neural circuits and mechanisms involved in Pavlovian fear contitioning: a critical review. *Neuroscience and Biobehavioral Reviews* 2006; **30:** 188-202.

27. LeDoux JE. Emotion circuits in the brain. *Annual Review of Neuroscience* 2000; **23:** 155-184.

28. Whalen PJ. Fear, vigilance, and ambiguity: Initial neuroimaging studies of the human amygdala. *Current Directions in Psychological Science* 1998; **7**(6)**:** 177-188.

29. Davidson RJ, Fox A, Kalin NH. Neuroal Bases of Emotion Regulation in Nonhuman Primates and Humans. In: Gross JJ (ed). *Handbook of Emotion Regulation*. The Guilford Press: New York, 2007.

30. Davis M, Whalen PJ. The amygdala: vigilance and emotion. *Molecular Psychiatry* 2001; **6**(1)**:** 13-34.

31. Morris JS, Ohman A, Dolan RJ. A subcortical pathway to the right amygala mediating unseen fear. *Proc Natl Acad Sci U S A*; **96:** 1680-1685.

32. Morris JS, Ohman A, Dolan RJ. Conscious and unconscious emotional learning in the human amygdala. *Nature* 1998; **393**(6684)**:** 467-470.

33. LeDoux J. The emotional brain, fear, and the amygdala. *Cellular and Molecular Neurobiology* 2003; **23**(4-5)**:** 727-738.

34. Sotres-Bayon F, Bush DEA, LeDoux JE. An update on prefrontal-amygala interactions in fear extinction. *Learn Mem* 2004; **11:** 525-535.

35. Klüver H, Bucy PC. Preliminary analysis of funcions of the temporal lobe in monkeys. *Archives of Neurological Psychiatry* 1939; **42:** 979-1000.

36. Bechara A, Tranel D, Damasio H, Adolphs R, Rockland C, Damasio AR. Double dissociation of conditioning and declarative knowledge relative to the amygala and hippocampus in humans. *Science* 1995; **269:** 1115-1118.

37. Labar KS, Ledoux JE, Spencer DD, Phelps EA. IMPAIRED FEAR CONDITIONING FOLLOWING UNILATERAL TEMPORAL LOBECTOMY IN HUMANS. *Journal of Neuroscience* 1995; **15**(10)**:** 6846-6855.

38. Buchel C, Dolan RJ. Classical fear conditioning in functional neuroimaging. *Curr Opin Neurobiol* 2000; **10**(2)**:** 219-223.

39. Manuck SB, Brown SM, Forbes EE, Hariri AR. Temporal stability of individual differences in amygdala reactivity. *American Journal of Psychiatry* 2007; **164**(10)**:** 1613-1614.

40. Johnstone T, Somerville LH, Alexander AL, Oakes TR, Davidson RJ, Kalin NH *et al.* Stability of amygdala BOLD response to fearful faces over multiple scan sessions. *Neuroimage* 2005; **25**(4)**:** 1112-1123.

41. Schwartz CE, Wright CI, Shin LM, Kagan J, Rauch SL. Inhibited and uninhibited infants "grown up": Adult amygdalar response to novelty. *Science* 2003; **300**(5627)**:** 1952-1953.

42. Ridderinkhof KR, van den Wildenberg WPM, Segalowitz SJ, Carter CS. Neurocognitive mechanisms of cognitive control: The role of prefrontal cortex in action selection, response inhibition, performance monitoring, and reward-based learning. *Brain and Cognition* 2004; **56**(2)**:** 129-140.

43. Quirk GJ. Prefrontal-Amygdala Interactions in the Regulation of Fear. In: Gross JJ (ed). *Handbook of Emotion Regulation*. The Guilford Press: New York, 2007.

44. Phelps EA, Delgado MR, Nearing KI, LeDoux JE. Extinction learning in humans: Role of the amygdala and vmPFC. *Neuron* 2004; **43**(6)**:** 897-905.

45. Gottfried JA, Dolan RJ. Human orbitofrontal cortex mediates extinction learning while accessing conditioned representations of value. *Nature Neuroscience* 2004; **7**(10)**:** 1145-1153.

46. Semendeferi K, Armstrong E, Schleicher A, Zilles K, Van Hoesen GW. Prefrontal cortex in humans and apes: A comparative study of area 10. *American Journal of Physical Anthropoloy* 2001; **114:** 224-241.

47. Kringelbach ML, Rolls ET. The functional neuroanatomy of the human orbitofrontal cortex: evidence from neuroimaging and neuropsychology. *Progress in Neurobiology* 2004; **72**(5)**:** 341-372.

48. Dias R, Robbins TW, Roberts AC. Dissociation in prefrontal cortex of affective and attentional shifts. *Nature* 1996; **380**(6569)**:** 69-72.

49. Rolls ET, Hornak J, Wade D, McGrath J. EMOTION-RELATED LEARNING IN PATIENTS WITH SOCIAL AND EMOTIONAL CHANGES ASSOCIATED WITH FRONTAL-LOBE DAMAGE. *Journal of Neurology Neurosurgery and Psychiatry* 1994; **57**(12)**:** 1518-1524.

50. Davidson RJ, Fox A, Kalin NH. Neural Bases of Emotion Regulation in Nonhuman Primates and Humans. In: Gross JJ (ed). *Handbook of Emotion Regulation*. The Guilford Press: New York, 2007.

51. Devinsky O, Morrell MJ, Vogt BA. CONTRIBUTIONS OF ANTERIOR CINGULATE CORTEX TO BEHAVIOUR. *Brain* 1995; **118:** 279-306.

52. Ochsner KN, Gross JJ. The Neural Architechture of Emotion Regulation. In: Gross JJ (ed). *The Handbook of Emotion Regulation*. The Guilford Press: New York, 2007.

53. Gehring WJ, Knight RT. Prefrontal-cingulate interactions in action monitoring. *Nature Neuroscience* 2000; **3**(5)**:** 516-520.

54. Buchnan SL, Powell DA. Cingulate cortex: its role in Pavlovian conditioning. *J Comp Physiol Psychol* 1982; **96:** 7555-7774.

55. Milad MR, Quirk GJ, Pitman RK, Orr SP, Fischl B, Rauch SL. A Role for the Human Dorsal Anterior Cingulate Cortex in Fear Expression. *Biological Psychiatry* 2007; **62**.

56. Quirk GJ, Likhtik E, Pelletier JG, Pare D. Stimulation of medial prefrontal cortex decreases the responsiveness of central amygdala output neurons. *Journal of Neuroscience* 2003; **23**(25)**:** 8800-8807.

57. Rosenkranz JA, Moore H, Grace AA. The prefrontal cortex regulates lateral amygdala neuronal plasticity and responses to previously conditioned stimuli. *Journal of Neuroscience* 2003; **23**(35)**:** 11054-11064.

58. Hamm AO, Greenwald MK, Bradley MM, Lang PJ. Emotional Learning, Hedonic Change, and the Startle Probe. *Journal of Abnormal Psychology* 1993; **102**(3)**:** 453-465.

59. Davis M, Astrachan DI. CONDITIONED FEAR AND STARTLE MAGNITUDE - EFFECTS OF DIFFERENT FOOTSHOCK OR BACKSHOCK INTENSITIES USED IN TRAINING. *Journal of Experimental Psychology-Animal Behavior Processes* 1978; **4**(2)**:** 95-103.

60. Hamm AO, Cuthbert BN, Globisch J, Vaitl D. Fear and the startle reflex: Blink modulation and autonomic response patterns in animal and mutilation fearful subjects. *Psychophysiology* 1997; **34**(1)**:** 97-107.

61. Davis M. The role of the amydala in conditioned fear. In: Aggleton J (ed). *The amygdala: Neurobiological aspects of emotion, memory, and mental dysfunction*. Wiley-Liss: NY, 1992, pp 255-306.

62. Davis M, Gendelman DS, Tischler MD, Gendelman PM. A Primary Acoustic Startle Circuit - Lesion and Stimulation Studies. *Journal of Neuroscience* 1982; **2**(6)**:** 791-805.

63. Hamm AO, Weike AI. The neuropsychology of fear learning and fear regulation. *International Journal of Psychophysiology* 2005; **57**(1)**:** 5-14.

64. Hitchcock JM, Davis M. EFFERENT PATHWAY OF THE AMYGDALA INVOLVED IN CONDITIONED FEAR AS MEASURED WITH THE FEAR-POTENTIATED STARTLE PARADIGM. *Behav Neurosci* 1991; **105**(6)**:** 826-842.

65. Dawson ME, Schell AM, Filion DL. The electrodermal system. In: Cacioppo JT, Tassinary LG, Berntson GG (eds). *Handbook of Psychophysiology*. Camebridge University Press: Camebridge, UK, 2000.

66. Lovibond PF, Shanks DR. The role of awareness in Pavlovian conditioning: Empirical evidence and theoretical implications. *Journal of Experimental Psychology-Animal Behavior Processes* 2002; **28**(1)**:** 3-26.

67. Tabbert K, Stark R, Kirsch P, Vaitl D. Dissociation of neural responses and skin conductance reactions during fear conditioning with and without awareness of stimulus contingencies. *Neuroimage* 2006; **32**(2)**:** 761-770.

68. Pavlov I. Conditioned Reflexes. Oxford University Press: London, 1927.

69. Mineka S, Zinbarg R. A contemporary learning theory perspective on the etiology of anxiety disorders - It's not what you thought it was. *American Psychologist* 2006; **61**(1)**:** 10-26.

70. Lissek S, Powers AS, McClure EB, Phelps EA, Woldehawariat G, Grillon C *et al*. Classical fear conditioning in the anxiety disorders: a meta-analysis. *Behaviour Research and Therapy* 2005; **43**(11)**:** 1391-1424.

71. Eysenck HJE. *Behavior therapy and the neuroses*. Pergamon Press: Oxford, England, 1969.

72. Eysenck HJ, Rachman S. *The causes and cures of neurosis*. Routledge and Kegan Paul: London, 1965.

73. Gorman JM, Kent JM, Sullivan GM, Coplan JD. Neuroanatomical hypothesis of panic disorder, revised. *American Journal of Psychiatry* 2000; **157**(4)**:** 493-505.

74. Öhman A, Flykt A, Lundqvist D. Unconscious emotion: Evolutionary perspectives, psychophysiological data and neuropsychological mechanisms. In: Lane R, Nadel L (eds). *The cognitive neuroscience of emotion*. Oxford University Press: New York, 2000, pp 296-327.

75. Bouton ME, Mineka S, Barlow DH. A modern learning theory perspective on the etiology of panic disorder. *Psychological Review* 2001; **108**(1)**:** 4-32.

76. Anderson KC, Insel BR. The promise of extinction research for the prevention and treatment of anxiety disorders. *Biological Psychiatry* 2006; **60**(4)**:** 319-321.

77. Foa EB, Franklin ME, Moser J. Context in the clinic: How well do cognitive-behavioral therapies and medications work in combination? *Biological Psychiatry* 2002; **52**(10)**:** 987-997.

78. Linden DEJ. How psychotherapy changes the brain - the contribution of functional neuroimaging. *Molecular Psychiatry* 2006; **11**(6)**:** 528-538.

79. Roffman JL, Marci CD, Glick DM, Dougherty DD, Rauch SL. Neuroimaging and the functional neuroanatomy of psychotherapy. *Psychological Medicine* 2005; **35**(10)**:** 1385-1398.

80. Furmark T, Tillfors M, Marteinsdottir I, Fischer H, Pissiota A, Langstrom B *et al.* Common changes in cerebral blood flow in patients with social phobia treated with citalopram or cognitive-behavioral therapy. *Archives of General Psychiatry* 2002; **59**(5)**:** 425-433.

81. Holmes A, Quirk GJ. Pharmacological facilitation of fear extinction and the search for adjunct treatments for anxiety disorders - the case of yohimbine. *Trends in Pharmacological Sciences*; **31**(1)**:** 2-7.

82. Norberg MM, Krystal JH, Tolin DF. A meta-analysis of D-Cycloserine and the facilitation of fear extinction and exposure therapy. *Biological Psychiatry* 2008; **63**(12)**:** 1118-1126.

83. Hofmann SG, Pollack MH, Otto MW. Augmentation treatment of psychotherapy for anxiety disorders with D-cycloserine. *Cns Drug Reviews* 2006; **12**(3-4)**:** 208-217.

84. Merrill KA, Steinmetz JE, Viken RJ, Rose RJ. Genetic influences on human conditionability: A twin study of the conditioned eyeblink response. *Behavior Genetics* 1999; **29**(2)**:** 95-102.

85. Hettema JM, Annas P, Neale MC, Fredrikson M, Kendler KS. A twin study of the genetics of fear conditioning. *Behavior Genetics* 2001; **31**(5)**:** 455-455.

86. Royce JR. AVOIDANCE CONDITIONING IN 9 STRAINS OF INBRED MICE USING OPTIMAL STIMULUS PARAMETERS. *Behavior Genetics* 1972; **2**(1)**:** 107-110.

87. Gordon JA, Hen R. Genetic approaches to the study of anxiety. *Annual Review of Neuroscience* 2004; **27:** 193-222.

88. Wehner JM, Radcliffe RA, Rosmann ST, Christensen SC, Rasmussen DL, Fulker DW *et al.* Quantitative trait locus analysis of contextual fear conditioning in mice. *Nature Genetics* 1997; **17**(3)**:** 331-334.

89. Caldarone B, Saavedra C, Tartaglia K, Wehner JM, Dudek BC, Flaherty L. Quantitative trait loci analysis affecting contextual conditioning in mice. *Nature Genetics* 1997; **17**(3)**:** 335-337.

90. Flint J, Corley R, Defries JC, Fulker DW, Gray JA, Miller S *et al.* A SIMPLE GENETIC-BASIS FOR A COMPLEX PSYCHOLOGICAL TRAIT IN LABORATORY MICE. *Science* 1995; **269**(5229)**:** 1432-1435.

91. Garpenstrand H, Annas P, Ekblom J, Oreland L, Fredrikson M. Human fear conditioning is related to dopaminergic and serotonergic biological markers. *Behav Neurosci* 2001; **115**(2)**:** 358-364.

92. Crisan LG, Pana S, Vulturar R, Heilman RM, Szekely R, Druga B *et al.* Genetic contributions of the serotonin transporter to social learning of fear and economic decision making. *Social Cognitive and Affective Neuroscience* 2009; **4**(4)**:** 399-408.

93. Batelaan NM, de Graaf R, Spijker J, Smit JH, van Balkom A, Vollebergh WAM *et al.* The course of panic attacks in individuals with panic disorder and subthreshold panic disorder: A population-based study. *Journal of Affective Disorders* 2010; **121**(1-2)**:** 30-38.

94. Kessler RC, McGonagle KA, Zhao SY, Nelson CB, Hughes M, Eshleman S *et al.* LIFETIME AND 12-MONTH PREVALENCE OF DSM-III-R PSYCHIATRIC-DISORDERS IN THE UNITED-STATES - RESULTS FROM THE NATIONAL-COMORBIDITY-SURVEY. *Archives of General Psychiatry* 1994; **51**(1)**:** 8-19.

95. Weissman MM, Bland RC, Canino GJ, Faravelli C, Greenwald S, Hwu HG *et al.* The cross-national epidemiology of panic disorder. *Archives of General Psychiatry* 1997; **54**(4)**:** 305-309.

96. Association AP. *Diagnostic and Statistical Maual of Mental Disorder - 4th Edition (DSM-IV)*. Psychiatric Press: Washington DC, 2000.

97. Wittchen HU, Nocon A, Beesdo K, Pine DS, Hofler M, Lieb R *et al*. Agoraphobia and panic. *Psychotherapy and Psychosomatics* 2008; **77**(3)**:** 147-157.

98. Bienvenu OJ, Stein MB, Samuels JF, Onyike CU, Eaton WW, Nestadt G. Personality disorder traits as predictors of subsequent first-onset panic disorder or agoraphobia. *Comprehensive Psychiatry* 2009; **50**(3)**:** 209-214.

99. Association AP. Panic disorder - proposed revision. *DSM-5 development (wwdsm5org) retrieved 15 march 2010* 2010.

100. Smoller JW, Tsuang MT. Panic and phobic anxiety: Defining phenotypes for genetic studies. *American Journal of Psychiatry* 1998; **155**(9)**:** 1152-1162.

101. van West D, Claes S. The genetics of panic disorder: state of the art. *Acta Neuropsychiatrica* 2004; **16**(2)**:** 68-78.

102. Gratacos M, Sahun I, Gallego X, Amador-Arjona A, Estivill X, Dierssen M. Candidate genes for panic disorder: insight from human and mouse genetic studies. *Genes Brain and Behavior* 2007; **6:** 2-23.

103. ShinnickGallagher P, Pitkanen A, Shekhar A, Cahill L, (eds). Brain imaging studies in mood and anxiety disorders - Special emphasis on the amygdala. *Proceedings of the Conference on the Amygdala in Brain Function*; Jun 24-26 2002; Galveston, Texas.

104. van den Heuvel OA, van de Wetering BJM, Veltman DJ, Pauls DL. Genetic studies of panic disorder: A review. *Journal of Clinical Psychiatry* 2000; **61**(10)**:** 756-766.

105. Hettema JM, Neale MC, Kendler KS. A review and meta-analysis of the genetic epidemiology of anxiety disorders. *American Journal of Psychiatry* 2001; **158**(10)**:** 1568-1578.

106. Smoller JW, Block SR, Young MM. GENETICS OF ANXIETY DISORDERS: THE COMPLEX ROAD FROM DSM TO DNA. *Depress Anxiety* 2009; **26**(11)**:** 965-975.

107. Kolassa IT, Kolassa S, Ertl V, Papassotiropoulos A, De Quervain DJF. The Risk of Posttraumatic Stress Disorder After Trauma Depends on Traumatic Load and the Catechol-O-Methyltransferase Val(158)Met Polymorphism. *Biological Psychiatry*; **67**(4)**:** 304-308.

108. Bryant RA, Felmingham KL, Falconer EM, Pe Benito L, Dobson-Stone C, Pierce KD *et al.* Preliminary Evidence of the Short Allele of the Serotonin Transporter Gene Predicting Poor Response to Cognitive Behavior Therapy in Posttraumatic Stress Disorder. *Biological Psychiatry* (in press).

109. Mitte K. A meta-analysis of the efficacy of psycho- and pharmacotherapy in panic disorder with and without agoraphobia. *J Affect Disord* 2005; **88:** 27-45.

110. Association AP. Practice guideline for the treatment of patients with panic disorder. Work group on panic disorder. *The American Journal of Psychiatry* 1998; **155**(5 Suppl.)**:** 1-34.

111. Ballenger JC, Burrows GD, Dupont RL, Lesser IM, Noyes R, Pecknold JC *et al*. ALPRAZOLAM IN PANIC DISORDER AND AGORAPHOBIA - RESULTS FROM A MULTICENTER TRIAL .1. EFFICACY IN SHORT-TERM TREATMENT. *Archives of General Psychiatry* 1988; **45**(5)**:** 413-422.

112. Riederer P, Lachenmayer L, Laux G. Clinical applications of MAO-inhibitors. *Current Medicinal Chemistry* 2004; **11**(15)**:** 2033-2043.

113. Klein DF. DELINEATION OF 2 DRUG-RESPONSIVE ANXIETY SYNDROMES. *Psychopharmacologia* 1964; **5**(6)**:** 397-408.

114. Westen D, Morrison K. A multidimensional meta-analysis of treatments for depression, panic, and generalized anxiety disorder: An empirical examination of the status of empirically supported therapies. *J Consult Clin Psychol* 2001; **69:** 875-899.

115. Bandelow B, Seidler-Brandler U, Becker A, Wedekind D, Ruther E. Meta-analysis of randomized controlled comparisons of psychopharmacological and psychological treatments for anxiety disorders. *World Journal of Biological Psychiatry* 2007; **8**(3)**:** 175-187.

116. Postel MG, de Haan HA, De Jong CA. E-therapy for mental health problems: a systematic review. *Telemed J E Health* 2008; **14:** 707-714.

117. Clark DM, Layard R, Smithies R, Richards DA, Suckling R, Wright B. Improving access to psychological therapy: Initial evaluation of two UK demonstration sites. *Behaviour Research and Therapy* 2009; **47**(11)**:** 910-920.

118. Carlbring P, Westling BE, Ljungstrand P, Ekselius L, Andersson G. Treatment of panic disorder via the Internet- a randomized trial of a self-help program. *Behav Therap* 2001; **32:** 751-764.

119. Schneider AJ, Mataix-Cols D, Marks IM, Bachofen M. Internet-guided self-help with or without exposure therapy for phobic and panic disorders. *Psychother Psychosom* 2005; **74:** 154-164.

120. Carlbring P, Bohman S, Brunt S, Buhrman M, Westling BE, Ekselius L *et al.* Remote treatment of panic disorder: A randomized trial of Internet-based cognitive behavioral therapy supplemented with telephone calls. *Am J Psychiatry* 2006; **163:** 21.

121. Klein B, Richards JC, Austin DW. Efficacy of internet therapy for panic disorder. *J Behav Ther Exp Psychiatry* 2006; **37:** 213-238.

122. Shandley K, Austin DW, Klein B, Pier C, Schattner P, Pierce D *et al.* Therapist-assisted, Internet-based treatment for panic disorder: can general practitioners achieve comparable patient outcomes to psychologists? *J Med Internet Res* 2008; **10**(2)**:** e14.

123. Carlbring P, Nilsson-Ihrfelt E, Waara J, Kollenstam C, Buhrman M, Kaldo V *et al.* Treatment of panic disorder: live therapy vs. self-help via the Internet. *Behaviour Research and Therapy* 2005; **43**(10)**:** 1321-1333.

124. Kiropoulos LA, Klein B, Austin DW, Gilson K, Pier C, Mitchell J *et al.* Is internet-based CBT for panic disorder and agoraphobia as effective as face-to-face CBT? *J Anxiety Disord* 2008; **22**(8)**:** 1273-1284.

125. Bergström J, Andersson G, Ljotsson B, Rück C, Andreewitch S, Karlsson A *et al.* Internet- vesrus group-administered cognitive behaviour therapy for panic disorder in a psychiatric setting: a randomizd trial. *submitted manuscript*.

126. Rapport MM, Green AA, Page IH. Serum Vasoconstrictor (Serotonin) .4. Isolation and Characterization. *Journal of Biological Chemistry* 1948; **176**(3)**:** 1243-1251.

127. Lucki I. The spectrum of behaviors influenced by serotonin. *Biological Psychiatry* 1998; **44**(3)**:** 151-162.

128. Azmitia EC. Serotonin and brain: Evolution, neuroplasticity, and homeostasis. *Pharmacology of Neurogenesis and Neuroenhancement*, vol. 77, 2007, pp 31-+.

129. Lesch KP. Linking emotion to the social brain - The role of the serotonin transporter in human social behaviour. *Embo Reports* 2007; **8:** S24-S29.

130. Kim JJ. Serotonin, Stress, and Conditioning. *Biological Psychiatry* 2008; (63)**:** 819-820.

131. Phelps EA. Emotion and cognition: Insights from studies of the human amygdala. *Annual Review of Psychology* 2006; **57:** 27-53.

132. Burghardt NS, Bush DEA, McEwen BS, LeDoux JE. Acute selective serotonin Reuptake inhibitors increase conditioned fear expression: Blockade with a 5-HT2C receptor antagonist. *Biological Psychiatry* 2007; **62**(10)**:** 1111-1118.

133. Burghardt NS, Sullivan GM, McEwen BS, Gorman JM, LeDoux JE. The selective serotonin reuptake inhibitor citalopram increases fear after acute treatment but reduces fear with chronic treatment: A comparison with tianeptine. *Biological Psychiatry* 2004; **55**(12)**:** 1171-1178.

134. Schmitt JAJ, Wingen M, Ramaekers JG, Evers EAT, Riedel WJ. Serotonin and human cognitive performance. *Current Pharmaceutical Design* 2006; **12**(20)**:** 2473-2486.

135. Walther DJ, Peter JU, Bashammakh S, Hortnagl H, Voits M, Fink H *et al.* Synthesis of serotonin by a second tryptophan hydroxylase isoform. *Science* 2003; **299**(5603)**:** 76-76.

136. Barnes NM, Sharp T. A review of central 5-HT receptors and their function. *Neuropharmacology* 1999; **38**(8)**:** 1083-1152.

137. Ramamoorthy S, Bauman AL, Moore KR, Han H, Yangfeng T, Chang AS *et al.* ANTIDEPRESSANT-SENSITIVE AND COCAINE-SENSITIVE HUMAN SEROTONIN TRANSPORTER - MOLECULAR-CLONING, EXPRESSION, AND CHROMOSOMAL LOCALIZATION. *Proc Natl Acad Sci U S A* 1993; **90**(6)**:** 2542-2546.

138. Bradley CC, Blakely RD. Alternative splicing of the human serotonin transporter gene. *Journal of Neurochemistry* 1997; **69**(4)**:** 1356-1367.

139. Uher R, McGuffin P. The moderation by the serotonin transporter gene of environmental adversity in the aetiology of mental illness: review and methodological analysis. *Mol Psychiatry* 2007; **13**(2)**:** 131-146.

140. Gelernter J, Kranzler H, Cubells JF. Serotonin transporter protein (SLC6A4) allele and haplotype frequencies and linkage disequilibria in African- and European-American and Japanese populations and in alcohol-dependent subjects. *Hum Genet* 1997; **101**(2)**:** 243-246.

141. Murphy DL, Lerner A, Rudnick G, Lesch KP. Serotonin transporter: Gene, genetic disorders, and pharmacogenetics. *Molecular Interventions* 2004; **4**(2)**:** 109-123.

142. Heils A, Teufel A, Petri S, Seemann M, Bengel D, Balling U *et al.* Functional promoter and polyadenylation site mapping of the human serotonin (5-HT) transporter gene. *Journal of Neural Transmission-General Section* 1995; **102**(3)**:** 247-254.

143. Reimold M, Smolka MN, Schumann G, Zimmer A, Wrase J, Mann K *et al.* Midbrain serotonin transporter binding potential measured with [C-11]DASB is affected by serotonin transporter genotype. *Journal of Neural Transmission* 2007; **114**(5)**:** 635-639.

144. Little KY, McLaughlin DP, Zhang L, Livermore CS, Dalack GW, McFinton PR *et al.* Cocaine, ethanol, and genotype effects on human midbrain serotonin transporter binding sites and mRNA levels. *American Journal of Psychiatry* 1998; **155**(2)**:** 207-213.

145. Van Dyck CH, Malison RT, Staley JK, Jacobsen LK, Seibyl JP, Laruelle M *et al.* Central serotonin transporter availability measured with [I-123]beta-CIT SPECT in relation to serotonin transporter genotype. *American Journal of Psychiatry* 2004; **161**(3)**:** 525-531.

146. Parsey RV, Hastings RS, Oquendo MA, Hu XZ, Goldman D, Huang YY *et al.* Effect of a triallelic functional polymorphism of the serotonin-transporter-linked promoter region on expression of serotonin transporter in the human brain. *American Journal of Psychiatry* 2006; **163**(1)**:** 48-51.

147. Kraft JB, Slager SL, McGrath PJ, Hamilton SP. Sequence analysis of the serotonin transporter and associations with antidepressant response. *Biological Psychiatry* 2005; **58**(5)**:** 374-381.

148. Wendland JR, Martin BJ, Kruse MR, Lesch KP, Murphy DL. Simultaneous genotyping of four functional loci of human SLC6A4, with a reappraisal of 5-HTTLPR and rs25531. *Molecular Psychiatry* 2006; **11**(3)**:** 224-226.

149. Hu XZ, Lipsky RH, Zhu GS, Akhtar LA, Taubman J, Greenberg BD *et al.* Serotonin transporter promoter gain-of-function genotypes are linked to obsessive-compulsive disorder. *Am J Hum Genet* 2006; **78**(5)**:** 815-826.

150. Martin J, Cleak J, Willis-Owen SAG, Flint J, Shifman S. Mapping regulatory variants for the serotonin transporter gene based on allelic expression imbalance. *Molecular Psychiatry* 2007; **12**(5)**:** 421-422.

151. Nakamura M, Ueno S, Sano A, Tanabe H. The human serotonin transporter gene linked polymorphism (5-HTTLPR) shows ten novel allelic variants. *Molecular Psychiatry* 2000; **5**(1)**:** 32-38.

152. Praschak-Rieder N, Kennedy J, Wilson AA, Hussey D, Boovariwala A, Willeit M *et al.* Novel 5-HTTLPR allele associates with higher serotonin transporter binding in putamen: A [C-11] DASB positron emission tomography study. *Biological Psychiatry* 2007; **62**(4)**:** 327-331.

153. Philibert RA, Sandhu H, Hollenbeck N, Gunter T, Adams W, Madan A. The relationship of 5HTT (SLC6A4) methylation and genotype on mRNA expression and liability to major depression and alcohol dependence in subjects from the Iowa Adoption Studies. *American Journal of Medical Genetics Part B-Neuropsychiatric Genetics* 2008; **147B**(5)**:** 543-549.

154. Murphy DL, Fox MA, Timpano KR, Moya PR, Ren-Patterson R, Andrews AM *et al.* How the serotonin story is being rewritten by new gene-based discoveries principally related to SLC6A4, the serotonin transporter gene, which functions to influence all cellular serotonin systems. *Neuropharmacology* 2008; **55**(6)**:** 932-960.

155. Wendland JR, Moya PR, Kruse MR, Ren-Patterson RF, Jensen CL, Timpano KR *et al.* A novel, putative gain-of-function haplotype at SLC6A4 associates with obsessive-compulsive disorder. *Human Molecular Genetics* 2008; **17**(5)**:** 717-723.

156. Sen S, Burmeister M, Ghosh D. Meta-analysis of the association between a serotonin transporter promoter polymorphism (5-HTTLPR) and anxiety-related personality traits. *American Journal of Medical Genetics Part B-Neuropsychiatric Genetics* 2004; **127B**(1)**:** 85-89.

157. Schinka JA, Busch RM, Robichaux-Keene N. A meta-analysis of the association between the serotonin transporter gene polymorphism (5-HTTLPR) and trait anxiety. *Molecular Psychiatry* 2004; **9**(2)**:** 197-202.

158. Sen S, Burmeister M, Ghosh D. 5-HTTLPR and anxiety-related personality traits meta-analysis revisited: response to Munafo and colleagues. *Molecular Psychiatry* 2005; **10**(10)**:** 893-895.

159. Munafo MR, Freimer NB, Ng W, Ophoff R, Veijola J, Miettunen J *et al.* 5-HTTLPR Genotype and Anxiety-Related Personality Traits: A Meta-Analysis and New Data. *American Journal of Medical Genetics Part B-Neuropsychiatric Genetics* 2009; **150B**(2)**:** 271-281.

160. Beevers CG, Gibb BE, McGeary JE, Miller IW. Serotonin transporter genetic variation and biased attention for emotional word stimuli among psychiatric inpatients. *Journal of Abnormal Psychology* 2007; **116**(1)**:** 208-212.

161. Osinsky R, Reuter M, Kupper Y, Schmitz A, Kozyra E, Alexander N *et al.* Variation in the serotonin transporter gene modulates selective attention to threat. *Emotion* 2008; **8**(4)**:** 584-588.

162. Pérez-Edgar K, Bar-Haim Y, McDermott JM, Gorodetsky E, Hodgkinson CA, Goldman D *et al.* Variations in the serotonin-transporter gene are associated with attention bias patterns to positive and negative emotion faces. *Biological Psychology*; (in press).

163. Fox E, Ridgewell A, Ashwin C. Looking on the bright side: biased attention and the human serotonin transporter gene. *Proceedings of the Royal Society B-Biological Sciences* 2009; **276**(1663)**:** 1747-1751.

164. Goodyer IM, Bacon A, Ban M, Croudace T, Herbert J. Serotonin transporter genotype, morning cortisol and subsequent depression in adolescents. *British Journal of Psychiatry* 2009; **195**(1)**:** 39-45.

165. Wust S, Kumsta R, Treutlein J, Frank J, Entringer S, Schulze TG *et al.* Sex-specific association between the 5-HTT gene-linked polymorphic region and basal cortisol secretion. *Psychoneuroendocrinology* 2009; **34**(7)**:** 972-982.

166. Gotlib IH, Joormann J, Minor KL, Hallmayer J. HPA axis reactivity: A mechanism underlying the associations among 5-HTTLPR, stress, and depression. *Biological Psychiatry* 2008; **63**(9)**:** 847-851.

167. Mueller A, Brocke B, Fries E, Lesch K-P, Kirschbaum C. The role of the serotonin transporter polymorphism for the endocrine stress response in newborns. *Psychoneuroendocrinology* (in press).

168. Alexander N, Kuepper Y, Schmitz A, Osinsky R, Kozyra E, Hennig J. Gene-environment interactions predict cortisol responses after acute stress: implications for the etiology of depression. *Psychoneuroendocrinology* 2009; **34**(9)**:** 1294-1303.

169. Caspi A, Hariri AR, Holmes A, Uher R, TE M. Genetic Sensitivity to the Environment: The Case of the Serotonin Transporter Gene and Its Implications for Studying Complex Diseases and Traits. *American Journal of Psychiatry* 2010; Epub ahead of print.

170. Hariri AR, Drabant EM, Munoz KE, Kolachana LS, Mattay VS, Egan MF *et al.* A susceptibility gene for affective disorders and the response of the human amygdala. *Archives of General Psychiatry* 2005; **62**(2)**:** 146-152.

171. Hariri AR, Mattay VS, Tessitore A, Kolachana B, Fera F, Goldman D *et al.* Serotonin transporter genetic variation and the response of the human amygdala. *Science* 2002; **297**(5580)**:** 400-403.

172. Bertolino A, Arciero G, Rubino V, Latorre V, De Candia M, Mazzola V *et al.* Variation of human amygdala response during threatening stimuli as a function of 5'HTTLPR genotype and personality style. *Biological Psychiatry* 2005; **57**(12)**:** 1517-1525.

173. Smolka MN, Buhler M, Schumann G, Klein S, Hu XZ, Moayer M *et al.* Gene-gene effects on central processing of aversive stimuli. *Molecular Psychiatry* 2007; **12**(3)**:** 307-317.

174. Dannlowski U, Ohrmann P, Bauer J, Deckert J, Hohoff C, Kugel H *et al.* 5-HTTLPR biases amygdala activity in response to masked facial expressions in major depression. *Neuropsychopharmacology* 2008; **33**(2)**:** 418-424.

175. Dannlowski U, Ohrmann P, Bauer J, Kugel H, Baune BT, Hohoff C *et al.* Serotonergic genes modulate amygdala activity in major depression. *Genes Brain and Behavior* 2007; **6**(7)**:** 672-676.

176. Surguladze SA, Elkin A, Ecker C, Kalidindi S, Corsico A, Giampietro V *et al.* Genetic variation in the serotonin transporter modulates neural system-wide response to fearful faces. *Genes Brain and Behavior* 2008; **7**(5)**:** 543-551.

177. Furmark T, Henningsson S, Appel L, Ahs F, Linnman C, Pissiota A *et al.* Genotype over-diagnosis in amygdala responsiveness: affective processing in social anxiety disorder. *Journal of Psychiatry & Neuroscience* 2009; **34**(1)**:** 30-40.

178. Canli T, Congdon E, Constable RT, Lesch KP. Additive effects of serotonin transporter and tryptophan hydroxylase-2 gene variation on neural correlates of affective processing. *Biological Psychology* 2008; **79**(1)**:** 118-125.

179. Furmark T, Tillfors M, Garpenstrand H, Marteinsdottir I, Langstrom B, Oreland L *et al.* Serotonin transporter polymorphism related to amygdala excitability and symptom severity in patients with social phobia. *Neuroscience Letters* 2004; **362**(3)**:** 189-192.

180. Domschke K, Braun M, Ohrmann P, Suslow T, Kugel H, Bauer J *et al.* Association of the functional-1019C/G 5-HT1A polymorphism with prefrontal cortex and amygdala activation measured with 3 T fMRI in panic disorder. *International Journal of Neuropsychopharmacology* 2006; **9**(3)**:** 349-355.

181. Munafo MR, Brown SM, Hariri AR. Serotonin transporter (5-HTTLPR) genotype and amygdala activation: A meta-analysis. *Biological Psychiatry* 2008; **63**(9)**:** 852-857.

182. Canli T, Omura K, Haas BW, Fallgatter A, Constable RT, Lesch KP. Beyond affect: A role for genetic variation of the serotonin transporter in neural activation during a cognitive attention task. *Proceedings of the National Academy of Sciences of the United States of America* 2005; **102**(34)**:** 12224-12229.

183. Canli T, Qiu M, Omura K, Congdon E, Haas BW, Amin Z *et al.* Neural correlates of epigenesis. *Proc Natl Acad Sci U S A* 2006; **103**(43)**:** 16033-16038.

184. Canli T, Lesch KP. Long story short: the serotonin transporter in emotion regulation and social cognition. *Nature Neuroscience* 2007; **10**(9)**:** 1103-1109.

185. Rao HY, Gillihan SJ, Wang JJ, Korczykowski M, Sankoorikal GMV, Kaercher KA *et al.* Genetic variation in serotonin transporter alters resting brain function in healthy individuals. *Biological Psychiatry* 2007; **62**(6)**:** 600-606.

186. Pezawas L, Meyer-Lindenberg A, Drabant EM, Verchinski BA, Munoz KE, Kolachana BS *et al.* 5-HTTLPR polymorphism impacts human cingulate-amygdala interactions: a genetic susceptibility mechanism for depression. *Nature Neuroscience* 2005; **8**(6)**:** 828-834.

187. Heinz A, Braus DF, Smolka MN, Wrase J, Puls I, Hermann D *et al.* Amygdala-prefrontal coupling depends on a genetic variation of the serotonin transporter. *Nature Neuroscience* 2005; **8**(1)**:** 20-21.

188. Canli T, Ferri J, Duman EA. GENETICS OF EMOTION REGULATION. *Neuroscience* 2009; **164**(1)**:** 43-54.

189. Pacheco J, Beevers CG, Benavides C, McGeary J, Stice E, Schnyer DM. Frontal-Limbic White Matter Pathway Associations with the Serotonin Transporter Gene Promoter Region (5-HTTLPR) Polymorphism. *Journal of Neuroscience* 2009; **29**(19)**:** 6229-6233.

190. Young KA, Holcomb LA, Bonkale WL, Hicks PB, Yazdani U, German DC. 5HTTLPR polymorphism and enlargement of the pulvinar: Unlocking the backdoor to the limbic system. *Biological Psychiatry* 2007; **61**(6)**:** 813-818.

191. Caspi A, Sugden K, Moffitt TE, Taylor A, Craig IW, Harrington H *et al.* Influence of life stress on depression: Moderation by a polymorphism in the 5-HTT gene. *Science* 2003; **301**(5631)**:** 386-389.

192. Risch N, Herrell R, Lehner T, Liang KY, Eaves L, Hoh J *et al.* Interaction Between the Serotonin Transporter Gene (5-HTTLPR), Stressful Life Events, and Risk of Depression A Meta-analysis. *JAMA-J Am Med Assoc* 2009; **301**(23)**:** 2462-2471.

193. Lesch KP. Serotonin transporter and psychiatric disorders: Listening to the gene. *Neuroscientist* 1998; **4**(1)**:** 25-34.

194. Lin PY. Meta-analysis of the association of serotonin transporter gene polymorphism with obsessive-compulsive disorder. *Progress in Neuro-Psychopharmacology & Biological Psychiatry* 2007; **31**(3)**:** 683-689.

195. Arango V, Huang YY, Underwood MD, Mann JJ. Genetics of the serotonergic system in suicidal behavior. *Journal of Psychiatric Research* 2003; **37**(5)**:** 375-386.

196. Colucci R, Blandizzi C, Bellini M, Ghisu N, Tonini M, Del Tacca M. The genetics of the serotonin transporter and irritable bowel syndrome. *Trends in Molecular Medicine* 2008; **14**(7)**:** 295-304.

197. Deckert J, Catalano M, Heils A, DiBella D, Friess F, Politi E *et al.* Functional promoter polymorphism of the human serotonin transporter: Lack of association with panic disorder. *Psychiatric Genetics* 1997; **7**(1)**:** 45-47.

198. Kim W, Choi YH, Yoon KS, Cho DY, Pae CU, Woo JM. Tryptophan hydroxylase and serotonin transporter gene polymorphism does not affect the diagnosis, clinical features and treatment outcome of panic disorder in the Korean population. *Progress in Neuro-Psychopharmacology & Biological Psychiatry* 2006; **30**(8)**:** 1413-1418.

199. Matsushita S, Muramatsu T, Kimura M, Shirakawa O, Mita T, Nakai T *et al.* Serotonin transporter gene regulatory region polymorphism and panic disorder. *Molecular Psychiatry* 1997; **2**(5)**:** 390-391.

200. Ishiguro H, Arinami T, Yamada K, Otsuka Y, Toru M, Shibuya H. An association study between a transcriptional polymorphism in the serotonin transporter gene and panic disorder in a Japanese population. *Psychiatry Clin Neurosci* 1997; **51**(5)**:** 333-335.

201. Olesen OF, Bennike B, Hansen ES, Koefoed P, Woldbye DP, Bolwig TG *et al.* The short/long polymorphism in the serotonin transporter gene promoter is not associated with panic disorder in a Scandinavian sample. *Psychiatric Genetics* 2005; **15**(3)**:** 159-159.

202. Blaya C, Salum GA, Lima MS, Leistner-Segal S, Manfro GG. Lack of association between the Serotonin Transporter Promoter Polymorphism (5-HTTLPR) and Panic Disorder: a systematic review and meta-analysis. *Behavioral and Brain Functions* 2007; **3**.

203. Maron E, Lang A, Tasa G, Liivlaid L, Toru I, Must A *et al.* Associations between serotonin-related gene polymorphisms and panic disorder. *International Journal of Neuropsychopharmacology* 2005; **8**(2)**:** 261-266.

204. Hamilton SP, Heiman GA, Haghighi F, Mick S, Klein DF, Hodge SE *et al.* Lack of genetic linkage or association between a functional serotonin transporter polymorphism and panic disorder. *Psychiatric Genetics* 1999; **9**(1)**:** 1-6.

205. Serretti A, Kato M, De Ronchi D, Kinoshita T. Meta-analysis of serotonin transporter gene promoter polymorphism (5-HTTLPR) association with selective serotonin reuptake inhibitor efficacy in depressed patients. *Molecular Psychiatry* 2007; **12**(3)**:** 247-257.

206. Perna G, Favaron E, Di Bella D, Bussi R, Bellodi L. Antipanic efficacy of paroxetine and polymorphism within the promoter of the serotonin transporter gene. *Neuropsychopharmacology* 2005; **30**(12)**:** 2230-2235.

207. Benes FM. Arvid Carlsson and the discovery of dopamine. *Trends in Pharmacological Sciences* 2001; **22**(1)**:** 46-47.

208. Schultz W. Getting formal with dopamine and reward. *Neuron* 2002; **36**(2)**:** 241-263.

209. Gorman JM. Discerning disorders and diseases. *Cns Spectrums* 2004; **9**(9)**:** 632-632.

210. Pezze MA, Feldon J. Mesolimbic dopaminergic pathways in fear conditioning. *Progress in Neurobiology* 2004; **74**(5)**:** 301-320.

211. Grace AA, Rosenkranz JA. Regulation of conditioned responses of basolateral amygdala neurons. *Physiology & Behavior* 2002; **77**(4-5)**:** 489-493.

212. Missale C, Nash SR, Robinson SW, Jaber M, Caron MG. Dopamine receptors: From structure to function. *Physiological Reviews* 1998; **78**(1)**:** 189-225.

213. Axelrod J, Tomchick R. ENZYMATIC O-METHYLATION OF EPINEPHRINE AND OTHER CATECHOLS. *Journal of Biological Chemistry* 1958; **233**(3)**:** 702-705.

214. Tunbridge EM, Harrison PJ, Weinberger DR. Catechol-o-methyltransferase, cognition, and psychosis: Val(158)Met and beyond. *Biological Psychiatry* 2006; **60**(2)**:** 141-151.

215. Lachman HM, Papolos DF, Saito T, Yu YM, Szumlanski CL, Weinshilboum RM. Human catechol-O-methyltransferase pharmacogenetics: Description of a functional polymorphism and its potential application to neuropsychiatric disorders. *Pharmacogenetics* 1996; **6**(3)**:** 243-250.

216. Tenhunen J, Salminen M, Lundstrom K, Kiviluoto T, Savolainen R, Ulmanen I. GENOMIC ORGANIZATION OF THE HUMAN CATECHOL O-METHYLTRANSFERASE GENE AND ITS EXPRESSION FROM 2 DISTINCT PROMOTERS. *European Journal of Biochemistry* 1994; **223**(3)**:** 1049-1059.

217. Jiang H, Ho SL, Xie T, Young LP. Distribution of catechol-O-methyltransferase expression in human central nervous system. *Neuroreport* 1998; **9**(12)**:** 2861-2864.

218. Fahndrich E, Coper H, Christ W, Helmchen H, Mulleroerlinghausen B, Pietzcker A. ERYTHROCYTE COMT-ACTIVITY IN PATIENTS WITH AFFECTIVE-DISORDERS. *Acta Psychiatrica Scandinavica* 1980; **61**(5)**:** 427-437.

219. Boudikova B, Szumlanski C, Maidak B, Weinshilboum R. CATECHOL O-METHYLTRANSFERASE (COMT) PHARMACOGENETICS - CORRELATION OF HEPATIC AND RED-BLOOD-CELL (RBC) THERMAL-STABILITY. *Clinical Pharmacology & Therapeutics* 1990; **47**(2)**:** 131-131.

220. Xie T, Ho SL, Ramsden D. Characterization and implications of estrogenic down-regulation of human catechol-O-methyltransferase gene transcription. *Molecular Pharmacology* 1999; **56**(1)**:** 31-38.

221. Heinz A, Smolka MN. The effects of catechol O-methyltransferase genotype on brain activation elicited by affective stimuli and cognitive tasks. *Reviews in the Neurosciences* 2006; **17**(3)**:** 359-367.

222. Palmatier MA, Kang AM, Kidd KK. Global variation in the frequencies of functionally different catechol-O-methyltransferase alleles. *Biological Psychiatry* 1999; **46**(4)**:** 557-567.

223. Weinshilboum RM, Otterness DM, Szumlanski CL. Methylation pharmacogenetics: Catechol O-methyltransferase, thiopurine methyltransferase, and histamine N-methyltransferase. *Annual Review of Pharmacology and Toxicology* 1999; **39:** 19-52.

224. Weinshilboum RM, Raymond FA. INHERITANCE OF LOW ERYTHROCYTE CATECHOL-O-METHYLTRANSFERASE ACTIVITY IN MAN. *Am J Hum Genet* 1977; **29**(2)**:** 125-135.

225. Harrison PJ, Tunbridge EM. Catechol-O-Methyltransferase (COMT): A Gene Contributing to Sex Differences in Brain Function, and to Sexual Dimorphism in the Predisposition to Psychiatric Disorders. *Neuropsychopharmacology* 2008; **33**(13)**:** 3037-3045.

226. Egan MF, Goldberg TE, Kolachana BS, Callicott JH, Mazzanti CM, Straub RE *et al.* Effect of COMT Val(108/158) Met genotype on frontal lobe function and risk for schizophrenia. *Proc Natl Acad Sci U S A* 2001; **98**(12)**:** 6917-6922.

227. Barnett JH, Jones PB, Robbins TW, Muller U. Effects of the catechol-O-methyltransferase Val(158)Met polymorphism on executive function: a meta-analysis of the Wisconsin Card Sort Test in schizophrenia and healthy controls. *Molecular Psychiatry* 2007; **12**(5)**:** 502-509.

228. Barnett JH, Scoriels L, Munafo MR. Meta-analysis of the cognitive effects of the catechol-O-methyltransferase gene val158/108Met polymorphism. *Biological Psychiatry* 2008; **64**(2)**:** 137-144.

229. Montag C, Hartmann P, Buckholtz JW, Merz M, Burk C, Hennig J *et al.* COMT genetic variation affects fear processing: Psychophysiological evidence. *Behav Neurosci* 2008; **122**(4)**:** 901-909.

230. Nolan KA, Bilder RM, Lachman HM, Volavka J. Catechol O-methyltransferase Val(158)Met polylmorphism in schizophrenia: Differential effects of Val and Met alleles on cognitive stability and flexibility. *American Journal of Psychiatry* 2004; **161**(2)**:** 359-361.

231. Bilder RM, Volavka J, Lachman HM, Grace AA. The catechol-O-methyltransferase polymorphism: Relations to the tonic-phasic dopamine hypothesis and neuropsychiatric phenotypes. *Neuropsychopharmacology* 2004; **29**(11)**:** 1943-1961.

232. Craddock N, Owen MJ, O'Donovan MC. The catechol-O-methyl transferase (COMT) gene as a candidate for psychiatric phenotypes: evidence and lessons. *Molecular Psychiatry* 2006; **11**(5)**:** 446-458.

233. Smolka MN, Schumann G, Wrase J, Grusser SM, Flor H, Mann K *et al.* Catechol-O-methyltransferase val(158)met genotype affects processing of emotional stimuli in the amygdala and prefrontal cortex. *Journal of Neuroscience* 2005; **25**(4)**:** 836-842.

234. Skuse D. Genetic influences on the neural basis of social cognition. *Philos Trans R Soc B-Biol Sci* 2006; **361**(1476)**:** 2129-2141.

235. Herrmann MJ, Wurflein H, Schreppel T, Koehler S, Muhlberger A, Reif A *et al.* Catechol-O-methyltransferase Val(158)Met genotype affects neural correlates of aversive stimuli processing. *Cognitive Affective & Behavioral Neuroscience* 2009; **9**(2)**:** 168-172.

236. Rosenkranz JA, Grace AA. Dopamine attenuates prefrontal cortical suppression of senory inputs to the basolateral amygdala of rats. *Journal of Neuroscience* 2001; **21:** 4090-4103.

237. Domschke K, Freitag CM, Kuhlenbaumer G, Schirmacher A, Sand P, Nyhuis P *et al.* Association of the functional V158M catechol-O-methyl-transferase polymorphism with panic disorder in women. *International Journal of Neuropsychopharmacology* 2004; **7**(2)**:** 183-188.

238. Rothe C, Koszycki D, Bradwejn J, King N, Deluca V, Tharmalingam S *et al.* Association of the Val158Met catechol O-methyltransferase genetic polymorphism with panic disorder. *Neuropsychopharmacology* 2006; **31**(10)**:** 2237-2242.

239. Evidence for a susceptibility locus for panic disorder near the catechol-O-methyltransferase gene on chromosome 22. *Proceedings of the 39th Annual Meeting of the American-College-of-Neuropsychopharmacology*; Dec 09-14 2000; San Juan, Puerto Rico.

240. Woo JM, Yoon KS, Yu BH. Catechol O-methyltransferase genetic polymorphism in panic disorder. *American Journal of Psychiatry* 2002; **159**(10)**:** 1785-1787.

241. Woo JM, Yoon KS, Choi YH, Oh KS, Lee YS, Yu BH. The association between panic disorder and the L/L genotype of catechol-O-methyltransferase. *Journal of Psychiatric Research* 2004; **38**(4)**:** 365-370.

242. Ohara K, Nagai M, Suzuki Y, Ochiai M, Ohara K. No association between anxiety disorders and catechol-O-methyltransferase polymorphism. *Psychiatry Research* 1998; **80**(2)**:** 145-148.

243. Domschke K, Deckert J, O'Donovan MC, Glatt SJ. Meta-analysis of COMT val158met in panic disorder: Ethnic heterogeneity and gender specificity. *American Journal of Medical Genetics Part B-Neuropsychiatric Genetics* 2007; **144B**(5)**:** 667-673.

244. Anttila S, Huuhka K, Huuhka M, Illi A, Rontu R, Leinonen E *et al.* Catechol-O-methyltransferase (COMT) polymorphisms predict treatment response in electroconvulsive therapy. *Pharmacogenomics Journal* 2008; **8**(2)**:** 113-116.

245. Szegedi A, Rujescu D, Tadic A, Muller MJ, Kohnen R, Stassen HH *et al.* The catechol-O-methyltransferase Val(108/158)Met polymorphism affects short-term treatment response to mirtazapine, but not to paroxetine in major depression. *Pharmacogenomics Journal* 2005; **5**(1)**:** 49-53.

246. Arias B, Serretti A, Lorenzi C, Gasto C, Catalan R, Fananas L. Analysis of COMT gene (Val 158 Met polymorphism) in the clinical response to SSRIs in depressive patients of European origin. *Journal of Affective Disorders* 2006; **90**(2-3)**:** 251-256.

247. Inada T, Nakamura A, Iijima Y. Relationship between catechol-O-methyltransferase polymorphism and treatment-resistant schizophrenia. *American Journal of Medical Genetics Part B-Neuropsychiatric Genetics* 2003; **120B**(1)**:** 35-39.

248. Poo MM. Neurotrophins as synaptic modulators. *Nature Reviews Neuroscience* 2001; **2**(1)**:** 24-32.

249. Lu B, Pang PT, Woo NH. The yin and yang of neurotrophin action. *Nature Reviews Neuroscience* 2005; **6**(8)**:** 603-614.

250. Chen ZY, Patel PD, Sant G, Meng CX, Teng KK, Hempstead BL *et al.* Variant brain-derived neurotrophic factor (BDNF) (Met66) alters the intracellular trafficking and activity-dependent secretion of wild-type BDNF in neurosecretory cells and cortical neurons. *Journal of Neuroscience* 2004; **24**(18)**:** 4401-4411.

251. Egan MF, Kojima M, Callicott JH, Goldberg TE, Kolachana BS, Bertolino A *et al.* The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. *Cell* 2003; **112**(2)**:** 257-269.

252. Shimizu E, Hashimoto K, Iyo M. Ethnic difference of the BDNF 196G/A (val66met) polymorphism frequencies: The possibility to explain ethnic mental traits. *American Journal of Medical Genetics Part B-Neuropsychiatric Genetics* 2004; **126B**(1)**:** 122-123.

253. Tyler WJ, Alonso M, Bramham CR, Pozzo-Miller LD. From acquisition to consolidation: On the role of brain-derived neurotrophic factor signaling in hippocampal-dependent learning. *Learn Mem* 2002; **9**(5)**:** 224-237.

254. Hariri AR, Goldberg TE, Mattay VS, Kolachana BS, Callicott JH, Egan MF *et al.* Brain-derived neurotrophic factor val(66)met polymorphism affects human memory-related hippocampal activity and predicts memory performance. *Journal of Neuroscience* 2003; **23**(17)**:** 6690-6694.

255. Rattiner LM, Davis M, Ressler KJ. Brain-derived neurotrophic factor in amygdala-dependent learning. *Neuroscientist* 2005; **11**(4)**:** 323-333.

256. Rattiner LM, Davis M, French CT, Ressler KJ. Brain-derived neurotrophic factor and tyrosine kinase receptor B involvement in amygdala-dependent fear conditioning. *Journal of Neuroscience* 2004; **24**(20)**:** 4796-4806.

257. Ou LC, Gean PW. Regulation of amygdala-dependent learning by brain-derived neurotrophic factor is mediated by extracellular signal-regulated kinase and phosphatidylinositol-3-kinase. *Neuropsychopharmacology* 2006; **31**(2)**:** 287-296.

258. Choi DC, Maguschak KA, Ye KQ, Jang SW, Myers KM, Ressler KJ. Prelimbic cortical BDNF is required for memory of learned fear but not extinction or innate fear. *Proc Natl Acad Sci U S A* 2010; **107**(6)**:** 2675-2680.

259. Hajcak G, Castille C, Olvet DM, Dunning JP, Roohi J, Hatchwell E. Genetic variation in brain-derived neurotrophic factor and human fear conditioning. *Genes Brain and Behavior* 2009; **8**(1)**:** 80-85.

260. Soliman F, Glatt CE, Bath KG, Levita L, Jones RM, Pattwell SS *et al.* A Genetic Variant BDNF Polymorphism Alters Extinction Learning in Both Mouse and Human. *Science* 2010; **327**(5967)**:** 863-866.

261. Frustaci A, Pozzi G, Gianfagna F, Manzoli L, Boccia S. Meta-Analysis of the Brain-Derived Neurotrophic Factor Gene (BDNF) Val66Met Polymorphism in Anxiety Disorders and Anxiety-Related Personality Traits. *Neuropsychobiology* 2008; **58**(3-4)**:** 163-170.

262. Nestler EJ, Barrot M, DiLeone RJ, Eisch AJ, Gold SJ, Monteggia LM. Neurobiology of depression. *Neuron* 2002; **34**(1)**:** 13-25.

263. Hashimoto K, Shimizu E, Iyo M. Critical role of brain-derived neurotrophic factor in mood disorders. *Brain Research Reviews* 2004; **45**(2)**:** 104-114.

264. Roffman JL, Weiss AP, Goff DC, Rauch SL, Weinberger DR. Neuroimaging-genetic paradigms: A new approach to investigate the pathophysiology and treatment of cognitive deficits in schizophrenia. *Harvard Review of Psychiatry* 2006; **14**(2)**:** 78-91.

265. Hashimoto K, Koizumi H, Nakazato M, Shimizu E, Iyo M. Role of brain-derived neurotrophic factor in eating disorders: Recent findings and its pathophysiological implications. *Progress in Neuro-Psychopharmacology & Biological Psychiatry* 2005; **29**(4)**:** 499-504.

266. Gratacos M, Gonzalez JR, Mercader JM, de Cid R, Urretavizcaya M, Estivill X. Brain-derived neurotrophic factor Val66Met and psychiatric disorders: Meta-analysis of case-control studies confirm association to substance-related disorders, eating disorders, and schizophrenia. *Biological Psychiatry* 2007; **61**(7)**:** 911-922.

267. Groves JO. Is it time to reassess the BDNF hypothesis of depression? *Molecular Psychiatry* 2007; **12**(12)**:** 1079-1088.

268. Domschke K, Lawford B, Laje G, Berger K, Young R, Morris P *et al.* Brain-derived neurotrophic factor (BDNF) gene: no major impact on antidepressant treatment response. *International Journal of Neuropsychopharmacology*; **13**(1)**:** 93-101.

269. Pezawas L, Verchinski BA, Mattay VS, Callicott JH, Kolachana BS, Straub RE *et al.* The brain-derived neurotrophic factor val66met polymorphism and variation in human cortical morphology. *Journal of Neuroscience* 2004; **24**(45)**:** 10099-10102.

270. Szeszko PR, Lipsky R, Mentschel C, Robinson D, Gunduz-Bruce H, Sevy S *et al.* Brain-derived neurotrophic factor val66met polymorphism and volume of the hippocampal formation. *Molecular Psychiatry* 2005; **10**(7)**:** 631-636.

271. Bueller JA, Aftab M, Sen S, Gomez-Hassan D, Burmeister M, Zubieta JK. BDNF val(66) met allele is associated with reduced hippocampal volume in healthy subjects. *Biological Psychiatry* 2006; **59**(9)**:** 812-815.

272. Montag C, Weber B, Fliessbach K, Elger C, Reuter M. The BDNF Val66Met polymorphism impacts parahippocampal and amygdala volume in healthy humans: incremental support for a genetic risk factor for depression. *Psychological Medicine* 2009; **39**(11)**:** 1831-1839.

273. Montag C, Reuter M, Newport B, Elger C, Weber B. The BDNF Val66Met polymorphism affects amygdala activity in response to emotional stimuli: Evidence from a genetic imaging study. *Neuroimage* 2008; **42**(4)**:** 1554-1559.

274. Sheehan DV, Lecrubier Y, Sheehan KH, Amorim P, Janavs J, Weiller E *et al.* The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *J Clin Psychiatry* 1998; **59 Suppl 20:** 22-33;quiz 34-57.

275. Grime SK, Martin RL, Holaway BL. INHIBITION OF RESTRICTION ENZYME CLEAVAGE OF DNA MODIFIED WITH 7-DEAZA-DGTP. *Nucleic Acids Research* 1991; **19**(10)**:** 2791-2791.

276. Livak KJ. Allelic discrimination using fluorogenic probes and the 5 ' nuclease assay. *Genetic Analysis-Biomolecular Engineering* 1999; **14**(5-6)**:** 143-149.

277. Logothetis NK, Pauls J, Augath M, Trinath T, Oeltermann A. Neurophysiological investigation of the basis of the fMRI signal. *Nature* 2001; **412**(6843)**:** 150-157.

278. Fox PT, Raichle ME. FOCAL PHYSIOLOGICAL UNCOUPLING OF CEREBRAL BLOOD-FLOW AND OXIDATIVE-METABOLISM DURING SOMATOSENSORY STIMULATION IN HUMAN-SUBJECTS. *Proc Natl Acad Sci U S A* 1986; **83**(4)**:** 1140-1144.

279. Ogawa S, Tank DW, Menon R, Ellermann JM, Kim SG, Merkle H *et al.* INTRINSIC SIGNAL CHANGES ACCOMPANYING SENSORY STIMULATION - FUNCTIONAL BRAIN MAPPING WITH MAGNETIC-RESONANCE-IMAGING. *Proc Natl Acad Sci U S A* 1992; **89**(13)**:** 5951-5955.

280. Stark CEL, Squire LR. When zero is not zero: The problem of ambiguous baseline conditions in fMRI. *Proc Natl Acad Sci U S A* 2001; **98**(22)**:** 12760-12765.

281. Worsley KJ, Friston KJ. ANALYSIS OF FMRI TIME-SERIES REVISITED - AGAIN. *Neuroimage* 1995; **2**(3)**:** 173-181.

282. Lundqvist D, Flykt, A. & Öhman, A. The Karolinska Directed Emotional Faces (KDEF) [CD-ROM]. Karolinska Institutet, Department of Clinical Neuroscience, Psychology Section: Stockholm, 1998.

283. Shear MK, Brown TA, Barlow DH, Money R, Sholomskas DE, Woods SW *et al.* Multicenter collaborative panic disorder severity scale. *Am J Psychiatry* 1997; **154**(11)**:** 1571-1575.

284. Montgomery SA, Åsberg M. A new depression scale designed to be sensitive to change. *Br J Psychiatry* 1979; **134:** 382-389.

285. Zigmond AS, Snaith RP. THE HOSPITAL ANXIETY AND DEPRESSION SCALE. *Acta Psychiatrica Scandinavica* 1983; **67**(6)**:** 361-370.

286. Spielberger CD. *Manual for the State-Trait Anxiety Inventory (Form Y)*. Consulting Psychologists Press: Palo Alto, CA, 1983.

287. Heinz A, Smolka MN, Braus DF, Wrase J, Beck A, Flor H *et al.* Serotonin transporter genotype (5-HTTLPR): Effects of neutral and undefined conditions on amygdala activation. *Biological Psychiatry* 2007; **61**(8)**:** 1011-1014.

288. Belsky J, Jonassaint C, Pluess M, Stanton M, Brummett B, Williams R. Vulnerability genes or plasticity genes? *Molecular Psychiatry* 2009; **14**(8)**:** 746-754.