Influence of genetic variants on serotonergic neurotransmission

Doctoral thesis at the Medical University of Vienna
in the program of Applied Medical Sciences N790
thematic program Clinical Neurosciences (CLINS)
for obtaining the medical degree

DOCTOR OF MEDICAL SCIENCE

submitted by

Pia Baldinger, MD

supervised by

Rupert Lanzenberger, Assoc. Prof, PD, MD

Functional, Molecular and Translational Neuroimaging Lab – PET & MRI
Department of Psychiatry and Psychotherapy
Medical University of Vienna
Währinger Gürtel 18-20, 1090 Vienna, Austria
http://www.meduniwien.ac.at/neuroimaging/

Vienna, 03/2015
DECLARATION

This thesis project was conducted at the Functional, Molecular and Translational Neuroimaging Lab – PET & MRI (head: Assoc. Prof. PD Dr. med. Rupert Lanzenberger) at the Department of Psychiatry and Psychotherapy (head: o.Univ.-Prof. Dr. h.c.mult. Dr. med. Siegfried Kasper), Clinical Division of Biological Psychiatry, Medical University of Vienna, Austria (www.meduniwien.ac.at/neuroimaging).

Positron emission tomography measurements were performed at the Department of Biomedical Imaging and Image-guided Therapy, Division of Nuclear Medicine (head: Univ. Prof. Dr. med. Markus Hacker), former Department of Nuclear Medicine (heads: o.Univ. Prof. Dr. med. Robert Dudczak and ao. Univ. Prof. Dr. med. Helmut Sinzinger), Medical University of Vienna, Austria Synthesis of radioligands was done by the working group of Radiopharmaceutical Sciences (www.radiopharmaceutical-sciences.net), Department of Nuclear Medicine, Medical University of Vienna, Austria (head: Assoc. Prof. PD Mag. Dr. Wolfgang Wadsak and PD Mag. Dr. Markus Mitterhauser).

Genotyping of DNA samples was conducted at the Genetics Research Center (head: Prof. Dr. med. Dan Rujescu), Department of Psychiatry and Psychotherapy (head: Prof. Dr. med. Peter Falkai), Ludwig-Maximilians-University, Munich, Germany (www.klinikum.uni-muenchen/Klinik-undPoliklinik-fuer-Psychiatrie-und-Psychotherapie/forschung/genetics/index.html).
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ABSTRACT (english) Influence of genetic variants on serotonergic neurotransmission

Psychiatric disorders were shown to be highly inheritable, for instance with rates of 40-50% for depressive disorder. The manifestation of a mental illness underlies multifactorial conditions including environment factors and the interplay of multiple gene variants, notably single-nucleotide-polymorphisms (SNPs), where each mutation taken individually is supposed to exert only a marginal effect. Genetic association studies including genome-wide investigations have led to the characterization of a multitude of risk genes thought to increase vulnerability for psychiatric disorders. In mood and anxiety disorders, main focus has been led on the exploration of the serotonergic neurotransmitter system and related gene variants, notably as drugs modulating the serotonin system have proven effective in the treatment of these diseases.

To date, the classification of psychiatric disorders is based on the observation and description of psychopathologic symptoms without taking into account etiologic factors. In biological psychiatry, to mitigate the issue of clinical heterogeneity, the concept of endophenotypes represents an attempt to reconcile genetic factors with measurable neurobiological correlates thought to underlie a certain clinical phenotype. In order to assess the implications of gene variants on serotonergic neurotransmission, we conducted three investigations using an imaging genetics approach combining allelic distribution patterns of genes of interest with imaging data retrieved from positron emission tomography studies investigating the serotonin-1A (5-HT$_{1A}$) receptor and the serotonin transporter (SERT), two major players in the modulation of the serotonin system. In the first study, we could determine an effect of catechol-O-methyltransferase gene variant (rs4680) on 5-HT$_{1A}$ receptor binding, where the high-activity allele (GG) is associated with higher receptor binding in healthy controls (HC). Secondly, we were able to replicate previous findings showing no effect of brain-derived-neurotrophic-factor Val66Met (rs6265) variant on 5-HT$_{1A}$ receptor in HC and SERT binding in both depressed and HC. Finally, we could establish a gene x gene interaction between a length polymorphism in SERT gene promoter region (5-HTTLPR) and a serotonin-1B receptor gene SNP (rs6296) affecting 5-HT$_{1A}$ receptor binding in HC with highest receptor densities in LL (5-HTTLPR) x CC (rs6296) carriers.

The publications arising from this thesis substantiate and expand our knowledge on the influence of SNPs on serotonergic neurotransmission using a promising research approach in the field of imaging genetics. Further studies are needed to confirm the gene-binding relationship and its role in the pathogenesis of psychiatric disorders. These studies represent a valuable step in search for a biologically-based classification of psychiatric disorders.
ABSTRACT (Deutsch) Einfluss genetischer Varianten auf die serotonerge Neurotransmission


Zum heutigen Zeitpunkt beruht die Klassifikation psychischer Erkrankungen auf der Beobachtung und Beschreibung psychopathologischer Symptome ohne Berücksichtigung ätiologischer Faktoren. Um das Problem der klinischen Heterogenität zu minimieren, hat sich im Bereich der biologischen Psychiatrie das Konzept der Endophänotypen durchgesetzt: Es handelt sich hierbei um einen Versuch, genetische Faktoren mit messbaren neurobiologischen Korrelaten in Verbindung zu bringen, die mutmaßlich bestimmten psychischen Störungen zugrunde liegen. Wir haben drei Studien durchgeführt, um die Effekte genetischer Varianten auf die serotonerge Neurotransmission, genauer gesagt auf den Serotonin-1A (5-HT_{1A}) Rezeptor und den Serotonintransporter (SERT), zwei wesentliche Akteure des Serotoninsystems, zu erfassen. Die Studien bedienen sich des Ansatzes der Imaging genetics, der auf der Kombination von Expressionsmustern genetischer Varianten und quantitativer Bildgebungssdaten, die mittels Positronen-Emissions-Tomographie erfasst wurden, beruht. In der ersten Studie konnten wir zeigen, dass eine Variante des Catechol-O-Methyltransferase Gens (rs4680) einen Effekt auf das 5-HT_{1A} Rezeptorbindungspotenzial bei Gesunden hat. Das Allel, das mit einer höheren enzymatischen Aktivität einhergeht (GG) ist mit einem höheren Bindungspotenzial vergesellschaftet. In der zweiten Studie konnten wir frühere Ergebnisse replizieren und zeigen, dass eine Variante des Brain-Derived-Neurotrophic-Factor Gens (Val66Met, rs6265) weder einen Einfluss auf das 5-HT_{1A} Rezeptorbindungspotenzial in Gesunden noch auf das SERT Bindungspotenzial bei depressiven Patienten und gesunden Kontrollprobanden hat. Drittens, konnten wir eine Gen x Geninteraktion zwischen dem Längenpolymorphismus der Promoterregion des SERT Gens (5-HTTLPR) und einer Variante
des Serotonin-1B Receptors (rs6296) etablieren, welche einen Einfluss auf das 5-HT$_{1A}$ Rezeptorbindungspotenzial ausübt. Eine hohe Rezeptorbindung konnte für jene Probanden festgestellt werden, die sowohl homozygote L (5-HTTLPR) und C (rs6296) Träger sind.

PUBLICATIONS ARISING FROM THIS THESIS

Publications


Related publications


<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>5-HT</td>
<td>serotonin</td>
</tr>
<tr>
<td>5-HT&lt;sub&gt;1A, 1B, 2A&lt;/sub&gt;</td>
<td>serotonin-1A, serotonin-1B, serotonin-2A</td>
</tr>
<tr>
<td>5-HTTLPR</td>
<td>serotonin-transporter-linked polymorphic region</td>
</tr>
<tr>
<td>CACNA1C</td>
<td>calcium channel, voltage-dependent, L type, alpha 1C subunit</td>
</tr>
<tr>
<td>BP</td>
<td>binding potential</td>
</tr>
<tr>
<td>BP&lt;sub&gt;ND&lt;/sub&gt;</td>
<td>binding potential, non-displaceable</td>
</tr>
<tr>
<td>BDNF</td>
<td>brain-derived neurotrophic factor</td>
</tr>
<tr>
<td>BOLD</td>
<td>blood oxygenation level dependent</td>
</tr>
<tr>
<td>COMT</td>
<td>catechol-O-methyltransferase</td>
</tr>
<tr>
<td>CNS</td>
<td>central nervous system</td>
</tr>
<tr>
<td>DEAF-1</td>
<td>deformed epidermal autoregulatory factor 1</td>
</tr>
<tr>
<td>GWAS</td>
<td>genome-wide association study</td>
</tr>
<tr>
<td>HTR1A</td>
<td>serotonin-1A receptor gene</td>
</tr>
<tr>
<td>HTR1B</td>
<td>serotonin-1B receptor gene</td>
</tr>
<tr>
<td>HTR2A</td>
<td>serotonin-2A receptor gene</td>
</tr>
<tr>
<td>ICD-10</td>
<td>International Classification of Diseases (10&lt;sup&gt;th&lt;/sup&gt; revision)</td>
</tr>
<tr>
<td>MAO</td>
<td>monoamine oxidase</td>
</tr>
<tr>
<td>MDD</td>
<td>major depressive disorder</td>
</tr>
<tr>
<td>MRI</td>
<td>magnetic resonance imaging</td>
</tr>
<tr>
<td>PET</td>
<td>positron emission tomography</td>
</tr>
<tr>
<td>SERT</td>
<td>serotonin transporter</td>
</tr>
<tr>
<td>SLC6A4</td>
<td>solute carrier family 6, member 4 (neurotransmitter transporter, serotonin)</td>
</tr>
<tr>
<td>SNP</td>
<td>single-nucleotide-polymorphism</td>
</tr>
<tr>
<td>SSRI</td>
<td>selective serotonin reuptake inhibitors</td>
</tr>
<tr>
<td>TPH1</td>
<td>tryptophan hydroxylase 1</td>
</tr>
<tr>
<td>TPH2</td>
<td>tryptophan hydroxylase 2</td>
</tr>
<tr>
<td>VNTR</td>
<td>variable number of tandem repeat</td>
</tr>
<tr>
<td>ZNF804A</td>
<td>zinc finger protein 804A</td>
</tr>
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This research was supported by funds of the Österreichische Nationalbank (Anniversary Fund, project number 11468, 12809, 13675) and the Austrian Science Fund (FWF P16549) to Rupert Lanzenberger and Siegfried Kasper, respectively, and an intramural grant of the Department of Psychiatry and Psychotherapy, Medical University of Vienna (Forschungskostenstelle).

I would like to express my sincere appreciation and gratitude to all the people who were involved in the accomplishment of this thesis. First, I would like to thank Rupert Lanzenberger, head of the Functional, Molecular and Translational Neuroimaging Lab at the Department of Psychiatry and Psychotherapy, for giving me the opportunity to start my working carrier in such a fruitful and inspiring environment, and – above all – for giving me the confidence to take the project forward. Furthermore, I would like to thank especially Christoph Kraus, Andreas Hahn and Georg Kranz for advising and helping me in the performance of the thesis project and the actual writing of the publications. Overall, I am grateful to the entire research team of the Functional, Molecular and Translational Neuroimaging Lab, for making it possible to work with such a positive and motivating group of people. Of course, I am very thankful to Siegfried Kasper, head of the Department of Psychiatry and Psychotherapy, who gave me the opportunity to complete my residency at the Medical University of Vienna.

In addition, I would like to thank the two members of my thesis committee Markus Mitterhauser and Martin Bilban for supporting me, as well as Johannes Hainfellner, the coordinator of the PhD program Clinical Neurosciences, carrying out this task with great commitment. Furthermore, I want to acknowledge our collaboration partners, the team of the Department of Biomedical Imaging and Image-guided Therapy, Division of Nuclear Medicine, Medical University of Vienna, and the working group of Radiopharmaceutical Sciences, especially Markus Mitterhauser, Wolfgang Wadsak, Johanna Ungersböck, Daniela Häusler and Christina Rami-Mark, as well as the team of the Genetics Research Center, Department of Psychiatry and Psychotherapy, Ludwig-Maximilians-University, Munich, Germany, particularly Dan Rujecsu, Annette Hartmann, Ina Giegling and Marion Friedl.

Finally and most importantly, I would like to say thank you to my family and friends for encouraging and accompanying me throughout the eventful last years and the challenging task of my doctoral studies.
1. **INTRODUCTION**

1.1. **General Introduction**

1.1.1. *New developments in the field of genetics*

Since the late 90s, remarkable methodological developments in genetic research have revolutionized our understanding of susceptibility to diseases and brought new insights in the causal involvement of biological factors in the occurrence of so-called multifactorial diseases. In the context of genetic research, the vast majority of studies focused on the characterization of risk alleles for complex disorders. Particular interest has been devoted to single-nucleotide-polymorphisms (SNPs), the most common genetic variations between members of the same species, e.g., in humans, which are defined by a variation of the DNA sequence occurring in at least 1% of the population in which a single nucleotide differs. There are approximately ten million common SNPs in the human genome (Consortium, 2003). It is assumed that complex diseases, such as bipolar disorder or diabetes, are the product of a combination of expressed risk alleles, where each single polymorphism displays only a very small effect size (Risch and Merikangas, 1996). In scientific research, this is commonly referred to as the “common disease, common variant” hypothesis (Manolio et al., 2009). Psychiatric disorders, to give an example, are highly inheritable with rates of 70-85% in schizophrenia for instance (Owen et al., 2000, Burmeister et al., 2008); however, heritability does not follow the rules of Mendelian inheritance, where a single gene can explain the occurrence of a disease (e.g., cystic fibrosis), but is rather determined by a pattern of multiple gene variants (Manolio et al., 2008). Polymorphisms thought to be implicated in the pathogenesis of complex disorders have been identified using genetic association studies with candidate genes presumably connected to the disorder, e.g., the gene encoding for the serotonin transporter *SLC6A4*, as the major target of antidepressants, was suggested to play a role in the development of anxiety disorders (Lesch et al., 1996). Additionally, linkage studies have been used to identify new gene loci which might be associated with the occurrence of disease using family-based designs with multiple affected individuals, e.g., in recurrent depression (Breen et al., 2011). This technique is based on the knowledge that neighbouring alleles on a chromosome are likely to be inherited together and thereby narrows the investigated DNA sequence following a hypothesis-driven approach assuming that genetic polymorphism are co-segregated with disease (Risch, 1990). Thirdly and of particular importance, a vast number of genome-wide association studies (GWAS) have been conducted
to explore the interplay of genetic variants and manifestations of disease (Burmeister et al., 2008).

**Figure 1:** GWAS are based on case–control studies in which SNPs across the human genome are genotyped (no hypothesis-driven approach). A) Presentation of 2 common variants (SNPs) on chromosome 9 in 3 subjects displaying individual allelic variants. B) The strength of association between each SNP and disease is calculated on the basis of the prevalence of each SNP in cases and controls. Here, SNPs 1 and 2 on chromosome 9 are associated with disease, with $p$ values of $10^{-12}$ and $10^{-8}$, respectively. C) Manhattan plot showing $p$ values for all genotyped SNPs that have survived a quality-control screen, with each chromosome shown in a different colour. The results implicate a locus on chromosome 9, marked by SNPs 1 and 2, which are adjacent to each other. Figure and legend from Manolio T.A. (Manolio, 2010).
As promising as GWAS were initially estimated, as sobering seem the achievements in research through GWAS at present. GWAS constitute case-control studies in enormous dimensions with hundreds of thousands of cases and controls undergoing DNA sequencing of more than 100,000 SNPs [see Figure 1 (Manolio, 2010)]. Due to the need for multiple testing corrections, statistical significance can be reached only for few SNPs. These findings might statistically be considered as robust; however, unfortunately, replication studies often fail to confirm the association of the concerned SNP and the investigated disease. Within this context, meta-analysis are considered a valuable tool to support the biological implication of SNPs that narrowly fail to reach significance in GWAS (Manolio, 2010). Up to date far more than 100 GWAS in different medical disciplines have been conducted (Goldstein, 2009) and about 100 loci of common polymorphisms have been identified (Figure 2) as potentially implicated in the pathophysiology of complex disorders (Hardy and Singleton, 2009, Manolio, 2010). All these SNPs have been meticulously characterized and classified according to different populations thanks to the HapMap project and these data are freely available online (Website, Manolio et al., 2008). As mentioned earlier, the effect sizes of a single SNP are rather modest [odds ratio 1.33 (Hindorff et al., 2009)] and the functionality of several SNPs detected in GWAS is questionable or seems at least rather unsuspected, e.g., CDKAL1 (Cyclin-dependent kinase 5 regulatory subunit associated protein 1-like 1) in type-2 diabetes (Scott et al., 2007, Manolio, 2010). Nonetheless, the advantage resulting from this non-hypothesis driven approach, is that important, hitherto unconsidered, pathophysiologic pathways cannot be overlooked (Hindorff et al., 2009).

Figure 2: Genomewide associations reported through March 2010. Circles indicate the chromosomal location of nearly 800 SNPs significantly associated (p<5×10⁻⁸) with a disease or trait and reported in the literature (545 studies published through March 2010 yielded the associations depicted). Each disease type or trait is coded by colour. Figure from Manolio T.A. (Manolio, 2010)
1.1.2. Psychiatric genetics

These new developments in genetics have also found application in psychiatric research. However, in psychiatry, clinicians are facing a major issue: Apart from organic mental disorders, where clinical tests - such as laboratory measurements and neuroimaging - might add important information, diagnostics are primarily based on the clinical assessment performed by the physician (Burmeister et al., 2008). The latter depends on anamnestic information, the observations made by the psychiatrist and most importantly verbal communication skills. The assessment relies on structured interviews using diagnostic classification manuals – in Europe mainly the International Classification of Diseases (ICD-10) – and psychometric scales requiring special training. Nonetheless, in clinical practice, diagnostic uncertainty remains in complex psychiatric issues due to overlapping symptoms in psychiatric disorders (Figure 3). In fact, a variety of symptoms are not disease-specific, such as hallucinations in delirium and schizophrenia or suicidal thoughts in personality disorder and major depression, and therefore, cannot be used as objective markers for mental illness. These diagnostic difficulties are most probably due to imprecise definitions, lacking biological tests and insufficient or approximate replications of biomarker investigations in the field of psychiatry (Kapur et al., 2012), e.g., inconsistent data regarding platelet serotonin transporter (SERT) function as a marker for serotonergic neurotransmission in the brain and major depression (Yubero-Lahoz et al., 2013).

Figure 3: Psychiatric disorders overlap and might be extremes of personality traits. Genetic vulnerabilities for psychiatric disorders are shown as emerging from the extreme end of normal population variations of personality, illustrated as different background shades of mood, anxiety, cognitive processing and volition. Genetic factors affecting levels of these underlying traits, in interaction with additional genetic and environmental factors, can lead to psychiatric disorders — shown here are bipolar disorder, schizophrenia, depression and anxiety disorders — the symptoms and genetic risk factors of which are in part unique and in part overlapping. Because not all disorders can be covered in two dimensions, interactions and overlaps exist in many more dimensions than can be represented here (for example, depression and anxiety are also present in schizophrenia). Figure and legend from Burmeister M. (Burmeister et al., 2008).
A great deal of hope was placed in the investigation of the genetic aetiology of psychiatric disorders (Owen and McGuffin, 1997). Based on twin studies, the heritability of major depression and panic disorder for instance is estimated at 40% (Owen et al., 2000). We must therefore assume a strong, potentially measurable, biological aspect in the development of these disorders. However, further complexities result from gene-gene (epistasis) and gene-environment interaction (epigenetics), which in turn include a myriad of variables difficult to consider entirely. Aside from rare cases with a monogenic heritability pattern, e.g., schizophrenia in DiGeorge syndrome (22q11.1 deletion syndrome) (Bassett et al., 2003), the development of psychiatric disorders is caused by the interplay of multiple genes (Owen et al., 2000). It is assumed that the more genes are involved in a phenotype, the more complex is the investigated phenotype and the required genetic analysis (Figure 4) (Gottesman and Gould, 2003).

Figure 4: Rationale for an endophenotype approach to genetic analysis of disorders with complex genetics. Figure from Gottesman I.I. and Gould T.D. (Gottesman and Gould, 2003).

To decrease complexity in the field of psychiatric genetics, Gottesman I.I. and Gould T.D. have introduced the concept of endophenotypes in psychiatry: “Endophenotypes, measurable components unseen by the unaided eye along the pathway between disease and distal genotype, have emerged as an important concept in the study of complex neuropsychiatric diseases” (Gottesman and Gould, 2003). These intermediate manifestations of disease are simpler to characterize and “closer to the site of primary causative agent” (genetics) (Flint and Munafo, 2007). They can vary widely in nature (e.g., neuropsychological or biochemical processes) (Gottesman and Gould, 2003). For instance, the endophenotype “neuroticism” assessed using a personality inventory (NEO-PI-R) was shown to be associated with a polymorphism in the serotonin transporter gene regulatory region 5-HTTLPR (Lesch et al., 1996). Based on this finding, one might conclude in a second step that this polymorphism plays a role in the occurrence of anxiety disorders but also major depression, as both diagnostic entities are strongly related to high neuroticism scores (Lesch et al., 1996), providing evidence
for further investigations in these disorders. Interestingly, anxiety- and depression-related diseases were found to be influenced largely by the same genes (Kendler et al., 1992).

GWAS in psychiatry have been advancing quickly thanks to modern and more affordable microarray technologies allowing for detection of up to 500,000 SNPs (tagSNPs) which in turn enable characterization of a multitude of other SNPs – not directly detected using an array – using linkage analyses (Risch and Merikangas, 1996, Visscher et al., 2012a). In schizophrenia and bipolar disorder for instance, despite of statistical issues in GWAS, some SNPs have been identified and replicated, such as the zinc finger binding protein 804A (ZNF804A) locus and the calcium channel, voltage-dependent, L type, alpha 1C subunit (CACNA1C) locus (O'Donovan et al., 2009), both proteins being parts of pathophysiologic pathways which had not been considered earlier in psychotic disorders. In contrast, in major depressive disorder (MDD), the search for liable SNPs or other genetic variations has been more challenging and up to date no gene could cleave its way into clinical practice as a diagnostic tool for MDD due to lacking replication by means of GWAS (Shyn et al., 2011, Wray et al., 2012). Additionally, despite all efforts, the detected SNPs in GWAS were shown to explain less than 1% of the variance in liability e.g., in the case of schizophrenia (Visscher et al., 2012b), a quite sobering finding. This has led to the introduction of the notion of “missing heritability”, which might be probably due to insufficient tagging of causal SNPs on the available microarrays or such small effect sizes that GWAS do not allow for detecting significant SNPs (Manolio et al., 2009). Unfortunately, up to date, GWAS have not facilitated a better differentiation of disease, such as schizophrenia and bipolar disorder, but rather emphasized similar genetic aetiologies (O'Donovan et al., 2009). As described earlier, this might also be due to artificially-created and inappropriate definitions of psychiatric disorders, which again underlines the necessity for endophenotypes in psychiatry. At present, while the gain of knowledge owed to GWAS seems indisputable regarding significant pathophysiologic pathways in the development of complex diseases, results retrieved from GWAS have not found application in clinical practice and no genetic tests are available to assess the risk of disease in clinical routine.
1.1.3. The role of serotonin in psychiatry

Serotonin, 5-hydroxytryptamine (5-HT), is a monoamine neurotransmitter available in the gastrointestinal tract, platelet and the central nervous system (CNS) (Muck-Seler and Pivac, 2011). Serotonin is primarily synthesized in enterochromaffin cells of the gastrointestinal tract - peripheral serotonin (95%) - by hydroxylation of the essential amino acid L-tryptophan, which must be provided by food. The enzyme catalysing this reaction is the tryptophan hydroxylase 1 (TPH1). In the brain, the synthesis of serotonin is performed by a second isoform of tryptophan hydroxylase, TPH2, in neurons in the raphe region of the brainstem (Walther et al., 2003). The most important enzyme in serotonin degradation is the monoamine oxidase (MAO). A serotonergic synapse is schematically illustrated in figure 5. Serotonin plays an important role in the regulation of many physiological, cognitive and behavioural functions, such as mood, aggression, appetite, sexuality, body temperature, circadian rhythms and sleep-wake cycle (Lucki, 1998). Therefore, it is not surprising that serotonin and serotonergic neurotransmission was shown to be implicated in the development of several psychiatric disorders, particularly anxiety disorders (Kahn et al., 1988) and MDD (Coppen, 1967). Particularly noteworthy is the so called “monoamine hypothesis of depression”, which was first mentioned in 1965 by J. Schildkraut in a review showing a clear association between elevation of catecholamine levels in the brain and antidepressant effects (Schildkraut, 1965).

![Figure 5: Schematic illustration of a serotonergic synapse. Serotonin (5-HT) is synthetized from tryptophan (Trp) and stored in vesicles, depleted in the synaptic cleft following an adequate stimulus. There, 5-HT functions as ligand of serotonin receptors (5-HTR) located both pre- and postsynaptically (e.g., 5-HT$_{1A}$ receptor) thereby influencing serotonergic neurotransmission. 5-HT is degraded by the enzyme monoamine oxidase (MAO) or removed in the presynaptic neuron by the serotonin transporter (SERT).]
In the brain, the vast majority of serotonin containing neurons is situated in the midbrain (median and dorsal raphe nuclei), projecting widely throughout the brain (Jacobs and Azmitia, 1992), including cortical regions of the forebrain (e.g., anterior cingulate cortex, prefrontal cortex) and the limbic system (e.g., hippocampus, amygdala). The diversity of serotonergic functions is mirrored in the complex structure of this neurotransmitter system. The serotonin system is modulated via at least 14 different serotonin receptor subtypes belonging to seven distinct receptor families (5-HT\textsubscript{1-7}) (Hoyer et al., 2002, Saulin et al., 2012), mostly G-protein coupled receptors with the characteristic seven transmembrane domains (see figure 5). Best explored by far are the 5-HT\textsubscript{1} and 5-HT\textsubscript{2} receptor families, due to their role in the mechanism of action of several psychoactive substances including antidepressant, antimigraine and antipsychotic drugs on one hand, but also thanks to the availability of specific radioligands to allow for visualizing them and investigating their function (Saulin et al., 2012). The 5-HT\textsubscript{1} class consists of five different receptor subtypes (5-HT1\textsubscript{A-F}) (Lanfumey and Hamon, 2004).

A great deal of attention has been devoted to the investigation of the 5-HT\textsubscript{1A} receptor, the major inhibitory serotonin receptor, present in the brain both as a presynaptic auto-receptor in the raphe nuclei, exercising a negative feedback on serotonergic neurons, and a hetero-receptor which inhibit mainly GABA- and glutamatergic neurons postsynaptically (Hannon and Hoyer, 2008). The 5-HT\textsubscript{1A} receptor is widely distributed throughout the brain with particularly high densities in the frontal and temporal cortical areas including the cingulate as well as the hippocampus and the midbrain (Saulin et al., 2012). The 5-HT\textsubscript{1A} receptor has been shown to be altered in a variety of psychiatric disorders, such as MDD (Parsey et al., 2006c, Hirvonen et al., 2008, Sullivan et al., 2009, Miller et al., 2013), anxiety disorders (Nash et al., 2008, Akimova et al., 2009), anorexia nervosa (Bailer and Kaye, 2011) and Alzheimer’s disease (Kepe et al., 2006), just to name a few. Staying with MDD, different pharmacological and non-pharmacological treatment modalities have been shown to result in changes of the 5-HT\textsubscript{1A} receptor underlining the crucial role of this receptor in the pathophysiology of depressive symptomatology (Spindelegger et al., 2008, Hahn et al., 2010, Lanzenberger et al., 2013).

Apart from the variety of serotonergic receptors, the SERT represents a key molecule of serotonergic neurotransmission. The SERT is located presynaptically on serotonergic neurons and manages the reuptake of serotonin from the extracellular space and the synaptic cleft into the neuron. Besides the raphe region, high levels of SERT are detectable in the thalamus, hypothalamus, striatum as well as the hippocampus and cingulate cortex (Huang et al., 2010, Saulin et al., 2012). The SERT is the primary target molecule of common antidepressants, e.g.
selective serotonin reuptake-inhibitors (SSRIs). It is assumed that via blocking of the SERT, SSRIs provoke an elevation of serotonin levels in the synaptic cleft which – among other effects – is accompanied by a reduction of depressive symptoms after a latency period of approximately 14 days (Gorman and Kent, 1999). The latter is thought to result from protracted adaptation processes of 5-HT receptor subtypes, for instance a desensitization of 5-HT₁A auto-receptors stimulated by increased 5-HT levels which leads to a disinhibition of serotonergic neurotransmission in the raphe region (Blier et al., 1990, Le Poul et al., 1997). This in turn induces an intensified serotonergic signal in projection areas. In line with these findings, SERT density was shown to be regionally altered in MDD and under treatment with SSRIs (Lira et al., 2003, Lanzenberger et al., 2012a, Gryglewski et al., 2014, Hahn et al., 2014). Overall, particularly within the context of MDD and anxiety disorders, the 5-HT₁A receptor and SERT have been subject to a vast number of studies including post-mortem investigations (Cheetham et al., 1990), in vivo brain micro-dialysis (Olivier et al., 2008), messengerRNA and protein expression (Jennings et al., 2006) and positron emission tomography (Savitz and Drevets, 2013), both in preclinical analyses as well as in humans (Akimova et al., 2009, Savitz et al., 2009). Therefore, it is not surprising that the description of the availability of both 5-HT₁A receptors and SERT in the human brain have been discussed as endophenotypes of psychiatric disorders, especially using neuroimaging methods in patients as compared to healthy control subject (imaging phenotypes) (Savitz and Drevets, 2009). To mention an example, several studies have aimed to characterize 5-HT₁A receptor density in MDD; the vast majority of the investigations point towards a reduced 5-HT₁A receptor when comparing different MDD patient cohorts with healthy subjects, e.g., in the hippocampus, amygdala, anterior cingulate cortex, mesiotemporal cortex, orbitofrontal cortex and prefrontal cortex (Drevets et al., 1999, Drevets et al., 2000, Sargent et al., 2000, Bhagwagar et al., 2004, Meltzer et al., 2004, Drevets et al., 2007, Hirvonen et al., 2008, Moses-Kolko et al., 2008). Based on these findings one might define reduced density of the 5-HT₁A receptor in certain brain regions as an imaging phenotype underlying MDD. However, using similar methods, some authors published opposite results (Parsey et al., 2006c, Miller et al., 2009, Parsey et al., 2010). In view of a clinical relevance of these findings, it can be noted that up to date measurements of 5-HT₁A receptor in depressed patients are not used as diagnostic tool in MDD due to lacking reference levels of receptor density and clinical trials with sufficient statistical power for stratification.
1.1.4. Relevant genetic variations

Serotonergic neurotransmission is controlled by the interplay of a variety of receptors, transporters and enzymes. Their expression and function, in turn, is mainly regulated by genes and their common polymorphisms (D'Souza and Craig, 2010). Hereafter, a – by no means exhaustive – selection of serotonergic proteins partly related to this thesis, their coding genes and important genetic variations in regard to psychiatric diseases are described.

**SLC6A4 5-HTTLPR**

The SERT gene SLC6A4 is located on chromosome 17 (17q11.1-q12) and contains a functional insertion-deletion polymorphism, known as 5-HTTLPR. Due to its location in the gene’s promoter region, the transcriptional activity of SLC6A4 depends partially on 5-HTTLPR (Lesch et al., 1996). In fact, promoter regions function as regulatory elements located upstream of the protein coding sequence allowing for binding of RNA polymerase, the enzyme responsible for DNA transcription. 5-HTTLPR results in two possible allelic variants differing in length: the short (S) and the long (L) allele, where the S allele was shown to be accompanied by lower transcriptional activity and expression rates of SERT (Lesch et al., 1996). In 2000, Nakamura et al. showed that the hitherto described as biallelic polymorphism actually comprises a triallelic locus (Nakamura et al., 2000), with two different manifestations of L, namely $L_A$ and $L_G$ [rs25531 (Kraft et al., 2005)], where the $L_G$ allele shows similar properties than S with lower SERT expression rates (Hu et al., 2006).

It is not surprising that 5-HTTLPR has been extensively investigated in psychiatric research with over 1000 publications listed in PubMed (http://www.ncbi.nlm.nih.gov/). The polymorphism has firstly been published in the context of affective disorders nearly 20 years ago by the group around Lesch K.P. with strikingly high frequencies of the S allele in depressive patients compared to healthy subjects (Collier et al., 1996). Since 1996, this polymorphism has been associated with a variety of psychiatric phenotypes, including e.g., anorexia nervosa (Hinney et al., 1997), bipolar disorder (Kunugi et al., 1997), autism (Klauck et al., 1997), seasonal affective disorder (Rosenthal et al., 1998), schizophrenia (Malhotra et al., 1998). Major focus has been laid on the characterization of so called gene x environment interactions involving 5-HTTLPR, e.g., the influence of 5-HTTLPR and child maltreatment for the occurrence of depression (Caspi et al., 2003), for review see Caspi et al. 2010 (Caspi et al., 2010). Moreover, this polymorphism
has also been investigated in relation to imaging endophenotypes, such as amygdala reactivity on harm avoidance using functional magnetic resonance imaging, showing an S-allele driven hyperreactivity of the amygdala (Hariri et al., 2005), a susceptibility factor of affective disorders (Siegle et al., 2002).

**HTR1A rs6295 C(-1019)G**

The 5-HT$_{1A}$ receptor gene (HTR1A) is located on chromosome 5 (Kobilka et al., 1987). Rs6295 is a common polymorphism located in the gene’s promoter region, thereby conceivably impacting on receptor expression (Wu and Comings, 1999, Lemonde et al., 2003). The C allele was shown to be recognized by transcription factors in the raphe nuclei, e.g., DEAF-1 (deformed epidermal autoregulatory factor 1) (Czesak et al., 2012), leading to a repression of gene transcription (Le François et al., 2008). In the case of GG homozygosity, insufficient binding of transcription factors leads to an abolition of the repression of HTR1A in the midbrain and increased inhibitory activity of 5-HT$_{1A}$ autoreceptors, thereby compromising serotonergic neurotransmission (Lemonde et al., 2003). This SNP is considered the most prominent HTR1A genetic variant involved in the occurrence of several psychiatric phenotypes, including mood disorders, anxiety disorders but also psychotic disorder (Drago et al., 2008). Lemonde et al. found a twofold incidence of GG homozygosity in depressed subjects compared to controls and yet fourfold incidence in suicide completers (Lemonde et al., 2003). Furthermore, in MDD, homozygous G allele carriers were shown to display a lower treatment response to antidepressants (Lemonde et al., 2004).

**HTR1B rs6296**

Similar to 5-HT$_{1A}$ receptor, the serotonin-1B (5-HT$_{1B}$) receptor is an inhibitory 5-HT receptor acting both as auto-receptor on serotonergic neurons in the raphe region and hetero-receptor in projection areas, namely the striatum, the occipital cortex and the basal ganglia (Barnes and Sharp, 1999, Saulin et al., 2012). 5-HT$_{1B}$ receptor was shown to play an important role in 5-HT cell firing and release in the midbrain (Adell et al., 2001). Moreover, evidence retrieved from knock-out studies suggests a role of 5-HT$_{1B}$ in aggressive and impulsive behaviour in rodents (Zhuang et al., 1999). 5-HT$_{1B}$ autoreceptors were shown to be involved in the antidepressant effect of SSRI as enhanced serotonin reuptake following long-term administration of
antidepressants seems to be accompanied by a 5-HT$_{1B}$ receptor down-regulation/desensitization in the raphe nuclei (Blier et al., 1988).

The gene encoding for 5-HT$_{1B}$ receptor is HTR1B, situated on chromosome 6. At present, several SNPs of HTR1B have been identified (Zouk et al., 2007, Conner et al., 2010); however, rs6296 (G861C), a synonymous SNP within the gene’s coding region, has by far been the most extensively investigated genetic variant of the 5-HT$_{1B}$ receptor. Findings obtained from association studies attribute a role to rs6296 in the development of manifold psychiatric disorders, such as substance abuse and major depression (Lappalainen et al., 1998, Huang et al., 2002). The C allele of rs6296 was reported to be associated with elevated depression and anxiety scores when in conjunction with recent stressful life events (Mekli et al., 2011).

**HTR2A rs6313**

In contrast to 5-HT$_{1A}$ and 5-HT$_{1B}$ receptor subtypes, the serotonin-2A (5-HT$_{2A}$) receptor is a major excitatory serotonergic receptor encoded by the HTR2A gene located on chromosome 13 (Hsieh et al., 1990). 5-HT$_{2A}$ receptors are widely distributed throughout the brain with highest densities in the cerebral cortex (Saulin et al., 2012). In the context of MDD, increased receptor densities have been determined in suicide victims (Stanley and Mann, 1983) and, consistent with this finding, administration of SSRI was shown to be accompanied by a down-regulation of 5-HT$_{2A}$ receptors in both rodents and humans (Maj et al., 1996, Meyer et al., 2001) suggesting a crucial role of 5-HT$_{2A}$ receptors in antidepressant treatment response.

A polymorphism of HTR2A at position T102C, rs6313, (Warren et al., 1993) was shown to affect treatment response to SSRI in MDD patients (McMahon et al., 2006, Kato et al., 2009). Furthermore, a positive relation between panic disorder and this SNP could be determined (Inada et al., 2003, Unschuld et al., 2007). Furthermore, rs6313 has been investigated in the context of alcohol dependence (Jakubczyk et al., 2012).

**COMT rs4860 Val158Met**

The catechol-O-methyltransferase (COMT) is the enzyme responsible for the degradation of catecholamines, including dopamine, encoded by the COMT gene (COMT) located on
chromosome 22 (Grossman et al., 1992). COMT represents a major player of dopamine clearance in the prefrontal cortex where dopamine transporter activity is of less significance (Tunbridge et al., 2004). Additionally, COMT activity seems to be directly influenced by serotonin in vitro (Tsao et al., 2012).

Several COMT polymorphisms have been discussed in the context of personality disorders, suicide risk and antidepressant treatment response (Arias et al., 2006, Calati et al., 2011, Schosser et al., 2012). The best studied allelic variant of COMT is rs4680, a common functional polymorphism resulting in an amino acid change of valine to methionine at codon 158 (Val158Met), where the A allele (Met) was shown to be associated with a three to four fold lower enzymatic activity (Lachman et al., 1996). Rs4680 was primarily investigated in connection with schizophrenia (Egan et al., 2001, Shifman et al., 2002), attention-deficit/hyperactivity disorder (Michaelovsky et al., 2008, Halleland et al., 2009) and bipolar disorder (Benedetti et al., 2010) due to a major involvement of dopamine in these psychiatric diseases (Rotondo et al., 2002, Craddock et al., 2006).

**BDNF rs6265 Val66Met**

Brain-derived neurotrophic factor (BDNF) is the most prominent member of the neurotrophin family of nerve growth factors and encoded by the gene BDNF on chromosome 11. BDNF is responsible for neuronal growth, development and survival. In the mature brain, BDNF promotes neuronal plasticity (Thoenen, 1995). BDNF has been proposed as a susceptibility locus for a variety of psychiatric phenotypes, including mood disorders (Green and Craddock, 2004). Furthermore, growing evidence suggests a major interplay of BDNF and serotonergic proteins (e.g., 5-HT1A receptor), sharing similar intracellular pathways and displaying reciprocal regulatory functions (Szapacs et al., 2004, Martinowich and Lu, 2007).

A functional SNP in the pro-domain of BDNF, rs6265, has been identified, leading to a valine to methionine substitution at codon 66 (Val66Met). The Met allele was shown to compromise intracellular trafficking and secretion of BDNF, leading to an impairment of CNS functions (Chen et al., 2004) and increased to susceptibility to neuropsychiatric symptoms and disorders, e.g., cognitive impairment (Egan et al., 2003, Hariri et al., 2003), Alzheimer's disease (Ventriglia et al., 2002), bipolar disorder (Neves-Pereira et al., 2002) and neuroticism as a risk factor for MDD (Sen et al., 2003).
**MAO-A rs6323 VNTR**

The monoamine oxidase A (MAO-A) is an isoenzyme of monoamine oxidase, and represents the major serotonin-degrading enzyme. MAO-A is encoded by MAO-A gene (MAOA) on chromosome X (Hotamisligil and Breakefield, 1991). Due to its role in the catalysation of biogenic amines, MAOA has been subject to intensive research in regard to behavioural traits and psychiatric disorders, e.g., aggressive behaviour in rodents (Cases et al., 1995) and delinquency in humans (Guo et al., 2008), respectively.

Sabol et. al. discovered a polymorphism located in the gene's promoter region, consisting in a 30 base pairs sequence repeated three to five times, each accompanied by a varying transcriptional activity, where alleles with 3.5 or four copies display a two to tenfold more efficient transcription (Sabol et al., 1998). This genetic variation is also known as variable number of tandem repeat (VNTR) polymorphism (rs6323) and was shown to be associated with impulsivity and suicidal behaviour (Huang et al., 2004).

**TPH2 rs120074175 Arg144His**

Tryptophan hydroxylase 2 (TPH2) is an isoenzyme of tryptophan hydroxylase responsible for the synthesis of serotonin from tryptophan in the CNS with highest expression in the midbrain raphe nuclei (Walther et al., 2003). TPH2 is encoded by tryptophan hydroxylase 2 gene (TPH2) on chromosome 12.

Zhang et al. detected a functional SNP resulting in an amino acid substitution of arginine to histidine on codon 144 (Arg144His) which leads to an 80% decreased 5-HT synthesis in vitro (Zhang et al., 2004). 144His has been shown to be associated with the occurrence of MDD (Zhang et al., 2005).
1.1.5. Positron emission tomography and imaging genetics

The research field of imaging genetics in psychiatry emerged in the last decade as a natural consequence of the wealth of data retrieved from neuroimaging studies on one hand and increasing methodological and financial opportunities of genome sequencing on the other hand. As the name indicates, imaging genetics consists of a combination of imaging endophenotypes (chapter 1.1.3.), gathered using primarily magnetic resonance imaging (MRI) or positron emission tomography (PET), and candidate genes thought to influence the structure and function of the brain.

The vast majority of imaging genetics publications involves MRI methodology (Hariri et al., 2006, Meyer-Lindenberg, 2012). In fact, compared to PET, MRI was shown to be more available and less expensive. Moreover, MRI is not accompanied by an exposure to radiation via an obligatory intravenous injection and thereby goes along with lower stress for the individual undergoing the measurement (Otte and Halsband, 2006). However, in this thesis, the focus will be laid on PET data providing the unique opportunity of measuring the availability of molecules in the brain, such as neurotransmitter receptors and transporters, in vivo. These proteins play the leading role in the transmission of neuronal information at the cellular membrane and the synapse, thereby substantially determining every process in the human brain, in health and in sickness (Heiss and Herholz, 2006). PET allows for comparing both clinical states on a molecular level.

PET represents a functional imaging technique in the field of nuclear medicine (Figure 6) that applies specific radiotracer consisting of a biological ligand labelled with a radioactive isotope which acts as positron emitter (e.g., $^{18}$Fluor, $^{11}$Carbon) (Wadsak and Mitterhauser, 2010). The radioligand is administered intravenously to the subject while lying in the PET device. Following application, positrons are emitted and collide after travelling a short distance - but without exiting the individual - with local electrons, a process called annihilation (Cherry, 2006). Subsequently, the annihilation results in the formation of two photons which are emitted in directly opposite directions ($180^\circ$) and detected simultaneously by the detector ring within the PET device. The entirety of these so called coincidence events is recorded and ultimately conflates to a final image (Otte and Halsband, 2006). To quantify specific radioligand binding and indirectly deduce e.g., the density of a neurotransmitter receptor, a tracer kinetic model using e.g., arterial blood sampling (Stilstein and Laruelle, 2001) or a reference model (Lammertsma, 2002) with a tissue devoid of specific binding sites (only non-specific binding), is needed. The assessed measure is among others the so called binding potential (BP (Innis et al., 2007)) that is defined by the ratio
of receptor density $B_{\text{max}}$ and the equilibrium constant $K_D$ (for a given radioligand), where the latter equals the reciprocal of the affinity. To be successful for PET, the radioligands have to fulfil certain criteria, namely stability of labelling, high affinity and selectivity for a given receptor/transporter molecule combined with low non-specific binding, sufficient lipophilicity for permeating the blood-brain barrier via passive diffusion and non-toxicity (Heiss and Herholz, 2006). In the context of brain imaging, manifold radiotracers are available to label proteins of interest, contributing to a better understanding of various psychiatric disorders (Heiss and Herholz, 2006).

Figure 6: Photograph of a GE Advance PET scanner (General Electroc Medical Systems, Milwaukee, Wisconsin, USA) at Department of Biomedical Imaging and Image-guided Therapy, Division of Nuclear Medicine Department of Nuclear Medicine, Medical University of Vienna.

Focusing on serotonergic neurotransmission, the majority of the imaging genetics investigations using PET are based on quantifications of the $5\text{-HT}_{1\text{A}}$ receptor as well as the SERT (Willeit and Praschak-Rieder, 2010) which among other things is due to the fact that suitable radioligands exist to visualize them, particularly $[^{\text{11}}\text{C}]\text{WAY-100635}$ (Wadsak et al., 2007) and $[^{\text{11}}\text{C}]\text{DASB}$ (Haeusler et al., 2009), respectively, to name the currently most reliable tracers. The most important findings retrieved from PET imaging genetics studies are attempted to be summarized in the following paragraph.

Increased SERT binding has repeatedly been demonstrated for homozygous $L_A$ carriers compared to non-$L_A/L_A$ carriers of $5\text{-HTTLPR}$ (SLC6A4) in several brain regions in healthy subjects (Praschak-Rieder et al., 2007, Reimold et al., 2007, Kalbitzer et al., 2010) and this is in accordance with the assumption that $S$ and $L_G$ carriers exhibit lower SERT expression rates (Hu et al., 2006). However, Parsey et al. reported no difference in SERT binding per genotype neither in healthy individuals nor in MDD patients (Parsey et al., 2006a). Interestingly, 5-
HTTLPR genotype status was shown to influence 5-HT_{1A} receptor binding such that S carriers exhibit lower BP in the healthy brain compared to LL carriers (David et al., 2005). Similarly, regarding the effect of rs6295 C(-1019)G (HTR1A) on 5-HT_{1A} receptor binding potential, findings seem rather inconclusive. While David et al. and Lothe et al. reported no effect on rs6295 on 5-HT_{1A} receptor BP (David et al., 2005, Lothe et al., 2010), Parsey et al. determined a higher 5-HT_{1A} receptor BP in G allele carriers (Parsey et al., 2006b, Parsey et al., 2006c). Homozygote Val carriers of BDNF Val66Met polymorphism were shown to display higher SERT BP in the anterior cingulate cortex of healthy individuals, but Val66Met had no effect on 5-HT_{1A} receptor binding (Henningsson et al., 2009). However, this findings was disputed by Lan et al. showing an association between Val66Met genotype and 5-HT_{1A} receptor BP, such that Met allele carriers exhibit lower 5-HT_{1A} receptor BP in healthy subjects but not MDD patients (Lan et al., 2014). Finally, MAOA VNTR genotype was shown to predict 5-HT_{1A} receptor BP in healthy women but not in men (Mickey et al., 2008).

To sum up and considering the results described above, imaging genetics applying PET is a promising research field in basic psychiatric research continually achieving new insights in the human brain’s complexity. However, at present, this area seems to be in its infancy with findings lacking replication and clinical implementation and further investigations are demanded to make clear statements regarding the association of genes and psychiatric pheno- and endophenotypes.
1.2. **Aims of the thesis**

A major issue arising from genetic studies is the required sample size to guarantee a sufficiently high statistical power. PET studies, by contrast, usually include rather low sample sizes and are still expected to provide reliable data to draw scientific conclusions regarding brain function. The pooling of PET data retrieved from a priori different investigations represents a promising solution to ensure a high statistical power when combining imaging and genetic data. Furthermore, as previously mentioned in the introduction, investigations applying an *imaging genetics* approach with PET remain rather scarce and lack replication. So far, only few gene x imaging endophenotype associations have been studied (chapter 1.1.5.), leaving room for yet unknown relations.

The aims of this thesis will be to show novel associations within the serotonergic system which are hitherto not recognized on the other hand and to replicate and expand earlier findings from the literature on the other hand by pooling data from previously published PET studies.

More precisely, the thesis will focus on the following associations between genetic variants thought to influence the serotonin system and the non-displaceable binding potential BP\_ND - an index for density - of two major serotonergic players, namely the 5-HT\_1A receptor and the SERT:

- Effect of *COMT* rs4680 on 5-HT\_1A receptor binding in healthy subjects.

- Effect of *BDNF* rs6265 Val66Met genotype on SERT and 5-HT\_1A receptor binding in healthy subjects and MDD patients.

- Effect of *HTR1B* rs6296 and *5-HTTLPR* on 5-HT\_1A receptor binding in healthy subjects.
2. MATERIALS AND METHODS

This thesis project was designed and performed by the “Functional, Molecular and Translational Neuroimaging Lab – PET & MRI” at the Department of Psychiatry and Psychotherapy, Clinical Division of Biological Psychiatry, Medical University of Vienna. The study protocol “Single-nucleotide-polymorphisms (SNPs) and molecular imaging of serotonin receptor subtypes and transporter” was approved by the Ethics Committee of the Medical University of Vienna and the General Hospital of Vienna (EK 720/2009).

Subjects recruited for this project had earlier participated in one of the following studies: “In vivo imaging of 5-HT1A receptors with PET in patients with anxiety disorders and healthy volunteers” (EK 318/2002 + Amendments) and “Longitudinal imaging of serotonin transporter occupancy using PET and [11C]DASB in patients with major depression treated with escitalopram or citalopram” (EK 578/2006 + Amendments).

Subjects were contacted by telephone and asked whether they would consider participating in a follow-up study, as they had already been enrolled in a PET study before. From each subject, willing to participate, informed consent was obtained after detailed information regarding study procedure and aim of the investigation. Subsequently, a blood sample (EDTA acid anticoagulated blood, 9ml) was drawn preferentially from a cubital vein for the genotypic analysis and the DNA was extracted using the QIAamp DNA Mini Kit from QIAGEN® (Hilden, Germany) and the provided handbook 11/2007 (Protocol: DNA purification from blood and other body fluids, http://www.qiagen.com/at/resources/search-resources/#filters=%7BF321478C-FDDA-437F-BE0B-87001D9936D3%7D). The DNA was then frozen and stored temporarily at our institute. Finally, the anonymized DNA samples were transported with dry ice to the Department of Psychiatry and Psychotherapy, Ludwig-Maximilians-University, Munich, Germany, where genotyping was performed for the SNPs of interest using single base primer extension coupled with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MassARRAY® platform by SEQUENOM®, San Diego, CA) as described by Oeth et al. (Oeth et al., 2009). For SLC6A4 5-HTTLPR length polymorphism genotyping was performed using real-time PCR. Quality assurance CEU HapMap Trios (Coriell Institute for Medical research, Camden, NJ) were included and compared with the HapMap-CEU population (www.hapmap.org).

The PET data from the above mentioned previous studies were then reassessed taking into account the newly obtained genetic data. Main outcome measures were either 5-HT1A receptor
binding potential (BP_{ND}) or SERT binding potential (BP_{ND}) in the whole brain (voxel-wise approach) or areas of interest (region-of-interest approach). Multivariate repeated-measures analyses of variances (ANOVA) were performed to uncover significant differences in 5-HT_{1A} receptor and SERT binding potential according to the genotype groups. Posthoc t-tests (p<0.05) were performed to investigate area-specific effects of genotypes. Correction for multiple testing was applied.

Further details regarding the acquisition of the PET data, radiochemistry of [^{11}C]DASB and [carbonyl-{^{11}}C]WAY-100635 and the analysis of the imaging data for quantification of serotonin-1A receptor and serotonin transporter binding potential are comprehensively described in previous publications (Lanzenberger et al., 2007, Lanzenberger et al., 2012a) and the papers arising from this thesis (Baldinger et al., 2014, Kraus et al., 2014, Baldinger et al., 2015).
3. RESULTS

3.1. First publication: Impact of COMT genotype on serotonin-1A receptor binding investigated with PET

Pia Baldinger¹, Andreas Hahn¹, Markus Mitterhauser², Georg S. Kranz¹, Marion Friedl³,⁴, Wolfgang Wadsak², Christoph Kraus¹, Johanna Ungersböck², Annette Hartmann³, Ina Giegling³, Dan Rujescu³,⁴, Siegfried Kasper¹, Rupert Lanzenberger¹

¹ Department of Psychiatry and Psychotherapy,
² Department of Biomedical Imaging and Image-guided Therapy, Division of Nuclear Medicine, Medical University of Vienna, Austria
³ Department of Psychiatry and Psychotherapy, Ludwig-Maximilians-University Munich, Germany
⁴ Department of Psychiatry, University of Halle, Germany

Accepted for publication in
BRAIN STRUCTURE AND FUNCTION
[IF 2013: 4.567]

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* Correspondence to: Rupert Lanzenberger, A/Prof, MD
  Functional, Molecular and Translational Neuroimaging Lab
  Department of Psychiatry and Psychotherapy
  Medical University of Vienna,
  Währinger Gürtel 18-20, 1090 Vienna, Austria
  rupert.lanzenberger@meduniwien.ac.at
  http://www.meduniwien.ac.at/neuroimaging/
3.1.1. **Abstract**

Alterations of the inhibitory serotonin-1A (5-HT₁A) constitute a solid finding in neuropsychiatric research, particularly in the field of mood and anxiety disorders. Manifold factors influencing the density of this receptor have been identified, e.g., steroid hormones, sunlight exposure and genetic variants of serotonin-related genes. Given the close interactions between serotonergic and dopaminergic neurotransmission, we investigated whether a common single-nucleotide-polymorphism (SNP) of the Catechol-O-methyltransferase (COMT) gene (VAL158MET or rs4680) coding for a key enzyme of the dopamine network that is associated with the pathogenesis of mood disorders and antidepressant treatment response, directly affects 5-HT₁A receptor binding potential. 52 healthy individuals (38 female, mean age ± standard deviation = 40.48 ± 14.87) were measured via positron emission tomography using the radioligand \([\text{carbonyl}^{11}\text{C}]\text{WAY-100635}\). Genotyping for rs4680 was performed using DNA isolated from whole blood with the MassARRAY platform of the software SEQUENOM®. Whole brain voxel-wise ANOVA resulted in a main effect of genotype on 5-HT₁A binding. Compared to A carriers (AA+AG) of rs4680, homozygote G subjects showed higher 5-HT₁A binding potential in the posterior cingulate cortex (\(F_{(2,49)}=17.7, p=0.05, \text{FWE corrected}\)), the orbitofrontal cortex, the anterior cingulate cortex, the insula, the amygdala and the hippocampus (voxel-level: \(p<0.01\) uncorrected, \(t>2.4\); cluster-level: \(p<0.05\) FWE corrected). In light of the frequently reported alterations of 5-HT₁A binding in anxiety and mood disorders, this study proposes a potential implication of the COMT genotype, more specifically the VAL158MET polymorphism, via modulation of the serotonergic neurotransmission.
3.1.2. Introduction

The growing availability of selective radioligands for positron emission tomography (PET) has enabled major advancements in the differentiation and definition of distinct neurotransmitter characteristics and thereby led to the emergence of molecular imaging genetics. To date, major clinical relevance is attributed to this link between genetics and neuroimaging data, which show a great deal of future potential. Basic neuropsychiatric research has contributed to a large body of evidence emphasizing a major role of the serotonin-1A (5-HT₁A) receptor in the development of psychiatric syndromes, particularly major depression and anxiety disorders (Lanzenberger et al. 2007; Akimova et al. 2009; Savitz et al. 2009; Lanzenberger et al. 2012a). Via its function as an auto-inhibitory serotonergic receptor in the midbrain raphe region, the 5-HT₁A receptor regulates neuronal signaling by modulating serotonergic cell firing and thus serotonin release (Barnes and Sharp 1999; Sharp and Hjorth 1990). In contrast, in subcortical and cortical projection areas, the 5-HT₁A heteroreceptors are located postsynaptically on glutamatergic and GABAergic neurons hereby mediating an inhibitory effect on non-serotonergic neurons (Sharp et al. 2007; Hahn et al. 2010). The repeatedly described monoamine hypotheses of depression suggests reduced extracellular levels of serotonin - alongside other neurotransmitters - as causal mechanism of lowered mood. Accordingly, the expression of the inhibitory 5-HT₁A receptor was shown to be altered during depressive states as compared to unaffected subjects (Schildkraut 1965). Using the selective 5-HT₁A receptor antagonist [carbonyl-¹¹C]WAY-100635, Drevets et al. determined a reduced 5-HT₁A receptor binding potential in the raphe, the mesiotemporal cortex, the occipital cortex and the postcentral gyrus in major depression when contrasting a group of unmedicated depressive persons and healthy subjects (Drevets et al. 2000; Drevets et al. 1999). This finding characterizes a distinct endophenotype of depression (Sargent et al. 2010; Hirvonen et al. 2008; Bhagwagar et al. 2004), namely a widespread diminished 5-HT₁A receptor binding potential compared to healthy individuals.

In recent years, growing interest has been attributed to the field of imaging genetics, which correlates data retrieved from magnetic resonance imaging and PET with allele distributions of genes of interest. The scope of this research consists of linking neuronal correlates of certain disorders with genes of interest, thereby circumventing the high complexity and subjectivity of clinical characterizations of psychiatric phenotypes (Scharinger et al. 2010). Within this context, a number of studies have been conducted in order to determine an association between 5-HT₁A receptor binding and potential risk alleles for depression, e.g., the 5-HT₁A receptor gene
(HTR1A, rs6295) (Wu and Comings 1999; Lemonde et al. 2003) and the serotonin transporter promoter polymorphism 5-HTTLPR (Lesch et al. 1996; Caspi et al. 2003).

Considering the high complexity of protein expression regulation, manifold additional factors were reported to influence the density of the 5-HT\textsubscript{1A} receptor, as e.g., sunlight exposure (Spindelegger et al. 2011) and steroid hormones (cortisol, progesterone) (Lanzenberger et al. 2010; Lanzenberger et al. 2011). Of major interest in regard to psychiatric phenotypes is the interplay of different neurotransmitter systems on an anatomical and functional level, particularly the modulatory interrelations of serotoninergic and dopaminergic signaling pathways. The latter have been brought into connection with e.g., mood disorders, cognitive decline but also novel therapeutic agents (Tsao et al. 2012; Wood and Wren 2008; Soeiro-de-Souza et al. 2012; Kocabas 2012). A key enzyme of dopamine metabolism is the catechol-O-methyltransferase (COMT), a ubiquitous enzyme responsible for the degradation of catecholamines, including dopamine, epinephrine and norepinephrine (Hong et al. 1998; Gogos et al. 1998). The COMT gene (COMT) on chromosome 22 has been closely investigated as a candidate for heightened susceptibility to psychosis and bipolar disorder. More specifically, a common SNP of COMT, rs4680, that leads to a G to A transition at codon 158 and thereby results in a valine to methionine (VAL158MET) substitution (G and A alleles correspond to VAL and MET amino acid respectively), has been associated with schizophrenia, bipolar and panic disorder (Craddock et al. 2006; Stein et al. 2005; Rotondo et al. 2002). MET/MET carriers have been shown to exhibit a 3 to 4 fold reduced enzymatic activity as compared to homozygous subjects for the VAL allele (Scanlon et al. 1979; Lachman et al. 1996). In this context, decreased red blood cell COMT activity has been demonstrated earlier in affective disorders, particularly in unipolar depression (Dunner et al. 1971; Ohara et al. 1998). Interestingly, serotonin was shown to directly inhibit COMT in vitro by binding at its active site, thereby influencing pain perception (Tsao et al. 2012). Hence, further modulatory effects of the serotonergic system on COMT, and vice versa, appear obvious, especially when it comes to the most important inhibitory serotonin receptor, the 5-HT\textsubscript{1A} receptor. Using imaging genetics techniques, the aim of our study was therefore to investigate a potential impact of the rs4680 genotype on 5-HT\textsubscript{1A} receptor binding in a sample of healthy subjects using [carbonyl\textsuperscript{11}C]WAY-100635.
3.1.3. Materials and Methods

Subjects

52 healthy Caucasian volunteers (38 female, 14 male) were included in this cross-sectional study after giving written informed consent at the screening visit. Subjects were aged between 21 and 63 (mean age ± standard deviation = 40.48 ± 14.87) and had partly served as control subjects in previous studies performed by our group (Hahn et al. 2012; Hahn et al. 2010; Lanzenberger et al. 2011; Fink et al. 2009; Stein et al. 2008a; Lanzenberger et al. 2007). The demographic data of the participants is summarized in table 1. Subjects were shown to be mentally healthy by an experienced psychiatrist using the Mini-International Neuropsychiatric Interview (M.I.N.I.) (Sheehan et al. 1998) or the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID). Any psychiatric disorder, including drug abuse, neurological illness or medication intake, was considered an exclusion criterion. In addition, in order to detect relevant abnormalities in physical health, each participant underwent a medical examination including general physical and neurological status, routine laboratory measurements as well as an electrocardiogram. The study was approved by the Ethics Committee of the Medical University of Vienna and the General Hospital of Vienna.

Genotyping

In a first step, 9ml EDTA acid anticoagulated blood samples were drawn from each participant and DNA was isolated following the “DNA Purification from Blood or Blood Fluids” protocol of the QIAamp DNA Mini and Blood Mini Handbook 11/2007 (QIAGEN®, Hilden, Germany). Genotyping of COMT rs4680 SNP was performed using the MassARRAY platform (SEQUENOM®, San Diego, CA) as described by Oeth et al. (Oeth et al. 2007). For genotyping quality assurance CEU HapMap Trios (Coriell Institute for Medical research, Camden, NJ) were included and compared with the HapMap-CEU population (www.hapmap.org). Genotyping frequencies were distributed in accordance with the Hardy-Weinberg Equilibrium (HWE) ($\chi^2$=3.78, df=1, p>0.05). Healthy subjects were partly genotyped previously for HTR1A rs6295 (50 subjects), for Brain-Derived Neurotrophic Factor gene (BDNF) SNP rs6265 and 5-HT$_{1B}$ receptor gene SNP rs6298 (46 subjects). These data were not included in our statistical analysis.
Each subject underwent one positron emission tomography scan acquired at the Department of Nuclear Medicine, Medical University of Vienna, Austria, using a GE Advance PET scanner (General Electric Medical Systems, Milwaukee, Wisconsin) as described previously (Lanzenberger et al. 2007). Briefly, to ensure the inclusion of the cerebellum in the field of view, the subjects’ heads were positioned in the scanner parallel to the orbitomeatal line by means of a laser beam system. Subjects were instructed not to speak nor move during the measurements. Furthermore, in order to minimize head movements, each subject’s head was fixated in a polyurethrane molded cushion with straps around the forehead and chin. Following 5-min transmission scan performed in two-dimensional mode for correction of tissue attenuation (68Ge rod sources), dynamic PET scans were acquired in three-dimensional mode. The measurements started simultaneously with bolus injection of [carbonyl-11C]WAY-100635 diluted in phosphate-buffered saline in a cubital vein. The radioligand was prepared at the Cyclotron Unit of the PET Center according to previously published methods (Wadsak et al. 2007). The average administered dose of the tracer was 296.62±92.96 MBq with a specific activity of 280.85±247.49 MBq/nmol and total acquisition time was 90 minutes. The emission data were scatter and attenuation corrected and the final images comprised a spatial resolution of 4.36 mm full-width at half-maximum 1cm next to the center of the field of view (matrix 128x128, 35 slices).

Data preprocessing and serotonin-1A receptor quantification

PET scans were motion-corrected and spatially normalized to a (in house) 5-HT1A receptor distribution template in Montreal Neurological Institute (MNI) space using Statistical Parametric Mapping (SPM8, Wellcome Trust Centre for Neuroimaging, http://www.fil.ion.ucl.ac.uk/spm) (Fink et al. 2009). Whole-brain 5-HT1A binding potential maps were computed in PMOD 3.3 (PMOD Technologies, Ltd., Zurich, Switzerland) as described previously (Hahn et al. 2010; Hahn et al. 2012). The multilinear reference tissue model 2 (MRTM2 (Ichise et al. 2003)) was applied to obtain voxel-wise estimates of 5-HT1A receptor binding (5-HT1A BPND (Innis et al. 2007; Hahn et al. 2010)). For each subject the individual clearance rate of the radiotracer from the reference region to plasma (k2’) was calculated from the insula (receptor-rich region) and cerebellum (receptor-poor region) (Ichise et al. 2003; Varnas et al. 2004). These regions of interest were taken from an automated anatomical labeling-based atlas (Tzourio-Mazoyer et al. 2002; Savli et al. 2012), whereas the cerebellar gray matter (excluding vermis and sagittal sinus) served as reference region due to negligible specific receptor binding in this area (Hall et al.
For the estimation of k2’, the time activity curves were extracted as average across left and right hemisphere. Hence, the regions comprised 4096 voxels (32.768 cm³) and 559 voxels (4.47 cm³) for the insula and cerebellar gray matter, respectively. This yields a sufficient signal to noise ratio for stable parameter estimation as well as high signal contrast between the two regions (Savli et al. 2012). As implemented in the pixel-wise modeling tool of PMOD 3.3, k2’ is first calculated using the MRTM model with a maximum error of 10%, and then inserted into MRTM2 for the computation of BP_{ND} maps (Savli et al. 2012; Ichise et al. 2003). For completeness, given the inconsistencies in the literature in regard to the cerebellar gray as reference region for 5-HT_{1A} receptor quantification (Parsey et al. 2005; Hirvonen et al. 2006), in a second step 5-HT_{1A} BP_{ND} was modeled using the cerebellar white matter.

**Statistical analysis**

In order to reveal a main effect of genotype on 5-HT_{1A} receptor distribution in the whole brain, voxel-wise analysis of variance (ANOVA) was computed using 5-HT_{1A} receptor binding potential as dependent variable and genotype (AA, AG and GG) as factor in SPM8. *Post-hoc* t-tests were performed with the objective of detecting a certain risk allele responsible for a major change in 5-HT_{1A} receptor binding potential. Comparisons of both AA homozygotes vs. G-carriers (AG+GG) and GG homozygotes vs. A-carriers (AA+AG) were drawn, testifying for the presence of a 5-HT_{1A} receptor binding modulating allele. To exclude a potential influence of age and sex on 5-HT_{1A} receptor binding potential, regression analyses were computed for both variables. All statistical tests were evaluated at a significance level of p<0.05 corrected for multiple comparisons with family wise error (FWE) at voxel-level. Regions of no interest (e.g., skull, cerebral blood vessels) were excluded from the statistical analyses. Additionally, we computed Cohen’s d in regard to effects sizes using the pooled standard deviation, as described previously (Kranz et al. 2012). Although whole-brain voxel-wise analyses clearly require correction for multiple comparisons, they imply major advantages over ROI-based evaluation, namely independence of choice, size and location of regions of interest (Lanzenberger et al. 2012a; Kranz et al. 2012; Lanzenberger et al. 2012b).
Average regional 5-HT$_{1A}$ receptor binding potential values of all subjects are summarized in the table 2. Among the study population comprising 52 healthy subjects, 10 were homozygote AA carriers, 9 were homozygote GG carriers and 33 subjects presented an AG allele combination for the COMT SNP rs4680 (see table 2). Whole brain voxel-wise ANOVA revealed a main effect of genotype on 5-HT$_{1A}$ receptor binding potential in a cluster encompassing the posterior cingulate cortex (PCC) (voxel-level: $x/y/z=10/-40/36$, $F_{(2,49)}=17.7$, $p=0.05$, FWE corrected) and in part the precuneus (see table 2). The inclusion of age and gender as covariates in the analysis lead to similar findings (voxel-level: same coordinates, $F_{(2,47)}=20.11$, $p<0.05$, FWE corrected). According to post-hoc t-test, homozygote GG subjects displayed significantly higher 5-HT$_{1A}$ receptor binding than A carriers in this brain region ($t=5.95$, $p<0.05$, FWE corrected, see figure 2 and table 2 for percentage change based on mean 5-HT$_{1A}$ binding potential of all A carriers). Furthermore, there was no significant difference in 5-HT$_{1A}$ receptor binding potential in any region when comparing AA and AG genotypes. Similarly, when pooling all G carriers and comparing them to homozygote AA carriers, voxel-wise ANOVA yielded no significant difference in 5-HT$_{1A}$ receptor binding potential (all $p>0.01$ uncorrected). For completeness, following an exploratory approach, post-hoc t-tests were computed comparing all three groups (AA, AG and GG). Significantly higher 5-HT$_{1A}$ receptor binding potential in GG vs. AA carriers ($x/y/z=10/-40/36$, $T=5.5$, $p<0.05$, FWE corrected) and AG carriers ($x/y/z=10/-40/36$, $T=5.28$, $p<0.05$, FWE corrected) in the PCC but no significant difference between AA and AG subjects ($p>0.05$, FWE corrected). Regarding the effect sizes, computations of Cohen’s d showed marked effects for the PCC when comparing AA vs. GG homozygotes (d=2.52) and AG vs. GG (d=1.91), but not for AA vs. AG carriers (d=0.53). The use of cerebellar white matter as reference region yielded similar results regarding the overall F-test (PCC: $F_{(2,49)}=17.63$; $p=0.06$ FWE-corrected voxel-level) and the post-hoc t-test (PCC: $t=5.94$; $p<0.05$ FWE-corrected voxel-level).

Regression analysis using age and sex as factors revealed a negative association of age and 5-HT$_{1A}$ receptor binding in 6 voxels scattered to 4 clusters ($x/y/z=32/-28/20$, $T=-5.31$, $k=1$; $x/y/z=-32/-32/20$, $T=-5.53$, $k=2$; $x/y/z=6/-80/-6$, $T=-5.24$, $k=2$; $x/y/z=34/-32/22$, $T=-5.21$, $k=1$, all $p<0.05$ FWE-corrected). As this effect is very limited and does not interfere regionally with the main finding regarding the influence of genotype on 5-HT$_{1A}$ receptor binding, this association was interpreted as a statistical artefact and therefore no further attention was paid to this outcome. Regarding gender, no significant association could be detected in any region.
For exploratory purposes, in a second step, we applied a less stringent correction for multiple comparisons to the voxel-wise ANOVA to provide a comprehensive view to the effect of rs4680 on 5-HT$_{1A}$ receptor binding. 5-HT$_{1A}$ binding potential reductions could be bilaterally equally noticed in a high number of brain regions, among them the orbitofrontal cortex, the anterior cingulate cortex, the insula, the amygdala and the hippocampus (voxel-level: p<0.01 uncorrected, t>2.4; cluster-level: p<0.05 FWE corrected, k>1293, see figure 1). Again, the post-hoc t-test pointed in the same direction as described for the PCC, with elevated 5-HT$_{1A}$ receptor binding potential in homozygote GG subjects (see table 2). Similarly, Cohen’s d computations for the entire cluster showed large effects when comparing AA vs. GG homozygotes (d=1.43) and AG vs. GG (d=1.08), but not for AA vs. AG carriers (d=0.33). Using cerebellar white matter as reference region for 5-HT$_{1A}$ BP$_{ND}$ quantification, ANOVA revealed a large cluster mostly overlapping with the cluster described above (t>2.4, voxel-level: p<0.01 uncorrected, t>2.4; cluster-level: p<0.05 FWE-corrected, k=47365 voxels).

Again, as gender was steadily shown to affect 5-HT$_{1A}$ receptor binding with higher 5-HT$_{1A}$ receptor density in females (Parsey et al. 2005; Parsey et al. 2002; Jovanovic et al. 2008), sex was included as covariate in the statistical analysis to test for possible confounders. No sex-difference could be detected and the computations lead to findings congruous to those initially obtained (PCC: t=5.85, p<0.05, FWE-corrected). Similarly, including age as variable of no interest (Stein et al. 2008b; Parsey et al. 2005; Parsey et al. 2002; Jovanovic et al. 2008) did not change our results (PCC: t=6.25, p<0.05, FWE-corrected).
3.1.5. Discussion

The findings described above show a significantly higher 5-HT$_{1A}$ receptor binding potential in several brain regions, in particular the posterior cingulate cortex, in homozygote GG carriers (VAL) versus A carriers (MET) of the rs4680 SNP (see figure 1) and support the assumption that COMT might act as major modulator of 5-HT$_{1A}$ receptor expression.

The 5-HT$_{1A}$ receptor has been subject to intense basic and clinical neuropsychiatric research in the past decades, especially within the framework of the search for biological markers for manifold psychiatric disorders as well as indicators of treatment response to antidepressants. In this context, starting with animal studies (Blier and de Montigny 1994) up to post-mortem investigations in suicide victims (Stockmeier et al. 1998) and finally all the way to PET studies in vivo (Lanzenberger et al. 2007; Parsey et al. 2006; Hirvonen et al. 2008), the 5-HT$_{1A}$ receptor was repeatedly demonstrated to be implicated in the pathogenesis of mood and anxiety disorders. One of the most consistent findings emphasizes a reduced 5-HT$_{1A}$ receptor binding in depression (Savitz et al. 2009; Hirvonen et al. 2008). Based on our results, which suggest a possible mechanism through which COMT may play a role in the pathophysiology of depression by modulating the 5-HT$_{1A}$ receptor, one might assume that homozygosis for the G allele in the COMT gene might represent a protective allele for affective disorder. Similarly, 5-HT$_{1A}$ receptor binding was shown to be reduced in patients suffering from social anxiety and panic disorder as compared to healthy subjects (Lanzenberger et al. 2007; Neumeister et al. 2004). Hence, one might carefully interpret the data to suggest that expression of the rs4680 A allele may be accompanied by an increased vulnerability to anxiety disorders compared to GG carriers. This plays in concert with the study performed by Woo et al. showing an association between the A allele of the rs4680 SNP, heightened trait-anxiety levels and increased risk for panic disorder (Woo et al. 2004). Accordingly, in a sample of 75 Japanese depressive disorder patients, the A allele was represented more frequently than in 135 healthy volunteers (Ohara et al. 1998). Nonetheless, a number of studies question the assumption that the A allele acts as risk factor for psychiatric disorders. Rather, evidence suggests an implication of the high-activity G allele (VAL) in anxiety disorders, particularly panic disorder, via enhanced COMT activity and thus lower prefrontal dopamine levels (Domschke et al. 2008; Domschke and Dannlowski 2010; Rothe et al. 2006; Hamilton et al. 2002), which stands in contrast to the earlier mentioned findings in Asian patient cohorts (Woo et al. 2004; Ohara et al. 1998). In the context of the present study, this would imply that the potential risk allele G is accompanied by elevated 5-HT$_{1A}$ receptor binding (see figure 2). Moreover, it should not remain unmentioned that there is data indicating
heightened 5-HT\textsubscript{1A} receptor levels in major depression. Parsey et al. determined a higher 5-HT\textsubscript{1A} receptor binding potential in medication-naïve depressed patients compared to depressed subjects with a history of antidepressant exposure and controls (Parsey et al. 2010). However, when grouping all depressed patients (drug-free and drug-naïve), no difference between patients and healthy controls could be shown. These contrasting findings might be explained by methodological issues, particularly the applied reference model for 5-HT\textsubscript{1A} BP\textsubscript{ND} computations (Parsey et al. 2010).

As COMT represents the major dopamine degrading enzyme in the prefrontal cortex (PFC), and rs4680 was shown to determine the catabolic activity of COMT, this SNP has been intensively investigated in regard to psychosis (Lachman et al. 1996). Higher activity rates can be observed with GG homozygotes, thereby leading to enhanced dopamine degradation in the PFC and, presumably, for the purpose of a negative feedback mechanism, elevated dopamine biosynthesis in the midbrain (Tunbridge et al. 2006; Akil et al. 2003; Scanlon et al. 1979). Schizophrenia, schizoaffective and bipolar disorders have repeatedly been shown to be associated with rs4680, whereby there is equally still disagreement whether the A or G allele represents one of the causal factors for developing such psychiatric conditions (Craddock et al. 2006). A robust finding concerning rs4680 is its modulatory effect on frontal lobe function, more specifically on cognitive performance (Craddock et al. 2006). Using neurophysiological, multimodal PET and functional magnetic resonance imaging (fMRI) methods, COMT was consistently shown to impact on prefrontal cortex activity (Winterer et al. 2006a; Winterer et al. 2006b; Meyer-Lindenberg et al. 2005). Impairment of PFC structure and function, more specifically its ventromedial part encompassing segments of the anterior cingulate and the orbitofrontal cortex, has been largely associated with mood disorders using fMRI (Scharinger et al. 2010). Additionally, cognitive decline equally represents a core symptom of major depression (Ravnkilde et al. 2002). Hence, one might assume that COMT - by influencing cognitive function - might impact on a certain intermediate phenotype, namely cognitive impairment, common to both psychotic and mood disorders (Soeiro-de-Souza et al. 2012).

The interplay of serotonin and dopamine has been shown to play an essential role in the pathophysiology of neuropsychiatric disorder including depression (Esposito et al. 2008; Alex and Pehek 2007). Initially, compared to serotonin and norepinephrine, the role of dopamine in depression has largely been neglected. However, there is a large body of research postulating the functional involvement of this neurotransmitter in at least two core symptoms of depression, namely anhedonia and motivational deficits (Yadid and Friedman 2008). Besides, preclinical
studies attribute a role to 5-HT\textsubscript{1A} autoreceptors in the raphe nuclei in the regulation of dopamine release, which is mirrored by the fact that both serotonin and dopamine were shown to determine reward-related behavior (Kranz et al. 2010; Yoshimoto and McBride 1992). Recent findings indicate an association of serotonin-dopamine interactions and antidepressant treatment response, presumably mediated via serotonin, which stimulates dopamine release in the nucleus accumbens and thereby leads to a diminution of depressive symptoms (Zangen et al. 2001). In line with these findings, \textit{COMT} VAL158MET polymorphism was repeatedly shown to impact on antidepressant treatment response (Benedetti et al. 2010a; Benedetti et al. 2009; Benedetti et al. 2010b), where carriers of the MET allele seem to exhibit a better treatment response than VAL carrier (Antypa et al. 2013). For excellent review see (Kocabas 2012).

Our study applies a similar approach as previously demonstrated by David et al., namely that polymorphisms of a distinct gene, in this case the 5-\textit{HTTLPR}, exhibit a certain modulatory influence on other functionally-related genes and their expression product, for example the 5-HT\textsubscript{1A} receptor (David et al. 2005). However, in the field of genetic association studies, it is well known that new findings should not be accepted without further questioning as there is evidence compromising the potential association of \textit{COMT} genotype and psychiatric phenotypes (Schosser et al. 2012; Rotondo et al. 2002; Craddock et al. 2006). Nonetheless, gene-gene interaction effects between \textit{COMT} and serotonin-related genes, such as 5-\textit{HTTLPR} (Tadić et al. 2009) and the monoamine oxidase A gene (\textit{MAO-A}) (Comasco et al. 2011) have been determined for psychiatric phenotypes emphasizing a potential regulatory mechanism of rs4680 on the serotonergic system. Systemic approaches to the development of psychiatric disorders consider complex receptor density alterations on a network level (Hahn et al. 2012) resulting in neuronal rearrangement and adaption processes of distinct functionally-related proteins as in this study the 5-HT\textsubscript{1A} receptor and \textit{COMT}. It is assumed that a number of feedback mechanism are involved in the regulation of the serotonergic system, hence, effects of single SNPs of serotonin-related genes might be compensated within the serotonergic network. These up- and down-regulation mechanisms might be more fine-tuned within a single neurotransmitter system than between different systems, such as the dopaminergic and serotonergic network, as presented in this study. Here, we assume that the expression of rs4680 – by regulating \textit{COMT} activity (Lachman et al. 1996) – determines dopamine turnover which in turn impacts on serotonergic neurotransmission. So far, direct interactions of \textit{COMT} and serotonin have been shown in the context of pain perception. Via binding to the catalytic site of \textit{COMT}, serotonin prevents methylation of \textit{COMT} substrates provoking pain hypersensitivity in mice (Tsao et al. 2012). Despite this finding, current scientific knowledge regarding a direct interplay of \textit{COMT}
and serotonergic proteins in psychiatric disorders remains unsatisfactory. The present PET study proposes a possible mechanism of action in vivo via modulation of 5-HT$_{1A}$ receptor binding by COMT, which is in line with the earlier described indirect associations of the serotonergic and the dopaminergic transmitter system. The relevance of our findings is even more pronounced as they concern brain structures – as the PCC, the PFC, the amygdala, the hippocampus (see table 2) – involved in emotion processing and consistently reported to be functionally altered in psychiatric diseases or antidepressant treatment (Hoffich et al. 2012; Lanzenberger et al. 2012a).

As a limitation of the study, one should not leave unmentioned the relatively small sample size of 52 subjects in the context of a study involving genetic variations. However, regarding the combination of imaging using PET and genetics, most previous molecular imaging studies included an even smaller number of individuals (Praschak-Rieder et al. 2007; David et al. 2005). In order to ensure a sufficiently high statistical power, pooled PET data from healthy subjects who had previously participated in several studies from our group were included in the analysis (Hahn et al. 2010; Lanzenberger et al. 2011; Fink et al. 2009). Our sample is therefore relatively heterogeneous, particularly in gender distribution, with women accounting for approximately two thirds of the study population, and age ranging between 21 and 63 years. However, the inclusion of both factors as covariates in the statistical analysis did not affect our findings.

Although clear evidence demonstrates the role of 5-HT$_{1A}$ receptor in the pathogenesis of mood disorder, we cannot attribute definitive causality to rs4680 in these processes with this dataset. In this regard, the implementation of psychological scales would have provided a gain in information. Moreover, the inclusion of serotonergic SNPs would have been of major interest, especially polymorphisms directly related to the serotonergic system such as the earlier mentioned (C-(1019)G). Subparts of the sample were genotyped for rs6295 (5-HT$_{1A}$ receptor), rs6298 (5-HT$_{1B}$ receptor gene) and rs6265 (BDNF gene). However, - as no complete genetic profiles were available for the entire set of healthy individuals and DNA material/blood samples being limited - these data were not included in the analyses. Initial computations indicated no significant effect of this SNP on 5-HT$_{1A}$ receptor binding (Lanzenberger et al. 2013).

Finally, no arterial blood sampling was carried out in the current work limiting the analysis to BP$_{ND}$. Although binding potential values relative to total (BP$_P$) or free (BP$_F$) radioligand in plasma are independent of differences in non-specific binding, this might be less of a problem for this study population. Specifically, only healthy controls were included and no treatment was applied, hence, it is reasonable to assume non-displaceable binding to be constant across subjects. Furthermore, binding potentials obtained from arterial blood sampling strongly correlate with
those from a reference region model in healthy subjects (Gunn et al. 1997). Concerning the quantification of 5-HT$_{1A}$BP$_{ND}$, there are methodological inconsistencies in the literature, e.g., the use of reference region, leading to opposing findings. Parsey et al. determined higher 5-HT$_{1A}$ receptor binding in depressed patients compared to control subjects using both cerebellar white and gray matter as reference (Parsey et al. 2010; Parsey et al. 2006; Parsey et al. 2005). Using cerebellar white matter in our computations, however, did not change our findings (see results).

3.1.6. Conclusions

To sum up, this PET study including 52 healthy volunteers shows a markedly higher 5-HT$_{1A}$ receptor binding potential in homozygote G subjects versus A carriers of the rs4680 SNP, pointing toward a crucial involvement of COMT in the modulation of 5-HT$_{1A}$ receptor density. Moreover, these findings provide a rationale for follow-up projects investigating the link between the COMT gene and affective and anxiety disorders, which were shown to be associated with altered levels of 5-HT$_{1A}$ receptor binding. Our results indicate a modulatory role of rs4680 in the development of neuropsychiatric disorders as altered 5-HT$_{1A}$ receptor binding which is accompanied by an elevated risk for depression and anxiety disorders. Further replication studies in patients might be needed to undermine this result and in view of the enlightenment of the multifaceted interplay of the different actors of the serotonergic neurotransmitter system.
ACKNOWLEDGMENTS

This research was supported by funds of the Oesterreichische Nationalbank (Anniversary Fund, project number: 11468, 12809) to R. Lanzenberger and S. Kasper, respectively, and an intramural grant of the Department of Psychiatry and Psychotherapy (Forschungskostenstelle). A. Hahn was recipient of a DOC-fellowship of the Austrian Academy of Sciences (OeAW) at the Department of Psychiatry and Psychotherapy. We thank Stefanie Pichler for clinical support and Marie Spies for linguistic revision of the manuscript. We thank the PET team at the Department of Nuclear Medicine for technical support.

CONFLICT OF INTEREST

Without any relevance to this work, S. Kasper declares that he has received grant/research support from Eli Lilly, Lundbeck A/S, Bristol-Myers Squibb, Servier, Sepraco, GlaxoSmithKline, Organon, and has served as a consultant or on advisory boards for AstraZeneca, Austrian Sick Fund, Bristol-Myers Squibb, GlaxoSmithKline, Eli Lily, Lundbeck A/S, Pfizer, Organon, Sepraco, Janssen, and Novartis, and has served on speakers' bureaus for AstraZeneca, Eli Lilly, Lundbeck A/S, Servier, Sepraco and Janssen. R. Lanzenberger received travel grants and conference speaker honoraria from AstraZeneca and Lundbeck A/S. M. Mitterhauser and W. Wadsak received speaker honoraria from Bayer.
3.1.7. References


Comasco E, Sylven SM, Papadopoulos FC, Sundstrom-Poromaa I, Orelend L, Skalkidou A


Innis RB, Cunningham VJ, Delforge J, Fujita M, Gjedde A, Gunn RN, Holden J, Houle S, Huang


Lanzenberger R, Mitterhauser M, Kranz GS, Spindelegger C, Wadsak W, Stein P, Moser U,


Parsey RV, Ogden RT, Miller JM, Tin A, Hesselgrave N, Goldstein E, Mikhno A, Milak M,


Scanlon PD, Raymond FA, Weinshilboum RM (1979) Catechol-O-methyltransferase:
thermolabile enzyme in erythrocytes of subjects homozygous for allele for low activity. Science 203 (4375):63-65


Stein P, Savli M, Wadsak W, Mitterhauser M, Fink M, Spindelegger C, Mien LK, Moser U,
distribution in healthy men and women measured by PET and [carbonyl-11C]WAY-

Stockmeier CA, Shapiro LA, Dilley GE, Kolli TN, Friedman L, Rajkowska G (1998) Increase in
serotonin-1A autoreceptors in the midbrain of suicide victims with major depression-
postmortem evidence for decreased serotonin activity. J Neurosci 18 (18):7394-7401

Dahmen N (2009) Interaction between gene variants of the serotonin transporter
promoter region (5-HTTLPR) and catechol O-methyltransferase (COMT) in borderline
personality disorder. American Journal of Medical Genetics Part B: Neuropsychiatric

Tsao D, Wieskopf JS, Rashid N, Sorge RE, Redler RL, Segall SK, Mogil JS, Maixner W,
Dokholyan NV, Diatchenko L (2012) Serotonin-induced hypersensitivity via inhibition of

and Psychosis: Val158Met and Beyond. Biological Psychiatry 60 (2):141-151.

Tzourio-Mazoyer N, Landeau B, Papathanassiou D, Crivello F, Etard O, Delcroix N, Mazoyer B,
macroscopic anatomical parcellation of the MNI MRI single-subject brain. NeuroImage 15

doi:10.1002/hbm.20035

Wadsak W, Mien LK, Ettlinger DE, Eidherr H, Haeusler D, Sindelar KM, Keppler BK, Dudczak R,
Kletter K, Mitterhauser M (2007) 18F fluoroethylations: different strategies for the rapid

Winterer G, Egan MF, Kolachana BS, Goldberg TE, Coppola R, Weinberger DR (2006a)
Prefrontal Electrophysiologic “Noise” and Catechol-O-Methyltransferase Genotype in

Winterer G, Musso F, Vucurevic G, Stoeter P, Konrad A, Seker B, Gallinat J, Dahmen N,
Weinberger DR (2006b) COMT genotype predicts BOLD signal and noise characteristics

disorder and the L/L genotype of catechol-O-methyltransferase. Journal of Psychiatric


TABLES

**TABLE 1**: Age, gender and genotype distributions of 52 healthy subjects

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<th>Genotype</th>
<th>AA</th>
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<th>GG</th>
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<tr>
<td></td>
<td>MET/MET</td>
<td>VAL/MET</td>
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<tr>
<td>N = 52</td>
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<td>33</td>
<td>9</td>
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<tr>
<td>Age (± SD)</td>
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<tr>
<td>Gender (M:F)</td>
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SD standard deviation; M male; F female
TABLE 2: Serotonin-1A receptor binding potentials (5-HT\textsubscript{1A} BP\textsubscript{ND}) showing significant differences between A carriers and GG homozygotes of the COMT rs4680 genotype. 5-HT\textsubscript{1A} receptor binding values are given as mean±SD. A regional selection of peak t-values is shown following voxel-wise post-hoc t-test of the ANOVA († t>5.18, p<0.05 FWE corrected; ** t>3.27 p<0.001 uncorrected; * t>2.4, p <0.01 uncorrected). Relative changes (%) are obtained when comparing 5-HT\textsubscript{1A} receptor binding of A carriers (AA+AG) versus GG carriers of rs4680. Regions of interest were taken from an automated anatomical labeling atlas (Tzourio-Mazoyer et al. 2002; Savli et al. 2012).

<table>
<thead>
<tr>
<th>Region of Interest</th>
<th>MNI coordinates (mm)</th>
<th>5-HT\textsubscript{1A} BP\textsubscript{ND} for rs4680 genotypes (mean ± SD)</th>
<th>5-HT\textsubscript{1A} BP\textsubscript{ND} AA+AG vs GG</th>
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MNI Montreal Institute of Neurology; 5-HT\textsubscript{1A} BP\textsubscript{ND} serotonin-1A receptor binding potential non-displaceable; SD standard deviation; L left; R right.
FIGURES

FIGURE 1: Differences in serotonin-1A receptor binding potential between AA+AG versus GG genotype carriers of the rs4680 SNP, presented on an axial and sagittal view, superimposed on a structural magnetic resonance image template. Shown are post-hoc t-test values of the ANOVA (voxel-level p<0.01 uncorrected, t>2.4, cluster-level p<0.05 FWE corrected, k>1293). The peak value can be observed in the posterior cingulate cortex (t>5.95, p<0.05 FWE-corrected, voxel-level). The color table indicates the t values. Cross is located at the coordinates x/y/z=-3/-42/41mm in MNI space.
FIGURE 2: Scatter plot showing the mean serotonin-1A receptor binding potential for each genotype of the rs4680 SNP in the posterior cingulate cortex for the peak difference between groups (x/y/z=10/-40/36, MNI space, t=5.95, p<0.05, FWE corrected). Binding potential values are highest for homozygote GG carriers (N=9) of the rs4680 SNP, where A carriers (AA: N=10; AG: N=33) display significantly lower values, for both applies p<0.001 (annotated with ***). There was no significant difference between AA and AG carriers (ns, p>0.1).
3.2. **Second publication: Exploring the impact of BDNF Val66Met genotype on serotonin transporter and serotonin-1A receptor binding**

Christoph Kraus¹, MD, **Pia Baldinger**¹, MD, Christina Rami-Mark², MSc, Gregor Gryglewski¹, Georg S. Kranz¹, PhD, Daniela Haeusler², PhD, Andreas Hahn¹, PhD, Wolfgang Wadsak², Assoc. Prof. PD PhD, Markus Mitterhauser², Assoc. Prof. PD, Dan Rujescu³, Prof. MD, Siegfried Kasper¹, Prof. MD, Rupert Lanzenberger¹*, Assoc. Prof. PD MD

¹ Department of Psychiatry and Psychotherapy,
² Department of Biomedical Imaging und Image-guided Therapy, Division of Nuclear Medicine, Medical University of Vienna, Austria
³ Department of Psychiatry, Medical University of Halle, Germany

Accepted for publication in
PLoS One
[IF 2013: 3.534]

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Figures: 2, Tables: 3

* Correspondence to: Rupert Lanzenberger, A/Prof, MD
Functional, Molecular and Translational Neuroimaging Lab
Department of Psychiatry and Psychotherapy
Medical University of Vienna,
Währinger Gürtel 18-20, 1090 Vienna, Austria
rupert.lanzenberger@meduniwien.ac.at
http://www.meduniwien.ac.at/neuroimaging/
3.2.1. Abstract

**Background:** The brain-derived neurotrophic factor (BDNF) Val66Met polymorphism (rs6265) may impact on the *in-vivo* binding of important serotonergic structures such as the serotonin transporter (5-HTT) and the serotonin-1A (5-HT_{1A}) receptor. Previous positron emission tomography (PET) studies on the association between Val66Met and 5-HTT and 5-HT_{1A} binding potential (BP_{ND}) have demonstrated equivocal results.

**Methods:** We conducted an imaging genetics study investigating the effect of Val66Met genotype on 5-HTT or 5-HT_{1A} BP_{ND} in 92 subjects. Forty-one subjects (25 healthy subjects and 16 depressive patients) underwent genotyping for Val66Met and PET imaging with the 5-HTT specific radioligand \[^{11}\text{C}]\text{DASB. Additionally, in 51 healthy subjects Val66Met genotypes and 5-HT} \textsubscript{1A} binding with the radioligand \[^{carbonyl-11}\text{C}]\text{WAY}-100635 were ascertained. Voxel-wise and region of interest-based analyses of variance were used to examine the influence of Val66Met on 5-HTT and 5-HT_{1A} BP_{ND}.

**Results:** No significant differences of 5-HTT nor 5-HT_{1A} BP_{ND} between BDNF Val66Met genotype groups (val/val vs. met-carrier) were detected. There was no interaction between depression and Val66Met genotype status.

**Conclusion:** In line with previous data, our work confirms an absent effect of BDNF Val66Met on two major serotonergic structures. These results could suggest that altered protein expression associated with genetic variants, might be compensated *in vivo* by several levels of unknown feed-back mechanisms. In conclusion, Val66Met genotype status is not associated with changes of *in-vivo* binding of 5-HTT and 5-HT_{1A} receptors in human subjects.
3.2.2. Introduction

The brain-derived neurotrophic factor (BDNF) is the most prominent member in the neurotrophin family and involved in development and activity-dependent regulation of neuronal structures [1]. Cumulating evidence demonstrated a functional interplay between BDNF and the neurotransmitter serotonin (5-HT), constituting common intracellular signaling pathways and transcription factors, BDNF control over the development and function of serotonergic neurons as well as serotonergic regulation of BDNF gene expression and signaling [2].

Briefly, BDNF is linked with at least three major intracellular signaling cascades: the phosphoinositide-3 kinase pathway enabling cell survival, the phospholipase-gamma pathway effecting synaptic plasticity and the mitogen-activated protein kinase pathway associated with neuronal differentiation and neurite outgrowth [3]. Beside the p75 neurotrophin receptor, which is activated by proBDNF and all other neurotrophins, BDNF releases its effects by binding to tropomyosin-kinase related receptor B (TrkB) [4-6]. Thereby, BDNF is a major factor in the proper development and plastic regulation of the central nervous system and highly active in limbic structures such as the hippocampus and the amygdala, where long-term potentiation, learning and memory are facilitated [7]. However, it should be stated here that most of the evidence of BDNF in this context is based on rodent data.

The BDNF gene is located at chromosome 11p13-14, including many splice sites and promoters. All BDNF mRNAs are initially translated into proBDNF and are then cleaved into mature BDNF [8]. The most investigated polymorphism of the BDNF gene exists in the codon 66 of proBDNF (Val66Met, rs6265) and consists of a valine to methionine substitution, which is associated with reduced intracellular proBDNF trafficking, synaptic secretion of BDNF, and thus a lower extracellular BDNF concentration in met-allele carriers [9]. Thought to trigger deficits in neuronal development and plasticity, the Val66Met polymorphism is of major interest in neuropsychiatric research [2, 7].

Interestingly, in humans the molecular connections between 5-HT and BDNF, and how alterations in one system affect the other are hardly known. Due to the lack of current methods to measure BDNF, TrkB or p75 in the living human brain, in vivo research in humans mainly focuses on the investigation of alterations of serotonergic structures thought to be mediated via changes in BDNF. In imaging genetics studies, serotonergic markers are labeled by radioligands and their binding is measured using PET. As yet, there exist three studies investigating alterations of BDNF, as represented by the Val66Met polymorphism, and its association with binding of 5-HT1A, 5-HT2A receptors as well as the 5-HTT in the human brain [10-12]. Two previous studies failed to detect links between Val66Met and binding of 5-HT1A and 5-HT2A.
receptors. On the other side, a recently published study reports lower 5-HT_{1A} binding in healthy subjects carrying the met-allele compared to val-homozygotes, a difference which was not observed in depressed subjects [12]. As far as 5-HTT is concerned, in one study, applying the serotonin transporter (5-HTT) specific radioligand \[^{11}\text{C}]\text{-MADAM (N=25)} with PET and \[^{123}\text{I}]\text{-ß-CIT (N=18)} with single photon emission tomography (SPECT) in two independent samples, the authors found increased 5-HTT binding in val-homozygote male subjects and compared to met-allele carriers [10]. On the other hand applying the radioligand \[^{11}\text{C}]\text{DASB (N=49)}, the second study failed to detect any effect of Val66Met genotype status on 5-HTT binding [11].

To resolve contradictory results we conducted an imaging genetics study investigating the association between 5-HTT binding using PET with the radioligand \[^{11}\text{C}]\text{DASB and the Val66Met genotype status in healthy subjects as well as in depressive patients. We also measured 5-HT}_{1A} receptor binding in healthy subjects genotyped for Val66Met, in order to resolve two equivocal findings. We hypothesized, that Val66Met impacts on 5-HTT binding in patients with major depression and healthy subjects. Furthermore, we hypothesized that significant differences are detected between BDNF genotype status and 5-HT_{1A} binding in healthy subjects.
3.2.3. Methods

Subjects

In a neuroimaging genetics study with a cross-sectional design in total 92 subjects, aged 18-65 years were included. The study was divided into two groups, in the first one 51 healthy adult volunteers (37 female) were included and measured with [carbonyl-\(^{11}\)C]WAY-10063. In the second group 25 healthy subjects (HS) and 16 currently depressed patients with an Hamilton Depression Rating Scale ≥ 16 (HAMD: 19.7±3.5, mean±SD) were included (for further details see table 1) and measured with \(^{11}\)C]DASB. None of the subjects received both radioligands. The study population originates from a pooled sample, which is part of previously published studies [13-16]. Genotyping data of BNDF were previously not published. All subjects underwent a psychiatric screening by the help of the complete Structured Clinical Interview for DSM-IV type disorders (SCID I+II), physical and neurological examination, clinical history, ECG, routine laboratory analysis, urinary drug and pregnancy screening. All subjects were at least three months free of any psychotropic medication. Every study subject was enrolled in study participation after detailed oral information about all study procedures and subsequent signing of a written informed consent form. The study and all study related procedures were approved by the Ethics Committee of the Medical University of Vienna.

BDNF Genotyping

All procedures were performed as previously described [13]. Briefly, DNA was isolated from peripheral blood mononuclear cells by the QIAamp DNA Mini-Kit (QIAGEN®, Hilden, Germany). Genotyping of BDNF rs6265 single nucleotide polymorphism (SNP) was conducted with the MassARRAY platform (SEQUENOM®, San Diego, CA) as described elsewhere [17]. PCR-primers were generated with the Assay Designer 4.0 software (SEQUENOM®). Multiplex PCR reactions were performed with 12.5ng of genomic DNA, 500μM dNTPs (ABgene®, Hamburg, Germany), 100nM PCR primers, 1.625mM MgCl₂ and 0.5U HotStar Taq polymerase (QIAGEN®). Shrimp alkaline phosphatase (SAP) treatment, an iPLEX reaction cocktail with extension primers (7-14μM), an iPLEX termination mix and an iPLEX enzyme (SEQUENOM®) were added to the PCR-products. The resulting extension products were desalted using SpectroCLEAN resin (SEQUENOM®), then spotted on SpectroCHIPs GenII (SEQUENOM®) and analyzed with the MassARRAY MALDI-TOF mass spectrometer. Typer 3.4 Software was used to identify allele specific extension products and resulting genotypes (SEQUENOM®). For
genotyping quality assurance CEU HapMap Trios (Coriell Institute for Medical research, Camden, NJ) were included and compared with the HapMap-CEU population (www.hapmap.org). For all analyses val/val homozygotes (=GG-carriers) were compared against met-carriers (AG- and AA-carriers).

Radiochemistry of $[^{11}\text{C}]$DASB and $[^{11}\text{C}]$WAY-100635 and PET Procedures

Radioligand synthesis and all PET measurements were conducted at the Department of Biomedical Imaging und Image-guided Therapy, Division of Nuclear Medicine at the Medical University of Vienna. PET measurements were performed with a GE Advance full ring PET scanner (General Electric Medical Systems, Waukesha, WI, USA). Subjects were placed with their head parallel to the orbitomeatal line guided by a laser beam system to ensure full coverage of the neocortex and the cerebellum in the field of view (FOV). A polyurethane cushion and head straps were used to minimize head movement and to guarantee a soft head rest during the whole scanning period.

For a complete description of $[^{11}\text{C}]$DASB radioligand synthesis see [18]. Mean injected dose was $358.97\pm70.47$ MBq, specific activity at time of injection was $49.00\pm38.10$ MBq/nmol and radiochemical purity was above 95%. After a 5 min transmission scan with retractable $^{68}\text{Ge}$ rod sources the 3D dynamic emission measurement was initiated simultaneously with the intravenous bolus injection of the radioligand $[^{11}\text{C}]$DASB. The total acquisition time (35 slices) was 90 min and reconstructed images comprised a spatial resolution of 4.36 mm full-width at half-maximum (FWHM).

For a complete description of $[^{11}\text{C}]$WAY-100635 please see [19, 20]. Mean injected dose was $312.04\pm105.84$ MBq, specific activity at time of injection was $285.47\pm251.22$ GBq/µmol and radiochemical purity was above 95%. Again, a 5 min transmission scan ($^{68}\text{Ge}$) was followed by 90 min dynamic scanning per subject at a spatial resolution of 4.36 mm FWHM.

Data preprocessing and calculation of binding potential

PET preprocessing was done in SPM8 (Wellcome Trust Centre for Neuroimaging, London, UK, http://www.fil.ion.ucl.ac.uk/spm/) using standard algorithms and parameters unless stated differently. After realignment to the mean image (quality = 1) scans of the entire time series were summed up and spatially normalized (affine regularization = average sized template) to a tracer-
specific template within standard MNI-space (Montreal Neurological Institute). Thereafter, the resulting transformation matrix was applied to each time frame.

We assessed in vivo target structure density as indexed by 5-HT\textsubscript{1A} receptor and 5-HTT binding potentials (BP\textsubscript{ND}), which represent the ratio at equilibrium of specifically bound radioligand to that of nondisplaceable radioligand in tissue [21]. All binding potentials were computed using the voxel-wise modeling tool in the PMOD 3.3 software package (PMOD Technologies, Ltd., Zurich, Switzerland) and applying the two-parameter linearized reference tissue model (MRTM2) [22], which provides advantages in signal-to-noise-ratio, especially for whole-brain voxel-wise analysis.

We modeled 5-HT\textsubscript{1A} BP\textsubscript{ND} as previously described by our group using the insula as receptor-rich region and the cerebellum as receptor-poor region [23]. The cerebellar gray matter excluding cerebellar vermis and venous sinus served as reference region. Serotonin transporter BP\textsubscript{ND} were modeled using the MRTM2 as previously described [16]. In short, k\textsubscript{2}’ was estimated from the striatum as 5-HTT-rich region and the cerebellar gray matter (excl. vermis and venous sinus) as 5-HTT-poor region. The cerebellar gray matter was chosen because it represents an optimal reference region for the quantification of the serotonin transporter with \textsuperscript{[11]}C]DASB [24, 25].

Regions of interest (ROI) for both radioligands were taken from an automated anatomical labeling-based (AAL) atlas [26] after normalization of BP\textsubscript{ND} maps to standard MNI-space. Values were averaged across both hemispheres. Due to inherent smoothness of PET data of the scanner and temporary smoothing during normalization we did not smooth during statistical processing.

Statistical Analysis

For normally distributed demographic variables and clinical measures student’s t-tests, for nominal variables chi-squared tests were performed. Significance was determined as p<0.05 and all tests were two-sided.

Differences of 5-HT\textsubscript{1A} and 5-HTT BP\textsubscript{ND} between BDNF Val66Met genotype groups were calculated using a voxel-wise and a ROI-based approach. For the voxel-wise analysis both in the 5-HTT and the 5-HT\textsubscript{1A} – groups an ANOVA was performed as implemented in SPM8. Grouped genotype status (val/val, vs. met-carrier = GG vs. A-carrier) served as factor and radioligand specific activity, sex and age served as covariates. In the 5-HTT-collective diagnosis was added as additional factor in a second step analysis. F-tests and group-wise post-hoc t-
tests between genotype groups were calculated and contrasted in SPM8. Additionally, in the 5-HTT-group an interaction between diagnosis and genotype status was contrasted by weighting contrast vectors in SPM according to group size. An absolute image threshold was set at 0.1 BP_{ND} to remove voxels with low signal-to-noise ratio and a cluster threshold was set at 50 voxels. A statistical level of p<0.05 corrected for multiple comparisons by the family-wise error rate (FWE) at voxel-level was considered significant, for subsequent explorative analysis an uncorrected threshold of p<0.001 was accepted.

In the ROI-based analyses differences between genotypes groups (val/val vs. met carrier) were calculated with a linear mixed model in SPSS 19 (IBM Corp. Released 2010. IBM SPSS Statistics for Windows, Version 19.0. Armonk, NY: IBM Corp.). Thereby, subject served as the random effect and BDNF genotype status, region, sex and age served as fixed effects. Ten representative regions were chosen due to their a priori known high distribution of 5-HT_{1A} receptors and 5-HTT and implications in psychiatric disorders (see tables 1,2 and figures 1,2). Diagnosis was taken as additional factor in the 5-HTT-study collective. Significance was determined as p<0.05. Post-hoc t-tests were conducted two-sided in 10 AAL ROIs (see tables 1,2 and figures 1,2).
3.2.4. Results

Out of the 51 HS in the 5-HT\textsubscript{1A}-group 30 carried GG, 18 carried AG and 3 AA. The 5-HTT-group had 25 HS with 19 carrying GG, 5 carried AG and 1 AA, whereas in the MDD group with 16 depressed patients 13 carried GG, 3 carried AG and 0 the AA allele (table 1). Allele frequencies of the BDNF gene in all study groups were distributed in accordance with the Hardy-Weinberg equilibrium [5-HT\textsubscript{1A}-group: \(X^2=0.02\), \(p=0.891\), 5-HTT-group HS: \(X^2=0.72\) \(p=0.4\), MDD patients \(X^2=0.17\) \(p=0.68\)]. The AA and AG+GG study groups did not differ in demographical, clinical measures or radiopharmaceutical measures (table 1). The allelic distribution was not associated with diagnosis in the 5-HTT-group (\(X^2=0.157\), \(p=0.692\)).

In the voxel-wise analysis there was no significant association of BDNF genotype (GG vs. A-carrier) status with 5-HT\textsubscript{1A} BP\textsubscript{ND} (F-test: all \(p>0.05\) FWE corr. and all \(p>0.001\) uncorr.). Furthermore, there was no significant association of BDNF genotype (GG vs. A-carrier) with 5-HTT BP\textsubscript{ND} (F-test: all \(p>0.05\) FWE corr. and all \(p>0.001\) uncorr.). There was no interaction between BDNF genotype status, diagnosis or sex and 5-HTT BP\textsubscript{ND} (t-test: all \(p>0.05\) FWE corr. and all \(p>0.001\) uncorr.).

The mixed model analyses of ROIs in the 5-HT\textsubscript{1A}-group, controlling for potential effects of sex, age and specific radioligand activity, yielded no significant difference of 5-HT\textsubscript{1A} BP\textsubscript{ND} in selected ROIs between GG homozygotes and A-allele carriers (\(F=0.342\), df=1,45, \(p=0.562\)). In the 5-HTT-group, the mixed model revealed no significant difference between 5-HTT BP\textsubscript{ND} in the selected ROIs between GG homozygotes and A-allele carriers (\(F=0.41\), df=1,33, \(p=0.526\)). There was no interaction between diagnosis and allele in the statistical model (\(p=0.989\)). Post-hoc t-tests and average BP\textsubscript{ND} values for both study groups are shown in table 2 and table 3, BP\textsubscript{ND}-values of allele groups are displayed in figure 1 and figure 2. Here, in the 5-HTT-group, a significant difference between GG and A-carriers was observed in HS in the midbrain (\(p=0.040\), uncorr., table 3) as well as between GG in HS and GG in MDD patients (\(p=0.034\), uncorr.), with BP\textsubscript{ND} increases in GG-carriers, respectively. All other post-hoc tests (5-HT\textsubscript{1A}: GG vs. A-carrier; 5-HTT HS: GG vs. A-carrier, MDD GG vs. A-carrier, HS vs. MDD GG, HS males GG vs. HS males A-carrier) did not yield significant results (all \(p>0.05\) uncorr.).
3.2.5. Discussion

In a voxel-wise analysis as well as in a ROI-based approach, we did not observe significant differences of 5-HT_{1A}-receptor BP_{ND} nor of 5-HTT BP_{ND} according to BDNF genotype status. There was no interaction between MDD diagnosis or sex and 5-HTT BP_{ND}. In the midbrain, weak increases of 5-HTT-BP_{ND} in healthy subjects between val-homozygotes and met-carriers were found. Furthermore, weak increases of 5-HTT BP_{ND} were observed in the midbrain in val-homozygote healthy subjects compared to val-homozygote MDD patients. There was no association between allelic distribution and major depression. To sum up, all voxel-wise and ROI-based testing yielded negative results and none of the post-hoc tests survived correction.

Our results are in concordance with a previous PET study applying [^{11}C]DASB in 49 healthy subjects, where the authors neither detected differences in 5-HTT binding in relation to BDNF genotype nor a correlation between blood BDNF levels and central 5-HTT binding [11]. Additionally, no effect on 5-HT_{2A} binding was shown in this work. Here, the authors calculated the radiotracer BP_{ND} similar to our study by applying a fully automated reference region model (MRTM2) [22] and an automated ROI-delineation. The only other currently published human PET-study investigating the impact of BDNF polymorphisms on 5-HTT binding reports differences in men and shows no effect of genotype status on 5-HT_{1A} binding [10]. Men homozygous for the val-allele exhibited significantly higher 5-HTT binding in regions such as the hippocampus, insula or dorsal raphe compared to met-carrier, while this effect was absent in women. Furthermore, reductions of 5-HTT binding in met-carrier (n=3) compared to val-homozygotes (n=6) in an independent [^{23}I]-ß-CIT-study with male suicide attempters was demonstrated, but this reduction was absent when pooled with healthy controls. The authors also used a reference region model with [^{11}C]-MADAM, a tracer exhibiting a comparable 5-HTT affinity to [^{11}C]DASB [27], the ROIs were manually delineated on individual magnetic resonance images (MRI). Notably, our group previously reported strong correlations of BP_{ND} values between automatically and manually delineated ROIs [23]. The radioligand and the method of ROI generation are on these grounds an unlikely source of variance leading to alternative results. Importantly, in search of arguments for this difference, one must mention that the number of male met-carriers in that collective was low (n=4), which makes the analysis vulnerable to outliers and hence may increase type-I errors. Likewise, our study exhibits a subgroup with a low subject number and indeed we saw an outlier in the MDD met-carrier group (n=3) when we plotted the individual BP_{ND} values (data not shown). Hence, our results in depressed patients have to be interpreted with caution. But the fact that both the study by Klein
et al., which exhibits a large sample size of healthy volunteers, as well as our study did not reproduce higher 5-HTT binding in val-homozygote healthy subjects, rather speaks for an absent effect of BDNF Val66Met on 5-HTT binding.

Apart from this, our study agrees with the data by Henningsson et al., on an absent effect of Val66Met on 5-HT1A receptor binding in healthy subjects [10]. Both studies apply the same radioligand, i.e. [carbonyl-11C]WAY-100635, exhibit an almost identical number of subjects (n=53 in Henningsson et al.), and modeled 5-HT1A binding by a reference region model (BPND). These results are in contradiction to a recent finding reporting 5-HT1A reductions in healthy met-allele carriers [12], which is not present in MDD patients. In this study 50 healthy subjects and 50 MDD patients were measured with the radioligand [carbonyl-11C]WAY-100635, yet 5-HT1A binding was calculated by an arterial input function (BP). Most interestingly, when the authors repeated their analysis with BPND values, the reduction of 5-HT1A binding in healthy met-carriers was not detectable, suggesting that this finding was associated with the method of radioligand modeling. Following the discussion of the authors, one cannot rule out that Val66Met causes differences of radioligand binding in the blood leading to a bias in the arterial input function. Although, our results are in agreement with all previous studies on 5-HT1A binding using reference tissue models [10, 12], validation by a different tracer not susceptible to modelling methodology is further needed. Taken together, while there are currently contradicting findings on the in vivo effect of BDNF Val66Met genotypes on 5-HTT binding [10, 11], this study adds data emphasizing the absence of such an effect. Moreover, this work corroborates previous results by reference tissue models demonstrating no association between BDNF Val66Met genotype status and 5-HT1A receptor binding [10, 12] and is in contradiction with a study reporting binding values modeled with arterial blood sampling [12].

Preclinical data report that BDNF promotes development and function of serotonergic neurons by enhancing survival and differentiation [28], increasing local 5-HT [29] modifying the firing pattern of serotonergic raphe neurons [28, 30] and altering the function of serotonergic receptors such as the 5-HT1A and 5-HT2A receptors and the 5-HTT [2, 29, 31]. Vice-versa, raised extracellular 5-HT levels occurring upon administration of SSRIs are thought to increase local BDNF levels by enhanced phosphorylation of serotonergic receptor coupled cAMP response element-binding (CREB) protein [32-34], a common target of BDNF and G protein-coupled serotonergic receptors [2]. Confronted with this evidence, one is puzzled upon the lack of strong evidence for an association between BDNF and serotonergic structures in humans in vivo. However, preclinical studies are not consistent and negative results regarding the expression of
5-HT receptors and transporter are reported [31, 35]. Although the interaction between the BNDF and 5-HT provides a promising bridge between structural and functional neuronal activity, and serves as explanatory hypothesis for neuronal plasticity deficits in neuropsychiatric disorders, exact mechanisms underlying the regulation of the cross connection between BDNF and 5-HT in humans remain unresolved [36]. Our data in concert with above referred work speak for a similar expression of 5-HTT and 5-HT1A receptors upon life-time BDNF reduction, but unfortunately do not illuminate the mechanisms leading to this observation. Theoretically, counter-regulatory or compensatory effects may have altered 5-HTT and 5-HT1A expression. Furthermore, it is possible that not absolute numbers but functional activity of serotonergic structures is altered by BDNF.

The evidence on connections between depression and BDNF genotype status is inconsistent as well. Meta-analytical research suggested an association of Val66Met with major depressive disorder antidepressant treatment response or hippocampal volume and a role of gender and ethnicity [37-39]. However, recent meta-analyses refuted these associations and detected power deficits in many trials [40-42]. Low serum levels of BDNF were suggested as potential peripheral marker of depression and increase of serum BDNF as response to the appropriate first-line treatment with selective 5-HT reuptake inhibitors (SSRIs). Likewise, this association is weaker than initially thought and there is no relationship between symptom severity and BDNF serum concentration [43]. Our results suggest no association between allelic distribution and diagnosis. Our small number of MDD subjects remains a limiting factor in that regard.

### 3.2.6. Limitations

Unfortunately a common problem of human PET studies is weak power resulting from low subject numbers, owed to the large effort of conducting PET-imaging. This is even more intrinsic to genetic PET studies reporting results based on genotype subgroups [44] and in SNP neuroimaging studies where pooling of rare genotype groups is common practice. The low subject number in the MDD met-carrier group could therefore be a limitation of our study. One elegant way to circumvent this issue in future studies would be pooling data between PET centers, which is already common in MRI studies. Second, mean age of genotype groups is heterogeneous, yet controlled for in all statistical analyses. Finally, we did not model PET data with an arterial input function [45], because arterial blood data were not collected. This would have been useful to confirm reported differences according to the methodology for calculating 5-
HT$_{1A}$ binding with [carbonyl-$^{11}$C]WAY-100635, an issue we are trying to resolve in future studies [46].

CONCLUSION

Although others have investigated the effects of the BDNF gene on 5-HT$^T$ and 5-HT$_{1A}$ binding with PET, this study adds data to the ongoing discussion about the cross connection between 5-HT and BDNF. While previous work in humans demonstrated contradicting results, due to this work the conclusion of an absent influence of Val66Met on 5-HTT and 5-HT$_{1A}$ has gained substantial support.

ACKNOWLEDGEMENTS

The authors are grateful to U. Moser, E. Akimova, P. Stein, M. Fink, C. Spindelegger, A. Höflich, I. Hofer-Irmler, S. Zgud, S. Pichler, A. Kautzky and D. Winkler for medical and administrative support, and M. Savli for technical support. We thank the PET team, especially G. Karanikas, T. Traub-Weidinger, L.-K. Mien, J. Ungersboeck, K. Kletter, L. Nics, and C. Philippe for technical support. Further, we thank the genetics team of D. Rujescu, especially M. Friedl, A. Hartmann, I. Giegling. The study is part of C. Kraus’ thesis “Serotonin and Neuroplasticity” supervised by R. Lanzenberger in the Clinical Neurosciences PhD program at the Medical University of Vienna, Austria. Parts of this study have been presented by P. Baldinger at the 19$^{th}$ at the 11$^{th}$ World Congress of Biological Psychiatry (WFSBP), June 23$^{rd}$ – 27$^{th}$, 2013, Kyoto, Japan.
3.2.7. References


### 3.2.8. Tables and Figures

#### TABLES

**TABLE 1:** Demographic variables of the entire study sample.

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<th></th>
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</thead>
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<td></td>
<td></td>
</tr>
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<td>[carbonyl-[^{11}\text{C}^{1}\text{C}]WAY-100635</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N (=51)</td>
<td>30</td>
<td>21</td>
<td>0.737</td>
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<tr>
<td>Age (years)</td>
<td>43.8 ± 13.1</td>
<td>45.1 ± 12.36</td>
<td>0.737</td>
</tr>
<tr>
<td>Sex (f/m)</td>
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<td>16/5</td>
<td>0.626*</td>
</tr>
<tr>
<td>weight</td>
<td>72.9 ± 17.1</td>
<td>67.1 ± 10.5</td>
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<tr>
<td>SA</td>
<td>296.9 ± 269.1</td>
<td>285.7 ± 197.3</td>
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</tr>
<tr>
<td>[^{11}\text{C}]\text{DASB}</td>
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<td></td>
<td></td>
</tr>
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<td>N (=25)</td>
<td>19</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Age (years)</td>
<td>31.0 ± 8.8</td>
<td>33.0 ± 13.2</td>
<td>0.672</td>
</tr>
<tr>
<td>Sex (f/m)</td>
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<td>1/5</td>
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<td>weight</td>
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<td></td>
</tr>
<tr>
<td>[^{11}\text{C}]\text{DASB}</td>
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<td>N (=16)</td>
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<td>3</td>
<td>0.925</td>
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<td>HAMD</td>
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<td>21± 3.5</td>
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<td>Age (years)</td>
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<td>46.7 ± 7.5</td>
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<td>Sex (f/m)</td>
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<td>weight</td>
<td>77.7 ± 21.3</td>
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<td>SA</td>
<td>63.9 ± 22.6</td>
<td>62.5 ± 16.7</td>
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Data are given as means±standard deviations (SD). P-values compare pooled BDNF Val66Met genotype groups with independent sample t-test, chi-square* or Mann-Whitney U test*.
**TABLE 2**: Post-hoc t-tests comparing serotonin-1A receptor (5-HT$_{1A}$) binding potential (BP$_{ND}$) according to BDNF Val66Met genotype status in 51 healthy subjects.

<table>
<thead>
<tr>
<th>Region</th>
<th>val/val (n=30)</th>
<th>met-carrier (n=21)</th>
<th>p</th>
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<tr>
<td>Anterior cingulate cortex</td>
<td>3.54 ± 1.14</td>
<td>3.63 ± 0.86</td>
<td>0.758</td>
</tr>
<tr>
<td>Amygdala</td>
<td>3.98 ± 1.23</td>
<td>4.17 ± 1.03</td>
<td>0.559</td>
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<tr>
<td>Medial cingulate cortex</td>
<td>2.9 ± 0.97</td>
<td>2.98 ± 0.65</td>
<td>0.723</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>3.64 ± 1.14</td>
<td>4.12 ± 0.94</td>
<td>0.118</td>
</tr>
<tr>
<td>Insula</td>
<td>4.46 ± 1.33</td>
<td>4.64 ± 0.91</td>
<td>0.596</td>
</tr>
<tr>
<td>Parahippocampus</td>
<td>5.41 ± 1.64</td>
<td>5.60 ± 1.14</td>
<td>0.596</td>
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<tr>
<td>Posterior cingulate cortex</td>
<td>2.2 ± 0.79</td>
<td>2.25 ± 0.58</td>
<td>0.822</td>
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<tr>
<td>Subgenual anterior cingulate</td>
<td>3.51 ± 0.96</td>
<td>3.85 ± 1.1</td>
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<tr>
<td>Temporal pole</td>
<td>4.65 ± 1.5</td>
<td>4.75 ± 0.93</td>
<td>0.786</td>
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<tr>
<td>Dorsal raphe nucleus</td>
<td>2.33 ± 0.87</td>
<td>2.29 ± 0.74</td>
<td>0.857</td>
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Regions of interest (ROIs) in standardized MNI space (Montreal Neurological Institute) were calculated by automatic anatomical labeling in both hemispheres and averaged. Data are given as 5-HT$_{1A}$ BP$_{ND}$ means ± standard deviations (SD) for each ROI and compared by post-hoc student’s t-tests, values correspond to bar charts in Fig.1.
TABLE 3: Post-hoc t-tests comparing serotonin transporter (5-HTT) binding potential ($\text{B}_\text{ND}$) according to BDNF Val66Met genotype status in 25 healthy subjects and 16 depressed patients.

<table>
<thead>
<tr>
<th>region</th>
<th>healthy subjects</th>
<th>MDD patients</th>
<th>p</th>
<th>healthy subjects</th>
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<tr>
<td></td>
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<td>Anterior</td>
<td>0.42 ± 0.08</td>
<td>0.40 ± 0.06</td>
<td>0.759</td>
<td>0.38 ± 0.14</td>
<td>0.32 ± 0.15</td>
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<tr>
<td>cingulate</td>
<td>1.24 ± 0.13</td>
<td>1.14 ± 0.17</td>
<td>0.167</td>
<td>1.06 ± 0.24</td>
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<td>Amygdala</td>
<td>0.40 ± 0.07</td>
<td>0.37 ± 0.08</td>
<td>0.431</td>
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<td>Medial cingulate</td>
<td>0.46 ± 0.08</td>
<td>0.41 ± 0.08</td>
<td>0.206</td>
<td>0.40 ± 0.10</td>
<td>0.44 ± 0.11</td>
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<td>Hippocampus</td>
<td>1.84 ± 0.21</td>
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<td>N. caudatus</td>
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<td>1.85 ± 0.27</td>
<td>0.756</td>
<td>1.75 ± 0.28</td>
<td>1.50 ± 0.30</td>
<td>0.248</td>
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<td>Putamen</td>
<td>2.07 ± 0.23</td>
<td>1.88 ± 0.11</td>
<td>0.071</td>
<td>1.88 ± 0.37</td>
<td>1.72 ± 0.45</td>
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<td>Thalamus</td>
<td>1.70 ± 0.16</td>
<td>1.66 ± 0.22</td>
<td>0.624</td>
<td>1.58 ± 0.25</td>
<td>1.37 ± 0.28</td>
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<tr>
<td>Striatum</td>
<td>2.91 ± 0.33</td>
<td>2.58 ± 0.31</td>
<td>0.040</td>
<td>2.62 ± 0.41</td>
<td>3.20 ± 1.80</td>
<td>0.382*</td>
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<tr>
<td>Midbrain</td>
<td>1.95 ± 0.3</td>
<td>1.82 ± 0.26</td>
<td>0.327</td>
<td>1.82 ± 0.30</td>
<td>1.67 ± 0.46</td>
<td>0.572</td>
</tr>
<tr>
<td>N. accumbens</td>
<td>1.95 ± 0.3</td>
<td>1.82 ± 0.26</td>
<td>0.327</td>
<td>1.82 ± 0.30</td>
<td>1.67 ± 0.46</td>
<td>0.572</td>
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</table>

Regions of interest (ROIs) in standardized MNI space (Montreal Neurological Institute) were calculated by automatic anatomical labeling in both hemispheres and averaged. Data are given as 5-HTT $\text{B}_\text{ND}$ means ± standard deviations (SD). T-tests or U-test (*) compare differences between val/val and met-carrier for each ROI.
FIGURES

FIGURE 1: Bar chart plotting serotonin-1A binding potential (5-HT$_{1A}$ BP$_{ND}$) according to BNDF Val66Met genotype status. Values at the y-axis represent 5-HT$_{1A}$ BP$_{ND}$ separated for val/val and met-carrier, respectively, x-axis shows regions of interest. Regions and values correspond to table 2. ACC: anterior cingulate cortex, AMY: amygdala, MCC: medial cingulate cortex, HIPP: hippocampus, INS: insula, paraHIPP: parahippocampus, PCC: posterior cingulate cortex, TempPole: temporal pole, DRN: dorsal raphe nucleus.
**FIGURE 2**: Bar chart plotting serotonin transporter binding potential \( (5\text{-HTT BP}_{ND}) \) according to BNDF Val66Met genotype status. Values at the y-axis represent \( 5\text{-HTT BP}_{ND} \) in pooled healthy subjects and depressive patients. Binding potential is separated for val/val and met-carriers, respectively, x-axis shows regions of interest. Because healthy subjects and depressive patients were pooled here, regions do, but values do not correspond to table 3. ACC: anterior cingulate cortex, AMY: amygdala, MCC: medial cingulate cortex, HIPP: hippocampus, CAUD: caudatum, PUT: putamen, THAL: thalamus, STRIA: striatum, MID: Midbrain, NACC: nucleus accumbens.
3.3. Third publication: Interaction between 5-HTTLPR and 5-HT_{1B} genotype status enhances cerebral 5-HT_{1A} receptor binding

Pia Baldinger^1*, Christoph Kraus^1*, Christina Rami-Mark^2, Gregor Gryglewski^1, Georg S. Kranz^1, Daniela Haeusler^2, Andreas Hahn^1, Marie Spies^1, Wolfgang Wadsak^2, Markus Mitterhauser^2, Dan Rujescu^3, Siegfried Kasper^1, Rupert Lanzenberger^1+

* both authors contributed equally

^1 Department of Psychiatry and Psychotherapy,
^2 Department of Biomedical Imaging und Image-guided Therapy, Division of Nuclear Medicine, Medical University of Vienna, Austria
^3 Department of Psychiatry, Medical University of Halle, Germany

Accepted for publication in:

NEUROIMAGE
[IF 2013: 6.132]

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* Correspondence to: Rupert Lanzenberger, Assoc. Prof, MD
Functiona, Molecular and Translational Neuroimaging Lab
Department of Psychiatry and Psychotherapy
Medical University of Vienna,
Währinger Gürtel 18-20, 1090 Vienna, Austria

rupert.lanzenberger@meduniwien.ac.at
http://www.meduniwien.ac.at/neuroimaging/
3.3.1. Abstract

Serotonergic neurotransmission is thought to underlie a dynamic interrelation between different key structures of the serotonin system. The serotonin transporter (SERT), which is responsible for the reuptake of serotonin from the synaptic cleft into the neuron, as well as the serotonin-1A (5-HT_{1A}) and -1B (5-HT_{1B}) receptors, inhibitory auto-receptors in the raphe region and projection areas, respectively, are likely to determine serotonin release. Thereby, they are involved in the regulation of extracellular serotonin concentrations and the extent of serotonergic effects in respective projection areas. Complex receptor interactions can be assessed in vivo with positron emission tomography (PET) and single-nucleotide-polymorphisms, which are thought to alter protein expression levels. Due to the complexity of the serotonergic system, gene x gene interactions are likely to regulate transporter and receptor expression and therefore subsequently serotonergic transmission. In this context, we measured 51 healthy subjects (mean age 45.5±12.9, 38 female) with PET using [carbonyl-^{11}C]WAY-100635 to determine 5-HT_{1A} receptor binding potential (5-HT_{1A} BP_{ND}). Genotyping for rs6296 (HTR1B) and 5-HTTLPR (SERT gene promoter polymorphism) was performed using DNA isolated from whole blood. Voxel-wise whole-brain ANOVA revealed a positive interaction effect of genotype groups (5-HTTLPR: LL, LS+SS and HTR1B: rs6296: CC, GC+GG) on 5-HT_{1A} BP_{ND} with peak t-values in the bilateral parahippocampal gyrus. More specifically, highest 5-HT_{1A} BP_{ND} was identified for individuals homozygous for both the L-allele of 5-HTTLPR and the C-allele of rs6296. This finding suggests that the interaction between two major serotonergic structures involved in 5-HT release, specifically the SERT and 5-HT_{1B} receptor, results in a modification of the inhibitory serotonergic tone mediated via 5-HT_{1A} receptors.
3.3.2. Introduction

Serotonin and interconnected neurotransmitter systems such as dopamine and glutamate are controlled by excitatory and inhibitory serotonergic receptors and the serotonin transporter (SERT). However, molecular interactions between receptors and the SERT within the serotonergic systems itself are hardly understood. In this regard, the serotonin-1A (5-HT$_{1A}$) receptor has been subject of numerous investigations, as this receptor was shown not only to play a role in the development of psychiatric disorders, but also in the effects of, and treatment-response to antidepressant medication (Hahn et al., 2010; Moret and Briley, 1990; Savitz et al., 2009; Spindelegger et al., 2009). Via its function as an auto-inhibitory receptor at presynaptic nerve terminals in the midbrain raphe region, the 5-HT$_{1A}$ receptor modulates the firing rate of serotonergic neurons and impacts on 5-HT synthesis and release (Barnes and Sharp, 1999; Bundgaard et al., 2006). As postsynaptic hetero-receptors on glutamatergic and GABAergic pyramidal neurons in cortical regions, the 5-HT$_{1A}$ receptor exerts an inhibitory influence on the serotonergic system (Savli et al., 2012; Varnäs et al., 2004).

On a fundamental level, the extracellular 5-HT concentration - which in turn impacts on 5-HT$_{1A}$ receptor function - is determined by the extent of serotonin release and reuptake into the neuron. Two serotonergic structures were shown to be substantially responsible for these mechanisms: SERT and the serotonin-1B (5-HT$_{1B}$) receptor. The SERT is mainly expressed in the midbrain and basal ganglia and is responsible for reuptake of extracellular 5-HT into the presynaptic neuron. Blockage of SERT represents the initial step in antidepressant drug effects of selective-serotonin-reuptake-inhibitors (SSRI). Moreover, the SERT was also shown to be involved in non-exocytotic 5-HT release via reversal of normal transporter flux (Baumann et al., 2014). 5-HT$_{1B}$ receptors exist as both auto- and hetero-receptors, are located in the raphe nuclei, basal ganglia and the occipital cortex and mediate 5-HT and other neurotransmitters’ release (Martin et al., 1992; Savli et al., 2012; Stamford et al., 2000). Based on these findings, it seems conceivable that both the SERT and 5-HT$_{1B}$ receptor modulate 5-HT$_{1A}$ receptor function.

Preclinical studies strongly support functional interactions between 5-HT receptors and the SERT. For instance, SSRI-related alterations of both the 5-HT$_{1A}$ and 5-HT$_{1B}$ receptors was found to cause a greater increase in 5-HT in the frontal cortex in rats than that achieved by separate antagonism (Sharp et al., 1997). Using a SERT knockout model, marked decreases in both 5-HT$_{1A}$ autoreceptor density and 5-HT$_{1A}$ autoreceptor-mediated responses could be noticed (Li et
al., 1999). Other knockout models suggest a reciprocal influence of SERT on 5-HT$_{1A}$ or 5-HT$_{1B}$ expression, but not a mutual influence of both receptors (Ase et al., 2001; Fabre et al., 2000).

Single nucleotide polymorphisms (SNPs) introduce an interesting new opportunity to study interactions within the 5-HT system in vivo in a minimally invasive manner in humans. The influence of interactions between receptor SNPs have been investigated in a variety of psychiatric disorders (Lebe et al., 2013; Mandelli et al., 2007; Wang et al., 2012). Most prominently, the 5-HTTLPR, a length polymorphism in the promoter region of the SERT gene, has gained major interest. The expression of its short “S” allele – compared to the long “L” allele – results in reduced mRNA transcription and diminished SERT synthesis and is associated with higher anxiety scores (Lesch et al., 1996). Similarly, the C allele of a SNP of the 5-HT$_{1B}$ receptor gene (HTR1B), rs6296 (G861C), has been linked to elevated depression and anxiety scores (Mekli et al., 2011). Considering that the SERT and 5-HT$_{1B}$ receptor are the main mediators of 5-HT availability (i.e., reuptake and release, respectively), we aimed to elucidate their combined impact on 5-HT$_{1A}$ receptor density using brain imaging in vivo. Based on the findings described above and earlier investigations describing the influence of gene x gene interactions on clinical phenotypes (Ressler et al., 2010), we hypothesized that the combined influence of 5-HTTLPR and 5-HT$_{1B}$ genotype status might impact on 5-HT$_{1A}$ receptor expression, serving as a psychiatric endophenotype.
3.3.3. Methods and Material

Subjects

In a neuroimaging genetics study with a cross-sectional design, 51 healthy subjects aged 18-65 years (mean 45.5±12.9, 38 female) were included (see table 1). The study population consists of subjects from previously published studies (Baldinger et al., 2013; Lanzenberger et al., 2010), but genotyping data of 5-HTT and 5-HT₁B receptor have not been previously published. All subjects underwent a psychiatric screening including the complete Structured Clinical Interview for DSM-IV type disorders (SCID I+II) to rule out lifetime axis I and II disorders, physical and neurological examination, a clinical history, ECG, routine blood analysis as well as urinary drug and pregnancy screening. All psychiatric disorders including drug abuse, neurological illnesses or previous intake of psychotropic medication were considered exclusion criteria. The study and all study related procedures were approved by the Ethics Committee of the Medical University of Vienna, Austria. All subjects provided written informed consent after detailed explanation of the study protocol and they were reimbursed for participation.

Genotyping

Blood samples and DNA extraction from mononuclear cells from peripheral blood were performed at the Department of Psychiatry and Psychotherapy, Medical University of Vienna, Austria. DNA was obtained using the QIAamp DNA Mini-Kit (QIAGEN®, Hilden, Germany). DNA samples were then transferred to the Department of Psychiatry and Psychotherapy, Ludwig-Maximilians-University, where genotyping was performed as previously described (Baldinger et al., 2013).

The 5-HTTLPR genotype with L, LS, and SS alleles was investigated by PCR. The primer sequences used for the 5-HTTLPR were 5k-CAACTCCCTGTACCCTCCTCA-3k (forward primer) and 5k-GGTTCAGGGGAGATCCTG-3k (reverse primer), resulting in a 147bp PCR product for S alleles, a 190bp PCR product for L alleles. The reaction mixture contained 5ng genomic DNA, 2.5mM MgCl₂, 60mM Tris-HCl, 15mM ammonium sulfate, 5µl Q-solution (QIAGEN®) 6,7nmol forward and 4,4nmol reverse primer, 40nmol dNTP, and 1 U Taq polymerase (Fermentas®). The temperature profile consisted of an initial denaturation at 94°C for 5 min, followed by 45 cycles of 30s at 94°C denaturation, 30s at 56.8°C annealing, and 60s
at 72°C elongation, followed by final elongation for 7 min at 72°C. 20µl PCR products were separated on a 2% agarose gel at 100V for 45 min.

The 5-HT$_{1B}$ receptor SNP (rs6296) was analyzed with a MassARRAY platform (SEQUENOM®, San Diego, CA) as described by (Oeth et al., 2006). PCR-primers were generated with the Assay Designer 4.0 software (SEQUENOM®) and are available upon request. Multiplex PCR reactions were performed with 12.5ng of genomic DNA, 500µM dNTPs (ABgene®, Hamburg, Germany), 100nM PCR primers, 1.625mM MgCl2 and 0.5U HotStar Taq polymerase (QIAGEN®). Shrimp alkaline phosphatase (SAP) treatment, an iPLEX reaction cocktail with extension primers (7-14μM), an iPLEX termination mix and an iPLEX enzyme (SEQUENOM®) were added to the PCR-products. The resulting extension products were desalted using SpectroCLEAN resin (SEQUENOM®), then spotted on SpectroCHIPs GenII (SEQUENOM®) and analyzed with the MassARRAY MALDI-TOF mass spectrometer. Typer 3.4 Software was used to identify allele specific extension products and resulting genotypes (SEQUENOM®). For genotyping quality assurance CEU HapMap Trios (Coriell Institute for Medical research, Camden, NJ) were included and compared with the HapMap-CEU population (www.hapmap.org).

In statistical testing S-carriers (LS- and SS-carriers homozygotes and G-carriers (GG- and GC-carriers) were pooled, respectively.

Subjects were partly genotyped for other SNPs - 50 subjects for rs6295 (5-HT$_{1A}$ receptor gene) and rs6265 (brain-derived neurotrophic factor gene) and 46 subjects for rs6298 (HTR1B) – and these data were not included in our statistical analysis.

**Radiochemistry of [carbonyl-$^{11}$C]WAY-100635 and PET Procedures**

Radioligand synthesis of [carbonyl-$^{11}$C]WAY-100635 and all PET measurements were conducted at the Department of Biomedical Imaging und Image-guided Therapy, Division of Nuclear Medicine at the Medical University of Vienna. A GE Advance full ring PET scanner (General Electric Medical Systems, Waukesha, WI, USA) was used for PET measurements. A laser beam was used to position subjects’ heads parallel to the orbitomeatal line to ensure full coverage of the neocortex and the cerebellum in the field of view. Subjects were instructed not to move and to speak only in case of emergency, while a polyurethane cushion and head straps
were used to minimize head movement, guaranteeing a soft head rest during the whole scanning period.

For a complete description of the synthesis of [carbonyl-$^{11}$C]WAY-100635 please see (Rami-Mark et al., 2013; Wadsak W. et al., 2007). Mean injected dose was 293.08±92.11 MBq, specific radioactivity at time of injection was 285.5±251.2 GBq/µmol and radiochemical purity was above 95%. A 5 min transmission scan ($^{68}$Ge) was followed by 90 min dynamic scanning per subject at a spatial resolution of 4.36 mm FWHM 1cm next to the center of the field of view (35 slices).

Data preprocessing and calculation of binding potential

All PET preprocessing was done in SPM8 (Wellcome Trust Centre for Neuroimaging, London, UK, [http://www.fil.ion.ucl.ac.uk/spm/](http://www.fil.ion.ucl.ac.uk/spm/)) using standard parameters unless otherwise stated. After realignment to the mean image (quality = 1) scans of the entire time series were summed up and spatially normalized (affine regularization = average sized template) to a tracer-specific template within standard MNI-space (Montreal Neurological Institute) as previously demonstrated (Hahn et al., 2010). This step includes correction for head movement. Thereafter, the resulting transformation matrix was applied to each time frame. For assessment of 5-HT$_{1A}$ in vivo binding we calculated 5-HT$_{1A}$ receptor binding potentials (BP$_{ND}$), which represent the ratio of specifically bound radioligand to that of nondisplaceable radioligand in tissue at equilibrium (Innis et al., 2007). All binding potentials were computed using the voxel-wise modeling tool in the PMOD 3.3 software package (PMOD Technologies, Ltd., Zurich, Switzerland) and applying the two-parameter linearized reference tissue model (MRTM2) (Ichise et al., 2003), which provides advantages in signal-to-noise-ratio, especially for whole-brain voxel-wise analysis. We modeled 5-HT$_{1A}$ BP$_{ND}$ as previously described by our group using the insula as receptor-rich region and the cerebellum as receptor-poor region (Savli et al., 2012). The cerebellar gray matter excluding cerebellar vermis and venous sinus served as reference region (Hahn et al., 2010; Hall et al., 1997). Due to inherent smoothness of PET data of the GE scanner and temporary smoothing during normalization we did not smooth during statistical processing. The final voxel size was 2x2x2 mm.

Statistical Analysis

For statistical testing, two groups were generated for each SNP, one group with “strong” homozygous and a second group with pooled “weak” alleles (5-HTTLPR: 1=LL and 2=LS + SS
carriers; \textit{HTR1B}, rs6296: 3=CC and 4=GC + GG carriers). Demographic variables and clinical measures from the four genotype-groups were compared with one-way analysis of variance (ANOVA) if normally distributed; chi-squared tests were performed where applicable. Significance was set at p<0.05 (two-sided).

For voxel-wise analysis of 5-HT\textsubscript{1A} binding potential (\textit{BP\textsubscript{ND}}) differences between the four genotype groups were assessed by an ANOVA as implemented in SPM8. Grouped genotype status (LL, LS+SS, CC, GC+GG) served as factor and specific radioactivity of the tracer, sex and age served as covariates of non-interest. An F-test was performed for the main model and a t-test was applied to assess the interaction of genotype groups. These tests, as well as group-wise post-hoc t-tests between genotype groups were calculated and contrasted in SPM8. Cohen’s \textit{d} was computed for post-hoc t-test as reported previously (Kranz et al., 2014). An image threshold was set at 0.1 (absolute value) and a cluster threshold was set at 50 voxels. A statistical level of p<0.05 corrected for multiple comparisons by the family-wise error rate (\textit{p\textsubscript{FWE, voxel-level}}) was considered significant, for more explorative analysis an uncorrected threshold of p<0.001 was accepted.

Region of interest (ROI) analyses provide additional information in small regions susceptible to higher signal to noise ratios. Because the same data were already included in voxel-wise testing and mass effect results generally overlap, we performed an additional ROI-analysis of the dorsal raphe nuclei (DRN) only. This was done by drawing a standardized ROI as described previously (Kranz et al., 2012). We then calculated a mixed model with all genotypes as well as their interaction as factors and sex, age and specific radioactivity of the tracer (SA) as covariates. A statistical level of p<0.05 was considered significant.
3.3.4. Results

Genotype groups did not differ according to age (F=2.03, p=0.123), SA (F0.58, p=0.63) and gender distribution ($\chi^2=2.26$, df=3, p=0.52). Allele distributions for both genes were in Hardy-Weinberg-Equilibrium ($5-HTTLPR$: $\chi^2=0.008$, p=0.93; rs6296: $\chi^2=2.44$, p=0.11).

A significant main effect was obtained for the ANOVA of $5-HT_1A$ BP$_{ND}$ between $5-HTTLPR$ and $5-HT_1B$ rs6296 genotype groups in the left and right anterior parahippocampal gyrus ($p_{FWE}<0.05$, left: F=13.77, right: F=12.56, see table 2). A positive interaction between genotype groups ($5-HTTLPR$: LL, LS+SS and $HTR1B$ rs6296: CC, GC+GG) and $5-HT_1A$ BP$_{ND}$ values was obtained with peak t-values in the right ($p_{FWE}<0.05$, t=5.02) and left parahippocampal gyrus (t=4.54, all following p<0.001), the right temporal pole (t=4.26), the bilateral fusiform gyrus (right: t=4.61, left t=4.22), the right superior (t=4.05) and inferior temporal cortices (t=3.69) as well as the right orbitofrontal cortex (t=3.94). There was no main effect of neither rs6296 nor $5-HTTLPR$ genotype, taken separately, on $5-HT_1A$ receptor binding potential (p>0.001, uncorrected). Mean regional $5-HT_1A$ receptor binding potential values for each genotype are summarized in table 3.

In a second step, the individual BP$_{ND}$ values were plotted at the point of maximal statistical significance of the interaction t-test in the right parahippocampal gyrus (MNI coordinates, x,y,z=18,0,24) to further investigate the variance between genotype groups. Highest BP$_{ND}$ values were obtained in the group with LL-alleles for $5-HTTLPR$ and CC-alleles for $5-HT_1B$ rs6296 ($5-HT_1A$ BP$_{ND}$= 7.54±1.94, mean±SD, Figure 1), followed by LL-alleles and CG+GG-alleles ($5-HT_1A$ BP$_{ND}$= 5.79±2.00), LS+SS-alleles and CG+GG-alleles (5.54±2.00) and finally LS+SS-alleles and CC-alleles (4.71±2.00). This implies that homozygosity for the C allele of rs6296 in LL-carriers is accompanied by a 30.3% higher $5-HT_1A$ BP$_{ND}$ compared to CG+GG-allele carriers (Cohen’s d=0.89) or a 60% higher $5-HT_1A$ BP$_{ND}$ compared to LS+SS and CC carriers, respectively (Cohen’s d=1.44). This effect is absent between CC and G-carriers for rs6296 in S-carriers for $5-HTTLPR$ (Figure 1).

Furthermore, t-contrasts were calculated in SPM between all genotype groups to further investigate topological differences. Strongest differences in $5-HT_1A$ BP$_{ND}$ were detected in LL-homozygotes between G-carriers ad CC-homozygotes, for $5-HTTLPR$ and rs6296 respectively, in a cluster covering the bilateral anterior parahippocampal gyrus to the amygdala, the temporal pole and the fusiform gyrus (right=5.51, left=5.39, all $p_{FWE} < 0.05$). A second significant cluster was observed in the anterior cingulate cortex spreading to the medial orbitofrontal cortex.
Additional results were obtained in both hemispheres in the insula (left $t=4.14$, right $t=4.11$) and the medial temporal cortex (left $t=4.23$, right $t=3.8$), as well as the medial frontal cortex ($t=4.56$), the precentral gyrus ($t=4.34$), the inferior temporal cortex ($t=4.09$), the olfactory cortex ($t=4.02$) and the gyrus rectus ($t=3.93$). For all results see Table 2 and for topological differences see Figure 2.

In addition, significant results with a similar topological pattern were observed between LL/CC-carriers and LS+SS/CC-carriers in the bilateral parahippocampal gyrus (right $t=5.54$, left $t=5.31$, all $p_{\text{FWE}}<0.05$. The difference between LL/CC-carriers and LS+SS/CG+GG-carriers again yielded a similar topological pattern, yet to a less pronounced extent (parahippocampus right $t=5.48$, $p_{\text{FWE}}<0.05$, left $t=4.63$ $p_{\text{uncorr}}<0.001$. All other possible comparisons did not yield statistically significant results.

There were no significant effects of neither genotype nor their interaction on DRN $\text{BP}_{\text{ND}}$ ($5'-HTTLPR$: $F=1.32$, $p=0.28$; rs6296: $F= 0.38$, $p= 0.68$; interaction: $F= 1.82$, $p= 0.17$).
This study investigates the combined influence of 5-HTTLPR and 5-HT<sub>1B</sub> genotype (rs6296) on 5-HT<sub>1A</sub> binding using PET and the radioligand [carbonyl-<sup>11</sup>C]WAY-100635 in 51 healthy subjects. Statistical analysis revealed a significant interaction on 5-HT<sub>1A</sub> receptor binding between both genes. More specifically, highest 5-HT<sub>1A</sub> receptor binding values were identified for individuals homozygous for both the L allele of 5-HTTLPR and the C-allele of HTR1B when compared to the remaining three genotype-constellations (LL and CG+GG, SL+SS and CG+GG, SL+SS and CC). The results were topologically most pronounced in the bilateral anterior and posterior parahippocampal gyrus as well as the amygdala, the temporal pole and the anterior cingulate cortex. This is the first study showing that an interaction between SERT and 5-HT<sub>1B</sub> receptor gene polymorphisms exerts an impact on 5-HT<sub>1A</sub> receptor density in humans, in vivo. The 5-HT<sub>1B</sub> receptor and SERT are key mediators of 5-HT release and reuptake- and – via their impact on extracellular serotonin concentrations - regulate the tone of the serotonergic system, while the 5-HT<sub>1A</sub> receptor is the major inhibitory serotonergic receptor.

In our analysis, there was no main effect on 5-HT<sub>1A</sub> binding when each gene variant was tested separately. Previous studies investigating the impact of the 5-HTTLPR polymorphism on 5-HT<sub>1A</sub> binding yielded contradictory results (Gryglewski et al., 2014; Willeit et al., 2003): While Lothe et al. postulated an increased 5-HT<sub>1A</sub> receptor binding in S allele carriers of 5-HTTLPR (Lothe et al., 2009), the opposite was found by other authors (Christian et al., 2013; David et al., 2005). Finally, Borg et al. detected no effect of 5-HTTLPR on 5-HT<sub>1A</sub> receptor binding (Borg et al., 2009). In regard to 5-HT<sub>1B</sub> receptor, up to now, there is no previous study on an association between 5-HT<sub>1B</sub> genes and 5-HT<sub>1A</sub> receptor binding in vivo. Nonetheless, growing literature in knockout animals points towards a modulation of SERT by 5-HT<sub>1B</sub> receptors (Montanez et al., 2014). Our findings suggest that – taken individually – effect sizes of rs6296 and 5-HTTLPR are too low to explain alterations of 5-HT<sub>1A</sub> receptor. According to our results, the impact on 5-HT<sub>1A</sub> receptor binding seems to be only noticeable when considering the interaction of both gene variants, which indicates that there might be a sort of reciprocal compensation mechanism. Indeed, the interaction of both genes is supported by one previous report of an association between 5-HTTLPR and HTR1B genotypes in regard to bone formation during psychopharmacological treatment with venlafaxine (Garfield et al., 2013). The authors report most prominent decreases of bone formation in subjects with the high expressing 5-HTTLPR genotype and the low expressing genotype of rs6296. Our study matches this observation as highest 5-HT<sub>1A</sub> BP<sub>ND</sub> values were detected in LL-carriers, which exhibit a 40-70% in vitro
increase of SERT (Lesch et al., 1996), in combination with CC-status, which results in a 20% reduction of 5-HT_{1B} receptor synthesis (Conner et al., 2010). The close interaction between SERT and 5-HT_{1B} receptors is in accordance with earlier findings in rats, showing that both increases and decreases of 5-HT_{1B} auto-receptor expression levels in brain tissue affect SERT activity (Hagan et al., 2012). Decreases of 5-HT clearance rates in the hippocampus were previously shown after antagonism of 5-HT_{1B} receptors (Daws et al., 1999; Daws et al., 2000) and might be mediated by differences in SERT trafficking to the synaptic membrane or by increases of SERT catalytic activity (Steiner et al., 2008). Such findings provide strong evidence that 5-HT_{1B} and SERT exhibit synergistic effects on synaptic 5-HT concentrations.

The heightened 5-HT_{1A} receptor binding potentials shown in our study could represent a response to reductions of synaptic 5-HT concentrations resulting from synergistic effects of the SERT and 5-HT_{1B} receptor. These effects are most pronounced in LL and CC-carriers, for 5-HTTLPR and rs6296, respectively. Small reductions were present in S-carriers in 5-HTTLPR and CC-homozygotes. While [carbonyl-^{11}C]WAY-100635 is not thought to be sensitive to endogenous 5-HT in physiological concentrations (Maeda et al., 2001), studies using the radioligand \[^{18}\text{F}\]-MPPF support 5-HT_{1A} receptor level increases through reduced synaptic 5-HT (Zimmer et al., 2003). Furthermore, 5-HT synthesis is negatively correlated with 5-HT_{1A} binding, indicating that lower 5-HT might be responsible for the observed effect (Frey et al., 2008). However, the finding that slight reductions of 5-HT_{1B} receptor expression, as caused by the C-allele of rs6296, should result in decreased 5-HT clearance (Conner et al., 2010), while 5-HTTLPR LL-carriers exhibit increases in 5-HT clearance (Lesch et al., 1996) is not in accordance with this explanation. The notion, that the LL and CC-alleles work in slightly opposite directions as far as synaptic 5-HT concentration is concerned, however, speaks against altered synaptic 5-HT levels as potential mechanism leading to these results.

Furthermore, the midbrain’s raphe nuclei could play an important role in mechanisms associated with our results. SERT knockout mice exhibit marked changes in 5-HT_{1A} and 5-HT_{1B} auto-receptors in the raphe nuclei (Fabre et al., 2000; Li et al., 2000), which in turn are associated with 5-HT_{1A} hetero-receptor densities in projection areas (Hahn et al., 2010). However, since the 5-HT_{1B} receptor and SERT interaction was not detectable in the midbrain’s raphe nuclei, there must be further mechanisms governing the observed effect. While our data do not permit causal inference on the underlying mechanisms, we believe that the interaction of SERT and 5-HT_{1B} observed in this study serves as valuable basis for further in vivo human research on auto-regulatory functions within the 5-HT system. It is to be expected that molecular interactions
between the three structures could be responsible for 5-HT₁A receptor increases. Although a molecular interaction between the SERT and 5-HT₁B receptors has yet to be demonstrated, pharmacological evidence from pulmonary fibroblasts and smooth muscle cells demonstrates that 5-HT, SERT and 5-HT₁B receptors utilize a common intracellular pathway via Rho-kinase (ROCK) and extracellular signal-regulated kinase (ERK) to stimulate proliferation (Liu and Fanburg, 2006; Liu et al., 2004; Mair et al., 2008).

All 5-HT₁A BP₉₀ values of genotype groups except LL and CC-carriers were in the range of the normative database of the serotonergic system previously published by our group (Savli et al., 2012). Significant increases were obtained in brain regions such as the parahippocampal gyrus, the temporal pole and the amygdala with maximum densities of 5-HT₁A receptors. These regions also exhibit a high amount of SERT and 5-HT₁B receptor, so that these results appear very plausible (Savli et al., 2012). An array of evidence emphasizes the role of the 5-HT₁A receptor in learning and memory (Ogren et al., 2008), which can be allocated regionally to the parahippocampal gyrus. Unfortunately, we did not collect neuropsychological data in this study; hence, we cannot describe phenotypical traits associated with our finding. Nevertheless, there are a number of previous studies indicating that interactions between 5-HTTLPR and other genetic polymorphisms are associated with clinical traits. One study reports that subjects with weak alleles in the 5-HTTLPR and the CC alleles in 5-HT₁B rs6296 are prone to alcoholism (Wang et al., 2012). Furthermore, interactions between the 5-HTTLPR and the catechol-O-methyl-transferase (COMT) are related to Borderline Personality Disorder (Tadic et al., 2009) and stressful life events in mood disorders (Mandelli et al., 2007), while brain-derived-neurotrophic-factor (BDNF) val66met and 5-HTTLPR were shown to mediate differences in lithium responses in patients with bipolar disorder (Rybakowski et al., 2007). This evidence suggests that gene x gene effects are useful for investigation of traits with complex inheritance. While we previously showed that a COMT SNP impacts on 5-HT₁A binding (Baldinger et al., 2013), this study adds that gene x gene interactions are important models to investigate alterations in complex neurotransmitter systems and beyond that, induce neurobiological alterations of potential clinical relevance.

As a limitation of this investigation, one must mention that 51 subjects can be considered small for a genetic investigation. However, the study size is comparable to other studies combining PET genetic data (David et al., 2005; Lothe et al., 2009). In order to ensure a sufficiently high statistical power, PET data from healthy subjects who had previously participated in performed studies from our group were pooled and included in the analysis (Baldinger et al., 2013; Hahn et
al., 2010). The retrospective study design and the limitation regarding statistical power do not allow for further computations of other serotonergic SNPs of interest (no genotyping performed), such as rs11568817, rs130058 (HTR1B), rs25531 and rs25532 (5-HTTLPR). One must not leave unmentioned that subparts of the study sample have been genotyped for rs6295 (5-HT1A receptor gene), rs6298 (HTR1B) and rs6265 (brain-derived neurotrophic factor gene). However, as no complete genetic profiles were available for the entire set of healthy individuals and DNA material being limited - these data were not included in the analyses. This has to be considered when interpreting the findings. The SNPs of interest investigated in this study were genotyped following a hypothesis-driven approach based on the existing literature.

In summary, we detected an elevated 5-HT1A receptor binding in healthy subjects homozygous for the L-allele in 5-HTTLPR and the C-allele in 5HT1B receptor rs6296. This suggests that the two major serotonergic structures involved in 5-HT reuptake and release are able to interact with each other to produce an increased inhibitory serotonergic tone. The consequences of receptor/transporter interactions within the serotonergic systems remain to be fully understood and this study might engage further investigations in humans in vivo. The described interrelation of different serotonergic structures modulating serotonin availability might represent an important auto-regulatory mechanism within the serotonergic system with therapeutic implications.
CONFLICT OF INTEREST

Without any relevance to this work, S. Kasper declares that he has received grant/research support from Eli Lilly, Lundbeck A/S, Bristol-Myers Squibb, Servier, Sepracor, GlaxoSmithKline, Organon, and has served as a consultant or on advisory boards for AstraZeneca, Austrian Sick Found, Bristol-Myers Squibb, GlaxoSmithKline, Eli Lilly, Lundbeck A/S, Pfizer, Organon, Sepracor, Janssen, and Novartis, and has served on speakers’ bureaus for AstraZeneca, Eli Lilly, Lundbeck A/S, Servier, Sepracor and Janssen. R. Lanzenberger has received travel grants and conference speaker honoraria from AstraZeneca, Lundbeck A/S and Roche Austria GmbH. P. Baldinger received travel grants from Roche Austria GmbH and AOP Orphan Pharmaceuticals AG, C. Kraus received travel grants and conference speaker honoraria from Roche Austria GmbH. G.S. Kranz received travel grants from Roche Austria GmbH and AOP Orphan Pharmaceuticals AG. W. Wadsak has received research support from Rotem GmbH, ABX, Iason, Advion and Raytest Austria and has served as a consultant/trainer for Bayer and THP. The authors M. Mitterhauser, C. Rami-Mark, G. Gryglewski, M. Spies, D. Haeusler, A. Hahn and D. Rujescu report no financial relationships with commercial interests.

ACKNOWLEDGEMENTS

This research was supported by funds of the Oesterreichische Nationalbank (Anniversary Fund, project number: 11468, 12809) to R. Lanzenberger and S. Kasper, respectively, and an intramural grant of the Department of Psychiatry and Psychotherapy, Medical University of Vienna, Austria (Forschungskostenstelle). The authors are grateful to A. Höflich, S. Pichler and A. Kautzky for medical and administrative support, and M. Savli for technical support. We thank the PET team, especially G. Karanikas, T. Traub-Weidinger, J. Ungersboeck, L. Nics, C. Philippe, T. Zenz and A. Krcal for technical support. Further, we thank the genetics team of D. Rujescu, especially M. Friedl, A. Hartmann, I. Giegling.

The study is part of P. Baldinger’s thesis “Influence of genetic variants on serotonergic neurotransmission” supervised by R. Lanzenberger in the Clinical Neurosciences PhD program at the Medical University of Vienna, Austria. Parts of this study have been presented by C. Kraus at the 10th International Symposium on Functional Neuroreceptor Mapping of the Living Brain, May 21st – 24th 2014, The Netherlands.
3.3.6. References


3.3.7. Tables and Figures

TABLES

**TABLE 1**: Subject characteristics: Demographic data of the study sample. Data are given as means ± SD. SA, specific radioactivity of the tracer

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**TABLE 2:** Voxel-wise analysis of variance (ANOVA) between 5-HT$_{1A}$ binding potential and 5-HTTLPR (rs4795541) and HTR1B (rs6296) genotype groups. Genotype status was calculated as factor while 5-HT$_{1A}$ BP was used as dependent variable. F-Test of ANOVA, t-Test of positive interaction between genotype groups as well as t-Test between 5-HTTLPR_rs4795541 LL carriers, HTR1B_rs6296 CC vs. G carriers are displayed (from top to bottom). FWE = family-wise error, peak level, R = right, L = left.

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TABLE 3: Mean regional $5$-HT$_{1A}$ receptor binding potential in 11 regions of interest (ROI) according to genotype status of $5$-HTTLPR (rs4795541, LL/LS/SS) and $HTR1B$ (rs6296, CC/CG/GG). SD, standard deviation; DRN, dorsal raphe nucleus; MRN, median raphe nucleus

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FIGURES

FIGURE 1: (Left) Differences in serotonin-1A (5-HT$_{1A}$) receptor binding potential between genotype groups of 5-HTTLPR (LL and LS/SS) and 5-HT$_{1B}$ receptor rs6296 (CC and CG/GG) presented on an axial view, superimposed on a structural magnetic resonance image template. A post-hoc t-test is displayed testing an interaction between all four genotype groups (p<0.001, uncorrected). The peak value can be observed in the right parahippocampal gyrus (t=5.02, p=0.008, FWE-corrected). The color table indicates the t-values. The crosshair is located at the overall peak t-value at the coordinates x/y/z=18/0/-24mm in standard MNI (Montreal Neurological Institute) space. (Right) Scatter plot showing the mean 5-HT$_{1A}$ receptor binding potential at the peak t-value in the right parahippocampal gyrus (x/y/z=18/0/-24, MNI space, t=5.02, p=0.008, FWE corrected) according to genotype groups. Highest binding potential values were obtained in the group with LL-alleles for 5-HTTLPR and CC-alleles for 5-HT$_{1B}$ rs6296 (5-HT$_{1A}$ BP$_{ND}$= 7.54±1.94, mean±SD), followed by LL-alleles and CG+GG-alleles (5.79±2), LS+SS-alleles and CG+GG-alleles (5.54±2) and finally LS+SS-alleles and CC-alleles (4.71±2). ** indicates peak level FWE-corrected p-values, * indicates p-values at <0.001 uncorrected as obtained by t-tests between genotype groups.
FIGURE 2: Serotonin-1A (5-HT$_{1A}$) receptor binding potential differences were most pronounced in homozygous LL-carriers (5-HTTLPR), when contrasting G-carriers to homozygous CC (HTR1B, rs6296) subjects (t-test, displayed at p<0.001, uncorrected). Increases in 5-HT$_{1A}$ binding were observed in the bilateral anterior and posterior parahippocampus, amygdala (p<0.05, FWE corrected) as well is in the anterior cingulate cortex (for further regions see Table 2). Coordinates represent standard MNI-Space.
4. DISCUSSION

4.1. General discussion

This doctoral thesis aimed at consolidating earlier findings retrieved from imaging and genetics studies on one hand, and to detect novel associations that might serve as new future research directions. Briefly to sum up our main findings, we could establish that COMT rs4680 influences serotonergic neurotransmission by affecting 5-HT₁A receptor binding, an indirect measure of receptor density, in a way that the high-activity allele (G, Val) is associated with higher 5-HT₁A receptor binding in healthy subjects [first publication (Baldinger et al., 2014)]. Secondly, we could substantiate previously published findings (Henningsson et al., 2009, Klein et al., 2010) showing that BDNF rs6265 does not influence 5-HT₁A receptor binding in healthy subjects nor SERT binding in both healthy subjects and individuals suffering from major depression [second publication(Kraus et al., 2014)]. Thirdly, we were able to determine a gene x gene interaction of 5-HTTLPR and HTR1B rs6296 affecting 5-HT₁A receptor binding in a healthy sample, with highest 5-HT₁A receptor densities in LL (5-HTTLPR, high transcriptional activity allele) x CC (rs6296) carriers [third publication (Baldinger et al., 2015)].

The interpretation of these results has to be done carefully considering that the majority of our findings were obtained in healthy individuals, especially when aiming at deducing clinically relevant conclusions. It should be stressed that, despite the relatively small sample size when compared to studies solely based in the field of genetics, the methodological approach chosen in these studies combining PET data (voxel-wise or regions-of-interest-based) and allelic distributions of gene variants withstands stringent statistical correction and leads to significant findings. This approach has been previously published several times (Willeit and Praschak-Rieder, 2010), nonetheless, the present project represents a further example underlining that this type of research is meaningful and promising. However, this begs immediately the question on the quantity of possible associations left to be determined and the answer might be probably countless. Are we on the right track?

What we can say with confidence is that this basic approach of directly linking measurable protein densities with gene expression while circumventing the complexity and heterogeneity of clinical phenotypes gives a unique possibility for simplification in search for an aetiological-based characterization of psychiatric disorders. At the same time, this advantage could also have a very inauspicious effect, namely an oversimplification of multifactorial entities, which are
mirrored in the sophisticated descriptions we can find in educational textbooks and clinical classification systems, such as the ICD-10. Perhaps, the concept of endophenotypes (Gottesman and Gould, 2003) is not sufficiently taking into consideration the impact of gene x gene and gene x environment interactions; it appears most improbable that in a system, where multi-genetic inheritance is assumed, one gene variant exerts significant effects by itself (Manolio et al., 2009). It is more likely that different gene variants exert reciprocal compensatory mechanism, e.g., regarding 5-HT_{1A} receptor density, thereby hindering the outbreak of a disease. If this system becomes unbalanced, negative consequences to healthy are inevitable. Furthermore, we cannot attribute a definite causal relationship between the SNPs and the intermediate imaging phenotypes, notable SERT and 5-HT_{1A} receptor binding, investigated in this thesis project. The implementation of psychological scales, e.g., recording of personality traits as described by Lesch et al. (Lesch et al., 1996), would have provided a major gain of information in regard to the clinical implications of the findings. It cannot be ruled out that the genetic and imaging variations are two independent systems, most probably playing a role in the development of psychiatric illness taken individually, as shown in multiple investigations, but not together.

Despite its limitations, the concept of endophenotypes has taken psychiatric research a major step forward since the beginning of the third millennium. When searching “endophenotype” in the medical database PubMed, more than 2500 articles pop up, the vast majority being psychiatric investigations with a clear emphasis on schizophrenia, where a biological aetiology has been assumed since the characterization as Dementia praecox by Emil Kraepelin at the end of the 19th century. The subsidiary concept of imaging genetics has gained recognition shortly after first references to endophenotypes, with now several available reviews, such as “Imaging genetics of mood disorders” (Scharinger et al., 2010), “Imaging genetics of anxiety disorders” (Domschke and Dannlowski, 2010), “Imaging genetics of schizophrenia” (Meyer-Lindenberg, 2010), “Imaging genetics in attention deficit hyperactivity disorder” (Durston, 2010) and many more. As opposed to the concept of biomarkers where it is assumed that disease or response to treatment is accompanied by variations of a measurable biological parameter, e.g., blood glucose concentration for the diagnosis and treatment of diabetes mellitus (Biomarkers Definitions Working, 2001), the concept of endophenotypes in psychiatry implies that the disorder is actually caused by neurobiologic factors, namely genes as well as structural and functional alterations of the brain, which are not necessarily specific for a psychiatric disease (Zobel and Maier, 2004).
Modelled after the work of Walters and Owens (Walters and Owen, 2007) as well as Kendler and Neale (Kendler and Neale, 2010), providing a conceptual analysis of the endophenotype construct in psychiatric research, the following scheme attempts to summarize the findings of the papers arising from this doctoral thesis regarding the 5-HT$_{1A}$ receptor in healthy subjects. One has to consider, that this system is based on the assumption that reduced 5-HT$_{1A}$ receptor binding represents an intermediate or imaging phenotypes for MDD (Drevets et al., 1999, Bhagwagar et al., 2004) and anxiety disorders (Neumeister et al., 2004, Lanzenberger et al., 2007) (as described in chapter 1.1.3).

One should not leave unmentioned that for BDNF rs6265, 5-HTTLPR and HTR1B rs6296 there is clear evidence provided by association studies supporting their role in the development of neuropsychiatric disorders (see chapter 1.1.4). However, taken individually, this effect seems not
to be caused by modulation of the 5-HT$_{1A}$ receptor (Kraus et al., 2014, Baldinger et al., 2015). It cannot be ruled out that not the quantity but rather the function and modulation of 5-HT$_{1A}$ receptor are altered by these genetic variants and that this effect is not apparent in PET studies. Furthermore, as discussed above, probably other genetic variants must be integrated in the model to uncover significant associations. In this regard, the HTR1A rs6295 might play an essential role, as it can be assumed that HTR1A directly impacts on 5-HT$_{1A}$ receptor binding. However, initial computations in this study sample have failed to show a significant association in this regard (Lanzenberger et al., 2012b), which can be due to a too small sample size to detect this effect (Parsey et al., 2006c) or compensatory mechanisms between somatodendritic 5-HT$_{1A}$ receptors in serotonergic projection areas and presynaptic 5-HT$_{1A}$ receptors in the raphe nuclei (Savitz et al., 2009, Czesak et al., 2012). Nonetheless, it might be expedient to include further genetic variants in statistical models to increase the probability that all variables essential to the system have been considered; in an investigation focusing on serotonergic neurotransmission it might therefore be required to include HTR1A, HTR2A, HTR1B, MAO-A, TPH2, etc. (see chapter 1.1.4). However, in such a model requiring correction for multiple testing, there is reason to fear that no effect will be determined at all, as each gene exhibits only a very low influence on the system. In imaging genetics, it seems therefore more appropriate to follow a hypothesis-driven approach in a more simplified model (gene $\rightarrow$ endophenotype $\rightarrow$ clinical phenotype) to detect actual effects which might pave the way for further investigations. However, it is fair to say that manifold possible gene x endophenotype and gene x gene x endophenotype interactions remain to be computed and interpreted.

Furthermore, one should not leave unmentioned that the imaging genetics approach was initially based on magnetic resonance imaging (MRI) data (Meyer-Lindenberg and Weinberger, 2006). Several robust imaging phenotypes have been characterized using MRI, such as the amygdala reactivity to emotional stimuli in anxiety disorders, but also hippocampal volumes for major depression, which have been extended through the inclusion of genetic data by Hariri et al. (Hariri et al., 2005) and Frodl et al. (Frodl et al., 2008), respectively. In both cases a major influence of 5-HTTLPR could be detected. However, the interpretation of these findings is challenging as the junction of allele distributions and for instance the BOLD (blood oxygenation level dependent) signal, measured in functional MRI studies, on one level of comparison seems inapplicable and difficult to interpret. The use of PET seems more immanent and appropriate as this approach relates genetic data and actual detectable molecules, such as neurotransmitter receptor and transporters.
4.2. Conclusion and further prospects

This thesis was centered on determining an influence of genetic variants on serotonergic neurotransmission, reflected in alterations in $5\text{-HT}_{1A}$ receptor and SERT binding potential as measured using PET. In the three publications arising from this project, two positive and one negative finding could be described. Notably, the effect of $\text{COMT}$ and the interaction of $5\text{-HTTLPR}$ and $\text{HTR1B}$ genotype on $5\text{-HT}_{1A}$ receptor binding have not been published earlier and represent novel findings, markedly enriching our knowledge on serotonergic neurotransmission and potential etiologic mechanisms involved in the development of neurotransmitter imbalances thought to underlie psychiatric disorders.

To date, none of the genetic associations shown in this thesis or described earlier in the wealth of literature on imaging genetics has been considered in the currently used diagnostic classification systems, including the revised DSM-5. The most promising candidate in this regard seems to be $5\text{-HTTLPR}$, particularly for mood and anxiety disorders. Actually, meanwhile there is the possibility to determine gene variants of interest in clinical practice, for instance at the Department of Psychiatry and Psychotherapy at General Hospital of Vienna using the so called Pharma-chip (http://intranet.akhwien.at/default.aspx?pid=3985&mid=5066&rid=1823). However, the focus of this diagnostic tool lies on the detection of genetics variations impacting on drug compatibility when following the application of several drugs, no clinical effect or on the contrary pronounced side-effects can be observed, particularly drug metabolism via CYP enzymes. Up to date, in spite of such possibility, the test is rarely applied. Among other factors, this might be primarily due to the fact that the results often do not match clinical observations but also that the finding ultimately fails to entail a consequence, e.g., switch to another drug. The technology still needs to mature and to win clinicians confidence.

In recent days, on international psychiatric and neuroscience conferences, genetics research in psychiatry, and particularly the relatively new and promising field of epigenetics (Petronis et al., 2000), represent an integral part. Despite the fact that a great deal of work remains to be done to uncover further significant associations of genetic variants and imaging phenotypes within the serotonergic system, the present thesis represents a valuable step forward in search of a biologically-based classification system of psychiatric disorders. This process seems substantial for destigmatization of mental illnesses and to guarantee adequate treatments.
5. REFERENCES


David SP, Murthy NV, Rabiner EA, Munafo MR, Johnstone EC, Jacob R, Walton RT, Grasby PM (2005) A functional genetic variation of the serotonin (5-HT) transporter affects 5-


Lan MJ, Ogden RT, Huang Y-y, Quendo MA, Sullivan GM, Miller J, Milak M, Mann JJ, Parsey RV (2014) Genetic variation in brain-derived neurotrophic factor val66met allele is


gene region and psychiatric disorders in individuals with 22q11.2DS. The International Journal of Neuropsychopharmacology 11:351-363.


Miller JM, Hesselgrave N, Ogden RT, Zanderigo F, Oquendo MA, Mann JJ, Parsey RV (2013) Brain Serotonin 1A Receptor Binding as a Predictor of Treatment Outcome in Major Depressive Disorder. Biological Psychiatry 74:760-767.


HT1A receptor binding in limbic regions in patients with anxiety disorders. Molecular psychiatry 14:1040-1050.


Visscher PM, Goddard ME, Derks EM, Wray NR (2012b) Evidence-based psychiatric genetics, AKA the false dichotomy between common and rare variant hypotheses. Molecular psychiatry 17:474-485.


6. APPENDIX

CURRICULUM VITAE
Pia Baldinger, MD

Functional, Molecular and Translational Neuroimaging Lab
Department of Psychiatry and Psychotherapy
Clinical Division of Biological Psychiatry
Medical University of Vienna
Währinger Gürtel 18-20
A-1090 Wien
Tel: 0043-1-40400-73882
e-mail: pia.baldinger@meduniwien.ac.at

Personal data
Date of birth: 04.08.1985
Place of birth: Oberndorf/Salzburg, Austria

Current position
05/2010 Researcher at the Functional, Molecular and Translational Neuroimaging Lab – PET&MRI (head: Assoc. Prof. Rupert Lanzenberger, MD PD) at the Department of Psychiatry and Psychotherapy, Medical University of Vienna
07/2010 Resident in psychiatry at the Department of Psychiatry and Psychotherapy, Medical University of Vienna (head: o. Univ. Prof. Dr. h.c.mult. Siegfried Kasper, MD)

Education
03/2010 – present Doctoral thesis program of applied medical sciences, thematic program Clinical Neurosciences (N790) at the Medical University of Vienna Wien (Supervisor: Assoc. Prof. Rupert Lanzenberger, MD, PD)
06/2009 Medical Doctor degree (Dr. med. univ.) at the Medical University of Vienna
2003 – 2009 Medical studies at the Medical University of Vienna, Austria.
2007 – 2009 Diploma thesis „Einfluss der lumbalen Sympathikusblockade auf die Schmerzmittelreduktion: eine retrospektive Studie“ at the Department of Anaesthesia, Critical Care and Pain Medicine, Medical University of Vienna
09/2007-07/2008 Medical studies at the Faculté de médecine Pierre et Marie Curie (Erasmus mobility program), Paris, France
06/2003 University entrance certificate “Matura” at the Lycée Français de Vienne; Austria

Special training and courses

04/2012 – present “Aufbaucurriculum” Cognitive-Behavioral Therapy (Psy-III) (Akademie für psychotherapeutische Medizin, Austrian Medical Association ÖAK)
02/2015 ECNP Internship, Prof. Laurence Lanfume, Centre des psychiatrie et de neurosciences, INSERM Paris, France
09/2010 – 06/2014 Propadeutic training in psychotherapy (ÖAGG)
03/2013 ECNP Workshop on Neuropsychopharmacology for Junior Scientists, Nice, France
07/2012 ECNP School of Neuropsychopharmacology, Oxford, United Kingdom
03/2012 „Basiscurriculum“ (Akademie für psychotherapeutische Medizin)

Additional skills
Languages: Fluent in english
            Fluent in french
            Mother tongue: german

Peer-reviewed grant support: 10 projects
Co-investigator: 3 projects

1. Effects of Electroconvulsive Therapy on Monoamine Oxidase A distribution volume in treatment-resistant depression investigated with PET

2. Influence of light exposure on cerebral Monoamine oxidase A in Seasonal Affective Disorder and Healthy Controls measured by PET

3. Effects of Silexan (WS® 1265) on the Serotonin-1A Receptor and Microstructure of the Brain: a randomized, Placebo-controlled, double-blind, cross-over Study with Molecular and Structural Neuroimaging
   Principal investigator: o. Univ. Prof. Dr. h.c.mult. Siegfried Kasper, MD.

Collaborator: 7 projects

1. Effects of sex steroid hormones on human brain function, structure and connectivity: A longitudinal study using 7 Tesla Ultrahigh-field Magnetic Resonance Imaging

2. Brain Network Dysfunction as a Model for Schizophrenia: Connectivity Alterations using Ketamine and pharmacological Magnetic Resonance Imaging

3. The Serotonin Transporter in Attention Deficit Hyperactivity Disorder Investigated with Positron Emission Tomography

4. The influence of hormone replacement therapy on serotonin$_{1A}$ receptor distribution and mood in postmenopausal women – A longitudinal study using Positron Emission Tomography (PET) and the radioligand [carbonyl-$^{11}$C]WAY-100635
   Funding: Austrian National Bank, Jubiläumsfonds Project # 12809, 2008 – 2011. Principal Investigator: o.Univ.-Prof. Dr.DDr. S.Kasper, MUW Austria

5. Effects of electroconvulsive therapy on serotonin-1A receptor binding in major depression

6. Effects of electroconvulsive therapy on serotonin-1A receptor binding in major depression investigated by positron emission tomography (PET)
7. The influence of sex steroid hormones on serotonin transporter binding in the human brain investigated by PET
Principal Investigator: Assoc. Prof. Rupert Lanzenberger, MD, PD

Honors and awards
2014 Mallinckrodt Award for Nuclear Medicine, Österreichische Gesellschaft für Nuklearmedizin und Molekulare Bildgebung, Zell am See, Austria
2013 ÖGPB Award for clinical psychiatry, Vienna Austria
2012 ECNP Travel Award, Vienna, Austria
2012 Travel grant „Förderungsprogramm Internationale Kommunikation“, Österreichischen Forschungsgemeinschaft (ÖFG)
2011 ECNP Travel Award, Paris, France
2011 ECNP Poster Award, Paris, France
2011 7th PhD Symposium – Best Oral Presentation, Vienna, Austria

Memberships in professional organizations
ÖGPB (Österreichische Gesellschaft für Neuropsychopharmakologie und Biologische Psychiatrie)
YSA (Young Scientist Association)
ECNP (European College of Neuropsychopharmacology)

Invited lectures and talks
02/2015 Bildgebung unter Lavendeleextrakt
Aromatherapiekongress der Österreichischen Gesellschaft für Physiotherapie, 22. Februar 2014, Wien, Österreich
11/2014 Optimizing outcome in schizophrenia
10/2014 EKT und Molekulare Bildgebung
14. EKT-Workshop und Treffen des DGPPN Referats, 24-25. Oktober 2014, Zürich, Schweiz
05/2014  Bildgebung bei psychiatrischen Erkrankungen
XX. Update für Psychiatrie und Psychotherapie, 23th May 2014, Vienna Austria

03/2014  Effekte von Silexan auf die Serotonin-1A Rezeptorbindung gemessen mit PET
Winterseminar “Biologische Psychiatrie”, 23th March 2014, Oberlech Austria

03/2013  Einfluss der Lichtexposition auf die zerebrale Monoaminoxidase A bei saisonal abhängiger Depression untersucht mittels PET
Winterseminar “Biologische Psychiatrie”, 11th March 2013, Oberlech Austria

06/2012  Genotype of serotonin-1B receptor affects serotonin-1A receptor binding in vivo
8th PhD Symposium, 13-14th June 2012, Vienna Austria

03/2012  Einfluss genetischer Varianten auf die serotonerge Neurotransmission
Winterseminar “Biologische Psychiatrie”, 11-16th March 2012, Oberlech Austria

11/2011  Behandlung psychischer Wechseljahresbeschwerden – Hormonersatztherapie, Workshop „Wirkung der Hormone auf Gehirn und Psyche“
13. Tagung der ÖGPB, 18th November 2011, Vienna Austria

10/2011  Die Psyche sichtbar machen
Science dabei!, 7th October 2011, Vienna Austria

06/2011  Decrease of 5-HT1A receptor binding in major depressive disorder after electroconvulsive therapy
7th PhD Symposium, 15-16th June 2011, Vienna Austria

03/2011  Einfluss genetischer Varianten auf die serotonerge Neurotransmission
Winterseminar “Biologische Psychiatrie”, 20-24th March 2011, Oberlech Austria
Publications:

First author:


Second author:


**Conference participation**
Aromatherapiekongress der Österreichischen Gesellschaft für Physiotherapie, February 2014, Vienna, Austria
14. EKT-Workshop und Treffen des DGPPN Referats, October 2014, Zürich, Switzerland
27th ECNP Congress, October 2014, Berlin, Germany
29th CINP Congress, June 2014, Vancouver, Canada
XX. Update Psychiatrie, May 2014, Vienna, Austria
26th ECNP Congress, October 2013, Barcelona, Spain
Winter Symposium of Biological Psychiatry, March 2013, Oberlech, Austria
Winter Symposium of Biological Psychiatry, March 2013, Oberlech, Austria
ECNP Workshop for Young Scientists in Europe, March 2013, Nice, France
8th PhD Symposium, June 2012, Vienna, Austria
Winter Symposium of Biological Psychiatry, March 2012, Oberlech, Austria.
25th ECNP Congress, October 2012, Vienna, Austria
28th CINP Congress, June 2012, Stockholm, Sweden
13th OeGPB Symposium, November 2011, Vienna, Austria
24th ECNP Congress, September 2011, Paris, France
7th PhD Symposium Medical University of Vienna, June 2011, Vienna, Austria
Winter Symposium of Biological Psychiatry, March 2011, Oberlech, Austria.
12th OeGPB Conference, November 2010, Vienna, Austria.
7th PhD Symposium Medical University of Vienna, June 2011, Vienna, Austria.

**Conference proceedings and published abstracts**
10 first-authorships, 27 co-authorships