THE IMPACT OF SEROTONIN TRANSPORTER GENE VARIATION ON NEURAL AND BEHAVIORAL CORRELATES OF GOAL-DIRECTED COGNITION

by

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To my daughter, Suzanne Kristina Vo,

who brings me joy and inspiration every day.

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LIST OF SELECTED ABBREVIATIONS

5-HT	Serotonin (5-hydroxytryptamine)
5-HTT	Serotonin transporter
5-HTTLPR	Serotonin transporter gene-linked polymorphic region
ACC	Anterior cingulate cortex
BOLD	Blood-oxygen-level dependent
DMPFC	Dorsal medial (or dorsomedial) prefrontal cortex
fMRI	Functional magnetic resonance imaging
G x E	Gene-environment interactions
HRF	Hemodynamic response function
L	Long allele (of the 5-HTTLPR)
L_A	Long allele (of the 5-HTTLPR), with A allele of the rs25531 SNP
L_G	Long allele (of the 5-HTTLPR), with G allele of the rs25531 SNP
MNI	Montreal Neurological Institute (template, xyz coordinates, etc.)
MPFC	Medial prefrontal cortex
MRI	Magnetic resonance imaging
MSIT	Multiple-source interference task
OFC	Orbital frontal (or orbitofrontal) cortex
PFC	Prefrontal cortex
PPI	Psychophysiologic interaction
ROI	Region of interest
rs25531	A-G SNP within the 5-HTTLPR
S	Short allele (of the 5-HTTLPR)
SNP	Single nucleotide polymorphism
STin2	Serotonin transporter intron 2 (polymorphism)
VMPFC	Ventral medial (or ventromedial) prefrontal cortex
VNTR	Variable number of tandem repeats (polymorphism)

ABSTRACT

In order to enable goal-directed behavior in a dynamically changing environment, the human brain must meet two competing requirements: (1) the goal representations must be stably and robustly *maintained* in the face of interference, and (2) the same goal representations must be rapidly and flexibly *adjusted* in response to changes in the environment. The amygdala–prefrontal cortex (PFC) circuitry is thought to be critically involved in balancing these two processing demands. It is therefore critical to elucidate the factors that modulate amygdala–PFC circuit function and that contribute to its dysregulation in psychopathology.

Growing evidence suggests that genetic factors impact the amygdala–PFC circuitry. In particular, several studies have shown that variation in the serotonin transporter (5-HTT) gene alters the response and functional connectivity within the amygdala–PFC circuit during emotion processing. In contrast, the effects of 5-HTT gene variation on the amygdala–PFC circuit function in cognition and goal-directed behavior are not well understood.

The purpose of this dissertation was to investigate the impact of two functional polymorphisms in the 5-HTT gene (the 5-HTT-linked polymorphic region [5-HTTLPR], including the rs25531 SNP, and the serotonin transporter intron 2 [STin2]) on the neural and behavioral correlates of goal-directed cognition, using behavioral genetics and imaging genetics approaches. The results of Study 1 suggest that 5-HTT gene variation modulates susceptibility to response interference from both neutral and emotionally

salient distracters during task performance (Requirement 1). In Study 2, employing an imaging genetics approach and a computer-tailored smoking-cessation intervention, we show that 5-HTT gene variation modulates the response and functional connectivity in the amygdala–PFC circuit when processing smoking-cessation messages in a manner that affects subsequent goal attainment, i.e., successful smoking cessation (Requirement 2).

Taken together, these studies demonstrate the impact of 5-HTT gene variation on two aspects of goal-directed cognition subserved by the amygdala–PFC circuit: resisting response interference from goal-irrelevant distracters, and updating a goal representation in response to goal-relevant stimuli. These findings add to our mechanistic understanding of the amygdala–PFC circuit involvement in goal-directed behavior and may shed light on the nature of dysregulation of this circuitry in psychopathology.

CHAPTER 1

INTRODUCTION

Compelling evidence suggests that the amygdala–PFC circuit is crucial to flexible goal-directed behavior and that it is dysregulated in several mental disorders. It is therefore critical to elucidate the factors that modulate amygdala–PFC circuit function and that contribute to its dysregulation in psychopathology. Growing evidence suggests that activity and connectivity in the amygdala–PFC circuit is modulated by variation in the serotonin transporter (5-HTT) gene.

We therefore investigated the impact of 5-HTT gene variation on two aspects of goal-directed cognition subserved by the amygdala–PFC circuit: resisting response interference from goal-irrelevant distracters, and updating a goal representation in response to goal-relevant stimuli.

We first review prior research documenting the involvement of the amygdala–PFC circuit in goal-directed behavior. Next, an overview of behavioral genetics and imaging genetics is given, followed by a review of literature on the effects of 5-HTT gene variation on behavior and brain function. In this context, we describe the aims of the two current studies investigating the impact of two functional polymorphisms in the 5-HTT gene (5-HTTLPR/rs25531 in the promoter and STin2 in intron 2) on the neural and behavioral correlates of goal-directed cognition.

1.1 Requirements for goal-directed behavior

In order to orchestrate goal-directed behavior in a dynamically changing environment, the human brain must meet two competing requirements: (1) the goal representations must be stably and robustly *maintained* in the face of interference, and (2) the same goal representations must be rapidly and flexibly *adjusted* in response to changes in the environment (Miller and Cohen, 2001). These two requirements apply whether the goal is *immediate*, such as a goal to correctly perform a task at hand, or *long-term*, such as a goal to quit smoking. On the one hand, it is adaptive to resist interference from task-irrelevant distracters when performing a demanding task, or to ignore smoking-related cues that may undermine one's resolution to stay abstinent. On the other hand, it is equally important to adjust one's task performance if the rules of the task change, or to update one's smoking-cessation strategies when participating in a tailored intervention.

However, these two processing demands of goal-directed behavior are in direct conflict. The processes that protect a goal representation from being disrupted by continuous interference from goal-irrelevant stimuli—are also the processes that prevent this goal representation from being updated by goal-relevant information. This is known as a paradox of robustness vs. adaptability. So how does the brain solve this problem?

1.2 Role of prefrontal cortex in goal-directed behavior

It has been proposed that both functions—the stable maintenance of goal representations and the dynamic updating of these goal representations—are carried out by the prefrontal cortex (PFC) (for reviews, see (Miller and Cohen, 2001; Sakai, 2008)).

Neurophysiological evidence from single-cell recordings in non-human primates suggests that neurons in the PFC encode abstract task rules that guide behavior towards specific goals (White and Wise, 1999; Wallis et al., 2001) and selectively

respond to goal-relevant stimuli (Rainer et al., 1998). Converging evidence for the involvement of the PFC in maintaining and updating goal representations comes from studies of human patients with prefrontal lesions (Bechara et al., 2000b) as well as human neuroimaging studies (Sakai and Passingham, 2006; Bengtsson et al., 2009). In particular, the orbitofrontal cortex (OFC), which partially overlaps with the ventromedial prefrontal cortex (VMPFC), is thought to encode both reward and aversive goal values, leading to seeking or avoidance behaviors, respectively (Plassmann et al., 2010). In fact, neuroimaging studies employing multivariate pattern-recognition techniques suggest that it is possible to decode the content of goal representations—whether representing immediate task sets (e.g., a planned motor response) or prospective intentions (e.g., a consumer decision)—from the patterns of prefrontal activity in people's brains while they reflect on these goals, a form of "mind reading" (Haynes et al., 2007; Soon et al., 2008; Tusche et al., 2010).

However, many questions remain unanswered, including the critical question of *how* the PFC balances the conflicting demands for stable maintenance and flexible updating of goal representations.

1.3 Role of amygdala–prefrontal cortex circuit in goal-directed behavior

One possibility is that the robustness-adaptability paradox is resolved at a circuit level. In particular, growing evidence suggests that the amygdala–prefrontal cortex (PFC) circuitry is critically involved in balancing the conflicting demands to stably maintain goal representations, but also dynamically update these representations in light of changing reward and punishment contingencies (for reviews, see (Bechara et al., 2000a; Holland and Gallagher, 2004; O'Doherty, 2004)).

The amygdala has been traditionally associated with emotion processing, including response to emotionally salient stimuli, emotional learning and memory, and expression of emotion. Amygdala involvement has been shown both in aversive processing, such as fear conditioning (LeDoux, 2000), and in appetitive or reward processing (Baxter and Murray, 2002). In humans as well as in lower species, the amygdala is critical to the rapid detection and appraisal of environmental stimuli in light of their biological significance to the organism, including both potential threat and potential reward, causing reorientation of attention and engagement of behavioral responses to these stimuli (LeDoux, 2000). This rapid detection and appraisal of environmental stimuli is possible because the amygdala receives sensory information through a fast subcortical pathway as well as through a slower cortical route (LeDoux, 2000), a finding supported by functional neuroimaging studies showing that the amygdala responds to threat stimuli that are outside of attentional focus or conscious awareness (Whalen et al., 1998; Vuilleumier et al., 2001). From an evolutionary perspective, in humans as in other species, such preferential processing of potential threat signals serves the adaptive function of facilitating rapid threat detection and fight-or-flight responses essential for survival—although the same system may produce hypervigilance, anxiety, and other maladaptive symptoms of psychopathology when it becomes hyperactive (Ohman and Mineka, 2001).

Neuroanatomical investigations in humans and non-human primates indicate that the amygdala has dense and reciprocal connections with the orbitofrontal cortex (OFC), which partially overlaps with the ventromedial prefrontal (VMPFC), as well as with the medial prefrontal cortex (MPFC) and the anterior cingulate cortex (ACC); in contrast, connections between the amygdala and other prefrontal regions are sparse (Ghashghaei et al., 2007). A complex interplay between the amygdala and PFC is critical to emotional

regulation and cognitive control, both engaged during goal-directed behavior (Barbas, 2000; Bechara et al., 2000a; Ochsner and Gross, 2005; Ghashghaei et al., 2007). Conversely, dysregulation of the amygdala–PFC circuit has been implicated in a number of mental disorders, such as mood and anxiety disorders (for reviews, see (Davidson et al., 2002; Bishop, 2007; Ressler and Mayberg, 2007)), and it has been proposed to underlie the impaired decision-making in addiction (the Somatic Marker Theory, reviewed in (Verdejo-Garcia and Bechara, 2009)).

1.4 Emotion-cognition interactions in goal-related behavior

One principle of brain function of fundamental importance to goal-directed behavior is that emotional and cognitive processes are closely interrelated, giving rise to complex and bidirectional *emotion-cognition interactions* (Davidson, 2003; Blair et al., 2007). Historically, emphasis has been placed on "cold" cognitive control over "hot" emotional reactivity. However, from an evolutionary standpoint, a dynamic balance between them is actually more adaptive because it allows goal-directed yet flexible and context-appropriate behavior (Mitchell et al., 2008). For example, it is adaptive that threat stimuli should capture attention and trigger fast and automatic coping responses when these stimuli signal a real threat—but it is equally adaptive to be able to ignore these threat stimuli if no real threat is present and performing a task at hand is more important. Analogously, emotionally salient stimuli may, on the one hand, trigger addictive behaviors, such as an urge to light a cigarette—and on the other hand, facilitate the formation of a long-term goal to abstain from smoking.

It is not an accident that converging evidence from several different areas of neuroscience—including neuroimaging, human lesion studies, and animal research—points to the amygdala–PFC circuit as a key locus of emotion-cognition interactions in the brain. Goal-directed behavior requires an efficient integration of emotional and

cognitive processes to guide behavioral choices. For example, goals critical for survival, such as avoiding injury or seeking nourishment, may be more robustly encoded and more immune to interference than more arbitrary goals, such as a goal to win a kick-boxing match or to follow a starvation diet. Emotion-cognition interactions also come into play during decision-making when two or more goal representations are in competition for control of behavior.

In sum, the evidence reviewed above strongly suggests that the amygdala–PFC circuit is crucial to flexible and context-appropriate goal-directed behavior, including the integration of emotional and cognitional influences on goal-directed action. Conversely, dysregulation of the amygdala–PFC circuit has been documented in major mental disorders, leading to a range of diverse clinical phenotypes, from apathy in major depression to compulsive drug-seeking in addiction. It is therefore critical to elucidate the factors that modulate the amygdala–PFC circuit function and that contribute to its dysregulation in psychopathology.

Growing evidence suggests that genetic factors modulate the amygdala–PFC circuitry. In particular, variation in the serotonin transporter (5-HTT) gene has been shown to modulate the function of the amygdala–PFC circuit during emotion processing. But the impact of the 5-HTT gene variation on *goal-directed cognition*—a term we use to denote all cognitive processes that subserve goal-directed behavior, including resisting interference and updating goal representations—has not been examined.

1.5 Gene structure and function

In the next several sections, an overview of behavioral genetics and imaging genetics is given, before prior research on the effects of the 5-HTT gene variation on behavior and brain function is reviewed.

All information necessary for the development and functioning of the human brain is contained in the human genome. The human genome consists of 46 chromosomes, including 22 pairs of autosomes and one pair of sex chromosomes (XX in females, XY in males). Each chromosome is one long molecule of deoxyribonucleic acid (DNA), composed of two intertwined polynucleotide chains held together by hydrogen bonds between complementary base pairs. These bases are adenine (A), thymine (T), cytosine (C), and guanine (G), the four letters of the genetic code. Adenine can only pair with thymine (A-T), and cytosine can only pair with guanine (C-G). Because of this specificity of base pairing, the sequence of one chain determines the sequence of its complementary partner chain. This base-pair specificity is the foundation of the two main functions of DNA in living organisms—to encode protein structure, and to transmit hereditary information via semi-conservative chain replication (Strachan and Read, 2004).

A *gene* is a basic functional unit of genetic information. According to the central dogma of genetics, DNA is transcribed into ribonucleic acid (RNA), and RNA is then translated into protein. But some genes encode only RNA molecules, with no protein products. Therefore, a gene is defined as a region of DNA that encodes a single protein, a single polypeptide chain, or a single RNA molecule, together with the associated non-coding regulatory sequences. The human genome contains estimated 22,000-25,000 genes. Each gene is characterized by its position (or *locus*) along a chromosome. In its linear structure, a gene typically contains a *promoter* and multiple *exons* and *introns*. The *promoter* is a regulatory non-coding region upstream of the gene, which contains the transcription start site as well as binding sites for transcription factors which regulate gene expression. *Exons* are regions of the gene which code for messenger RNA and determine the amino-acid sequence of the protein product. In contrast, *introns* are the

intervening non-coding regions of the gene which are removed from mature RNA via splicing. Intronic sequences also play a role in transcriptional regulation (Strachan and Read, 2004).

1.6 Genetic variation

With the exception of genes located on the major parts of the X and Y chromosomes, each individual carries two copies of each gene—one copy at the corresponding locus on each homologous chromosome, with one copy inherited form the mother and one from the father. In addition, genes can exist in different forms (or *alleles*) in the population due to sequence variation produced by *de novo* or inherited mutations. If an individual carries two identical alleles at a locus, he is said to be a *homozygote* for that allele; if alleles are different, the individual is a *heterozygote*. Thus, at each locus, the individual carries a combination of alleles, or a *genotype*. For a bi-allelic locus with alleles A and B, the possible genotypes are: A/A, A/B, and B/B. Individuals with either A/A or A/B genotype (i.e., carrying at least one copy of the A allele) can also be described as A allele *carriers*. The expected relative proportion of these three genotypes in the population under conditions of equilibrium (i.e., in the absence of mutation, selection, non-random mating, and other forces that affect allele and genotype frequencies) is described by the Hardy-Weinberg equation: $A^2 + 2AB + B^2$, where A is the frequency of allele A and B is the frequency of allele B in the population. The Hardy-Weinberg law states that the proportion of heterozygotes will always be greater than the proportion of either homozygote groups (e.g., if the frequencies of alleles A and B both equal 50%, then 50% of the population will be A/B heterozygotes, 25% will be A/A homozygotes, and 25% will be B/B homozygotes). In addition, both allele and genotype frequencies can vary greatly between ethnic groups (Strachan and Read, 2004).

Rare alleles (i.e., present at <1% in the population) are referred to as *variants* or *mutations*, while more common alleles (i.e., present at >1% in the population) are referred to as *polymorphisms*. Three common classes of polymorphisms are *single nucleotide polymorphisms*, *insertion/deletion polymorphisms*, and *variable numbers of tandem repeats*, described in more detail below. Because of negative selection against deleterious alleles and because coding sequences account for only 1.5% of the human genome, all three classes of polymorphisms are much more frequently found in non-coding regulatory regions—where most polymorphisms have no functional effects but some may affect gene expression and/or splicing—than in the coding regions of the genome, where they may alter protein structure and function (Strachan and Read, 2004).

1.6.1 Single nucleotide polymorphisms

Single nucleotide polymorphisms (SNPs) involve a substitution of a single nucleotide by a different nucleotide (e.g., A→G). SNPs are the most common type of genetic variation. The human genome contains approximately three billion base pairs, and on average, 1 in every 1000 bases contains a SNP, resulting in an estimated number of three million SNPs in the human genome. Typically, SNPs have only two alleles. A SNP located in a coding region but not resulting in a change of amino-acid sequence of the protein (due to the redundancy of the genetic code) is termed *synonymous*. A SNP which is located in a coding region of the gene and which alters the amino-acid sequence of the protein product of that gene is referred to as a *non-synonymous* SNP. However, as stated above, a majority of SNPs are located in non-coding regulatory regions where they may affect gene expression by altering the sequence of binding sites for transcription factors (Strachan and Read, 2004).

1.6.2 Insertion/deletion polymorphisms

Insertion/deletion (*indel*) polymorphisms involve an insertion or a deletion of one or more nucleotides in the DNA sequence. When occurring in coding regions, indel polymorphisms can produce a shift in the reading frame and thus disrupt protein synthesis. However, as for SNPs, a majority of indel polymorphisms are located in non-coding regulatory regions where they may alter the binding sites of transcription factors and thus affect gene expression (Strachan and Read, 2004).

1.6.3 Variable numbers of tandem repeats

Variable numbers of tandem repeats (VNTRs) consist of tandemly repeated copies of a short DNA sequence. Each number of repeats is an allele. VNTRs are divided into *microsatellites* (repeats of 1-9 nucleotides) and *minisatellites* (repeats of >9 nucleotides). Unlike SNPs and indels, VNTRs often have multiple alleles. But similarly to SNPs and indels, VNTRs typically occur in non-coding regulatory regions of the gene (Strachan and Read, 2004).

1.7 Behavioral genetics

While *genotype* refers to the genetic make-up of an organism, *phenotype* describes the physical expression of that genotype. Thus, different genotypes result in different phenotypes. Height, eye color, and insulin sensitivity are all examples of phenotypes that demonstrate large and measurable individual differences. However, in addition to influencing physical characteristics and physiology, genetic variation is also believed to account for a large portion of individual differences in complex behavioral traits (Plomin et al., 1994). The field of genetics concerned with elucidating the genetic contributions to behavior, personality, and susceptibility to psychiatric disorders is *behavioral genetics*.

1.7.1 Heritability

The first step in determining the genetic basis of a trait is to establish that this trait is heritable. Heritability (h^2) of a trait is the proportion of the total variance in that trait that is due to genetic factors, as opposed to environmental factors. Heritability estimates are population-specific. In other words, the heritability measure informs us about how much of the differences in a given trait between people in a given population at a given time are caused by their genetic differences, compared to by their different environments. Heritability of a trait is typically estimated by comparing the concordance rates between *monozygotic* (MZ) twins, who are genetically identical, and *dizygotic* (DZ) twins (preferable same-sex), who on average share 50% of their genome, the same as any pair of siblings. If the trait has a genetic component, the concordance rates will be higher among MZ twins compared to DZ twins. The distinct contributions of genetics and environmental factors can be further dissociated via studies of MZ twins separated at birth and raised in different environments (Strachan and Read, 2004).

1.7.2 Identification of causal genetic variants

Once the heritability of a trait has been demonstrated, the next step is to identify specific genetic variants that underlie the variation observed in that trait in the population. *Family-based linkage studies* use known genetic markers interspersed across the genome in order to map the genomic regions which co-segregate (or are *linked*) with the trait in families. These genomic regions can then be investigated further to identify the putative causal variants. In an analogous manner, *population-based association studies* aim to identify genetic variants which correlate (or are *associated*) with the trait in the population. *Whole-genome association studies* (GWAS) search for genetic variants associated with a trait across the whole genome with no *a priori*

hypotheses. In contrast, *candidate-gene association studies* investigate genetic variants that are *a priori* hypothesized to be associated with a specific trait based on prior genetic evidence (e.g., prior linkage studies) or based on existing knowledge of neurobiological pathways involved in the expression of that trait (Strachan and Read, 2004).

For any genetic variant to affect behavior (or any other complex phenotype), this genetic variant must first have functional consequences at the molecular and cellular level, and these can be tested *in vitro*. Thus, a non-synonymous SNP located in an exon of a gene may produce a truncated or misfolded protein or no detectable protein product at all. Similarly, a VNTR polymorphism located in the promoter of a gene may lead to increased or decreased expression of that gene. In both cases, demonstrating that a given genetic variant is *functional* (i.e., affects gene expression or protein synthesis *in vitro*) would indirectly support the potentially causal impact of this variant on a complex phenotype such as behavior (Strachan and Read, 2004).

1.7.3 Challenges to genotype-phenotype mapping

There are many challenges to successfully mapping of behavioral phenotypes to their underlying causal genotypes (for a recent review, see (Burmeister et al., 2008)). Early genetic studies focused on monogenic Mendelian traits, i.e., traits that are largely determined by a single genetic factor whose influence is fully manifested in each individual. However, most behavioral phenotypes are thought to be *polygenic* (i.e., shaped by multiple genetic factors) and *heterogenic* (i.e., the same phenotype can be produced by different genotypes at distinct loci involved in the same or interacting neurobiological pathways). Conversely, one genotype can contribute to multiple phenotypes (*pleiotropy*). In addition, the impact of any genetic variant on behavior may be modified by gene-gene interactions (*epistasis*) as well as gene-environment

interactions (*G x E*). Behavioral phenotypes often demonstrate *incomplete penetrance* (i.e., a genotype imparts only a susceptibility to a phenotype, so the phenotype may not be expressed in all individuals or in all environments). Finally, compared to the rapid development of sophisticated genetic techniques, the development of precise and neurobiologically-based phenotypic measures has lagged behind. Not surprisingly, in the face of these challenges, the progress in identifying and characterizing genetic variants that underlie individual differences in behavior, personality, and susceptibility to mental disorders has been slower than expected (Burmeister et al., 2008).

1.8 Endophenotypes

If the path from genes to behavior is highly complex, then one approach to reducing this complexity is to focus on intermediate phenotypes—or *endophenotypes*—which are postulated to lie closer to the genes than behavioral or clinical phenotypes (Gottesman and Gould, 2003; Bearden and Freimer, 2006; Cannon and Keller, 2006). Endophenotypes can be biochemical (e.g., a rate of enzymatic reaction), physiological (e.g., heart rate variability), neuroanatomical (e.g., hippocampus volume), neuropsychological (e.g., response inhibition), or other. Because endophenotypes are more directly related to the underlying causal genetic variants, they are thought to reflect the impact of these genetic variants to a greater extent than the more removed behavioral phenotypes. Therefore, it should be easier to detect an association of a candidate variant with a related endophenotype than with a behavioral phenotype.

An endophenotype should meet the following key criteria: (1) be at least moderately heritable; (2) be associated with a behavioral or clinical phenotype of interest (e.g., depression) in the population; (3) occur in non-affected family members of affected individuals at higher rates than in the general population (reflecting susceptibility); (4) be

trait-like rather than state-dependent; and (5) be reliably and accurately measured (Gottesman and Gould, 2003). In addition, endophenotypes should (6) be associated with causes rather than effects of disorders or their treatment (i.e., be part of the causal path from genes to behavior), and (7) vary continuously in the population to further increase power in statistical analyses (Bearden and Freimer, 2006; Cannon and Keller, 2006). In psychiatry, endophenotypes have been proposed to aid in the dissection of the genetic basis of mental disorders by serving as heritable biomarkers of these disorders that can be reliably and accurately measured and recreated in animal models for further study (Gottesman and Gould, 2003; Bearden and Freimer, 2006).

1.9 Imaging genetics

One class of endophenotypes that has gained prominence in human genetics is neuroimaging endophenotypes. Neuroimaging technologies, including functional and structural magnetic resonance imaging (MRI) and positron emission tomography (PET), allow a measurement of brain function and structure in humans *in vivo*. The approach that integrates neuroimaging and genetics to assess the impact of genetic variation on the brain is termed *imaging genetics* (Hariri and Weinberger, 2003; Hariri et al., 2006). The aim of an imaging genetics study is to test the association of a specific genetic variant with a specific measure of brain function or structure, rather than with a behavioral trait or a disorder (Hariri and Weinberger, 2003).

Neuroimaging endophenotypes are postulated to be particularly useful in uncovering the genetic basis of behavior because the impact of genetic variants on any behavior is likely to be mediated by specific brain processes underlying this behavior. By definition, neuroimaging endophenotypes lie closer to the underlying genetic variants than behavioral phenotypes, so genetic effects should be more robust and easier to

detect at the level of the brain than at the level of behavior (Hariri and Weinberger, 2003). In addition, measures of brain function and structure may be more objective, precise, and reliable than some behavioral assays (e.g., those allowing the use of different strategies) and personality measures (e.g., self-report questionnaires) (Hariri and Weinberger, 2003). Overall, imaging genetics is a powerful new approach to investigating the effects of genetic variation on the functional and structural characteristics of brain circuits underlying complex behaviors, and as such complements the more traditional approach of behavioral genetics.

1.9.1 Functional MRI

One of the most widely used neuroimaging techniques is *functional magnetic resonance imaging (fMRI)*. The development of fMRI methodology in the last two decades gave neuroscience researchers an unprecedented opportunity to non-invasively measure the human brain function *in vivo.* Unlike PET imaging, which relies on the injection of radioactive tracers to produce the images, MRI-based imaging takes advantage of the natural magnetic properties of hydrogen nuclei in the human body, making it safe for human subjects to undergo even prolonged or repeated MRI scanning (Huettel et al., 2004). Because it is safe and can be flexibly adapted to a variety of experimental paradigms, MRI-based imaging, particularly fMRI, has become a standard neuroimaging technique in human subject research. One important consideration in MRI-based imaging, however—which applies to all human neuroimaging—is the high cost of scanning per subject. This is particularly relevant to imaging genetics research, which typically requires large sample sizes.

Unlike traditional structural MRI, which produces images of brain *structure*, functional MRI (fMRI), measures the changes in brain *function* over time. The most

prominent fMRI technique is the blood-oxygenation-level dependent (BOLD) fMRI, which relies on the difference in magnetic properties between the *oxygenated* hemoglobin (which is diamagnetic, or has a weak repulsion to a magnetic field) and the *deoxygenated* hemoglobin (which is paramagnetic, or attracted to a magnetic field). This difference in magnetic properties between the oxygenated and deoxygenated blood, which can be measured with MR pulse sequences sensitive to T_2* contrast, is the basis of the BOLD contrast measured with the BOLD fMRI. (The T_1 and T_2 contrasts are used for anatomical images.) (Huettel et al., 2004).

Although the BOLD fMRI does not directly measure neuronal activity, it measures physiological processes which correlate with neuronal activity. Specifically, the BOLD fMRI measures the increased metabolic requirements of active neurons and the increased cerebral blood flow which supplies glucose and—critically—oxygen (bound to hemoglobin molecules) to the active brain areas. This change in the MR signal in response to neuronal activity is referred to as a *hemodynamic response*. The hemodynamic response is much more sluggish than the neuronal activity, peaking approximately 4 seconds after the event (such as a stimulus presentation) and returning to the pre-event baseline within 10 seconds, while the neuronal response occurs on a scale of tens or hundreds of milliseconds. To account for the sluggishness of the BOLD signal, the time-series of functional images is convolved with a *hemodynamic response function (HRF)*, often with a time derivative to accommodate between-subject and between-voxel variability in the response peak. With that correction, a well designed BOLD fMRI experiment can achieve a temporal resolution of hundreds of milliseconds to a few seconds, although the ability to discriminate between events occurring close in time is limited. The spatial resolution of the images is determined by the size of the

voxels collected: functional image voxels are typically 3 cubic mm, while the high-resolution structural image voxels are 1 cubic mm.

Following the required preprocessing steps, the analysis of the functional time-series is *massively univariate*, in that the MR signal is fitted to the HRF using a General Linear Model (GLM) separately in each single voxel of the brain. Due to the intrinsic relativity of the BOLD signal (i.e., the absence of absolute baseline against which to measure a signal change), fMRI experiments typically employ a *subtraction method*, in which the experimental condition is compared to a control condition, which (ideally) matches the experimental condition in all attributes except the psychological process of interest (Huettel et al., 2004). Finally, the patterns of stimulus- and task-related neural activity revealed in individual subjects by fMRI studies appear stable and replicable over time, heritable, and trait-like—meeting all key requirements for endophenotypes.

1.9.2 Candidate gene approach: focus on functional polymorphisms

Although the imaging genetics approach is starting to be applied to a genome-wide search for new candidate genes associated with complex behavioral traits, a majority of imaging genetics studies have focused on known functional polymorphisms hypothesized to affect brain neurotransmitter systems, such as the serotonin system (Hariri and Weinberger, 2003; Hariri, 2009). In addition, common polymorphisms (i.e., those with relatively high minor allele frequencies in the population) require smaller numbers of subjects to achieve balanced genotype frequencies, and therefore are easier to investigate using neuroimaging than rare variants.

1.10 Serotonin system

Serotonin, or 5-hydroxy-tryptamine (5-HT), is a major modulatory neurotransmitter in the mammalian brain. Serotonin is crucially involved in a range of

brain processes necessary for survival, including stress response, arousal, appetite, sexual drive, motor activity, mood, and sleep (Jacobs and Azmitia, 1992). Serotonin is also involved in brain processes associated with flexible, goal-directed behavior, including attention, cognitive control, emotion regulation, reward processing, and decision making (for recent reviews, see (Cools et al., 2008; Dayan and Huys, 2009; Kranz et al., 2010)). Dysregulation of the brain serotonin system has been reported in a number of mental disorders, including mood and anxiety disorders (Meltzer, 1989; Owens and Nemeroff, 1994; Ressler and Nemeroff, 2000), schizophrenia, obsessive-compulsive disorder, and drug addiction.

In the human brain, the neurons that synthesize and release serotonin arise primarily from the raphe nuclei in the brainstem, and project to all areas of the brain (Jacobs and Azmitia, 1992; Hensler, 2006). Serotonergic neurons with cell bodies in the dorsal and median raphe nuclei innervate all regions of the forebrain, with particularly dense serotonergic innervation observed in the cortical and subcortical structures of the limbic system, including prefrontal and cingulate cortices, amygdala, hippocampus and the adjacent entorhinal cortex, ventral striatum, and hypothalamus (Hensler, 2006). In contrast, a smaller group of serotonergic neurons with cell bodies in the caudal raphe nuclei project to the brainstem, cerebellum, and spinal cord (Hensler, 2006).

Serotonin exerts its multiple effects through 7 different types and 14 subtypes of receptors (5-HT1R – 5-HT7R). All receptor are metabotropic, and exert their effects on the cell through second-messenger signal-transduction cascades, with the exception of the ionotropic 5-HT3 receptors. Furthermore, even the same type of receptors may vary in their function depending on their synaptic localization (pre- or post-synaptic) and their localization to different types of neurons in the brain (e.g., glutamatergic or dopaminergic). This variety of receptor types, coupled with their distinct patterns of

anatomical distribution in the brain, accounts for both global and specific effects of serotonin on brain function and behavior (Hensler, 2006).

1.10.1 Serotonin synthesis

Serotonin was initially identified in blood serum (thus the name) and it is also present in the intestinal tract. Serotonin is a monoamine, specifically an indoleamine, with a very simple chemical structure. In the brain as well as in the rest of the body, serotonin is synthesized from the essential amino acid tryptophan obtained from the diet, and dietary acute tryptophan depletion (ATD) has been shown to be effective in reversibly reducing the levels of serotonin in human subjects. Serotonin is synthesized from tryptophan in two steps. In the first, rate-limiting step, tryptophan is converted to 5-hydroxy-tryptophan (5-HTP) by the enzyme tryptophan hydroxylase (TPH). TPH has two isoforms encoded by two different genes: TPH2 is the brain-specific isoform, while TPH1 is found in other tissues. In the second step, 5-HTP is immediately converted to serotonin (5-HT) by the enzyme amino-acid decarboxylase (AADC). After it is synthesized, serotonin is stored in synaptic vesicles in serotonergic axon terminals and released into the synaptic cleft to act on its receptors. Serotonin is degraded by the enzyme monoamine oxidase, of which two isoforms exist (MAO-A and MAO-B).

1.11 Serotonin transporter gene

A key component of the serotonin system and a regulator of serotonin signaling is the serotonin transporter (5-HTT). The 5-HTT is a trans-membrane transporter that is responsible for active reuptake of serotonin from the extracellular space back into the presynaptic neuron, terminating the action of serotonin at its receptors. The 5-HTT is also the proximal target of a range of psychoactive drugs, including anti-depressant

drugs such as tri-cyclics and selective serotonin reuptake inhibitors (SSRIs) and drugs of abuse such as cocaine and amphetamines.

The 5-HTT protein is encoded by a single gene (the solute carrier family 6, member 4, *SLC6A4*; also referred to as *5-HTT* or *SERT*) located on chromosome 17q11.1 – 17q12, spanning 31 kilobases and composed of 14 exons (Ramamoorthy et al., 1993; Lesch et al., 1994). Ever since it was identified and cloned, the 5-HTT gene has been a prime candidate gene in research on the genetic basis of behavior, personality, and susceptibility to mental disorders. Twin studies confirm that the 5-HTT function is modulated by genetic factors (Meltzer and Arora, 1988). Sequencing of the protein-coding exonic regions of the gene did not reveal any common functional variants for further study. However, several common, functional polymorphisms have been found in the non-coding regulatory regions of the gene, including two insertion/deletion VNTR polymorphisms described below.

1.11.1 STin2 polymorphism in intron 2

The first polymorphic region identified in the 5-HTT gene was an insertion/deletion VNTR polymorphism in intron 2 (serotonin transporter intron 2, or STin2), containing 9, 10, 11, or 12 copies of a 17 base-pair repeat element (Lesch et al., 1994; Ogilvie et al., 1996). The 12-repeat allele has been shown to be more efficiently transcribed than the 10-repeat allele, using a reporter-gene expression assay *in vitro* (Fiskerstrand et al., 1999), demonstrating that STin2 is a functional polymorphism. The transcription efficiency of the 9-repeat allele exceeded that of either the 10-repeat or the 12-repeat allele (Lovejoy et al., 2003). In Caucasians, the 12 allele is the most common (frequency of ~60%), followed by the 10 allele (frequency of ~40%), whereas the 9 allele is rare (frequency ~1%) (Ogilvie et al., 1996).

The mechanism by which the STin2 polymorphism affects the 5-HTT gene expression is not known. Ogilvie et al. (Ogilvie et al., 1996) initially speculated that the STin2 VNTR may affect transcription through an adjacent Activator Protein-1 (AP-1) binding site. Because the length of the STin2 repeats is sufficient to specify binding sites for transcription factors, it is has also been proposed that the STin2 VNTR itself functions as a transcriptional regulatory domain, containing both positive and negative regulatory elements (Fiskerstrand et al., 1999). Finally, adding to the complexity, Lovejoy et al. (Lovejoy et al., 2003) showed that individual repeats vary in their primary sequence and support different levels of gene expression, suggesting that the impact of the STin2 VNTR may reflect synergistic or additive effects of individual repeat elements, possibly acting in a tissue-specific manner.

1.11.2 5-HTTLPR/ rs25531 promoter polymorphisms

The most studied 5-HTT gene polymorphism is an insertion/deletion VNTR located upstream of the transcription start site in the promoter region (5-HTT-linked polymorphic region, or 5-HTTLPR), containing variable copies of a 20-23 base-pair repeat element (Heils et al., 1996). The short (S) allele consists of 14 repeats, while the long (L) allele consists of 16 repeats. Like STin2, the 5-HTTLPR polymorphism is a functional polymorphism. Compared to the L allele, the S allele has been shown to reduce the transcriptional efficiency of the promoter by ~65% in a reporter-gene assay, leading to decreased membrane expression of the transporter and decreased 5-HT uptake *in vitro* (Heils et al., 1996; Lesch et al., 1996). In Caucasians, the L allele frequency is approximately 60%, while the S allele is less common, at approximately 40% (Lesch et al., 1996). In Asians, these relative allele frequencies are reversed, with the S allele being by far the most common (~80%) compared to the L allele (~20%) (Nakamura et al., 2000).

In addition, the sequencing of the 5-HTTLPR identified a common A→G SNP (rs25531) within the larger insertion/deletion VNTR region (Nakamura et al., 2000; Hu et al., 2006). The G allele is the minor allele (frequency of 9-15%) in Caucasians (Hu et al., 2006). The combination of the 5-HTTLPR (L allele or S allele) and the rs22531 (A or G) produces four possible alleles (*tetra-allelism*): L_A, L_G, S_A and S_G. However, the combination of the rs25531 G allele and the 5-HTTLPR S allele (i.e., S_G allele) is very rare (Nakamura et al., 2000; Hu et al., 2006). Therefore, the 5-HTTLPR/ rs25531 polymorphism is sometimes described as *tri-allelic*, i.e., consisting of only three alleles: L_A, L_G and S (Hu et al., 2006).

The combined 5-HTTLPR/ rs25531 polymorphism is also functional. Relative to the L_A allele, the L_G allele showed a two-fold reduction in transcriptional efficiency of the promoter, rendering the L_G allele functionally equivalent to the S allele (Hu et al., 2006). As a result, in some studies, the L_G and S alleles are grouped together as the low transcriptional-efficiency alleles, compared to the high transcriptional-efficiency L_A allele.

As with STin2, the mechanism by which the 5-HTTLPR/ rs25531 polymorphism affects the 5-HTT gene expression is not fully understood. The 5-HTT promoter activity is controlled by interactions of transcription factors at several positive and negative regulatory elements, and it has been hypothesized that the 5-HTTLPR affects these interactions by altering the sequence of these response elements (Heils et al., 1996). In addition, the L_G allele has been shown to create a binding site for the AP-2 transcription factor, resulting in transcriptional suppression (Hu et al., 2006).

1.11.3 Linkage disequilibrium between STin2 and 5-HTTLPR

Both STin2 and 5-HTTLPR polymorphisms have been shown to be functional, i.e., to affect gene transcription *in vitro* (Lesch et al., 1996; Fiskerstrand et al., 1999). But

because most studies to date examined the effects of STin2 and 5-HTTLPR separately, it is still unclear whether their effects are independent of each other. The question is difficult to answer because the two loci are in *linkage disequilibrium* (LD) with each other in some populations, including Caucasians (Gelernter et al., 1999; Kazantseva et al., 2008), i.e., specific alleles at these loci are transmitted together as a *haplotype*, or a chromosomal block, more often than expected by chance.

One group reported combined (or additive) effects of the low-expressing alleles (10 and S) on relative 5-HTT mRNA levels in lymphoblast cell lines *in vitro* (Hranilovic et al., 2004). Specifically, cells with no low-expressing genotype at either locus (12/12 L/L) showed the highest mRNA levels, cells with low-expressing genotype at one locus (L/L 10 carriers and S carriers 12/12) showed intermediate mRNA levels, and cells with low-expressing genotypes at both loci (S carriers 10 carriers) showed the lowest mRNA levels (Hranilovic et al., 2004). On the other hand, Kazantseva and colleagues (2008) examined the effects of STin2 – 5-HTTLPR haplotype on personality traits, and found opposite effects of S10 and S12 haplotypes (as well as a main effect of STin2 genotype) on harm avoidance, suggesting a relatively larger impact of STin2 polymorphism. Finally, it is also possible that another unmeasured variant in the 5-HTT gene, in LD with STin2 or 5-HTTLPR, is the true causal variant producing the effects.

1.12 Effects of serotonin transporter gene variation on emotion

In the following two sections, a concise review of literature on the impact of the functional variation in the 5-HTT gene on emotion and cognition, both from the perspective of behavioral genetics and imaging genetics, will be given.

Previous research has provided compelling evidence that the 5-HTTLPR genotype modulates emotional reactivity and sensitivity to stress (for reviews see (Hariri

and Holmes, 2006; Canli and Lesch, 2007; Caspi et al., 2010)). Relative to the L allele, the S allele has been associated with higher measures of anxiety-related personality traits, particularly neuroticism (Lesch et al., 1996), as assessed with the NEO personality inventory (NEO-PI-R) (Costa et al., 1992), a finding confirmed by a meta-analysis (Schinka et al., 2004; Sen et al., 2004). The S or L_G allele has been associated with an increased attentional bias to negative emotional stimuli, such as aversive images (Osinsky et al., 2008), angry faces (Perez-Edgar et al., 2010), or anxiety-related words (Beevers et al., 2007). Individuals with the S allele also show an increased hypothalamic-pituitary-adrenal (HPA) axis response to experimental stressors (Gotlib et al., 2008). In studies focusing on gene-environment (G x E) interactions, individuals with the S allele demonstrate greater susceptibility to depression, depressive symptoms, and suicide following adverse early-life experiences or stressful life events, relative to the L/L genotype group (Caspi et al., 2003; Eley et al., 2004; Kendler et al., 2005; Taylor et al., 2006; Zalsman et al., 2006), with a recent meta-analysis supporting the view that the 5-HTTLPR moderates the relationship between stress and depressive phenotypes (Karg et al., 2011).

Some evidence also suggests that the 5-HTTLPR genotype modulates the reactivity to *positive* emotional stimuli, although results have been mixed. On the one hand, the L_A allele has been linked to an increased attentional bias towards positive emotional images, compared to the S or L_G allele (Fox et al., 2009; Perez-Edgar et al., 2010). On the other hand, G x E studies suggest that, while they are more vulnerable to depression in harsh, stressful life conditions, individuals with the S allele also benefit more from protective, nurturing environments, in which their risk of depressive symptoms is actually lower than the risk in the L/L group (Caspi et al., 2003; Eley et al., 2004; Taylor et al., 2006). In fact, it has been argued in the literature that, rather than

modulating specifically the impact of adverse stimuli, the 5-HTTLPR genotype may impart differential susceptibility to *all* environmental influences, whether positive or negative (Uher, 2008; Belsky and Pluess, 2009), a trait described as *hypervigilance* (Homberg and Lesch, 2010). Such genetic modulation of global reactivity to the environment could explain robust gene-environment interactions in the absence of genetic main effects (particularly if the environmental influences obscure the genetic effects or are not included in the analysis at all), and thus explain some of the inconsistent results.

Converging evidence for the effects of the 5-HTT gene variation on emotional and stress reactivity, as well as a plausible mechanism underlying these effects, has come from imaging genetics research of the 5-HTTLPR and other serotonergic gene polymorphisms. The most robust and consistent finding has been the association between the 5-HTTLPR S allele and increased amygdala reactivity to emotionally salient stimuli relative to neutral stimuli, first reported by Hariri et al. (Hariri et al., 2002), and since then replicated in several independent studies and using a variety of experimental paradigms and stimuli (e.g., (Canli et al., 2005; Hariri et al., 2005; Heinz et al., 2005; Smolka et al., 2007)), and confirmed by a meta-analysis (Munafo et al., 2008). Compared to the L/L homozygotes, the S allele carriers display a greater amygdala response to human facial expressions signaling a threat (angry or fearful faces) and to negative emotional pictures (e.g., images of war, mutilation, and pain). This genetically-driven enhancement of amygdala reactivity to threat signals is observed in both healthy individuals and patients with affective disorders such as anxiety or major depression, and whether the threat stimuli are attended to or outside of the attentional focus, and consciously perceived or subliminally detected. Moreover, the association with increased

amygdala reactivity to negative emotional stimuli has also been demonstrated for the 5-HTTLPR/ rs25531 L_G allele (Dannlowski et al., 2008; Dannlowski et al., 2010).

Furthermore, imaging genetics studies also demonstrated that the 5-HTTLPR genotype affects the functional connectivity in the amygdala–PFC circuit during the processing of emotionally salient stimuli (Heinz et al., 2005; Pezawas et al., 2005), although the direction of the association appears to depend on the specific prefrontal region involved. Specifically, Heinz and colleagues (Heinz et al., 2005) showed that the functional connectivity between the amygdala and VMPFC (Brodmann Area (BA) 10) was greater in the S allele carriers compared to the L/L homozygotes, a finding replicated by another study (Pezawas et al., 2005), which also showed that the S allele carriers had a reduced functional connectivity between the amygdala and perigenual ACC, particularly rostral ACC. The association with an increased functional coupling in the amygdala–PFC circuit (BA 10) was also shown for the carriers of the 5-HTTLPR/ rs25531 L_G allele (Friedel et al., 2009), consistent with the two studies above (Heinz et al., 2005; Pezawas et al., 2005).

Of note, the 5-HTTLPR genotype has also been shown to modulate the response and the functional connectivity during the processing of emotionally salient stimuli of other brain regions (besides the amygdala and the PFC), including the fusiform gyrus (Smolka et al., 2007; Surguladze et al., 2008).

In stark contrast to the extensive literature on the 5-HTTLPR, very little is known about the effects of STin2 genotype on emotion processing either at the level of behavior or brain function. The 12-repeat allele has been shown to be associated with increased risk of bipolar disorder relative to the 10-repeat allele (Collier et al., 1996a; Collier et al., 1996b). The rare 9-repeat allele has also been associated with increased risk of unipolar

depression (Ogilvie et al., 1996). An association of STin2 genotype with anxiety-related personality traits has also been reported, with 12/12 homozygotes scoring higher than 10 allele carriers on measures of neuroticism and harm avoidance (Kazantseva et al., 2008). STin2 genotype effects on brain correlates of emotion processing remain unknown.

1.13 Effects of serotonin transporter gene variation on cognition

Because emotion and cognition closely interact in guiding goal-directed behavior, genetic variants that modulate emotion processing are also likely to modulate cognitive processing. As a result, more recent investigations of the impact of the 5-HTT gene variation (primarily the 5-HTTLPR) on behavior and brain function turned their focus from emotion processing to different aspects of cognitive processing. However, compared to emotion processing (operationalized as reactivity to emotionally salient stimuli), cognition is a very broad concept and encompasses a number of diverse processes, including (but not limited to) decision-making processes and the processes subserving cognitive control, such as response inhibition and interference resolution. In part due to this sheer diversity of cognitive processes, the scope and character of the effects of the 5-HTT gene polymorphisms on cognition remain poorly understood (reviewed in (Homberg and Lesch, 2010)).

With respect to decision-making, a growing body of evidence suggests that the 5-HTTLPR modulates decision-making processes across a range of experimental paradigms (Roiser et al., 2006; Blair et al., 2008; da Rocha et al., 2008; Homberg et al., 2008; Roiser et al., 2009). Group differences between the 5-HTTLPR genotypes have been demonstrated in studies employing the Iowa Gambling Task (IGT), in which subjects try to accumulate as much money as possible by choosing from advantageous

(moderate rewards and low losses) and disadvantageous (high rewards but also sudden high losses) decks of cards. A high score on the IGT indicates that a person chose from advantageous decks more often than from disadvantageous decks, resulting in a higher net gain. Typically, subjects' performance on the IGT improves in the course of the task due to learning. Recent evidence suggests that, relative to the L_A allele, the S or L_G allele of the 5-HTTLPR is associated with impaired decision-making in the IGT: these individuals choose more disadvantageously overall, are slower to improve their performance, and achieve a lower net score (da Rocha et al., 2008; Homberg et al., 2008). Other studies examined the 5-HTTLPR genotype differences in susceptibility to decision-making biases induced by external cues, such as *framing effects* (e.g., choosing a sure option when it is framed in terms of gains, and a gamble option when it is framed in terms of losses). In an elegant study that included neuroimaging measures of decision-making-related activity and functional connectivity in the amygdala–PFC circuit, Roiser and colleagues (Roiser et al., 2009) demonstrated that the S/S homozygote group were more susceptible to framing effects than the L_A/L_A homozygotes, and that they exhibited greater amygdala response when making decisions in accord with the framing effects as opposed to counter to the framing effects, a relationship not observed in the L_A/L_A group. Conversely, the L_A/L_A group showed greater functional connectivity between the amygdala and the anterior cingulate cortex (ACC) when making decisions counter to the framing effects compared to in accord to the framing effects, whereas the S/S group showed no such relationship (Roiser et al., 2009). Importantly, the authors interpreted these results in terms of impaired amygdala–PFC circuit function (specifically, a failure of the ACC to regulate amygdala responses to contextual cues that trigger the framing effects) in the S/S group relative to the L_A/L_A group (Roiser et al., 2009).

In contrast, with respect to cognitive-control processes required for flexible goal-directed behavior, including response inhibition and interference resolution, evidence for the 5-HTTLPR modulation has been inconclusive. One source of this variability may be the presence or absence of emotionally salient stimuli in the experimental paradigm. Specifically, the 5-HTTLPR could affect cognitive task performance either *indirectly*, via its impact on reactivity to emotionally salient stimuli, or *directly*, by modulating the susceptibility to response interference irrespective of the emotional salience of the stimuli presented. For example, one study (Roiser et al., 2007) showed that the S/S homozygotes outperformed the L/L homozygotes in the affective go/no-go task (i.e., made fewer omission errors), a continuous-performance test of response inhibition, in which subjects are asked to execute or inhibit a motor response based on the emotional valence of the stimuli. In contrast, no behavioral effect of the 5-HTTLPR genotype on response inhibition was found in similar continuous-performance paradigms in the absence of emotionally salient stimuli (Fallgatter et al., 1999; Clark et al., 2005).

However, some evidence also suggests that the 5-HTTLPR modulation extends to cognitive processes in the absence of emotionally salient stimuli. One study showed that, relative to the L_A/L_A group, the S or L_G carriers display altered post-error behavioral adjustments in a flanker interference task, in which subjects indicate the direction of the target middle arrow flanked by arrows pointing in the same (the congruent condition) or opposite direction (the incongruent condition) (Holmes et al., 2010). Specifically, the L_A/L_A group showed improved accuracy on post-error trials relative to post-correct trials, while these behavioral adjustments were not observed in the S or L_G carriers. The neuroimaging data collected in the same study (Holmes et al., 2010) showed that the genotype groups also differed in the patterns of brain response, with a decreased conflict-related response in the dorsal ACC, and an increased error-related response in

the rostral ACC, in the S or L_G carriers compared to the L_A/L_A group. These results are consistent with previous studies using event-related potentials (ERP), showing increased error-related negativity (ERN) signal, localized to the ACC, in the S allele carriers compare to the L/L group (Fallgatter et al., 2004; Althaus et al., 2009).

As with emotion processing, the impact of STin2 polymorphism on cognitive function, both at the level of the brain and behavior, remains mostly unknown.

1.14 Purpose of this dissertation

The purpose of this dissertation was to investigate the impact of two functional polymorphisms in the serotonin transporter gene (5-HTTLPR/ rs25531 in the promoter and STin2 in intron 2) on behavioral and neural correlates of goal-directed cognition, using behavioral genetics and imaging genetics approaches.

In Study 1, we examined the impact of the promoter polymorphism in the 5-HTT gene (5-HTTLPR/ rs25531) on the behavioral indices of susceptibility to response interference from neutral and emotionally salient distracters during a cognitive task. The results are reported in Chapter 2.

In Study 2, we employed an imaging genetics approach and a computer-tailored smoking-cessation intervention to determine whether the variation in the 5-HTT gene (5-HTTLPR/ rs25531 and STin2) modulates the activity and functional connectivity within the amygdala–PFC circuit during the processing of smoking-cessation messages, and whether this genetic modulation of neural response in turn affects the relevant goal-directed behavior, i.e., post-intervention quitting outcome. In Chapter 3, we report the results of the mediation analyses focused on the neural processing of smoking-cessation messages in the amygdala as the *a priori*, anatomically defined region of interest. In Chapter 4, moving to the circuit level, we examine the impact of the intronic

polymorphism in the 5-HTT gene (STin2) on the functional connectivity in the amygdala–PFC circuit during the processing of smoking-cessation messages, and the relevance of this impact to subsequent quitting outcome. In Chapter 5, we test whether the neural response to smoking-cessation messages in the MPFC, previously implicated in the processing of tailored and persuasive health communications, also serves as a neural mediator of STin2 genotype on the post-intervention smoking-cessation outcome. Because MPFC is a key region in the self-related processing network, we also examined whether these mediation effects were specific to the processing of smoking-cessation communications or extended to other tasks involving self-related processing.

Finally, in Chapter 6 (Conclusions), we provide a general discussion of the results of both studies in the context of prior research on the effects of the 5-HTT gene variation on behavior and brain function. We discuss the limitations of the current research, as well as future directions in behavioral and imaging genetics research on the impact of genetic variation in the serotonin system on cognitive function and emotion-cognition interactions relevant to goal-directed behavior. We conclude with a discussion of the potential translational relevance of the current and future research aimed at elucidating the brain processes mediating the effects of genetic risk factors on maladaptive and pathological behaviors.

As will probably be clear, this is a “staple dissertation,” i.e., the studies presented in this dissertation were designed and conducted as separate and independent entities. Every effort has been made to provide a theoretical framework linking the two studies into a coherent, unified research project. Unfortunately, because of the nature of a “staple dissertation,” the Introduction and Methods sections of individual data chapters have substantial overlap because we are hoping to publish them separately. Other

asymmetries and redundancies between the chapters also remain. Those will be addressed by planned future studies, outside the scope of this dissertation.

CHAPTER 2

SEROTONIN TRANSPORTER GENE PROMOTER POLYMORPHISM MODULATES SUSCEPTIBILITY TO RESPONSE INTERFERENCE

2.1 Goals

The goal of Study 1 was to determine the impact of serotonin transporter gene variation on behavioral indices of cognitive processing and emotion-cognition interactions relevant to goal-directed behavior, specifically, the susceptibility to response interference from neutral and emotionally salient distracters during a cognitive task performance.

2.2 Introduction

The ability to successfully carry out a task despite interference from task-irrelevant stimuli is a crucial requirement for goal-directed behavior. According to accepted models of selective attention and cognitive control, task-irrelevant stimuli interfere with task performance by competing for attentional and response-selection resources with task-relevant stimuli (Desimone and Duncan, 1995; Miller and Cohen, 2001). Moreover, this interference can come from both neutral and emotionally salient stimuli, highlighting the importance of both cognitive and emotional control processes in goal-directed action. Because impaired control processes are a hallmark of several brain disorders, elucidation of the sources of individual differences in susceptibility to response interference can inform our understanding of the etiology of these disorders.

Extensive evidence supports the involvement of serotonin (5-hydroxytryptamine, 5-HT), in a range of behavioral control processes required for goal-directed behavior (Cools et al., 2008). In the human brain, serotonergic neurons arise from the raphe nuclei in the brainstem and project to all areas of the brain (Jacobs and Azmitia, 1992), with particularly dense serotonergic innervation in the anterior cingulate cortex (ACC), ventromedial prefrontal cortex (VMPFC) and the amygdala (Hensler, 2006), the key brain circuits involved in resolving interference (Carter et al., 1999) as well as integrating emotional and cognitive influences on behavior (Barbas, 2000; Bechara et al., 2000a).

Serotonin signaling is regulated by the serotonin transporter (5-HTT), which is encoded by the 5-HTT gene (*SLC6A4*) (Ramamoorthy et al., 1993; Lesch et al., 1994) . The 5-HTT gene contains a well-studied functional polymorphism in the promoter region (5-HTT-linked polymorphic region, or 5-HTTLPR), with a variable number of copies of a 20-23 base-pair repeat (Heils et al., 1996). The short (S) allele, consisting of 14 repeats, has been shown to reduce the transcription efficiency of the promoter, leading to decreased transporter expression and decreased 5-HT uptake *in vitro*, compared to the long (L) allele with 16 repeats (Heils et al., 1996; Lesch et al., 1996). In addition, an A→G single nucleotide polymorphism (SNP) within the 5-HTTLPR (rs25531) produces L_A and L_G alleles, with L_G allele functionally equivalent to the S allele (Hu et al., 2006). (See **Sections 1.11.1** and **1.11.2** for details about both polymorphisms.)

Previous studies demonstrated the impact of the 5-HTTLPR on emotional reactivity and sensitivity to stress, both at the level of behavior and at the level of brain function (Caspi et al., 2010) (see **Section 1.12** for a more detailed review). Briefly, the S allele has been associated with greater reactivity to negative emotional stimuli (Beevers et al., 2007; Osinsky et al., 2008) and greater stress sensitivity (Caspi et al., 2003; Eley et al., 2004; Taylor et al., 2006; Zalsman et al., 2006; Gotlib et al., 2008), possibly due to

a heightened amygdala response (Hariri et al., 2002; Hariri et al., 2005; Munafo et al., 2008) and an altered functional connectivity between the amygdala and the prefrontal regions during the processing of threat stimuli (Heinz et al., 2005; Pezawas et al., 2005).

In contrast, the reports of the 5-HTTLPR effects on cognitive function have been inconsistent (Homberg and Lesch, 2010). One source of this variability may be the presence or absence of emotionally salient stimuli in the experimental paradigm. Specifically, the 5-HTTLPR could affect cognitive task performance either *indirectly*, via its impact on reactivity to emotionally salient stimuli, or *directly*, by modulating the susceptibility to response interference irrespective of the emotional salience of the stimuli presented. For example, Roiser et al. (2007) showed that the S allele carriers performed better than the L/L homozygotes in the affective go/no-go task, in which subjects are asked to inhibit a response based on the emotional valence of the stimuli. However, no behavioral effect of the 5-HTTLPR genotype on response inhibition was found in similar paradigms in the absence of emotionally salient stimuli (Fallgatter et al., 1999; Clark et al., 2005).

On the other hand, some evidence also suggests that the 5-HTTLPR modulation extends to cognitive processes in the absence of emotionally salient stimuli. Holmes and colleagues (Holmes et al., 2010) recently showed that, relative to the L_A/L_A group, the S or L_G carriers display altered post-error behavioral adjustments in a flanker interference task, in which subjects indicate the direction of the target middle arrow flanked by arrows pointing in the same (the congruent condition) or opposite direction (the incongruent condition). Specifically, the L_A/L_A group showed improved accuracy on post-error trials relative to post-correct trials, while these behavioral adjustments were not observed in the S or L_G carriers (Holmes et al., 2010). These effects were observed in the absence of any emotional stimuli in the experimental paradigm and in the absence of differences

in self-reported mood between the genotype groups (Holmes et al., 2010). However, no genotype differences were observed in the primary measure of the efficiency of interference processing in the task, i.e., flanker interference effects either in accuracy or in reaction times. This leaves open the possibility that the magnitude of the 5-HTTLPR genotype effects on interference processing depends on the emotional salience of the stimuli used.

In the current study, we set out to test two hypotheses regarding the impact of the 5-HTTLPR on cognitive task performance. The 5-HTTLPR could modulate the magnitude of response interference produced specifically by emotionally salient stimuli (Hypothesis 1). If that was the case, we would *not* expect genotype differences in interference effects in the absence of emotionally salient distracters. Conversely, the 5-HTTLPR could modulate response interference irrespective of the emotional salience of the stimuli (Hypothesis 2). In that case, there *would* be genotype differences in interference effects both in the presence and in the absence of emotionally salient distracters. In order to dissociate the response interference produced by neutral vs. emotionally salient stimuli, we employed a cognitive-interference task modified to include threat and neutral distracters.

2.3 Methods

2.3.1 Subjects

Seventy-one healthy Caucasian females aged 18 to 34 years (M = 23.0 years, SD = 4.0 years) participated in the study. All subjects were right-handed and had normal or corrected-to-normal vision. Exclusion criteria included any serious medical condition, head injury or trauma, lifetime diagnosis of psychiatric illness, current use of a

psychoactive medication, and smoking. The study was approved by the University of Michigan Medical School IRB and all subjects provided written informed consent.

2.3.2 Genotyping procedures

Genomic DNA was obtained from saliva using the Oragene saliva collection system and extracted using the protocol provided (Genotek, Ontario, Canada). The extracted DNA samples were genotyped for the 5-HTTLPR and the rs25531 in two steps, according to Wendland and colleagues (Wendland et al., 2006). In the first step, the 5-HTTLPR was amplified via polymerase-chain reaction (PCR) using site-specific forward and reverse primers, yielding "short" (14- repeat, 375 bp) and "long" (16-repeat, 419 bp) products. In the second step, the PCR product from the first step was digested with Hpa II restriction enzyme to genotype the A→G SNP (rs25531) by identifying L_G (305 bp) and L_A alleles**.** All PCR products were visualized via gel electrophoresis on a 3% agarose gel using ethidium bromide under ultraviolet (UV) light.

2.3.3 Task: Threat-Distracter MSIT

We employed a modified version of the Multiple-Source Interference Task (MSIT) (Bush et al., 2003; Bush and Shin, 2006). The MSIT is a validated response-interference paradigm which combines the sources of interference from Erikson, Stroop, and Simon tasks, in order to maximally tax the interference processing associated with the dorsal anterior cingulate cortex (dACC) (Bush et al., 2003). The MSIT has been shown to produce a robust and temporally stable *interference effect* both in reaction times and in error rates (Bush et al., 2003).

In the MSIT, subjects were presented with a set of three numbers from 0 to 3, one of which was different from the other two (the oddball number). Subjects were instructed to indicate the identity of the oddball number with a corresponding key press:

a key press with the index finger if the oddball number was “1”, with the middle finger if the oddball number was “2”, and with the ring finger if the oddball number was “3.” On *congruent* trials, the identity of the oddball number corresponds to its location and the other two numbers are 0’s, not related to any valid key press response. An example of a congruent trial is “020,” where the oddball number is “2” and the correct response is a key press with the middle finger. On *incongruent* trials, the identity of the oddball number is incongruent with its position and the other two numbers are related to competing key press responses, resulting in stimulus-response incompatibility. An example of an incongruent trial is “311,” where the oddball number is “3” and the correct response is a key press with the ring finger (*not* the competing response tendency to press with the index finger, based on the position of the oddball number). The *Incongruent condition* – *Congruent condition* contrast yields the interference effect in reaction times and in accuracy. The magnitude of the interference effect is interpreted as an index of the efficiency of interference resolution, with a greater interference effect signaling a lower efficiency of interference resolution.

To address our specific aims, we modified the MSIT to include 3 categories of task-irrelevant flanker distracters: Threat, Neutral, and Scrambled. As Threat distracters, we used images of human faces signaling the presence of a threat (angry or fearful expression). To isolate the effects specific to threat information, we included Neutral distracters (images of human faces with neutral expression) and Scrambled distracters (images retaining the basic oval shape of a face but no facial features). Face stimuli were carefully selected from standardized sets (Ekman, 1976; Gur et al., 2002; Tottenham et al., 2009). Angry and fearful faces displayed intense emotion and showed bared teeth and/or open mouth as an additional perceptual homogeneity criterion. In contrast, all neutral faces had closed mouths. All faces were Caucasian, to optimally

control for potential sources of variability in emotional responses. All images were presented in grayscale, with hair and background cropped to yield an oval shape. Scrambled distracters were generated from the human face stimuli used in the other two distracter conditions, while preserving their oval shape and average brightness.

2.3.4 Experimental protocol

A timeline of events in a single trial is shown in **Figure 2.1**. The MSIT stimuli and two identical flanking distracter images were presented simultaneously for 500 ms, followed by a black screen for 1000 ms, and then a fixation cross for another 500 ms. The durations of these three events added up to the overall response limit of 2000 ms. A black screen presented for 100 ms separated two consecutive trials. Subjects were instructed to respond as fast and as accurately as they could. The task stimuli were presented and the key press responses collected using the E-Prime 2.0 software implemented on the Lenovo ThinkPad T61 series computer.

After a self-timed tutorial in the task and a short practice run, subjects completed a total of 640 trials, divided into 2 runs, 4 blocks per run, 80 trials per block. A short intermission separated Run 1 (blocks 1-4, the total of 320 trials) from Run 2 (blocks 5-8, the total of 320 trials). Each block lasted approximately 3 minutes and consisted of 40 congruent and 40 incongruent trials. Within the sets of 40 congruent and 40 incongruent trials, 10 trials included Threat distracters (5 Angry faces, 3 female, 2 male or 2 female, 3 male; and 5 Fearful faces, 3 female, 2 male or 2 female, 3 male), 10 trials included Neutral distracters (5 female, 5 male), 10 trials included Scrambled distracters, and 10 trials were no-distracter trials (i.e., with MSIT stimuli only). The order of the trials was pseudo-randomized within each block, with the provision that no two consecutive trials

1) had the same correct response or 2) both included Threat distracters. The whole experiment lasted approximately 30 minutes.

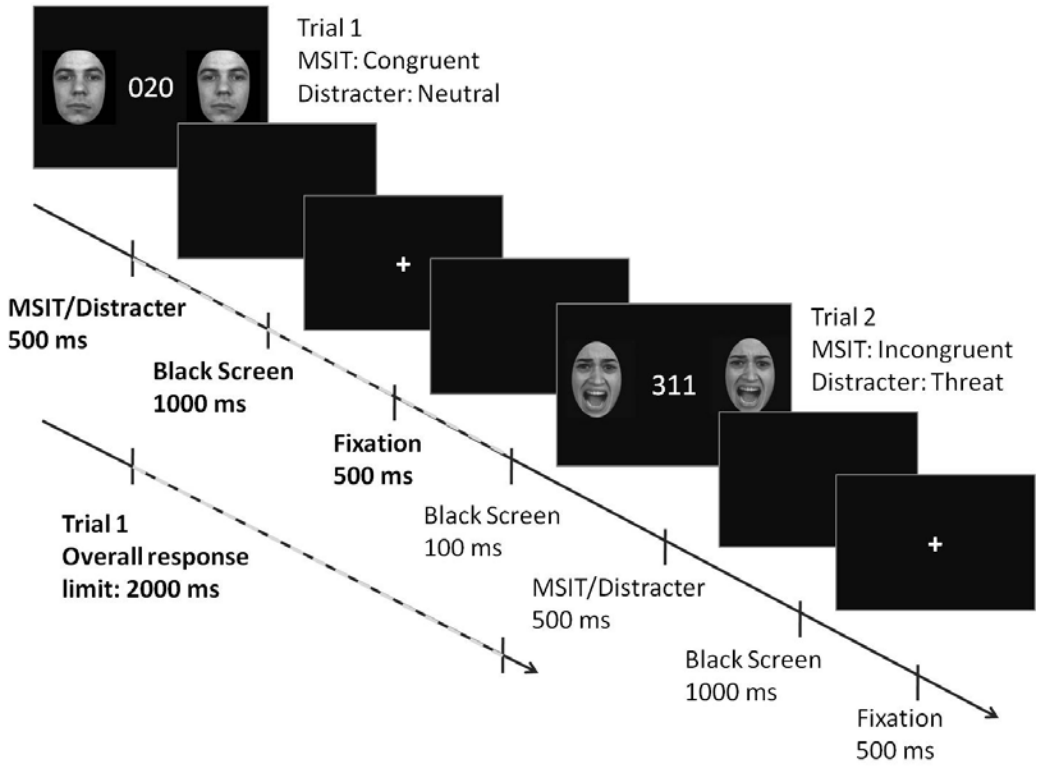

Figure 2.1 The anatomy of a trial in the Threat-Distracter MSIT. The MSIT stimuli and two identical flanking distracter images were presented simultaneously for 500 ms, followed by a black screen for 1000 ms, and then a fixation cross for another 500 ms. The durations of these three events added up to the overall response limit of 2000 ms. A black screen (100 ms) separated two consecutive trials. Face images reproduced with permission from Gur et al., 2002.

2.3.5 Statistical Analyses

The data were analyzed in a series of steps using repeated-measures Analysis of Variance (ANOVA) and *t*-tests as implemented in SPSS 17.0. We used two behavioral indices of task performance, reaction times (RTs) on correct trials and accuracy rates, as dependent variables. In addition, the MSIT interference effects in reaction times and in

accuracy were used as a global measure of the efficiency of interference processing, with greater interference effects indicating less efficient interference resolution. We used a statistical significance threshold of $p < 0.05$ throughout. The *t*-tests are two-tailed unless stated otherwise.

2.4 Results

2.4.1 Final sample

Out of the 71 subjects who participated in the study, the data from two subjects were excluded from analysis due to concerns about task compliance and performance accuracy. One subject did not follow the task instructions and responded to the position of the oddball number rather than to its identity ($M = 0.05$ accuracy on Incongruent trials), an occurrence reported in approximately 5% of participants in prior work using the original version of the MSIT (Bush and Shin, 2006). Another subject had the mean accuracy of 0.34 on incongruent trials, corresponding to a chance level of responding in a 3-choice task. The data from the final sample of 69 subjects were analyzed and are reported below.

2.4.2 Genotyping results

The genotyping results are summarized in **Table 2.1**. We observed the following 5-HTTLPR genotype counts (and frequencies): 25 (0.35) L/L homozygotes, 35 (0.49) L/S heterozygotes, and 11 (0.16) S/S homozygotes. We also analyzed the combined 5-HTTLPR/rs25531 functional genotypes, which were grouped as follows: 23 (0.32) subjects were L_A/L_A, 36 (0.51) subjects were functional L_A/L_GS (2 L_A/L_G and 34 L_A/S_A), and 12 (0.17) subjects were S/S (1 L_G/S and 11 S/S). The observed genotype frequencies did not deviate from the Hardy-Weinberg Equilibrium. The genotype groups did not differ in age, education, or socio-economic status.

Table 2.1 Distribution of 5-HTTLPR and 5-HTTLPR/rs25531 alleles and genotypes. S allele and L_G allele are denoted as functional S alleles.

5-HTTLPR Genotype Count (Frequency)						**5-HTTLPR Allele Count (Frequency)**		
L/L	**L/S**		**S/S**			**L**	**S**	
25 (0.35)	35 (0.49)		11 (0.16)			85 (0.60)	57 (0.40)	
5-HTTLPR/rs25531 Genotype Count (Frequency)						**5-HTTLPR/rs25531 Allele** Count (Frequency)		
Func L/L	**Func L/S**		**Func S/S**			**Func L**	**Func S**	
23 (0.32)	36 (0.51)		12 (0.17)			82 (0.58)	60 (0.42)	
L_A/L_A	**L_A/L_G**	**L_A/S**	**L_G/L_G**	**L_G/S**	**S/S**	**L_A**	**L_G**	**S**
23 (0.32)	2 (0.03)	34 (0.48)	0	1 (0.01)	11 (0.16)	82 (0.58)	3 (0.02)	57 (0.40)

2.4.3 Behavioral data

TD-MSIT effects: Threat distracters transiently potentiate response interference

To verify that the task engaged the processes of interest, we first examined whether threat distracters increased the interference effect in the MSIT independent of genotype. We also examined whether the effects of distracters changed over the course of the experiment.

Consistent with previous reports (Bush et al., 2003; Bush and Shin, 2006), MSIT produced robust interference effects in both measures of task performance (in RTs: M = 219.0 ms, SD = 67.3 ms, $t(68) = 27.04$, $p < 0.0001$; in accuracy: M = 0.15, SD = 0.11,

$t(68) = 11.06$, $p < 0.0001$): subjects were less accurate and slower to correctly respond in the incongruent condition compared to the congruent condition.

Using a 2 x 4 within-subject ANOVA, we found a significant 2-way interaction of run number and distracter type on the interference effect in RTs, $F(3, 69) = 14.81$, $p < 0.0001$, partial eta squared = 0.18 (**Figure 2.2A**), as well as on the interference effect in accuracy rates, $F(3, 69) = 5.15$, $p = 0.002$, partial eta squared = 0.07 (**Figure 2.2B**). In Run 1, Threat distracters potentiated the interference effects in RTs relative to Neutral distracters ($t(68) = 4.31$, $p < 0.0001$), Scrambled distracters ($t(68) = 2.38$, $p = 0.020$), and no distracters ($t(68) = 7.36$, $p < 0.0001$). In contrast, in Run 2 (following the intermission), the interference effects in RTs observed in the Threat-distracter condition were lower than in the presence of Neutral distracters ($t(68) = -3.87$, $p < 0.0001$) or Scrambled distracters ($t(68) = -3.28$, $p = 0.002$), and comparable to the no-distracter condition (**Figure 2.2A**). Similarly, in Run 1, Threat distracters potentiated the interference effects in accuracy relative to Neutral distracters ($t(68) = 3.03$, $p = 0.004$), Scrambled distracters ($t(68) = 1.74$, $p = 0.09$), and no distracters ($t(68) = 3.73$, $p < 0.0001$). In contrast, in Run 2, the interference effects in accuracy elicited by Threat distracters appeared to be lower than those elicited by Neutral distracters ($t(68) = -1.78$, $p = 0.080$) or Scrambled distracters ($t(68) = -3.24$, $p = 0.002$), and again comparable to the interference effects observed in the no-distracter condition (**Figure 2.2B**).

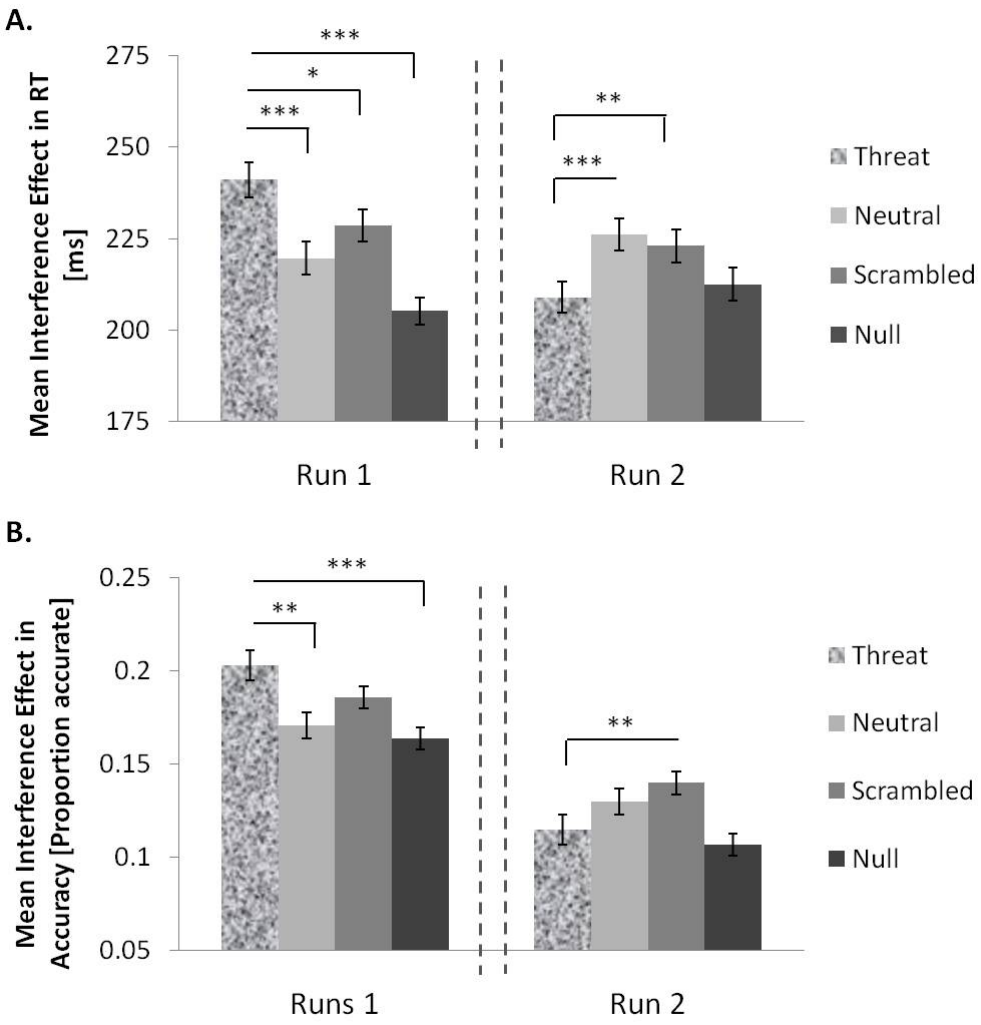

Figure 2.2 The effects of different distracter types on MSIT interference effects over time. Threat distracters potentiated interference effects in RTs (**A**) and in accuracy (**B**) in Run 1 but these effects were abolished in Run 2. Error bars show standard errors of the mean. The dashed lines denote an intermission. Significant two-tailed t-tests: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.0001$.

In sum, threat distracters produced a greater increase in the interference effect than neutral or scrambled distracters in all subjects, but the effects of threat distracters were the most robust in the first half of the experiment and diminished in the second half. As a result, we focused exclusively on the first half of the experiment (Run 1) when testing for the 5-HTTLPR genotype effects on the susceptibility to response interference in the presence of different distracter types.

5-HTTLPR genotype modulates response interference irrespective of emotional salience

Once we verified that the task successfully engaged the processes of interest, we could test the two hypotheses regarding the impact of the 5-HTTLPR genotype on response interference. The 5-HTTLPR could either modulate response interference produced specifically by threat distracters, in which case would see genotype effects in the Threat-distracter condition, but *not* in Neutral-, Scrambled-, or Null-distracter conditions (Hypothesis 1). Alternatively, the 5-HTTLPR could modulate response interference irrespective of the emotional salience of distracters, in which case we would see genotype effects in *all* distracter conditions (Hypothesis 2).

We examined whether the 5-HTTLPR genotypes modulated the susceptibility to response interference as a function of distracter type using a between-subject ANOVA (3 genotype groups: L/L, L/S, and S/S). We found a non-significant trend of main effects of the 5-HTTLPR genotype on interference effects in accuracy in the Threat, Neutral, and Null distracter conditions in Run 1 (Threat: $F(2, 68) = 2.07$, $p = 0.13$; Neutral: $F(2, 68) = 2.25$, $p = 0.11$; Null: $F(2, 68) = 2.28$, $p = 0.11$; Scrambled: ns) (**Figure 2.3**). Compared to the L allele carriers, the S/S homozygotes showed greater interference effects in accuracy irrespective of the distracter condition (Threat: $t(67) = 2.02$, $p = 0.023$; Neutral: $t(67) = 2.10$, $p = 0.020$; Scrambled: $t(67) = 1.72$, $p = 0.045$; Null: $t(67) = 2.07$, $p = 0.021$; all one-tailed *t*-tests), while the L/L and L/S genotype groups did not differ (**Figure 2.3**). The genotype effects remained significant or marginally significant with the inclusion of the rs25531 SNP (L_A alleles carriers vs. S or L_G homozygotes: Threat: $t(67) = 1.57$, $p = 0.061$; Neutral: $t(67) = 1.81$, $p = 0.038$; Scrambled: $t(67) = 1.65$, $p = 0.052$; Null: $t(67) = 1.72$, $p = 0.045$; all one-tailed *t*-tests). In contrast, the genotype groups did not differ in the interference effects in RTs in any distracter conditions.

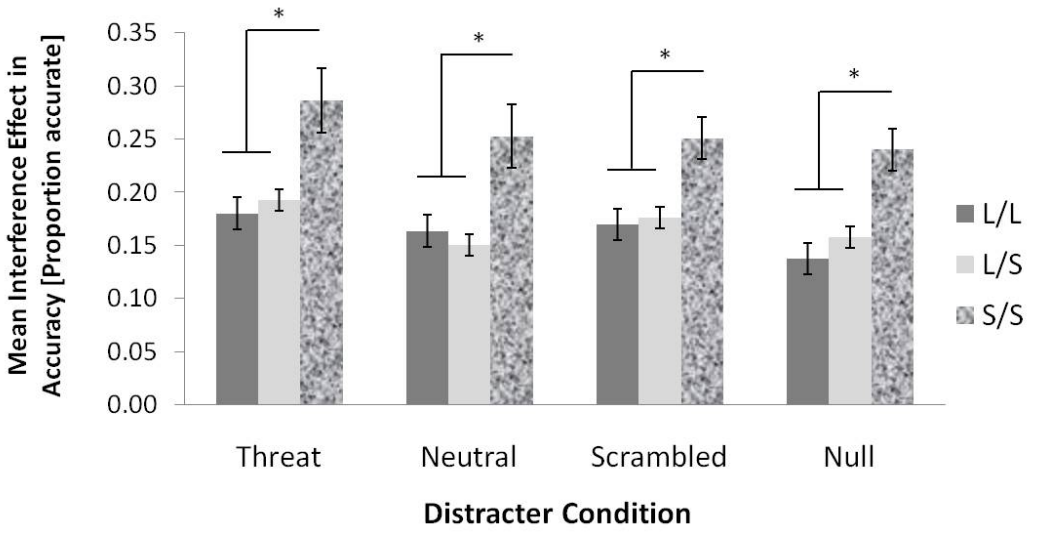

Figure 2.3 The 5-HTTLPR genotype modulates interference effects in accuracy. The S/S homozygotes showed greater interference effects in accuracy irrespective of emotional salience of task-irrelevant stimuli, compared to the L allele carriers. Error bars show standard errors of the mean. Significant one-tailed *t*-tests: * $p < 0.05$.

2.5 Discussion

Previous studies provided compelling evidence that the 5-HTTLPR genotype modulates emotional and stress reactivity, possibly by altering the reactivity and functional connectivity in the amygdala-prefrontal cortex circuitry (Caspi et al., 2010). Some studies also showed that the 5-HTTLPR modulation extends to cognitive processes, but the results have been inconsistent. One source of this variability may be the presence or absence of emotionally salient stimuli in the experimental protocol.

In the current study, we employed the Multiple-Source Interference Task (MSIT) modified to include emotionally salient and neutral distracters in order to test two alternative hypotheses regarding the impact of the 5-HTTLPR on task performance. The 5-HTTLPR could affect task performance by modulating response interference elicited

specifically by emotionally salient stimuli (Hypothesis 1). Alternatively, the 5-HTTLPR could modulate response interference irrespective of the emotional salience of the stimuli (Hypothesis 2).

Our data support Hypothesis 2: the 5-HTTLPR modulation of response interference was not specific to threat distracters but extended to neutral and scrambled distracters and was also observed in the no-distracter condition. Across all four distracter conditions, the S/S homozygotes showed greater interference effects in accuracy (i.e., a greater impairment in task performance) compared to the L allele carriers. This pattern of results suggests that the S/S homozygotes are more susceptible to response interference from task-irrelevant stimuli, irrespective of the emotional salience of these stimuli. These results are particularly intriguing in light of the robust (if transient) potentiation of interference effects by threat distracters observed in all subjects, collapsing across genotypes.

Our results are consistent with the view that, rather than modulating specifically the impact of adverse stimuli, the 5-HTTLPR genotype may affect susceptibility to environmental influences in general (Uher, 2008; Belsky and Pluess, 2009), a trait described as *hypervigilance* (Homberg and Lesch, 2010). Thus, the S allele is associated with worse behavioral and clinical outcomes in the context of adverse environmental conditions, such as childhood maltreatment or stressful life events, but it can also lead to more favorable outcomes in protective, nurturing environments, relative to the L allele (Caspi et al., 2003; Eley et al., 2004; Taylor et al., 2006).

Our results also have clinical implications. Increased susceptibility to environmental stimuli, and to response interference these stimuli may elicit, is a feature of several psychiatric disorders which are also associated with alterations in the

serotonin system, including affective disorders and substance abuse. In our experiment, the S allele (the hypothesized “risk” variant) was associated with a greater susceptibility to response interference from both threat and neutral task-irrelevant stimuli, resulting in greater interference effects in accuracy and impaired task performance, relative to the L allele. Thus, our findings suggest that genetic risk variants in the serotonin system may contribute to the risk of mental disorders by imparting a greater susceptibility to response interference produced by external stimuli.

CHAPTER 3

AMYGDALA RESPONSE TO SMOKING-CESSATION MESSAGES MEDIATES SEROTONIN TRANSPORTER GENE EFFECTS ON SUBSEQUENT SMOKING CESSATION

3.1 Goals

In this study (Study 2), we employed an imaging genetics approach and a computer-tailored smoking-cessation intervention to determine whether serotonin transporter (5-HTT) gene variation (5-HTTLPR/rs25531 and STin2) modulates activity and functional connectivity within the amygdala–PFC circuit during the processing of smoking-cessation messages, and whether this genetic modulation of neural processing in turn affects relevant goal-directed behavior, i.e., post-intervention quitting outcome. The analyses presented in this chapter focused on the neural processing of smoking-cessation messages in the amygdala as the *a priori*, anatomically defined region of interest.

3.2 Introduction

There is a vital need for more effective smoking-cessation treatments. Many smokers attempt to quit smoking but a majority relapse within 6 months (Quaak et al., 2009). While environmental factors such as stressors and smoking-related cues undoubtedly play a major role, twin data demonstrate that approximately half of the

variance in smoking-cessation outcomes may be explained by genetics (Xian et al., 2003). But specific genetic variants that affect a smoker's risk of relapse following a quit attempt have been difficult to identify, and the brain processes that mediate these genetic influences on smoking cessation are even less well understood. Such knowledge of underlying neurobiology could be used to more effectively tailor smoking-cessation interventions to individual smokers (Quaak et al., 2009).

In the current study, we employed an imaging genetics approach, which combines neuroimaging and genetics (Hariri and Weinberger, 2003; Hariri et al., 2006; Hariri, 2009) (see **Section 1.9** for details), and a computer-tailored smoking-cessation intervention to examine the effects of variation in the 5-HTT gene on brain response to smoking-cessation intervention messages and on subsequent quitting outcome.

Serotonin (5-hydroxytryptamine, or 5-HT) is a major modulatory neurotransmitter in the mammalian brain, and it is crucially involved in a range of brain processes, including stress, arousal, motor activity, appetite, and mood (Jacobs and Azmitia, 1992)—but also cognitive control, emotion regulation, and reward processing (Cools et al., 2008; Dayan and Huys, 2009; Kranz et al., 2010). Dysregulation of the brain serotonin system has been reported in a number of mental disorders, including mood and anxiety disorders (e.g., (Meltzer, 1989; Owens and Nemeroff, 1994). The serotonin transporter protein (5-HTT), responsible for reuptake of serotonin from the synapse back into the presynaptic neuron for degradation, serves as a key regulator of serotonergic signaling. Twin studies suggest that the 5-HTT function is modulated by genetic factors (Meltzer and Arora, 1988), and several polymorphisms in the 5-HTT gene have been identified, although their effects on the brain and behavior are not well understood.

We focused on two common, functional polymorphisms in the regulatory regions of the 5-HTT gene. The 5-HTT-linked polymorphic region (5-HTTLPR) is a 44-bp insertion/deletion polymorphism in the promoter region, with the short allele (S) less efficiently transcribed than the long allele (L) (Heils et al., 1996; Lesch et al., 1996). In addition, 5-HTTLPR includes an A→G single nucleotide substitution (rs25531), with the L_G allele being functionally equivalent to the S allele (Hu et al., 2006) (see **Section 1.11.2** for more details on the 5-HTTLPR and rs25531). The second functional polymorphism, serotonin transporter intron 2 (STin2), is a 17-bp insertion/deletion polymorphism in intron 2, with the 12-repeat allele more efficiently transcribed than the 10-repeat allele (Lesch et al., 1994; Fiskerstrand et al., 1999) (see **Section 1.11.1** for more details on STin2). Both S and 12 alleles have been linked to anxiety-related personality traits (Lesch et al., 1996; Sen et al., 2004; Kazantseva et al., 2008) and increased risk of affective disorders (Collier et al., 1996a; Caspi et al., 2003), suggesting that they may act as "risk" alleles that impart increased susceptibility to mental disorders.

It is plausible that these genetic variants increase risk by affecting neural function. One brain structure that has been implicated in the processing of smoking-related cues in smokers (Due et al., 2002), as well as alcohol- and cocaine-related cues in their respective users (Childress et al., 1999; Schneider et al., 2001), is the amygdala. More generally, the amygdala is critical to the rapid detection and appraisal of environmental stimuli in light of their biological significance to the organism, including both potential threat and potential reward, as well as to stimulus-outcome learning (LeDoux, 2000; Baxter and Murray, 2002) (see **Section 1.3** for a more detailed discussion of amygdala involvement in goal-directed behavior).

Growing evidence from imaging genetics also points to the amygdala as a candidate brain mediator of the 5-HTT gene effects on behavior and risk for disease. In

particular, the 5-HTTLPR/ rs22531 polymorphism has been consistently shown to modulate amygdala response to emotionally salient stimuli. In adult Caucasians, the S or L_G carrier status is associated with a greater amygdala response to threat signals compared to L_A/L_A homozygotes (5-HTTLPR only: (Hariri et al., 2002; Hariri et al., 2005; Munafo et al., 2008); 5-HTTLPR/rs25531: (Dannlowski et al., 2007; Dannlowski et al., 2010)).

In the current study, we examined the amygdala response to smoking-cessation messages, and the impact of 5-HTT gene variation on this response, in smokers trying to quit. We hypothesized that the STin2 and 5-HTTLPR/ rs25531 polymorphisms in the 5-HTT gene would modulate amygdala response to smoking-cessation messages in smokers trying to quit smoking. We further hypothesized that amygdala response to smoking-cessation messages would act as a brain mediator of serotonin transporter gene effects on smoking cessation.

3.3 Methods

3.3.1 Subjects

We tested our hypotheses in a sample of 91 heavy smokers interested in quitting. Smokers were eligible to participate if they smoked a minimum of 10 cigarettes per day and at least 100 cigarettes in their lifetime. Subjects were not enrolled in other smoking-cessation programs or taking pharmacological treatments for smoking cessation during study enrollment. All subjects were native English speakers, had normal vision and hearing, and had no history of head injury or mental illness. The study protocol was approved by the University of Michigan Medical School IRB and all subjects provided written informed consent. We present the results from the final sample

of 84 participants (mean age 37.5 ± 11.5 years; 40 females, 44 males; 65 (77%) Caucasian) for whom genotyping, fMRI, and outcome data were available.

3.3.2 Study design

The study involved 3 sessions plus a follow-up phone interview. In Session 1, subjects completed a baseline assessment of their smoking history and other health, demographic, and psychosocial characteristics relevant to smoking cessation. The responses were used to create tailored smoking-cessation messages for the subsequent intervention. In Session 2, subjects completed a Messages Task during functional MRI (fMRI). In Session 3, scheduled within one week from their fMRI session, subjects completed a web-based computer-tailored smoking-cessation intervention (Strecher et al., 2008) and started their quit attempt. All subjects received a 10-week supply of nicotine patches (6 weeks of 21-mg, 2 weeks of 14-mg, and 2 weeks of 7-mg patches), as recommended by the manufacturer. All subjects also donated saliva for DNA extraction and genotyping. Four months after the intervention session, subjects were interviewed on the phone to determine their smoking-cessation status. The primary outcome measure was 7-day point-prevalence abstinence ("Did you smoke a cigarette, even a puff, in the past 7 days?") (Velicer and Prochaska, 2004).

3.3.3 Computer-tailored smoking-cessation intervention

All subjects completed a computer-tailored web-based smoking-cessation intervention developed at the University of Michigan's Center for Health Communications Research (UM-CHCR) (*Project Quit*) (Strecher et al., 2008). Computer-tailored interventions employ data-matching algorithms to tailor the communication content to each individual user based on a baseline assessment (Krebs et al., 2010). The content of the intervention was based on cognitive-behavioral methods of smoking cessation and

relapse prevention, including motivational, instructional, personalization, and feedback content tailored to each individual smoker. “Tailoring” refers both to (1) the assessment of individual characteristics relevant to the desired health-behavior outcome and (2) the algorithms that use the assessment data to generate intervention messages specific to each user. The tailored smoking-cessation content included reinforcement of motives for quitting, self-efficacy enhancement, and advice on how to cope with tempting situations and emotions.

3.3.4 Messages Task

The Messages Task was used to simulate a simple version of a message-based smoking-cessation intervention in the scanner, and it involved viewing and listening to smoking-cessation messages and neutral control messages presented in a blocked design. Subjects were instructed to pay attention to the messages but no response was required. Four broad types of Smoking-Cessation messages were used: Personalization/Feedback, Motivation, and Instruction messages were tailored to individual smokers, while Smoking-Related Health Information messages were relevant to all smokers. The Personalization/Feedback, Motivation, and Instruction messages varied between subjects; but all subjects received the same Smoking-Related Health Information messages and the same Neutral messages. All subjects completed 5 runs of the Messages Task, with 2 blocks of each message type per run, 5 messages per block, for a total of 250 messages presented. Blocks were separated by fixations lasting between 4 and 10 seconds (an average of 7 seconds). Runs also started and ended with a 10-second fixation. We assessed the neural response to smoking-cessation messages compared to neutral messages using the blood-oxygenation level dependent (BOLD) signal and the *Smoking-Cessation Messages > Neutral Messages* contrast.

The following are examples of messages used. Personalization/Feedback: *You want to quit because you are tired of spending your money on cigarettes; You feel like your friend will help you stay on track once you quit; You have a very strong urge to smoke when you first wake*. Motivation: *Quitting smoking will help you gain more control over your life; The sooner you quit, the less damage you'll do to your skin*; *Children brought up in a non-smoking home are less likely to become smokers themselves.* Instruction: *Don't have meals with friends who smoke around meal time, until you feel secure that you won't smoke; If you think something will make you feel too anxious, don't do it; Before, during, and after you go out to social events, remind yourself that you are a nonsmoker.* Smoking-Related Health Information: *Many people quit with another person so they can support each other; Many people relapse due to stress, alcohol, and cravings; Most people need to try more than once to quit smoking for good.* Neutral: *The longest duration of a solar eclipse was 7 minutes, 31 seconds; Bali attracts more tourists than any other Indonesian island; Global warming caused the recent collapse of an Antarctic ice shelf.*

3.3.5 Genotyping procedures

Genomic DNA was obtained from saliva samples using Oragene collection system and extracted using the protocol provided (Genotek, Ontario, Canada). STin2 and 5-HTTLPR/ rs25531 polymorphisms were genotyped using polymerase chain reaction (PCR) and oligonucleotide primers. 5-HTTLPR was genotyped using primers from Yonan et al. (Yonan et al., 2006). PCR products were analyzed on 1.5% TBE agarose gels (expected band sizes: S – 415bp, L – 459bp). To additionally genotype the A-G SNP (rs25531), product was digested with MspI. Digest products were resolved on 3% TBE agarose gels (expected band sizes: L_A – 331bp, 66 bp, and 62 bp; L_G –157 bp, 174 bp, 66 bp, and 62 bp; S – 287 bp, 66 bp, and 62 bp). STin2 was genotyped using

primers from Kaiser et al. (Kaiser et al., 2001). PCR products were resolved for size on 1.5% agarose gels. Gels were visualized using ethidium bromide under UV light and reviewed by two independent people, with 100% concordance. Six of the samples were verified by Sanger sequencing, with 100% concordance.

3.3.6 Image acquisition

Scanning was performed on a 3T GE Signa Excite 2 scanner (Milwaukee, Wisconsin), beginning with a structural T1-overlay image (repetition time [TR] = 250 ms, echo time [TE] = 7 ms, flip angle [FA] = 75 degree, field of view [FOV] = 220 mm, 43 oblique axial slices, 256 x 256, slice thickness 3.0 mm). Functional scans were collected using a T2*-weighted spiral-in acquisition sequence (gradient echo, TR = 2000 ms, TE = 30 ms, FA = 90^{o}, FOV = 220 mm, 64 x 64, slice thickness 3.0 mm) (Noll et al., 1998). High-resolution T1 scans were also obtained for precise anatomical localization (3D spoiled-gradient echo [3D-SPGR] with inversion recovery prep, time of inversion = 400 ms, TR = 9.0 ms, TE = 1.8 ms, FA = 15^{o}, FOV = 260 mm, 128 slices, 256 x 256, 1.2 mm slice).

3.3.7 Image preprocessing

All functional scans were slice-time-corrected, motion-corrected, and realigned to the first scan using the MCFLIRT program (FSL Analysis Group, FMRIB, Oxford, UK). Subsequent processing was done using SPM (Wellcome Institute of Cognitive Neurology, London, UK). The T1-overlay was co-registered with a functional scan. The high-resolution 3D-SPGR image was co-registered to the T1-overlay and anatomically normalized to the Montreal Neurological Institute (MNI) 152 template. The resulting transformation parameters were then applied to the co-registered functional volumes. All

functional volumes were smoothed with a Gaussian kernel with a full width at half maximum (FWHM) of 7 mm (5 mm at the first level, 5 mm at the second level).

3.3.8 Data analyses

After pre-processing, the functional data were analyzed using a modified General Linear Model (GLM) and a blocked design. Regressors of interests were convolved with a canonical hemodynamic response function (HRF) with a time derivative to account for between-subject and between-voxel variability in the response peak. Movement parameters were included as covariates.

Statistical analyses were conducted in a series of steps using a random-effects model. First, anatomically defined ROI masks of right and left amygdalae were constructed using WFU PickAtlas (Maldjian et al., 2003) (right amygdala: 87 voxels; left amygdala: 75 voxels). For each individual subject, we extracted the average parameter estimates (betas) for *Smoking-Cessation Messages – Neutral Messages* contrast for right and left amygdala ROIs. These individual parameter estimates, together with genotyping and outcome data, were then entered into second-level group analyses in SPSS 17.0. The following measures were included as covariates in all analyses: race (Caucasian or not), gender, cigarettes smoked per day prior to the intervention, age when started smoking, length of use of nicotine patch following the intervention, motivation to quit, and confidence in quitting. We used a statistical significance threshold of $p < 0.05$ throughout.

3.3.9 Mediation analyses

To test for mediation effects in our data, we adopted the theoretical framework outlined by Baron and Kenny (Baron and Kenny, 1986). A mediation relationship is illustrated by a three-variable model with three causal pathways: Path a represents the

effect of the predictor on the mediator; Path b represents the effect of the mediator on the outcome; and Path c represents the total effect of the predictor on the outcome. For mediation to occur, Paths a, b, and c must first each be significant. In addition, Path c' denotes the direct effect of the predictor on the outcome, controlling for the effect of the mediator. Evidence for mediation is obtained if we can reject the null hypothesis of no difference between the total effect (c) and the direct effect (c'), that is, $c - c' \neq 0$, demonstrating that the predictor affects the outcome at least in part through the mediator.

The mediation analyses were conducted on the extracted average parameter estimates for the *Smoking-Cessation Messages – Neutral Messages* contrast from the amygdala ROIs for all individual subjects using the Sobel test of mediation and non-parametric bootstrapping approach (Preacher and Hayes, 2008). Bootstrapping is a non-parametric method of estimating effect sizes and hypothesis testing, and it involves sampling with replacement to test the null hypothesis of no mediation effect in a large number of samples taken from the data.

3.4 Results

3.4.1 Smoking-cessation outcome

Smoking-cessation outcome was assessed 4 months after the intervention using 7-day point-prevalence abstinence measure (Velicer and Prochaska, 2004). Forty-five subjects were abstinent (and were classified as Quitters) and thirty-nine were smoking (and were classified as Non-Quitters). The two outcome groups did not differ in any pre-intervention demographic or smoking-related measures, including motivation to quit and confidence in quitting, except for a higher initial number of cigarettes smoked per day in Non-Quitters compared to Quitters ($p < 0.05$). Quitters and Non-Quitters also did not

differ in nicotine patch use following the intervention. These data are summarized in **Table 3.1**.

Table 3.1 Summary of demographic and smoking-related characteristics of Quitter and Non-Quitter groups.

	Quitters (n = 45)	**Non-Quitters (n = 39)**	**P-value***
Age (years)	36.4 ± 11.4	38.4 ± 12.0	0.446
Gender (females); N	18 (40%)	22 (56%)	0.133
Race (Caucasian); N	35 (77%)	30 (76%)	0.926
Age when Started Smoking (years)	17.8 ± 6.0	17.9 ± 5.1	0.949
Cigarettes Smoked Per Day	15.6 ± 5.3	18.4 ± 6.2	**0.031**
Pack Years	15.1 ± 11.9	19.7 ± 13.9	0.104
Previous Attempts to Quit	1.4 ± 0.9	1.5 ± 1.0	0.368
Motivation to Quit (a 10-point scale)	8.9 ± 1.2	9.1 ± 1.1	0.446
Confidence in Quitting (a 10-point scale)	8.2 ± 1.73	8.3 ± 1.7	0.791
Post-intervention Nicotine Patch Use (days)	54.2 ± 20.9	46.9 ± 28.5	0.186

* Chi-square or two-tailed independent-sample t-tests were performed where appropriate. Significant tests are shown in bold. Group means and standard deviations are given (mean ± SD).

3.4.2 Genotyping results

We observed the following counts (and frequencies) of STin2 genotypes: thirteen (0.15) subjects were 10/10 homozygotes, thirty-eight (0.45) were 10/12 heterozygotes, and thirty-three (0.39) were 12/12 homozygotes. The observed 5-HTTLPR/ rs25531 genotypes counts (and frequencies) were functionally grouped as follows: twenty-five

(0.30) subjects were L_A/L_A homozygotes, forty-one (0.49) were L_A/L_GS heterozygotes (i.e., L_A/S or L_A/L_G), and eighteen (0.21) were L_GS/ L_GS homozygotes (i.e., S/S, L_G/S or L_G/L_G). The allele and genotype distribution in Quitter and Non-Quitter groups is given in **Table 3.2**. No deviations from the Hardy-Weinberg Equilibrium were noted.

Table 3.2 Distribution of STin2 and 5-HTTLPR/ rs25531 alleles and genotypes.

	STin2 allele Count (Frequency)			**STin2 genotype Count (Frequency)**					
	10	12		10/10	10/12	12/12			
Total	64 (0.38)	104 (0.62)		13 (0.15)	38 (0.45)	33 (0.39)			
Quitters	40 (0.44)	50 (0.56)		8 (0.18)	24 (0.53)	13 (0.29)			
Non-Quitters	24 (0.31)	54 (0.69)		5 (0.13)	14 (0.36)	20 (0.51)			
	5-HTTLPR/rs25531 allele Count (Frequency)			**5-HTTLPR/rs25531 genotype** Count (Frequency)					
	L_A	L_G	S	L_A/L_A	L_A/L_G	L_A/S	L_G/L_G	L_G/S	S/S
Total	91 (0.54)	8 (0.05)	69 (0.41)	25 (0.30)	4 (0.05)	37 (0.44)	1 (0.01)	2 (0.02)	15 (0.18)
Quitters	53 (0.59)	3 (0.03)	34 (0.38)	15 (0.33)	2 (0.04)	21 (0.47)	0	1 (0.02)	6 (0.13)
Non-Quitters	38 (0.49)	5 (0.06)	35 (0.45)	10 (0.26)	2 (0.05)	16 (0.41)	1 (0.03)	1 (0.03)	9 (0.23)

Consistent with previous reports (Gelernter et al., 1999; Kazantseva et al., 2008) (see **Section 1.11.3** for more details), the STin2 and 5-HTTLPR/rs25531 loci were in linkage disequilibrium ($\chi^2 = 31.13$, $p < 0.0001$) (**Table 3.3**). Based on a previous report suggesting a relatively larger impact of STin2 polymorphism on personality traits (Kazantseva et al., 2008), we focused on testing STin2 effects. We present the results both with and without the 5-HTTLPR/rs25531 genotype as a covariate.

Table 3.3 Linkage disequilibrium between the STin2 and 5-HTTLPR/ rs25531 loci.

		5-HTTLPR/rs25531 Number of S or L_G Alleles			Total
		0	**1**	**2**	
STin2 Number of 12 Alleles	**0**	11	2	0	13
	1	7	26	5	38
	2	7	13	13	33
Total		25	41	18	84

3.4.3 Mediation results

STin2 genotype predicts bilateral amygdala response to smoking-cessation messages (Path a)

We first asked whether the STin2 genotype (10/10, 10/12, 12/12) modulated amygdala response to smoking-cessation messages, using the anatomically defined regions of interest (ROI) in right and left amygdala (**Figure 3.1A**). The STin2 genotype (number of 10 alleles) was a significant predictor of the neural response to smoking-cessation messages vs. neutral messages in bilateral amygdala (linear regression β = 0.44, *SE* = 0.16, p = 0.009, Path a in **Figure 3.1B**), demonstrating that Path a (from genes to the brain) was significant in our model. Specifically, the number of 10 alleles was positively correlated with amygdala response magnitude (r = 0.40): 10/10 > 10/12 > 12/12. The STin2 genotype remained a predictor of amygdala response when controlling for the 5-HTTLPR/rs22531 genotype (linear regression β = 0.40, *SE* = 0.18, p = 0.032). In contrast, neither the 5-HTTLPR/rs25531 genotype, nor any of the other covariates, predicted amygdala response to smoking-cessation messages.

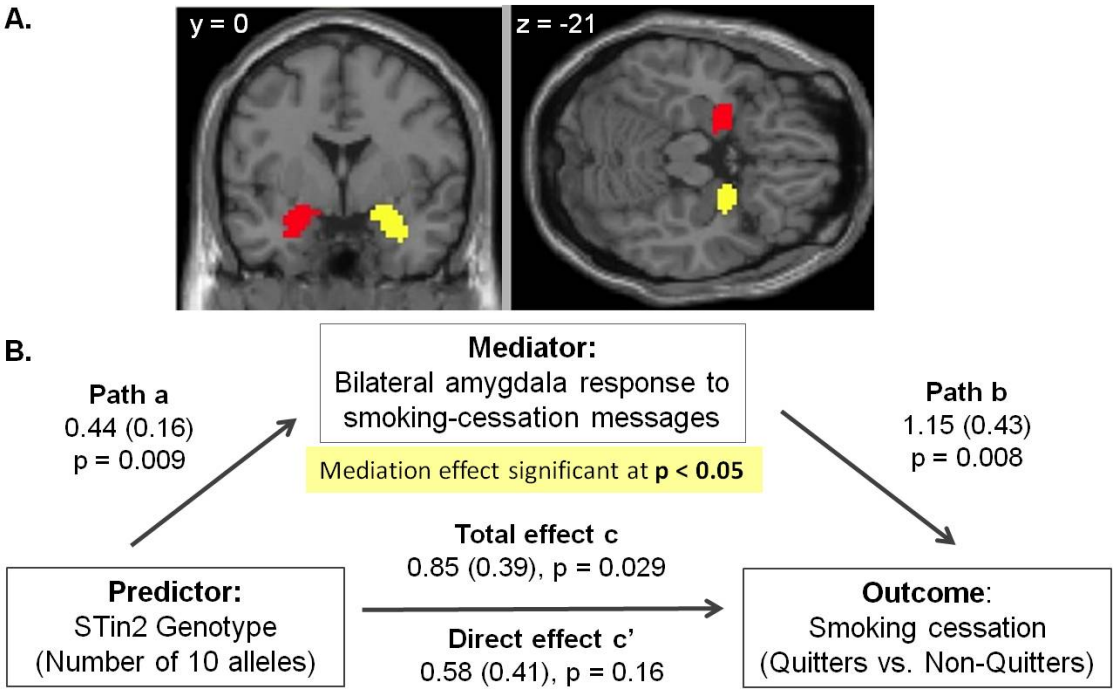

Figure 3.1 Bilateral amygdala response to smoking-cessation messages vs. neutral messages mediates the effects of STin2 genotype on subsequent smoking cessation. **A**. Anatomically defined ROI in right and left amygdala shown against MNI 152 template. **B**. Path diagram of the mediation results. Regression coefficients, standard errors (in parentheses), and *p* values are given. A greater number of STin2 10 alleles is associated with a relatively greater magnitude of amygdala response, and this greater amygdala response is in turn associated with better odds of quitting success. Path b denotes the association of bilateral amygdala response with smoking-cessation outcome, controlling for the effects of STin2 genotype. Direct effect c' denotes the effect of STin2 genotype on smoking-cessation outcome, controlling for the mediation effect in bilateral amygdala.

Amygdala response to smoking-cessation messages predicts quitting (Path b)

Next, we asked whether bilateral amygdala response to smoking-cessation messages vs. neutral messages predicted subsequent real-life quitting—i.e., whether amygdala response differentiated Quitters from Non-Quitters, irrespective of genotype. We found that bilateral amygdala response to smoking-cessation messages was a robust predictor of quitting outcome when controlling for STin2 genotype (logistic

regression β = 1.15, *SE* = 0.43, p = 0.008; Path b in **Figure 3.1B**) and for both STin2 and 5-HTTLPR/ rs22531 genotypes (logistic regression β = 1.17, *SE* = 0.44, p = 0.008). Specifically, a greater magnitude of amygdala response to smoking-cessation messages was associated with better odds of quitting success. This result demonstrated that Path b (from the brain to behavior) was significant in our model. In both cases, the model correctly predicted 80.0% of Quitters, for the overall prediction accuracy of 74.4%. The only other measure that also predicted quitting was the number of cigarettes smoked per day prior to the intervention (logistic regression β = -0.11, *SE* = 0.05, p = 0.040), consistent with previous literature.

STin2 genotype predicts quitting (Path c)

Next, we tested whether the STin2 genotype (10/10, 10/12, 12/12) predicted quitting outcome. We found that the STin2 genotype (number of 10 alleles) was a significant predictor of quitting (logistic regression β = 0.85, *SE* = 0.39, p = 0.029; Total effect c in **Figure 3.1**), consistent with an additive model of allele action. Specifically, a higher number of STin2 10 alleles was associated with better odds of quitting success. The model that included the STin2 genotype and other covariates (but not amygdala response) correctly predicted 75.6% of Quitters, for the overall accuracy of 68.3%. The STin2 genotype remained a marginally significant predictor of quitting when controlling for the 5-HTTLPR/rs25531 genotype (logistic regression β = 0.81, *SE* = 0.42, p = 0.057). In contrast, the 5-HTTLPR/rs25531 genotype was not a predictor of quitting in our data, consistent with previous reports (Munafo et al., 2006; David et al., 2008).

Amygdala response to smoking-cessation messages mediates STin2 effects on quitting

In the final step of our analyses, we tested whether bilateral amygdala response to smoking-cessation messages mediated the impact of STin2 genotype on smoking-cessation outcome following the intervention. The STin2 genotype (i.e., the number of 10 alleles) was the predictor variable, the extracted cluster-averaged parameter estimate (betas) for the bilateral amygdala was the mediator variable, and the smoking status at the 4-month follow-up (Quitter vs. Non-Quitter) was the outcome variable.

Consistent with our main hypothesis, bilateral amygdala response to smoking-cessation messages vs. neutral messages mediated STin2 effects on subsequent smoking-cessation outcome (bootstrap results: mean mediation effect value = 0.65, *SE* = 0.44, 95% CI: 0.05 – 1.51; $p < 0.05$) (Path diagram in **Figure 3.1B**). Because the confidence interval for the mediation effect (c – c') did not include zero when using the bootstrapping test of mediation effect, we could reject the null hypothesis of no difference between the total effect (c) and the direct effect (c') in favor of the alternative hypothesis of mediation. Controlling for mediation effect in the bilateral amygdala reduced the association between the STin2 genotype and smoking cessation (total effect c: logistic regression $\beta = 0.85$, *SE* = 0.39, $p = 0.029$; direct effect c': logistic regression $\beta = 0.58$, *SE* = 0.41, $p = 0.16$). The mediation effect for the STin2 genotype remained significant when the 5-HTTLPR/rs25531 genotype was added as a covariate (bootstrap results: mean mediation effect value = 0.60, *SE* = 0.39, 95% CI: 0.01 – 1.25; $p < 0.05$), with the total effect of the STin2 genotype on quitting outcome still marginally significant (total effect c: logistic regression $\beta = 0.81$, *SE* = 0.42, $p = 0.057$).

3.5 Discussion

Tailored smoking-cessation interventions show promise in helping smokers quit (Strecher et al., 2008; Krebs et al., 2010). However, the efficacy of these traditional tailoring techniques (i.e., based on demographic, psychosocial, and smoking-related

self-report measures) could be improved by accounting for the neurobiological factors that affect an individual smoker's quitting outcome. In particular, optimal intervention tailoring could incorporate knowledge of genetic basis of susceptibility to relapse after a smoking-cessation intervention as well as of the brain mechanisms mediating these genetic influences on smoking behavior (Quaak et al., 2009).

In the current study, we focused on the amygdala as a potential brain mediator of the 5-HTT gene effects on real-life quitting outcome following an intervention. The amygdala is critically involved in the detection and appraisal of salient environmental stimuli, both aversive and appetitive, as well as in stimulus-outcome learning (LeDoux, 2000; Baxter and Murray, 2002). The amygdala has also been previously implicated in the processing of smoking-related cues in smokers (Due et al., 2002), as well as alcohol- and cocaine-related cues in their respective users (Childress et al., 1999; Schneider et al., 2001).

In a recent fMRI study (Janes et al., 2010), smokers who subsequently slipped in their quit attempt showed greater pre-quit amygdala reactivity to smoking-related cues than those who stayed abstinent. In an interesting contrast, our data suggest that a greater pre-quit amygdala response to smoking-cessation messages is predictive of *better* odds of quitting success. We propose that this apparent discrepancy may be explained by a fundamental difference between smoking-related cues (intended to *trigger* smoking behavior) and smoking-cessation messages (intended to *inhibit* such behavior). We further speculate that, while the amygdala engagement by smoking-related cues may trigger the over-learned, incentive-sensitized stimulus-response pathways underlying compulsive drug-seeking—the amygdala engagement by smoking-cessation communications may serve an opposite function of conveying motivational salience to the prefrontal regions involved in representing prospective goals and in

inhibiting prepotent, stimulus-driven behavior. Future studies, including those planned and currently conducted by our group, will help explain the role of the amygdala in both promoting and overcoming addictive behaviors such as smoking.

The serotonin system has been another prominent focus of research on neurobiology of addiction and related behaviors. In the current study, we examined two functional polymorphisms in the 5-HTT gene (STin2 and 5-HTTLPR/rs25531). Because the two loci were in linkage disequilibrium, we focused on STin2, which may have a relatively larger impact on behavior (Kazantseva et al., 2008), while including the 5-HTTLPR/rs25531 genotype as a covariate in all analyses.

Previous imaging genetics research (Hariri et al., 2002; Canli et al., 2005; Hariri et al., 2005; Heinz et al., 2005; Smolka et al., 2007) demonstrated that the 5-HTTLPR genotype modulates amygdala response to threat stimuli relative to neutral stimuli, with the S allele associated with a greater amygdala response, a finding confirmed by a meta-analysis (Munafo et al., 2008). In the current study, we report that the STin2 genotype, another functional polymorphism in the 5-HTT gene, is a robust predictor of bilateral amygdala response to smoking-cessation messages, with a greater number of 10 alleles associated with a relatively greater amygdala response.

To our knowledge, this is the first report of STin2 effects on brain function. Of note, the direction of the STin2 effects on amygdala function in our study is consistent with that previously reported for the 5-HTTLPR in the context of emotion processing: the high-transcription alleles (12 allele, L_A allele) are associated with a lower amygdala response than the low-transcription alleles (10 allele, S or L_G allele). However, our results should be considered preliminary and interpreted with caution until replicated. Future studies could also directly compare the effects of both polymorphisms on

amygdala response to different classes of salient stimuli, including smoking-related cues and smoking-cessation messages. Finally, if the STin2 12 allele is more akin to the 5-HTTLPR L allele than to the "risk" S allele in terms of transcription efficiency, frequency in Caucasians (~60%), and, as our results suggest, amygdala reactivity—it is also puzzling why the 12 allele and the S allele appear to have comparable impact on behavior and risk for disease? Here one possibility is that the two polymorphisms may differ in gene-environment interactions. It is also possible that another, unmeasured variant in linkage disequilibrium with the STin2 and 5-HTTLPR loci is responsible for the observed effects.

Consistent with our main hypothesis, our results also suggest that bilateral amygdala response to smoking-cessation messages is a partial mediator of the impact of STin2 genotype on subsequent real-life quitting outcome following a tailored smoking-cessation intervention. Specifically, a higher number of STin2 12 alleles (or, conversely, a lower number of STin2 10 alleles) was predictive of a lower bilateral amygdala response to smoking-cessation messages, and this lower magnitude of amygdala response was in turn predictive of a higher risk for relapse to smoking following the intervention.

Finally, we illustrate the use of mediation analysis in imaging genetics research to explicitly link genes, brain, and behavior, as a first step towards explaining and predicting individual differences in complex behavioral traits and susceptibility to mental disorders (Hariri, 2009). Importantly, one prior study (Fakra et al., 2009) demonstrated that a promoter polymorphism in another serotonergic gene (HTR1A), coding for the 5-HT1A autoreceptor, has a significant indirect effect on trait anxiety by biasing amygdala reactivity to threat stimuli in healthy individuals. While a direct effect of this polymorphism on trait anxiety (Path c in a mediation model) was not detected, Fakra et

al. (2009) provided crucial evidence that common genetic polymorphisms in the serotonin system may affect complex behavioral traits by modulating amygdala response to salient environmental stimuli. Our current study extends the findings to a clinical context by showing that amygdala response to smoking-cessation intervention mediates the effects of STin2 genotype on subsequent smoking-cessation outcome in individuals with nicotine dependence.

To conclude, in the current study, by linking sequence variation in the serotonin transporter gene (predictor) with bilateral amygdala response to smoking-cessation messages (mediator) and a real-life post-intervention quitting success (outcome), we have identified a gene-brain-behavior pathway relevant to smoking cessation. These results may be relevant to the design and selection of smoking-cessation interventions, and point to the possibility of intervention tailoring based on genetic and neural-response profiles on individual smokers for optimal intervention efficacy.

CHAPTER 4

GENETIC MODULATION OF FUNCTIONAL CONNECTIVITY IN THE AMYGDALA–VMPFC CIRCUIT DURING PROCESSING OF SMOKING-CESSATION MESSAGES

4.1 Goals

The overall goal of Study 2 was to examine the impact of 5-HTT gene variation on amygdala–PFC circuit function during the processing of smoking-cessation messages, and the significance of this genetic modulation of neural function for goal-directed behavior, i.e., post-intervention quitting outcome. In Chapter 3, we reported that the neural responses to smoking-cessation communications in one component of the amygdala–PFC circuit, the amygdala, mediated the impact of the intronic STin2 polymorphism on quitting. In this chapter, we tested the impact of STin2 on functional connectivity in the amygdala–VMPFC circuit when processing smoking-cessation messages, and the relevance of this impact to the post-intervention quitting outcome.

4.2 Introduction

Converging evidence from several different areas of neuroscience—including neuroimaging, human lesion studies, and animal research—suggests that the amygdala–prefrontal cortex (PFC) circuitry is critical to cognitive and affective control critical for goal-directed behavior, and a dysregulation of this circuit may underlie key aspects of psychopathology, including addictive behaviors. Knowledge of the genetic

factors that affect the function of the amygdala–PFC circuitry, and the scope of this genetic modulation, will add to our understanding of brain function in health and disease.

The amygdala has dense and reciprocal anatomical connections with the ventromedial PFC (VMPFC), which partially overlaps with the orbitofrontal cortex (OFC), as well as the dorsomedial PFC (DMPFC) and the anterior cingulate cortex (ACC) (Ochsner and Gross, 2005; Ghashghaei et al., 2007). A complex interplay between the amygdala and PFC is critical to emotional regulation and cognitive control, which in turn enable flexible, context-appropriate and goal-directed behavior (Barbas, 2000; Bechara et al., 2000a; Ghashghaei et al., 2007). Conversely, dysregulation of the amygdala–PFC circuit has been reported in a number of mental disorders, including mood and anxiety disorders (for reviews, see (Davidson et al., 2002; Ressler and Mayberg, 2007)), and it has been proposed to underlie the impaired decision-making in addiction (the Somatic Marker Theory, reviewed in (Verdejo-Garcia and Bechara, 2009)).

Growing evidence from imaging genetics suggests that serotonin transporter (5-HTT) gene variation modulates functional connectivity in the amygdala–PFC circuit during emotion processing (Heinz et al., 2005; Pezawas et al., 2005), although the direction of the association appears to depend on the specific prefrontal region involved. One study (Heinz et al., 2005) showed that functional connectivity between the amygdala and ventromedial PFC (VMPFC), specifically Brodmann Area (BA) 10, was greater in the S allele carriers compared to the L/L homozygotes when processing threat stimuli. This finding was replicated by another study (Pezawas et al., 2005), which also showed that the S allele carriers had reduced functional connectivity between the amygdala and perigenual anterior cingulate cortex (ACC), particularly rostral ACC. The association with increased functional coupling in the amygdala–PFC circuit (BA 10) was

also shown for the carriers of the 5-HTTLPR/ rs25531 L_G allele (Friedel et al., 2009), consistent with the two studies above (Heinz et al., 2005; Pezawas et al., 2005).

Recent evidence also suggests that the 5-HTTLPR genotype modulates functional connectivity in the amygdala–PFC circuit (in this case, the subgenual and supragenual ACC) during economic decision-making, and contributes to individual differences in decision-making biases triggered by contextual cues, such as framing effects (Roiser et al., 2009). In particular, the S/S homozygotes, but not the L_A/L_A group, exhibited greater amygdala response when making decisions in accord with framing effects (i.e., choosing a sure option when it is framed in terms of gains, and a gamble option when it is framed in terms of losses) as opposed to counter to the framing effects. Conversely, the L_A/L_A homozygotes, but not the S/S group, showed greater functional connectivity between the amygdala and the anterior cingulate cortex (ACC) when making decisions counter to framing effects compared (Roiser et al., 2009).

In the context of message-based health-behavior interventions, the MPFC has been shown to respond to tailored (Chua et al., 2009) or persuasive health messages (Falk et al., 2009). In addition, the neural responses to health communications in the ventral MPFC (VMPFC) (Falk et al., 2010b; Falk et al., 2010a) and dorsal MPFC (DMPFC) (Chua et al., in press) have been shown to predict subsequent health-behavior change. The specific involvement of the VMPFC (or the OFC) in computing goal values during decision-making is also supported by neuroimaging investigations of decision-making (for reviews, see (O'Doherty, 2004, 2007)), and extends to both appetitive and aversive goal values (Plassmann et al.) and to a range of different goal categories (Chib et al., 2009).

The analyses presented in this chapter were inspired by the following line of reasoning. Based on the evidence outlined above, one role of the prefrontal regions within the amygdala–PFC circuitry during the processing of tailored and persuasive health messages could be to encode a long-term goal aimed at a positive health-behavior change, such as abstaining from cigarettes. The efficiency of this goal encoding could be modulated by the strength of functional coupling between the amygdala and its prefrontal partners when processing health messages. The strength of the functional coupling within the amygdala–PFC circuit could in turn be modulated by genetic variation in the serotonin system, particularly the functional polymorphisms in the 5-HTT gene, because the 5-HTT protein serves as a key regulator of the serotonin transmission in the brain.

More specifically, we hypothesized that functional connectivity in the amygdala–PFC circuit during the processing of smoking-cessation messages would be: (1) modulated by the intronic STin2 polymorphism in the 5-HTT gene, and (2) predictive of subsequent real-life quitting outcome.

4.3 Methods

4.3.1 Subjects

Subjects were 91 heavy smokers interested in quitting who participated in Study 2, as described above (see **Section 3.3.1** for details). The results reported below are from the final sample of 84 participants (mean age 37.5 ± 11.5 years; 40 females, 44 males; 65 (77%) Caucasian) for whom genotyping, fMRI, and outcome data were available.

4.3.2 Study design

The study involved: a baseline assessment (Session 1); performance of the Messages Task during fMRI (Session 2; see **Section 3.3.4** for details and examples of messages used); saliva donation for DNA extraction and completion of a web-based computer-tailored smoking-cessation intervention, which marked the start of a quit attempt (Session 3; see **Section 3.3.3** for details); and a 4-month follow-up phone interview to determine the smoking-cessation outcome using a 7-day point-prevalence abstinence measure.

4.3.3 Genotyping of STin2 polymorphism

Genomic DNA was obtained from saliva samples using Oragene collection system and extracted using the protocol provided (Genotek, Ontario, Canada). STin2 was genotyped using polymerase chain reaction and oligonucleotide primers from Kaiser et al. (2001) (see **Section 3.3.5** for details).

4.3.4 Image acquisition and preprocessing

Details on image acquisition and preprocessing in Study 2 are given in **Section 3.3.6** (image acquisition) and **Section 3.3.7** (image preprocessing).

4.3.5 Data analyses

The preprocessed functional data were analyzed using a modified GLM and a blocked design. Regressors of interest were convolved with a canonical HRF with a time derivative to account for between-subject and between-voxel variability in the response peak. Movement parameters were included as covariates.

Statistical analyses were conducted in a series of steps using a random-effects model. At the first level, individual *Smoking-Cessation Messages – Neutral Messages*

contrast images were created. These individual contrast images were then carried to the second-level for group analysis.

4.3.6 Functional connectivity assessed with Psychophysiologic Interaction (PPI)

We assessed whether the functional connectivity between amygdala and other brain regions is altered during processing of smoking-cessation messages compared to neutral messages. We used the Psychophysiological Interaction (PPI) approach (Friston et al., 1997), including the deconvolution-reconvolution procedure (Gitelman et al., 2003).

The goal of Psychophysiological Interaction (PPI) analyses is to determine the degree to which the physiological activity in two brain regions covary as a function of the task condition which forms the psychological context (Friston et al., 1997). One region is selected *a priori* as the seed region. The activity in the *seed* region is hypothesized to modulate the activity of one or more *target* regions in a task-dependent manner (i.e., in the experimental condition A but not in the control condition B). Evidence of psychophysiological interaction is obtained if the degree to which the activity in the seed and target regions covaries is significantly different in condition A relative to condition B (Friston et al., 1997). In order to assess this interaction more accurately based on the underlying neural response of both regions, a hemodynamic deconvolution procedure is used (Gitelman et al., 2003). This procedure involves three steps: (1) the BOLD signal in the seed and target regions is *deconvolved* (i.e., separated) from the assumed hemodynamic response, in order to obtain an estimated neuronal time-course of activity in these regions; (2) a psychophysiological interaction (PPI) term is calculated by multiplying the resulting estimated neuronal time-courses in the seed and target regions; and (3) the PPI term is then *reconvolved* with a hemodynamic response function (HRF)

(Gitelman et al., 2003). The PPI results using the deconvolution step give a more accurate measure of task-dependent functional connectivity between two brain regions because a PPI term calculated based on deconvolved neuronal time-courses is more sensitive to the relative onsets of events than a PPI term calculated based on the BOLD signal.

In the current analyses, we focused on the right-lateralized amygdala–VMPFC circuit (with the right amygdala as the seed region and the right VMPFC as the target region). We focused on the VMPFC region based on prior literature suggesting that the VMPFC has the greatest anatomical connectivity with the amygdala. More specifically, the seed region was the anatomically defined ROI mask for the right amygdala (87 voxels), constructed with WFU PickAtlas (Maldjian et al., 2003) (see also **Section 3.3.8**). As before, the analyses were conducted in a series of steps using a random-effects model. The PPI term for the *Smoking-Cessation Messages – Neutral Messages* contrast was calculated for each individual subject. The individual contrast images were then carried to the second-level group for further analyses in SPM5. Because of our *a priori* focus on the right VMPFC as the hypothesized functional-connectivity partner of the right amygdala, we constructed an anatomically defined target ROI mask of right VMPFC using WFU PickAtlas (Maldjian et al., 2003). The VMPFC target ROI encompassed Brodmann Area 11 and Orbital Gyrus, both right-lateralized, for the total extent of 510 voxels (see **Figure 4.1**). The average parameter estimates (betas) for the PPI term for the *Smoking-Cessation Messages – Neutral Messages* contrast were then extracted from the VMPFC target ROI for all individual subjects. These individual PPI betas, together with genotyping and outcome data, were entered into second-level group analyses in SPSS 17.0. We tested whether the right amygdala–VMPFC circuit

connectivity was modulated by STin2 (linear regression) and whether it predicted quitting (logistic regression). We used a statistical significance threshold of $p < 0.05$ throughout.

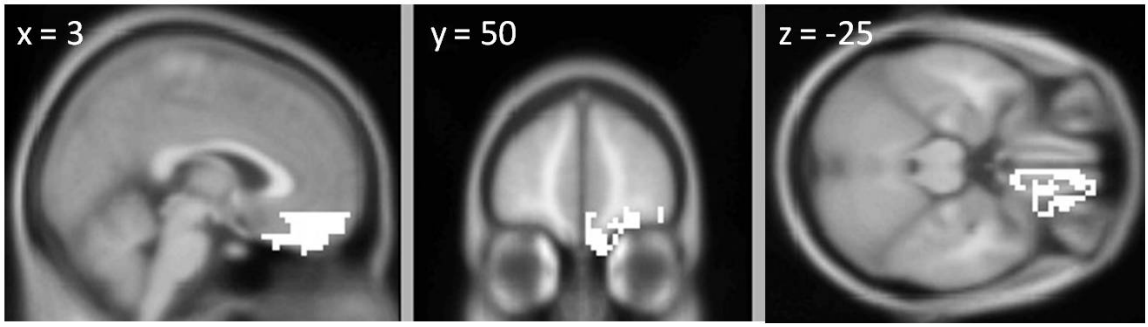

Figure 4.1 Anatomically defined right VMPFC target ROI (510 voxels) used in the functional connectivity analyses (seed ROI: right amygdala). The VMPFC target ROI encompassed Brodmann Area 11 and Orbital Gyrus, both right-lateralized.

4.4 Results

4.4.1 Smoking-cessation outcome

Using a 7-day point-prevalence abstinence measure at 4-month follow-up, forty-five subjects were abstinent (and were classified as Quitters) and thirty-nine were smoking (and were classified as Non-Quitters). For a comparison of Quitters and Non-Quitters on demographics, smoking-related measures, and post-intervention nicotine patch use, see **Section 3.4.1** and **Table 3.1**.

4.4.2 STin2 genotyping results

We observed the following counts (and frequencies) of STin2 genotypes: thirteen (0.15) subjects were 10/10 homozygotes, thirty-eight (0.45) were 10/12 heterozygotes, and thirty-three (0.39) were 12/12 homozygotes. For allele distribution, see **Table 3.2**.

4.4.3 Functional connectivity results

STin2 genotype modulates amygdala–VMPFC functional connectivity during processing of smoking-cessation messages

We first tested whether the functional connectivity between the right amygdala and right VMPFC during the processing smoking-cessation messages, as indexed by the individual PPI betas extracted from the whole anatomically defined VMPFC target ROI, was modulated by the STin2 genotype. Indeed, the STin2 genotype was a significant predictor of the amygdala–VMPFC functional connectivity in our data (linear regression $\beta = 0.06$, $SE = 0.03$, $p < 0.05$).

When directly compared with a one-tailed independent-samples *t*-test (*STin2 10 allele carriers > STin2 12/12 homozygotes*) within the larger VMPFC target ROI, the STin2 10 allele carriers showed a significant increase in amygdala–VMPFC functional coupling relative to the STin2 12/12 group in a right VMPFC cluster (MNI *x, y, z*: 9, 57, -24; cluster extent $k = 23$; $T = 3.39$; $Z = 3.26$; family-wise error (FWE) corrected $p = 0.045$, uncorrected $p = 0.001$) (**Figure 4.2A**). ANOVA conducted on the extracted PPI betas from the smaller VMPFC cluster confirmed a main effect of the STin2 genotype (10/10, 10/12, 12/12) on the functional connectivity between the amygdala and the VMPFC ($F(2,82) = 5.98$, $p = 0.004$). Specifically, the STin2 10 carriers combined, and the STin2 10/12 genotype group separately, showed an increase in amygdala-VMPFC coupling during processing of smoking-cessation messages relative to the STin2 12/12 genotype group (**Figure 4.2B**).

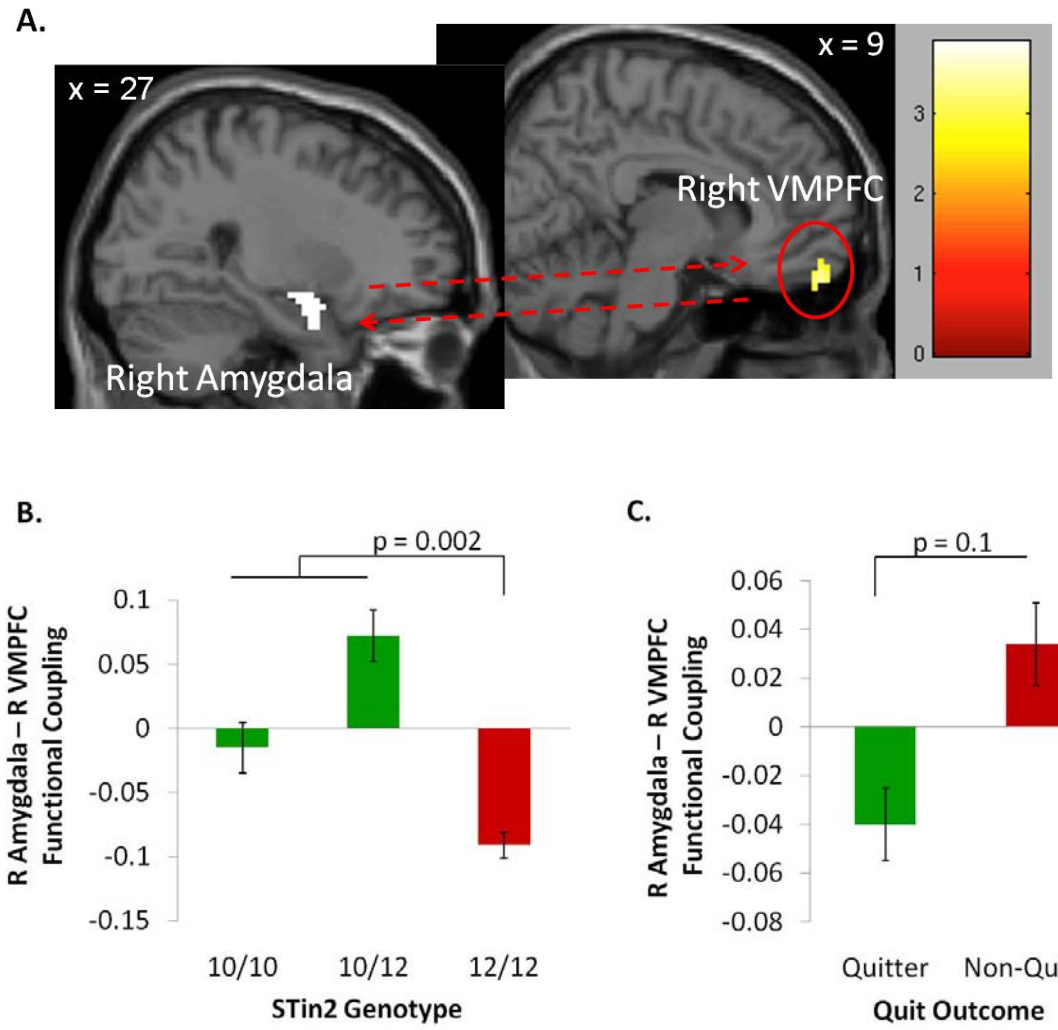

Figure 4.2 STin2 genotype modulates the functional connectivity between right amygdala and right VMPFC during processing of smoking-cessation messages vs. neutral messages. **A**. Left panel: The anatomically defined right amygdala seed ROI. Right panel: The right VMPFC cluster (MNI *xyz*: 9, 57, -24, cluster extent *k* = 23 voxels *T* = 3.39, *Z* = 3.26, family-wise error corrected *p* = 0.045), functionally defined by the STin2 modulation of the psychophysiological interaction (PPI) with the right amygdala seed during the processing of smoking-cessation messages (the *STin2.10 carriers* > *STin2.12/12 homozygotes* contrast). The scale shows one-tailed independent-sample *t*-test values. Both ROI clusters are shown against MNI 152 template. The VMPFC cluster is located within the larger, anatomically defined right VMPFC ROI (510 voxels). **B**. STin2 10 allele carriers show an increase in amygdala–VMPFC functional coupling when processing smoking-cessation messages relative to the STin2 12/12 homozygotes. **C**. Subsequent Quitters show a non-significant trend towards a decrease in amygdala–VMPFC functional coupling when processing smoking-cessation messages compared to Non-Quitters. Error bars show standard errors of the mean. Red bars denote "risk" groups with respect to smoking-cessation outcome. R, right.

Amygdala-VMPFC functional coupling during processing of smoking-cessation messages predicts quitting

Next, we examined whether the functional connectivity between the right amygdala and right VMPFC during the processing smoking-cessation messages (*i.e.*, the individual PPI betas extracted from the whole anatomically defined VMPFC target ROI) was a predictor of subsequent quitting. We did not find evidence of such predictive relationship for the whole VMPFC ROI, which encompassed the entire right-lateralized Brodmann Area 11 and Orbital Gyrus (for the total of 510 voxels). However, the amygdala coupling with the smaller VMPFC cluster, functionally defined by STin2 modulation within the main VMPFC ROI, did predict subsequent quitting when controlling for the STin2 effects (logistic regression β = -3.07, *SE* = 1.30, p = 0.02). Interestingly, an increase in amygdala–VMPFC coupling when processing smoking-cessation messages was associated with an increased risk of failing in the quit attempt. When the outcome groups were directly compared, subsequent Quitters showed a non-significant trend towards a decrease in amygdala–VMPFC coupling when processing smoking-cessation messages relative to subsequent Non-Quitters ($t(82) = 1.62$, p = 0.1) (**Figure 4.3C**).

4.5 Discussion

The goal of the analyses presented in this chapter was to determine the impact of the STin2 genotype on functional connectivity in the amygdala–VMPFC circuit during the processing of smoking-cessation messages, and the relevance of this impact for subsequent quitting outcome in smokers. We assessed functional connectivity using the psychophysiological interaction (PPI) approach, with the anatomically defined right amygdala as the seed ROI and the anatomically defined right VMPFC as the target ROI.

As hypothesized, the results suggest that functional coupling between the right amygdala (the seed ROI) and the right VMPFC during the processing of smoking-cessation messages was both modulated by the STin2 genotype and predictive of the post-intervention smoking-cessation outcome. The STin2 12/12 genotype (the hypothesized “risk” genotype) showed a reduced functional coupling between the right amygdala and right VMPFC compared to the STin2 10 allele carriers. Intriguingly, subsequent Non-Quitters showed an increased functional coupling between these two regions compared to Quitters. The significance of this result is not clear and additional evidence may be needed before it can be interpreted.

These results support previous imaging genetics evidence that the 5-HTT gene variation modulates the response and functional coupling within the amygdala–PFC circuit. Previous studies documented this genetic modulation of the amygdala–PFC circuit during emotion processing (Heinz et al., 2005; Pezawas et al., 2005; Friedel et al., 2009) and during economic decision-making (Roiser et al., 2009). The current results extend this evidence to the processing of health messages in the context of health-behavior interventions, where the 5-HTTLPR genotype may modulate the processes underlying the maintenance and updating of health-related goal representations. We also provide the first evidence that, in addition to the previously demonstrated modulation by the 5-HTTLPR/rs25531 polymorphism, the function of the amygdala–PFC circuit is also modulated by the STin2 polymorphism in the 5-HTT gene.

CHAPTER 5

BILATERAL DMPFC RESPONSE TO SMOKING-CESSATION MESSAGES MEDIATES STIN2 EFFECTS ON SUBSEQUENT SMOKING CESSATION

5.1 Goals

The overall goal of Study 2 was to examine the impact of 5-HTT gene variation on amygdala–PFC circuit activity during the processing of smoking-cessation messages, and the significance of this genetic modulation of neural function for goal-directed behavior, i.e., post-intervention quitting outcome. In Chapter 3, we demonstrated that the amygdala response to smoking-cessation communications mediated the impact of the intronic STin2 polymorphism on quitting. In Chapter 4, we showed that the STin2 genotype also modulated functional connectivity between amygdala and VMPFC when processing smoking-cessation communications. The specific goal of the analyses presented in this chapter was to test whether the STin2 genotype also modulated neural response to smoking-cessation messages in the dorsal portion of the MPFC (DMPFC), previously shown to predict smoking cessation in our data (Chua et al., in press), in a manner that affected subsequent smoking-cessation outcome.

5.2 Introduction

Message-based smoking-cessation interventions tailored to individual smokers show a promise in helping smokers quit (Strecher et al., 2008; Krebs et al., 2010).

However, the effectiveness of these interventions varies between individuals. Elucidating the biological factors that determine message efficacy could help in the design and selection of smoking-cessation interventions (as well as a range of other health-behavior interventions) optimally tailored to individual users, for maximum intervention efficacy.

Several recent neuroimaging studies focused on the neural correlates of message efficacy in eliciting a positive health-behavior change. These studies show that both tailored communications (Chua et al., 2009) and communications judged as persuasive (Falk et al., 2009) engage the *self-related processing network* in the brain, which encompasses the medial prefrontal cortex (MPFC) and the precuneus/ posterior cingulate cortex along the midline, as well as the temporal cortices (for a meta-analysis of neuroimaging studies of self-related processing, see (Northoff et al., 2006)).

Self-relevance and other aspects of self-related processing have long been hypothesized to play a key role in the enhanced effectiveness of tailored-message interventions compared to one-size-fits-all interventions to bring about a positive health-behavior change (Brug et al., 1996; Dijkstra, 2005; Strecher et al., 2006). Tailored messages make references to an individual's unique needs, characteristics, and life experiences, as well as to their specific obstacles to achieving a desired health-behavior outcome. Therefore, by design, tailored messages should be appraised as self-relevant and thus engage self-related processes in the brain. In turn, a heightened perception of self-relevance has been linked to enhanced learning and memory, possibly due to a greater elaboration, deeper encoding, and a more systematic organization of self-relevant information in the brain (for a meta-analysis of the self-reference effect in memory, see (Symons and Johnson, 1997)).

Recent neuroimaging evidence supports the view that self-related processing is a key brain mechanism underlying the enhanced efficacy of tailored health-behavior interventions. Three independent studies have demonstrated that the degree to which health messages engage the MPFC—a key region within the self-related processing network in the brain—is a predictor of subsequent health-behavior change (Falk et al., 2010b; Falk et al., 2010a; Chua et al., in press). Of particular relevance to this dissertation, the MPFC response to smoking-cessation communications has been shown to predict real-life smoking-cessation outcome as assessed with self-report of abstinence (Chua et al., in press), and real-life reductions in smoking as assessed with self-report and exhaled carbon monoxide (Falk et al., 2010a).

But what factors are responsible for the individual differences in the magnitude of MPFC response to health-behavior interventions? Genetic factors are a strong candidate, given that a variety of measures of both brain function and brain structure show substantial heritability (see the Imaging Genetics Special Issue of Biological Psychiatry for reviews (de Geus et al., 2008)). More specifically, growing evidence from imaging genetics suggests that functional variation in the serotonin transporter (5-HTT) gene affects the activity and functional connectivity of in the amygdala–PFC circuitry when processing salient emotional stimuli (Heinz et al., 2005; Pezawas et al., 2005; Friedel et al., 2009), as well as during economic decision-making (Roiser et al., 2009).

In the current analyses, we focused on STin2, a functional polymorphism in intron 2 of the 5-HTT gene (Lesch et al., 1994; Ogilvie et al., 1996; Fiskerstrand et al., 1999). Specifically, we combined functional MRI (fMRI) data, genotyping results, and a smoking-cessation outcome data following a computer-tailored smoking-cessation intervention, in order to test whether the STin2 genotype modulated the MPFC response

to smoking-cessation messages in a manner that affected the subsequent quitting success in smokers.

5.3 Methods

5.3.1 Subjects

Subjects were 91 heavy smokers interested in quitting who participated in Study 2, as described above (see **Section 3.3.1** for details). The results reported below are from the final sample of 84 participants (mean age 37.5 ± 11.5 years; 40 females, 44 males; 65 (77%) Caucasian) for whom genotyping, fMRI, and outcome data were available.

5.3.2 Study design

The study involved: a baseline assessment (Session 1); performance of the Messages Task (see **Section 3.3.4** for details and examples of messages used) and the Self-Appraisal Task (details below) during fMRI (Session 2); saliva donation for DNA extraction and completion of a web-based computer-tailored smoking-cessation intervention, which marked the start of a quit attempt (Session 3; see **Section 3.3.3** for details); and a 4-month follow-up phone interview to determine the smoking-cessation outcome using a 7-day point-prevalence abstinence measure.

5.3.3 Self-Appraisal Task

The Self-Appraisal Task (Schmitz and Johnson, 2006) included two task conditions: the Self-Appraisal condition (designed to engage self-related processing) and the Valence Judgment condition (a linguistically equivalent control condition). In the task, subjects read adjectives and made simple keypress responses to indicate whether an adjective described them or not (Self-Appraisal condition), or whether an adjective was

of positive valence or not (Valence Judgment condition). Examples of adjectives used include “shy,” “happy,” and “analytical.” Subjects completed two runs of the task, 5 blocks of each task condition per run, 6 adjectives per block. Adjectives were presented for 3 seconds plus a 1-second inter-stimulus interval. The order of the task conditions was counterbalanced across subjects and across runs. The task lasted approximately 9 minutes. We assessed the neural response when making self-referential judgments compared to valence judgments using the blood-oxygenation level dependent (BOLD) signal and the *Self-Appraisal condition* > *Valence Judgment condition* contrast.

5.3.4 Genotyping of STin2 polymorphism

Genomic DNA was obtained from saliva samples using Oragene collection system and extracted using the protocol provided (Genotek, Ontario, Canada). STin2 was genotyped using polymerase chain reaction and oligonucleotide primers from Kaiser et al. (2001) (see **Section 3.3.5** for details).

5.3.5 Image acquisition and preprocessing

Details on image acquisition and preprocessing in Study 2 are given in **Section 3.3.6** (image acquisition) and **Section 3.3.7** (image preprocessing).

5.3.6 Data analyses

The preprocessed functional data were analyzed using a modified GLM and a blocked design. Regressors of interest were convolved with a canonical HRF with a time derivative to account for between-subject and between-voxel variability in the response peak. Movement parameters were included as covariates.

Statistical analyses were conducted in a series of steps using a random-effects model. First, at the first level, individual *Smoking-Cessation Messages – Neutral*

Messages contrast images were created. These individual contrast images were then carried to the second-level for group analysis. Because the neural response to tailored smoking-cessation messages in a dorsal MPFC (DMPFC) cluster was predictive of smoking-cessation outcome in our data (Chua et al., in press), we focused on the DMPFC in the current analyses to test for genetic modulation. We constructed an anatomically defined ROI mask of bilateral DMPFC using WFU PickAtlas (Maldjian et al., 2003). The ROI mask encompassed the overlapping portions of the medial portion of the Superior Frontal Gyrus and Brodmann Area 9, for the total extent of 210 voxels (see **Figure 5.3A** below). The average parameter estimates (betas) for *Smoking-Cessation Messages – Neutral Messages* contrast were then extracted from the bilateral MPFC ROI for all individual subjects. These individual parameter estimates, together with genotyping and outcome data, were then entered into second-level group analyses in SPSS 17.0. We used a statistical significance threshold of $p < 0.05$ throughout.

The same analyses were conducted on the fMRI data from the Self-Appraisal Task, to test whether any observed effects generalized to other tasks involving self-related processing, such as self-appraisal, or were specific to the processing of smoking-cessation messages. Here, our main contrast of interest was the *Self-Appraisal condition – Valence Judgment condition* contrast.

5.3.7 Mediation analyses

To test for mediation effects, we adopted the theoretical framework outlined by Baron and Kenny (Baron and Kenny, 1986) (see **Section 3.3.9** for details on a mediation relationship and requirements for mediation effects). The mediation analyses were conducted on the extracted average parameter estimates (betas) for the *Smoking-Cessation Messages – Neutral Messages* contrast and the *Self-Appraisal condition – Valence Judgment condition* contrast from the anatomically defined bilateral DMPFC

ROI for all individual subjects, using linear and logistic regression and a non-parametric bootstrapping approach (Preacher and Hayes, 2008) (see **Section 3.3.9** for details). Gender, number of cigarettes smoked per day (prior to the quit attempt), and body-mass index were included as covariates.

5.4 Results

5.4.1 Smoking-cessation outcome

Using a 7-day point-prevalence abstinence measure at 4-month follow-up, forty-five subjects were abstinent (and were classified as Quitters) and thirty-nine were smoking (and were classified as Non-Quitters). The two outcome groups did not differ in any pre-intervention measures, nor in nicotine patch use following the intervention, except for a higher pre-intervention number of cigarettes smoked per day in Non-Quitters compared to Quitters ($p < 0.05$) (see **Section 3.4.1** and **Table 3.1** for details).

5.4.2 STin2 genotyping results

We observed the following counts (and frequencies) of STin2 genotypes: thirteen (0.15) subjects were 10/10 homozygotes, thirty-eight (0.45) were 10/12 heterozygotes, and thirty-three (0.39) were 12/12 homozygotes. For allele distribution, see **Table 3.2**.

5.4.3 Neural response to smoking-cessation messages

Before testing for genotype differences in the DMPFC response to the Messages Task, we first verified whether the task engaged self-related processing regions (critically, the DMPFC) in the whole sample, collapsing across genotypes. Indeed, as assessed with the *Smoking-Cessation Messages – Neutral Messages* contrast and one-sample *t*-test (thresholded at family-wise error (FWE) corrected $p = 0.01$, minimum 5 contiguous voxels), smoking-cessation messages produced a robust response in a large

extent of the MPFC (MNI *x, y, z:* 3, 63, 3; cluster extent *k* = 1479 voxels; *T* = 8.47; *Z* = 7.09) and in the precuneus /posterior cingulate regions (MNI *x, y, z*: 3, -66, 39; *k* = 1071; *T* = 9.28; *Z* = 7.56) along the midline, as well as in the bilateral middle frontal gyrus, inferior frontal gyrus, temporal gyrus, inferior parietal lobule, and cerebellum (**Figure 5.1**).

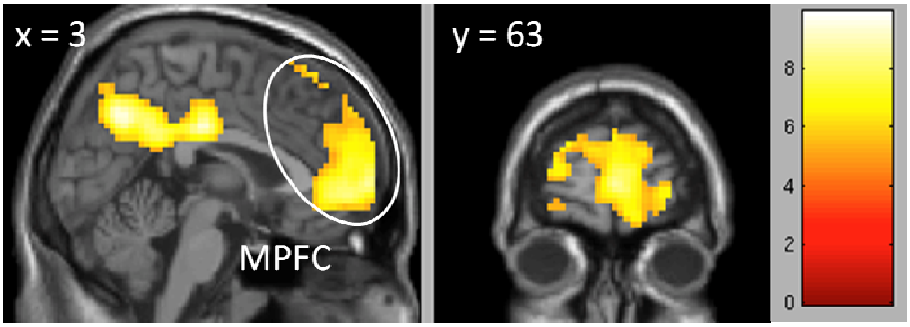

Figure 5.1 Neural response to smoking-cessation messages vs. neutral messages in the whole sample, collapsing across genotypes (one-sample *t*-test thresholded at family-wise error (FWE) corrected *p* = 0.01, corresponding to *T* = 5.51, minimum 5 contiguous voxels). MPFC, medial prefrontal cortex.

5.4.4 STin2 genotype modulates DMPFC response to smoking-cessation messages

When directly compared to STin2 12/12 homozygotes, STin2 10 allele carriers showed a significantly greater response to smoking-cessation messages in two mirror-like clusters within the anatomically defined, bilateral DMPFC mask (ROI analyses: left DMPFC cluster: MNI *x, y, z*: -9, 57, 39; cluster extent *k* = 30 voxels; *T* = 3.45; *Z* = 3.31; false-discovery rate (FDR) corrected *p* = 0.040; uncorrected *p* < 0.0001; right DMPFC cluster: MNI *x, y, z*: 12, 60, 33; cluster extent *k* = 51 voxels; *T* = 2.79; *Z* = 2.72; FDR corrected *p* = 0.050; uncorrected *p* = 0.003; one-tailed *t*-test; expected false discovery rate <= 0.11) (**Figure 5.2**).

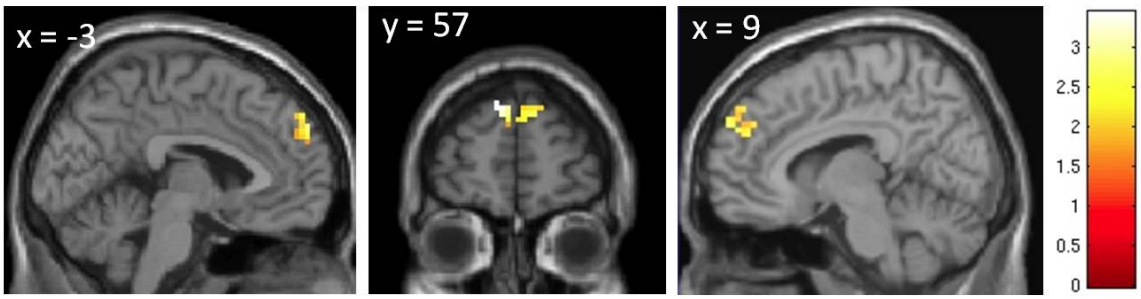

Figure 5.2 STin2 genotype modulates the neural response to smoking-cessation messages within the anatomically defined, bilateral DMPFC ROI, as assessed with a one-tailed independent-sample *t*-test (*STin2 10 allele carriers* > *STin2 12/12 homozygotes*). **Left panel**: Left DMPFC cluster (MNI *x, y, z*: -9, 57, 39; *k* = 30 voxels; *T* = 3.45; *Z* = 3.31; FDR corrected *p* = 0.040; uncorrected *p* < 0.0001). **Center panel**: A coronal section showing both left and right MPFC clusters. **Right panel**: Right DMPFC cluster (MNI *x, y, z*: 12, 60, 33; *k* = 51 voxels; *T* = 2.79; *Z* = 2.72; FDR corrected *p* = 0.050; uncorrected *p* = 0.003). Clusters are shown against MNI 152 template. The scale shows *t*-test values.

5.4.5 Mediation results

A mediation path diagram is shown in **Figure 5.3B**. The STin2 genotype (10 allele carriers vs. 12/12 homozygotes) was the predictor, the mean betas for the *Smoking-Cessation Messages – Neutral Messages* contrast extracted from the bilateral MPFC ROI were the hypothesized mediator, and smoking cessation was the outcome.

Path a: from genes to the brain. The STin2 genotype (10 allele carriers vs. 12/12 homozygotes) was a significant predictor of the response to smoking-cessation messages in the anatomically defined, bilateral DMPFC ROI (linear regression $\beta = 0.75$, $SE = 0.37$, $p < 0.05$). Specifically, the STin2 12/12 homozygote status was associated with reduced DMPFC response to smoking-cessation messages relative to the STin2 10 allele carrier status. In contrast, we found no evidence of genetic modulation of the self-related processing during the Self-Appraisal task in the same bilateral DMPFC ROI

(linear regression β = 0.10, *SE* = 0.09, $p > 0.3$). Thus, the genetic effects appeared specific to the processing of smoking-cessation messages and did not generalize to self-appraisal.

Path b: from the brain to behavior. The neural response to smoking-cessation messages in the anatomically defined, bilateral DMPFC ROI was in turn a significant predictor of subsequent quitting, when controlling for STin2 genotype effects (logistic regression β = 0.35, *SE* = 0.18, $p < 0.05$). Here, reduced DMPFC response to smoking-cessation messages was associated with increased risk of relapse to smoking. Compared to Quitters, Non-Quitters showed a reduced DMPFC response to smoking-cessation messages (*M* ± *SE*: Quitters = 1.76 ± 0.25; Non-Quitters = 0.80 ± 0.25; $t(82) = -2.70$, $p = 0.008$). In contrast, the engagement of the same DMPFC ROI during self-appraisal did not predict smoking cessation (logistic regression β = 0.19, *SE* = 0.58, $p > 0.7$), suggesting that the effects were specific to the processing of smoking-cessation messages.

Path c: from genes to behavior. In our data, the STin2 12/12 homozygote status was associated with a significantly greater risk of failing in the quit attempt following the intervention relative to the STin2 10 allele carrier status (logistic regression β = -1.04, *SE* = 0.50, $p < 0.05$).

Mediation effects. We tested for mediation effects in the anatomically defined, bilateral DMPFC ROI using a non-parametric bootstrapping approach as described in the methods above (**Section 5.3.7**). The DMPFC response to smoking-cessation messages was a significant partial mediator of the effects of STin2 genotype on real-life smoking cessation (bootstrap mediation results: estimated mean effect value = 0.31, estimated *SE* = 0.23; estimated 95% CI: 0.001 – 0.886, $p < 0.05$). Because the

confidence interval for the mediation effect (c – c') did not include zero, we could reject the null hypothesis of no difference between the total effect (c) and the direct effect (c') in favor of the alternative hypothesis of mediation. The association of STin2 genotype with smoking cessation was reduced when controlling for the mediator effects in the DMPFC during processing of smoking-cessation messages (total effect c = 1.04, *SE* = 0.50, $p < 0.05$; when controlling for DMPFC mediation: direct effect c' = 0.85, *SE* = 0.51, $p = 0.1$). In contrast, the DMPFC response during self-appraisal was not a mediator of STin2 effects on quitting.

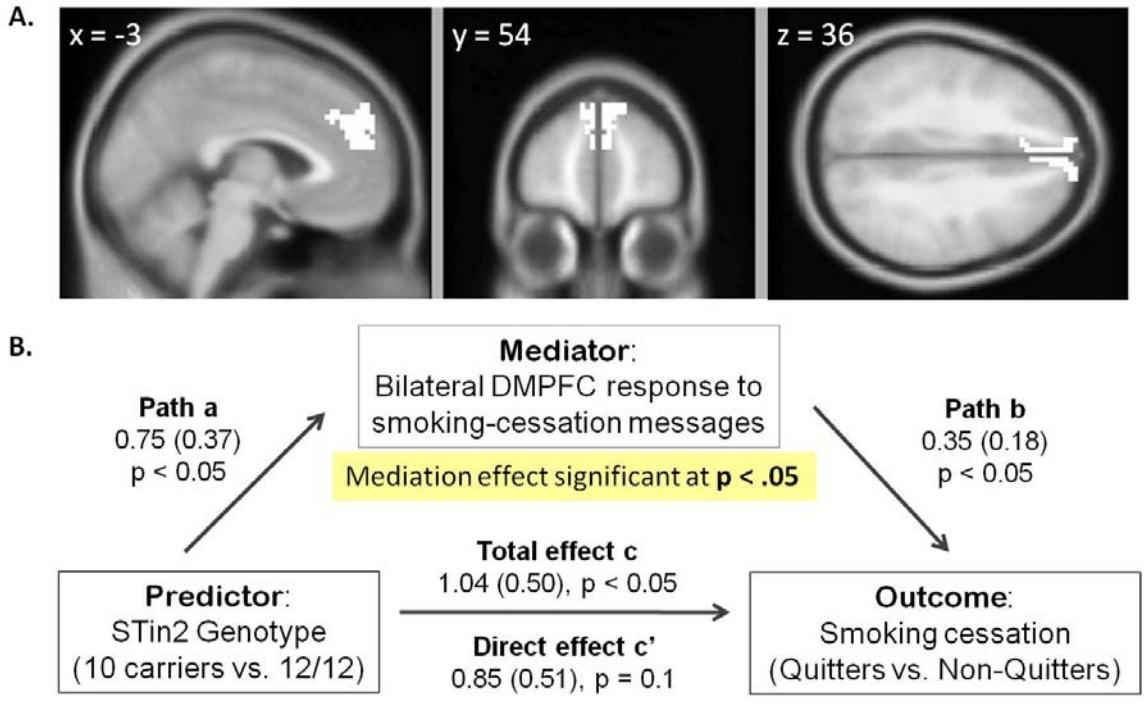

Figure 5.3 DMPFC response to smoking-cessation messages mediates the effects of STin2 genotype on subsequent smoking cessation. **A**. Anatomically defined, bilateral DMPFC ROI (210 voxels). **B**. Path diagram of the DMPFC mediation results. Regression coefficients, standard errors (in parentheses), and *p* values are given. Path b denotes the association of bilateral DMPFC response with smoking-cessation outcome, controlling for the effects of STin2 genotype. Direct effects c' denote the effect of STin2 genotype on smoking-cessation outcome, controlling for the DMPFC mediation effects.

5.5 Discussion

Previous neuroimaging studies demonstrated that self-relevance, as indexed by the degree to which health communications engage the MPFC and other self-related processing regions in the brain, predict subsequent health-behavior outcome (Falk et al., 2010a; Chua et al., in press). But genetic factors that influence the magnitude of MPFC response to health communications—and potentially also the efficacy of these communication to elicit a desired health-behavior change—have not yet been identified.

The goal of the analysis presented in this chapter was to test whether the neural response to smoking-cessation messages in an anatomically defined, bilateral DMPFC ROI served as a mediator of 5-HTT gene effects on real-life post-intervention quitting outcome in smokers. We also tested for mediation effects in the same DMPFC ROI during self-appraisal, in order to determine if the mediation effects were specific to the processing of smoking-cessation messages or extended to other tasks involving self-related processes.

We found evidence that the DMPFC response to smoking-cessation messages partially mediated the effects of the STin2 genotype on post-intervention quitting outcome. Analogously to the mediation effects in the right amygdala reported in Chapter 3, the STin2 12/12 homozygotes (the hypothesized “risk” group) showed a reduced DMPFC response to smoking-cessation messages compared to neutral messages relative to the STin2 10 allele carriers, and this reduced DMPFC response was in turn predictive of a higher risk of relapse to smoking. Furthermore, the observed mediation effects in the DMPFC appeared to be specific to the processing of smoking-cessation messages, because the neural response in the same anatomically defined ROI during

self-appraisal was neither modulated by the genotype nor predictive of the quitting outcome, and thus did not serve as a mediator of STin2 effects on quitting.

The current results support previous imaging genetics evidence that 5-HTT gene variation influences brain function in both subcortical structures, such as the amygdala, and cortical regions, such as the PFC, and extend this evidence to the context of message processing and health-behavior interventions. We also provide the first evidence that the neural response in the prefrontal regions is modulated by the STin2 genotype.

Our results have implications for the growing field of communication neuroscience research (Falk, 2010), which is broadly concerned with the neural correlates of messages persuasion and message efficacy in eliciting behavior change. We demonstrate that the response of the prefrontal regions (here, the DMPFC) involved in message processing, and in mediating the impact of message-based interventions on subsequent behavior change, is modulated by genetic variation in the serotonin system. We further show that this genetic modulation in turn contributes to the individual differences in responsiveness and efficacy of message-based interventions to produce a desired behavior change. These findings may have implications for the design and selection of a range of message-based health-behavior interventions, and may help in the development of more effective and optimally tailored interventions in the future.

CHAPTER 6

GENERAL DISCUSSION

6.1 Summary of results and significance

The purpose of this dissertation was to investigate the impact of the functional variation in the serotonin transporter (5-HTT) gene on behavioral and neural correlates of cognitive processing and emotion-cognition interactions underlying goal-directed behavior, using behavioral genetics and imaging genetics approaches. We conducted two studies to examine genetic modulation of two aspects of goal-directed cognition: maintaining goal representations despite interference from task-irrelevant distracters (Study 1), and updating these goal representations in response to goal-relevant stimuli (Study 2).

Study 1 examined the impact of the promoter polymorphism in the 5-HTT gene (5-HTTLPR) on the behavioral indices of goal-directed cognitive processing and emotion-cognition interactions, specifically, the susceptibility to response interference from neutral and emotionally salient distracters during a cognitive task performance. Using a response-interference task modified to include threat and neutral distracters, we demonstrated that the S/S homozygotes (the hypothesized “risk” group) showed greater interference effects in accuracy irrespective of the distracter condition, compared to the L allele carriers. This pattern of results suggests that the S/S homozygotes may be more susceptible to response interference from all task-irrelevant stimuli, whether emotionally

salient or neutral. This global modulation of interference susceptibility by the 5-HTTLPR genotype was observed despite the fact that threat distracters induced greater interference effects than neutral distracters in the whole sample, collapsing across the 5-HTTLPR genotypes.

The results of Study 1 extend previous findings of the 5-HTTLPR modulation of emotion reactivity by showing that the 5-HTTLPR genotype also modulates cognitive task performance. These results support the view that the 5-HTTLPR may modulate the susceptibility to environmental influences in general, rather than modulating specifically the impact of adverse stimuli (Uher, 2008; Belsky and Pluess, 2009), a trait described as *hypervigilance* (Homberg and Lesch, 2010). Clinically, increased susceptibility to environmental stimuli, and to response interference that these stimuli may elicit, is a feature of several psychiatric disorders which are also associated with alterations in the serotonin system, including affective disorders and substance abuse. Thus, our findings suggest that genetic risk variants in the serotonin system may contribute to the risk of mental disorders by imparting a greater susceptibility to response interference produced by external stimuli.

In Study 2, we employed the imaging genetics approach and a computer-tailored smoking-cessation intervention to identify brain processes through which the variation in serotonin transporter gene (5-HTTLPR/rs25531 and STin2) affects the subsequent smoking-cessation outcome. Collectively, the analyses of the neuroimaging data focused on the impact of 5-HTT gene variation on the amygdala–prefrontal cortex (PFC) circuit, which is critically involved in the integration of emotional and cognitive influences on goal-directed behavior.

In Chapter 3, we report the results of the mediation analyses linking the intronic polymorphism in the serotonin transporter gene (STin2), the neural processing of smoking-cessation messages in the amygdala as the *a priori*, anatomically defined region of interest, and the real-life quitting outcome 4 months following a tailored smoking-cessation intervention. We found that the STin2 genotype was a robust predictor of bilateral amygdala response to smoking-cessation vs. neutral messages, with a larger number of 12 alleles associated with a lower amygdala response. Bilateral amygdala response to smoking-cessation messages was also a predictor of subsequent quitting outcome when controlling for the STin2 genotype effects, with a greater amygdala response associated with better odds of quitting success. Finally, consistent with our main hypothesis, the results of a mediation analysis suggested that bilateral amygdala response to smoking-cessation messages was as a mediator of STin2 effects on real-life quitting.

To our knowledge, the results presented in Chapter 3 are the first evidence of brain mediation of genetic effects on a clinically relevant behavior. In addition, we extend previous imaging genetics literature on the 5-HTT gene effects on amygdala function (Hariri and Holmes, 2006; Munafo et al., 2008; Hariri, 2009) in two ways. First, we show that the 5-HTT gene modulation of amygdala response to emotionally salient stimuli extends to emotionally salient health-behavior messages. And second, we demonstrate that another functional polymorphism in the same gene, STin2, also modulates amygdala response to salient stimuli.

The results presented in Chapter 3 also add to our understating of the multifaceted role of the amygdala in goal-directed behavior—in this case, in both promoting and potentially overcoming addictive behaviors. While previous research showed greater amygdala engagement by smoking-related cues in smokers who would

subsequently fail in their quit attempt (Janes et al., 2010), our data suggest that a greater pre-quit amygdala response to smoking-cessation messages is predictive of *better* odds of quitting success. In our view, this apparent discrepancy may be explained by a fundamental difference between smoking-related cues (intended to *trigger* smoking behavior) and smoking-cessation messages (intended to *inhibit* such behavior). The amygdala engagement by smoking-related cues may trigger the over-learned, incentive-sensitized stimulus-response pathways underlying compulsive drug-seeking. The amygdala engagement by smoking-cessation communications may serve an opposite function of conveying motivational salience to the prefrontal regions involved in representing prospective, intentional goals and in inhibiting prepotent, stimulus-driven behavior.

In Chapter 4, moving to the circuit level, we examined the impact of the STin2 genotype on the functional connectivity in the amygdala–PFC circuit during the processing of smoking-cessation messages, and the relevance of this impact to subsequent quitting outcome. We used the psychophysiological interaction (PPI) approach to assess the functional coupling between the right amygdala (as the anatomically defined seed ROI) and the right ventromedial PFC (VMPFC) (as the anatomically defined target ROI) during the processing of smoking-cessation messages relative to neutral messages. We demonstrate that amygdala–VMPFC functional connectivity was both modulated by the STin2 genotype and predictive of the post-intervention smoking-cessation outcome. Interestingly, while the STin2 12/12 genotype (the hypothesized “risk” genotype) showed a reduced functional coupling between the right amygdala and a right VMPFC cluster relative to the STin2 10 allele carriers, the subsequent Non-Quitters showed an increased functional coupling between these two regions relative to Quitters.

The results presented in Chapter 4 support previous imaging genetics evidence that the 5-HTT gene variation modulates the response and functional coupling within the amygdala–PFC circuit, and extend this evidence to the context of message processing and health-behavior interventions. We also provide the first evidence that, in addition to the previously demonstrated modulation by the 5-HTTLPR/rs25531 polymorphism, the function of the amygdala–PFC circuit is also modulated by the STin2 polymorphism in the 5-HTT gene.

Finally, in Chapter 5, we tested whether the neural response to smoking cessation messages in the prefrontal components of the amygdala–PFC circuitry also served as a neural mediator of STin2 effects on post-intervention smoking cessation. Prior communication neuroscience research showed that the MPFC response to tailored and persuasive health communications predicts subsequent health-behavior change (Falk et al., 2010a; Chua et al., in press). In the current analyses, we tested for mediation effects in an anatomically defined, bilateral DMPFC ROI. We report evidence that the DMPFC response to smoking-cessation messages mediated the impact of the STin2 genotype on the post-intervention quitting outcome. Analogously to the amygdala mediation results, the STin2 12/12 homozygotes (the hypothesized “risk” group) showed a reduced response to smoking-cessation messages compared to neutral messages in the anatomically defined, bilateral DMPFC cluster relative to the STin2 10 allele carriers, and this reduced DMPFC response was in turn predictive of a higher risk of relapse to smoking. Because DMPFC is a key region in the self-related processing network, we also examined whether the DMPFC mediation effects were specific to the processing of smoking-cessation communications or extended to other tasks involving self-related processing, such as self-appraisal. We found no evidence that the DMPFC response

during self-appraisal mediated STin2 effects on quitting, suggesting that the observed mediation effects may be specific to the processing of smoking-cessation messages.

The results presented in Chapter 5 further support previous imaging genetics evidence that the 5-HTT gene variation influences brain function in both subcortical structures, such as the amygdala, and cortical regions, such as the PFC, and extend this evidence to the context of message processing and health-behavior interventions. We also provide the first evidence that, in addition to the previously shown modulation by the 5-HTTLPR/rs22531 genotype, neural response in the prefrontal regions is also modulated by the STin2 genotype. These results are relevant to the growing field of communication neuroscience, which is broadly concerned with the neural correlates of messages persuasion and message efficacy in eliciting behavior change. We demonstrate that the response of prefrontal regions (here, the DMPFC) involved in message processing, and in mediating the impact of message-based interventions on subsequent behavior change, is modulated by genetic variation in the serotonin system. We further show that this genetic modulation in turn contributes to the individual differences in responsiveness and efficacy of message-based interventions to produce a desired behavior change. The results presented in Chapter 5 may have implications for a range of tailored health-behavior interventions, and point to the possibility of intervention tailoring based on genetic and neural-response profiles of individual users.

Overall, the results of Study 2 add to our understanding of the role of amygdala–PFC circuit in goal-directed cognition, or the cognitive processes which enable flexible goal-directed behavior. The amygdala has been traditionally associated with emotion processing, both aversive processing, such as fear conditioning (LeDoux, 2000), and appetitive or reward processing (Baxter and Murray, 2002). However, growing evidence suggests that via its dense and bidirectional connections with prefrontal cortices

(Barbas, 2000; Bechara et al., 2000a; Ghashghaei et al., 2007), the amygdala is more broadly involved in orchestrating flexible, goal-directed behavior (Damasio, 1994; Phelps, 2006). More specifically, the amygdala has been postulated to modulate the prefrontal processes in response to emotionally salient cues (Phelps, 2006). Because a key function of the prefrontal cortex is to *maintain* goal representations and the behavioral strategies to attain these goals (Miller and Cohen, 2001), one role of the amygdala could be to signal the need to *update* these goal representations (i.e., enhance, alter, or inhibit them) in response to a change in reward or threat contingencies in the environment.

In this theoretical framework, we offer one possible interpretation of the Study 2 results. Smoking-cessation messages may be interpreted as emotionally salient by smokers attempting to quit smoking, and as such, these messages engage the amygdala. Because the participants enrolled in the study were motivated to quit smoking, they were also likely to interpret the smoking-cessation messages as relevant to their current goals, which would elicit activation in the prefrontal regions involved in representing goals and goal values. A resulting communication between the amygdala and the prefrontal regions in response to smoking-cessation messages would enhance an already encoded goal to abstain from cigarettes. In addition, an abstract goal representation is believed to encompass subgoals related to specific behavioral strategies for achieving the overall goal (Miller and Cohen, 2001). Thus, amygdala activation in response to smoking-cessation messages could also facilitate the encoding of novel smoking-cessation strategies (subgoals) related to the overall abstract goal to quit. In both cases, genetic modulation of amygdala response to smoking-cessation messages would have a downstream impact on the stability and robustness of the prefrontal representation underlying the resolution to quit, leading to differential quitting

outcomes at 4-month follow-up. Specifically, individuals with the STin2 12 “risk” allele do not sufficiently engage the amygdala when processing smoking-cessation messages, and therefore also do not sufficiently encode the goal to quit, or the behavioral-strategy subgoals, putting them at a higher risk for failing in their quit attempt. This interpretation is obviously speculative. Future studies are needed to more fully elucidate the role of amygdala in mediating the impact of genetic variation on smoking-cessation outcome following a tailored intervention, as well as the role of amygdala in the maintenance and updating of goal representations more generally.

Our findings also extend previous communication neuroscience research, showing that a number of cortical regions, including prefrontal, posterior cingulate, and temporal cortices, preferentially respond to tailored messages (Chua et al., 2009) and to messages judged as persuasive (Falk et al., 2009), and predict subsequent behavior change (Falk et al., 2010b; Falk et al., 2010a; Chua et al., in press). In Study 2, we extend these prior findings by showing that smoking-cessation messages also engage subcortical regions, specifically the amygdala. In fact, our data suggest that amygdala response to smoking-cessation messages, particularly response in the right amygdala, is a robust predictor of subsequent real-life quitting outcome. We also integrate communication neuroscience with imaging genetics by showing that genetic polymorphisms in the 5-HTT gene, and their modulation of amygdala and prefrontal cortex response to health messages, may contribute to individual differences in the efficacy of message-based interventions to produce a positive health-behavior change.

6.2 Future directions

Some limitations of both studies should be acknowledged. First, the subjects in Study 1 were all Caucasian females, making it difficult to generalize the results to males

or to other ethnicities. Future studies in subjects of both genders and of other ethnicities will allow a generalization of the current results to the population.

In Study 2, all the subjects participating in the fMRI experiment were heavy smokers prior to their quit attempt. As in Study 1, the fact that all the subjects were a specific population subgroup precludes a generalization of the current results to the general population until the results are replicated in other subgroups, including healthy never-smokers. However, it should be noted that the tailored smoking-cessation intervention, together with the subsequent smoking-behavior change it elicited, was a crucial component of the study and cannot be easily replicated in non-smokers, for obvious reasons. Future studies could examine the brain mediation of genetic effects on behavior in the context of tailored interventions targeting other clinical conditions (e.g., anxiety) and other health-behavior problems (e.g., weight management, physical exercise).

In our view, both Study 1 and Study 2 demonstrated the effects of the 5-HTT variation on cognitive function in the broader context of emotion-interactions, because emotion processing was engaged alongside cognitive processing in both paradigms. In Study 1, threat distracters, which were deliberately selected for their negative emotional salience, elicited an emotional response to threat signals. In Study 2, the smoking-cessation messages were likely perceived as emotionally salient due to their self-relevance for the subjects and the negative connotations of smoking for the subjects' health and well-being. In both studies, emotion processing may have also been engaged in more subtle ways. The wish to perform well on the response-interference task may have acted as a stressor, producing performance anxiety. Similarly, although no overt response was required in the Messages Task, passive viewing and listening to smoking-cessation messages may have caused the smokers to experience anxiety about the

efficacy of the intervention and the outcome of their smoking-cessation attempt. Future studies, employing novel experimental paradigms, will be needed to further dissociate the effects of the genetic variation in the serotonin system on emotional and cognitive processes, respectively. However, because of the centrality and pervasiveness of emotion-cognition interactions at the level of brain function and behavior, it may not be possible to fully dissociate the impact of any genetic variant on emotion and cognition.

Regarding the mediation analyses in Study 2, our aim was to identify a gene-brain-behavior pathway relevant to real-life smoking cessation following an intervention. The mediation effects reported in Chapters 3 and 5 provide preliminary evidence linking sequence variation in the serotonin transporter gene (predictor) with brain response to smoking-cessation messages (mediator) and a real-life post-intervention quitting success (outcome). Although these mediation results are correlational in nature, and therefore do not demonstrate causation, we can cautiously infer the temporal direction of the observed associations as going from the genes through the brain to behavior. However, the current results demonstrate neither necessity nor sufficiency. Future studies could examine the causal nature of the gene-brain-behavior associations outlined above more directly by experimentally manipulating the levels of the mediator variable via pharmacology, transcranial magnetic stimulation (TMS), cognitive training, neurofeedback or other means, to show that by changing the brain response it is possible to abolish or even reverse the genetic effects on behavior.

Finally, the serotonin transporter gene is known to interact with other genes (e.g., brain-derived neurotrophic factor, BDNF (Pezawas et al., 2008)) and with environmental factors (Caspi et al., 2010) in its impact on brain function and behavior. Therefore, future behavioral and imaging genetics studies, employing appropriate designs and sample sizes, will be needed to systematically examine gene-gene and gene-environment

interactions on the function of the amygdala–PFC circuit in goal-directed cognition and emotion-cognition interactions.

6.3 Translational relevance

Elucidation of gene-brain-behavior pathways can add to our basic mechanistic understanding of the neurobiological processes underlying goal-directed behavior and their dysregulation in psychopathology. However, this knowledge may also have a tremendous translational impact in the near future, leading to improved treatments and prevention programs for a range of maladaptive and pathological behaviors. Specifically, a characterization of a gene-brain-behavior pathway relevant to a specific risk behavior suggests two levels of intervention aimed at correcting or preventing this behavior: an intervention could target the genetic risk variants—or it could target the brain processes influenced by these genetic risk variants and mediating their impact on behavior. Given the prohibitively complex pleiotropic and epistatic genetic landscape of complex traits and diseases, it may not be possible or desirable to “repair” the genetic risk variants present in an individual’s genome. Instead, if we understand the brain processes through which a given genetic risk variant leads to risk behaviors, we may be able to remedy the behavior by directly modulating the brain processes involved. Thus, the brain processes mediating the impact of genetic risk factors on behavior become potential therapeutic targets.

To conclude, the goal of this dissertation was to add to our understanding of the impact of genetic variation in the serotonin system on the function of the amygdala–PFC circuit in goal-directed cognition. Continued research in this area of neuroscience will not only advance our knowledge of the mechanistic principles of brain function, but it may

also lead to novel, biologically tailored and more effective treatments and prevention programs for a range of maladaptive and pathological behaviors, such as addiction.

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