Thesis for doctoral degree (Ph.D.)
2010

Emotional Orientation, Brain Function and Genetics in Adults and Children: Implications for Development, and Psychopathology

Kara M. Lindstrom

Karolinska Institutet

200 År
1810 – 2010

Thesis for doctoral degree (Ph.D.) 2010

Emotional Orientation, Brain Function and Genetics in Adults and Children: Implications for Development, and Psychopathology Kara M. Lindstrom

Karolinska Institutet

200 År
1810 – 2010

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

EMOTIONAL ORIENTATION, BRAIN FUNCTION AND GENETICS IN ADULTS AND CHILDREN: IMPLICATIONS FOR DEVELOPMENT, AND PSYCHOPATHOLOGY

Kara M. Lindstrom

Karolinska Institutet

Stockholm 2010

Published by Karolinska Institutet. Printed by Reproprint

ISBN 978-91-7457-140-0

ABSTRACT

The ability to attend or avoid emotional stimuli is important to our survival. Attending to potential threats can help us avoid danger; while attending to positive stimuli is important for our social function. For example, when we see a man with a knife it is important to run away, or avoid the threat so we are not harmed. Just as the knife warns us of the threatening stiuation, a smiling face indicates a friendly person. We are drawn to this cue to possibly receive a rewarding social interaction. Attention orientation to both negative and positive stimuli may be impacted by development, psychopathology and genetics. The dot probe task yields both behavioral and neural indices of attention biases towards or away from an emotional cue (angry or happy face). This thesis includes three studies to determine the effects of development, psychopathology, and genetics on attention orientating.

In Study I, we examined age-related correlations in attention-orienting biases to negative and positive faces in a healthy sample using functional magnetic resonance imaging (fMRI) and a dot probe task. Behavioral response data indicated a positive correlation between age and attention bias towards happy faces, such that younger participants showed less bias towards happy, relative to neutral, faces, than older subjects. Attention bias towards angry faces did not correlate with age. Relative to older, younger participants demonstrated greater activation in the left cuneus and left caudate on the contrast of trials used to assess happy-face attention bias.

In Study II, using the dot probe task in a home setting, we studied parents that were highly exposed to the attack on the World Trade Center in 2001 and their children. We found that psychiatrically healthy parents who experienced severe trauma showed greater attention bias towards threat than parents experiencing no such trauma, but trauma experienced by parents did was not predictive of attention bias in their children.

In Study III, using an fMRI on 5-HTTLPR genotyped adults performing dot probe task; we compared amygdala response to threat bias contrasts. The 5-HTTLPR has been previously linked to amygdala reactivity and the amygdala has been implicated in the orienting of attention towards threat. Behavioral data indicated no difference between the two genotyped subject populations for the 5-HTTLPR polymorphism (l/l and s-carrier). However, fMRI data did reveal between-group differences in the amygdala activation. Specifically, relative to l/l, s-carriers showed greater right amygdala activation to trials with angry faces. Because similar levels of threat bias were found in the two genotype groups, these findings suggest that s-carriers exhibit a lower threshold for engaging the amygdala within the context of the task.

In total, these three studies explore the effect of both the environment and genes on behavior and brain function. Studies I and II focus on environment, specifically, how their environment affects their emotional orientation. On the genetic side, Study III focuses on the effect of genetics on emotional orientation.

LIST OF PUBLICATIONS

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

I **Lindstrom, K.M.**, Guyer, A.E., Mogg, K., Bradley, B.P., Fox, N.A., Ernst, M., Nelson, E.E., Leibenluft, E., Britton, J.C., Monk, C.S., Pine, D.S. & Bar-Haim, Y. (2009). Normative data on development of neural and behavioral mechanisms underlying attention orientation toward social-emotional stimuli: An exploratory study. *Brain Research*, 1292, 61-70.

II **Lindstrom, K.M.,** Mandell, D.J., Musa, G.J., Sankin, L., Mogg, K., Bradley, B.P., Ernst, M., Doan, T., Bar-Haim, Y., Leibenluft, E., Pine, D.S. & Hoven, C.W. (2010). Attention orientation in parents exposed to the 9/11 terrorist attacks and their children. *Psychiatry Research, in press*

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

Publications by the author, which are not included in the thesis:

Thomason, M.E., Henry, M.L., Hamilton, J. P., Joormann, J., Pine, D.S., Ernst, M., Goldman, D., Mogg, K., Bradley, B.P., Britton, J.C., **Lindstrom, K.M.**, Monk, C.S., Sankin, L.S., Louro, H.M. & Gotlib, I.H. (2010). Neural and behavioral responses to threatening emotion faces in children as a function of the short allele of the serotonin transporter gene. *Biological Psychology,* 85(1), 38-44.

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

Golkar A., Lonsdorf T.B., Olsson, A., **Lindstrom, K.M.,** Fransson, P., Schalling, M., Ingvar, M., Öhman A. Separation of maintenance of instruction and emotional regulation. *submitted manuscript*

Thomas, L., Bones, B. , Milch, H., **Lindstrom, K.M.,** Marsh, A. A., Blair, R.J.R., Pine, D., Leibenluft, E. Differential brain engagement to emotional faces in youth with bipolar disorder, severe mood dysregulation, and controls while performing an implicit face processing task. *Manuscript under review*

CONTENTS

1 INTRODUCTION 1

1.1 ANXIETY DISORDERS 3

1.2 THREAT 5

1.3 REWARD 6

1.4 TRAUMA 7

1.5 SEROTONIN 8

2 AIMS 11

2.1 OVERVIEW OF STUDIES 12

3 METHODS 14

3.1 RESEARCH PARTICIPANTS 14

3.2 DOT PROBE TASK 16

3.3 BEHAVIORAL DATA ANALYSIS 18

3.4 fMRI 19

3.5 fMRI DATA ACQUISITION 21

3.6 fMRI DATA PROCESSING AND ANALYSIS 21

3.7 DNA EXTRACTION AND GENOTYPING 23

4 RESULTS AND DISCUSSION 24

4.1 STUDY I 24

4.2 STUDY II 27

4.3 STUDY III 30

5 CONCLUSIONS 33

6 GENERAL DISCUSSION 34

7 FUTURE DIRECTIONS 37

8 ACKNOWLEDGEMENTS 38

9 REFERENCES 42

LIST OF ABBREVIATIONS

5-HT	Serotonin
5-HTT	Serotonin Transporter
5-HTTLPR	5-HTT linked polymorphic region
AFNI	Analysis of Functional NeuroImaging
BOLD	Blood Oxygenated Level Dependent
CIDI	Compostite International Diagnostic Interview
CS	Conditioned Stimulus
DISC	Dominance Influence Steadiness Conscientiousness
DNA	Deoxyribose nucleic acid
DSM	Diagnostic and Statistical Manual of Mental Disorders
EEG	Electrophanlography
EPI	Echo Planar Imaging
erfMRI	Event related functional magnetic resonance imaging
fMRI	functional magnetic resonance imaging
FOV	Field of View
IAPS	International Affective Picture Set
ICD	International Classification of Diseases
IQ	Intelligence Quotient
K-SADS	Kiddie- Schedule for Affective Disorders and Schizophrenia
MDD	Major Depressive Disorder
MEG	Magnetoencephalography
MRI	Magnetic Resonance Imaging
NIH	National Institutes of Health
PET	Positron Emission Topography
PTSD	Post traumatic stress disorder
RT	Reaction Time
TE	Echo Delay Time
TR	Repetition Time
WASI	Wechsler Abbreviated Scale of Intelligence
WTC	World Trade Center

1 INTRODUCTION

Humans live in a dynamic, stimulus-rich environment filled with threat and rewarding stimuli. An example of threat stimuli is an angry face or a snake and rewarding stimuli may be a happy face or a cute puppy. Human survival and quality of life depend on the ability to respond appropriately to these threatening or rewarding situations. Our attention to these emotional stimuli and subsequent orientation toward or away from the situation is indicative of adaptation to threat or reward. Inability to orient toward, or away, from emotional stimuli may indicate a psychopathological issue, such as anxiety or depression.

Considerable theoretical and empirical work demonstrates a relationship between trauma vulnerability and cognitive functioning. Perhaps the strongest, most consistent findings emerge from work implicating perturbed attention orienting in post-traumatic anxiety (Bar-Haim, Lamy et al. 2007). Cross-sectional and prospective results both suggest that attention bias predicts vulnerability to anxiety following trauma (Bar-Haim, Lamy et al. 2007). Moreover, experimental work suggests that these attention biases can arise through the effects of experience (MacLeod, Mathews et al. 1986). This has given rise to models suggesting that trauma produces anxiety by altering attention bias.

Previous studies raise questions on age-related differences in the associations among trauma, vulnerability, and dot probe performance. The most consistent findings document greater attention bias *towards* various threats in adults with PTSD, as compared to healthy adults (Elsesser, Sartory et al. 2004). Findings in children are far less consistent and vary based on the age of the subjects, the features of the attention paradigm, and the nature of psychopathology. However, one particularly notable study in highly-anxious, traumatized children used a version of the dot probe paradigm where anxious adults consistently exhibit an attention bias towards threat and healthy adults exhibit no bias. This study found a bias away from angry faces in traumatized children, in contrast to a bias towards angry faces in healthy children. Thus, relationships among anxiety, trauma, and attention bias may vary with age. However, this study did not directly compare adults and children. Moreover,

traumatized children in this study had been exposed to domestic abuse. Under these circumstances, bias away from angry faces could be interpreted as idiosyncratic reaction to cues highly similar to the traumatic exposure. It is important to examine attention bias using this task in children and adults simultaneously exposed to trauma.

The function of the central serotonin system plays a critical role in the modulation of emotions. There is a functional variation associated with serotonin reuptake called 5-HTTLPR. Variants in this site have been associated with anxiety related traits and behaviors similar to anxious populations. The 5-HTTLPR s-carrier demonstrated an attentional bias towards negative threatening emotional stimuli such as, faces, IAPS pictures, spiders and anxiety related words (Beevers, Gibb et al. 2007; Fox, Ridgewell et al. 2009; Perez-Edgar, Bar-Haim et al. 2010). In contrast, 5-HTTLPR l-l genotype has a bias towards rewarding, postive emotional stimuli including positive IAPs pictures and happy faces (Fox, Ridgewell et al. 2009; Perez-Edgar, Bar-Haim et al. 2010). These studies imply that attention bias may be affected be a person's genetic background.

In summary, the aim of this thesis is to explore how emotional orientation may be impacted by development, psychopathology and genetics. First, given the dramatic developmental change in the brain during during adolescence into adulthood, in particular the neural circuitry supporting social function, attention orienting negative and positive facial cues and neural substrates that subserve this attention orientating may differ in relatively young or old individuals. However, virtually no research has examined such age-related differences in the neural architecture supporting attention orientation to emotional faces. Secondly, while trauma affects both parents and their children, the role of information-processing perturbations remains unclear in shaping unique or similar responses to trauma experienced by parents and their children. Finally, the 5-HTTLPR polymorphism; however, little is know about the effects of the 5-HTTLPR geneotype (long vs. short allele), on the behavior and the neural correlates of orienting towards threat in a normative population.

1.1 ANXIETY DISORDERS

Feelings of anxiety are a common aspect of everyday life. It may be an apprehensive uneasiness in anticipation over an upcoming event, such as a dreaded test or an oral defense of a PhD thesis. However, these feelings are classified as a mental illness if they cause distress and impaired daily function.

Anxiey is marked by excessive anxiety or worry about different situations. The symptoms include behavioral disturbances and cause marked impairment. Approximately 40 million American adults ages 18 and older have been diagnosed with an anxiety disorder. Most people with one anxiety disorder also have another anxiety disorder or comorbidity. Nearly three-quarters of those with an anxiety disorder will have their first episode by age 21.5 (Kessler, Chiu et al. 2005). Anxiety disorders include panic disorder, obsessive-compulsive disorder, post-traumatic stress disorder, generalized anxiety disorder, and phobias (social phobia, agoraphobia, and specific phobia). For characterization of anxiety subtypes, see table1.

One of the characteristics of anxiety is abnormal attention to threat. Adults with high levels of anxiety tend to orient toward threats, whereas adults with low levels of anxiety tend to either avoid threats or allocate similar levels of attention to threats and neutral cues (MacLeod, Mathews et al. 1986; Mogg and Bradley 1998; Fox, Russo et al. 2001; Bar-Haim, Lamy et al. 2007). Brain imaging studies among adults suggest that these between-group differences relate to underlying differences in neural function. Anxious adults also tend to show an enhanced sensitivity within the fear circuit for threats. This is manifested as increased activation within the amygdala and ventral prefrontal cortex (Bishop 2009).

Threat orientation findings among children and adolescents show some inconsistencies with findings among adults (MacLeod, Mathews et al. 1986; Williams, Mathews et al. 1996; Mogg and Bradley 1998; Calvo and Castillo 2005). Two studies appear particularly relevant in this respect: Pine et al. (2005) found that children with post-traumatic stress disorder (PTSD) tended to avoid spatial locations where threatening faces appeared, whereas children without PTSD tended to monitor locales where threatening faces appeared. Similarly, Monk et al. (2006) found that adolescents

with generalized anxiety disorder also tended to avoid threats, whereas healthy adolescents tended to monitor threats. Moreover, this study from was performed in the MRI scanner, allowing an examination of brain regions engaged during threat processing. Monk et al. (2006)found that adolescent generalized anxiety disorder was associated with enhanced engagement of the ventrolateral prefrontal cortex during threat-monitoring events.

The cause of the normal anxious behavior developing into a mental illness has been a popular research topic. It is still unclear how anxiety disorders are formed but as previously mentioned, there is evidence that there is a genetic component as well as an environmental component. In this thesis we will explore both of these components and their effect on attention orientation.

Table 1. Classification of the subtypes of Anxiety Disorder and symptoms that characterize the anxiety subtypes.

Disorder	**Symptoms**
Panic Disorder	Recurrent, unexpected panic attacks Persistent worry or concern about continuing attacks and the outcome of the attacks
Panic Disorder with agoraphobia	Features of Panic Disorder with the addition of fear or avoidance of situations where a panic attack may occur
Social Phobia	Fear or avoidance of social situations due to the possibility of humiliation or embarrassment
Specific Phobia	Fear or avoidance of specific objects of situations (e.g. heights, spiders, enclosed spaces)
Generalized Anxiety Disorder	Chronic excessive, uncontrollable worry about a number of event or activities
Obsessive-Compulsive Disorder	Recurrent, intrusive thoughts, images or impulses Repetitive behaviors or mental acts aimed at reducing distress or "neutralizing" an obsession
Post-Traumatic Stress Disorder	Persistent re-experiencing, distress, and avoidance of stimuli associated with prior exposure to extreme stress

Note: DSM-IV *Diagnostic and Statistical Manual of Mental Disorders,* fourth edition (American Psychiatric Association, 1994).

1.2 **THREAT**

A threat is an expression of intention to inflict evil, injury or damage. When animals are confronted with a threat, they have a 'fight or flight response.' The animal will react to the threatening stimuli by either confronting the perceived threat or fleeing away from the threat to safety. In humans, this behavior has evolved from prehistic times when humans were confronted with a predator they would fight or flee to safety, to modern times where the fight behavior may be classified as anger or aggressive behavior and flight behavior may be classified as social withdrawl or avoidance.

Research on fear conditioning suggests that the affective valence of a stimulus influences the degree to which the stimulus can be conditioned. Ohman (1986) proposed that orientation towards a threat in the environment is modulated by a biologically prepared mechanism sensitive to threat stimuli such as angry faces. Thus, expectation of adverse events associated with one or another potential conditioned stimulus may affect the degree to which this stimulus becomes conditioned. Further support for this idea was found in dot probe tasks where subjects orient their attention towards threatening stimuli (Armony and Dolan 2002).

Orientation of attention towards threat in anxious adults is important both for the consistency of the observation and for its connection to work on the neural correlates of threat orientation. This latter work provides insights on neural factors that might mediate between group differences among adults in attention orienting towards or away from threats (Thomas, Drevets et al. 2001; Amaral, Behniea et al. 2003; Hariri, Mattay et al. 2003; Bishop, Duncan et al. 2004; Suslow, Ohrmann et al. 2006; McClure, Monk et al. 2007). Using comparable paradigms employed in research on individual differences, neuroimaging and lesion studies implicate a distributed neural circuit in spatial orienting during threat processing.

Most work on emotion-attention interaction examines threat attention interactions, emphasizing findings related to anxiety and associated patterns of perturbed amygdala function. Age-related changes in behavior, such as increases in risk-taking and novelty-seeking behavior may be accompanied by changes in sensitivity to threat (Spear 2000; Nelson, Leibenluft et al. 2005). Changes in threat sensitivity may reflect age-related changes in a dedicated neural architecture for processing threat in situations that create fear. This architecture is though to incompass the amygdala and an associated network in the ventral prefrontal cortex and parietal lobes, a network specifically implicated in threat-attention interactions (LeDoux 2000).

1.3 **REWARD**

A reward is a stimulus administered to an organism following a correct or desired response that increases the probablilty of the occurance of the response. For example,

if we were training a dog we would reward the dog with doggie treats after they preformed a desired act, such as 'sit,' to reinforce that behavior. Eventually, the dogs will learn that if they obey the 'sit' command they will receive more doggie treats. Animals, dogs and humans alike, seek out rewarding stimuli.

Although studies of attention bias have focused mainly on the threat system, principally because of their focus on neurocognitive mechanisms of anxiety, attention to appetitive stimuli also represents a key survival function and is as important to understand as threat bias. Changes in life circumstances with age (e.g., dependency to independence) may be accompanied by changes in responsivity of the threat and reward systems. The reward system has been associated with the dopamine system, in particular the mesolimbic dopamine pathway which encompasses the ventral striatum, including nucleus accumbens and orbitofrontal cortex (Haber, Kim et al. 2006). Although most studies of reward processes focus on the nucleus accumbens, ample evidence implicates the caudate nucleus and putamen, which are classically assigned to the motor processes and habit formation. Ventral regions of these structures are particularly responsive to appetitive stimuli (Fudge, Breitbart et al. 2005).

Effects of reward on cognition are thought to arise through effects on the mesolimbic dopamine pathway and its influence on the striatum (Fudge, Breitbart et al. 2004; Fudge, Breitbart et al. 2005; Kleijer, Garritsen et al. 2006). Similar to the neuronal network associated with processing threat, these striatal influences are also thought to impact ventral prefrontal cortex and parietal areas to modulate attention. Accordingly, age-related changes in this mesolimbic-striatal system may relate to age-related changes in reward-attention interactions.

1.4 **TRAUMA**

Trauma predicts a high risk for psychiatric symptoms, particularly symptoms of mood and anxiety disorders (Breslau 2002; Pine and Cohen 2002; Pine 2003; Hoven, Duarte et al. 2005). These associations manifest with diverse traumas in various age groups. Variability occurs in the response to trauma, which is thought to reflect the unique circumstances of particular traumatic exposures, coupled with individual differences in vulnerability. One major question emerging from this research concerns

the relationship between age and vulnerability. Some studies suggest that traumatized children are more likely than their traumatized parents to exhibit psychopathology, whereas other studies suggest the reverse (Pynoos, Steinberg et al. 1999; Laor, Wolmer et al. 2001; Pine and Cohen 2002; Pine, Costello et al. 2005). Additional work suggests that parents shape their children's response to trauma, given that parental response is one of the best predictors of the child's psychiatric outcome following trauma (Pine, Costello et al. 2005). However, many children in families exposed to trauma show signs of resilience, even when their parents manifest psychopathology. This work raises core questions on age-related differences in vulnerability among children and adults living in families exposed to trauma.

Notably, few studies examine relationships between severe trauma exposure and attention bias in a non-clinical sample. Far more work using the dot probe task compares anxious-traumatized subjects or subjects with PTSD to subjects with neither anxiety nor trauma (Bar-Haim, Lamy et al. 2007). As such, these studies predominantly examine people who are actively struggling with their reactions to trauma, either because trauma has occurred recently or because they have failed to overcome their initial reactions to prior trauma. These studies are not able to predict if the impact of previously experienced severe trauma may endure, even when clinical effects are not detected via psychiatric assessments. It is unknown whether previously experienced, severe trauma influences attention even when it does not appear to influence psychopathology.

1.5 **SEROTONIN**

Serotonin (5-HT) plays a critical role in the modulation of emotions (Lucki 1998). It has been associated with fear conditioning and stress response (Kim 2008). The serotonin transporter (5-HTT) reuptakes serotonin in the synaptic cleft and thereby terminates synaptic transmission. The gene coding for the 5-HTT harbors a common 43bp ins/del in its promoter region, referred to as the 5-HTT-linked polymorphic region (5-HTTLPR). This variation yields two alleles; a long (l) and a short (s) allele. The s-allele has been associated with reduced 5-HTT expression in vitro (Heils, Teufel et al. 1995) which would ultimately lead to increases in synaptic 5-HT levels. However, human studies failed so reveal a consistent functional effect on 5-HTT availability in

vivo (Van Dyck, Malison et al. 2004; Parsey, Hastings et al. 2006; Reimold, Smolka et al. 2007) and post-mortem (Little, McLaughlin et al. 1998) and consequently more complex mechanisms like receptor up- and downregulation, differences in methylation patterns or developmental effects may underlie the functional effects associated with the 5-HTTLPR on the behavioral and neural level.

Recently, a functional A→G SNP (db number 25531) has been identified just upstream of the 5-HTTLPR. The minor G-allele is nearly always in phase with the 5-HTTLPR l-allele has been shown to reduce 5-HTT expression to a level similar to that associated with the 5-HTTLPR s-allele. It has become standard in the literature to study the effect of this mini-haplotype (in the literature referred to as "triallelic 5-HTTLPR"), which is thought to better capture the functional variability of the 5-HTT gene, rather than the 5-HTTLPR alone.

Genetic varitation has been associated with contributing to many psychiatric diseases, including, but not limited to anxiety, depression and schizophrenia (Meyer-Lindenberg and Weinberger 2006; Thomason, Henry et al. 2010). However, the mechanism of the gene's effect on the behavior has long seemed elusive. Behavioral and imaging studies of genotyped populations have attemped to clarify the genetic influence on behavior associated with psychiatric disorders. Using the dot probe paradigm, studies have revealed behavioral differences based on one's genotype such that 5-HTTLPR s-carriers demonstrate an attentional bias towards negative threatening emotional stimuli including, faces, IAPS pictures, spiders and anxiety related words (Beevers, Gibb et al. 2007; Fox, Ridgewell et al. 2009; Perez-Edgar, Bar-Haim et al. 2010). In contrast, the 5-HTTLPR l-l genotype has a bias towards rewarding, positive emotional stimuli including positive IAPS pictures and happy faces (Fox, Ridgewell et al. 2009; Perez-Edgar, Bar-Haim et al. 2010).

One of the neural areas associated with the attentional bias behavior is the amygdala. Genetic imaging studies have demonstrated increased amygdala reactivity in 5-HTTLPR s-carriers compared with non-carriers (l-l) when performing various task, such as passive viewing of affective faces and neutral faces and a matching of angry and neutral faces (Hariri, Tessitore et al. 2002; Hariri, Drabant et al. 2005; Smolka, Buhler et al. 2007; Munafo, Brown et al. 2008). 5-HTTLPR has been shown to affect the function of the amygdala and its connection to the prefrontal cortex (Heinz, Braus

et al. 2005). As previously mentioned in section 1.2 these neural regions have been associated with threat-attention interactions (LeDoux 2000).

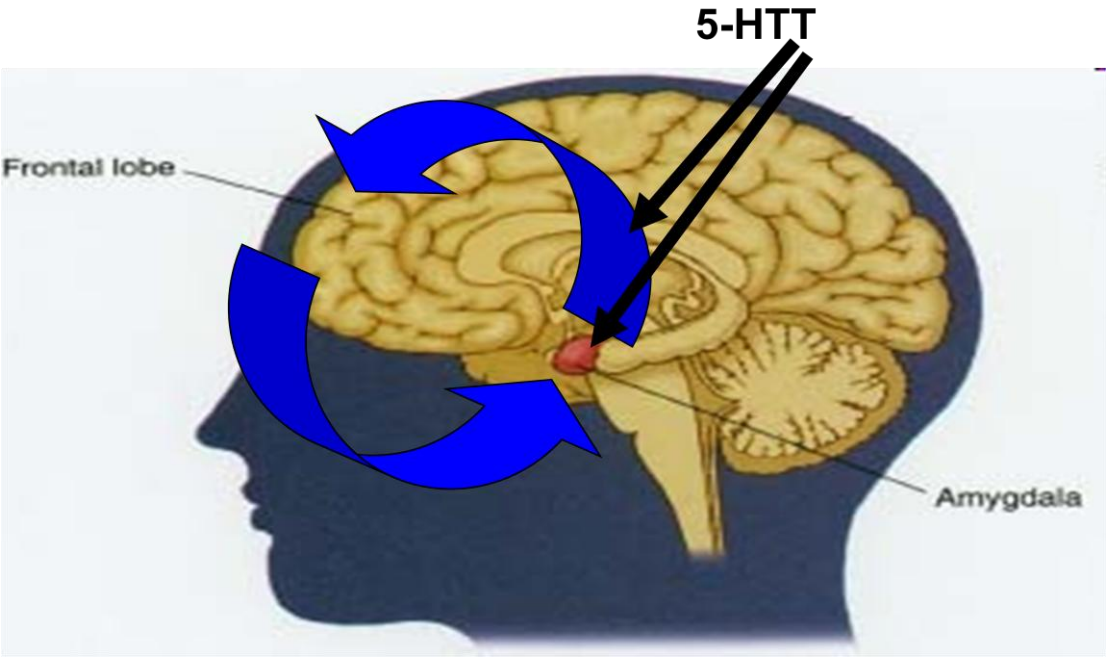


Figure 1. A schematic representation of the effect of 5-HTT on the brain regions associated with threat orientation. [figure was modified from a image downloaded from Wikimedia Commons. No know restrictions on publication]

2 AIMS

The overall goal is to examine the behavioral and neural correlates of attention orientation to emotional stimuli. The work presented in this thesis brings together insights from the fields of neuroscience, psychology and genetics.

The specific objectives were to identify:

age-related correlations in attention-orienting biases to positive and negative face emotions (**Study I**). To address this aim we used fMRI and a dot probe task.

the associations among levels of trauma exposure, psychopathology and attention bias in parents and their children (**Study II**). To address this aim we used psychological inventories and a dot probe task.

the association between 5-HTTLPR allelic variation and responses to fearful and angry faces in adult participants (**Study III**). To address this aim, we used fMRI, genotyping and a dot probe task.

2.1 OVERVIEW OF STUDIES

STUDY I

We examined associations between age and either reward-related or threat-related attention bias. Two main sets of findings emerged. First, on the dot probe task, implemented in the fMRI setting, reward-related, happy-face bias showed a positive correlation with age, a finding consistent with previous literature utilizing other attention-related tasks (Mather and Carstensen 2005). Age also showed a negative correlation with neural activation for happy-face/reward bias, specifically in the striatum and cuneus, two brain regions previously implicated in attention control (Pessoa, McKenna et al. 2002). Second, while a threat-related attention bias emerged across the entire sample, there was no evidence of an association between threat bias and age. Moreover, age showed no association with neural activation on angry incongruent versus angry congruent events. In summary, age relates to both behavioral and neural indicators of biased attention allocation to rewarding, happy faces, but not to attention allocation to threatening, angry faces.

STUDY II

We examined associations among trauma, threat bias, and psychopathology. Four-to-five years separated the traumatic exposure from the assessments of psychopathology and attention bias. While traumatized parents with low lifetime rates of psychopathology did exhibit threat bias, lifetime rates of psychopathology were determined through retrospective reports. Higher rates of psychopathology in severely traumatized parents might be expected with repeated, prospective assessments, beginning immediately following severe exposure. Therefore, the absence of associations with psychopathology may reflect limitations in the assessment techniques. Moreover, recent studies suggest that relationships among trauma, attention bias, and psychopathology evolve over time following traumatic exposure (Bar-Haim, Holoshitz et al. 2010).

STUDY III

Using event-related, functional magnetic resonance imaging (erfMRI) paired with a visual-probe threat-orienting task, we compared amygdala response to angry faces in

psychiatrically healthy 5-HTTLPR genotype selected adults. In this threat-orienting task, participants viewed angry/neutral face pairs. After viewing each face pair, participants indicated by button-press whether a subsequent probe appeared on the same (congruent) or opposite (incongruent) side as the angry face. RT differences between congruent and incongruent face trials provided a measure of attention orientation towards or away from angry faces. Slower RT's to angry faces on the opposite visual field (incongruent) and a faster RT to angry faces on the same visual field (congruent) indicate an attention bias towards threat. Behavioral data indicated no difference between the two genotyped subject populations (l-l and s-carrier). However, erfMRI data did reveal between-group differences in the amygdala. Specifically, relative to l-l, s-carriers showed greater right amygdala activation to trials containing angry faces. Because similar levels of threat bias were found in the two genotype groups, these findings suggest that s-carriers exhibit a lower threshold for engaging the amygdala within the context of the task.

3 METHODS

3.1 RESEARCH PARTICIPANTS

All participants had no reported history of brain injury, no behavioral indications of possible mental impairment, no past or present Axis I disorders. At all three sites, Maryland, USA; New York, USA and Stockholm, Sweden, the participants were compensated for their time. Parents and adolescents gave informed consent and assent, respectively, as approved by the NIH, Columbia University and Karolinska Institute Institutional Review Boards.

In **Study I**, participants were 37 healthy paid volunteers (18 males, mean age: 21.51 ± 8.69). A total of 12 subjects were excluded from the study either due to movement over 3mm in any direction, failure to perform under a 25% error rate on the dot-probe task or technical complications in the acquisition of data. Psychiatric history was assessed using a structured psychiatric interview, the Kiddie-Schizophrenia-and-Affective-Disorders-Schedule (K-SADS) (Kaufman, Birmaher et al. 1997) in youth and the Structured Clinical Interview for DSM-IV-TR (SCID) (Spitzer, Williams et al. 1992) in adults. Experienced clinicians trained to achieve high reliability for all diagnoses (kappa>0.75) administered these interviews to each participant, as well as to one parent of each participating youth. Each participant had an IQ > 70 based on the Wechsler Abbreviated Scale of Intelligence (WASI) (Wechsler 1999), mean IQ = 115, range = 87-142.

Study II had 45 parent and child sets (parents: 4 males, mean age=46.3 ± 5.5). This study examined families living near the site of the 9/11 World Trade Center attacks. The families consisted of a parent who was the primary caregiver and one biological child between 9-15 years old. Forty-five parent and child sets were divided into two groups: i) 14 families with a parent highly exposed to the 9/11 World Trade Center attacks and; ii) 31 families with a parent with lower levels of exposure. The exposure rating scale that was used to divide the groups is described in detail in the publication. Highly-exposed parents scored greater than two on an established trauma-severity scale; low-exposed parents scored less than or equal to two.

Psychopathology was assessed in the parent and the child using the Composite International Diagnostic Interview (CIDI) and the Diagnostic Interview Schedule for Children (DISC), respectively. The CIDI is a structured interview previously used in many large-scale epidemiological studies of adults (Kessler, Demler et al. 2005). The version used here assessed both lifetime and current prevalence according to the definitions and criteria of ICD-10 and the DSM-IV. The CIDI assessment of parents measured mood, anxiety, and substance-related problems. The DISC is a highly structured psychiatric assessment tool designed to be administered to children by lay interviewers and is used extensively in epidemiological investigations (Shaffer et al., 1996). The current study utilized the DISC to assess both current and lifetime presence or absence of eight disorders previously linked to trauma: PTSD, major depression, generalized anxiety disorder, separation anxiety disorder, panic, agoraphobia, conduct disorder, and substance-use disorders (Hoven et al., 2005).

Rates of current psychopathology were low in both parents and children. As a result, findings are presented for lifetime presence or absence of disorders. In addition, symptom counts were used to assess symptom severity for two conditions, PTSD and MDD, where considerable prior work notes associations with trauma. The parent symptom counts for PTSD and MDD indexed DSM-IV-TR (2000) positively-endorsed CIDI questions related to diagnostic criteria for each disorder. The child symptom counts used established DISC algorithms derived from established methods (Lucas et al, 2001). These measures were used to examine the relationship between psychopathology severity and attention bias.

Study III had 64 paid volunteers (27 males, mean age: 24 ± 2.6). From a larger population of 600 genotyped volunteers, participants were selected based on their 5-HTTLPR rs25531 to obtain an age and gender matched sample. Participants were grouped into 5-HTTLPR s-carrier vs. long-long. Of note, participants with a 5-HTTLPR lg- of Sg- allele were not selected. All included participants had a constant rs25531 A/A genotype background. In additional the COMTval158met was balanced in the two 5-HTTLPR genotype groups. Exclusion criteria included non-Caucasian ancestry, lifetime psychiatric and neurological disorders, pregnancy and the presence of metal in the body. The final study sample included 60 subjects after excluding 2

subjects for not completing the study, one subject having metal in their body and one subject for having pathological brain anatomy.

3.2 DOT PROBE TASK

Attention orienting tasks present threat or reward stimuli in spatially confined locations (e.g. on the left and right sides of a visual display) and require individuals to identify probes that appear in corresponding locations. These paradigms provide a behavioral measure of orienting bias, either towards or away from an emotional stimulus. Based on such paradigms, studies indicate that threat vs. non-threat stimuli have a greater influence on orienting in anxious vs. non-anxious adults (Mogg and Bradley 1998; Bradley, Mogg et al. 1999; Fox, Russo et al. 2001; Mogg and Bradley 2002; Mogg, Philippot et al. 2004).

The dot probe task was used to assess attention biases. The task was administered on a laptop (Study I) or projected (via Avotec or MRI compatible googles in Studies I and III respectively) during an fMRI scan. During the task, angry-neutral trials, happy-neutral trials, and neutral-neutral trials were presented. Trial presentation order is fully randomized for each participant.

Each trial began with a centrally located fixation cross, followed by a pair of faces that appeared on the left and right sides of the screen. The faces were replaced by an probe (either an asterisk * probe, or a spatial discrimination probe : or ..) which appeared in right or left visual fields. Participants were instructed to press one of two buttons as quickly and as accurately as possible to indicate the location of the probe (left or right) or the orientation of the two dots (either vertical : or horizontal ..). Figure 2 below depicts the two key trial types contained in the task. In congruent trials, the probe occupied the location of the emotional face in emotion-neutral pairs. In incongruent trials, the probe occupied the location of the neutral face in the emotion-neutral pair.

The attentional bias toward or away from the emotional faces was calculated by subtracting the congruent trial from the incongruent trials. For example,

to calculate the threat bias, the results of the angry-neutral congruent trials are subtracted from the angry-neutral incongruent trials. To calculate the happy bias, the happy-neutral congruent trials are subtracted from the happy-neutral incongruent trials.

In Studies I and III we relied on a rapid-event-related design, as opposed to a slow-event-related design. In this rapid design, jitter in the timing of fMRI acquisition is accomplished by the inclusion of randomly-appearing null events or so-called "blank trials". We included 40 such blank trials, randomly interspersed among the face trials. Beyond ensuring jittering of timing, these trials also provide a comparison condition with minimal stimulation to serve as the implicit baseline for analyses. In these blank trials, a fixation cross was presented for 500 ms followed by a blank screen for 1600 ms. Thus, with this design, the fMRI activation during each trial type can be randomly sampled, so that unbiased comparisons can be made of brain regions engaged across specific trial types. Prior to scanning, all participants received practice trials on the dot probe task using a different set of faces than the stimulus set used in the experiment. Practice continued until participants were comfortable performing the task properly (typically 10-30 practice trials).

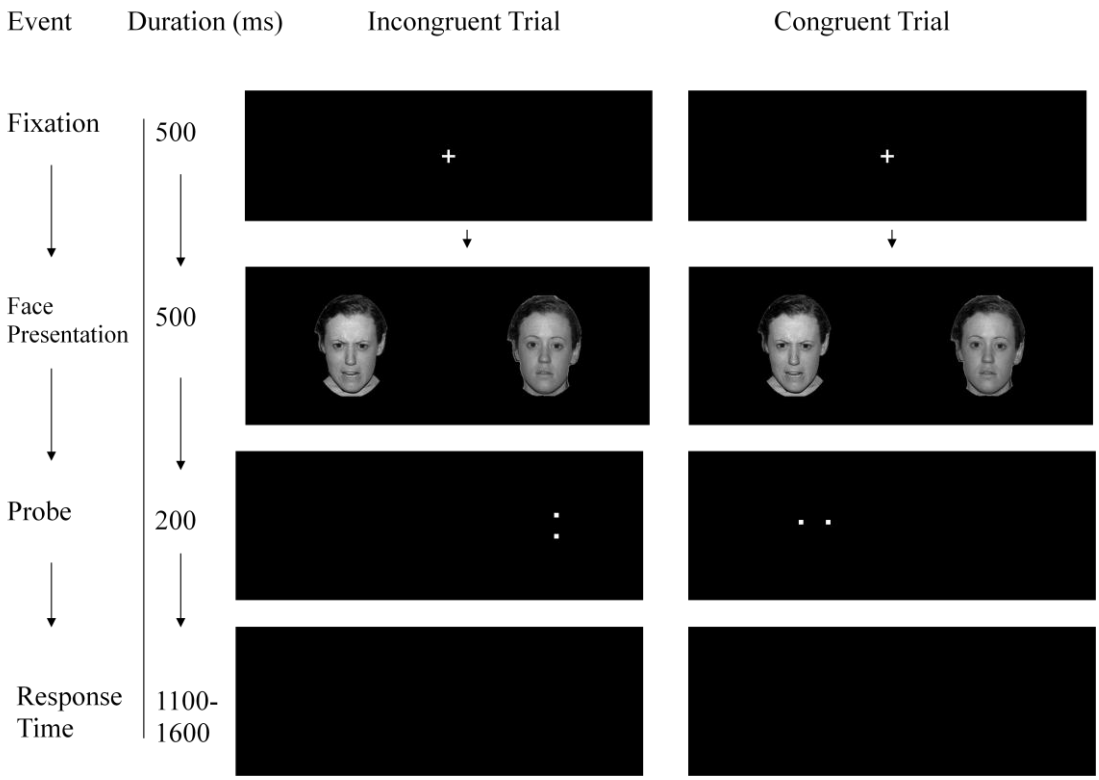


Figure 2. In the dot probe paradigm there are two types of trials included in the dot probe paradigm, incongruent and congruent trials.Congruent trials occurred if the probe was located on the same side as the emotional face (angry or happy) and incongruent trials occurred if the probe was located on the opposite side as the emotional face.

3.3 **BEHAVIORAL DATA ANALYSIS**

Participants were required to perform correctly on at least 75% of task trials; subjects unable to meet this standard were excluded. Individual task trials were excluded from analyses if participants responded in less than 200 ms or more than 800 ms after probe presentation (Monk, Nelson et al. 2006; Monk, Telzer et al. 2008). As in prior studies, attention bias for emotionally-evocative faces was calculated by subtracting the mean reaction time (RT) of trials in which the target probe appeared at the location of the emotional face instead of the neutral face (i.e., congruent trials) from the mean RT of trials in which the target appeared at the location of the neutral face instead of the emotional face (i.e., incongruent trials). Positive values indicate bias towards emotion, whereas negative values indicate bias away from emotion. Separate bias scores were calculated for the angry and happy face-event types.

In **Study I**, analysis generated correlations between age and performance for two behavioral parameters of interest: overall mean RT and percent error. This analysis was considered secondary since most prior research on emotion-attention interactions examines bias scores. Additional analysis used paired t-tests between congruent and incongruent events in smaller sub-samples formed by including subjects in relatively narrow age ranges (age ranges: 9-12, 13-17, 18-30, 31-40). This analysis determined if bias scores differed from zero in each specific age-related sub-sample. These analyses were considered secondary in light of low statistical power, given the small sample sizes and expectation of small point estimates for bias scores, based on prior work with the dot probe task.

In **Study II,** group differences (trauma exposed parents vs low exposed parents and children of exposed parents vs children of non-exposed parents) in attention bias were examined using independent sample t-tests. Significant bias scores within each exposure to trauma group were tested using one-sample t-tests. Group differences in trauma severity were examined using Chi-square, Fisher's Exact Test. Significant correlations between symptom severity and attention bias scores were tested using Pearson's correlation. All significant findings were denoted using alpha=0.05.

In **Study III,** the bias scores and mean RTs for each condition and the overall RT across the experiment were calculated for the entire group and separately for the two genotyped groups (s-carriers and l-l). In SPSS, one way ANOVA using genotype as a between group factor compared the RT's for the accuracy rate, RTs and biases between s-carriers and l-l genotypes. A one way ANOVA was used to determine significant attention bias (i.e. attention bias > 0). Statistical significance was determined by p=0.05.

3.4 **FMRI**

Functional Magnetic Resonance Imaging (fMRI) is a technique that is used to detect the localized changes in blood flow and blood oxygenation that occurs in the brain in response to neural activation. This imaging technique is one of the most

common tools used to investigate the association between a person's behavior and the neural architecture supporting that behavior.

Work in the basic sciences emphasizes the need to use developmental approaches to extend research on threat orienting among mature organisms. Individual differences in threat-related behaviors among rodents and non-human primates are powerfully shaped by developmental factors, including both environmental and genetic influences (Gross and Hen 2004). Therefore, the behavior of the adult organism when confronted with a threat reflects the ontogenetic influences of early-life experiences. Efforts to extend this line of research to human children are limited by the invasiveness of most methods used in developmental psychobiology. The availability of functional magnetic resonance imaging (fMRI) has dramatically enhanced the ability to conduct studies of human neural development. While relatively few studies examine developmental aspects of threat processing, the available literature raises major questions. For example, some studies find comparable engagement of fear-circuitry structures in children, adolescents, and adults during threat exposure. Other studies document robust developmental differences. Methodological limitations preclude firm conclusions in this area. In particular, more developmental imaging studies are needed that employ behavioral paradigms relevant to fear processing during the acquisition of fMRI data.

One of the reasons this technique is so popular is that it is non-invasive and can be performed many times on the same participant. The spatial resolution, referring to the ability to identify brain activation in deep structures, is superior to other imaging techniques such as EEG or MEG, although the temporal resolution referring to the ability to identify brain activation over time; is relatively inferior.

Once fMRI data has been collected and preprocessed (see section 3.5 and 3.6 for details), the data is modeled to the general linear model. The general linear model is an equation that links our observations (data) to our expectations (our experiment). The model is estimated for very voxel, a unit of space, in the brain along time that is compartmentalized into different conditions determined by the design of the experiment. Is is assumed that the errors are indepentant and normally distributed across all subjects and conditions.

3.5 FMRI DATA ACQUISITION

In **Study I**, images were collected on a General Electric 3 Tesla scanner (Waukesha, WI). During the scanning session, both anatomical and functional images were obtained. Parameters for T2 weighted functional echo planar image (EPI) data collection were: 240mm field of view (FOV), matrix size of 64 x 64, 29 continuous slices 3.3mm thick, repetition time (TR) of 2300ms, echo time (TE) of 23ms, and a 90 degree flip angle. The parameters for the anatomical images were: 256mm field of view (FOV), 256 x 256 matrix size, 180 continuous slices, 1.0 mm slice thickness, TR: 11.4 ms, TE: 4.4 ms, time to return to equilibrium (TI) 300ms. Imaging data were processed and analyzed using AFNI software version 2, 2006_06_30_1332 (Cox 1996). Movement was monitored for each participant and data from those participants who moved more than 2.5mm in any plane were excluded from the analysis.

In **Study III,** images were collected on a General Electric Signa Echo Speed 1.5 Tesla scanner (Waukesha, WI) at the MR Center/Stockholm Brain Institute in Stockholm, Sweden using an 8 channel head coil. During the scanning sessions, both anatomical and functional images were collected. Parameters for T2 weighted functional echo planar image (EPI) data collection were: 240mm field of view (FOV), matrix size of 64 x 64, 32 continuous slices 3mm thick, repetition time (TR) of 2500ms, echo time (TE) of 40ms, and a 90 degree flip angle. The parameters for the anatomical images were: 250mm field of view (FOV), 256 x 192 matrix size, 200 continuous slices 1.0 mm thick, TR: 24 ms, TE: 6ms.

Study II was a home based behavioral study and did not use fMRI.

3.6 FMRI DATA PROCESSING AND ANALYSIS

Each participant's EPI data were motion corrected, registered to the anatomical image, and concatenated across runs. Functional data were smoothed with a 6 mm full width at half maximum isotropic Gaussian filter. After preprocessing, individual-level data were analyzed using multiple regression. Five conditions were specified as regressors: angry-congruent, angry-incongruent, happy-congruent, happy-incongruent,

and neutral. Trials with errors were included as events of no interest. Contrast values were created based on the five conditions. Given the prior literature on attention bias, our main interest was on contrasts of incongruent (relative to congruent) event classes for both anger and happy emotion conditions. This contrast focused on the happy and angry attention biases. After individual models were created, participant's anatomical and functional data were converted into Talairach space (Talairach and Tournoux 1988).

In **Study I,** a random effects model was used to analyze fMRI data at the group level using a general linear model with a two-tailed, whole-brain corrected *p*-value < 0.05. The AFNI *3dRegAna* program was used to regress whole brain contrast values with age for the selected contrasts. These analyses examined two contrasts: i) happy incongruent vs. happy congruent and ii) angry incongruent vs. angry congruent. Analyses relied on an initial whole-brain Monte Carlo simulation set at $p = 0.005$ for each contrast of interest, examining specifically the regressions between age and activation. Using this initial $p = 0.005$ threshold in the simulation, for each contrast, the whole-brain-corrected significant $p < 0.05$ threshold, based on 1000 Monte Carlo simulations, was determined to require a cluster size of 809 voxels. Thus, the final t-maps are corrected at the whole-brain level. After defining whole-brain significant clusters, mean percent signal change values were extracted from the caudate and cuneus clusters for both contrasts corresponding to happy and threat bias and correlated with age for illustrative purposes. Secondary analyses were designed to parallel secondary analyses of behavioral data in smaller sub-samples including subjects in relatively narrow age ranges (age ranges: 9-12, 13-17, 18-30, 31-40 years old).

In **Study III,** after preprocessing, individual-level data were analyzed using multiple regression. Six conditions were specified as regressors: angry-congruent, angry-incongruent, happy-congruent, happy-incongruent, neutral and incorrect trials. Five conditions were specified as regressors of interest. Incorrect trials and six motion parameters were also included in the model as covariates of non interest. Regression coefficients were calculated in the model. Contrasts of coefficients were generated to represent angry bias (angry incongruent –angry congruent) and happy bias (happy i-happy c), angry vs. baseline, happy vs baseline based of 5-HTTLPR genotype groups.

The region of interest (ROI), in **Study III** was the amygdala. We extracted percent signal change, i.e. averaged brain activation, for all voxels in the anatomically defined amygdala, and analyzed these data using conventional statistical tests. Using a similar approach as the behavioral data, we examined the amygdala percent signal change in response to attention bias. Here, an ANOVA compared activation between genotype groups, for the contrast of angry-incongruent minus angry-congruent trials. For other brain regions beyond the amygdala, we relied on voxel-wise methods to compare our activation between the 5-HTTLPR genotype groups using 3dttest. A whole brain Monte Carlo was preformed to correct for multiple corrections in the whole brain using a grey matter mask. We used the AlphaSim procedure in AFNI to determine how many significant spatial clusters are needed to achieve a significant corrected activation within the whole brain. Based on 1000 Monte Carlo simulations and initial cluster threshold of $p < 0.01$ we determined that the significant cluster size for the whole brain to be 56 voxels.

3.7 DNA EXTRACTION AND GENOTYPING

In **Study III**, DNA was extracted from either whole blood or saliva samples. Saliva samples were collected using a DNA genotek ORANGENE DNA kit. Genotyping of 5-HTTLPR/rs25531 was preformed as previously described (Lonsdorf, Ruck et al. 2009). Genotyping for COMTval158met genotypes was performed using the 5' Nuclease (TaqMan®) assay (Livak, 1999). The TaqMan® assays were ordered from Applied Biosystems and the kits contain two allele specific probes, complementary to the teo alleles of the respective SNP. Fluorescence measurements were performed using an ABI7900 DNA analyzer (Applied Biosystems) and the SDS package. Genetic data was not used in studies I and II.

4 RESULTS AND DISCUSSION

4.1 STUDY I

Normative data on development of neural and behavioral mechanisms underlying attention orientation toward social-emotional stimuli: An exploratory study.

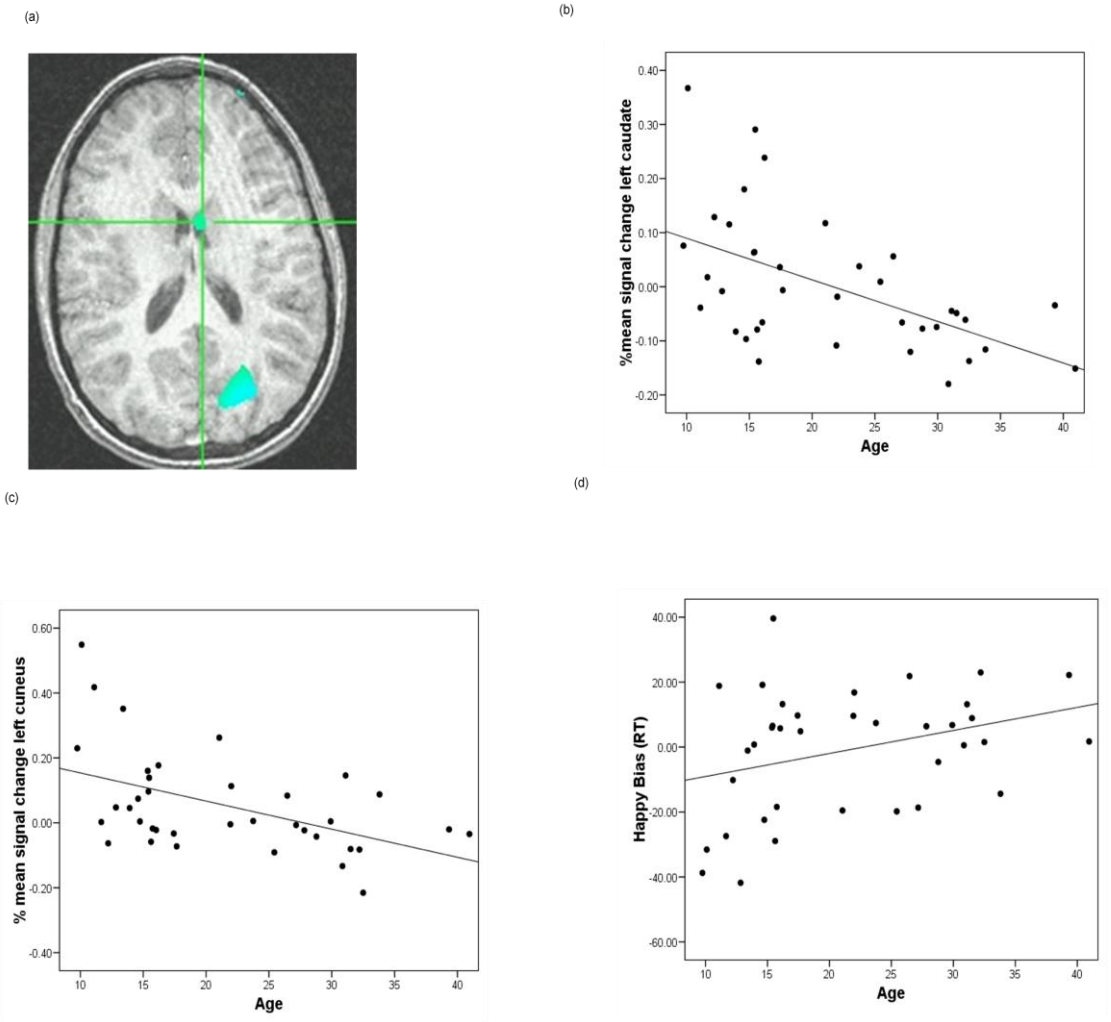
In **Study I**, we examined age-related correlations in attention-orienting biases to positive and negative face emotions in a healthy sample (9 to 40 years old) using functional magnetic resonance imaging and a dot probe task. The dot probe task in an fMRI setting yields both behavioral and neural indices of attention biases towards or away from an emotional cue (happy or angry face). Behavioral response data indicated a positive correlation between age and happy-face bias: younger participants showed less bias toward happy, relative to neutral, faces than did older subjects. Angry-face attention bias scores did not correlate with age. Relative to older participants, younger participants demonstrated greater activation in the left cuneus and left caudate on the contrast of trials used to assess happy-face attention bias.

Whole Sample Behavioral Data

One sample *t*-tests indicate that overall, independent of age, participants displayed a small (9 ms) but significant attention bias toward the angry faces relative to the neutral faces, $t(37) = 3.09$, $p < 0.05$. No such bias was detected for the happy faces $t(37) < 1$.

The main behavioral analyses examined correlations between age and each bias score. Happy face bias scores correlated positively with age, $r(37) = 0.32$, $p < 0.05$, such that older participants showed greater bias towards happy, relative to neutral, faces. Angry face bias scores did not correlate with age, $r(37) = 0.17$, $p = 0.94$.

Figure 2. (a) Activation map depicting a negative correlation between age and activation in the left cuneus and left caudate for the contrast that yields happy bias (happy incongruent vs. happy congruent trials). Cross-hairs are centered on the left caudate (x = -1.8, y = 7.7, z = 16.9); also shown is the left cuneus (x = -24.4, y = -74.1, z = 19.4). Percent signal change values were extracted from each region of interest and graphed for illustrative purposes. (b) Correlation between age and percent signal change in the caudate, Pearson r (37) = -0.54, p = 0.001 (c) Correlation between age and percent signal change in the cuneus, Pearson r (37) = -0.49, p = 0.002, across all participants for the happy bias contrast. (d) Scatter plot of age and happy bias (happy incongruent – happy congruent)



Whole Sample fMRI Data

To explore associations between age and brain activity during performance on the dot probe task, we first used age as a predictor of activation, across the brain, in two contrasts: angry incongruent vs. angry congruent and happy incongruent vs. happy congruent (i.e., the same events used to calculate angry bias and happy bias). Following a whole-brain Monte Carlo procedure correcting for multiple comparisons at the $p < 0.05$ level, no associations were found between brain activation and age for the angry-bias contrast. However, for the happy-bias contrast, activation within the left caudate and left cuneus correlated negatively with participant's age. Secondary analyses clarified factors contributing to age-related patterns for happy bias. Analyses for mean percent signal change within the two largest clusters, the caudate and the cuneus, indicated that as age decreases, activation increases in these regions (Pearson r (37) = -0.54 and -0.49, p = 0.001 and 0.002, for the left caudate and left cuneus, respectively). Follow-up analyses were conducted to decompose the nature of these interactions. For both areas, the correlation between age and happy bias reflected negative correlations between age and BOLD signal in the happy congruent trials (i.e., trials in which the target probe replaced the happy face of a happy-neutral face pair), and positive correlations between age and BOLD signal in the happy incongruent trials (i.e., trials in which the target probe replaced the neutral face of a happy-neutral face pair). Finally, we also used these extracted BOLD values to examine correlations between RT measures of bias and brain activation. There was no significant correlation between the happy-face attention bias (derived from the RT data) and the corresponding contrast of BOLD values in the left cuneus and left caudate (Pearson r (37) = -0.16 and -0.08, p = 0.35 and 0.63, for the left caudate and left cuneus, respectively).

Behavioral and fMRI Data in Narrowly-Defined Age Groups

Results from primary analyses revealed associations between reaction time and neural response in the cuneus and caudate and age, across the entire sample. A set of secondary analyses examined the degree to which these specific age-related

behavioral or fMRI patterns on reward-related events (happy faces) emerged in more narrowly-construed, age-delimited samples. This secondary analysis divided the subjects into four unique subgroups. Briefly summarized, the youngest subjects (age range: 9 -12 years old) showed a trend (t (6) = 2.3; p = 0.056) for an attention bias away from happy faces (as indicated by RT data), but bias did not differ significantly from zero in any other subgroup. For fMRI data, the oldest subjects (age range: 31-40 years old) showed a statistically significant caudate activation to happy bias, with lower signal in incongruent relative to congruent happy-face trials. No other subgroup showed significant activations in response to attention bias contrasts in fMRI data.

4.2 STUDY II

Attention Orientation in Parents Exposed to the 9/11 Terrorist Attacks and Their Children

Parents exposed to the 9/11 World Trade Center attacks and their children were recruited from the New York Metropolitan area. Using home-based procedures, psychiatric symptoms, trauma exposure, and attention bias to threat as measured with the dot probe task were each assessed in 90 subjects, comprising 45 parents and their children. Associations among levels of trauma exposure, psychopathology, and attention bias were examined in parents and their children separately. In addition, the association between attention bias in parents and children was examined.

Trauma and Attention Bias in Parents and Children

Partipant groups are based on the presence or absence of severe 9/11-related trauma in the parent. Groups did not differ on demographic variables, and rates of psychopathology were low in both groups of parents and their children, not differing as a function of exposure status in parents. There were relatively low occurances of major depressive disorder and PTSD, two disorders frequently linked to trauma in prior research. Analyses available on request did examine associations between measures of psychopathology and attention bias, both in parents and their offspring. While these revealed no associations between any measure of bias and psychopathology, the

analyses are limited by the low rates of psychopathology in both parents and their offspring.

Behavioral data was calculated on sample as a whole as well as in the high and low-exposure groups of parents and their children, classified based on trauma in the parent. Both groups of parents and their children performed the task well, as indicated by generally low error rates. The key analysis for the dot probe task was for the threat-bias score. This key analysis showed that high-exposure in parents predicted bias towards angry-face cues in parents ($t_{14.6}$=-2.2; $p< 0.05$). Among parents, no group differences occurred in response to happy faces. No relations emerged between trauma in parents and bias in children.

Table 2. Dot probe performance in exposed and non-exposed parents and their children

*- p<.05; Parent diagnosis and symptom counts are from CIDI; Child diagnosis and symptom counts are from DISC; FET=Fisher Exact Test

	All Parents	Exposed Parents	Non-exposed Parents	Statistics	All Children	Children of Exposed Parents	Children of Non-exposed Parents	Statistics
Number	45	14	31		45	14	31	
DOT PROBE								
Threat Bias	4.4 ± 36	26.8± 49	-5.0 ± 24	t_{43} =-2.2*	-0.2 ± 25	1.4 ± 33	-0.9± 22	t_{43} = -0.3
Happy Bias	8.9 ± 34	-0.4 ±35	12.8±33.4	t_{43} = 1.2	10.6 ±30	4.7 ± 26	13.4±32	t_{43} = 0.9
Neutral RT	551±104	520±69	565±114	t_{43} = 1.3	483±88	478±59	486±99	t_{43} = 0.3
%Errors	2.2 ± 3.4	2.5 ± 5.0	2.1± 2.5	t_{43} = -0.4	1.3 ± 3.5	0.5 ± 0.8	1.7 ± 4.1	t_{43} = 1.0

A final set of analyses compared children with and without severe 9/11-related trauma exposure, based on their own personal (as opposed to parental) exposure. This analysis was limited by the small number of children with severe, personal exposures (n=4). Much like data in parents with high exposure, these four children did exhibit a bias towards angry faces (23.0 ± 28.1), whereas children with low exposure showed no tendency for such bias (-2.5 ± 24.9). However, this difference was not statistically significant (t_{42}= -1.9; p= 0.06). Bias to happy faces was highly similar in children with (14.0 ± 25.7) or without (10.3 ± 31.0) high exposure.

Attention Bias in Parents and Children

No association emerged between bias measures in parents and children. Considering all 90 subjects (45 pairs) together, parent-child attention bias measures showed non-significant negative correlations, both for threat bias (r=-0.24) and for happy bias (r=-0.16). Non-significant relationships also emerged in secondary analyses examining parent-child bias-score correlations in sub-sets of families, formed by stratifying families based on levels of trauma exposure.

Parents experiencing severe trauma showed greater attention bias towards threat than parents experiencing no such trauma, but trauma experienced by parents did not predict attention bias in their children. Moreover, attention bias in parents showed no association with attention bias in their children. Family-based research can feasibly assess trauma exposure, psychopathology, and attention bias in the home. Assessed with such methods, trauma exposure experienced by a parent predicts attention bias in a parent but not in their child. This attention bias was found four-to-five years after 9/11, suggesting that the cognitive effects of trauma on threat processing endure. Larger, prospective studies might use home-based assessments to examine relationships within families among traumatic exposures, psychopathology, and information-processing functions.

4.3 STUDY III

5-HTTLPR genotype influence on right amygdala activation during threat orientation

Using event-related, functional magnetic resonance imaging (erfMRI) paired with a visual-probe threat-orienting task, we compared amygdala response to angry faces in psychiatrically healthy 5-HTTLPR genotyped adults (n=60). In this threat-orienting task, participants viewed angry/neutral face pairs. After viewing each face pair, participants indicated by button-press whether a subsequent probe appeared on the same (congruent) or opposite (incongruent) side as the angry face. Response time differences between congruent and incongruent face trials provided a measure of attention orientation towards or away from angry faces. Slower RT's to angry faces on

the opposite visual field (incongruent) and a faster RT to angry faces on the same visual field (congruent) indicate an attention bias towards threat.

Behavioral Results

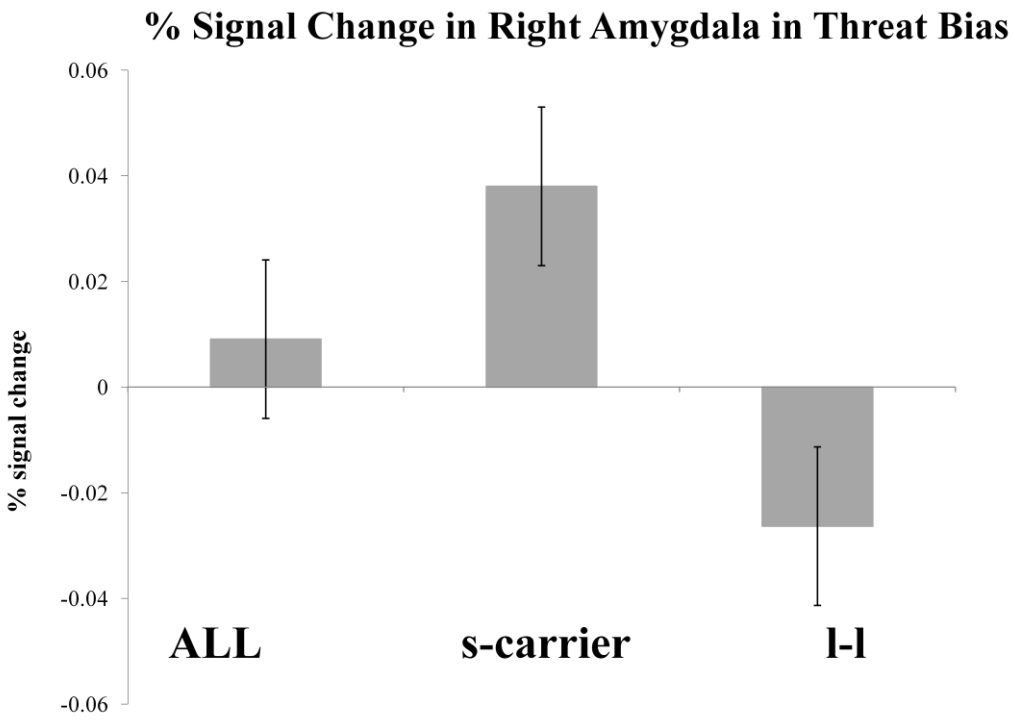
Behavioral data indicated no differences between the two genotyped subject populations (s-carrier and l-l) on any measure ($F<0.53$ for all comparisons). Specifically, there was no difference between s-carrier (mean=-2.04ms ± 26.90) and l-l genotypes (mean=-3.25 ± 27.01) in threat bias scores ($F_{59}=0.03$, $p=0.86$). In addition, there was no difference of the threat bias in the population overall when compared to zero (mean =-2.58 ±26.73; $t_{59}=-0.74$, $p=0.45$).

fMRI Results

Using the extracted data from the right amygdala anatomical mask, s-carriers, compared to non-carriers (l-l) exhibited increased amygdala activation during the threat bias contrast (angry incongruent-angry congruent) (one-way ANOVA, $F= 4.85$, $p=0.03$). The pattern of activation of the amygdala during the threat bias condition in the s-carrier group was driven by an increased activation during the angry incongruent trials compared with the slight deactivation in the angry congruent trials.

In addition, we conducted whole brain analyses corrected for multiple comparisons at the whole-brain $p = 0.05$ level on the whole group, not separate by genotype. In the threat bias contrast (angry incongruent- angry congruent), the right cuneus (178 voxels) and left middle occipital cortex (185 voxels) surpassed the significant cluster size threshold of 56 voxels.

Figure 4. **% Signal Change in Right Amygdala to Angry Faces**
Bar graph of % signal change extracted from an anatomical right amygdala mask in the threat bias (angry incongruent-angry congruent) condition. The subjects are grouped as a whole and by the 5-HTTLPR genotypes, s-carrier and l-l.



Relative to l-l, s-carriers showed greater right amygdala activation to trials containing angry faces. Because similar levels of threat bias were found in the two genotype groups, these findings suggest that s-carriers exhibit a lower threshold for engaging the amygdala. These results have implications for the susceptibility to psychopathology and information on the genetic factor on anxiety behavior and the underlying neural structure.

5 CONCLUSIONS

Angry-face attention bias scores did not correlate with age, and age did not correlate with brain activation to angry faces. However, age did positively correlate with attention bias towards happy faces; age also negatively correlated on a happy-bias fMRI contrast with activation in the left cuneus and left caudate. Secondary analyses suggested the presence of age-related changes in attention to happy faces, which shifts with increasing age from attention avoidance to attention monitoring, in tandem with increasing neural deactivation.

Parents exposed to trauma allocated attention towards threatening angry faces. Their children did not have a bias toward threat. These findings indicate that trauma has a long lasting effect on threat orientation. Although the parents were affected by trauma, they did not transfer this behavior to their children who where not exposed to trauma.

Compared to l-l, s-carriers showed greater right amygdala activation to trials containing angry faces of the dot probe task. Because similar levels of threat bias were found in the two genotype groups, these findings suggest that s-carriers exhibit a lower threshold for engaging the amygdala within the context of the task.

6 GENERAL DISCUSSION

The aim of this thesis was to examine the behavioral and neural correlates of emotional orientation. First, what is the normative maturation of emotional orientation? Second, how does exposure to trauma affect emotional orientation? Finally, is there association between 5-HTTLPR allelic variation to threat orientation in psychiatrically healthy adults?

In Study I, increasing age was associated with a greater bias towards happy faces. By contrast, older individuals showed less neural engagement to the happy bias contrast. Such an opposing pattern for behavioral and neural data, in terms of associations with age, might reflect diverse underlying factors. For example, the greater attention bias to happy faces in older subjects, occurring in tandem with the lower levels of neural engagement in the cuneus and caudate, might reflect differences in efficiency of activation-behavior associations. From this perspective, lower fMRI activation with age, in the context of similar or even opposite-appearing behavioral response patterns, may reflect the requirement of greater brain activation to support one or another behavioral repertoire in the younger individuals, whose engagement of brain regions may be "less efficient" (Ochsner, Bunge et al. 2002). This pattern might suggest that rewards have the capacity to engage the brain at particular points in development. This idea is consistent with prior work suggesting the importance of developmental change in the salience of rewarding social stimuli (Nelson, Leibenluft et al. 2005).

Unlike the happy bias finding, analyses of both behavioral and fMRI data for angry faces were consistent in that neither revealed an association with age. Interpretation of null results is qualified by many constraints, particularly small sample sizes. Nevertheless, the null finding cannot be completely discounted, given that the failure to observe associations with age emerged in the context of a significant overall threat bias in the sample as a whole. This finding supports suggestions that attention to threat is a core function that facilitates survival and adaptive social behavior (Damasio 1999; Ohman 2002). Although the present finding of an overall threat bias is consistent with this view, it appears to contrast with the findings of a meta-analysis of face-based

dot-probe tasks, indicating no bias for angry faces in non-anxious participants (Bar-Haim, Lamy et al. 2007). Nevertheless, previous work also indicates that state-related factors (e.g., situational stressors) can increase threat bias (Bar-Haim, Lamy et al. 2007). In addition, Helfinstein et al. (2008) found that even mildly aversive social threat primes can induce a bias towards threat in healthy non-anxious subjects.

An example of a situational stressor is the exposure to a traumatic event such as the attack on the WTC on 9/11/2001. Study II classified adults and their children based on their own, personal experiences with exposure to trauma. The main finding, concerning heightened threat bias in parents with severe trauma exposure, can be placed in the context of considerable previous research. Trauma-related and other forms of anxiety consistently predict biased allocation of attention to threat on the dot probe and other attention bias tasks (Bar-Haim et al., 2007; Williams et al., 1996). Such associations emerge in research on diverse clinical, anxiety-related constructs, including trait anxiety, personality measures, and clinical diagnoses, such as PTSD. The most consistent findings document a bias towards threats, such as angry faces or anxiety-related words, in anxious subjects, which significantly differs from the profile in non-anxious subjects, who typically show no bias either towards or away from threats.

Few previous studies have examined relationships between severe trauma exposure and attention bias in a non-clinical sample. Far more work using the dot probe task compares anxious-traumatized subjects or subjects with PTSD to subjects with neither anxiety nor trauma (Bar-Haim, Lamy et al. 2007). As such, these studies predominantly examine people who are actively struggling with their reactions to trauma, either because trauma has occurred recently or because they have failed to overcome their initial reactions to prior trauma. The unique circumstances of Study II extend this prior work. Parents in the current study were exposed to severe trauma four-to-five years prior to the assessment of attention, and, unlike in prior studies, the majority was free of psychopathology. Thus, the current results suggest that the impact of long-ago experienced severe trauma may endure, as is reflected in measures of threat bias, even when clinical effects are not detected via psychiatric assessments.

Study II demonstates the effect of the environment on behavior without the presence of psychopathology. In Study III we examined the effects genetics on

behavior and the brain without the presence of psychopathology. Prior studies in healthy subjects find with some consistency that s-allele carries exhibit an attention bias towards threat cues (Beevers, Gibb et al. 2007; Perez-Edgar, Bar-Haim et al. 2010). As a result, the absence of such a finding Study III requires an explanation. Namely, the point estimates for attention bias scores in both 5-HTTLPR appeared nearly identical, and neither group showed any evidence of a bias towards threat. While this is consistent with prior results in healthy subjects, one would expect some evidence of a bias towards threat in s-allele carriers, even in studies with limited statistical power.

Considered individually, the current behavioral and imaging findings can be reconciled with prior findings for the 5-HTTLPR. However, questions arise related to the discordance between the two sets of findings in the current study. Why do s-allele carries differ in terms of amygdala engagement but not in terms of behavioral performance? Based on the frequency with which such "brain-behavior" dissociations arise, major questions exist in fMRI research on the nature of relationships between behavior and activation. Within the context of face-processing tasks and the 5-HTTLPR, observations of amygdala activation differences in the absence of behavioral differences have been the rule, rather than the exception (Meyer-Lindenberg and Weinberger 2006). Prior explanations generally view such findings as reflecting the enhanced sensitivity of brain imaging measures to detect gene-related between-group differences. This view, in turn, emerges from the contention that gene-to-behavior relationships arise through two other mediated relationships, one connecting genes to brain function and a second connecting brain function to behavior.

7 FUTURE DIRECTIONS

To further explore the effects of a person's environment and innate characteristics, such as genes, on emotional orientation additional research is needed.

The three studies highlighted in this thesis use fMRI and behavioral testing to explore the behavior and neural correlates of emotional orientation. These tools provide important information about the underlying neural mechanisms realted to attention orientation. Future studies may use MEG to further explore the temporal effects of emotional orientation. With MEG we will be able to gain the temporal resolution needed to separate the neural reaction to the face presentation from the attentional orientation to the neutral and angry or happy face. This attentional decision to attend or avoid is yet to be fully explained.

As the fields of neuroimaging, genetics, psychology and psychiatry are developing, more cross field research is needed. We know that emotional orientation is affected by age, but how is the normative maturation of emotional orientation affected when a trauma occurs. In Study II, we examined 4 children who were exposed to trauma, yet this group is too small to gain statistical significance. Future studies may study a broad age range of psychiatrically healthy children and adult exposed to trauma to examine how trauma affects the normative maturation of emotional processing.

8 ACKNOWLEDGEMENTS

The processof obtaining a PhD can be a lonely and frustrating task. I feel that I am one of the lucky ones- I have had a team of people spanning the world that have helped me achieve this goal.

To my graduate mentors, Martin Ingvar and Danny Pine, I sincerely thank you for sharing you knowledge with me. I appreciate your patience, support and willingness to share your mind with me.

To all my subjects worldwide, I thank you for your time and commitment in participating in my studies. Without you my questions would be left unanswered.

To my NIH lab (former and present), Eric Nelson, Amanda Guyer, Chris Monk, Ellen Leibenluft, Jen Lau, Yair Bar-Haim, Monique Ernst, Sven Mueller- Thank you for your guidance and support.

To Jen Britton, Thank you for your friendship, mentoring, invalueable advice and editing.

To the 'KATs' Armita and Tina- Thank you for all of your help and for keep me smiling through the long scanning nights.

To myNIH and KI assistants- Michelle, Lindsey, Eric, Andrew, Markus, Katerina, Christin, Anders- Thank you all for your assistance on scanning and data management our research projects.

To the 'Brain Toys' group- Karin, Sissela and Valeria, Thank you for our discussions and for your contagious excitement.

To the Stingfellows gang- Jeremy, Jonathon, Armita, Fredrik, Thank you for the enlightened discussions- scientific…. and not.

To Peter Fransson and Predrag Petrovic - Thank you for our discussions and your encouragement.

To the SBI Juniors- Anke, Orjan, Linda, Lea, Simon C, Deepak, Kattis, Julia, Stina, Fredrick, Jonathan, Jeremy, Armita, Tina, Natalie, Jonas, Jesper, Fiona, Sissela,Valeria, - Thank you for our discussions over many conferences and for sharing your science.

To Mimmi, Thank you for guiding me through the Swedish paperwork with ease and for taking this American chick under your wing.

To my fellow NIH-KI students- Carolyn- We started this journey together and we will finish it together. Thank you for your friendship and a warm place to stay. Tracy Jill- Thank you for your advice and for reminding me that 'it is all good.' Theresa, Catherine, Jeff, David, Cynthia, Garth and Nick- Thank you for being trail blazers for the rest of us to follow.

To Brenna Argall, Thank you for your friendship and for loaning me your overanalytical mind for editing assistance. We have many more adventures in our future.

To Devon Oskvig, Thank you for reading this thesis and our NIH coffee break talks.

To my brother and sister in law, John and Becca- Thank you for your support and the early visit to Sweden.

To my new family, Bob Brink, Debby Brink and John and Eliza Dermody- Thank you for your support, editing, feeding me, housing me… Thank you for welcoming me into your family.

To my parents, Bill and Lullo Lindstrom- You are only as strong as your foundation. Thank you for love and support.

To my David, Thank you for your sacrifice and your constant support. You have given my heart a permenent home. I love you.

9 REFERENCES

Amaral, D. G., H. Behniea, et al. (2003). "Topographic organization of projections from the amygdala to the visual cortex in the macaque monkey." Neuroscience **118**(4): 1099-1120.

Armony, J. L. and R. J. Dolan (2002). "Modulation of spatial attention by fear-conditioned stimuli: an event-related fMRI study." Neuropsychologia **40**(7): 817-826.

Bar-Haim, Y., Y. Holoshitz, et al. (2010). "Life-threatening danger and suppression of attention bias to threat." Am J Psychiatry **167**(6): 694-698.

Bar-Haim, Y., D. Lamy, et al. (2007). "Threat-related attentional bias in anxious and nonanxious individuals: A meta-analytic study." Psychological Bulletin **133**(1): 1-24.

Bar-Haim, Y., D. Lamy, et al. (2007). "Threat-related attentional bias in anxious and nonanxious individuals: a meta-analytic study." Psychol Bull **133**(1): 1-24.

Beevers, C. G., B. E. Gibb, et al. (2007). "Serotonin transporter genetic variation and biased attention for emotional word stimuli among psychiatric inpatients." J Abnorm Psychol **116**(1): 208-212.

Bishop, S., J. Duncan, et al. (2004). "Prefrontal cortical function and anxiety: controlling attention to threat-related stimuli." Nat Neurosci **7**(2): 184-188.

Bishop, S. J. (2009). "Trait anxiety and impoverished prefrontal control of attention." Nat Neurosci **12**(1): 92-98.

Bradley, B. P., K. Mogg, et al. (1999). "Attentional bias for emotional faces in generalized anxiety disorder." Br J Clin Psychol **38 (Pt 3)**: 267-278.

Breslau, N. (2002). "Epidemiologic studies of trauma, posttraumatic stress disorder, and other psychiatric disorders." Can J Psychiatry **47**(10): 923-929.

Calvo, M. G. and M. D. Castillo (2005). "Processing of threat-related information outside the focus of visual attention." Span J Psychol **8**(1): 3-11.

Cox, R. W. (1996). "AFNI: software for analysis and visualization of functional magnetic resonance neuroimages." Comput Biomed Res **29**(3): 162-173.

Damasio, A. R. (1999). The Feeling of What Happens. New York, Harcourt Brace.

Elsesser, K., G. Sartory, et al. (2004). "Attention, heart rate, and startle response during exposure to trauma-relevant pictures: a comparison of recent trauma victims and patients with posttraumatic stress disorder." J Abnorm Psychol **113**(2): 289-301.

Fox, E., A. Ridgewell, et al. (2009). "Looking on the bright side: biased attention and the human serotonin transporter gene." Proc Biol Sci **276**(1663): 1747-1751.

Fox, E., R. Russo, et al. (2001). "Do threatening stimuli draw or hold visual attention in subclinical anxiety?" J Exp Psychol Gen **130**(4): 681-700.

Fudge, J. L., M. A. Breitbart, et al. (2005). "Insular and gustatory inputs to the caudal ventral striatum in primates." J Comp Neurol **490**(2): 101-118.

Fudge, J. L., M. A. Breitbart, et al. (2004). "Amygdaloid inputs define a caudal component of the ventral striatum in primates." J Comp Neurol **476**(4): 330-347.

Gross, C. and R. Hen (2004). "The developmental origins of anxiety." Nat Rev Neurosci **5**(7): 545-552.

Haber, S. N., K. S. Kim, et al. (2006). "Reward-related cortical inputs define a large striatal region in primates that interface with associative cortical connections, providing a substrate for incentive-based learning." J Neurosci **26**(32): 8368-8376.

Hariri, A. R., E. M. Drabant, et al. (2005). "A susceptibility gene for affective disorders and the response of the human amygdala." Arch Gen Psychiatry **62**(2): 146-152.

Hariri, A. R., V. S. Mattay, et al. (2003). "Neocortical modulation of the amygdala response to fearful stimuli." Biol Psychiatry **53**(6): 494-501.

Hariri, A. R., A. Tessitore, et al. (2002). "The amygdala response to emotional stimuli: a comparison of faces and scenes." Neuroimage **17**(1): 317-323.

Heils, A., A. Teufel, et al. (1995). "Functional promoter and polyadenylation site mapping of the human serotonin (5-HT) transporter gene." Journal of Neural Transmission-General Section **102**(3): 247-254.

Heinz, A., D. F. Braus, et al. (2005). "Amygdala-prefrontal coupling depends on a genetic variation of the serotonin transporter." Nat Neurosci **8**(1): 20-21.

Helfinstein, S. M., L. K. White, et al. (2008). "Affective primes suppress attention bias to threat in socially anxious individuals." Behaviour Research and Therapy **46**(7): 799-810.

Hoven, C. W., C. S. Duarte, et al. (2005). "Psychopathology among New York city public school children 6 months after September 11." Arch Gen Psychiatry **62**(5): 545-552.

Kaufman, J., B. Birmaher, et al. (1997). "Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version (K-SADS-PL): initial reliability and validity data." J Am Acad Child Adolesc Psychiatry **36**(7): 980-988.

Kessler, R. C., W. T. Chiu, et al. (2005). "Prevalence, severity, and comorbidity of 12-month DSM-IV disorders in the National Comorbidity Survey Replication." Arch Gen Psychiatry **62**(6): 617-627.

Kessler, R. C., O. Demler, et al. (2005). "Prevalence and treatment of mental disorders, 1990 to 2003." N Engl J Med **352**(24): 2515-2523.

Kim, J. J. (2008). "Serotonin, stress, and conditioning." Biol Psychiatry **63**(9): 819-820.

Kleijer, W. J., V. H. Garritsen, et al. (2006). "Prenatal diagnosis of citrullinemia and argininosuccinic aciduria: evidence for a transmission ratio distortion in citrullinemia." Prenat Diagn **26**(3): 242-247.

Laor, N., L. Wolmer, et al. (2001). "Mothers' functioning and children's symptoms 5 years after a SCUD missile attack." Am J Psychiatry **158**(7): 1020-1026.

LeDoux, J. (2000). "Emotion circuits in the brain." Annu Rev Neurosci **23**: 155-184.

Little, K. Y., D. P. McLaughlin, et al. (1998). "Cocaine, ethanol, and genotype effects on human midbrain serotonin transporter binding sites and mRNA levels." American Journal of Psychiatry **155**(2): 207-213.

Lonsdorf, T. B., C. Ruck, et al. (2009). "The symptomatic profile of panic disorder is shaped by the 5-HTTLPR polymorphism." Prog Neuropsychopharmacol Biol Psychiatry **33**(8): 1479-1483.

Lucki, I. (1998). "The spectrum of behaviors influenced by serotonin." Biol Psychiatry **44**(3): 151-162.

MacLeod, C., A. Mathews, et al. (1986). "Attentional bias in emotional disorders." J Abnorm Psychol **95**(1): 15-20.

Mather, M. and L. L. Carstensen (2005). "Aging and motivated cognition: the positivity effect in attention and memory." Trends Cogn Sci **9**(10): 496-502.

McClure, E. B., C. S. Monk, et al. (2007). "Abnormal attention modulation of fear circuit function in pediatric generalized anxiety disorder " Arch Gen Psychiatry **64**: 97-106.

Meyer-Lindenberg, A. and D. R. Weinberger (2006). "Intermediate phenotypes and genetic mechanisms of psychiatric disorders." Nat Rev Neurosci **7**(10): 818-827.

Mogg, K. and B. P. Bradley (1998). "A cognitive-motivational analysis of anxiety." Behav Res Ther **36**(9): 809-848.

Mogg, K. and B. P. Bradley (2002). "Selective orienting of attention to masked threat faces in social anxiety." Behav Res Ther **40**(12): 1403-1414.

Mogg, K., P. Philippot, et al. (2004). "Selective attention to angry faces in clinical social phobia." J Abnorm Psychol **113**(1): 160-165.

Monk, C. S., E. E. Nelson, et al. (2006). "Ventrolateral prefrontal cortex activation and attentional bias in response to angry faces in adolescents with generalized anxiety disorder." Am J Psychiatry **163**(6): 1091-1097.

Monk, C. S., E. H. Telzer, et al. (2008). "Amygdala and ventrolateral prefrontal cortex activation to masked angry faces in children and adolescents with generalized anxiety disorder." Arch Gen Psychiatry **65**(5): 568-576.

Munafo, M. R., S. M. Brown, et al. (2008). "Serotonin transporter (5-HTTLPR) genotype and amygdala activation: a meta-analysis." Biol Psychiatry **63**(9): 852-857.

Nelson, E. E., E. Leibenluft, et al. (2005). "The social re-orientation of adolescence: a neuroscience perspective on the process and its relation to psychopathology." Psychological Medicine **35**: 163-174.

Ochsner, K. N., S. A. Bunge, et al. (2002). "Rethinking feelings: An fMRI study of the cognitive regulation of emotion." J Cogn Neurosci **14**(8): 1215-1229.

Ohman, A. (1986). "Face the beast and fear the face: animal and social fears as prototypes for evolutionary analyses of emotion." Psychophysiology **23**(2): 123-145.

Ohman, A. (2002). "Automaticity and the amygdala: Nonconscious responses to emotional faces." Current Directions in Psychological Science **11**(2): 62-66.

Parsey, R. V., R. S. Hastings, et al. (2006). "Effect of a triallelic functional polymorphism of the serotonin-transporter-linked promoter region on expression of serotonin transporter in the human brain." American Journal of Psychiatry **163**(1): 48-51.

Perez-Edgar, K., Y. Bar-Haim, et al. (2010). "Variations in the serotonin-transporter gene are associated with attention bias patterns to positive and negative emotion faces." Biol Psychol **83**(3): 269-271.

Pessoa, L., M. McKenna, et al. (2002). "Neural processing of emotional faces requires attention." Proc Natl Acad Sci U S A **99**(17): 11458-11463.

Pine, D. S. (2003). "Developmental psychobiology and response to threats: relevance to trauma in children and adolescents." Biol Psychiatry **53**(9): 796-808.

Pine, D. S. and J. A. Cohen (2002). "Trauma in children and adolescents: risk and treatment of psychiatric sequelae." Biol Psychiatry **51**(7): 519-531.

Pine, D. S., J. Costello, et al. (2005). "Trauma, proximity, and developmental psychopathology: the effects of war and terrorism on children." Neuropsychopharmacology **30**(10): 1781-1792.

Pynoos, R. S., A. M. Steinberg, et al. (1999). "A developmental psychopathology model of childhood traumatic stress and intersection with anxiety disorders." Biol Psychiatry **46**(11): 1542-1554.

Reimold, M., M. N. Smolka, et al. (2007). "Midbrain serotonin transporter binding potential measured with [C-11]DASB is affected by serotonin transporter genotype." Journal of Neural Transmission **114**(5): 635-639.

Smolka, M. N., M. Buhler, et al. (2007). "Gene-gene effects on central processing of aversive stimuli." Mol Psychiatry **12**(3): 307-317.

Spear, L. P. (2000). "The adolescent brain and age-related behavioral manifestations." Neurosci Biobehav Rev **24**(4): 417-463.

Spitzer, R. L., J. B. Williams, et al. (1992). "The Structured Clinical Interview for DSM-III-R (SCID). I: History, rationale, and description." Arch Gen Psychiatry **49**(8): 624-629.

Suslow, T., P. Ohrmann, et al. (2006). "Amygdala activation during masked presentation of emotional faces predicts conscious detection of threat-related faces." Brain Cogn **61**(3): 243-248.

Talairach, J. and P. Tournoux (1988). Co-planar Stereotaxic Altas of the Human Brain. Stuttgart, Georg Thieme Verlag.

Thomas, K. M., W. C. Drevets, et al. (2001). "Amygdala response to facial expressions in children and adults." Biol Psychiatry **49**(4): 309-316.

Thomason, M. E., M. L. Henry, et al. (2010). "Neural and behavioral responses to threatening emotion faces in children as a function of the short allele of the serotonin transporter gene." Biol Psychol **85**(1): 38-44.

Van Dyck, C. H., R. T. Malison, et al. (2004). "Central serotonin transporter availability measured with [I-123]beta-CIT SPECT in relation to serotonin transporter genotype." American Journal of Psychiatry **161**(3): 525-531.

Wechsler, D. (1999). Wechsler Abbreviated Scale of Intelligence (WASI). San Antonio, TX, Harcourt Assessment, Inc.

Williams, J. M., A. Mathews, et al. (1996). "The emotional Stroop task and psychopathology." Psychol Bull **120**(1): 3-24.