

5-6-2009

# Stress, Serotonin Transporter Genotype, and Emotion Processing in Children

Amy Meadows

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**Stress, Serotonin Transporter Genotype, and Emotion Processing in Children**

A Thesis Submitted to the

Yale University School of Medicine

in Partial Fulfillment of the Requirements for the Joint

Degree of Doctor of Medicine and Master of Health Science

By

Amy Lynn Meadows

2008

### **Abstract**

STRESS, SEROTONIN TRANSPORTER GENOTYPE, AND EMOTION PROCESSING IN CHILDREN. Amy L. Meadows, John Herrington, and Joan Kaufman. Child Study Center and Department of Psychiatry, Yale University School of Medicine, New Haven, CT.

Child maltreatment is a significant contributor to psychopathology, including depressive disorders and Post-Traumatic Stress Disorder (PTSD). Multiple studies have demonstrated important changes in emotion processing and regulation in children exposed to early life stress, but the underlying neural mechanism is unknown. There is significant variability in outcome in children exposed to early life stress which may be moderated by genetic polymorphisms in the serotonin transporter. This study was designed as a functional Magnetic Resonance Imaging project using dichotic listening to study emotion-processing pathways and interhemispheric transfer.

Eighteen children were recruited in a two-by-two factorial design with maltreatment and serotonin genotype as factors. Main effects of genotype were seen in increased activation in the amygdala and orbitofrontal cortex (OFC) to attended angry stimuli. No main effects of trauma (either as a categorical or continuous variable) were seen, and there was no interaction of trauma and genotype. Non-significant differences were found in the pattern of emotion processing between the maltreatment groups seen in the superior temporal gyrus. The results are consistent with a growing body of literature that has identified genotype as an important factor in neural pathways. The increased amygdala and OFC activity was seen in the controls as well as the traumatized children with the vulnerable genotype. An emerging question is how these differences lead to psychopathology in some instances and not others.

### **Acknowledgements**

I would like to begin by thanking my parents and family, whose unwavering support and untiring sacrifice have made all of the achievements in my life possible.

I also gratefully acknowledge the guidance of my mentors, Joan Kaufman, Ph.D. and Jim Leckman, M.D. The mentor of the following projects, Dr. Kaufman, has graciously opened her clinical and research group to me, helping me to understand complex clinical and scientific issues and to provide for a particularly underserved segment of the psychiatric population. As my Cohen Fellowship mentor, Dr. Leckman has helped connect me to the immense possibilities of psychiatry practice and research from our very first meeting. Dr. Leckman played an enormous role in defining my interests in traumatized children and was the first to suggest Dr. Kaufman as a potential resource.

Moreover, the following projects were possible because of the time-intensive support of Heather Douglas-Palumberi, M.A. Ms. Douglas-Palumberi has a gentle way of making the most difficult situations into intensely positive, rewarding, and empowering moments, especially for all the families which have participated. Of course, significant acknowledgement also goes to John Herrington, Ph.D. who took time to build my knowledge and understanding of fMRI research up literally from the foundation. Dr. Herrington's participation in the practical details of these projects was a crucial step.

Thanks to Andrés Martin, M.D. and Rebecca Hommer, M.D. who both reviewed an earlier version of this thesis. Thank you also to the Office of Student Research, Doris Duke Foundation, and the Yale Child Study Center who all generously supported—financially and practically—my participation in the research.

## Table of Contents

Abstract.....	2
Acknowledgements.....	3
Table of Contents.....	4
Introduction.....	6
Early Life Stress.....	6
Emotional Regulation.....	7
Prior Magnetic Resonance Imaging in Trauma.....	9
Structural MRI.....	9
Functional MRI.....	10
Novel Paradigm for Studying Affective Processing and Interhemispheric Transfer.....	12
Genetic Moderation of the Effects of Trauma.....	13
Imaging Genetics.....	15
Statement of Purpose.....	16
Methods.....	18
Participants.....	18
Table 1: 2x2 Factorial Design.....	19
Recruitment and Informed Consent.....	19
Measures Available at Time of Recruitment.....	20
Stress History.....	20
Clinical Diagnoses.....	21
Research Camp.....	21
Genotype.....	22
Assessments Obtained for fMRI Study.....	22
Clinical Update.....	22
MRI Procedures and Training in the Mock Scanner.....	23
Auditory Stimuli.....	24
Dichotic Listening Task: Training and Task Description.....	24
Pilot Feasibility.....	25
MRI Data Acquisition.....	25
Imaging Processing and Statistical Analysis.....	26
Author Contributions.....	28
Results.....	29
Clinical Data.....	29
Accuracy of Participants.....	30
Table 2: Accuracy of Participants in fMRI Protocol.....	30
Imaging Data Analysis.....	30
Analysis 1: Voxel-Based Analysis: Main Effects of Emotion.....	30
Table 3: Clusters of Activity in Anger to Neutral Comparison.....	31
Analysis 2: Interhemispheric Transfer: Superior Temporal and Broca's Area Correlation.....	31
Figure 1: Correlation between Broca and STG Activation in Low Stress Controls (N=6).....	32
Figure 2: Correlation between Broca's and STG Activation in High Stress (N=12) .....	33

Analysis 3: Anger in the Attended Ear versus Non-Attended Ear.....	34
Analysis 4: Examination of Genetic and Environmental Stress Effects of Amygdala and OFC Activation .....	35
Table 4: Effect of Genotype Amygdala and OFC Activation in Attended v. Non-Attended Angry Trials .....	35
Figure 3: Amygdala Activation in High Risk “s/s” Group in Attended v. Non-Attended Anger (N=7).....	36
Figure 4: OFC Activation in Low Risk “l/l” (N=11) vs. High Risk “s/s” (N=7) Genotype Contrast in Attended v. Non-Attended Anger.....	36
Analysis 5: Correlation between Amygdala and OFC Activation.....	37
Figure 5: Correlation between OFC and Amygdala Activation in the Attended vs. Non-Attended Contrast (N=18) .....	38
Analysis 6: Clinical Correlates of Brain Activation .....	38
Figure 6: Correlation of PTSD score with STG Activation to Angry Stimuli.....	39
Figure 7: Correlations between Subscales of SCARED and Amygdala Activation .....	40
Discussion.....	40
Limitations .....	43
Conclusion .....	44
References.....	46
Appendix 1: Stimulus Characterization .....	55
Methods.....	55
Participants.....	55
Table A.1: Behavioral Study Participant Characteristics .....	55
Table A.2: Race and Ethnicity of Behavioral Study Participants.....	55
Table A.3: 5HTTLPR Genotype.....	56
Recruitment and Informed Consent.....	56
Baseline Assessments .....	56
Dichotic Listening Stimuli.....	57
Data Analysis.....	58
Author Contributions .....	58
Results.....	58
Table A.4: Ratings of Angry and Neutral Stimuli .....	59
Table A.5: Correlation of Angry and Neutral Stimuli with Age .....	59
Table A.6: Emotion Processing by Genotype.....	59
Table A.7: Ratings of Angry and Neutral Stimuli Used in fMRI Protocol .....	60
Figure A.1: Ratings of Individual Stimuli .....	60
Table A.7: Percentage of Participants Rating “Angry” fMRI Stimuli a 4 or 5 ....	61
Table A.8: Percentage of Participants Rating “Neutral” fMRI Stimuli a 1 or 2...	61
Appendix 2: Participant Characteristics.....	63
Table A.9: Participant Characteristics .....	63
Table A.10: Race and Ethnicity of Participants.....	63

## Introduction

### **Early Life Stress**

Studies have shown that early life stress, including community, interpersonal, and intrafamilial violence, occurs at epidemic rates. In a longitudinal, community-based survey of adolescents, 40 percent had been exposed to trauma as a child.<sup>1</sup> A separate sample of urban adolescent females presenting for primary care reported upwards of ninety percent had been exposed to some form of trauma.<sup>2</sup> Even restricting the definition of trauma to intrafamilial violence, the rates are still very high. According to the National Incidence Study, approximately 1.5 million children are abused each year.<sup>3</sup> In clinical samples, 30 percent of child psychiatric outpatients and over 55 percent of inpatients had a history of child maltreatment.<sup>4-6</sup> Because of the high prevalence of trauma in clinical populations, child maltreatment, including neglect, physical and sexual abuse, and witnessing interpersonal violence, is now widely recognized as a significant public health burden.<sup>7, 8</sup>

Given the wide exposure of children to traumatic events, early life stress has been increasingly studied and recognized as a risk factor for lasting alterations in a number of domains in both clinical and pre-clinical studies.<sup>9-11</sup> Although not all children who are exposed to early life stress develop problems, the sequelae of early trauma can include Post Traumatic Stress Disorder (PTSD) as well as other significant medical and psychiatric morbidities.<sup>8, 12-15</sup> For instance, childhood trauma has been associated with depression, anxiety, substance use, chronic pain disorders, personality disorders, and suicidality.<sup>16-19</sup> In one prospective longitudinal sample, 80% of children who had been abused were diagnosed at age 21 with at least one psychiatric disorder, including PTSD.<sup>20</sup>

Additionally, the Adverse Childhood Experiences survey, with nearly 10,000 respondents from patients at a large Health Maintenance Organization (HMO), found child abuse associated with an increased risk of adult diseases (such as obesity and heart disease) and health risk factors with a dose-response relationship between severity of familial dysfunction and later risk.<sup>21</sup>

Consequences of childhood trauma frequently include Post Traumatic Stress Disorder (PTSD).<sup>22</sup> Although different definitions of trauma have been proposed, the Diagnostic and Statistical Manual (DSM-IV-TR) describes traumatic experiences sufficient to meet criteria for PTSD as the following: 1) the person experienced, witnessed, or was confronted with an event or events that involved actual or threatened death or serious injury, or a threat to the physical integrity of self or others and 2) the person's response involved intense fear, helplessness, or horror.<sup>23</sup> Trauma that meet these criteria are known as "Criterion A" traumatic experiences, so named for the criteria in the diagnostic code. PTSD has three sets of core symptoms; it is characterized by symptoms of hyperarousal, re-experiencing of the traumatic event, and avoidance.<sup>24</sup> Consistent with these core symptoms, many studies on children and adults with traumatic experiences have focused on understanding the effects of trauma on emotion processing and regulation.<sup>25</sup>

### **Emotional Regulation**

Previous work has established associations between experience of stress and changes in emotional response in both children and adults with trauma or PTSD, although the pathophysiology behind this association remains unclear.<sup>26</sup> In one study, preschoolers



who were maltreated demonstrated more blunted and mixed emotion response patterns than non-maltreated controls.<sup>27</sup> Another study of preschoolers from 3 to 5 years of age found that maltreated children were less able to distinguish between the negative facial expressions of anger, sadness and fear and were less accurate at identifying emotional expressions overall.<sup>28</sup> Other studies have found that maltreated children from 8 to 15 years have faster processing time in recognizing morphed angry faces, with no difference in the ability to identify the emotion.<sup>29</sup> One study of children with maltreatment found that the severity of maltreatment predicted avoidance of angry faces compared with neutral or happy faces.<sup>30</sup> These findings are consistent with the pattern of emotional avoidance characteristic of PTSD.

Other studies have found emotional avoidance as a core feature of traumatic response. For instance, the inability to describe one's own emotions is known as alexithymia, an emotion processing deficit frequently found in patients with traumatic experiences.<sup>31-33</sup> Emotional numbing symptoms in PTSD have been correlated with alexithymia in combat veterans.<sup>32</sup> Another study found the degree of alexithymia in adults with Borderline Personality Disorder and PTSD correlated with severity of physical or sexual abuse experienced in childhood.<sup>34</sup> A separate study in a group of Holocaust survivors found that alexithymia was correlated with PTSD symptom severity.<sup>35</sup> Moreover, several studies have commented on the neuroendocrine overlap between patients with PTSD and those with alexithymia alone.<sup>36,37</sup>

Other deficits in emotion regulation more closely relate to the hyperarousal aspects of PTSD. One study of children found that children from "high-conflict" homes exhibited more behavioral distress when watching a videotaped argument.<sup>38</sup> On the other

hand, another study of abused four year olds found that while they were not more aroused (as measured by heart rate and sweat gland response) by the onset of angry voices than control children; rather they became more aroused during an ambiguous period in which the angry voices had ceased but were unresolved.<sup>39</sup>

Similar behavioral findings of hyperarousal have been noted in adults. One study of adult, female survivors of childhood abuse who were seeking treatment found difficulties in emotion regulation predicted functional impairment when controlling for PTSD symptoms.<sup>40</sup> Studies of male war veterans with PTSD have consistently found emotion processing deficits, including less ability to experience happy or neutral emotions, and a tendency to experience negatively valenced emotions. One study showed that veterans with PTSD reported greater arousal to negative visual stimuli than control veterans.<sup>41</sup> Another study of war veterans found that those with PTSD rated neutral images more negatively than controls and also showed less positive emotion during positive stimuli after being exposed to visual trauma cues, as measured by electromyography.<sup>42</sup> Therefore, across different groups with a variety of traumatic experiences, changes in emotional processing have been repeatedly demonstrated, and are a central feature of the PTSD construct.

## **Prior Magnetic Resonance Imaging in Trauma**

### *Structural MRI*

Structural neuroimaging in adults with trauma histories has demonstrated volumetric changes in core areas associated with affect processing. Specifically, decreases in hippocampal volume in adults with combat-related PTSD have been replicated in several

studies.<sup>43,44</sup> Adult survivors of childhood physical and sexual abuse with PTSD similarly have shown decreased hippocampal volume.<sup>45</sup> Moreover, other structural changes in adults have been identified, such as decreased corpus callosum volume.<sup>46</sup> One small study (N=9) in female, adult survivors of childhood abuse reported decreased posterior corpus callosum volume (relative to overall callosal size).<sup>47</sup> The corpus callosum is an interhemispheric tract; the medial and caudal portions carry fibers from the auditory cortex as well as the superior temporal sulcus, cingulate, and other limbic and paralimbic areas; these areas in turn have wide connections to prefrontal areas that mediate the processing of emotional stimuli, memory function, and may play a role in alexithymia.<sup>48</sup> <sup>49</sup> Additionally, a recent meta-analysis of adult MRI studies found that subjects with PTSD also had decreased volumes in the anterior cingulate cortex and amygdala.<sup>50</sup>

In children with PTSD, prior structural neuroimaging studies have document different findings, reporting instead smaller cortical volumes and no statistically significant changes in hippocampal volume.<sup>51,52</sup> In fact, one study has found increased, not decreased, hippocampal volumes in children with PTSD.<sup>53</sup> Overall, the most replicated finding in children with PTSD is that of corpus callosum atrophy.<sup>51, 54, 55</sup>

### *Functional MRI*

Whereas structural MRI which is sensitive to changes in brain anatomy, functional MRI provides a map of brain structures are active at a particular moment in time. Functional MRI measures blood-oxygen level-dependent (BOLD) hemodynamic changes in particular brain regions. In BOLD fMRI, brain activation is inferred by

comparing hemoglobin oxygenation levels during the completion of one task compared with another (or to a rest period).<sup>56</sup>

Given the emotion processing deficits and alexithymia reported among traumatized children and adults, areas of potential investigation in brain imaging include the affective processing networks and cross-hemispheric transfer. However, prior functional neuroimaging, including fMRI and Positron Emission Tomography (PET) scanning, in adults with PTSD have used one of a limited number of paradigms:

- symptom provocation by providing a trauma-specific script, which has been the most common but lead to variable outcomes,
- using nonspecific affective stimuli as probes, which has led to greater consistency in activation patterns,
- functional connectivity analysis which statistically calculates brain regions likely to be acting together based on the time of activation, but is relatively newer with fewer studies.<sup>57</sup>

Prior studies have identified numerous areas potentially involved in emotion processing in PTSD compared with trauma controls without PTSD, however there has been little consistency among reported regions of activation.<sup>58</sup> Several studies have reported decreased anterior cingulate activation, although the finding has not been widely replicated.<sup>59, 60</sup> Others have reported increased amygdala activity.<sup>61-63</sup> Still others have reported increased orbitofrontal cortex activation.<sup>61</sup> Amygdala and orbitofrontal cortex (OFC) activation in traumatized individuals may reflect corticolimbic connections that allow emotional stimuli to influence attention.<sup>64</sup>

All of the studies mentioned above relied on adult samples. In fact, currently, there is no published brain imaging study focusing on pediatric patients with a history of maltreatment. There is one study of adolescent earthquake victims who were exposed to trauma-related pictures during fMRI; the adolescents with PTSD had less activation of the anterior cingulate compared with trauma controls.<sup>65</sup>

More consistency has been found in the fMRI studies using affective probes such as fearful and happy faces.<sup>66, 67</sup> Adults with either chronic or acute PTSD consistently show greater activation of the amygdala than controls when shown the faces with fearful expressions, even when the faces are masked.<sup>66-69</sup>

However, faces of various races which are commonly used for cognitive activation studies may have variable outcomes, including a differential effect in the emotion processing areas of the brain such as the amygdala particularly among an ethnically diverse sample.<sup>70, 71</sup> For example, during one fMRI study using facial probes, the activation of the amygdala was found to be related to the degree of prejudice toward different racial groups.<sup>72</sup> Therefore, facial probes may complicate the interpretation of emotion regulation processes in an ethnically diverse sample, whereas auditory probes may avoid this confound.

#### *Novel Paradigm for Studying Affective Processing and Interhemispheric Transfer*

One possible auditory probe has been behaviorally studied in children.<sup>73</sup> An event-related fMRI using these auditory probes of angry or neutral nonsense words has been used to study emotion processing in adults.<sup>74, 75</sup> The study implemented a dichotic listening task, in which different stimuli were presented in either ear. For example, an

angry voice would be presented in the left ear and a neutral voice presented in the right ear. In a non-clinical sample, participants were instructed to selectively attend to one ear, and determine the gender of the speaker in that one ear. When attention was on the angry utterances, increased activity was observed in amygdala and orbitofrontal cortex (OFC).<sup>74, 75</sup> Other brain areas may also be activated by the task. For instance, Broca's area and the superior temporal regions have been found to be important in prosody processing<sup>76, 77</sup>. Because the task involves dichotic listening, prior studies indicate potential involvement of the corpus callosum in interhemispheric transfer of information.<sup>78, 79</sup>

### **Genetic Moderation of the Effects of Trauma**

Despite the large literature documenting widespread psychological and neurobiological consequences of trauma, not all people who experience significant stress or abuse will develop problems.<sup>80</sup> Recent studies have raised the possibility that the development of psychopathology subsequent to trauma may be mediated by other factors, including genetic susceptibility.<sup>81</sup> Although many genes may play a role in the neurohormonal response to trauma, a functional variation in the serotonin transporter gene has been shown in a number of studies to be clinically relevant to some of the outcomes common in trauma, such as depression.<sup>82</sup> Since the development of the anti-depressant drugs, the selective serotonin reuptake inhibitors (SSRIs), much attention has been focused on the serotonin neurotransmitter system in the pathophysiology of mood disorders and depression.<sup>83</sup> In fact, the serotonin transporter (SERT), which is the site of action of SSRIs, has been theorized as a link between the neuroendocrine response to

stress and depression.<sup>84</sup> There are two alleles of promoter region of the transporter, or 5HTTLPR, and the short and long versions of a variable number tandem repeat (VNTR) segment within a particular gene, SLC6A4, that affect the size of the serotonin transporter promoter region. The short version (“s”) of the allele leads to reduced transcription and transporter capacity, and interferes with a reuptake feedback loop integral to the regulation of serotonin function in the nervous system (via the termination of the action of serotonin in the synapse). The long version (“l”) of the allele is associated with normal transcription and functional capacity.<sup>85</sup>

In both population and family-based association studies, the short version of the 5HTTLPR site has been associated with an increased risk for anxiety-related traits and depressive disorders.<sup>86-88</sup> These findings have been replicated in pediatric and adolescent populations.<sup>89,90</sup> Other studies have related the serotonin transporter polymorphism to temperament and coping style, with the short allele conferring risk for higher anxiety temperaments.<sup>91,92</sup> However, other studies that have failed to find a clear association.<sup>93,94</sup> One possible reason for the variability in outcomes is that the genetic susceptibility is modified by the stress of the environment, i.e., a gene-by-environment interaction.

In the area of gene-by-environment interactions, a landmark study was published in 2003 by Caspi and colleagues characterizing the interaction of 5HTTLPR genotype and early life stress.<sup>95</sup> The Caspi study was a longitudinal epidemiological study of predictors of psychopathology, including depression, and the high risk “s/s” allele conferred increased risk for a major depressive episode at 26 years of age only when adults had also been maltreated as children.<sup>95</sup> Since that report, a number of studies have been published regarding the interaction of genotype and stress. A similar gene-by-

environment interaction was identified in a population of children who had been removed from their caregivers due to allegations of abuse or neglect by child protective services; however, the higher depression scores in the high-risk, high-stress group was moderated by the availability of social support.<sup>96</sup> Anxiety-related traits show a gene-by-environment interaction with child maltreatment and the serotonin transporter in pediatric age group, as well.<sup>97</sup>

Some studies have noted that SLC6A4 may be triallelic; there is a gain-of-function mutation within the “l” allele, an A to G (noted as La and Lg) polymorphism that causes functional expression to be similar to that of the “s.” While the Lg variant is rare in Caucasian populations, it has higher prevalence in some minority populations.<sup>98-</sup><sup>100</sup> However, in an exploratory analysis of previously published gene-by-environment interactions in a population similar to that being studied in the following experiments, the triallelic reclassification was not informative and accounted for only a small proportion of the variability (Kaufman, personal communication.)

### **Imaging Genetics**

In a new line of research, the serotonin transporter gene has been found to impact brain structure and function. In a recent post-mortem analysis, patients with schizophrenia, bipolar, and major depressive disorder as well as controls with the s/s genotype were found to have increased serotonin transporter in the pulvinar nucleus of the thalamus, which receives projections from the limbic system.<sup>101</sup> Older adults with major depression and the high risk polymorphism were found to have an increase in



caudate nucleus size by structural MRI compared with those with major depression and the lower risk polymorphism.<sup>102</sup>

In functional neuroimaging, there is increased amygdala activation in response to emotionally negative stimuli with an s/s or s/l genotype, which is consistent with the structural findings. Numerous studies have replicated the findings of increased amygdala reactivity in both healthy and depressed adults.<sup>103-111</sup> In fact, these effects have been demonstrated using a variety of fMRI paradigms, including: fearful faces<sup>103, 104, 106, 109-111</sup>, unpleasant pictures<sup>105, 107</sup>, and aversive words.<sup>108</sup> The examination of the serotonin transporter polymorphism on functional neuroimaging may contribute to the eventual development of a “phenotypic assay” for vulnerability to particular stress-related psychiatric disorders.<sup>112</sup>

### **Statement of Purpose**

Child maltreatment is a widespread and significant contributor to psychopathology, including depressive disorders and PTSD, even though there is significant outcome variability for children who have experienced early life stress. Multiple studies have demonstrated important changes in emotion processing and regulation in children exposed to early life stress, but few studies have examined the related neural pathways. In fact, there are no currently published fMRI studies in children with maltreatment.

Recent studies suggest particular patterns of brain activation involved in the emotion-processing pathways are moderated by genetic polymorphisms in the serotonin system. The short allele of the serotonin transporter gene is associated with increased

activation to emotionally negative stimuli. Imaging genetics is an increasingly recognized tool to examine the contribution of particular genetic polymorphisms to the development, mechanism, and maintenance of psychopathology; however, there is no literature utilizing imaging genetics in a pediatric population.

Many of the currently published studies examine fearful faces as a probe of the affective neural pathways. Although face stimuli have yielded consistent findings, but these stimuli may be inappropriate in ethnically diverse samples, as participant attitudes and diverse racial characteristics influence the emotional response to the stimuli. Therefore, an auditory (i.e., dichotic listening) task using nonspecific emotional stimuli may overcome some of the earlier limitations of using fearful faces. Moreover, the dichotic listening task requires interhemispheric transfer of information and may therefore illustrate the functional implications of observed corpus callosum deficits seen in abused children. Building on prior work regarding changes seen in children with a history of stressful life experiences, the goal of this study is to use fMRI in maltreated children to study deficits in emotion processing and the related neural correlates of these changes. The genetic and environmental influences on brain circuitry will be studied using a two-by-two factorial design using the novel dichotic listening task.

The primary aims and hypotheses were to:

1. To utilize a fMRI dichotic listening task to examine the effects of trauma and genotype on emotional processing in children. *Hypothesis:* A history of maltreatment (high stress) and two “s” alleles of the serotonin transporter gene are both expected to be associated with increased amygdala and orbitofrontal activation in response to angry

stimuli, with greatest amygdala and OFC activation expected in maltreated children with the s/s genotype.

2. To conduct a fMRI study in maltreated children with a task that requires interhemispheric transfer through the posterior corpus callosum, a region found to be reduced in traumatized samples in multiple prior pediatric neuroimaging studies.

*Hypothesis:* When compared to demographically matched controls with no history of significant lifetime trauma (low stress), maltreated (high stress) children will show reduced activation in regions that receive inputs from axons that cross the hemispheres through the posterior corpus callosum, such as the superior temporal gyrus.

## **Methods**

### **Participants**

The eighteen participants in the fMRI task were grouped according to a 2 x 2 factorial design (see Table 1) including environmental stress and genetic risk factors. Functional Magnetic Resonance Imaging data from twenty-two children were obtained while they completed the Dichotic Listening Task. However, data from four participants was excluded from the final analysis: two participants were excluded due to excessive motion and two participants failed to respond to a large number of trials. The final sample of eighteen children and adolescents had an age range of 7-17 years (average  $13.2 \pm 2.8$ ); 50% of the sample was female. 16.7% of the sample was Caucasian, 50% African American, 22.2% Hispanic, and 11.1% biracial. An ANOVA indicated that none of the groups differed significantly in age or gender. The groups did not differ by racial/ethnic categories (as indicated by Fischer's exact test).

*Table 1: 2x2 Factorial Design*

	<b>High Risk “s/s”</b>	<b>Low Risk “l/l”</b>	<b><i>Total</i></b>
<b>High Stress</b>	4	8	<i>12</i>
<b>Low Stress</b>	3	3	<i>6</i>
<b><i>Total</i></b>	<i>7</i>	<i>11</i>	<i>18</i>

# of Participants in each condition.

### **Recruitment and Informed Consent**

The Yale University Human Investigations Committee approved this study. Children in the high stress group were either removed from their caregivers due to allegations of neglect or abuse and had temporary custody awarded to the State of Connecticut Department of Children and Families (DCF) by court order, or were involved with DCF for substantiated child abuse or neglect (e.g., witnessing domestic violence or experiencing physical or sexual abuse). Community comparison participants (low stress group) were recruited either through advertisements or from prior research studies, including the summer research camp (described below). Controls and maltreated children were matched according to age, gender, and demographics. Annual household income was <\$30,000 for all participants. All participants (maltreated and community controls) were able to participate in a summer camp as part of a study on the Genetic and Environmental Predictors of Depression in Children. Participants were also recruited from the sample used in a study of SAFE Homes and placement permanency.<sup>113</sup> Dr. Kaufman is the primary investigator on both of these projects. However, recruitment for the Genetic and Environmental Predictors of Depression has been on hold while the State of Connecticut decides its policy regarding genetic studies of children in state care. The moratorium, in place since August 2006, has limited the pool of available participants.

Exclusion criteria for all groups included major medical diagnoses, history of seizures, mental retardation, and traumatic brain injury with loss of consciousness, pregnancy, and standard MRI exclusion criteria. Comparison children had no history of involvement in the child protective services system and no current Axis I pathology (see below for clinical measures). Informed consent was obtained by guardians of all participants, and assent was provided by all participants. If appropriate, biological parents were asked to provide assent for participants when children were in the custody of the Connecticut Department of Children and Families.

### **Measures Available at Time of Recruitment**

#### *Stress History*

For children in the high stress group, history of maltreatment was obtained from multiple sources including biological parents, DCF caseworker, and the protective services computerized records. Parents also completed *Partner Violence Inventory*<sup>114</sup> and children and parents completed the *Traumatic Events Screening Inventory—Parent Report Revised*.<sup>115</sup> Parent and child reports of trauma history were cross-referenced with the state database of involvement in the child protective services. Data from the various sources was compiled into a coding system for various types and severity of abuse (for example, physical abuse, sexual abuse, neglect, emotional abuse, and exposure to domestic violence).<sup>116</sup> The total number of familial and non-familial traumas experienced by the children was tallied.

### *Clinical Diagnoses*

Detailed clinical diagnostic information was available from the *Kiddie Schedule for Affective Disorders and Schizophrenia for School-Age Children—Present and Lifetime Version [K-SADS-PL]* which is a semi-structured diagnostic clinical interview administered by a master's level clinician separately to each parent informant and child.<sup>117</sup> At the conclusion of the K-SADS-PL, researchers gave each participant a *Child Global Assessment of Functioning Scale [C-GAF]* score.<sup>118</sup> On a scale of 0-100, the C-GAF rates the child's highest level of functioning and the most severe level of impairment over the prior year. Given all of the information collected, final diagnoses were assigned using a best estimate psychiatric diagnosis procedure described elsewhere.

119

### *Research Camp*

Children participating in the interlocking studies were eligible to attend a one-week summer research camp. Approximately one to two hours per day were spent on research assessments once per day one-on-one with a Master's or Doctoral level clinician. The group also completed two computer tasks over the week-long camp. The rest of the day was spent in typical camp activities, such as recreation, sports, art, and music. The camp provided a naturalistic setting in which to observe strengths and deficits in a number of areas, including social and emotional development.<sup>120</sup> At camp, children completed the *Wechsler Intelligence Scale for Children—Revised Short Form [WISC-R-SF]*. The short form includes the Information, and Block Design subtests and correlates highly (0.89) with full scale IQ.<sup>121, 122</sup> Children also completed trauma measures, and the child report portion of the *K-SADS*.

### *Genotype*

DNA collection was performed at the summer research camp. Participants had provided saliva for buccal cell DNA extraction and genotyping. Children were provided with Scope mouthwash (Original Mint Scope, Proctor and Gamble, Cincinnati, Ohio) and instructed to swish the mouthwash and then spit into a 50mL tube. Specimens were then refrigerated and DNA was extracted using Puregene kits (Gentra, Minneapolis, Minnesota). Genetic variation in the serotonin transporter gene is measured at the site of the SLC6A4 (the transporter protein) allele.<sup>123</sup> A variable-number tandem repeats polymorphism was genotyped in the laboratory of Joel Gelernter using agarose gel size fractionation. Alleles were characterized according to the number of repeats: 14 repeats was characterized as a “short” or “s” allele and 16 or more repeats was characterized as a “long” or “l” allele.

### **Assessments Obtained for fMRI Study**

#### *Clinical Update*

After MRI study enrollment, a research associate traveled to each participant’s house to describe the project and obtain consent. At that time, the parental or guardian informant and participant would provide any updated clinical information. Self-reports of symptoms were obtained from children using the *Screen for Child Anxiety Related Emotional Disorders*, a forty-one item rating scale developed to assess anxiety symptoms in children and adolescents.<sup>124</sup> Children also completed the *Mood and Feelings Questionnaire*, a thirty-three-item self-report measure that assesses depressive

symptomatology in children.<sup>125</sup> Each of the items was read by the research personnel to the child. All participants were asked about recent exposure to trauma and filled out a *Children's PTSD Checklist* which is a twenty-question self-report on PTSD symptoms.<sup>126</sup> Additionally, participant handedness was assessed via the *Edinburgh Handedness Inventory*.<sup>127</sup> Guardians also provided updated clinical and trauma information on their children using a *Child Behavior Checklist* and a *Life Event Checklist [LEC]* which is a forty-six item checklist assessment recording recent stressful life events.<sup>128</sup>

#### *MRI Procedures and Training in the Mock Scanner*

Participants and families (typically siblings and parents) would come to Yale New Hospital and meet with two research associates on the day of participation. Families and children would be able to eat dinner with the research personnel who would be administering their scan, in the scanner room, or filling out any missing assessments before walking to the MRI Center.

In order to minimize participant discomfort and movement-related image artifacts, all participants underwent a mock scanning procedure. On the night of the scan, the family of the participant was invited into a room in the Yale Magnetic Resonance Resource Center in which a defunct MRI machine was present. The child had the opportunity to be in the defunct machine with guardian present, and he or she was presented with scanner noises via a stereo system. Participants were trained to remain as still as possible and received real-time feedback during training.



### *Auditory Stimuli*

The emotion processing task utilizes two nonsense words (e.g. “goster” and “niuenci”) recorded in angry and neutral tones by speakers of different genders.<sup>129</sup> In a behavioral study, thirty-four children (age range: 6-13) rated the angry stimuli used in the fMRI protocol as an average 4.2 on a 1-5 scale where 5 was “angry” and 1 was “neutral.” In contrast, the “neutral” stimuli used in the scanner were rated a 2.0 on average. Children were able to reliably distinguish between angry and neutral stimuli by paired samples t-test (See Appendix 1 for more information)

### *Dichotic Listening Task: Training and Task Description*

All participants were introduced to the stimuli outside of the scanner. Each participant listened to all auditory stimuli for approximately three minutes, and then practiced the gender judgment for approximately two minutes immediately before scanning. In the scanner, stimulus presentation followed a blocked design during which two sounds are presented simultaneously, one to each ear: (anger/neutral [AN], neutral/anger [NA], or neutral/neutral [NN] on the left/right side) on each trial, in pseudorandom order. Participants were instructed to selectively attend to either the left or right ear during two successive blocks (signaled by an “L” or “R” presented on the monitor in front of them) and then are asked indicate the gender of the voice heard on the target side.

During the scan, auditory stimuli (750 ms duration) were delivered via MR-compatible headphones and were always presented with a varying jitter during the silent gap between each EPI volume. Each trial consisted of a delay period (465 ms duration)

after presentation of the stimulus. There were 24 different events for each experimental condition, plus 48 null events without auditory stimulation as a baseline condition. Rest periods lasted 12 seconds between events, and a tone indicated that word trials were to begin. A total of 120 trials are presented to each participant. Trials were blocked according to emotional valence and attended ear. Participants respond via button presses with their right index and middle fingers.

### *Pilot Feasibility*

Before data were collected in the present study, one adult control and two normal child control participants participated in a pilot feasibility study in which they performed the task in the fMRI but data was not included in the overall sample. The participants were asked qualitatively about the various stimuli, and the fMRI task was subsequently purged of some of the more ambiguous and more difficult to identify angry stimuli based on the participant feedback and preliminary examination of the imaging data.

### *MRI Data Acquisition*

Participants underwent MRI using a Siemens Magnetom Trio 3-Tesla MRI Scanner (Siemens Medical Solutions, Erlangen, Germany) at the Magnetic Resonance Resource Center at Yale University School of Medicine. At the beginning of the scan, two standard structural localizers were collected. Functional images were acquired using a gradient-echo EPI sequence (TR=4000ms, TE/Flip = 25 ms/60-degrees, FOV = 225 mm, base resolution = 64 x 64) sensitive to BOLD (Blood Oxygenation Level Dependency) contrast. Image volumes consisted of 40 contiguous 3.5 mm axial slices

acquired parallel to the anterior and posterior commissures. In order to present auditory stimuli without background scanner noise, the sequence followed sparse imaging procedure in which the effective acquisition time (TA) was 2320 ms of each 4000 ms repetition. Two structural scans were also acquired in order to register fMRI data into standard space: one a T1-weighted SPGR sequence in the same plane as the fMRI data, and the other a MPRAGE sequence with full-head coverage (1x1x1 mm isovoxel; TE/Flip = 3.66 ms/7 degrees, TR= 2530ms, TI= 1100ms, matrix = 256 x 256).

Overall, scanning took approximately 60 minutes per participant. Each of three functional runs lasted 6.4 minutes. Throughout the MRI procedure, a member of the research study team was available inside the scanning room and the participant was in verbal communication with a scanning technician and a member of the research team operating the stimuli.

### *Imaging Processing and Statistical Analysis*

MRI processing and initial analyses were carried out using BrainVoyager (Brain Voyager QX, Brain Innovation BV, The Netherlands) and locally developed Matlab software. The machine is equipped with an 8 channel phased array head coil for parallel imaging. Functional data were inspected for adequate signal to noise and acceptable participant motion. A movement spike distance of less than half of a voxel (1.75 mm) between volumes was used as the threshold for useable fMRI data, although spike removal software was used when isolated movement spikes were present. Other corrections included linear detrending and motion correction prior to analyses. Standard procedures were used to register the fMRI data to structural data in Talairach space.<sup>130</sup> Of

the three runs typically obtained for the study, two without significant motion were chosen for inclusion in the group general linear model (GLM) since not all participants had usable data over all three runs. All MRI analyses and determination for inclusion were blinded to stress and genotype group until the final GLM was calculated.

General linear model (GLM) analyses were carried out on each intracerebral voxel and analyses were focused on a priori predictions of increased amygdala activity and orbitofrontal cortex (OFC) activity and decreased superior temporal sulcus/gyrus (STS/STG) for angry words in the high stress, high genetic risk groups. The a priori predictions were based on regions of interest reported in the Sander et. al. paper implementing the same dichotic listening task in an adult non-clinical sample.<sup>131</sup> In addition to the areas of interest, an image-wide search was conducted using a voxel-wise significance threshold employed by Sander et al ( $p < 0.001$ ) in order to examine other potential areas related to the emotion processing pathways. Each of the six trial types based on stimuli type to each ear and ear to be attended (ANL, NAL, NNL, NAR, ANR, NNR) was included as an explanatory variable (EV) in the GLM. Parameter estimate maps were calculated representing multiple key contrasts between the EVs: emotion versus neutral words (ANL/NAL/NAR/ANR vs. NNL/NNR), attended versus unattended emotional words (ANL/NAR vs. NAL/ANR), and attended angry versus neutral words (ANL/NAR vs. NNL/NNR). All conditions versus rest were also examined in order to check for overall auditory cortex activity.

The contrast maps were used as the variables for random-effects (RFX) ANOVAs looking at Stress and Genetic Risk effects on patterns of activation. Activation clusters that were greater than  $5 \text{ mm}^3$  voxels were considered for subsequent analysis. Each area

identified was also cross-referenced with the Talarach and Tournoux brain atlas coordinates.<sup>130</sup>

When significant clusters of activation were identified in a priori areas of interest after whole brain analysis, the average parameter estimates (betas) for each condition were extracted for further analysis and interpretation. This approach allowed for the simultaneous examination of stress, genetics and their interactions. (Few fMRI analysis programs, including BrainVoyager, really allow for second and third-order interactions to be examined on a voxel-wise basis; John Herrington, Personal Communication). Correlation analysis was also examined to investigate the association in the different the identified areas of interest. Finally, an ANOVA was implemented following a two-by-two design with stress (high, low) and genotype (s/s, l/l) as factors. The correlations and ANOVAs were calculated via SPSS 15.0 (SPSS, SPSS Inc., Chicago, Illinois).

Participant responses were recorded by button box when they were performing the gender discrimination task according to attended ear. Behavioral accuracy data were analyzed using SPSS with a 2 x 2 ANOVA using the genetic risk and the environmental stress as independent variables.

#### *Author Contributions*

All genetic analyses were performed in the laboratory of Joel Gelernter. The dichotic listening task and variation for use in the fMRI scanner was altered by Patrik Vuilleumier and colleagues. Heather Douglas-Palumberi consented all participants and guardians for the scanner, and completed all MRI-specific assessments. John Herrington and Joan Kaufman performed an intermediate analysis of data with 11 participants. The

author was present during most of the data collection, meeting families before the scanning task, assisting in the behavioral task, and assisting in the analysis of the imaging data. Group-level analyses were done in BrainVoyager by both the author and John Herrington. Additional analyses on the voxel-based beta-parameter estimate values were done in SPSS by Joan Kaufman.

## **Results**

### *Clinical Data*

Despite the absence of child maltreatment or the experience of intrafamilial violence, all of the demographically matched, low-income, low stress children had at least one “Criterion A” traumatic experience, including community violence, severe illness, or an unexpected death; one of the low stress children had two traumatic experiences that met the criteria. The children in the maltreatment group, in contrast, had a mean of 6 “Criterion A” traumatic experiences. The genetic and risk groups did not differ significantly on Full Scale IQ (range: 74-120; mean  $96.4 \pm 13.0$ ) or depressive symptomatology as measured by the *MFQ* (range: 1-25; mean:  $9.9 \pm 6.9$ ) and *SCARED* (range: 4-37;  $15.2 \pm 10.1$ ). The genetic groups, however, were significantly different on the *Child PTSD Checklist*, independent of maltreatment history, with no significant gene-by-environment interaction (overall range: 0-57; mean:  $11.5 \pm 13.1$ ). The low risk “l/l” group had a mean PTSD score of  $5.7 \pm 4.3$ ; the high risk “s/s” group had a mean of  $20.6 \pm 17.3$  (See Appendix 2 for group means).

### *Accuracy of Participants*

While participants were in the fMRI machine, they were asked to perform a behavioral discrimination of whether a male or female voice was producing the sound in a particular attended ear. Overall accuracy was measured during fMRI scanning for participants. On average, participants were 72.8% accurate, significantly better than chance (one-tailed t-test on behavioral accuracy over all subjects was  $p < 0.001$ ). An ANOVA was performed on the accuracy of all participants and found a non-significant trend of stress (62.8% correct for the high stress group vs. 82.2% correct for the low stress group;  $p=0.054$ ). However, there was no main effect of genotype ( $p=0.452$ ) and no interaction of stress by genotype ( $p=0.497$ ).

*Table 2: Accuracy of Participants in fMRI Protocol*

	<b>High Risk “s/s”</b>	<b>Low Risk “l/l”</b>	<b>Total</b>
<b>High Stress</b>	60.8±15.8	71.9±16.5	68.2±16.4*
<b>Low Stress</b>	81.9±8.7	82.4±11.4	82.2±8.9*
<b>Total</b>	69.8±16.6	74.7±15.4	72.8±15.6

*Note:* Percentage Correct ± SD. \*Trend for the high stress participants to be less accurate in determining the attended-side compared with the low stress participants.

### *Imaging Data Analysis*

#### *Analysis 1: Voxel-Based Analysis: Main Effects of Emotion*

Because the task had not yet been used in a child sample, one primary goal was to examine the validity and appropriateness of the task in children and adolescents by examining brain regions responding to emotional prosody. Therefore, the first analysis examined the main effect of angry prosody, regardless of the target side (AN+NA), compared to neutral prosody (NN). Overall, angry words activated many more brain regions than neutral words (Table 3).

*Table 3: Clusters of Activity in Anger to Neutral Comparison*

<b>Brain Region</b>	<b>Finding</b>	<b>Side</b>	<b>Peak Coordinates</b>	<b>Peak Significance</b>
Superior Temporal Gyrus (STG)	Anger>Neutral	R	57, -8, 0	0.0002
Superior Temporal Sulcus (STS)/STG	Anger>Neutral	L	-56, -29, 0	<0.0001
STG	Anger>Neutral	L	57, -31, 10	0.0001
Peri-amygdala	Anger>Neutral	L	-27, -11, -11	0.0004
Anterior Cingulate	Anger>Neutral	R	2, 34, 19	0.0007
Insula	Anger>Neutral	L	-27, 12, -3	0.0003
Broca's Area	Anger>Neutral	L	-51, 17, 13	<0.0001
Dorsolateral Prefrontal Cortex (DLPFC)	Anger>Neutral	R	-32, 46, 10	<0.0001

*Note:* Peak coordinates are in standard Talairach space. Whole brain voxel-wise analysis significant at  $p < 0.001$ . Areas of activation observed in children in the study were consistent with the areas when the task was administered to adults.

Results revealed numerous similarities to the results reported in adults.<sup>131</sup> Relative to neutral stimuli, angry stimuli activated the left STG/STS ( $x, y, z = -56, -29, 0$ ;  $p = 0.0001$ ) as well as a homologous area of the right STG ( $x, y, z = 57, -8, 0$ ;  $p = 0.0002$ ). Other similar areas identified included the right Anterior Cingulate ( $x, y, z = 2, 34, 17$ ;  $p = 0.0007$ ) and left Inferior Frontal Gyrus, also known as Broca's Area ( $x, y, z = -51, 17, 13$ ;  $p < 0.0001$ ). Additionally, several left peri-amygdala areas were identified in the analysis that met the level of significance ( $x, y, z = -27, -11, -11$ ;  $p = 0.0001$ ).

*Analysis 2: Interhemispheric Transfer: Superior Temporal and Broca's Area Correlation*

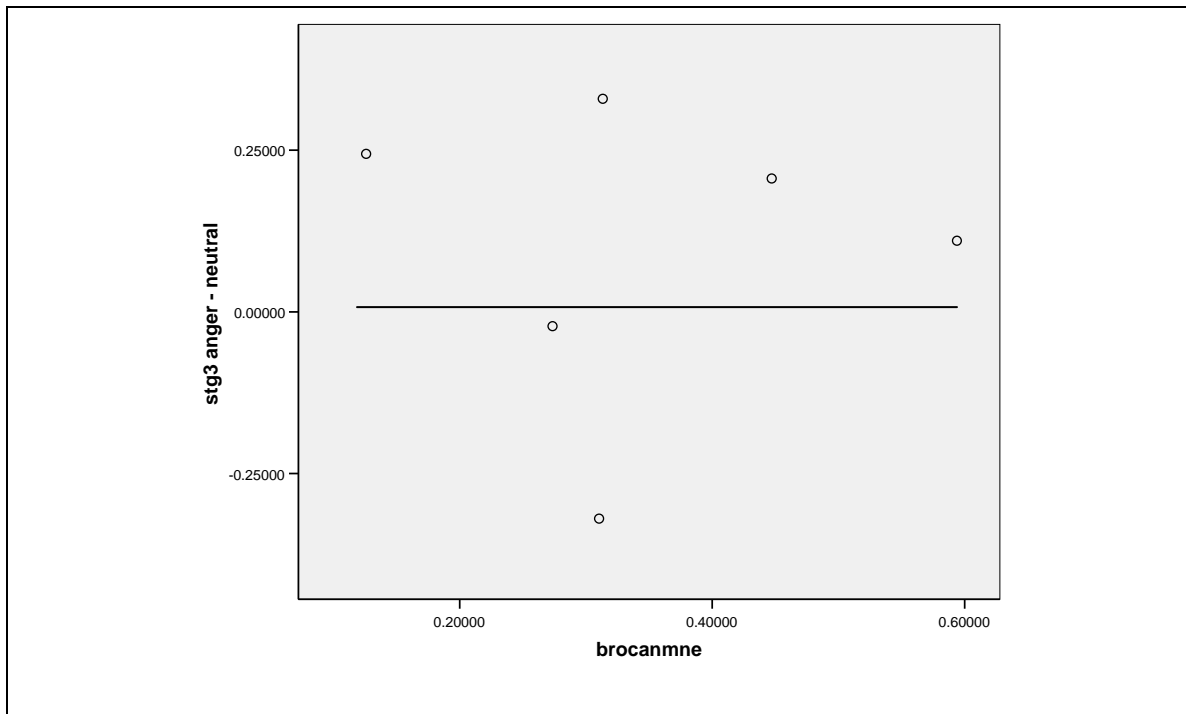
Because children and adolescents with PTSD have been found to have structural changes to the posterior corpus callosum, one of the primary hypotheses related to interhemispheric transfer of information between Broca's area and the Superior Temporal



areas. Specifically, average betas within Broca's Area and STG (a priori areas isolated in Analysis 1) were extracted. The specific association between activation in Broca's area and the STG could be probed using correlation analyses. Additionally, prior studies have indicated that maltreatment has an effect on the corpus callosum, the group ("high stress" versus "low stress") differences in activation pattern could also be examined.

The overall correlation for the entire sample between activation in Broca's area and the STG was nonsignificant with an  $R = +0.33$ . However, looking at the groups separately, the correlation between the two areas had a different pattern. In the non-maltreated low stress group ( $N=6$ ), the correlation between activation in Broca's area and the STG approached zero at  $R = -0.01$ . However, the low stress group shows a positive association between the two regions when the anger is presented in the attended ear ( $R = +0.27$ ) and negative if the anger is presented in the unattended ear ( $R = -0.34$ ) (Figure 1).

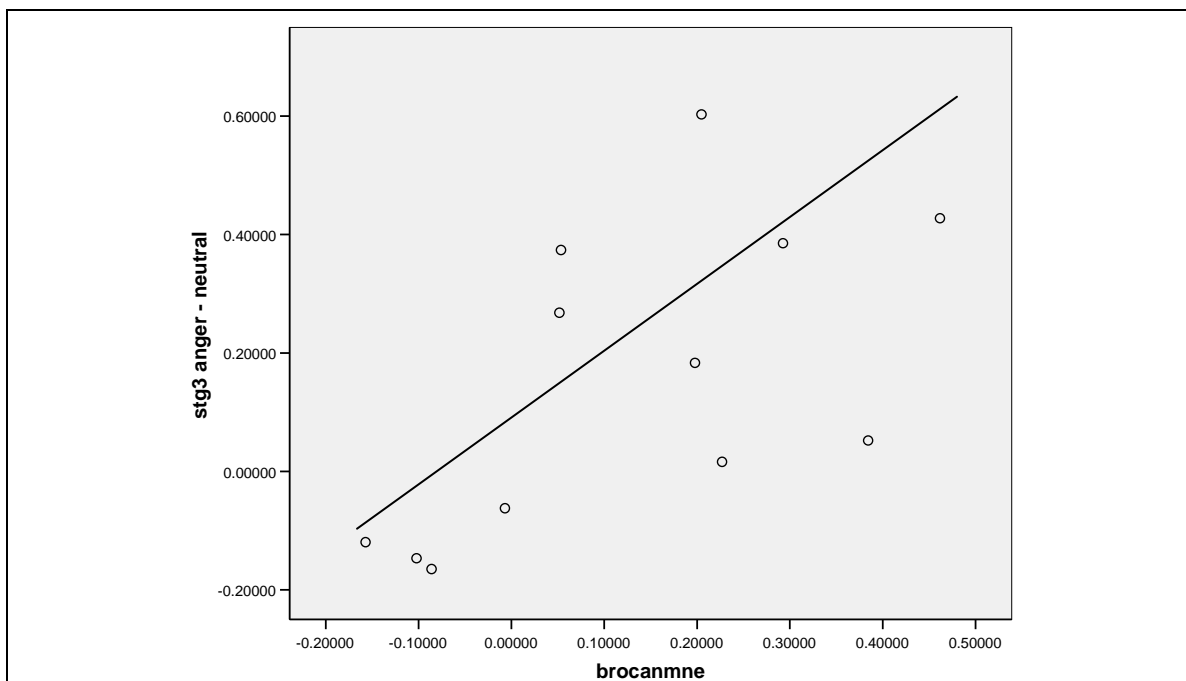
*Figure 1: Correlation between Broca and STG Activation in Low Stress Controls (N=6)*



*Note:* Correlation overall between activation Broca's Area and the STG (Angry words v. Neutral words) is  $R = -0.01$  in non-maltreated, low-stress controls. Different patterns are seen if anger is in the attended (positive correlation) v. non-attended ear (negative correlation.)

In contrast to the low stress control children, high stress maltreated children ( $N = 12$ ) showed a positive correlation between Broca's area and the STG ( $R = +0.61$ ,  $p < 0.04$ ) (Figure 2). Moreover, the maltreated children showed a different pattern of activation in that there were positive correlations whether the anger was in the attended or unattended ear.

*Figure 2: Correlation between Broca's and STG Activation in High Stress ( $N=12$ )*



*Note:* Activation in Broca's area and in the Superior Temporal Gyrus are significantly correlated ( $R = +0.61$ ,  $p < 0.04$ ) in maltreated, high-stress children. Activation during angry words is compared with activation to neutral words. The high stress, maltreated children had a positive association whether or not they were instructed to attend to the words.

However, the difference in correlations (as measured via Fischer z-score transformation) was not statistically significant; it was likely due to lack of statistical

power (i.e., due to the small sample.) Given the patterns of activation observed, it was estimated that a sample size of 18 participants in each of the stress categories would be required for results to reach statistical significance.

Because of the findings from structural imaging, our primary hypotheses in these regions concerned the maltreatment history of participants. However, a similar pattern emerged when examining the correlations between Broca's area and the STG for subjects with the high risk "s/s" genotype (N=7; R = +0.61) and the low risk "l/l" genotype (N=11; R = +0.18).

### *Analysis 3: Anger in the Attended Ear versus Non-Attended Ear*

Consistent with the analytic strategy of Sander and colleagues, the next set of voxel-based analyses examined areas of attendant-dependent emotional activation by contrasting activation in response to anger in the attended versus non-attended ear.<sup>131</sup> A voxel-based analysis showed increased activation in the right OFC and the left amygdala. In the amygdala, there was a 6 mm<sup>3</sup> voxel area with peak coordinates at -18, -11, -14 with a peak p value of < 0.0001 (See Figure 3).

There were also a priori predictions of changes in the orbitofrontal cortex, and coordinates were examined in the frontal lobes close to those reported in adults.<sup>131</sup> A 197 mm<sup>3</sup> voxel peak of activity was found in the orbitofrontal cortex at -6, 41, -8 with a peak p value of < 0.0001.

However, when the data were analyzed to investigate potential effects of stress, there were no clusters in either the amygdala or the OFC that met the threshold. Using stress as a covariate, a 4 mm<sup>3</sup> voxel peak was noted in the right peri-amygdala at 12, -7, -

17 with a peak  $p$  value of 0.0008, however, this did not meet the  $5 \text{ mm}^3$  voxel size threshold.

*Analysis 4: Examination of Genetic and Environmental Stress Effects of Amygdala and OFC Activation*

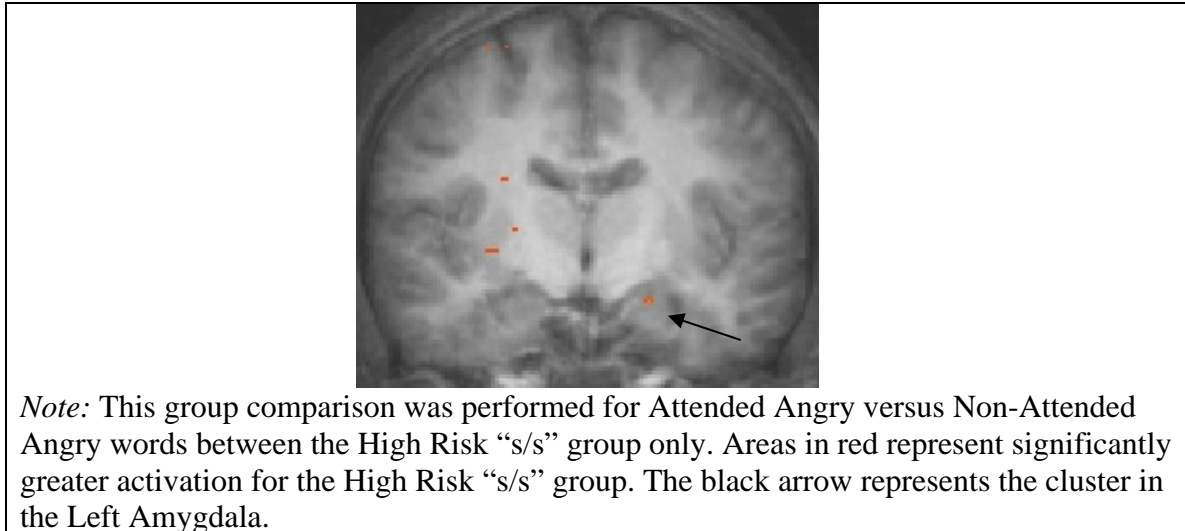
Average cluster betas were again extracted from the identified regions of interest, the amygdala and OFC, to conduct a two-by-two multivariate analysis of variance (MANOVA). Examining the effects of attended versus unattended anger on the beta parameter estimate maps in the amygdala and OFC, there was a main effect of genotype (as had been previously identified on a voxel-wise basis using BrainVoyager.) However, there was not a main effect of maltreatment history nor an interaction between genes and environment. The genotype was significant by Wilks' Lambda test ( $F = 5.79$   $p < 0.02$ ). However, since the genotype was the only significant effect identified by 2x2 MANOVA, the subsequent tests were performed as a MANOVA and follow-up ANOVAs only looking at gene effects on the activation patterns in the OFC and amygdala. The results of the follow-up ANOVAs are presented in the table below and depicted in Figures 3 and 4.

*Table 4: Effect of Genotype Amygdala and OFC Activation in Attended v. Non-Attended Angry Trials*

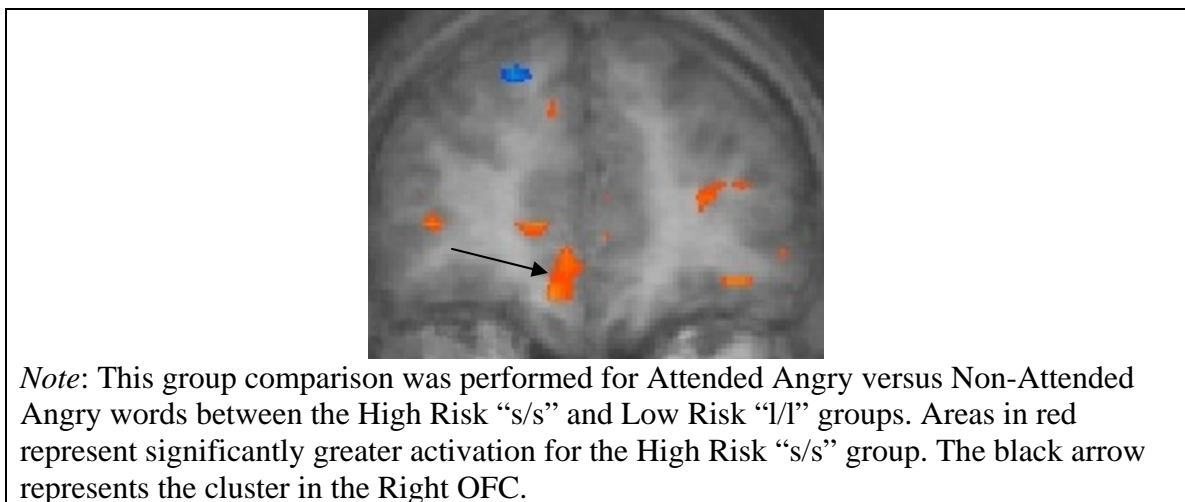
<b>Area of Interest</b>	<b>F</b>	<b>Sig.</b>
Amygdala Angry Attended – Non-Attended	6.03	.03
OFC Angry Attended – Non-Attended	12.46	.003

*Note:* Children with high risk genotype (“s/s”) showed greater activation in the amygdala and OFC than children with the low risk genotype (“l/l”) when attending to angry stimuli.

*Figure 3: Amygdala Activation in High Risk “s/s” Group in Attended v. Non-Attended Anger (N=7)*



*Figure 4: OFC Activation in Low Risk “l/l” (N=11) vs. High Risk “s/s” (N=7) Genotype Contrast in Attended v. Non-Attended Anger*



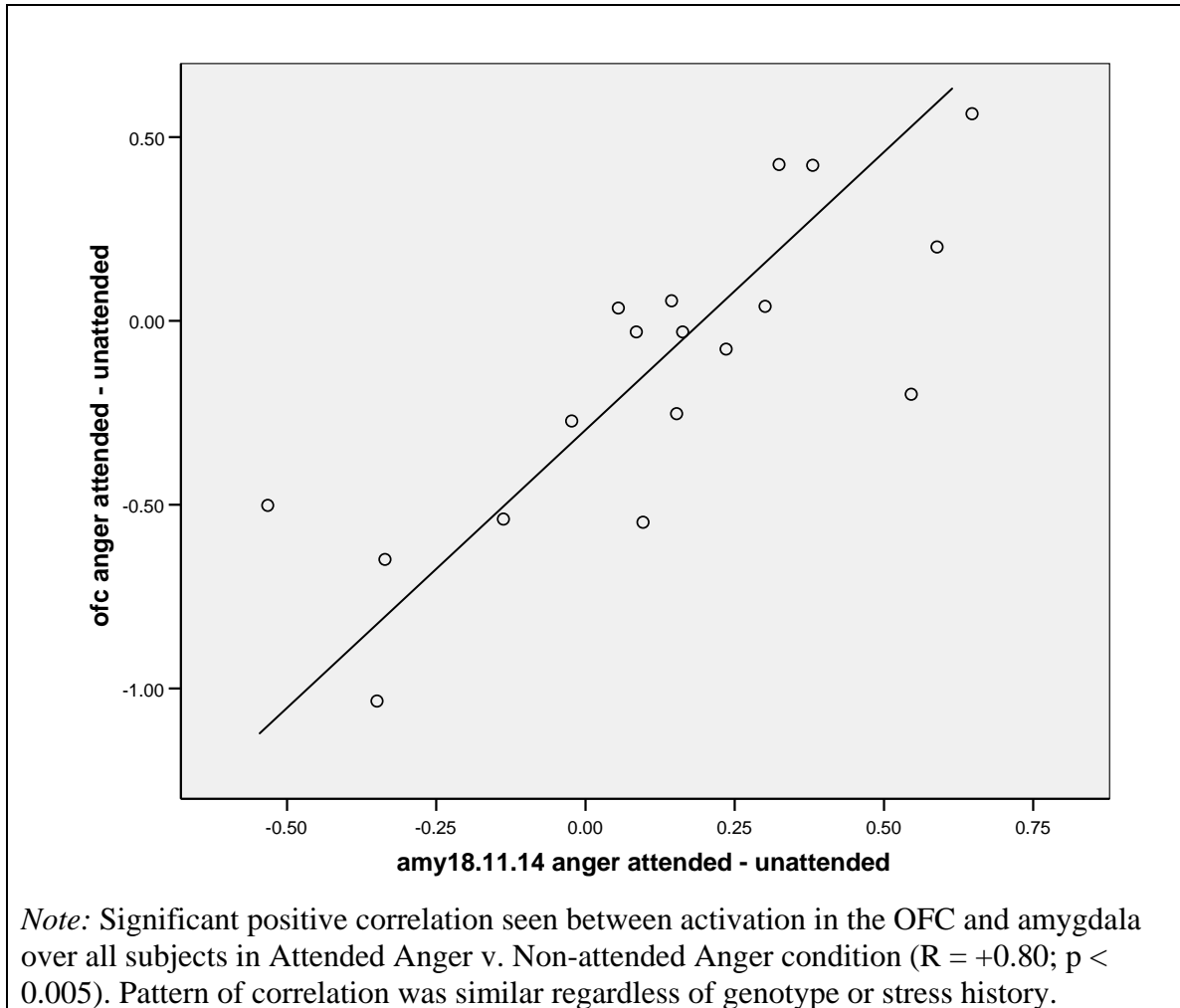
To investigate whether the lack of maltreatment effects in the amygdala was due to insufficient power (i.e., a small sample size), a power analysis was conducted for both genotype and maltreatment group. Based on the differences in the betas in the amygdala

between the maltreatment groups (high stress 0.19 vs. low stress 0.17), the small effect size ( $f = 0.07$ ) means that a sample size of 100 per group would only lead to a 16% power to detect a difference (two-tailed, alpha of 0.05) between the groups. In contrast, the larger difference in amygdala activation between groups based on genotype (“high risk” s/s: 0.35 vs. “low risk” l/l: 0.01) leads to a larger effect size ( $f = 1.13$ ) and therefore a sample of only 10 per group gives a 100% power to detect a difference between the groups.

#### *Analysis 5: Correlation between Amygdala and OFC Activation*

The beta extraction also allowed the examination of correlation between the OFC and amygdala across all subjects. There was a significant correlation between the activation in the OFC and amygdala in the attended versus non-attended contrast ( $R = +0.80$ ;  $p < 0.005$ ) (Figure 5). There was no difference when analyses were done separately by group or by genotype.

Figure 5: Correlation between OFC and Amygdala Activation in the Attended vs. Non-Attended Contrast (N=18)

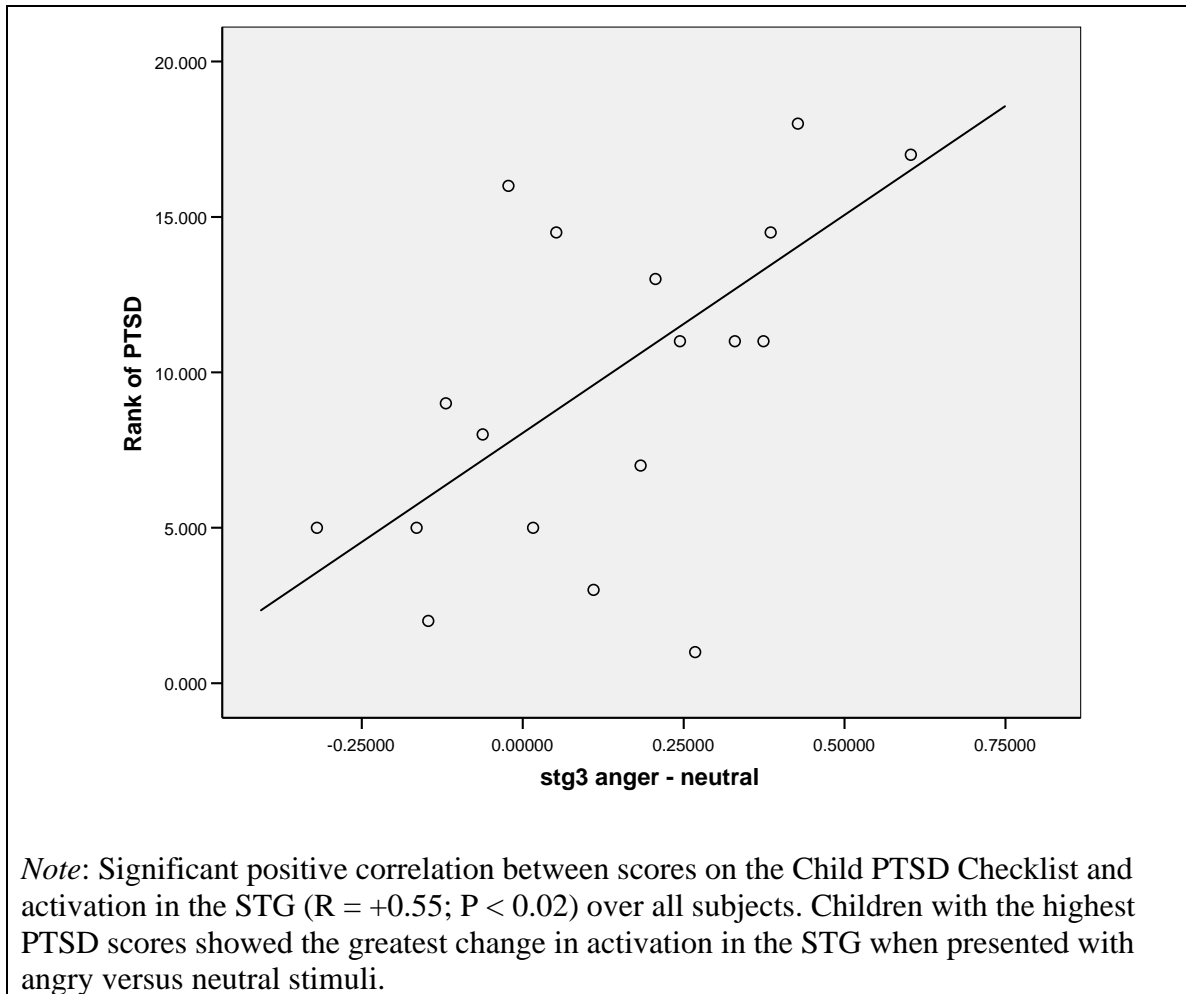


#### Analysis 6: Clinical Correlates of Brain Activation

PTSD fundamentally involves alterations in emotion processing and behavior. Therefore, the clinical assessment scores that had been collected on the children before participation in the fMRI protocol were correlated with the brain regions of interest. There was a correlation between PTSD scores on the PTSD Checklist and changes in STG activation to Angry v. Neutral contrast ( $R = +0.55$ ;  $P < 0.02$ ). Examining the

subtests of the PTSD checklist, the correlation is mostly due to the avoidance scores (Figure 6).

*Figure 6: Correlation of PTSD score with STG Activation to Angry Stimuli*

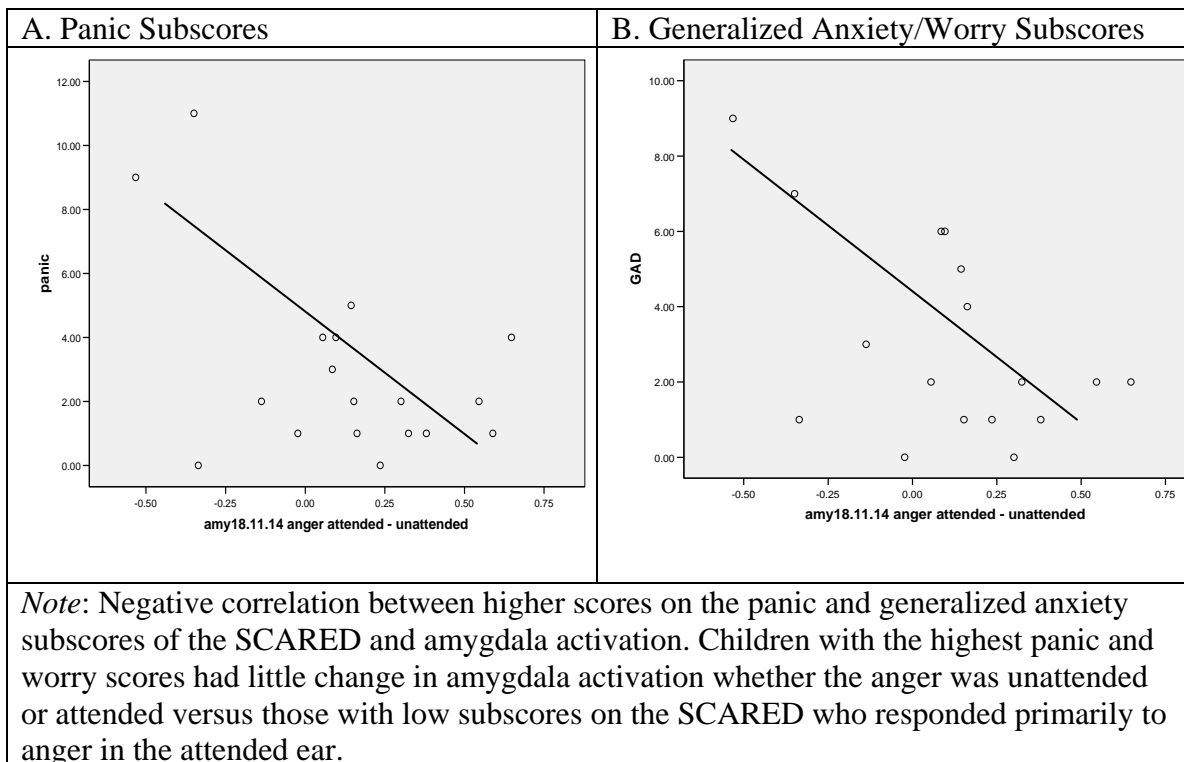


Additionally, scores on the SCARED subscales were examined for correlation with changes in amygdala activation for the Attended Anger vs. Non-Attended contrast. There was a negative correlation between scores on the panic and generalized anxiety subtests of the SCARED ( $R = -0.50$  and  $-0.53$ , respectively) (Figure 7). Children with higher scores on the SCARED subscales showed higher activation in the amygdala during non-attended trials than children with lower scores; therefore, the children who



had more anxious traits had less change in the amygdala activation when comparing the attended to the non-attended angry trials. The measures on the two subtests were also significantly correlated (0.789).

*Figure 7: Correlations between Subscales of SCARED and Amygdala Activation*



### **Discussion**

The current study is the first to use fMRI in maltreated children and the first imaging genetics study to examine brain circuitry in traumatized individuals. When simultaneously examining the impact of genetics and stress on patterns of brain activation, only main effects of genetics were found. Given the previous findings in structural neuroimaging in pediatric PTSD, it was surprising that there were no main effects of trauma in any of our a priori regions of interest. In fMRI studies with

traumatized adults, enhanced activation in the amygdala and OFC had been reported in several studies, but even when examining a continuous measure of stress, differences did not emerge based on stress.

However, the finding of a main effect of genetics is consistent with an in press study in adults with an active episode of MDD versus control subjects which also only found main effects of the serotonin transporter risk gene, and no effect for depression status or the two in interaction in accounting, for activation pattern differences in the amygdala during an emotion processing task.<sup>132</sup> To date, there have been 17 studies (14 published, and 3 unpublished) that have examined the effect of the serotonin transporter polymorphism on amygdala activation during fMRI in either controls without psychopathology or cohorts of clinical (social phobia, panic disorder, or major depression) patients.<sup>133</sup> The studies have consistently reported increased amygdala activation across a range of paradigms in association with the “s” allele of the serotonin transporter gene.<sup>133, 134</sup>

In addition to finding genetic effects on amygdala activation, there were also effects on orbitofrontal cortex activation. The orbitofrontal cortex modulates attention, especially to emotional stimuli.<sup>64, 135</sup> Attention effects are particularly important as the orbitofrontal cortex is understood as involved in the “executive” functions.<sup>136</sup> Genetically susceptible children “s/s” activated the amygdala and OFC more than the less genetically susceptible “l/l” children when the anger was on the attended side. Based on prior studies, these were the predicted directions of findings, indicating increased activation of the emotion processing or “limbic” system. Over all subjects there was a positive correlation between the amygdala and OFC. Given the expected connectivity between the areas, the

positive correlation is an intriguing finding, suggesting that when children are activating the orbitofrontal cortex they are also activating the amygdala.

Examination of group differences in the STG failed to reach statistical significance, but there was a difference in the pattern of neural activity. Because one of the major interests was to examine interhemispheric transfer of the information, we examined the interaction between Broca's and the Superior temporal area. Both stress groups, the high stress, maltreated participants and the low-stress controls activated to attend anger. However, maltreated children in the high stress group consistently activated Broca's and the STG regardless of attention to anger, perhaps displaying less of an ability to filter affectively charged information. This finding may relate to the trend toward differences in performance on the percent correct between the high stress and low stress groups. The low stress group only activated Broca's and the STG together during trials when they were attending to anger. However, low-stress showed a negative correlation when they were not attending to the anger, implying higher activation in the Broca's was associated with lower activation in the STG during non-attended trials. Because maltreated children have been found to have structural changes in the corpus, the differences in the pattern of correlation between the two areas may be a reflection of decreased posterior corpus integrity or size.<sup>137</sup>

Moreover, when examining clinical characteristics, the activation in the STG was related to PTSD symptoms. In fact, children with higher PTSD scores had significantly more activation in the superior temporal gyrus in the angry versus neutral condition. Given that the high risk genotype "s/s" children had higher PTSD scores than the low risk genotype "l/l," the genotype may impact both the perception of stressful events as well as

the activation in the superior temporal gyrus, which is highly sensitive to prosodic (e.g., angry) information. Another aim of the present study was to examine the overall pattern of activation in children and adolescents when completing the task that has previously only been administered to adults. Using the findings in adults as a template, there were many similarities in brain activation.<sup>131</sup> Children performing a dichotic listening task with emotionally salient nonsense words preferentially activated the amygdala, STS, STG, Broca's area and the anterior cingulate (as compared with neutral words) independent of attention. The amygdala has consistently been identified as being involved in the processing of emotion through both visual tasks and those that involve listening to emotional stimuli.<sup>138, 139</sup> Additionally, the superior temporal areas have been associated with processing of emotional speech, preferentially compared with non-affectively salient speech.<sup>140, 141</sup> Moreover, it seems as though the STS/STG may be preferentially processing paralinguistic information, such as emotional prosody.<sup>141-143</sup> Broca's area, in the left inferior temporal gyrus, has been known for many years to be involved in the comprehension and production of emotionally salient speech due to studies in patients who had infarcted the area.<sup>76</sup> Anterior cingulate is involved in attention and the control of emotions.<sup>144</sup> The data from this study suggests that the task robustly elicits activation in relevant areas in juvenile populations and is an excellent alternative emotion processing task which avoids race biasing effects inherent in face processing tasks.

## **Limitations**

There were several important limitations to the current studies. First, there was a limited sample available from which to recruit, but hopefully future studies may be able

to include more participants. There was also an absence of demographically matched controls with a complete absence of trauma. In fact, all children in the study had at least one “criterion A” traumatic experience. Moreover, none of the maltreated children were in the acute phase of their traumatic experience. All were in stable placements, either back in the home with their biological parents or in a stable foster placement. Therefore, including a wider range of stressful experiences (i.e., those with no stressful experiences and those with acutely stressful experiences) may yield differences. Finally, the analyses were restricted primarily to the a priori regions of interest. In the future, a whole-brain voxel-based analysis may identify other stress-related areas. Additionally, other methods of probing the connectivity between areas likely will be employed as our findings indicate activation in the regions of interest are correlated.

## **Conclusion**

Genetic variation is associated with individual differences in neural processing, and these differences are observed in people with and without psychopathology. Studies are only beginning to uncover the robustness of genetic variations’ effects on particular pathways. However, it remains to be investigated why “hard-wired” individual differences may lead to particular outcomes in some but not all people. Many potential explanations remain to be studied. For one, connectivity and the integrity of the synaptic connections may play a role in the potential for development of psychopathology. Gene-gene interactions may be another way of examining the variability in outcomes, and with gene microchip array technologies, multiple genes may eventually be studied in behavioral genetics. Finally, there may be a more complicated relationship to other

environmental risk or protective factors than the science yet understands. Overall, the field of imaging genetics and the influence of genetics on emotions, behavior, and psychopathology remain an active area for future research.

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## Appendix 1: Stimulus Characterization

The specific aims of the Stimulus Characterization project were to examine the appropriateness of an emotionally salient prosody-processing task in a pediatric sample. The hypothesis was that the participants would rate the previously characterized angry stimuli as significantly angrier than the neutral stimuli. Moreover, the individual stimuli were characterized by the number of “angry” responses vs. “neutral” and separated by those used in the subsequent fMRI project.

Data was collected in the behavioral study after the inception of the neuroimaging project and will be used to further examine the dichotic listening task behaviorally in the pediatric age range.

### *Methods*

#### *Participants*

Thirty-four participants (age range: 6-12 years old) participated in a behavioral study rating the sounds used in the dichotic listening task. Participants were approximately equally distributed across genders (see Table A.1).

*Table A.1: Behavioral Study Participant Characteristics*

<b>Age</b>	<b>Gender</b>	<b>MFQ</b>	<b>SCARED</b>	<b>CBCL</b>	<b>FSIQ</b>
9.1 ± 2.0	.50 F	12.8 ± 7.4	17.8 ± 10.6	51.4 ± 11.5	94 ± 13

All information presented as mean ± standard deviation except gender. F = female. *MFQ* = Mood and Feelings Questionnaire (depression screen); *SCARED* = Screen for Child Anxiety Related Disorders; *CBCL* = Child Behavior Checklist (normed to mean of 50±10); *FSIQ* = Full Scale Intelligence Quotient (normed to mean of 100±15).

*Table A.2: Race and Ethnicity of Behavioral Study Participants*

<b>Race/Ethnicity</b>	<b>Percentage</b>
<i>Caucasian, Non-Hispanic</i>	11.8



<i>African-American</i>	58.8
<i>Hispanic</i>	26.5
<i>Biracial</i>	2.9

*Table A.3: 5HTTLPR Genotype*

<b>s/s</b>	6
<b>l/l</b>	15

Note: Genotype not available for 13 participants.

### *Recruitment and Informed Consent*

The Yale University Human Investigations Committee approved this study. The children in this behavioral study were participants in a larger study examining the genetic and environmental predictors of childhood depression. Parents or guardians gave consent to participation in the research camp; children gave written assent to participate. Families were recruited through advertisements in the local newspaper and through mailings to state-certified day-care providers in the greater New Haven area to participate in research through a week-long summer day camp. Inclusion in the study was limited to children with no current major medical diagnoses precluding camp participation. Informed consent was obtained by guardians of all participants, and assent was provided by all participants.

### *Baseline Assessments*

Informed consent and baseline assessments were conducted by Masters-level research personnel either at Yale University facilities or the participant's home. An initial visit was carried out with the parental or guardian informant detailing demographic and clinical information. After enrollment in the study, the parent or guardian was given a number of baseline assessments and reported detailed demographic information. Parents

were asked to fill out the *Child Behavior Checklist [CBCL] Parent Report Form*: a one-hundred and thirteen item parent report measure which assesses internalizing (e.g. depression, anxiety) and externalizing (e.g. aggression) symptomatology in children and adolescents. It yields a Total Behavior Problem Score, Internalizing Factor Score, and Externalizing Factor Score.<sup>145</sup> The CBCL was completed at the first baseline assessment.

The second baseline assessment was conducted with two research personnel, one conducting a psychiatric diagnostic interview with the parent or guardian about the participant, and the other, an initial demographic, history, and clinical interview with the participant. At the second baseline assessment meeting, self-reports of symptoms were obtained from children using the *Screen for Child Anxiety Related Emotional Disorders*<sup>124</sup> which is a forty-one item rating scale developed to assess anxiety symptoms in children and adolescents. They were also asked *Mood and Feelings Questionnaire*<sup>125</sup> which is a thirty-three-item self-report measure that assesses depressive symptomatology in children. Each of the items was read by the research personnel to the child.

#### *Dichotic Listening Stimuli*

The task was administered to a group of four children at a time by three trained research assistants. Participants were presented with the stimuli bilaterally through headphones using E-Prime software (E-Prime, Psychology Software Tools Inc., Pittsburgh, Pennsylvania). The emotion processing task utilizes two nonsense words (e.g. “goster” and “niuvenci”) recorded in angry and neutral tones by speakers of different genders.<sup>129</sup> The auditory stimuli are presented bilaterally through a headset. All auditory stimuli were randomized independently for each participant. Participants were instructed to rate the stimuli according to how angry the stimulus sounded. Responses were

recorded via keyboard buttons one through five, labeled with cartoons of neutral to angry facial expressions.

### *Data Analysis*

Data from E-Prime were analyzed using SPSS 15.0 statistical software (SPSS, SPSS Inc., Chicago, Illinois). The stimuli had been previously characterized as angry or neutral prosody in an adult sample; therefore, a paired samples t-test compared the ratings of angry and neutral stimuli across all participants. A Pearson correlation was completed to look at the effects of age on angry and neutral ratings. Additionally, the range of ratings for individual stimuli, including those selected for inclusion in the fMRI task, was examined. Ratings of the stimuli were analyzed with all stimuli and with the stimuli that were specifically chosen during the pilot feasibility for the fMRI task were also compared using a pairwise t-test. Genetic differences in angry and neutral words were compared using an independent samples t-test.

### *Author Contributions*

The author was involved in the recruitment, assessment, and clinical interviewing for many of the participants, and was present when all participants completed Study 1 at the Summer Research Camp described above. John Herrington coded the behavioral E-Prime task and abstracted the data from E-prime for all participants so that it could be analyzed by the author. All included statistical analyses for Stimulus Characterization were conducted by the author.

### *Results*

Given that the stimuli had been characterized in an adult sample, our primary goal was to ensure that participants in a younger age range could differentiate the 20 “angry”

from the 20 “neutral” prosody words in the stimuli set. In the first analysis, participants (N=34) were able to distinguish between angry (average rating: 3.4) and neutral (average rating: 2.0) stimuli on a scale of 1-5 (Table A.4). Additionally, there were small, but nonsignificant correlations between the average rating and age (Table A.5). In another analysis of the data, the ratings of the angry and neutral stimuli were not found to differ by genotype, although only 21 subjects had available genotypic information (Table A.6).

*Table A.4: Ratings of Angry and Neutral Stimuli*

	<b>Angry Stimuli</b>	<b>Neutral Stimuli</b>	<b>Sig. (2-tailed)</b>
Ratings	3.4 ± 0.4	2.0 ± 0.7	<0.001

Mean ± SD.

*Table A.5: Correlation of Angry and Neutral Stimuli with Age*

	<b>Age α Angry Stimuli</b>	<b>Age α Neutral Stimuli</b>
<b>Correlation Coefficient</b>	0.140	-0.287
<b>Sig. (2-tailed)</b>	0.430	0.100

*Table A.6: Emotion Processing by Genotype*

	<b>Angry Stimuli</b>		<b>Neutral Stimuli</b>	
	<b>s/s</b>	<b>l/l</b>	<b>s/s</b>	<b>l/l</b>
<b>Ratings</b>	3.6 ± 0.4	3.5 ± 0.4	1.8 ± 0.9	2.2 ± 0.6
<b>Sig. (2-tailed)</b>	0.671		0.352	

Mean ± SD.

Another goal of the behavioral study was to examine the ratings of the stimuli used in the fMRI experiment, which had been determined by the pilot feasibility study. From the beginning sample of 40 stimuli, the first three fMRI Pilot Feasibility participants chose 8 of the most unambiguous angry stimuli and 18 of the most unambiguous neutral stimuli. As with the overall stimuli, participants were able to discriminate between the angry (average rating: 4.2) and neutral stimuli (average rating:

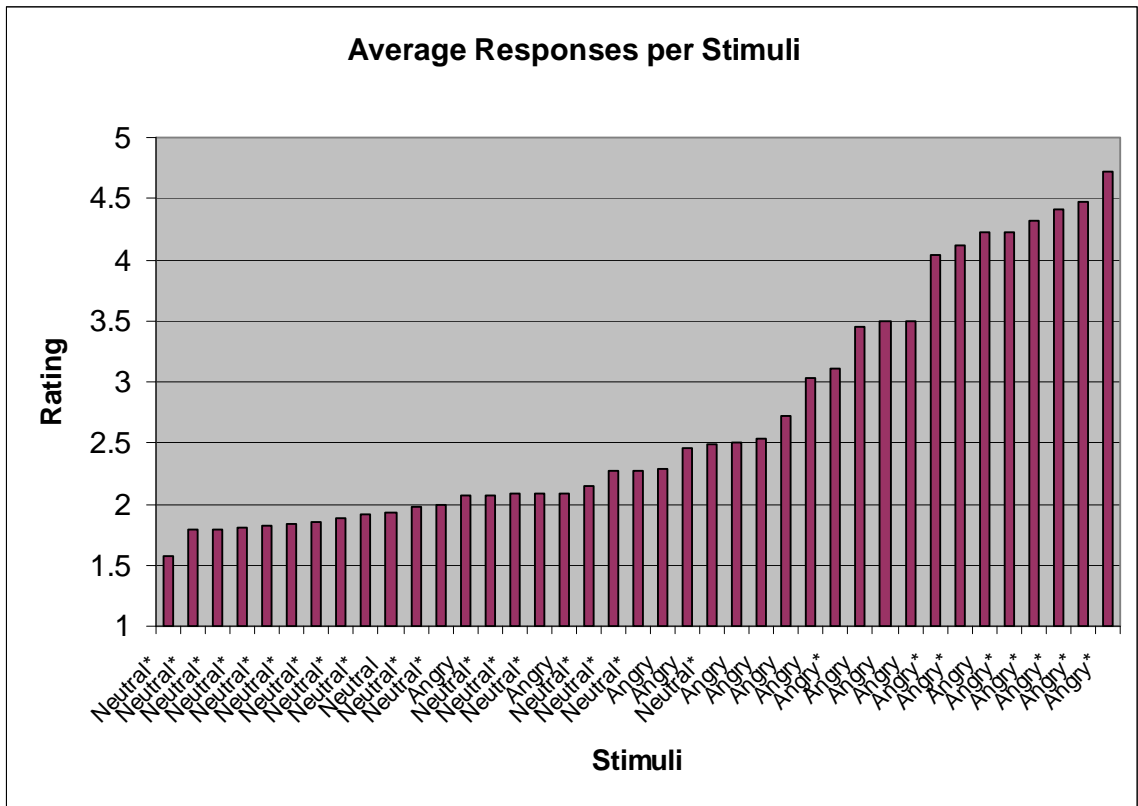
2.0) used in the fMRI protocol (Table A.7). When using only the stimuli that were then used in the scanner, ratings of angry stimuli increased from 3.4 to 4.2. The stimulus ratings were also visually examined to ensure stimulus disambiguation (Figure A.1).

*Table A.7: Ratings of Angry and Neutral Stimuli Used in fMRI Protocol*

	<b>Angry Stimuli</b>	<b>Neutral Stimuli</b>	<b>Sig. (2-tailed)</b>
Ratings	4.2 ± 0.5	2.0 ± 0.7	<0.001

Mean ± SD.

*Figure A.1: Ratings of Individual Stimuli*



Affective ratings of nonlinguistic human sound stimuli. An emotion rating of “1” indicates a neutral sound; a rating of “5” indicates a negative/unhappy sound. Note: Starred stimuli were used in the fMRI experiment.

*Table A.7: Percentage of Participants Rating “Angry” fMRI Stimuli a 4 or 5*

<b>Stimulus</b>	<b>%4or5</b>
Ang1	20.7
Ang2	69.0
Ang3	74.2
Ang4	80.0
Ang5	83.3
Ang6	85.2
Ang7	86.7
Ang8	92.6
<b>Average</b>	<b>74.0</b>

*Table A.8: Percentage of Participants Rating “Neutral” fMRI Stimuli a 1 or 2*

<b>Stimulus</b>	<b>%1 or 2</b>
Neut1	78.1
Neut2	78.1
Neut3	78.8
Neut4	80.0
Neut5	81.5
Neut6	83.9
Neut7	87.9
Neut8	88.5
Neut9	90.0
Neut10	90.0
Neut11	92.0
Neut12	92.0
Neut13	92.9
Neut14	92.9
Neut15	93.1
Neut16	95.5
Neut17	100.0
Neut18	100.0
<b>Average</b>	<b>88.6</b>

Given the individual visual examination of the stimuli used in the fMRI study, only one of the angry stimuli was ambiguous. Looking at the first time the stimulus was rated by participants in the study, only 20.7% of participants rated the ambiguous “angry” stimulus as a 4 or a 5. Overall, participants were able to identify the angry stimuli used in

the fMRI study as a 4 or a 5 74.0% of the time upon first presentation. Additionally, 88.6% participants were able to identify the neutral stimulus as a 1 or a 2.

## Appendix 2: Participant Characteristics

N= 18 Participants in the fMRI Study

*Table A.9: Participant Characteristics*

		<b>Age</b>	<b>Gender</b>	<b>MFQ</b>	<b>SCARED</b>	<b>PTSD</b>	<b>FSIQ</b>
<b>Low Stress</b>	<b>l/l</b>	15.0±1.7	.67 F	4.3 ± 5.8	11.0 ± 8.9	6.3 ± 4.9	97.7±16.7
	<b>s/s</b>	13.7±2.1	.67 F	11.7 ± 6.5	12.3 ± 10.1	12.7±4.6	89.0 ± 4.6
<b>High Stress</b>	<b>l/l</b>	13.6±2.1	.50 F	9.0 ± 5.7	17.3 ± 9.6	5.5±4.4	95.5±13.8
	<b>s/s</b>	10.0±3.5	.25 F	14.8 ± 8.8	16.5 ± 14.2	26.5±21.7	97.9±13.8
<b>Sig.(2-tailed) (stress, gene)</b>		0.063, 0.174	0.308, 0.657	0.269, 0.072	0.358, 0.958	0.259, <b>0.027*</b>	0.416, 0.920

All information presented as mean ± standard deviation except gender. F = Female. *MFQ* = Mood and Feelings Questionnaire; *SCARED* = Screen for Child Anxiety Related Disorders; *PTSD* = Child PTSD Checklist; *FSIQ* = Full Scale Intelligence Quotient (normed to a mean of 100 ± 15).

*Table A.10: Race and Ethnicity of Participants*

<b>Race/Ethnicity</b>	<b>Stress Level</b>	<b>High Risk “s/s”</b>	<b>Low Risk “l/l”</b>
<b>White, Non-Hispanic</b>	<b>High Stress</b>	2	1
<b>African-American</b>	<b>High Stress</b>	1	4
	<b>Low Stress</b>	1	1
<b>Hispanic</b>	<b>High Stress</b>	1	3
	<b>Low Stress</b>	1	1
<b>Biracial</b>	<b>Low Stress</b>	1	1