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Shared Genetic and Environmental Influences on Fear, Anxiety, Posttraumatic Stress, and Brain Morphometry

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Shared Genetic and Environmental Influences on Fear, Anxiety, Posttraumatic Stress, and Brain Morphometry

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Virginia Commonwealth University

by

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List of Abbreviations

A ..... Additive genetic factor

Ac..... Common additive genetic factor

ACC ..... Anterior cingulate cortex

Ads..... Anxiety disorders

AIC .....Akaike information criterion

ANML ..... Fear of animals

As .....Specific additive genetic factor

C ..... Familial environment

Cc ..... Common familial environment

cACC ..... Caudal anterior cingulate cortex

CFA .....Confirmatory factor analysis

CFM .....Correlated factors model

CPM ..... Common pathway model

CRIT ..... Fear of failure and criticism

Cs ..... Specific familial environment

CxE ..... Familial environment x environment interaction

DF ..... Degrees of freedom

DNA .....Deoxyribonucleic acid

DSM .....Diagnostic and Statistical Manual

DZ ..... Dizygotic twin pair

E ..... Unique environment

Ec ..... Common unique environment

EP ..... Estimated parameters

Es ..... Specific unique environment

ExE ..... Unique environment by environment interaction

FIML ..... Full information maximum likelihood

FDR ..... False discovery rate

fMRI ..... Functional magnetic resonance imaging

FSSC-R ..... Fear survey schedule for children revised

FSSC-RSF ..... Fear survey schedule for children revised short form

GAD ..... Generalized anxiety disorder

GWAS ..... Genome-wide association study

GxE ..... Gene by environment interaction

IPM ..... Independent pathway model

JAS ..... Juvenile Anxiety Study

lOFC ..... Lateral orbitofrontal cortex

M ..... Moderator

MED ..... Medical fears

MGH ..... Massachusetts General Hospital

mOFC ..... Medial orbitofrontal cortex

MRI ..... Magnetic resonance imaging

MZ ..... Monozygotic twin pair

NIH ..... National Institute of Health

NIMH ..... National Institute of Mental Health

OEF ..... Operation Enduring Freedom

OIF ..... Operation Iraqi Freedom

OND ..... Operation New Dawn

PAN ..... Panic disorder

PFC ..... Prefrontal cortex

PTSD ..... Posttraumatic stress disorder

rA ..... Genetic correlation

rACC ..... Rostral anterior cingulate cortex

rC ..... Familial environment correlation

RDoC ..... Research Domain Criteria

rE ..... Unique environment correlation

RMSEA ..... Root mean square error approximation

SCARED ..... Screen for Child Anxiety Related Emotional Disorders

SD ..... Standard deviation

SE ..... Standard error

SEM ..... Structural equation modeling

SEP ..... Separation anxiety

SOC ..... Social anxiety

T ..... Trait

TE ..... Echo time

TI ..... Inversion time

TR ..... Repetition time

UCSD ..... University of California, San Diego

UNKN ..... Fear of the unknown

VCU-JAS ..... Virginia Commonwealth University Juvenile Anxiety Study

VETSA ..... Vietnam Era Twin Study of Aging

vmPFC ..... Ventromedial prefrontal cortex

Abstract

THE GENETIC AND ENVIRONMENTAL INFLUENCES ON FEAR, ANXIETY, AND POSTTRAUMATIC STRESS SHARED WITH BRAIN MORPHOMETRY

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A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Virginia Commonwealth University.

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Anxiety disorders (ADs) and stress-related disorders are some of the most common psychiatric disorders in the United States. Like other complex psychiatric illness, genetics and neuroimaging research has focused on understanding their underlying neurobiology. Areas within the fear-network play important roles in threat perception, fear conditioning/learning, cognitive processing, and modulation of fear responses including contextual modulation and extinction and have been implicated in ADs as well as stress disorders such as posttraumatic stress disorder (PTSD). The primary gap in the current search for underlying biological mechanisms is in whether biomarkers associated with disorders share genetic influences with the disorders they index. Therefore, the aims of this dissertation are: 1) to examine the shared etiology of PTSD and threat-related brain regions while accounting for trauma using a large sample of male twins who served in the

military during the Vietnam War; 2) to elucidate the shared and specific risk factors (genetic, familial environment and unique environment) and their roles amongst fear and anxiety domains in children; and 3) to examine whether brain regions previously implicated in fear processing and anxiety are significantly associated with a genetic factor score indexing fear and anxiety measures in a child sample. Using biometrical twin modeling this dissertation produced several novel findings regarding etiology of PTSD, threat-related domains and associated brain morphometry. Analyses investigating brain morphometric differences as potential endophenotypes for PTSD provided preliminary evidence that their phenotypic association is largely accounted for by environmental influences, specifically trauma exposure. However, sample size-induced model instability limits the ability to make definitive conclusions. Examining domains of fear and anxiety in children suggested a substantial genetic overlap between the two. Finally, the incorporation of a genetic factor score derived from the results of the biometrical modeling of fear and anxiety provided preliminary evidence for a genetic relationship between fear/anxiety and brain regions of interest. Although these results should be interpreted within the context of important limitations, they provide clear evidence that additional research into the genetic relationship between brain regions and disorders with larger sample sizes is justified.

Chapter 1: Global Introduction

Anxiety disorders (ADs) such as panic, generalized anxiety, social phobia, and specific phobias are some of the most common psychiatric disorders in the United States. Although now in a separate section in the 5th edition of the Diagnostic and Statistical Manual[1] (DSM-5), posttraumatic stress disorder (PTSD) was once considered part of the AD[2] group and retains a high degree of comorbidity with the ADs. Psychiatric neuroimaging research has focused on understanding the underlying neurobiology of these disorders.

Before we can begin to examine the underlying biology, it is important to first describe what exactly is being examined. There are two main phenotypic paradigms researchers use when studying psychopathology: diagnoses from the DSM and other diagnostic classification systems, and systematic domains and constructs from the National Institute of Mental Health (NIMH) Research Domain Criteria (RDoC) initiative. Until recently DSM diagnoses, and to a lesser extent symptom counts, were the primary foci of inquiry. However, the NIMH has emphasized the need to broaden research of mental health outcomes beyond diagnostic boundaries via creation of the RDoC initiative. RDoC was created to implement Strategy 1.4 of the 2008 NIMH Strategic Plan: "Develop new ways of classifying disorders based on dimensions of observable behaviors and brain functions" and assumes that mental illness is a disorder of brain circuits, with biosignatures detectable via genetics and clinical neuroscience

research which will ultimately be used to augment symptom assessment for diagnosis and treatment planning.[3] This shift beyond diagnoses may also help researchers find underlying biological mechanisms that are ultimately shared across diagnoses and in part account for the high comorbidity seen between certain disorder groups. The search for underlying biological mechanisms is heavily focused on identifying whether a biomarker meets the criteria for an endophenotype of the disorder. To be considered an endophenotype a biomarker must: 1) associate with the disorder in the population; 2) be heritable; 3) be state-independent (i.e., is present whether disorder is active or not); 4) co-segregate with the illness within families; and 5) also present in unaffected family members of affected individuals at a rate higher than that of the general population.[4] Endophenotypes are thought to form part of a neurobiological bridge between phenotypes and genotypes. The focus on endophenotypes and biological mechanisms/markers shared across diagnoses may be especially effective for neuroanatomic magnetic resonance imaging (MRI) as the functional MRI (fMRI) studies are task specific and as such are not as amenable to comparisons across disorders compared to structural MRI, which does not require task activation. Currently, research involving fMRI uses case-control group comparisons to examine differences in task-elicited activation of specific regions. Task-based analyses are designed to elicit activation in either a specific structure or a specific network of structures[5] (e.g. the fear network) and as such, it becomes difficult to disentangle whether differences between studies were due to the different tasks used or represent distinct findings. To a more minor point, the possible variations in definitions of a 'case' (e.g., only generalized anxiety patients (GAD), GAD plus other anxiety disorders such as social anxiety, or panic, or GAD with or without major depression comorbidity) can further complicate aggregations of study findings.

Hallmarks of ADs in general include excessive fear, avoidance, and worry in response to specific stimuli and absent of any imminent danger[1]. ADs are some of the most common psychiatric disorders within the community[6], and neuroimaging research has focused on understanding their underlying neurobiology. Since excessive fear is a core element of ADs symptomatology, research into their neurobiology has largely derived from the study of fear circuits in animal models. Key components of this fear circuitry include the amygdala, hippocampus, hypothalamus, insular cortex, anterior cingulate cortex (ACC) and areas of the prefrontal cortex (PFC) which can go by many labels including the ventromedial PFC, and the medial or lateral orbitofrontal cortex (mOFC; lOFC). These areas play important roles in threat perception, fear conditioning/learning[7,8], cognitive processing, and modulation of fear responses including contextual modulation[9] and, in some circumstances, extinction[10]. The theory is that by understanding the mechanisms related to the symptoms more effective treatments can be developed or refined. Figure 1.1 illustrates the divisions of the OFC and ACC while figure 1.2 shows the structure of the hippocampus and amygdala.

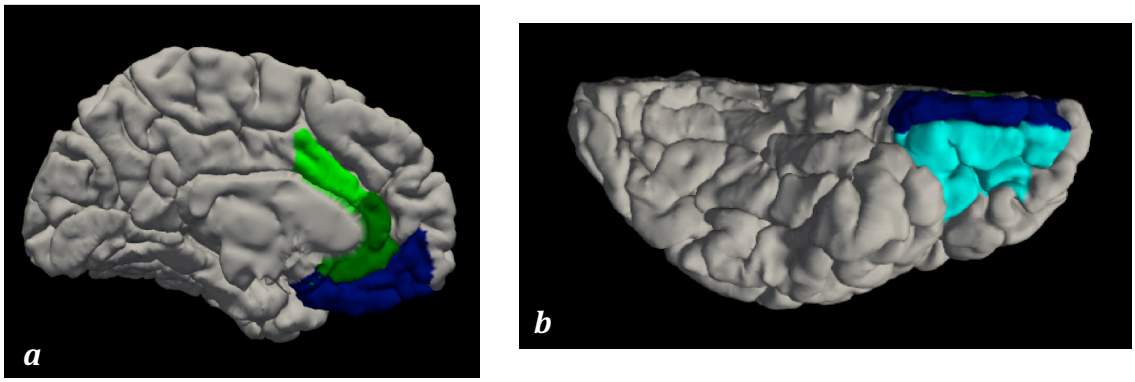

Figure 1.1 Divisions of the left orbitofrontal and anterior cingulate cortices
Panel *a* shows a sagittal cross section and *b* shows an inferior view of the left hemisphere. Medial orbitofrontal cortex (OFC) is illustrated in dark blue, lateral OFC in light blue. Caudal anterior cingulate cortex (ACC) is in light green and rostral ACC is in dark green.

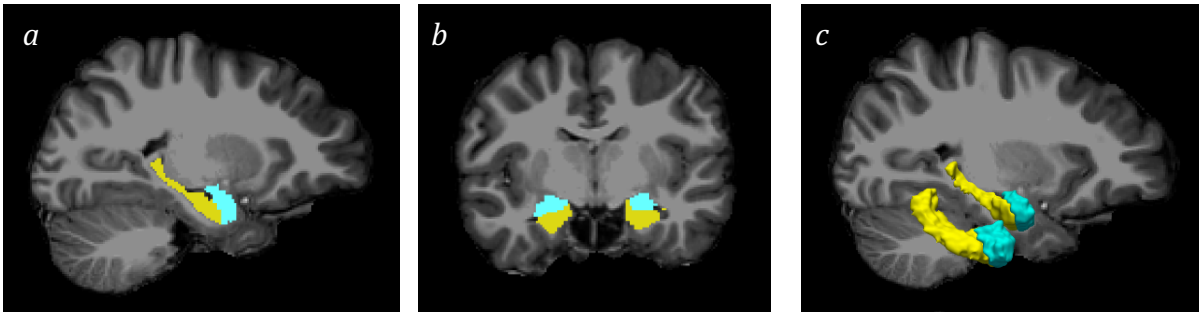

Figure 1.2 Bilateral Structures of the Hippocampus and Amygdala
Panel *a* shows a sagittal view of the hippocampus (light blue) and amygdala (yellow), *b* shows a coronal view and *c* shows a 3-dimensional bilateral reconstruction of the structures with a sagittal cut of the left hemisphere to provide relative spatial context.

The basic areas of the fear-related neurocircuitry are a useful place to begin examining anxiety-related neurocircuitry. It should be noted that the specific roles of many brain regions have not been unequivocally established. Generally speaking, studies examining these areas have found increased amygdala activation in response to disorder-relevant stimuli in posttraumatic stress[11,12], social phobia[13–16], and specific phobias[17,18]. There is some evidence to suggest reduced hippocampal volumes may be unique to PTSD in comparison to ADs but is not unique across all psychiatric disorders. Additionally, positron emission tomography and fMRI studies of PTSD have found a decrease in[19,20], or failure to activate[21] the PFC (including ACC) when presented with trauma related stimuli[19,20], and fear extinction[21] tasks. Whereas disorder-relevant stimuli have elicited hyperactivation of these areas in GAD[22], and phobia patients.[23] Evidence also suggests that morphometric differences in these PFC areas are associated with these disorders to varying degrees. Thus far it is not clear whether these functional and morphometric differences are the cause or the effect of specific disorders, and further research is needed to untangle them. Given the accumulation of studies and meta-analyses that associate

PTSD and ADs with morphometric differences in the brain, it is a logical next step to investigate potential shared etiology between PTSD/ADs and regions of interest (ROIs) with the goal of identifying whether ROI morphometries meet the criteria for endophenotypes of PTSD/ADs.

The data available for this dissertation did not have all three phenotypes of interest (PTSD, fear, and anxiety) available in one sample. Therefore, to capitalize on available data, lines of inquiry were split between an adult and child sample. ADs are some of the most common psychiatric disorders within a community whether examining adult or child prevalence rates, whereas PTSD is less so in adults and children. Additionally, many ADs begin in childhood. Therefore, this dissertation will focus on PTSD and fear-network ROIs within an adult sample and will use a child sample to investigate fear and anxiety as they relate to fear-network ROIs.

Posttraumatic Stress Disorder and Trauma Exposure

PTSD Prevalence and Etiology

Exposure to accidental and interpersonal forms of trauma, such as a car accident or physical and emotional abuse, respectively, is associated with many negative consequences including the possible development of PTSD.[24] PTSD involves the persistent reexperiencing of a traumatic event through nightmares, intrusive memories, or flashbacks, persistent negative thoughts and emotions, and hyperarousal or excess reactivity after the event. According to the World Mental Health Survey Consortium, the United States has one of the highest rates of trauma exposure with 82% of participants from the United States endorsing at least one traumatic event.[25] Whereas, most European countries had endorsement rates below 80%, and Asian countries generally had even

lower rates (52% endorsement in China, and 60% endorsement in Japan). While estimates of the prevalence of trauma in the United States range from 60-90%[26], the lifetime prevalence of PTSD is 10-12% in women and 5-6% in men with approximately 8% of the total United States population developing PTSD at some point in their lifetime.[27] Therefore, it is possible to conceptualize a PTSD diagnosis as an inability to recover from the effects of trauma. Subsequently, understanding the role of trauma exposure on the biological systems involved in the development of PTSD may help to improve prevention and treatment for individuals at risk for PTSD after exposure to trauma.

PTSD is a moderately heritable (30-72%) condition across a range of trauma types[28–31], and several biological systems may be involved in its development. PTSD is highly comorbid with anxiety disorders and major depressive disorder.[31–34] The high comorbidity may be explained, in part, by substantially overlapping genetic influences as is the case with PTSD and MDD in women[31] and men[34].

Trauma Types

Trauma exposure can be categorized into a few main types including: interpersonal, accidental, military, and childhood. A major distinction in trauma types as identified via principal components analysis is between assaultive (or interpersonal) and non-assaultive trauma.[30] In the context of civilian trauma, assaultive trauma includes experiences such as being robbed, held captive, sexual assault, and other life threatening experiences.[30,35] Whereas non-assaultive trauma encompasses experiences such as sudden family death, car accident, fire, and natural disasters (i.e. tornado, flood, earthquake etc.) and are generally thought to be more random in nature.[30,35] In general,

individuals exposed to assaultive traumatic events are at a higher risk to develop PTSD compared to events without an interpersonal component.[35–38]

Men and women experience these types of trauma at differing rates with men generally experiencing overall higher rates of trauma exposure with 61% of men compared to 51% of women reporting at least one traumatic experience.[27] Women are more likely to be exposed to interpersonal traumas[25,27,35,39], specifically sexual assaults, and men are more likely to experience accidents, interpersonal violence, and combat-based traumas.[25,27,39] Additionally, while men experience more events, women were at a higher risk for PTSD when controlling for trauma type in a community based sample[35] as well as in a meta-analysis.[39]

Trauma due to combat exposure during military service is experienced in roughly equal proportions between men and women. Of those who served during the Vietnam era 30% served in southeast Asia, and 15% of vets are thought to have been directly involved in combat[40] with an additional 14% exposed to combat hazards while serving.[41] Within samples of more recent veterans of Operation Enduring Freedom (OEF), Operation Iraqi Freedom (OIF), and Operation New Dawn (OND), the rate of combat exposure is substantially higher, with 95%[42] of returning veterans reporting exposure to at least one combat trauma event.

Trauma exposure that occurs during childhood is thought to have an especially strong influence on the development of psychopathology within child- and adulthood to a greater extent than experiencing traumatic events as an adult. This is supported by distinct epigenetic[43,44] and neurobiological[45] alterations that are associated with childhood but not adult trauma. Various studies have reported the prevalence of exposure to different trauma types during these developmentally sensitive time periods. According

to the Nation Survey of Children's Exposure to Violence depending on how trauma is defined and the reporter used, 8-12% of American youths (0 - 17 years old) have experienced at least one sexual assault, 9-19% have experienced physical abuse by a caregiver, and 38-70% have been witness to serious violence within the community.[46] Additionally, 20% of all youths had experienced more than one type of trauma. Studies have shown that higher levels of trauma are linked to more severe forms of distress in adolescents.[47,48]

When examining the etiology of trauma types, it was found that assaultive trauma, being related to human behavior, is influenced by genetic factors, while non-assaultive traumas were not. Heritability of assaultive trauma varies greatly within this broad category. 'High-risk' traumas had a heritability estimate of 60%[31], combat exposure within a male sample estimated heritability at 35-54%[49] with the highest heritability estimates being associated with receiving combat decoration, and a heritability estimate of 20% for a general measure of assaultive trauma.[30] It has been theorized that these genetic influences may be working through differences in heritable traits such as personality[50–52]. For example, personality may influence an individual's choices resulting in selecting into environments that ultimately lead to an increase in risk of exposure to a traumatic event, such as after volunteering for military service. Previous studies have found various personality traits to be associated with increased likelihood for trauma exposure, such as neuroticism[53], psychoticism[51] and conduct disorder[51,54] (which could be an early indicator of antisocial personality traits). These are examples of gene-environment correlations and have implications for research examining genetic influences on behaviors and are discussed in more detail later.

The heritability of exposure to various traumatic experiences highlights the role of complex interactions between potential risk factors, and the trauma exposure/situational stressors that engenders PTSD development. Premorbid risk factors, or diatheses, represent an individual's predisposition towards development of PTSD and/or other psychopathology and represent things such as genetic vulnerability, social support and previous trauma exposure. Under the diathesis-stress model as diatheses accumulate the severity threshold needed to instigate the development of PTSD decreases.[55] The tipping point for a given individual varies depending on the interaction between the level of stress/trauma experienced and number of risk factors present prior to the stressor. Therefore, those who are most liable for PTSD development have a greater accumulation of diatheses, to the point where they would not require a very severe stressor to reach the PTSD development tipping point. However, this also means that a person with a higher level of risk factors would not develop PTSD until they experience a sufficiently severe stressor.

Within this diathesis-stress model the interactions between environmental and biological risk factors and the catalytic exposure to stress are complex in nature (e.g. the previous example of personality and potentially selecting into environments with greater potential for exposure to traumatic experiences). Research into the stressor/trauma exposure aspect of this model found that repeated or prolonged exposures to traumatic experiences greatly increases the likelihood of PTSD development. Broadly, it is thought that a cumulative exposure to trauma increases not only risk for development of psychopathology[56,57] but also symptom severity[58] in a dose-dependent manner. Within the context of military service, a strong dose-response relation between severity of combat exposure and PTSD symptoms has been observed in both Vietnam and OEF/OIF veteran

samples.[54,59–63] This dose-response relationship between trauma and PTSD outcomes could potentially be acting via several biological pathways associated with responses to stress including via brain circuits responsible for fear learning.

PTSD and Brain Regions of Interest

Current neurobiological models of PTSD implicate the amygdala, hippocampus, anterior cingulate cortex (rACC) and ventromedial prefrontal cortex (vmPFC) as regions of interest (ROIs).[64,65] The amygdala is hypothesized to be hyper-responsive in PTSD potentially explaining the hyperarousal, amplified fear responses, and traumatic reexperiencing. Alternatively, the vmPFC and rostral anterior cingulate cortex (rACC) are hypo-active, suggesting that they do not properly inhibit amygdala activation.[65–67] It is not currently understood which of these areas is responsible for the overall disorder-based fear response outcomes, but it is possible for both situations (underactive vmPFC and ACC, and overactive amygdala) to lead to deficits in fear extinction, emotion regulation, and contextual processing.[68] Changes to hippocampal function are thought to contribute to the deficits in contextual processing in addition to the changes commonly observed in memory and neuroendocrine regulation. Additionally, the insula appears to be hyper-responsive in PTSD and other anxiety disorders and is thought to mediate susceptibility to anxiety.[69,70]

Most recent psychiatric neuroimaging research has focused on fMRI compared to structural MRI. The limited structural literature appears to be split regarding reduction in hippocampal volume in PTSD, with several supporting reduced volumes[71–87] and others not.[88–94] Both camps appear to have support regardless of population examined, type of trauma exposure, or measure of PTSD (symptom counts or diagnoses), but apart from the

meta-analyses the studies have small sample sizes. There have been a few studies on amygdala morphology in PTSD. Two meta-analyses reported reduced amygdala volumes,[84,95] but several other studies with smaller sample sizes found no significant differences between cases and controls.[74,83,88,92,96] Reduction in ACC volumes appears to have more consistent support across different studies[67,97,98] and meta-analyses[78,79,84,95,99], with one monozygotic discordant twin design study suggesting the reduction in grey matter density in the ACC is an acquired disorder indicator (e.g. a stress-induced reduction) rather than a pre-existing risk factor.[67] This is further supported by another study that showed that, while PTSD and ACC volume were associated with measures of threat sensitivity and threat response, they also significantly interacted to predict both outcomes. This suggests that ACC volume may play a moderating role regarding both threat sensitivity and threat response through impaired habituation in trauma exposed individuals. Furthermore, morphometric studies have also reported reduced grey matter density in the insular cortex.[67,78,95,97,100] Recently, one study found that a reduction in cortical thickness of the prefrontal cortex was associated with PTSD symptom load which remained significant after controlling for potential confounds including medication status[101], supporting the findings of meta-analyses based on PTSD diagnoses.[95,99] Although more limited in scope, these morphometric-based analyses implicate similar regions as fMRI studies and overall show that in some capacity the ACC, prefrontal cortex, amygdala, insula, and potentially the hippocampus are associated with PTSD. Further research is now needed to determine if these differences in neuroanatomy represent true endophenotypes.

Resilience to Trauma Exposure and Brain Regions of Interest

When investigating the association between PTSD and ROIs it can be difficult to distinguish what constitutes a potential risk factor present prior to a traumatic event or PTSD and what brain morphology differences are due to effects of trauma exposure and/or PTSD disease processes. One way to untangle this is to incorporate participants exposed to trauma who did not develop PTSD or other psychopathology into analyses. In addition to examining PTSD compared to experience-matched controls, it is also possible to compare the trauma-exposed controls to healthy controls to identify potential protective factors.

One study found greater cortical thickness in the right temporal cortex in a group who experienced a single traumatic event (Tsunami) compared to healthy controls.[102] A twin study by Gilbertson et al.[82] compared monozygotic twins discordant for service in the Vietnam War and found smaller hippocampi of both the deployed twin and the one who did not serve predicted the PTSD symptom load of the deployed twin. These results suggest smaller hippocampal volume is a risk factor for PTSD rather than an effect of PTSD-related neuroprocesses. However, their findings are contradicted by two meta-analyses[78,84] that found bilateral reduction in hippocampal volume in trauma-exposed controls versus healthy controls, with one study showing even further reduction in hippocampal volume in the PTSD group versus the trauma-exposed controls, suggesting that trauma exposure and development of PTSD is responsible for the reduction in hippocampal volume.[84] It should be noted that reduced hippocampal volume is found in major depression, bipolar disorder[103], schizophrenia[104] and chronic hypercortisolemia[105,106], which is related to chronic stress. Given these results, reduction in hippocampal volume may be a generalized marker for mental health disorders rather

than a disorder-specific indicator. Another PTSD-focused meta-analysis showed reduced amygdala volume in trauma-exposed controls compared to healthy controls.[79] A rather large Australian study with 265 participants focused on early trauma exposure rather than PTSD found those with 2 or more adverse childhood events had smaller ACC and caudate nuclei volumes compared to those with no adverse events and may implicate the influence of early trauma exposure on the developing brain.[107]

In summary, most PTSD MRI studies found morphological differences in the amygdala, hippocampus, PFC and ACC and these regions have been observed in trauma survivors without PTSD as well as in individuals who experienced adverse childhood experiences without later psychopathology. One hypothesis is that these ROI might be associated with risk-resilience factors rather than occurring secondary to neuropathological processes associated with PTSD.

Fears and Phobias

Prevalence and Etiology

Fear represents the emotional-behavioral response to the perception of immediate danger, leading one to avoid the threat for discernible survival value.[108] Various phobic fears are common throughout adulthood, with a lifetime prevalence of 7-12%[6], and it is consistently found that girls report more fears than boys during childhood and adolescence with reliable patterns of waxing and waning in response to developmental changes.[109–112]

For researching fears, self-report surveys are a quick, convenient, and inexpensive way to assess a wide range of fears and provide researchers and clinicians with a wealth of information. Although there are many fear survey instruments available, the mostly

widely used are revisions of Scherer and Nakamura's Fear Survey for Children.[113–116] The Fear Survey Schedule for Children-Revised (FSSC-R) is a commonly used self-report measure for measuring fears and fearfulness in children and adolescents.[114,117,118] Five subscales have been consistently found: 'fear of failure and criticism', 'fear of the unknown', 'fear of small animals', 'fear of danger and death', and 'medical fears', but they are mutually correlated supporting the use of the total score as a general fearfulness index.[117] Overall, general fearfulness as defined by the total score of the FSSC-R has been found to be moderately heritable (0.29), with heritability of specific subscales ranging from 0.46-0.12.[119]

Twin studies conducted by our group have found phobias in adults to be moderately heritable (30-40%) with phobia subtypes having overlapping genetic and environmental influences as well as subtype-specific factors.[120–122] These overlapping influences help explain the high comorbidity amongst fears and phobias.[123] To our knowledge, no genetic studies of phobic diagnoses have been conducted in children. However, individual fear symptoms in children have been reported as moderately heritable with modest familial environmental influences and a greater role for unique environment.[124–126] There has been limited genetic research on the comorbidity structure of fear symptoms in children, with one twin study reporting a shared latent genetic factor that influenced all fear symptom clusters in addition to fear-specific factors.[124] This overlap in genetic influence found in child and adult twin studies could be indexing possible shared biological underpinnings, and this dissertation aims to further understand their potential brain structure endophenotypes.

Fears, Phobias and Brain Regions of Interest

Many neurobiological models of phobias and their symptom counts ('fears') focus on mechanisms of fear conditioning and fear extinction and as such primarily implicate the amygdala and vmPFC. This approach is likely not the complete picture given that 1) many individuals with phobias do not report a conditioning event, and 2) only a small number of common stimuli or situations are the focus of phobias.[127] Despite these known shortcomings, fear conditioning and extinction models have provided useful insights into the roles of the amygdala, vmPFC, and insula in phobias. Within fear conditioning paradigms an increase in insular cortex[128,129], amygdala[128,129], and hippocampal activation are commonly reported, with mixed findings for rACC[18,23,129–131] activation changes. Several morphological differences between cases and controls have been associated with specific phobias including increased rACC cortical thickness[132,133], bilateral increase in insular cortical thickness[132,133], and increased grey matter volume in the left orbitofrontal cortex[132] (lOFC), an area within the vmPFC, and reduced hippocampal[134,135] and amygdalar[134,135] volumes. The amygdala, insula, and OFC appear to be hyper-responsive when presented with phobia-related stimuli with a possible corresponding increase in size. These differences in morphometric measures tend to disappear in scans following successful treatment providing additional evidence of their involvement with phobic neuroprocesses.[17,18,136]

Anxiety

Prevalence and Etiology

Anxiety disorders (ADs) often have a basis in normal anxious concerns; however, the degree of anxiety and associated symptoms becomes excessive, uncontrollable, and

impairing to an individual's life. The AD group is the most prevalent class of psychiatric disorders in US adults with a lifetime prevalence of 28.8%.[6] Community prevalence rates of any current AD in children range between 3 and 9.5%[137–139] and the cumulative prevalence reaches as high as 31% in adolescence.[140] Similar to fears, girls are found to have higher rates of ADs throughout both childhood and adulthood.[137,141]

As with fears, an efficient way for researchers to assess common anxiety disorders is through self-report measures such as the Screen for Child Anxiety Related Emotional Disorders (SCARED). The SCARED was originally developed to screen for anxiety disorders within clinical samples[142,143] but has also been widely used in community and research studies.[144,145] It assesses five clusters of childhood anxiety symptoms: panic/somatic (PAN), generalized anxiety (GAD), social anxiety (SOC) and separation anxiety (SEP) as well as school avoidance. Several twin studies have examined the heritability of the SCARED subscales and found the subscales to be moderately heritable (0.53-0.60) with no familial environmental influence.[146] Additionally, the covariance between the SCARED subscales is also almost entirely explained by genetic factors.[147]

In general, twin studies have demonstrated that ADs are also moderately heritable.[148] Like fears and phobias, ADs are highly comorbid with each other, and adult twin studies suggest this comorbidity may be due, in part, to genetic risk factors shared between disorders.[149,150] This high rate of comorbidity is also seen in children, where 40% to 60% of children with one AD are estimated to meet criteria for additional ADs[151,152] and suggests potential shared underlying biological mechanisms.

Anxiety and Brain Regions of Interest

ADs are a prevalent problem in the community and neuroimaging research could provide insights that may ultimately be used to help inform development of new treatments or possibly predict an individual's response to various treatment types. It is not surprising that current research into the neurocircuitry of anxiety disorders is closely linked to fear circuits in animal models. Both fear and anxiety have a basis in threat response, with fear corresponding to more acute and imminent threats, and anxiety related to the concern about potential and long-term threats.[153]

There has been limited research on differences in volumetric measures associated with anxiety disorders. According to one study with a limited sample size, smaller bilateral amygdala volumes were found in panic disorder compared to healthy controls.[154] However, another study reported larger amygdala volumes in pediatric generalized anxiety disorder patients compared to healthy controls.[155] Within the functional neuroimaging literature there appears to be a general consensus of exaggerated amygdala activation to a variety of disorder-specific stimuli across many anxiety disorders such as panic disorder[156,157], social phobia[13–16], generalized anxiety.[22,158,159] Here again the volumetric literature is sparse, but some studies and meta-analyses suggest a reduction in grey matter volumes in the rACC across multiple disorders including panic, social anxiety, generalized anxiety and specific phobia[160–162] and a decrease in cortical thickness of the ACC and OFC in older generalized anxiety patients.[163] Reduction in left medial OFC (mOFC) thickness or grey matter volume has also been associated with panic disorder in a few smaller studies.[164–166] OFC is often examined in anxiety disorders (primarily panic and generalized anxiety) due to its reciprocal connections with the amygdala. It should be noted that a common limitation across several of these anxiety neuroimaging studies

is the inclusion of participants taking psychiatric medications, and the analyses were not corrected for this potential confound. Research has shown some medications are associated with changes in volumes of specific structures.[167] Despite these limitations it appears that fear neurocircuitry is involved in some capacity in our current understanding of the biological mechanisms of ADs.

This dissertation examines the relationship between brain ROI and threat-related psychopathology in adults (PTSD) and children (anxiety and fears) with the ultimate goal of beginning to connect dimensional measures of psychopathology to basic biological components of mental health using genetically informative samples. Based on the previous literature presented above, this dissertation will focus on six main regions in each hemisphere of the brain: hippocampus, amygdala, rACC, cACC, lOFC, and mOFC (which combined with the lOFC capture a commonly studied area of the PFC). These areas have been associated with PTSD and/or ADs and have a known involvement with fear neurocircuitry in animal models. The broad aims of this dissertation will bridge the current gaps between heritability of psychopathology, heritability of ROIs, and morphometric changes associated with psychopathology. This will be accomplished by: 1) examining the etiological relationship between PTSD, specific brain regions, and the role of trauma in that relationship, 2) investigate the shared and specific risk factors (genetic, familial environment and unique environment) and their roles amongst fear and anxiety domains in youth, and 3) determine whether genetic factors shared with fear and anxiety are associated with specific brain regions. These aims and the required analytic approaches are summarized below.

The Role of Trauma in the Etiology of PTSD and ROIs

Chapter 2 addresses the first aim of this dissertation by examining the strength of the etiological relationships between PTSD and ROIs previously implicated via functional and structural MRI studies. Several areas involved in the processing of threatening stimuli have been associated with PTSD. PTSD[28–31] and ROI morphologies[168–170] are both considered moderately to highly heritable. Given their heritability estimates and known phenotypic association, it is plausible that PTSD and ROIs have a shared genetic etiology.

Another important consideration in examining this potential etiological relationship is gene-environment correlation, because it could falsely appear as a gene by environment interaction.[171] Previous studies using the Vietnam Era Twin Registry show evidence of such gene-environment correlation between PTSD and combat exposure.[57-59] Also, given that exposure to adverse childhood experiences is associated with morphometric differences in areas such as the ACC and hippocampus, areas also associated with PTSD, it is important to account for trauma exposure when examining etiological overlap between PTSD and ROIs. Morphological differences may in fact be risk factors for PTSD development rather than an effect of trauma exposure and PTSD neuroprocesses.

Accordingly, the aim of chapter 2 is to examine the shared etiology of PTSD and ROIs while accounting for trauma using a large sample of male twins who served in the military during the Vietnam War. There are several potential models available to fit (e.g.

Cholesky, correlated factors, simplex etc.), and the model ultimately chosen depends on the underlying theory being tested. Since this chapter aims to understand the degree of etiological overlap between PTSD and specific ROIs the correlated factors model is the best choice for these analyses.[172] As shown in Figure 1.3 the correlated factors approach decomposes variances of variables into genetic (A) and environmental (C and E) factors separately, and the correlations of the factors across the variables are estimated (rA, rC, rE). The correlated factors approach allows the estimating of latent sources of variance for both PTSD symptoms and ROI measures as well as the correlation between the latent sources of each variable. Despite substantiated associations found between PTSD and specific brain ROIs and their moderate heritability estimates, we still do not know if covariation of these two phenotypes is due to overlapping genetic factors. Without understanding the nature of this relationship, it will be more difficult to comprehend how underlying mechanisms and genetic endophenotypes lead to psychopathology. Therefore, the contribution of this chapter is significant because it will be the first to formally test these ROIs as potential endophenotypes of PTSD.

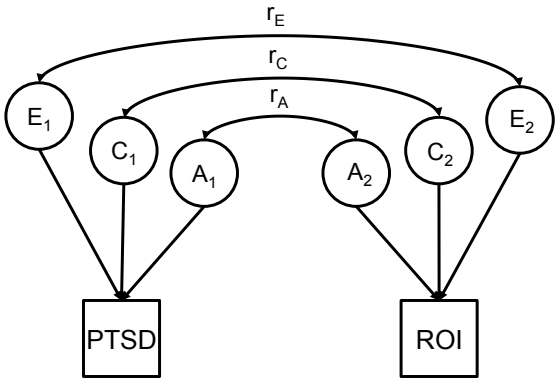

Figure 1.3 Correlated Factors Twin Model

Next, the role of trauma will be accounted for in this model via moderation on the individual variance components. Since trauma is known to influence both PTSD and ROI measures, we aim to understand PTSD and ROIs in the context of trauma exposure. By understanding the interplay of trauma, genetic factors, ROIs, and PTSD in adults we will

be able to better identify potential endophenotypes/risk-resilience factors present in non-trauma exposed adults.

The Shared Etiology of Fear and Anxiety in Juvenile Twins

Fear and anxiety are conceptualized as responses to acute or potential threat, respectively. Adult twin studies have found substantial interplay between genetic and environmental factors influencing fear disorders (phobias) and anxiety disorders. Research in children, however, has largely examined these factors independently. Thus, there exists a substantial knowledge gap regarding the underlying etiologic structure of these closely-related constructs during development. Given the partial distinction between risk factors for phobias and other ADs in adults, it is important to examine their childhood precursors. Furthermore, early fear and anxiety disorders are the strongest predictors of later psychiatric comorbidity.[173]

Chapter 3 addresses this gap by examining measures of fear (as indexed by the FSSC-RSF) and anxiety (as indexed by the SCARED) in a juvenile twin sample. The aim of this chapter is to elucidate the shared and specific risk factors (genetic, familial environment and unique environment) and their roles amongst fear and anxiety domains in children. First, chapter 3 focuses on understanding the etiology shared between anxiety symptom clusters on the one hand and the etiology shared between fear symptom clusters on the other. Second, etiology shared between the anxiety and fear clusters is examined via a correlated factors model similar to that in chapter 2. Lastly, an independent pathway model provides a more flexible and detailed representation of the covariance structure between all of the clusters beyond the simple factor correlations estimated in the correlated factors model. This set of analyses will investigate the

underlying etiology of fear and anxiety symptoms and provide a possible reason for the highly diffuse symptom patterns seen during development.

The Shared Etiology of Fear and Anxiety with Cortical and Subcortical Structural Measures

One of the primary goals of the RDoC framework is to incorporate multiple levels of information from genomics to circuits to self-report in order to understand the nature of psychiatric illnesses. To incorporate this concept into this dissertation the last part of this dissertation will incorporate the genetic findings from the previous chapter on self-report measures with preliminary structural neuroimaging data. This will test whether ROIs are potential endophenotypes for fear and anxiety in children.

To be considered an endophenotype a biomarker must be proven to associate with the disorder, be heritable, and have a genetic relationship to the disorder identified through either family, twin, or measured genotype based analyses.[4] The neuroimaging data available for this chapter is not sufficient for a well-powered twin study into the heritability of ROIs specifically. However, by incorporating the results from chapter 3 it is possible to create a genetic factor score indexing latent liability to fear/anxiety, which can then be used to test whether ROIs have a genetically-based relationship with fear/anxiety in children.

The genetic score is thought to be more proximal than phenotypic symptom measures to the biological processes related to fear and anxiety measures, and as such may provide a stronger link between fear and anxiety with ROIs. To test this hypothesis, genetic factor scores indexing an individual's latent liability to fear/anxiety are incorporated into a mixed effect regression to predict ROI measures. A mixed effect linear

regression allows for the control of the non-independence of twin pairs by clustering based on family and zygosity. This allows for more accurate estimations of confidence intervals that would otherwise be artificially tighter due to the non-independence of participants. Site of scanner, age, sex, and total intracranial volume will also be added to the regression as fixed effect covariates. By examining the genetic factor score, chapter 4 aims to assess whether the ROIs are potential endophenotypes for fear and anxiety in children.

As a post-hoc follow-up to these analyses, we will also test whether the fear and anxiety total scale sum scores predict hippocampal volumes given prior findings. This may elucidate whether either one of these scales is the main driving force behind findings from the genetic score regressions. However, given our sample size and the complex nature of both acquisition of neuroimaging data in children and highly comorbid internalizing disorders, we are cautiously optimistic about any potential findings remaining significant after multiple testing corrections. Regardless, these analyses would be the first to examine these ROIs as potential endophenotypes for fear and anxiety using a dimensional approach in children.

Overall, this dissertation will elucidate the etiological relationship between PTSD and related traits (anxiety and fear) with specific brain ROIs in a trans-diagnostic framework. The primary gap in the current understanding of brain morphometry endophenotypes is whether specific regions of interest (ROIs) have a genetic relationship to disorders to which they are phenotypically associated. Investigating whether ROIs meet endophenotypic criteria for PTSD, fear and anxiety will begin to fill these critical gaps within the PTSD and child anxiety literatures. This knowledge will be particularly

useful as the fields of neuroimaging and genetics continue to integrate and larger neuroimaging datasets become publicly available.

Chapter 2: The Moderating Role of Trauma on the Shared Etiology of Post-Traumatic Stress Disorder and Brain Regions of Interest

This chapter addresses the first aim of this dissertation by examining the strength of the relationship between post-traumatic stress disorder (PTSD) and brain regions of interest previously implicated via functional and structural magnetic resonance imaging (MRI). Given the accumulation of studies and meta-analyses that associate PTSD with morphometric differences in the brain, it is a logical next step to investigate potential shared etiology between PTSD and regions of interest (ROIs) with the goal of identifying whether ROI morphometries meet the criteria for endophenotypes of PTSD. To identify whether a biomarker is an actual endophenotype it must associate with the disorder, be heritable, and have a genetic relationship to the disorder identified through either family, twin, or measured genotypic analyses.[4] The aim of this chapter is to assess whether ROIs implicated in stress response meet the criteria for an endophenotype. Traumatic stress is also associated with lasting changes in these areas and, as such, understanding the interplay of trauma, genetics, ROIs, and PTSD in adults will better inform the ability to identify potential endophenotypes/risk factors present in non-trauma exposed adults.

Regions of Interest

Thus far, functional neuroimaging studies have mainly examined differences between PTSD and healthy controls with regards to activation of the fear-network and

related cortical and subcortical areas.[174] The more limited structural imaging literature has also predominately used case/control study designs. PTSD symptoms are thought to be the behavioral manifestation of stress-induced changes in function and structure of these areas, which may also underlie the changes in endocrine and immune systems associated with PTSD. As reviewed in Chapter 1, the hippocampus[78,84,86], amygdala[84,95], areas of the PFC[95,99], and ACC[78,79,84,95,99] have varying degrees of support for their involvement in PTSD, with generally smaller subcortical volumes (hippocampus and amygdala) and thinner cortical thicknesses (vmPFC and ACC) found to be associated with PTSD compared to healthy and/or trauma-exposed controls. The hippocampus and vmPFC have conflicting or limited support, respectively, for their structural differences associated with PTSD. By contrast, the amygdala has gained more recent and consistent support from large meta-analyses.[84,95] Finally, the ACC has the most conclusive evidence for grey matter atrophy associated with PTSD[67,78,84,95,97–99,175]. Given the previous literature, it is hypothesized that PTSD will be significantly associated with reduction in volumes of the hippocampus and amygdala as well as thinner average cortical thicknesses for the ACC and areas of the PFC: the lOFC, and mOFC. The literature appears to be fairly consistent regarding an inverse direction of association for amygdala and ACC with PTSD and as such these areas appear to hold the most potential for significant findings in these data. There appears to be more conflict in the literature regarding hippocampus and PFC areas (lOFC and mOFC), therefore non-significant findings in these areas would not be surprising.

Trauma

Exposure to a traumatic experience is necessary but not sufficient for a diagnosis of PTSD.[1] Previous research shows there are heritable risk factors for PTSD, with some twin studies estimating that 30-72% of the variance of PTSD is accounted for by genetic factors with the remaining variance accounted for by environmental factors unique to each twin.[28–31,176] Trauma in the context of combat exposure is known to partially account for the prevelance[177] and chronicity[178] of PTSD, with those who experienced the highest levels of combat exposure continuing to experience elevated PTSD symptom levels up to 25 years after the exposure. Likewise, in noncombat trauma children with the highest levels of exposure to a hurricane experienced higher levels of PTSD symptoms in a dose-response manner.[179] Given that not all who are exposed to traumatic events develop PTSD, it is plausible that these experiences interact with facets of an individual and affect their liability towards PTSD.

According to the diathesis-stress model of PTSD, an individual's premorbid risk factors interact with a stressor to produce a PTSD outcome.[55] One possible route for this interaction is if a traumatic event specifically interacts with underlying genetic liability for PTSD and increases the risk for the disorder. This would be an example of gene by environment interaction (GxE). In this case GxE implies the effect of exposure to trauma is conditional on a person's genetic liability towards the disorder. Trauma is thought to change the heritability of PTSD via two hypothetical routes.[180] In one, combat exposure could increase the heritability of PTSD by causing the underlying differences in genetic risk to manifest their effects. Only under certain environmental conditions might a genetic predisposition manifest. A study using the full Vietnam Era Twin Registry found just that when examining the moderating role of an ordinalized combat exposure measure

on the heritability of PTSD diagnoses.[180] In this study, as theorized, the influence of trauma exposure was stronger in those with higher levels of trauma. In the other route, combat exposure could ultimately decrease heritability of PTSD in a scenario where the severity of the trauma is great enough that it overwhelms any potential genetic effects so that essentially anyone who is exposed develops PTSD, regardless of their genetic predisposition.[181]

In addition to genetic liability interacting with combat exposure to affect risk of PTSD, the influence of environmental factors on PTSD risk may vary depending on the level of combat exposure. These environmental effects can be shared between twins (familial environment CxE) or unique to an individual (unique environment ExE). Twin studies that incorporate moderation allow for the examination of GxE, CxE and ExE effects. Combat exposure could increase the influence of other environmental factors such as previous trauma from childhood or civilian life. Similar to genetic influences, the environmental influences may decrease in the presence of combat exposure due to extreme exposure overriding any other environmental protective/risk factors for PTSD. One study that previously examined the effect of trauma due to combat exposure on PTSD found the influence of heritability and unique environment increased at higher levels of combat exposure.[180] That is to say, those with the most severe combat exposure levels were at an increased risk of PTSD due to interactions with both genetic and environmental factors.

The effects of trauma exposure on brain morphology and circuitry without a later diagnosis of PTSD or other psychopathology are not commonly examined. However, well-designed studies do compare PTSD to healthy controls as well as to trauma-exposed controls, and this is often where understanding of trauma-specific effects derives. Of

studies conducted in this manner, meta-analyses have found bilateral reduction in hippocampal volume[78,84] in trauma-exposed controls versus healthy controls as well as reduced amygdalar volumes. [79] Those exposed to trauma early in life had smaller ACC volumes compared to controls and suggests this early trauma exposure may influence the developing brain.[107] Studies have shown that trauma is associated with morphometric differences in the brain, and a twin study demonstrated trauma's moderation of the genetic and environmental influences on PTSD. Therefore, it is important to account for the potential role of trauma when examining the relationship between PTSD and ROIs.

Study Aims

The aims of the present chapter are to 1) identify brain ROIs previously implicated in PTSD that are significantly associated with PTSD sum scores in this sample; 2) examine the genetic and environmental bases for significantly associated ROIs and PTSD symptom sum scores; and 3) examine the extent to which trauma interacts with the shared genetic and environmental factors of PTSD and ROIs associated with PTSD. Aim 3 allows the examination of competing hypotheses about the effect of trauma on PTSD and ROIs. These aims will be addressed by using mixed effect linear regressions and correlated factor twin models.

Methods

Participants

Participants in this study are middle-aged male twins who participated in Wave 2 of the Vietnam Era Twin Study of Aging[182] (VETSA) with a mean age of 61.72 (SD = 2.45) at time of assessment. All VETSA participants served in some branch of the military between 1965 and 1975 with a mean age of entry to the military of 19.30 years (SD = 1.38),

with the majority of the sample not serving in combat or in south east Asia. This sample is 88.3% non-Hispanic white, 5.3% African American, 3.4% Hispanic, and 3% "other", and is very similar to American men in this age range with respect to health and lifestyle characteristics.[183] There was no selection criteria for this sample from the Vietnam Era Twin Registry beyond safety measures required for the MRI portion of the protocol, such as no metal present in the body. The University of California San Diego ethics committee approved this study, and written consent was obtained from all participants.

Measures

PTSD

PTSD symptom counts were measured using the PTSD symptom checklist at wave 2 assessment. This scale consists of 17 retrospective items of symptoms experienced within the last month on a Likert scale (1 = 'not at all' to 5 = 'extremely'). Sum scores were calculated for those missing less than 10% of their responses by prorating for the number of non-missing responses, with less than 2% of the sample exceeding this missingness threshold. Sum scores range from 17 to 84 (Mean= 26.36 SD= 10.66, skew= 2.02) with a higher score indicating higher levels of PTSD symptoms and had good internal consistency with a Cronbach's alpha = 0.94. Within this sample a total of 94 participants (7.8%) meet criteria for a probable PTSD diagnosis based on DSM- IV criteria, which is consistent with PTSD rates seen in the general population as well as in other samples of Vietnam era

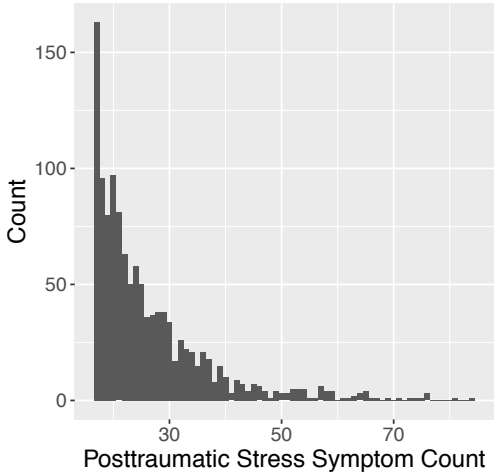

Figure 2.1 Histogram of Posttraumatic Stress Symptom Counts

veterans.[184,185] This sample contains both individuals who served in southeast Asia as well as those stationed elsewhere and as such a lower prevalence rate is expected when compared to samples of only Vietnam theater veterans or more contemporary veteran populations.

Combat Trauma Exposure

To quantify combat trauma, the 18- item Combat Exposure Index was used, which participants completed by mail as part of a prior study, the Survey of Health. Participants completed this index at a mean age of 37.46 years old (SD = 2.49) with a mean time since military service of 18.16 years.. The mean age at military service was 19.30 years (SD = 1.38). This index assessed personal history of specific combat roles and experiences that an individual could experience during the Vietnam War including flying in aircraft or helicopter attacks, serving on river patrols, receiving incoming fire, and being captured or wounded. The Combat Exposure index has demonstrated good internal consistency and predictive validity.[186] Total number of endorsed experiences ranged from 0 to 16 (Mean = 1.48, SD = 2.82, skew = 2.16). Figure 2.2 shows a histogram of total endorsed combat experiences. Civilian and childhood trauma were not assessed by measures used in these analyses. Additionally, this measure only accounted for combat situations experienced in the Vietnam theater. However, only 30% of this sample were stationed in southeast Asia at

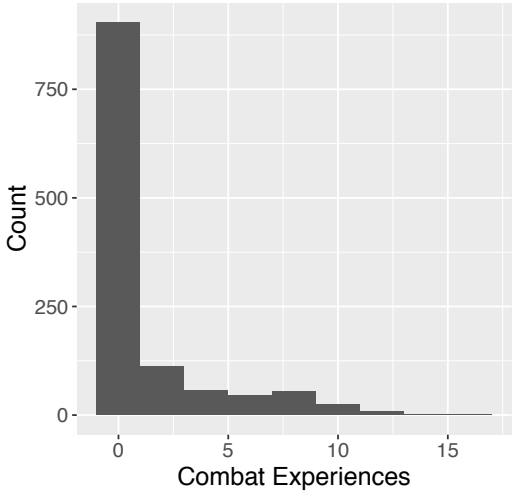

Figure 2.2 Histogram of Combat Experience Counts

some point during their military service, which partly accounts for the lower level of trauma exposure compared to more contemporary veteran samples. Of those stationed in southeast Asia the mean number of combat exposures was 5.49 (SD = 3.13, skew = .63). Due to the moderating role of trauma, those without combat exposure data (incomplete or missing combat exposure index data) were excluded from these analyses. A total of 1,207 individuals have PTSD and combat exposure data.

MRI acquisition

MRI scans were collected as part of the wave 2 assessments at two sites: University of California, San Diego (UCSD) and the Massachusetts General Hospital (MGH). Imaging and questionnaire data were assessed during the same visit and both twins were present on the same days. At UCSD a General Electric 3T Discovery 750 scanner with an eight-channel phase array head coil was used. Imaging protocol included sagittal 3D fast spoiled gradient echo (FSPGR) T1-weighted volume optimized for maximum gray/white matter contrast with the following parameters: TE= 3.164 msec; TR= 8.084 msec; TI=600 msec; flip angle=8°; pixel bandwidth=244.141; FOV=24 cm; frequency=256; phase =192; slices=172; slice thickness=1.2mm. At MGH a Siemens Tim Trio with a 32-channel head coil was used. The imaging protocol included a 3D magnetization-prepared rapid gradient-echo (MPRAGE) T1-weighted volume optimized for maximum gray/white matter contrast with the following parameters: TE=4.33 msec; TR=2170 msec; TI= 1100 msec; flip angle=7°; pixel bandwidth= 140; slices= 160; slice thickness=1.2mm. A total of 584 twins were scanned across the two sites.

MRI Processing

The processing of the structural MRI images is described elsewhere in further detail[169]. Processing of images was performed using standard, automated procedures available in the Freesurfer image analysis software suite, which is freely available for download and fully documented (Version 6.0, http://surfer.nmr.mgh.harvard.edu/). Processing consisted of motion correction[187], correction of distortion due to gradient nonlinearity and B1 field inhomogeneity, image intensity normalization[188], removal of non-brain tissue using a hybrid watershed/surface deformation procedure[189], and automated Talairach transformation. FreeSurfer software package routines[190,191] were used to define gray matter, white matter, segmentation of subcortical structures, and cerebral spinal fluid segmentation. The procedures used for cortical thickness measurement have been validated against histological analysis[192] and manual measurements[193,194]. After image processing subcortical volume and average cortical thickness data were available from 447 twins (110 monozygotic twin pairs, 75 dizygotic twin pairs and 77 singletons). The most common reason for exclusion of a scan was due to excessive motion in the scanner which prohibited accurate assessment of brain morphometry.

Cortical and Subcortical Measures

Prior to all analyses, all ROIs were regressed on age, scan site, and estimated intracranial volume to remove the fixed effects of these covariates, i.e., the residuals were used in subsequent analyses. The ROIs examined in this chapter include hippocampal volume (N = 398), amygdala volume (N = 402), rostral anterior cingulate cortex (rACC; N = 397) mean thickness, caudal anterior cingulate cortex (cACC; N= 397) mean

thickness, lateral orbitofrontal cortex (lOFC; left hemisphere N = 396, right hemisphere N = 397) mean thickness, and the medial orbitofrontal cortex (mOFC; N = 397) mean thickness. Due to bilateral asymmetry within the brain the left and right hemispheres of each of these regions were analyzed separately, for a total of 12 ROIs.

Statistical Analyses

Mixed Effect Linear Regression

As a preliminary analysis, mixed effect linear regressions were used to identify which brain ROIs previously implicated in PTSD were associated with the PTSD symptom sum score in this data. Given the inconsistent nature of the extant literature significant associations for all ROIs were not expected in this dataset. Therefore, preliminary analyses were needed to identify significant ROIs to perform the primary analyses of this chapter. Random effects models were used to adjust for possible effects of correlated observations in the twin data. In each model, family ID and zygosity respectively denoted family membership and whether the pair was monozygotic or dizygotic and were entered as random effects. The *umx* R package[195] was then used to obtain 95% confidence intervals for all standardized beta estimates.

Twin Modeling

This study used the classic twin design, which leverages the differences between MZ and DZ twin types to decompose phenotypic variation into additive genetic (A), common environmental (C), and unique environmental (E) factors[196]. Because MZ twins share 100% of their genes and DZ twins share, on average, 50% of their segregating genes, genetic factors contribute twice as much to the MZ twin correlation than to the DZ twin

correlation. Common environmental factors are shared factors that make twin pairs more similar, regardless of their zygosity. Unique environmental factors are specific to the individual and represent experiences not shared by twins and contribute to neither MZ nor DZ twin correlations. The unique environment component also captures measurement error.

Correlated Factors Twin Model. There are several potential bivariate models available to fit (e.g. Cholesky, correlated factors, simplex etc.) and the model chosen should depend on the underlying theory being tested[172]. Since this chapter hypothesizes there are genetic and environmental overlaps between PTSD and specific ROIs, the correlated factors model is the best choice for these analyses. It is possible to directly test whether ROIs qualify as PTSD endophenotypes with this correlated factors approach. In the correlated factors model, variances of variables are decomposed into genetic and environmental factors separately, and the correlations of the factors across the variables are estimated (rA, rC, rE). The correlated factors approach is specified for each latent source of variance (genetic [A], familial environment [C], and unique environment [E]) and allows estimation of latent sources of variance for PTSD symptoms, ROI measures, and the correlation between the latent sources of each variable. A low genetic correlation would suggest PTSD and ROIs were influenced primarily by separate genes, and likewise a high environmental correlation would suggest there exist environmental events that influence both PTSD and ROIs.

Moderation. An underlying assumption of the classic twin model is that genetic and environmental variance is consistent across environmental conditions (i.e. homoscedastic). Heteroskedasticity arises when genetic and environmental factors vary as a function of a moderator and represents genetic sensitivity to the environment.

Moderators can be purely environmental (e.g., earthquakes), or another trait also under some degree of genetic control (e.g., personality traits). Gene-environment interactions can be parameterized in this model by having the variance decomposition of the trait (T) as a linear function of a moderator (*M*), after accounting for the main effect of the moderator variable on the trait. The moderator has a main effect on the trait (as seen in the moderation of the mean of T), in addition to a moderating effect on the residual A, C, and E variance components of the trait. In addition to A, C, and E estimates, β parameters (accounting for the moderating effect of *M* on each path) are also estimated. A β coefficient significantly different from zero would indicate the presence of moderation on that variance component, with a larger β indicating a greater degree of moderation. It is important to remember that not only is PTSD liability heritable, but trauma exposure is also moderately heritable.[30,49] This is an example of gene-environment correlation.[197] To account for gene-environment correlation the mean of T is regressed on *M* for both twins. This approach reduces false positive GxE effects for two main circumstances 1) when *M* and T are correlated with each other and 2) when *M* is correlated across twins. False positive inflation can occur when the moderator is correlated between twins.[198]

Three primary biometrical models were examined that investigated: 1) the etiology of PTSD; 2) the shared etiology of PTSD and ROIs; and 3) the shared etiology of PTSD, ROIs moderated by trauma. For each of the primary models, submodels were tested by dropping parameters and comparing the fit statistics to the full model (Model 1) to determine the best-fitting model. A full information maximum likelihood approach for raw data implemented in the OpenMx software was used.[199] Model fits were compared using the difference in negative two log-likelihood (Δ-2LL) for nested models, and with Akaike Information Criterion (AIC) for non-nested models with lower values indicating a

better fit.[200] Under certain regularity conditions, Δ-2LL is distributed as χ^2 with degrees of freedom equal to the models' difference in the number of free parameters.[201] AIC is an index that balances explanatory power with parsimony. Parsimony is an important consideration in maximum likelihood approaches because log-likelihoods will continue to decrease with additional parameters estimated, resulting in "overfitting". AIC penalizes models with many parameters once they improve fit by less than 2LL units, and provides an appropriate balance between model complexity and explanatory power as manifest by the degree of misfit.[202]

Results

Mixed Effect Regression Analyses

Preliminary analyses were used to identify brain ROI associated with PTSD sum scores from a list of potential brain ROI based on the extant literature and as such do not correct for multiple-testing. Correlations between all variables are shown in Table 2.1. Each ROI was examined in a separate analysis, and Table 2.2 summarizes the results. All 12 ROIs (six in each hemisphere) are listed with their corresponding standardized beta estimate, confidence interval (CI), *t*- and *p*-values. Only three regions (right rACC, left lOFC, and left mOFC) had confidence intervals that did not include zero and were significantly associated with PTSD sum scores after accounting for the non-independence of twin pairs. These three areas were then examined in bivariate twin analyses.

Table 2.1 Correlations Between PTSD, Trauma, and Brain Morphometry Variables

Variables	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1. PTSD														
2. Trauma	.23 *<.001*													
3. Age	-.01 *.66*	.20 *<.001*												
4. L Hipp	-.08 *.12*	-.03 *.56*	-.05 *.32*											
5. R Hipp	-.11 *.02*	-.04 *.42*	-.04 *.47*	.73 *<.001*										
6. L amyg	-.01 *.81*	.03 *.52*	.00 *.97*	.40 *<.001*	.36 *<.001*									
7. R amyg	-.03 *.57*	.03 *.55*	-.05 *.29*	.37 *<.001*	.33 *<.001*	.58 *<.001*								
8. L cACC	-.04 *.45*	.00 *.96*	.11 *.02*	.01 *.88*	.01 *.85*	.00 *.95*	-.06 *.21*							
9. R cACC	-.06 *.25*	.00 *.95*	.08 *.13*	.04 *.43*	-.03 *.52*	-.10 *.04*	-.08 *.10*	.37 *<.001*						
10. L rACC	-.04 *.42*	.14 *.01*	.11 *.03*	.08 *.13*	.01 *.78*	.03 *.49*	-.01 *.87*	.37 *<.001*	.34 *<.001*					
11. R rACC	-.14 *<.001*	0.4 *.42*	.12 *.01*	-.03 *.50*	-.06 *.22*	-.10 *.05*	-.08 *.10*	.31 *<.001*	.41 *<.001*	.36 *<.001*				
12. L lOFC	-.11 *.03*	-.08 *.03*	.00 *.94*	.14 *<.001*	.08 *.10*	.17 *<.001*	.07 *.14*	.25 *<.001*	.33 *<.001*	.38 *<.001*	.28 *<.001*			
13. R lOFC	-.07 *.17*	-.03 *.63*	.05 *.28*	.04 *.43*	-.03 *.58*	.02 *.65*	.00 *.96*	.31 *<.001*	.29 *<.001*	.36 *<.001*	.25 *<.001*	.65 *<.001*		
14. L mOFC	-.11 *.03*	.06 *.02*	.08 *.13*	.02 *.71*	.02 *.75*	-.02 *.75*	-.04 *.44*	.29 *<.001*	.34 *<.001*	.45 *<.001*	.36 *<.001*	.45 *<.001*	.36 *<.001*	
15. R mOFC	-.07 *.18*	.00 *.94*	.12 *.01*	.03 *.61*	-.03 *.53*	-.01 *.81*	-.05 *.37*	.24 *<.001*	.37 *<.001*	.42 *<.001*	.48 *<.001*	.49 *<.001*	.51 *<.001*	.43 *<.001*

Correlations are reported in plain text, with *p* values reported below in italics for all variables.

Table 2.2 ROIs predicting PTSD Sum Scores in Separate Analyses

Region of Interest	β [95% CI]	*t*(151)	*p*
L Hippocampus	0 [0,0]	-1.68	.095
R Hippocampus	0 [-0.01, 0]	-2.22	.028
L Amygdala	0 [-0.01, 0]	-0.28	.744
R Amygdala	0 [-0.01, 0]	-0.78	.397
L rACC	-1.48 [-5.56, 2.59]	-0.72	.473
R rACC	-5.52 [-9.63, -1.20]	-2.54	.011*
L cACC	-1.27 [-4.95, 2.42]	-0.68	.498
R cACC	-2.03 [-5.95, 1.88]	-1.03	.307
L lOFC	-6.65 [-12.78, -0.41]	-2.10	.037*
R lOFC	-4.79 [-10.90, 1.31]	-1.55	.123
L mOFC	-6.44 [-12.59, -0.29]	-2.07	.040*
R mOFC	-3.41 [-8.92, 1.96]	-1.27	.221

β = standardized beta estimates, 95% CI = 95% confidence interval, L = left, R = right, rACC = rostral anterior cingulate cortex, cACC = caudal anterior cingulate cortex, lOFC = lateral orbitofrontal cortex, mOFC = medial orbitofrontal cortex. Using data from the Vietnam Era Twin Study of Aging mixed effect linear regressions were performed

Twin Analyses of PTSD and Specific ROIs

To quantify the genetic and environmental influences on PTSD and each of the three significantly associated brain ROIs, univariate and bivariate twin models were fitted. Table 2.3 outlines the univariate models fitted to PTSD and each ROI. The first section of Table 2.3 shows the results of fitting of univariate twin models to the PTSD sum scores. The best fitting model for PTSD was the AE model with a standardized heritability (a^2) estimate of 0.36 (95% Confidence Intervals [95%CI]: 0.27, 0.44), and a unique environment estimate of 0.64 (95%CI: 0.55, 0.73). The best fitting model for the right rACC model was also AE; the heritability was 0.21 (95%CI: 0.0, 0.42), and unique environment was 0.79 (95% CI: 0.60, 1.00). Lastly, the best-fitting models for the left lOFC and mOFC were the AE models with heritabilities of 0.47 (95%CI: 0.31, 0.60) and 0.41 (95%CI: 0.25, 0.54), respectively, and unique environment estimates of 0.53 (95%CI: 0.39, 0.68) and 0.59 (95%CI: 0.44, 0.73), respectively.

Table 2.3 Model fit statistics for Univariate PTSD and ROI Twin Models

Model	Factors	Δdf	-2LL	AIC	p
Posttraumatic Stress Disorder Sum Score					
I	ACE	1159	8753.63	6435.63	-
II	**AE**	**1**	**8753.69**	**6433.69**	**.822**
III	CE	1	8760.16	6440.16	.010
IV	E	2	8803.09	6481.09	<.001
Right Rostral Anterior Cingulate Cortex					
I	ACE	388	-67.25	-843.25	-
II	**AE**	**1**	**-67.18**	**-845.18**	**. 693**
III	CE	1	-67.10	-845.10	. 786
IV	E	2	-63.30	-843.30	. 139
Left Lateral Orbitofrontal Cortex					
I	ACE	387	-374.25	-1148.25	-
II	**AE**	**1**	**-374.23**	**-1150.23**	**.898**
III	CE	1	-371.31	-1147.31	.086
IV	E	2	-346.06	-1124.06	<.001
Left Medial Orbitofrontal Cortex					
I	ACE	388	-357.87	-1133.87	-
II	**AE**	**1**	**-357.85**	**-1135.85**	**.880**
III	CE	1	-355.85	-1133.85	. 155
IV	E	2	-334.95	-1114.95	<.001

-2LL = -2 log-likelihood, Δdf = change in degrees of freedom from full model (I), ΔAIC = change in Akaike Information Criterion from full model (I). For AIC and -2LL, smaller or more negative values indicate a better fit compared to the full model (Model 1). *p* is related to the statistical difference of -2LL values between full and sub models. Best fitting models are designated in bold text for each section of analyses.

Bivariate correlated-factors models were fitted to PTSD and each of the three significantly associated brain ROIs (Right rACC, Left lOFC, and Left mOFC) from the first aim with model results shown in Table 2.4. Similar to the PTSD univariate model, an AE model fit best in all three bivariate models (right rACC, left lOFC, and left mOFC). Figure 2.1 shows an example of the etiological structure of PTSD and each ROI, with standardized variance and correlation information for each model shown in Table 2.5. All three ROIs have minimal, nonsignificant genetic correlations with PTSD (0.05 to 0.10) and moderate unique environmental correlations (-0.25 to -0.26).

Table 2.4 Model Fit Statistics for Bivariate Correlated Factors Model of PTSD and ROIs

Model	Factors	Δdf	-2LL	AIC	p
PTSD & Right rACC Bivariate Model					
I	ACE	1544	8676.72	5586.72	-
II	**AE**	**3**	**8676.96**	**5580.96**	**.971**
III	CE	3	8684.64	5588.64	.047
IV	E	6	8732.68	5630.68	<.001
PTSD & Left lOFC Bivariate Model					
I	ACE	1543	8371.72	5283.72	
II	**AE**	**3**	**8374.74**	**5280.74**	**.387**
III	CE	3	8380.03	5286.03	.039
IV	E	6	8452.81	5352.81	<.001
PTSD & Left mOFC Bivariate Model					
I	ACE	1544	8388.41	5298.41	
II	**AE**	**3**	**8390.43**	**5294.43**	**.567**
III	CE	3	8396.32	5300.32	.042
IV	E	6	8464.32	5362.32	<.001

-2LL = -2 log-likelihood, Δdf = change in degrees of freedom from full model (I), AIC = Akaike Information Criterion. rACC= rostral anterior cingulate cortex, lOFC= lateral orbitofrontal cortex, mOFC= medial orbitofrontal cortex. For AIC and -2LL, smaller or more negative values indicate a better fit compared to the full model (Model 1). *p* is related to the statistical difference of -2LL values between full and sub models. Best fitting models are designated in bold text for each section of analyses.

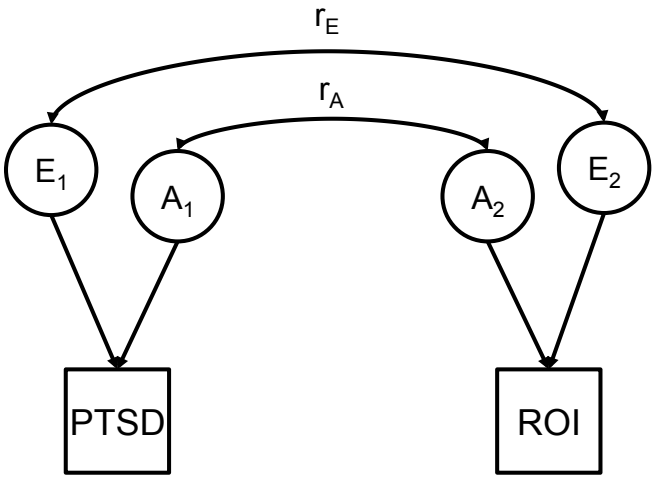

Figure 2.3 Example Bivariate Correlated Factors Model for PTSD and ROIs. A= Genetic Factor, C= Familial Environment Factor, E= Unique Environment Factor, r_A = genetic correlation between PTSD and ROI, r_E = environmental correlation between PTSD and ROI

Table 2.5 Standardized Variance and Correlation Estimates for Bivariate Correlated Factors Models between PTSD and each ROI

	Genetic Factors			Unique Environment Factors		
Model	A_1 (95%CI)	A_2 (95%CI)	rA (95%CI)	E_1 (95%CI)	E_2 (95%CI)	rE (95%CI)
R rACC	.36 (.27; .44)	.19 (.03, .38)	.12 (-.48, .21)	.64 (.55, .73)	.80 (.61, .98)	-.16 (-.26, -.04)
L lOFC	.36 (.27, .44)	.45 (.28, .60)	.05 (-.27, .42)	.64 (.55, .73)	.54 (.41, .71)	-.26 (-.43, -.06)
L mOFC	.36 (.27; .44)	.38 (.22, .53)	.07 (-.28, .51)	.64 (.55, .73)	.63 (.55, .73)	-.25 (-.42, -.05)

rACC= rostral anterior cingulate cortex, lOFC= lateral orbitofrontal cortex, mOFC= medial orbitofrontal cortex. L = left, R = right, 95%CI = Confidence intervals. A_1 and E_1 factors load onto the PTSD sum score, A_2 and E_2 factors load onto the ROI listed in the Model column. rA and rE are the correlations between each factor. . Each model listed is the best-fitting model from the corresponding sections of Table 2.2.

Moderated Bivariate Twin Analyses of PTSD, Specific ROIs, and Trauma

To address the final aim of this chapter, a moderated bivariate correlated factors model was fit to the data with trauma as the moderator on PTSD and ROI variance components as well as on the means. Given that there was no evidence for shared genetics across PTSD and the ROIs (noted by the large confidence intervals that cross zero in Table 2.5) these covariances were constrained to zero as the models failed to converge when they were freely estimated. Table 2.6 shows the fit statistics for models testing moderated correlated factor models for PTSD and each ROI. The moderators on variance components were tested individually by adding them into the model one at a time rather than starting with all being freely estimated. This is due to the fact the sample size was too small to provide model stability when all moderators were freely estimated in the full model. Therefore, model fitting began with an environment only (E) model and built up to the AE model while adding moderation to a source of variance at each step as outlined in Table 2.6. For the right rACC, models did not converge when incorporating moderation

on the genetic factors, while for the left mOFC only moderation on the genetic factor of the mOFC could be examined. For the left lOFC genetic moderation of both PTSD and the ROI simultaneously was examined, but models did not converge when moderation on both genetic and environmental factors was specified. The first section of Table 2.6 shows that model I, an E model with moderation on the means and the environmental factors fit best for PTSD and the right rACC. The best-fitting model of the left lOFC was model IV, an AE model with moderation on the means and genetic factors. Lastly, model IV, an AE model with moderation on the means and on the genetic variation in mOFC was the best-fitting model for the mOFC. Table 2.7 shows the path estimates for the best-fitting moderated model for each ROI. Figure 2.4 shows an example moderated bivariate model with path labels corresponding to the estimates in Table 2.7.

Table 2.6 Twin Model Fit Statistics for Moderated Bivariate Correlated Factors Model of PTSD and ROIs

Model	Factors	Moderation	DF	-2LL	AIC
PTSD and Right rACC					
I	**E**	**E**	**1447**	**8117.94**	**5223.94**
II	AE	-	1547	8676.96	5580.96
III	AE	E	1445	8164.48	5274.48
PTSD & Left lOFC					
I	E	E	1447	11155.40	8261.40
II	AE	-	1547	8374.74	5280.74
III	AE	E	1445	10078.18	7186.17
IV	**AE**	**A**	**1445**	**7934.21**	**5044.21**
PTSD and Left mOFC					
I	E	E	1447	7888.40	4994.40
II	AE	-	1547	8390.43	5294.43
III	AE	E	1445	7911.042	5021.04
IV	**AE**	**A_{OFC}**	**1446**	**7718.45**	**4826.45**

-2LL = -2 log-likelihood, DF = degrees of freedom, AIC = Akaike Information Criterion. rACC= rostral anterior cingulate cortex, lOFC= lateral orbitofrontal cortex, mOFC= medial orbitofrontal cortex. For AIC and -2LL, smaller or more negative values indicate a better fit compared to the full model (Model 1). *p* is related to the statistical difference of -2LL values between full and sub models. Best fitting models are designated in bold text for each section of analyses.

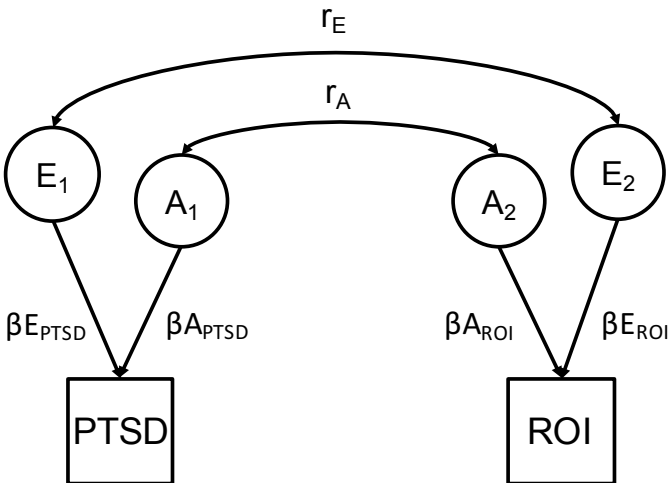

Figure 2.4 Example Moderated Bivariate Correlated Factors Model for PTSD and ROIs.
A= genetic factor, E= unique environment factor, r_A = genetic correlation between PTSD and ROI, r_E = environmental correlation between PTSD and ROI, β A = combat trauma moderation on genetic factor, β E = combat trauma moderation on unique environment factor
β E= combat trauma moderation on Unique Environmental Factor

Table 2.7 Standardized Parameter Estimates For Each Moderated Bivariate Model

Parameter	Bivariate rACC	Bivariate Left lOFC	Bivariate Left mOFC
	Estimate [SE]	Estimate [SE]	Estimate [SE]
A_{PTSD}	-	0.03 [.08]	0.03 [0.08]
A_{ROI}	-	0.32 [.002]	0.40 [.002]
E_{PTSD}	1.00 [.43]	0.97 [.04]	0.97 [0.04]
E_{ROI}	1.00 [.08]	0.68 [.002]	0.60 [.001]
rE	0.0 [.09]	0.00 [.08]	0.00 [.08]
β A_{PTSD}	-	0.045 [.19]	-
β A_{ROI}	-	-0.43 [.06]	.15 [.05]
β E_{PTSD}	0.06 [.01]	-	-
β E_{ROI}	0.02 [.05]	-	-

A = additive genetic factor; E = unique environmental factor; β= moderation beta estimate on specified variance component; PTSD = posttraumatic stress disorder, ROI = region of interest; SE = standard error

Discussion

The overall aim of this chapter was to assess whether ROIs implicated in stress response meet the criteria for an endophenotype of PTSD. This was done by examining the moderating role of trauma on the shared genetic and environmental structure of PTSD and brain regions associated with PTSD. First, by using mixed effect linear regressions, it was possible to identify brain ROIs associated with PTSD sum scores from a list of potential brain ROIs based on the extant literature. Next, modeling of the shared etiology of PTSD with each of the 3 surviving ROIs showed familial environment was not significant for any of the phenotypes examined. Table 2.5 shows there was minimal to no genetic correlation between PTSD and ROIs, and a small but significant negative correlation between unique environmental influences of PTSD and each ROI. Lastly, we examined whether trauma moderated the genetic or unique environmental influences on PTSD and ROIs.

In the preliminary analyses of 12 potential ROIs only three were significantly associated with PTSD, and had non-zero standardized beta estimates. All of the significantly associated ROIs had the expected direction of association: a reduction in cortical thicknesses as PTSD sum scores increased. The mixed effect regressions found the right rACC, left lOFC, and mOFC associated with PTSD symptoms. This is consistent with previous meta-analyses which found reduced grey matter volume in the right anterior cingulate gyrus (a sub-region of the ACC)[163] and two other meta-analyses that found significant associations with thinner OFC regions.[95,99]

For all non-moderated genetic modeling, the AE models provided the best fit, suggesting that both genetic and unique environmental influences, but not shared environmental influences contribute to the etiology of PTSD and brain morphology. This

is consistent with previous studies that separately examined the etiology of PTSD[28–30], and brain morphology[170]. In the bivariate models PTSD and ROIs were found to have overlapping unique environmental influences, but did not share genetic influences as noted by the wide confidence intervals that cross zero. Within the statistical power of this study, this finding eliminates ROI volume as a potential endophenotype for PTSD. This is the first study to examine the potential shared etiology of PTSD symptoms and brain morphology and, therefore, these results represent novel findings for the field of neuroimaging genetics.

Analyses for the final aim added moderation to the previous bivariate models with trauma as the moderator on PTSD and ROI variance components as well as on the means. These models tested whether trauma exposure accounted for the associations observed between PTSD and ROIs. Given that there was no evidence for shared genetic factors across PTSD and the ROIs these covariances were constrained to zero as the models failed to converge when included. The best fitting model for the ACC was an environment only model (E), with moderation on the means and variances. An AE model was the best-fitting model for both the lOFC and mOFC, with moderation on both genetic factors for the model including lOFC , and moderation on the ROI genetic factor only for the one including mOFC.

Although there was significant genetic moderation specifically on PTSD within one of the bivariate models, it was substantially smaller than previous studies.[180] When examined in a univariate model as a follow-up analysis, the moderation of trauma on the heritability of PTSD was -0.42 (SE = 0.80), which was still roughly half of previous findings. However, at minimum it does provide support for a diathesis-stress model of PTSD together with the previous findings of combat exposure's moderating role on PTSD.

However, the strength of this relationship is not as strong in these analyses. There are several potential reasons for this discrepancy such as a small sample size and timing difference between assessment of combat exposure and PTSD symptoms.

The extended time between the assessments of trauma and PTSD may also further reduce the ability to detected significant moderation. Although trauma recall is fairly accurate, even with over a decade since the exposure[203], there is a known slight inflation in exposure reports when there is an increase in reexperiencing symptoms. Within a latent class analysis of PTSD symptoms across time 4 main trajectories were identified: delayed-onset, improving, elevated-recovering, and stable low symptom. All but one class showed either low levels or decreasing levels of symptoms for both active and veteran military personnel within a large sample (N = 22,080).[204] This reduction in PTSD symptoms across time combined with potential reporting biases of combat exposure based on the current level of symptoms being experienced could negatively impact the ability of this study to obtain accurate results.

Using an all-male sample may influence these findings as well. Volumetric differences between sexes[205] and their potential implications for functional differences may contribute, in part to the differential rate of specific symptom clusters between men and women with women reporting more re-experiencing, avoidance, and hyperarousal symptoms[206]. Additionally, sex steroids are involved in structural plasticity of regions involved in the stress response[207], i.e. the hippocampus and amygdala, leading to slight differences in the physiological stress response between the sexes.[208] Based on previous literature of known sex differences in symptoms, brain morphometry, and physiological stress response these analyses could have different results within a sample of women.

However, of the results obtained in this study the most interesting result of the moderated models is that inclusion of the combat exposure moderator completely removes the previous environmental correlations between PTSD and each ROI. This supports the hypothesis that the correlations between PTSD and ROIs are to some degree accounted for by combat exposure experienced by the twins. For the lOFC it appears that combat exposure decreases the heritability estimates at higher levels of combat exposure. For the mOFC, combat exposure increases the heritability of the mOFC at higher levels of combat exposure.

Although close in proximity, the lateral and medial sub-regions of the OFC are cyto-architecturally distinct, displaying different connectivity patterns[209–217], and there is support for divergent functions in learning and decision-making tasks[218] between them. However, their functionality has not been unequivocally established in humans or other animal models. In a meta-analysis of connectivity modeling, the lOFC showed co-activation with other regions in the PFC involved in cognitive functions and memory. It is possible that in addition to being one of the regions with higher heritability[219,220], the mOFC is also more sensitive to trauma exposure. Given the greater degree of genetic influence and the relationship with learning and memory, individuals who were genetically vulnerable to trauma exposure would be more sensitive to the pathological effects of trauma. For the mOFC this would likely manifest as impairments in and failure to re-establish fear regulation[221] and lead to PTSD-like symptoms.

This chapter is unable to directly test mechanisms by which combat exposure modifies the strength of genetic and environmental liabilities for PTSD. However, epigenetics is one possible mechanism[222] by which environmental factors could affect genetic influences on a trait (i.e. GxE) and has been previously implicated in PTSD.[223,224]

Potentially, combat exposure could function through epigenetic mechanisms to cause gene expression changes that affect neuroprocesses, which then lead to atrophy of cortical thickness in areas involved in the processing of traumatic events (i.e. the ROIs examined in this chapter) and ultimately lead to the development of PTSD. If this were to happen, PTSD and ROIs would appear to be environmentally correlated, however once trauma exposure (and indirectly its effect on epigenetic mechanisms) is accounted for, the correlation would disappear. Further research involving thorough phenotyping of trauma type and timing, as well as epigenetics and neuroimaging is necessary to further test this possible explanation of these results.

These findings should be interpreted in the context of several potential limitations. First, this sample contained only male middle-aged participants, so the results may not generalize to women, or younger populations. Second, all MRI data were obtained at two sites. This was accounted for in analyses by regressing out any contributions related to site of scan, but this does not include possible random effects of site (the number of sites is too few for this type of correction). It should also be noted that each twin was scanned at the same site as their co-twin, and there were equal mixes of MZ and DZ twin pairs scanned at each site. Additionally, participants were assessed for PTSD symptom severity and combat exposure levels at separate time points, approximately 50 and 30 years after service in the Vietnam War. Although the PTSD items assessed symptomatology in the past 30 days, the delay between combat exposure and its measurement for this study does raise questions about recall bias in participant self-report measures of combat exposure. Lastly, although overall VETSA has a relatively large twin sample, PTSD is not as common as other psychopathology[27], therefore this study has reduced power to detect significant findings in ROI-based analyses of PTSD compared to other psychiatric disorders.

However, this is a common issue for samples that are not explicitly enriched for phenotypes. This issue became especially apparent when fitting the last series of models containing moderation, as the larger models with more parameters did not converge. Therefore the results from the final aim should be interpreted with the understanding that they are most likely underpowered. Larger consortia-based analyses, such as those associated with the enhancing neuroimaging genetics through meta analyses (ENIMGA) consortium may be better suited to obtained more precise estimates of shared etiology.

Chapter 3: The Genetic and Environmental Structure of Fear and Anxiety in Juvenile Twins

Fear and anxiety are adaptive responses to acute or potential threat, respectively.[153] When symptoms become dysregulated, excessive, or interfere with functioning and quality of life, fear and anxiety symptoms are considered clinical phobias or other anxiety disorders, respectively.[1] As disorders of threat response with some shared features, psychiatric nosology traditionally includes phobias, generalized anxiety, and panic within the anxiety disorder domain. Both domains have roots in childhood but commonly expand and persist into adolescence and adulthood, accounting for a substantial proportion of lifetime psychiatric illness.[6] However, due to their individually broad but partially distinguishable features and complex unfolding across development, researchers have often separately investigated various aspects of their symptomatology at different ages.

Fear represents the emotional-behavioral response to the perception of immediate danger, leading one to avoid the threat for discernible survival value.[108] Fears and phobias are highly comorbid[123], and twin studies suggest this may be explained, in part, by overlapping genetic and environmental influences[120,225]. One twin study investigating the comorbidity structure of fear symptoms in children reported a common genetic factor that influenced all clusters in addition to fear-specific factors.[124]

Anxiety disorders (ADs) often have a basis in normal anxious concerns; however, the degree of anxiety and associated symptoms may become excessive, uncontrollable, and impairing to an individual's life. ADs are highly comorbid with each other, and adult twin studies suggest that this comorbidity may be due, in part, to genetic risk factors shared between disorders.[149,150] This comorbidity pattern is also seen in children, where 40% to 60% of children with one AD are estimated to meet criteria for additional ADs.[151,152]

In a non-twin study, Muris and colleagues[226] report substantial correlations between subscales of the Fear Survey Schedule for Children-Revised (FSSC-R)[114] and the Screen for Child Anxiety-Related Emotional Disorders (SCARED).[143] However, only a few studies of children have examined the liability structure of DSM-based anxiety dimensions[146] or phobic fear symptoms.[119,124] No child twin studies have explored the potential sources of shared etiology of these two threat response domains. Given the partial distinction between risk factors for phobias and other ADs in adults, it is important to examine their childhood precursors. Furthermore, early fear and anxiety disorders are the strongest predictors of later psychiatric comorbidity.[173] Therefore, this chapter aims to explicate the shared and specific risk factors (genetic, familial environment and unique environment) and their roles amongst fear and anxiety domains in youth. Due to the moderate level of correlation between the FSSC-R and SCARED subscales described above, partial overlap between genetic and environmental factors across these scales is predicted. By leveraging symptom sum scores rather than diagnostic criteria, the statistical power to detect meaningful patterns of shared and specific variance is likely to be increased.

Methods

Participants

The twins included in these analyses comprised the VCU Juvenile Anxiety Study (VCU-JAS).[227] Using twin families recruited by the Mid-Atlantic Twin Registry[228], VCU-JAS enrolled twins aged 9-14 across two sites (VCU and the National Institute of Mental Health; NIMH) to participate in a study of internalizing phenotypes. Only Caucasian twins were recruited to minimize heterogeneity within the sample for the genetic aims of the overall study. The Institutional Review Boards at VCU and NIMH approved this study, and parents of all participants provided informed consent before participating. Self-report data available for this study came from 746 youths (N=130 monozygotic (MZ) twin pairs and N=243 dizygotic (DZ) twin pairs) consisting of 388 female and 358 male twins. Zygosity was determined using parental responses to standard questions about physical appearance of the twins and DNA testing as described in detail elsewhere.[227]

Measures

Fear

The FSSC-R[114] is a widely used questionnaire for assessing common fears in children.[112,118,229] It uses a 3-point Likert scale (1='none', 2='some', 3='a lot') for each of 80 feared stimuli or situations. The shortened 25-item form (FSSC-RSF) has a 5-factor structure similar to the full scale[230], and a confirmatory factor analysis (CFA) using Mplus version 7.4[231] demonstrated an adequate fit for our data (CFI= .88, RMSEA= .06) consistent with previous literature. The five subscales included fear of failure and criticism (CRIT), fear of the unknown (UNKN), fear of animals (ANML), fear of danger

and death (DEATH), and medical fears (MED). A sum score was calculated for all subscales with the following means (standard deviations): CRIT 8.51 (2.43), UNKN 7.68 (2.38), ANML 7.28 (2.02), DEATH 12.96 (3.41), and MED 6.85 (2.06). Figure 3.1 shows histograms for each subscale and the total scale, with the mean of each denoted by a blue line. Prior studies have found the FSSC-RSF has good internal consistency, total score Cronbach's alpha=0.91, and subscale alpha=0.74-0.82[230], with full-scale alpha=0.96 and two-week test-retest reliability=0.78 found in our sample[227].

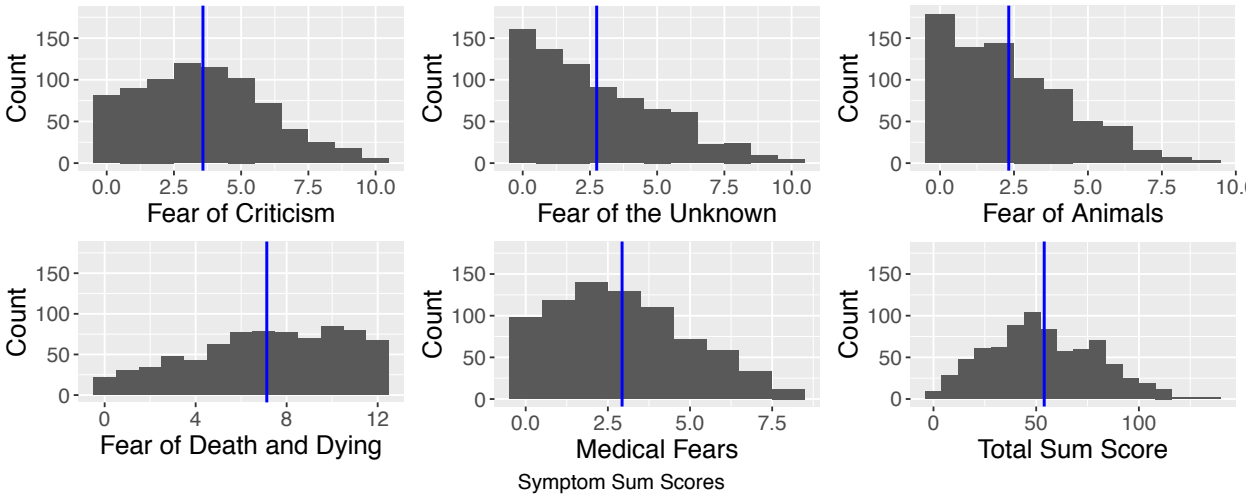

Figure 3.1 Histograms FSSC-RSF Subscales and Total Sum Score Counts
Sum scores for each subscale and total sum score are shown with blue line indicating the mean of the subscale.

Anxiety

The SCARED was developed to screen for ADs within clinical samples[142,143] but has also been widely used in community and research studies.[144,145] It assesses five clusters of childhood anxiety symptoms: panic/somatic [PAN], generalized anxiety [GAD], social anxiety [SOC] and separation anxiety [SEP] as well as school avoidance. The 41-item version[142] assesses symptoms on a 3-point Likert scale. (0='almost never', 1='sometimes',

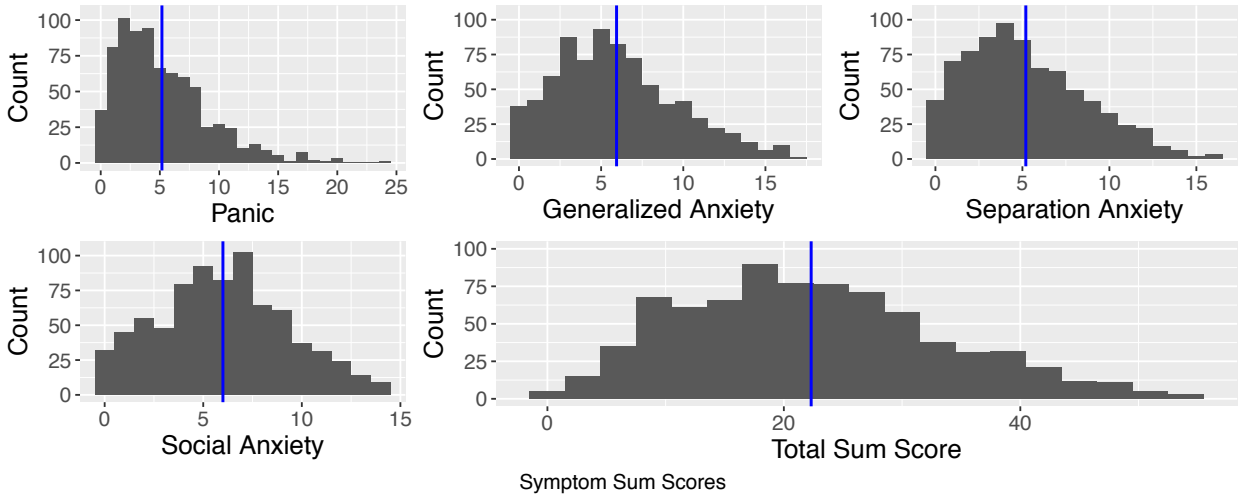

Figure 3.2 Histograms SCARED Subscales and Total Sum Score Counts
Sum scores for each subscale and total sum score are shown with blue line indicating the mean of the subscale.

2='often'). As intended, the factor structure for the scale yielded five subscales: PAN, GAD, SEP, SOC, and School Avoidance. Since School Avoidance is not related to a specific DSM-IV disorder, we did not use it for our analyses. A CFA excluding the school avoidance items showed a four-factor model fit our data adequately (CFI=.92, RMSEA=.04) consistent with previous literature.[232] A sum score was calculated for each of the four DSM-related subscales with the following means (standard deviations) in VCU-JAS: PAN 5.17 (3.86), GAD 5.94 (3.69), SEP 5.20 (3.42), and SOC 6.00 (3.29); for more information see.[227] Figure 3.2 shows histograms for each subscale and the total scale, with the mean of each denoted by a blue line. Previous studies found a high degree of internal consistency of the SCARED (Cronbach's alpha=0.74-0.93) and good test-retest reliability (intraclass correlation coefficients=0.70-0.90).[142,143] Our sample found a similar full-scale Cronbach's alpha=0.90 and two week test-retest reliability=0.89.

Statistical analyses

Analyzing the similarity of MZ and DZ twins can elucidate the roles of additive genetic (A), familial environmental (C), and unique environmental (E) effects. Variance is partitioned into underlying genetic and environmental influences by leveraging the difference in genetic relatedness between twin types. Additive genetics (A) reflects the latent cumulative effects of individual genetic loci influencing a trait. Familial environment (C) captures non-genetic influences that make twins more similar to each other compared to the general population. Unique environment (E) describes influences that contribute to the differences seen between co-twins, including measurement error. Models were fitted by full information maximum-likelihood (FIML) using the OpenMx package.[199]

In multivariate structural equation modeling, ACE components can be specific to each subscale (e.g., As_1 in Figure 3.3) or common to multiple subscales (e.g., Ac_1). Age and sex have a substantial effect on fear and anxiety measures and were included as fixed-effect covariates for all phenotypic means. Due to sample size we do not have the power to examine sex effects on variances, however inclusion of age and sex as covariates on the means is a step towards reducing the heterogeneity introduced by these covariates. Significance of individual parameters was tested by comparing the fit of a model to that of a constrained submodel. Likelihood ratio χ^2 tests are used to determine if the constrained model fits the data significantly worse than the saturated model. AIC is based on twice the difference in log-likelihood between higher order and submodels with a penalty for degrees of freedom, with lower AIC denoting a better balance of model fit and parsimony.[202]

An increasingly complex series of hypothetical models were fit to the data. Multivariate independent pathway models (IPMs), and common pathway models (CPMs) were estimated separately for each scale to estimate their sources of variance and covariance. Second, we tested whether the common ACE factors for the best-fitting FSSC-RSF were correlated with those of the SCARED via a correlated factors model (CFM). Non-zero correlations provide evidence for shared etiology across fear and anxiety domains in children. The final model, a combined IPM covering both sets of symptom clusters, provided a more nuanced representation of the covariance structure beyond the factor correlations in the CFM. We tested the need for multiple sets of common ACE sources of covariance to explain the observed data.

Results

Fear

The Fear sections of Table 3.1 displays the fit statistics for the independent and common pathway models of the FSSC-RSF. To test for genetic and environmental factors common to all subscales, we began with a model including single common A, C, and E factors plus specific ACE factors with age and sex covariates for each subscale mean. Significance of common and then specific factors were sequentially tested by iteratively constraining parameters to zero. Common pathway models consisting of 1-, 2-, and 3-factors were fit to the data as well. As indicated in Table 3.1, Model 1b was determined to be the best fitting and most parsimonious model. It included a single set of common ACE factors and subscale specific A and E factors. Females had the expected pattern of higher mean subscale scores compared to males across all subscales, depicted in Figure 3.3 as paths from the sex moderator (lower right circle) loading on each subscale. A slight

decrease in means as age increases for all scales except for criticism is consistent with decreases in childhood fears over development. The influence of common and specific genetic factors accounted for 10-34% of the total of variance of each subscale with the remaining variance accounted for primarily by subscale specific, unique environmental factors. As a follow up to the two-factor common pathway model, which fit almost as well as Model 1b in Table 2.1 of the independent pathway series, a series of independent pathway models were fit to the data with two sets of common ACE factors. However, they were unable to converge on a final solution. As such analyses moved forward with the best-fitting model containing a single set of ACE factors.

Anxiety

The Anxiety sections of Table 3.1 displays the fit statistics for the independent and common pathway models of the SCARED. Similar to fear, we found model 1b of the independent models fit best with single set of common ACE factors and subscale specific A and E. Age and sex influenced the means of each subscale in a similar pattern to fear. GAD was the exception for which the opposite age trend was found, consistent with clinically observed increased risk of GAD with age. The total influence of all genetic factors accounted for 18-35% of each subscale's variance with remaining variance accounted for primarily by subscale specific unique environmental factors. The genetic factor common to all subscales accounted for 5-19% of the variance for PAN, GAD and SEP. Only SOC did not share genetic influences with the other symptom clusters. Figure 3.4 depicts the path estimates from the best fitting model for the SCARED.

Table 3.1 Model-Fitting Results for Multivariate Independent Pathway Models of Fear and Anxiety

Model	Common Factors	Specific Factors	EP	df/Δ	Model Fit -2LL	Model Fit AIC	*P*
Fear Independent Pathway Models							
1	$A_1C_1E_1$	All ACE	45	3612	15553.6	8349.6	-
1a	**$A_1C_1E_1$**	**All AE**	**40**	**5**	**15553.6**	**8339.6**	**.999**
1b	$A_1C_1E_1$	All CE	40	5	15558.6	8344.6	.413
1c	$A_1C_1E_1$	All E	35	10	15569.2	8345.2	.110
2	C_1E_1	All ACE	40	5	15569.7	8355.7	.000
3	A_1E_1	All ACE	40	5	15560.8	8346.8	.204
4	A_1C_1	All ACE	40	5	15714.8	8500.8	.000
5	-	All ACE	30	15	16455.7	9221.7	.000
Fear Common Pathway Models							
6	1 Factors	All ACE	38	3609	15572.5	8354.5	-
7	2 Factors	All ACE	46	3601	15552.8	8350.8	-
8	3 Factors	All ACE	54	3593	15538.6	8352.6	-
Anxiety Independent Pathway Models							
1	$A_1C_1E_1$	All ACE	36	2900	14824.0	9024.0	-
1a	**$A_1C_1E_1$**	**All AE**	**32**	**4**	**14824.0**	**9016.0**	**.999**
1b	$A_1C_1E_1$	All CE	32	4	14837.6	9029.7	.008
1c	$A_1C_1E_1$	All E	28	8	14852.6	9036.7	.000
2	C_1E_1	All ACE	32	4	14833.4	9025.4	.005
3	A_1E_1	All ACE	32	4	14836.1	9028.1	.001
4	A_1C_1	All ACE	32	4	14951.4	9143.4	.000
5	-	All ACE	24	12	15544.1	9720.1	.000
Anxiety Common Pathway Models							
6	1 Factors	All ACE	31	2905	14836.7	9024.7	-
7	2 Factors	All ACE	38	2898	14827.1	9031.1	-
8	3 Factors	All ACE	45	2891	14823.0	9041.0	-

Abbreviations: EP=estimated parameters, df /Δ= degrees of freedom for model and change in degrees of freedom for submodels, -2LL = twice the negative log likelihood of model, AIC = Akaike information criterion of model. For AIC and -2LL, smaller values indicate a better fit compared to the full model (Model 1). *p* is related to the statistical difference of -2LL values between full and sub models. Bold designates the overall best fitting model.

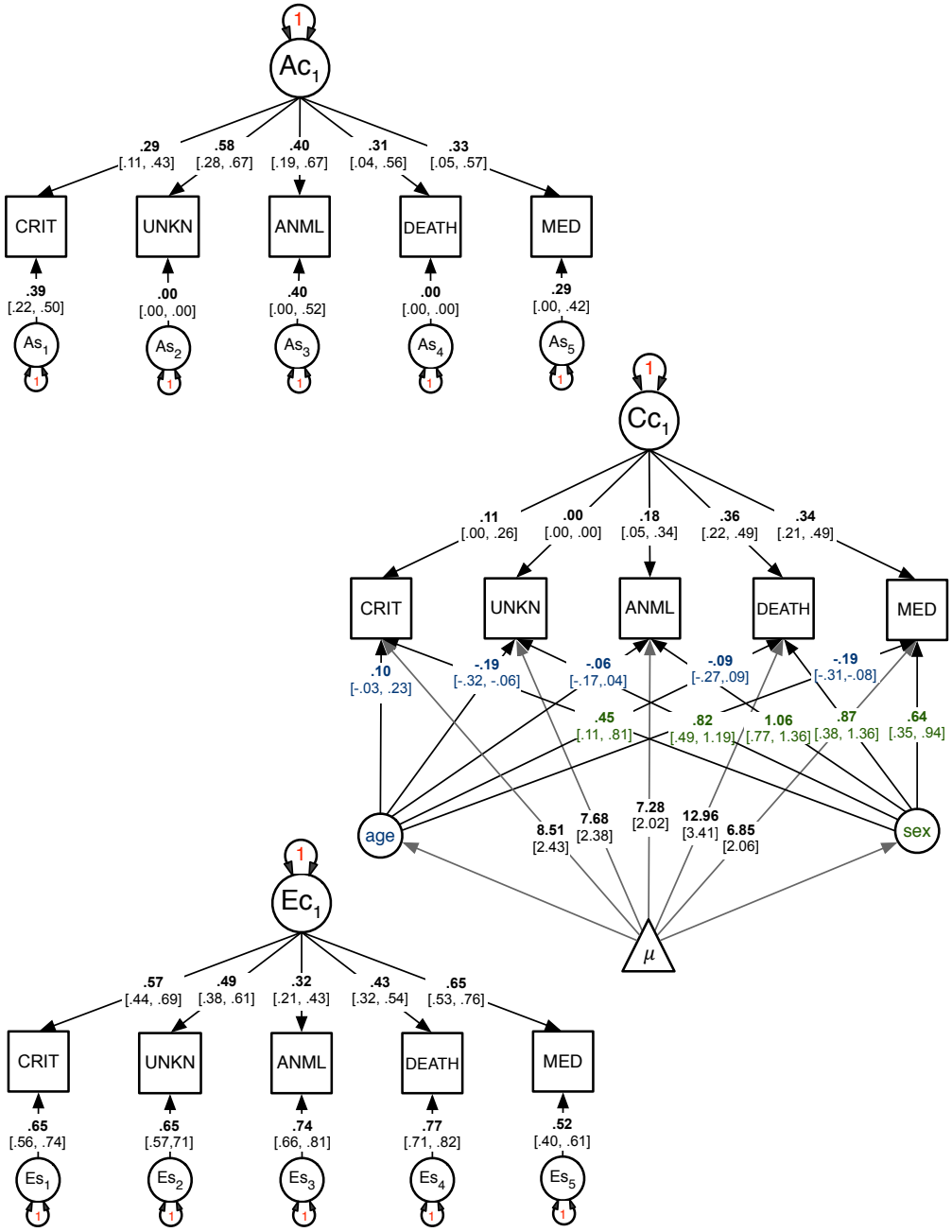

Figure 3.3 Best-Fitting Model for Fear Subscales
The model contains 1 common additive genetic factor (Ac_1), 1 common familial environmental factor (Cc_1), and 1 common unique environmental factor (Ec_1). Only subscale specific additive genetic, and unique environmental factors were found to be significant, and thus retained in the final model. Path coefficients representing standardized estimates are listed above 95% confidence intervals for each path for fear of failure and criticism (CRIT) fear of the unknown (UNKN), fear of animals (ANML), fear of danger and death (DEATH), and medical fears (MED). Triangles in the middle figure denote age and sex moderators on the means for all subscales, with 95% CIs listed below the standardized path estimate. Triangle (μ) represents the means [S.D.] of the four subscales in addition to loading onto the covariates age and sex [95% CI]

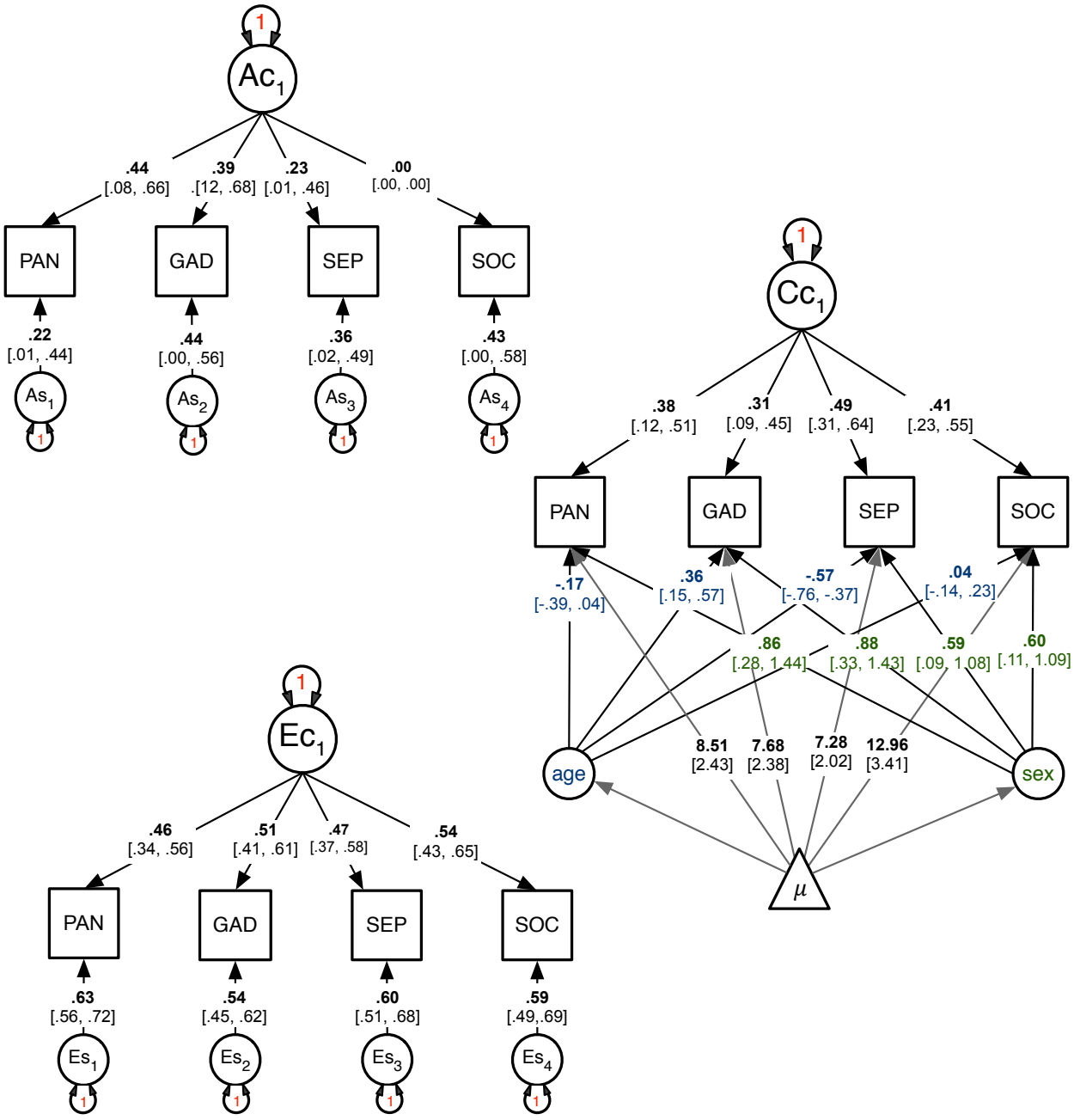

Figure 3.4 Best-Fitting Model for Anxiety Subscales
The model contains 1 common additive genetic factor (Ac_1), 1 common familial environmental factor (Cc_1), and 1 common unique environmental factor (Ec_1). Only subscale specific additive genetic, and unique environmental factors were found to be significant, and thus retained in the final model. Path coefficients representing standardized estimates are listed above the 95% confidence intervals (CIs) for each path for panic disorder (PAN), generalized anxiety disorder (GAD), social phobia (SOC) and separation anxiety (SEP). Triangle (μ) represents the means [SD] of the four subscales in addition to loading onto the covariates age and sex [95% CI]

Modeling Across Fear and Anxiety

Correlated Common Factors Model

We used the best fitting individual IPMs of Fear and Anxiety to examine the overlap in their etiology via correlations between the two sets of common ACE factors. Each subscale retained specific ACE factors to capture residual variance not otherwise accounted for by common factors. Model 1 in Table 2 freely estimated all correlations, and submodels tested significance of each correlation by constraining to 1 (fully shared) or 0 (no sharing) and comparing fit to the full model. Model 12 had a slightly better fit to these data, with the correlation between common familial environment factors and the correlation between common genetic factors constrained to 1 plus a common unique environmental factor correlation estimated at r=0.67. Consistent with the single scale models, it included significant age and sex effects on the means. Statistically stable specific influences on the subscales and highly correlated influences common to all fear and anxiety clusters motivates a more detailed examination of the risk structure via the combined independent pathway model.

Table 3.2 Model-Fitting Results for Correlated Common Factor IPM of Fear and Anxiety

	Fixed			Model Fit		
Model	Correlations	EP	df/Δ	-2LL	AIC	*P*
1	-	84	6499	30065.0	17067.0	-
2	rA, rC, rE = 0	65	3	30377.6	17373.6	< .001
3	rA, rC, rE = 1	65	3	30109.6	17105.6	< .001
4	rA = 1	83	1	30047.6	17047.6	.999
5	rA = 0	83	1	30049.7	17049.7	.999
6	rC= 1	83	1	30038.0	17038.0	.999
7	rC= 0	83	1	30044.4	17044.4	.999
8	rE= 1	83	1	30097.5	17097.5	< .001
9	rE=0	83	1	30087.4	17087.4	< .001
10	rA=1 rC=0	82	2	30045.4	17043.4	.999
11	rA=0 rC=1	82	2	30052.3	17050.3	.999
12	**rA=1 rC=1**	**82**	**2**	**30038.6**	**17036.6**	**.999**
13	rA=0 rC=0	82	2	30096.9	17039.1	< .001

Table 2 shows the fit statistics for all models tested. Model 1 is the full model with all three latent correlations freely estimated. To test the significance of correlations subsequent models constrained the correlations to 1 and 0. Table abbreviations: IPM = Independent Pathway Models, rA= correlation between common latent genetic factors, rC= correlation between common latent familial environmental factors, rE= correlation between common latent unique environmental factors. Bold designates the overall best fitting model.

Combined Independent Pathway Model

To examine which subscales were driving the correlations between latent factors and explore a larger set of possible risk structures among fear and anxiety, we examined an IPM with two sets of latent common ACE factors. To ensure model identification and a unique solution, for each set of ACE common factors, we dropped one variable to designate one set as the 'anxiety' set and the other as the 'fear' set. I.e., all SCARED subscales load on the 'anxiety' ACE factors and all subscales except criticism from FSSC-RSF do so as well, and the reverse with all FSSC-RSF and all SCARED except GAD loading onto the 'fear' ACE set (as seen in Figure 3.5 in the C and E factor loading illustrations). When testing a single common factor model (i.e., Ac_1, but no Ac_2), we allowed all subscales

to load onto that single factor. In a test similar to fixing correlations to one in the correlated common factors model, we successively dropped one of the common genetic factors (Ac) then one each of the common environmental factors (Cc and Ec) to find the most parsimonious model. While one of the two latent Ac factors could be eliminated without a significant deterioration in fit, we were unable to remove any of the four common environmental factors (i.e., keeping two each for Cc and Ec). The best fitting was Model 3b in Table 3 with similar effects of age and sex as before.

Table 3.3 Model Fitting Results for Independent Pathway Model Including All Symptom Clusters

	Common	Specific			Model Fit		
Model	Factors	Factors	EP	df/ Δ	-2LL	AIC	*P*
1	$A_1A_2C_1C_2E_1E_2$	All ACE	102	6481	30146.6	17184.6	-
2	$A_1A_2C_1E_1E_2$	All ACE	95	7	30051.4	17085.4	.999
3	$A_1C_1C_2E_1E_2$	All ACE	95	7	30024.8	17048.8	.999
3a	$A_1C_1C_2E_1E_2$	All CE	86	16	30040.1	17046.1	.032
3b	**$A_1C_1C_2E_1E_2$**	**All AE**	**86**	**16**	**30024.8**	**17030.8**	**.999**
3c	$A_1C_1C_2E_1E_2$	All E	77	25	30069.0	17057.0	< .001
4*	$A_1A_2C_1C_2E_1$	All ACE	95	7	-	-	-
5	$A_1C_1E_1$	All ACE	81	21	30105.4	17101.4	.999
6	C_1E_1	All ACE	72	30	30196.9	17202.9	.014
7	A_1E_1	All ACE	72	30	30170.8	17148.8	.762
8	A_1C_1	All ACE	72	30	30381.5	17359.5	< .001
9	-	All ACE	54	48	31999.8	18941.8	< .001

Table 3 shows the fit statistics for all models tested. Common Factors are the ACE latent factors shared between all observed variables. Specific Factors are the nine sets of ACE latent factors that each only load onto one observed variable, respectively. Specific factors were tested by dropping a class at a time (e.g., all specific A latent factors dropped at same time). Bold designates the overall best fitting model.

* Model 4 was unable to converge to a final solution

The proportion of variance in liability accounted for by each source of variance is listed in Table 4. Figure 3.5 illustrates the larger role of shared genetic influences on the anxiety subscales, with limited influence of familial environment common to all subscales and the largest proportion of unique environmental influences originating from subscale specific factors. This partitioning is reflected in Table 4 where the total genetic influences across shared and specific components account for 15-40% of the variance, whereas the total variance accounted for by common and specific familial environment is markedly lower (0-17%); the remainder was accounted for by some common but predominantly specific unique environment (47-74%).

Table 3.4 Proportion of Variance in Liability to Anxiety and Fear Symptom Clusters from Common and Specific Genetic and Environmental Risk Factors*

Symptom Cluster	Genetic Factors			Familial Environmental Factors			Unique Environmental Factors			
	Ac	As	**Total**	Cc_1	Cc_2	**Total**	Ec_1	Ec_2	Es	**Total**
PAN	.28	.12	**.40**	.03	.02	**.05**	.16	.00	.39	**.55**
GAD	.50	.03	**.53**	.00	-	**.00**	.12	-	.35	**.47**
SEP	.21	.12	**.33**	.02	.10	**.12**	.21	.04	.30	**.55**
SOC	.14	.26	**.40**	.02	.00	**.02**	.18	.02	.38	**.58**
CRIT	.37	.00	**.37**	-	.00	**.00**	-	.19	.44	**.63**
UNKN	.14	.00	**.14**	.02	.15	**.17**	.04	.27	.38	**.69**
ANML	.03	.18	**.21**	.06	.01	**.07**	.00	.18	.54	**.72**
DEATH	.14	.02	**.16**	.10	.00	**.10**	.00	.15	.59	**.74**
MED	.15	.04	**.19**	.15	.00	**.15**	.00	.26	.30	**.66**

Table 4. Panic disorder (PAN), generalized anxiety disorder (GAD), social phobia (SOC) and separation anxiety (SEP), fear of failure and criticism (CRIT), fear of the unknown (UNKN), fear of animals (ANML), fear of danger and death (DEATH), and medical fears (MED), Ac (Common A factor), As (Specific A factor), Cc_1 (First Common C factor) Cc_2 (Second Common C factor), Ec_1 (First Common E factor), Ec_2 (Second Common E factor) Es (Specific E factor). Bolded columns designate proportion of total variance accounted for by the combined common and specific etiological sources of variance for each subscale.
*Best-fitting IPM model 3b from Table 3

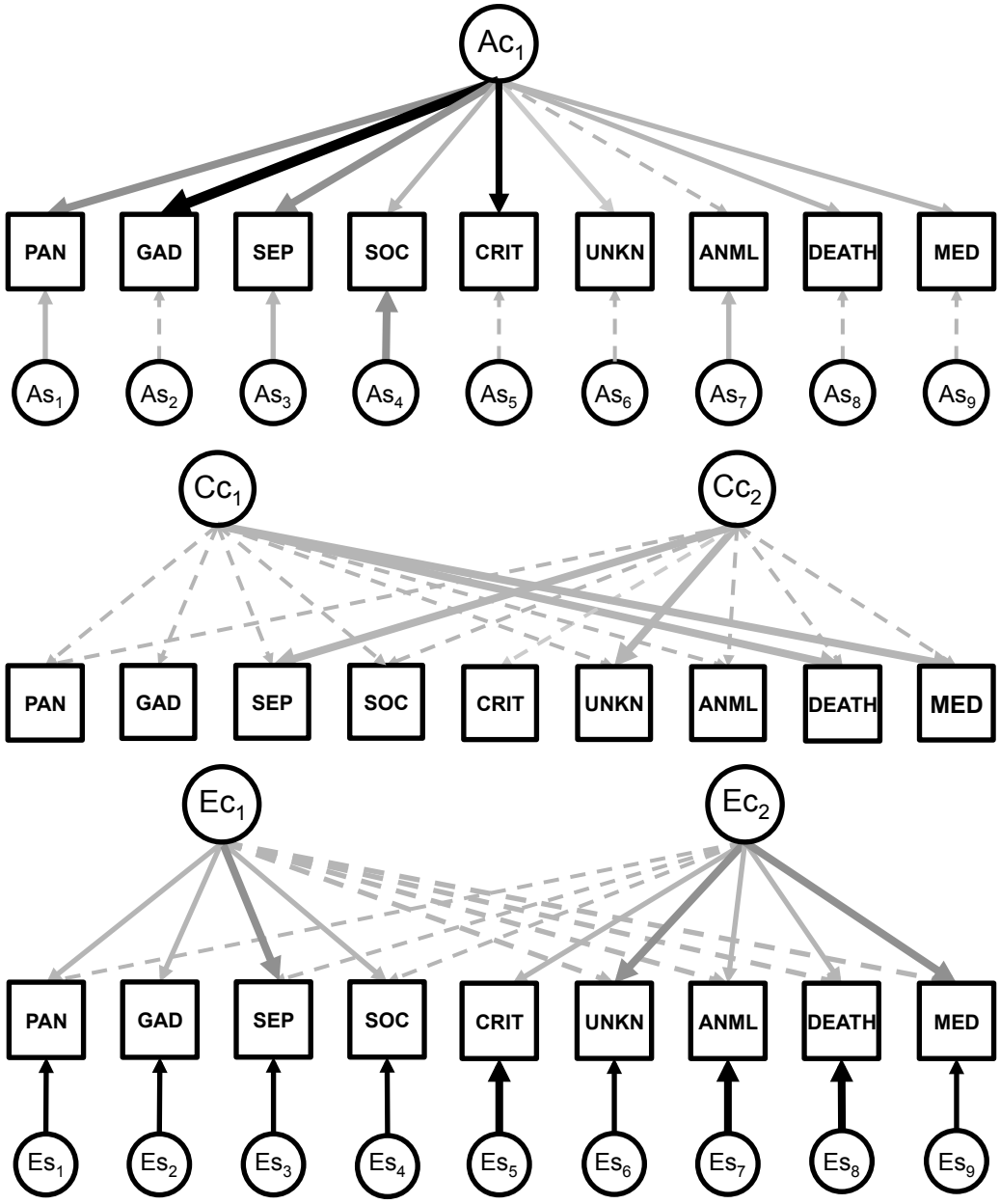

Figure 3.5 Best-Fitting Model for Fear and Anxiety Symptom Clusters
The model contains one common additive genetic factor (Ac_1), two common familial environmental factors (Cc_1 and Cc_2), and two common unique environmental factors (Ec_1 and Ec_2). Only disorder specific additive genetic and unique environmental factors were found to be significant and retained in the final model. The darker lines indicate a stronger influence of the latent factor on the observed variable. Lighter lines indicate a standardized path estimate less than 0.10. Table 3 provides the proportion of variance accounted for by each of these pathways.

Discussion

We used multivariate SEM to examine the structure of genetic and environmental risk factors that underlie the associations between fear and anxiety symptoms in a juvenile twin sample. We first separately examined each scale's genetic and environmental factors. Fear and anxiety each respectively displayed an overall similar etiologic covariance structure that included moderate influences of genetic plus familial and unique environmental factors common to all clusters (Figure 3.5). The remaining influences were due to outcome-specific genetic and unique environmental effects. Prior childhood studies have primarily focused on the etiology of particular fears[119] and their longitudinal changes over development[124] or the etiology shared between fears or phobias.[126] Whether measuring diagnoses or symptom counts, extant studies reported moderate genetic influences that were partially shared with other fears or phobias plus a predominance of unique environmental influences. Our findings of significant fear-specific genetic and unique environmental effects, but little to no familial environmental influences, replicate those of prior studies examining these domains independently.[125,126,233,234]

Within AD symptom domains, a latent genetic factor Ac accounted for a modest proportion of variance shared by all anxiety subscales except SOC. This is generally consistent with the findings of Ogliari and colleagues.[235] Differences between these two studies are largely accounted for by our finding of an additional common familial factor Cc, with modest influence on the covariance of subfactors that the other study did not include in their final models. Overall, our findings of significant moderate genetic and unique environmental influences are similar to previous independent studies of SOC, SEP, and PAN in children.[125,147,233]

Considering previous twin research that examined fear and anxiety separately in children, our study provides novel insights into the potential etiological underpinnings responsible for the high comorbidity and relatively parallel developmental sequence observed between phobic fear and anxiety domains in youth. The correlated common factors model yielded a structure consistent with very highly correlated common A and C factors plus moderately correlated common E factors across symptom domains. The combined IPM, with its greater flexibility in determining overall risk structure compared to the correlated common factors model, then allowed a more detailed representation of the covariance structure to emerge.

Within the combined IPM (Figure 3.5), risk across domains was variably influenced by a single genetic factor (Ac) in addition to domain-specific familial environment (Cc_1 and Cc_2), plus those unique to each individual (Ec_1 and Ec_2). The proportion of variance accounted for by Ac is lower for fear symptoms (3-37%) than for anxiety symptoms (14-50%). Only Ac accounts for greater than 30% of the variance for any of the clusters affecting both fear of criticism and GAD, the most genetically influenced of each domain (37% and 50% heritability, respectively).

While our best fitting model included multiple C and E factors, they only partially distinguished between fear and anxiety clusters. Familial environment was not strongly influential, and the pattern that emerged was not simply fear versus anxiety. As Figure 3.5 shows, C_1 has a modest degree of cross-loading on anxiety symptoms and fear of death and medical fears, although the influence on anxiety symptoms is minimal. Only SEP and fear of the unknown load onto C_2 with the remaining items receiving little to no influence. This suggests an underlying relationship between responses to seemingly distinct threats such as separation anxiety and fear of the unknown. However, both tap into related

constructs of basic survival threat: the former due to separation from a source of safety (caregiver) and the latter of facing unknown situations that nonspecifically threaten basic survival without a caregiver's protection. Notably, familial environment plays little role in the comorbidities of internalizing disorders in adults.[236]

The only common factors to show an arguable distinction between the domains were the unique environmental influences. Figure 3.5 shows all the anxiety symptoms clustering together on Ec_1 and all the fear symptoms on Ec_2 with minimal cross loadings from the other domain (represented by dashed lines in the figure). Since fear and anxiety are differential responses to acute versus potential threat, their overall environmental influences are likely to separately cluster. Fear is a more primitive, instinctive defensive reaction primarily involving the amygdala and its recruitment of other subcortical regions that develop early, while anxiety requires more complex responses dependent upon cortical involvement which reaches maturity later than subcortical regions.[237] In the context of brain development these environmental influences may be more a reflection of a child's current ability to respond to a fear cue, whereas anxiety would require brain regions that are not yet developed, thus a potentially biologically mediated process appears as separate environmental influences. Furthermore, given that normative fears are variably expressed within certain developmental windows (fear of strangers, separation, the dark, animals, etc.), it is more likely for their environmental influences to cluster according to exposures by age that make them more highly correlated with each other than with environmental influences on anxiety symptoms. That is, while predisposing genetic influences of fear and anxiety largely overlap, their environmental influences may be differentially moderated by age and neurodevelopmental stage.

Our results further suggest that the etiological structures of fear and anxiety in children are not as clearly differentiated as in adults. Prior adult twin studies report substantial continuity of etiological influences among fears and their corresponding phobias.[121] Furthermore, both adult phenotypic[238] and twin[149] studies find correlated but partially distinct structural relationships between phobias and other anxiety disorders. Thus, while our finding of moderate levels of genetic influences common to all symptom clusters is not unexpected, the degree of sharing seen here is notable. This reflects, and likely helps explain, clinical observations in which children are substantially more likely to have a complex, changing pattern of syndromes compared to adults.[6,141,151,152]

The results of this analysis should be interpreted within the context of several limitations. While we were able to control for fixed effects of age and sex in the analyses, this sample does not possess sufficient power to examine their detailed influences on the latent genetic and environmental factors.[239] However, previous studies have indicated conflicting results regarding age and sex having a moderating effect on the variance of fear and anxiety measures[124,146,147]; thus, we covaried for them at the means level to minimize these biases. Second, although it might limit generalizability to clinical samples, a dimensional approach that reflects symptom measures increases the statistical power to detect the influences of etiologic significance over use of categorical diagnoses. Generalizability is also limited due to the exclusive use of Caucasian twin pairs driven by the aim to minimize genetic heterogeneity introduced when sampling from multiple ethnicities. Most prior twin studies were also conducted in Caucasian twins, maximizing our comparability with them.

The findings of this analysis have implications for investigating the risk mechanisms underlying fear and anxiety symptoms in childhood and beyond. From a

trans-diagnostic perspective, these findings help explain and potentially validate the high rates of comorbidity among internalizing disorders in children. Longitudinal research in developmental psychopathology would benefit from incorporating both threat response domains, given their close links in childhood. Studies in adults show a clearer distinction between the two domains and their sources of covariation, while their expression in children is more diffuse and malleable. From an etiological perspective this could be due to the greater degree of shared genetic influences expressed during child development coupled with developmentally specific environmental influences that help disentangle fear and anxiety. A longitudinal study extending into late adolescence would further inform the temporal unfolding of fear and anxiety risk factors as they merge into those seen in adulthood.

Chapter 4: Shared Etiology of Fear and Anxiety with Brain Morphometry

As discussed more thoroughly in chapters 1 and 3, fear and anxiety are adaptive responses to threat; with fear being focused on more imminent danger that is linked to a drive to survive, whereas anxiety is primarily focused on potential or long-term threats.[237] Both threat responses have their basis in deep-seated motivations of continued survival. However, misplaced, excessive, and unwarranted fear and anxiety can be maladaptive, and clinically recognized as phobias and panic or generalized anxiety disorder (GAD). This chapter considers the neurobiology of these fundamental threat responses and their potential shared etiologies with fear-network related brain regions.

Many animal model research studies have focused on Pavlovian fear conditioning and extinction as testable, although simplistic, processes relevant to anxiety-related disorders such as phobias, and posttraumatic stress disorder. This translational model has provided insights into the importance of the amygdala, hippocampus, anterior cingulate cortex (ACC), and areas of the prefrontal cortex (PFC) in fear conditioning and extinction.[240] In humans, activation of these areas is also associated with tasks directly investigating fear conditioning/extinction.[10] Additionally, differences in morphometry[132,133] and functional activation[18,23,128–131] of these areas have been associated with phobias and other anxiety disorders, primarily in adults. The current neurocircuitry-based understanding of many anxiety disorders such as GAD, panic, and

posttraumatic stress disorder largely concerns over-activation and recruitment of the 'fear network' including the amygdala, hippocampus, and some brainstem structures, with more recruitment of cortical areas such as the ACC, and ventromedial PFC (vmPFC), compared to activation patterns of phobias. The latter two areas are associated with anxiety disorders more focused on worry and other cognitive processes such as generalized anxiety and social anxiety. Within the functional neuroimaging literature there appears to be a general consensus that amygdala activation is exaggerated in response to a variety of disorder-specific stimuli across many anxiety disorders such as panic disorder[156,157], social phobia[13–16], generalized anxiety[22,158,159], and posttraumatic stress disorder.[11,12]

Published studies on structural brain differences between anxiety patients and controls in adults are limited, and those of children more so. The latter are limited both in number of studies and sample sizes, with most studies having 50 or fewer participants. In three studies smaller hippocampal volumes were associated with childhood anxiety disorders[241] or symptoms[242,243], whereas others found no significant differences.[155,244–246] There are similarly conflicting findings for associations with amygdala volume. Some studies report larger[155,247] volumes associated with pediatric anxiety disorders, others smaller[241,245,248], and yet others finding no significant association.[244,246] When investigating areas of the prefrontal cortex, four studies reported smaller cortical thicknesses (vmPFC/medial orbitofrontal cortex [mOFC]) among children with anxiety disorders[243,246–248], while another study found greater vmPFC thickness associated with generalized anxiety.[249] While these areas were originally identified for their involvement in fear conditioning and emotional regulation, structural neuroimaging studies have had limited success in finding associations between structural differences (i.e. thinner cortical

thickness, or smaller volumes) in these regions and childhood anxiety disorders. Two of the more recent studies used continuous measures of anxiety symptom severity with varying degrees of success[242,243], but most of the neuroimaging literature continues to use case/control analytic approaches.

The aim of the present chapter is to test for an endophenotypic relationship between brain regions of interest (ROIs) previously implicated in fear processing and anxiety. This is accomplished by examining whether the ROIs are significantly associated with a genetic factor score indexing fear and anxiety measures in this sample. While a whole brain voxel-wise approach would be ideal, this chapter uses pilot imaging data from a child anxiety study. In order to maximize its statistical power and limit the number of statistical tests, a small number of preselected brain regions were analyzed. As such this chapter focuses on the hippocampus, amygdala, ACC, lOFC, and mOFC of the left and right hemispheres. The prefrontal region of the brain is divided into many sub-regions, and depending on the brain atlas used in analyses, the same location in two studies can be labeled as different areas. For these analyses the lateral and medial OFC labels refer to the PFC and vmPFC of previous studies. Based on the previous literature and that two of the larger loadings on the genetic factor score were for anxiety subscales, it is hypothesized that this genetic factor score will be associated with an increase in hippocampal and amygdalar volumes and a decrease in OFC and ACC sub-region cortical thicknesses. The literature is ambiguous regarding direction of effect for some regions, so these hypotheses were chosen based on representative prior studies that had measures similar to this study.

In contrast to the prior studies that focused on anxiety case-control differences, this chapter use a dimensional genetic factor score phenotype based on the best-fitting

model from chapter 3 that indexes fear and anxiety outcomes. It is hypothesized that this score is more proximal to the biological processes related to fear and anxiety measures. Additionally, since the genetic factor score indexes a latent liability to both fear and anxiety measures, an exploratory aim seeks to investigate whether associations found between ROI volumes and the genetic factor score are driven by a specific scale. This final exploratory aim is designed to replicate a previous study[243] that used anxiety symptom sum scores to predict hippocampal volumes.

Methods

Participants

The twins included in these analyses are a subset from the Virginia Commonwealth University Juvenile Anxiety Study (VCU-JAS). VCU-JAS enrolled twins aged 9-14 across two sites (VCU and the National Institute of Mental Health; NIMH) to participate in a study of internalizing phenotypes. Only Caucasian twins were recruited to minimize heterogeneity within the sample for the genetic aims of the overall study. The Institutional Review Boards at VCU and NIMH approved this study, and parents of all participants provided informed consent before participating. Self-report and neuroimaging data came from 105 youths (N=20 monozygotic (MZ) twin pairs, N= 24 dizygotic (DZ) twin pairs, and 17 singletons) aged 9-14 years old consisting of 60 female and 45 male participants. These participants were recruited post-hoc from those who participated in the larger VCU-JAS sample through an additional funding protocol. Zygosity was determined using parental responses to standard questions about physical appearance of the twins or DNA testing as described in detail elsewhere.[227] For safety, children were excluded from the imaging protocol if they had metal braces or other metal

objects present in the body as an additional exclusionary criteria beyond those of the primary study. The only other exclusion criterion was the participant's general tendency to be fidgety during the VCU-JAS assessments, since motion within the scanner disrupts imaging signals.

Measures

Fear

The shortened 25-item form of the Fear Survey Schedule for Children Revised[230] (FSSC-RSF) was used for the analyses in this chapter. The short form has a 5-factor structure similar to the full scale: fear of failure and criticism, fear of the unknown, fear of animals, fear of danger and death, and medical fears. Further details on this measure can be found in chapter 3. For consistency with prior studies, a single sum score was calculated for this measure and used as a general index of overall fearfulness with a mean of 46.70 (SD = 26.14, and skew = 0.65).

Anxiety

The 41-item version of the Screen for Child Anxiety-Related Emotional Disorders[143] (SCARED) was used. This scale contains five subscales: panic (PAN), generalized anxiety (GAD), separation anxiety (SEP), social anxiety (SOC), and School Avoidance. Since School Avoidance is not related to a specific DSM-IV disorder, it is not use in these analyses. Chapter 3 contains a more detail introduction to this scale. A single sum score was calculated from the four DSM-related subscales and used as a general index of overall anxiousness with a mean of 20.06 (SD = 10.78, and skew = 0.49).

MRI acquisition

Structural images were collected at two sites: Virginia Commonwealth University (VCU); and the National Institute of Health (NIH). At VCU a Philips Ingenia 3.0T scanner with a 32-channel head coil was used. Imaging protocol included 3D magnetization-prepared rapid gradient-echo (MPRAGE) T1-weighted volume optimized for maximum gray/white matter contrast with the following parameters: flip angle=6°; FOV=24 cm; slices=160; slice thickness=1mm; 240x240 matrix; repetition time [TR]= 8.1ms; echo time [TE]= 3.7ms). At NIH a General Electric 3.0T scanner with an eight-channel head coil was used. The imaging protocol included a 3D MPRAGE T1-weighted volume optimized for maximum gray/white matter contrast with the following parameters: flip angle=7°; FOV=25.6 cm; slices= 176; slice thickness=1mm; 256x256 matrix; TR= 7.7ms; TE= 3.4ms.

MRI Processing

Processing of images was performed using standard, automated procedures available in the Freesurfer image analysis software suite, which is freely available for download and fully documented (Version 6.0, http://surfer.nmr.mgh.harvard.edu/). Processing consisted of motion correction[187], correction of distortion due to gradient nonlinearity and B1 field inhomogeneity, image intensity normalization[188], removal of non-brain tissue using a hybrid watershed/surface deformation procedure[189], and automated Talairach transformation. FreeSurfer software package routines[190,191] were used to define gray matter, white matter, segmentation of subcortical structures, and cerebral spinal fluid segmentation. After cortical models were created, the Desikan-Killiany[250] probabilistic atlas was used to assign neuroanatomical labels to each

voxel[251,252] followed by subcortical volume and cortical thicknesses calculation using this parcellation. The procedures used for cortical thickness measurement have been validated against histological analysis[192] and manual measurements[193,194]. The protocols used to obtain cortical thickness and subcortical volumes measurements have been used in previous child psychiatric research[243,249,253–256].

Cortical and Subcortical Measures

Prior to all analyses all ROIs were regressed onto age, sex, scan site, and intracranial volume to account for the fixed effects of these covariates. ROIs examined in this chapter include hippocampal volume, amygdala volume, rostral anterior cingulate cortex (rACC) mean thickness, caudal anterior cingulate cortex (cACC) mean thickness, lateral orbitofrontal cortex (lOFC) mean thickness, and the medial orbitofrontal cortex (mOFC) mean thickness separately in both the left and right hemispheres of the brain, for a total of 12 ROIs examined in these analyses. Figures 4.1 and 4.2 illustrate these areas in the left hemisphere.

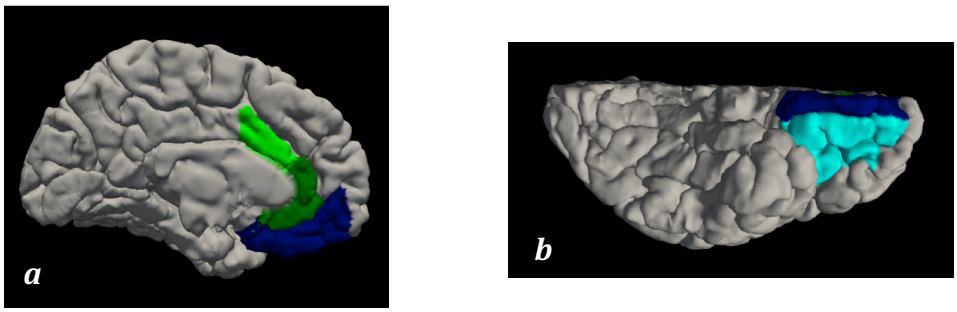

Figure 4.1 Divisions of the left orbitofrontal and anterior cingulate cortices Panel *a* shows a sagittal cross section and *b* shows an inferior view of the left hemisphere. Medial orbitofrontal cortex (OFC) is illustrated in dark blue, lateral OFC in light blue. Caudal anterior cingulate cortex (ACC) is in light green and rostral ACC is in dark green.

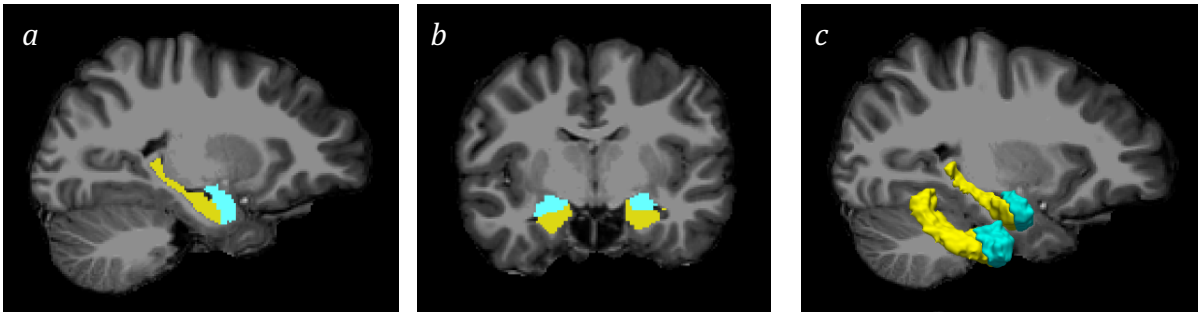

Figure 4.2 Bilateral Structures of the Hippocampus and Amygdala
Panel *a* shows a sagittal view of the hippocampus (light blue) and amygdala (yellow), *b* shows a coronal view and *c* shows a 3-dimensional bilateral reconstruction of the structures with a sagittal cut of the left hemisphere to provide relative spatial context.

Statistical Analyses

Factor Scores

Genetic factor scores were created in OpenMx based on the final best-fitting twin model from chapter 3. This twin model included a single common genetic factor for the fear and anxiety measures described above. This factor score was created using a regression predictor. In this approach the estimated parameters from a factor analysis are used to define linear combinations of observed variables, which then generate the factor scores. Specifically, the Thomson-Thurstone regression method[257–259] was used, which defines the factor score as the product of the factor loading matrix, the inverse of the data covariance matrix, and a vector containing the data. Factor scores were calculated for the entire sample using the unstandardized factor loadings.

Mixed Effect Linear Regression

The random effects within a mixed effect linear regression were used to adjust for possible effects of correlated observations in the twin data. In each regression, family ID and zygosity denoted family membership and whether the pair was monozygotic or

dizygotic, respectively, were entered as random effects. The *umx*() package was used to obtain 95% confidence intervals for all standardized beta estimates.[260]

Results

Associations between genetic factor scores and 12 ROIs implicated in fear and anxiety in adults were examined. Specifically, the genetic factor scores were used to predict volumes of sub-cortical ROIs and mean thickness of cortical ROIs. A false discovery rate (FDR) was used to account for testing of the 12 ROIs.[261] Although none of the results remained significant after multiple testing corrections, there were two regions with unadjusted $p < 0.05$, the left and right hippocampi (Table 4.1). As a final exploratory aim, the association between fear and anxiety total scale sum scores and the left and right hippocampal volumes were examined, but no significant associations prior to FDR correction were found (Tables 4.2 and 4.3, respectively).

Table 4.1 Genetic Factor Score predicting ROIs Measures in Separate Analyses

Region of Interest	β [95% CI]	t value	*p*	FDR *p*
L Hippocampus	76.86 [9.84, 143.88]	2.32	.026	.312
R Hippocampus	83.41 [6.92, 159.91]	2.20	.033	.396
L Amygdala	-21.7 [-61.13, 17.73]	-1.11	.273	.999
R Amygdala	-1.16 [-46.67, 44.34]	-0.05	.959	.999
L rACC	-0.03 [-0.1, 0.04]	-0.88	.382	.999
R rACC	0.00 [-0.07, 0.07]	-0.13	.900	.999
L cACC	-0.04 [-0.1, 0.02]	-1.41	.165	.999
R cACC	-0.02 [-0.07, 0.04]	-0.59	.560	.999
L lOFC	0.02 [-0.02, 0.06]	0.95	.346	.999
R lOFC	0.02 [-0.02, 0.06]	1.06	.296	.999
L mOFC	0.01 [-0.04, 0.05]	0.26	.794	.999
R mOFC	0.00 [-0.05, 0.04]	-0.21	.832	.999

β = standardized beta estimates, 95% CI = 95% confidence interval, FDR p= false discovery rate adjusted p value, L = left, R = right, rACC = rostral anterior cingulate cortex, cACC = caudal anterior cingulate cortex, lOFC = lateral orbitofrontal cortex, mOFC = medial orbitofrontal cortex. Using data from VCU-JAS mixed effect linear regressions were performed to predict subcortical volumes and cortical thicknesses based on genetic factor scores that index latent liability to fear and anxiety within the sample

Table 4.2 Continuous Measure of Fear Symptoms Predicting Hippocampal Volumes

Variables	(95% CI)	t value	p value
L hippocampus	1.71 [-0.26, 3.69]	1.75	.087
R hippocampus	2.09 [-0.31, 4.48]	1.76	.086

β = standardized beta estimates, 95% CI = 95% confidence interval, L = left, R = right. Using data from VCU-JAS mixed effect linear regressions were performed to predict hippocampal volumes based on fear symptom sum scores

Table 4.3 Continuous Measure Anxiety Symptoms Predicting Hippocampal Volumes

Variables	(95% CI)	t value	p value
L hippocampus	1.97 [-2.68, 6.62]	0.86	.396
R hippocampus	4.48 [-0.81, 9.77]	1.71	.095

β = standardized beta estimates, 95% CI = 95% confidence interval, L = left, R = right. Using data from VCU-JAS mixed effect linear regressions were performed to predict left and right hippocampal volumes based on anxiety symptom sum scores

Discussion

Mixed effect linear regressions were used to examine whether specific ROIs were associated with a genetic factor score indexing fear and anxiety within this child sample. Prior to multiple-testing correction using FDR, the left and right hippocampal volumes were significantly associated with greater genetic liability towards fear and anxiety. After FDR correction no significant associations were found between the ROIs and a genetic factor score indexing fear and anxiety. Simpler individual-based fear and anxiety measures were less informative.

Larger mean hippocampal volume has been previously found in adults with anxiety[262]. However several child[241–243] and adult[134,135] anxiety studies have found either decreases in hippocampal volumes, or no differences at all in child[155,244–246] or adult[263–265] samples. Although most prior studies have implemented an anxiety disorder cases versus healthy controls study design, two previous studies also examined anxiety

symptom severity as a continuous measure associated with hippocampal volume[242,243]. The first study by Koolschijn[242] and colleagues examined the association between hippocampal and amygdalar volumes with an internalizing disorder score derived from the child behavior check list[266]. They found lower left hippocampal volume was significantly associated with higher internalizing symptom scores. In a similar approach Gold and colleagues[243] found higher SCARED total scale sum scores were significantly associated with smaller right hippocampal volumes. Both studies are relatively large for a child imaging study, and the Gold study using the SCARED is roughly the same size as the current study (N = 108 and N = 105, respectively) with approximately the same uncorrected p-values (0.02, and 0.03, respectively), although more regions were examined in this study and none of the findings survived multiple-testing correction. Since these analyses were focused on the genetic score which incorporates the SCARED and FSSC-RSF scales and the potential associations found were in the opposite direction of previous work, the next step was to specifically test whether hippocampal volumes were associated with SCARED and FSSC-RSF total scale scores. In these follow-up analyses no significant results were found that corroborated or contradicted the findings of Gold et al.

There are several potential reasons for these null results. First, the morphometric differences observed in adult and child anxiety disorder patients versus healthy controls could be the result of neuroprocesses related to the specific disorders, and thus there may not be a predisposing difference that is detectible prior to the onset of a clinically significant symptom threshold. This seems unlikely as a justification applicable to all anxiety disorders, however for some disorders these morphometric differences tend to disappear with successful treatment such as with phobias.[18,129,136] Second, this sample is

still early in their onset trajectories for some of the ADs, with panic, generalized anxiety, and posttraumatic stress (the last of which is not examined in this juvenile sample but is of interest to this dissertation at large) having substantially later age of onset compared to the other disorders such as specific phobias, separation anxiety, and social phobias.[267] Given that panic and generalized anxiety with later onset ages were two of the largest loadings on the latent genetic factor from chapter 3 it is possible that the genetic factor score captured in these data does not fully account for the genetic variance expressed across both developmental and disease trajectories and, as such, limits the ability to detect significant associations. Lastly, these ROIs were originally selected as they are frequently examined within adult anxiety functional neuroimaging research. However, these ROIs are not consistently implicated in the more limited number of structural studies of child anxiety disorders. Most adult and child studies are also limited by small sample sizes, which further reduces their statistical power to detect significant results, especially when examining all brain regions rather than a pre-specified list. Future imaging studies with larger sample sizes are needed to fully address these research questions in a more comprehensive manner than the extant literature offers. The Adolescent Brain Cognitive Development study (ABCD study) is in the process of recruiting 11,500 9-10 year olds, including 800 twin pairs and will hopefully provide a sample size large enough to examine structural differences associated with fear and anxiety symptoms with adequate statistical power. The enhancing neuroimaging genetics through meta analyses (ENIMGA) consortium may also be better suited to obtain and properly harmonize the largest collection of psychiatric focused neuroimaging samples. ENIGMA has already identified genetic loci associated with brain morphometry[268] and

has several working groups currently developing projects across a variety of phenotypes including an anxiety focused working group (ENIGMA-ANX).

These findings, or rather the lack thereof, should be interpreted in the context of several key limitations. First, the exclusive use of Caucasian twins pairs to minimize the heterogeneity introduced with multiple ethnicities limits the generalizability of these results. Second, it is possible that the most anxious and fearful participants from the full sample chose not to participate in this imaging portion due to the potentially stressful nature of additional imaging protocols. In fact, a 2-sample t-test shows a significant difference ($p = 0.04$) between the SCARED sum score means of this subsample (mean = 20.06) and the full sample (mean = 22.31) as well as significant differences ($p = .007$) between the fear sum score mean of the subsample (mean = 46.70) and the full sample (mean = 53.91). These differences may have artificially limited the upper bound of the fear and anxiety measures compared to the full sample and reduced the information available for the regression analyses by removing participants with the most extreme scores, which could be contributing to the null results of this study. Lastly, these results may not generalize to clinical samples, because the measures of fear and anxiety are dimensional measures rather than being based on clinical diagnoses of anxiety disorders. In principle, this approach using full distributions of quantitative traits vs. categorical cut-offs should have increased the power to detect associations between genetic liability to fear/anxiety and ROIs. To conclude, it is still possible that ROIs differ between anxiety diagnoses and healthy controls. A study with a larger sample size may find the differences sought in this chapter.

Chapter 5: Global Discussion

The broad aim of this dissertation was to identify whether differences in specific fear-network related brain morphometries were endophenotypes for PTSD, fear, and anxiety. This was addressed by examining the etiological relationships between brain morphologies, PTSD in adults, and fear/anxiety in children. The primary gap in the current understanding of brain morphometry endophenotypes is whether specific regions of interest (ROIs) have a genetic relationship to disorders to which they are phenotypically associated. This dissertation focused on establishing these genetic relationships through twin data modeling and related analyses. In the case of PTSD, trauma is known to be associated with adverse outcomes such as a PTSD diagnosis, but it has also been associated with morphometric differences in the brain between trauma-exposed and healthy individuals. In order to examine the etiological relationship between PTSD and ROIs trauma, exposure must also be taken into account. The knowledge gained from these analyses will be particularly useful as the fields of neuroimaging and genetics continue to integrate, and larger neuroimaging datasets become publicly available.

In chapter 2, biometrical SEM was used to examine the shared etiology of PTSD and ROIs while accounting for trauma using a large population-based sample. It was found that thinning of fear-network related cortical areas, specifically the right ACC, left

lOFC, and left mOFC, were associated with increased PTSD symptom sum scores. Univariate twin models suggest that both genetic and unique environmental influences, but not familial environmental influences, contribute to the etiology of PTSD and brain morphology. Examination of the overlapping etiology of PTSD and each of these three ROIs found that they had overlapping unique environmental influences, but they did not have overlapping genetic influences, as reflected by the wide confidence intervals that cross zero. These results would suggest that while ROI differences may be useful as potential biomarkers for PTSD, they currently do not meet the criteria for endophenotypes based on these analyses. To be considered an endophenotype a biomarker must be proven to associate with the disorder, be heritable, and have a genetic relationship to the disorder identified through either family, twin, or measured genotype based analyses.[4] Although they did not share genetic influences, PTSD and ROIs did demonstrate overlapping environmental influences that may be worth further study. As a final step, the etiological relationship between PTSD and ROIs was examined in the context of moderation by combat exposure. It appears the phenotypic associations between them is entirely accounted for by moderation of combat exposure due to the fact that inclusion of the combat exposure moderator completely removed the previous environmental correlations between PTSD and each ROI. However, the moderation estimates were small, and the instability of some of the models suggests the sample is underpowered to make any definitive conclusions on the role of trauma in the etiology of PTSD and ROIs.

Despite the limited sample size of this study, the differences in morphometry of fear-network related brain regions do not appear to meet criteria for endophenotypes of PTSD at this time, as there was no genetic overlap found in this study. Further research

is needed via larger twin samples or measured-genotype based analyses to replicate and extend these findings. One study has already had success identifying overlapping genetic risk for obsessive-compulsive disorder with genetic influences on subcortical brain structures.[269] Future analyses similar to this study are currently in progress with the Enhancing NeuroImaging Genetics Through Meta Analyses (ENIGMA) consortium. Examination of epigenetic changes associated with trauma exposure may provide a causal link between trauma exposure and the differences observed in brain morphometry. Previous work in epigenome-wide studies has implicated methylation changes on genes involved in immunity[270] and methylation age[271] with PTSD. The methylation age was also negatively associated with neural integrity of the corpus callosum and nominally associated with lower neural integrity of the left rACC, providing further support of epigenetic effects of PTSD affecting brain morphometry. Differences in gene expression patterns between PTSD and trauma-exposed controls have been identified and primarily aggregate in genes associated with cortisol response[272] (which is known to be dyrsegulated in PTSD) and immunity.[270] Further research into shared measured genotype and methylation is being pursued by the PTSD working group of the Psychiatric Genomics Consortium. This working group is focused on pooling PTSD cases and trauma-exposed controls across many studies with the aim of finding genetic loci, methylation sites, and differences in gene expression associated with PTSD. Ultimately combining work from ENIGMA and the PGC may provide a causal mechanism that explains the current phenotypic associations of PTSD and its brain biomarkers.

Chapter 3 investigated the shared etiology of fear and anxiety in children. The measures of fear and anxiety were found to have an overall similar etiologic covariance structure that included moderate influences of genetic plus familial and unique

environmental factors common to all subscales of fear or anxiety with the remaining influences due to outcome-specific genetic and unique environmental effects. When examined together, the final best-fitting model for chapter 3 showed a single genetic factor was common to all subscales of fear and anxiety. Familial environment was not strongly influential, with many subscales showing minimal to no influence from the familial environment. The only common factor to demonstrate a degree of distinction between fear and anxiety was the unique environmental factor. Overall, these findings suggest that although predisposing genetic influences for fear and anxiety largely overlap, their environmental influences may be the distinguishing wedge that separates the presentation of fear and anxiety from each other in children.

The results of chapter 3 suggest the high comorbidity of anxiety disorders (including phobias) seen in community samples may be due, in part, to these highly shared genetic influences. While studies in adults show a clearer distinction between the two domains and their sources of covariation, their expression in children appears to be more diffuse and malleable. The pattern of 'same genes but, different environments' found in this chapter is also found in the relationship between two other comorbid disorders, depression and generalized anxiety.[273,274] Fears, anxiety, and depression are all considered internalizing disorders so it is possible that this shared genetic influence may be tapping into something larger than just fear and anxiety. The genetic influence in this chapter might be indexing something much more global such as a predisposition for internalizing negative behaviors. Examination of additional internalizing symptoms and behaviors would provide further insight into the interplay of fear and anxiety in a broader context. Future gene finding efforts involving pediatric samples may benefit from the

inclusion of multiple anxiety disorders or from the allowance of comorbidity with other anxiety disorders in case status individuals.

Finally, chapter 4 tested whether ROIs previously implicated in fear processing and anxiety have a genetically-based relationship with fear/anxiety in children. To test this hypothesis the results from chapter 3 were used to create a genetic factor score indexing latent liability to fear/anxiety. This genetic score is thought to be more proximal to the biological processes related to fear and anxiety measures and may provide a stronger link between self-report measures of fear and anxiety with ROIs. To test this hypothesis, genetic factor scores indexing an individual's latent liability to fear/anxiety were incorporated into a mixed effect regression to predict ROI measures. After multiple-testing correction no significant associations were found between the ROIs and the genetic factor score, and simpler individual-based fear and anxiety measures were less informative. There are two main potential reasons for these null results, including: 1) morphometric differences may be due to disease related neuroprocesses and as such are not detectible prior to the onset of a clinically significant symptom threshold; and 2) these participants might be too early in their onset trajectories for a genetic factor score to fully account for genetic variance expressed across developmental and disease trajectories, which limits the ability to detect significant associations. While the genetic factor score was unable to conclusively identify endophenotypes, it did provide preliminary evidence for a genetic relationship between fear/anxiety and ROIs. Consortia and larger studies that integrate genetically informative methods and neuroimaging, such as ENIGMA and ABCD, are better situated to address the sample size limitations seen in this chapter. Future research from these groups may benefit from using several different approaches to incorporate genetics into neuroimaging research, such as implementing a classic twin

design, or using polygenic risk scores created from large genome-wide association studies (GWAS) of psychiatric disorders. Given the increase in genome-wide significant signals from anxiety and stress-related GWAS[275–281], Mendelian randomization is also an option to examine potential causal effects of specific loci. However, identification of SNPs associated with brain morphology is still a bit further behind compared to the field of psychiatric genetics.

Limitations

The findings of this dissertation must be interpreted within the context of several limitations of these analyses. Although detailed more thoroughly in previous chapters, some of these limitations do provide a framework for future research into the intersection of psychopathology, genetics and neuroimaging.

A limitation for all phenotypic measures across both samples is that they were assessed via self-report questionnaires. This presents limitations in a few different manners. First, the measure of trauma is dependent on participant report of combat experiences 30 years prior, which raises concerns of recall biases. Second, results from this dissertation may not generalize to clinical samples, because the measures of fear, anxiety, and PTSD are dimensional measures rather than being based on clinical diagnoses of disorders. Although, in principle, this approach, using full distributions of quantitative traits vs. categorical cut-offs, should increase the power to detect associations between phenotypes. Next, for both the adult and child samples all MRI data were obtained at two sites, which was partly accounted for in analyses by regressing out any contributions related to site of scan. Additionally, for both samples each twin was scanned at the same site as their co-twin, and there were equal mixes of MZ and DZ twin

pairs scanned at each site. The adult twin sample contained only male middle-aged participants, so the results may not be generalizable to women, younger populations, or individuals with trauma exposures other than combat experience. While it was possible to control for fixed effects of age and sex within the analyses of the child sample, this sample does not possess sufficient power to examine their detailed influences on the latent genetic and environmental factors. Additionally, previous studies indicated conflicting results regarding age and sex having a moderating effect on the variance of fear and anxiety measures[124,146,147], so they were covaried for at the means level to minimize these biases. Generalizability with results from the child sample is also limited due to the exclusive use of Caucasian twin pairs, which was driven by the aim to minimize genetic heterogeneity introduced when sampling from multiple ethnicities.

Conclusions

This dissertation used a trans-diagnostic framework to examine the shared etiology of PTSD, fear, anxiety, and fear-network related brain morphometries. There were several novel findings regarding etiology of threat-related domains, and associated brain morphometry. Analyses investigating brain morphometric differences as potential endophenotypes for PTSD provided preliminary evidence that their association is largely accounted for by environmental influences, specifically trauma exposure. However, the small sample size caused model instabilities, which in turn limited the ability to make definitive conclusions. Examining domains of fear and anxiety in children found a substantial genetic overlap between the two. Lastly, incorporating a genetic factor score derived from the results of the previous chapter on fear and anxiety provided preliminary evidence for a genetic relationship between fear/anxiety and ROIs.

Although this dissertation extensively examined brain morphology of fear-network related regions, there are still many alternative imaging modalities available for examining the association between brain morphometry/functioning and psychiatric disorders, and these may provide further insight into potential psychiatric endophenotypes. Further research is needed to identify endophenotypes across these modalities with the ultimate goal of linking disorder outcomes to genetic, epigenetic, and gene expression changes. This understanding of biological pathways and mechanisms that result in psychiatric disorders could eventually help identify potential prevention or treatment options.

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