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A GENETICALLY INFORMED STUDY OF ACUTE THREAT ENDOPHENOTYPES FOR CALLOUS-UNEMOTIONAL TRAITS

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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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[1] Manuscript has been previously published and modified for inclusion in this dissertation.

ABSTRACT

A GENETICALLY INFORMED STUDY OF ACUTE THREAT ENDOPHENOTYPES FOR CALLOUS-UNEMOTIONAL TRAITS

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Introduction. Callous-unemotional (CU) traits predict socially debilitating outcomes including Antisocial Personality Disorder and violent crime in adulthood. Despite significant research, the etiology of CU traits is not well understood. This dissertation incorporates genetic, physiological, neuroanatomical, and self-report measures to investigate the etiology of CU traits. Specifically, this project focuses on measures previously found to associate with impaired fear-processing observed in individuals high on CU. Brain morphometry for paralimbic regions of interest (ROIs) and electromyographic facial eyeblink reflex to startle and fear-potentiated startle probes were

investigated as potential endophenotypes for CU traits. **Methods.** Two genetically informative (ages 9-20) twin samples (*N=1696 individuals; 848 twin pairs*) were used to estimate the changing heritable and environmental influences on CU over the age range of 9-20 using age-moderated biometric structural equation modeling (SEM). To determine potential endophenotypes, shared genetic variance with CU was examined for baseline and fear-potentiated startle reflex and morphometric measures of brain ROIs. **Results.** The heritability of CU increases over the ages of 9-20, from approximately 34% at age 9 to 47% at age 20. Therefore, environmental mechanisms for CU are most influential at younger ages. Although there were no significant associations after correction for multiple testing, there was some evidence to suggest potential positive associations between CU traits and baseline and fear-potentiated startle in younger (9-14) females. There was also evidence suggesting potential negative associations between CU traits and right anterior cingulate cortex thickness as well as right posterior cingulate cortex thickness in females only. There was no genetic covariance between CU and any of the examined physiological or neuroanatomical phenotypes. **Discussion.** These results suggest that middle childhood may be the most salient time for environmental interventions associated with preventing or ameliorating CU traits. Furthermore, these results suggest that the cingulate cortex may play a role in the development of CU traits, possibly in females specifically. The cingulate cortex may influence CU traits through its roles in emotional processing, learning, and memory. Larger samples will likely be needed to determine the genetic relationship between CU traits and the structural development of the cingulate cortex.

CHAPTER 1. INTRODUCTION[2]

I. AN INTRODUCTION TO CALLOUS-UNEMOTIONAL TRAITS

Conduct disorder (CD) is a psychiatric disorder of childhood and adolescence that reflects socially debilitating psychopathology (American Psychiatric Association, 2013). CD has been associated with a variety of negative health outcomes including poorer physical health (Bardone et al., 1998; Odgers et al., 2007), premature mortality (Laub and Vaillant, 2000), co-morbid psychiatric conditions (Kim-Cohen et al., 2003), and increased risk for legal problems (Simonoff et al., 2004). It is estimated that 12% of males and 7% of females will meet criteria for CD at some point in their lifetime (Nock, Kazdin, Hiripi, & Kessler, 2006).

There appears to be substantial heterogeneity in the developmental trajectories, corollaries, and treatment outcomes associated with CD (Frick, 2012). This observed heterogeneity has contributed to a substantial literature on subtypes and features of CD

[2] Some passages from this chapter are taken verbatim from the author's previous work, including: (a) Moore, A. A., Blair, R. J., Hettema, J. M., & Roberson-Nay, R. (2019). The genetic underpinnings of callous-unemotional traits: A systematic research review. *Neuroscience and Biobehavioral Reviews, 100*, 85-97. (b) Moore, A. A., Carney, D., Moroney, E., Machlin, L., Towbin, K. A., Brotman, M. A., . . . Hettema, J. M. (2017). The inventory of callous-unemotional traits (ICU) in children: Reliability and heritability. *Behavior Genetics, 47*, 141-151.

that may delineate those individuals who are at the highest risk for future psychopathic behavior. Some of the most prominent subtyping efforts involve grouping individuals based on age of onset (Moffitt et al., 2008), whether they display aggressive behaviors (Tackett et al., 2005), socialized vs. undersocialized constructs (American Psychological Association, 1980), and callous-unemotional (CU) traits (Frick, Ray, Thornton, & Kahn, 2014).

CU traits have been described as the "core" features of psychopathy (Frick & Morris, 2004; Fowles & Dindo, 2009). Psychopathic traits describe a set of characteristics that are interpersonal (e.g., egocentrism, social manipulation), emotional (e.g., lack of guilt, shallow affect), and behavioral (e.g., impulsivity, aggression, antisociality; Hare & Neumann, 2005; Salekin, 2017). Although the term 'psychopath' is not generally used to describe children, some psychopathic characteristics are clinically observed in childhood including the emotional deficits known as CU traits.

CU traits are widely recognized as a risk factor for future psychopathic traits and antisocial behavior, and an analog of CU traits was recently introduced into the DSM-5 (American Psychiatric Association, 2013). This CD specifier, "with limited prosocial emotions," requires that children display two of the following four characteristics: lack of guilt/remorse, lack of empathy, deficient affect, and/or lack of concern about performance (American Psychiatric Association, 2013). These *emotional traits* supplement the *behavioral characteristics* described by CD. Approximately 10-32% of individuals meeting criteria for CD will qualify for the limited prosocial emotions (CU) specifier (Kahn, Frick, Youngstrom, & Kogos Youngstrom, 2012).

The recent inclusion of CU traits in the DSM-5 is not surprising given the usefulness of these traits in delineating youth at the highest risk for severe conduct problems (Frick, 2012; Frick et al., 2014). Specifically, CU traits appear to be associated with proactive aggression (Centifanti, Fanti, Thompson, Demetriou, & Anastassiou-Hadjicharalambous, 2015; Thornton, Crapanzano, & Terranova, 2013), violent crime (Kruh, Frick, & Clements, 2005; Vitacco & Vincent, 2006) and antisocial personality disorder (ASPD) in adulthood (McMahon, Witkiewitz, & Kotler, 2010). The ability of CU traits to classify youth into those at highest risk for future externalizing behavioral disorders has established CU traits as a construct of specific interest to developmental researchers, particularly those interested in the sequelae associated with adult psychopathy.

CU traits are frequently associated with several core deficits: (i) an impaired ability to recognize the emotional expressions (particularly the distress cues of fear and sadness) of others (Dawel, O'Kearney, McKone, & Palermo, 2012; Marsh & Blair, 2008; Wilson, Juodis, & Porter, 2011); (ii) reduced autonomic arousal to emotional stimuli (e.g., Anastassiou-Hadjicharalambous, & Warden, 2008; Blair, 1999; de Wied, van Boxtel, Matthys, & Meeus, 2012; Kimonis et al., 2008); (iii) reduced augmentation of the startle reflex by visual primes (e.g., Fanti, Panayiotou, Lazarou, Michael, & Georgiou, 2016). These deficits have been hypothesized to reflect dysfunction within the amygdala and connected regions and to interfere with social learning and moral development leading to the behavioral manifestations of CU traits (Blair, 1995; Blair 2013). There are also data indicating that CU traits are associated with insensitivity to punishment – either self-report (Fanti, Panayiotou, Lazarou, Michael, & Georgiou, 2016) or task-based (O'Brien & Frick,

1996). However, other data indicate that reinforcement insensitivity is a feature related to conduct problems more generally rather than CU traits specifically (e.g., White et al., 2014).

Despite the fact that the impairments described above are also noted in adults with psychopathy, whether or not the behavioral and emotional manifestations of psychopathy are consistent across age is still a widely debated topic (e.g., Anderson & Kiehl, 2014). Some research suggests that the construct of psychopathy is longitudinally invariant (i.e., symptoms index the same construct) across adolescence and adulthood (Hawes, Mulvey, Schubert, & Pardini, 2014). However, longitudinal invariance is less clear when it comes to the transition from childhood to adolescence, although it has been suggested that callousness is invariant from around age 11 onward (Obradović, Pardini, Long, & Loeber, 2007). For these reasons, a research focus on callousness and unemotionality in childhood is often preferred over the construct of psychopathy.

It is clear that CU traits and their associated trajectories represent socially debilitating emotions and pathology. Unfortunately, the etiology of CU traits is not completely understood (e.g., Viding et al., 2013), thereby hindering effective treatment and prevention efforts.

II. THE DEVELOPMENT OF CALLOUS-UNEMOTIONAL TRAITS

Although the construct of psychopathy is well-established in adults, research in children has only recently grown its own research field (for a review, see Salekin & Frick, 2005). Several reasons for this lag in extant research exist. First, researchers and clinicians are cautious of describing children using a pejorative term (such as

'psychopath') that generally implies a severe and intractable disorder (Salekin & Frick, 2005). This concern has been somewhat alleviated by the now widespread use of 'CU traits' to describe a subset of psychopathic traits in children. Second, it is possible that CU/psychopathic traits may actually represent qualitatively different constructs in childhood and adulthood. This concern over construct invariance was expressed by Anderson and Kiehl (2014) in their review of developmental psychopathy: "… certain perceived psychopathic traits in youth may simply be a consequence of immature behavioral controls, which usually improve with time and guidance" (p. 106). Empirical research suggests that psychopathic traits are longitudinally invariant (i.e., the constructs mean the same thing at different ages) across adolescence and adulthood (Hawes et al. 2014). However, the data is less clear for CU traits across childhood and adolescence. For example, Obradović, Pardini, Long, & Loeber (2007) described a longitudinal invariance study of callousness in which invariance was supported from around age 11 onward, although these invariant developmental periods differed slightly depending on the type of reporter (parent vs. teacher).

Despite the fact that longitudinal invariance has not yet been well-established, additional support for the construct of psychopathy in childhood is demonstrated by the numerous research studies reporting its longitudinal stability. Specifically, the constructs of psychopathy and callous-unemotionality have been widely reported as temporally stable across the periods of childhood to adolescence (Frick, Kimonis, Dandreaux, & Farell, 2003; López-Romero, Romero, & Villar, 2014; Lynam et al., 2009; Obradović et al., 2007) and adolescence to adulthood (Blonigan, Hicks, Krueger, Patrick, & Iacono, 2006; Forsman, Lichtenstein, Andershed, & Larsson, 2008; Loney, Taylor, Butler, &

Iacono, 2007; Lynam, Caspi, Moffitt, Loeber, & Stouthamer-Loeber, 2007). However, despite the predominance of evidence for temporal stability, some studies do demonstrate individual change over time. Specifically, in one study of 3-7 year olds, approximately 23% of the sample demonstrated increasing or decreasing CU trajectories over time (Klingzell et al., 2016). Furthermore, in a similar study of 7-12 year olds, approximately 27% of the sample had an increasing or decreasing CU trajectory (Fontaine, Rijsdijk, McCrory, & Viding, 2010).

Although research suggests that the emotional characteristics of psychopathy (i.e., callousness) are generally stable over the period spanning from childhood to emerging adulthood, the associated antisocial behavioral characteristics observed in adult psychopathy are known to display great fluctuation during this period. In fact, the long observed, dramatic increase in criminal offenses around age 17, followed by a continuous decrease until around age 30, spawned its own developmental theory of conduct disorder (Moffitt, 1993). Importantly, the research that Moffitt's theory helped generate elucidated the fact that earlier age-of-onset for these behavioral characteristics are indicative of a more severe disorder course and poorer prognosis (e.g., Moffitt, Caspi, Harrngton, & Milne, 2002; Odgers et al., 2007; Silberg, Moore, & Rutter, 2015). Although technically these are distinct constructs, the comorbidity of behavioral and affective characteristics observed in adult psychopathy suggests that earlier age of onset for CU traits may potentially indicate more severe occurrences of the disorder.

The research on the developmental course of psychopathic/CU traits has only just begun to scratch the surface of this complicated topic (Salekin & Frick, 2005). However, given the observed within-individual changes observed during this time period, paired

with the potential negative consequences of earlier age-of-onset, it is clear that the development period spanning childhood to emerging adulthood is extremely important to the development of CU traits.

III. SEX DIFFERENCES IN CALLOUS-UNEMOTIONAL TRAITS

Externalizing psychopathology (including conduct disorder, antisocial behavior, and psychopathy) is almost uniformly found to be more prevalent in males than females (for a review see Hipwell and Loeber, 2006). Furthermore, females tend to have a delayed-onset compared to males for early behavioral problems (Silverthorn & Frick, 1999; Moore, Silberg, Roberson-Nay, & Mezuk, 2017). In terms of CU traits, the prevalence trends for antisocial behavior hold true – females less frequently display high levels of CU traits (e.g., Essau, Sasagawa, & Frick, 2006; Fanti, Demetriou, & Kimonis, 2013). The mean level of CU traits in the general population, as measured by the Inventory of Callous-Unemotional Traits (ICU), has been reported as significantly higher in males than females (27.1 vs. 21.6, respectively; Essau et al., 2006). Furthermore, the CU gender discrepancy is even more striking in individuals with very high levels of CU. Fanti and Colleagues (2013) reported a ratio of approximately 8-10:1 for adolescent boys and girls belonging to a latent class characterized by high levels of CU traits.

Despite the lower female prevalence of antisocial behavior, females clinically referred for these traits often demonstrate more severe symptoms, correlates, and co-morbid psychopathology (for a review see Hickwell & Loeber, 2006). For example, highly aggressive adolescent girls demonstrate significantly higher levels of CU traits than their highly aggressive male counterparts (Stickle, Marini, & Thomas, 2012). Females

diagnosed with CD are also more likely than males with CD to be diagnosed with co-morbid ODD (Loeber, Burke, Lahey, Winters, & Zera, 2000; Hipwell et al., 2011). This greater severity despite lower prevalence has been referred to as the 'gender paradox' of antisocial behavior (Loeber and Keenan, 1994; Keenan, Wroblewski, Hipwell, Loeber, & Southamer-Loeber, 2010), and some authors have used this paradox to hypothesize that females require a greater loading of risk factors before antisocial psychopathology emerges (McClellan, Farabee, & Crouch, 1997; Hicks et al., 2012). One potential mechanism for this paradox is females' possession of higher baseline levels of traits that protect against antisocial behavior (Decety, Yoder, & Lahey, 2015; Freitag et al., 2017) such as increased levels of empathy (Jolliffe and Farrington, 2006; Freitag et al., 2017). Given the relatively recent inclusion of CU traits in diagnostic criteria (American Psychiatric Association, 2013) as well as the all-male samples often used to research psychopathic traits, the gender paradox has not yet been extended to include CU traits. However, given the trends seen for other components of antisociality (Loeber and Keenan, 1994; Keenan et al., 2010), it is entirely possible that females with CU may represent individuals with more severe psychopathology when compared to males who more frequently display high levels of CU traits.

Despite significant sex differences in severity of correlates and symptomatology of antisocial behavior, measures of CU traits appear to index the same construct in males and females. Essau and colleagues (2006) examined the factor structure of the self-report ICU and found the same factor structure in adolescent males and females. Furthermore, the parent-report ICU appears to display measurement invariance across male and female adolescents (Horan, Brown, Jones, & Aber, 2015). Furthermore, a meta-analysis

of 10 studies comprising almost 6,000 individuals revealed that CU traits appear to be a 'marker' for increased severity of antisocial behavior in both males and females (Longman, Hawes, & Kohlhoff, 2016).

IV. GENETIC AND ENVIRONMENTAL INFLUENCES ON CALLOUS-UNEMOTIONAL TRAITS

The observed variation in CU traits can be decomposed into factors representing genetic and environmental influences. Using variations of the classical twin study (Neale & Cardon, 1992), CU traits have been shown to be moderately to highly heritable, with estimates of additive genetic variance ranging from 25-80% of the total trait variance (for a complete review, see chapter 3). The highest of these estimates (63-80%) come from samples drawing upon the extreme end of the phenotypic distribution (e.g., top 10%; Fontaine et al., 2010; Humayun, Kahn, Frick, & Viding, 2014; Larsson, Viding, & Plomin, 2008; Viding, Blair, Moffitt, & Plomin, 2005). Given twin modeling's reliance on the assumption of distributional normality (Neale & Cardon, 1992), the heritability estimates reported in these studies may be upwardly biased (Neale, Eaves, Kendler, & Hewitt, 1989). Therefore, 36-67% may represent a more accurate range of heritability estimates (Moore et al., 2019).

An estimate of heritability reflects the relative importance of genes and environment in a specific sample, and therefore heritability can easily vary with the range of ages considered, highlighting the fact that genes influence behavior in a developmentally dynamic way. One way to examine these developmentally dynamic age-effects is to compare heritability estimates across different ages; however, more

sophisticated statistical techniques are generally used. Blonigan and colleagues (2006) demonstrated one of these techniques when they longitudinally examined the genetic influences on both the stability and change in callous-unemotional traits from data collected at ages 17 and 24. This study reported that 58% of the stable variance across these assessments was due to additive genetic effects. This same technique was used to examine the heritable influence on the stable variance at ages 7 and 12 which was estimated at approximately 89% (Henry et al., 2018a). Another technique used to estimate heritable age-effects uses large cross-sectional samples of a relevant age range to estimate age-moderated effects on heritability (Purcell, 2002). This allows for the estimation of heritability across strata of ages, although this technique has not yet been used to investigate the development of CU/psychopathic traits. Therefore, our current knowledge about the heritable developmental effects for psychopathic traits is limited to the fact that genetic effects appear to influence multiple measurements of psychopathy across various assessments in adolescence and young adulthood, leaving the developmental period of childhood ripe for exploration.

Some studies have also begun to investigate how genetic influences on CU traits vary by sex. These effects may take the form of either quantitative sex effects or qualitative sex effects. Qualitative sex effects indicate that entirely- or partially-distinct sets of genes influence CU traits in males and females. Conversely, quantitative sex effects indicate that while the same set of genes influence CU traits in males and females, the proportion of genetic and environmental effects differs by sex. Although most twin studies are underpowered to detect qualitative sex effects, there is some preliminary evidence that suggests this effect is not present for CU traits (Larsson et al., 2006; Ficks

et al.,2014). However, several studies suggest that quantitative sex effects exist for CU traits. Two separate samples ranging from 7-10 years old report the heritability of CU traits is higher in boys (.64-.67) than girls (.48-.49; Bezdjian, Raine, Baker, & Lynam, 2011; Viding et al., 2007), and there is some evidence to suggest that common environment is a salient factor for girls only, accounting for 20% of the variance in CU traits (Viding et al., 2007). Furthermore, one study of longitudinal CU trajectories over the ages of 9-12 found that the class characterized by stable and high CU traits was influenced primarily by genetic factors in males but primarily by common environment in females (Fontaine et al., 2010). However, these results are not entirely consistent, and the *absence* of quantitative sex effects has also been reported (Larsson et al., 2006; Ficks et al., 2014; Tuvblad, Fanti, Andershed, Collins, & Larsson, 2017). Chapter 3 provides a detailed review of 39 current genetic studies of callous-unemotional traits, both twin and molecular.

Heritability reflects the *proportion* of variance due to genetic factors, and therefore the remaining variance is due to environmental factors. The influence of family, school, and community environments on CU/psychopathic traits has been widely investigated. It is generally accepted that harsh familial environments in early life play a role in the development of CU/psychopathic traits. These environmental factors include abuse/neglect (Kimonis, Fanti, Isoma, & Donoghue, 2013), low maternal sensitivity/warmth (Mills-Koonce et al., 2016; Waller, Shaw, Forbes, & Hyde, 2015), household chaos (Hicks et al., 2012; Kahn, Deater-Deckard, King-Casas, & Kim-Spoon, 2016; Mills-Koonce et al., 2016), and low SES (Mills-Koonce et al., 2016). These factors have been hypothesized to affect the development of brain and HPA-axis functioning

which leads to increased risk for CU/psychopathic traits (Blair, 2013; Daversa, 2010; Gostisha et al., 2014). The peer and social environments also appear to play a role in the development of CU/psychopathic traits, including level of academic achievement/engagement (Hicks et al., 2012) and prosocial vs. antisocial peer affiliation (Burt & Klump, 2014; Hicks et al., 2012). Although community-level factors have not been found to directly influence CU/psychopathic traits, some evidence suggests that these factors interact with biological risk to increase the likelihood of antisocial behavior and psychopathy (Baskin-Sommers et al., 2016; Lei, Simons, Edmond, Simons, & Cutrona, 2014).

V. AN INTRODUCTION TO ENDOPHENOTYPES

Endophenotypes are defined as "measurable components unseen by the unaided eye along the pathway between disease and distal genotype" (Gottesman & Gould, 2003, p. 636). The rationale for the use of endophenotypes in psychiatric research stems from the fact that most psychiatric traits are highly polygenic in nature (Plomin, Haworth, & Davis, 2009; Sullivan, Daly, & O'Donovan, 2014). Furthermore, more genes contributing to a phenotype likely leads to more complex and difficult genetic analysis (Egan & Goldberg, 2003; Leboyer, 2003; Sullivan et al., 2014). A more basic and intermediary phenotype (an endophenotype) is theorized to be influenced by fewer genes and therefore provides "a means for identifying the 'downstream' traits or facets of clinical phenotypes, as well as the 'upstream' consequences of genes" (Gottesman & Gould, 2003, p. 637). Therefore, identification of endophenotypes has the potential to elucidate novel biological disease pathways.

In their seminal paper on psychiatric endophenotypes, Gotteman & Gould (2003) suggested four primary criteria for determining if a putative endophenotype represents a true endophenotype lying on the biological pathway between genotype and psychiatric trait. First, the endophenotype should be associated with the trait of interest (i.e., phenotypic correlation). Second, the endophenotype should be heritable. Third, the endophenotype and trait should co-segregate within families (i.e., genetic covariance). Finally, when interested in a psychiatric illness the putative endophenotype should be state-independent manifesting even when the illness is not active. An additional criterion for traits with complex patterns of inheritance was also included, suggesting that the endophenotype should be seen in non-affected family members at rates higher than the general population.

The classical twin study is one potential avenue for determining if a putative endophenotype meets the above criteria. That is, a twin study can examine phenotypic correlations, heritability of the trait and endophenotype, as well as genetic covariance *between* the trait and endophenotype. This may improve the ability of researchers to determine causal mechanisms underlying mental illness, especially for complex traits where other lines of genetic research have been unsuccessful.

The concept of endophenotypes is not without its limitations. The usefulness of endophenotypes in genetic research stems from the assumption that the genetic architecture of an endophenotype is simpler than a psychiatric trait or diagnosis. However, researchers have pointed out that this may not be the case for most endophenotypes. In a meta-analysis of genetic effect sizes for the COMT Val158Met polymorphism (*rs4680*), Flint and Munafò (2006) found that the effect sizes for

endophenotypes were no larger than the effect sizes for other phenotypes. In a review of 17 psychophysiological endophenotype studies, Iacono and colleagues (2014) reached similar conclusions. That is, psychophysiological endophenotypes such as electrodermal activity and EEG p3 amplitude do not have simpler genetic architecture than clinical phenotypes. However, the authors also state that after genetic markers are discovered for a clinical phenotype, endophenotypes may be useful for uncovering physiologic and neural mechanisms important for understanding disease pathology (Iacono, Vaidyanathan, Vrieze, & Malone, 2014).

Despite these limitations, the National Institute of Mental Health has emphasized the need for research on endophenotypes by way of their Research Domain Criteria (RDoC) initiative. In an effort to identify phenotypes that more closely align with the mechanisms underlying psychopathology, the NIMH launched the RDoC project to "better understand basic dimensions of functioning underlying the full range of human behavior" (National Institute of Mental Health, 2015). RDoC serves as a framework for new approaches to psychopathological research using fundamental dimensions that cut across traditional disorder categories and putatively reflect endophenotypes (Miller & Rockstroh, 2013). The RDoC system is organized in a matrix consisting of 6 overarching domains (negative valence systems, positive valence systems, cognitive systems, social processes, sensorimotor systems, and arousal and regulatory systems) each made up of several smaller constructs. Several endophenotypes have been proposed for CU traits/psychopathy that lie within the acute threat construct in the negative valence domain of the matrix. Specifically of interest to this dissertation are several neuroanatomical circuits and physiological responses mentioned within the acute threat construct.

VI. STARTLE REFLEX AS AN ENDOPHENOTYPE FOR CALLOUS-UNEMOTIONAL TRAITS

The eyeblink startle reflex (SR) is a physiological reaction to a sudden or unexpected stimulus that elicits the contraction of muscles in the face and neck region. This reaction is thought to protect the vulnerable areas of the body (eyes, neck, etc.) from potential harm (Lang, 1995). The startle reflex is often greater when the individual is in the presence of an aversive, threatening, or frightening situation. This exaggerated startle reflex is termed fear-potentiated startle (FPS). Baseline and fear-potentiated startle have been studied in relation to psychiatric phenotypes associated with both fearfulness (e.g., anxiety; Lake, Baskin-Sommers, Li, Curtin, & Newman, 2011; Lau et al., 2008; Lissek et al., 2005; Vaidyanathan, Patrick, & Cuthbert, 2009) and fearlessness (e.g., psychopathy; Lake et al., 2011; Loomens, Tulen, & van Marle, 2015; Patrick, 1994).

The fearlessness theory states that reduced autonomic reactivity in response to aversive stimuli results in a fearless phenotype (Raine, 1993; Raine, Venables, & Mednick, 1997). While fearlessness can be protective against internalizing disorders (Ross, Benning, Patrick, Thompson, & Thurston, 2009), high levels of fearlessness are risk factor for antisocial behavior, including psychopathic/CU traits (Frick & Morris, 2004; Frick & Viding, 2009). In the seminal text on psychopathy, Hervey Cleckley (1941) describes psychopaths as lacking emotional responses to fearful stimuli. Descriptions of underlying fear deficits have continued to pervade modern descriptions of the disorder (Fowles, 1980; Hare, 1965). It is, therefore, not surprising that SR and FPS have emerged as significant areas of inquiry when examining the underlying etiology of psychopathic or CU traits.

In addition to anecdotal accounts of decreased fear responding, research has demonstrated that individuals high on psychopathic traits have an overall lower SR than non-psychopathic individuals. In a study comparing 24 controls to 25 psychopathic individuals from high-security forensic treatment facilities, Herpertz and colleagues (2001) noted that a higher percentage of psychopathic individuals showed an absence of the startle reflex as defined by electrodermal activity and EMG corrugator muscle activity. It has also been demonstrated that criminal psychopaths display lower overall EMG eyeblink SR compared to healthy individuals (Rothemund et al., 2012). An additional study of adults with ASPD and psychopathic traits did not demonstrate a unique effect of psychopathy on SR but did determine that individuals with ASPD and/or psychopathy showed blunted SR compared to healthy individuals (Loomans, Tulen, & van Marle, 2015).

Associations between psychopathic traits and overall SR have also been replicated in juvenile samples. In a study of 40 juvenile offenders and 52 control subjects aged 12-18 years, psychopathic juvenile offenders showed lower overall SR than their non-psychopathic counterparts (Syngelaki, Fairchild, Moore, Savage, & van Goozen, 2013). Although no studies have yet shown direct associations between CU traits and overall SR, Dackis, Rogosch, & Cicchetti (2015) recently studied 132 children ages 8-12 years and found that overall SR was blunted in children who displayed CU traits and no history of maltreatment compared to a higher startle response in children with CU and history of maltreatment. This distinction between maltreatment subtypes is paramount to the ‘primary’ vs ‘secondary’ psychopathy distinction where the primary variant is thought to be more biologically based and the ‘secondary’ variant is thought to be environmentally

mediated, potentially acting as a coping mechanism for traumatic experiences (e.g., Kimonis, Frick, Cauffman, Goldweber, & Skeem, 2012).

FPS has been examined in psychopaths using two types of measures. The first type measures relative EMG startle reflex in the presence of negative, neutral, and positively valenced sets of images, often referred to as "affect modulated startle" (AMS). The second type uses classical conditioning to condition participants to fear an otherwise neutral stimulus (NS) and measures the EMG startle reflex in the presence of the conditioned stimulus (CS+) vs. an unconditioned stimulus (NS or CS-), referred to as "fear-conditioned startle" (FCS). Patrick, Bradley, & Lang (1993) were the first to demonstrate an aberrant AMS in criminal psychopaths. In this sample of 54 incarcerated sexual offenders, non-psychopaths had an increasing EMG response to positive, neutral, and negatively valenced images (a linear pattern), whereas psychopaths had lower EMG responses to both positive *and* negatively valenced images compared to neutral images (a quadratic pattern). They also found that PCL-R factor 1 scores (related to emotional detachment) were the strongest predictor of this AMS pattern. The quadratic trend for psychopathic individuals, as well as the relationship between emotional detachment and decreased AMS was replicated in an independent sample of 36 incarcerated individuals (Levenston, Patrick, Bradley, & Lang, 2000). A more recent study extended the relationship between psychopathic traits and decreased AMS in an undergraduate sample indicating that the boldness facet of psychopathy, akin to unemotional traits, was uniquely associated with decreased AMS (Esteller, Poy, & Moltó, 2006). Decreased FCS has also been demonstrated in criminals with ASPD and psychopathic traits (Loomans et al., 2015; Rothemund et al., 2012) with one study noting that EMG amplitude revealed a

lack of differentiation between the CS+ and CS- in the psychopathic group (indicative of impaired fear-learning; Rothemund et al., 2012).

The impaired AMS observed in psychopathic adults has also been replicated in youth with high levels of CU traits. Although one study indicated a non-significant relationship between AMS and CU traits (Dackis et al., 2015), most studies report a significant negative relationship between CU traits and EMG response to negatively valenced images indexing fear (Fanti, Panayiotou, Lazarou, Michael, & Georgiou, 2016), violence/victimization (Fanti et al., 2017; Kimonis, Fanti, Goulter, & Hall, 2017; Kyranides, Fanti, Sikki, & Patrick, 2017), and threat (Kimonis et al., 2017). However, no studies have yet investigated FPS and CU traits using a fear-conditioning paradigm.

In addition to phenotypic association, a putative endophenotype must also be a heritable trait. The heritability of SR has been well established and is estimated to lie between 34-70% (Anokhin, Golosheykin, & Heath, 2007; Anokhin, Heath, Myers, Ralano, & Wood, 2003; Dahmija, Tuvblad, Dawson, Raine, & Baker, 2017; Savage et al., 2019; Vaidyanathan, Malone, Miller, McGue, & Iacono, 2014). The lowest of these heritability estimates, 34%, comes from the only study to use an air puff startle probe (Savage et al., 2019), whereas auditory startle probes appear to provide somewhat higher heritability estimates in the range of 49-70% (Anokhin et al., 2003; Anokhin et al., 2007; Dahmija et al., 2017; Vaidyanathan et al., 2014). Only one of these studies reported sex differences in heritability with SR variance in 14-15 year old twins accounted for primarily by genetics (49%) in males but common environment (53%) in females (Dahmija et al., 2017).

Only one study has investigated the heritability of FCS and was not able to identify unique genetic influences after accounting for those that influence baseline SR (Savage

et al., 2019). That is, in a multivariate heritability model a single genetic factor was the primary source of genetic variance for all aspects of the startle task including SR and FPS. This indistinguishable genetic effect is not unique to FPS; the genetic variance of AMS also appears to be indistinguishable from baseline SR in multiple studies (Anokhin et al., 2007; Dhamija et al., 2017; Vaidyanathan et al., 2014). Although some of these studies report point estimates of around .1 for the unique genetic influences on FPS/AMS they are likely underpowered to detect these small effect sizes (Savage et al., 2019; Vaidyanathan et al., 2014).

In terms of meeting endophenotypic criteria, a putative endophenotype must first be associated with the trait of interest. FPS clearly meets this criterion. Although SR and CU have not been explicitly associated, evidence for the relationship between psychopathy and SR is well established and hints at a plausible relationship between SR and CU traits. In terms of the second criterion, SR but not FPS meets the requirement that a putative endophenotype must be heritable. However, research into the co-segregation within families (i.e., shared genetic etiology; third endophenotypic criterion) has not yet been examined. Currently, no studies have investigated the common genetic underpinnings of CU traits and EMG startle reflex, leaving a significant gap in the literature that must be filled before SR or FPS can be considered an endophenotype for psychopathy or CU traits.

VII. BRAIN MORPHOMETRY AS AN ENDOPHENOTYPE FOR CALLOUS-UNEMOTIONAL TRAITS

Research into the brain-related changes associated with psychopathic and CU traits reveals the importance of both paralimbic and limbic structures, sometimes referred to as “the paralimbic system” (Kiehl, 2006). Specifically, this system includes the orbitofrontal cortex (OFC), insula, anterior cingulate cortex (aCC), posterior cingulate cortex (pCC), amygdala, parahippocampal gyrus, and superior temporal gyrus. Within this system, volumetric measures for several regions have been associated with psychopathy, including the aCC (Rijsdijk et al., 2010), pCC (Ermer, Cope, Nyalaanti, Calhoun, & Kiehl, 2013; Rijsdijk et al., 2010), amygdala (Yang, Raine, Narr, Colletti, & Toga, 2009), and OFC (Ermer et al., 2013; Fairchild et al., 2013). Regions within the amygdala, aCC, and OFC are included within the ‘circuits’ units of analysis for the acute threat construct in the RDoC matrix, highlighting the importance of these regions in explaining the fear deficits observed in psychopathy/CU.

The amygdala is an almond-shaped subcortical structure within the temporal lobe of the brain (Swanson and Petrovich, 1998). The amygdala appears to play a major role in emotion and affect, particularly fear (Davis and Whalen, 2001; Feinstein, Adolphs, Damasio, & Tranel, 2011). Specifically, the amygdala plays a role in fear conditioning and fear recognition, both traits that are deficient in individuals with high levels of CU/psychopathic traits (Blair, 1995; Feinstein et al., 2011; Kiehl, 2006). In a study of 27 highly psychopathic individuals and 32 matched controls, Yang and colleagues (2009) found significant reductions (17-18%) in bilateral amygdala volume in the psychopathic groups. When examining individual psychopathy facets, the affective dimension of

psychopathy (the dimension closest to CU traits) emerged as one of the strongest predictors of reduced amygdala volume.

The OFC is a region of the frontal lobe located just above the ocular orbits (Conn, 2016). This region is involved in affect, decision-making, and stimulus-reinforcer learning (Rolls and Grabenhorst, 2008). The OFC has been proposed to play a key role in the emotional processing relevant to fear and emotion-based learning (Bechara, Damasio, & Damasio, 2000) that is often associated with psychopathic traits. However, volumetric analyses of the OFC and psychopathic/CU traits have been mixed. In one study of 22 female adolescents (14-20 years old) with CD and 21 matched controls CU traits were positively correlated with bilateral OFC volume even after controlling for CD (Fairchild et al., 2013). Conversely, in a study of 191 male adolescents (mean age = 17.3 years) from a youth detention facility, psychopathy scores were negatively associated with grey matter volume in the OFC. However, no significantly associated brain regions emerged in the whole-brain analysis after correction for multiple testing (Ermer et al., 2013).

The cingulate cortex is a large, multifaceted region in the medial portion of each cerebral hemisphere adjacent to the corpus callosum (Stevens et al., 2011). It plays a role in emotion, empathy, and social cognition (Phan, Wagner, Taylor, & Liberzon, 2002; Hadland, Rushworth, Gaffan, & Passingham, 2003). The aCC plays specific roles in a range of social and emotional behaviors including processing emotional imagery (Phan et al., 2002), affect regulation, pain perception (Bush, Luu, & Posner, 2000), and maternal behavior (Vogt, Finch, & Olson, 1992). The pCC plays specific roles in evaluating sensory input and monitoring behavior (Vogt et al., 1992). In a study of 191 male adolescents (mean age = 17.3 years) from a youth detention facility, psychopathy scores were

negatively associated with grey matter volume in the pCC. However, as stated above, no significantly associated brain regions emerged in the whole-brain analysis after correction for multiple testing (Ermer et al., 2013). However, in a study of 125 males aged 10-13 psychopathic traits were positively associated with grey matter concentration in the left pCC and right dorsal aCC (Rijsdijk et al., 2010).

Twin-based structural imaging studies reveal that brain structure is highly heritable with individual regions displaying heritable influences on the order of 60-80% (for a review see Jansen, Mous, White, Posthuma, & Polderman, 2015). However, only one study has thus far investigated the shared genetic etiology of neuroanatomy and psychopathy (Rijsdijk et al., 2010). This study examined grey matter concentrations in 23 brain regions and their genetic relationship to psychopathy in a sample of 61 male twin pairs aged 10-13 years old. They found two regions that fulfilled the third criterion for putative endophenotypes (i.e., shared genetic etiology), with both the left pCC and right dorsal aCC showing moderate overlap of genetic influences common to psychopathy (r_G=.42 & .37, respectively). However, the genetic models in this study included multiple parameters that were fixed at estimates taken from the extant literature and were, therefore, not derived from the actual study data.

In terms of meeting endophenotypic criteria, a putative endophenotype must first be associated with the trait of interest. Several neuroanatomical regions (OFC, amygdala, pCC, & aCC) clearly meet this criterion. In terms of the second criterion, brain morphometry demonstrates high heritability, meeting the requirement that a putative endophenotype be heritable. However, research into the co-segregation within families (i.e., shared genetic etiology; third endophenotypic criterion) has only just begun.

Currently, only one study has investigated the common genetic underpinnings of neuroanatomy and psychopathy. However, additional research is needed to replicate these results and extend the findings in a mixed-sex sample investigating CU traits specifically.

CHAPTER 2. SAMPLE, PARTICIPANTS, AND MEASURES[3]

I. SAMPLES

The Juvenile Anxiety Study (JAS)

The Juvenile Anxiety Study (JAS) is a genetically informed twin sample of 398 non-Hispanic Caucasian twin pairs (*N* = 796) aged 9-14. JAS was designed to assess internalizing psychopathology and related negative valence system constructs. As part of the larger study, researchers obtained various data, including self-report, parent-report, psychophysiological, biological, and laboratory-based tasks. However, only the specific measures listed below were used in the current dissertation. Only Caucasian twins were recruited to reduce genetic heterogeneity for the molecular aims of the overall JAS study.

[3] Some passages from this chapter are taken verbatim from the author's previous work, including: (a) Moore, A. A., Blair, R. J., Hettema, J. M., & Roberson-Nay, R. (2019). The genetic underpinnings of callous-unemotional traits: A systematic research review. *Neuroscience and Biobehavioral Reviews, 100*, 85-97. (b) Moore, A. A., Carney, D., Moroney, E., Machlin, L., Towbin, K. A., Brotman, M. A., . . . Hettema, J. M. (2017). The inventory of callous-unemotional traits (ICU) in children: Reliability and heritability. *Behavior Genetics, 47*, 141-151. (c) Moore, A. A., Rappaport, L. M., Blair, R. J., Pine, D. S., Leibenluft, E., Brotman, M. A., . . . Roberson-Nay, R. (In press). Genetic underpinnings of callous-unemotional traits and emotion recognition in children, adolescents, and emerging adults. *The Journal of Child Psychology and Psychiatry.*

Participants were recruited through the Mid-Atlantic Twin Registry (Lilley & Silberg, 2013) and were drawn from the Mid-Atlantic region of the United States. Data was collected at one of two sites located in Washington, D.C., and Richmond, Virginia. The Institutional Review Board of Virginia Commonwealth University approved the study, and all participants provided informed consent (parents) and assent (children) before participating. Twins and parents were monetarily compensated for their participation in the study.

Participants were excluded from the studies if they were intellectually disabled, had an autism spectrum disorder diagnosis, had experienced a psychotic episode, were currently using anxiolytic or antidepressant medication, or had been diagnosed with any medical condition which might have adversely impacted participants' safety or ability to complete the study, including aspects of the study not described here (for detailed description of study procedures see Carney et al., 2016).

A sub-sample of JAS was recruited post-hoc to participate in an additional MRI protocol. This sample consisted of *N* = 109 participants (*N* = 43 twin pairs and *N* = 17 singletons). In addition to the exclusionary criteria for the larger study, participants chosen for this MRI protocol needed to meet two additional criteria: 1) due to safety protocols associated with the MRI scanner, participants could not have metal braces or any other metal objects present in their body; 2) since unnecessary motion inside the scanner results in poor quality images, participants were excluded if the JAS research staff noticed a high level of fidgeting, twitching, or restlessness during the regular JAS tasks.

The Adolescent and Young Adult Twin Study (AYATS)

The Adolescent and Young Adult Twin Study (AYATS) is a genetically informed twin sample consisting of 430 non-Hispanic Caucasian, Hispanic, and African American twin pairs (*N* = 860) aged 15-20 years. AYATS was designed to overlap with elements of JAS and assesses internalizing psychopathology and related negative valence systems. As part of the larger study, various data was obtained, including self-report, parent-report, psychophysiological, biological, and laboratory-based tasks. However, only the specific measures listed below were used in the current dissertation.

Participants were recruited through the Mid-Atlantic Twin Registry (Lilley & Silberg, 2013), and were drawn from the Mid-Atlantic region of the United States. Data was collected at one site located in Richmond, Virginia. The Institutional Review Board of Virginia Commonwealth University approved the study, and all participants provided informed consent (parents and adult twins) or assent (children) before participating. Twins and parents were monetarily compensated for their participation in the study.

Participants were excluded from the studies if they were intellectually disabled, had an autism spectrum disorder diagnosis, had experienced a psychotic episode, were currently using anxiolytic or antidepressant medication, or had been diagnosed with any medical condition which might have adversely impacted participants' safety or ability to complete the study, including aspects of the study not described here (for detailed description of study procedures see Cecilione et al., 2018).

II. PARTICIPANTS

The combined JAS/AYATS sample consists of 828 twin pairs (*N* = 1,656) between the ages of 9-20. Only those twins with ICU data were included in the current dissertation, resulting in an analytic sample size of *N* = 1,448. The sample included *N* = 109 monozygotic male-male (MZM) twin pairs, *N* = 156 monozygotic female-female (MZF) twin pairs, *N* = 115 dizygotic male-male (DZM) twin pairs, *N* = 137 dizygotic female-female (DZF) twin pairs, *N* = 202 dizygotic opposite-sex (DZOS) twin pairs, and *N* = 5 twin pairs of unknown or undetermined zygosity. The sample was comprised of 44.9% male participants and 55.1% female participants. The mean age was 14.1 years (SD = 3.1 years; range = 9.2 - 20.3 years). Participant race was as follows: *N* = 1,363 (94.5%) non-Hispanic White; *N* = 46 (3.2%) Black; *N* = 31 (2.2%) Hispanic; *N* = 3 (0.2%) unknown.

The imaging subsample of JAS consists of *N* = 112 participants. Only those individuals with ICU data were included in the current dissertation, resulting in an analytic sample size of *N* = 109. The analytic imaging sample included *N* = 7 monozygotic male-male (MZM) twin pairs, *N* = 13 monozygotic female-female (MZF) twin pairs, *N* = 7 dizygotic male-male (DZM) twin pairs, *N* = 5 dizygotic female-female (DZF) twin pairs, *N* = 11 dizygotic opposite-sex (DZOS) twin pairs, *N* = 3 twin pairs of unknown or undetermined zygosity, and *N* = 17 singletons. The imaging sample was comprised of 42.2% male participants and 57.8% female participants. The mean age was 11.2 years (SD = 1.3 years; range = 9.2 – 14.2 years). Participant race was *N* = 109 (100%) non-Hispanic White. The subsample of JAS with imaging data did not differ significantly from the full JAS sample on sex (t = 1.3, df = 148.33, p = .19), age (X^2 = 1.43, df = 1, p = .23), or ICU sum score (t = .94, df = 156.71, p = .35).

III. MEASURES

Zygosity

In both JAS and AYATS a parent or legal-guardian answered a set of standard questions used to assess the physical similarities of their twins (Nichols and Bilbro, 1966; Peeters et al., 1998), and these were used to determine zygosity classification (monozygotic [MZ] or dizygotic [DZ]). For a subset of twin pairs in AYATS (N = 82 twin pairs), zygosity was verified using an assay of single nucleotide polymorphisms, and this molecular-derived zygosity metric was highly concordant with the self-report algorithm-assigned zygosity (κ = 0.95). For a subset of JAS (N = 42 twin pairs), the self-report algorithm-assigned zygosity agreed highly with zygosity from placental/DNA testing reported by parents (κ = 1.00).

The Inventory of Callous-Unemotional Traits (ICU)

For each twin, a parent or legal-guardian completed the parent-report Inventory of Callous-Unemotional Traits (Frick, 2004), a 24-item measure assessing traits relating to callousness, carelessness, and emotionless. Table 2.1 lists ICU item questions and wording. Parents ranked each item on a 4-point Likert scale, from 0 (not at all true) to 3 (definitely true). Scores were summed across all items to create single continuous ICU variable for each participant, with a potential range of 0-72. All ICU items load onto a single factor under substantial genetic control (Henry, Pingault, Boivin, Rijsdijk, & Viding, 2016). Therefore, ICU sum scores are valid constructs for measuring the underlying genetic structure of CU traits. The ICU has demonstrated good test-retest reliability, internal consistency and convergent validity (e.g., Kimonis et al., 2008; Feilhauer, Cima, & Arntz, 2012; Moore et al., 2017). The mean ICU score for the entire sample was 19.3

(SD = 8.8; range = 0 – 55). The mean ICU score in the imaging subsample of JAS was 16.7 (SD = 7.1; range = 1 - 36). The distribution of ICU scores in the entire sample is displayed in Figure 2.1.

Table 2.1. Items from the parent-report version of the Inventory of Callous-Unemotional Traits (ICU)

Item #	Item Wording
1	Expresses his/her feelings openly.*
2	Does not seem to know “right” from “wrong”.
3	Is concerned about schoolwork.*
4	Does not care who he/she hurts to get what he/she wants.
5	Feels bad or guilty when he/she has done something wrong.*
6	Does not show emotions.
7	Does not care about being on time.
8	Is concerned about the feelings of others.*
9	Does not care if he/she is in trouble.
10	Does not let feelings control him/her.
11	Does not care about doing things well.
12	Seems very cold and uncaring.
13	Easily admits to being wrong.*
14	It is easy to tell how he/she is feeling.*
15	Always tries his/her best.*
16	Apologizes (“says he/she is sorry”) to persons he/she has hurt.*
17	Tries not to hurt others’ feelings.*
18	Shows no remorse when he/she has done something wrong.
19	Is very expressive and emotional.*
20	Does not like to put the time into doing things well.
21	The feelings of others are unimportant to him/her.
22	Hides his/her feelings from others.
23	Works hard on everything.*
24	Does things to make others feel good.*

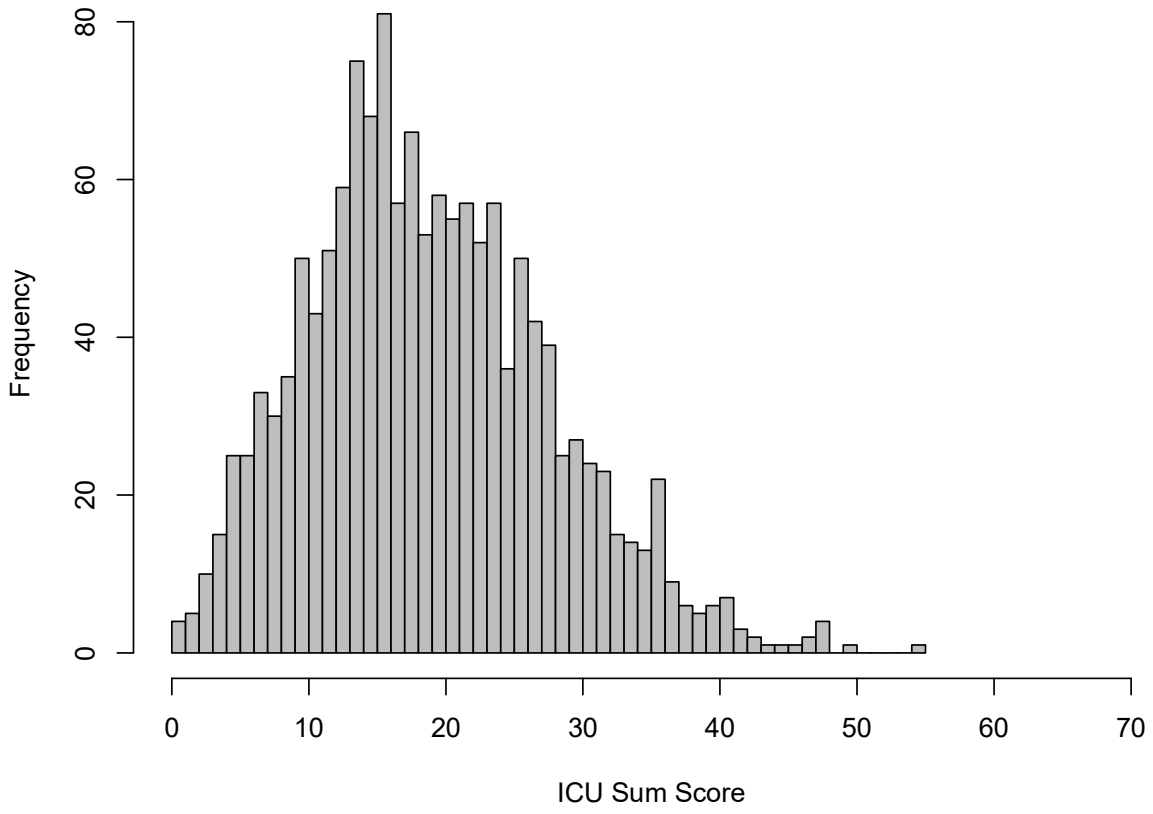
Note: * indicates item is reverse coded

Electromyographic Startle Reflex

“The screaming lady” paradigm (JAS). In the JAS study, baseline startle and fear-potentiated startle were assessed with the classical fear-conditioning paradigm developed by Dr. Daniel Pine and colleagues at the National Institute of Mental Health

(i.e., “The Screaming Lady”; for a full description see Lau et al., 2008). A mechanical air puff (40 ms, 3 psi of compressed room air) directed at the center of participants’ foreheads served as the startle probe. This air puff was administered through a polyethylene tube affixed 1cm from the skin by way of a headpiece worn by participants. An aversive, loud (90 dB, 500 ms) scream served as the unconditioned stimulus (UCS) and was paired with one of two distinct photographs of female faces (neutral stimuli [NS]). Twins were counterbalanced and conditioned to one of the two women, resulting in one of the two faces serving as the conditioned stimulus (CS+).

Figure 2.1. Histogram of ICU Sum Score in Combined JAS/AYATS Sample



During the task participants sat in a comfortable chair facing a computer screen on which images were presented. Participants wore in-ear headphones for UCS delivery. After a 2-minute acclimation period participants underwent the paradigm consisting of three phases: 1) habituation (a.k.a. “pre-acquisition”), 2) fear acquisition, and 3) fear extinction. Only data from the habituation and fear acquisition phases of the study are used in the current analyses. During the habituation phase, 12 startle probes were presented without the UCS. Four were paired with a blank screen during the intertrial interval (ITI) and four were paired with images of each of two women with neutral facial expressions. During the fear acquisition phase 30 startle probes were paired with the ITI and the neutral images of the two women (10 presentations of each). One face (CS+) was paired with the USC on 8 of the 10 presentations. During the CS+ presentations the UCS occurred immediately after the presentation of the neutral image, which was then morphed into an image of the same face displaying a fearful expression. The second face (CS-) was never paired with the UCS.

Throughout the paradigm images were presented on-screen for a total of 8 seconds. Startle probes were administered 5 to 6 seconds after image onset. ITI periods were variable, lasting approximately 30 seconds. Startle probes during the ITI were administered 15 to 20 seconds into the ITI. Four randomization schedules determined the CS+/CS- designation and startle probe presentation order. These schedules were the same within twin pairs but counterbalanced across twin pairs.

Data recording. Startle probes were automatically administered using E-Prime software (Psychology Software Tools, 2016). Data were recorded with a BIOPAC MP150 system and AcqKnowledge software (BIOPAC Systems Inc., Goleta, CA). The unfiltered

EMG channel was acquired and sampled at a rate of 1,000 Hz. Before recording, participant's skin was prepared with an exfoliant (NuPrep, Weaver and Company, Aurora, CO) to ensure that impedance levels were not higher than 20 kOhms. Electromyographic (EMG) response of the orbicularis oculi muscle (located under the left eye) was measured after each startle probe to determine the magnitude of the eyeblink startle reflex. EMG activity was recorded via two reusable 4mm Ag/AgCl electrodes affixed 1cm apart below the participants left eye. Each electrode was filled with high-conductivity electrode gel and attached with trimmed, double-sided adhesive collars. A ground electrode was placed in the center of the participant's forearm.

Data cleaning and variable construction. Data files were manually inspected and a total of $N = 57$ files (8.4%) were removed due to bad signal and/or recording problems. The remaining files were processed by applying a finite impulse response (FIR) band-pass filter (28 Hz – 500 Hz). AcqKnowledge's Derive Average Rectified EMG procedure was used with a 20-ms moving window to obtain average EMG values.

Startle probes were identified using the stimuli channel input and EMG startle response to each probe was calculated by subtracting the average EMG value in the 50 ms pre-probe window from the maximum value in the 20 - 150 ms post-probe window. Individual startle probes were considered to be indistinguishable from baseline noise and removed from the file if the standard deviation of the pre-probe baseline was more than three times the standard deviation of all pre-probe baselines for that individual. Individual startle probes were also removed if there was a non-response trial, defined as a post-probe magnitude less than 1 standard deviation above the pre-probe magnitude.

Participants files were excluded from analysis if they quit the task prior to the fear acquisition phase, if more than 20% of their individual trials were removed during data cleaning procedures listed above, or if their mean startle reflex was more than 3 standard deviations above the average reflex for all participants. These data cleaning procedures resulted in removal of *N* = 49 participants (7.3%).

Two variables were computed for the current analyses. First, baseline startle reflex (SR) was defined as the average raw EMG response to all startle probes in the habituation phase before the UCS was presented. Second, fear-potentiated startle (FPS) was defined as the average raw EMG response to all CS+ startle probes in the fear acquisition phase. T-scores and/or differential scores were not used in the current analyses due to evidence that transformed startle metrics produced more biased heritability estimates than raw metrics. (Savage et al., 2019).

Fear generalization paradigm (AYATS). In the AYATS study, baseline startle and fear-potentiated startle were assessed with the fear conditioning and generalization paradigm developed by Dr. Christian Grillon and colleagues at the National Institute of Mental Health (for a full description see Lissek et al., 2008). An aversive burst of white noise (50 ms, 102 dB with near-instantaneous rise time) served as the startle probe. The white noise burst was administered biaurally through a set of headphones worn by participants. A 100 ms electrical shock delivered to the left wrist served as the unconditioned stimulus (UCS). The UCS ranged from 3 – 5 mA depending on the shock level that each participant rated 'highly uncomfortable but not painful.' The UCS was paired with pictures of rings ("O" shapes) of various sizes. Either the largest ring (with a diameter of approximately 4.7 inches) or the smallest ring (with a diameter of

approximately 2.6 inches) served as the CS+, with the other serving as the CS-. Inter-trial intervals (ITIs) and presentations of a “V” shape were also included to determine whether responses to the “O” shapes were specific responses to that shape or general responses to any on-screen presentation.

During the task participants sat in a comfortable chair facing a computer screen on which images were presented. Participants wore headphones for startle probe delivery. The paradigm consisted of three phases: 1) pre-acquisition (a.k.a. “habituation”), 2) fear acquisition, and 3) fear generalization. Only data from the habituation and fear acquisition phases of the study are used in the current analyses. During the habituation phase, there were 16 total presentations, 4 each of the CS-, CS+, “V,” and ITI. During the fear acquisition phase there were 48 total presentations, 12 each of the CS-, CS+, “V,” and ITI. One ring (CS+) was paired with the UCS on 8 of the 12 presentations (66.6% reinforcement rate). The second ring (CS-) was never paired with the UCS. Startle probes accompanied 50% of the presentations and on the remaining 50% of presentations participants were asked to rate the perceived likelihood of shock. Only startle probe presentations are used for the analyses in this dissertation.

Throughout the paradigm images were presented on-screen for a total of 8 seconds. Startle probes were administered 4 to 5 seconds after image onset. ITIs lasted 16 seconds. Four randomization schedules determined the CS+/CS- designation and startle probe presentation order. These schedules were the same within twin pairs but counterbalanced across twin pairs.

Data recording. Startle probes were automatically administered and data were recording using PSYCHLAB (Contact Precision Instruments, Cambridge, MA). The

unfiltered EMG channel was acquired and sampled at a rate of 1,000 Hz. Before recording, participant's skin was prepared with an exfoliant (NuPrep, Weaver and Company, Aurora, CO) to ensure that impedance levels were ≤ 10 kOhms. Electromyographic (EMG) response of the orbicularis oculi muscle (located under the left eye) was measured after each startle probe to determine the magnitude of the eyeblink startle reflex. EMG activity was recorded via two reusable 6mm Sn electrodes affixed below the participants left eye. Each electrode was filled with standard electrolyte gel (SignaGel, MFI Medical, San Diego, CA) and attached with trimmed, double-sided adhesive collars. A ground electrode was placed in the center of the participant's forearm.

Data cleaning and variable construction. *N* = 851 data files were manually inspected and a total of *N* = 43 files (5.1%) were removed due to bad signal and/or recording problems. The remaining files were processed by applying a finite impulse response (FIR) band-pass filter (30 Hz – 500 Hz). Startle EMG was rectified and smoothed using a 20-ms moving window.

Startle probes were identified using the stimuli channel input and EMG startle response to each probe was calculated by subtracting the average EMG value in the 50 ms pre-probe window from the maximum value in the 0 - 120 ms post-probe window. Individual startle probes were considered to be indistinguishable from baseline noise if there was a difference of 0 between the pre- and post-probe EMG magnitude. Participants' files were excluded from analysis if they were identified as a non-responder (defined as 100% of fear acquisition trials with value of '0') resulting in the removal of *N* = 53 participants (6.2%).

Two variables were computed for the current analyses. First, baseline startle reflex (SR) was defined as the average raw EMG response to all startle probes in the habituation phase before the UCS was presented. Second, fear-potentiated startle (FPS) was defined as the average raw EMG response to all CS+ startle probes in the fear acquisition phase. For both computed variables, values greater or less than 3 standard deviations from the sample mean were removed from the data. T-scores and/or differential scores were not used in the current analyses due to evidence that transformed startle metrics produced more biased heritability estimates than raw metrics. (Savage et al., 2019).

Brain Morphometry

Neuroanatomical data were collected at two sites: the Functional Magnetic Resonance Imaging Facility (FMRIF) at the National Institute of Mental Health (NIMH) in Bethesda, MD and the Collaborative Advanced Research Imaging (CARI) center at Virginia Commonwealth University (VCU) in Richmond, VA. Extensive time was dedicated to preparing each participant for the MRI procedure via mock-scans and education about head movement.

VCU image acquisition protocol. Scanning was performed in a Philips Ingenia 3.0T scanner with a 32-channel head coil. T1-weighted images were acquired using magnetization-prepared rapid acquisition gradient-echo (MPRAGE) sampling with the following parameters: flip angle = 6°; field of view (FOV) = 24 cm; slices = 160; slice thickness = 1mm; 240 x 240 matrix; repetition time (TR) = 8.1ms; echo time (TE) = 3.7ms).

NIH image acquisition protocol. Images were obtained with a General Electric 3.0T scanner with an 8-channel head coil. T1-weighted images were acquired using MPRAGE sampling with the following parameters: flip angle = 7°; FOV = 25.6 cm; slices

= 176; slice thickness = 1mm; 256 x 256 matrix; TR = 7.7ms; TE = 3.4ms.

Image processing. T1-weighted images were processed using the Freesurfer image analysis software suite, version 6.0. Freesurfer and its associated processing routines, with full documentation, are available for download at http://surfer.nmr.mgh.harvard.edu. Detailed information about Freesurfer's automated routines have been published elsewhere. Briefly, the automated pre-processing pipeline consisted of motion correction (Reuter, Rosas, & Fischl, 2010), intensity normalization (Sled, Zijdenbos, & Evans, 1998), removal of non-brain tissue (e.g., skull; Segonne et al., 2004), and Talairach transformation (Dale, Fischl, & Sereno, 1999). Intensity gradients were used to automatically place boundaries ("surfaces") at optimal locations between the gray/white matter ("white matter surface") and between the gray matter and cerebrospinal fluid ("pial surface"; Dale et al., 1999; Dale and Sereno, 1993; Fischl and Dale, 2000). Subcortical white and gray matter was automatically segmented into volumetric subcortical structures and labeled (Fischl et al., 2002; Fischl et al., 2004a), and the Desikan-Killiany probabilistic atlas was used to automatically parcellate and label cortical regions (Desikan et al., 2006).

The author (AAM) and one additional graduate student (CKS), both with previous training in neuroanatomy, performed visual image processing. Both individuals also underwent extensive training in structural imaging processing via Freesurfer at the NIH for the specific purpose of processing JAS neuroimaging data. Visual processing of individual files included manual inspection of all images to ensure that Freesurfer's automated processes had correctly labeled all cortical and subcortical regions and that pial and white matter surface maps were correctly placed. When required, voxels were

added/deleted and/or processing parameters were changed to ensure that the automatic segmentation and parcellation were maximally accurate.

CHAPTER 3. THE GENETIC UNDERPINNINGS OF CALLOUS-UNEMOTIONAL TRAITS: A SYSTEMATIC RESEARCH REVIEW [4]

I. SPECIFIC AIM

Given the severity of the adult outcomes associated with high levels of CU traits, discovering the etiological contributions to these traits is of utmost importance to better inform clinical and translational work in the fields of intervention and prevention. Although specific *causal* mechanisms for CU traits have not yet been elucidated, several researchers have proposed precise neurohormonal models of CU etiology, specifically as they relate to oxytocin, serotonin, and amygdala dysfunction (e.g., Dadds & Rhodes, 2008; Moul, Killcross, & Dadds, 2012). Furthermore, there has been a substantial amount of research into the etiological sources of variance (genetic vs. environmental) and molecular genetic mechanisms associated with CU traits. In 2012, Viding and McCrory conducted a review of published genetic and neurocognitive studies of CU traits. In the 6 years since this study was published, the number of genetically-informed studies of CU traits has nearly doubled - with an especially rapid growth in the number of molecular

[4] This chapter was modified from a manuscript originally published as: Moore, A. A., Blair, R. J., Hettema, J. M., & Roberson-Nay, R. (2019). The genetic underpinnings of callous-unemotional traits: A systematic research review. *Neuroscience and Biobehavioral Reviews, 100,* 85-97.

genetic studies. Given this rapid expansion and productivity in the field of behavior genetics, a reassessment of the genetic underpinnings of CU traits is warranted with a particular focus on molecular genetic mechanisms.

The aims of the current chapter were to compile, review, and discuss the extant literature on the genetic underpinnings of CU traits including both quantitative genetic and molecular genetic studies. Quantitative genetic studies seek to identify the relative contribution of genes and environment to a trait of interest and generally report the "heritability" of a trait (i.e., the proportion of trait variance in the population that is explained by genetic variation). Researchers generally prescribe to the belief that heritability should be established before the search for molecular genetic mechanisms begins. There are a variety of methods for uncovering molecular genetic mechanisms including candidate gene studies, genome-wide methods, and epigenetic methods. A review of each of these methodologies is included in the methods section of the current review. Considering both quantitative and molecular studies, we conducted a systematic review of the extant CU literature using two relevant databases: PubMed and PsychINFO. The following section describes our systematic review of the literature.

II. ANALYSES

Due to the often-technical nature of the studies reviewed here, Table 3.1 defines key terms that have been bolded throughout the manuscript. To be included in the current review manuscripts must have met three criteria: 1) The manuscript must have described original research (i.e., reviews and meta-analyses were not included), 2) The manuscript must have focused on quantitative (e.g., **heritability**) and/or molecular

Table 3.1. Glossary of Key (Bolded) Terms

Term	Definition
Additive Genetic Effects (*A*)	The additive effect of all polymorphic SNVs across the genome.
BDNF	A protein-coding gene for brain derived neurotropic factor, which plays a role in synaptic transmission and plasticity.*
Biomarker	An objective biological measure that is associated with a phenomenon, disease, or trait.
Biometrical Structural Equation Modeling	A statistical method used to quantify the influence of genes and environment on a trait or behavior, usually via twin and family studies.
Candidate Gene (CG) Study	A type of study where one or more genetic variant(s) is chosen a priori and the association with a phenotype of interest is examined.
Common/Shared/Family Environmental Effects (*C*)	The overall effect of the environmental factors that are shared between twins.
COMT	A protein-coding gene for catechol-O-methyltransferase, which plays a role in the clearance of catecholamine neurotransmitters (dopamine, norepinephrine, epinephrine) from the synaptic cleft.*
CpG Site	Refers to a location in the genome where a cytosine (C) nucleotide is followed by a guanine (G) nucleotide in the 5' to 3' direction. These CpG sites can be methylated to form 5-methylcytosine, which reflects the process underlying epigenetic methylation.
Dominant Genetic Effects (*D*)	The non-additive, or multiplicative, effect of all polymorphic SNVs across the genome.
Epigenetic	Refers to processes that effect gene expression, but do not involve changes in the nucleotide sequence of DNA. Includes both chromatin and DNA modifications.
Exome	The portion of the genome that is formed by exons, which are sections of genes that code for a final mRNA product. Comprises approximately 1% of the genome.
Gene x Environment Interaction	An interaction between a genotype and an environment, such that different genotypes respond differently to the same environmental process.
Gene Expression	The process by which DNA is synthesized (transcribed, spliced, translated, etc.) to produce a gene product/protein.
Genetic Correlation	A measure of similarity between sets of genes influencing separate phenotypes. This statistic indicated the degree to which the same genes influence two or more traits.
Genome	All the genetic information contained by an organism.
Genome-Wide Association (GWA) Study	An examination of a large number of SNVs across the genome to determine which are associated with a phenotype.
Genome-Wide Complex Trait Analysis (GCTA)	A method of estimating heritability using non-family samples, by directly estimating the small degree of genetic relatedness for each pair of individuals in the dataset (via measured genetic variants).
Heritability	The proportion of a trait's phenotypic variance that is due to genetic variance in the population.
Heterozygous	Indicates the possession of two different alleles (for example, G/T or A/C) for a specific SNV.
Homozygous	Indicates the possession of two identical alleles (for example, C/C or T/T) for a specific SNV.
HTR1B	A protein-coding gene for 5-hydroxytryptamine (serotonin) receptor 1B, which acts as a G-protein coupled receptor for serotonin.*
HTR2A	A protein-coding gene for 5-hydroxytryptamine (serotonin) receptor 2A, which acts as a G-protein coupled receptor for serotonin.*

Table 3.1 - Continued. Glossary of Key (Bolded) Terms

Term	Definition
MAOA	A protein-coding gene for monoamine oxidase A, which is involved in the metabolism of amine neurotransmitters (dopamine, norepinephrine, epinephrine, histamine, serotonin).*
Methylation	The process by which a methyl group is added to a CpG site, rendering a region of DNA less accessible to the cellular machinery responsible for transcription.
MZ-Differences Design	A methodology that uses differences between members of an MZ-pair on traits of interest to investigate unique/non-shared environmental effects. This design leverages the fact that MZ twins theoretically share all of their genes and shared/family environment, and therefore differences are thought to be due entirely to unique/non-shared environment.
Next-Generation Genome Sequencing	A fast method of genomic sequencing that simultaneously sequences millions of small DNA fragments and then uses bioinformatic techniques to piece the respective fragments of genetic code together.
OXT	A protein-coding gene for oxytocin/neurophysin I prepropeptide, which acts as a precursor protein for oxytocin and neurophysin I.*
OXTR	A protein-coding gene for the oxytocin receptor, which acts as a G-protein coupled receptor for the neurotransmitter oxytocin.*
Phenotype	An observable characteristic or set of observable characteristics (in the case of a disease or disorder).
Polygenic	A term referring to a phenotype that is causally influenced by more than one SNV.
Polygenic Risk Score	A metric, usually derived from GWA summary statistics, that is used to quantify an individual's level of genetic risk for a specific phenotype.
Precursor	An inactive protein that can be turned into an active protein via modification (such as the addition or removal of a molecule).
Polymorphic Allele	When a mutation in a gene produces more than one variation of that gene in the population, each form is a polymorphic allele.
Qualitative Sex Effects	A term used to describe the phenomenon where the variance of a trait is influenced by different sets of genes in males and females.
Quantitative Sex Effects	A term used to describe the situation in which a trait is influenced by the same set of genes in males and females, although to a different degree (stronger heritability in one sex vs. the other).
Receptor	A neurotransmitter receptor is a protein within neuronal cellular membranes that binds with neurotransmitter to trigger electrochemical signal transmission from one neuron to another.
Rs Number	The references SNV cluster ID, or rs number, is an identifier used by researchers and databases to refer to a specific genomic SNV.
Single Nucleotide Variant (SNV)	A variation of a single base pair at a specific genomic location.
SLC6A4	A protein-coding gene for a serotonin transporter, which clears serotonin from the synaptic cleft and transports it back to the pre-synaptic terminal.*
Transporter	A protein that clears neurotransmitter from the synaptic cleft and transports it back to the pre-synaptic terminal.
Unique/Non-Shared Environmental Effects (*E*)	The overall effect of environmental factors that are unshared between twins, plus error.
Variable Number Tandem Repeat (VNTR)	A section of the genome that is repeated a variable number of times within the population.

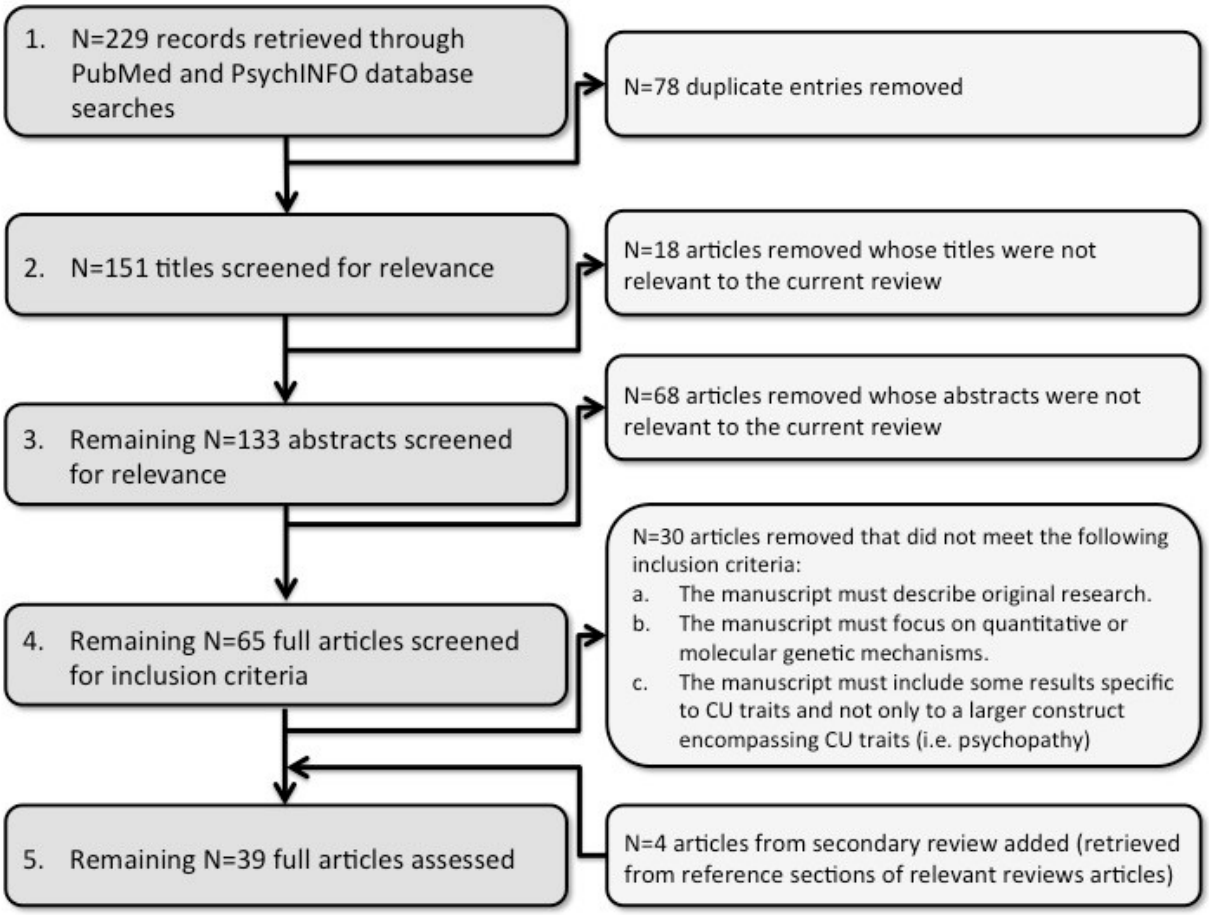
Note: * Information retrieved from *GeneCards* human gene database (Weizmann Institute of Science, 2018).

genetic mechanisms (e.g., **candidate gene**, **genome-wide association [GWA]**, **methylation**, etc.) of CU traits, and 3) The manuscript must have described CU traits in a unitary context and not only as it relates to other (or larger) constructs. For example, in a hypothetical study on the larger construct of psychopathy, the study would only be eligible for inclusion in the current review if it also included results on a psychopathy sub-factor that was described as "callous," "affective," or "callous-unemotional"; however, if only details on the larger psychopathy construct were included then it was not eligible for the current review.

Studies were selected by searching, in August of 2018, the PubMed and PsychINFO databases with the search term algorithm *("callous" OR "callous-unemotional") AND ("twins" OR "heritab*" OR "geneti*" OR "genom*" OR "epigen*")*. This algorithm ensured the inclusion of quantitative, genetic, genomic, and **epigenetic** studies. Wildcard operators (*) were used to include all possible suffixes on a relevant search term (such as the terms *'genomic'* and *'genome'* captured by the wildcard operator *'genom*'*). Titles and abstracts were screened to determine if the studies were eligible for inclusion. If questions about eligibility remained then the entire article was reviewed to determine if inclusion criteria were met. This resulted in a total of 35 studies. Additionally, references of relevant review articles were evaluated to ensure no articles were missed. This secondary review resulted in the inclusion of 4 additional studies for a total of 39 studies meeting the eligibility criteria listed above. Figure 3.1 displays a flow-chart of the article review process.

The studies reviewed below generally fall into one of two categories: 1) Quantitative genetic studies or 2) molecular genetic studies. Of the 39 reviewed studies,

Figure 3.1. Flow-chart of article review process



24 included quantitative components and 16 included molecular components (one study included both quantitative and molecular results). A brief review of the methodology most frequently used in these studies is included below.

Quantitative Genetic Studies

Quantitative genetic studies seek to determine the relative contribution of several genetic and environmental sources of variance to a phenotype of interest: **Additive genetic (*A*), dominant genetic (*D*), common/shared/family environmental (*C*), and unique/non-shared environmental (*E*).** *A* reflects the additive effect of all **polymorphic**

alleles. *D* reflects the non-additive effects of all polymorphic alleles, including phenomena like gene-gene interaction (i.e., "epistasis"). *C* refers to environmental factors that make family members more alike compared to random pairs of individuals, and *E* refers to environmental factors that are unique to each individual, plus measurement error. **Biometrical structural equation modeling** (SEM; a.k.a. "twin modeling") is the method most often used to decompose the observed variation in a trait into these sources of variance.

The classical twin model uses two correlations, one between pairs of monozygotic (MZ) twins and the other between pairs of dizygotic (DZ) twins, to decompose the variance of a trait into A, C, & E, or A, D, & E factors. MZ twins theoretically share 100% of their polymorphic alleles, 100% of their shared/family environment, and 0% of their non-shared environment, so the MZ correlation can be represented as $r_{MZ} = A + C$. DZ twins share, on average, 50% of their polymorphic alleles, 100% of their shared/family environment, and 0% of their non-shared environment, so the DZ correlation can be represented as $r_{DZ} = ½A + C$. Using these two equations as its basis, biometrical SEM compares several models for which different etiological influences are considered (for example, ACE, AE, CE, & E models) and -2 log-likelihood (-2LL) fit statistic values are compared to determine the best-fitting most parsimonious model (Neale & Cardon, 1992). These procedures form the foundation of the classical twin model and are the basis for the quantitative genetic (twin) studies reviewed below. However, some quantitative studies include more complicated methodology, including examination of genetic sex effects, **genetic correlation**s between more than 1 trait, and/or heritability estimated directly with molecular data via **genome-wide complex trait analysis** (GCTA).

Genetic sex effects can take the form of either **quantitative sex effects** or **qualitative sex effects**. The presence of quantitative effects indicates that the *proportion,* or *amount*, of genetic influences is different in males and females. On the other hand, qualitative effects indicate that different *sets of genes* are influencing CU traits in males and females. **Genetic correlations** (r_G) are statistics that reflect the degree to which the groups of genes influencing two separate phenotypes are correlated (i.e., include the same genes). Finally, GCTA is a method used to compute heritability without the use of twins. In this method, the small degree of genetic relatedness among individuals that would normally be considered unrelated is estimated via common SNVs measured with GWA methodology (Yang, Lee, Goddard, & Visscher, 2011).

Molecular Genetic Studies

Molecular genetic studies are generally performed after determining that a phenotype is heritable and these studies seek to determine the specific genetic mechanisms underlying the trait of interest. These studies are undertaken using a wide variety of methods, but modern studies mostly fall into three broad categories of interest to the current review: 1) candidate gene (CG) studies, 2) genome-wide association (GWA) studies, and 3) epigenetic and **gene expression** studies.

Candidate gene (CG) studies. A CG study uses 1 or more pre-selected genetic single nucleotide variant(s) (SNV; a.k.a. “variant”) for which the sample is genotyped. This genotypic data is then used to examine the association between the number of alleles (0, 1 or 2) of a genetic variant and the trait of interest. Most researchers choose variants for CG studies based on some underlying hypothesis about the biological etiology of the phenotype. For example, a variant located in a gene that plays a role in the re-uptake of

serotonin may be a biologically plausible mechanism to study in regards to a phenotype that is hypothesized to be causally related to a dysfunctional serotonin system. Once variants are determined and genotyped in a sample, linear or logistic regression is used to examine potential associations (van der Sluis & Posthuma, 2008), where the genetic predictor variables may be binary (e.g., 0 alleles vs. 1 or 2 alleles) or ordinal (e.g., 0 alleles vs. 1 allele vs. 2 alleles) in nature.

A concept that is frequently discussed in CG studies is **gene x environment (GxE) interaction**. Gene-environment interaction can be conceptualized as different genotypes responding differently to the same environmental process. Alternatively, it can also be conceptualized as differential effects of genotype based on environmental processes.

Genome-wide association (GWA) studies. In contrast to CG studies, GWA studies simultaneously examine a large number of variants across a participant's genome. Associations are tested between a phenotype and hundreds of thousands of common genetic variants without any *a priori* hypotheses. The statistical methods used are much the same as that of CG studies (Sullivan & Purcell, 2008). However, a single GWA study variant must meet a stringent multiple testing burden *p*-value, generally set at $5x10^{-8}$ (Clark et al., 2011; Pe'er, Yelensky, Altshuler, & Daly, 2008). This stringent significance threshold and lack of *a priori* hypotheses means that significant GWA results are generally perceived as more credible than CG results. However, replication of findings in multiple independent samples is still needed before any conclusions can be drawn from GWA (or CG) results.

Epigenetic studies. Epigenetics refers to DNA or chromatin modifications that can influence gene expression but do not influence gene structure. The amount of protein

a gene produces can vary due to epigenetic factors that regulate the gene's expression (sometimes referred to as turning a gene "on" or "off," although the process is actually much more complex). It is important to remember that while DNA sequence is inherited, epigenetic modifications to the genome are most frequently mediated by environmental processes and not inherited directly from one's parents. Currently, the most commonly studied epigenetic mechanism is DNA methylation, whereby a methyl group is added to a specific DNA site making the gene less accessible to the cellular machinery responsible for gene transcription (the process of copying DNA into RNA). The blockage of the transcriptional machinery can decrease the expression of a particular gene (Allis & Jenuwein, 2016). A study that assesses epigenetic mechanisms can take the form of either a CG or GWA study by assessing modifications at one (or a few) DNA site(s), or across the entire genome, respectively.

III. RESULTS

Quantitative Genetic Studies

Table 3.2 lists the 24 reviewed quantitative genetic studies of CU traits and includes relevant sample descriptives and a brief presentation of the main results. These 24 studies use a variety of instruments and methods, and the reported heritability of CU traits ranges from 25-80%. Several of these studies use selected samples (e.g., selected for a behavioral disorder, in the top 10% of the CU trait distribution, etc.), and these studies tend to report the highest heritabilities (63-80%; Fontaine, Rijsdijk, McCrory & Viding, 2010; Humayun, Kahn, Frick, & Viding, 2014; Larsson, Viding, & Plomin, 2008; Saunders et al., 2018; Viding, Blair, Moffitt, & Plomin, 2005). It is important to note that the concept

Table 3.2. Quantitative Genetic Studies, Chronologically within Sample

Sample	Authors & Date	N	Sex	Age (years)	Instrument(s)	Selected sample	Results
Boston University Twin Project	Flom & Saudino, 2017a	*N* = 628 (314 twin pairs)	53% male	2-3	Child Behavior Checklist 1½-5 (PR)	N	CU @ 2 years old *A* = 72%, *E* = 28%. CU @ 3 years old *A* = 65%, *E* = 35%. 42% of genetic variance at age 2 persisted to age 3.
	Flom & Saudino, 2017b	*N* = 628 (314 twin pairs)	53% male	2-3	Child Behavior Checklist 1½-5 (PR)	N	At age 2, CU *A* = 71% (27% specific to CU, 44% shared with ADHD and ODD), *E* = 29% (25% specific to CU, 4% shared with ADHD and ODD).
Child and Adolescents Twin Study in Sweden (CATSS)	Saunders et al., 2018	*N* = 426	58% male	15	Youth Psychopathic Traits Inventory (SR)	Y	CU A = 63%, E = 37%. r_G = .77 for CU and CD, .36 for CU and hyperactivity, and -.23 for CU and emotional problems.
Georgia Twin Registry	Ficks, Dong, & Waldman, 2014	*N* = 1770 (885 twin pairs)	49% males	4-17	Antisocial Process Screening Device (PR)	N	CU factor *A* = 49%, *E* = 51%. No quantitative or qualitative sex effects.
Minnesota Twin and Family Study	Taylor, Loney, Bobadilla, Iacono, & McGue, 2003	*N* = 796 (398 twin pairs)	100% male	16-18	Minnesota Temperament Inventory (SR)	N	CU factor *A* = 40%, *E* = 60%. r_G = .78 for CU traits and antisocial behavior.
	Blonigen, Hicks, Krueger, Patrick, & Iacono, 2005	*N* = 1,252 (626 twin pairs)	46% male	17	Multidimensional Personality Questionnaire (SR)	N	CU factor *A* = 45%, *E* = 55%. r_G = .16 for CU traits and antisocial behavior.
	Blonigen, Hicks, Krueger, Patrick, & Iacono, 2006	*N* = 1,252 (626 twin pairs)	46% male	17, 24	Multidimensional Personality Questionnaire (SR)	N	CU factor *A* = 42%, *E* = 58%. Genetic factors accounted for 58% of the stability in CU traits from age 17 to 24.
Preschool Twin Study in Sweden (PETSS)	Tuvblad, Fanti, Andershed, Colins, & Larsson, 2017	*N* = 1,189	50% male	5	Child Problematic Traits Inventory (TR)	N	CU factor *A* = 25%, *C* =17%, *E* = 58%. No quantitative sex effects.
Quebec Newborn Twin Study (QNTS)	Henry et al. 2018a	*N* = 1,324 (662 twin pairs)	Unknown	7-12	Inventory of Callous-Unemotional Traits (TR) & Antisocial Process Screening Device (TR)	N	CU @ age 7 *A* = 46%, *E* = 54%. CU @ age 9 *A* = 59%, *E* = 41%. CU @ age 10 *A* = 39%, *E* = 61%. CU @ age 12 *A* = 50%, *E* = 50%. In both longitudinal models (growth and Cholesky) genetic factors accounted for the majority of the stability across age.

Table 3.2 - Continued. Quantitative Genetic Studies, Chronologically within Sample

Sample	Authors & Date	N	Sex	Age (years)	Instrument(s)	Selected sample	Results
	Henry et al., 2018b	*N* = 1,324 (662 twin pairs)	Unknown	7-12	Inventory of Callous-Unemotional Traits (TR) & Antisocial Process Screening Device (TR)	N	CU *A* = 65%, *E* = 35%. Heritability of CU decreased as warm-rewarding parenting increased (gene-environment interaction).
Southern California Twin Project	Bezdjian, Raine, Baker, & Lynam, 2011	*N* = 1,219 (605 sets of twins and triplets)	49% male	9-10	Child Psychopathy Scale (SR; PR)	N	Callous/disinhibited factor *A* = 64% in boys, *A* = 49% in girls, remaining variance accounted for by *E*.
	Tuvblad, Bezdjian, Raine, & Baker, 2014	N = 1,208 (604 twin pairs)	49% male	14-15	Child Psychopathy Scale (SR; PR) & Antisocial Process Screening Device (SR;PR)	N	Callous/disinhibited factor of CPS (SR) *A* ≈ 42%, *E* ≈ 58%. Callous/disinhibited factor of CPS (PR) *A* ≈ 46%, *E* ≈ 54%. CU factor of APSD (SR) *A* ≈ 47%, *E* ≈ 53%. CU factor of APSD (SR) *A* ≈ 63%, *E* ≈ 37%.
The Swedish Twin Study of Child and Adolescent Development	Larsson, Andershed, & Lichtenstein, 2006	*N* = 2,180 (1,090 twin pairs)	48% male	16	Youth Psychopathic Traits Inventory (SR)	N	Callous/unemotional factor *A* = 43%, *E* = 57%. No qualitative or quantitative sex effects.
	Kendler, Patrick, Larsson, Gardner, & Lichtenstein, 2013	N = 884 (442 twin pairs)	100% male	16-17	Youth Psychopathic Traits Inventory (SR)	N	Callous/unemotional factor *A* = 36%, *E* = 61%, & *C* = 3%. 68% of CU's genetic variance was specific to CU, while the other 32% was attributable to a genetic factor common to several externalizing traits (delinquency, grandiosity, impulsivity, criminality, etc.)
The Texas Twin Project	Mann, Briley, Tucker-Drob, & Harden, 2015	*N* = 535 (264 sets of twins and triplets)	50% male (as of 2012)	13-21	Inventory of Callous-Unemotional Traits (SR)	N	ICU *A* = 40%, *E* = 60%.
Twins Early Development Study (TEDS) [+]	Viding, Blair, Moffitt, & Plomin, 2005	Total *N* = 7,374, Analytic *N*s = 808-1,071	47% male	7	Antisocial Process Screening Device (TR) and Strengths and Difficulties Questionnaire (TR)	Y	Extreme CU traits *A* = 67%, *E* = 33%. Extreme antisocial behavior with extreme CU traits *A* = 81%, *E* = 19%. Extreme antisocial behavior without extreme CU traits *A* = 30%, *E* = 70%.

Table 3.2 - Continued. Quantitative Genetic Studies, Chronologically within Sample

Sample	Authors & Date	N	Sex	Age (years)	Instrument(s)	Selected sample	Results
	Viding, Frick & Plomin, 2007	Total *N* = 6,464, (3,232 twin pairs)	47% male	7	Antisocial Process Screening Device (TR) and Strengths and Difficulties Questionnaire (TR)	N*	Quantitative sex differences for overall model including both CU and CD. For males CU *A* = 67%, *C* = 4%, *E* = 29%, CU/CD r_G = .57. For females, CU *A* = 48%, *C* = 20%, *E* = 32%, CU/CD r_G = .65.
	Larsson, Viding, & Plomin, 2008	*N* = 4,430 (Analytic N differed for each analysis)	47% male	7	Antisocial Process Screening Device (TR) and Strengths and Difficulties Questionnaire (TR)	Y	Extreme CU traits without extreme antisocial behavior *A* = 68%, *E* = 32%. Extreme CU traits with extreme antisocial behavior *A* = 80%, *E* = 20%.
	Fontaine, Rijsdijk, McCrory, & Viding, 2010	*N* = 9.462	47% male	7, 9, 12	Antisocial Process Screening Device (TR) and Strengths and Difficulties Questionnaire (TR)	Y	Quantitative sex-differences for stable-high CU group. In boys *A* = 78%, *C* = 01%, *E* = 21%. In girls *C* = 75%, *E* = 25%.
	Viding et al., 2013**	*N* = 5,772 (2,886 twin pairs)	47% male (Total sample)	7, 9, 12	Antisocial Process Screening Device (TR) & Strengths and Difficulties Questionnaire (TR)	N	Via twin modeling, CU *A* = 64%, *E* = 36%. Via GCTA, CU *A* = 7%, environmental variance (*C* & *E*) = 93%.
	Humayun, Kahn, Frick, & Viding, 2014	Total *N* = 7,374, Analytic *N*s = 210-992	47% male	7	Antisocial Process Screening Device (TR) and Strengths and Difficulties Questionnaire (TR)	Y	Extreme CU traits without extreme anxiety *A* = 75%, *E* = 25%. Extreme CU traits with extreme anxiety *A* = 66%, *E* = 34%.
	O'Nions et al., 2015	Total N = 14,556 (7,278 twin pairs)	47% male	7	Antisocial Process Screening Device (TR) and Strengths and Difficulties Questionnaire (TR)	N	CU *A* = 66%, *E* = 34%. 74% of the genetic variance in CU was specific to CU, with the remaining 26% attributable to a genetic factor influencing CU, social interaction, and social communication difficulties.
	Henry, Pingault, Boivin, Rijsdijk, & Viding, 2016	*N* = 10,184 (5,092 twin pairs)	47% male	16	Inventory of Callous-Unemotional Traits (PR)	N	General factor *A* = 58%. Callous-uncaring factor *A* =70%. Unemotional factor *A* = 79%. Remaining variance due to *E*.
Virginia Commonwealth University Juvenile Anxiety Study (VCU-JAS)	Moore et al., 2017	*N* = 678 (339 twin pairs)	48% male	9-14	Inventory of Callous-Unemotional Traits (PR)	N	CU *A* = 39%. Accounting for measurement error, liability to CU *A* = 46%. Remaining variance due to E.

*Table 3.2 Note: A = additive genetic effects. C = shared/family environmental effects. E = non-shared environmental effects. r_G = genetic correlation. SR = self-report. PR = parent-report. TR = teacher-report. Y/N = yes/no to whether or not these samples were selected for high levels of CU. ⁺In the TEDS sample, "extreme" phenotypes mean the proband twin was in the top 10% of the TEDS distribution. *A Selected sample was used for some analyses; however, those results are not included here. **Viding et al. (2013) study reported both quantitative (twin, GCTA) analysis as well as molecular (GWAS) analysis and is therefore included in tables 1 & 2.*

of heritability is specific to the population under study and only assesses the genetic influences on trait variability in members of that specific population. Therefore, the studies using selected samples indicate that *among those with very high levels of CU traits*, genetic factors influence a high proportion of the variability *within these individuals*. However, these studies do not provide any information on the proportion of genetic influences on trait variation in the general population. Importantly, these studies do not provide information about the differences between individuals with high and low CU traits, which is generally how the term heritability is conceptualized. Although these studies provide information about variation among individuals with pathological levels of CU traits, care should be taken to describe these results as separate from general population studies as to not upwardly bias heritability estimates (e.g., Neale, Eaves, Kendler, & Hewitt, 1989).

Of the remaining quantitative genetic studies using non-selected samples to estimate heritability, the greatest variation in heritability estimates is observed in the youngest samples (ages 2-5), which is not surprising given the infrequent investigation of CU in very young samples and the questions about whether or not CU/psychopathic traits are tapping into the same construct in young children. The youngest of these samples (Flom & Saudino, 2017a; Flom & Saudino, 2017b) used data from the parent-report Child Behavior Checklist (CBCL; Achenback & Rescorla, 2001) in *N* = 628 twins aged 2-3 years and estimated the heritability of CU traits at age 2 and 3 at 72% and 65%, respectively.

Conversely, in a slightly older sample (age 5; Tuvblad, Fanti, Andershed, Colins, & Larsson, 2017), the teacher-report Child Problematic Traits Inventory (CPTI; Colins et al., 2014) in *N* = 1,189 twins generated the lowest reported estimate of heritability: 25%. The variation in heritability estimates for young children could be due to the different measures and reporters used, and these issues may be compounded by longitudinal non-invariance. That is, there is a distinct possibility that researchers using very young samples may be tapping into a psychological construct that differs from the traditional conceptualization of CU traits seen in adolescents and adults (e.g., Hawes et al., 2014; Obradović et al., 2007)

The picture of CU trait heritability among general population samples of individuals in late childhood, adolescence, and early adulthood (aged 7-19) is much less variable, ranging from 36-67% (Bezdjian, Raine, Baker, & Lynam, 2011; Blonigan, Hicks, Kreuger, Patrick, & Iacono, 2006; Ficks, Dong, & Waldman, 2014; Henry et al., 2018a; Henry et al., 2018b; Henry, Pingault, Boivin, Rijsdijk, & Viding, 2016; Kendler, Patrick, Larsson, Gardner, & Lichtenstein, 2013; Larsson, Andershed, & Lichtenstien, 2006; Moore et al., 2017; O'Nions et al., 2015; Taylor, Loney, Bobadilla, Iacono, & McGue, 2003; Mann, Briley, Tucker-Drob, & Harden, 2015; Tuvblad, Bezdjian, Raine, & Baker, 2014; Viding et al., 2013; Viding, Frick, & Plomin, 2007). Furthermore, two studies of this age range suggest that genetic factors account for a substantial proportion of *stable variation* in CU traits across time; 58% from age 17 to 24 (Blonigan et al., 2006) and up to 89% across ages 7-12 (Henry et al., 2018a). However, no study has yet investigated whether or not the heritability of CU traits changes dynamically throughout development. Such changes in heritability have been observed in other externalizing psychopathology of adolescence

such as substance abuse (for a review see Dick, Adkins, & Kuo, 2016) and may be a relevant phenomenon to consider for CU traits.

Although almost all studies demonstrate that CU traits are significantly influenced by genetics; only three of the 24 quantitative studies report a significant influence of shared/family environment. First, Tuvblad and colleagues (2017) report 17% of the variance in CU is accounted for by shared/family environment among a sample of *N* = 1,189 5-year-old twins. The other two studies report a significant influence of shared/family environment in girls only. One of these studies used latent trajectory analysis to analyze a stable-high CU group in *N* = 9,462 children aged 7-12, and found the variance in this group to be influenced primarily by genetics in males (A = 78%) but primarily influenced by shared/family environment in females (C = 75%; Fontaine, Rijsdijk, McCrory, & Viding, 2010). The final study investigated the relationship between CU and CD in a sample of over 6,000 7-year-olds and found that shared/family environment accounted for 20% of the CU variance in girls, but was an insignificant factor for boys' CU. Interestingly, although the influence of C is a common finding in antisocial behavior (for a review see Rhee & Waldman, 2002) it is a relatively rare finding in studies of CU traits. Therefore, the significant influence of shared/family environment found in these three studies may stem from study-specific data collection and/or analyses procedures or they may simply be spurious findings.

The examination of potential sex-differences in CU trait heritability has been neglected by the majority of studies. However, a few studies have begun to investigate genetic sex effects on CU traits. Some studies have reported the presence of quantitative sex effects (Bezdijian et al., 2011; Fontaine et al., 2010; Viding et al., 2007). These studies

report a greater influence of genetic factors on CU traits in males as compared to females. However, others have failed to replicate these findings (Larsson et al., 2006; Ficks et al., 2014; Tuvblad et al., 2017), suggesting no *consistent* indication of quantitative sex effects. Only two studies have formally tested for qualitative sex effects (different sets of genes influencing CU in males vs. females), and neither found evidence for such differences (Larsson, Andershed, & Lichtenstein, 2006; Ficks, Dong, & Waldman, 2014).

In regard to the different measures used to assess CU traits, the majority of quantitative genetic studies reviewed above rely on measures of CU traits derived from instruments originally designed to assess psychopathic traits and/or personality more broadly (e.g., APSD [Frick & Hare, 2001], Child Psychopathy Scale [CPS; Lynam, 1997], CPTI, Minnesota Temperament Inventory [MTI; Loney, Taylor, Butler, & Iacono, 2002], Multidimensional Personality Questionnaire [MPQ; Tellegen & Waller, 2008], SDQ [Goodman, 1997], Youth Psychopathic Traits Inventory [YPI; Andershed, Kerr, Stattin, & Levander, 2002]). Such studies usually perform a series of factor analyses and/or extract a score for a handful of items corresponding to the authors' conceptualization of CU traits. However, several problems with these methods have been noted. Specifically, these extracted measures often include only a small number of items (sometimes as few as 4) with limited response options resulting in negatively skewed responses. These measurement issues often result in significant psychometric issues such as poor internal consistency (For a review of these issues, see Frick & Ray, 2014). The use of a scale designed specifically to assess CU traits is one potential way to avoid several of these shortcomings and also increase the comparability of results across studies.

The Inventory of Callous-Unemotional Traits (ICU; Frick, 2004; Kimonis et al., 2008) is one measure that was designed to assess CU traits as a unitary construct. However, the ICU is not without its flaws. Psychometric properties of the ICU, specifically the unemotional subscale, have been inconsistent (e.g., Hawes et al., 2014; Henry et al., 2016; Moore et al., 2017) and problems with the directionality of wording have been noted (e.g., Hawes et al., 2014; Ray, Frick, Thornton, Steinberg, & Cauffman, 2015). However, the ICU is still one of the most frequent measurements used to provide a more complete assessment of CU traits in children and adolescents (Fanti, Frick, & Georgiou, 2009). Three studies have thus far examined the heritability of CU traits using the ICU (Henry et al., 2016; Mann et al., 2015; Moore et al., 2017). In a sample of over 10,000 16-year old twins, Henry and colleagues (2016) reported a bifactor model for the parent-report ICU consisting of a general, callous-uncaring, and unemotional factor, and estimated the heritability of these factors at 58%, 70%, and 79%, respectively. Moore and colleagues (2017) used data from *N* = 678 9-14 year old twins assessed at two time points approximately 3 weeks apart to estimate heritability while controlling for measurement error (which is usually included in estimates of non-shared environment). This study estimated parent-report ICU heritability at 39% before measurement error was accounted for, with this estimate increasing to 46% when error was accounted for in the model. Using *N* = 535 twins and triplets aged 13-21 years, Mann and colleagues (2015) found a similar heritability using self-report ICU: approximately 40%.

A molecular methodological alternative to twin studies, Genome-wide Complex Trait Analysis (GCTA), was recently used to estimate the heritability of CU traits in *N* = 2,930 children aged 7-12 years (Viding et al., 2013). This GCTA analysis estimated the

heritability of CU traits, as measured by the APSD and SDQ, at 7%; far less than the 40-60% reported in twin studies. However, large discrepancies between twin- and GCTA-based heritability estimates are not uncommon. For example, GCTA-based heritability for parent-report psychopathy and teacher-report ASPD were recently estimated at 14% and 8%, respectively, while the corresponding twin-based heritabilities in the same samples were 47% and 62%, respectively (Cheesman et al., 2017). Furthermore, even for anthropomorphic and cognitive traits, GCTA-based heritability estimates are about half of the twin-based estimates (Plomin et al., 2013). These differences in GCTA- vs. twin-based heritability likely stem from the fact that GCTA methodology does not capture the full range of genetic architectures (e.g., common vs. rare variants, additive vs. multiplicative effects, etc.; Gibson, 2012; Chatterjee et al., 2013; Wray et al., 2013). Researchers have noted that GCTA-based heritability estimates reflect the ceiling for genetic effects discovered in genome-wide methodology (e.g., Cheesman et al., 2017). Therefore, researchers should consider uncovering the underlying genetic architecture one of the most important future directions for genetic studies of CU traits.

Although most quantitative genetic studies focus on CU traits as a unitary context, a subset of these studies also report genetic correlations between CU traits and related phenotypes. Unsurprisingly, CU traits are highly correlated with CD at the genetic level. Genetic correlations for these two phenotypes have been estimated at r_G = .77 in a mixed-sex sample (Saunders et al., 2018), and estimated separately for females and males at r_G = .65 and r_G = .57, respectively (Viding et al., 2007). The genetic correlation between CU and antisocial behavior has been estimated at r_G = .78 in adolescent males (Taylor et al., 2003). However, the genetic correlation appears much lower, r_G = .16, when estimated

in a mixed-sex sample (Blonigan et al., 2005) indicating potential sex-differences in the genetic covariance between CU and antisocial behavior. Finally, Kendler and colleagues (2013) investigated the genetic correlation between a range of externalizing phenotypes, and found that 32% of the genetic variance in CU traits was shared with a general externalizing factor that also influenced traits such as delinquency, grandiosity, impulsivity, and criminality. Together, these studies suggest that there is substantial genetic overlap between CU traits and other behavioral, impulsive, and antisocial traits. However, a substantial portion of CU's genetic variance is unique to CU, suggesting a partially distinct etiology.

Molecular Genetic Studies

Table 3.3 lists the 16 reviewed molecular genetic studies of CU traits and includes relevant sample descriptives/methods and a brief presentation of the main results. Of these 17 studies, 11 were traditional CG studies, 2 were traditional GWA studies, and 3 were CG methylation studies.

Candidate gene (CG) studies. CG studies have potentially implicated several genes in the etiology of CU traits, including ***BDNF, COMT***, ***HTR1B***, ***HTR2A***, ***MAOA***, ***OXTR***, & ***SLC6A4***. Most significant candidate gene findings thus far are associated with genes belonging to the serotonin and oxytocin systems.

Several variants in genes involved in coding proteins for the serotonin **receptor**s have been investigated as potential candidates for CU traits. The biological plausibility for these variants is clear, especially given that manipulation of the serotoninergic system (for example, via tryptophan depletion) can induce features central to psychopathy (e.g., reduced fear recognition and reduced response to punishment; Blair et al., 2008; Finger

Table 3.3. Molecular Genetic Studies, Chronologically

Authors & Date	N	Sex	Age (years)	Instrument(s)	Methods	Genes	Results
Fowler et al., 2009	*N* = 147	93% male	12-19	Psychopathy Checklist (SR)	CG	COMT, MAOA, SLC6A4	In youth with ADHD, callousness associated with val/val at COMT val158met (no rs provided by authors, although one assumes rs4680), short/short 5-HTTLPR alleles, & homozygous high-risk (2 & 3 repeat) versions of MAOA 30bp VNTR.
Sadeh et al., 2010	Study 1: *N* = 118 Study 2: *N* = 178	Study 1: 42% male Study 2: 45% male	Study 1: mean = 14.3 Study 2: mean = 10.8	Study 1:Antisocial Process Screening Device (SR) Study 2: Inventory of Callous-Unemotional Traits (SR)	CG; GxE	SLC6A4	For both studies, 5-HTTLPR long allele positively associated with CU in children, but only in those with low SES.
Viding et al., 2010	*N* = 600 (discovery); *N* = 586 (replication)	48% male	7	Antisocial Process Screening Device (TR) & Strengths and Difficulties Questionnaire (TR)	GWAS	Genome-wide	No genome-wide significant results.
Beitchman et al., 2012	*N* = 162	65% male	6-16	Psychopathy Screening Device (PR)	CG	OXT, OXTR	Among aggressive youth, A/A rs237885 (in OXTR gene) positively associated with CU.
Malik, Zai, Abu, Nowrouzi, & Beitchman, 2012	*N* = 236	69% male	6-16	Psychopathic Screening Device (PR)	CG	OXT, OXTR	Examining OXT and OXTR variants among aggressive youth, no significant associations with respect to CU behaviors.
Willoughby, Mills-Koonce, Propper, & Waschbusch, 2013	*N* = 171	63% male	0-3	Achenbach System of Empirically Based Assessment (PR)	CG; GxE	BDNF	Early harsh-intrusive parenting was associated with CU at age 3, but only among those with G/A or A/A at rs6265 in the BDNF gene.
Cecil et al., 2014	*N* = 84	50% male	Birth, 7, 9, 13	6-item questionnaire (PR), highly correlated with CU scale of the Antisocial Process Screening Device	Epigenetic; CG	OXTR	Among youth with early-onset persistent CD, OXTR methylation level (12 probes examined) at birth was positively was associated with CU at age 13, but only for those without internalizing problems.
Dadds, Moul, Cauchi, Hawes, & Brennan, 2013	*N* = 210	77% male	3-16	Antisocial Process Screening Device (PR, TR, SR) & Strengths and Difficulties Questionnaire (PR, TR, SR)	CG; GWAS Replication	13 SNVs from Viding (2010) within 20kb of a gene	Examining 13 suggestive SNVs from Viding (2010) GWAS, no replicated SNVs in terms of CU (although some significant findings for CD).

Table 3.3 - Continued. Molecular Genetic Studies, Chronologically

Authors & Date	N	Sex	Age (years)	Instrument(s)	Methods	Genes	Results
Hirata, Zai, Nowrouzi, Beitchman, & Kennedy, 2013	*N* = 144	72% male	6-16	Psychopathy Screening Device (PR)	CG	COMT	Examining variants in the COMT gene, no significant results.
Moul, Dobson-Stone, Brennan, Hawes, & Dadds, 2013	*N* = 35-157 (depending on analysis)	100% male	3-16	Antisocial Process Screening Device (PR) & Strengths and Difficulties Questionnaire (PR)	CG	HTR1A, HTR1B, HTR2A, HTR3B, TPH1, TPH2, SLC6A4	Among boys with conduct problems, high CU associated with G/T at rs11568817 (in HTR1B gene), and C/C at rs6314 (in HTR2A gene); High CU also associated with lower serum serotonin.
Viding et al., 2013*	*N* = 2,930	47% male (total sample)	7, 9, 12	Antisocial Process Screening Device (TR) & Strengths and Difficulties Questionnaire (TR)	GWAS	Genome-wide	No genome-wide significant results.
Dadds et al., 2014a	*N* = 121 (discovery) *N* = 59 (replication)	71% male (discovery) 78% male (replication)	4-16	Antisocial Process Screening Device (PR, TR, SR) & Strengths and Difficulties Questionnaire (PR, TR, SR)	CG	OXTR	Among youth with conduct problems, T/T rs10427778 (in OXTR gene) was associated with CU traits (Bonferroni-corrected); this finding was replicated in a separate sample.
Dadds et al., 2014b	*N* = 37-156 (depending on analysis)	100% male	4-16	Diagnostic Interview Schedule – CU CD Specifier (PR; PR & SR when > 8)	Epigenetic; CG	OXTR	Among 9-16 year olds with CD, OXTR methylation level (11 probes examined) was positively associated with CU. In a partially-overlapping sample plasma oxytocin was negatively associated with CU traits. Neither of these associations held true in the 4-8 year old group.
Moul, Dobson-Stone, Brennan, Hawes, & Dadds, 2015	*N* = 117	100% male	3-16	Antisocial Process Screening Device (PR) & Strengths and Difficulties Questionnaire (PR)	Epigenetic; CG; GxE	HTR1B	CU was positively associated with heterozygous (G/T) status for rs11568817 in the HTR1B gene. CU was positively associated with HTR1B methylation level (19 sites examined) only among individuals heterozygous (G/T) for rs11568817.
Brammer, Jezoir, & Lee, 2016	*N* = 230	69% male	5-10	Antisocial Process Screening Device (PR)	CG	SLC6A4	Number of 5-HTTLPR long alleles positively associated with CU traits.
Hirata et al., 2016	*N* = 123	78% male	6-16	Antisocial Process Screening Device (PR)	CG	PRL, PRLR	Two PRL gene SNVs and 3 PRLR gene SNVs were examined. No significant associations with CU traits.

*Table 3.3 Note: SR = self-report. PR = parent-report. TR = teacher-report. GWAS = genome-wide association study. GCTA = genome-wide complex trait analysis. CG = candidate gene study. GxE = gene-environment interaction study. var = proportion of variance accounted for. CD = conduct disorder. *Viding et al. (2013) study reported both quantitative (twin, GCTA) analysis as well as molecular (GWAS) analysis and is therefore included in both tables.*

et al., 2007; Marsh et al., 2006). Furthermore, recent research has demonstrated a negative association between peripheral blood levels of serotonin and CU traits (Moul, Dobson-Stone, Brennan, Hawes, & Dadds, 2013). Genes that code for serotonin receptors have been associated with CU traits in a sample of *N* = 157 males with CD (age 3-16), including G/T **heterozygous** status at rs11568817 in the *HTR1B* gene and C/C homozygous status at rs6314 in the *HTR2A* gene (Moul et al., 2013).

In studies examining associations between CU traits and the 5-HTTLPR promoter polymorphism in the *SLC6A4* gene that codes for the serotonin **transporter**, results have been mixed. In a study of 147 adolescents with ADHD, Fowler and colleagues (2009) found significant associations with several genetic variants and callousness as measured by the psychopathy checklist (PCL-YV; Forth, Kosson, & Hare, 2003), including **homozygous** status for the 5-HTTLPR short allele. Contrary to the direction of this reported association, another study found a *positive* association between the number of 5-HTTLPR long alleles and CU traits in a sample of *N* = 230 children aged 5-10 years (Brammer, Jezoir, & Lee, 2016). Adding even more uncertainty to the picture of 5-HTTLPR genotype, Sadeh and colleagues (2010) found no overall association between 5-HTTLPR and CU traits in two separate samples (*N* = 118 & 178) using two different methods of measuring CU. However, they did report a gene-environment interaction such that individuals with the long/long 5-HTTLPR genotype had higher CU traits in the presence of low socioeconomic status. These inconsistent 5-HTTLPR genotypic associations may indicate that some significant results are false-positives. However, the

presence of an interaction effect may also suggest that the serotonin transporter exerts differential effects under various environmental circumstances (i.e., gene x environment interaction).

Some of the more recent genetic associations with CU traits involve variants in the oxytocin system (involved in coding protein for oxytocin **precursor**s and oxytocin receptors). Oxytocin represents another biologically plausible mechanism for CU traits, specifically given its role in modulating amygdala activity (e.g., Gorka et al., 2015) and the association between psychopathic traits and peripheral blood levels of oxytocin (Dadds et al., 2014b). In a sample of *N* = 162 aggressive youth aged 6-16 years, the A/A genotype at rs237885 in the *OXTR* gene was positively associated with CU traits (Beitchman et al., 2012). Furthermore, another nearby *OXTR* variant, rs1042778, was positively associated with CU traits as measured by the APSD and SDQ in two independent samples (*N*= 121 & 59) of youth aged 4-16 with conduct problems (Dadds et al., 2014a). Given their close proximity to one another in the genome (within 1000 base pairs), these two variants are likely indexing the same genetic signal. However, these results are tempered by the fact that the associations between CU traits and oxytocin variants have not been universally replicated. One recent study used a sample of *N* = 236 aggressive youth (aged 6-16) to examine a set of 8 SNVs within two oxytocin genes (*OXTR* and ***OXT***) and was unable to find any significant associations with CU traits (Malik, Zai, Abu, Nowrouzi, & Beitchman, 2012).

Several other genetic variants have also been investigated in CG studies of CU traits although less frequently. A significant gene x environment interaction for CU traits was reported in a sample of *N* = 171 children in which harsh/intrusive parenting predicted

early (age 3) CU traits but only among children possessing a specific *BDNF* genotype (G/A or A/A at rs6265; Willoughby, Mills-Koonce, Propper, & Waschbusch, 2013) that codes protein for brain-derived neurotropic factor (BDNF), which is involved in synaptic transmission and plasticity (Weizmann Institute of Science [WIS], 2008). Two additional significant associations were reported by Fowler and colleagues (2009) in their study of *N* = 147 adolescents with ADHD. First, there was a significant association between CU and the high-risk allele (2-3 repeats) of the 30bp **variable number tandem repeat** in the *MAOA* gene that codes protein for monoamine oxidase A, which is involved in the metabolism of amine neurotransmitters such as dopamine, norepinephrine, and serotonin, among others (WIS, 2008). Second, Fowler and colleagues (2009) reported a significant association between CU and val/val homozygous status at the val158met SNV in the *COMT* gene responsible for the clearance of catecholamine neurotransmitters from the synaptic cleft by catechol-O-methyltransferase (WIS, 2008; the authors did not include the **rs number** for the *COMT* SNV, but one assumes they are referring to the oft-researched rs4680). Unfortunately, some of Fowler and colleagues' (2009) results have failed to replicate. For example, in another CG study of *N* = 144 individuals (age 6-11), researchers examined associations between CU traits and several variants in the *COMT* gene, including rs4680, and no significant associations were found (Hirata, Zai, Nowrouzi, Beitchman, & Kenndey, 2013).

Genome-wide association (GWA) studies. Given the large sample sizes required for GWA studies to be sufficiently powered, the two preliminary GWA studies that have been conducted (Viding et al., 2010; Viding et al., 2013) are both likely quite underpowered to detect relevant effects. In the first GWA study of CU traits Viding and

colleagues (2010) used data from a total of *N* = 1,186 individuals aged 7 years, while Viding and colleagues (2013) more than doubled their original sample size to *N* = 2,930 children aged 7-12 years. Neither study identified any significant genome-wide associations with CU traits as measured by the APSD and SDQ. However, the authors ranked SNVs based on their associated *p*-values and suggested that future genetic studies consider these top SNVs as potential research targets. Following this advice, a separate research group investigated the top 13 SNVs from Viding (2010) in *N* = 213 individuals aged 3-16 years. Although one variant was associated with CD, none were significantly associated with CU traits (Dadds, Moul, Cauchi, Hawes, & Brennan, 2013). Therefore, no significant findings have thus far emerged from genome-wide methods investigating the etiology of CU traits.

Epigenetic studies. The epigenetic mechanisms involved in CU traits, like other psychiatric phenotypes, are only just beginning to be studied. The serotonin and oxytocin systems are receiving the most attention, which is not surprising given the results of the CG studies reviewed above and the neural mechanisms associated with serotonin and oxytocin. More specifically, oxytocin appears to modulate amygdala activity (e.g., Gorka et al., 2015), serotonin depletion appears to induce some of the core symptomatology of psychopathy (e.g., Marsh et al., 2006), and emerging evidence suggests that peripheral blood levels of serotonin and oxytocin may serve as **biomarkers** for CU traits (Moul et al., 2013; Dadds et al., 2014b).

HTR1B, a serotonin receptor gene that has been investigated as a CG for CU traits, has also been investigated for potential associations between methylation level and CU traits. Using a sample of *N* = 117 youth (aged 3-16) researchers found that CU traits

measured by the APSD and SDQ are positively associated with *HTR1B* methylation (at 19 examined **CpG site**s), but only among individuals who are heterozygous (G/T) at a specific SNV (rs11568817) in the *HTR1B* gene (Moul, Dobson-Stone, Brennan, Hawes, & Dadds, 2015). This unique finding indicates a genotype x methylation interaction in the *HTR1B* gene, a phenomenon that is currently not well characterized. However, the authors of this study hypothesize that increased HTR1B methylation "may serve to counteract the increased transcription of HTR1B that is created by the presence of the minor allele at rs11568817" (Moul et al., 2015, p.11).

Two additional studies have reported associations between CU traits and methylation of the *OXTR* gene in late childhood and early adolescence. First, in a sample of *N* = 84 13-year-olds with CD, level of *OXTR* methylation at birth (at 12 examined CpG sites) was positively associated with CU traits, but only among those without internalizing problems (Cecil et al., 2014). The authors of this study hypothesize that there are distinct etiological pathways to CU traits in individuals with and without associated internalizing psychopathology (Cecil et al., 2014). Second, in two partially overlapping samples (*N* = 156 & 37) of children and adolescents with CD, *OXTR* methylation level (at 11 examined CpG sites) was positively associated with CU traits as well as lower plasma levels of oxytocin. However, these associations were present only in older children (9-16 years old compared to 4-8 years old; Dadds et al., 2014b) which highlights the developmentally dynamic nature of epigenetic modifications.

IV. DISCUSSION

A review of the extant literature revealed 39 quantitative and/or molecular genetic studies on CU traits. Twenty-four of these studies included quantitative components, and the range of heritability reported was 25-80%. However, upon further inspection it appears that the heritability of CU traits in the *general population* of individuals in middle childhood, adolescence, and adulthood (where the construct of CU appears longitudinally invariant [e.g. Obradović et al., 2007]) lies between 36-67%, which is similar to most other temperament and personality traits of childhood and adolescence (for a review, see Polderman et al., 2015). Sixteen studies reviewed here included molecular components. Several SNVs, particularly those involved in the serotonin and oxytocin systems, have been implicated in CU traits. However, replicating significant CG findings has not been particularly successful. Furthermore, no GWA study has thus far identified any associated SNV at a genome-wide level of significance.

One factor that influences the large range of heritability estimates for CU traits is the frequent use of selected samples. This is not surprising given the relative rarity of CU traits in children as well as the historical tendency to consider psychopathology in terms of categorical constructs (APA, 2013). However, many researchers advocate for taking a dimensional approach to psychopathology research (e.g., Widiger and Gore, 2014). Specifically, in genetic analyses where heritability is estimated based entirely on variation between individuals within a specific sample, sample selection at a distribution's tail will produce biased estimates (e.g., Neale et al., 1989). For this reason, it is important to study the heritability of 'extreme' phenotypes (such as CU and psychopathy) in appropriate samples, such as those measured either as continuous or as case-control assuming an

underlying normal distribution. These approaches allow for the study of variation among the general population, including psychopathic and non-psychopathic individuals, as opposed to studying the variation among only extremely psychopathic individuals. At the risk of overgeneralizing, studying the heritability among a case-control or community sample is akin to comparing a psychopath to a normal individual, whereas studying heritability among a highly selected sample is akin to comparing one psychopath to another psychopath – two very different research questions.

Although most quantitative studies indicate that CU traits are substantially heritable, the search for associated molecular genetic variants has not been particularly successful. Although CG studies tend to implicate genes that play important roles in the serotonin and oxytocin systems, these results are infrequently replicated and the percentage of variance accounted for is small. The difficulty in replicating CG results is not unique to CU traits. The limitations of CG studies have been widely noted for some time, especially for complex psychiatric phenotypes whose etiology is likely **multifactorial** and **polygenic** (for a review, see Duncan & Keller, 2011).

The massively polygenic nature of most psychiatric phenotypes, influenced by hundreds to thousands of SNVs of very small effect, serves to highlight the core problems of CG studies; they are underpowered, infrequently replicated, and create unfortunate noise in the literature (Duncan & Keller, 2011). The era of CG studies has been referred to as the “dark era” of psychiatric genetics (Moore, Sawyers, Adkins, & Docherty, 2017), and the National Institute of Mental Health (NIMH) has recently advanced this view in their updated research policies by emphasizing “the need for robust evidence of [genetic] association, generally resulting from adequately powered genome wide association

studies, as opposed to candidate gene approaches." (National Institute of Mental Health, 2017).

Although most researchers believe that CG studies represent obsolete methodology, there is at least some evidence of potential biological plausibility for the serotonin and oxytocin systems given the emerging evidence that peripheral blood levels of these neurotransmitters may serve as biomarkers for CU traits (Moul et al., 2013; Dadds et al., 2014b). However, despite the potential biological plausibility, the research to date is insufficient to suggest a genetic association between these genes and CU traits. Much work is needed in this area, and it is our view that researchers should focus on the genome-wide methodology that will allow for statistically sound inferences to be drawn.

GWA studies are one potential way to use genome-wide molecular data to elucidate novel genetic etiology. However, only two studies have examined CU traits using this methodology, and neither resulted in successful identification of associated variants. These null results are not entirely surprising given the relatively small sample sizes of these studies (N < 3,000) compared to other successful GWA studies. This is especially true considering the first genome-wide significant variants in schizophrenia GWA studies were only identified after data on more than 21,000 individuals had been analyzed (The Schizophrenia Psychiatric Genome-Wide Association Study Consortium [Schizophrenia PGC], 2011). However, the success of schizophrenia GWA, as evidenced by gene identification, replication, and phenotype prediction (e.g., Docherty et al., 2017; Hamshere et al., 2013; Schizophrenia PGC, 2011; Vassos et al., 2017), may not be obtainable for traits where amassing such large samples is unlikely.

Despite the very large samples required for GWA studies, some newer genetic techniques allow for the examination of genetic associations with more modestly sized samples (for a review see Moore et al., 2017). One of these methods, the **polygenic risk score (PRS)**, uses summary statistics from previous well-powered GWA studies to assign individuals a quantitative risk-score that can be used to predict the same, or a different, phenotype (Wray et al., 2014; Moore et al., 2017). However, this approach does not allow the discovery of new risk variants.

Another genetic methodology that has recently become more cost-effective is **next-generation genome sequencing**, which sequences the whole **genome** or **exome** of individuals with the primary aim of identifying rare causal mutations responsible for disease (ten Bosch & Grody, 2008). This is a particularly interesting avenue for future research on CU traits, especially in light of the discrepancy between heritability reported in twin studies (25-80%) vs. GCTA (7%; Viding et al., 2013). Since GCTA only indexes *common* genetic variants, one potential explanation for the discrepancy in heritability estimates across study methodologies is the presence of *rare* genetic variants of large effect, which would potentially be probed in sequencing studies. However, sequencing can require even larger sample sizes than GWA since even more variants are tested.

Given the current scarcity of significant replicated genetic findings, it is important to remember that a large proportion of the variance in CU traits (20-75%, depending on the study) is accounted for by environmental factors. Environmental contributions to CU etiology are arguably easier to identify and are certainly more easily modifiable with clinical intervention. For example, parenting behaviors are one potential environmental mechanism influencing CU traits. In a recent study by Hyde and colleagues (2016),

positive reinforcement provided by an adoptive mother was found to be protective of CU traits even in the presence of significant genetic risk (i.e., a biological mother with antisocial traits). Furthermore, a similar study used an **MZ-differences design** to control for genetic effects and determined that negative parental discipline, while a unique environmental risk factor for CD, was not a salient factor in the development of CU (Viding, Fontaine, Oliver, & Plomin, 2009). Taken together, this research suggests that specific parenting practices such as increased positive reinforcement, but not negative reinforcement, represent targetable environmental experiences that may potentially counteract one's genetic risk for CU. Therefore, specific parenting practices represent important targets for future clinical and prevention research.

Future Directions

Based on the reviewed literature, it is clear that the search for molecular genetic mechanisms underlying CU traits has only just begun. Most researchers have thus far chosen to focus on candidate genes studies, and these results have been infrequently replicated. Therefore, we recommend researchers focus on genome-wide approaches to understanding CU traits, including GWA and PRS studies. This type of research will require increasingly large sample sizes, and collaboration between research groups will be advantageous. Researchers with genome-wide data on CU traits should consider following the lead of other groups and forming a CU consortium, in the style of the psychiatric genomics consortium (PGC; www.med.unc.edu/pgc) or genetics of personality consortium (GPC; www.tweelingenregister.org/GPC/). Such collaborations will increase the speed and quality of the discoveries regarding the underlying genetic mechanisms of CU traits.

Conclusions

Although CU trait variance appears to be substantially influenced by genetic factors, the search for replicable genetic mechanisms has been, thus far, largely unsuccessful. Given the current lack of molecular genetic associations, paired with the heterogeneous and pathological nature of CU traits, it is likely that very large sample sizes and advanced genetic methodology will need to be employed in the search for its underlying genetic etiology. Future research should seek to elucidate relevant genetic etiology while also striving to identify environmental factors that are targetable mechanisms for prevention and intervention.

CHAPTER 4. THE IMPACT OF AGE AND SEX ON THE GENETIC AND ENVIRONMENTAL ETIOLOGY OF CALLOUS-UNEMOTIONAL TRAITS

I. SPECIFIC AIM

Chapters 1 and 3 describe the differences in CU traits based on age and sex as well as the potential environmental and genetic mechanisms underlying these traits. Despite the vast amount of research into genetic and environmental influences on CU/psychopathic traits, how these specific influences vary by age and sex is still not well-understood. The current study will seek to elucidate the etiological nuances of CU traits via the following aims: 1) To examine and replicate the phenotypic associations between age, sex, and CU traits; 2) To examine potential qualitative and quantitative effects of sex on the heritability of CU traits; 3) To examine age-moderated heritability of CU traits, estimating the relative influence of genes and environment along a continuum of ages ranging from 9-20 years old.

II. ANALYSES

As a first step in our analyses, and because age and sex are primary variables of interest in the current study, the parent report ICU scale was examined for measurement invariance across our variables of interest: sex (male vs. female) and study/age (JAS [9-

14] vs. AYATS [15-20]). This was accomplished via multi-group confirmatory factor analysis (CFA) in the Amos program (Arbuckle, 2014), whereby sets of parameters are constrained across groups in order to test their equivalence. To account for the non-independence of twin-based observations, these analyses were performed for a random half of the sample (one twin from each pair) and replicated in the second half of the sample. This semi-independent replication sample allows us to decrease potentially spurious results by reporting only those findings that emerge in both samples. Furthermore, our large sample (N > 1,400) means that loss-of-power due to splitting the sample was not a major concern.

We next examined potential demographic associations with ICU sum score, including sex, age, and their interaction. These associations were examined via linear regression in the R software environment (R Core Team, 2015). In order to account for the non-independence of twin observations, the “geeglm” function from the GEE package (Carey, 2015) was used with an exchangeable correlation structure.

Heritability of ICU sum score was examined using the classical twin model (i.e., biometrical structural equation modeling [SEM]; Neale & Cardon, 1992) via the OpenMx package (Neale et al., 2016) for the R program (R Core Team, 2015). Biometrical SEM generally decomposes trait variance into three constituent parts: Additive genetic effects (A), common/shared environmental effects (C), and unique/non-shared environmental effects (E). A reflects the additive effect of all genetic alleles that vary within the population (i.e., polymorphic alleles). Theoretically, MZ twins share 100% of their polymorphic alleles, whereas DZ twins share, on average, 50% of their polymorphic alleles. Therefore, the effects of A contribute twice as much to the correlation (i.e., similarity) of MZ twins vs.

the correlation of DZ twins. C reflects environmental factors that are shared between twins (e.g., socioeconomic status, parenting practices, geographic location, etc.) and therefore equally contributes to MZ and DZ correlations. E reflects environmental influences unique to one twin and also includes measurement error. Therefore, E is uncorrelated for both MZ and DZ twins. In order to determine which sources of variance are significant, models with specific parameters constrained to equal zero are compared to a full, unconstrained, model. These comparisons examine differences in degrees of freedom (Δ df) and -2 log-likelihood (Δ -2LL) between models, which follow a χ^2 distribution. A significant χ^2 value for these comparisons indicates that the constrained model significantly deteriorates the fit of the full model, and the constrained model is therefore rejected.

Quantitative sex effects on heritability reflect differences in the *amount* of genetic influence on trait variance in males vs. females. Alternatively, qualitative sex effects refer to *different sets of genes* influencing trait variance in males and females. To test for qualitative sex effects, a model with a decay parameter (r_G) that allows the opposite-sex genetic correlation to be estimated at less than .5 (the typical DZ genetic correlation) was compared to a model without this parameter. Because quantitative sex effects are not interpretable in the presence of qualitative sex effects, quantitative effects were only examined if qualitative effects were not supported. Quantitative sex effects are tested by constraining the A, C, and E paths to be equal for males and females and comparing this to a model where these parameters are allowed to vary.

The best-fitting model with appropriate sex-effects was moved to another phase of model testing for age-effects on heritability of ICU score. In these analyses, age was used as a moderator (i.e., definition variable) on both the mean level of the trait as well as the

variance paths (A, C, and E). To test for significant moderation, models without mean and/or variance moderation were compared to a model where both these moderators were included.

Based on the above analyses, the best-fitting heritability model with appropriate sex- and age-effects was further constrained to test the significance of individual sources of variance (A & C). This was accomplished by constraining paths for specific sources of variance and comparing these models to the best-fitting sex- and age-effects model determined above. A final best fitting model was determined from these analyses.

III. RESULTS

Invariance Across Sex and Age

The parent-report ICU was examined for measurement invariance across sex (male vs. female) and age (JAS [9-14] vs. AYATS [15-20]) using multi-group CFA. The factor structure used in all invariance testing was the bi-factor model previously reported as best-fitting in the JAS dataset (see Moore et al., 2017). This model consists of one general factor loading onto all 24 ICU items and 2 residual factors loading on 5 items corresponding to items indexing features of unemotionality & 6 items indexing features of apathy or lack of concern about performance, respectively. This model was tested in the AYATS sample before invariance testing was performed, and fit was adequate to good (CFI = .982; TLI = .979; RMSEA = .068).

For each comparison (male vs. female & younger vs. older), three omnibus χ^2 tests were conducted where a set of parameters (i.e., factor loadings, factor variances/covariances, and item residuals) were constrained to be equal across groups,

and these models were compared to an unconstrained model. These tests were performed in a hierarchical manner such that each level of constrained parameters contained the level(s) that had been tested previously. Table 4.1 displays the χ^2 tests across both comparison groups in both split-half samples. Overall, 11 of the 12 omnibus tests were significant at $p < .05$.

Table 4.1. Model Fit Statistics for Invariance Testing via Constrained Parameters, Assuming Unconstrained Model is Correct.

Parameters Constrained	Sample 1			Sample 2		
	DF	X^2	*p*	*DF*	X^2	*p*
Comparison: Males vs. Females						
Factor Loadings	32	54.117	.009	32	64.125	.001
Factor Variances/Covariances	36	64.221	.003	36	70.170	.001
Item Residuals	60	178.347	.032	60	169.470	< .001
Comparison: Younger vs. Older						
Factor Loadings	32	59.945	.002	32	83.920	.014
Factor Variances/Covariances	36	76.225	< .001	36	100.693	.016
Item Residuals	60	354.255	< .001	60	313.205	.051

Note: Each level of constrained parameters also contains constrained parameters for all previous levels.

However, it is important to note that for each overall invariance test (male vs. female & younger vs. older) there were two split-half samples used, and each model contained 60 parameters, for a total of 240 individual parameter comparisons. Table 4.2 lists the associated z-values for all 240 comparisons. Due to the large number of comparisons and our large sample size, differences in individual parameter values across groups were examined for significance using a Bonferroni-correction for 60 tests ($p <$

Table 4.2. Z-Values for Individual Parameter Comparisons

Parameter	Comparison			
	Males vs. Females (sample #1)	Males vs. Females (sample #2)	Younger vs. Older (sample #1)	Younger vs. Older (sample #2)
Factor Variance/Covariance Parameters				
Variance of 'General'	1.268	1.968	-0.348	-0.316
Variance of 'Unemotional'	-0.696	0.911	0.598	-1.025
Variance of 'Apathetic'	0.485	1.367	1.088	0.857
Covariance of 'Apathetic' & 'Unemotional'	-1.613	-2.644	-0.086	-1.803
Factor Loadings				
'General' Item #1	NA	NA	NA	NA
'General' Item #2	-0.939	-0.116	1.444	1.515
'General' Item #3	-1.410	-1.026	1.71	1.099
'General' Item #4	-2.127	-1.570	1.453	0.911
'General' Item #5	-1.171	-1.306	0.861	0.165
'General' Item #6	0.626	-1.974	2.030	2.484
'General' Item #7	0.566	-1.385	1.663	2.048
'General' Item #8	-1.015	-1.523	0.709	0.407
'General' Item #9	-1.378	-1.382	1.814	0.985
'General' Item #10	-0.217	-1.08	-0.061	1.068
'General' Item #11	0.033	-0.761	1.726	1.292
'General' Item #12	-1.601	-1.494	2.258	2.102
'General' Item #13	-1.239	-1.500	1.074	0.223
'General' Item #14	-0.439	-2.168	1.109	0.832
'General' Item #15	-0.747	-1.086	1.357	1.108
'General' Item #16	-0.869	-1.615	0.620	0.421
'General' Item #17	-1.289	-1.534	0.738	0.689
'General' Item #18	-1.249	-1.630	1.317	0.537
'General' Item #19	0.169	-1.570	0.010	1.918
'General' Item #20	-0.553	-0.983	1.566	0.886
'General' Item #21	0.302	-0.494	-0.139	1.818
'General' Item #22	-1.021	-0.577	2.249	1.491
'General' Item #23	-1.353	-1.159	1.262	0.954
'General' Item #24	-0.695	-1.484	0.687	0.888
'Unemotional' Item #1	NA	NA	NA	NA
'Unemotional' Item #6	2.308	0.706	2.447	**3.645**
'Unemotional' Item #14	0.872	-0.719	0.211	1.690
'Unemotional' Item #19	1.292	-0.904	-0.067	1.993
'Unemotional' Item #22	0.265	-1.687	0.791	1.528
'Apathetic' Item #3	NA	NA	NA	NA
'Apathetic' Item #7	-0.056	0.570	0.791	-0.800
'Apathetic' Item #11	1.508	0.713	-1.805	-0.151
'Apathetic' Item #15	0.016	-1.795	-0.421	-0.602

Table 4.2 - Continued. Z-Values for Individual Parameter Comparisons

	Comparison			
Parameter	Males vs. Females (sample #1)	Males vs. Females (sample #2)	Younger vs. Older (sample #1)	Younger vs. Older (sample #2)
'Apathetic' Item #20	2.194	0.108	-2.330	-1.380
'Apathetic' Item #23	-0.137	-2.223	-1.285	0.061
Item Residuals				
Item #1	-0.380	-2.434	1.656	**3.503**
Item #2	**-3.224**	-1.895	**4.442**	**3.839**
Item #3	-0.834	-1.814	-1.263	-0.478
Item #4	**-4.800**	2.837	**6.703**	**4.322**
Item #5	-0.149	-2.230	0.803	1.848
Item #6	-0.349	-0.183	2.847	**3.997**
Item #7	2.562	-0.780	0.666	-0.2630
Item #8	1.213	2.401	2.646	**3.717**
Item #9	**4.119**	-2.401	**3.691**	**6.391**
Item #10	-0.183	1.193	-0.930	0.458
Item #11	**3.310**	2.374	0.173	2.543
Item #12	0.701	1.966	**7.074**	**3.870**
Item #13	0.487	1.322	-0.169	1.123
Item #14	-0.275	0.028	0.582	0.680
Item #15	2.003	2.211	-0.616	-0.270
Item #16	0.253	2.309	0.939	2.405
Item #17	0.253	2.070	-0.832	-0.670
Item #18	2.457	2.087	-1.292	-0.737
Item #19	-0.063	0.510	1.005	-0.927
Item #20	-0.833	2.129	**4.193**	0.430
Item #21	**-3.199**	-2.847	-0.113	**-3.458**
Item #22	0.812	1.626	**4.433**	2.073
Item #23	1.555	2.523	0.094	-1.472
Item #24	-0.680	-0.526	2.809	0.318

Note: *Z*-values > 3.145 are bolded, indicating statistical significance after Bonferroni-correction for 60 comparisons ($p < .00083$). NAs represent factor loadings that were constrained to equal 1.

.00083). At the corrected significance threshold, no significant parameter differences were replicated in the two split-half samples across males and females. Furthermore, only 4 item residuals (items #2, 4, 9, # 12) were significantly different across the younger and older group in both split-half samples. Therefore, the ICU appears to be invariant across

sex and younger vs. older cohorts with the exception of a few item residuals that do not change the overall interpretation of the scale.

Phenotypic Associations

Linear regression was used to examine the associations between ICU sum score and the predictors of age, sex, and the interaction of age and sex. The results of the individual and multiple regressions are presented in Table 4.3. In individual regressions, both age and sex significantly predicted ICU sum score. Females demonstrated an average ICU score that was approximately 3.1 units lower than males (β = -3.077; p = 1.4 x 10^{-9}). Furthermore, each successive year of age predicted an increase of approximately 0.6 units in ICU score (β = 0.5813; p = 5.2 x 10^{-12}).

Table 4.3. Linear Regression Estimates for Age and Sex Predicting ICU Sum Score

Predictor	β	*p*
Individual Regressions		
Sex	-3.077	1.4 x 10^{-9}
Age	0.5813	5.2 x 10^{-12}
Multiple Regression		
Sex	2.358	.311
Age	1.216	1.4 x 10^{-5}
Sex*Age	-0.395	.020

When these predictors plus their interaction were included in a single multiple regression, the results were generally consistent. The effect of age remained significant,

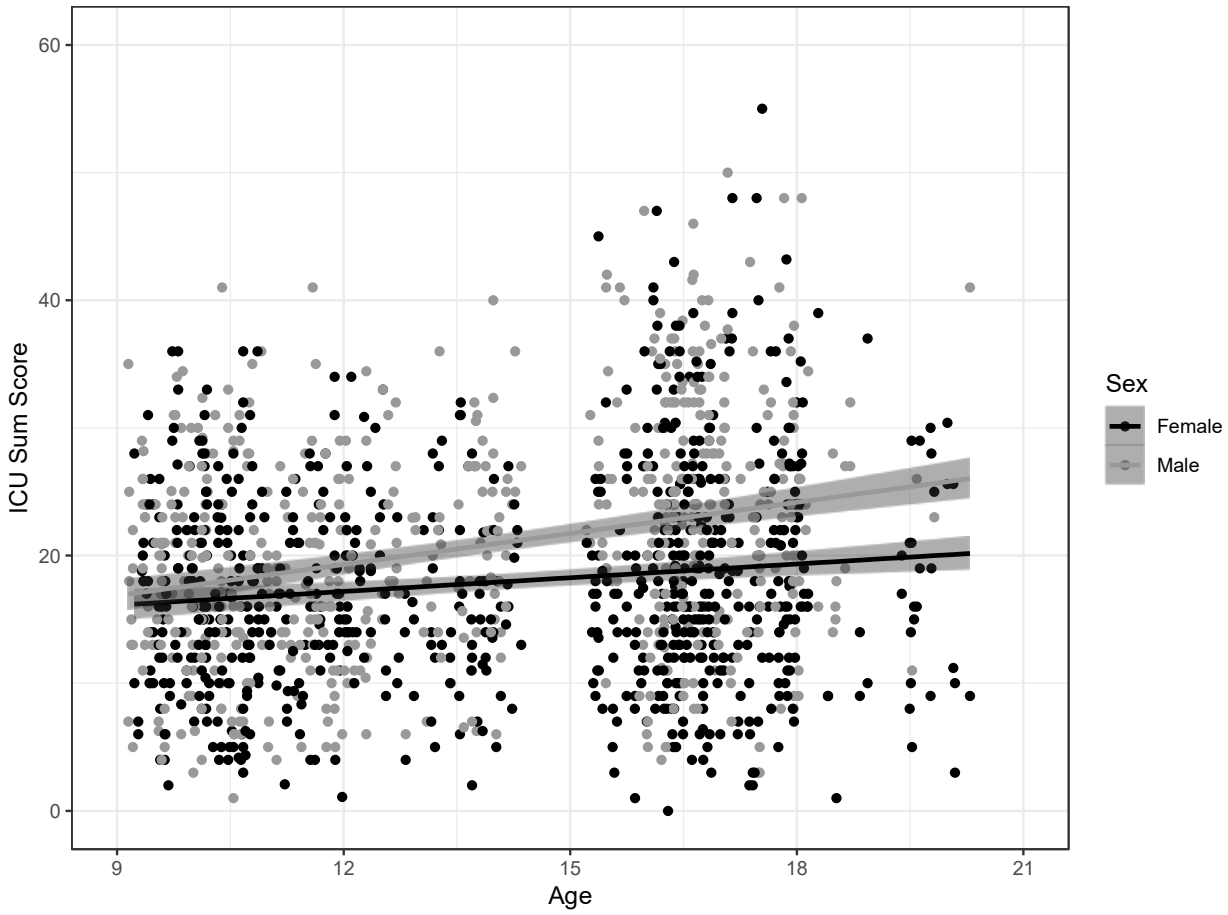
albeit slightly stronger, with each year of age predicting an increase of approximately 1.2 units in ICU score (β = 1.216; p = 1.4 x 10^{-5}). Although the main effect of sex was no longer significant in this model (β = 2.358; p = .311), there was a significant interaction of sex and age (β = -0,395; p = .020).

ICU score was regressed on age in males and females separately, and although both regressions coefficients were significant, the relationship between age and ICU score for males (β = .824, p = 9.8 x 10^{-11}) was approximately twice as strong as females (β = .419, p = .002). In order to visualize this interaction, Figure 4.2 plots all participants ICU score by their age, with separate colors used for males (gray) and females (black). The regression lines for males and females are overlaid. This figure demonstrates that the average ICU score at age 9 is approximately equal (around 17) for males and females. Although ICU score tends to increase over the developmental period of 9-20, this increase is substantially faster for males, who increase an average of approximately 6 points, whereas the average female ICU score only increases by approximately 3 points.

Sex-Moderated Heritability

Table 4.4 presents the cross-twin correlations for ICU sum score presented for each of the five zygosity groups (MZM, MZF, DZM, DZF, DZOS). The point estimates from this table indicate the potential presence of genetic sex effects. That is, both male-male and female-female correlations across DZ twins is approximately .2. However, the male-male and female-female correlations across MZ twins are approximately .57 & .38, respectively, indicating potential quantitative effects. Furthermore, the opposite-sex DZ correlation, .06, is lower than the same-sex DZ correlations, around .2, which is indicative of potential qualitative sex effects. However, the confidence intervals for these

Figure 4.2. Age x Sex interaction for ICU Sum Score in Combined JAS/AYATS Sample



correlations are too large to suggest that these estimates are significantly different from one another.

The upper panel of Table 4.5 displays the results of model fitting to determine the significance of quantitative sex effects, qualitative sex effects, and sex-effects on the mean. Constraining the r_G parameter to indicate the absence of qualitative sex effects (model II), did not significantly deteriorate (p = .231) the fit of the full model (model I), and therefore the presence of qualitative sex effects was not supported. Next, a model without

Table 4.4. Cross-Twin Correlations for ICU Sum Score by Zygosity

	Female-Female *r* (95% CI)	Male-Male *r* (95% CI)	Opposite Sex *r* (95% CI)
MZ	.376 (.227-.507)	.566 (.416-.685)	NA
DZ	.196 (.024-.356)	.198 (.009-.374)	.063 (-.077-.201)

Note: DZ = dizygotic; MZ = monozygotic

quantitative or qualitative sex effects was compared to the full model. This was accomplished by constraining r_G to indicate the absence of qualitative sex effects and constraining the variance component paths to be equal across sex. This model (model III) did not significantly deteriorate (p = .052) the fit of the full model, and therefore the presence of quantitative sex effects was not supported. Finally, dropping mean-level sex effects (model IV) significantly deteriorated the fit ($p = 1.9 \times 10^{-6}$) of the full model, and therefore a mean-level sex effect was supported. The only sex-specific parameter that was moved into the next phase of model fitting (age-moderation analyses) was effect of sex on mean ICU score.

Age-Moderated Heritability

The middle panel of Table 4.5 displays the model fitting results to determine the significance of age-moderation on the variance paths and age effects on the mean. Dropping the age-moderation parameters on the variance components (model VI) significantly deteriorated the fit ($p = 4.1 \times 10^{-5}$) of the full model (model V), indicating

Table 4.5. Model-Fitting Results for Determining the Significance of Sex-Moderation, Age-Moderation, and Components of Variance on ICU Score.

		Sex Moderation			Age Moderation							
Model	Variance Comp.	Means	Qual. Var.	Quant. Var.	Means	Variance Comp.	# Param.	df	-2 LL	Δ df	Δ -2 LL	*p*
Testing Sex Moderation												
I	ACE	+	+	+	-	-	10	1365	9790.030	NA	NA	NA
II	ACE	+	-	+	-	-	9	1366	9790.779	1	0.749	.387
III	**ACE**	**+**	**-**	**-**	**-**	**-**	**6**	**1369**	**9795.106**	**4**	**5.076**	**.280**
IV	ACE	-	+	+	-	-	9	1366	9812.718	1	22.688	1.9×10^{-6}
Testing Age Moderation												
V	**ACE**	**+**	**-**	**-**	**+**	**+**	**9**	**1370**	**9758.916**	**NA**	**NA**	**NA**
VI	ACE	+	-	-	+	-	6	1373	9781.898	3	22.981	4.1×10^{-5}
VII	ACE	+	-	-	-	+	8	1371	9801.167	1	42.251	8.0×10^{-11}
Testing Variance Components												
VIII	ACE	+	-	-	+	+	9	1370	9758.916	NA	NA	NA
IX	**AE**	**+**	**-**	**-**	**+**	**+**	**7**	**1372**	**9758.925**	**2**	**0.008**	**.996**
X	CE	+	-	-	+	+	7	1372	9774.313	2	15.397	4.5×10^{-4}

Note: Bold indicates best-fitting model (most parsimonious without significant deterioration in fit) for each level of testing. Qual. Var. = qualitative sex effects on variance components. Quant. Var. = quantitative sex effects on variance components. Means = sex- or age-moderation on means. Variance Comp. = components of variance. # param. = number of parameters. df = degrees of freedom. A = additive genetic effects. C = common/shared environmental effects. E = unique/non-shared environmental effects.

significant age-moderation on genetic and environmental factors influencing ICU variance. Dropping the age effects on the mean (model VII) also significantly deteriorated the fit (p = 8.0 x 10^{-11}) of the full model, and therefore the presence of a mean-level age effect was supported. Therefore, the best-fitting model thus far included moderation of the variance components by age as well as mean-level effects of both age and sex.[5] This model was then moved into our final set of heritability analyses to determine the significance of individual components of variance.

Significance of Variance Components

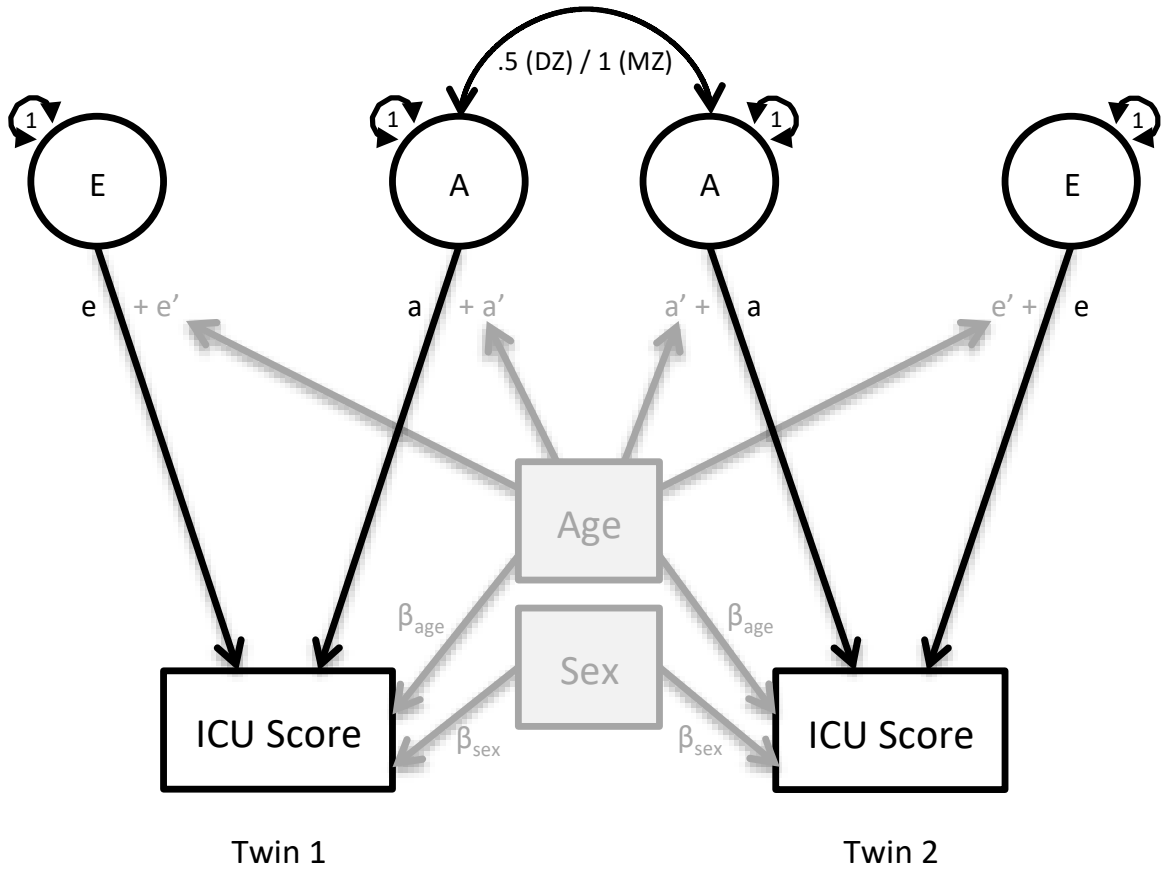
The lower panel of Table 4.5 displays the model fitting results to determine the significance of additive genetic and common environmental components of variance (because E contains sources of measurement error, dropping it from the model is generally ill-advised). Model VIII reflects the best-fitting model from analyses performed in the previous two sections. Constraining C to equal zero (model IX) did not significantly deteriorate the fit (p = .996) of the full model (model VIII), and indicates that C is not a salient factor influencing CU trait variance in the current model. However, constraining A to equal zero (model X) significantly deteriorated the fit (p = 4.5 x 10^{-4}) of the full model, indicating that A is a salient factor influencing CU trait variance in the current model.

Effects of Genes, Environment, Sex, and Age on ICU Score

The final model reflecting the influences of age and sex on the etiological factors contributing to ICU variance is presented in Figure 4.3. This model reflects the fact that

[5] Due to the small number of 20 year olds (N = 3) in the current sample, all age-moderation analyses were repeated with these 3 individuals removed, and results were very consistent with the results reported here.

Figure 4.3. Best-Fitting Biometrical Model For ICU Score Including All Significant Tested Parameters



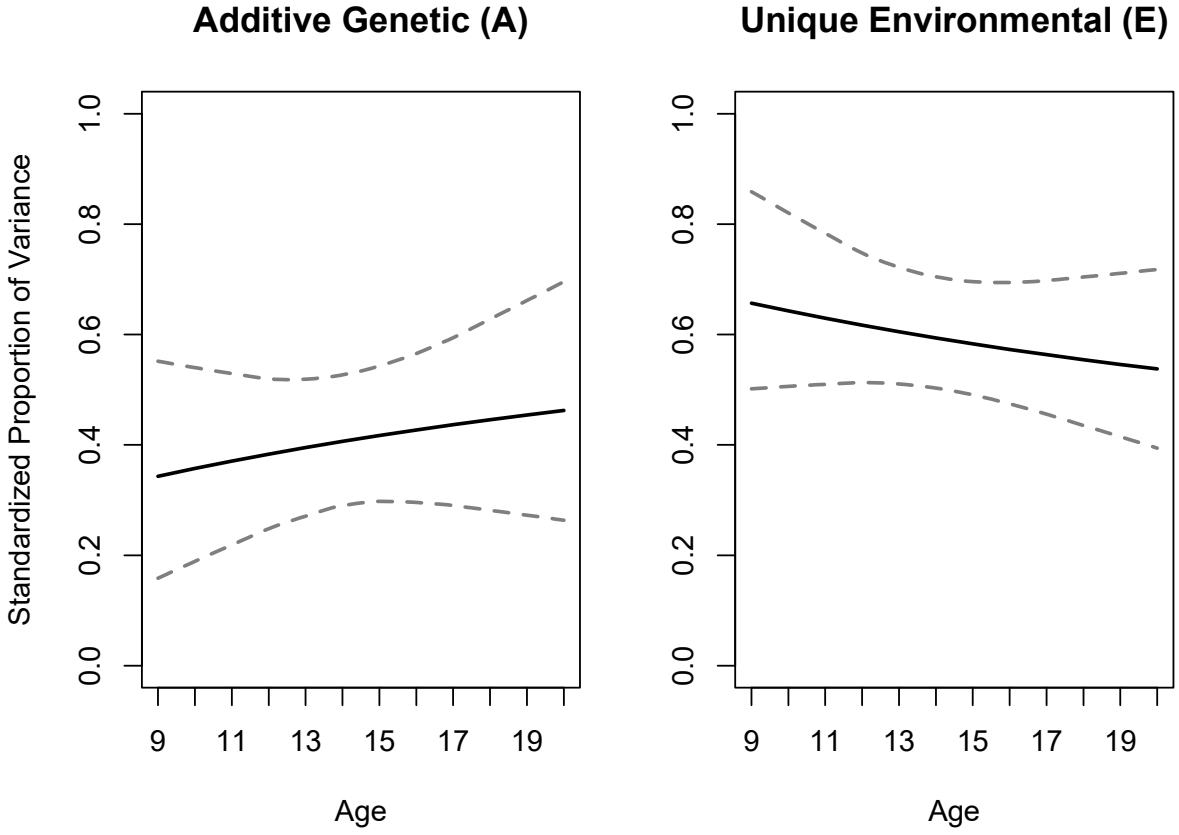
additive genetic (A) and unique environmental (E) factors explained the variance ICU score, and common environmental factors (C) did not. Furthermore, there is significant age-moderation on these components of variance, and age and sex also influence mean ICU score.

In the best-fitting biometrical model (see Figure 4.3), females had an average ICU score 3 units lower than males (β_{sex} = - 2.983; 95% CIs: -3.933, -2.037). Furthermore,

each year increase in age predicted an increase in ICU score of approximately .5 units (β_{age} = 0.550; 95% CIs: 0.386, 0.713). As expected, these results closely mirror the phenotypic regressions reported earlier in this study.

To interpret the age-moderated variance components, estimates of standardized and raw variance were plotted, along with their 95% confidence intervals at each year of age between 9-20. Figure 4.4 displays the standardized genetic (A) and unique environmental (E) sources of variance between 9 and 20 years of age. Over this time

Figure 4.4. Standardized Proportions of Variance in ICU Sum Score Between Ages 9 and 20

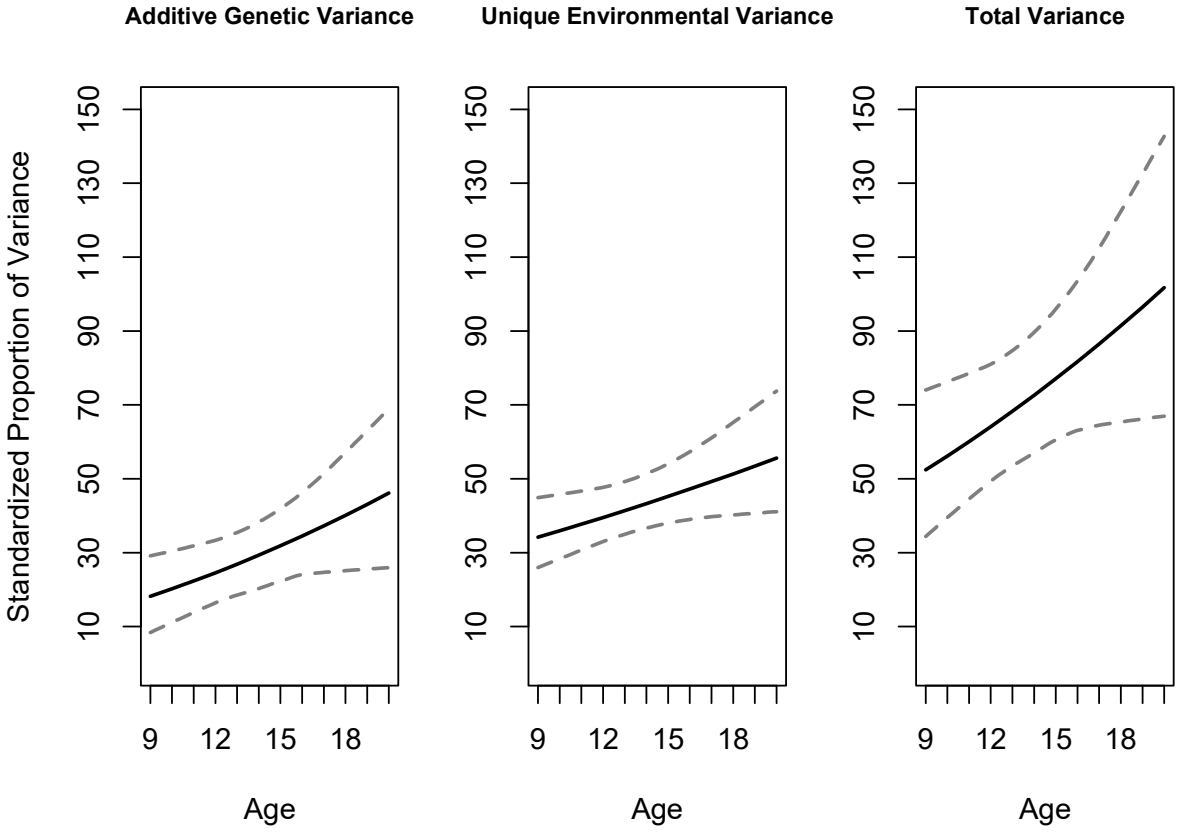


period, the influence of additive genetics increases slightly, from 34.3% at age 9 to 46.2% at age 20. Conversely, the influence of unique environment decreases slightly, from 65.7% at age 9 to 53.8% at age 20. Figure 4.5 displays the raw variance (additive genetic, unique environmental, and total variance) between 9 and 20 years of age. This graph provides us with more information about why the *relative* proportion of additive genetic variance increases over time. As seen in the right panel of Figure 4.5, total ICU variance increases substantially over this time period, from 52.4 at age 9 to 101.7 at age 20. Within this raw total variance, both additive genetic and unique environmental variance increase, although additive genetic does so with a slightly steeper slope, from 18.2 at age 9 to 46.12 at age 20 (compared to E variance which increases from 33.2 at age 9 to 55.6 at age 20). Therefore, the *relative* proportion of additive genetic influence (i.e., heritability) increases slightly over the developmental period of 9-20 years of age.

I. DISCUSSION

We have reported here the results from a study on the effects of sex and age on CU trait etiology. We used a large, cross-sectional sample of twins (N = 1,384) and the methodology originally described by Purcell (2002) for examining gene-environment interactions in twin samples. Both age and sex were significantly associated with mean level of CU traits, with males and older participants having higher ICU scores. Furthermore, there was an interaction between age and sex predicting ICU score, such that males had a steeper increase of CU traits over the ages of 9-20 compared to females. A series of nested structural equation models were fit to examine the sources of variance

Figure 4.5. Raw Variance in ICU Sum Score Between Ages 9 and 20



influencing ICU score across development. Results indicated a significant mean-moderation by age and sex and a significant moderating effect of age on the genetic and environmental components contributing to the observed variation in CU traits. That is, the proportion of ICU variance due to genes and environment appears to vary depending on the age of an individual. These results are consistent with the concept of gene-environment interplay (Rutter, Moffitt, & Caspi, 2006) which suggests that the heritability of a trait is often developmentally dynamic, as is often the case during the period of

adolescence (e.g., Dick, Adkins, & Kuo, 2016). Furthermore, and consistent with previous literature (e.g., Moore et al., 2019), only the influence of additive genetics (A) and unique environment (E) significantly contributed to the variance in ICU score across development.

The heritability (i.e., the proportion of genetic influence) of CU traits increased from around 34% at age 9 to around 46% at age 20. Inspecting the raw variance throughout development reveals that although environmental variance does increase substantially (almost doubling from around 33 at age 9 to around 56 at age 20), the genetic variance increases at a slightly faster rate (from around 18 at age 9 to around 46 at age 20). This slightly faster increase in genetic variance means that the *proportion* of variance due to genetic effects (i.e., the heritability) increases over this time period.

There are several potential reasons for the increased genetic variation across development. One potential cause of increased genetic variation may actually be related to increases in environmental variation. That is, as children become adolescents they are exposed to an increasing number of social behaviors through media, school, peers, and the community. This increased environmental variation might reflect a loosening of environmental control over children's behavior, allowing an adolescent's underlying genetic predisposition to be expressed. Another explanation for the increased genetic variation is active r_{GE}, whereby adolescents select environments, such as antisocial or callous friend groups, and these selected environments elicit underlying genetic predispositions. Although this is the first study to demonstrate increased genetic variation during adolescence for the *emotional* characteristics of psychopathy, a body of research has demonstrated increasing genetic variation in adolescence for related *behavioral*

characteristics, such as antisocial behavior, aggression, and delinquency (for a review, see Bergen, Gardner, & Kendler, 2007).

The genetic principals described above are similar to those described by Albert Bandura's Social Cognitive Theory (Bandura, 1986). A core component of this theory is *triadic reciprocal determinism*, which is the interaction between the person's behavior (B), the environment (E), and the person's biological and psychological characteristics (P), which are likely genetically mediated. Bandura also proposed three categories of environments: Those that are created via our behavior, those that are imposed upon us, and those that we actively select. These environmental categories are reminiscent of the three types of gene-environment correlations (evocative, passive, and active, respectively) described by Rutter, Moffitt, and Caspi (2006). Of particular importance to CU traits, Bandura proposed *vicarious reinforcement* as a learning mechanism, similar to operant conditioning, whereby children observe and internalize the rewards other children receive for their behavior (Bandura, 1986). In the case of CU traits, children and adolescents may observe CU traits and antisocial behavior in others as a set of characteristics that elicit social power/status, attention from others, and even physical rewards (money, stolen property, etc.). These callous and antisocial characteristics, as displayed by the self and others, represent the behavioral and environmental components of a triadic interaction. These two factors might interact with Bandura's third proposed factor, the person's psychology/biology, such that individuals' underlying genetic predispositions are able to be are expressed. Bandura's theoretical framework may help to explain the observed increase in genetic variation during adolescence and young adulthood.

Limitations and Future Directions

The results of the current study should be interpreted in light of several limitations. First, our sample was relatively homogenous and will likely limit the generalizability of the reported results. Specifically, our sample was recruited from a single region (Mid-Atlantic) in the United States and was comprised primarily of Caucasian individuals. Caucasians were purposefully oversampled in the two datasets due to the planned inclusion of molecular genetic tests (where statistical power is maximized in relatively small samples by including only one ancestry group.) Biological and environmental factors contributing to CU traits may differ in different populations, so replication across a range of cultures and ethnicities is an important consideration for future research.

Second, although our study methodology was able to assess differences due to age, it should be reiterated that our study design was cross-sectional in nature. This leaves open the possibility that the greater heritability observed in older individuals could be a cohort effect. Therefore, future research should replicate the findings reported here in longitudinal samples that are more suited for disentangling age and cohort effects.

Conclusions

We have reported here the results from the first study to examine the relative proportion of genetic and environmental influences on CU traits over a large age range (9-20 years) that is of significant importance for the emergence and development of CU traits. Our results indicate that the heritability of CU traits increases with age, from around 34% at age 9 to around 46% at age 20. However, raw estimates indicate a substantial increase over this time period in both genetic and environmental variance. Researchers should seek to clarify this developmental process in longitudinal samples. Furthermore,

clinicians should stress the importance of early environmental intervention for CU traits, especially in light of the current results suggesting the relative importance of environmental factors is highest in childhood.

CHAPTER 5. EXAMINING ELECTROMYOGRAPHIC STARTLE REFLEX AS A POTENTIAL ENDOPHENOTYPE FOR CALLOUS-UNEMOTIONAL TRAITS

I. SPECIFIC AIM

Chapter 1 details the body of research suggesting that decreased autonomic arousal to fearful stimuli, including electromyographic (EMG) startle reflex, is associated with CU and psychopathic traits. Although the phenotypic relationship between these traits is well established, no study has yet investigated startle as a potential endophenotype for CU or psychopathic traits. Therefore, the aims of the current chapter are two-fold. First, to examine the phenotypic association between CU traits, age, sex, baseline startle reflex and fear-potentiated startle. Second, to examine the genetic relationship between CU traits and startle phenotypes in the current sample using a multivariate model of genetic covariance.

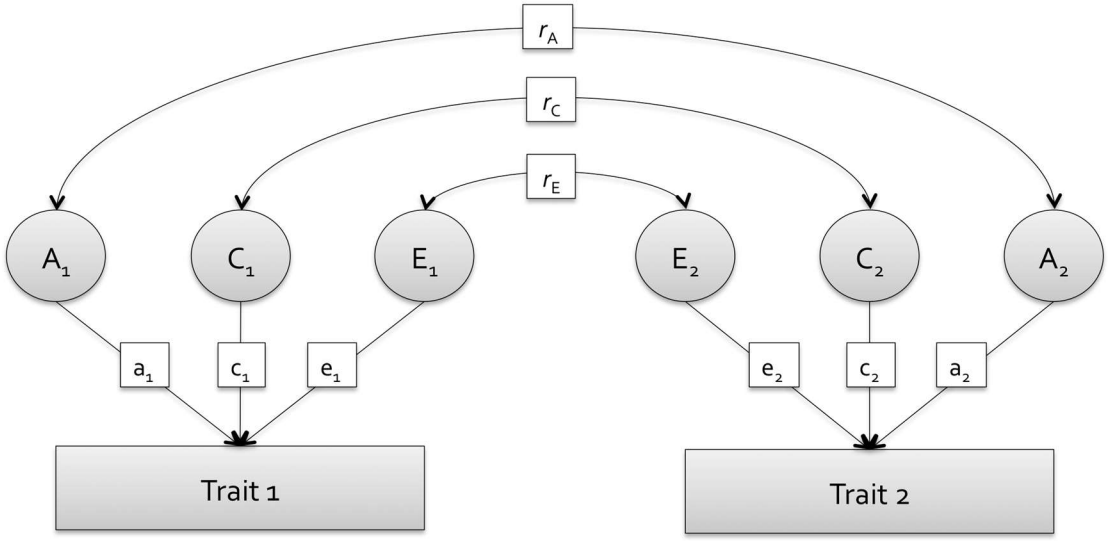
II. ANALYSES

The relationship between CU traits and startle measures (SR and FPS) were examined via multiple linear regressions. Each multiple regression controlled for age, sex, and non-independence of twin observations (familial clustering). For each test, age- and sex-moderation were also examined. Due to the different startle paradigms used in JAS

and AYATS, all analyses in this chapter were performed separately for each study. A *p*-value less than .05 was used to determine suggestive significance, and a Bonferroni-corrected *p*-value less than .025 (corrected for 2 tests corresponding to two studies) was used to determine significance. A Bonferroni-correction for two tests was chosen because although baseline and fear-potentiated startle represent two separate tests in two separate samples, previous research (in addition to the current study) demonstrates a very high correlation between these two startle measures (Savage et al., 2019). Therefore, measures of SR and FPS are not likely indexing independent phenotypes.

Studies (JAS or AYATS) with startle measures significantly associated with CU traits were moved into a multivariate genetic analysis to examine the genetic covariance/correlational structure between CU traits, baseline startle, and fear potentiated startle. A correlated factors model (CFM) was chosen over other bivariate genetic models (i.e., Cholesky model) to accurately represent the endophenotypic relationship being tested whereby two or more traits are hypothesized to share all or some of their genetic underpinnings. An example of the CFM is depicted in Figure 5.1. In this model, the variance of each trait is decomposed into its constituent sources of variance (A, C, and E factors) in the same fashion as the classical twin model. In addition, the CPM uses cross-twin cross-trait correlations to estimate the correlation between sources of variance for each trait (r_A, r_C, r_E). To establish the endophenotypic criterion of genetic covariance there must be a significant r_A pathway. A correlation of 0 for this path would indicate that the genetic factors underlying the two traits are entirely separate. A correlation of 1, on the other hand, would indicate that the genetic factors underlying the two traits are identical.

Figure 5.1. Correlated Factors Model (CFM)



Note: Model depicted for 1 twin only. A = additive genetics; C = common/shared/family environment; E = unique/non-shared environment; r_A = genetic correlation; r_C = common environment correlation; r_E = unique environment correlation.

III. RESULTS

Phenotypic associations

JAS "screaming lady" paradigm. Table 5.1 presents the results of the multiple regressions examining the associations between CU traits and startle reflex metrics in JAS. Neither startle metric significantly predicted CU traits, nor were there any significant interactions by age. However, there was a significant interaction between sex and SR, as well as between sex and FPS, in predicting ICU sum score. Table 5.2 displays these regression estimates separately for males and females. As noted in Table 5.2 and depicted graphically in Figure 5.2 there was not a significant relationship between SR and CU traits in males. However, there was a significant (p = .002) relationship in females

Table 5.1. Startle measures predicting ICU sum score in JAS

	β (*p*)
Baseline startle	
Baseline/SR	0.419 (.085)
Baseline/SR * age	0.108 (.455)
Baseline/SR * sex	**1.171 (.014) ***
Fear potentiated startle	
Fear-potentiated/FPS	0.263 (.303)
Fear-potentiated/FPS * age	0.180 (.261)
Fear-potentiated/FPS * sex	**1.157 (.022) ***

Note: Each cell represents a separate multiple regression controlling for age, sex, and non-independence of twin pairs (family clustering); ^ = $p < .05$; * = $p < .025$

Table 5.2. Startle measures predicting ICU sum score in JAS males and females separately

	β (*p*)
Males	
Baseline/SR	-0.229 (.517)
Fear-potentiated/FPS	-0.341 (.347)
Females	
Baseline/SR	**0.943 (.002) ***
Fear-potentiated/FPS	**0.732 (.027) ^**

Note: Each cell represents a separate multiple regression controlling for age and non-independence of twin pairs (family clustering); ^ = $p < .05$; * = $p < .025$

such that each unit increase in SR predicted an increase of approximately .9 units in ICU sum score. As noted in Table 5.2 and depicted graphically in Figure 5.3 there was not a significant relationship between FPS and CU traits in males. However, there was a significant (p = .027) relationship in females such that each unit increase in FPS predicted an increase of approximately .7 units in ICU sum score.

Figure 5.2. Sex Interacts with Baseline Startle to Predict ICU Sum Score

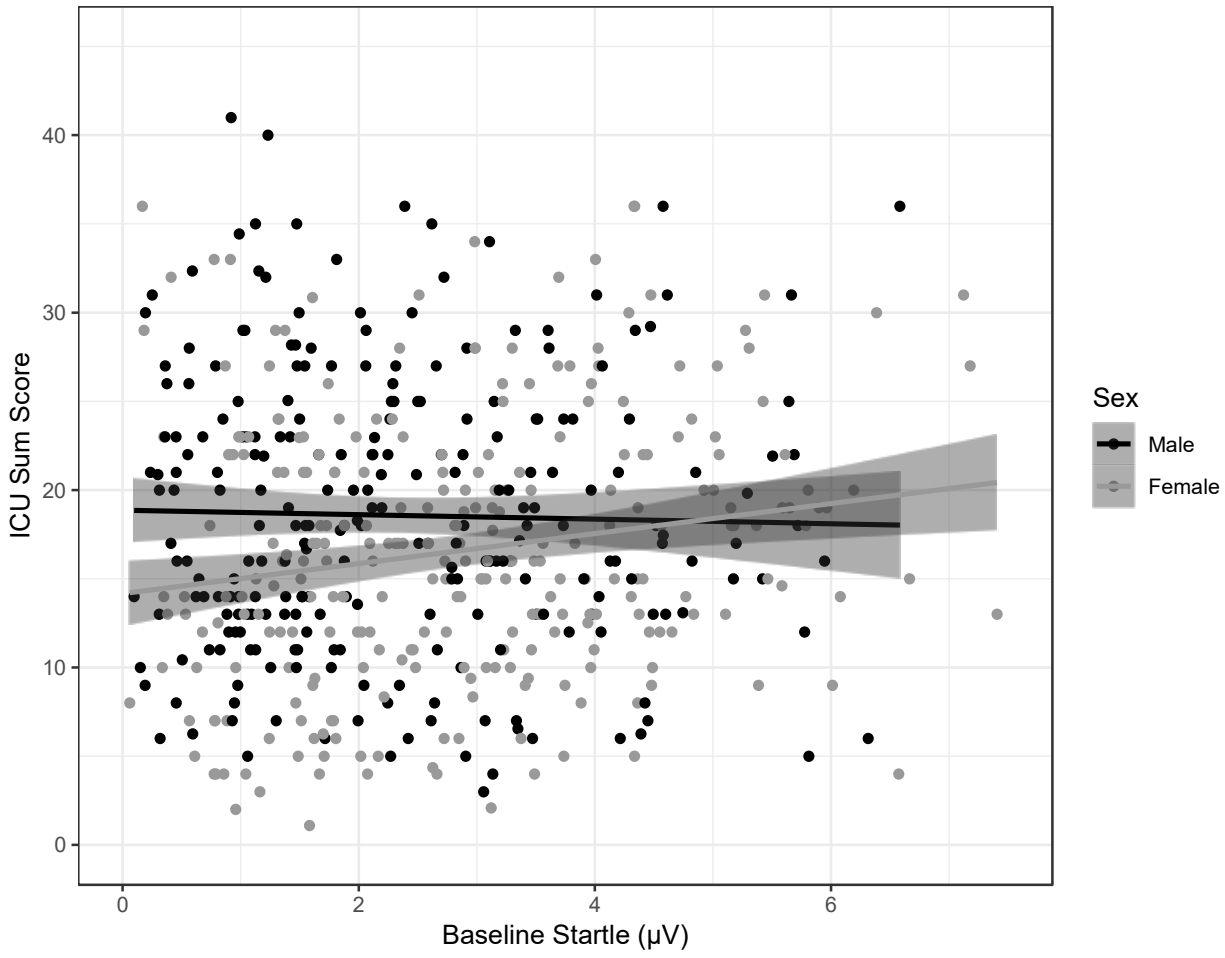
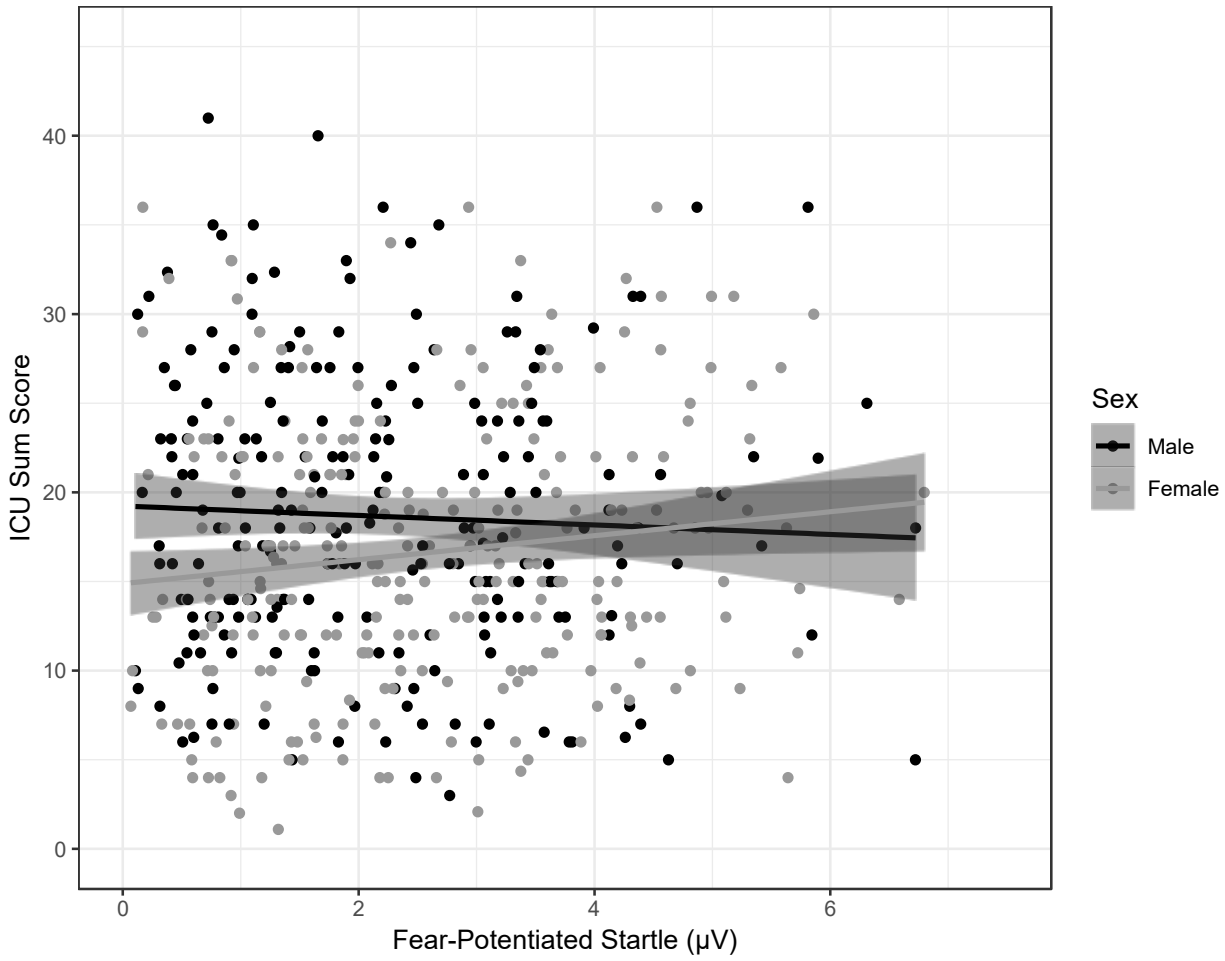


Figure 5.3. Sex Interacts with Fear-Potentiated Startle to Predict ICU Sum Score



AYATS fear generalization paradigm. Table 5.3 presents the results of the multiple regressions examining the associations between CU traits and startle reflex metrics in AYATS. Neither startle metric significantly predicted CU traits, nor were there any significant interactions by age or sex.

Table 5.3. Startle measures predicting ICU sum score in AYATS	
	β (*p*)
Baseline startle	
Baseline (overall habituation)	-0.027 (.163)
Baseline * age	-0.003 (.830)
Baseline * sex	-0.022 (.572)
Fear potentiated startle	
Fear-potentiated (acquisition CS+)	-0.013 (.483)
Fear-potentiated * age	-0.015 (.331)
Fear-potentiated * sex	-0.031 (.431)

Note: Each cell represents a separate multiple regression controlling for age, sex, and non-independence of twin pairs (family clustering); ^ = $p < .05$; * = $p < .025$

Genetic covariance CU and ROIs

JAS "screaming lady" paradigm. CU traits, baseline startle, and fear-potentiated startle were investigated in a biometrical CFM. Table 5.4 presents the model fit statistics for this CFM. Dropping all additive genetic effects from the model did not result in significant model deterioration ($p = .059$). All common environmental effects could also be dropped from the model without significant deterioration in fit ($p = .957$). However, dropping both A and C from the model resulted in significant deterioration ($p < .001$). Therefore, an ACE model was chosen as the best-fitting from which to derive parameter estimates and confidence intervals, displayed in Figure 5.4 with significant pathways bolded. The common genetic influences on baseline startle, fear potentiated startle, and CU traits were 47%, 43%, & 39%, respectively. Unique environmental influences on

Table 5.4. Model fit statistics for CFM (baseline startle, fear-potentiated startle, & CU traits) in JAS

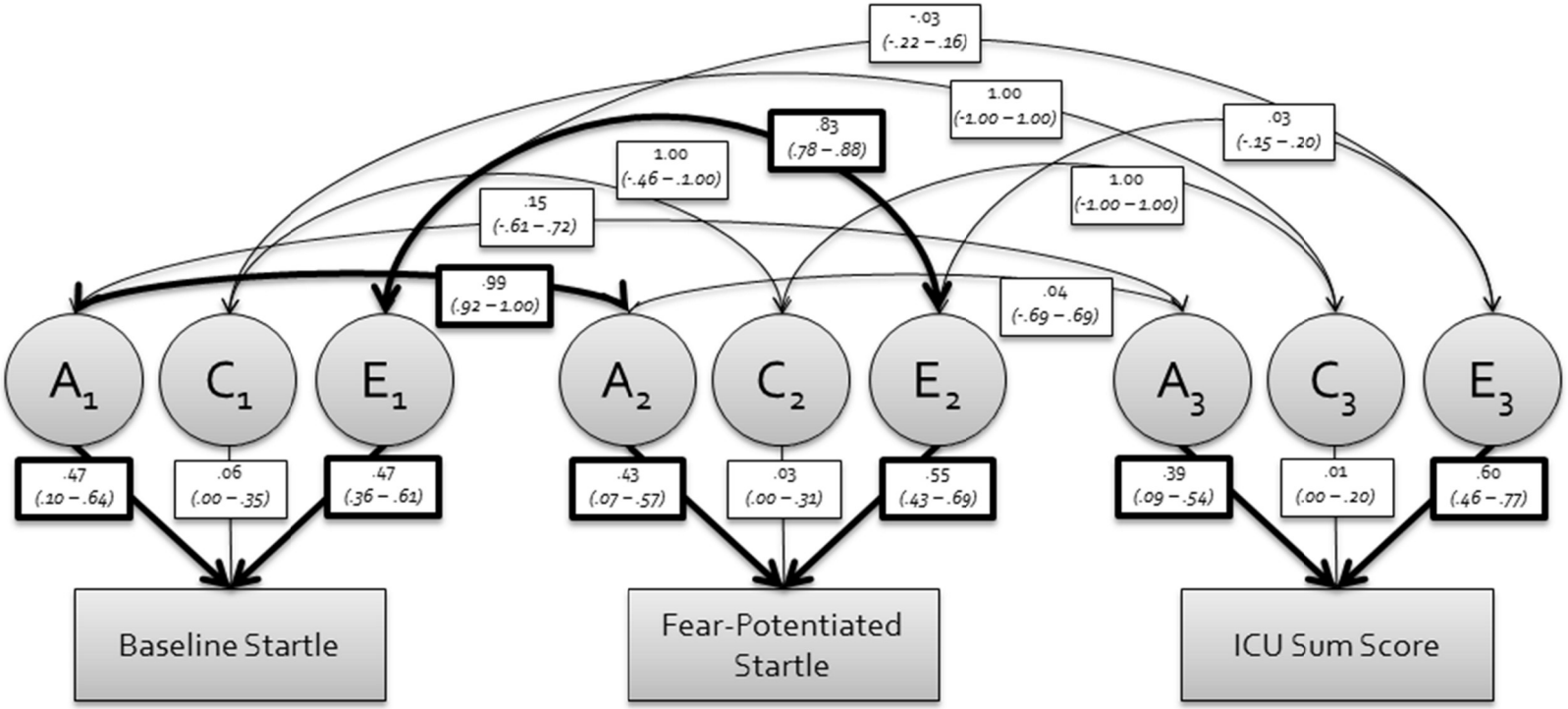
Model	# Param.	-2LL	df	Δ -2LL	Δ df	p
ACE	21	7116.862	1654	-	-	-
AE	18	7117.180	1657	.317	3	.957
CE	18	7124.298	1657	7.436	3	.059
E	15	7169.572	1660	52.710	6	< .001

Note: # Param. = number of parameters; -2LL = -2 log likelihood; df = degrees of freedom; Δ = change.

baseline startle, fear potentiated startle, and CU traits were 47%, 55%, & 60%, respectively. All remaining variance was accounted for by C although none of these pathways were significant as demonstrated by confidence intervals overlapping 0. There were significant etiological correlations between baseline and fear potentiated startle estimated at r_A = .99 and r_E = .83.

AYATS fear generalization paradigm. CU traits, baseline startle, and fear-potentiated startle were investigated in a biometrical CFM. Table 5.5 presents the model fit statistics for this CFM. Dropping all common environmental effects from the model did not result in significant model deterioration (p = 1.0). However, dropping additive genetic effects from the model resulted in significant deterioration (p = .008). Therefore, an AE model was chosen as the best-fitting, most parsimonious from which to derive parameter estimates and confidence intervals, displayed in Figure 5.5 with significant pathways bolded. The common genetic influences on baseline startle, fear potentiated startle, and CU traits were 50%, 47%, & 41%, respectively. All remaining variance was accounted for by unique environmental influences. There were significant etiological correlations between baseline and fear potentiated startle estimated at r_A = 1.0 and r_E = .51.

Figure 5.4. Correlated Factors Model for Baseline Startle, Fear-Potentiated Startle, and ICU Sum Score in JAS



Note: All path estimates have been standardized and squared to represent proportions of variance (single-headed arrows) and correlations (double-headed arrows). Bolded paths are significant based on 95% CIs that do not overlap 0.

Table 5.5. Model fit statistics for CFM (baseline startle, fear-potentiated startle, & CU traits) in AYATS

Model	# Param.	-2LL	df	Δ -2LL	Δ df	p
ACE	21	16065.66	1952	-	-	-
AE	18	16065.66	1955	< .001	3	1.00
CE	18	16077.46	1955	11.792	3	.008
E	15	16123.31	1958	57.642	6	< .001

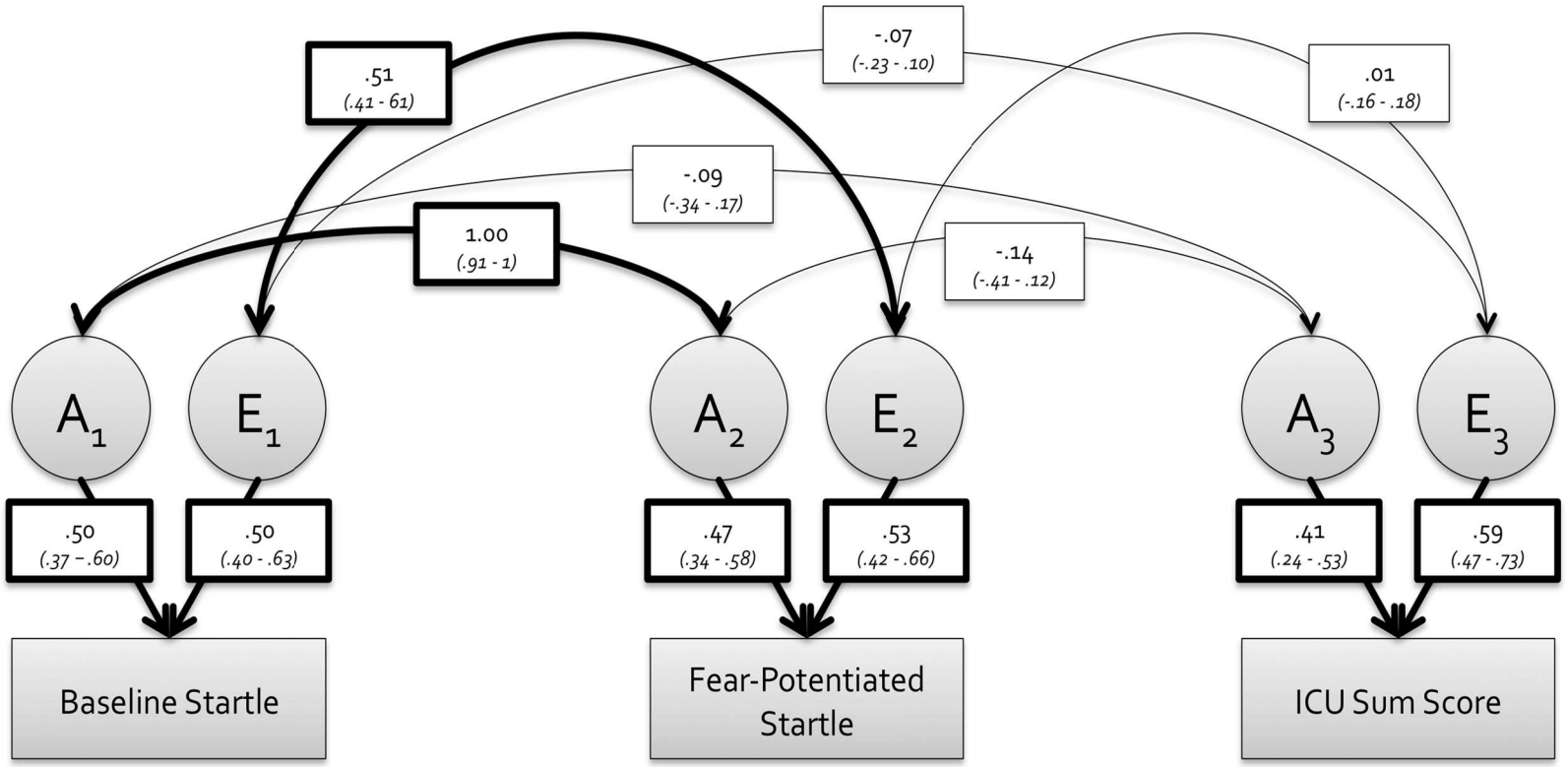
Note: # Param. = number of parameters; -2LL = -2 log likelihood; df = degrees of freedom; Δ = change.

I. DISCUSSION

The current study sought to investigate baseline startle reflex (SR) and fear-potentiated startle reflex (FPS) as potential endophenotypes for CU traits. Although previous research has determined a phenotypic relationship between psychopathic/CU traits and startle metrics, this is first to examine the phenotypic and genetic covariance between startle metrics and CU traits. We also sought to replicate the observed phenotypic relationship between startle and CU in two genetically informative juvenile twin samples. Due to previous relationships established between age, sex, and CU traits (see chapter 4), age and sex interactions were also examined in the relationship between startle and CU.

In the older sample (15-20 years; AYATS) there were no significant relationships between CU and any startle metric. However, the younger sample (9-14; JAS) revealed two significant interactions, such that there was a significant relationship between CU and both SR and FPS in females but not in males. In females, each μV unit increase for SR

Figure 5.5. Correlated Factors Model for Baseline Startle, Fear-Potentiated Startle, and ICU Sum Score in AYATS



Note: All path estimates have been standardized and squared to represent proportions of variance (single-headed arrows) and correlations (double-headed arrows). Bolded paths are significant based on 95% CIs that do not overlap 0.

and FPS predicted an increase in ICU sum score of approximately .7 - .9 units. This positive association is unexpected, considering most previous research reveals a negative association between startle and CU traits. However, much of this research has been performed in males only (Fairchild et al., 2008; Herpertz et al., 2001; Kimonis et al., 2017; Levenston et al., 2000; Loomans et al., 2015; Patrick et al., 1993; Rothemund et al., 2012; Syngelaki et al., 2013). Among the research that has been performed in mixed-sex samples (Esteller et al., 2016; Fanti et al., 2016; Fanti et al., 2017; Kyranides et al., 2017), most have not explicitly examined the effect of sex. The only study that has examined these phenomena in a female-only sample (Fairchild, Strobbe, van Goozen, Calder, & Goodyer, 2010) reported no significant relationship between SR and CU in females. They did, however, report a significant relationship between conduct disorder and decreased SR (Fairchild et al., 2010).

One study that may shed light on our findings is a recent study by Dackis and colleagues (2015). In this study, SR was blunted in children with CU and no history of maltreatment, whereas children with CU and a history of maltreatment showed a higher startle reflex. The distinction is meant to differentiate between the primary and secondary variants of psychopathy, with primary psychopathy indicative of a biologically based disorder characterized by a pattern of hypoarousal. Secondary psychopathy, on the other hand, is characterized by more internalizing characteristics, a pattern of normative or hyperarousal, and is hypothesized to be a potential coping mechanism for traumatic experiences (e.g., Cleckley, 1941; Lykken, 1995). Although our study was unable to investigate the primary/secondary distinction, it is worth noting that more females than males often fit into the secondary category (Lee & Salekin, 2010; Sevecke, Lehmkuhl, &

Krischer, 2008; Vaughn, Edens, Howard, & Smith, 2009), and this may be one potential explanation for our findings.

We also examined the genetic covariance of CU traits, SR, and FPS using a multivariate CFM. Although each separate phenotype was significantly heritable (39% - 50%) there was no significant genetic covariance between CU and either startle metric in either of the two samples. This is not surprising, however, given the non-significant phenotypic associations in the older sample and among males in the younger sample. Also, we were unable to distinguish genetic influences that were unique to FPS above and beyond those that account for SR (r_G = .99 & r_G = 1.0 in JAS and AYATS, respectively). These findings are similar to previous heritability studies of SR and FPS using both an FCS paradigm in the JAS study (Savage et al., 2019) and other studies measuring baseline and modulated startle using AMS paradigms (Anokhin et al., 2007; Dhamija et al., 2017; Vaidyanathan et al., 2014).

Limitations and Future Directions

The results of the current study should be interpreted in light of several limitations. First, our sample was relatively homogenous and will likely limit the generalizability of the reported results. Specifically, our sample was recruited from a single region in the United States and was comprised mostly of Caucasian individuals. Caucasians were purposefully oversampled in the two datasets due to the planned inclusion of molecular genetic tests (where statistical power is maximized in relatively small samples by including only one ancestry group.) Biological and environmental factors contributing to CU traits may differ in different populations, so replication across a range of cultures and ethnicities is an important consideration for future research.

Second, although we examined the startle reflex across a range of ages, the unique startle paradigms used in each study made it infeasible to combine the two studies into a single set of results. Specifically, JAS used an air puff startle probe and an auditory UCS, whereas AYATS used an auditory startle probe and an electrical shock UCS. Furthermore, JAS data was collected via the BIOPAC system and AYATS was collected with PSYCHLAB. These different paradigms and data recording procedures resulted in substantially larger EMG responses in the AYATS study. It is also possible that the level of conditioning to the CS+ varied due to these different paradigms. In the future, data harmonization procedures may be used to combine these different paradigms into a larger sample in order to increase statistical power.

Third, although sex differences in startle reflex were discovered in the younger sample, our sample size was underpowered to detect genetic sex-effects. Therefore, sex differences in the genetic relationship between startle and CU remain unexamined. Increasingly large samples will be needed to uncover both the unique genetic influences on FPS and to disentangle potential genetic sex effects on startle variance and startle/CU covariance.

Conclusions

This study was unable to replicate the previous findings of negative associations between startle and CU/psychopathic traits. Furthermore, we found a positive association between CU traits and both SR and FPS in younger females. We are the first to find this effect, although previous research on startle and CU in females is limited. Future research should focus on disentangling this relationship in females specifically.

Our study was also the first to examine the genetic covariance, a required endophenotypic criterion, between CU and startle. In our sample, there was no genetic covariance between CU and either startle metric (SR or FPS). Taken in combination with previous research (see chapter 1), it appears as if EMG FPS does not meet several criteria (heritability, genetic covariance) and is, therefore, not suitable as a putative endophenotype. The only study to date to find a heritable component to FPS used an alternate measure to EMG, electrodermal activity (i.e., skin conductance; Hettema, Annas, Neale, Kendler, & Fredrikson, 2003), highlighting the importance of choosing a heritable measure when examining potential endophenotypes in psychiatric research.

CHAPTER 6. EXAMINING BRAIN MORPHOMETRY AS A POTENTIAL ENDOPHENOTYPE FOR CALLOUS-UNEMOTIONAL TRAITS

I. SPECIFIC AIM

Chapter 1 details the previous studies implicating the paralimbic system in the development of psychopathic/CU traits. However, only one study, conducted in males, has examined brain morphometry as a potential endophenotype for psychopathy. No studies have yet investigated brain morphometry as a potential endophenotype for CU traits nor in a mixed-sex sample. The aims of the current chapter are three-fold. The first aim was to examine the phenotypic association between CU traits, age, sex, and four paralimbic regions of interest (ROI) previously associated with CU/psychopathic traits (amygdala, orbitofrontal cortex [OFC], anterior cingulate cortex [aCC], posterior cingulate cortex [pCC]). The second aim was to examine the heritability of associated areas. The third aim was to examine the genetic correlation between CU traits and those ROIs determined to be heritable and associated with CU in the current sample.

II. ANALYSES

The relationship between CU traits and ROIs was examined via multiple linear regression. Each multiple regression controlled for age, sex, total intracranial volume, site

of scan (NIH vs. CARI), and non-independence of twin pairs (family clustering). Each of the four regions (amygdala, OFC, aCC, & pCC) were examined separately for both the right and left hemispheres. Analyses of the three cortical regions (OFC, aCC, & pCC) could be subdivided into cortical thickness, surface area, and their combined measure: cortical volume. However, for the subcortical region (amygdala) only volume is available as a measure of morphometry. Therefore, 20 multiple regressions (10 for each hemisphere) were performed in total. For each test, age- and sex-moderation were also examined. A *p*-value less than .05 was used to determine suggestive significance, and a Bonferroni-corrected *p*-value less than .0125 (corrected for 4 tests corresponding to four ROIs) was used to determine significance. Although all 20 of the tests performed are not entirely independent, we recognize that correction for only four tests may be insufficient to reduce type 1 error. However, as the first study of brain morphometry as an endophenotype for CU traits, we chose a relatively liberal significance threshold for an initial exploration.

ROIs meeting the threshold for suggestive significance ($p < .05$) were examined to determine if they were heritable phenotypes. Procedures for estimating heritability are detailed in chapter 3 of the current dissertation. Once heritability was determined, ROIs were moved into a bivariate genetic analysis to examine genetic covariance/correlation with CU traits. A correlated factors model (CFM) was used to determine if CU traits and ROIs shared significant genetic covariance required for meeting endophenotypic criteria. The rationale behind choosing the CFM and an explanation of genetic correlations is presented in chapter 5. An example of the CFM is depicted in Figure 5.1. For this set of analyses, the entire sample (not just the neuroimaging subsample) was included to

improve the accuracy of the path estimates for CU traits where information from additional participants was available.

III. RESULTS

Phenotypic associations

Table 6.1 presents the results of the 20 multiple regressions examining the associations between CU traits and paralimbic brain morphometry. Only CU traits and right aCC thickness were suggestively associated (p = .03). As shown in Table 6.1 and graphically depicted in Figure 6.1, each additional millimeter (mm) of right aCC thickness predicts a decrease of approximately 2.7 units in ICU sum score.

Table 6.1. ROI morphometry predicting ICU sum score

ROI	Left hemisphere β (p)	Right hemisphere β (p)
Amygdala volume	0.0048 (.323)	0.0023 (.533)
Posterior cingulate cortex volume	0.0001 (.951)	-0.0011 (.418)
Posterior cingulate cortex thickness	-3.1500 (.371)	-5.8800 (.102)
Posterior cingulate cortex area	0.0024 (.660)	-0.0006 (.861)
Orbitofrontal cortex volume	0.0001 (.551)	< 0.0001 (.898)
Orbitofrontal cortex thickness	0.6780 (.793)	1.3400 (.588)
Orbitofrontal cortex area	0.0009 (.609)	-0.0006 (.679)
Anterior cingulate cortex volume	-0.0002 (.735)	-0.0007 (.125)
Anterior cingulate cortex thickness	0.1900 (.887)	**-2.7200 (.030) ^**
Anterior cingulate cortex area	-0.0011 (.709)	-0.0027 (.339)

Note: Each cell represents a separate multiple regression controlling for age, sex, total intracranial volume, site of scan (NIH vs. CARI), and non-independence of twin pairs (family clustering). ^ p < .05; * p < .0125

Figure 6.1. Right Anterior Cingulate Cortex Thickness Predicts ICU Sum Score

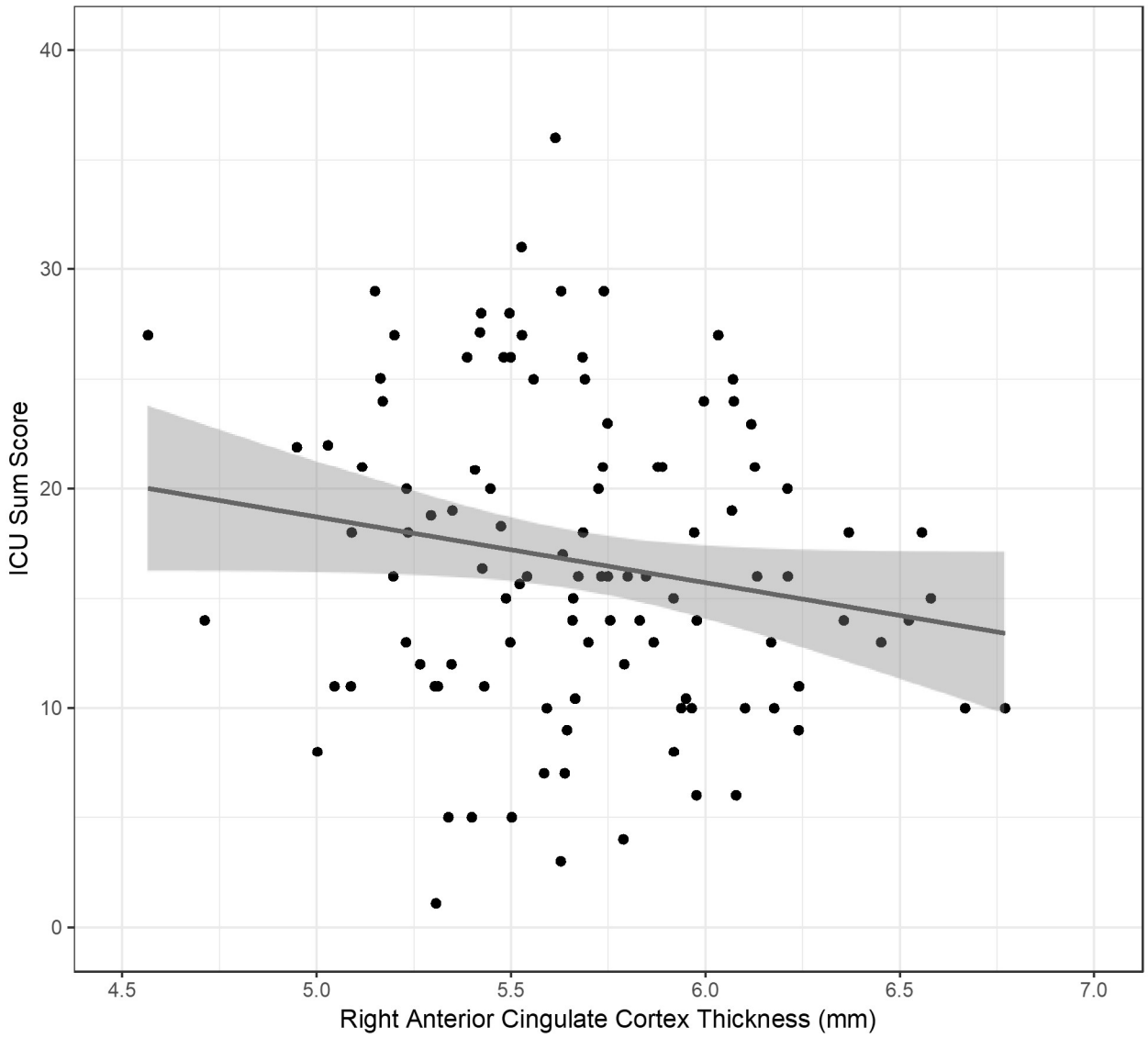


Table 6.2 presents the results of the regressions for the interaction of age and ROI predicting ICU sum score. There were no significant age by ROI interactions. Table 6.3 presents the results of the regressions for the interaction of sex and ROI predicting ICU sum score. There was a suggestively significant association (p = .02) between CU traits

and the interaction of sex and right pCC thickness. Post-hoc multiple regressions were performed for this ROI in males and females separately and the results of these regressions are presented in Table 6.4. As shown in Table 6.4 and graphically depicted in Figure 6.2, the relationship between right pCC thickness and CU traits in males was not significant. However, in females each additional millimeter (mm) of right pCC thickness predicted a decrease of approximately 15 units in ICU sum score (p = .00079).

Table 6.2. ROI morphometry * age interactions predicting ICU sum score

ROI	Left hemisphere β (p)	Right hemisphere β (p)
Amygdala volume	0.0013 (.526)	0.0016 (.440)
Posterior cingulate cortex volume	-0.0008 (.565)	-0.0011 (.191)
Posterior cingulate cortex thickness	-3.1100 (.327)	-2.1400 (.327)
Posterior cingulate cortex area	0.0022 (.556)	-0.0014 (.453)
Orbitofrontal cortex volume	< 0.0001 (.874)	< 0.0001 (.757)
Orbitofrontal cortex thickness	-2.7300 (.050)	-2.4100 (.144)
Orbitofrontal cortex area	0.0011 (.190)	0.0007 (.312)
Anterior cingulate cortex volume	0.0001 (.708)	-0.0004 (.728)
Anterior cingulate cortex thickness	-1.7300 (.146)	0.1800 (.830)
Anterior cingulate cortex area	0.0001 (.941)	0.0010 (.550)

Note: Each cell represents a separate multiple regression controlling for age, sex, total intracranial volume, site of scan (NIH vs. CARI), and non-independence of twin pairs (family clustering). ^ p < .05; * p < .0125

Table 6.3. ROI morphometry * sex interactions predicting ICU sum score

ROI	Left hemisphere β (*p*)	Right hemisphere β (*p*)
Amygdala volume	-0.0010 (.890)	-0.0011 (.870)
Posterior cingulate cortex volume	-0.0039 (.330)	-0.0026 (.350)
Posterior cingulate cortex thickness	-4.7400 (.400)	**-11.5000 (.020) ^**
Posterior cingulate cortex area	-0.0026 (.800)	0.0050 (.443)
Orbitofrontal cortex volume	< 0.0001 (.870)	-0.0002 (.560)
Orbitofrontal cortex thickness	-2.2900 (.450)	-4.2400 (.250)
Orbitofrontal cortex area	0.0010 (.690)	-0.0006 (.780)
Anterior cingulate cortex volume	-0.0003 (.710)	-0.0009 (.240)
Anterior cingulate cortex thickness	0.6840 (.800)	-2.5700 (.331)
Anterior cingulate cortex area	-0.0018 (.720)	-0.0041 (.380)

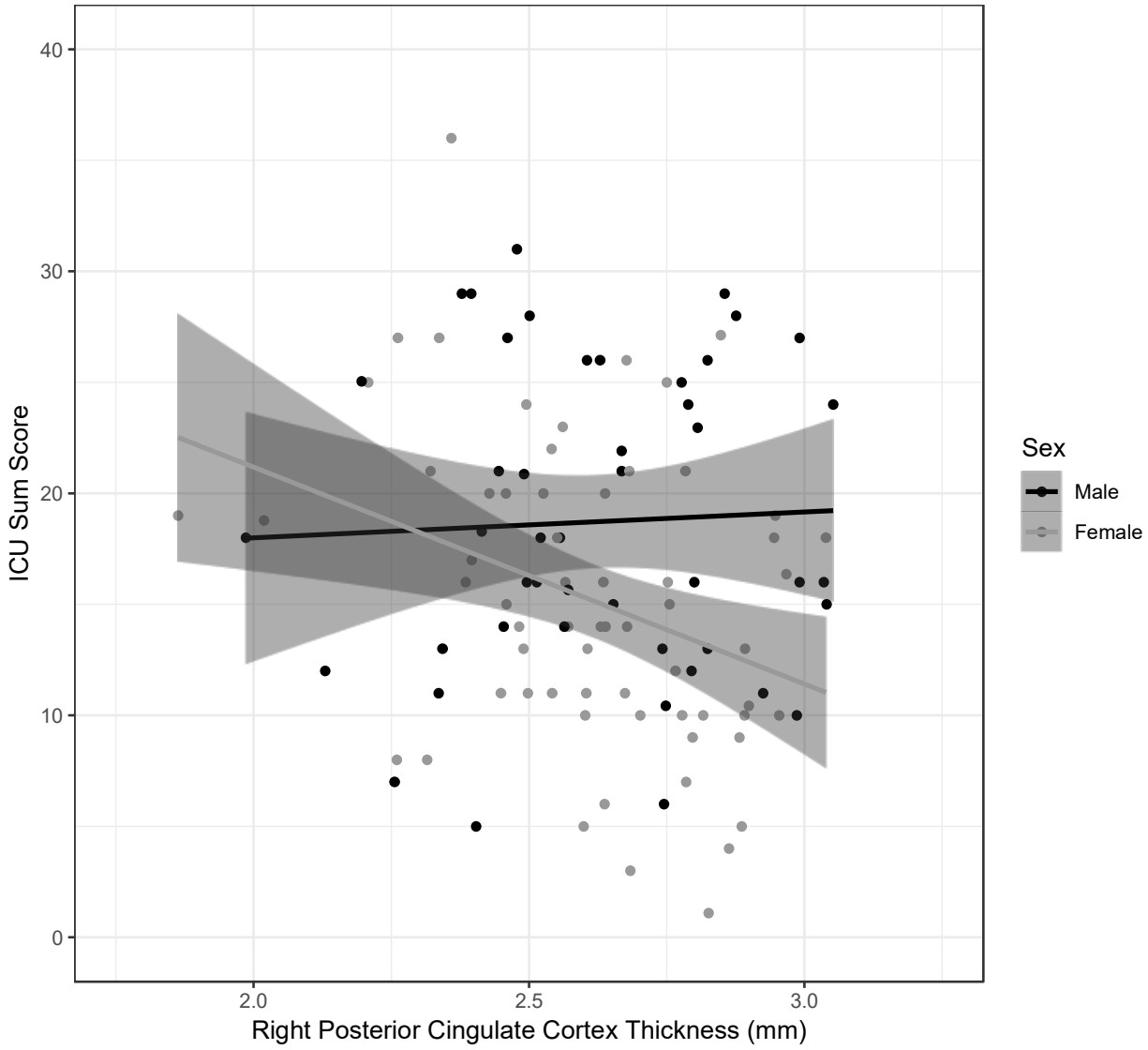
Note: Each cell represents a separate multiple regression controlling for age, sex, total intracranial volume, site of scan (NIH vs. CARI), and non-independence of twin pairs (family clustering). ^ $p < .05$; * $p < .0125$

Table 6.4. Right Posterior Cingulate Cortex Thickness Predicting ICU Sum Score for Males and Females

Sex	β (*p*)
Females	**-15.00 (.00079) ***
Males	5.43 (.31300)

Note: Each cell represents a separate multiple regression controlling for age, total intracranial volume, site of scan (NIH vs. CARI), and non-independence of twin pairs (family clustering). ^ $p < .05$; * $p < .0125$

Figure 6.2. Sex Interacts with Right Posterior Cingulate Cortex Thickness to Predict ICU Sum Score



Heritability of ROIs

The two ROIs measures demonstrating suggestively significant association with CU traits (right aCC thickness and right pCC thickness) were examined to verify that these measures were heritable in the current sample. Model fit statistics are presented in Table 6.5 and Table 6.6 for right aCC thickness and right pCC thickness, respectively. For both

Table 6.5. Model fit statistics for right aCC thickness

Model	# Param.	-2LL	df	Δ -2LL	Δ df	p
ACE	4	99.7304	96	-	-	-
AE	3	99.7304	97	< .0001	1	1.00
CE	3	102.8144	97	3.0839	1	.079
E	2	107.3172	98	7.5868	2	.023

Note: # Param. = number of parameters; -2LL = -2 log likelihood; df = degrees of freedom; Δ = change.

Table 6.6. Model fit statistics for right pCC thickness

Model	# Param.	-2LL	df	Δ -2LL	Δ df	p
ACE	4	-20.3591	96	-	-	-
AE	3	-19.1202	97	1.2389	1	.266
CE	3	-19.7657	97	0.5934	1	.441
E	2	-0.6890	98	19.6701	2	< .001

Note: # Param. = number of parameters; -2LL = -2 log likelihood; df = degrees of freedom; Δ = change.

regions, there was insignificant power to disentangle the familial effects of genes and shared environment. That is, for both morphometric measures we could drop A from the model without significant deterioration in model fit (p = .079 & .441, respectively), and we could also drop C from the model without significant deterioration in model fit (p = 1.0 & .266, respectively). However, both A and C could not be dropped from the model simultaneously without significantly deteriorating the model fit (p = .023 & p < .001, respectively). For that reason, the ACE models were used to extract the heritability

estimate for each measure. For right aCC thickness, additive genetic factors accounted for approximately 52.4% of the variance with the remaining variance (47.6%) accounted for by unique environmental effects. For right pCC thickness, additive genetic factors accounted for approximately 26.4% of the variance, 36.9% of the variance was accounted for by common environmental effects, and the remaining variance (36.8%) was accounted for by unique environmental effects.

Genetic covariance CU and ROIs

The two ROIs measures demonstrating suggestively significant associations with CU traits (right aCC thickness and right pCC thickness) both demonstrated significant heritability and were therefore moved into the CFM analyses. Each ROI measure was examined in a CFM with CU traits to examine potential genetic covariance.

Table 6.7 presents the model fit statistics for the CFM of CU traits and right aCC thickness. Dropping all additive genetic effects from the model resulted in significant model deterioration (p = .037). However, all common environmental effects could be dropped from the model without significant deterioration in fit (p = 1.0). Therefore, an AE model was chosen as the best-fitting and most parsimonious model. Figure 6.3 displays the AE model with standardized and squared path coefficients to represent proportions of variance and etiological correlations. Ninety-five percent confidence intervals are also presented in Figure 6.3. In this model additive genetic factors accounted for 39% and 48% of the variance in CU traits and right aCC thickness, respectively, with the remaining variance accounted for by unique environmental effects. The correlation point estimates for r_A and r_E were -.41 and -.04, respectively. However, confidence intervals for both correlations overlapped with 0 and were therefore non-significant.

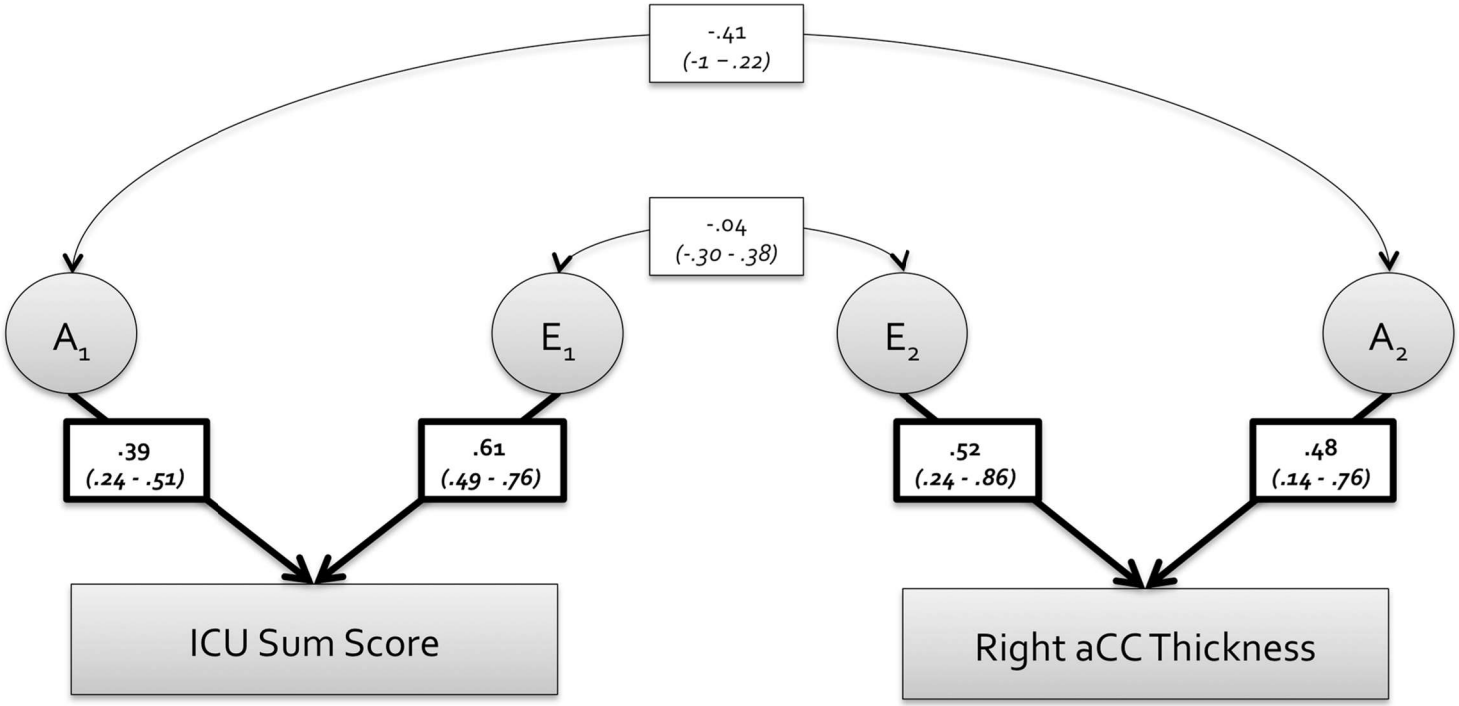
Table 6.7. Model fit statistics for CFM (right aCC thickness & CU traits)

Model	# Param.	-2LL	df	Δ -2LL	Δ df	p
ACE	11	4857.471	787	-	-	-
AE	8	4857.471	790	< .001	3	1.00
CE	8	4865.943	790	8.472	3	.037
E	5	4888.305	793	30.834	6	< .001

Note: # Param. = number of parameters; -2LL = -2 log likelihood; df = degrees of freedom; Δ = change.

Table 6.8 presents the model fit statistics for the CFM of CU traits and right pCC thickness. Dropping all additive genetic effects from the model did not result in significant model deterioration (p = .788). All common environmental effects could also be dropped from the model without significant deterioration in fit (p = .097). However, dropping both A and C from the model resulted in significant deterioration (p < .001). Therefore, an ACE model was chosen as the best-fitting model. Figure 6.4 displays the ACE model with standardized and squared path coefficients to represent proportions of variance and etiological correlations. Ninety-five percent confidence intervals are also presented in Figure 6.4. In this model, significant pathways that do not include zero (bolded in figure 6.4) indicate that 36% of CU trait variance was accounted for by A, and 62% was accounted for by E. Of the variance in right pCC thickness, 35% was accounted for by E. Common environmental influences on both CU traits and right pCC thickness had confidence intervals overlapping 0 and were therefore non-significant. Furthermore, additive genetic influences on right pCC thickness also had confidence intervals indicating

Figure 6.3. Correlated Factors Model for ICU Sum Score and Right aCC Thickness



Note: All path estimates have been standardized and squared to represent proportions of variance (single-headed arrows) and correlations (double-headed arrows). Bolded paths are significant based on 95% CIs that do not overlap 0.

Table 6.8. Model fit statistics for CFM (right pCC thickness & CU traits)

Model	# Param.	-2LL	df	Δ -2LL	Δ df	p
ACE	11	4732.116	787	-	-	-
AE	8	4733.170	790	1.0542	3	.788
CE	8	4738.443	790	6.3273	3	.097
E	5	4775.881	793	43.7646	6	< .001

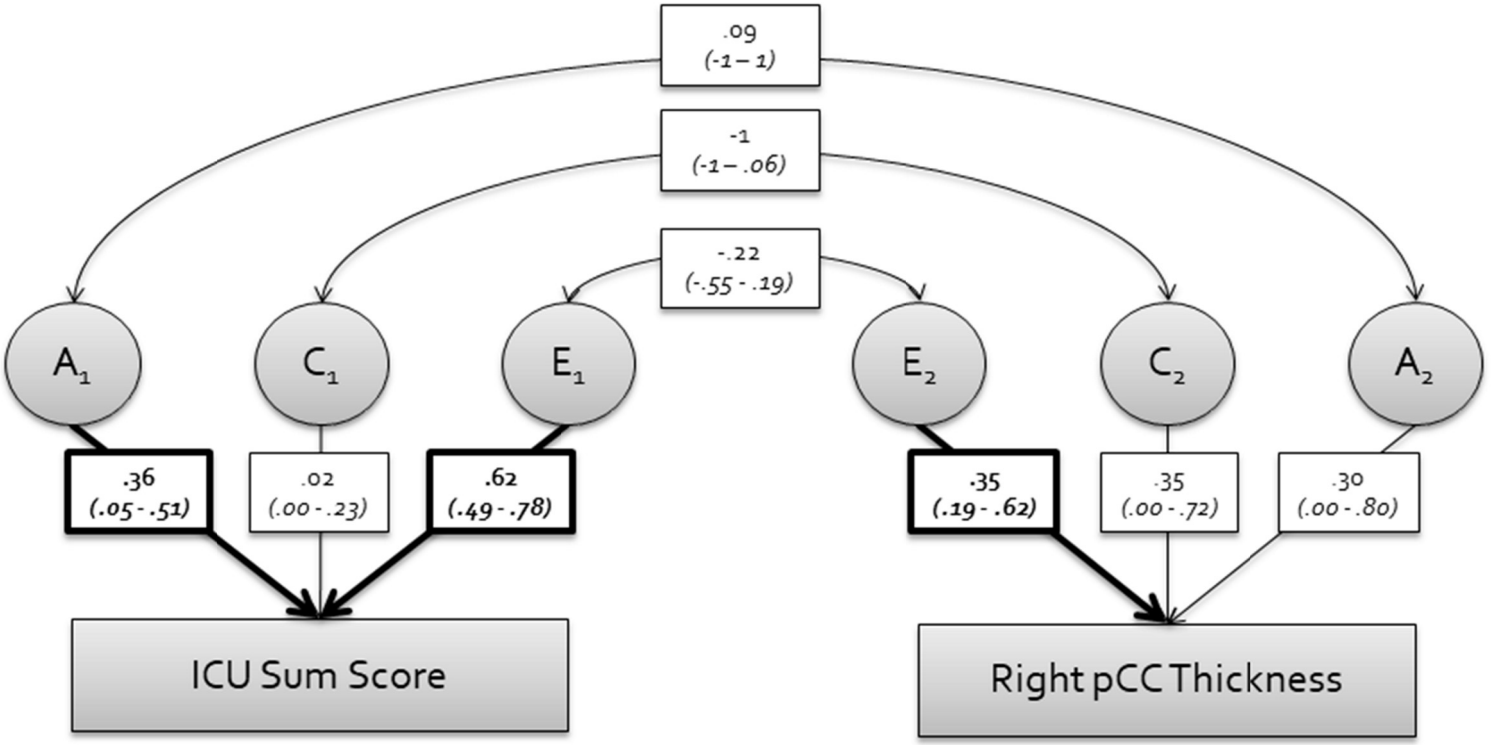
Note: # Param. = number of parameters; -2LL = -2 log likelihood; df = degrees of freedom; Δ = change.

non-significance. The correlation point estimates for r_A, r_C and r_E were .09, -1, and -.22, respectively. However, confidence intervals for all three correlations overlapped with 0 and were therefore non-significant.

I. DISCUSSION

The current study sought to examine brain morphometry in four paralimbic ROIs as potential endophenotypes for CU traits. Although previous research has determined a phenotypic relationship between psychopathic/CU traits and brain morphometry, only one prior study has examined the genetic covariance of brain morphometry and psychopathy in a sample of male juveniles. This study is the first to examine brain morphometry as an endophenotype for CU traits and in a mixed-sex sample. We used a genetically informative twin sample to assess Gottesman and Gould's (2003) primary endophenotypic criteria: that the trait and the endophenotype are associated, that the endophenotype is heritable, and that the trait and the endophenotype share genetic covariance.

Figure 6.4. Correlated Factors Model for ICU Sum Score and Right pCC Thickness



Note: All path estimates have been standardized and squared to represent proportions of variance (single-headed arrows) and correlations (double-headed arrows). Bolded paths are significant based on 95% CIs that do not overlap 0.

CU traits were suggestively associated with right aCC cortical thickness with each mm of thickness predicting a decrease of about 3 units of ICU sum score. There were no significant age interactions, however there was a suggestively significant interaction between sex and right pCC thickness in predicting CU traits. As such, each mm of right pCC thickness predicted a decrease of approximately 15 units of ICU sum score in females only. Both the pCC and aCC have been previously identified as associated with psychopathy in males during adolescence (Ermer et al., 2013) and middle childhood (Rijsdijk et al., 2010). Interestingly, our child sample identified nearly identical regions as the only other study using child participants, which identified the right dorsal aCC and left pCC as significantly associated with CU traits (Rijsdijk et al., 2010). However, our directions of effect are in the opposite direction; whereas we identified negative associations between CU and aCC/pCC, Rijsdijk and colleagues (2010) identified positive associations. This is especially surprising given that the measures used in these two studies, cortical thickness and grey matter concentration, have been reported as highly correlated (e.g., Narr et al., 2004). However, some research indicates sex differences in the development of various morphometric brain measurements, with females displaying lower cortical volume but higher grey matter concentration across development (Gennatas et al., 2017). Therefore, reasons for study discrepancies in direction of effect may stem from differences in study design, such as our use of a mixed-sex sample, specific morphometric measurements, and/or the use of CU traits vs. psychopathy.

Both suggestively associated morphometric measures, right aCC thickness and right pCC thickness, were determined to be significantly heritable with estimates equal to approximately 52% and 26%, respectively. The genetic correlations (r_A) between right

aCC thickness and CU traits as well as right pCC thickness and CU traits were estimated at -.41 and .09, respectively. However, the 95% confidence intervals for both estimates of r_A overlapped with 0 and were, therefore, non-significant. Despite similar sample sizes, we were unable to replicate the genetic covariance between CU and pCC/aCC established in an earlier study. However, unlike Rijsdijk and colleagues (2010) who fixed multiple model parameters at estimates taken from the extant literature, all our estimates were derived from the study dataset. Therefore, we were likely underpowered to detect all but very large genetic covariances.

Limitations and Future Directions

The results of the current study should be interpreted in light of several limitations. First, our sample was relatively homogenous and will likely limit the generalizability of the reported results. Specifically, our sample was recruited from a single region in the United States and was comprised entirely of Caucasian individuals aged 9-14. Biological and environmental factors contributing to CU traits and brain morphometry may differ in different populations, so replication across a range of cultures and ethnicities is an important consideration for future research.

Second, although our sample size was large for a neuroimaging study, it was quite small for a twin study, and we were, therefore, likely underpowered to detect all but very large effects. This issue is complicated by the fact that our study revealed sex differences in the relationship between CU and right pCC thickness. If sex differences in heritability exist, then it is possible that the inclusion of both sexes may have diluted these effects on genetic covariance. However, as our study is already underpowered to detect genetic covariance the possibility of investigating sex-differences in heritability is unjustified.

Future studies should seek to disentangle the effects of sex on the relationship between psychopathic/CU traits and morphometry of the paralimbic system.

Conclusions

Taken together with previous research, our results indicate that aberrant development of the cingulate cortex may impact the development of psychopathic/CU traits. This is not surprising given this region's role in social and emotional behaviors. However, our results also raise several important questions regarding sex differences and highlight the need for researchers to pay special attention to sex when investigating the relationship between psychopathy/CU and brain structure.

This study was the first to investigate brain morphometric measures as potential endophenotypes for CU traits. We were unable to replicate the genetic covariance observed previously in a male sample assessing psychopathic traits. Although these results may seem to eliminate brain morphometry as a plausible endophenotype for CU traits, firm conclusions should not be drawn until these results are replicated in larger samples with greater power to detect genetic covariance. Given the sex differences observed in our phenotypic relationships, heritable sex differences should also be examined further.

CHAPTER 7. CONCLUSIONS AND FUTURE DIRECTIONS

I. SUMMARY

This dissertation aimed to investigate the genetic etiology of CU traits. Specifically, this included 1) a literature review of all quantitative and/or molecular genetic studies of CU traits, 2) an investigation into the effects of age and sex on the genetic and environmental etiology of CU traits, 3) an investigation of baseline and fear-potentiated startle reflex as potential physiological endophenotypes for CU traits, and 4) an investigation of neuroanatomy in four paralimbic ROIs as potential endophenotypes for CU traits. The results from each of these components are discussed below.

A review of the extant literature revealed 39 quantitative and/or molecular genetic studies on CU traits. Twenty-four of these studies included quantitative components, and 16 studies included molecular components (one included both). The heritability of CU ranged from 25-80% depending on the type of sample, measurement instrument, and range of ages examined. When considering only those estimates from non-selected samples in middle childhood, adolescence, and adulthood (where the construct of CU appears longitudinally invariant [e.g. Obradović et al., 2007]) heritability is estimated between 36-67%. Despite this significant heritability, the search for associated molecular

genetic variants has not been particularly successful. Several SNVs in the serotonin and oxytocin systems have been implicated in CG studies. Although most researchers believe CG studies represent obsolete methodology, there is at least some evidence that these markers may influence CU traits, specifically based on early evidence that peripheral blood levels of these neurotransmitters may serve as biomarkers for CU (Moul et al., 2013; Dadds et al., 2014b). However, the research to date is insufficient to suggest a genetic association between these genes and CU traits. Furthermore, no GWA study has thus far identified any associated SNV at a genome-wide level of significance.

In terms of how the etiological influences on CU change based on age and sex, the current analyses demonstrate that mean levels of CU vary based on age and sex, although CU trait etiology varies based only on age. That is, males and older individuals tend to have higher ICU scores and there is also an interaction between these two variables such that ICU score over the ages of 9-20 tends to increase faster in males compared to females. However, no sex-differences in the etiology of CU traits were discovered. Heritability did, however, increase slightly across the age range of 9-20, from 34% at age 9 to 46% at age 20, with a compensatory decrease in the contribution of unique environment.

Because significant heritability estimates have not translated into significant molecular genetic findings, some researchers have suggested the use of endophenotypes as alternatives to self-report measures of psychopathology (e.g., Gottesman & Gould, 2003). As such, we investigated eyeblink startle measures of SR and FPS as potential endophenotypes for CU traits in two separate age groups and startle paradigms. There was no significant phenotypic relationship between startle and CU in

the older (ages 15-20) sample. However, the younger (ages 9-14) sample revealed two significant interactions, such that there was a significant positive association between CU and both SR and FPS in females but not in males. Although this direction of effect is not expected, little prior research has taken sex into account in their analysis of the startle reflex in individuals with psychopathic/CU traits. In a multivariate CFM CU, SR, and FPS were significantly heritable (39%-50%), although there was no significant genetic covariance between CU and either startle metric in either of the two samples, calling into question the use of SR or FPS as potential endophenotypes for CU. In line with previous research, we were also unable to distinguish genetic influences that were unique to FPS above and beyond those that account for SR, calling into question the use of FPS as an endophenotype for any trait.

We continued to investigate potential endophenotypes for CU traits by examining neuroanatomical measures of four ROIs within the paralimbic system previously associated with psychopathic/CU traits. CU traits were suggestively negatively associated with right aCC thickness. Additionally, there was a suggestively significant interaction between sex and right pCC thickness predicting CU. Specifically, the relationship between right pCC thickness and CU was negatively associated and highly significant in females but non-significant in males. Although these regions have been previously identified as putative endophenotypes for psychopathic traits in males, the direction of effect was different - again highlighting potential sex differences in the biological underpinnings of CU. Finally, neither right aCC nor right pCC thickness shared genetic covariance with CU traits, although we were likely underpowered to detect these genetic effects in the small subsample of participants with neuroimaging data.

II. LIMITATIONS AND FUTURE DIRECTIONS

This dissertation should be interpreted in light of several limitations. First, the samples for the empirical analyses in this dissertation were relatively homogeneous and, therefore, the generalizability of the current results is limited. That is, the samples were recruited from a single region of the United States (the mid-Atlantic) and were comprised almost entirely of Caucasian participants. Caucasians were purposefully oversampled in the two datasets due to the planned inclusion of molecular genetic tests (where statistical power is maximized in relatively small samples by including only one ancestry group.) However, biological and environmental factors contributing to CU traits may differ in different ancestral populations, so replication across a range of cultures and ethnicities is an important consideration for future research.

Second, our results revealed sex to be an important factor in participants' overall level of CU traits as well as in the relationship between CU traits and both physiological and neuroanatomical endophenotypes. However, our sample size was underpowered to detect all but very large genetic sex effects. Specifically, we only examined sex-limited heritability in our analyses of CU traits because we were able to use the combined JAS/AYATS sample and, therefore, had an adequate sample size (N = 1,448) for sex-limited heritability analyses. Still, we did not find any significant etiological differences based on sex. In the analyses with smaller samples, such as chapters 5 and 6, we were unable to examine genetic sex effects with any precision. However, given the importance of sex in our phenotypic and endophenotypic results, we argue that it is paramount to disentangle the effects of sex when examining research questions relating to the

underlying biology of CU traits in males and females. Increasingly large sample sizes will be required to do so, however, and the cost of collecting such samples in physiological or neuroimaging research is potentially prohibitive.

Another limitation that is also related to sample size stems from our neuroimaging analyses. Only a small subsample of the JAS dataset (*N* = 109) had neuroimaging data available. Although this is a relatively large sample size for neuroimaging, it is quite small for a twin study, consisting of only 20 and 23 complete MZ and DZ pairs, respectively. Therefore, our bivariate CFM analyses were underpowered to detect all but the largest genetic covariance between neuroanatomy and CU traits. As such, the non-significant genetic covariances should not be interpreted as the absence of a relationship but, rather, as insufficient to establish a relationship (potential Type II error). The issue of sample size is compounded by the fact that potential sex differences were revealed for the relationship between CU and right pCC thickness, which, as discussed above, could not be moved into our examination of etiology based on our prohibitively small sample.

Finally, our analyses of the eyeblink reflex were examined in two different studies with two different startle paradigms, making data combination infeasible. Specifically, AYATS used an auditory startle probe and an electrical shock as the UCS, whereas JAS used an air puff startle probe and an auditory UCS. Furthermore, JAS data was collected with BIOPAC and AYATS with PSYCHLAB. These different paradigms and data recording procedures resulted in substantially larger EMG responses in the AYATS study. Although not examined in the current analyses, it is also possible that conditioning varied across studies. In the future, data harmonization procedures may be used to combine

these different paradigms into a larger sample in order to increase statistical power to unearth relevant effects.

III. DISCUSSION AND CONCLUSIONS

Overall, the results of the current dissertation reveal a complex relationship between sex and the biological underpinnings/correlates of CU traits. Specifically, mean levels of CU traits appear to increase during adolescence in males, but not females. These results are in line with previous research, which indicates that externalizing psychopathology (including conduct disorder, antisocial behavior, and psychopathy) is more prevalent in males than females (for a review see Hipwell and Loeber, 2006) with females displaying a delayed-onset of these behaviors compared to males (e.g., Silverthorn & Frick, 1999; Moore, Silberg, Roberson-Nay, & Mezuk, 2017).

The relationship between CU and potential endophenotypes also appears to vary based on sex. That is, the screaming lady startle paradigm showed a significant association between CU and both SR and FPS in females but not males. Furthermore, there was a suggestively significant but large association between right pCC thickness and CU traits in females only. Unfortunately, most of the current endophenotypic research for psychopathic/CU traits has used exclusively male samples, which means we have little research regarding the different biological correlates/endophenotypes of CU traits among females.

Despite little endophenotypic research on CU traits in females, evolutionary psychology may offer insights into plausible reasons for biological differences across sex in the development of CU traits. In a review by Glenn, Kurzban, & Raine (2011),

psychopathy is proposed to have several evolutionary advantages including mating success, ability to gain resources, and resilience to stress. Specifically, promiscuous sexual behavior and the willingness to engage in sexual violence (rape) offer an advantage in mating success; instrumental aggression and the ability to coerce/manipulate others offer an advantage in gaining and protecting resources; and a shallow or deficient emotional experience (characteristics associated with CU traits) may offer resilience to stress, anxiety, and depression (Glenn et al., 2011). These same traits also have disadvantages, including lack of family stability stemming from sexual promiscuity, lack of harmonious interpersonal relationships stemming from aggression, and poor nurturing of offspring associated with reduced emotional experience (Glenn et al., 2011).

The increased investment in parenting required among the females of a species is a primary influence on evolutionary sexual selection (Trivers, 1972). Therefore, the disadvantages conferred by CU traits (poor nurturing of offspring) may result in evolutionary selection *against* CU traits in females. Furthermore, since male parenting behaviors are *not* a driver of sexual selection, the advantages associated with CU traits (resilience to stress, anxiety, and depression) may lead to the evolutionary selection *for* CU traits in males. The process of sexual selection, therefore, offers a potential mechanism for the different biological correlates and underpinnings of CU traits observed in males and females.

Although the analyses and results presented in this dissertation cannot substantiate a theory of sexual selection in CU traits, it does offer a jumping off point for researchers interested in the biological differences in CU among males and females.

There are clearly differences in CU trait prevalence and associations between CU and physiology/neurobiology. Therefore, researchers should continue to investigate whether these phenotypic and biological differences are due to differences in genetic makeup, environmental exposures, and/or cultural expectations.

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VITA

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