THE DEVELOPMENT OF ANTISOCIAL BEHAVIOR: GENES, BRAIN AND ENVIRONMENT AS DIRECT AND INTERACTIVE PREDICTORS OF ANTISOCIAL BEHAVIOR FROM EARLY CHILDHOOD TO YOUNG ADULTHOOD

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Antisocial behavior (AB), including physical and sexual aggression, destruction of property, theft, and violation of serious rules, has been of particular interest to researchers and the general public because of its large cost to society and negative impact on perpetrators and victims, its chronic nature and trajectory, and the difficulty in preventing and treating AB. Although recent views of AB have emphasized the complex interplay between biology and the environment (Jaffee et al., 2005; Reiss, 2005; Rutter, 1997), little empirical work has connected genetic variability, neural reactivity and environmental risk in understanding the development of AB in early adulthood. Thus, the current study sought to advance our understanding of AB in an ethnically diverse sample of 310 young men followed prospectively from age 1.5 to age 20 through measurement of amygdala reactivity to threat, variability in genes affecting serotonin signaling, cumulative environmental risk during early childhood and early adolescence, and measures of AB during adolescence and at age 20. Contrary to our hypotheses, we found that AB across adolescence and at age 20 was related to lower amygdala reactivity to threat, regardless of the level of callous traits also present. Also contrary to our hypotheses, we found that variants in serotonin genes previously linked to lower amygdala reactivity were related to

callous traits as well as AB in the presence of high callousness. Imaging genetics models that linked variability in specific serotonin genes, amygdala reactivity, and AB were not supported. Similarly, little support was found for Imaging Gene by Environment interactions in which the interactions between genetic variability and environmental risk were linked to AB via their association with neural reactivity. Results highlight the difficulty in testing complex models of the likely interactions between genes, brain and environment in understanding AB and suggest a specific role of amygdala reactivity in AB.

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PREFACE

I owe incredible debt and gratitude to too many people to name in helping me to complete this dissertation and my PhD. Many thanks to my committee members for all of their expertise and help with this document. Special thanks for Daniel S. Shaw, PhD, my primary advisor, who has been the best advisor I could ever ask for. He has helped guide me with an incredible amount of wisdom, knowledge and patience, and I certainly would not have completed this project without him. Thank you to all of those within the Pitt, UPMC and CNCB community for all of your support in my career. Big thanks also to my family and friends for supporting me during the writing of this dissertation, especially to my fiancée Kelley M. Kidwell, PhD.

1.0 INTRODUCTION

A long history of research on children, adolescents, and adults has emphasized multiple pathways in the development and maintenance of antisocial behavior (AB). This heterogeneous group of behaviors, including physical and sexual aggression, destruction of property, theft, and violation of serious rules, has been of particular interest to researchers and the general public because of its large cost to society and negative impact on perpetrators and victims, its chronic nature and trajectory, and the difficulty in preventing and treating AB. Etiologic theories of AB from a wide array of disciplines have emphasized the contributions of biological (e.g., neurologic, genetic; DiLalla & Gottesman, 1991; Raine, 2002a; Rowe, 2002) and/or environmental (e.g., parenting, poverty, peers; Dishion, Patterson, Stoolmiller, & Skinner, 1991; Patterson, Reid, & Dishion, 1992) mechanisms, with recent nuanced views emphasizing the complex interplay between biology and the environment (See Figure 1; Jaffee et al., 2005; Reiss, 2005; Rutter, 1997).

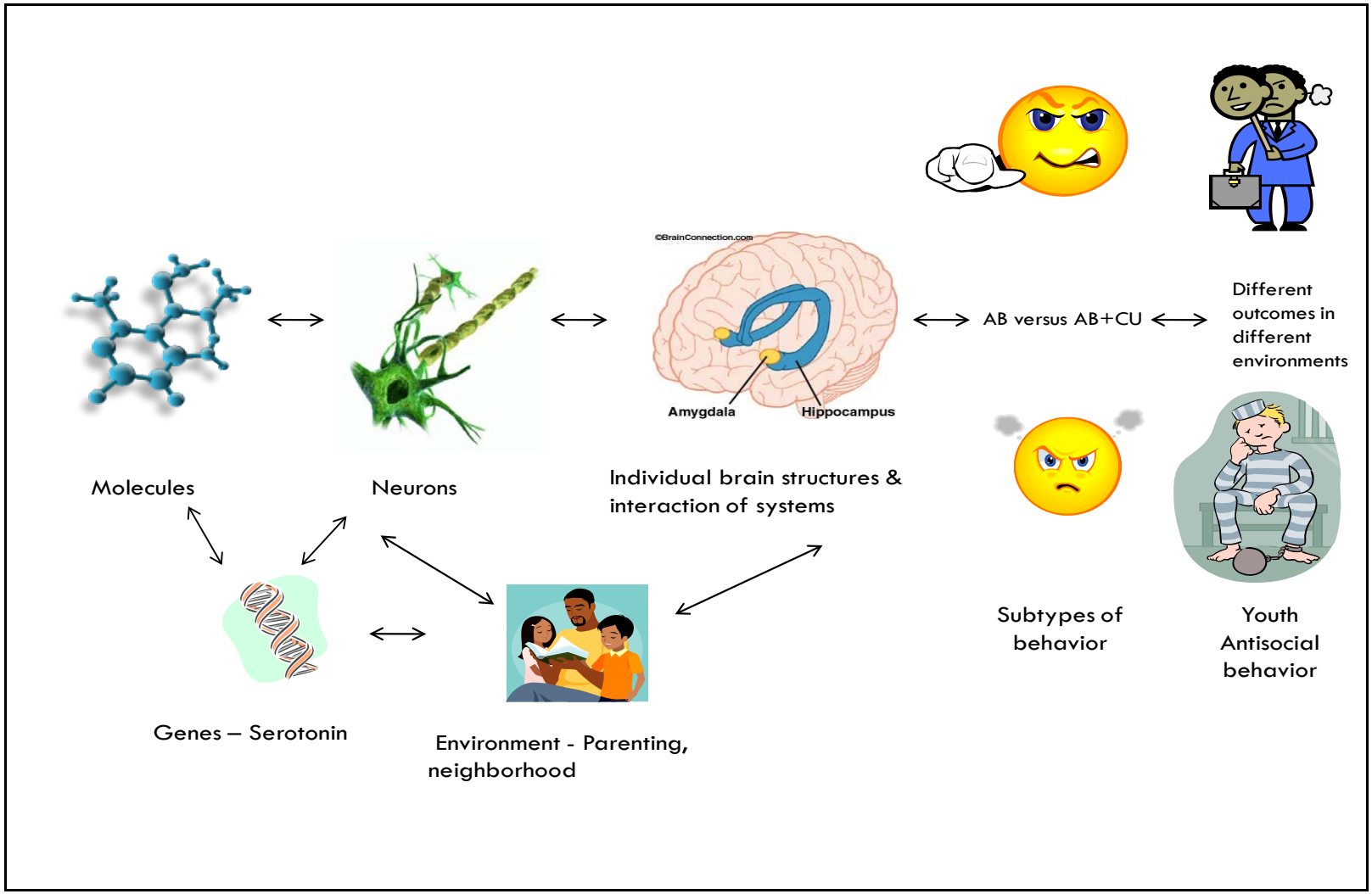


Figure 1: The interaction of biology at multiple levels as it may inform understandings of youth antisocial behavior.

In the past three decades, methodological advances in neuroscience and genetics have advanced our ability to measure specific biological processes. Popularization of techniques such as functional magnetic resonance imaging (fMRI) and advanced molecular genetics assays have made studies incorporating these techniques more practical in larger and more diverse samples, which in turn, has increased our understanding of the brain's role in psychopathology by allowing us to link biology to behavior.

Research that applies neuroscience to the study of AB is just beginning to emerge on adults and adolescents. Several recent studies have linked dysfunction of interconnected brain areas to adult psychopathy (Yang & Raine, 2008) using a variety of different tasks to probe specific behaviors implicated in the disorder. In studies on AB in adolescents, a small and varied literature on neural correlates has recently emerged. In particular, several studies on adolescents ranging in age from 10 to 17 with both AB and callous-unemotional (CU) traits have demonstrated differences in brain areas subserving arousal and emotion such as the amygdala (Jones, Laurens, Herba, Gareth, & Viding, 2009; Marsh et al., 2008). While these studies have helped to identify particular areas of dysfunction, they have been limited by the use of small samples with wide age ranges, the use of groups high on both AB and CU, and inconsistent results (Decety, Michalska, Akitsuki, & Lahey, 2009; Herpertz et al., 2008). Thus, because of an inconsistent pattern of findings and use of extreme groups of adolescents with high co-occurring levels of CU and AB, many issues warrant further investigation, especially the possibility of differentiating the role of brain function in AB versus CU.

In terms of genetic perspectives on AB, various genetic approaches have yielded links to behavior. AB has been shown to be at least moderately heritable using genetically-informed research designs, including twin, family, and adoption studies (DiLalla & Gottesman, 1991;

Raine, 2002a; Rhee & Waldman, 2002), and highly heritable when the phenotype is AB with callous-unemotional traits (Viding, Blair, Moffitt, & Plomin, 2005). Moreover, through both animal and human work, specific genes that code for proteins affecting neural transmission have been identified to correlate with AB and related behaviors and traits such as impulsivity (Blonigen & Krueger, 2006; Holmes, 2008). Unfortunately, these genetic studies generally have not been contemporaneously linked to other measures of biological functioning (i.e., neuroimaging), and thus interpreting the results from a mechanistic standpoint is often challenging. Consequently, even when specific genes are linked to AB it is unclear how these genes bias the biology of the individual to increase the probability of AB.

While both genetic and neuroimaging approaches to studying AB have informed our understanding of these behaviors (Crowe & Blair, 2008; DiLalla & Gottesman, 1991), these approaches have been particularly useful in understanding biological components of the etiology of psychopathology when they are considered together. Imaging data provide plausible mechanisms for underlying genetic influence and specific genes provide insights into how differential neural functioning may be affected by genetically driven differences in neuromodulatory systems (Hariri, 2009). Thus, an imaging genetics approach accomplishes two goals: it uses brain functioning to explain genetic linkages to behavior and uses genes to explain brain-behavior links at a molecular level. These goals are accomplished by measuring specific genes with known biological function (i.e., genes affecting serotonin transmission), while probing brain function in regions of interest (i.e., the amygdala) in samples of those at risk for AB (see Figure 2; Viding, Williamson, & Hariri, 2006).

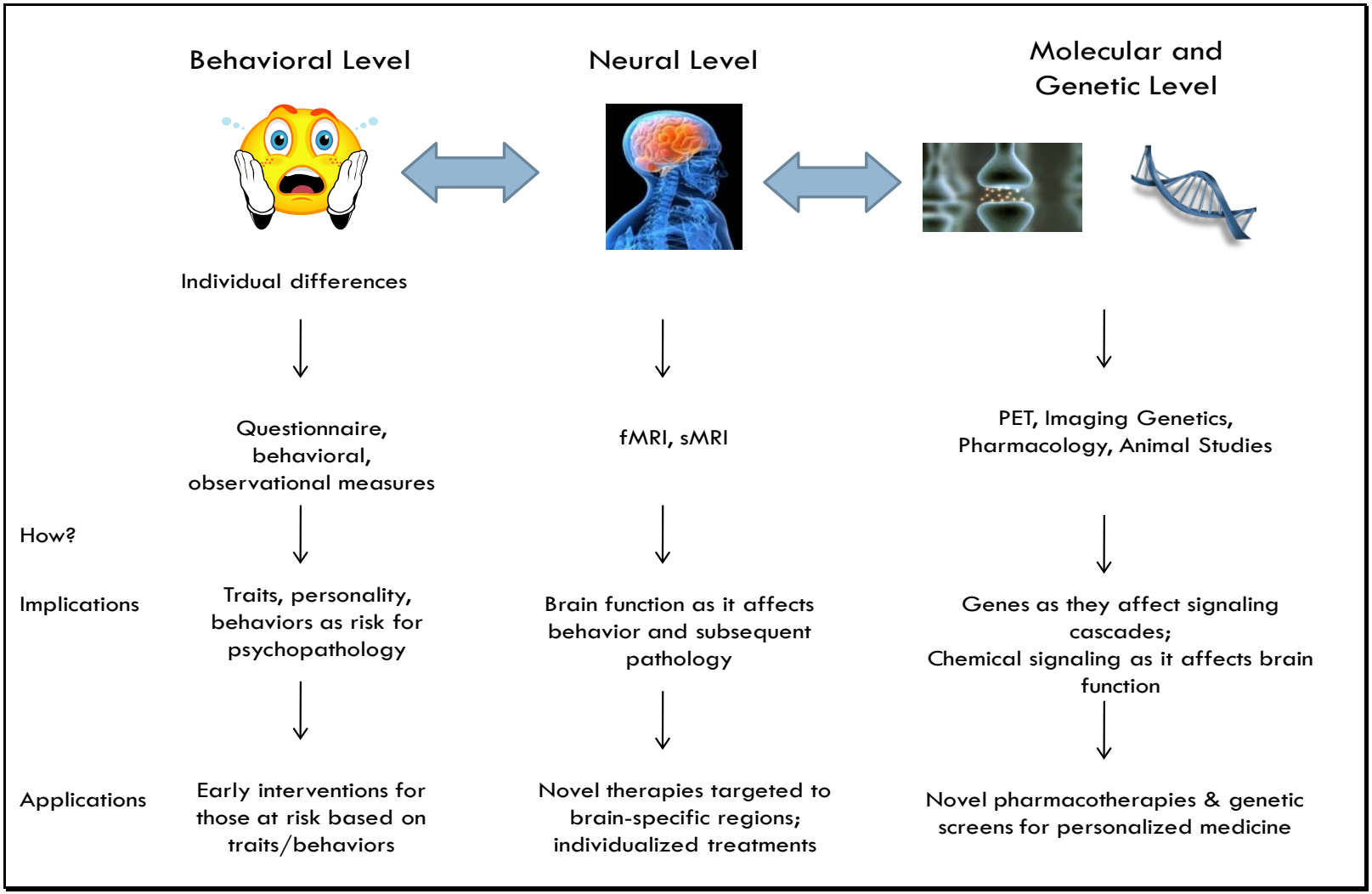


Figure 2: Integrative neuroscience: how analysis at multiple levels can inform etiology and treatment.

Finally, as implicated in more recent theories of AB (Rutter, 1997), biological links to behavior are likely qualified by an individual's environmental context. Recent gene by environment interaction (GxE) studies suggest the salience of the interplay between biology and environment in the etiology of AB (Caspi et al., 2002; Jaffee et al., 2005; Tuvblad, Grann, & Lichtenstein, 2006). Although GxE studies have changed our understanding of the role of biology and environment in psychopathology, few studies to date have combined this approach with neuroimaging. Just as imaging genetics studies have the potential to inform our understanding of biology at the mechanistic level, *imaging gene environment interactions* (IGxE) studies have the potential to inform our understanding of how genes affect brain functioning and subsequent behavior differentially across environmental contexts (Caspi & Moffitt, 2006; Hyde, Bogdan, & Hariri, 2011). In this approach, links between genes and neural reactivity or links from genes to AB through neural reactivity may be stronger or weaker in environments characterized by risk for AB (Kim-Cohen et al., 2006; Raine, 2002b).

The present study focuses on neural, genetic, and contextual correlates of AB and aims to advance our understanding of AB by applying imaging genetics and developmental psychopathology perspectives to the development of AB using an ethnically diverse cohort of 310 low-income children followed prospectively from early childhood to early adulthood. First, this study will extend previous studies by examining both neural and genetic correlates of AB from early adolescence to young adulthood (see Figure 3). Second, this study will apply an imaging genetics approach to understanding AB in which specific genes are postulated to have an indirect effect on AB through their effect on brain functioning. Third, environmental risk factors will be examined using IGxE during two pivotal periods, early childhood and early adolescence, as moderators of biological links to AB, with the expectation that genetic effects on

brain function and subsequent AB will be amplified based on the levels of environmental risk at two salient developmental periods.

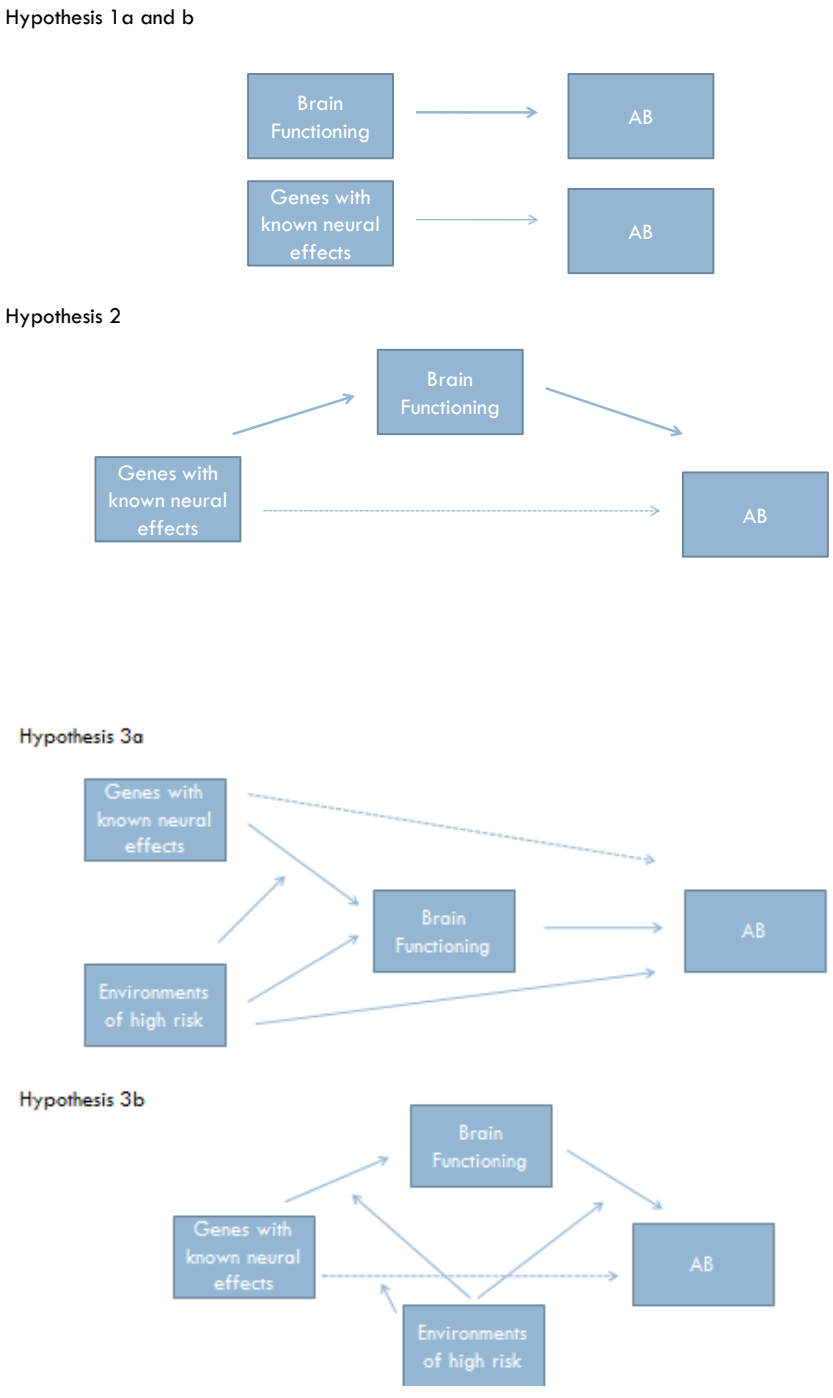


Figure 3: Broad hypotheses

1.1 ANTISOCIAL BEHAVIOR AND BIOLOGY

AB is a prevalent and pervasive problem in youth (Nock, Kazdin, Hiripi, & Kessler, 2006). AB can be described by a host of terms in children, adolescents, and adults, including legal definitions (delinquency), broad behavioral definitions (externalizing), and specific types of behaviors (aggression). In the *Diagnostic and Statistical Manual of Mental Disorders*, Fourth Edition (DSM-IV) (American Psychiatric Association, 1994), youth AB is categorized into oppositional-defiant disorder (ODD) and conduct disorder (CD), with ODD more focused on early age-inappropriate angry and oppositional behaviors, and CD focused on severe aggression and behaviors that involve inflicting pain on others (e.g., initiating fights, sexual assault), denying the rights of others (e.g., stealing), and status offences (e.g., truancy) (Hinshaw & Lee, 2003). When these behaviors are persistent in adults, they are categorized on Axis II as Antisocial Personality Disorder (APD), with the diagnosis requiring prior CD. These disorders are quite common: a recent study has estimated the lifetime prevalence of CD in the United States to be 9.5% of the population (12 % among males, and 7% among females), with a median age of onset of 11.6 years (Nock et al., 2006).

Within both child and adult antisocial populations, heterogeneity of symptoms is prevalent, often causing researchers to either subdivide these behaviors or study them individually. For example, researchers have proposed dividing subgroups based on age of onset (Moffitt, 1993), type of behavior (reactive versus proactive or overt versus covert) (Loeber & Stouthamer-Loeber, 1998; Vitaro, Brendgen, & Barker, 2006), or presence of early markers of particularly problematic traits such as CU traits (Frick et al., 2003). Early onset AB and AB with

CU traits have been found to represent more homogenous groups with a particularly chronic and delinquent course (Frick & White, 2008; Moffitt, Caspi, Harrington, & Milne, 2002). In adults, a major distinction has been made between criminality (and APD) and a more severe form of personality called psychopathy, the latter involving a parasitic and antisocial lifestyle as well as interpersonal and affective deficits such as lack of empathy, guilt, and remorse along with superficial charm and manipulativeness (Patrick, 2007).

A long history of antisocial propensity theories (Gottfredson & Hirschi, 1990; Lahey, Waldman, & McBurnett, 1999) has emphasized stable, early appearing dispositions and traits that increase risk for developing AB. These traits are thought to be genetic in their initial origin and interact with the environment over time to increase risk for AB. Heritability estimates from genetically informed designs support the idea that some substantial portion of the risk for AB is genetically driven (DiLalla & Gottesman, 1991; Rhee & Waldman, 2002). Interestingly though, heritability increases with age (the later AB is measured, the more heritable) (Jacobson, Prescott, & Kendler, 2002) and those with early onset AB demonstrate higher heritability (Taylor, Iacono, & McGue, 2000). Beyond genetically informed designs, a plethora of studies have demonstrated that those high on AB differ across a range of biological measures (DiLalla & Gottesman, 1991; Nelson, 2006; Raine, 2002a). As these behaviors likely have some genetic origin, and as all behaviors eventually reflect differences in brain functioning at some level, researchers have begun to identify differences in functioning in specific brain areas and genes linked to these destructive behaviors to help understand the biological components of the etiology of AB.

1.2 NEUROIMAGING APPROACHES TO AB

Given the longstanding theory of biological and neural differences in those demonstrating higher levels of AB, it is not surprising that as more direct measures of central nervous system (neural) functioning such as fMRI have become more accessible, studies have begun to examine the neural correlates of AB, using past models and studies that examined peripheral nervous system differences (e.g., galvanic skin response, startle blink potentiation, heart rate) to identify and posit neural differences.

1.2.1 Adults

In adults, most of the research on neural functioning in relation to AB has been examined in studies focused on psychopathy. This disorder has been linked to differences in peripheral nervous system functioning across many different measures (e.g., skin conductance, heart rate, startle blink potentiation) with implications for central nervous system functioning. These differences, reflecting lower autonomic arousal, particularly in response to threatening and arousing images and situations, are thought to reflect core features present in psychopathy, including fearlessness, physiological under-reactivity, and emotional detachment (Scarpa & Raine, 2006).

Based on findings using the aforementioned peripheral measures of biological function, emerging research using neuroimaging has emphasized the link between psychopathy and threat, emotion, and arousal centers in the brain (i.e., the amygdala), as well as other regulatory areas (i.e., the prefrontal cortex) that are responsible for executive functioning and planning. For example, studies have emphasized decreased activity in the amygdala and broader amygdala-

hippocampal formation in criminal psychopaths versus healthy controls during aversive classical conditioning paradigms (Birbaumer et al., 2005; Veit et al., 2002) and during an affective lexical task contrasting emotional phrases to neutral phrases (Kiehl et al., 2001). Twenty healthy male college students scoring high on a trait measure of psychopathy displayed similar attenuated amygdala response during an emotional faces paradigm (Gordon, Baird, & End, 2004) and when their cooperation was not reciprocated in a prisoner's dilemma game (Rilling et al., 2007). Reduced amygdala reactivity has also been correlated with levels of psychopathic traits during a moral decision-making task among a sample of 17 adults from the community (Glenn, Raine, & Schug, 2009).

However, while amygdala *under*-reactivity in this population has been emphasized in research and theory (Davidson, Putnam, & Larson, 2000; Kiehl, 2006), several studies have found *greater* amygdala reactivity in this population in response to emotional picture viewing and classical conditioning tasks (Muller et al., 2003; Schneider et al., 2000), leading to some ambiguity in the field as to the direction of the relationship with amygdala reactivity. While the direction of correlation is unclear, focus on the amygdala is not surprising. The amygdala has been implicated in emotional learning, fear response and classical conditioning, memory consolidation, and general arousal (LeDoux & Sciller, 2009), all of which have been shown to be disrupted in those with high levels of AB (Glenn & Raine, 2008). Hence, the amygdala is a particularly important structure in understanding possible neuropathology in AB as it may progress through development: early disruptions in amygdala reactivity could plausibly interact with environmental risk to start a cascade leading to diminished emotional response to others (i.e., empathy) and difficulty with learning consequences of problematic behaviors (Blair, Peschardt, Budhani, & Pine, 2006b).

Beyond the amygdala, in many of these same studies, functional differences have been noted in other regions of the brain, particularly in various areas of the prefrontal cortex (PFC). Decreased orbital frontal cortex (OFC) functioning has been found in psychopaths versus healthy controls and among healthy individuals scoring higher on trait measures of psychopathy during the tasks described above (Birbaumer et al., 2005; Glenn et al., 2009; Gordon et al., 2004; Rilling et al., 2007; Veit et al., 2002). These findings of OFC dysfunction fit with neuropsychological findings implicating the OFC specifically in psychopathy (Blair, Newman, et al., 2006). They are also consistent with theory suggesting amygdala-OFC functioning to be a critical dysfunction in psychopathy (Blair, 2003) and OFC dysfunction to be important broadly in aggression and AB (Blair, 2004). Beyond the OFC, several studies have found differences in dorsal-lateral PFC functioning in relation to trait and criminal psychopathy (Gordon et al., 2004; Rilling et al., 2007; Schneider et al., 2000; Veit et al., 2002). Differences in neural activation have also been found for other regions, including the anterior cingulate, insula, and ventral striatum (Birbaumer et al., 2005; Buckholtz et al., 2010; Kiehl et al., 2001; Veit et al., 2002). Differences in the functioning of these brain areas have been posited to reflect the neural correlates of disruptions in decision making, inhibition of behavior, reward dependence, and empathy found in antisocial populations (Blair, 2003; Buckholtz et al., 2010; Decety et al., 2009).

These neuroimaging studies on adults have focused primarily on psychopathy as an endpoint either in criminal populations (versus controls) or in samples of college students (using trait measures of psychopathy). Generally, these studies have used dichotomous groups and have not focused on community samples at high risk where AB or psychopathy can be measured continuously, nor have they examined AB outside of its role in psychopathy. The age range of these studies has also varied widely without focus to a specific developmental period (except for

those studies of college students). Whereas some of these studies have suggested reduced amygdala (and PFC) functioning in response to salient affective and conditioning paradigms, results have been mixed.

Finally, beyond these studies, a few other intriguing findings bear mentioning. First, in a study of 10 adults characterized by impulsive aggression (i.e., a diagnosis of Intermittent Explosive Disorder), those in the patient group displayed increased amygdala reactivity to angry faces in comparison with controls (Coccaro, McCloskey, Fitzgerald, & Phan, 2007). Second, in a study of 20 healthy college students mentioned above, those scoring high on factor 1 psychopathy (emotional, interpersonal dimension) displayed *attenuated* amygdala reactivity to emotional faces, but those scoring high on factor 2 psychopathy (social deviance, impulsive aggression) displayed *increased* amygdala reactivity (Gordon et al., 2004). These studies suggest that within adult AB, different facets (factor 1 psychopathy or callousness versus impulsive aggression) may correlate differently with neural reactivity, particularly in the amygdala. Thus more research is needed that focuses on amygdala reactivity across different types of AB.

In sum, studies in adults have generally implicated amygdala and prefrontal cortex dysfunction in psychopathy and AB. While most theory and empirical work has emphasized *hypo*-reactivity in the amygdala in this population, there have been some studies showing the opposite relationship, especially for those adults demonstrating AB without psychopathy which is likely characterized by reactive rather than proactive aggression.

1.2.2 Adolescents.

In adolescents the pattern of findings has been similar: an emphasis on the amygdala and mixed findings regarding the direction of the association between neural reactivity and behavior.

Several research groups have recently explored the link between amygdala functioning and youth AB using fMRI paradigms that generally contrast negative stimuli to neutral stimuli. For the most part, based on the literature above with adult psychopaths, these research teams have proceeded with the general hypothesis that children with AB would show less amygdala reactivity than controls to negative as compared with neutral stimuli due to a deficiency in general arousal. In the first study of its kind, Sterzer and colleagues (2005) initially found no differences in amygdala functioning comparing a group of 13 conduct disordered adolescent boys, ages 9 to 16 years old, with 14 healthy controls when contrasting negative to neutral pictures from the International Affective Picture System (IAPS). However, the authors noted a high degree of anxiety/depression symptomatology in the sample and when they controlled for these symptoms, they found that the CD group displayed less left amygdala reactivity to the negative to neutral contrast than the control group (Sterzer, Stadler, Krebs, Kleinschmidt, & Poustka, 2005).

In two studies with very similar methods (Jones et al., 2009; Marsh et al., 2008), 17 and 12 boys ages 10-12 and 10-17, respectively, high on both AB and CU traits were found to have less right amygdala reactivity than controls in a task contrasting fearful faces to neutral faces. These three studies taken together have been interpreted as evidence that adolescents with AB demonstrate amygdala *hypoactivity*, at least when CU traits are also present. As youth with AB and CU traits are seen as having an earlier form of psychopathy (Frick & White, 2008), these findings further support theory of diminished arousal in psychopathy across several developmental periods (early adolescence, mid-adolescence, late adolescence/early adulthood, adulthood). In addition and more recently, a study of older adolescents (age 16-21) within a larger sample (n = 75) of both early and late starting AB, found *less* bilateral amygdala reactivity

(and decreased activity across many other related areas including the OFC, vmPFC, and insula among others) in tasks contrasting angry faces to neutral faces and sad faces to neutral faces (Passamonti et al., 2010). Interestingly, in this study CU traits were not related to amygdala reactivity suggesting that AB itself may be driving the amygdala hypoactivity. However, based on this study's extreme group design CU and AB symptoms might have been confounded. Clearly studies are needed that can separate these two constructs to examine their possible differential association with amygdala reactivity.

In contrast, several studies of adolescents have examined the neural correlates of AB without also examining CU traits. First, in a study of 22 boys ages 12-17 with Conduct Disorder, the patient group displayed *greater* left amygdala reactivity than controls to a paradigm contrasting negative and neutral images (Herpertz et al., 2008). Second, in a study of eight adolescents examining the role of empathy in early starting CD (Decety et al., 2009), participants watched animations of other people experiencing pain caused either by accident or on purpose versus people not experiencing pain. Although both groups displayed increased activity in brain areas associated with pain (Jackson, Rainville, & Decety, 2006), the CD group showed greater activation compared to controls in limbic and some frontal regions (amygdala, temporal pole, striatum) and lesser activation in other frontal areas (dorso-lateral PFC, right superior gyrus). Within the CD group, aggressive CD symptoms and dimensions of daring and sadism showed a correlation with activity in the amygdala. Thus, in the CD group, the amygdala showed *greater* reactivity during this paradigm and the CD group was found to have less PFC-amygdala connectivity, which fits well with theories linking aggression with difficulties regulating negative emotions (Raine, 2002b). However, these findings conflict with studies on children with AB and CU, who showed under-activation of the amygdala compared to non-AB children.

In sum, studies on adolescents have emphasized differences in neural reactivity across the amygdala and various PFC areas (among others). Several studies suggest that adolescents with AB and CU show decreased amygdala reactivity to emotional paradigms, while several others suggest that in non-CU focused samples with CD, the reverse association may hold. Finally, additional studies suggest differences in other brain areas (especially the PFC) without replicated findings. These findings mirror work done in adults and may suggest an opposite pattern of amygdala reactivity for those with AB versus the small group of youth with AB and CU, and broad but inconsistent differences in PFC functioning. However, these studies suffer from many of the same issues as those in adults – small samples that are extreme on traits and behaviors, wide age ranges (which is particularly important during adolescence), the inability to separate CU versus AB in correlations with neural reactivity, and a variety of different imaging tasks. Beyond simply using large age ranges of youth, much of this research has ignored developmental models of AB. Although using CU traits as a way to identify a particularly homogenous and severe subgroup is gaining momentum (www.dsm5.org), these studies have not addressed a growing emphasis of person centered approaches that identify subgroups based on developmental trajectories (Bergman & Magnusson, 2003; Emde & Spicer, 2000) or approaches aimed at examining pathology across a continuum (Plomin, Haworth, & Davis, 2009). Studies that can examine AB and CU traits contemporaneously as continuous variables across a wide range or that can classify more homogenous groups based on trajectories of behaviors using person centered approaches can help to extend this work so that we can understand the relationship between neural reactivity (especially amygdala reactivity) and AB in a more complex and nuanced way throughout development.

1.3 GENETIC AND NEUROMODULATOR APPROACHES

As AB has been shown to be at least moderately heritable (DiLalla & Gottesman, 1991; Rhee & Waldman, 2002), researchers have aimed to identify specific genes and genetically regulated neuromodulators (e.g., hormones, peptides, and neurotransmitters) linked to AB, aggression and other related behaviors (e.g., CU traits, empathy). Targets have included monoamines (e.g., serotonin, dopamine, norepinephrine) and steroidal hormones (e.g., androgens, cortisol, estrogen) among others (Nelson, 2006). There have been few genome wide association study (GWAS) on this topic and those that do exist fail to find any consistent findings (see Gunter, Vaughn, & Philibert, 2010). For example, a recent GWAS on a selected sample (AB+CU+) failed to demonstrate consistent and statistically significant genetic associations across two samples (Viding et al., 2010). While some authors have suggested pursuing genetic and molecular targets associated with both AB and brain functioning (Blair, Peschardt, Budhani, & Pine, 2006c; Viding et al., 2006), few studies have done so. Based on developmental work implicating the role of threat, physiological arousal, and empathy in AB (Blair, Peschardt, et al., 2006c) and the aforementioned neuroimaging work on AB, the current study focuses specifically on serotonin (5-HT) and its role in AB and neural functioning. As 5-HT has been linked widely to aggression and AB, has a clear role in modulating neural reactivity, especially in limbic targets (such as the amygdala highlighted above), and has been central to theory on neuroimaging and AB (Blair, Peschardt, et al., 2006c), examination of genetic variation in this neurotransmitter system as it relates to AB and neural reactivity may be crucial for understanding the biology of AB (Gunter et al., 2010).

1.3.1 The role of 5-HT

Across animal and human studies, lower serotonin (5-HT) levels have been implicated theoretically (Coccaro, 1996; Coccaro & Kavoussi, 1996; Soubrie, 1986; Spoont, 1992) and empirically (for reviews see Higley et al., 1992; Manuck, Kaplan, & Lotrich, 2006; Mehlman et al., 1994; Tuinier, Verhoeven, & Van Praag, 1995) with higher levels of aggression and impulsivity, although studies in youth have been mixed (van Goozen, Fairchild, Snoek, & Harold, 2007). From a genetic standpoint, within rodent models and human linkage studies, variation in genes coding for 5-HT receptors (1A, 1B, 2A, 3, and 7) and molecules important for the synthesis (tryptophan hydroxylase 1 and 2 - TPH), reuptake (5-HT transporter), and degradation of 5-HT (monoamine oxidase A and B– MAOA and MAOB) have been linked to impulsivity and aggression (Holmes, 2008; Lesch & Merschdorf, 2000). This literature is important when considering neuroimaging studies of AB because 5-HT is a critical modulator of many neural circuits implicated in AB: 5-HT neurons emanating from the raphe nuclei project to forebrain targets implicated in AB including the amygdala and PFC (Azmitia & Gannon, 1986; Holmes, 2008; Sterzer & Stadler, 2009). Thus, it is not surprising that 5-HT has been hypothesized to be a critical component of several neurobiological models of youth and adult AB (Blair, Peschardt, Budhani, & Pine, 2006a; Siegel, Bhatt, Bhatt, & Zalcman, 2007; van Goozen et al., 2007).

When linking 5-HT genes to youth AB, a few important studies bear closer examination. First, several G x E studies have demonstrated links between individual variability in a common variant in the promoter of the MAOA gene (that affects degradation of monoamines including 5-HT and others) and AB in youth and adults who have experienced abuse or maltreatment (Caspi et al., 2002; Kim-Cohen et al., 2006). Similarly, variants in genes for TPH and 5-HTT have also

been linked to aggression and AB in adults and youth (Beitchman et al., 2006; Beitchman et al., 2003; Manuck et al., 1999; Sadeh et al., 2010; Young & Leyton, 2002), but sometimes in contradictory directions. Interestingly, some of these same variants (e.g., 5-HTTLRP and *MAOA* VNTR) have also been linked to functioning of the amygdala and PFC – areas highlighted earlier in this review as vital to our understanding of youth AB (Brown et al., 2005; Buckholtz et al., 2008; Hariri et al., 2002; Pezawas et al., 2005). For example, Buckholtz and colleagues have linked the pattern of neural reactivity that was related to variation in *MAOA* (Buckholtz et al., 2008) to patterns of neural reactivity seen in aggressive and violent populations (Buckholtz & Meyer-Lindenberg, 2008; Meyer-Lindenberg et al., 2006): Those with low expressing *MAOA* alleles (the allele linked in many G x E studies to increased AB) displayed increased functional activity in the left amygdala and decreased response across various cortical areas (e.g., BA25 and 32, lateral OFC and insula).

However, theses links are not as straightforward as they seem. Several of the specific alleles linked to AB (e.g., 5-HTTLPR S allele, low expressing *MAOA* alleles) have been associated with *greater* reactivity in brain areas such as the amygdala (Buckholtz et al., 2008; Buckholtz & Meyer-Lindenberg, 2008; Hariri et al., 2002), whereas much of the literature reviewed emphasizes *lesser* amygdala reactivity in AB, particularly in AB with CU or psychopathy.

One intriguing hypothesis is that the link between 5-HT genes and behavior may not be the same for all groups of antisocial individuals. Similar to neuroimaging studies already reviewed, such associations may differ drastically when comparing subgroups of those engaged in AB (e.g., those high on CU or psychopathy vs. those with more reactive and impulsive AB) (Glenn, 2011), which may help explain a pattern of contradictory findings. Glenn (2011) noted

that although the 5-HTTLPR S allele is thought of as the "risk" allele and has been linked to depression and anxiety, as well as *impulsive* aggression, the L allele has been related to many intermediate phenotypes (e.g., *decreased* amygdala reactivity, decreased skin conductance during fear conditioning, deficits in passive avoidance learning) that have also been linked to psychopathy (though see Fowler et al., 2009). The hypothesis linking the L allele to psychopathy has been subsequently supported in a recent study of adults with alcohol dependence (Herman et al., 2011) and in a GxE study of youth (Sadeh et al., 2010). In the GxE study, individuals with the S allele evidenced more impulsivity, but those with the L allele and low socioeconomic status had greater CU traits (Sadeh et al., 2010). A similar argument has been made in regards to *MAOA*: GxE findings (Caspi et al., 2002) and literature on early maltreatment in humans and animals (Kaufman & Charney, 2001; Pollak & Sinha, 2002) suggest that low expressing *MAOA* alleles, especially in the presence of early maltreatment, could lead to greater amygdala reactivity and later reactive violence (Hanson et al., 2010; Tottenham et al., 2011; Viding & Frith, 2006). However, high expressing *MAOA* alleles could be linked with proactive aggression and CU traits similar to that described for the 5-HTTLPR L allele (in both the 5-HTTLPR short allele and the MAOA low expressing alleles result in reduced transcriptional efficiency, which could lead to less breakdown and clearance of 5-HT from the synapse). Thus, it is possible that there may be subgroups of youth with AB who show divergent patterns of genetic and neuroimaging associations, although presently, this hypothesis is still quite speculative and needs to be tested empirically.

1.3.2 Specific 5-HT genetic targets

A particularly promising approach to understanding the role of genetics and 5-HT more

specifically, would be to explore 5-HT genes that are connected several important steps in the neurotransmission of 5-HT: biosynthesis (*TPH2*), autoregulation (*HTR1A*), transport (5-HTT), and degredation (MAOA) (See Figure 4). One important note to consider in examining the possible biological effect of these genes is that the relationship between acute genetic effects and the ultimate effects on 5-HT signaling are likely to be quite complicated. For example, if a specific genetic variant leads to less of a protein that upregulates 5-HT signaling, this different variant could cause a cascade of events leading to up or down regulation of other 5-HT regulators (e.g., proteins regulating synthesis, reuptake of 5-HT). Moreover, as this process begins *in utero*, these variants could ultimately affect adult or adolescent neural transmission through effects on brain structure, connectivity, and/or immediate or long term neural chemistry. Additionally, these effects could vary based on developmental stage, other genetic variants and environmental experiences. With this caveat in mind, four candidate genes are reviewed below, highlighting important studies in regards to the current study though this literature is not reviewed exhaustively (for a more comprehensive review see: Gunter et al., 2010).

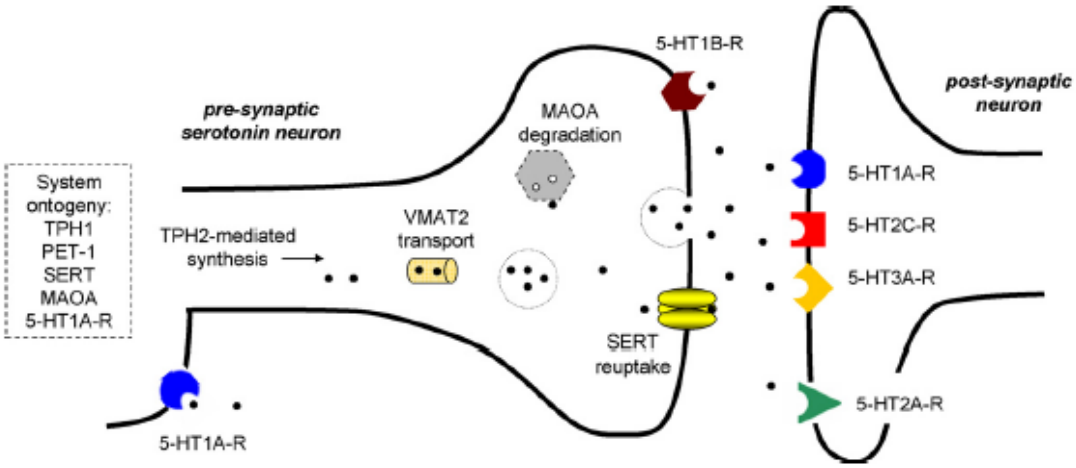


Figure 4: Illustration of the role of various molecules in the 5-HT signaling cycle (Holmes, 2008)

1.3.2.1 *MAOA*

As noted above, MAOA is a key enzyme in the metabolism of monoamines (e.g., 5-HT, dopamine), and the rate at which MAOA degrades 5-HT is one determinant of 5-HT availability. MAOA knock-out mice show large increases in aggression and increased brain 5-HT and norepinephrine (Cases et al., 1995; Seif & De Maeyer, 1999). Additionally, a very rare point mutation of this gene found in humans (which is essentially a knock-out) was linked to abnormal monoamine metabolism and a marked increase in impulsive aggression and AB in a Dutch kindred with 24 affected men (Brunner, Nelen, Breakefield, Ropers, & Van Oost, 1993; Brunner, Nelen, Van Zandvoort, et al., 1993). A more common repeat sequence in the human gene has been linked to transcriptional activity in the MAOA promoter. This VNTR contains "low" and "high" *MAOA* activity variants (originally described in Sabol et al., as low = 3 and 5 repeats, high = 3.5 and 4 repeats) with "low" activity variants having decreased transcriptional efficiency (Sabol, Hu, & Hamer, 1998). Those with the "high" activity allele displayed greater dispositional aggressiveness and impulsivity, and less central 5-HT responsivity in a community sample of 110 men (Manuck, Flory, Ferrell, Mann, & Muldoon, 2000), although opposite results have been shown in another study (Williams et al., 2009). The low-expressing variant (i.e., less transcription of *MAOA* which would theoretically lead to less degradation of 5-HT) has been linked to increased amygdala and hippocampal response and attenuated PFC response to threatening faces, as well as changes in brain volume across limbic and frontal areas of the brain (Buckholtz et al., 2008; Buckholtz & Meyer-Lindenberg, 2008). Finally, in GxE studies, the low-expressing variant has been linked to AB in those experiencing abuse (e.g., Caspi et al., 2002); though not in all studies (e.g., Young et al., 2006). Thus, variations in the *MAOA* gene appear to be linked to amygdala reactivity (i.e., "low" expression *MAOA* alleles are linked to greater

amygdala reactivity) and AB (at least via G x E mechanisms); however, the direction of these associations may vary depending on which types of aggression or AB are examined.

1.3.2.2 *HTR1A*

The 5-HT 1A receptor is an autoreceptor that regulates neuron activity in the dorsal raphe and is a postsynaptic receptor mediating 5-HT activation on corticolimbic regions, including the medial PFC and amygdala. The 1A receptor, therefore, is an essential mechanism by which the 5-HT system can self-regulate (Holmes, 2008). While variation in the 1A receptor has traditionally been linked to anxiety behaviors in mice and humans (Fakra et al., 2009; Holmes, 2008), some studies have shown 1A receptor agonists have anti-*aggressive* effects in rats, apparently through their reduction of 5-HT neurotransmission during combative social interaction (de Boer & Koolhaas, 2005). Importantly, density of 5-HT1A autoreceptors accounts for 30% to 40% of variability in amygdala reactivity in healthy adults (Fisher, Meltzer, Ziolko, Price, & Hariri, 2006), and a relatively common SNP in the promoter region of this gene was demonstrated to affect transcriptional regulation (Lemonde et al., 2003). Consequently, the 1A receptor is an essential target in understanding 5-HT's role in amygdala reactivity and has been linked to amygdala reactivity in humans (Fakra et al., 2009).

1.3.2.3 5-HTT

The 5-HT transporter (5-HTT) removes 5-HT from the synaptic cleft and thus determines the magnitude and duration of post-synaptic signaling. The 5-HTT contains functional variability in the promoter region of the gene (*SLC6A4*) coding of the transporter (5-HTTLPR) and this variability has been widely studied and linked to psychopathology and personality broadly, and specifically to traits linked to both affective disorders and anger and aggression (Munafò et al.,

2009).The 5-HTT has been linked to aggression in mice (Holmes, Murphy, & Crawley, 2002) and recent reports link 5-HTTLPR low activity alleles (S allele) to aggression in children (Beitchman et al., 2006; Beitchman et al., 2003). Moreover, the 5-HTTLPR has been linked to reactivity in the amygdala and other important limbic structures across several studies in which the S allele has been linked to greater amygdala reactivity (Hariri et al., 2005; Hariri et al., 2002; Pezawas et al., 2005). As noted above, there is some suggestion that although the S allele has been linked to aggression and impulsivity, the L allele has recently been linked to psychopathy (Glenn, 2011; Herman et al., 2011; Sadeh et al., 2010). Thus research is needed that can address possible subgroups within youth and adults with AB.

1.3.2.4 *TPH2*

The first step in 5-HT biosynthesis in neurons is catalyzed by the rate-limiting enzyme TPH (tryptophan hydroxlase). Altering levels of tryptophan (and thus serotonin) artificially can induce higher levels of aggressive responding (Young & Leyton, 2002) and in a community sample of 251 adults, (non-functional) variation in a TPH gene (*TPH1*) predicted a wide range of measures of aggression, anger, and AB while also predicting a measure of central nervous system 5-HT activity (Manuck et al., 1999). Though results have been mixed, variability in *TPH2* genotype has been linked to suicidality, often seen as a measure of impulsive aggression (Gunter et al., 2010). Additionally, variability within the functionally relevant gene TPH2 has been linked to amygdala reactivity (Brown et al., 2005).

In sum, these four gene targets cover important steps in 5-HT transmission and re-uptake, each gene has been linked to aggression and antisocial behavioral targets in animals and/or humans, and each gene has shown links to differences in neural reactivity, particularly in limbic areas such as the amygdala. As a result, exploring these genes in concert can help their

association with AB and their impact on brain functioning as it may in turn link to AB.

1.4 IMAGING GENETICS APPROACHES

Imaging genetics is one tool within a multimodal neuroimaging approach to understand the biology underlying behavior, personality, and pathology at multiple levels of biological analysis (see Figure 1; Hariri, 2009). Imaging genetics links genetic variability, in the form of common genetic polymorphisms, to variability in brain functioning in response to well-characterized imaging paradigms. In turn, variability in brain functioning is linked to behavior (Hariri, Drabant, & Weinberger, 2006; Munoz, Hyde, & Hariri, 2009). Classically, this technique can be seen in studies where a genetic polymorphism (i.e., variation in genes coding for an autoreceptor in the serotonin system) indirectly predicts behavior (i.e., trait anxiety) through its effect on brain functioning (i.e., amygdala reactivity) (Fakra et al., 2009). This model is intuitively appealing – genes that affect neurotransmitter levels affect brain functioning, which in turn affects behavior. The genes used in imaging genetics research typically have an established link to some functional effect at the molecular and synapse level and are thus proxies, not just for genetic make-up, but for what is occurring neurochemically at the synapse. As a result, while the approach lends itself to understanding brain function as the mediator of a genetically driven effect, it is also important to understand that the genetic variation being studied should have been previously established as affecting neurotransmission at a molecular level. Therefore, the genes in question can help explain individual differences in brain function and links to behavior at the molecular level. An imaging genetics approach has been used to help understand risk for affective disorders. Serotonin genes (e.g., 5-HTT, 5-HTR1A) have been related to increased

amygdala reactivity (Hariri et al., 2005; Hariri et al., 2002), and amygdala reactivity has been associated with outcomes such as trait anxiety and depression (Fakra et al., 2009; Monk et al., 2008; Siegle, Thompson, Carter, Steinhauer, & Thase, 2007).This technique has also been used to understand a variety of endpoints from impulsivity to schizophrenia to autism (Hariri, 2009; Meyer-Lindenberg et al., 2008; Meyer-Lindenberg & Weinberger, 2006).

As emphasized above, an imaging genetics approach could be very important in understanding the role of genes and brain functioning in AB. However, few studies have utilized such a perspective in relation to AB. As noted previously, Meyer-Lindenberg, Buckholtz and colleagues have used this technique to link genetic variation in MAOA to neural structure and function (Buckholtz et al., 2008; Meyer-Lindenberg et al., 2006). However, it should be noted that while these studies do, as a whole, provide links from genes to brain to behavior, links were only drawn to personality variables (i.e., harm avoidance, reward dependence), and not actual AB or history of violence. Thus, studies that draw out all three connections concurrently are needed. Moreover, beyond these studies, the imaging genetics approach has not been applied to the study of AB, nor used within a developmental psychopathology framework (Viding et al., 2006). Therefore, studies with molecular genetics, neuroimaging, and behavioral data are needed to address the possible and probable links among genes (particularly those in the 5-HT system), neural reactivity (particularly the amygdala), and AB (or subtypes such as AB and CU) (Blair, Peschardt, et al., 2006c; Viding et al., 2006).

In sum, while an imaging genetics approach has the potential to inform our understanding of the biology of AB at multiple levels of analysis (i.e., from neural correlates to neurochemistry to genetic influences on brain and behavior), as it has for disorders such as anxiety and depression, little work has applied the approach to the study of AB. Although studies examining

either genetics or neuroimaging have been inconsistent, there is some evidence that two biological pathways may emerge in imaging genetics studies of AB. One pathway of genetic variants linked to less amygdala reactivity and subsequent AB with CU (or more proactive aggression), while another path would involve genetic variants linked to greater amygdala reactivity and subsequent AB (without CU) or impulsive and reactive behaviors.

1.5 ENVIRONMENTAL CONTRIBUTORS TO AB

Although there is clear evidence for the role of biology in the etiology of AB, there is also a plethora of literature implicating environmental causes and contributors. Genetically informed designs highlight heritable factors, but they also implicate both shared and non-shared environmental influences on AB (Rhee & Waldman, 2002). Several domains of the environment have been consistently linked to the development of AB, some becoming more robust as children move into school-age and adolescence (e.g., neighborhood risk, monitoring of child behavior, exposure to deviant peers), with others evident from early childhood (parenting, parental depression). Risk factors across childhood and adolescence such as harsh parenting and lack of nurturance (Shaw, Gilliom, Ingoldsby, & Nagin, 2003), poor parental monitoring (Stattin & Kerr, 2000), inter-parental conflict (Fantuzzo et al., 1991), child maltreatment and physical discipline (Dodge, Bates, & Pettit, 1990; Lansford et al., 2011), poverty and dangerousness neighborhoods (Leventhal & Brooks-Gunn, 2000; McCabe, Lucchini, Hough, Yeh, & Hazen, 2005), single parenthood and teen parenthood (Conseur, Rivara, Barnoski, & Emanuel, 1997), parent-child conflict (Trentacosta et al., 2011), maternal depression (Shaw et al., 2003), low maternal education (Nagin & Tremblay, 2001), overcrowding in the home (Martino, Ellickson,

Klein, McCaffrey, & Edelen, 2007), and parental antisocial behavior (Jaffee, Moffitt, Caspi, & Taylor, 2003) have all been linked to later AB in adolescence or adulthood (for reviews see: Loeber & Dishion, 1983; Yoshikawa, 1994). While these studies and others have found strong effects of individual factors on concurrent and later AB, studies of cumulative risk indicate broadly that as the number of risk factors accumulate, child AB increases (Appleyard, Egeland, Dulmen, & Sroufe, 2005; Deater-Deckard, Dodge, Bates, & Pettit, 1998). Often the accumulation of risk has been found to be more salient and predictive than looking at individual factors alone (Deater-Deckard et al., 1998). Some recent studies have also shown that examining different domains of risk (e.g., in-home family risk factors versus socioeconomic/community-level risk factors) can be a helpful approach in unpacking how the accumulation of risk in more proximal versus more distal areas may have different effects on later AB (Deater-Deckard et al., 1998).

Two particularly salient domains of risk leading to later AB are the caregiving environment and socioeconomic context. Parenting and caregiving have held a central place in many developmental models of AB (Aguilar, Sroufe, Egeland, & Carlson, 2000; Patterson et al., 1992; Shaw & Bell, 1993). For example, constructs such as physical abuse, harsh and rejecting parenting, ineffective discipline, and lack of nurturance in the caregiving environment have all be related to later AB even after controlling for many confounding variables (Jaffee, Caspi, Moffitt, & Taylor, 2004; Lansford et al., 2002; Patterson, DeBaryshe, & Ramsey, 1989; Shaw, Bell, & Gilliom, 2000; Shaw et al., 2003; Shaw, Keenan, & Vondra, 1994). Whereas associations between dimensions of caregiving and AB have been found across most developmental periods, such dimensions vary in importance based on children's developmental status. For example, during the toddler period when children often test caregivers' patience because of limited

cognitive and emotional abilities in the context of increasing physical mobility, the use of inordinate control and harsh parenting and lack of nurturance has been found to be particularly important in relation to emerging AB (Campbell, Pierce, Moore, & Marakovitz, 1996; Patterson et al., 1992; Shaw et al., 2000; Shaw, Winslow, Owens, & Hood, 1998). In early adolescence, as children begin to spend more time away from home with peers and again show increases in physical mobility, parental knowledge and monitoring of their child's activities are crucial (Dishion & McMahon, 1998; Stattin & Kerr, 2000). Beyond direct measures of parenting, other factors within the caregiving context such as maternal depression (Aguilar et al., 2000; Shaw et al., 2003), inter-parental conflict (Cummings, Pellegrini, Notarius, & Cummings, 1989; Fantuzzo et al., 1991), criminal activities within the home (Jaffee et al., 2003), and parent-child conflict (Criss, Shaw, & Ingoldsby, 2003; Trentacosta et al., 2011) have also been shown to predict AB, presumably though the effects of these factors on relationships within the family and parents' personal and emotional resources for parenting.

Beyond the caregiving environment, more distal factors such as the socioeconomic circumstances of the child's family and the characteristics of the neighborhood and broader community a child inhabits have also been a focus in understanding the development of AB and many other negative outcomes such as school readiness and achievement and internalizing disorders (Hinshaw, 1992; Leventhal & Brooks-Gunn, 2000). Specific sociodemographic factors such as low family income (Cote, Vaillancourt, LeBlanc, Nagin, & Tremblay, 2006; Shaw, Winslow, & Flanagan, 1999), low maternal education (Cote et al., 2006; Harachi et al., 2006; Nagin & Tremblay, 2001), teen parenthood (Jaffee, Caspi, Moffitt, Belsky, & Silva, 2001; Nagin & Tremblay, 2001), single parent status (Conseur et al., 1997; Harachi et al., 2006), and overcrowding in the home (Martino et al., 2007; Richman, Stevenson, & Graham, 1982) have all

been linked to later child AB (Beck & Shaw, 2005). From a broader perspective, neighborhood environments characterized by high levels of crime and danger, poverty, and exposure to deviant peers and adults have been repeatedly shown to be linked to youth AB, typically beginning after the preschool period (Leventhal & Brooks-Gunn, 2000; Trentacosta et al., 2008). For example, exposure to community violence has been linked in many studies to later AB among school-age children and adolescents even after accounting for the effects of other confounding variables such as child maltreatment, SES, and intimate partner violence (McCabe et al., 2005). Other factors such as neighborhood impoverishment, structure, and dangerousness have also been shown to predict later youth AB (Beyers, Bates, Pettit, & Dodge, 2003; Ingoldsby et al., 2006; Trentacosta, Hyde, Shaw, & Cheong, 2009). In sum, parenting and caregiving context quality as well as sociodemographic and neighborhood adversity have been broadly implicated in the etiology of AB, and many studies have demonstrated that many more specific factors within these contexts are robustly related to AB.

These studies and others have formed a broad empirical base for identifying contextual risk factors for AB. In addition, some recent research has highlighted interactions between such environmental risk factors and child factors (i.e., temperament, dispositions) in relation to later AB. For example, in one study using 289 low-income boys within the current sample, neighborhood dangerousness amplified the magnitude of association between child daring and later AB, and high levels of parental monitoring magnified the protective effects of child prosociality on later AB (Trentacosta et al., 2009). In two related studies, the link between youth impulsivity and callous traits and AB was amplified in the context of living in neighborhoods characterized by lower income or lower collective efficacy in samples ranging in size from 80 – 85,000 participants (Lynam et al., 2000; Meier, Slutske, Arndt, & Cadoret, 2008). These types of

studies emphasize the dynamic interaction between environmental and child factors; however, they are not able to directly address the interaction between biology and environment, and inform a truly biological-environmental interaction model of AB.

1.6 GENE BY ENVIRONMENT INTERACTIONS

GxE studies that have emerged over the last two decades have provided evidence for models positing a dynamic interaction between biology and environment in understanding psychopathology (Caspi et al., 2002; Caspi et al., 2003; Kendler et al., 1995; Rutter, 1997). Within the context of AB, both quantitative genetic studies exploring heritability estimates across different environments (i.e., genetically informed designs such as twin and adoption studies) and molecular genetics studies have been conducted. The few existing quantitative genetic approaches to GxE in AB have emphasized that heritability of AB may vary across environments in both childhood (Jaffee et al., 2005) and adolescence (Tuvblad et al., 2006) using twin designs. While these studies emphasize the varying contribution of heritability broadly across environments, they are not able to specify specific risk genes as they interact with the environment.

Studies using molecular genetics designs to understand youth AB have focused for the most part on two variables: genetic variability in MAOA and child maltreatment. In the first study focusing on this interaction, MAOA genotype (high activity versus low activity) was shown to moderate the effect of maltreatment on later conduct disorder diagnosis (ages 10 – 18), convictions for violent offenses (by age 26), dispositions toward violence (at age 26), and antisocial personality disorder symptoms (at age 26) in a large representative sample of 442

males (Caspi et al., 2002). For all of these outcomes, the correlation between child maltreatment and later AB was statistically significant in those with the low activity variant but only marginally significant or nonsignificant in those with the high activity MAOA variant. Moreover, whereas MAOA genotype did not directly predict AB, maltreatment did. Therefore, this study can be interpreted as showing that the main effect of child maltreatment is amplified by risky genetic variation or alternatively, that the high activity MAOA variation is protective in this risky environment.

Since the publication of the Caspi et al. (2002) study, the aforementioned finding has been replicated or partially replicated in at least 10 other studies (for a review see: Weder et al., 2009), although there have been several nonreplications (Haberstick et al., 2005; Young et al., 2006). These replication studies have generally shown that the link between maltreatment and later AB is only significant in those with the low-activity MAOA allele (Weder et al., 2009). The MAOA x maltreatment interaction effect has been demonstrated in studies of children (Kim-Cohen et al., 2006), adolescents (Foley et al., 2004; Nilsson et al., 2006), adults (Ducci et al., 2007; Widom & Brzustowicz, 2006), in epidemiological samples (Foley et al., 2004), and in forensic and psychiatric samples (Frazzetto et al., 2007; Reif et al., 2007), and even in non-human primates (Newman et al., 2005). The results have been extended to a wide variety of antisocial behaviors such as criminality and violent crimes (Nilsson et al., 2006), conduct disorder (Foley et al., 2004), alcohol use disorders and antisocial personality disorder (Ducci et al., 2007), impulsivity (Huang et al., 2004), as well as broad composites of mental health (Kim-Cohen et al., 2006). Moreover, several studies have extended the breadth of environmental risks measured from physical maltreatment to psychosocial risk indexes comprised of parental neglect, exposure to inter-parental violence, and inconsistent discipline (Foley et al., 2004); type of

residence and exposure to home and community violence (Nilsson et al., 2006); and social status, family structure and climate, and school education (Reif et al., 2007).

Most of these studies have focused only on males as MAOA is x-linked, with studies including females finding less consistent results than has been found for males (Ducci et al., 2007; Frazzetto et al., 2007; Sjöberg et al., 2006). Additionally, the majority of these studies have examined this GxE interaction only in Caucasians, although one study has corroborated this effect to a retrospective sample of 291 Native American adult women (Ducci et al., 2007). The few studies that have used samples with multiple races have demonstrated conflicting results: one replication was found for across a sample of 114 Caucasian, African American, Hispanic, and biracial children (Weder et al., 2009), another found replication in a sample of 803 "white versus non-white" adults in a mixed gender sample (Widom & Brzustowicz, 2006), and another study found a main effect of maltreatment but no interaction with MAOA genotype in a sample of 247 male Caucasian, African American, and Hispanic adolescents (Young et al., 2006). Finally, it should be noted that while several of these studies have larger sample sizes than imaging studies, many of these studies, particularly those using adult participants, have been retrospective and self-report in their assessment of early adversity (Ducci et al., 2007; Haberstick et al., 2005; Nilsson et al., 2006; Reif et al., 2007; Young et al., 2006) and thus prospective longitudinal designs are needed, particularly in high risk and diverse samples.

Although these studies have already helped inform models of AB, they can be extended in two major ways. First, they can be expanded to explore genes that affect 5-HT signaling beyond MAOA. Second, and particularly relevant for the current study, they can begin to address mediating biological factors such as neural reactivity on the association between GxE interactions and AB. Theoretically, both the direct effects of genes and GxE interactions on AB

should be reflected in individual variability in brain function, yet no studies to date have attempted to apply such an imaging genetics perspective to the study of AB.

In addition, whereas many studies have examined MAOA, only two appear to have examined other 5-HT genes (Reif et al., 2007; Sadeh et al., 2010). In one (retrospective) study of a forensic sample of 184 Caucasian male adults, the 5-HTTLPR was also examined, and those individuals with a short allele (the allele commonly linked to greater amygdala reactivity) were more likely to be grouped in the violent group, particularly in the context of an adverse childhood environment. In those with two copies of the long allele, there was no significant correlation between childhood adversity and later violence (Reif et al., 2007). The results, although based on retrospective reporting of the child environment, suggest that the GxE findings may extend broadly to 5-HT signaling genes and emphasize the need to examine other 5-HT genes. The second study (Sadeh et al., 2010), as noted above, found that the 5-HTTLPR L allele was related to psychopathy but only for those in living low SES environments. This result suggests that links between 5-HT genes and AB may be qualified by the type of AB examined.

1.7 IMAGING GENE ENVIRONMENT INTERACTIONS

Finally, as noted above, although GxE studies have changed the conceptualization of how genes and environments may contribute to the development of psychopathology, little empirical work has addressed how biological factors such as neural reactivity may mediate the effect of genes and environments on subsequent behavior. Although theoretically an IGxE approach holds great promise (Caspi & Moffitt, 2006; Hyde, Bogdan, et al., 2011), the way in which these variables may interact is complex and involves multiple intereaction points (see Figure 3 – hypothesis 3).

In the first model (*a neural mediated model*), each predictor variable (genetic variation, environmental risk, GxE interaction) is seen as directly affecting brain functioning and subsequent behavior through an indirect or mediated pathway (genes, environment, and their interaction predicts behavior indirectly through their effect on the brain) (Figure 3 – hypothesis 3a). This approach emphasizes the main effects of environmental risk on both brain function and on subsequent behavior. The direct effect of environment on brain functioning has not been extensively demonstrated, but is plausible based on studies examining environmental effects on protein translation from genes (e.g., epigenetics: Meaney, 2010; van Vliet, Oates, & Whitelaw, 2007), hormone and neuropeptide signaling (Fries, Ziegler, Kurian, Jacoris, & Pollak, 2005; Tarullo & Gunnar, 2006), and studies showing effects of psychotherapy on brain functioning (Brody et al., 2001; Dichter et al., 2009; Fu et al., 2008). From this standpoint, risky genes would have a greater effect on neural reactivity at high levels of environmental adversity and genetic and environmental risk could also have direct, noninteractive effects on neural reactivity. These effects on neural reactivity would then increase risk for AB. In this model all effects are posited to affect neural functioning, and the neural functioning to AB relationship is seen as static across risk. Moreover, a continuous interaction among variables is emphasized. This model addresses the question of how genes and environments may directly and interactively affect brain functioning and indirectly predict subsequent risk for AB.

In a second model (*moderated imaging genetics model*), an imaging genetics pathway (genes to brain function to behavior) is moderated by environmental risk (Figure 3 – hypothesis 3b), such that both the gene-brain link and the brain-behavior link can be moderated by the environment. While environmental risk moderating the gene to brain link is more intuitive based on GxE studies, it is possible that brain-behavior links may also be qualified by external or

environmental factors (Hyde, Manuck, & Hariri, 2011). This model addresses the question of whether gene to brain to behavior links are stronger or weaker in different environments. If both gene to brain and brain to behavior links are moderated indirect effects can be tested across environments to tests if the indirect effect "fits" in some environments but not in others. Both models are valid; however, they address slightly different, albeit overlapping questions, and would be tested statistically in different ways (moderating one versus two paths). Consequently, studies are needed to determine if either or both models are valid.

In sum, GxE findings suggest that genetic links to behavior may be exacerbated or qualified by environmental risk. Clear evidence exists for an MAOA x maltreatment interaction predicting AB. Some studies indicate that these results may extend to other genes affecting 5-HT signaling and to specific and cumulative domains of environmental risk. Just as imaging genetics has greatly expanded our understanding of neuroimaging and genetic studies, so too can IGxE studies expand our understanding of GxE studies through addition of a mediating biological variable such as brain functioning. Moreover, IGxE studies can approach understanding the interaction of environment with biology in two similar yet distinct ways by emphasizing the mediating role of the brain or the relative strength of biological links across environments.

2.0 STATEMENT OF PURPOSE

When combining an imaging genetics and developmental psychopathology perspective to understand psychopathology, research linking genes, brain functioning, environmental risk and their interactions can help inform models of the development of AB. Various studies have implicated neural functioning, genes, and environmental risk in the etiology of AB, yet the vast majority of studies measuring neural and/or genetic correlates of AB have failed to carefully measure proximal and more distal environmental risk across time, particularly beginning in early childhood. Furthermore, studies that measure phenomenon at multiple levels of biological analysis (i.e., genes, brain functioning) concurrently are needed to advance our understanding of AB from a mechanistic standpoint. Finally, studies examining biological links as they interact with environmental risk are needed to advance our current understanding of the development of AB from a developmental psychopathology perspective.

The current study aims to advance our understanding of the etiology and development of AB longitudinally using imaging genetics and IGxE approaches. First, current studies linking neural reactivity and 5-HT genes to AB were extended by examining these relationships in a sample of 249 low income boys/young men at risk for AB followed longitudinally from 1.5 to 20 years using measures of youth/adult behavior and environmental risk obtained from multiple informants, contexts, and methods, as well as biological measures of 5-HT genes and neural reactivity to threat. Second, an imaging genetics approach was applied for the first time to the

study of AB in an effort to link 5-HT genes to neural reactivity to AB. Third, the nascent IGxE perspective was used to examine the moderation of these links by environmental risk context across two important developmental periods (early childhood and early adolescence) and two important contextual domains (proximal risk - caregiving context quality, and distal risk - socioeconomic context adversity). Analyses were enriched through the use of a well validated neural reactivity design and measures of AB that are informed by multiple waves of measurement and other relevant attributes (e.g., CU traits) to define more homogenous subgroups of young adults.

2.1 HYPOTHESES

Based on previous findings and theories, the following hypotheses were tested.

2.1.1 Hypothesis 1: Biological correlates of early adult AB.

2.1.1.1 Hypothesis 1a – Neural correlates.

Based on a broad literature implicating amygdala and broader limbic function in AB (Blair, Peschardt, et al., 2006c; Kiehl, 2006), it was hypothesized that a persistent pattern of AB across adolescence and emerging adulthood would be related to reactivity in the amygdala. More specifically, based on literature linking amygdala reactivity positively to AB without CU (Coccaro et al., 2007; Decety et al., 2009) and negatively to CU and AB (Marsh et al., 2008), AB was posited to be related to greater amygdala reactivity, whereas AB in the context of CU traits

and CU traits themselves (i.e., regardless of levels of AB) was expected to be related to lesser amygdala reactivity (see Figure 3 – Hypothesis 1a).

2.1.1.2 Hypothesis 1b: Genetic 5-HT correlates.

Based on literature linking AB to genes known to affect 5-HT functioning (Holmes, 2008; Lesch & Merschdorf, 2000; Manuck et al., 2006), AB was theorized to be related to genetic variants that theoretically would confer greater amygdala reactivity (e.g. 5-HTTLPR short alleles, *MAOA* low alleles, *5-HTR1A* CC homozygotes, *TPH2* T carriers), whereas AB in the context of CU traits and CU traits themselves were expected to be related to variants possibly conferring lesser amygdala activity (e.g.,. 5-HTTLPR LL homozygotes) (see Figure 3 – Hypothesis 1b; Figure 5).

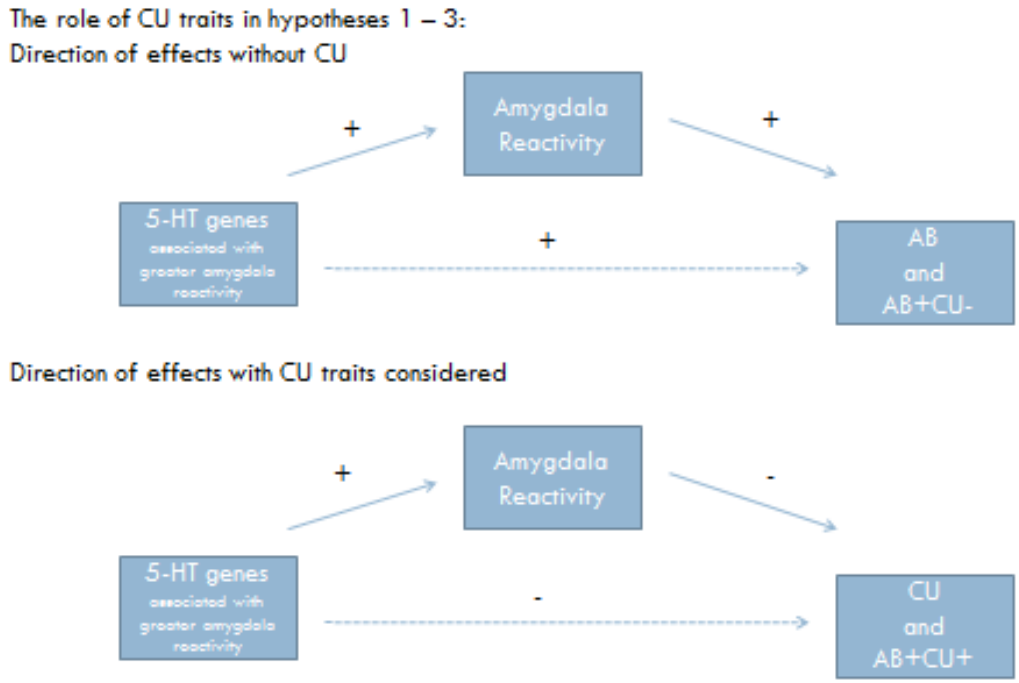


Figure 5: The role of CU in hypotheses

2.1.2 Hypothesis 2: Imaging genetics approach to early adult antisocial behavior

As literature has linked the above 5-HT genes to greater amygdala reactivity (Fakra et al., 2009; Hariri et al., 2002), and greater amygdala reactivity has been linked to higher levels of AB (Coccaro et al., 2007; Decety et al., 2009), individual genes previously related to greater amygdala reactivity were posited to be linked to greater amygdala reactivity, which in turn was hypothesized to be positively linked to AB through an indirect pathway (see Figure 3 – Hypothesis 2). Moreover, consistent with hypothesis 1a and 1b, genes previously linked to lower amygdala reactivity were hypothesized to be related to lower amygdala reactivity, which in turn was hypothesized to be related to higher levels of AB in the context of CU traits and CU traits themselves (see Figure 5).

2.1.3 Hypothesis 3: Environmental moderation of biological pathways

2.1.3.1 Gene and environment interaction, brain functioning and AB

Based on literature illustrating that risky environments exacerbate genetic risk for psychopathology, particularly AB (Kim-Cohen et al., 2006), and theory urging the combination of GxE and neuroscience approaches such as fMRI and imaging genetics (Caspi & Moffitt, 2006; Hyde, Bogdan, et al., 2011), the interaction of genetic risk (i.e., 5-HT genes) and environmental risk (i.e., based on cumulative indices of the caregiving context and the socioeconomic context of the child during both early childhood and early adolescence) was hypothesized to contribute independent variance to the prediction of amygdala reactivity above

and beyond their direct effects, which in turn is expected to predict AB (see Figure 3 – Hypothesis 3a).

2.1.3.2 Environmental risk moderates the gene-brain-behavior link.

As literature suggests the importance of GxE in understanding brain functioning and behavior (Caspi & Moffitt, 2006), and as emerging evidence makes possible that the brain-behavior link may be moderated by external factors (Hyde, Manuck, et al., 2011), both the link between genes and brain, and brain and behavior were expected to be moderated by the degree of environmental risk. Specifically, as most GxE work has emphasized exacerbation of genetic risk by the environment (Jaffee et al., 2005), it was expected that the association between 5-HT genes and amygdala reactivity, and the association between amygdala reactivity and AB would be stronger under conditions of high environmental risk (see Figure 3 – Hypothesis 3b). The indirect effect of 5-HT genes on AB through amygdala reactivity would therefore also be stronger under conditions of greater environmental risk.

3.0 METHODS

3.1 PARTICIPANTS

Participants in this study are part of the Pitt Mother and Child Project (PMCP), an ongoing longitudinal study of child vulnerability and resiliency in low-income families. In 1991 and 1992, 310 infant boys and their mothers were recruited from Allegheny County Women, Infant, and Children (WIC) Nutrition Supplement Clinics when the boys were between 6 and 17 months old (Shaw et al., 2003). At the time of recruitment, 53% of the target children in the sample were European-American, 36% were African-American, 5% were biracial, and 6% were of other races (e.g., Hispanic-American or Asian-American). Two-thirds of mothers in the sample had 12 years of education or less. The mean per capita income was $241 per month ($2,892 per year) and the mean Hollingshead SES score was 24.5, indicative of a working class to impoverished sample. Thus, a large proportion of the boys/men in this study could be considered at high risk for antisocial outcomes because of their socioeconomic standing.

Retention rates have generally been high at each of the time points from age 1.5- to 17-years old, with 90-94% of the initial 310 participants completing visits at ages 5 and 6, some data are available on 89% or 275 participants at ages 10, 11, or 12, and some data available on 87% or 272 participants at ages 15 or 16. The retention rate at age 17 was 251 (81%). When compared with those who dropped out at earlier time points, participants who remained in the study at ages

15, 16, or 17 did not differ on the CBCL externalizing scores at ages 2, 3.5, or 5, maternal age, income or educational attainment (*ps* = 0.20 to .93). At age 20, behavioral data as available on 249 young men and 182 of those men participated in the neuroimaging portion of the visit. After image analysis and processing, 159 young men were included in fMRI analyses. Table 1 contains a breakdown of why men did not complete the visit (e.g., unable to locate, incarcerated), did not complete the neuroimaging portion (e.g., head injury, bullets/metal fragments in body), or were excluded from final imaging analysis (e.g., poor coverage of the amygdala, poor task performance).

Table 1: Summary of available data for analyses

	Number lost	Participants with data
Original sample		**311**
Sample with behavioral data at age 20		**249**
- Parent requested drop out	11	
- Target youth requested drop out	3	
- Incarcerated	10	
- In the military	5	
- Deceased	1	
- Unable to locate	11	
- Hard to contact	5	
- Probable drop outs	6	
- On the schedule but not yet visited	1	
- Data collected but not yet available	7	
- Data collection error/permanently missing	2	
Total lost	62	
Sample with imaging data at age 20		**182**
- Concussion/head injury	24	
- Bullets/metal fragments	15	
- Braces	2	
- Phone interviews (out of the area)	5	
- MRI portion refused	7	
- Living at home/treatment facility (too ill to participate – schizophrenia, autism, car accident)	4	
- Claustrophobic	6	
- Left before scanning portion or wanted to stop scan	2	
- Did not physically fit in the bore	1	
- Reported being currently on drugs/rescheduled	1	
Total Lost	67	
Sample with usable imaging data at age 20		**159**
- Incidental findings on sMRI	2	
- Poor amygdala coverage (< 90%) or visual overlap	15	
- Poor performance on task (< 75%)	1	
- No amygdala reactivity or processing errors	1	
- Slept during scan	1	
- Excessive movement/outliers	1	
- Psychosis	1	
- Appeared to be on drugs and not responding to task	1	
Total Lost	23	

3.1.1 Visit procedures

Target children and their mothers were seen for two- to three-hour visits at ages 1.5, 2, 3.5, 5, 5.5, 6, 8, 10, 11, 12, 15, and 17 years old. Data were collected in the laboratory (ages 1.5, 2, 3.5, 6, 11, 20) and/or at home (ages 2, 5, 5.5, 8, 10, 12, 15). Adolescents participated in a brief phone interview at ages 16 and 18. Target children (now adults) participated in a lab assessment at age 20 alone. During home and lab assessments, parents completed questionnaires regarding sociodemographic characteristics, family issues (e.g., parenting, family member's relationship quality, maternal well-being), and child behavior. In addition, parents, other family members (siblings, alternative caregivers), and friends of the target child were videotaped interacting with each other and/or the target child in age-appropriate tasks, including mother-son clean-up tasks in early childhood, sibling play or discussion tasks during preschool and school-age periods, and peer discussion of problematic topics at age 15 and 17. Youth provided DNA via saliva at age 17. At age 20, target adults participated in a lab visit that included questionnaires, a psychiatric interview, and an fMRI scanning session. Participants were reimbursed for their time at the end of each assessment. All assessments and measures have been approved by the IRB of the University of Pittsburgh.

Measures and other procedures to be used in the current study are described below. They were selected based on the above literature and in an effort to measure the following: 1. Genes with known biological implications for brain functioning, specifically genes involved in 5-HT at several stages of neurotransmission, 2. Neural reactivity to ecologically valid stimuli in regions of interest in the brain (the amygdala), 3. Measures of AB that create valid subgroups or dimensions likely to have more homogenous biology, 4. Environmental variables measured at

developmentally sensitive periods (early childhood and early adolescence) that have been directly linked to youth AB.

3.1.2 Neuroimaging Procedures

3.1.2.1 Amygdala reactivity paradigm.

The experimental fMRI paradigm consists of four blocks of a face processing task interleaved with five blocks of a sensorimotor control task (Brown, Manuck, Flory, & Hariri, 2006; Brown et al., 2005; Manuck, Brown, Forbes, & Hariri, 2007; Manuck et al., 2010). During the face processing task, subjects view a trio of faces (expressing one of four emotions) and select one of two faces (bottom) identical to a target face (top; see Figure 6). Each face processing block consists of six images, balanced for sex, all derived from a standard set of pictures of facial affect (Ekman & Friesen, 1976). Each of the four face processing blocks consists of a different expressed affect (anger, fear, surprise, neutral) and participants were randomly assigned to one of four different orders of block presentation. During the sensorimotor control blocks, subjects view a trio of simple geometric shapes (circles, vertical and horizontal ellipses) and select one of two shapes (bottom) identical to a target shape (top). Each sensorimotor control block consists of six different shape trios. All blocks are preceded by brief instructions (‘‘Match Faces’’ or ‘‘Match Shapes’’) lasting 2 s. In the face processing blocks, each of the six face trios is presented for 4s with a variable interstimulus interval of 2—6s (X = 4 s) for a total block length of 48s. In the sensorimotor control blocks, each of the six shape trios is presented for 4s with a fixed inter-stimulus interval of 2s for a total block length of 36s. Total task time is 390s. Subject performance (accuracy and reaction time) was monitored during all scans. The inclusion of four different expressions differs from previous studies with this

paradigm (Manuck et al., 2010). These additional expressions were added to allow for estimation of neural sensitivity and selectivity to affect (e.g., anger > neutral), while retaining the overall structure (i.e., alternation with simple geometric shape matching) that contains power to elicit a robust response (e.g., all faces > shapes) in regions of interest such as the amygdala.

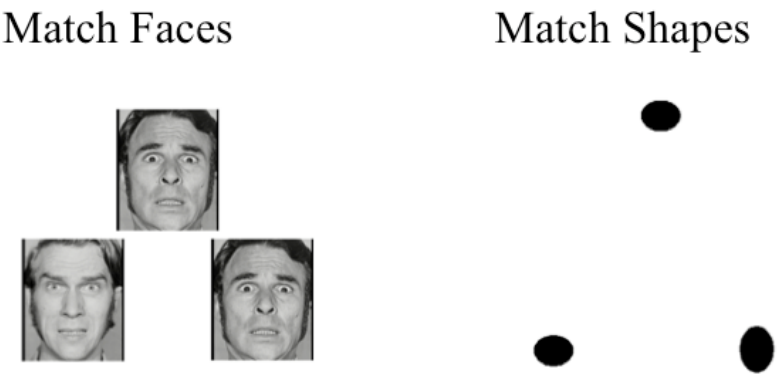


Figure 6: Example of the emotional face processing task

3.1.2.2 Bold fMRI acquisition parameters

Each participant underwent scanning at the Magnetic Resonnance Research Center (MRRC) of the Presbyterian University Hospital of UPMC Pittsburgh. Data were collected with a Siemens 3-T Tim Trio (Siemens Medical Solutions, Erlangen, Germany). Blood oxygenation level–dependent (BOLD) functional images are acquired with a gradient-echo echoplanar imaging (EPI) sequence (repetition time/echo time=2000/25 milliseconds, field of view = 20 cm, matrix = 64 x 64), which covered 34 interleaved axial slices (3-mm slice thickness) aligned with the AC-PC plane and encompassing the entire cerebrum and most of the cerebellum (with a goal of maximum coverage of limbic structures). All scanning parameters were selected to optimize the quality of the BOLD signal while maintaining a sufficient number of slices to acquire whole-brain data. Before collecting fMRI data for each participant, a reference echoplanar imaging scan

was acquired and visually inspected for artifacts (e.g., ghosting) and good signal across the entire volume of acquisition, including the amygdala. Additionally, an autoshimming procedure was conducted before the acquisition of BOLD data in each participant to minimize field inhomogeneities. To maximize data collection, additional higher-order shimming was implemented as needed for subjects with poor signal-to-noise ratio in our regions of interest.

3.1.2.3 Image processing and analysis

Whole-brain image analysis was completed using the general linear model of SPM8 (Wellcome Department of Imaging Neuroscience, London, England). Images for each participant were grey matter segmented, realigned to the mean volume in the time series and unwarped to correct for head motion, co-registered to high resolution structural scans (using an MPRAGE structural scan), spatially normalized into a standard stereotactic space (Montreal Neurological Institute template) using a 12-parameter affine model, and smoothed to minimize noise and residual difference in gyral anatomy with a gaussian filter set at 6 mm full-width at half-maximum. Voxelwise signal intensities are ratio-normalized to the whole-brain global mean. After preprocessing, the Artifact detection Tools (ART) software package (MIT, Boston, MA, USA) was used to detect global mean intensity and translation or rotational motion outliers (> 4.5 SD from the mean global brain activation) within each participant's data and omitted them from subsequent statistical analyses These preprocessed data sets were analyzed using second level random-effects models that account for both scan-to scan and participant-to-participant variability to determine task specific regional responses.

Following preprocessing, linear contrasts employing canonical hemodynamic response functions were used to estimate condition-specific (i.e., faces > shapes) BOLD activation for each individual and scan. These individual contrast images (i.e., weighted sum of the beta

images) were then used in second-level random effects models that account for both scan-to-scan and participant-to-participant variability to determine mean condition-specific regional responses using one-sample t-tests. As the main goal of this study was to examine amygdala reactivity to specific contrasts within ROIs, the following contrasts were estimated and extracted from SPM8 based on specific hypotheses for the study: a.) all faces > shapes (robust amygdala engagement); b.) anger > shapes & fear > shapes (to explore differential responding to these emotions in this population); c.) neutral > shapes (to explore the possibility of differential interpretation of neutral faces as threatening in those higher in AB) (Dodge, Pettit, Bates, & Valente, 1995; Marsh & Blair, 2008), d.) fear and anger > neutral e.) fear > neutral, f.) anger > neutral (to compare to studies using this paradigm in this population) (Jones et al., 2009; Marsh et al., 2008). All ROI analyses were extracted and thresholded at $p < .05$ corrected for multiple comparisons using the FWE correction within SPM8.

3.1.2.4 Regions of Interest.

BOLD contrast estimates were extracted from functional clusters to delineate anatomy specific effects without risk of double correlation when these clusters are extracted and used in regression and structural equation models (Viviani, 2009; Vul, Harris, Winkielman, & Pashler, 2009). BOLD contrast estimates were extracted from functional clusters exhibiting a main effect of task using the above threshold within anatomically defined ROIs (Manuck et al., 2010). Separate ROIs containing the amygdala's basolateral region (latero-basal amygdala: LB) and central-medial region (centro-medial amygdala: CM) were constructed using masks created from probabilistic amygdala definitions (Amunts et al., 2005). These masks were defined originally using an SPM toolbox that uses a probabilistic algorithm to define the probability each voxel is within a certain ROI (Eickhoff et al., 2005). The specific amygdala ROIs were created such that

the LB region contains the basolateral, basomedial, basoventral and lateral nuclei, while the CM regions contains the central and medial nuclei (Amunts et al., 2005). Additionally, a whole amygdala ROI was created using the AAL definition of the bilateral amygdala with the WFU PickAtlas Tool, version 1.04 (Wake Forest University School of Medicine, Winston-Salem, NC).

3.1.3 5-HT genes identification

Genomic DNA from all participants was collected when youth were age 17 and isolated from saliva samples using the Oragne^{TM} DNA self-collection kid following the manufacturer's instructions (DNA genotek, Inc, 2006). DNA was extracted from the saliva using standard extraction methods and stored at -80^{o}C. Single Nucleotide Polymorphisms (SNPs) were identified using TaqMan allelic discrimination assays and variable number tandem repeat (VNTR) sequences were identified using polymerase chain reaction and gel electrophoresis. The specific gene variants to be identified were as follows: 1.) *HTR1A* (SNP C(-1019)G, rs6295), 2.) *TPH2* (SNP G(-844)T, rs4570625), 3.) 5-HTTLPR (VNTR in the promoter region of *SLC6A4*), and 4.) *MAOA* (VNTR 30-bp variable number of tandem repeats). All genotypes were found to be in Hardy-Weinberg Equilibrium (except MAOA which was not tested based on it more complex allele distribution). Note that for 3 of the variants, the genotyping was done and labeled as in past studies. For the *MAOA* VNTR genotyping resulted in variants that were reported differently than in some other publications. This genotyping resulted in 4 variants with lengths: 2.5, 3.5, 4.5, and 5.5. Though this scheme differs from others, it matches a recent publication that have noted possible drawbacks of previous schemes (Das et al., 2006). Thus, these variants are most likely to match previous variants as follows: 2.5 = 2, 3.5 = 3 & 3.5, 4.5 = 4, 5.5 = 5. The allele frequencies reported in this sample were consistent with this translation of genotypes

(Sabol, Hu, & Hamer, 1998). Thus, those with the 4.5 length were classified as "high" on MAOA, while those with other lengths (2.5, 3.5, 5.5) were classified as "low". Though the classification (particularly of the extreme lengths – 2.5, 5.5) is still of debate, the number of individuals with these lengths is quite small in this sample (9 participants, 5% of the sample have 2.5 or 5.5 length variants; of those with 5.5 variants 3 of the 4 did not participate in the scanning session at age 20) and thus unlikely to have a large impact on the results. The *in vitro* results as to whether the 5.5 allele has increased or decreased transcription is still of debate (Beach et al., 2010), and thus the 5.5 variant was included in the "non 4.5 group" to maintain the 4.5 group as most homogenous. Importantly, the main contrast taken in most studies (those with the 3 versus 4 variant is the focus of this coding scheme) is essentially retained. Results were reanalyzed using only those with 3 and 4 length variants and the results did not change.

The following gene variants have shown evidence of increased amygdala reactivity and thus will be summed to create a cumulative index of 5-HT genes that ranges from 0 to 4 and biologically could theoretically range from lowest 5-HT to highest 5-HT transmission (though in practice these range from lowest to highest amygdala reactivity): *HTR1A* (C/C), *TPH2* (T carrier), 5-HTTLPR (short carrier), & *MAOA* (low). This cumulative sum was then used to predict neural reactivity and AB just as the individual genes were. Hence this cumulative 5-HT index can interrogate the cumulative versus the individual effect of 5-HT signaling and hypothesized amygdala reactivity effects.

3.2 MEASURES

3.2.1 Outcomes

3.2.1.1 Antisocial Behavior

AB was assessed based on boys' self-report using the Self-report of Delinquency Questionnaire (SRD) (Elliott, Huizinga, & Ageton, 1985) at age 10,11, 12, 15, 16, 17, 18, & 20. The SRD is a semi-structured interview that contains 33 items (at age 10, 11, 12) or 62 items (at age 15, 16, 17, 18) and assess the frequency with which an individual has engaged in aggressive and delinquent behavior, alcohol and drug use, and related offenses. Using a 3-point rating scale (1 = never, 2 = once/twice, 3 = more often), children rate the extent to which they engaged in different types of antisocial activities (e.g., stealing, throwing rocks at people, drug use). Across ages 10 -17 internal consistency was high (α = .79 - .92). At age 20, the measure was shortened to 53 items by removing items not still appropriate for adults (i.e., have you had sex?, have you smoked a cigarette?). For purposes of constructing groups "high" on AB and other traits (i.e., AB+CU+ groups), those men with scores in the highest quartile on each measure (AB and CU) were considered "high", while those below the mean of the group will be "low". Hence three different groups were constructed: AB+/CU+ (high on both; n = 24), AB+/CU- (high AB, low CU; n = 25), AB-/CU- (low on both AB and CU; n = 76).

3.2.1.2 Trajectories of delinquency

Using the above described SRD, trajectories were formed across adolescence (ages 10-18) using Nagin's semiparametirc group based Proc Traj in SAS 9.2 (Nagin, 2005). Trajectory group models were evaluated using the following criteria: BIC scores, no groups smaller than

4% of the sample, and high posterior probability of group membership (Shaw, Hyde, & Brennan, 2012). These analyses yielded 4 distinct groups: a low group, a late-starting moderate group, an early desisting group, and a high group (See Figure 7). These trajectory groups have been shown to be valid as they discriminated both court involvement and behavior disorder diagnosis at age 17. Given the small size of the "desisting" group and evidence that these youth may be under-reporting (see Shaw et al., 2012), the early starting group and desisting group were collapsed into an overall early starting/high AB group (n = 43).

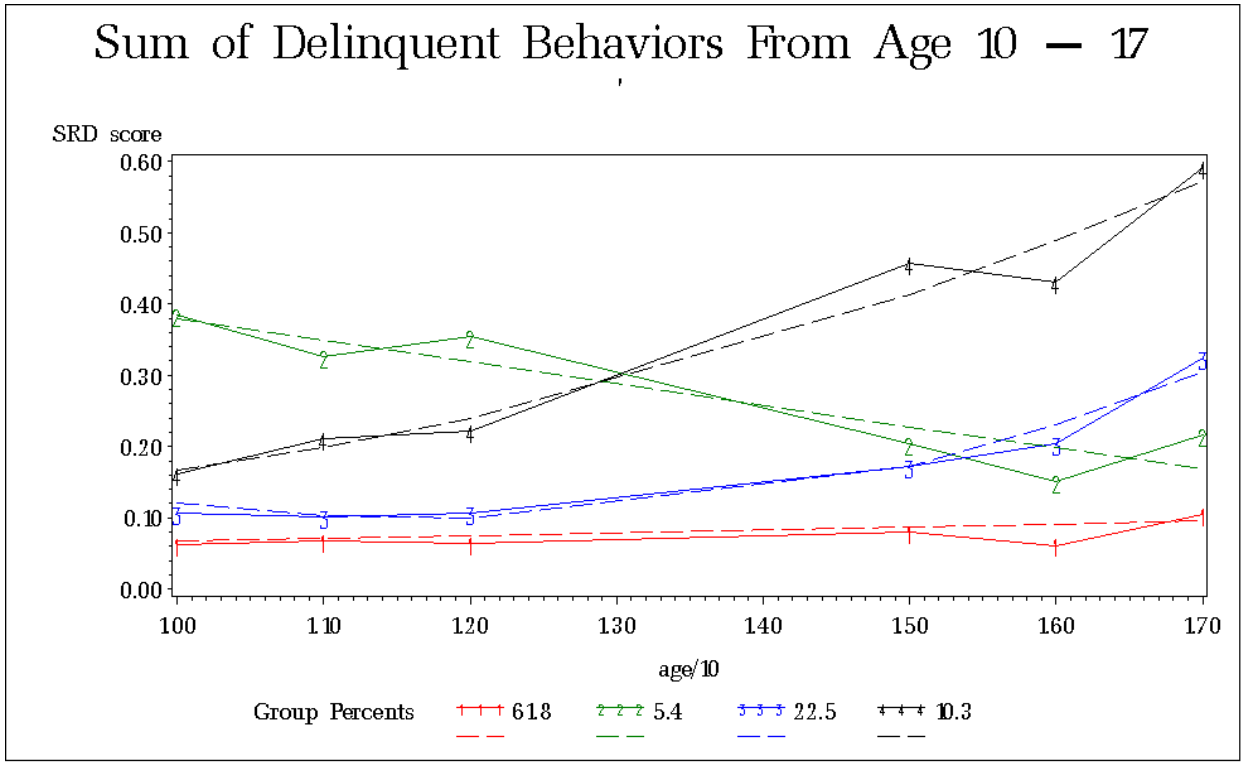


Figure 7: Trajectories of delinquent behavior across adolescence (Shaw et al., 2012)

Note: Dashed lines and numbers denote predicted trajectories, while solid lines denote each group mean at each age. Red lines denoted the "low" group, blue lines denote the "late" group, black lines note the "early-high" group and green lines denote the "desisting" group which was subsequently combined into the "early-high" group.

3.2.1.3 Callous/Unemotional Traits

CU traits were measured via self-report at age 20 using the six-item CU factor from the Antisocial Process Screening Device (APSD) (Frick, Bodin, & Barry, 2000) and items from the Child and Adolescent Dispositions Scale (CADS) (Lahey et al., 2008; Waldman et al., 2011). These 6 items from the APSD assess lack of empathy and affect, and callousness (e.g. you are concerned about the feelings of others) on a 3-point rating scale (0 = not at all true, 1 = sometimes true, 3 = definitely true). However, as internal consistency of this measure has been debated (e.g., Dillard, Salekin, Barker, & Grimes, 2012) and at the age 20 visit it was lower than desired (α = .47), these items were added to items from the CADS measure and the best items from both scale were used to create a CU scale. The prosociality/empathy scale of the CADS has been shown to predict later AB in this sample (Trentacosta et al., 2009) and contains items germane to callousness such as "Do you feel bad for other's when they get hurt?". The items from both the CU scale of the APSD and the prosociality scale from the CADS were entered together in an exploratory factor analysis to gain one factory representing the CU construct with acceptable factor loadings and internal consistency. After excluding items that had poor face validity or poor loadings, a final scale of 7 items (2 from the APSD, 5 from the CADS) was constructed that contained items with high loadings (>.60), had high internal consistency (α = .86), and reflected a lack of empathy and callousness (see Table 2). For purposes of constructing groups "high" on CU (i.e., AB+CU+ groups), those men with scores in the top quartile on this measure will be considered "high" and those below the median are "low".

Table 2: Summary of exploratory factor analysis results for constructing a CU scale

Note: items in **BOLD** are those included in the final scale

Item	Details on exclusion	Loading in 1 factor EFA with all possible items	Loading in final EFA
CADS3 – I do things to help others without being asked	included	.658	.819
CADS9 – I share things with others	poor face validity poor loading	.437	
CADS10 – I feel bad for others when they get hurt	included	.756	.773
CADS16 – I try to cheer others up when they are upset	included	.656	.679
CADS18 – I feel sorry for kids who get picked on	included	.720	.748
CADS20 – I want everyone to follow the rules including me	poor face validity poor loading	.495	
CADS21 – I care about other's feelings	included	.814	.826
CADS22 – I enjoy learning new and interesting things	Poor face validity (excluded from initial EFA)		
CADS26 – I am concerned about right and wrong	Poor face validity poor loading	.369	
APD1 – You are concerned about the feelings of others	included	.795	.819
APD2 – You feel bad or guilty when you do something wrong	included	.670	.664
APD3 – You care about how well you do at school or work	poor face validity poor loading to APSD in other published reports (excluded from initial EFA)		
APD4 – You are good at keeping promises	poor face validity poor loading	.381	
APD5 – You hide your feelings or emotions from others	poor loading	-.081	
APD6 – You keep the same friends	poor loading	.228	

3.2.1.4 Cumulative Risk Index

Environmental risk factors for AB were identified from previous work in this sample and others (Nagin & Tremblay, 2001; Rutter 1979; Sameroff, Seifer, Zax, & Barocas, 1987) and are summarized in Table 3 and 4. Each factor has been linked in studies to youth AB, and most of these factors have been used individually or in cumulative risk indices within this sample and others and linked to AB (e.g. Beck & Shaw, 2005; Shaw et al., 2003; 2004). Risk factors were drawn from two domains (caregiving context and socioeconomic risk) thought to reflect proximal and distal risk respectively. Moreover, these factors were measured at two critical age periods (early childhood and early adolescence) and as such risk was examined in the following ways: total risk, early versus late risk, and proximal versus distal risk. Note that "early risk" would contain both distal and proximal scales but only those scales as measured in early childhood. Similarly, "proximal risk" would contain proximal risk factors during both early childhood and early adolescence. Risk factors were dichotomized based on the child or family being in the highest (or lowest) quartile or another specified cutoff to reflect those at highest risk and to have approximately 25% of families in the study in the "risk" category for each risk factor (i.e., mother had her first child when she was a teenager). Hence within each domain and age period, youth received a score of 0 or 1 on each risk factor, and these scores were summed within each domain (e.g., for early childhood proximal risk the score can vary from 0 to 5). These domains were then summed to generate an overall cumulative environmental risk score (ranging from 0 to 22) with the ability to unpack this risk by domain and developmental period. Unless otherwise specified, all factors described below were dichotomized as follows: '1' = highest quartile, and '0' = all those in the bottom three quartiles. As some families were missing data at some points, generally when a measure was collected at multiple time points, the risk must be

present at 50% or greater of the available time points (2 of 3, 1 of 2, or 1 of 1 time points are coded ‘1’) to be coded as ‘1’ in the index.

Table 3: Cumulative risk index variables in early childhood

Construct	Measure	Citation	Child age when collected	Reporter	Cutoff
Early childhood caregiving context					
Rejecting parenting	Early Parent Coding System (EPCS)	(Shaw et al., 2003; Shaw, Lacourse, & Nagin, 2004)	18 & 24 months	Coder global and molecular codes	Highest quartile
Parental nurturance	Home Observation for Measurement of the Environment (HOME) – Nurturance factor	(Bradley & Caldwell, 1984; Shaw et al., 2004)	24 months	Examiner assessment global ratings	Bottom quartile
Inter-parental Conflict	Conflict Tactics Scale (CTS) – verbal and physical aggression factors	(Hyde, Shaw, & Moilanen, 2010; Straus, 1979)	42 months	Primary caregiver	Highest quartile
Physical discipline attitudes	Adolescent Parenting Inventory	(Bavolek, Kline, McLaughlin, & Publicover, 1979)	24 months	Primary caregiver	Highest quartile
Parenting Hassles	Parenting Daily Hassles	(Crnic & Greenberg, 1990)	18, 24, & 42 months	Primary caregiver	Highest quartile
Early childhood socioeconomic risk					
Very low family income	Total household income per month	(Shaw et al., 1999)	18, 24,& 42 months	Primary caregiver	Bottom quartile at 50% or more timepoints
Low maternal education	What grade have you you finished?	(Shaw et al., 2003; Shaw et al., 2004)	18 months	Primary caregiver	Never finished high school or GED
Young maternal age at first birth	Age when first child was born	(Shaw et al., 2003; Shaw et al., 2004)	24 months	Primary caregiver	< 19 years old for first child
Overcrowding in the home	Demographic questions regarding size of the house and those living in it	(Trentacosta et al., 2008)	18, 24, & 48 months	Primary caregiver	4 or more children or fewer rooms than people
Single parent	Marital status asked on demographic questionnaire	(Shaw et al., 1999)	18, 24, & 42 months	Primary caregiver	Not married or living together at 50% or more timepoints
Neighborhood dangerousness	Me and my neighborhood	(Beck & Shaw, 2005; PYS, 1991)	24 months	Primary caregiver	Highest quartile

Table 4: Cumulative risk index variables in early adolescence

Construct	Measure	Citation	Child age when collected	Reporter	Cutoff
Early adolescence caregiving context					
Parental monitoring and knowledge	Parenting interview	(Dishion et al., 1991; Trentacosta et al., 2009)	12 years	Target Child	Lowest quartile
Inter-parental conflict	Conflict Tactics Scale (CTS) – verbal and physical aggression	(Hyde et al., 2010; Straus, 1979)	10, 11, & 12 years	Primary caregiver	Highest quartile
Child-parent conflict	Adult-Child Relationship Scale	(Pianta, 2001; Trentacosta et al., 2011)	10, 11, & 12 years	Primary caregiver	Highest quartile
Parent physical discipline	2 parenting interview items: frequency of "spanking" and "slap or hit with hand, fist, or object"	(Lansford et al., 2011)	10, 11, & 12 years	Primary caregiver	Highest quartile
Harsh parenting	8 items from the HOME[1]	Items picked for face validity and high inter-correlation (Bradley & Caldwell, 1984)	10, 11, & 12 years	Examiner assessment global ratings	Highest quartile
Early adolescence socioeconomic risk					
Very low family income	Total household income	(Shaw et al., 1999)	10, 11, & 12 years	Primary caregiver	Bottom quartile at 50% or more timepoints
Low maternal education	What grade did you finish?	(Shaw et al., 2003; Shaw et al., 2004)	10 years	Primary caregiver	Never finished high school
Overcrowding in the home	Demographic questions: size of the house and those living in it	(Trentacosta et al., 2008)	10, 11, & 12 years	Primary caregiver	4 or more children or fewer rooms than people
Single parent	Marital status asked on demographic questionnaire	(Shaw et al., 1999)	10, 11, & 12 years	Primary caregiver	at 50% or more timepoints
Neighborhood dangerousness	Me and my neighborhood questionnaire	(Ewart & Suchday, 2002; Trentacosta et al., 2009)	11 years	Primary caregiver	Highest quartile

[1] Items: "expresses hostility at the child", "shouts at the child", "initiates negative physical contact with the child", "appears to have an inappropriate relationship with the child", "good control of the child" (reversed), "accepting of the child" (reversed), "supervises carefully" (reversed), & "disciplines appropriately" (reversed).

3.2.1.5 Early Childhood Proximal Risk

To assess early childhood proximal risk the following risk factors were included in an index of caregiving context risk. These risk factors have all been linked to AB in this sample and others, and were drawn from measures available at assessment points when the children were 1.5, 2 and 3.5 years old: Rejecting Parenting was assessed using global and molecular codes using the Early Parent Coding System (EPCS) on videotapes of a cleanup task at ages 1.5 and 2 (Shaw et al., 2003; Shaw et al., 2004). For this study (and many previous studies), two molecular ratings – *verbal/physical approval* and *critical statements*, and three global ratings – *hostility*, *warmth*, and *punitiveness* (all with high inter-rater reliability, $\kappa > .79$) were combined using principal components analysis to yield a single factor score averaged across the two age periods. Parental Nurturance was assessed using scores from age 2 on the Home Observation for the Measurement of the Environment (HOME) (Bradley & Caldwell, 1984; Shaw et al., 2004) which assess the quality and quantity of support and stimulation in the child's home environment using a parent interview and a semi-structured observation (based on trained graduate student and research assistant interviewers' ratings after the entire home visit). The parental nurturance score is derived by adding scores from the responsivity and acceptance factors of the measure. For this measure (and all others coded in the "positive" direction), children at "risk" was defined as those in the *bottom* quartile. Inter-parental conflict was assessed using the Conflict Tactics Scale (CTS – Form N) (Hyde et al., 2010; Straus, 1979) which asks mothers to report about verbal reasoning, verbal aggression and violence between adult partners and was administered at age 3.5. The sum of the verbal and physical aggression scores was used, with those in the top quartile considered at risk. Parental discipline attitudes were assessed using the Adolescent Parenting Inventory (Bavolek et al., 1979). This scale includes 32 items and asks mothers to

report on their attitudes on topics such as physical discipline, comforting a child and developmental expectations. Those youth in the highest quartile on parental discipline attitudes were considered at risk. Parenting hassles was assessed using the Parenting Daily Hassles scale (Crnic & Greenberg, 1990), a 20 item scale that assesses the frequency of daily hassles a parent contends with and how much of a hassle these events are. Youth in the highest quartile were considered at risk.

3.2.1.6 Early Childhood Distal Risk

The following risk factors included in this index of sociodemographic and neighborhood risk have all be used in this sample and linked to AB, and are drawn from measures available at assessment points when the children were 1.5, 2 and 3.5 years old: Primary caregivers reported on teen parent status at the 1.5 year assessment, and received a score of '1' scored if they were under 18 years of age at their first child's birth (Shaw et al., 2004). At each of the 3 assessments (age 1.5, 2, 3.5 years), primary caregivers also reported on single parent status ('1' = single adult in the home at 50% or more of the time points) (Shaw et al., 1999); household overcrowding ('1' = 4 or more children in the home *or* fewer rooms than people at 50% or more of the time points) (Trentacosta et al., 2008); very low family income ('1' = bottom quartile of the sample at more than 50% of time points which is approximately $500 per month total income) (Beck & Shaw, 2005), and low maternal education ('1' = less than a high school degree or no GED by age 2) (Shaw et al., 2003). Neighborhood dangerousness was assessed using a sum of all items on the Neighborhood Questionnaire (PYS, 1991), a 17-item measure of problematic and dangerous activities within a family's neighborhood as perceived by the primary caregiver at the age 2 visit (Beck & Shaw, 2005). Those in the highest quartile were considered at risk.

3.2.1.7 Early Adolescence Proximal Risk

To measure early adolescent proximal risk the following risk factors will be included in an index of the caregiving context and have all been linked to AB in the current sample and others (Beck and Shaw, 2005; Trentacosta et al., 2008; 2009, and all are drawn from measures available at assessment points when the children were 10, 11, and/or 12 years old: Parental monitoring and knowledge was assessed using an interview developed at the Oregon Social Learning Center (Dishion et al., 1991). Interviewers asked children a series of questions about their parent's knowledge of their whereabouts and discipline practices. The knowledge factor is based on five items of the boys' whereabouts, plans, and interests (Trentacosta et al., 2009). Those boys who scored in the lowest quartile mean were coded as '1'. Inter-parental conflict was measured as described in the early childhood risk index but with these measures again collected at ages 10, 11, and 12. Child-parent conflict was assessed using the Adult-Child Relationship Scale (ACRS), an adapted version of the Student-Teacher Relationship Scale (Pianta, 2001; Trentacosta et al., 2011). Primary caregivers were asked about their feelings about the child and attachment-related behavior through the ACRS at ages 10, 11, and 12. For this study, the 10 item "conflict" scale was used. This scale has been shown to have acceptable internal reliability and stability over time, and it predicts later AB in this sample (Trentacosta et al., 2011). Those youth in the highest quartile were considered at risk. Parent physical discipline was assessed from 2 items within a structured interview of the primary caregiver at ages 10, 11, and 12. These items ask for the frequency of the parent "spanking" the child and "slapping or hitting with hand, fist or object" (Lansford et al., 2011). Youth in the highest quartile were considered at risk. Harsh parenting was assessed using 8 items of observer report modeled after items from the early childhood version of the HOME (see Table 4 for individual items; Bradley

& Caldwell, 1984). These items are global ratings made after each home and lab visit (ages 10, 11, and 12) by trained graduate students and research assistants and were selected for their similarity to items from the early childhood version of parental nurturance and the rejecting parenting construct, and for face validity to the construct of harsh parenting. Preliminary analyses indicated high inter-correlation among items and satisfactory internal consistency of the measure at each age (α's > .7). Youth in the highest quartile were considered at risk.

3.2.1.8 Early Adolescence Distal Risk

Factors selected to index socioeconomic risk during the early adolescent period (ages 10, 11, and 12) are all nearly identical to those used in early childhood. Very low family income (bottom quartile now approximately $1257/month), overcrowding in the home, and single parent status, are all the same measures, conceptualized and coded in the same way but using data from these later three time points. Low maternal education was measured from the same demographic questionnaire at age 10 to ascertain stability or change in education since age 2. Neighborhood dangerousness was measured using primary caregiver report at age 11 on the Me and My Neighborhood questionnaire which is an adaption of the City Stress Inventory (Ewart & Suchday, 2002) and contains the factor 'exposure to violence' containing 7 items assessing the frequency of dangerous events in the neighborhood (Trentacosta et al., 2009).

3.3 DATA ANALYTIC PLAN

The primary goal of the proposed research is to investigate the relations among genes affecting 5-HT transmission, neural functioning (i.e., threat related amygdala reactivity measured in early adulthood), environmental risk (i.e., proximal and distal cumulative risk during early childhood and early adolescence), and AB (as assessed in early adulthood, across adolescence, and with and without CU traits). Analyses focused on 5-HT genes and neural reactivity as predictors of AB, genetic indirect effects on AB through neural reactivity, and interactions between cumulative environmental risk and biological factors (i.e., 5-HT genes, neural reactivity) in predicting AB. A summary of hypotheses and measures is described in Figure 8 for a summary and specific analytic techniques are described within the results for each hypothesis. Broadly, analyses were done with SPSS using listwise deletion, given that many analyses only contained 2-3 variables and thus methods to account for missing data (e.g., multiple imputation, maximum likelihood) with this amount of missingness was inappropriate. Also of note, as racial background may affect genetic findings, every analysis containing genotypes was analyzed broadly in the whole sample and within more homogenous racial categories (i.e., all analyses were computed for the entire sample, the White subsample and the Black subsample).

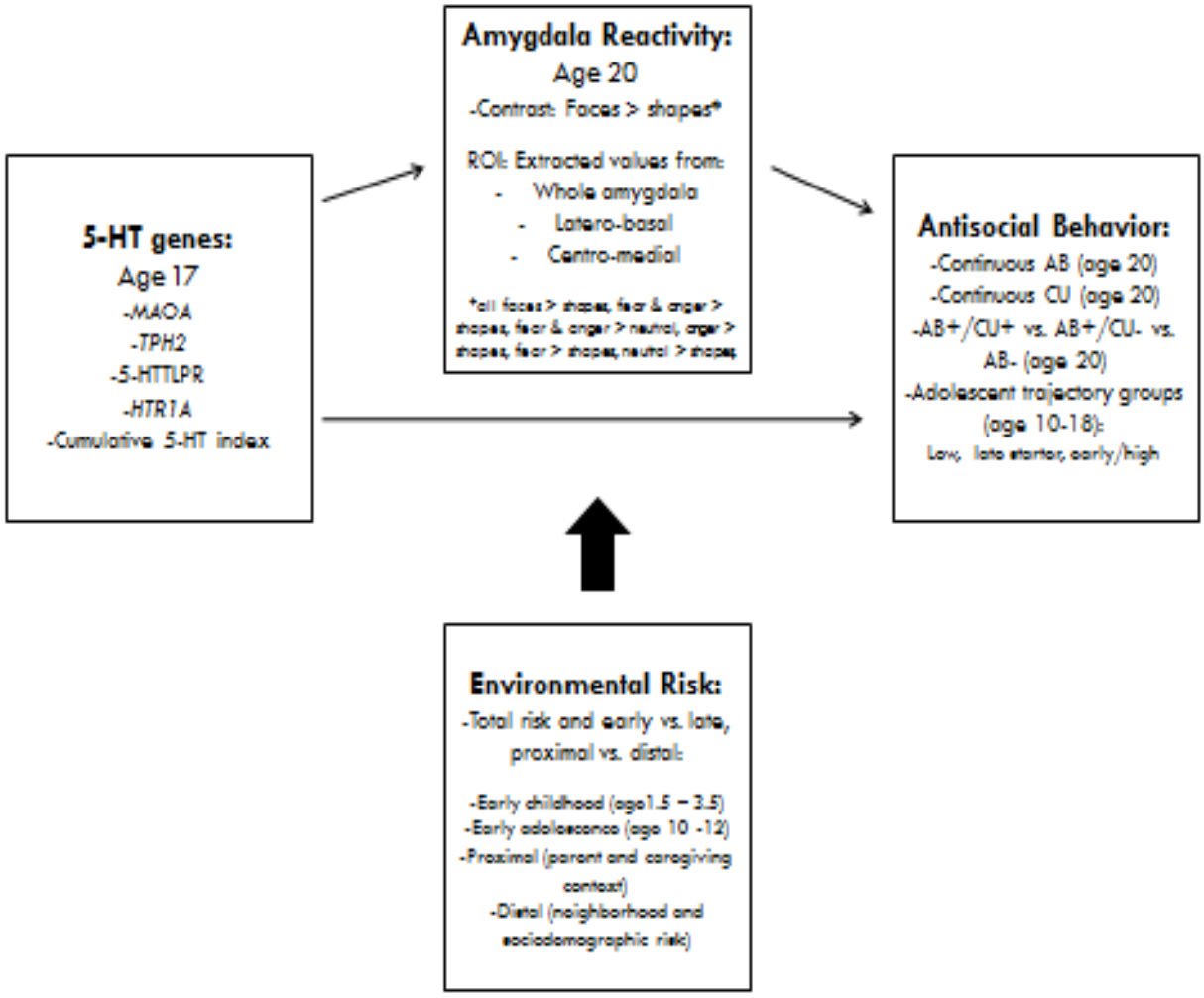


Figure 8: Summary of variables and age of collection

4.0 RESULTS

Prior to presenting result for each of the study's 3 main hypotheses, descriptive statistics and preliminary analyses are presented for variables that contributed to evaluating each of the hypotheses.

4.1 DESCRIPTIVE STATISTICS AND PRELIMINARY ANALYSES

Descriptive statistics appear in Table's 5-8. Table 5 contains the distributions of the allele frequencies for each genetic variant of interest. All genes were found to be in Hardy-Weinberg Equilibrium (HWE) across the entire sample. In addition, within White and Black subsamples all allele distributions were generally consistent with previous published reports where available (*MAOA* was not tested for HWE but sample distributions were similar to those reported within and across races in Sabol et al. 1998). Table/Figure 6 contains a distribution of the number of cumulative risk factors reported by study families. As can be seen in this figure, most families have at least some risk factors, albeit only moderate amounts (0 – 7), while a small portion of families have as many as 19 of the total possible 21 risk factors present.

In terms of outcomes, as can be seen in Table 7, age 20 self-reports of delinquency and CU were examined between young men based on their adolescent delinquency trajectory group and AB/CU group as a means of confirming the differences expected between these groups.

Adolescent trajectory group status predicted age 20 AB ($F(2, 225) = 43.7$, $p < .001$) and CU ($F(2, 224) = 3.5$, $p < .05$) as expected. Late starting youth and early starting/high youth were higher on AB than low delinquency youth but did not differ from each other statistically. Although late starting and early starting/high youth appeared to be higher on CU than low delinquency youth, these differences became nonsignificant once corrected for multiple comparisons. This result is notable as much CU literature suggests that early starters would be expected to have the highest levels of CU and this assumption was not supported within this sample in these preliminary analyses.

As expected (based on how groups were made), the AB/CU groups differed on AB ($F(2, 122) = 167.7$, $p < .001$) and CU ($F(2, 124) = 218.0$, $p < .001$). Although the AB+CU+ group had the highest levels of CU, the AB+CU- and AB-CU- groups did not differ statistically on level of CU. Additionally, the AB+CU- and AB+CU+ groups were higher on AB than the AB-CU- group but did not differ from each other statistically.

Finally, Table 8 presents descriptive statistics and correlations for major study variables. Of note within this table, non-White participants were found to have higher levels of cumulative risk across childhood and adolescence. AB and CU were correlated with each other, but at a lower level than expected ($r = .17$, $p < .01$). In terms of genes, non-White participants were more likely to be T carriers of the *TPH2* variant and White participants were more likely to be LL carriers of the 5-HTTLPR. *MAOA* high alleles were more likely to be S carriers of the 5-HTTLPR.

Table 5: Gene Distributions

Gene	Allele	Whole sample		White		Black	
		N	%	n	%	n	%
5HTTLPR	SS	36	17	20	18	10	12
	SL	85	41	55	59	27	33
	LL	88	42	35	32	45	55
TPH2	TT	19	8	7	6	10	11
	TG	80	35	36	30	35	39
	GG	130	57	78	65	44	49
HTR1A	CC	67	29	33	27	25	28
	CG	107	46	55	45	47	52
	GG	59	25	35	29	18	20
MAOA	2.5 (low)	5	3	0	0	5	6
	3.5 (low)	73	36	32	31	35	42
	4.5 (high)	121	60	70	67	41	49
	5.5 (low)	4	2	2	2	2	2

Note: all genes in HWE across sample and subsamples (*MAOA* not tested for HWE).

Table 6: Distribution of the total cumulative risk variable

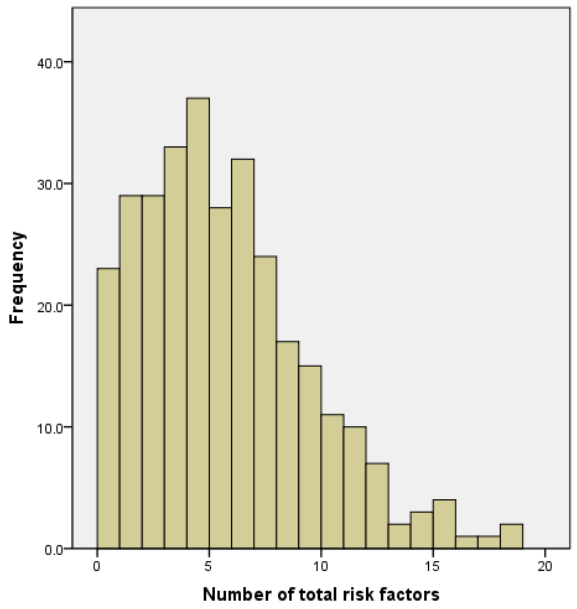


Table 7: Descriptive statistics of outcome groups

Variable	Group	# participants in group (% of total)	Mean AB at age 20	Mean CU at age 20
Adolescent Delinquency Trajectories	Low	171 (64%)	1.13[a]	.97
	Late starting	54 (20%)	1.30[b]	1.21
	Early Starting/ High	43 (16%)	1.31[b]	1.20
AB/CU groups	AB-CU-	76 (61%)	1.10[a]	.50[a]
	AB+CU-	25 (20%)	1.42[b]	.56[a]
	AB+CU+	24 (19%)	1.46[b]	1.92[b]

Note: numbers with different superscript letters differ from each other statistically using post-hoc tests corrected for multiple comparisons (Tukey test)

Table 8: Correlations and descriptive statistics of selected study variables

Variable	Mean (SD) or coding	1	2	3	4	5	6	7	8	9
1. Race	0 = White 1 = non=White									
2. Cumulative Risk total	5.7 (3.8)	.43**								
3. Age 20 AB	1.20 (.17)	-.05	.06							
4. Age 20 CU	1.04 (.64)	.15*	.17**	.18**						
5.Right Amygdala	.26 (.24)	-.05	-.04	-.17*	.13					
6. Left Amygdala	.27 (.28)	.02	.03	-.11	.09	.70***				
7. *HTR1A*	0 = G car 1 = CC	.05	.11+	.04	.09	-.02	-.04			
8. *TPH2*	0 = GG 1 = T car	.16*	.10	-.10	.05	.05	.06	-.06		
9. 5-HTTLPR	0 = LL 1 = S Car	-.22**	-.07	.01	-.17*	-.06	-.09	.01	-.05	
10. *MAOA*	0 = high 1 = low	.16*	.01	.15*	.04	.10	.14	-.06	.00	-.20**

+ p < .10, * p < .05, ** p < .01, *** p < .001

4.2 MAIN EFFECTS OF THE NEUROIMAGING TASK

To examine amygdala reactivity to various contrasts within the threat paradigm, individual subjects' values were extracted from SPM for each contrast of interest (e.g., all faces vs. shapes; fear faces vs. shapes) and for each ROI (i.e., the total amygdala, the centro-medial region, the latero-basal region). These ROIs were extracted for all voxels within the ROI that were above the threshold of $p < .05$ FWE (Family-Wise Error) corrected for multiple comparisons across the entire brain volume. The size of each cluster and the coordinates and statistical strength of the peak voxel within each cluster are presented in Table 9. Figure 9 presents examples of activation patterns within each ROI. As seen in this table, all five main study contrasts (faces, fear, neutral, and anger versus shapes) yielded statistically significant clusters within the whole amygdala bilaterally and these clusters were generally relatively large (41-178 voxels), with peaks showing a robust and significant response. When examining subregions of the amygdala, the same was generally true, although some contrasts and regions (i.e., the CM region) did not yield effects above the statistical threshold (right CM region to the fear > shapes contrast, bilateral CM region to the neutral > shapes contrast). Furthermore, some contrasts that did yield clusters above threshold were quite small (i.e., the left CM fear > shapes cluster was 2 voxels, the right LB neutral > shapes cluster was 9 voxels, right CM anger > shapes cluster was 2 voxels, and the left CM anger > shapes cluster was 1 voxel). Overall, as expected, the faces > shapes contrast appeared to result in the most robust response across the bilateral amygdala and within sub-regions in terms of size and strength of the clusters found.

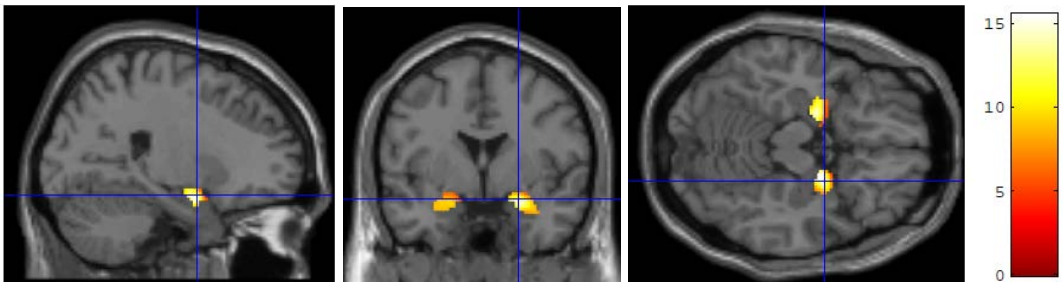
Whereas the contrasts described above were the main focus of the study, several other contrasts also were examined for extraction. Fear > neutral, anger > neutral, and fear & anger > neutral were explored, as these contrasts have been used in much of the previous AB literature (e.g., Jones et al., 2009; Marsh et al., 2008). Additionally, to be consistent with much of the past imaging genetics literature (e.g., Hariri et al., 2005), a fear & anger > shapes contrast was also explored. Although these contrasts have been used in other studies, several of the contrasts did not yield main effects above the threshold for multiple comparisons across the whole brain: fear > neutral, anger > neutral, and fear & anger > neutral. The fear & anger > shapes contrast did yield bilateral main effects (right: 135 voxels with a peak at MNI coordinates 22, -4, -16; left: 154 voxels with a peak at MNI coordinates -20, -6, -16). As these contrasts were not of primary interest in this study, only whole amygdala ROIs (and not LB or CM regions) were extracted. Moreover, as the fear & anger > shapes contrast was not of primary focus to the study and was found not to be associated with any primary outcomes of the study (i.e., age 20 AB and CU, adolescent trajectory groups, AB/CU groups, all genetic variants), data from this contrast is not presented any further.

Table 9: Summary of Main Effects of Contrasts

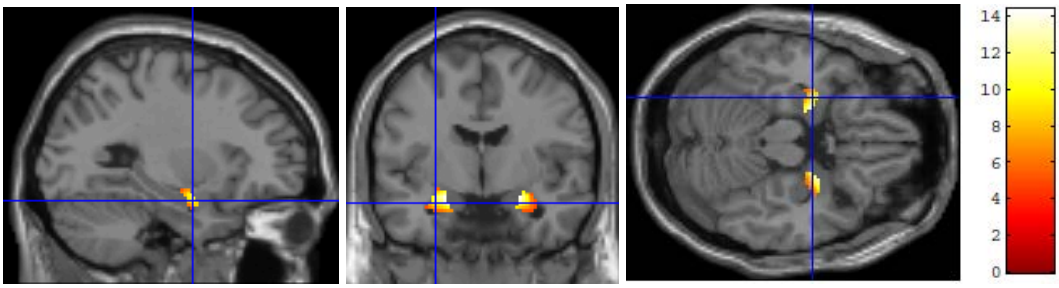
Contrast	Side	Cluster mask	Coordinates (MNI)	Number of voxels	Peak voxel (Z)
Faces > Shapes	Right	AAL	22, -2, -18	178	inf
		LB	26, -2, -18	138	inf
		CM	24, -6, 18	36	inf
	Left	AAL	-20, -4, -18	179	inf
		LB	-20, -6, -18	143	inf
		CM	-28, -4, -14	25	inf
Fear > Shapes	Right	AAL	22, -4, -16	52	7.38
		LB	24, -4, -16	20	6.66
		CM	n/a	n/a	None above threshold
	Left	AAL	-18, -6, -18	95	inf
		LB	-20, -6, -16	73	7.79
		CM	-20, -6, -10	2	5.12
Neutral > Shapes	Right	AAL	20, -4, -16	41	6.62
		LB	24, -4, -16	9	5.76
		CM	n/a	n/a	None above threshold
	Left	AAL	-20, -4, -16	36	5.57
		LB	-20, -6, -16	21	5.51
		CM	n/a	n/a	None above threshold
Anger > Shapes	Right	AAL	22, -4, -16	97	7.64
		LB	24, -4, -16	49	7.63
		CM	24, -6, -10	2	5.57
	Left	AAL	-22, -4, -16	121	inf
		LB	-22, -4, -16	67	inf
		CM	-28, -4, -14	1	5.10

Note: AAL = AAL definition of the whole amygdala; LB = Latero-Basal region of the amygdala; CM = Centro-Medial region of the amygdala; Inf = infinity. All clusters were above a threshold of $p < .05$ FWE corrected across the entire brain volume for multiple comparisons. N = 159.

AAL whole amygdala mask (22, -2, -18):



LB amygdala region mask (-20, -6, -18):



CM amygdala mask (24, -6, -10):

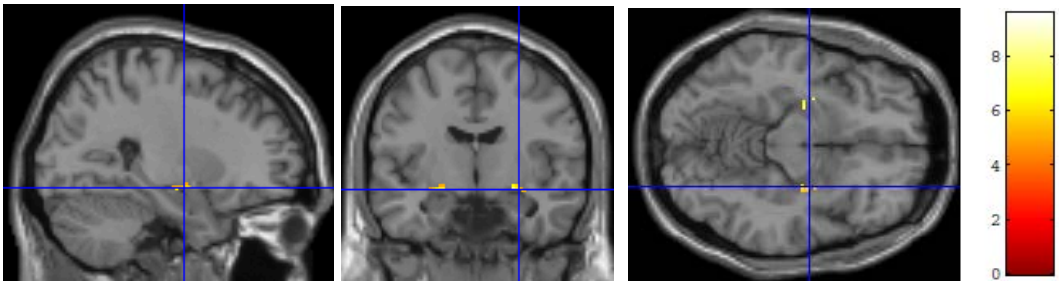


Figure 9: Sample images of amygdala regions from which values were extracted

(all for contrast Faces > Shapes)

4.3 HYPOTHESIS 1: BIOLOGICAL CORRELATES OF EARLY ADULT ANTISOCIAL BEHAVIOR

4.3.1 Hypothesis 1a – Neural correlates

To examine the hypothesis that AB across adolescence and in early adulthood would be related to amygdala reactivity at age 20 (AB would be related to greater amygdala reactivity, except in the presence of CU in which case AB+CU+ would be related to lower amygdala reactivity), associations were tested between behavioral measures of AB and CU and extracted neural reactivity in each ROI (i.e., whole amygdala, as well as centro-medial and basolateral amygdala, values based on the extracted main effects of task) and to each contrast of interest (i.e., all faces > shapes, fear > shapes, anger > shapes, neutral > shapes). Pearson correlations were used to examine the relationship between continuously measured AB (using the Self-Report of Delinquency) and CU (using the constructed scale of CU) and extracted neural reactivity across contrasts and ROIs. For categorical measures of AB (AB/CU groups, adolescent delinquency trajectory groups), a series of ANOVAs were computed with group as the independent variable and neural reactivity as the dependent variable. In the case of significant omnibus differences, post hoc comparisons were examined using Tukey tests to correct for multiple comparisons. Results for analyses involving the main ROI (i.e., the whole amygdala) are presented in Table 10 with graphs of significant ANOVA results presented in Figure 10 for clarity of interpretation of group differences. Results from associations exploring amygdala subregions (i.e., the baso-

lateral (BL) and centro-medial (CM) subregions) are presented in Table 11 with significant ANOVA results presented in Figure 11.

These analyses indicated that, in contrast to our hypotheses, right amygdala reactivity to a contrast of faces > shapes was negatively related to AB (greater AB was correlated with *less* amygdala reactivity). Surprisingly, within correlations of self-reported AB at age 20, there was a significant negative correlation between amygdala reactivity and AB (r = -.17, $p < .05$), meaning that youth with greater AB demonstrated less right amygdala reactivity to faces versus shapes. When examining relationships by groups, right amygdala reactivity to faces > shapes differed by group in directions contrary to our hypotheses. In the case of AB/CU groups, young men high on AB and CU (AB+CU+) were shown to have *greater* amygdala reactivity to this contrast, and this group was significantly higher in reactivity than men reporting to be high on AB but low on CU (AB+CU-) (F (2, 71) = 4.7, $p < .05$). The group low on AB and CU was in the middle in terms of amygdala reactivity to faces > shapes, but was not statistically different than either group. When examining groups based on adolescent AB trajectory, young men differed in their right amygdala reactivity to faces > shapes depending on their trajectory of adolescent AB (F (2, 148) = 4.1, $p < .05$). In this analysis, in contrast to expectations, young men with a history of early starting/high adolescent delinquency had the lowest amygdala reactivity of the three groups; however no pairwise comparisons were statistically significant. There was not a significant relationship between continuously measured CU and amygdala reactivity across any contrast, nor was there a relationship between continuously measured AB and amygdala reactivity on the left side to the faces > shapes contrast, nor bilaterally for any other contrasts of interest. Thus, overall when examining the main ROIs and contrasts of interest, there was little association between AB and amygdala reactivity, with the exception of an association between

right amygdala reactivity to faces > shapes and various measures of AB. However, within this association the results were in contrast to our hypotheses: young men with high and early starting delinquency were shown to be *lowest* on amygdala reactivity to this contrast, as were young men reporting the highest levels of concurrent continuously measured AB at age 20. At the same time, youth reporting to be high on AB *and* CU were found to have the *highest* amygdala reactivity to this same contrast.

When examining subregions of the amygdala, a similar pattern of results was found, albeit with greater numbers of statistically significant associations. In both the CM and LB regions, right amygdala reactivity to the face > shapes contrast was negatively correlated with age 20 AB (r = -.25, p < .01; r = -.14, p < .10, respectively). Right amygdala reactivity to faces > shapes within these regions also differed by AB/CU group in the LB region (F(2, 71) = 4.4, p < .05) and the CM region (F(2, 71) = 3.2, p < .05) in the same pattern as seen in the entire amygdala: men reporting being high on AB but low on CU had the lowest amygdala reactivity (AB+CU+ had the highest). When examining these subregions, men within different adolescent AB trajectory groups also appeared to differ in the same pattern as results from the whole amygdala, but in both the CM and LB this pattern of results was not statistically significant (p > .10).

Analyses delving into these subregions also uncovered several other associations with AB that were not present when using whole amygdala ROIs. Consistent with findings on the right side, *left* amygdala reactivity to faces > shapes within the CM region negatively correlated with age 20 AB (r = -.16, p < .05), and men differed in left amygdala reactivity to this contrast when compared by adolescent trajectory groups (i.e., the early/high group had the lowest amygdala reactivity though pairwise comparisons yielded no significant differences between

groups; F (2, 148) = 3.2, $p < .05$). Left amygdala reactivity in the CM region to the contrast of fear > shapes also correlated with age 20 amygdala reactivity in the same direction as seen with the faces > shapes contrast (r = -.23, $p < .01$).

In sum, amygdala reactivity to most contrasts (fear, neutral, and anger > shapes) was not shown to be associated with AB measured continuously and through subgroups. However, when examining the contrast of faces > shapes, right amygdala reactivity was negatively correlated with AB (at age 20), and men that had reported early and high levels of adolescent AB were observed to have the lowest level of amygdala reactivity to the faces > shapes contrast. Interestingly, when men were divided based on their AB and CU, those reporting high levels of AB but not CU continued to have the lowest level of amygdala reactivity, but those that also had high levels of CU had the *highest* levels of amygdala reactivity to this contrast. These findings appeared to generalize to both the LB and CM regions within the amygdala but may have been slightly stronger with the CM region. These results are in direct contrast to our hypothesis in which we argued that overall young men with higher levels of AB would show *higher* levels of amygdala reactivity to faces > shapes unless they also showed high levels of CU traits, in which case they would show the *lowest* level of amygdala reactivity. Moreover, although not the primary focus on these analyses, the lack of findings to other contrasts suggests that the overall contrast of faces > shapes generated the most robust amygdala response across participants and individual variability in this response was the most highly related to AB phenotypes.

Table 10: Associations between neural reactivity and antisocial behavior

Variable	N	Faces > Shapes		Fear > Shapes		Neutral > Shapes		Anger > Shapes	
		R	L	R	L	R	L	R	L
Correlations between neural activation and behavioral outcome (r)									
Age 20 AB	159	-.17*	-.11	-.11	-.11	-.01	-.00	.10	.00
Age 20 CU	159	.13	.09	.08	.09	-.04	.03	.05	.001
ANOVA with behavioral variables as grouping variable and neural activity as outcome (F)									
AB-/CU- vs. AB+/CU- vs. AB+/CU+	73	4.7*	.52	.68	.81	.06	.06	1.63	.62
Adolescent trajectory groups	148	4.1*	1.77	.50	.09	.64	.81	.74	1.3

Note: * $p < .05$

Table 11: Associations between neural reactivity and antisocial behavior divided by regions of the amygdala

		Faces > Shapes				Fear > Shapes				Neutral > Shapes				Anger > Shapes			
		LB		CM		LB		CM		LB		CM		LB		CM	
Variable	N	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L
Correlations between neural activation and behavioral outcome (r)																	
Age 20 AB	159	-.14+	-.08	-.25**	-.16*	-.07	-.07	n/a	-.23**	.05	-.01	n/a	n/a	.08	-.00	.03	-.01
Age 20 CU	159	.08	.01	.04	.10	.03	.07	n/a	.12	.03	-.01	n/a	n/a	.02	.00	.04	.04
ANOVA with behavioral variables as grouping variable and neural activity as outcome (F)																	
AB-/CU- vs. AB+/CU- vs. AB+/CU+	73	4.4*	.50	3.2*	.36	2.4+	.45	n/a	1.6	1.2	.05	n/a	n/a	.34	.21	.26	.92
Adolescent trajectory groups	148	2.3	1.5	2.2	3.2*	.21	.20	n/a	.40	2.1	.81	n/a	n/a	.08	1.8	.08	1.9

Note: + p<.10, * p < .05, ** p <.01; Note: n/a columns – no significant main effects of the contrast in this region and thus cannot be examined with behavioral data. LB = latero-basal amygdala; CM = centro-medial amygdala.

Figure 10a: Young adults differ on amygdala reactivity at age 20 based on their reports of AB and CU. F (2, 71) = 4.7, p< .05; AB+CU+ > AB+CU- at p< .05 using a Tukey test for multiple comparisons.

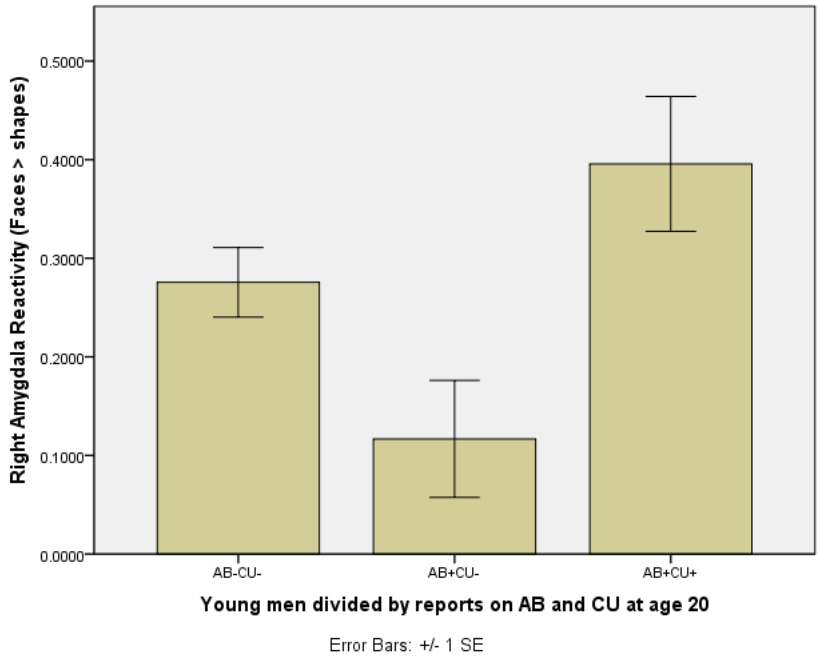


Figure 10b: Young adults differ on amygdala reactivity based on their adolescent history of self-reported delinquency. F (2, 148) = 4.1, p < .05; No pairwise comparisons were significant at p < .05 when using a Tukey correction for multiple comparisons.

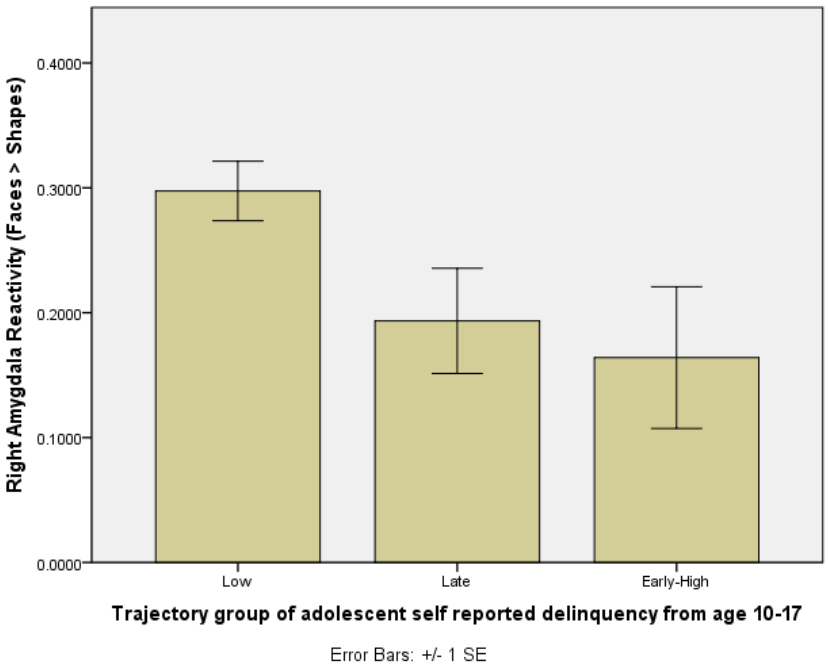


Figure 10: Amygdala reactivity at age 20 by grouping of youth

Figure 11a: Young adults differ on right latero-basal amygdala reactivity at age 20 based on their reports of AB and CU. F (2, 71) = 4.4, p< .05; AB+CU- < AB-CU-, AB+CU+ at p< .05 using a Tukey test for multiple comparisons.

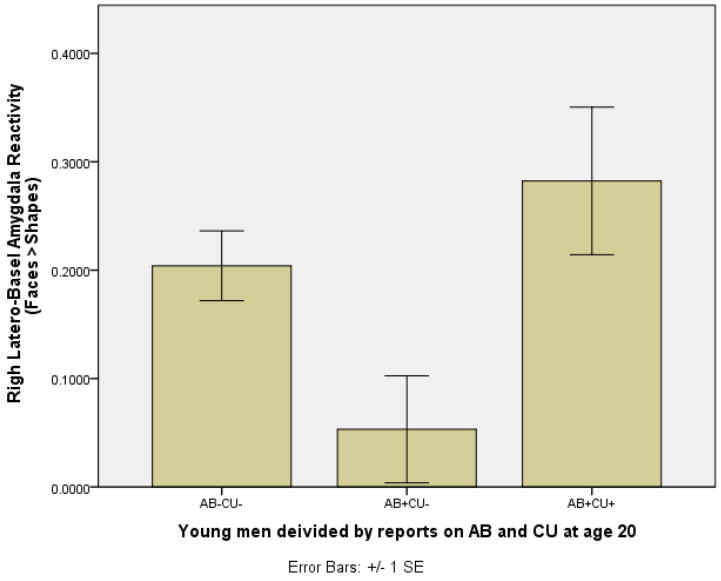


Figure 11b: Young adults differ on right central-medial amygdala reactivity at age 20 based on their reports of AB and CU. F (2, 71) = 3.3, p< .05; AB+CU- < AB-CU- at p< .05 using a Tukey test for multiple comparisons.

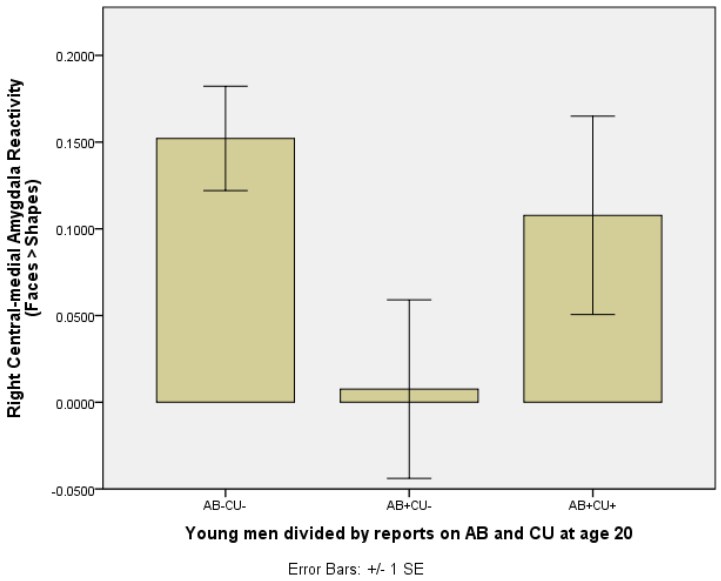


Figure 11c: Young adults differ on amygdala reactivity based on their adolescent history of self-reported delinquency. F (2, 148) = 4.1, $p < .05$; No pairwise comparisons were significant at $p < .05$ when using a Tukey correction for multiple comparisons.

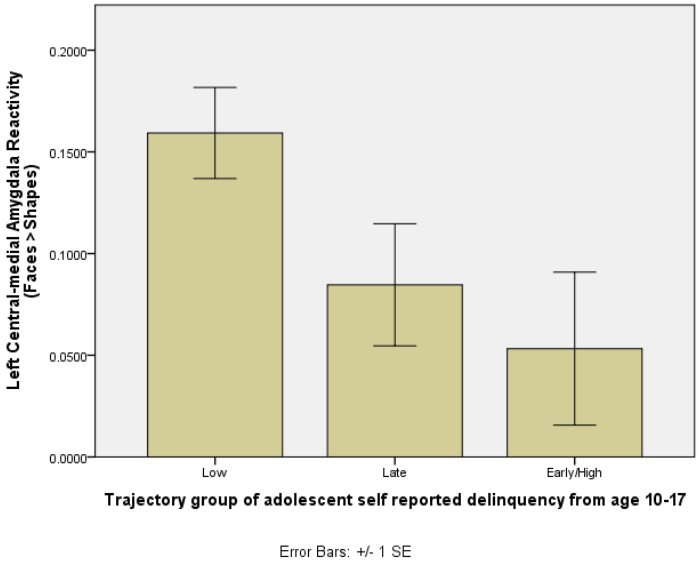


Figure 11: Group differences in amygdala reactivity by regions of the amygdala

4.3.2 Hypothesis 1b – Genetic 5-HT correlates

To examine the hypothesis that AB would be related to genetic variants previously linked to *greater* amygdala reactivity, while AB in the context of CU traits (and CU traits themselves) would be related to variants previously linked to less amygdala reactivity, point biserial and Pearson correlations were computed between individual 5-HT genes (and an index of 5-HT transmission) and continuous self-reports of AB (i.e., the Self-Report of Delinquency) and CU (CU factor scores) at age 20. For categorical outcomes, the frequency of risk alleles for each gene (and total 5-HT index) was also compared using Fisher Exact tests between adolescent AB trajectory groups and groups high versus low on CU and AB. Fisher exact tests were used instead of chi-square tests because chi-square tests require at least 5 individuals in each cell, which was violated in many cases within these analyses. Additionally, as gene distributions and

their associations with behavioral variables can vary by race, all associations were tested for the entire sample, within those reporting to be White, and then within those reporting to be Black (participants reporting Hispanic, Biracial, or Other were not examined separately as these groups were too small for analyses).

Results from these analyses are reported in Table 12 and significant group findings are displayed with graphs in Figure 12. Additionally as genetic effects do not necessarily follow past grouping methods (e.g., grouping 5-HTTLPR genes into S carriers versus LL), three group models (dominant homozygous, heterozygote, recessive heterozygotes) of each gene were explored to examine possible allele load effects and are presented in Table 13.

As can be seen within these tables and figures, there were no relationships between *HTR1A* genotype and AB, nor between *TPH2* genotype and AB using two group or three group classification schemes for the genotype and this null finding held across the entire sample as well as the White and Black subsamples. The cumulative 5-HT risk variable was not associated with any of the antisocial phenotypes in the entire sample, or in the White and Black subsamples.

In terms of 5-HTTLPR, unexpectedly, S carriers reported greater callousness at age 20 ($F(1, 184) = -5.6$, $p < .05$) than those with the LL genotype. This effect was significant in the White subsample ($F(1, 99) = 4.4$, $p < .05$) but not the Black subsample ($F(1, 69) = .00$, $p > .05$). This same pattern was seen using a three group genotype scheme, but the effect was only marginally significant in the whole sample ($F(2, 183) = 2.8$, $p < .10$), and was not significant in the White subsample ($F(2, 98)=2.3$, $p > .10$). When examining AB/CU groups, within the Black subsample, in contrast to our hypotheses, S carriers were over-represented in the AB+CU+ group (i.e., almost all of the Black participants in the AB+CU+ group were S carriers). This result did not hold across the entire sample, or across the White subsample. Finally, within the Black

subsample, again in contrast to our hypotheses, there was a trend towards LL homozygotes reporting greater AB at age 20 ($F(1, 69) = 3.2$, $p < .10$). Overall, the results examining 5-HTTLPR were opposite of the hypothesized directions: although LL homozygotes were expected to be higher on CU, with S carriers expected to be higher on AB (when not in the presence of CU), results indicated that S carriers reported the highest CU (particularly within the White subsample) and within the Black subsample those in the AB+CU+ group were most likely to be S carriers.

MAOA genotype was shown to be related to some AB phenotypes. When examining age 20 AB, as expected those with the low genotype were found to have high self-reported AB across the entire sample ($F(1, 180) = 4.0$, $p < .05$) with a trend towards this same pattern in Black participants ($F(1, 69) = 2.9$, $p < .10$) but not White participants ($F(1, 96) = 1.6$, $p > .2$). CU and adolescent delinquency status were not related to *MAOA* genotype, although AB/CU group status was (Fisher exact test = 11.4, $p < .01$). In contrast to our hypotheses, individuals in the AB+CU+ group were more likely to have a low allele than the high allele (i.e., there were only 2 individuals in the AB+CU+ group with high alleles). This pattern was statistically significant in the Black subsample (Fisher exact test = 6.3, $p < .05$), but not the White subsample (Fisher exact test = .27, $p > .05$).

In sum, *HTR1A* and *TPH2* genetic variants were not related to antisocial phenotypes in this sample, nor in the subsamples. 5-HTTLPR (and *MAOA* to some extent) was related to some antisocial phenotypes but in opposite directions as hypothesized: S carriers (5-HTTLPR) were shown to be higher on self-reported CU, and S carriers and low carriers (*MAOA*) were over-represented among the AB+CU+ group. LL carriers of the 5-HTTLPR among Black participants were higher on AB. These results were in direct contrast to the hypothesis that AB would be

related to S carrier and low carrier status except in the presence of CU, in which LLs and high carriers would be overrepresented. Whereas the majority of the significant findings were opposite of hypotheses, one *MAOA* result was in the expected direction: low carriers reported greater AB at age 20.

Figure 12a: Men with *MAOA* VNTR high alleles (4.5) report less delinquency at age 20 than those with low alleles (2.5, 3.5, 5.5) (across the entire sample). F (1, 180) = 4.0, p < .05.

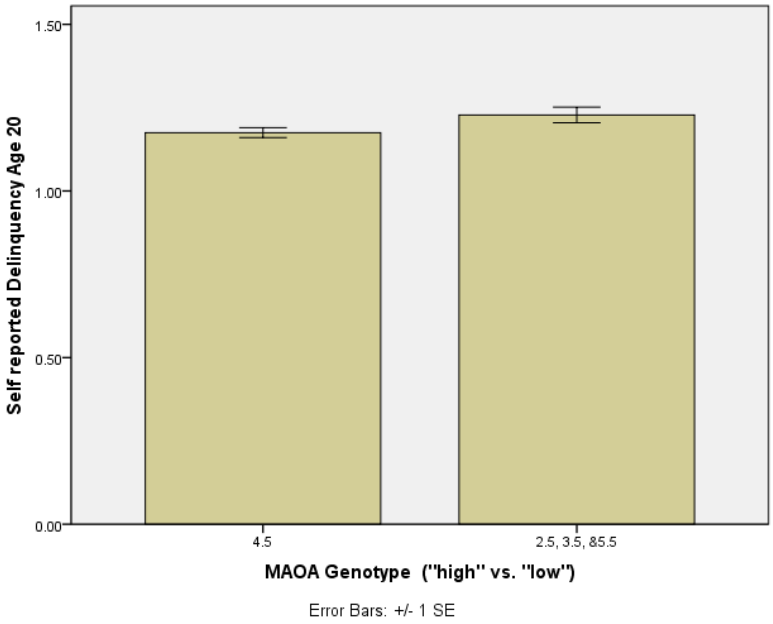


Figure 12b. 5-HTTLPR S carriers report greater callousness at age 20 than those with LL genotype (across the entire sample). F (1, 184) = 5.6, $p < .05$. Results were similar when examined only in White participants (S carriers > L homozygotes; F (1, 99) = 4.4, $p < .05$) but not Black participants (F (1, 69) = .00, $p > .05$).

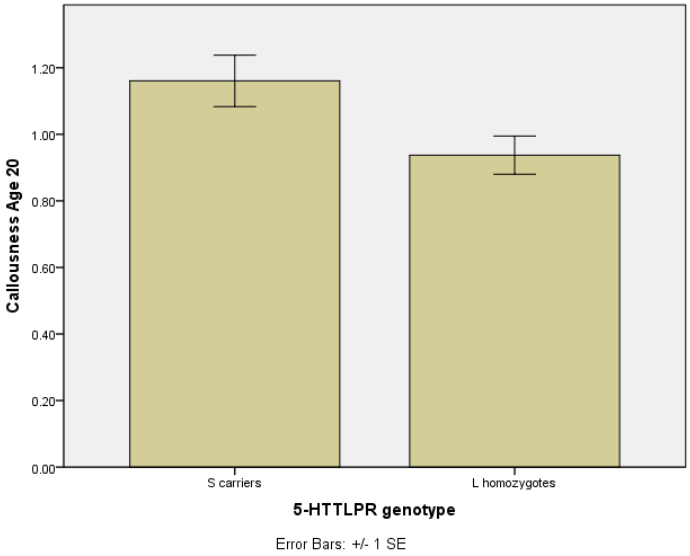


Figure 12c. Men with high levels of self-reported AB and CU at age 20 are more likely to have low efficiency *MAOA* alleles (2.4, 3.5, 5.5); Fisher's exact test = 11.4, $p < .01$ across all participants. Black participants were observed to have the same pattern of relationship between AB/CU groups and *MAOA* allele distribution (Fisher's exact test = 6.3, $p < .05$; AB+CU+ group has a great proportion of low variants) but with White participants this pattern was not statistically significant.

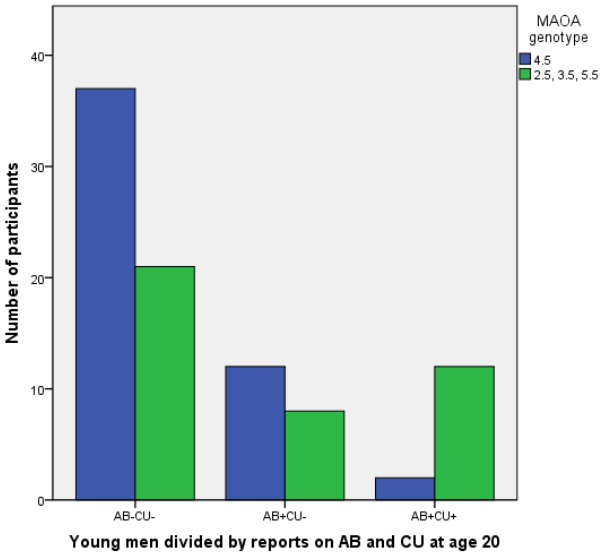


Figure 12d. Black men with high levels of self-reported AB and CU at age 20 are more likely to be S carriers of the 5-HTTLPR (and those with low levels of AB and CU are more likely to be L homozygotes); Fisher's exact test = 5.8, $p < .05$. The pattern of results was opposite in Whites (more S carriers in the AB-CU- group, less S carriers in the AB+CU+ group) but this difference was not statistically significant (Fisher's exact test = 3.3, $p > .05$).

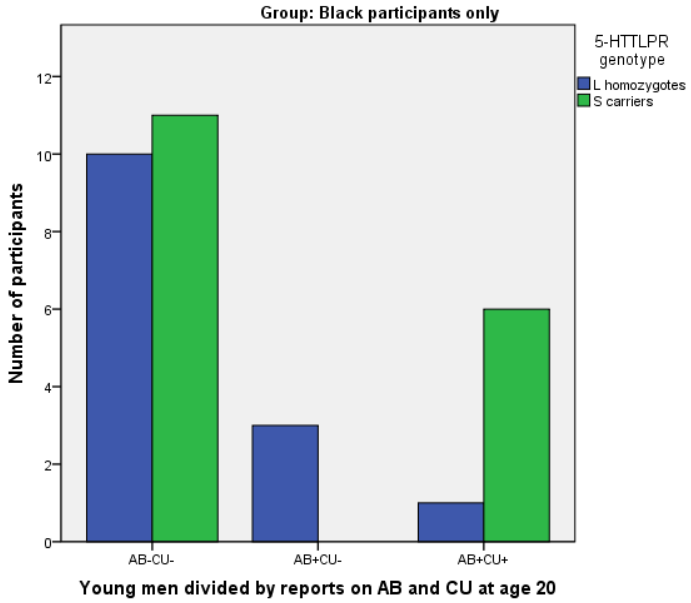


Figure 12: Relationships between young men's antisocial behavior and genotype

Table 12: 5-HT gene associations with AB and CU using genes in 2 groups

Outcome		*HTR1A* (CC)			*TPH2* (T carrier)			HTTLPR (S carrier)			MAOA (low carrier)			Cumulative 5-HT signaling		
	Analysis type	White n = 111	Black n = 77	All n = 206	White n = 109	Black n = 76	All n = 202	White n = 100	Black n= 70	All n = 185	White n = 96	Black n = 70	All n = 180	White n = 115	Black n = 78	All n = 210
Age 20 AB	ANOVA with AB as outcome	F = .09	F = .33	F = .28	F = .27	F = 2.5	F = 2.2	F = .55	F = 3.2+	F = .00	F = 1.6	F = 2.9+	F = 4.0*	r = -.06	r = .14	r = .01
Age 20 CU	ANOVA with CU as outcome	F = .90	F = .56	F = 1.8	F = .11	F = .08	F = .46	F = 4.4*	F = .00	F = 5.6*	F = .16	F = .00	F = .34	r = .00	r = .04	r = .03
AB-/CU- vs. AB+/CU- vs. AB+/CU+	Fisher exact test	.98	.79	.48	.84	2.7	.72	3.3	5.8*	1.6	2.7	6.3*	11.4**	F = .05	F = 1.5	F = .81
Adolescent trajectory groups	Fisher exact test	1.3	1.1	3.4	.76	.34	1.3	.27	1.1	4.0	1.1	1.5	1.7	F = .57	F = .14	F = .84

Note: + p <.10, * p < .05. For cumulative 5-HT signaling a Pearson correlation was used with continuous variables and an ANOVA was used for categorical variables.

Table 13: 5-HT gene association with AB using genes in three groups

Outcome		*HTR1A*			*TPH2*			HTTLPR		
	Analysis type	White	Black	All	White	Black	All	White	Black	All
Age 20 AB	ANOVA;	F = .18	F = .45	F = .14	F = .20	F = 1.3	F = 1.1	F = .35	F = 2.4+	F = .56
Age 20 CU	ANOVA	F = 1.6	F = 1.7	F = .89	F = 1.8	F = .16	F = .87	F = 2.3	F = .12	F = 2.8+
AB-/CU- vs. AB+/CU- vs. AB+/CU+	Fisher exact test	2.4	2.8	1.4	1.3	3.4	2.1	4.9	6.1	2.4
Adolescent trajectory groups	Fisher exact test	3.7	3.6	7.6+	1.6	1.4	1.5	1.8	2.7	5.2

Note: +p < 0.10, * p < 0.05

4.4 HYPOTHESIS 2: IMAGING GENETICS APPROACH TO EARLY ADULT ANTISOCIAL BEHAVIOR

To examine the hypothesis that individual genes previously linked to greater amygdala reactivity would be positively linked to greater amygdala reactivity, which in turn would be expected to be positively linked to AB through an indirect pathway, a series of correlational analyses was conducted to assess for possible mediated or indirect effects. Before being able to test mediated or indirect effects, two conditions must be met. First, the independent variable (IV; i.e., genetic variability) must be significantly related to the mediator (i.e., neural reactivity). Second, the mediator must be related to the dependent variable (DV; i.e., AB) while controlling for the IV. If these two conditions are met, the indirect pathway can be tested, or if the independent variable is related to the dependent variable, mediation can be tested.

Thus, to examine potential indirect effects these conditions were explored. First, in Step 1, 5-HT genes (using both 2 group and 3 group classification schemes and the cumulative 5-HT index) were correlated with amygdala reactivity (from each ROI: whole, CM, and LB region; right and left sides) from each contrast (all faces, fear, anger, and neutral > shapes) in the entire sample, as well as in the White and Black subsamples (see Table 14). The majority of these correlations did not reach statistical significance (i.e., only 16 out of 576 possible correlations were significant: 3%). As the percentage of statistical tests that were significant (i.e., 3%) was less than that expected by chance (i.e., 5%), follow-up probes for identifying possible indirect effects were not conducted based on the scant as evidence of indirect effects with these variables across the entire sample or within each subsample.

Table 14: First steps to examining possible mediated/indirect relationships from genes to neural reactivity to behavior across the entire sample (results within the White and Black subsample not shown).

Variable	N	Faces > Shapes		Fear > Shapes		Neutral > Shapes		Anger > Shapes	
		R	L	R	L	R	L	R	L
Correlations between neural activation and genotype (r)									
5-HTR1A (1 = CC, 0 = G car)	138	-.02	-.04	-.11	-.01	.03	.05	.02	-.01
TPH2 (1 = T car; 0 = GG)	135	.05	.06	-.08	-.12	-.04	.03	.07	-.02
5-HTTLPR (1 = S carrier; 0 = LL)	124	-.06	-.09	-.12	-.17	.-03	-.16	-.09	-.06
MAOA (1 = 2.5, 3.5, 5.5; 0 = 4.5)	120	.10	.14	.03	.02	.06	.08	.11	.10
Cumulative 5-HT signaling	140	.07	.06	-.13	-.11	-.01	-.03	.-6	.01

4.5 HYPOTHESIS 3: ENVIRONMENTAL MODERATION OF BIOLOGICAL PATHWAYS

To examine the hypothesis that the interaction of genetic and environmental risk predicts amygdala reactivity above and beyond their direct effects, which in turn predicts AB, a series of moderation and moderated mediation models were examined. Just as in Hypothesis 2, a series of steps was followed leading up to test this final hypothesis. First, interaction terms were generated between each genotype (2 and 3 group genotype models) and each cumulative

environmental risk variable (i.e., total risk, proximal risk, distal risk, early risk, late risk). A set of Pearson correlations was then computed between each interaction term and each neural outcome (e.g., reactivity to each of 4 contrasts in the whole, CM, and LB amygdala and on the right and left side) in the whole sample, as well as the White and Black subsamples. As the number of statistical tests was high (i.e., 2520 correlations were tested), this first step of correlations was used instead of full interaction regressions to provide an initial statistical threshold before exploring interactions for two reasons. First, it is relatively rare for an interaction term to be related to an outcome while accounting for the variance from the main effects of each independent variable but to be unrelated to the outcome when the main effects are not in the model (i.e., suppression effects are rare within interactions). Second, interactions in which there are suppression effects are more difficult to interpret and draw meaningful conclusions from. After exploring these correlations, the second step was to explore significant correlations between the interaction term and neural reactivity within a traditional regression framework, examining interaction terms that remained significant after accounting for the main effects of genotype and cumulative risk. Third, any regression that contained a significant interaction was explored within PROCESS, a macro for SPSS that can explore moderated mediation and examine conditional indirect effects (Hayes, 2012).

In step 1, a series of correlations between possible interaction terms and neural reactivity yielded 92 significant correlations (92 out of 2520 possible: 4%). Of these significant correlations 26 out of 840 (3%) were found in the whole sample, five out of 840 were found in the White subsample (0.6%) and 61 out of 840 were found in the Black subsample (7%). In step 2, these interaction terms were tested in a regression also controlling for the main effects of cumulative risk and genotype. Twenty of the 92 possible interactions remained significant (22%)

when controlling for main effects. However, as these 20 significant interactions represented 0.7% of the total interactions tested (e.g., 20 out of a possible 2520), we did not go further in testing full moderated mediation models. As the percentage of significant effects was below that expected by chance, we concluded that there was little evidence for moderated mediation with these variables in this sample. In sum, there were very few interactions present in which G x E predicted neutral reactivity within this sample and thus we could not tested for IGxE models.

5.0 DISCUSSION

Broadly, the purpose of the present study was two-fold. The first goal was to examine biological (neural and genetic) correlates of AB in emerging adulthood focusing on amygdala reactivity and specific subgroups of youth with AB, among an ethnically diverse sample of low-income males followed from ages 1.5 to 20. A second goal was to extend findings linking individual variability in 5-HT genes and amygdala reactivity to AB by testing models linking genetic variability to AB through amygdala reactivity (i.e., imaging genetics models) and examining the potential moderating role of the environment on these mechanisms (i.e., IGxE models).

Across all three hypotheses there was a dearth of findings. When statistically reliable associations were found, many of them were in the opposite direction as hypothesized. Most associations were also of small to medium in effect size (e.g., r = .17 - .25) (Cohen, 1992). Although statistically significant findings were rare, the findings that did emerge supported relationships between neural reactivity to threat and AB.

In terms of brain-behavior links, amygdala reactivity to the faces > shapes contrast was related to various measures of AB, albeit in opposite directions than hypothesized. Right amygdala reactivity was negatively correlated with AB at age 20, and young men in the early/high adolescent delinquency trajectory demonstrated the lowest amygdala reactivity in this contrast. Moreover, when CU was examined as a potential moderator of the relationship between neural reactivity and AB, the results continued to be in the unexpected direction --

young men with high levels of AB but low CU had the *lowest* amygdala reactivity and young men high on AB and CU had the *highest* amygdala reactivity. In terms of the link between 5-HT genes and AB, results continued to be sparse and in the opposite direction as expected. S carriers of the 5-HTTLPR reported to be higher on CU. S carriers and *MAOA* low carriers were more likely to be in the AB+CU+ group. Both of these findings were in direct contrast to the hypothesis that this AB+CU+ group would be more likely to have LL and high genotypes of the 5-HTTLPR and *MAOA* polymorphisms, respectively.

When testing imaging genetics links, although many possible associations were tested, there was little evidence of reliable indirect effects. Similarly, when testing IGxE models, again many models were tested but few significant results were found to support statistically reliable IGxE models.

Note that in the following discussion of results, we focus much of the discussion on Hypothesis 1a – neural correlates of AB – because this hypothesis yielded the greatest number of consistent results, and because more literature exists to contextualize these findings. Certainly many of the points explored in regards to these findings can, and do, apply to other hypotheses. Thus, first we discuss the results for this hypothesis in more detail before moving to other hypotheses and discussing limitations of the study and possible clinical implications.

5.1 AMYGDALA REACTIVITY AND AB IN ADOLESCENCE AND EMERGING ADULTHOOD

Contrary to expectation, AB appeared to be *negatively* associated with amygdala reactivity. This finding was true for the outcome of age 20 self-reported delinquency, adolescent trajectory

groups, and those high on AB and low on CU. CU was not generally related to amygdala reactivity and when it was considered in combination with AB, those high on AB and CU had *greater* amygdala reactivity than those high on AB but low on CU. These results are inconsistent with the findings of several prominent studies in which those high on AB and CU were found to have the lowest level of amygdala reactivity to faces (Jones et al., 2009; Marsh et al., 2008), and much theory in the field (along with our own line of reasoning in the introduction) proposing that AB+CU- would be associated with greater amygdala reactivity to threat, while AB+CU+ would be associated with lesser amygdala reactivity (Blair, Peschardt, et al., 2006a; Viding, Fontaine, & McCrory, 2012).

However, upon closer inspection of the limited literature, this pattern of findings may not be wildly discrepant from the current state of empirical research. The most recent, largest and most comparable study to the current study examined older adolescents (age 16-21) within a larger sample (n = 75) and found that both early and late starting antisocial youth had *less* amygdala reactivity to emotional faces and that amygdala reactivity was *not* related to CU traits (Passamonti et al., 2010). Although we argued in the introduction that CU and AB were confounded in this sample, it is just as possible that low amygdala reactivity is a mark of severe AB rather than of AB and CU together or CU in and of itself. Past studies on youth with AB and CU (Jones et al., 2009; Marsh et al., 2008) and adult psychopaths (e.g., Gordon et al., 2004; Kiehl et al., 2001) that found reduced amygdala reactivity in antisocial populations were unable to separate the contribution of AB versus CU or psychopathy. In these studies the presence of CU may only indicate more severe AB. In the only study on adults that focused exclusively on AB and found increased amygdala reactivity to anger faces (Coccaro et al., 2007), the sample was comprised of individuals diagnosed with Intermittent Explosive Disorder; it remains an open

question as to the overlap between Intermittent Explosive Disorder and AB in young adults. Thus, it is possible that previous studies on AB when combined with CU or psychopathy that found decreased amygdala reactivity were simply measuring the association between severe AB and amygdala reactivity, with level of CU being relatively unimportant to amygdala reactivity. Certainly the current findings and those of Passamonti and colleagues support the notion that AB is associated with decreased amygdala reactivity to emotional faces.

5.1.1 Considerations in comparing these findings to others

It is possible that this interpretation, that AB is broadly related to low amygdala reactivity regardless of the level of CU, may fit with some of the current empirical literature (albeit certainly contrasts with much of the theory and some studies in the field). However, there are several aspects of the study of note that could have affected the pattern of results and account for the differences in comparison to other studies. As we have argued elsewhere (Hyde, Shaw, & Hariri, under-review), these small details may have a large bearing on the direction and strength of the findings.

First, the measure of CU used in this study was unique and created specifically within this study. The items comprising the CU factor (see Table 2) rest heavily on empathy. While empathy is a core component to the CU construct, the current measure of CU may not tap the same underlying construct as previous reports using the CU scale of the Antisocial Process Screening Device. However, as the psychometric properties of the Antisocial Process Screening Device CU scale were unacceptable for the current sample, it could not be used on its own. As the measurement of CU has been improved recently with the Inventory of Callous Unemotional traits (Kimonis et al., 2008), it would be helpful to use this newer CU measure with this sample

in the future, or use adult measures of psychopathy or callousness (e.g., the Psychopathy Checklist, the Self-Report of Psychopathy).

A second important point was that measures of CU and AB were correlated more modestly with each other than in some prior research (e.g., r = .18 vs. .38-.52 in prior research with children) (Frick et al., 2000). However, it is important to note that little work has explored CU in early adulthood and recent reviews suggest that CU is more important in defining a subgroup of youth with a different course of AB, rather than as a strong correlate of AB (Frick & White, 2008). Thus, the relatively modest magnitude of the relationship between CU and AB was both a strength and limitation of the study. The low correlation between CU and AB meant that these two variables were not confounded as they often are in other studies (e.g., Marsh et al., 2008; Passamonti et al., 2010), and therefore we were able to separate the two constructs. Relatedly, high CU traits were evenly distributed across adolescent delinquency trajectory groups, meaning that the effects seen in those groups were likely related to trajectory of delinquency rather than confounded CU. This lack of confounding between variables was a strength in exploring the relationship between AB, CU and other variables, but may limit the generalizability of the current findings to other populations. This lower correlation between AB and CU could reflect our specific measure of CU as more empathy focused, or it could reflect a different relationship between AB and CU within this sample of young men. Most prior studies examining CU have been conducted with children or early adolescents in normative, clinic referred or forensic samples (Frick & White, 2008). Given that the construct of CU was designed for youth and it was measured with a sample of 20 year olds, it could be that the construct has a different relationship with AB as adolescents become young adults and their patterns of AB change, often desisting or becoming more severe.

Third, the method of analyzing the neural reactivity data in this study was quite different than other similar studies. Because several of the hypotheses involved multiple variables and more complex statistical models, we focused on using extracted values from main effects within the amygdala at stringent statistical thresholds (family wise error corrected across the entire brain volume). Most other comparable studies have examined brain-AB links within neural imaging software using lower statistical thresholds (e.g., small volume correction) and with the ability to search the brain volume for only voxels that are demonstrating peaks to the variable of interest (e.g., AB/CU groups). This analysis difference is relevant because several other studies have focused only on the contrast of anger > neutral and fear > neutral; however, in the current study no amygdala voxels showed main effects above our high threshold to be extracted and thus we did not explore this contrast in relationship to behavioral outcome. This lack of main effects to these specific contrasts in the current study could stem from the higher statistical thresholds or the focus on extracting main effects across the entire amygdala. Alternatively, it could also have to do with the presentation of multiple types of faces (i.e., fear, anger, surprise, neutral) across only 4 blocks (one of each face type), which may have decreased the power to examine specific faces when contrasted with neutral faces. Moreover, as suggested by a recent publication (Passamonti et al., 2010), neutral faces may be driving differences in AB groups rather than other emotions within in a contrast. Thus a contrast of anger > neutral may be as much a product of response to anger as to neutral (and neutral may be potent in driving the amygdala based on novelty).

For the most part, many of the significant findings in relating neural reactivity to AB came from the faces > shapes contrast. This emphasis on all faces suggests that there was little important difference between face types in predicting neural reactivity, that each face type

presented drove the amygdala due to novelty, or that there was insufficient power to detect these differences within the current task and analytic strategy. Thus, although the ability to examine different faces types versus both shapes and neutral faces was a strength of the study, issues with power to detect the effects based on task and extraction method may have limited the ability to examine these more fine-grained associations.

This point is also quite important in interpreting the results linking reactivity to these faces to AB. Previous studies have focused specifically on the neural reactivity to fear versus neutral (Jones et al., 2009; Marsh et al., 2008; Passamonti et al., 2010) because of findings that antisocial youth (especially those high on CU) have particular trouble identifying fear faces (Marsh & Blair, 2008). According to theory in this area of research, one might not expect a divergence between AB+CU- and AB+CU+ groups to neural reactivity to all faces but only to fearful faces (Blair, Colledge, Murray, & Mitchell, 2001; Viding et al., 2012). Without a statistically significant cluster to the contrast of fear > neutral, it is difficult to assess the extent to which the current results can be compared to other studies and the extent to which previous findings are really specific to fearful rather than any emotional face. At the same time, there was one correlation between reactivity to fear > shapes in the left CM region of the amygdala to age 20 AB. This correlation was positive and significant, suggesting that some of the effect to all faces may be driven by the fear faces block and that the present results may be addressing the effect of fearful faces. Moreover, albeit not significant, the correlation between age 20 CU and left CM reactivity to fear > shapes was *positive*. To the extent that the current results in regards to fear > shapes can be compared to previous studies using fear > neutral, the results continue to suggest that neural reactivity to fear is correlated negatively with *AB* rather than CU.

Fourth, the population represented by this sample may represent a very different population than other comparable studies. It is possible that neural correlates of AB may be very different in the context of an ethnically homogeneous sample of middle-class boys and girls (Marsh et al., 2008) than in the present sample of low-income, ethnically diverse young men. Though we hypothesize that the level of environmental risk may moderate brain-behavior links and explain differences between the current study and others that are lower risk (e.g., biological factors are more important in lower risk settings), there was little evidence of moderation by SES or risk of the brain-behavior relationship within the current study. Exploratory analyses (not shown) in which family SES at age 18 months and total cumulative risk was a moderator of the relationship between amygdala reactivity (faces > shapes) and AB and CU, found little evidence for statistically significant moderation. All but one interaction was non-significant. The one significant interaction found that the relationship between amygdala reactivity and CU was more closely linked at *higher* rather than lower risk (see Figure 13). Thus, our hypothesis that we found fewer brain-behavior links because of the higher risk of this sample was not supported within the range of risk in the current study. However, as the range of environmental risk in this sample is restricted to relatively high risk, it is still possible that levels of risk between studies could contribute to different patterns of results. Finally, beyond the issue of risk, developmental processes, both environmental and neurodevelopmental, may mean that findings in adolescence do not readily generalize to emerging adulthood (age 20 in this study).

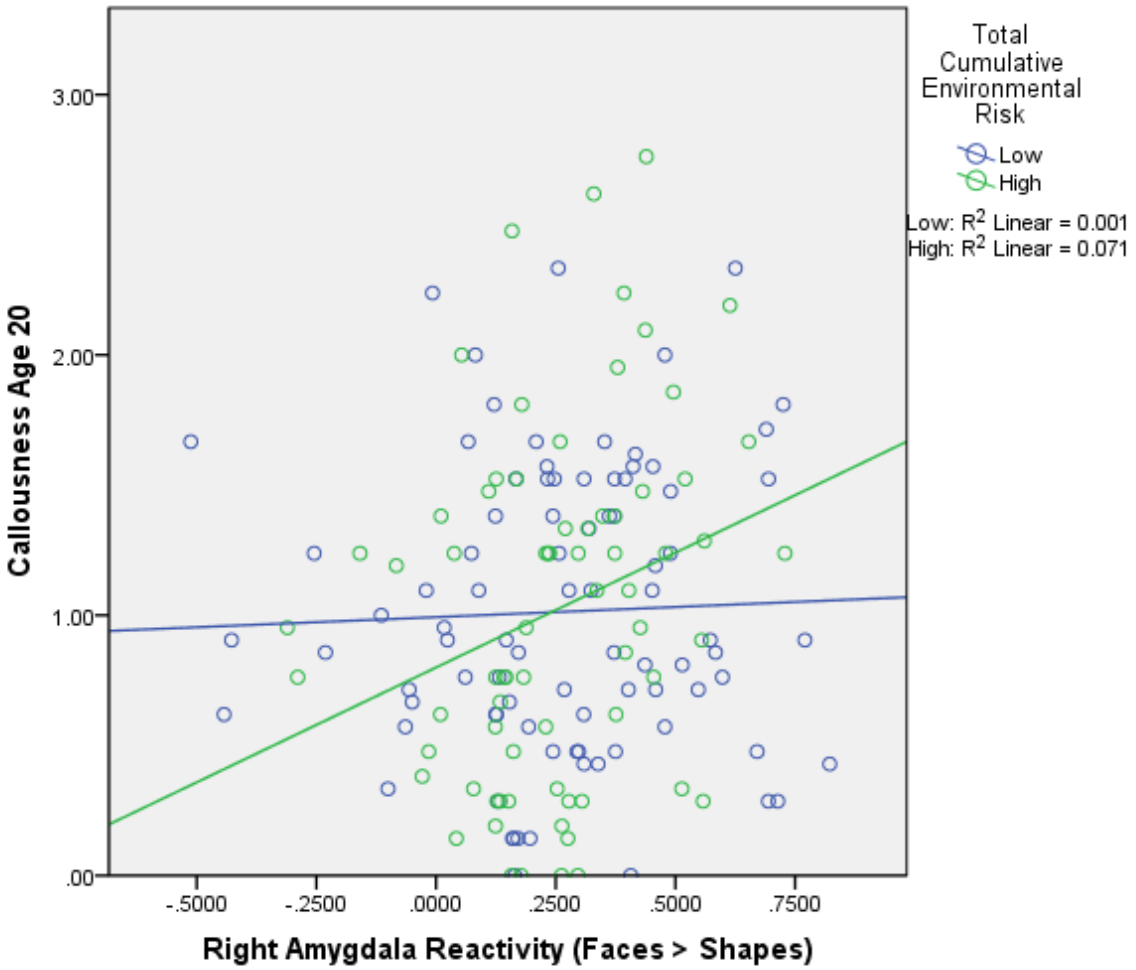


Figure 13: Total Environmental Risk Moderates the Link Between Amygdala Reactivity and Callous-Unemotional Traits

One final note to consider in comparing our findings to others: a very recent study by our group explored relationships between 4 facets of self-reported psychopathy and amygdala reactivity to fear > shapes and anger > shapes in a sample of 200 young adults aged 18-21 (Carre, Hyde, Neumann, Viding, & Hariri, 2012). The results of this recent study were consistent with the overall hypotheses of the current study: When considering path models controlling for the overlap of the 4 facets of psychopathy, the interpersonal facet, which is similar to CU, was *negatively* associated with amygdala reactivity to *fear*, and the lifestyle facet,

which is loosely related to AB, was *positively* associated with amygdala reactivity to *anger*. Though this study is quite difficult to compare to the current one based on sample make-up (i.e., co-ed college students versus low income inner city males), measure of AB (i.e., the Self-Report of Psychopathy versus measures of CU and self-reported delinquency), and analytic approach (i.e., path models emphasizing suppression effects of the four facets versus CU and AB versus AB and CU tested in different models), it does suggest that future analyses in this sample that examine the overlapping and unique contributions of AB versus CU to amygdala reactivity might find different relationships between these variables and amygdala reactivity to fear and anger faces. Certainly the findings from this recent study stand in contrast to the findings of the current study.

In sum, although the current finding that emerging adults high on AB or those with a history of high and early starting adolescent delinquency behavior have less amygdala reactivity to emotional faces than their peers was contrary to our hypotheses, the finding is consistent with a recent and comparable study (Passamonti et al., 2010). Moreover, the current study differs in many ways (e.g., measure of CU, methods of data analysis, sample characteristics) that may make comparison to other samples difficult. The results also suggest that the theoretical link establishing the prominence of the link between CU and amygdala reactivity may be worth revisiting in samples in which AB and CU are not confounded and within the context of socioeconomic adversity.

5.1.2 Location within the amygdala

One way in which the current study improves on the current literature is in examining the differential contribution of different major subareas of the amygdala. When examining the

relationship between right amygdala reactivity and measures of AB, both LB and CM regions were associated with measures of AB (age 20 AB and AB/CU groups). However, in the left hemisphere in response to faces > shapes, only reactivity in the CM region was related to measures of AB (age 20 AB, trajectory groups). Moreover, in relation to the contrast of fear > shapes, only reactivity in the left CM region was correlated with AB (age 20 AB). Thus, some of the results appear to extend to both LB and CM regions, but in the case of fear > shapes and faces > shapes in the left hemisphere, the CM region appeared to be a more reliable predictor of AB. Based on work suggesting that the CM region is more involved in impulsivity and rapid emotional responses to stimuli due to its connections to brainstem targets which affect heart rate and arousal, whereas the LB region is involved in more elaborate planning and motor responses due to its connections to striatum (Brown et al., 2006; Cain & LeDoux, 2008), it may be that these neural differences in antisocial youth and adults are driven by regions of the amygdala (i.e., the CM region) more associated with impulsivity. This finding would fit nicely with data suggesting that a majority of youth high on AB are quite impulsive (Farrington, 1995; Luengo, Carrillo-De-La-Pena, Otero, & Romero, 1994) and diagnostic criteria that include impulsivity within AB diagnoses (American Psychiatric Association, 1994). It also suggests that exploring the role of impulsivity as a mediator between neural reactivity and AB could be helpful in understanding some forms of AB. Moreover, this finding suggests that neural studies of youth AB should also explore the overlap of AB with ADHD and other disorders involving impulsivity (e.g., substance use).

5.1.3 Understanding the current results within existing neuroscientific models of antisocial behavior

This study was driven by theory and research on antisocial behavior that emphasizes the role of the amygdala in youth AB and adult psychopathy, and suggests the possibility of differential correlates of AB+CU+ versus AB+CU- (Blair, Peschardt, et al., 2006c; Crowe & Blair, 2008; Viding et al., 2012). In terms of the focus on the amygdala, all broader theories in this field have emphasized the role of the amygdala in AB. For example, various theories postulate the importance of the OFC and amygdala in psychopathy (e.g., Blair, 2007), broad "paralimbic dysfunction" in psychopathy, including the amygdala (Kiehl, 2006), and distributed dysfunction across multiple brain systems (e.g., some areas of the prefrontal cortex, the amygdala, insula, and cingulate) linked specifically to emotional deficits, antisocial behaviors, and lying in psychopathy (e.g., Glenn & Raine, 2008; Glenn et al., 2009; Raine, 2002b; Yang & Raine, 2008). Implicit in all of these theories' focus on the amygdala has been that psychopathy or CU traits are driving the lowered amygdala reactivity seen in antisocial populations, rather than AB itself. However, the current study, through decoupling CU and AB constructs, suggests that lower amygdala reactivity to threat may be a correlate of broad AB, rather than of CU, psychopathy, or AB only in the presence of CU. In this study, CU was not correlated with lowered amygdala reactivity and when it was examined as a moderator of the relationship between amygdala reactivity and AB, it was the group of young men lower on CU and high on AB that had the lowest amygdala reactivity. Although the differences in our findings could be due to sample differences or different methods of assessing CU, it is also possible that because most previous studies have focused on individuals high on AB that also were high on CU and psychopathy (e.g., Jones et al., 2009; Kiehl et al., 2001; Marsh et al., 2008) and thus

relationships between amygdala reactivity and CU and psychopathy may have been attributable to CU's correlation or confounding with AB. According to the current results, lowered amygdala reactivity to threat may be a correlate of broad AB (consistent with the recent study by Passamonti et al., 2010) rather than CU or AB+CU+.

One other important issue to consider is why amygdala reactivity itself may be important to the development of AB. Early developmental studies of amygdala reactivity in problem behavior in young children do not exist, so it is unclear if differences in amygdala reactivity in this population are the cause or sequelae of trajectories of youth AB. Blair (1995) has proposed a theory of the developmental consequences of early amygdala dysfunction, which is postulated to affect a violence inhibition mechanism (VIM) that accounts for both the blunted amygdala response to distress cues and instrumental aggression displayed by psychopaths. According to the VIM, moral socialization occurs through the pairing of distress cues (unconditioned stimuli, US; e.g., sad or fearful faces) with representations of the acts leading to the distress (conditioned stimuli, CS; e.g., hitting another person). The inability to learn such CS-US pairings, a critical function of the amygdala, could lead to a dysfunctional VIM and heightened instrumental aggression. However, the extent to which amygdala function mediates instrumental aggression is currently unclear. Moreover, as studies have not examined amygdala functioning early in life, we know little about how early differences may emerge in this population or how they could affect development over time. Regardless, as findings from the current study did not support a strong relationship between CU and amygdala reactivity, the current results do not appear to be consistent with this theory.

5.2 GENETIC CORRELATES OF EMERGING ADULT AB WITHIN THE 5-HT SYSTEM

Although there were no direct relationships between *HTR1A* and *TPH2* SNPs and measures of AB, variability in 5-HTTLPR and *MAOA* was correlated with some measures of AB and CU, particularly in the Black subsample, albeit mostly in unexpected directions. In the whole sample, the *MAOA* findings appeared to be in the expected direction (i.e., low genotype correlated with greater AB), but analyses within the AB/CU groups suggested that this effect may be driven by the AB+CU+ group, particularly in the Black subsample. For findings with the 5-HTTLPR, CU was related to being an S carrier but AB was related to being an LL, both in the opposite direction as expected. Examining subgroups indicated that the White subgroup may be driving the effect for CU, as AB findings were only present in the Black subgroup. These complexities highlight the difficulty of examining direct gene to behavior relationships within a racially diverse sample and the difficulty of connecting genetic variability directly to behavior in any sample. Clearly, low income minority youth are at increased risk for AB (Farrington, 2005; Patterson et al., 1989) and yet less work has been done to connect 5-HT genes to AB with historically underrepresented minority groups. As discussed below, this issue is particularly problematic given that some studies suggest that the same allele within different genetic/racial backgrounds may have very different correlations with outcome variables. At the same time, it is difficult to recruit and fund large and diverse samples with sufficiently large homogenous subgroups to analyze separately. Even within this moderately sized sample, when splitting between groups, the Black subsample only contained 70 subjects for gene to behavior analyses, which provides little power to detect the effects of each genetic variant. Moreover, without including environmental moderators or more proximal mediators (e.g., neural reactivity), it is

particularly challenging to detect candidate gene associations with a complex phenotype such as AB (Uher, 2011).

With those caveats in mind, the findings fit with some of the inconsistent literature in this area. Whereas we hypothesized that the *MAOA* low alleles would be related to AB when not in the presence of CU, our "unexpected" findings were consistent with a study by Fowler and colleagues that found an association between the low allele and psychopathy trait scores in a sample of adolescents previously diagnosed with ADHD (Fowler et al., 2009). Based on the numerous G x E studies linking the low allele to AB in the presence of maltreatment (Caspi et al., 2002), perhaps the low allele is a risk factor for AB broadly and also for subgroups such as those high on CU or psychopathy. Interestingly, in the Fowler study the low allele predicted the affective components of psychopathy and was not strongly related to AB.

When considering the similarly unexpected results with 5-HTTLPR – that S carriers reported more CU and were more likely to be in the AB+CU+ group – a similar explanation can be offered to that found for *MAOA*. Consistent with prior research (Glenn, 2011; Sadeh et al., 2010), we had hypothesized that S carriers would be more likely to be "impulsively antisocial," suggesting that S carriers would be higher on AB and that LL carriers would be high on CU. Yet, our results were more consistent with findings from the study by Fowler and colleagues (2009), in which SS homozygotes were higher on total psychopathy scores, especially the affective component that is most similar to CU.

Ignoring the issues of inconsistent findings between the Black and White subsamples, although the results related to 5-HTTLPR and *MAOA* were consistent with one other study, these findings highlight issues raised in a recent review by Gunter and colleagues (2010). Gunter and colleagues conclude that findings in this area have been highly inconsistent, with the use of

different types of samples and many different measures of AB and CU making interpreting results difficult at best. It should also be noted that the cumulative 5-HT index did not appear to correlate with any outcome of interest. The cumulative model makes intuitive sense, is a way of addressing multiple related genes simultaneously, and has been recently applied to the dopamine system within an imaging genetics model (Nikolova, Ferrell, Manuck, & Hariri, 2011). However, in the present sample, the specific 5-HT genes may have non-linear or non-additive effects, with some variants (e.g., *MAOA*) affecting multiple neurotransmitter systems, and may have limited utility for predicting AB. The hypothesized cumulative effects may simply not be important in understanding AB or it may be that this model would work better with different sets of variants involved within the 5-HT or other important neurotransmitter systems.

One final point in regards to the genetic findings: we did not have measures of ancestry informative markers (AIMs) and thus could not explore for occult genetic substructure within the White or Black subsample. It is possible that many of the null or unexpected findings were driven by unobserved subgroups within these broad "White" or "Black" groups. The extent to which these two subgroups are homogenous or heterogeneous likely bears on the pattern of results. These broad self-reported racial categories may not reflect homogenous genetic groups that share similar distributions of alleles.

5.3 IMAGING GENETICS APPROACHES TO AB

When applying an imaging genetics approach to link candidate polymorphisms to amygdala reactivity to AB, we found no significant results. Thus, the major question remaining to address

is why there were no significant findings linking candidate genes to amygdala reactivity. Four issues are relevant to discuss in reference to these null findings.

First, as there few links from gene to behavior, from gene to brain, and from brain to behavior, there were few opportunities to test for indirect pathway from gene to brain to behavior. Second, the lack of findings in hypothesis 1 was compounded by a lack of gene to brain relationships. Each genetic variant used in this study had been related to amygdala reactivity in at least one other study, but in the current study few relationships between genetic variability and amygdala reactivity emerged. In many cases, it is possible that these gene-to-brain effects were muddled when computed for the whole sample because of very different correlations within the White and Black subsamples. Finding results in opposite directions between Black and White subsamples would not have been completely surprising based on prior work suggesting that race moderates the relationship between genetic variants such as 5-HTTLPR and behavioral outcomes (Gelernter, Kranzler, Coccaro, Siever, & New, 1998), central nervous system serotonin function (Williams et al., 2003), and neural reactivity to an emotional faces task (Lee & Ham, 2008). As much of the imaging genetics literature has focused on Whites (e.g., Hariri et al., 2005; Hariri et al., 2002), there is less empirical literature to guide our understanding of gene to neural reactivity associations in Blacks or other non-White groups where allele frequencies may vary from those studies in Whites. That being said, there were so few relationships between gene and neural reactivity, even within Black or White subsamples, that these relationships could not be trusted as statistically credible.

Third, the power to detect mediated or indirect effects in this sample was modest. Even using bootstrapped standard errors, samples of at least 100 individuals are needed to approach acceptable levels of power to detect small to medium sized indirect effects (MacKinnon,

Lockwood, & Williams, 2004). Given the need to partition analyses by race, the small to medium observed effects, the conservative methods used to extract neural data, and subject loss at each level of data collection, it is not surprising that we did not find relationships between variables that could have led to testing any statistically significant indirect effects. Moreover, most "imaging genetics" studies have focused only on the link from genetic variant to brain structure or function (e.g., Hariri et al., 2006; Hariri et al., 2002; Manuck et al., 2010). As the focus of the study was on AB, imaging genetics models set up to be tested from gene to brain to behavior. This three-variable indirect pathway is the ideal in imaging genetics but has actually only been tested and supported in a few studies (e.g., Fakra et al., 2009; Furmark et al., 2008). Thus, the expectation to find an indirect or mediated mechanism may have been overly optimistic based on the sample size.

Fourth, as emphasized in the introduction and by G x E research, gene to brain to behavior links are likely affected by the environment (Hyde, Bogdan, et al., 2011). Testing purely imaging genetics models without an appreciation of the effect of the environment may result in null findings. In some cases correlations may exist between these variables but only at certain levels of environmental risk. Without accounting for such moderators, the current models may have missed detecting such relationships. This point is especially important in a low-income sample where youth are likely exposed to a large variety and high intensity of risk factors likely to affect neural development and behavior.

5.4 IGXE APPROACHES TO UNDERSTANDING AB

Overall, there were few significant models when testing IGxE relationships. As noted above, testing this complex relationship with variables became difficult when splitting analyses by race and when using listwise deletion across four variables. Just as in hypothesis 2, there were so few statistically significant findings in the steps leading up to testing full IGxE models, we decided not to test full IGxE models. Again, based on the sparse findings across the hypotheses leading up to hypothesis 3, it should not be surprising that few moderated mediation models were supported. Only a handful of previous publication has even linked G x E to neural reactivity (Bogdan, Williamson, & Hariri, 2012; Canli et al., 2006; Ursini et al., 2011), and no studies have ever extended the effect of this interaction through to behavior. Thus, it should not be surprising that we did not find these complex relationships in a moderate-sized high-risk sample with racial heterogeneity.

5.5 LIMITATIONS

The current study was ambitious in testing a series of hypotheses built on each other and tested models of risk and resilience not yet tested in the literature. In reviewing the findings and in particular, the general lack of support for most of the hypotheses, it is important to reiterate the importance of several limitations that have been highlighted throughout the discussion. We briefly discuss overarching issues that emerged across hypotheses.

The most striking limitation of the current study is the limited power and concurrent alpha inflation, as many, sometimes hundreds of, statistical tests were computed to test each

hypothesis. Power to detect associations was limited by sample size, and specifically by the need to subdivide the sample by race for genetic analyses and by the accumulation of data loss across each measure (e.g., fMRI, molecular genetics). On the other hand, for a neuroimaging study with genetics on an ethnically diverse sample, the current sample size was actually quite large in comparison to many past neuroimaging studies. However, those studies and the current one were clearly underpowered given the complexity of the models being tested. Power analyses of moderated mediation statistical models suggest that to obtain sufficient power (β = .80), samples of at least 300-500 are needed (Preacher, Rucker, & Hayes, 2007) and this size could be a low estimate when candidate genes are used as one predictor variable. As larger scale fMRI studies are becoming more common, achieving samples of this size are becoming more feasible than they were even as recently as 5 to 10 years ago, and will be needed to model the complex relationships between biology and experience.

In terms of alpha inflation, the many statistical tests conducted would have undermined any findings in models in hypothesis 2 or 3. Thus, we did not test these full models once steps leading up to these models yielded far fewer statistically significant findings that would be expected to be identified by chance. The number of tests increased quickly in attempting to address many weaknesses of the current literature, such as not addressing multiple types of contrasts during fMRI, not examining the different contributions of different regions of the amygdala, and not examining different subgroups or measures of AB. Although each particular variable used to measure a construct was justified, the combination of multiple variables within each construct led to exponentially more analyses. This approach appears somewhat justified based on the study's preliminary and exploratory nature, but any significant findings would have needed to be replicated repeatedly before placing stock in their credibility. Overall, hypothesis 1a

appeared to have the most convincing results that do fit with some other literature and were less subject to the same scope of alpha inflation (i.e., for the whole amygdala 3 out of 8 statistical tests were significant using the faces > shapes contrast, 3 out of 32 tests were significant across all contrasts; for the LB and CM regions, 6 out of 16 statistical tests were significant using the faces > shapes contrast, 8 out of 52 tests were significant across all contrasts). However, support for hypotheses 2 and 3 was consistently weak, especially considering the percentage of significant findings that emerged in expected directions.

On a related point, because the ultimate goal of the study was to explore complex moderated mediation pathways, no hypothesis was studied very intensively. For example, hypothesis 1a could be followed-up in more detail by exploring findings within neuroimaging software (e.g., using SPM8) at lower statistical thresholds to increase comparability with data from previous studies. However, had these analyses been conducted using this alternative method, any significant findings would have been difficult to examine in hypotheses 2 and 3. For example, had a cluster been found in the amygdala that was correlated with the main effects of the task and a measure of AB, and then extracted to be used in mediation pathways, we would have risked double correlation in having already selected voxels in the brain that were biased to be related to the outcome (e.g., Vul et al., 2009).

Another previously mentioned limitation is that the study was carried out in the context of much theoretical but limited empirical work within this area of neural and genetic correlates of AB. Given that hypotheses 2 and 3 were completely novel and that little work has explored gene to brain or behavior links among Black samples or among high risk/low SES samples, it is difficult to evaluate how unexpected the current results really are. Clearly more research is needed among these populations.

5.6 CLINICAL IMPLICATIONS

It may be a bit foolhardy to discuss the clinical implications of this work based on its exploratory nature, the dearth of findings, and the overall alpha inflation. However, the study exemplifies a way forward in exploring the interaction between genetic and environmental factors and their impact on behavior via their impact on the brain. This study also exemplifies the many challenges to this approach. The ultimate goals of this work are as follows: First, by understanding subgroups within the heterogeneous group of youth and adults with AB through biological measures, we may be able to identify ways in which youth whose behavior appears to be the same may have very different etiologies. For example, if youth with CU traits do have a different and more biologically driven etiology, they may need different types of interventions or have similar interventions adapted to suit their individual needs. Dadds and colleagues have written compellingly about how basic studies demonstrating differences between AB+CU+ and AB+CU- youth on measures of neural functioning, eye gaze, emotion understanding, and emotional reactivity can inform changes in standard treatments for AB (e.g., parent management training) and inform the creation of new interventions aimed at developing empathy and emotion understanding in +CU youth (Dadds et al., 2012; Dadds, El Masry, Wimalaweera, & Guastella, 2008; Dadds et al., 2006; Dadds & Rhodes, 2008; Hawes & Dadds, 2005). As the results from this study suggest that levels of CU in this sample are *not* driving neural differences, more research is needed to establish the extent to which youth with the AB+CU+ profile may actually be different behaviorally and physiologically. Second, understanding which genes or environments or their combinations puts youth at greatest risk for AB can help inform prevention efforts (e.g., Dishion et al., 2008). With limited resources, prevention trials or community services could be targeted to those at greatest risk. Finally, models that examine the interaction

of genes and environments can help inform the scientific community and the general public that neither genes nor environments are destiny. Studies demonstrating the exacerbating effects of environmental risk could help spur more public health and policy initiatives to abate community level risks and help address iatrogenic messages that youth with CU or adults with psychopathy are untreatable and unlikely to change. Ultimately, models that are closer to the complexity of nature are likely to better inform basic and applied science.

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