Amygdala – Ventral Prefrontal Network Influences on Emotion Regulation in Adolescence and Adulthood

by

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Chapter 1. Overall Introduction

Across the centuries, the role of emotion in human behavior has been contested. In the Stoic tradition of early philosophy, emotion was regarded as a liability that interfered with rational thought (Long, 2004). Emotions have also been found to be an ally in behavioral outcomes, as they provide us with motivation to 'jump' toward opportunities, as well as to approach obstacles energetically (Lazarus, 1991). Emotions may both interfere with our performance in an important interview and keep us from riding a roller coaster with our peers, but also take the form of the excitement that motivates us to apply to that ideal position and the apprehension that keeps us away from dangerous situations. Whereas the exact role of emotions in our daily behavior has interested and puzzled psychologists as early as James (1890), research on emotion regulation, or the explicit or implicit control individuals can have over the reaction that emotions can provoke in their behavior, is a relatively new and growing field (Gross, 2013).

Emotion regulation has implications for our behavior throughout the lifespan and plays a large role in normative social behavior (Gross, 2013). In childhood, emotion regulation can, for example, be seen in its extrinsic form when a parent comforts or calms their child, or in its intrinsic form when a child covers their eyes as the cartoon's villain comes out (Eisenberg, Hofer, Sulik, & Spinrad, 2014). Meanwhile, in adolescence, emotion regulation takes a major role in socioemotional behavior, as individuals find themselves in a state of internal and external adjustment (Steinberg, 2005), and at greater risk for the onset of psychopathology (Pine, 2001). In adulthood, cognitive reappraisal, or re-evaluating one's reaction to a situation (Gross &

Thompson, 2007), is employed at a greater rate (John & Gross, 2004), possibly influencing the higher levels of subjective well-being observed in late adulthood (Carstensen, Gross, & Fung, 1997; Gross & John, 2003).

Whereas the role of emotion regulation is evident in typically developing populations, equally or more important is the effect of modulating emotions in individuals who experience difficulties in social behavior. Such is the case of individuals with autism spectrum disorder (ASD). ASD is characterized by deficits in social behavior and communication, as well as restricted/repetitive behavior and interests (American Psychiatric Association, 2013). Emotion regulation is particularly important in autism because it is implicated in the distinctive social behavior and communication abnormalities exclusive to ASD (Mazefsky et al, 2013). In contrast, individuals with mental retardation and those with obsessive-compulsive disorder share certain restricted/repetitive behavior symptoms (Richler, Huerta, Bishop, & Lord, 2010 & Lord, 2010). Investigating what is unique about this disorder might be a more informative route towards finding an etiology for ASD.

Different populations experience emotional regulation problems in very different ways. For example, adolescents exhibit increased sensitivity and response to social stimuli, which affects decision making and increases the risk for affective disorders (Steinberg & Morris, 2001), whereas individuals with ASD exhibit difficulties in appropriately interpreting and responding to facial expressions (Losh et al., 2009). Despite differences in the expression of emotional regulation problems, researchers specializing in these diverse populations have converged on the idea that perturbations in the amygdala-ventral prefrontal cortex (vPFC) network might explain the distinct symptoms underlying aberrant behavior.

The present dissertation focuses on the functional and structural connection between the amygdala and the vPFC and how this network affects emotion regulation in typically developing (TD) children, adolescents, and adults, as well as children and adolescents with ASD. Specifically, the research studies in this dissertation investigate the effect of amygdala –vPFC network structural development on amygdala reactivity to emotional faces, as well as the effect of genetic variations on functional connectivity between the amygdala and vPFC in ASD and TD individuals. In this introduction, I will cover background research on the function of the amygdala and the vPFC in these and other populations, focusing on processing and regulation in response to social stimuli. I will also discuss theoretical models on the interplay of these structures in socioemotional processing, thus providing a theoretical basis for the research conducted.

General processing of goal-related stimuli in the amygdala

The amygdala is a brain structure in the limbic system involved in many aspects of stimulus perception. In the most general sense, the role of the amygdala is to provide information regarding salience and/or relevance of a stimulus, linking perception to cognition and behavior (Adolphs, 2001, 2010; Santos, Mier, Kirsch, & Meyer-Lindenberg). Amygdala reactivity informs us how salient and relevant a stimulus may be for biological goal achievement, such as social situations and situations important for survival (Sander, Grafman, & Zalla, 2003). For example, the amygdala is involved in the interpretation of emotions (Adolphs, 2002); a task that has great implications during adolescence and for individuals with ASD. Evidence for the role of the amygdala in social and survival situations is that non-human animals with lesioned amygdalae lack the cautiousness and distrust animals show toward novel or frightening stimuli which may be a sign of amygdala involvement in unpredictability (Adolphs, 2010; Machado, Kazama, &

Bachevalier, 2009; Mason, Capitanio, Machado, Mendoza, & Amaral, 2006). Additionally, the amygdala is responsive to the reward value of stimuli as shown by a decrease in activation in response to food, once hunger is satiated (Murray, 2007). Thirdly, the amygdala is responsive to both positive and negative emotional faces (Hamann, Ely, Hoffman, & Kilts, 2002), but is particularly responsive to faces that are more unpredictable and require vigilance (Santos et al., 2011). As such, the amygdala is more responsive to fearful faces than to angry faces (Fusar-Poli et al., 2009), and is more impaired in response to fearful and surprised faces in patients with amygdala damage (Adolphs & Tranel, 2004). These studies lend support to a theory where the amygdala is constantly evaluating stimuli and is also flexible to the changes within a stimulus (Adolphs, 2010). Such abilities are thought to be important in emotion regulation strategies such as cognitive reappraisal (McRae et al., 2012). However, stimulus reappraisal would necessitate top-down regulation (Kim et al., 2011). It is here where a brain region involved in regulating amygdala activation, such as the ventral prefrontal cortex, comes into play.

The role of the ventral prefrontal cortex in emotion regulation

Social properties in a stimulus (e.g. faces, gaze, body orientation) are encoded in specific regions of the temporal visual cortex. Permanent features, such as the identity of a stimulus, are activate the fusiform gyrus whereas changing features, such as emotional expression activate the superior temporal sulcus (Adolphs, 2001). The salience and relevance of social stimuli are then further processed by the amygdala as described above, in conjunction other limbic and prefrontal structures (Adolphs, 2001). Finally, reaction to a stimulus is modulated through the mutual communication of the amygdala and areas of the vPFC, as evidenced by fear extinction and recall studies conducted on rodents (Milad & Quirk, 2012).

The interplay between the amygdala and the vPFC in emotion regulation would be further clarified by delineating the development of these structures and their connections in typical development as well as in populations exhibiting affective and developmental disorders. However, the influence of development on emotion regulation is not yet fully understood.

Amygdala-vPFC network in typical development (Theoretical Basis for Ch. 2)

Affective and regulatory brain structures undergo a relatively parallel rate of development after infancy and during childhood (Casey et al., 2005). However, these structures begin to develop at different rates during adolescence (Casey et al., 2008; Yurgelun-Todd, 2007). The influence of this drift in developmental maturation rate between the amygdala and the vPFC is subject to great discussion in emotional regulation research. Yurgelun-Todd (2007) discuss that linear increases in prefrontal development are primarily what is reflected in adolescent behavior. In contrast, Casey et al. (2008) posit that increases in risk –taking during adolescence compared to childhood and adulthood are evidence for a non-linear pattern of emotional regulation, where immature prefrontal regulation is overwhelmed by increased amygdala reactivity in emotionally salient situations.

We offer an alternative theory that would explain the data supporting these two distinct theories. Amygdala activity in response to emotional faces generally decreases in a linear fashion from childhood to adulthood (Gee et al., 2013; Swartz, Carrasco, Wiggins, Thomason, & Monk, 2014). However, since adolescents have also been shown to exhibit greater amygdala activation than children and adults in response to certain tasks (Hare et al., 2008), we suggest that specific stimuli may shift this pattern. In line with this theory, adolescents experience increases in peer interaction, which may be relatively salient at this stage, often having a role in impulsivity and risky decision-making (Casey et al., 2008). We also believe that the development of emotional

regulation mechanisms in the brain increases with prefrontal maturation. White matter pathways between the amygdala and the vPFC increase in a linear fashion from childhood to adulthood, supporting the increase in the speed of communication between the amygdala and the vPFC (Giedd et al., 1999; Lebel, 2008; Lebel and Beaulieu, 2011). A linear increase of fiber growth in the neural connection between the amygdala and the vPFC has also been found in rats (Cunningham, 2002). Additionally, emotion regulation strategies employ the amygdala-vPFC network and the activation of these structures exhibits a linear increase from childhood to adulthood when using cognitive reappraisal strategies (McRae et al., 2012). Furthermore, individuals also exhibit a linear shift from positive to increasingly negative functional connectivity between the amygdala and the vPFC, in response to emotional faces spanning from childhood to adulthood (Gee et al. 2013; Wu et al., 2016). Individuals with anxiety, an affective disorder characterized by emotion regulation inefficiency, exhibit a shift from negative to positive connectivity of these two structures during adolescence (Kujawa et al., 2016). In such a model, emotional regulation increases with prefrontal maturation, and plays a major role in the reactivity of the amygdala. However, amygdala reactivity is dependent on the external mechanisms influencing regulation. The topic of the second chapter in this dissertation is the effect of white matter development in the amygdala – vPFC network on amygdala activation in adolescents and adults. There we will cover the relationship between white matter and amygdala reactivity to emotional faces in more depth.

Amygdala-vPFC functioning in autism spectrum disorder (Theoretical Basis for Ch. 3)

The amygdala – vPFC network has also been implicated in autism spectrum disorder as a possible explanatory construct in the abnormal social functioning of individuals with this developmental disorder. Individuals with ASD display increased amygdala activation to

emotional faces (Kleinhans et al., 2009, 2010; Weng et al., 2011). Additionally, this population exhibits decreased amygdala habituation. Habituation is defined as a decrease in amygdala activation in response to repeated or prolonged exposure to a stimulus (Kleinhans et al., 2009; Swartz, Wiggins, Carrasco, Lord, & Monk, 2013), and is linked to the amygdala-vPFC network (Hare et al., 2008; Swartz et al., 2013). Moreover, studies employing diffusion tensor imaging, an imaging method designed to test the diffusion of molecules along white matter tracts, have linked the amygdala-vPFC network to ASD. Based on DTI measurements, individuals with ASD show increased mean diffusivity, interpreted as compromised white matter, in the tracts connecting the amygdala to the vPFC (Shukla, Keehn, & Müller, 2011).

Relatedly, variations in the serotonin transporter gene influence social functioning in various populations (Canli & Lesch, 2007; Pezawas et al., 2005; Hariri et al., 2005) as well as social communication in ASD (Brune et al., 2006). This has led researchers to study the influence of the serotonin transporter-linked polymorphic region variant (5-HTTLPR) on amygdala functioning in ASD (Wiggins et al., 2014;Wiggins & Monk, 2013). 5-HTTLPR is a functional polymorphism found in the promoter region of the gene that encodes the serotonin transporter, a protein that modulates serotonin function (Hu et al., 2006; Canli et al., 2007). Typically developing adults with the low expressing short (S) variant of 5-HTTLPR exhibit greater amygdala activation and greater functional connectivity between the amygdala and the vPFC during the presentation of aversive stimuli (Heinz et al., 2005), as well as increased levels of anxiety (Canli et al., 2007). The S variant is present at higher rates in individuals with ASD (Devlin et al., 2005), and at an even greater rate to individuals with ASD who are more severely impaired (Tordjman et al., 2001). Individuals with ASD and the 5-HTTLPR low-expressing genotype displayed decreased habituation to emotional faces compared to those of the high-

expressing genotype as well as typically developing individuals showing the low expressing genotype (Wiggins et al., 2014a). This interaction between diagnosis group (ASD vs. TD) and genotype (low vs. high expressing) thus has been shown to affect amygdala function. However, the role of vPFC regulation in this relationship has not been examined. For this reason, Chapter 3 of this dissertation will use fMRI connectivity and genetic analyses to examine the effects of 5-HTTLPR genotype on connectivity between the amygdala and the vPFC.

The effects of amygdala-vPFC connectivity on emotional regulation

The research chapters of this dissertation will investigate the structural and functional connection between the amygdala and the vPFC. We believe that investigating the network connecting these structures may shed light on the factors behind emotional regulation in typically developing populations as well as in ASD. Our chapters reflect a theoretical stance where the amygdala serves as a general 'informant' about the salience and relevance of stimuli for biological goal achievement (Sander et al., 2003). The amygdala is responsive to the intensity of a stimulus but is agnostic about the valence (positive or negative). The development of the amygdala-vPFC network has and will reflect a linear pattern of maturation and, consequently, of emotional regulation efficiency. However, the intensity of amygdala activation as well as genetic factors also affect emotional regulation, thus not allowing behavioral profiles to reflect the linearity of emotional regulation increases

Chapter 2 - The relationship between white matter development and amygdala activity in response to emotional faces

Introduction

Emotion regulation is a deliberate or involuntary process that influences an individual's reaction towards an emotion (Gross, 1998). Whereas decreased regulation in response to emotional faces is linked to internalizing symptoms, such as anxiety and depression symptoms, during adolescence (Gee et al., 2013; Hare et al., 2008; Swartz et al., 2014), the transition from adolescence to adulthood overall is characterized by an increase in emotion regulation. Thus, studying the neural mechanisms related to emotional regulation during this period will help to provide useful information about the protective factors behind normative emotional development. Earlier work indicates that emotion regulation difficulties are linked to the differences in maturation between the amygdala, a limbic brain structure with an important role in emotional processing, and the ventral prefrontal cortex (vPFC), an area of the frontal cortex involved the emotion regulation process (Casey et al., 2008; Hare et al., 2008). More recent theoretical work posits a more complex developmental model that urges researchers to consider the variety of systems connected to these structures, and to make use of multiple methods of data analysis in order to account for this complexity (Crone and Dahl, 2012; Pfeifer and Allen, 2012). In the present study, we used functional MRI (fMRI) and diffusion tensor imaging (DTI) to assess this network's relationship to amygdala activation in response to emotional faces showing

specific basic emotions and to examine the development of the structural connection between the amygdala and the vPFC.

To better understand the differences in emotion regulation functioning between adolescents and adults, it is useful to map structural and functional changes in the brain occurring between these developmental periods. The ability to differentiate between social and non-social stimuli develops in early childhood and does not undergo changes during adolescence (Pavlova, Krageloh-Mann, Sokolov, & Birbaumer 2001; Nelson, Leibenluft, McClure, & Pine, 2005), whereas brain structures that are responsive to the emotional significance of a stimulus, such as the amygdala, undergo structural and functional changes with the advent of puberty (Romeo, Richardson, & Sisk, 2002; Stevens, 2002). The amygdala is highly responsive to salient and relevant stimuli (Adolphs, 2010). Stimuli may be salient because of their affective properties (Anderson and Phelps, 2001), or because of their novelty (Laine, Spitler, Mosher, & Gothard, 2009). Relevant stimuli are those that have contextual or goal-dependent value, such as human faces for their social value, or threatening stimuli for their survival value (Adolphs, 2010). Adolescents display greater amygdala activation than adults in their reaction to fearful faces vs. neutral faces (Monk et al., 2003; Guyer et al., 2008). Additionally, amygdala response to emotional faces decreases from ages 9-19 in response to sad and happy faces, (Swartz et al., 2014), as well as to fearful faces in the transition from adolescence to adulthood (Gee et al., 2013). In line with these findings, adolescents display increased amygdala activation compared to adults in response to a go-no go paradigm where fearful faces were the target conditions and calm faces were non-targets (Hare et al., 2008). In summary, these findings establish a possible difference in emotional regulation between adolescence and adulthood.

Structures within the ventral prefrontal cortex (vPFC) are involved in the regulation of the amygdala (Casey, Pattwell, Glatt, & Lee, 2013; Milad & Quirk, 2012). Relevant to developmental changes, gonadal hormone receptor density is lower in the vPFC compared to the amygdala (Stevens, 2002). Whereas hormone onset may affect amygdala maturation and reactivity early on in adolescence, developmental changes of the vPFC are seen in the form of increased white matter integrity (i.e. myelination, fiber organization, or axonal packing; Beaulieu, 2002) in existing white matter networks connecting this area to other brain structures. Cunningham et al. (2002) found an increase in the density of the fibers connecting the amygdala and vPFC as rats mature into early adulthood. Relatedly, the uncinate fasciculus (UF) (the white matter tract spanning the area between prefrontal and limbic regions; Von Der Heide, Skipper, Klobusicky, & Olson, 2013) shows a pattern of increasing white matter integrity in humans from adolescence to adulthood (Lebel & Beaulieu, 2011). In sum, the protracted growth of the connection between amygdala and vPFC seems to match increasing emotion regulation increases in the transition from adolescence to adulthood.

In line with this theory, adults activate relevant regulatory regions of the vPFC to a greater extent than adolescents across multiple attention tasks (i.e. determining how afraid one is, judging nose width, passive viewing) involving emotional face stimuli (Monk et al., 2003). However, there are studies that find increased amygdala activation but no differences in amygdala-vPFC functional connectivity in adolescents compared to adults (Guyer et al., 2008). This result may point to a complex interplay between structural and functional connectivity. Therefore, it may be useful to look at the research done on functional and structural connectivity across adolescent development. In terms of functional connectivity, Gee et al. (2013) and Wu et al., (2016) posit that a switch from positive to negative amygdala-vPFC functional connectivity

may underlie amygdala reactivity changes from childhood to young adulthood (Gee et al., 2013; Wu et al., 2016). In other words, an increasingly negative correlation between the activation of the amygdala and that of the vPFC is thought to reflect increasing emotion regulation.

Gee and colleagues (2013) found that decreasing amygdala activation from childhood to adulthood is paralleled by a switch in connectivity valence happening early in adolescence, and growing stronger in the transition from adolescence to adulthood. This switch in valence is seen in response to fearful faces (Gee et al., 2013) as well as to fearful, angry and happy faces (Wu et al., 2016). In support of this theory, individuals with anxiety, a population that displays increased amygdala reactivity compared to typically developing individuals (Killgore and Yurgelun-Todd, 2005; McClure et al., 2007; Monk et al., 2008; Thomas et al., 2001), exhibit connectivity that switches from negative to positive from ages 7-25, whereas typically developing individuals in the same age range display a switch from positive to negative amygdala-vPFC connectivity (Kujawa et al., 2016). These findings highlight the functional correlates that may underlie the increase in amygdala regulation in the transition to adulthood. However, a developmental examination of the white matter pathways through which the amygdala and the vPFC communicate, could provide structural evidence to support this hypothesis.

Structural connectivity between the amygdala and vPFC has been studied using DTI, and more specifically fractional anisotropy (Barnea-Goraly et al., 2004). Fractional anisotropy (FA), is a widely used DTI measure of white matter integrity that indexes the directionality of water diffusion along white matter tracts (Thomason & Thompson, 2011), and increased FA is interpreted as greater white matter integrity. Kim and Whalen (2009) showed that increased amygdala activity in response to fearful vs. neutral faces was related to the FA of a white matter pathway within the uncinate fasciculus. Additionally, FA values extracted from this structure

were related to trait anxiety levels. Swartz et al. (2014) used fMRI and DTI to analyze the effects of white matter development on amygdala reactivity to emotional faces in a sample ranging from ages 9-19. Uncinate fasciculus FA increase was related to a decrease in amygdala activation in response to emotional faces compared to a baseline condition. Moreover, the relationship between specific emotions and FA was also studied. Sad faces and happy faces were compared to neutral faces and related to FA. Uncinate fasciculus FA negatively predicted amygdala activation in the comparison of sad vs. neutral faces and happy vs. neutral faces. Finally, activation to sad faces compared to neutral faces predicted increased internalizing symptoms in adolescence.

We posit that a linear increase in uncinate fasciculus FA contributes to improved regulation of the amygdala from childhood to adulthood. The effect of FA on emotion processing has been analyzed in the transition from childhood to adolescence, but is yet to be studied in the transition from adolescence to adulthood. The present study bridges this gap in research by studying the effect of uncinate fasciculus FA changes on amygdala activation from ages 12-25, in a normally developing, non-clinical population. To date, no other study has examined the relationship between structural and functional connectivity during the transition to adulthood. Additionally, we collected DTI data using a more advanced protocol than previous studies (64 non-linear directions compared to 15 in Swartz et al., 2014, and 32 in Kim and Whalen, 2009). We hypothesized that amygdala activation would decrease with age. We also predicted that uncinate fasciculus FA would increase from adolescence to adulthood, replicating Lebel and Beaulieu (2011). Finally, we hypothesized that lower amygdala activation would be related to uncinate fasciculus FA increase, in line with Kim and Whalen (2009) and Swartz et al. (2014). In an exploratory analysis, we will analyze how FA levels are related to amygdala activation in

response to specific positive and negative emotions (fearful, happy, and sad) compared to neutral faces.

Methods

Participants

Our final sample consisted of 29 typically developing participants (69% female) with acceptable data in both fMRI and DTI modes. Participant ages range from 12 to 24 years of age (Mean Age = 18.62, SD = 3.08). Participants were recruited from a University of Michigan research participation website or through the posting of flyers around the community. Individuals considered for the study had verbal and non-verbal IQ scores above 85 (Mean Verbal IQ = 112.97, SD =11.23, Range = 89-135; Mean Non-verbal IQ = 110.21, SD= 13.41, Range = 86-133) as measured by the Peabody Picture Vocabulary Test (PPVT; Dunn & Dunn, 1997) and Raven's Progressive Matrices (Raven, 1960) respectively. Out of the total 55 individuals who gave consent to participate in the study, four were excluded due to excessive head motion (>3 mm translation or 3° rotation) in any direction during the scan, three did not complete the fMRI and DTI data collection visit, seven were excluded because of white pixel artifacts, eight were excluded due to technical problems in the DTI scan, two were excluded due to past neurological conditions, one participant was excluded due to high depressive symptoms, and finally, one participant was removed due to scoring below our accuracy cutoff (80%) in the gender identification task performed in the scanner. The University of Michigan Medical School Institutional Review Board (IRBMED) approved all procedures performed in this study. Adult participants and parents of minors signed informed consent forms. Minors also gave assent to participate. Participant demographic and behavioral measures were obtained in a data-collection visit prior to their MRI scan. Pubertal development was measured using the Pubertal

Development Scale (Petersen et al., 1988). Verbal and non-verbal cognitive function tests were measured using the Peabody Picture Vocabulary Test (Dunn et al., 1997), the Differential Ability Scales (DAS; Elliot, Murray, & Pearson, 1990), the Stanford-Binet Intelligence Scales (Thorndike, Hagen, & Sattler, 1986), the Wechsler Intelligence Scale for Children (Wechsler, 1949), or the Ravens Progressive Matrices (Raven, 1960). Aside from MRI safety exclusion, we also assessed for mental health conditions using the Child Depression Inventory (CDI; Kovacs, 1992), Beck Depression Inventory (Beck, Steer, and Brown, 1996), Multidimensional Anxiety Scale for Children (MASC; March et al., 1997), Beck Anxiety Inventory (Steer and Beck, 1990), Child Behavior Checklist (CBCL; Achenbach, 1991), and Adult Self Report (ASR; Rescorla and Achenbach, 2004). Individuals who were above clinical cutoff scores were excluded from our study and referred to a clinician.

Behavioral Tasks

The amygdala activation task consisted of a set of 112 facial expression stimuli from the NimStim Set (Tottenham et al., 2009). Faces were presented in a pseudo-randomized order so that participants would see all four expressions (happy, sad, fearful, neutral) every four trials in a randomized order. Faces presented were model numbers 1, 3, 6, 7, 9-13, 15-21, 23, 24, 26, 27, 33, 34, 36, 38, and 39-42. Model genders were 50% male and 50% female. Each model was presented once per emotion. The breakdown of model ethnicities was as follows: 14 models were European-American, 8 models were African-American, 5 models were Asian-American, and the remaining model was Latino-American.

E-prime software (Psychological Software Tools, Pittsburgh, PA) was used to present the stimuli in the scanner and record data. As illustrated in Figure 1, every trial began with a black screen and a fixation cross presented for 500 ms. After this, faces were presented for 250 ms in

order to limit fluctuations in attention and to compare with previous tasks done in our lab (Swartz et al., 2014; Weng et al. 2011; Wiggins et al.). Participants were asked to respond to gender of the facial stimulus, answering whether the face presented was male or female on a hand-held response apparatus. Participants were given 1500 ms to respond by pressing the appropriate button on the response device, and each answer was followed by a randomized intertrial interval that ranged from 0 to 6000 ms with intervals of 2000 ms. This intertrial period was chosen as our baseline condition.

Emotion recognition accuracy was tested by a similar protocol outside the scanner. Using E-Prime, we presented participants with the same 112 stimuli as in the fMRI task, but this time in a randomized order. Participants were asked to identify the emotion seen in each trial. The task started with a black screen and a fixation cross that was presented for 500 ms. Faces were once again presented for 250 ms. Participants were then shown a screen where they were asked to identify the emotion in a multiple-choice format and at their own pace.

fMRI Data Acquisition

Functional MRI data were collected using a GE Discovery MR750 3.0 T scanner and an 8-channel head coil. A total of 300 T2* weighted blood oxygen level dependent (BOLD) images were acquired using a reverse spiral sequence (Glover & Law, 2001;TR=2000 ms, TE=30 ms, flip angle=90°, FOV=22 cm, 64° x 64 matrix, 40 contiguous axial 3 mm slices). The AC-PC line was used as reference for slice acquisition. Structural images were composed of a 2D T1 axial overlay (TR=400, TE=14, flip angle=90°, FOV=22 cm, slice thickness=3 mm, 40 slices; matrix=256 x160) acquired for anatomical localization, and a axially acquired high-resolution spoiled gradient-recalled acquisition in steady state (SPGR) image (flip angle=15°, FOV=26 cm, 1.4 mm slice thickness, 110 slices) acquired for co-registration of the functional images.

fMRI Data Analysis

Imaging data were partially preprocessed at the University of Michigan Functional MRI Laboratory using their standard processing pipeline. Magnetic field inhomogeneity distortions were removed from k-space data using field map correction during data reconstruction. K-space outliers greater than two standard deviations from the mean were removed from the raw data and were replaced with the average of the contiguous time points. Slice timing correction was performed using local sinc interpolation (Oppenheim, Schafer, & Buck, 1989) using the middle slice as a temporal reference point. Realignment and motion correction are performed with MCFLIRT in FMRIB Software Library using the 10th functional image as reference (Jenkinson, Bannister, Brady, & Smith, 2002). We used SPM8 (http://www.fil.ion.ucl.ac.uk/spm/) for subsequent data processing. Structural images are co-registered to functional images in order to convert functional images to a standard anatomical space. Images are then normalized to Montreal Neurological Image (MNI) space and smoothed using an 8 mm full width at half maximum (FWHM) Gaussian kernel.

Canonical hemodynamic response function (HRF) and HRF temporal derivative were modeled to each time emotional face stimulus presentation (Friston et al., 1997). A separate regressor was computed for each emotion, yielding 4 regressors of interest at the individual level of analysis. Incorrect responses in responding to the gender of the facial stimulus were treated as a separate regressor and excluded in this process.

Addressing Head Motion

We excluded participants exceeding 3 mm in any direction. The six motion parameters (x, y, z, roll, pitch, yaw) were included in our individual level analysis as regressors of non-interest.

DTI Data Acquisition

Diffusion tensor imaging data are collected after the fMRI scan using spin-echo EPI diffusion sequence (scan parameters: TR = 9.05 s, TE = minimum, FOV = 35 cm, 59 slices, thickness = 2.7 mm, 5 diffusion-weighted acquisitions with b = 1000 s/mm^2, and 64 non-linear directions). One non-diffusion weighted image (b = 0 s/mm^2) was collected for transforming data into MNI template space.

DTI Data Analysis

Diffusion weighted images were pre-processed and analyzed using MrDiffusion and Quench, part of the open-source mrVista package (https://white.stanford.edu/software/). This process consisted of correcting for head motion using eddy current correction and linear registration to a non-diffusion weighted image. T1 files for each subject were aligned with the anterior and posterior commissure and checked for white pixel or other forms of artifact. Individuals who displayed white pixel artifacts in 8 or more directional volumes were dropped from the analyses. FA images were processed using tract-based spatial statistics (TBSS; Smith et al., 2006) in FSL (Smith et al., 2004), realigned to FMRIB standard-space, and transformed into MNI standard space. FSL was used to create a mean FA skeleton with a threshold of .2. FA values were extracted from left and right, uncinate fasciculus tracts. We also extracted FA values from the superior and inferior longitudinal fasciculi, and the corticospinal tract, major white matter tracts chosen as controls to test whether FA development in other structures affects amygdala activity. White matter tracts were extracted using regions of interest created using the Johns Hopkins University White Matter Tractography Atlas (Mori et al., 2005) as performed by Swartz et al. (2014).

Group Analyses

Amygdala activation and age

Multiple regression analyses using SPM8 were conducted to test the contribution of age to amygdala activation. Significance level was set at $p < 0.05$ voxelwise family-wise error (FWE), small volume corrected using anatomically defined left and right amygdalae as regions of interest (ROI), as defined by the Wake Forest University PickAtlas (WFU PickAtlas; Maldjian et al., 2003). In order to test whether our conditions replicated previously found patterns of amygdala response to emotions (Swartz et al., 2014), we first tested whether age predicted amygdala activation in response to all faces (fearful, happy, sad, neutral) vs. baseline. We corrected for multiple comparisons (age predicting left amygdala activation and right amygdala activation) by setting voxelwise Bonferroni correction to $p < 0.025$. Based on these results, we conducted multiple regression analyses where age predicted amygdala activation in response to fearful vs. neutral faces, sad vs. neutral faces and happy vs. neutral faces.

FA analyses

White matter integrity development was statistically tested with FA values extracted from the uncinate fasciculus. SPSS software, version 21 was used to conduct Pearson's correlations in order to analyze the relationship between FA and age. Multiple regression analyses using SPM8 were then conducted to test the influence of FA on amygdala activation levels. We first analyzed whether uncinate fasciculus FA predicted amygdala activation when comparing all faces vs. baseline. We, again, corrected for multiple comparisons (left uncinate fasciculus FA predicting left amygdala activation and right and right uncinate fasciculus predicting right amygdala activation) by setting Bonferroni correction to $p < 0.025$. Based on these results, we conducted multiple regression analyses where right uncinate fasciculus FA predicted amygdala activation in

response to fearful vs. neutral faces, sad vs. neutral faces and happy vs. neutral faces. We first conducted an overall *F* test on our three emotions vs. neutral. This provides an omnibus test of the hypothesis concerning the influence of our three chosen emotions vs. neutral. Significant results from the omnibus tests were then followed up with *t*-tests in order to analyze whether FA positively or negatively predicted amygdala activation. Finally, we tested whether FA values extracted from control white matter tracts predicted right amygdala activation, in order to test whether the relationship between FA and amygdala activation was specific to the uncinate fasciculus.

Age and FA analyses

Multiple regression analyses were conducted to test the influence of FA on amygdala activation, when removing the variance associated to age. These analyses included amygdala activation as our dependent variable, FA as a covariate of interest, and age as our covariate of non-interest. These analyses were followed by age x FA interaction analyses in SPM.

Additional analyses

We tested whether amygdala activation was related to internalizing symptom measures. Because our sample contained both adolescents and adults, we compared z-scores from age-appropriate measures. The variables selected were internalizing symptom scores (z-scores obtained from the CBCL for minors and from the ASR for adults), as well separate analyses of anxiety scores (z-scores obtained from the MASC for minors and from the BAI for adults), and depression scores (z-scores obtained from the CDI for minors and from the BDI for adults). Significant results were tested for interactions with FA on amygdala activation using multiple regression analyses in SPM.

Results

Amygdala and age

Multiple regression analyses showed that age negatively predicts right amygdala activation in response to all faces vs. baseline, $t(27)=3.41$, $p= 0.022$, $xyz = 34, -2, -28$, corrected for multiple comparisons for right and left amygdala at a voxelwise threshold of $p < 0.025$. Age did not significantly predict left amygdala activation. For this reason, the remaining analyses were conducted only on the right amygdala. Follow-up results showed that age negatively predicts activation to sad vs. neutral faces in the right amygdala, $t(27)=3.32$, $p= 0.028$, $xyz = 24, 0, -20$ (Figure 2). However, this result did not survive Bonferroni correction for multiple comparisons at a threshold of $p < .05/3 = 0.0167$ (three comparisons - fearful vs. neutral, happy vs. neutral, sad vs. neutral). The relationship between age and activation to fearful vs. neutral faces and happy vs. neutral faces was not significant.

FA and age

A significant correlation between right uncinate fasciculus FA and participant age was observed, $r(29)=.372$, $p= 0.047$ (Figure 3).

FA and amygdala activation

Greater right uncinate fasciculus FA predicts lower amygdala activation in response to all faces compared to a baseline condition, $t(27)=3.41$, $p= 0.022$, $xyz = 34, -2, -28$ (Figure 4). In terms of FA affecting the amygdala reactivity to specific faces, omnibus F tests yielded a significant overall relationship between right uncinate fasciculus and right amygdala activation in response to fearful faces compared to neutral faces, $F(1,27) = 11.37$, $p= 0.002$, $xyz = 28\ -8\ -12$, Bonferroni corrected for multiple comparisons. F-tests also yielded a significant overall relationship between right uncinate fasciculus and right amygdala activation in response to sad

faces compared to neutral faces, $F(1,27)=16.95$, $p= 0.01$, $xyz = 30, 4, -20$. Omnibus F-tests analyzing the relationship between right uncinate fasciculus and right amygdala activation in response to happy faces vs. neutral faces were non-significant.

Follow-up t-tests showed that greater right uncinate fasciculus FA predicts lower right amygdala activation to fearful faces compared to neutral faces, $t(27)=3.37$, $p= 0.025$, $xyz = 28\ -8\ -12$ (Figure 5). Greater right uncinate fasciculus FA also predicts lower right amygdala activation to sad faces compared to neutral faces, $t(27)=4.12$, $p= 0.005$, $xyz = 30, 4, -20$ (Figure 6).

Control White Matter Tracts and Amygdala Activation

Omnibus F tests analyzing the relationship between right amygdala activation and our control white matter tracts yielded a non-significant relationship in response to fearful vs. neutral (right superior longitudinal fasciculi, $F(1,27) = 2.07$, $p= 0.239$, $xyz = 34\ -2\ -28$; right inferior longitudinal fasciculi, , $F(1,27) = 8.03$, $p= 0.136$, $xyz = 28\ -8\ -12$, and right corticospinal tract, *no suprathreshold data*) and sad vs. neutral faces (right superior longitudinal fasciculi, $F(1,27) = 5.33$, $p= 0.312$, $xyz = 30\ 4\ -20$; right inferior longitudinal fasciculi, , $F(1,27) = 6.46$, $p= 0.224$, $xyz = 30\ 4\ -20$, and right corticospinal tract, *no suprathreshold data*).

Removing variance associated with age

Multiple regression analyses in which age was entered as a covariate of non-interest remained significant for the comparison of sad vs. neutral faces, $t(26)=3.53$, $p= 0.019$, $xyz = 30, 4, -20$. However, the FA x age interaction analysis was non-significant. The relationship between FA and fearful vs. neutral faces removing the variance associated with age approached significance, $t(26)=2.93$, $p= 0.061$, $xyz = 28, -8, -12$.

Affective symptom measures, amygdala activation, and FA

Amygdala activation in response to fearful versus neutral, happy versus neutral, and sad versus neutral faces was not significantly related to CBCL and ASD standardized internalizing symptoms, MASC and BAI standardized anxiety measures, nor CD and BDI standardized depression symptoms.

Discussion

The present study examined the relationship between uncinate fasciculus FA integrity and amygdala activation to emotional faces in a sample of adolescents and young adults. There are three main findings. First, participant age predicted amygdala activation in response to faces in general, but not in the comparison of specific emotion categories. Specifically, younger subjects exhibited greater amygdala activation than older subjects. Second, FA increased with age in our cross-sectional sample of adolescents and young adults. Third, higher uncinate fasciculus FA was related to lower amygdala activation in response to both fearful and sad faces compared to neutral faces, but not to happy vs. neutral faces.

This investigation adds to the extant literature by finding that greater FA of the right uncinate fasciculus predicts lower amygdala activation, and that greater FA predicts less amygdala activation. This finding supports and extends work by Kim and Whalen (2009), who found that amygdala predicts FA in a subsection of the uncinate fasciculus in a sample ranging from ages 18-25. The specific contrasts were fearful vs. neutral and sad vs. neutral. Furthermore, our work is consistent with longitudinal results from Lebel and Beaulieu (2011), showing that uncinate fasciculus FA increases with age. This result had previously been replicated by Swartz et al. (2014) in a cross-sectional sample ranging from ages 9 to 19, and is here found in a cross-sectional sample ranging from ages 12-25. The present study also finds that FA development in

other major white matter tracts does not predict amygdala activation, extending results seen in Swartz et al. (2014) to an older sample.

Our sample of typically developing individuals did not show significant effects of FA on internalizing symptoms, anxiety, or depression. Nevertheless, the finding of a relationship between increased FA and decreased amygdala activation to specific categories of facial expressions, suggests that white matter integrity may play a role in increased emotion regulation with age. We posit that an increase in white matter integrity (i.e. myelination, fiber density) would speed up the regulatory communication occurring between the vPFC and the amygdala, ultimately increasing the regulatory efficiency. Indeed, a decreasing pattern of amygdala activation from childhood to adulthood has been observed by certain studies (Gee et al., 2013; Swartz et al., 2014). However, the pattern of amygdala regulation across development measured in response to an emotional stimulus may be altered by certain emotions or scenarios. Evidence for this are studies adolescents display increased amygdala activation in adolescents compared to children and adults (Hare et al., 2008). The laterality of our results may also point to the specific neural effect of certain emotions, as happy conditions (a condition that did not show a significant relationship with FA) produce more left-sided activations, possibly as a result of the social approach behavior that is present in most happy situations, while withdrawal behavior (such as fearful faces and in some cases sad faces) activates right sided activation (Davidson, Ekman, Saron, Senulis, and Friesen, 1990).

To frame this developmentally and in the context of extant research, we offer the following theoretical proposition of emotional regulation. In parallel with the early development of the amygdala (Casey et al., 2008), decreased acceptance of extrinsic emotional regulation from parents (Gross, 2013), and in the context of increased peer interaction and sensation

seeking (Steinberg, 2008), the need for amygdala regulation increases. This need for regulation is highlighted by research showing that increased amygdala activation to specific categories of emotional expressions is linked to internalizing symptoms (Hare et al., 2008; Kim & Whalen, 2009; Swartz et al., 2014). Frequently used connections increase in myelination, while those that are less frequently used are pruned. The process of white matter development is relatively protracted (Nelson et al., 2005). As the need for regulation increases, white matter integrity increases with the use of the amygdala - vPFC network. Evidence for this comes from white matter increases with tool-use in primates (Quallo et al., 2009) and language learning in human work by Lovden et al. (2010). Additionally, Gee et al. (2013) and Wu et al. (2016) find that in late childhood, functional connectivity valence is positive. Myelination increases the speed of communication, which, we speculate, might relate to a switch in connectivity valence from a positive to an increasingly negative relationship. In line with this theory, individuals with anxiety disorders display lower uncinate fasciculus FA compared to controls (Phan et al., 2009; Tromp et al., 2012), and switch in amygdala-vPFC connectivity valence opposite to that seen in typically developing individuals (Kujawa et al., 2016). As individuals reach adulthood, there is an established decrease in affective disorder onset (Pine, 2001; Steinberg, 2005), paralleled by a plateau in uncinate fasciculus development (Lebel and Beaulieu, 2011). Although not taking into account other cortical emotion regulatory mechanisms, additional brain structures involved in emotional processing, as well as the influence of neurotransmitters, we speculate that uncinate fasciculus FA increase is triggered by amygdala development and activity and also, that its development may contribute to decreases in amygdala activity from adolescence to adulthood.

Whereas we find that FA is related to amygdala reactivity in response to fearful and sad faces, we do not find this relationship in response to happy faces. This may be related to the

salience or intensity of the emotions, and not their valence. The amygdala is responsive to both positive and negative stimuli (Hamann, 2002), and whereas the relationship between vPFC and amygdala activation has been reliably found in response to negative emotions (Hare et al., 2008; Monk et al., 2003; Kim et al., 2009), the relationship between FA and amygdala activation has also been linked to happy faces in the transition from childhood to adolescence (Swartz et al., 2014). We speculate that the specific effect of each emotion on amygdala activation may have an effect on how efficient the speed of communication between amygdala and vPFC may be for emotion regulation. For example, fearful faces have been shown to non-consciously activate the amygdala with more intensity than happy faces (Whalen et al., 1998). We speculate that negative faces require more emotion regulation and thus may show the effect of FA change on amygdala activation to a greater extent. Future studies would benefit from obtaining participant self-reported intensity ratings, as was done by Kim and Whalen (2009), in their comparison of fearful vs. neutral faces.

Additionally, the hypothesized effect of age on activation to specific emotional expressions did not survive correction for multiple comparisons, and we only see the effect of age on activation in response to an all faces vs. baseline contrast. However, we believe that this may be due to the size of our sample. Age is a proxy for various complex developmental mechanisms and, with enough statistical power, should capture the maturation of various developmental changes, including those in white matter integrity. In fact, we do see a positive relationship between age and FA.

Age by FA interaction analyses were conducted but did not yield significant results. The relationship between these two variables on amygdala activation may be multicollinear as seen in

our analyses removing the variance associated to age, where the effects of FA on activation to fearful vs. neutral faces do not remain significant.

In future research, it would be informative to conduct the current analyses using a longitudinal sample. Following the effect of uncinate fasciculus FA on amygdala activation longitudinally would help establish the link between white matter and emotional regulation by allowing mediation analyses, which must be performed on data collected at multiple times. While conceptually we may be able to identify associations between FA, amygdala activation and age, longitudinal analyses may be able to get at causal relationships. Future research would also benefit from testing the influence of FA on vPFC activation, and on functional connectivity between the amygdala and the vPFC. Variability in the white matter integrity of this pathway is implicated in a variety of emotional disorders, both internalizing (e.g. Phan et al., 2009; Tromp et al., 2012) and externalizing (e.g. Passamonti et al., 2012). As amygdala activation (Hare et al., 2008) and FA within this tract (Kim et al., 2009) predict trait anxiety, white matter integrity may serve as a reliable structural correlate of an increased risk for affective disorders during adolescence.

Limitations of this study include the following. Due to the various reasons for data exclusion described previously, our sample size was significantly reduced from the original sample. However, fMRI and DTI data are in line studies performed across the development from childhood to adolescence (Gee et al., 2013), as well as with large longitudinal studies (Lebel and Beaulieu, 2011). It has a smaller number of participants than previous studies linking amygdala activation to DTI, but were collected using a more advanced DTI acquisition (Swartz et al., 2014, Kim et al., 2009). Additionally, this was as cross-sectional study and, therefore, it was not possible to analyze changes in FA and amygdala activity within individuals over time. However,

our conclusions converge with the established longitudinal increase of FA in the uncinate fasciculus (Lebel et al., 2011). In addition, the reduction of amygdala activity in response to emotions in the transition to adulthood has been replicated in previous studies (Hare et al., 2008; Gee et al., 2013). A remaining question for future research would be whether FA is related to the switch in connectivity valence occurring during adolescence (Gee et al., 2013; Wu et al., 2016). It would also be beneficial to study this effect in the transition into, as well as out of, adolescence.

The present study employed multi-modal methods to study the relationship between white matter development and amygdala activation. We also bridge a gap in research on emotional development from adolescence to adulthood finding a possible structural basis for a reduction in emotional reactivity that is characteristic of risk for affective disorders. Finally, we propose a model of emotional regulation, which considers structural, functional, and contextual development from childhood to adulthood.

Chapter 3 – The Influence of 5-HTTLPR Transporter Genotype on Amygdala-Ventromedial Prefrontal Cortex Connectivity in Autism Spectrum Disorder

Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental condition characterized by deficits in social interaction and communication (American Psychiatric Association, 2013). Social deficits in ASD have been linked to abnormalities in the functioning of the amygdala, a brain structure in the limbic system involved in processing salient, often emotionally charged stimuli. However, this disorder is increasingly characterized as a disorder of networks (Müller et al., 2011; Nomi and Uddin, 2015). Multiple studies have focused on the connection between the amygdala and the regulatory structures within the region known as the ventral prefrontal cortex (vPFC; Milad and Quirk, 2012; Ray and Zald, 2012). Extant research in typically developing (TD) individuals linked greater amygdala activation and greater functional connectivity between the amygdala and the vPFC to serotonin transporter linked polymorphic region (5-HTTLPR) variant expression during the presentation of aversive stimuli (Heinz et al., 2005). Similarly, amygdala habituation rates (i.e., decreased activation over time) in individuals with ASD differ based on 5-HTTLPR expression (Wiggins et al., 2014a). The current study further characterizes differences in brain function among individuals with ASD and TD individuals by examining connectivity between the amygdala and the vPFC based on 5-HTTLPR expression.

Amygdala –vPFC connection in ASD

The amygdala is implicated in emotion processing because its primary role is to link the perception of stimuli to a cognitive or behavioral reaction by ascribing emotional significance to it (Adolphs, 2010). Additionally, damage to this area impairs the ability to process and identify emotions (Adolphs et al., 1994). This structure is responsive to salient and relevant stimuli (Adolphs, 2010) of both positive and negative valence (Yang et al., 2002). A reciprocal neural connection between amygdala activity and the vPFC has been established in human and rodent research (Milad and Quirk, 2012; LeDoux, 2000). The amygdala receives sensory input then relays signals to cortical regions such as the vPFC. The vPFC simultaneously receives information from sensory cortices, hippocampus, insula, and subcortical regions in order to inform the modulation of amygdala response while also receiving signals from the amygdala regarding the salience and relevance of the stimulus for biological goals (Hariri, 2015).

Early fMRI studies focusing on the amygdala in ASD reported amygdala hypoarousal in response to emotional facial gestures (Baron-Cohen et al., 1999; Critchley et al., 2000; Pierce et al., 2001), whereas newer ASD studies found amygdala hyperarousal when limiting stimulus presentation time (Monk et al., 2010; Weng et al., 2011). This change in methods was in response to research linking lower social functioning in ASD to amygdala hyperarousal models (Nacewicz et al., 2006) and gaze fixation studies linking attention to the eyes to increased amygdala activation with shorter stimulus presentation duration (Dalton et al., 2005;Kliemann, Dziobek, Hatri, Baudewig, & Heekeren, 2012). In order to better characterize this hyperactivation of the amygdala in response emotional faces, Swartz et al. (2013) analyzed amygdala habituation patterns in ASD and controls. Swartz et al. (2013) found that TD individuals showed amygdala habituation whereas those with ASD exhibited an increase in

activation over the course of a face-processing task. Habituation in the TD sample was also related to connectivity between the amygdala and the vPFC (Brodmann's area 25). Additionally, individuals with ASD show increased mean diffusivity, interpreted as white matter compromise, in the white matter tracts connecting the amygdala to the vPFC (Shukla, Keehn, & Müller, 2011).

5-HTTLPR effects on ASD brain function

5-HTTLPR is a functional polymorphism found in the promoter region of the gene that encodes the serotonin transporter, a protein that modulates serotonin function (Hu et al., 2006; Canli & Lesch, 2007). The serotonin transporter serves to uptake serotonin from the synaptic cleft into the presynaptic neuron, terminating its effect (Nelson, 1998). The 5-HTTLPR polymorphism directly affects serotonin transporter expression and consequently serotonin function in that individuals with the short variant (S) produce less serotonin transporter mRNA and protein than individuals with the long (L) variant, thus creating increased levels of serotonin in the synaptic cleft. TD individuals who are carriers of 5-HTTLPR S alleles exhibit increased anxiety related traits (Canli et al., 2007), amygdala activation, and positive functional connectivity coupling between the amygdala and the vPFC during the presentation of aversive stimuli (Heinz et al. 2005). Past research consistently reported associations between ASD and 5-HTTLPR S and L alleles. The S allele is transmitted at higher rates to individuals with this disorder (Devlin et al., 2005), and is more highly present in individuals with ASD who are more severely impaired (Tordjman et al., 2001). However, 5-HTTLPR can be subdivided into further allelic variants (Nakamura et al., 2000). Short (S) alleles and long (L) alleles with genotypes that contain the G (i.e. S_G and L_G) variants are associated with decreased serotonin transporter expression, compared to individuals with long alleles and genotypes that contain the A variant

(i.e. L_A; Hu et al., 2006). With relation to ASD, an increase in whole-blood serotonin levels (i.e. hyperserotonemia) has been found by multiple studies to be present in 25% to 50% of individuals with this disorder (Cook and Leventhal, 1996; Veenstra-VanderWeele et al., 2002; Anderson et al., 2002). Finally, linking serotonin to the amygdala, Whitaker-Azmitia (2005) found that a serotonin agonist mimicking hyperserotonemia caused the loss of serotonin terminals in rat amygdalae. Recent studies have grouped S/S, S/L_G and L_G/L_G genotypes as low expressing genotypes of the 5-HTTLPR polymorphism (Wiggins 2014a; 2014b). Wiggins et al. (2013b) examined the influence of 5-HTTLPR on resting state connectivity in ASD and found that youth with the low expressing genotypes exhibited stronger connectivity in the default network than high expressing groups, whereas the opposite was true for the TD group. In addition, youth with low expressing genotypes show decreased amygdala habituation (i.e., more sustained activation over time) in response to emotional faces (Wiggins et al., 2014). In sum, individuals with ASD and low expressing 5-HTTLPR genotypes tend to display more severe impairments in symptoms and differences in brain function, when compared with individuals with ASD and higher expressing genotypes as well as TD individuals.

Hypothesis

Based on the prior work identifying differences in social functioning and amygdala regulation between low and higher expressing individuals with ASD, we hypothesized a diagnosis (ASD vs. TD) by 5-HTTLPR genotype (low vs. higher) interaction on amygdala-vPFC connectivity to emotional faces. In an exploratory analyses, we tested whether specific emotions elicited distinct patterns of activation between the groups.

Method

Participants

We recruited 187 participants. The final sample consisted of 44 individuals with ASD (Mean age=13.5, SD=3.26) and 65 TD individuals (Mean age = 14.7, SD = 3.73), ranging from 8 to 19 years of age. Reasons for data exclusion were: excessive head motion (>2.25 mm translation or 2.25° rotation) in any direction compared to the initial position, lack of amygdala or vPFC coverage (below 70%), scoring below our accuracy cutoff (80%) in the gender identification task performed in the scanner, absent or incomplete magnetic resonance imaging (MRI) scan due to discomfort, fMRI technical problems, or because participants did not provide a saliva sample for genotyping (See Table 3 for details). Three participants with ASD were excluded due to amygdala responses above 2.5 standard deviations from the mean. Participants considered for the study did not have metal in their bodies and other physical or neurological conditions that are contrary to the MRI safety guidelines. Preliminary analyses yielded no significant differences in verbal and non-verbal cognitive function tests between diagnosis groups (ASD and TD, low and higher expressing genotypes; see Table 3 for additional detail). These subjects' data overlap with prior studies from our research group (Swartz et al., 2013, 2014; Weng et al., 2010, 2011; Wiggins et al., 2014a, 2014b).

Individuals with ASD were diagnosed at the University of Michigan Autism and Communication Disorders Center (UMACC). Diagnoses were based on the Autism Diagnostic Observation Schedule (ADOS; Lord et al., 2000), the Autism Diagnostic Interview- Revised (ADI-R; Lord et al., 1994), and clinical expertise (Lord et al., 2006). TD individuals were recruited using flyers posted in approved posting areas around Ann Arbor, Michigan. The Institutional Review Boards of the University of Michigan Medical School oversaw and

approved the methods and procedures conducted by this study. Adult participants and parents of minors signed informed consent forms. Participants under the age of 18 also gave assent.

Participant demographic and behavioral measures were obtained in a data-collection visit prior to their MRI scan. Pubertal development was measured using the Pubertal Development Scale (Petersen et al., 1988). Verbal and non-verbal cognitive function tests were measured using the Peabody Picture Vocabulary Test (Dunn et al., 1997), the Differential Ability Scales (DAS), the Stanford-Binet Intelligence Scales, the Wechsler Intelligence Scale for Children, or the Ravens Progressive Matrices (Raven, 1960). Aside from MRI safety exclusion, we also assessed for developmental, emotional, and behavioral conditions using the Child Depression Inventory (CDI; Kovacs, 1992), Multidimensional Anxiety Scale for Children (MASC; March et al., 1997), Child Behavior Checklist (CBCL; Achenbach, 1991), and Spence Children's Anxiety Scale (Spence, 1998). Preliminary tests looking at these behavioral measures among diagnosis groups and 5-HTTLPR genotypes found no significant differences within diagnosis groups (low versus higher; Table 3).

5-HTTLPR Procedures

Genetic analyses were performed using saliva samples collected with Genotek Oragene DNA kits (DNA Genotek, Kanata, Canada). The first step in the analysis was to differentiate between S and L variants of 5-HTTLPR. For this, we used polymerase chain reaction (PCR) and agarose gel electrophoresis. To find whether the L variant presented an A or a G SNP, we used Sanger sequencing (Hu et al., 2006). Individuals with ASD and TD individuals were subdivided into low expressing and higher expressing genotype groups. Low expressing groups were composed of individuals with S/S, S/L_G and L_G/L_G genotypes. The higher expressing groups were composed of individuals with medium and high expressing variants L_A/L_A, S/L_A, and

L_A/L_G genotypes. S and L_G variants were grouped as they have been shown to drive 5-HTTLPR expression nearly equivalently (Hu et al., 2006). These groups were also chosen to address sample-size concerns that stem from subdividing by L variants into smaller groups, similar to past studies (Cicchetti et al., 2007) as well to correspond to the data from our previous studies (Wiggins et al. 2013b; Wiggins et al., 2014a). Fifteen individuals with ASD displayed low expressing genotypes and 29 displayed higher expressing genotypes. Within the TD group, 23 individuals displayed low expressing genotypes and 42 displayed higher expressing genotypes. Hardy-Weinberg Equilibrium tests were performed on 5-HTTLPR genotypes (S/S, S/L, L/L) within each group. Hardy-Weinberg equilibrium was met in the TD group, χ^2 (1, N=65) = 5.53, p=0.02). Hardy-Weinberg equilibrium was not met in the ASD group, χ^2 (1, N=44) = 0.36, p=0.55.

Behavioral Tasks

A face-processing task (Figure 1) was presented to the participants inside the scanner. Faces used were part of the NimStim Set (Tottenham et al., 2009). Faces presented were model numbers: 1, 7, 10, 12, 15, 16, 17, 20, 23, 25, 30, 34, 38, 40, and 42 of this stimulus set. Each expression was presented 15 times, each time by a different model in a randomized order, for a total of 60 faces. The expressions presented were fearful, happy, neutral, or sad faces. Half of the faces presented were male and half were female. Eight models were European-American, four were African-American, and the remaining three models were Asian-American.

Faces were presented for 250 ms to avoid group differences in attention found in studies where stimuli were presented for longer periods of time (Klin et al., 2002; Pelphrey et al., 2002). We used E-prime (Psychological Software Tools, Pittsburgh, PA) to display the stimuli and record the responses. Before every face presentation, a black screen with a white fixation cross

was presented for 500 ms. Participants were given an additional 1500 ms after every 250ms face presentation to identify the gender of the person in the stimulus via button press, making the total response interval 1750 ms. Reaction time constituted the time from stimulus onset until button press. A randomized intertrial interval (Fusar-Poli et al.) followed the response and ranged from 0 to 6000 ms with intervals of 2000 ms. The ITI constituted the implicit baseline. Univariate ANOVAs were used to test differences in reaction time and accuracy in our face-processing task among the four diagnosis-by-genotype subgroups.

After the scanning procedure, we acquired emotion recognition accuracy data by presenting participants with 120 trials showing the same expressions as in the fMRI task. Each emotion was shown 30 times. Participants were given a laptop equipped with E-Prime and were instructed to indicate what emotion they saw in every trial. Each trial consisted of the same fixation cross screen presented for 500 ms followed by the emotional stimulus for 250 ms. Participants then viewed a multiple choice screen where they were asked to indicate whether the emotion they saw was fearful, happy, neutral or sad. A repeated-measures ANOVA was used to test a genotype (low vs. high) x diagnosis (ASD vs. TD) x emotion (happy vs. neutral; sad vs. neutral; fearful vs. neutral).

fMRI Data Acquisition

Imaging data were acquired using a 3-T GE Signa scanner at the University of Michigan Functional MRI Laboratory. A reverse spiral sequence was used to acquire a total of 300 T2* weighted blood oxygen level dependent (BOLD) images (Glover and Law, 2001;TR=2000 ms, TE=30 ms, flip angle=90°, FOV=22 cm, 64° x 64 matrix, 40 contiguous axial 3 mm slices). Slices were obtained parallel to the AC-PC line. A 3D T1 axial overlay (TR=8.9, TE=1.8, flip angle=15°, FOV=26 cm, slice thickness=1.4mm, 124 slices; matrix=256 x160) acquired for

anatomical localization, and a sagitally acquired high-resolution spoiled gradient-recalled acquisition in steady state (SPGR) image (flip angle=15°, FOV=26 cm, 1.4 mm slice thickness, 110 slices) acquired for coregistration of the functional images, were used for the structural images.

fMRI Data Analysis

Imaging data were preprocessed as part of the standard processing procedure at the University of Michigan. K-space outliers greater than two standard deviations from the mean were removed from the raw data and were replaced with the average of the contiguous time points. K-space data were also reconstructed using field map correction to remove magnetic field inhomogeneity distortions. Slice timing differences were corrected for using local sinc interpolation (Oppenheim et al., 1989) with the middle slice as the temporal reference point. Finally, images were realigned and corrected for motion with MCFLIRT in FMRIB Software Library (Jenkinson et al., 2002) using the 10^{th} functional images as reference.

The SPM8 Matlab toolbox (Wellcome Department of Cognitive Neurology, London, UK; http://www.fil.ion.ucl.ac.uk) was used to further preprocess the data. Co-registration to the functional images was performed on the anatomical images. These were then smoothed using an isotropic 8 mm full width at half maximum (FWHM) Gaussian kernel. Time-courses for every voxel were low-pass filtered to reduce sources of noise (Biswal et al., 1995). Individual subject level condition effects were run through the general linear model using SPM. Conditions for each participant were modeled with the SPM canonical hemodynamic response function. A high pass filter of 128 seconds was also used to remove the influence of linear drift. Incorrect trials were considered as a separate condition and were entered as regressors of non-interest. Using SPM, we conducted a psychophysiological interaction (PPI) analysis to investigate whether or

not there were differences in connectivity in response to facial stimuli displaying the four chosen emotions (fearful, happy, sad, and neutral). We created a 6 mm sphere around a voxel located in the left amygdala (*xyz* -30, -6, -14), as the seed region to generate functional connectivity images. This sphere was chosen because of amygdala regulation interactions found at this voxelwise peak, between group (ASD vs. TD) and genotype (low vs. higher) by our previous study (Wiggins et al., 2014). The mask chosen for small volume correction as vPFC was Left Brodmann Area 25 (Left BA 25), defined structurally by Wake Forest University Pickatlas (Maldjian et al., 2003); this area was previously used in human and non-human animal research linking amygdala functioning to vPFC connectivity (Swartz et al., 2013; Fisher et al., 2009).

Group analyses were conducted using SPM8 to create a statistical model that tested whether the low expressing ASD group showed significantly greater connectivity than the higher expressing ASD group as well as both TD expression groups. The model included accuracy and total Euclidian distance of motion parameters (x, y, z, roll, pitch, yaw) as covariates. This analysis was performed for fearful compared to neutral, happy compared to neutral, as well as sad compared to neutral stimuli. After small volume correction using Left BA 25, we controlled for multiple comparisons of stimuli using a the voxelwise threshold of $p < 0.0125$. This small-volume correction searched within BA25 and applied a family-wise error correction based on the size of the left BA 25 mask (Worsley et al., 1996).

Post-hoc analyses were performed to test the effect of social interaction scores on the relationship between amygdala-vPFC connectivity. We tested each of the four groups (diagnosis x genotype) on emotion contrasts where significant differences were found. Total SRS raw scores, as well as SRS social awareness, social cognition, social motivation, and social communication subscales were used to measure social interaction. A one-way ANCOVA was

conducted to determine if the four groups differed on connectivity values, controlling for SRS scores.

Addressing Head Motion

We have excluded participants exceeding 2.25 mm in XYZ directions (2.25° in the roll, pitch, or yaw directions) as indicated by Power et al. (2015) and Satterthwaite et al. (2012). A one-way ANOVA showed no significant differences between the four ASD/TD 5-HTTLPR expression groups in total Euclidian distance between the six motion parameters. A one-way ANCOVA showed no effect of age and puberty on total Euclidian distance in head motion (see Appendix A). We included head motion in our connectivity analyses by including the X, Y, Z, the mean Euclidean distance between these three directions, roll, pitch, and yaw values as regressors of non-interest in order to remove variance associated with head motion.

Results

Behavioral Results

Participant ability to identify emotions was tested outside the scanner. There were no group differences in the accuracy to identify each of the emotions presented (Diagnosis x Genotype x Emotion; $F(1, 101) = .435$, $p = 0.76$). There were no group differences in the accuracy of gender identification for our face-processing task inside the scanner (Diagnosis x Genotype interaction; $F(1, 105) = 1.68$, $p = 0.18$). Analyses of reaction time in response to the face-processing task showed no significant differences in reaction time. However, the p value suggested a trend towards faster reaction time in the TD Low group relative to the remaining 3 groups, $F(1, 103) = 2.61$, $p = 0.06$. To better understand this trend, we conducted an analysis of whether face processing reaction time differed among expression types. Reaction times were lower in TD individuals with low expressing genotypes compared to all other groups in response

to fearful faces ($F(3, 103) = 3.16$ $p = 0.03$). There were also trends for lower reaction time to happy ($p = 0.078$) and neutral faces ($p = 0.107$) in TD individuals with low expressing genotypes. There were no significant differences among the four groups in reaction time to sad faces ($p = .257$). Our sample differed in age ($F(1, 105) = 4.73$, $p = 0.04$) and pubertal development ($F(1, 105) = 4.08$ $p = 0.01$) among the four diagnosis-by-genotype subgroups (see Table 3 for means).

Connectivity Results

A significant group-by-genotype interaction on amygdala – vPFC connectivity was observed (xyz = -4, 22, -12, $t_{102} = 3.58$, $p = 0.008$, corrected for multiple comparisons; Figure 7). Specifically, individuals with ASD and low expressing 5-HTTLPR genotypes showed significantly greater amygdala-vPFC connectivity than the other groups (ASD higher expressing group and both TD 5-HTTLPR expression groups) in the contrast of happy vs. neutral faces after controlling for multiple comparisons of emotion. Both sad vs. neutral and fearful vs. neutral contrasts did not show group by genotype connectivity interactions in Left BA 25.

Post-hoc Analyses

Post-hoc analyses were conducted in SPSS using values extracted from structural Left BA 25. Connectivity values from the happy vs. neutral contrast for the ASD low expressing group were significantly different from all other groups. A more conservative Bonferroni correction was then applied. Only the difference between individuals with ASD and low-expressing 5-HTTLPR and TD individuals with low-expressing genotypes survived this more conservative analysis, although ASD low-expressing vs. ASD high-expressing approached significance ($p = .05$). We then compared happy vs. baseline and neutral vs. baseline in order to clarify these results. Individuals with ASD and low expressing 5-HTTLPR genotypes showed

significantly greater connectivity than the ASD higher expressing, TD higher expressing, and TD lower expressing groups in response to happy faces compared to our baseline condition (*xyz* = -4, 22, -12, t_{102} = 3.22, *P*= 0.02; see Figure 8). There were no significant differences between the four ASD/TD 5-HTTLPR expression groups in the neutral vs. baseline comparison.

Weng et al. (2011) showed that individuals with ASD yielded increased amygdala activation using this same task. To rule out the possibility that the present result was observed due to amygdala reactivity differences and not connectivity between the amygdala and the vPFC, we included amygdala activation as a covariate. Connectivity continued to be significantly greater for the low expressing ASD group in response to happy versus neutral faces, *xyz* = -4, 22, -12, t_{101} = 3.24, *P*= 0.02. In separate analyses, we tested the influence of age and puberty on connectivity. Again, connectivity continued to be significantly greater for the low expressing ASD group in response to happy versus neutral faces, *xyz* = -4, 22, -12, t_{101} = 3.66, *P*= 0.06

Post-hoc analyses tested the effect of social interaction scores in each of the four groups on the relationship between amygdala-vPFC connectivity. There was a non-significant effect of total SRS raw scores, as well as SRS social awareness, social cognition, social motivation, and social communication subscales on amygdala-vPFC connectivity values in response to happy faces (see Appendix B).

Discussion

The present study examined the relationship between amygdala-vPFC functional connectivity and 5-HTTLPR genotype in ASD. We hypothesized a diagnosis (ASD vs. TD) by genotype (low vs. higher expressing serotonin transporter gene variant) interaction on amygdala-vPFC connectivity. We expected greater connectivity in individuals with ASD and low expressing 5-HTTLPR genotypes. Consistent with this hypothesis, individuals in the low

expressing ASD group showed significantly greater connectivity than the ASD higher expressing group and both TD groups in response to happy relative to neutral faces. The interaction was not observed in response to sad and fearful faces.

These findings add to the body of literature documenting that individuals with ASD and low expressing 5-HTTLPR genotypes exhibit marked differences in brain function in comparison to other groups. Using the same sample as the present study, Wiggins and colleagues (2014a) found that participants with ASD and low expressing genotypes displayed decreased amygdala habituation (i.e., more sustained activation over time) during the same face-processing task as the present study and stronger age-related increases in default network connectivity values during rest (Wiggins et al., 2013b) relative to those with ASD and high expressing genotypes as well as controls.

There are many complex steps between functioning of a gene variant and brain function. Therefore, it is currently not possible to identify the neurochemical mechanisms that affect amygdala-vPFC connectivity throughout development. Furthermore, the reason for differential effects of 5-HTTLPR genotypes between individuals who are typically developing and those with autism spectrum disorders is yet unknown. Nevertheless, one possible mechanism may be that amygdala-vPFC connections are affected in individuals with low expressing 5-HTTLPR genotypes through a decrease in serotonin transporter early in development. The serotonin transporter serves to regulate the levels of serotonin in the synaptic cleft (Nelson, 1998), and a lack of serotonin level control is associated with enduring effects on behavior (Veenstra-VanderWeele et al., 2012). Disruptions of serotonin transporter function early in central nervous system development affect adult emotional responses to novelty and stress in mice (Ansorge et al., 2004), and may affect cortical circuit organization as shown by Jitsuki et al. (2011). We

speculate that the connection between amygdala and vPFC may be additionally affected by abnormal serotonin levels in individuals with ASD and low expressing genotypes, as individuals with this variant show abnormal serotonin levels (Canli et al., 2007) and 30% of individuals with ASD exhibit hyperserotonemia (Veenstra-VanderWeele et al., 2012). Whereas it has been found that 5-HTTLPR genotype is not related to the level of total blood serotonin (Betancur et al., 2002; Anderson et al., 2002), a possible compounded effect of decreased serotonin reuptake and increased serotonin levels may affect the cortical organization of individuals with ASD and low expressing genotypes. In line with this conjecture, serotonin transporter knockout rats show reduced social play behavior (Homberg et al., 2007) and selective serotonin reuptake inhibitors (SSRIs) as well as serotonin transporter agonists ($5\text{-}HT_{1A}$) affect sociability traits in BTBR T+tf/J (BTBR) mice, a strain of mice that exhibits behavior reminiscent of ASD characteristics (Gould et al., 2011). Future studies may benefit from monitoring the effect of 5-HTTLPR genotype and serotonin levels on structural connectivity differences between individuals with ASD and TD individuals.

We interpret happy faces to show positive regard and invitation to a social encounter. We speculate that the observed gene-by-diagnosis interaction in connectivity may be specific to happy faces because the emotional regulation system in individuals with ASD and low expressing genotypes responds abnormally to social stimuli, possibly due to decreased serotonin transporter. Individuals with ASD show greater physiological arousal to faces (Joseph et al., 2008) and greater avoidance of gaze to the eyes in face stimuli, compared to TD individuals (Klin et al., 2002). However, it is this particular genotype group that experiences higher levels of social interaction deficits, as measured by the ADI-R (Brune et al., 2006).

In contrast to the current findings, Wiggins et al. (2014a) found an interaction showing decreased amygdala habituation to *sad faces* but not in happy faces, in the same group of individuals with ASD and low expressing genotypes. They suggested that not knowing the social protocol in response to individuals who are sad (i.e. whether to approach them or to leave them alone), could be particularly aversive for individuals with ASD, and underlying reduced amygdala habituation. This finding is in line with research showing decreased habituation rates in response to sad vs. happy faces (Swartz et al., 2013) in individuals with ASD. We conjecture that if the amygdala were more highly activated by sad faces than happy faces, because of the ambiguity in social approach protocol toward sad faces, the effects of an abnormally functioning emotional regulation system would not be as apparent when only focusing on amygdala activation. This would make the effects of aberrant connectivity to emotion regulation less apparent in response to happy faces. Alternatively, the gene-by-diagnosis interaction showing an increase in connectivity for happy faces may stem instead from a more efficient regulatory mechanism. If the vPFC serves to regulate amygdala activation when individuals find themselves in a positive social encounter, an increase in communication between amygdala-vPFC in response to happy faces may decrease amygdala response. This, in turn, may explain why the same individuals do not exhibit increased connectivity to sad faces nor decreased habituation in response to happy faces.

Sample size was limited in the current findings, but was comparable to previous imaging research analyzing genetic effects on socioemotional processing (Battaglia et al., 2012; Roiser et al., 2009; Thomason et al., 2010). Additionally, the subjects' subjective feeling towards the specific emotional expression categories was not tested in the present study. Such a measure could help us interpret the reason behind the differences in connectivity to specific emotions.

Our sample differed in age and pubertal development among the four diagnosis-by-genotype subgroups. To control for this, we tested whether including age and puberty as a covariate affected our group analysis. Results continued to show that the ASD group with low expressing genotypes had significantly greater amygdala-vPFC connectivity than ASD higher expressing genotypes and both TD genotype groups. Developmental transitions from childhood into adolescence have been shown to be important components in determining whether individuals with ASD display amygdala over-connectivity or under-connectivity in comparison to TD individuals (Nomi and Uddin, 2015). Analyses that consider the effects of genotype on connectivity across development will require a larger sample. Such an approach would provide a more complete understanding of neural changes occurring during this important developmental transition. Furthermore, the current analyses were only conducted in the left hemisphere. Previous data from our lab have found links between left amygdala- left BA25 connectivity and left amygdala habituation, as well as decreased left amygdala habituation in a low expressing ASD group compared to a high expressing ASD group and TD individuals. We speculate that the left amygdala may be showing more differences in response to our rapid event-related task, as it has been found that right amygdala habituates quickly to rapid face stimulus presentations, whereas the left amygdala may not (Wright et al., 2001).

Future research may wish to investigate participant interpretation of the emotions presented. Our construal of the participants' reaction in response to happy faces (i.e. an aversion to happy faces) was not tested. Future studies may add to the present results by testing the relationship between distress to social contact and happy faces in ASD using various sources. This could be assessed through skin conductance response to test for physiological arousal and eye tracking to test for avoidance of gaze to the eyes. Moreover, the 5-HTTLPR polymorphism

does not operate alone in its relationship with brain function disparities within individuals with ASD. Examining differences in connectivity based on other neurotransmitters and hormones, in addition to the 5-HTTLPR polymorphism, would give us a more informed picture of genetic influences on brain function. Finally, a diffusion tensor imaging (DTI) study would inform our understanding of structural connectivity between the amygdala and the prefrontal cortex and its relationship to 5-HTTLPR genotype. Such research may highlight the relevance of the present finding to the differences in cortical connection size and number observed between ASD and TD individuals linked to serotonin (Casanova et al., 2002; Janušonis et al., 2004).

Conclusions

The present findings are consistent with previous research showing that individuals with ASD and low expressing 5-HTTLPR genotypes display differences in brain function compared to high expressing genotypes and TD individuals. Disparities in neural functioning have been found in terms of decreased amygdala habituation (Wiggins et al., 2014a) and increased default network connectivity with age (Wiggins et al., 2013b). The present work contributes to the literature by revealing a gene-by-diagnosis interaction where individuals with ASD and low expressing 5-HTTLPR genotypes exhibit increased amygdala-vPFC connectivity. This is the first ASD study to combine genetic polymorphism analyses and functional connectivity in the context of a social task. The current findings point to imaging as useful method for understanding the interplay between neural and genetic influences on ASD.

Chapter 4: Overall Discussion

Taken together, the research presented in this dissertation focused on the role of structural and functional connectivity between the amygdala and the vPFC. These findings have implications on the role of this network in emotional regulation difficulties. The populations that we have focused on in these studies, namely typically developing adolescents and individuals with ASD, both display differences in the functioning of the amygdala – vPFC network in comparison to their control population. The findings of this dissertation support a theoretical stance in which the role of the amygdala is to process general salience of stimuli (Sander et al., 2003). Our research is also in line with theories positing that emotional reactivity and regulation reflects a linear pattern of maturation and development (Yurgelun-Todd, 2007). Finally, our data reflect the role that contextual factors and genetic expression have on the mechanisms underlying emotional regulation, which may still partially reflect work by Casey (2008) by showing that contextual changes in adolescence may make this a period of suboptimal decision making and increased risk for the onset of affective conditions. The following sections will highlight the ways in which each of the chapters supports these points.

Uncinate fasciculus FA increase with age and in relation to amygdala activation

In the examination of uncinate fasciculus FA development on amygdala activation, higher uncinate fasciculus FA was related to decreased amygdala reactivity in response to fearful faces and sad faces. Moreover, FA increased with age, supporting Lebel et al., (2008), Lebel and Beaulieu (2011), and Swartz et al. (2014). Findings in regards to sad and fearful faces reflect the

context specificity of amygdala reaction, and may possibly suggest that the amygdala responds to salience and relevance, and more specifically ambiguity as either reaction requires a previous stimulus of which the participant is unaware, whereas happy faces are a more common and normative reaction that is not necessarily seen in response to a particular stimulus. However, the present findings do not provide direct support to this idea, as we did not obtain measures of subjective reactions to the different emotions, which would be useful for future studies.

Moreover, the linearity of FA increase with age in this and past studies supports claims of linear increases in emotional regulation (Yurgelun-Todd, 2007). Children show greater amygdala activation than adolescents in response to certain tasks (Gee et al., 2013; Swartz et al., 2014), and positive connectivity switches to negative in late childhood/early adolescence, and less uncinate fasciculus FA than adolescents (Lebel & Beaulieu, 2011). However, it is still true that adolescents display the highest levels of affective disorder onset. We believe that the environment of adolescents compared to children, creates decision making difficulties where at the same time, adult extrinsic regulation may be unwelcome (Gross, 2013). Adolescence is a period of adjustment to new social situations, more independent to the structure and regulation provided by adults during childhood (Steinberg, 2005). This decrease in extrinsic regulation, along with increased sensation-seeking (Steinberg, 2008) may help explain increased risky decision making.

5-HTTLPR genotype influence on amygdala-vPFC connectivity in ASD

Individuals with ASD and low-expressing genotypes display increased amygdala-vPFC connectivity as found in Chapter 3 of this dissertation. This result is in line with research showing genetic influence on brain function related to social processing (Canli et al., 2007; Hariri et al., 2005; Pezawas et al., 2005). Reports of worse social symptoms (Brune et al., 2006)

and decreased amygdala habituation (Wiggins et al., 2014) led us to the hypothesis that the individuals exhibiting the highest apparent emotional regulation would also exhibit differences in connectivity between amygdala and vPFC, and that this difference would be in response to specific faces. However, the directional hypothesis of what differences would look like was a bit more complex. The amygdala is responsive to both positive and negative faces (Hamann et al., 2002), although negative faces may me more salient and relevant because of their survival value (Brune et al., 2006). Social behavior in ASD is much less understood. Individuals with ASD show greater avoidance in visual contact (Klin et al., 2002) and high levels of social anxiety even in high-functioning cases (Bellini, 2004). These levels of anxiety mediate amygdala response in response to emotional faces (Kleinhans et al., 2010). Happy faces, which are thought to be indicators of impending social contact, may be particularly salient and relevant for individuals with ASD, especially since the low expressing group exhibits worse social symptoms (Brune et al., 2006).

Limitations

The investigation conducted for this dissertation had limitations worth mentioning, aside from the specific limitations mentioned within the chapters. Both studies had sample size problems that may have been exacerbated with the use of multiple methods. The various reasons for exclusion may have influenced our findings. In ASD, for example, it is often those who exhibit more severe impairments that are excluded from fMRI studies because of their movement inside the scanner or their need to end participation before completion. However, it is through the use of these methods that we were able to add to the extant literature on emotion regulation in TD and ASD in relation to structural and functional connectivity, as well as genetic influences. Moreover, the developmental implications of these studies may be constrained by our cross-

sectional methods. Longitudinal measures would allow us to conduct mediation analyses in order to test the influence of FA on amygdala activation across development. Finally, imaging methods continue to be inexact in their spatial and temporal reliability. Measures with better spatial resolution would make the study of amygdala subsections possible. However, current fMRI methods allow us to conduct research on the functioning of these complex human brain networks and with replication and conservative statistical methods, we believe that reliable results may be found.

Future Directions

Emotional regulation studies on adolescence and ASD may benefit from further multi-modal investigations. A remaining question in the field of ASD study is whether structural connectivity affects amygdala habituation and activation, the way it does in TD populations. Additionally, future studies may employ eye-tracking methods, which would allow investigators to consider individual differences (from genetics, disorder, and age) in participant gaze (fixations and saccades), gaze aversion to the emotional face stimulus, as well as pupil dilation and other useful measures tracking participant implicit or explicit visual reactions. Moreover, we have assessed the role of 5-HTTLPR in our study, but as we know, this variant is one of many complex actors in the emotional regulation system. Future studies may complement the present findings by investigating the role of other genes and their networks.

All in all, emotional regulation systems have significant daily effects in the daily life of individuals of all ages, and in any behavioral and cognitive condition. The use of multi-modal imaging methods has allowed researchers to come closer to understanding the underlying mechanisms behind a process that can happen in a spectrum that ranges from explicit to implicit, and from effortful to effortless (Gross, 2013). With the use of fMRI, DTI, and genetic work, the

present dissertation contributes to the understanding of a topic that affects every interaction we may have.

Table 1

Participants' demographic characteristics (Chapter 2).

	Frequency	Percentage
Gender		
Female	20	69.0%
Male	9	31.0%
Handedness		
Right	25	86.2%
Left	4	13.8%
Race		
Asian	7	24.1%
Black or African-American	1	3.4%
White	19	65.5%
Mixed Race - Black/White	2	6.9%
Ethnicity		
Hispanic or Latino	1	3.4%
Non-Hispanic/Non-Latino	28	96.6%
Father's Education		
High School/GED	2	6.9%
Some College, no degree	4	13.8%
Associate Degree	2	6.9%
Bachelor's Degree	8	27.6%
Master's Degree	5	17.2%
Doctorate/Professional	8	27.6%
Mother's Education		
High School/GED	1	3.4%
Some College, no degree	2	6.9%
Associate Degree	6	20.7%
Bachelor's Degree	11	37.9%
Master's Degree	5	17.2%
Doctorate/Professional	4	13.8%

Note. Total number of participants = 29.

Table 2

Participant Accuracy and RT to Emotion Recognition Task (Chapter 2)

	Fear	Happy	Sad	Neutral
Accuracy M (SD)	.91 (.11)	.96 (.08)	.944 (.05)	.894 (.08)
RT M (SD)	1285 (256)	983 (171.96)	1140 (211)	1288 (341)

Note. Accuracy and RT were missing for one participant.

Table 3. Participant Characteristics (Chapter 3)

	Autism Spectrum Disorder Group									Control Group								
	Low			Higher						Low			Higher					
5-HTTLPR Genotype	**S/S**	**S/LG**	**LG/LG**	**LA/LA**	**S/LA**	**LA/LG**				**S/S**	**S/LG**	**LG/LG**	**LA/LA**	**S/LA**	**LA/LG**			
Number of Participants	10	4	1	8	20	1				20	2	1	20	21	1			
Total N		15			29						23			42				
Gender (F:M)		1:14			4:25						5:18			11:31				
Handedness[a](L:R)		3:11			4:20						4:19			3:36		**F**	**df**	**p**
fMRI task accuracy		95.2% (4.17%)			94.9% (5.00%)						96% (4.45%)			96.7% (3.23%)		1.68	1,105	0.18
fMRI task RT (ms)		799 (159)			771 (125)						801 (149)			698 (139)		2.61	1,103	0.06
ER accuracy		86.4% (7.6%)			88.1 (6.8%)						86.8% (10.1%)			87.3 (10.3%)		0.24	1,105	0.50
ER RT (ms)		1338 (367)			1307 (340)						1245 (305)			1167 (230)		2.67	1,105	0.11
Age		12.9 (2.37)			14.1 (2.24)						13.8 (3.20)			15.1 (2.08)		4.37	1,105	0.04*
Puberty		1.99 (0.93)			2.58 (1.00)						3.01 (.71)			2.41 (1.01)		4.08	1,105	0.01*
Verbal CF[b]		115 (25.3)			111 (18.5)						112 (12.4)			115 (14.0)		0.43	1,105	0.74
Non-verbal CF		109 (18.7)			104 (20.9)						104 (10.7)			99.7 (14.0)		0.21	1,101	0.21
							t	**df**	**p**							**t**	**df**	**p**
CDI		7.67 (5.18)			8.62 (6.07)		0.52	42	0.61		5.43 (3.59)			4.55 (5.39)		0.80	42	0.43
OCI-R		20.0 (16.4)			17.0 (11.1)		0.69	38	0.50		10.87 (7.9)			10.3 (8.9)		0.48	38	0.63
MASC		42.5 (21.6)			45.6 (16.2)		0.50	37	0.62		30.9 (14.3)			30.1 (16.3)		0.51	37	0.61
CBCL-Internal		63.4 (8.71)			63.4 (9.01)		0.00	38	1.00		46.2 (8.96)			46.6 (8.89)		0.13	38	0.90
CBCL-External		52.4 (8.92)			56.7 (12.0)		1.20	38	0.24		45.7 (7.06)			43.0 (8.35)		1.34	38	0.19
CBCL Total		61.4 (8.09)			64.7 (8.90)		1.17	38	0.25		44.52 (8.46)			43.55 (8.68)		1.01	38	0.32
SRS		73.9 (11.9)			77.1 (11.3)		0.86	42	0.40		44.1 (7.50)			42.5 (6.95)		0.91	42	0.37

Note.. Chart displays participant genotype, gender, and handedness distributions across both ASD and TD groups. We also report means and standard deviations (in parenthesis) for behavioral tests, as well as between diagnosis group (ASD and TD, low and higher genotypes) ANOVA results, and within diagnosis group (low versus higher) independent samples T-Test results. fMRI task accuracy refers to the accuracy of identifying the gender of the person in the stimulus presented. Puberty refers to the Pubertal Development Scale, CF refers to cognitive functioning, for which we utilized The Peabody Picture Vocabulary Test (PPVT; Verbal CF) and the Ravens Progressive Matrices (Non-verbal CF) with TD individuals. Cognitive functioning in participants with ASD was assessed using the PPVT (Verbal), as well as the Raven's Progressive Matrices, the Differential Ability Scales II – School Age, the Stanford-

Binet Intelligence Scales, the Wechsler Intelligence Scale for Children IV, or the Wechsler Abbreviated Scale of Intelligence (Non-Verbal).

[a] 9 Participants Missing Handedness, 5 Missing Accuracy, 1 Missing Puberty, 4 Missing Non-verbal CF, 1 Missing CDI, 4 Missing OCI-R, 8 Missing MASC, 6 Missing CBCL, 1 Missing SRS, 8 Missing SCQ, 2 Missing Reaction Time.

[b] CDI = Children's Depression Inventory (Kovacs, 1992), OCI-R = Obsessive Compulsive Inventory – Revised, MASC = Multidimensional Anxiety Scale for Children, CBCL = Child Behavior Checklist, CBCL Internal = Child Behavior Checklist - Internalizing Subscale, CBCL External = Child Behavior Checklist- Externalizing Subscale, SRS = Social Responsiveness Scale, SCQ = Social Communication Questionnaire – Lifetime.

*Significant diagnosis x genotype differences in age and puberty were found. Additional analyses to test the influence of these differences on our main finding were performed and reported. The non-significant trend in reaction time was also further investigated.

Table 4. Participant Racial and Ethnic Characteristics (Chapter 3)

	Frequency	Percentage
ASD Low		
White	14	93.3
Mixed Black and White	1	6.7
ASD High		
White	27	93.1
Black or African-American	2	6.9
TD Low		
White	18	78.3
Black or African-American	2	8.7
Asian	3	13
TD High		
White	32	76.2
Black or African-American	7	16.7
Mixed Asian/White	2	4.8
Mixed American Indian Alaska Native/White	1	6.7

*Figure 1***.** Emotional faces task (Chapters 1 and 2). Depiction of face-processing task displays the order and duration of each stage in the procedure. Gender identification stage was the time participants were given to indicate the gender of the person displayed in the stimulus presentation. Intertrial interval ranged from 0 to 6000 ms with intervals of 2000 ms. A total of 60 faces (15 per emotion) were presented. The stimuli used were obtained from the NimStim Set (Tottenham et al., 2009).

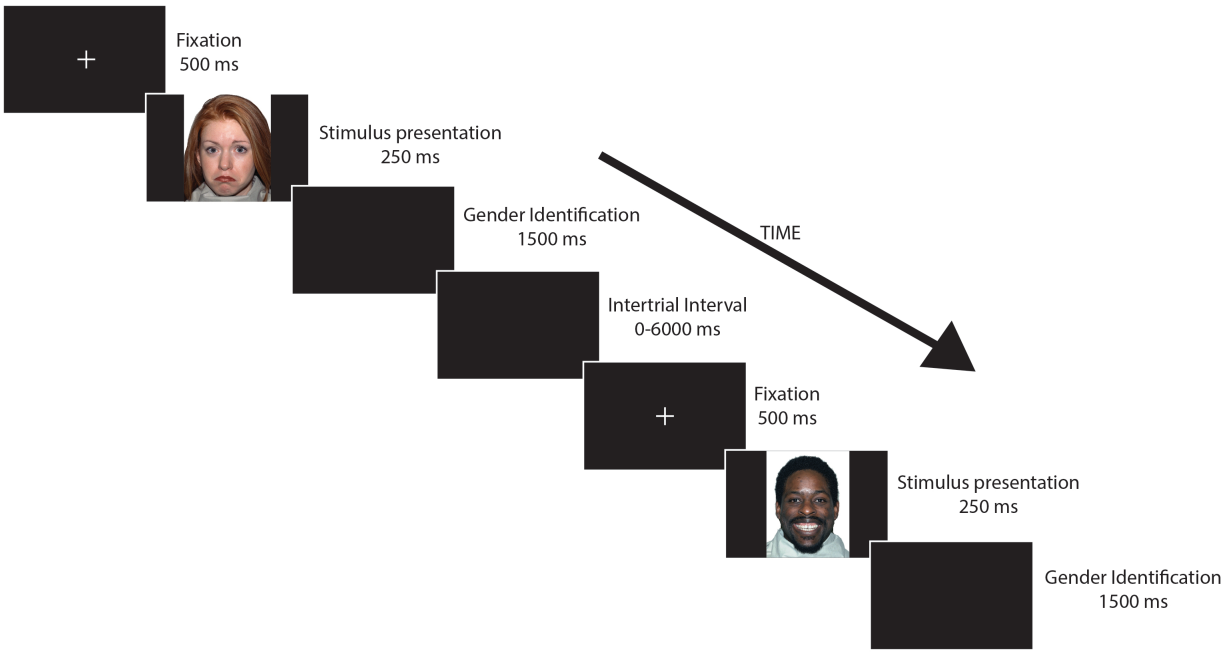
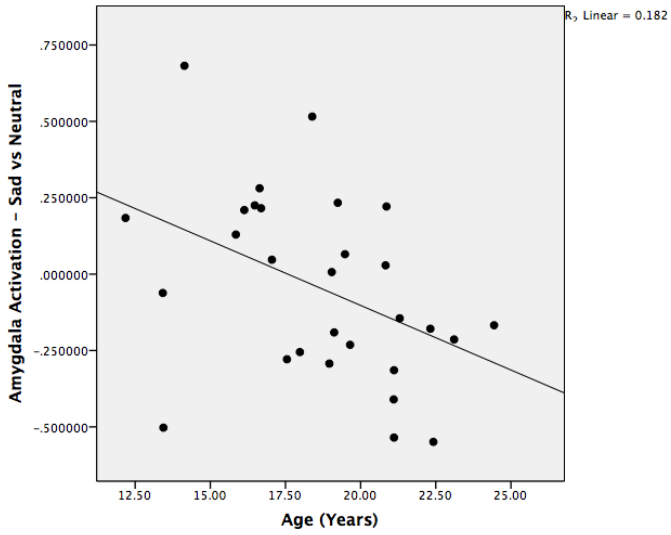


Figure 2. Relationship between amygdala activation to sad vs. neutral faces and age (Chapter 2). A. Scatterplot showing the relationship between age and amygdala activation in the sad vs. neutral contrasts. Amygdala activation values were extracted from a 6 mm sphere around peak activation voxel for visualization purposes. B. Brain images show peak at *xyz* = 24, 0, -20 in right amygdala, where age negatively predicts amygdala activation to sad v. neutral contrast at a $p < 0.05$ threshold.

A.



B.

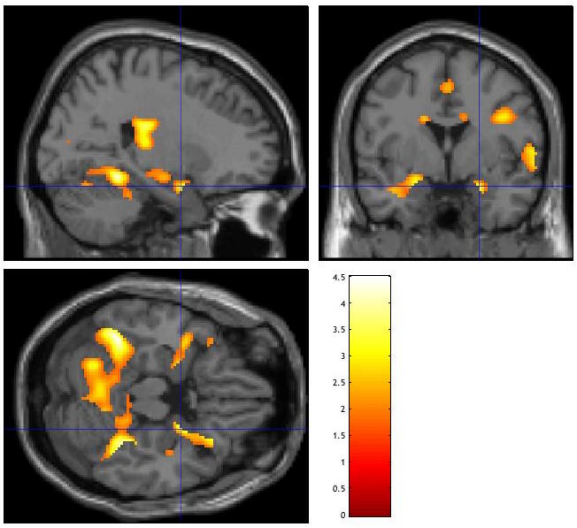


Figure 3. Relationship between uncinate fasciculus FA and age (Chapter 2). Scatterplot showing the relationship between right uncinate fasciculus FA and participant age.

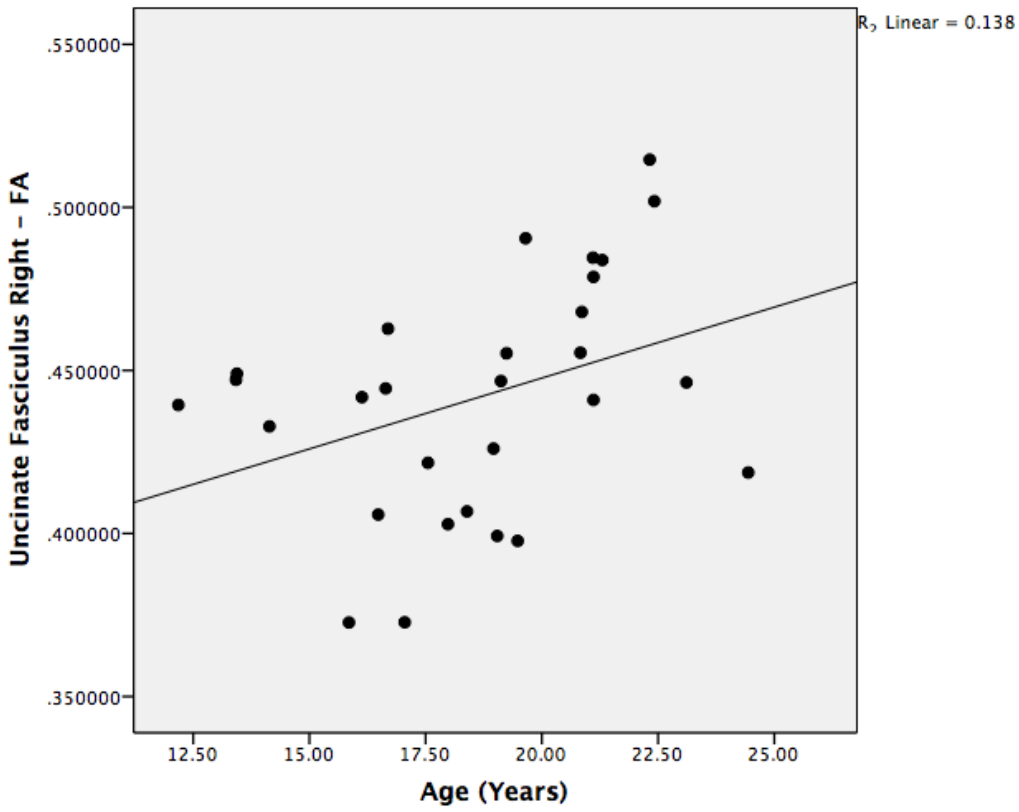
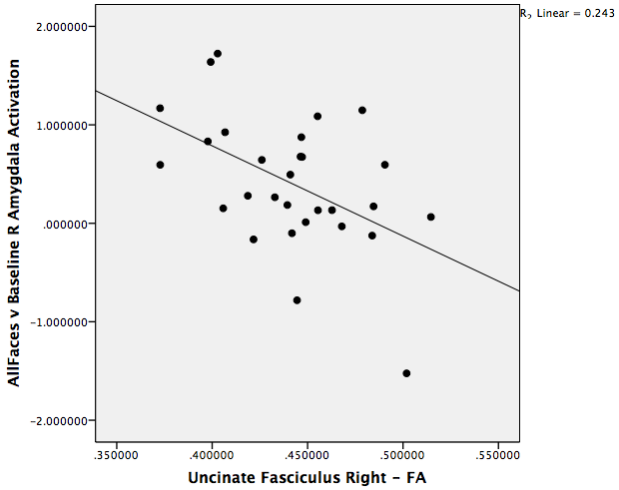


Figure 4. Relationship between amygdala activation to all faces vs. baseline and uncinate fasciculus FA (Chapter 2). A. Scatterplot showing the relationship between right uncinate fasciculus FA and right amygdala activation in response to all faces compared to baseline condition. Amygdala activation values were extracted from a 6 mm sphere around peak activation voxel for visualization purposes. B. Brain images show peak at *xyz* = 34, -2, -28 in right amygdala, where lower FA scores significantly predict positive activation at a $p < 0.05$ threshold.

A.



B.

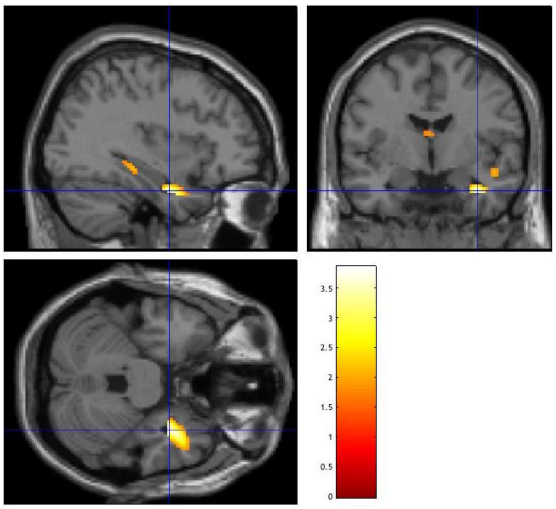
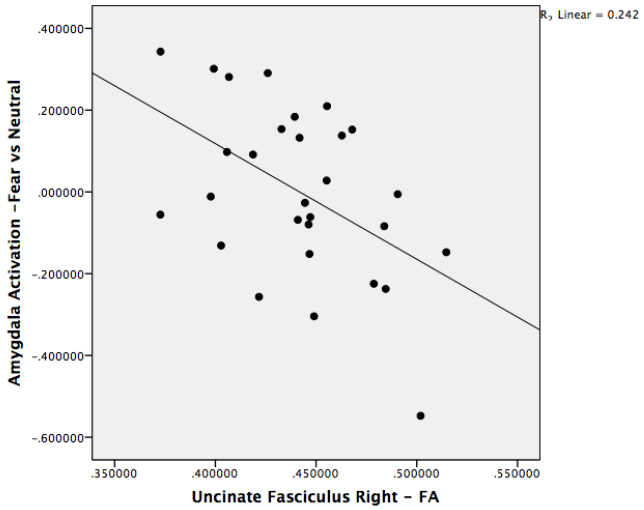


Figure 5. Relationship between amygdala activation to fearful vs. neutral faces and age (Chapter 2). A. Scatterplot showing the relationship between right uncinate fasciculus FA and right amygdala activation in response to fearful faces compared to neutral faces. Amygdala activation values were extracted from a 6 mm sphere around peak activation voxel for visualization purposes. B. Brain images show peak at *xyz* = 28, -8, -12 in right amygdala, where lower FA scores significantly predict positive activation at a $p < 0.05$ threshold.

A.



B.

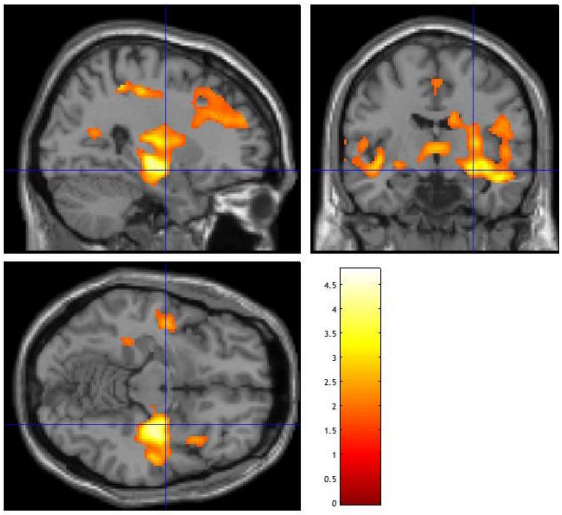
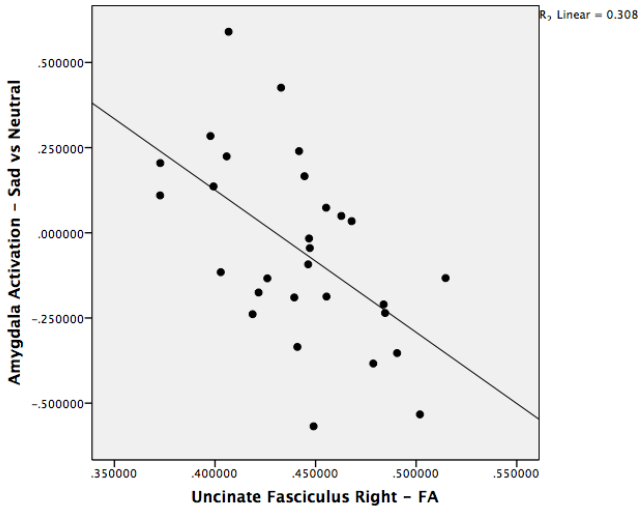


Figure 6. Relationship between amygdala activation to sad vs. neutral faces and uncinate fasciculus FA (Chapter 2). A. Scatterplot showing the relationship between right uncinate fasciculus FA and right amygdala activation in response to sad faces compared to neutral faces. Amygdala activation values were extracted from a 6 mm sphere around peak activation voxel for visualization purposes. B. Brain images show peak at *xyz* = 30, 4, -20 in right amygdala, where lower FA scores significantly predict positive activation at a $p < 0.05$ threshold.

A



B.

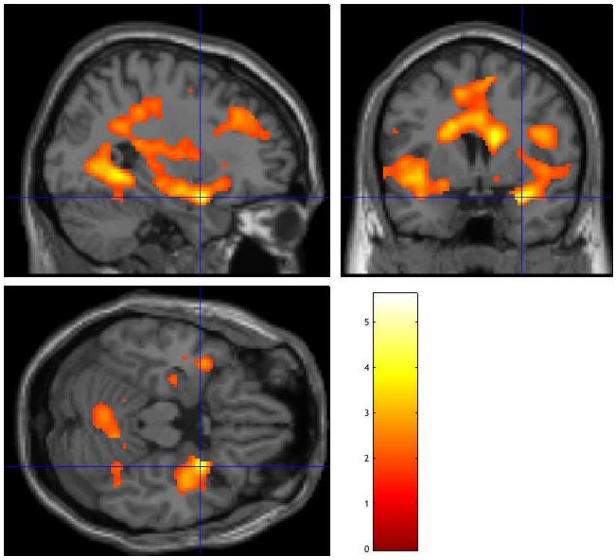


Figure 7. Differences in left amygdala-vPFC (Left BA 25) connectivity across groups contrasting happy versus neutral conditions (Chapter 3). Brain image displays area where differences were observed (peak xyz= -4, 22, -12) within Left BA 25 (small-volume corrected; threshold of .05 inclusively masked within left BA 25). Graph displays connectivity values extracted from structural left BA 25 and shows that the low expressing ASD group exhibits significantly greater connectivity than the ASD higher expressing group as well as both TD genotype groups. The difference in connectivity between the ASD low expressing group and the TD low expressing group remained significant after Bonferroni correction for multiple comparisons.

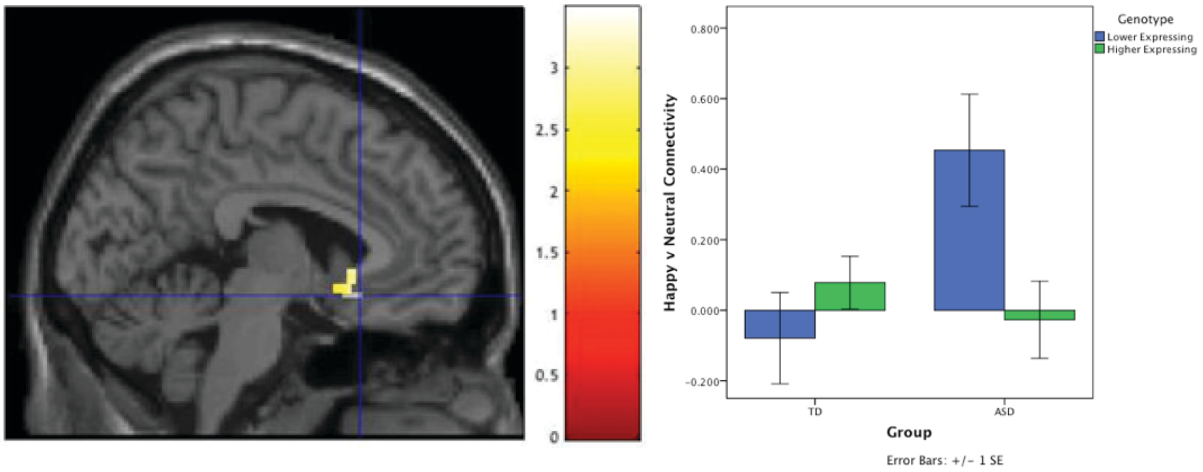
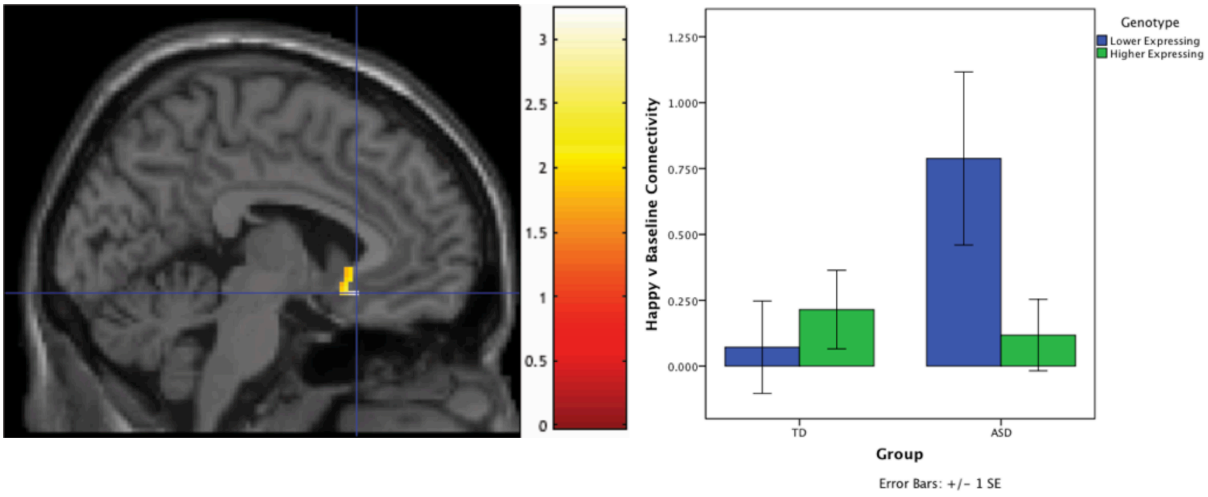


Figure 8. Differences in amygdala-vPFC (Left BA 25) connectivity across groups contrasting happy versus baseline conditions (Chapter 3). The figure shows a pattern similar to the happy versus neutral condition, where the low expressing ASD group exhibits significantly greater connectivity than all ASD higher expressing as well as both TD genotypes. Values for graph were extracted from structural left BA 25, defined structurally by the Wake Forest University Pickatlas (Maldjian et al., 2003).



Appendix A

Supplementary head motion analyses. A one-way ANOVA was conducted to test for differences in total Euclidian distance in head motion between the four diagnosis-by-genotype groups. No significant differences were found between the four groups ($F(1, 105) = 1.62$, $p = 0.19$). A one-way ANCOVA tested the influence of age on total Euclidian distance in head motion. There was no significant effect of age on total Euclidian distance in head motion ($F(1, 104) = 0.36$, $p = 0.55$). A one-way ANCOVA tested the influence of puberty on total Euclidian distance in head motion. There was no significant effect of puberty on total Euclidian distance in head motion ($F(1, 104) = 1.78$, $p = 0.185$).

Appendix B

Supplementary symptom analyses. A one-way ANCOVA was conducted to test the effect of social interaction scores on connectivity values in response to happy vs. neutral faces, controlling for SRS scores. There was no effect of raw SRS social awareness subscale scores ($F(1, 104) = 0.46$, $p = 0.23$), SRS social cognition subscale raw scores ($F(1, 104) = 3.48$, $p = 0.65$), raw SRS social motivation scores ($F(1, 104) = 0.52$, $p = 0.47$), SRS social communication subscale raw scores ($F(1, 104) = 2.93$, $p = 0.09$), or total SRS raw scores ($F(1, 104) = 3.07$, $p = 0.08$) on amygdala –vPFC connectivity.

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