Molecules to Mind: The Construction of Emotion

by

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Dr. Michael De Bellis

Dissertation submitted in partial fulfillment of
the requirements for the degree of Doctor
of Philosophy in the Department of
Psychology and Neuroscience in the Graduate School
of Duke University

2009
ABSTRACT

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Abstract

In recent years, there has been increasing scientific interest in the biological basis of emotion. By characterizing the neural and genetic basis of affective functioning, new research has the potential to contribute to our scientific understanding of both typical social development and aberrant trajectories for emotional disorders. The four studies detailed here investigated the biological substrates of affective functioning from a multiple-levels-of-analysis perspective in order to understand potential interactions of genes, the brain, personality and behavior in emotion. The first study examined the relationship between personality and the ways in which individuals look at faces. Results indicated a robust positive correlation between the personality trait of neuroticism and the amount of time spent looking at the eyes of faces, especially the eyes of fearful faces. A follow up study found that subjects high in neuroticism also fixated most on fearful faces placed within an array of objects. This effect remained strong even when controlling for negative mood state. The second study involved an experimental manipulation of activity in the face processing system of individuals with autism. The results showed that by manipulating visual scanpaths to involve increased fixation on the eye region of a face, the hypoactivation of the amygdale and fusiform gyri, an established characteristic of social brain functioning in autism, was temporarily reversed. A third study investigated the neural correlates of emotion regulation across
development using functional magnetic resonance imaging (fMRI). Results revealed increases in anterior cingulate to amygdale connectivity during episodes of regulatory demand. Magnitude of ACC activity was correlated with both age and levels of fearful temperament in children. Finally, the last study integrated the results of previous experiments and illustrated interactions among a common polymorphism in the serotonin transporter gene (5HTTLPR), brain activity, personality, and visual scanpaths. Results contribute to the growing body of literature characterizing the development of individual differences in the perception, feeling, and regulation of emotion. In addition, these findings have the potential to inform our understanding of abnormal emotional development by detailing a complex system in which genetic vulnerability produces increased attention to emotionally arousing aspects of the environment through differential brain activation.
Dedication

Para Óscar que me ha apollado siempre…
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List of Abbreviations

5HTTLPR = Serotonin Transporter Gene

ACC = Anterior Cingulate

AOIs = Areas of Interest

DLPFC = Dorsolateral Prefrontal Cortex

EEG = Electoencephalogragram

ERP = Event-Related Potential

fMRI = Functional Magnetic Resonance Imaging

FFA = Fusiform Face Area

FFG = Fusiform Gyrus

OFC = Orbitofrontal Cortex

PET = Positron Emission Tomography

PFC = Prefrontal Cortex

ROIs = Regions of Interest

VMPFC = Ventromedial Prefrontal Cortex
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1. General Introduction

Important variation in human interaction can be attributed to individual differences in emotion. A prominent topic in the psychological literature during the last 20 years, emotion has become a critical area for scientific study for several reasons. First, emotion is the primary communicator of our thoughts, feelings, and desires, making it quite important for social communication with others. From a developmental perspective, emotion emerges early as a method for human interaction. Even pre-verbal infants are capable of communicating their needs and desires through facial expressions and vocalizations of emotion. Finally, understanding emotional processes is key to our knowledge of clinical disorders. Nearly every incidence of childhood and adult psychopathology can be related to some type of emotional disturbance. In emotional disorders, such as anxiety and depression, dysregulation of fear and sadness in both adults (Mennin, Heimberg, Turk, & Fresco, 2002) and children (Cole, Zahn-Waxler, Fox, Usher, & Welsh, 1996) is often described as the mechanism for impaired functioning. Developmental disorders along the Autism spectrum have also been related to impaired emotional interaction (Loveland & Tunali, 1991). Therefore, understanding the mechanisms for human emotional functioning can serve to aid not only our scientific knowledge, but our future treatments of those with clinical needs. In following, research into human emotion has experimentally related this construct to that of personality and individual differences.
My studies detailed herein sought to further investigate the emotional brain. Specifically, my research questions are posed from a perspective of individual differences. I am interested in the mechanisms involved in emotional behavior and how both typical and aberrant development of these mechanisms can underlie variation in effective emotional functioning and emotional disorders. My studies were intended to investigate the following core aims:

**Aim 1: To replicate and extend previous findings investigating biological mechanisms for the perception, experience, and regulation of emotion**

Although it is a widely studied in cognitive neuroscience, many questions still remain as to the specific biological mechanisms underlying emotion and its development. One of the main theoretical challenges surrounding this research involves separating the concepts of emotional perception, experience, and regulation. While each of these processes can certainly occur in unison, or in relationship to the same stimulus, emotion researchers have always grappled with the need to define and separate these constructs. For example, when does the rise and fall of emotional reactivity become the process of emotion regulation? Conversely, is regulation inherent in the experience of emotion? In this set of studies, I seek to disentangle the constructs of affective perception, experience and regulation. In Study 1, I investigate the visual perception of emotional faces and how scanpaths (saccadic eye movements which direct the fovea to
fixate upon areas of interest) vary with personality. Study 2 focuses on emotional experience by investigating the brain’s activity to a fearful face as a function of the amount of eye contact. In Study 3, I investigate emotion regulation in the brain by looking at the activity to emotional faces as a function of changing levels of induced frustration.

**Aim 2: To identify typical and atypical pathways for emotional brain development**

A second aim of this research is to investigate the development of the emotional brain both from a typically and atypically developing perspective. Although the brain mechanisms for emotion have been widely studied, few investigations have focused on how these mechanisms develop. Therefore, my set of studies seeks to understand changes in the brain (e.g. activation and connectivity) across age in association with continued emotional development. These studies specifically examine the processing of emotional faces in order to extend our knowledge of the development of brain response to social stimuli. In Study 1, I investigate the visual scanning of emotional faces in a typically-developing adult population. In Study 3, emotion regulation is studied in adults and as a function of age in children ages 5-11.

In addition, this research focuses on the abnormal development of the emotional brain by specifically looking at a disorder of social and emotional functioning. Adults with autism are studied to probe the effects of aberrant pathways of emotional brain
development on adult social functioning. In Study 2, I examine deficits in brain activity to emotional faces by investigating the effects of atypical visual scanpaths.

Aim 3: To investigate the role of individual differences in emotional brain development.

Both personality and childhood temperament are hypothesized to be related to individual differences in emotional functioning. Therefore, this research is intended to investigate neural level individual differences that may underlie these remarkably stable traits. Specifically, this set of studies is intended to illuminate brain level differences in emotional perception, reactivity, and regulation that can moderate individual variation in emotional behavior. In Study 1, I investigate the visual scanning of emotional faces and how fixation on various facial features relates to adult personality. In Study 3, child temperament is examined in relation to brain mechanisms for emotion regulation. Finally, Study 4 probes adult personality as one link in a complex chain of genes, the brain, personality and behavior in emotion.

Aim 4: To understand the mechanisms for emotional development from a multilevel perspective (genes, the brain, personality, behavior)

Finally, my collected set of studies seeks to identify the mechanisms for typical and atypical emotional development from a multi-level approach. My work investigates
genes, the brain, personality, and behavior in the construction of emotion. This multilevel approach serves to link all aspects of a complex system to better understand the biological basis of emotional behavior. All of these studies investigate some aspect of this multi-layer approach. Study 4, however, seeks to integrate all three of the previous studies by integrating genetic, fMRI, personality, and eye-tracking data.
2. What is the Emotional Brain?

My studies investigate the role of the perception, experience, and regulation of emotional arousal in the brain as a possible mechanism underlying visual attention to emotional stimuli. Specifically, I seek to investigate the role of activity in the amygdala, prefrontal cortex, and anterior cingulate cortex as well functional connectivity among these brain regions in relation to individual differences in the typical and atypical development of emotion.

2.1 The Amygdala

Much of the literature on emotion and emotional modulation describes the amygdala as a central structure involved in affective processes (see Davis & Whalen, 2001 for a review). Shown to activate to negative facial expressions and scenes (Adolphs, Tranel, Damasio, & Damasio, 1995; Hariri, Tessitore, Mattay, Fera, & Weinberger, 2002; Morris, Frith, Perrett, Rowland, Young, Calder, et al., 1996), dynamic changes in facial expression (Grahm, Devinsky, & LaBar, 2007; LaBar, Crupian, Voyvodic, & McCarthy, 2003; Sato, Kubota, Okada, Murai, Yoshikawa, & Sengoku, 2002), and conditioned fear acquisition and extinction (LaBar, Gatenby, Gore, LeDoux, Phelps, 1998; Whalen, 1998), the amygdala is primarily implicated in the processing of negative emotion. Most commonly, researchers have reported associations between the amygdala and fearful stimuli, (Alolphs et al., 1995; Calder, Young, Rowland, Perrett,
indicating it may be a critical structure in perception and vigilance to environmental threat.

Evidence from brain lesion studies support the critical role of the amygdala in the recognition of fearful facial expression. For example, studies of patient SM, describe difficulty in the identification and representation of fearful facial expressions in an individual suffering from bilateral amygdala damage. Patient SM was unable to identify the emotion of fear in pictures of human faces or draw a fearful face, although other emotional facial expressions were identified at generally normal levels of performance (Adolphs, Tranel, Damasio, & Damasio, 1994). Until recently, however, possible mechanisms explaining the role of the amygdala in processing fearful faces were largely unknown. A recent eye tracking study demonstrated that Patient SM displays a lack of spontaneous fixation on the eye region of all faces, which hinders her judgment of fearful expressions (Adolphs, Gosselin, Buchanan, Tranel, Schyns, & Damasio, 2005). Indeed, the eye region is known to be the most important indication of emotion in a fearful face (Ekman, & Friesen, 1978). Further, when SM is explicitly directed to examine the eyes of facial expressions, her rate of fear recognition normalizes. Taken together, these findings suggest the importance of the amygdala in direction of eye gaze and the processing of fear related stimuli.

Similarly, fMRI studies find a strong association between the amygdala and the processing of fearful stimuli. Multiple studies suggest an increase in amygdala
activation in response to posed images of fear (Baird et al., 1999; Breiter et al., 1996; Morris et al., 1996). It seems as though the amygdala responds consistently to these stimuli, which suggest a possible threat, but are not necessarily arousing themselves. Amygdala activity is not often linked to the self-report of being “afraid” in study participants (Davis & Whalen, 2001), and subjects have been shown to display amygdala activation even when not consciously aware of having seen a fearful face (Morris, Ohman, & Dolan, 1998; Whalen, Rauch, Ectoff, McInerney, Lee, & Jenike, 1998). In sum, these findings imply the importance of the amygdala in perceiving emotional threat, which hints at the role of this brain region in affective vigilance.

The results of previous studies suggest that the regulation of visual attention may be an important mediator in the relationship between genetics, amygdala activity and personality. That is, the amygdala may be crucial in the visual perception of emotionally arousing stimuli. Variations in the regulation of visual attention may have a genetic basis and be related to personality. My studies seek to further examine the role of the amygdala and its connectivity with other brain regions in these multi-level effects.

### 2.2 The Prefrontal Cortex (PFC)

The prefrontal cortex (PFC) is located in the anterior division of the frontal lobe. Most accounts of the PFC describe this large structure as critical in processes of executive function including decision making, attention, planning, conflict monitoring, and suppression of behavior. One account of the PFC (Krawczyk, 2002) divides the PFC
into three smaller divisions. First, the orbitofrontal (OFC) and ventromedial areas (VMPFC) are highly involved in evaluation of reward and affective processing related to decision making. Second, the dorsolateral prefrontal cortex (DLPFC) is hypothesized to be the structure most related to integrating multiple sources of information to perform higher level tasks. Finally, the anterior cingulate cortex (ACC), described in greater detail below, is noted for its role in conflict monitoring and the integration of cognition and emotion.

Miller and Cohen’s (2001) Integrative Theory of PFC Functioning describes top-down role of the PFC as critical for the representation and achievement of goals. Their “bias signals” sent to other brain structures to guide neural activity along pathways that help the individual in accomplishing a task. These bias signals modulate multiple brain functions, such as sensory processing, memory retrieval, response execution, and emotional monitoring. Their theory states that the PFC manages neural inputs and connections to allow cognitive control of our actions. The OFC is, therefore, a critical structure involved in nearly all aspects of our daily behavior.

Most germane to my current studies, however, the PFC is hypothesized to be an important link in the circuitry involved in producing and regulating both positive and negative affect. Various fMRI studies have noted activation in the PFC in response to stimuli of both positive and negative valence (Gray, Braver, & Raichle, 2002; Lane, Reiman, Ahern, Schwartz, & Davidson, 1997; Lane, et al., 1997), however the PFC is
most often noted in the anticipation of emotional consequences. Lesion studies show that individuals with bilateral damage to the PFC are unable to anticipate the positive or negative consequences of their actions, although they are sensitive to immediate rewards and punishments (Bechara, Damasio, Damasio, & Anderson, 1994). The PFC seems to be important in guiding emotional behavior through motivation. It has been implicated in representing the means by which to achieve emotional goals and sends signals to other brain regions to facilitate-task appropriate responses when faced with emotionally ambiguous information (Davidson, & Irwin, 1999; Davidson, Pizzagalli, Nitschke, & Kalin, 2003).

Beyond its function in emotional anticipation, learning, and motivation, the PFC has been implied in the regulation of affect. Mega, Cummings, Salloway, & Malloy (1997) report two parallel pathways by which emotional stimuli are processed in the amygdala may interface with the PFC. First, the canonical medial circuit links the basal amygdala with the ventromedial orbitofrontal cortex, rostral insula, and anterior cingulate gyrus. Second, the lateral pathway connects the ventrolateral prefrontal cortex and anterior cingulate with the basal amygdala and inferotemporal cortex. Further studies support the segregation of PFC emotional function into dorsal and ventral streams (Yamasaki, LaBar, & McCarthy, 2002). The PFC, therefore, seems to be in good position to regulate the amygdala through its anatomical connectivity. Indeed, studies have reported an increase in activation in the PFC in correlation with a decrease in
activity to the amygdala. When subjects simply viewed fearful stimuli, such as facial expressions and scenes, they displayed peak amygdala activation. However, when they were asked to label the emotional stimulus, blood flow to the PFC increased and the response within the amygdala attenuated (Hariri, Bookheimer, & Mazziotta, 2000; Hariri, Mattay, Tessitore, Fera, & Weinberger, 2003). The task of labeling seems to activate the PFC, often implied in cognitive functioning, which, in turn modulates the response of the amygdala. The PFC, therefore, may represent the human ability to regulate our own emotion through labeling, reasoning, and rationalizing our behavior and experiences.

2.3 The Anterior Cingulate Cortex (ACC)

The anterior cingulate cortex (ACC) is hypothesized to act as a bridge between attention and emotion. The ACC is composed of spindle shaped neurons, which branch out to other parts of the brain and further help integrate cognitive and affective processes (Allman, Hakeem, Erwin, Nimchinsky, & Hof, 2001). Comprised of two sections, the ACC processes cognitive and affective stimuli separately (Davidson, et al., 2003). The dorsal “cognitive” division, interconnected with the PFC and other motor and cognitive brain areas, is most activated in cognitively demanding tasks. Similarly, the rostral-ventral “affective” division maintains connections with the amygdala and other emotion related brain areas, and is most often activated during affect-related tasks, including studies of emotional processing (Bush, Luu, & Posner, 2000). In an oddball
task with emotional distractors, for example, Yamasaki, et al (2002), found that the ACC was the only region that responded to both cognitive/attentional stimuli like the PFC and emotional stimuli like the amygdala. With connections to both cognitive and emotional brain regions, the ACC is likely involved in top-down regulatory system between motivation, attention, and emotion (Bush et al., 2000).

The ACC may be involved in the regulation of emotion through the modulation of attention. The reciprocal suppression model observes that activation in the dorsal "cognitive area" attenuates in response to intense emotional states, while activation in the rostral-ventral "affective area" increases. In following, the response decreases in the affective area and heightens in the cognitive area when emotion is regulated (Drevets & Raichle, 1998). Behaviorally, this regulatory process may occur through a shift in attention away from the emotionally arousing stimulus. Like the PFC in the emotional processing tasks described above, the ACC has been found to respond to cognitive evaluation of emotional stimuli rather than perceptual processing (Hariri, Mattay et al., 2003). ACC and PFC activation during emotional processing likely reflect those regions’ modulatory influence over amygdala activation. Indeed, the ACC has been implicated in both the normal and dysfunctional regulation of emotion (Allman et al., 2001).

2.4 Interactions Among Brain Regions

The amygdala, PFC, and ACC have been implicated in a circuit that is thought to serve to regulate emotion in the brain (Davidson et al., 2003). Specifically, amygdala
activation to emotional stimuli is attenuated by increased activity in the PFC and ACC. Hariri et al., (2000) and Hariri, Mattay et al., 2003, for example, found that perceptual evaluation of a negative emotional image resulted in activation of the amygdala. Cognitive processing of the same image, however, evoked greater activation of the PFC and ACC and attenuated amygdala activation. When simply matching a negative emotional scene or face to a target picture, participants experienced high levels of emotional reactivity (indicated by amygdala activation and a surge in skin conductance). However, when participants were asked to match a linguistic label to the emotional stimulus, emotional activity lessened. The authors hypothesize that cognitive processing forces a top-down regulation of the amygdala through the PFC and ACC, a process that may underlie behavioral emotion regulation.

Similarly, this regulatory effect has been observed in investigations of emotional attention. Most, Chun, Widders, and Zald (2005) demonstrated an "attentional blink" for stimuli that closely follow emotionally charged scenes. They presented subjects with series of pictures in which the subjects had to search for a single target. An irrelevant, emotional, negative picture preceded the target by either 2 or 8 items (200-800 ms). At the shorter lag, negative pictures spontaneously induced greater deficits in target processing than did neutral pictures. Hence attentional distraction toward emotional information induced a temporary inability to process stimuli that people were actively attempting to process. A recent fMRI study indicates that regulatory brain circuitry may
underlie this effect (Most, Chun, Johnson, & Kiehl, 2006) and that it further varies with personality. Participants' inability to detect a target after an emotionally salient image was related to greater amygdala activation and lower ACC activation. Further, participants high in harm avoidance, a personality measure associated with trait anxiety (Cloninger, Przybeck, & Svrakie, 1991), experienced greater amygdala activation and lower ACC activation when not given specific attentional set. This effect may relate to a lack of spontaneous top-down regulation in participants high in harm avoidance.

A major focus of this collection of studies concerns the role of individual differences in the emotional brain, both from a typically and atypically developing perspective. Below, personality is defined from the perspective of the five factor model and related to biological differences in emotional behavior. My work focuses on the personality trait of neuroticism, which is defined and described below.

3.1 Defining Personality

Social psychological theories emphasize that personality is composed of five broad traits, or “super factors”; extraversion, neuroticism, conscientiousness, agreeableness, and openness (McCrae & Costa, 2003). One’s personality can be conceptualized as a complex tapestry of varying levels of all five attributes. Further, each individual factor has been related to individual differences in positive and negative emotional experience as well as mechanisms for emotion regulation. Neuroticism, for example, has been linked to both trait (enduring) and state (temporary) negative affect including fear, anger, and anxiety (Costa & McCrae, 1980; Meyer & Shack, 1989). People with high levels of the neurotic personality trait, therefore, may attend more to emotionally negative aspects of the environment and respond to hostile surroundings with negative emotionality. Relating specifically to emotion regulation, individuals high in the personality trait of neuroticism are hypothesized to make fewer, and less
effective, attempts at regulating emotion and are more likely to lack the attentional resources needed for effective modulation of arousal (Gross & John, 2003; John & Gross, 2007).

Previous behavioral studies suggest that our personality shapes the way in which we absorb the emotional information in our environment. Specifically, neuroticism has been positively correlated with the processing of negative emotional information including increased attention to negative stimuli (Derryberry & Reed, 1994), better recall of negative words (Gomez, Gomez, & Cooper, 2002), and greater amygdala activation to negative visual scenes (Canli, Zhao, Desmond, Kang, Gross, & Gabrieli, 2001). Collectively, these data suggest a bias in processing and attention to negative affective states.

Development, including that of personality, is part of a multi-level system (Gottlieb, 1991), hierarchically organized into multiple levels including genetic activity, neural activity, behavior, and the environment. These levels mutually influence each other in a bidirectional system that is neither exclusively from the genetic level up nor from the behavior level down. When examining the development of personality from this viewpoint, it is believed that personality is composed of genetic variation, differences in brain activation and circuitry, variability in behavior, and dynamic changes in the environment. Genetic activity can influence the development of personality from the bottom up by affecting biological differences in brain response and
subsequent behavior. Further, our behavior can provoke changes in our environment. Similarly, the environment can galvanize developmental changes in this multi-level system by encouraging behavioral change and further influence physiological activity and genetic expression.

### 3.2 The Serotonin Transporter Gene and Personality

Personality traits are hypothesized to be related to important differences in the genetic composition of individuals. Levels of neuroticism and harm avoidance, specifically, have been linked to a variation in the serotonin transporter gene (5-HTTLPR). Lesch, et al. (1996) first reported that the 5-HTT polymorphism has a dominant-recessive type of association with the personality variable of neuroticism. Individuals with either one or two copies of the s form of the 5-HTTLPR scored higher in neuroticism than did those who were homozygous for the l variant of 5-HTTLPR. No differences were found between the l/s and s/s genotypes in this large (n = 505), Caucasian sample. The authors concluded that the 5-HTT polymorphism accounts for 3 to 4 percent of the total variation in anxiety-related personality traits, however important differences do exist in both the rates of the s allele and its relation to specific personality traits in samples of varying ethnicity (Gelernter, Kranzler, Coccaro, Siever, & New, 1998; Katsuragi et al., 1999).

Subsequent attempts to replicate these findings have proved to be inconsistent (Sen et al., 2004). Katsuragi et al. (1999) and Melke et al. (2001) reported associations
between variations in the 5-HTTLPR and anxiety like traits in smaller, Japanese and Swedish samples. Further, differences were reported between the l/s and s/s genotypes. Participants homozygous for the s form of the 5-HTTLPR scored higher on anxiety related personality traits than did the participants possessing the l/s genotype. Other studies (Deary, Battersby, Whiteman, Connor, Fowkes, & Harmar, 1999; Ebstein, Gritsenko, Nemanov, Osher, & Belmaker, 1997; Flory, Manuck, Ferrell, Dent, Peters, & Muldoon, 1999), however, have been unable to replicate these findings, reporting no relationship between variations in the serotonin transporter gene and neuroticism/anxiety measures in Scottish, Israeli, and American samples respectively. Links between biological and self-report measures can be difficult to detect without large sample sizes and consistent methods of measurement (Hariri & Weinberger, 2003). Indeed, in a meta-analysis on the topic, Sen et al., (2004) found that the s allele was related to neuroticism as measured by the NEO Five-Factor Personality Inventory (Costa & McCrae, 1991), but not by other self-reported measures of neuroticism.

3.3 The Serotonin Transporter Gene and Brain Function

The production and reuptake of serotonin is critical to brain functioning. The serotonin transporter allele has previously been related to both serotonin function and synaptic levels in humans (Garpenstrand, Annas, Ekblom, Oreland, & Fredrikson, 2001) and non-human animals (Holmes, Yang, Murphy, & Crawley, 2002). Specifically, individuals homozygous for the l allele display greater levels of 5-HTT and nearly twice
as much synaptic 5-HT as those possessing one or two copies of the s variant. Further, serotonin production and reuptake is highly important to amygdala functioning. 5-HTTLPR has been related to fear conditioning (Garpenstrand et al., 2001), which is known to be mediated by the amygdala (LaBar, et al. 1998; Phillips & LeDoux, 1992). Garpenstrand et al. (2001) found that participants possessing one or two copies of the s allele were quicker to acquire a conditioned fear response to shock than their homozygous l allele counterparts. It, therefore, seems that while links between 5-HTTLPR and personality are somewhat inconsistent, there may be a more reliable link between the serotonin transporter allele and amygdala activity.

Recent studies in the field of imaging genetics (Hariri & Weinberger, 2003) have examined the role of variation in gene polymorphism in relation to the brain, behavior, and personality. Hariri, Mattay et al. (2002) found that individuals who possess one or two copies of the s variant of the 5-HTTLPR exhibit an increased amygdala response to fearful stimuli, a finding that has been replicated (e.g. Canli, Omura, Haas, Fallgatter, Constable, & Lesch, 2005; Hariri,Munoz et al., 2003). Specifically, when asked to match a fearful emotional face to a face displaying the same fearful emotion, l/s or s/s individuals display heightened activity in the right amygdala. Therefore, while direct links between the long and short allele of 5-HTTLPR and neuroticism may be less consistent, amygdala activity to fearful stimuli may partly mediate the relationship between genes and personality.
3.4 Variation in Brain Function and Personality

Varying personality traits have been related to both brain structure and function. Canli et al., (2001) reported that diverse patterns in brain activation in response to emotional stimuli correlate highly with aspects of personality. Specifically, extraversion correlated with brain activity to positive stimuli in localized regions (e.g. amygdala, temporal lobe, cingulate gyrus), while neuroticism was correlated with response to negative stimuli in localized regions (e.g. temporal gyrus, frontal gyrus). Further studies report strong amygdala and ACC activity in response to happy faces within the trait of extraversion (Canli, 2004; Canli, Amin, Haas, Omura, & Constable et al., 2004; Canli, Sivers, Whitfield, Gotlib, & Gabrieli, 2002), while neuroticism and childhood anxiety, specifically, have been negatively correlated with right amygdala gray matter volume (De Bellis et al., 2000; Omura, Constable, & Canli, 2005). These findings suggest a hypothesis about the importance of the amygdala in anxiety related personality traits. When combined with eye-gaze behavior to emotional stimuli, further research may suggest a multilevel pathway by which genes, the brain, and behavior relate to our personality.

3.5 Defining Temperament

Temperament is commonly defined as the aspect of personality which is biologically based (Buss & Plomin, 1984; Thomas & Chess, 1977). Although there are certainly aspects of temperament which are influenced by the environment,
temperament is largely considered an innate construct. Therefore, most studies of temperament focus on infancy through early childhood years. Thomas, Chess, & Birch (1968) conducted the first longitudinal study examining the stability of temperament throughout the lifespan. Following infants throughout childhood, they identified nine characteristics of temperament closely linked to biological functioning: activity level, regularity of sleep and eating patterns, initial reaction, adaptability, intensity of emotion, mood, distractibility, persistence and attention span, and sensory sensitivity. Since the Thomas et al. (1968) study, the construct of temperament has been modified (Kagan, 1997; Rothbart, Ahadi, Hershey, & Fisher, 2001; Thomas & Chess, 1977) but still focuses on stable, biological mechanisms that influence social behavior.

Mary Rothbart and her colleagues (Rothbart, Ahadi, & Evans, 2000; Rothbart & Derryberry, 1981; Rothbart & Sheese, 2007) have related temperament to individual differences in emotional reactivity and regulation. Specifically, they define temperament as individual differences in reactivity and self-regulation assumed to have a constitutional basis (Rothbart & Derryberry, 1981), meaning that temperament relates to the enduring physiological excitability of the organism and modulation patterns of that arousal. Due to the clearly defined links between temperament and the biological bases of emotion, investigation of the brain mechanisms related to this construct have the potential to shape our understanding of the biological bases of emotion.
4. Study 1: The Effects of Personality on Visual Scanpaths

My first study contains two experiments which are intended to examine the biological and behavioral basis of emotional perception (Aim 1) and to quantify the role of individual differences as a mechanism for this process (Aim 3). In these experiments, subjects with varying personality types were shown images of emotional faces while their eye movements were tracked. I examined individual differences in the personality trait of neuroticism in relation to visual scanpath. Findings are discussed as a possible mechanism for the relationship between neuroticism and previous genetic and brain findings.

4.1 Experiment 1: Introduction

It is widely accepted that personality results from many complex interactions between genes and the environment and that it is an important aspect of who we are and how we perceive the world (Bouchard, 1994). Multiple models of personality have been put forward to account for individual differences in human social behavior (e.g., Eysenck, 1970; McCrae & Costa, 2003). However, it has been argued that specific personality traits account for only a moderate proportion of the variance in social behavior, with human interaction being largely affected by situational factors (Mischel, 1977; Vernon, 1964). Determining the ways in which personality traits interact with contextual determinants to shape social behavior remains an important empirical
enterprise (Mischel, 2004; Mischel, Shoda, & Mendoza-Denton, 2002). Here I sought to evaluate a potential mechanism whereby personality might shape how we perceive, and interact with, our social world.

A trait-congruency perspective, whereby specific personality traits predispose individuals to seek out and process information that is congruent with those characteristics (Bargh, Lombardi, & Higgins, 1988; King & Sorrentino, 1988), provides one explanation for how personality and environmental context may interact to impact social behavior. To illustrate, optimism, an established personality trait (Scheier, Carver, & Bridges, 1994), has been related to the selective processing of trait-congruent emotional information. Segerstrom (2001) found that highly optimistic people demonstrated increased attention to positive words in an emotional Stroop task and slower latency to a skin conductance response for negative words than their more pessimistic counterparts. Further, a similar effect has been found for individual differences in visual scanpaths (Isaacowitz, 2006). Optimists are more likely to divert their eye gaze away from images of skin cancer than are pessimists, underscoring a regulatory component of gaze in which visual attention is directed toward information that will help a person achieve his or her goals and away from stimuli that will not (Isaacowitz, 2005).

The relationship between personality and trait congruent attention to social stimuli has been well documented. Highly anxious individuals exhibit increased
hypervigilance to negative social stimuli (Bradley, Mogg, & Millar, 2000; Mogg & Bradley, 2002; Mogg, Millar, & Bradley, 2000). For example, during a visual probe task, participants high in trait anxiety are fastest to respond to probes presented in the same spatial location of masked threatening rather than neutral faces (Mogg & Bradley, 2002). Further eye-tracking studies confirmed that participants high in state anxiety (Bradley, Mogg, & Millar, 2000), as well as those diagnosed with generalized anxiety disorders (Mogg, Millar, & Bradley, 2000), are quicker than those low in anxiety to orient to threatening faces. Overall, this program of research indicates increased vigilance to threatening stimuli in those with anxious personalities and anxiety disorders.

My study sought to extend prior work by characterizing the relationship between individual differences in personality and an essential human social behavior: eye contact with social partners. Humans have the most prominent eyes of any species with regard to determining direction of gaze (Kobayashi & Koshima, 1997), which has been linked to our advanced and perhaps unique social cognition abilities (Tomasello, Hare, Lehmann, & Call, 2007). Typically developing adults fixate the eye region more than other facial features (Adolphs, 2006; Pelphrey, Sasson, Reznick, Paul, Goldman, & Piven, 2002; Walker-Smith, Gale, & Findlay, 1977). Further, the eye region of the face contributes greatly to our understanding of emotion in others (Adolphs, 2006; Ekman & Friesen, 1975; Emery, 2000). Fixation on the eyes is critical in the perception of emotion and the communication of our own affective state (Adolphs, 2006). However, the eye
region of the face is more important for perceiving and communicating some emotions (e.g., fear) than others (Adolphs et al., 2005). Finally, fixation upon the eyes of others is an early developing social skill. Neonates orient more to a moving face than other classes of stimuli (Goren, Sarty, & Wu, 1975; Johnson, Dziurawiec, Ellis, & Morton, 1991) and infants begin to attend preferentially to the eyes of faces during social interaction as early as seven weeks of age (Haith, Bergman, Moore, 1977).

In the present study, I used eye tracking to quantify overt visual attention to the eyes of faces. I measured the visual scanpaths of individuals of varying personality while they viewed emotional facial expressions. I focused our investigation on the specific personality trait of neuroticism, which is characterized as the tendency to experience negative affect and anxiety, and to inadequately cope with emotional distress (Costa & McCrae, 1980). In accordance with the trait congruency hypothesis (Bargh et al., 1988), I hypothesized that participants high in neuroticism would seek out the most arousing aspects of emotional faces. We predicted that individuals high in neuroticism would attend preferentially to the eyes of fearful facial expressions.

### 4.2 Experiment 1: Method

#### 4.2.1 Subjects

Thirty-three adult volunteers (20 female, 13 male; mean age = 22.35 years; range = 18-35 years) participated in this experiment. Data from three participants were not
included in the analysis due to poor equipment calibration leaving a sample of 30 subjects.

4.2.2 Stimuli

Stimuli consisted of photographs of six individuals (3 male, 3 female) posing each of seven prototypical emotions (happy, sad, fear, anger, surprise, disgust) taken from the Ekman stimulus set (Ekman & Friesen, 1975).

4.2.3 Experimental Procedure

Emotional photographs, occupying the majority of the 17 inch LCD screen, appeared for 5s with a fixation point in the center of the screen appearing for 3s between images. Participants were seated 60 cm from the computer screen and told to freely view the images. Eye movements were recorded at 50 Hz using a remote infrared eye-tracking system (1750, Tobii Technology). Prior to the eye tracking procedure, participants completed the NEO Five Factor Inventory (NEO-FFI; Costa & McCrae, 1991) to assess dimensions of personality. The NEO-FFI is a short version (60 items) of the standard NEO Personality Inventory (NEO-PI; 300 items) which is designed to measure the five main dimensions of adult personality: Neuroticism, Extraversion, Openness, Agreeableness, and Conscientiousness.
Figure 1: Illustration of Areas of Interest (AOI) of facial features. AOIs here created individually for each photograph in the stimulus set.

The location and duration of fixations were calculated from areas of interest (AOIs) drawn around the eye, nose, and mouth regions, as well as the entire face of the face image (see Figure 1). The duration of fixation for each AOI was calculated separately for each image and collapsed across emotions. To adjust for individual differences in looking time due to blinking or momentary distraction from the screen, analyses were preformed on the proportion of fixation time spent looking at each AOI within the time spent looking within the whole-face AOI. See Tables 1 and 2 for
summary statistics regarding fixations on each region of the face for each emotional expression and personality variables respectively.

Table 1: Percentages of the total time spent fixating on each region of the face for each emotional facial expression (stimulus duration = 5 s)

<table>
<thead>
<tr>
<th>Facial Expression</th>
<th>Area of Interest</th>
<th>Face</th>
<th>Eyes</th>
<th>Nose</th>
<th>Mouth</th>
<th>Non-AOI - Face</th>
</tr>
</thead>
<tbody>
<tr>
<td>Happy</td>
<td>Face</td>
<td>56%</td>
<td>25%</td>
<td>12%</td>
<td>8%</td>
<td>0%</td>
</tr>
<tr>
<td>Sad</td>
<td>Face</td>
<td>56%</td>
<td>26%</td>
<td>15%</td>
<td>4%</td>
<td>0%</td>
</tr>
<tr>
<td>Anger</td>
<td>Face</td>
<td>57%</td>
<td>24%</td>
<td>14%</td>
<td>5%</td>
<td>0%</td>
</tr>
<tr>
<td>Fear</td>
<td>Face</td>
<td>55%</td>
<td>25%</td>
<td>16%</td>
<td>5%</td>
<td>0%</td>
</tr>
<tr>
<td>Surprise</td>
<td>Face</td>
<td>55%</td>
<td>26%</td>
<td>13%</td>
<td>6%</td>
<td>0%</td>
</tr>
<tr>
<td>Disgust</td>
<td>Face</td>
<td>57%</td>
<td>22%</td>
<td>16%</td>
<td>4%</td>
<td>0%</td>
</tr>
<tr>
<td>Neutral</td>
<td>Face</td>
<td>57%</td>
<td>26%</td>
<td>15%</td>
<td>2%</td>
<td>0%</td>
</tr>
</tbody>
</table>

Table 2: Descriptive statistics for the NEO-FFI personality variables (n = 30)

<table>
<thead>
<tr>
<th>Personality Traits</th>
<th>Neuroticism</th>
<th>Extraversion</th>
<th>Openness</th>
<th>Agreeableness</th>
<th>Conscientiousness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>20.3</td>
<td>30.2</td>
<td>31.73</td>
<td>31.60</td>
<td>33.70</td>
</tr>
<tr>
<td>SD</td>
<td>8.94</td>
<td>6.7</td>
<td>5.31</td>
<td>7.27</td>
<td>8.79</td>
</tr>
<tr>
<td>Range</td>
<td>33</td>
<td>27</td>
<td>24</td>
<td>29</td>
<td>42</td>
</tr>
</tbody>
</table>

4.3 Experiment 1: Results

A significant, moderate, positive correlation was found between level of neuroticism and fixation on the eyes for the total stimulus set (r = .37, p = .044 two tailed, p = .022, one tailed). Individual correlations for each of the five emotions suggested that the strength (but not the form) of the effect varied by emotion. In particular, two-tailed
correlation analyses indicated that neuroticism scores correlated significantly with fixation on the eyes for fearful \((r = .60, p < .001 \text{ two tailed, } p < .0005 \text{, one tailed; Figure 2})\), happy \((r = .37, p = .04 \text{ two tailed, } p = .02 \text{, one tailed})\), and sad faces \((r = .41, p = .03 \text{ two tailed, } p = .02 \text{, one tailed})\). Significant correlations were not found for angry \((r = .23, p = .22 \text{ two tailed, } p = .11 \text{, one tailed})\), disgusted \((r = .21, p = .26 \text{ two tailed, } p = .13 \text{, one tailed})\), surprised \((r = .14, p = .45 \text{ two tailed, } p = .23 \text{, one tailed})\), nor neutral faces \((r = .21, p = .27 \text{ two tailed, } p = .14 \text{, one tailed})\). T-tests of dependent correlations (Cohen, Cohen, West, & Aiken, 2003) revealed that the correlation between neuroticism and fixation upon the eyes of fearful faces was significantly higher than that of happy \((t(27) = -1.84, p = .039, \text{ one tailed})\) and sad faces \((t(27) = -1.73, p = .048, \text{ one tailed})\).

Figure 2: Scatterplot illustrating the correlation between level of Neuroticism and the percent of time spent looking at the eyes of the fearful faces.
Further, in an unexpected finding, conscientiousness was also negatively correlated with time spent looking at the eyes of fearful ($r = -.44, p < .05$), happy ($r = -.39, p < .05$), and sad ($r = -.33, p < .10$) faces. This finding appeared to be driven by a negative correlation between the neurotic and conscientious personality traits ($r = -.54, p < .01$) in our sample. When a semi-partial correlation was computed to control for level of neuroticism, the correlation between time spent looking at the eyes and conscientiousness was no longer apparent for fearful ($sr = -.18, p = .36$), happy ($sr = -.24, p = .21$), nor sad ($sr = -.14, p = .46$) faces. Semi-partial correlations were then computed to control for level of conscientiousness within my previous neuroticism correlations. In this case, only the correlation between level of neuroticism and fixation upon the eyes of fearful faces remained significant ($sr = .48, p < .01$). Those for happy ($sr = .21, p = .27$) and sad faces ($sr = .29, p = .13$) dropped out. A principal component analysis was then performed to account for the shared variance between neuroticism and conscientiousness (Bartlett’s test of sphericity; $X^2 = 80.557, p < .001$, KMO = .5). A single factor was extracted to account for 76.8% of the shared variance between these two personality variables. Once correlated with time spent looking at the eyes of emotional faces, this common factor, which may be related to anxious concern for emotional outcome (McCrae & Costa, 2003), was found to correlate positively with time spent looking at the eyes of fearful ($r = .59, p < .001$), happy ($r = .43, p < .05$), and sad ($r = .42, p < .05$) faces. It is important to note, however, that only the correlation between
neuroticism and fixations to the eyes of fearful faces remained significant ($r = .60$, $p < .0005$) after a Bonferroni adjustment for multiple comparisons (105 comparisons; 5 personality traits × 7 emotions × 3 facial regions). See Tables 3 and 4 for complete correlations between all facial expressions, AOIs, and personality types.

Table 3: Correlations among personality traits and proportion of time spent fixating on each facial area of interest.

<table>
<thead>
<tr>
<th>Personality Traits</th>
<th>Neuroticism</th>
<th>Extraversion</th>
<th>Openness</th>
<th>Agreeableness</th>
<th>Conscientiousness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eyes</td>
<td>.37*</td>
<td>.06</td>
<td>.17</td>
<td>.12</td>
<td>-.29</td>
</tr>
<tr>
<td>Nose</td>
<td>-.44*</td>
<td>-.04</td>
<td>-.31</td>
<td>-.17</td>
<td>.18</td>
</tr>
<tr>
<td>Mouth</td>
<td>.30</td>
<td>-.03</td>
<td>.09</td>
<td>-.21</td>
<td>-.16</td>
</tr>
</tbody>
</table>

N = 30, *p < .05, two tailed

4.4 Experiment 1: Discussion

The results of my study suggest that personality is related to one of our most basic and earliest developing social behaviors: eye contact with faces. As illustrated in Figure 3, individuals high in the personality trait of neuroticism attend more to the most emotionally arousing and/or most informative features of the fearful face (the eyes), while those low in neuroticism spend less time doing so. Individuals high in neuroticism may perceive a salient emotional image signaling a threat in the immediate environment, while those low in neuroticism may perceive a stimulus less laden with emotional content. In this way, personality may affect not just how individuals interpret
and think about what they see, but what emotionally salient contextual information they attend to in the first place.

Table 4: Correlations among personality traits and proportion of time spent fixating on the eyes of each facial expression.

<table>
<thead>
<tr>
<th>Facial Expression</th>
<th>Neuroticism</th>
<th>Extraversion</th>
<th>Openness</th>
<th>Agreeableness</th>
<th>Conscientiousness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Happy</td>
<td>.37*</td>
<td>-.04</td>
<td>.00</td>
<td>.10</td>
<td>-.39*</td>
</tr>
<tr>
<td>Sad</td>
<td>.41*</td>
<td>.004</td>
<td>.22</td>
<td>.02</td>
<td>-.33#</td>
</tr>
<tr>
<td>Anger</td>
<td>.23</td>
<td>.18</td>
<td>.26</td>
<td>.12</td>
<td>-.08</td>
</tr>
<tr>
<td>Fear</td>
<td>.60**</td>
<td>-.19</td>
<td>.16</td>
<td>.00</td>
<td>-.44*</td>
</tr>
<tr>
<td>Surprise</td>
<td>.14</td>
<td>.21</td>
<td>.18</td>
<td>.30</td>
<td>-.03</td>
</tr>
<tr>
<td>Disgust</td>
<td>.21</td>
<td>.07</td>
<td>.10</td>
<td>.16</td>
<td>-.32</td>
</tr>
<tr>
<td>Neutral</td>
<td>.21</td>
<td>.14</td>
<td>.07</td>
<td>-.03</td>
<td>-.07</td>
</tr>
<tr>
<td>Total</td>
<td>.37</td>
<td>.06</td>
<td>.17</td>
<td>.12</td>
<td>-.29</td>
</tr>
</tbody>
</table>

N = 30, #p < .10, *p < .05, **p < .001, two tailed

My findings are consistent with a trait congruency model (Bargh et al., 1988) in which individuals seek out information that is congruent with their personality traits and avoid information that is not. Neuroticism has been linked to both trait (enduring) and state (temporary) dysregulated negative affect including fear and anxiety (Costa & McCrae, 1980). This effect is consistent with prior behavioral studies that have documented an attentional bias towards trait congruent, highly arousing stimuli (Derryberry & Reed, 1994; Gomez et al., 2002; Reed & Derryberry, 1995). The highly neurotic subjects seemed to be most attracted to the eyes of fearful faces, a stimulus that is congruent with their more negative personalities.
My unexpected finding of the negative relationship between conscientiousness and attention to the eyes of emotional faces led to the investigation of a common factor between neuroticism and conscientiousness in the current sample. Although the big five personality traits are hypothesized to be orthogonal, the trait of neuroticism and conscientiousness share a commonality in anxious concern for emotional outcome (McCrae & Costa, 2003). Both of these personality types display a high level of attention to emotional details and anxiety for negative consequence. Those high in neuroticism seem to be attracted to negative emotionality while those high in conscientiousness are generally apt to avoid it (McCrae & Costa, 2003). My data showed that a common factor between these two traits correlated with attention to fearful eyes. However, consistent with their attention to or avoidance of negative emotional situations, high neuroticism subjects tended to look towards this highly arousing stimulus while high conscientiousness subjects looked away.
Figure 3: Top panel, map illustrating the regions of this fearful face fixated upon based on the top 1/3 and bottom 1/3 scores on levels of Neuroticism in my sample. The green-to-red colormap indicates the number of fixations over each pixel. Bottom panel, “cut out” images depicting the functional stimulus as a function of membership in the two groups of high and low levels of neuroticism.

Further, my data are relevant to prior findings from neuroimaging and genetic studies. Neuroticism has been associated with the short variant of the serotonin transporter allele (Lesch et al., 1996; Sen et al., 2004) relating to lower serotonergic production and reuptake (Garpenstrand et al., 2001). In addition, trait neuroticism has been linked to increased right amygdala gray matter concentration (Omura, et al., 2005) and amygdala hyper-activity in response to facial expressions of fear (Hariri, Mattay et
Further, recent research suggests increased amygdala activity to threatening faces in individuals high in personality traits characterized by elevated levels of negative emotionality (Beaver, Lawrence, Passamonti, & Calder, 2008).

Other evidence highlights the key role of amygdala functioning in directing visual attention to the eyes of faces. SM, a rare neuropsychological patient with bilateral amygdala damage, displays a lack of spontaneous fixation on the eyes of faces, contributing to her deficits in recognizing fearful facial expressions (Adolphs et al., 2005). Similarly, individuals with autism, who fail to make and maintain eye contact with others (Klin, Jones, Schultz, Volkmar, Cohen, 2002; Pelphrey et al., 2002), display abnormally low levels of amygdala activation while viewing emotional facial expressions (Pelphrey, Morris, McCarthy, & LaBar, 2007).

These results may reflect a behavioral mechanism in the relationships among gene variation, amygdala activity, and neuroticism. The present findings support a model whereby people with high levels of neuroticism have a bias towards increased activity in the amygdala. This bias could lead to the recruitment of attentional resources to redirect gaze towards the eyes (Adolphs et al., 2005), whereby more information might be obtained about the signaler of an emotion. This effect is particularly strong for fearful faces because facial expressions of fear are especially good activators of the amygdala and/or because fearful faces demand attention to the eye region for successful emotion identification. Although further research is needed to untangle the
directionality of these relationships, it seems that eye gaze may be one behavioral link in a complex relationship between genes, brain function, and personality.

The individual differences in visual scanpaths observed here underscore an important methodological issue. Individuals display different visual scanpaths in response to faces as a function of individual differences in personality. It follows that individuals of various personality types may perceive varying levels of emotional content in presented stimuli. Thus, there may be a disparity between the nominal and functional value (Bartlett, 1932) of any emotional stimulus in a standard neuroimaging study: although all participants in a study might be presented with the same image, variation in image exploration could result in differential perception based on the personality of the each participant. Consistent with the trait congruency hypothesis, for example, when subjects are shown scenes containing a negative situational context, those high in neuroticism may seek out the most negative information and thus perceive a more salient emotional image than those subjects high in optimism, who may only selectively attend to more positive aspects of the image.

In sum, I found evidence that visual attention to emotional faces varies with the personality trait of neuroticism. However, my conclusions are tempered by some limitations to the current study. First, my stimulus set was limited to static images of facial expression of emotion. It is not clear whether differences in scanpaths would be observed for other types of emotionally salient images or dynamic face stimuli. Nor is it
clear whether the differences in attention observed here would generalize to other modalities, such as emotional sound clips. Second, in the present study, data on the current emotional state of the participants was not collected. It may be the case that fleeting individual differences, such as variation in mood state, may also play a role in selective attention to emotional information. Future studies are planned to address this possibility.

4.5 Experiment 2: Introduction

The study described above (Perlman, Morris, Vander Wyk, Green, Doyle, & Pelphrey, under review) investigated the effects of personality on visual examination of faces. I found that subjects high in the personality trait of neuroticism, characteristic of attention to emotionally arousing environmental signals, high anxiety, and negative affect, spent more time looking at the eyes of emotional fearful faces than subjects low in neuroticism. These findings support the trait congruency hypothesis by finding that subjects who are generally high in negative affect selectively attend to environmental stimuli that are congruent with their highly aroused emotional state. Although my findings will serve to extend and inform the relevant literature on personality, face processing, and imaging genetics, there exist some important limitations to my work. The current study was designed to replicate the effects of my previous work, but also to address its short comings.
First, in my previous study, the face image displayed on the screen was large, which intended to mimic face perception during social interaction. Consequently, the face occupied the entire computer screen. Although realistic in size, my stimuli did not intend to represent a naturalistic environment where objects and scenery enter the visual field. Other stimuli may serve as competing points of visual interest, which might wash out the effects of personality seen in the previous study. Therefore, in this experiment, a single face stimulus was “jumbled” with an array of everyday objects. The face occupied only a small portion of the screen to mimic the subjects’ visual field when scanning the environment. I was interested in the amount of time those high in neuroticism would spend fixating upon the face, in comparison to their low neuroticism counterparts, as well as their latency to disengage from the emotional stimulus and examine other objects in the array. Increased fixation upon emotional faces would suggest the seeking out of emotional information, which is both consistent with this personality type and likely to increase emotional brain activity.

Second, my earlier experiment interlaced a central fixation point with the face image presented to subjects. This central crosshair served to constrain attention to the center of the screen between face trials. Although eye position on the facial stimuli varies by such factors as head size, sex, and race, the eyes of the emotional face generally appear at or near the center of the screen, very near to the central fixation cross. It may be, therefore, that my findings are more related to ineffective attention modulation and
shifting then to the seeking out of trait-congruent stimuli. That is, it is possible that those high in neuroticism are simply less skilled at disengaging their visual attention from the central fixation point and, therefore, maintain fixation near the center of the screen (the eye area) at all times. The current study sought to eliminate this possibility by varying the position of the emotional stimulus. After every fixation cross, an array of objects appeared with a randomly placed face somewhere on the screen. Subjects were, therefore, required to disengage from the central fixation point to visually attend to the emotional stimulus if they chose.

Finally, one important confound of my previous work is that we did not control for current emotional state. Subjects high in neuroticism are also highly emotional and are prone to negative mood. It may be the case, therefore, that the high neuroticism subjects in my sample were feeling more negative emotions during testing than their low neuroticism counterparts. The effect, in fact, may have been due to state negative affect rather than trait personality. In the current study, I collected information on participants’ current mood at the time of testing. I sought to disentangle the effects of trait personality from affective state when examining emotional faces.

I hypothesized that all subjects would spend a considerable amount of time fixating upon the face. Although it occupied a small portion of the screen, faces are salient social stimuli to which typically developing subjects selectively attend. We expected those high in the personality trait of neuroticism to spend the most time fixated
upon the emotional face and to display a longer latency to disengage from the emotional stimulus to examine the rest of the objects in the array. Further, due to the salience of fear in the neurotic personality type and to my previous results, I expected this effect to be larger for fearful faces than for other emotions.

4.6 Experiment 2: Method

4.6.1 Subjects

Thirty-three adult volunteers, recruited through internet advertisements, participated in this experiment. Data from four participants were not included in the analysis due to poor equipment calibration leaving a sample of 29 subjects (19 female, 10 male; mean age = 23.76 years; range = 18-44 years). All participants signed informed consent as specified by protocols approved at the University of Pittsburgh and Carnegie Mellon University.

4.6.2 Stimuli

Stimuli consisted of 15 different images containing a scattered array of common objects (e.g. chairs, shoes, balls). Within each individual object array, an emotional face appeared in a distinct location (see Figure 4 for a depiction of my study design). Each array was presented four times throughout the experiment (60 total images) each containing a single image of a model posing one of four emotional facial expressions (Happy, Angry, Fearful, or Neutral). Emotional expression stimuli were taken from the NimStim stimulus set (Tottenham et al., in press).
4.6.3 Experimental Procedure

Object arrays, occupying the full 17 inch LCD screen, appeared for 6s with a fixation point in the center of the screen appearing for 2s between images. Participants were seated approximately 60 cm from the computer screen and told to freely view the images during the 8 minute long experiment. Eye movements were recorded at 50 Hz using a remote infrared eye-tracking system (T60, Tobii Technology). Prior to the eye tracking procedure, participants completed the NEO Five Factor Inventory (Costa & McCrae, 1991) to assess dimensions of personality and the Positive Affect Negative Affect Scale to assess state affect (Watson, Clark, & Tellegen, 1988).

The location and duration of fixations were calculated from (AOIs) drawn around the emotional face of each image. This region of interest occupied exactly 6.5% of the screen. The duration of fixation for the face AOI was calculated separately for
each image and collapsed across emotions. To adjust for individual differences in looking time due to blinking or momentary distraction from the screen, analyses were preformed on the proportion of fixation time spent looking at the face within the time spent looking within the whole array. The total amount of time spent looking at the screen did not correlate with neuroticism \(r(27) = -.003, p = .99\). See Tables 5 and 6 for summary statistics regarding the NEO-FFI and PANAS respectively.

**Table 5: Descriptive statistics for the NEO-FFI personality variables \(n = 29\)**

<table>
<thead>
<tr>
<th>Personality Traits</th>
<th>Neuroticism</th>
<th>Extraversion</th>
<th>Openness</th>
<th>Agreeableness</th>
<th>Conscientiousness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>20.34</td>
<td>31.48</td>
<td>32.48</td>
<td>32.14</td>
<td>30.66</td>
</tr>
<tr>
<td>SD</td>
<td>8.26</td>
<td>5.65</td>
<td>5.65</td>
<td>4.60</td>
<td>6.28</td>
</tr>
<tr>
<td>Range</td>
<td>28</td>
<td>22</td>
<td>21</td>
<td>17</td>
<td>29</td>
</tr>
</tbody>
</table>

**Table 6: Descriptive statistics for the PANAS state emotion variables \(n = 29\)**

<table>
<thead>
<tr>
<th>Emotional State</th>
<th>Positive Emotion</th>
<th>Negative Emotion</th>
<th>Fear</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>27.62</td>
<td>14.24</td>
<td>7.79</td>
</tr>
<tr>
<td>SD</td>
<td>7.43</td>
<td>5.96</td>
<td>3.49</td>
</tr>
<tr>
<td>Range</td>
<td>30</td>
<td>26</td>
<td>18</td>
</tr>
</tbody>
</table>

### 4.7 Experiment 2: Results

A significant, moderate, positive correlation was found between level of neuroticism and fixation on all faces for the full stimulus set \(r = .42, p = .022\) two tailed, \(p = .011\) one tailed. Individual correlations for each of the four emotions suggested that
the strength (but not the form) of the effect varied by emotion. In particular, two-tailed correlation analyses indicated that neuroticism scores correlated significantly with fixation on the eyes for fearful \((r = .53, p = .003 \text{ two tailed}, p = .002 \text{ one tailed; Figure 5})\), happy \((r = .37, p = .047 \text{ two tailed}, p = .024 \text{ one tailed})\), angry \((r = .39, p = .036 \text{ two tailed}, p = .018 \text{ one tailed})\), and neutral faces \((r = .36, p = .053 \text{ two tailed}, p = .027 \text{ one tailed})\). T-tests of dependent correlations (Cohen et al., 2003) revealed that the correlation between neuroticism and fixation upon fearful faces was significantly higher than that of happy \((t = -.1.80, p = .04, \text{ one tailed})\) and neutral faces \((t(26) = -.1.82, p = .04, \text{ one tailed})\) and marginally significantly higher than that of angry faces \((t(26) = -.1.53, p = .07, \text{ one tailed})\). It is important to note, however, that only the correlation between neuroticism and fixations upon fearful faces remained significant \((r = .53, p = .003)\) after a Bonferroni adjustment for multiple comparisons \((20 \text{ comparisons; } 5 \text{ personality traits } \times 4 \text{ emotions})\). See Tables 7 for complete correlations between all facial expressions and personality types.
Figure 5: Scatterplot illustrating the correlation between level of Neuroticism and the percent of time spent looking at the fearful faces ($r = .53, p < .01$). Level of trait personality (Neuroticism) remained significantly correlated with the percentage of time spent fixating upon the fearful face even when controlling for state personality (Negative Affect; $sr = .49, p < .01$).

In order to investigate the effects state personality on the visual examination of emotional stimuli, I computed each participant’s self report of current positive and negative emotions using the PANAS scale. These scores were then correlated with the percent of time spent looking at emotional faces within the object array. Neither positive nor negative emotional state was correlated with the percent of time spent fixating upon faces of any emotion. In addition to overall negative affect, the specific emotion of fear has been implicated as a prominent aspect of the neurotic personality type (Costa & McCrae, 1980). Therefore, I examined the participants self-report of state fear in
correlation with the visual fixation upon faces. Fearful state was not significantly correlated with the percent of time spent fixating upon any emotional facial expression.

Table 7: Correlations between NEO-FFI personality variables and attention to emotional facial expressions

<table>
<thead>
<tr>
<th>Expression</th>
<th>Neuroticism</th>
<th>Extraversion</th>
<th>Openness</th>
<th>Agreeableness</th>
<th>Conscientiousness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fear</td>
<td>.53**</td>
<td>-.12</td>
<td>-.9</td>
<td>-.28</td>
<td>-.10</td>
</tr>
<tr>
<td>Happy</td>
<td>.37*</td>
<td>-.15</td>
<td>-.11</td>
<td>.00</td>
<td>.07</td>
</tr>
<tr>
<td>Angry</td>
<td>.39*</td>
<td>-.21</td>
<td>-.15</td>
<td>-.15</td>
<td>-.06</td>
</tr>
<tr>
<td>Neutral</td>
<td>.36*</td>
<td>-.10</td>
<td>-.10</td>
<td>-.01</td>
<td>.02</td>
</tr>
<tr>
<td>Total</td>
<td>.42*</td>
<td>-.15</td>
<td>-.12</td>
<td>-.11</td>
<td>-.01</td>
</tr>
</tbody>
</table>

** p < .01, * p ≤ .05, two tailed

Finally, to account for state personality within trait personality effects, I computed a semi-partial correlation to control for current negative affect in the relationship between neuroticism and attention to emotional faces. In this case, only the correlation between level of neuroticism and fixation upon the fearful faces remained significant (sr = .49, p < .01). Those for happy (sr = .36, p = .06), angry (sr = .35, p = .07), and neutral faces (sr = .34, p = .08) dropped to below the standard (p < .05) level of significance. See Table 8 for complete semi-partial correlations between all facial expressions and neuroticism.
Table 8: Semi-partial correlations accounting for PANAS state negative affect in the correlation between neuroticism and attention to emotional faces.

<table>
<thead>
<tr>
<th>% Fixation on Facial Expression</th>
<th>Fear</th>
<th>Happy</th>
<th>Angry</th>
<th>Neutral</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neuroticism</td>
<td>.49**</td>
<td>.36+</td>
<td>.35+</td>
<td>.34+</td>
<td>.39*</td>
</tr>
</tbody>
</table>

** p < .01, *p < .05, +p < .10, two tailed

4.8 Experiment 2: Discussion

The results of my second study provide additional support for the trait-congruency hypothesis. As seen in Figure 6, subjects highest in neuroticism spent the most time fixated upon the emotional face stimulus. These subjects were less likely to visually examine the other objects within the visual array in comparison to low neuroticism participants who also attended to the face, but examined the other objects in addition. This effect was especially significant for fearful faces. Like my earlier findings of heightened attention to the arousing eye area of the fearful face, I found that the personality trait of neuroticism correlated with fixation upon fearful faces more than any other emotion. Fearful faces were, indeed, the only stimuli to maintain a significant correlation for looking time after adjustment for multiple significance tests.

This experiment sought to imitate a realistic environment by including everyday objects, which are as visually complex as faces, as part of my stimuli. This gave the participants the opportunity to look at the face as long as it interested them, but to also be visually distracted by other interesting objects. I found that subjects high in neuroticism maintained fixation upon the face and generally did not examine the rest of
the array. This finding relates to those of my previous study in that subjects high in neuroticism seem to preferentially attend to the most emotionally arousing stimuli in immediate view. In the earlier case, subjects were drawn to the highly arousing fearful eyes when only a face was in view, but here it is demonstrated that they attend preferentially to the only emotional stimulus available to them, even when other objects are available for viewing. Future studies will be needed to test whether or not this effect is solely related to faces or whether subjects high in neuroticism will attend to other types of emotional images.

Figure 6: Map illustrating the regions of this object array (containing a fearful face) fixated upon based on the top 1/4 and bottom 1/4 scores on levels of Neuroticism in my sample. Fixation times are calculated based on the amount of time spent looking at the screen, which was equal for both groups of subjects \( t(12) = -.85, p = .41 \). The colormap indicates the amount of time spent fixated on each pixel. Red represents the longest total fixation time on a specific pixel across subjects: 8.58 seconds for the high neuroticism group and 2.34 seconds for the low neuroticism group.
This study also tested the hypothesis, and possible alternative interpretation of my past work, that the personality trait of neuroticism might correlate positively with difficulty in disengaging visual attention. If this hypothesis proved true, subjects high in neuroticism would only attend preferentially to the eyes of fearful faces because their attention had been directed to the center of the screen by a central crosshair. The current study placed the face stimulus in varying locations within the object array, necessitating attentional disengagement from the central crosshair in order to fixate upon the face. Subjects high in neuroticism spent more time fixating on faces (especially fearful faces) rather than a centrally placed object, indicating that my previous effects are due to preferential attention to emotional stimuli in neuroticism rather than a lack of attentional disengagement.

Finally, my study investigated not only trait personality and its relationship to visual attention to faces, but state personality as well. Before participation in the eye tracking study, subjects provided information on their current emotional state. I hypothesized that, in accordance with the trait-congruency hypothesis, participants highest in negative mood (likely those who are also highest in neuroticism) would be most attracted to the negatively arousing face stimuli. I did not find a significant correlation between current mood state (general positive, general negative, or fearful) and attention to any type of face. Further, when controlling for negative emotional state,
the correlation between neuroticism and attention to fearful faces remained significant, indicating that mood state is not an important contributor to fixation upon facial stimuli. Further studies will be needed to better understand the effects of state personality on visual attention to emotional stimuli.
5. Study 2: Experimental Manipulation of Eye Contact

The above studies examined the personality trait of neuroticism and its links to the visual examination of emotional faces. Although I found striking differences between subjects high and low in neuroticism in the sample, it must be acknowledged that neuroticism is not a clinical trait. Variation in neuroticism is common in the typically developing population and is not related to aberrant social functioning.

Regardless of neuroticism status, all subjects in my study displayed a typical triangular scanpath. The following study was designed to investigate abnormal emotional brain processing of social/emotional stimuli (Aim 2). Individuals with Autism Spectrum Disorders have been noted to display abnormal visual scanning of faces, which has been linked to deficits in brain activity and social interaction.

Although Autism spectrum disorders are characterized by multiple deficits (DSM-IV-TR, American Psychiatric Association, 1994), including delays in speech and language and impaired motor behavior, the disorder is also related to deficits in aspects of emotional functioning. In 1943, Leo Kanner referred to childhood Autism as a “disturbance of affective contact”, noting that the children he observed seemed emotionally detached from others. He describes one specific case as a child who does not note that others are coming or leaving, does not physically display affection, and does not interact with other children. In addition, several more recent studies have noted a lack of social/emotion understanding that typically-developing individuals find
inherent in faces (e.g. Adolphs, Sears, & Piven, 2001; Baron-Cohen, Leslie, & Frith, 1985; Hobson, Ouston, & Lee, 1988a,b).

It is, therefore, important to understand the mechanisms by which individuals with Autism view faces and how this atypical development of face processing might influence brain mechanisms for the perception of emotional facial expression, and possibly deficits in social behavior. In the following study, I manipulated visual scanpaths in individuals with autism to quantify normalization of brain activity (Perlman, Hudac, Pegors, Minshew, & Pelphrey, under review).

5.1 Introduction

Autism is a pervasive neurodevelopmental disorder characterized by pathognomic social deficits (Kanner, 1943; Wing & Gould, 1979). A deficit in eye contact is among the most striking of these social impairments and is one of the diagnostic criteria for the disorder. This deficit appears to be among the earliest behavioral manifestations of autism. For example, in a study of first birthday videos, looking at others was found to be among four social behaviors that differentiated children who would go on to be diagnosed with autism later in childhood (Osterling & Dawson, 1994). Further, eye-tracking studies have served to quantify and characterize the developmental nature of the eye-to-eye gaze impairment (Jones, Carr, & Klin, 2008; Klin et al., 2002; Pelphrey et al., 2002). For instance, Jones and colleagues (2008) quantified looking to the eyes of others in two-year-old children with autism (currently the earliest
possible point of reliable diagnosis), typically developing children, and developmental
delayed children without autism. They found that looking at the eyes of others was
significantly decreased while looking at mouths was increased in two-year-olds with
autism, in comparison with typically developing and developmentally delayed but
nonautistic children. Jones and colleagues (2008) concluded that “diminished and
aberrant eye contact is a lifelong hallmark of disability” (pg.1).

Given the unique nature of the social deficits in autism, it is not surprising that
many (and perhaps most) of the available functional neuroimaging studies of
individuals with autism have examined aspects of the human face processing system
(e.g. Critchley et al., 2000; Pierce, Müller, Ambrose, Allem, & Courchesne, 2001; Schultz
et al., 2000). In comparison to typically developing participants, hypoactivation of the
amygdale (e.g. Baron-Cohen et al, 1999; Ogai et al, 2003) and the “fusiform face area”
(FFA), a region of the lateral fusiform gyrus (FFG) (e.g. Critchley et al., 2000; Schultz et
al. 2000) that is specialized for face perception, have both been observed in participants
with autism. Note, however, that the question of whether or not hypoactivation in these
regions is an aspect of the brain phenotype in autism remains an open and widely
debated question.

A recent literature search revealed 17 studies of the face processing system in
individuals with autism published between 1999 and 2008 (see Table 9). Of those 17
studies, 14 reported data from the FFG and 11 reported data from the amygdala. Ten
(Critchley et al., 2000; Dalton et al. 2000; Humphreys, Hasson, Avidan, Minshew, & Behrmann, 2008; Koshino, Kana, Keller, Cherkassky, Minshew, & Just, 2008; Hubl et al., 2003; Pelphrey et al., 2007; Pierce et al., 2001; Piggot et al., 2004; Schultz et al., 2000; Wang, Dapretto, Hariri, Sigman, & Bookheimer, 2004) out of 14 studies of the FFG reported hypoactivation in participants with autism compared to neurotypical participants, while four reported equivalent FFG activity (Bookheimer, Wang, Scott, Sigman, & Dapretto, 2008; Hadjikhani et al., 2004; Pierce, Haist, Sedaghat, & Courchesne, 2004; Pierce & Redcay, 2008) in participants with and without autism. A particularly elegant study of children with and without autism reported hypoactivation for unfamiliar faces but equivalent activation for familiar and/or highly salient faces (i.e., pictures of the child’s mother) (Pierce & Redcay, 2008). Eight (Ashwin, Baron-Cohen, Wheelwright, O’Riordan, Bullmore, 2007; Baron-Cohen et al., 1999; Bookheimer et al., 2008; Critchley et al., 2000; Pelphrey et al., 2007; Pierce et al., 2004; Pierce et al., 2001; Wang et al., 2004) of 11 studies reported hypoactivation of the amygdala in participants with autism as compared to typically developing participants. Two reported equivalent amygdala activity in affected and unaffected individuals (Pierce et al., 2004; Piggot et al., 2004). One study reported amygdala hyperactivation (Dalton et al., 2005) in participants with autism relative to typically developing participants.
### Table 9: Neuroimaging studies (1999-2008) investigating abnormal face processing in autism spectrum disorders

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Journal</th>
<th>Task</th>
<th>Fusiform</th>
<th>Amygdala</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baron-Cohen et al.</td>
<td>1999</td>
<td>European J of Neuroscience</td>
<td>Emotion/TOM judgment of eyes only</td>
<td>N/A</td>
<td>Hypoactivation</td>
</tr>
<tr>
<td>Schulz et al.</td>
<td>2000</td>
<td>Arch of General Psychiatry</td>
<td>Perceptual discrimination</td>
<td>Hypoactivation</td>
<td>N/A</td>
</tr>
<tr>
<td>Critchley et al.</td>
<td>2000</td>
<td>Brain</td>
<td>Explicit and implicit face processing, Emotion and sex judgment task</td>
<td>Hypoactivation</td>
<td>Hypoactivation</td>
</tr>
<tr>
<td>Pierce et al.</td>
<td>2001</td>
<td>Brain</td>
<td>Button press in response to female faces</td>
<td>Hypoactivation</td>
<td>Hypoactivation</td>
</tr>
<tr>
<td>Hubl et al.</td>
<td>2003</td>
<td>Neurology</td>
<td>Emotion and sex discrimination</td>
<td>Hypoactivation</td>
<td>N/A</td>
</tr>
<tr>
<td>Ogai et al.</td>
<td>2003</td>
<td>NeuroReport</td>
<td>Emotion discrimination</td>
<td>N/A</td>
<td>Hypoactivation</td>
</tr>
<tr>
<td>Pierce et al.</td>
<td>2004</td>
<td>Brain</td>
<td>Presentation of familiar faces, Maintain fixation on center of screen, Button press in response to females</td>
<td>Equal</td>
<td>Equal</td>
</tr>
<tr>
<td>Hadjikahani et al.</td>
<td>2004</td>
<td>NeuroImage</td>
<td>Passive viewing of faces, Subjects maintain central fixation</td>
<td>Equal</td>
<td>N/A</td>
</tr>
<tr>
<td>Wang et al.</td>
<td>2004</td>
<td>J Am Acad Child Adolesc Psychiatry</td>
<td>Emotional expression identification</td>
<td>Hypoactivation</td>
<td>Lack of modulation due to task demands in ASD</td>
</tr>
<tr>
<td>Dalton et al.</td>
<td>2005</td>
<td>Nature Neuroscience</td>
<td>Emotion and familiar vs. unfamiliar identity discrimination</td>
<td>Hypoactivation</td>
<td>Hyperactivation</td>
</tr>
<tr>
<td>Pelphrey et al.</td>
<td>2007</td>
<td>SCAN</td>
<td>Observation of dynamic emotional expression</td>
<td>Hypoactivation</td>
<td>Hypoactivation</td>
</tr>
<tr>
<td>Ashwin et al.</td>
<td>2007</td>
<td>Neuropsychologia</td>
<td>Observation of varying degrees of fearful faces</td>
<td>N/A</td>
<td>Hypoactivation</td>
</tr>
<tr>
<td>Koshino et al.</td>
<td>2008</td>
<td>Cerebral Cortex</td>
<td>Face recognition N-back task</td>
<td>Hypoactivation</td>
<td>N/A</td>
</tr>
<tr>
<td>Humphreys et al.</td>
<td>2008</td>
<td>Autism Research</td>
<td>Viewing of line drawings and movies of faces</td>
<td>Hypoactivation</td>
<td>N/A</td>
</tr>
<tr>
<td>Bookheimer et al.</td>
<td>2008</td>
<td>J Inter Neuropsych Society</td>
<td>Upright vs. inverted faces recognition task</td>
<td>Equal</td>
<td>Hypoactivation</td>
</tr>
<tr>
<td>Pierce &amp; Redcay</td>
<td>2008</td>
<td>Biological Psychiatry</td>
<td>Viewing of familiar vs. unfamiliar faces</td>
<td>Equal</td>
<td>N/A</td>
</tr>
</tbody>
</table>
Potentially critical methodological differences exist between those studies that have reported hypoactivation versus equivalent activity in the FFG. The majority of studies reporting FFG hypoactivation involved free viewing and/or very modest task demands. In contrast, two of the three studies reporting equivalent activation in the FFG constrained the viewing patterns of the participants by placing a fixation cross in the center of the stimulus (e.g., on the bridge of the nose between the eyes) (Hadjikhani et al., 2004; Pierce et al., 2004). A third study reporting equivalent FFG activation involved a relatively demanding face recognition task in the context of upright and inverted faces (Bookheimer et al., 2008). Finally, Pierce and Redcay (2008) observed equivalent FFG activity in children with and without autism when the children viewed highly familiar and salient pictures of their mothers. It can be argued that the underlying mechanism uniting these studies is a task manipulation that increased visual attention to the face, and particularly the eyes of the faces, either by requiring participants to maintain a fixation placed between the eyes (Hadjikhani et al., 2004; Pierce et al., 2004) thereby removing the confound of altered visual scanpaths by increasing the salience of the face (Pierce & Redcay, 2008) or increasing the relevance of the core facial features to the task at hand (Bookheimer et al., 2008).

As can be appreciated from the review above, the state of the literature on the face processing system in individuals with autism is currently quite unsettled. As expressed by Klin (2008), all of the prior studies can be criticized, albeit on different grounds, for
the approaches they have taken to the issue of potentially confounding differences in overt visual attention. One the one hand, studies that have involved free viewing can be criticized for ignoring known differences in visual attention in individuals with autism. On the other hand, the two studies that have controlled for differences in visual scanpaths by constraining fixation to a central cross may be criticized because their design does not allow us to evaluate experimentally the exact mechanism underlying the observed outcome. That is, it is possible that constraining visual fixation to the center of the face inadvertently alters the task for the typically developing participants who are forced to attend to a fixation point that, in turn, may reduce their experiences of faces as such. In this circumstance, the observed lack of FFG abnormalities in individuals with autism might actually reflect reduced FFG activation in control participants rather than increased activity in participants with autism.

Indeed, a previous study of typically developing individuals experimentally investigated this issue via the direct manipulation of visual scanpaths (Morris, Pelphrey, & McCarthy, 2006). Participants visually tracked a small crosshair moving across a static face. When the crosshair followed an “atypical” scanpath (landing on eyes less than 12% of the time), decreased FFA activation was observed as compared to that recorded during a “typical” scanpath (landing on eyes approximately 80% of the time). This study demonstrates that by making typically developing individuals scan the eyes
less or by constraining their fixation to a central cross we can actually reduce levels of FFA activity.

Only one study to date has directly measured visual attention to faces during fMRI scanning. Dalton and colleagues (2005) reported a strong, positive correlation in participants with autism between the number of fixations upon the eyes of faces and the level of activation in the FFG and left amygdala, suggesting a link between visual scanpaths and hypoactivation in these components of the face processing system. This association could mean that individuals with more FFG and left amygdala activity are the ones who look more at the eyes of faces. Alternatively, the correlation could reflect that when subjects with autism happen to look more at the eyes, they in turn exhibit greater activity in face processing regions. This latter explanation could indicate that direct eye contact is experienced as aversive for individuals with autism. The available data cannot adjudicate between these two plausible mechanisms because scanpaths were not experimentally manipulated.

In the present study, I sought to manipulate experimentally activity in the face processing system of individuals with autism by compelling them to look at the eyes of faces to varying degrees. I hypothesized that requiring individuals with autism to visually scan the eyes of an emotionally expressive face would serve to increase activation in key components of the face processing system. To this end, I directly manipulated visual scanpaths across four incremental experimental conditions: free
viewing, low, medium, or high amounts of fixating the eyes in a group of high-functioning adults with autism during whole-brain functional magnetic resonance imaging (fMRI) at 3.0 Tesla.

5.2 Methods

5.2.1 Subjects

I studied a group of 12 adults with autism (mean age = 25.5, 11 male) and 7 typically developing adults (mean age = 28.57, 7 male) matched on age and verbal and performance IQ (see Table 10). Written informed consent was obtained from each participant for a protocol approved by the University of Pittsburgh’s Institutional Review Board. All individuals with autism met DSM-IV criteria for autism, as based on a history of clinical diagnosis of autism, expert evaluation, parental interview (ADI-R; Lord, Rutter, & Le Couteur, 1994), and proband assessment (ADOS-Module 4; Lord et al., 2000). Typically developing individuals completed either the ADOS (n = 3) or the SCQ (Rutter, Bailey, Berument, LeCouteur, Lord, & Pickles, 2003) (n = 4) to ensure they did not meet criteria for a diagnosis of autism. The two groups did not differ significantly on any of the matching variables, including age \[t(1,17) = 1.08, p = .29\], verbal IQ \[t(1,17) = 1.86, p = .08\] and performance IQ \[t(1,17) = 1.56, p = .14\]. See Table 10 for subject demographic and matching information.
Table 10: Subject demographics and diagnostic scores

<table>
<thead>
<tr>
<th></th>
<th>Autism (n = 12)</th>
<th>Typically Developing (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N Female : N Male</td>
<td>11 : 1</td>
<td>7 : 0</td>
</tr>
<tr>
<td>Age Range (Years)</td>
<td>18-37</td>
<td>22-37</td>
</tr>
<tr>
<td>RH : LH:</td>
<td>11 : 1</td>
<td>7 : 0</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Age</td>
<td>25.5 (7.47)</td>
<td>28.57 (5.74)</td>
</tr>
<tr>
<td>Verbal IQ</td>
<td>106.7 (11.7)</td>
<td>114.8 (4.4)</td>
</tr>
<tr>
<td>Performance IQ</td>
<td>102.2 (15.8)</td>
<td>112.0 (6.7)</td>
</tr>
<tr>
<td>ADI Social</td>
<td>21.6 (3.2)</td>
<td>-</td>
</tr>
<tr>
<td>ADI Communication Verbal</td>
<td>16.5 (4.1)</td>
<td>-</td>
</tr>
<tr>
<td>ADI Communication Nonverbal</td>
<td>9.0 (3.0)</td>
<td>-</td>
</tr>
<tr>
<td>ADI Stereotyped Behaviors</td>
<td>6.1 (2.4)</td>
<td>-</td>
</tr>
<tr>
<td>ADOS Communication</td>
<td>4.8 (1.2)</td>
<td>1.0 (0.8)</td>
</tr>
<tr>
<td>ADOS Social</td>
<td>8.5 (2.8)</td>
<td>0.3 (1.3)</td>
</tr>
<tr>
<td>ADOS Com + Social Total</td>
<td>13.3 (3.8)</td>
<td>1.3 (1.6)</td>
</tr>
<tr>
<td>ADOS Stereotyped Behaviors</td>
<td>1.4 (1.3)</td>
<td>0.0 0.7</td>
</tr>
<tr>
<td>SCQ</td>
<td>- -</td>
<td>3 (2.8)</td>
</tr>
</tbody>
</table>

5.2.2 Stimuli and Procedure

Participants viewed a single fearful face in full color from the NimStim set of facial expressions (Tottenham et al., in press) in the center of the screen, throughout the experiment. Participants were asked to follow visually a small crosshair as it made small jumps across the face every 500 ms so that they made a saccade and fixated upon the crosshair at each new location. As illustrated in Figure 7, three types of 12-s blocks were designed to simulate scanpaths with varying amounts of eye fixation (Low = 32%, Medium = 48%, High = 56%). These percentages were chosen to reflect normal fixation
on the eye region of faces in typically developing subjects. I also employed a Free Viewing condition in which participants were allowed to look at the faces as they typically would in the absence of a moving crosshair. Each block (6 blocks of each condition) alternated with a Fixation block during which the crosshair made small jumps around the nose area for 6 seconds. To ensure compliance with our instructions, participants were asked to press a button upon seeing the rare (two times across the experiment) event of the crosshair changing from red to blue. This color change occurred for the duration of one crosshair jump (500 ms). All of the participants were able to comply with this instruction 100% of the time.

Figure 7: Experimental design. Each face image shows fixations from a block condition in which participants’ attention was drawn to the eye area of the emotional face for varying amounts of time. During free viewing, participants were instructed to view the face as they normally would.
5.2.3 fMRI Data Acquisition

Scanning was performed on a Siemens 3 Tesla Allegra head-only scanner (Siemens, Erlangen, Germany). High-resolution, T1-weighted anatomical images were acquired using an MPRAGE sequence (TR = 1630 ms; TE = 2.48 ms; FOV = 20.4 cm; $\alpha$ = 8°; image matrix = 256$^2$; voxel size = 0.8 × 0.8 × 0.8 mm; 224 slices). Whole-brain functional images were acquired using a single-shot, gradient-recalled echoplanar pulse sequence (TR = 2000 ms; TE = 30 ms; $\alpha$ = 73°; FOV = 20.4 cm; image matrix = 64$^2$; voxel size = 3.2 × 3.2 × 3.2 mm; 35 slices) sensitive to blood oxygenation level-dependent (BOLD) contrast. Runs consisted of the acquisition of 225 successive brain volumes beginning with 2 discarded RF excitations to allow for steady-state equilibrium.

5.3 Results

I analyzed the data at the whole brain level and later examined a priori regions of interest. First, I compared the brain activity of the individuals with and without autism during Free Viewing using an ANOVA with random effects in the Brain Voyager QX software package (Brain Innovation, Maastricht, the Netherlands). I found that regions localized to the right lateral FFG and bilateral amygdala were significantly more active in the typically developing participants as compared to individuals with autism during Free Viewing [$t(72) \geq 2.04$]. These regions are illustrated in Figure 8 (top left panel). For this and all subsequent analyses, I used the false discovery rate procedure (Genovese, Lazar, & Nichols, 2002) [$\text{FDR}(q) < .05$] to control for multiple statistical comparisons.
Next, I created regions of interest where activity in typically developing individuals was greater than for those with autism in the right FFG and bilateral amygdala. Within these regions, for the group of individuals with autism, I compared

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1 Some figures illustrating fMRI data are masked to display a priori regions of interest only.
the High and Free Viewing conditions to identify regions exhibiting an effect of manipulating scanpaths. As illustrated in Figure 8 (bottom left panel), this contrast revealed regions within the right lateral FFG and left amygdala in which participants with autism demonstrated significant High > Free Viewing activity $[t(72) \geq 2.09, \ q < .05]$. To further investigate the form of this effect, we computed the average $t$-score for each participant with autism within each region of High > Free Viewing activity. Repeated-measures ANOVAs revealed a significant effect of condition (Free, Low, Med, High) for the right FFG $[F(3,33) = 12.311, \ p < .0001]$ and left amygdala $[F(3,33) = 3.04, \ p < .05]$. For the right FFG, the effect of condition was linear $[F(1,11) = 26.63, \ p < .0001]$. As shown in Figure 8 (right panels), activation in these regions increased from negative during Free Viewing to strongly positive during the High condition. Notably, positive activity in the left amygdala was only observed during the High condition.

The significant linear effect for fixation condition that I found within the autism group was not present in our typically developing subjects. When a group X condition interaction was computed, I observed several areas in which subjects with autism showed increased activity when fixating for longer durations on the eyes of the fearful face while typically developing subjects did not (see Figure 9). Amongst these regions, small areas within the bilateral amygdala and right FFG displayed an interaction effect $[F(4,68) \geq 2.51, \ p < .05]$.
Finally, within the regions High > Free Viewing described above, I contrasted activity in the High condition for participants with autism with activity in the Free Viewing condition for typically developing participants. This contrast revealed a region of activation within the right lateral FFG in which activity for the participants with autism was significantly higher than that observed in the typically developing participants \( t(72) \geq 2.04 \) (see Figure 10). The other regions of interest did not differ between the two groups.

Figure 9: A group by condition interaction shows areas of the bilateral amygdala and right FFG in which subjects with autism show an effect of fixation condition, but typically-developing subjects do not. X = -25, Y = -2, Z = -14.
Figure 10: T-map of a region of activation within the rFFG (Peak voxel: $t = 5.36$; Talairach coordinates: $35x, -64y, -8z$) in which activity for the participants with autism during the High condition was significantly higher than that observed in the typically developing participants during normal Free Viewing.

5.4 Discussion

By identifying hypoactivation in two components of the face processing system in people with autism during unrestricted viewing of faces, my results replicate numerous studies (e.g. Critchley et al., 2000; Pierce et al., 2001). My findings concerning increases in FFG activity when participants with autism are compelled to look at the eyes are consistent with the two prior studies that constrained fixation to a central crosshair (Hadjikhani et al., 2004; Pierce et al., 2004). However, I significantly extend those prior studies by demonstrating that activity in the right FFG increases from a slightly below zero free-viewing baseline to levels slightly greater than that observed in the right FFG of typically developing participants during Free Viewing. This increase is directly
attributable to my experimental manipulation of looking at the eyes. In essence, by simply manipulating the amount of visual attention to the eyes, I was able to “normalize” activity in components of the face processing system as compared to the level of activity in the same participants during Free Viewing, and the level of “normalization” was tightly coupled with the amount of time spent viewing the eyes. Furthermore, we demonstrate that the effect of eye contact on activity in the FFG and left amygdala of individuals with autism is a true effect that is specific to the group of participants with autism. That is, my findings from the free viewing condition reveal that the effect is not attributable to decreases in activity in the FFG of the neurotypical participants as a function of artificially constraining their visual scanpaths.

My results may speak to the question of whether the previously observed correlation between fixations upon the eyes and the level of activation in the FFG and left amygdala (Dalton et al., 2005) should be interpreted as reflecting that individuals with more FFG and left amygdala activity are the ones who look more at the eyes of faces or if looking at the eyes of faces causes increased activation in these components of the face processing system in people with autism because they are experiencing these stimuli as aversive. It is tempting to interpret the greater-than-normal (defined here as the level of activity observed in typically developing participants during free viewing) level of activity observed in the high eyes condition as reflecting hyperactivity resulting from the experience of mild aversion upon being compelled to spend a high percentage
of the time inspecting the eyes. However, in the absence of within-subject corroborating data (e.g., increased in skin-conductance response or self-reports of finding the stimuli aversive when compelled to look more at the eyes), I can only speculate about this possibility. Nevertheless, in speculating as such, it is interesting to note Joseph and his colleagues (Joseph, Ehrman, McNally, & Keehn, 2008) found that skin conductance responses to faces with direct gaze where higher in individuals with autism relative to typically developing controls. These results, combined with the prior findings of Dalton et al. (2005) and my current results provide tentative support for the hypothesis that direct eye contacting is overly arousing for people with Autism Spectrum Disorders.

Although this study revealed important findings concerning the normalization of activity in the face processing system in individuals with autism, I acknowledge an important limitation to my data set. During fMRI scanning, I was not able to collect eye-tracking data. Therefore, although previous studies indicate that those with autism spectrum disorder lack fixation upon the eye region of faces (Jones et al., 2008; Klin et al., 2002; Pelphrey et al., 2002), I was not able to experimentally verify this effect. It is, therefore, possible that subjects with autism did look at the eyes in more in the free viewing condition than one or more of the other conditions. In addition, all subjects were able to comply with my instructions of pressing a button when the crosshair changed to blue, but without eye tracking, I cannot be sure that subjects with Autism and non-autistic subjects followed the crosshair in the same manner. Although I do
believe that this study contributes to the growing literature of face processing in autism, future studies are planned to include eye-tracking measures in this paradigm.

In addition, my findings point to important repercussions for theoretical debates concerning the nature of the FFA and the Amygdala. Some researchers have argued that the FFA is not specialized for faces per se, but rather the seemingly selective response to faces reflects the development of a high level of visual “expertise” for faces, relative to other categories of objects, in typically developing individuals (Gauthier, Behrmann, & Tarr, 1999; Tarr, & Gauthier, 2000). This theoretical camp has seized upon the findings of FFA hypoactivation in autism as evidence in favor of their theory of visual processing (e.g. Grelotti, Gauthier, Schultz, 2002). Specifically, they argue that a lack of attention to the eyes leads to a failure to develop a FFA in individuals with autism. My results are not easily reconciled with the expertise view of face processing deficits in autism. I do find that the FFA is hypoactive in individuals with autism, but show here that this hypoactivation can be reversed with a simple manipulation of fixation to the eyes. It appears that an FFA is present in individuals who presumably lack the necessary “expertise” with faces to have developed such a region.

When examining increased amygdala and FFG activation to the fearful face as subjects increase fixation upon the eye region, I found a group x condition interaction in which activation incrementally increased in subjects with Autism but did not in controls. One prominent theory of the amygdala describes this region as a “threat detector”
(LeDoux, 2000) in which input to this region can escape conscious perception though a fast subcortical pathway. In support of this theory, studies have shown that the amygdala is responsive to masked fearful faces presented at a rapid rate (~30 ms) and not consciously noted by the research subject (e.g. Whalen et al., 1998). Other results are conflicting with this perspective (e.g. Pessoa, Japee, & Ungerleider, 2006) indicate that some subjects may consciously perceive rapidly presented stimuli, which may explain this effect independent of a fast pathway response. In my study, we found that typically developing subjects displayed normal amygdala and FFG activation to the face regardless of time spent fixating upon the highly arousing eye-region. Subjects with autism did not display this level of amygdala and FFG activation when not explicitly instructed to attend to the emotional eyes. These results may, therefore hint at a fast pathway to the amygdala that is present in control subjects without explicit attention to emotional stimuli. This mechanism for an emotional brain response may not be present in individuals with autism.

The present results add a higher degree of specificity to our understanding of social brain dysfunction in autism, however, pivotal questions are raised for future research: If these regions can be engaged in individuals with autism, why are they not engaged organically and what limits the implementation of these regions in everyday situations? Finally, my results offer important implications for behavioral interventions which may help those with autism to develop higher levels of social functioning through
increased attention to the eyes of social partners. The present findings offer some preliminary insights into the mechanisms by which the common clinical practice of encouraging eye contact might serve to shape the development of the social brain. Requiring individuals with autism, even high-functioning adults, to fixate the eyes appears temporarily to “normalize” activity within previously silent components of the face processing system. Future research will be needed to determine if this kind of manipulation can have any lasting effects on brain activity beyond the time frame of a single experimental session.
6. Study 3: Emotion Regulation in the Brain

My previous studies focused on perception of emotional facial expression and emotional brain reactivity in both typically and atypically developing populations. These studies examined the behavioral and brain effects of the perception and experience of emotion and how these effects might deviate in abnormally developing samples. However, adept social interaction is dependent upon, not only the experience of emotion, but both the internal and external control of that emotional arousal as well. Deviant mechanisms for emotion regulation, at both the behavioral and physiological level, have been related to abnormal development and emotional functioning (Cole et al., 1996). Thus, understanding the mechanisms for atypical development of emotion regulation can aid in our knowledge and treatment of psychopathology.

Due to continuous and dynamic changes in our social world, regulation of the self (emotion regulation, specifically) is imperative for negotiating the give and take of social reciprocity (Cicchetti & Tucker, 1994). In Autism Spectrum Disorders a failure both to correctly identify what others are feeling and to adapt individual emotional expression to that affective display could lead to the breakdown in reciprocal interaction that is commonly observed in this disorder (Loveland & Tunali, 1991). In following, it is of clear importance that we seek to identify the mechanisms for emotion dysregulation in Autism, not only to better understand the emotional breakdowns often noted in this disorder (Kanner, 1943), but to also aid individuals with Autism in repairing impaired
social reciprocity. Indeed impairments in emotion regulation in autism have been related to disfunction in the brain’s orbitofrontal to amygdala circuit (Bachevalier & Loveland, 2006).

The following experiments test a new paradigm for quantifying brain mechanisms for emotion regulation in typically-developing adults and children (Aim 3). Future research is planned to examine similar brain circuitry in Autism Spectrum Disorders.

6.1 Experiment 1: Introduction

In recent years, there has been increasing scientific interest in emotion regulation. This construct has captured the attention of the scientific community, not only because of its importance in general human social interaction (Gross, 1998), but also because of its relevance to psychopathology and abnormal social functioning (Bachevalier & Loveland, 2006; Davidson, Putnam & Larson, 2000). The definition, measurement, and general validity of emotion regulation as an area for psychological study are now well-established (see Cole, Martin, & Dennis, 2004). A general consensus regards emotion regulation as a process separate from the experience of emotion itself, depicting regulation as a modulator of affective reactivity (Campos, Frankel, & Camras, 2004; Dodge, 1989). Humans spend most of their time in a neutral emotional state. We feel an emotion when one is elicited by an internal or external stimulus. The emotion we experience varies in intensity according to the situation. The process of emotion
regulation manages all aspects of that emotion, including, but not limited to its subjective feeling and behavioral display. Appropriate emotion regulation is a necessity for managing both heightened and attenuated positive and negative emotions so that an individual can successfully function in the social world (Calkins, 1994; Kopp, 1989; Thompson, 1994).

The conceptual two-part model separating emotional regulation from the emotional activation itself has encouraged multiple biological systems perspectives focusing upon the role of the human brain and its development in the regulation of affect (e.g. Davidson, Putnam et al., 2000; Lewis & Todd, 2007; Thompson, Lewis, & Calkins, 2008). Much of the work that characterized emotional brain reactivity and linked it to specific emotional brain regions (e.g. Adolphs et al., 1994; Calder et al., 1996; Morris et al., 1996) has been followed with studies implicating brain mechanisms for control of these regions in association with regulation of emotion (e.g. Ochsner, Bunge, Gross, & Gabrieli, 2002; Hariri et al., 2000). Both lesion (e.g. Adolphs et al., 1994) and neuroimaging studies (e.g. Morris et al., 1996) point to the role of the bilateral amygdale as critical for the perception and experience of positive and negative emotion, while the anterior ACC and regions of the PFC, have been implicated in the modulation of this emotional arousal (e.g. Beauregard, Lévesque, & Bourgouin, 2001; Hornack et al., 2003; Ochsner et al., 2002). To date, there exist several theoretical contributions to the literature conceptualizing frontal areas as regulators of the brain’s emotional arousal.
(e.g. Dahl, 2001; Davidson, Fox, & Kalin, 2007; Davidson, Jackson, & Kalin, 2000; Davidson, Putnam et al. 2000; Fox, 1994; Goldsmith & Davidson, 2004; LeDoux, 2000; Lewis & Stiben, 2004; Lewis & Todd, 2007; Ochsner & Gross, 2004; Ochsner & Gross, 2005; Ochsner & Gross, 2007; Thompson et al., 2008) and at least a dozen empirical studies have quantified brain changes in relation to meaningful regulatory behaviors (Beauregard et al., 2001; Goldin, McRae, Ramel, & Gross, 2008; Hare, Tottenham, Davidson, Glover, & Casey, 2005; Hariri et al., 2000; Hariri, Mattay, et al., 2003; Lange et al. 2003; Lévesque et al., 2004; Ochsner et al., 2002; Ochsner et al., 2004, Schaefer, Jackson, Davidson, Aguirre, Kimberg, & Thompson-Schill, 2002; Simpson, Drevets, Snyder, Gusnard, & Raichle, 2000).

Most of the prior studies have found that increased frontal activation associated with cognitive and attentional control mechanisms coupled with decreased amygdala activation underlies the brain’s ability to regulate emotion. To illustrate, Ochsner and colleagues (2002) presented images of negatively arousing scenes to their subjects during functional magnetic resonance imaging (fMRI). Subjects were instructed to view the image and to experience any feelings that the image produced during the first few seconds of presentation. Subjects were then instructed to either “attend” to those emotions or try to reduce negative feelings by “reappraising” the content of the image. Reappraisal is thought to be an effective mechanism for the attenuation of negative feelings accomplished by recasting a negative event in a positive or unemotional way.
Ochsner and colleagues (2002) found less right amygdala and orbitofrontal cortex (OFC) activation when subjects were instructed to reappraise the negative scene rather than attend to their induced emotional state. Further, subjects’ self report of reduced negative feeling correlated with increased ACC activation. The authors suggest that their findings support the critical role of the PFC and ACC in the down-regulation of emotional distress.

Although the current empirical literature defining emotion regulation in the brain generally supports the findings of Ochsner and colleagues (2002), it must be noted that nearly all of the available research studies employ a similar technique of measuring cognitive regulation of emotion. That is, the majority of studies approach the question of emotion regulation in the brain from a perspective of conscious control of emotional arousal, including reappraisal, suppression, and attentional shift strategies (Beauregard et al., 2001; Goldin et al., 2008; Hariri et al., 2000; Hariri et al., 2003; Lange et al., 2003; Lévesque et al., 2004; Ochsner et al., 2002; Ochsner et al., 2004, Schaefer et al., 2002). This strategy serves to eliminate individual differences in emotion regulation by ensuring consistent strategy use across all subjects. To date, few studies have attempted to induce positive or negative emotional states in subjects, and then measured natural regulatory changes independent of experimenter instructions. This gap in the experimental literature is likely due to difficulty accomplishing mood inductions outside of a natural environment, and within the confines of the scanner, and to the challenge of quantifying
regulatory processes in the absence of direct instructions to subjects. Nevertheless, an account of the natural mechanisms for the brain’s regulation of emotional feeling and behavior would be a useful addition to our existing body of knowledge, both to understand individual differences in emotion regulation abilities and possibly to impact our understanding of emotional disorders.

A small number of studies have employed emotion induction techniques during positron emission tomography (PET) scanning to investigate the neural mechanisms of emotional change (Dougherty et al., 1999; George, Ketter, Parekh, Herscovich, & Post, 1996; Kimbrell et al., 1999). These studies have relied on participant generation of emotional scripts, such as recall of previous experience, to galvanize emotional brain activation. Dougherty and colleagues (1999), for example, generated stories based on their subjects’ self report of emotional experiences in their lives. Subjects experienced hyperactivation of the OFC and ACC while listening to their own anger, relative to neutral, experiences, in conjunction with an increase in self-reported anger. It must be taken into account that, although successful in inducing negative emotions, this paradigm does not measure the regulation of emotion directly. That is, it is unknown if some subjects regulated emotion more than others, what regulatory mechanisms were employed, and whether emotion modulation was even necessary in the first place (but see Thompson et al., 2008 for a discussion of regulatory processes embedded within
emotional reactivity). Measurement of individual differences in cerebral mechanisms for emotion regulation requires a different approach.

One developmental study (Lewis, Lamm, Segalowitz, Stieben, & Zelazo, 2006) elegantly measured the regulatory brain effects of emotion induction using event-related potentials (ERPs). Children (ages 5-16 years), engaged in a go/no-go task in which they gained and lost points towards a desired prize. A temporary loss of all points triggered self-reported negative emotions, as well as an increase in frontal P3 response. In addition, source modeling revealed increasing activity in frontal midline regions across age. This effect is hypothesized to parallel increased functioning of the ACC across development, possibly indicating incremental changes in use of this region to regulate emotion. The design of this task allowed for measurement of more automatic and implicit brain mechanisms for emotion regulation, as well as the successful induction of negative emotions during an ecologically valid event relative to the children’s everyday lives: loss of points during a computer game. The present study employed similar procedures within a novel, emotional task to measure adult regulatory brain changes during fMRI scanning.

Finally, the well-replicated findings of increased ACC and PFC activation paired with decreased amygdala activation during regulatory tasks suggest increased functional connections between these key areas. It may be, therefore, that individual differences in efficient functional brain connectivity underlie successful regulatory
functioning. Enhanced understanding of functional connectivity has greatly improved our knowledge of the neural correlates of human information processing and cognitive operations (Finglekurts, Finglekurts, & Kähkönen, 2005; Friston, 1994; Friston, Frith, Liddle, & Frackowiak, 1993). Modern developments in techniques for studying functional and effective connectivity (e.g., Just, Cherkassky, Keller, & Minshew, 2004; Meyer-Lindenberg et al., 2005; Roebroeck, Formisano, & Goebel, 2005) now allow neuroscientists to move toward brain-based, mechanistic theories of cognitive and emotional functioning. However, few studies exist which quantify both regional brain activation and inter-region connectivity in relationship to emotional brain regulation. My study represents an attempt to measure emotional brain activation and effective connectivity in response to regulatory demand. See figure 11 for a depiction of my regions of interest for effective connectivity analysis.

Figure 11: Hypothesized regions of interest for effective connectivity analysis
6.2 Experiment 1: Method

6.2.1 Subjects

Subjects were 25 (13 male, 12 female) English-speaking adults ages 19-41 years with normal or corrected to normal vision. Subjects were recruited from local community postings and internet advertisements and were paid for their participation. In addition, they earned a $10 gift certificate to a local merchant as part of their task participation (see below). Ethical approval for the project was obtained from the Institutional Review Boards of the University of Pittsburgh and Carnegie Mellon University. All subjects provided written informed consent.

6.2.2 Stimuli and Experimental Procedure

Before data collection and practice of the task, subjects were told that they would be playing to win a prize: a $10 gift certificate to the store of their choice. After choosing their desired local merchant, they were told that they would need to earn a large amount of points in order to keep their gift certificate and that they would earn points based on speed and accuracy. Subjects were not told the specific number of points which they would need to win, but if questioned, the experimenter replied, “You need a lot of points. Over a thousand, at least.”. They were reminded that they would not be told whether they won or lost until the end of the game. Subjects, then, practiced the emotional go/no-go task outside of the scanner to ensure understanding during fMRI data collection.
Subjects participated in a novel, event-related within a block designed, emotion regulation task partially adapted from Garavan, Ross, & Stein (1999) and Lewis and colleagues (2006). The emotional go/no-go task was presented using E-prime software (Psychological Software Tools, Pittsburgh, PA) and is depicted in Figure 12. Throughout the paradigm, a stream of pictures of common objects (e.g. balls, shoes, umbrellas) was presented at a rapid rate. Subjects were instructed to press a button when the object was presented in a green frame, but to inhibit their response when the object was presented in a red frame. Incorrect responses to both go and no-go trials were followed by a large “X” on the center of the screen and the sound of a buzzer. Periodically, fearful and neutral faces from the NimStim set of facial expressions (Tottenham et al., in press),
appeared for a one second duration 6-15 seconds apart (120 total trials), accompanied by a short chime. The presentation of the face stimulus represented my point of measurement for the event-related analysis. Subjects were also notified of their total number of accumulated points approximately every 30 seconds throughout the task. As point totals were presented, subjects heard a “slide whistle” sound indicative of point loss or point gain.

Table 11: Stimulus presentation time and point adjustment algorithm for each of three emotion induction blocks. Subjects won points during Block 1, but then lost all earnings in Block 2. During Block 3, which had the same task difficulty level as Block 1, subjects regained their points to win their desired prize.

<table>
<thead>
<tr>
<th>Stimulus Presentation</th>
<th>Block 1 - Winning</th>
<th>Block 2 – Losing</th>
<th>Block 3 – Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correct No-Go Response</td>
<td>-35 ms</td>
<td>-75 ms</td>
<td>-35 ms</td>
</tr>
<tr>
<td>Incorrect No-Go Response</td>
<td>+50 ms</td>
<td>+30 ms</td>
<td>+50 ms</td>
</tr>
<tr>
<td>Points for Go Trial Correct/Incorrect</td>
<td>+1/0</td>
<td>0/-15</td>
<td>+15/+1</td>
</tr>
<tr>
<td>Points for No Go Trial Correct/Incorrect</td>
<td>+2/0</td>
<td>0/-15</td>
<td>+25/+1</td>
</tr>
</tbody>
</table>

This task was designed to maintain the same level of difficulty for all subjects, therefore, an algorithm was built into the design to keep all subjects functioning at approximately the same error rate (50 ± 10%). The error rate for the task was maintained by adjusting the stimulus duration dynamically (see Table 11). Stimulus duration increased with each error made on a no-go trial and decreased with each correct response. Unbeknownst to subjects, the task contained three blocks designed to induce
different types of emotion. Each block lasted approximately six minutes. During Block 1 (Winning), subjects saw their points steadily increase to well over 1000 with a stimulus interval set between 600 – 950 ms. This block was designed to induce positive emotions in my subjects. The task was designed to be difficult throughout, however, changes in the point-adjustment algorithm and stimulus presentation speed (400 – 800 ms) caused increased task difficulty and point loss during Block 2 (Losing), which was intended to induce negative emotion. Subjects lost all of their earlier points and even went into negative territory. With a return to the more generous algorithm in Block 3 (Recovery), subjects regained their points and ultimately won their desired prize. Although there was notable point gain, I designed this block to represent a recovery period after an emotionally negative event. Each of the six-minute blocks served as points of measurement for my block analysis. The experiment was designed with three long blocks in order to allow subjects the necessary time to feel the induced positive or negative emotions due to lengthily periods of gaining or losing points, respectively.

6.2.3 fMRI Data Acquisition

Scanning was performed on a Siemens 3 Tesla Allegra head-only scanner (Siemens, Erlangen, Germany). High-resolution, T1-weighted anatomical images were acquired using an MPRAGE sequence (TR = 1630 ms; TE = 2.48 ms; FOV = 20.4 cm; $\alpha = 8^\circ$; image matrix = 256$^2$; voxel size = $0.8 \times 0.8 \times 0.8$ mm; 224 slices). Whole-brain functional images were acquired using a single-shot, gradient-recalled echoplanar pulse...
sequence (TR = 2000 ms; TE = 30 ms; α = 73°; FOV = 20.4 cm; image matrix = 64²; voxel size = 3.2 × 3.2 × 3.2 mm; 35 slices) sensitive to blood oxygenation level-dependent (BOLD) contrast. Runs consisted of the acquisition of 224 successive brain volumes beginning with 2 discarded RF excitations to allow for steady-state equilibrium.

The BrainVoyager QX software package (Brain Innovations, Maastricht, The Netherlands) was used for all analyses. The following preprocessing procedures were performed on raw images prior to data analysis: slice scan time correction (using cubic spline interpolation), high-pass temporal filtering to remove nonlinear drifts of three or fewer cycles per time course, spatial data smoothing using a Gaussian kernel with a 4 mm full width at half-maximum, and three-dimensional motion correction to detect and correct for small head movements by spatial alignment of all volumes to the first volume by rigid body transformation. Head movements never exceeded 3 mm for all subjects included in this analysis. Functional data were coregistered to the anatomical volume by alignment of corresponding points to obtain optimal fit and were then transformed into Talairach space.

To test my signal coverage across the full brain, data from all adult and child subjects (see experiment 2) were tested at a low threshold for statistical significance. As shown in Figure 13, I had full coverage of the amygdalae in both child and adult subjects. The data did display significant signal loss in the OFC.
Figure 13: Full-brain data of adult and child subjects set at a low threshold. I was able to successfully image the amygdalae, however, signal loss in the OFC was observed.

6.3 Experiment 1: Results

6.3.1 Manipulation Check

In order to ensure that subjects felt a mix of both positive and negative emotions, a separate sample of 23 subjects filled out a brief questionnaire after participation in the emotional go/no-go task. One hundred percent of subjects reported that they had felt at least some positive emotion during the task. Sixteen subjects (70%) reported that they had felt negative emotions during the task. Eighty-three percent reported that there were moments when they did not believe that they would win their prize. More than half of subjects (61%) reported that they felt the need to regulate their emotions at some
point during the task. Finally, when specifically examining regulatory strategy use, 78% reported that they used a suppression strategy (i.e. tried to suppress negative feelings) at some point during the task. In addition, 81% of subjects reported the use of a reappraisal strategy for emotion regulation (i.e. tried to change negative thoughts into positive thoughts).

6.3.2 Event-Related Analysis

I first analyzed all data at the whole brain level. Figure 14 displays full brain activation to the one second presentation of fearful faces across blocks. Significant activation from baseline was found in posterior visual areas in response to the face presentation. Also, deactivation from baseline was observed in prefrontal region such as the dorsal ACC and DLPFC. This deactivation may represent a default network (see Raichle & Snyder, 2007) during a one second break from a taxing cognitive task. The deactivation to faces observed in my study was centered at the DLPFC, an area known to be highly active in go/no-go tasks (see Simmonds, Pekar, & Motofsky, 2008). One second fixation upon the face may have caused subjects to briefly disengage from the task, leading to a brief but powerful deactivation of this region.
Next, it employed a hypothesis-driven anatomical region-of-interest (ROI) approach to analysis of this data set. Using the standard Analysis of Functional Neuroimages ROIs (AFNI; National Institutes of Mental Health; Bethesda, MD), I localized areas within the bilateral amygdala and lateral FFG that were responsive to the presentation of faces (regardless of emotion) across the entire task \([t = 2.28, p < .03, q < .05]\). Areas that were active to both neutral and fearful faces are depicted in Figure 15.

For this analysis, from which I derived my functional ROIs for subsequent analyses, I used the false discovery rate procedure (Genovese et al. 2002) \([\text{FDR}(q) < .05]\) to control for multiple statistical comparisons. Within my functional ROIs, I found greater activity in a region of the right amygdala for fearful, relative to neutral, faces \([t = 2.58, p < .01, \text{uncorrected}]\).
Next, I looked at each block individually to investigate the effects of emotion induction on the processing of fearful and neutral faces (40 faces per block, 20 fearful/20 neutral). All three blocks localized a region of the bilateral FFG that was responsive to the brief face presentation [Block 1: $t = 2.54, p < .02, q < .05$; Block 2: $t = 2.60, p < .01, q < .05$; Block 3: $t = 2.22, p < .03, q < .05$; see Figure 16]. This activation was essentially uniform, producing no significant differences between blocks [rFFG: $F = 1.49, p = .18$; lFFG: $F = 1.140, p = .34$]. Within the bilateral amygdala, we found activation to faces,
however, this activation differed as a function of block [right amygdala: $F = 3.11, p < .001$; left amygdala: $F = 2.68, p < .01$]. The amygdala were significantly less active to faces in Block 2 (Losing) than to faces presented in Blocks 1 and 3 [$t = 1.96, p < .05$, uncorrected]. Finally, a comparison between Block 1 (Winning) and Block 3 (Recovery) revealed significantly greater face-related activation of the right and left amygdala in Block 3 [$t = 1.96, p < .05$, uncorrected].

In addition, I found that differential activation to fearful versus neutral faces changed across blocks. In Block 1, the bilateral amygdala responded more to fearful than neutral faces [$t = 1.96, p < .05$, uncorrected]. However, in Block 2, both the right and left amygdala displayed equal activation to fearful and neutral faces. Finally, during Block 3, I again found that the bilateral amygdala responded more to fearful than neutral faces [$t = 1.96, p < .05$, uncorrected].
A second event-related analysis was completed to investigate brain activation unrelated to the presentation of the face. Here, I examined the 1 second period of time preceding the face presentation for each block. As expected, in comparison to the face presentation, I saw significantly less activation in the bilateral amygdala and bilateral FFG in the 1 second preceding the face \((t = 2.76, p < .006, q < .05)\). In addition, I found notable change in prefrontal activation across blocks. In Block 2 (Losing), I observed an increase in ventromedial prefrontal cortex (VMPFC) and dorsal and subgenual ACC activity before the face was presented in comparison to the Winning Block 1 \((t = 1.96, p < .05, \text{uncorrected})\). Comparing Block 1 (Winning) and the similarly challenging Block 3 (Recovery), I saw increased activation in the pre-face period in Block 3 in both the
VMPFC and bilateral amygdala ($t = 1.96, p < .05, \text{uncorrected}$), but no significant difference was observed in the ACC.

**6.3.3 Block Analysis**

In order to inspect differences in brain functional connectivity between task blocks, an analysis was performed comparing activity between the Winning, Losing, and Recovery blocks. Within the emotional go/no-go task, each block lasted approximately 6.25 minutes. To ensure that sufficient emotion was induced during my time of measurement, the first minute of each block was excluded from analysis. Therefore, taking into account individual differences in subject task duration, Block 1 (Winning) was defined as minutes 1-6.25, Block 2 (Losing) was defined as minutes 7.25-12.35, and Block 3 was defined as minutes 13.35-18.70.
Figure 17: Granger causality mapping was employed to estimate differences in effective connectivity across blocks. Green represents active areas preceding right amygdala activity in each block shown in axial, sagital, and coronal orientations \((x = 3, y = 43, z = -6)\). Peak ACC connectivity voxel (Block 2: BA 32, \(x = -3, y = 41, z = -13\)).

The right amygdala activation related to presentation of faces across blocks (see Figure 15) was selected as a reference region to map sources of influence. Granger Causality theory states that a discrete time series \(X\) “Granger-causes” a discrete time
series $Y$ if the past values of $X$ improve the prediction of the current value of $Y$, given that all other sources of influence have been taken into account (Roebroeck et al., 2005). Temporal information from the data is thus used to define direction of influence without establishing a model of assumed regional connectivity. There is some debate in the literature concerning the validity of the Granger Causality method for measuring temporal precedence amongst functionally connected regions (see David et al., 2008). Due to variation in the hemodynamic response between regions, GCM may determine temporal precedence between regions in which neuronal firing is instantaneously coupled. This limitation to the GCM method can, however, be mitigated by examining differences in GCM maps between two or more experimental conditions. This approach was taken in the current study.

My Granger Causality analysis was conducted at the group level to generate a $t$ statistic image of the Granger Causality Map for each block. For each granger map, $p$-values were subjected to a multiple-comparison correction (FDR($q$) < .05; Genovese et al., 2002). A priori hypotheses led me to focus interest on the ACC. During Block 1, I did not observe significant effective connectivity between the amygdala and ACC or any other regions (see Figure 17). However, in Block 2, I observed a striking increase in connectivity, indicating that activity in the ACC/VMPFC preceded the rise and fall of the amygdala. During Block 3, significant ACC connectivity remained and did not return to Block 1 levels. Activity in a portion of the ACC preceded amygdala activity. I
also examined the portion of the rFFG that was active to faces across blocks (see Figure 15) as a seed region for effective connectivity to and from the amygdala and ACC. I did not observe significant connectivity between the rFFG and amygdala or ACC. See Figure 18 for a depiction of connectivity among regions for each block.

Figure 18: Connectivity amongst regions of interest during each block. Score represents peak connectivity voxel.

6.4 Experiment 1: Discussion

My study provides additional support for earlier models of brain mechanisms for emotion regulation by replicating findings of decreased amygdala activation coupled
with increased prefrontal activation during emotional demand. Amygdala responses to both fearful and neutral faces varied as a function of emotion induction across the three blocks of my task. My study also addressed hypotheses of increased connectivity during emotion regulation. Effective connectivity analyses found heightened coupling of the amygdala and frontal regions during episodes of increased regulation in response to task demands.

Replicating the findings of previous studies (e.g. Ochsner et al., 2002), I found changes in amygdala activation in relation to moments of emotion regulation during my novel, emotion induction task. Although I found equivalent FFG responses to faces across all three blocks of the task, amygdala activation varied throughout. During Block 1, while subjects were steadily gaining points and likely experiencing positive emotions, the bilateral amygdala was responsive to all faces. In addition, the amygdala differentiated fearful and neutral faces and displayed the typical response of increased activation to fearful faces (e.g. Morris et al., 1996). During Block 2, however, task difficulty increased and subjects lost all previously earned points. Right amygdala activation decreased to baseline levels in response to the presentation of faces, likely reflecting down-regulation of the amygdala driven by increased activity in frontal regions. The left amygdala was equally responsive to both fearful and neutral faces. Finally, when task difficulty returned to the more generous Block 1 levels, I observed a surge in both left and right amygdala activity. As subjects regained points towards their
desired prize in Block 3, the bilateral amygdala again responded more to fearful than neutral faces. It is interesting to note that although Blocks 1 and 3 were equal in terms of task difficulty, emotional context differentiated these episodes. During Block 3, subjects had recently experienced frustration during point loss and were unsure that they would win their desired prize. In the absence of frontal down-regulation, due to lowered task difficulty, a tense/nervous emotional state may have caused the observed surge in amygdala response to faces.

Although my data replicate the findings of previous studies relating to amygdala modulation during regulation of emotion (e.g. Ochsner et al., 2002; Lange et al., 2003; Schaefer et al., 2001), my study differed in that it employed a unique method to investigate the affective change in the absence of cognitive control. That is, the studies cited above encouraged subjects to consciously regulate emotion through the modification of task instructions (e.g. Lange et al., 2003), reappraisal of negative images (e.g. Ochsner et al., 2002), or even directly instructed subjects to regulate emotion (e.g. Schaefer et al., 2001). My study staged a realistic situation (loss of points during a computer game) to manipulate mood and lead subjects to regulate emotion in a more implicit fashion. While I did note significant brain changes across emotion induction blocks, my study relied upon self-directed mechanisms for emotion regulation and, thus, was not able to measure specific affective modulation techniques such as reappraisal or attentional deployment used by individual subjects. Therefore, my results may be
diluted somewhat by large, unmeasured individual differences in strategy use. Future studies are planned to address the role of individual differences in brain mechanisms for emotion regulation independent of task instructions, which may serve to elucidate differential functioning in emotional disorders.

My study also extended previous experimental designs by measuring the brain’s reaction to task-irrelevant, but highly significant social stimuli. Whether employing images of facial expressions (e.g. Hariri et al., 2000; Lange et al., 2003), emotional scenes (e.g. Lévesque et al., 2004; Ochsner et al., 2002), personal narratives (e.g. Dougherty et al., 1999; Kimbrell et al., 1999), or emotional movies (e.g. Goldin et al., 2008) other fMRI studies to date have focused on the brain’s regulation of affect induced by task-related stimuli. During my emotional go/no-go task, subjects were directed to respond to the colored frames surrounding images of everyday objects. When presented with a task-irrelevant faces (with no color frame), subjects were told to simply view the face as they normally would. Both lesion (Malloy, Bihrir, & Duff, 1993) and fMRI studies (Casey et al., 1997) have documented the importance of frontal brain regions during go/no-go tasks, however, it has previously been unknown exactly how frontal regulation due to task demands would affect the brain’s processing of non-task related social stimuli. My results demonstrate that increased task difficulty and/or negative mood might affect emotional brain responses to a task irrelevant stimulus (the amygdala), but not necessarily the cortical response to the stimulus itself (the FFG).
In contrast to previous studies (e.g., Hariri et al., 2000; Lange et al., 2003), my paradigm utilized emotional face stimuli not as a method of mood induction, but as a point of measurement for the effects of induced affect on processing of an emotional stimulus. Faces, especially fearful faces, are known to be potent activators of the amygdala (e.g., Morris et al., 1996). My study found some evidence of attenuated amygdala activity and lessened differentiation of emotion as a function of the manipulation of frustration. During both winning and recovery, the bilateral amygdala responded more to fearful than neutral faces, however this was not the case during the more frustrating, emotionally demanding losing block. The lateral FFG, which is known to be a face-selective region (Kanwisher, McDermott, & Chun, 1997; Puce, Allison, Asgari, Gore, & McCarthy, 1996), responded equally to face stimuli regardless of induced mood. It may be, therefore, that regulation of emotion changes our processing of the emotional states of others, but not necessarily the identification of faces in the environment. It must be noted, however, that this study does not necessarily qualify amygdala response as related specifically to social stimuli. Both the amygdala and skin conductance responses are greater to emotional facial expressions than emotional scenes, possibly relating to the social and evolutionary importance of a fearful face (Hariri, Tessitore et al., 2002), but my study did not test the effects of mood induction on the brain’s processing of other types of emotional stimuli. Future research will be needed to ascertain the nature of emotion induction on social processing and to possibly extend
these results to other emotionally relevant stimuli such as scenes, recall of negative personal events, or sound clips.

My study investigated enhanced functional brain connectivity as a mechanism for emotion regulation. Using a relatively new method for quantifying effective connectivity to the amygdala (Granger Causality; Roebroeck et al., 2005) I found that ACC activity immediately preceded amygdala activation, but only during episodes in which subjects were expected to regulate emotion. During Block 1, I did not observe effective connectivity between the amygdala and ACC, nor any other region. In response to the change in task difficulty and loss of desired points during Block 2, the GCM analysis produced a section of the dorsal/ventral ACC in which activation immediately preceded the attenuated response to emotional faces. Finally, in Block 3, task demand lessened to Block 1 levels, but the ACC connectivity remained present, coupled with a surge in amygdala response to faces. These changes in connectivity between the amygdala and ACC occurred independently of general ACC activation which was equal in Blocks 1 and 3, but demonstrated a slight increase during the more challenging Block 2. The ACC is hypothesized to be a brain region specialized for performance monitoring and error detection (Carter, Braver, Barch, Botvinick, Noll, & Cohen, 1998), but also important in the processing and top-down regulation of emotion (Bush et al., 2000). Therefore, increased ACC-amygdala connectivity in Block 2 may have been driven by increased ACC activation in relation to error monitoring during the
exceptionally difficult episode. However, Block 1 and Block 3 demonstrated a marked difference in ACC-amygdala connectivity despite task difficulty and error rate being equal across these blocks. Increased effective ACC connectivity in Block 3 may represent regulation of emotional brain regions during negative mood, in comparison to the positive mood induced in Block 1.

In conclusion, my study employed a novel emotion regulation paradigm to replicate previous findings of variability in amygdala reactivity during emotion regulation, but I also demonstrated a relationship between modulation of induced mood and ACC-amygdala connectivity. My study implicates increased connectivity as a mechanism for regulation of emotion in the brain. Given previous research relating abnormal functional connectivity to cognitive deficits in disorders such as Autism (Just et al, 2004), Schizophrenia (Meyer-Lindenberg et al., 2001), and Alzheimer’s Disease (Grady, Furey, Pietrini, Horwitz, & Rapoport, 2001) my findings have the potential to inform our understanding of regulatory brain deficits in emotional disorders. Future research is planned to address how regulatory brain connectivity might vary with personality, what genetic bases may underlie these changes, and how the child brain might develop increased connectivity to support both well-regulated and disordered emotional behavior.
6.5 Experiment 2: Introduction

Proficiency in the management of affective arousal is a critical aspect of early emotional development. During childhood, infants move from a necessary dependence upon the emotional support of their caregivers (Rothbart, Ziaie, & O’Boyle, 1992) to the development of self-sufficient mechanisms for emotion regulation at school age (Denham, 1998). Thus, characterizing individual differences in the typical and atypical development of affective regulation has become a well-established area of psychological study (see Cole, Martin, & Dennis, 2004). Behavioral studies have examined both children’s control of overt emotional behavior (Cole, 1986; Saarni, 1984) and modulation of subjective emotions (Harris, Olthof, & Meerum Terwogt, 1981). However, it is not until recently that researchers have begun to examine brain mechanisms for the development of emotion regulation (for reviews see Lewis & Stiben, 2004; Lewis & Todd, 2007; Thompson et al., 2008).

Numerous investigations have characterized emotional reactivity and regulation in the adult brain. Both lesion (e.g. Adolphs et al., 1994) and neuroimaging studies (e.g. Morris et al., 1996) point to the role of the bilateral amygdale as critical for the perception and experience of positive and negative emotion, while the ACC and regions of the PFC, have been implicated in the modulation of this emotional arousal (e.g. Beauregard et al., 2001; Lange et al., 2003; Ochsner et al., 2002). To illustrate, Ochsner and colleagues (2002) presented images of negatively arousing scenes to their subjects
during fMRI. Subjects were instructed to view the image and to experience any feelings
that the image produced during the first few seconds of presentation. Subjects were
then instructed to either “attend” to those emotions or try to reduce negative feelings by
“reappraising” the content of the image. Ochsner and colleagues (2002) found less right
amygdala and OFC activation when subjects were instructed to reappraise the negative
scene rather than attend to their induced emotional state. Further, subjects’ self report of
reduced negative emotion correlated with increased ACC activation. A similar study
conducted with child subjects (Lévesque et al., 2004) also found evidence for increased
PFC and ACC activation during suppression of emotion while watching sad film clips.
Taken together, these findings support the critical role of the PFC and ACC in the down-
regulation of emotional distress in both adults and children; however, the mechanisms
by which these regulatory processes develop have yet to be determined.

One developmental study (Lewis et al., 2006) elegantly measured the regulatory
effects of emotion induction using event related potentials (ERPs). Children (ages 5-16
years), engaged in a go/no-go task in which they gained and lost points towards a
desired prize. A temporary loss of all points triggered self-reported negative emotions,
as well as an increase in frontal P3 response. In addition, source modeling suggested
localization of this increased response to frontal midline regions across age. This effect
was hypothesized to parallel increased functioning of the ACC across development,
possibly indicating incremental changes in the recruitment of this region to regulate
emotion. Notably, the design of this task allowed for measurement of more automatic and implicit brain mechanisms for emotion regulation, as well as the successful induction of negative emotions during a relatively ecologically valid event: loss of points during a computer game.

A recent fMRI study conducted in my own laboratory employed a modified version of this novel task in a sample of adults to identify brain mechanisms for emotion regulation (Perlman & Pelphrey, under review). Adapting the paradigm of Lewis et al. (2006), participants played a game in which fearful faces were presented throughout blocks of winning and losing points towards a desired prize. I conceptualized difference in brain reactivity across block to be representative of emotion regulation. When observing the responses of the brain’s face processing system to the emotional faces, I replicated the pattern of increased prefrontal activity and decreased amygdala activation during episodes in which subjects were required to regulate emotion. In addition, I used effective connectivity analyses (Roebroeck et al., 2005) to investigate differential correlations between the activity profiles of key emotional brain areas in response to the regulatory demands of my task. We found reliable increases in effective connectivity between the ACC and the amygdala during periods of increased demand for enhanced emotion regulation. The current study employed a similar paradigm, with modifications to make it more effective for children, to investigate the development of
and individual differences in brain mechanisms for emotion regulation in a sample of school-age children.

Brain-based, biological accounts have, thus far, emphasized two theoretical approaches to characterizing the development of emotion regulation. First, researchers have probed the influence of individual differences in temperament on the brain’s ability to control emotional arousal in infancy and childhood (Rothbart & Sheese, 2006). Fox and his colleagues (for reviews see Fox, 1994; Fox & Calkins, 2003) have implicated the construct of electroencephalogram (EEG) asymmetry in individual differences in children’s temperament, emotional reactivity, and regulation. Specifically, right-lateralized frontal EEG asymmetry has been related to increased fearful temperament and impaired regulation of fear during social interaction (Fox et al., 1995). In a related line of fMRI research, adolescents who had been temperamentally classified as behaviorally inhibited displayed heightened amygdala activation while rating the emotional content of face stimuli (Pérez-Edgar et al., 2007). This amygdala hyperprocessing of emotional faces may be related to their difficulties in effective regulation during social engagement, either by underlying deficits in emotional behavior or resulting from them. Taken together, these studies describe a theoretical viewpoint in which the development of emotion regulation is represented through stability in children’s temperament and its related brain changes.
The second approach to understanding the development of the neural basis of emotion regulation concerns the development of the PFC and its relationship to executive functioning (see Casey, Geidd, & Thomas, 2000). The PFC is the last brain area to fully develop, with continued synaptogenesis (Rakic, Bourgeois, & Goldman-Rakic, 1994) and increasing white matter volume (Pfefferbaum, Mathalon, Sullivan, Rawles, Zipursky, & Lim, 1994; Shaw et al., 2006) until late adolescence or early adulthood. This late occurring growth has been linked to the development of various complex cognitive skills (Caviness, Kennedy, Richelme, Rademacher, & Filipek, 1996; Diamond, 1988; Shaw et al., 2006), which are known to be important for the modulation of affective arousal (Gross, 2002). Various fMRI studies investigating the development of self-regulation have demonstrated that differential recruitment of regions of the PFC may underlie decreased cognitive control relative to adult subjects (Bunge, Dudukovic, Thomason, Vaidya, & Gabrieli, 2002; Casey et al., 1997; Luna et al., 2001). Casey and colleagues (1997), for example, found that children recruited the same prefrontal regions as adults during a go/no-go task. Activation was, however, greater in children than adults, demonstrating inefficient recruitment of the PFC in development, a finding replicated by Lewis et al. (2006) in their ERP go/no-go task. Further, activity in the ACC correlated with error rates, a finding supported by error monitoring accounts of the ACC (Carter et al., 1998). In the present study, I incorporated aspects of each theoretical approach in designing a novel emotion regulation paradigm for fMRI to characterize the regulatory
roles of the PFC and ACC across childhood as well as to investigate the influence of individual differences in effects of temperament on mechanisms for affective regulation.

6.6 Experiment 2: Method

6.6.1 Subjects

Subjects were 20 (9 male, 11 female) English-speaking children ages 5-11 years (average age = 8 years, 2 months) with normal or corrected-to-normal vision. An additional 6 children were scanned, but not included in data analyses due to excessive head movement (4), inability to complete the scan (1), or drowsiness (1). These subjects did not differ from subjects with usable data in age \[t(24) = -.19, p = .85\] or on any dimension of temperament (see below) \[t(24) = -.50-1.83, p = .08-.97\]. Subjects were recruited from local community postings and internet advertisements and were paid for their participation. In addition, they earned an age appropriate prize as part of their task participation (see below). Ethical approval for the project was obtained from the Institutional Review Boards of the University of Pittsburgh and Carnegie Mellon University. A parent or legal guardian of each subject provided written informed consent for their child’s participation. Each child provided written assent.

6.6.2 Stimuli and Experimental Procedure

Before data collection and practice of the task, children were told that they would be playing to win a prize: their choice of toy from a large toy collection. After choosing their desired prize, children were given a few minutes to play with the toy and show it
to their parent or siblings. They were then told that they would need to earn a large amount of points during the game in order to keep their prize and that they would earn points based on speed and accuracy. Children were not told the specific number of points which they would need to win, but if questioned, the experimenter replied, “You need a lot of points. Over a thousand, at least.”. They were reminded that they would not be told whether they won or lost until the end of the game. Subjects, then, practiced the emotional go/no-go task outside of the scanner to ensure understanding during fMRI data collection.

Before scanning began, children participated in a “mock scanning” session to ensure compliance with the requirement to remain motionless during data collection. Children were trained to remain still while watching a children’s video inside a replica of my MRI scanner. During practice, custom-written software received input from a head motion sensor worn by the child and used that input to play a sound when they moved outside of a set threshold (3mm). In addition to viewing approximately 10 minutes of the video, children, again, practiced the go/no-go task inside the mock scanner. With the addition of realistic scanner sounds played during children’s practice session, I was able to reproduce the scanning environment and acclimate my young subjects to fMRI scanning procedures.
Table 12: Speed and point adjustment algorithm for child emotional go/no-go task.

<table>
<thead>
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<tr>
<td>Points for No Go Trial Correct/Incorrect</td>
<td>+7/0</td>
<td>0/-15</td>
<td>+25/+1</td>
</tr>
</tbody>
</table>

Subjects participated in a novel, event-related within a block designed, emotion regulation task which was similar to the study described above (Perlman & Pelphrey, under review). The task, however, was modified for child participation. The design of the speed and point adjustment algorithm for this task was normed on 25 5-year-olds outside of the scanner to ensure ability to complete this game at the 5-year-old level (see Table 12 for details of the speed and point adjustment algorithm for children). Each block lasted approximately four minutes long for children and was designed to induce similar emotions to adult version.

6.6.3 Temperament Data Collection

During children’s participation in the project, caregivers we asked to provide information on their children’s temperament. They completed the Child Behavior Questionnaire (CBQ)- Long Form (Rothbart et al., 2001) which is an experimentally-validated and commonly used caregiver assessment of 15 dimensions of children’s temperament.
6.6.4 Genetic Data Collection

Eighteen participants donated saliva as deoxyribonucleic acid (DNA) samples. DNA extraction from buccal epithelial cells was performed using the Oragene DNA isolation kit and high molecular weight DNA was extracted using the manufacturer’s instructions. Subjects were genotyped for the 5-HTTLPR variable-number-tandem-repeat polymorphisms by the method of Wendland, Martin, Kruse, Lesch, & Murphy (2006) using PCR/RFLP and resolution on agarose gel to classify the LA, LG and S alleles. Alleles were classified as either high-(LA) or low-expressing (LG and S). Using this functional allelic nomenclature, we will explored the differential activity across genotype classifications: “high expressing” (La/La), “intermediate” (La/S, La/Lg) and “low expressing” (S/S, Lg/S, Lg/Lg).

6.6.5 fMRI Data Acquisition

fMRI data Acquisition for this study was equal to the procedure described in the previous experiment. Due to the absence of a standard child brain template, child data were normalized to the adult brain in Talairach space. Previous studies have determined that child brain normalization to an adult template results in only marginal between group differences differences. This negligible variability is unlikely to produce spurious effects in child structural (Burgund et al., 2002) or functional (Kang, Burgund, Lugar, Petersen, & Schlaggar, 2003) imaging data.
6.7 **Experiment 2: Results**

6.7.1 **Manipulation Check**

In addition to adult reports of emotion induction and regulation described above, my child subjects reported on their impressions of the task. One hundred percent of children recalled losing all their points during the game and 95% (all except one child) reported feeling negative emotions during that episode. Ninety percent reported feeling positive emotions when they discovered that they would, indeed, win their prize. Finally, 74% of children reported an overall enjoyment of the game.

6.7.2 **Event-Related Analysis**

I completed all analyses at the whole brain level, but then employed a hypothesis-driven anatomical region-of-interest (ROI) approach. Using the standard Analysis of Functional Neuroimages ROIs (AFNI; National Institutes of Mental Health; Bethesda, MD), I localized areas within the bilateral amygdala and lateral fusiform gyrus (FFG) that were responsive to the presentation of faces (regardless of emotion) across the entire task \[ t = 2.07, p < .04, q < .05 \]. Areas that were active to fearful faces are depicted in Figure 19. For this analysis, from which I derived my functional regions of interest for subsequent analyses, I used the false discovery rate procedure (Genovese et al., 2002) \[ \text{FDR}(q) < .05 \] to control for multiple statistical comparisons.
Figure 19: Regions of the child face processing system active to the 1 second presentation of faces across all blocks. Left amygdala peak voxel (x = -21, y = -4, z = -11); Lateral rFFG peak voxel (x = 36, y = -46, z = -14).

Next, I looked at each block individually to investigate the effects of emotion induction on the processing of fearful faces (20 faces per block). In each of the three blocks, I was able to localize a region of the rFFG that was responsive to the brief presentation of faces [Block 1: $t = 2.46, p < .01, q < .05$; Block 2: $t = 2.39, p < .02, q < .05$; Block 3: $t = 2.44, p < .01, q < .05$; see Figure 20]. Within the left amygdala, I found activation to faces, however, this activation differed as a function of block. Significant activation was found in Blocks 1 and 2, but not in Block 3 [Block 1: $t = 2.46, p < .01, q < .05$; Block 2: $t = 2.39, p < .02, q < .05$].
Figure 20: Regions of the child face processing system active to the 1 second presentation of faces for each emotion induction block. FFG activity remained equal across blocks, but amygdala activity varied according to task demand.

A second event-related analysis was completed to investigate brain activation unrelated to the presentation of the face. Here, I examined the 1 second period of time preceding the face presentation for each block. As expected, in comparison to the face presentation, I saw significantly less activation in the left amygdala and rFFG in the 1 second preceding the face ($t = 2.34$, $p < .02$, $q < .05$). In addition, I found notable change in prefrontal activation across blocks. In Block 2 (Losing), I observed an increase in VMPFC and OFC activity before the face was presented in comparison to the Winning Block 1 [$t = 1.96$, $p < .05$, uncorrected]. Comparing Block 1 (Winning) and the similarly challenging Block 3 (Recovery), saw increased activation in the pre-face period in Block
3 in the VMPFC \( [t = 1.96, p < .05, \text{uncorrected}] \). No significant differences across blocks were found in the ACC.

### 6.7.3 Age and Temperament Correlations

#### Age

Using an ANCOVA with random effects, I investigated differences across blocks in my specified regions of interest as a function of age and temperament. Here I looked at the difference between Block 3 and Block 1 to examine regions sensitive to my mood induction (see Figure 20). Child age (in months) was first included as a covariate. This analysis identified a section of the dorsal ACC in which the level of activity correlated positively with a child’s age \( [r(18) \geq .50, p < .05] \), as well as a section of the subgenual ACC in which activity levels correlated negatively with age \( [r(18) \geq -.50, p < .05] \). In addition, the change in face related activity from Block 1 to Block 3 in portions of the bilateral amygdale correlated positively with child age \( [r(18) \geq .50, p < .05] \).

#### Fearful Temperament

In addition, I examined the fearful dimension of temperament as indexed by parent report on the CBQ (Rothbart et al., 2001) in relation to activity evoked by the presentation of faces during mood induction. My analysis revealed a portion of the dorsal ACC in which activity levels correlated negatively with fearfulness \( [r(18) \geq -.50, p < .05] \) and a portion of the ventral ACC in which activity correlated positively with this dimension of temperament \( [r(18) \geq .50, p < .05] \). Activity in a small segment of the right
amygdala correlated negatively with the change in activity from Block 1 to Block 3.

Child age and fearful temperament were uncorrelated in my sample \( r(18) = -0.04, p = 0.87 \).

Figure 21: Correlations between age and temperament and the change in activation between winning and recovery blocks. Red represents positive correlation and blue represents negative correlation.

\textit{Serotonin Transporter Gene}

Finally, I examined correlations between the serotonin transporter gene and activation change from Block 1 to Block 3 (see Figure 22). I found portions of the ventral
and subgenual ACC and a small portion of left amygdala which correlated positively with serotonin transporter gene expression. Higher expressers displayed increased activation in these areas. When examining only Block 2 (losing) I found areas of the ventral ACC which correlated positively, along with a small region of the right amygdala which correlated negatively with the serotonin serotonin transporter gene expression.

Figure 22: Correlations between brain activation and serotonin transporter expression. Red represents positive correlation and blue represents negative correlation.
6.7.4 Block Analysis

To evaluate differences in brain functional connectivity between task blocks, an analysis was performed comparing activity among the Winning, Losing, and Recovery blocks. Within the emotional go/no-go task, each block lasted approximately 4 minutes. To ensure that sufficient emotion was induced during my time of measurement, the first 30 seconds of each block were excluded from analysis. Therefore, taking into account individual differences in subject task duration, Block 1 (Winning) was defined as minutes 0.5-4, Block 2 (Losing) was defined as minutes 4.5-8, and Block 3 was defined as minutes 8.5-12.

The left amygdala activation that was found to be related to the presentation of faces across blocks (see Figure 19) was first selected as a reference region in order to map sources of influence. *A priori* hypotheses led me to focus interest on amygdala to ACC/VMPFC connectivity. During Block 1, I did not observe significant effective connectivity between the left amygdala and ACC or any other regions (see Figure 23). However, in Block 2, I observed a striking increase in connectivity, indicating that activity in the VMPFC preceded the rise and fall of the left amygdala. During Block 3, significant ACC connectivity emerged as VMPFC connectivity increased. Activity in the ACC and VMPFC preceded left amygdala activity.

I also examined the portion of the rFFG that was active to faces across blocks (see Figure 19) as a seed region for effective connectivity to and from the amygdala and
ACC. I found evidence for connectivity in the bilateral amygdalae and hippocampus in all blocks. In Block 3, this connectivity seemed to decrease slightly and was localized to the right amygdala/hippocampus. Right FFG activity preceded activity in the amygdala and hippocampus throughout the task.

Figure 23: Granger causality mapping was employed to estimate differences in effective connectivity across blocks. Green represents active areas preceding left amygdala activity in each block. Peak ACC connectivity voxel (Block 3: BA 32, x = -6, y = 38, z = 13). Blue represents active areas following rFFA activity in each block. Peak right amygdala/hippocampus connectivity voxel (Block 3: x = 27, y = -19, z = -17).
6.8 Experiment 2: Discussion

My study provides additional support for earlier adult models of brain mechanisms for emotion regulation by replicating findings of decreased amygdala activation coupled with increased prefrontal activation during emotional demand in children. Amygdala responses to fearful faces varied as a function of emotion induction across the three blocks of my task. Further, I found support for differential prefrontal activation relating to both children’s age and temperament. Finally, my study addressed hypotheses of increased connectivity during emotion regulation in children. Effective connectivity analyses found heightened coupling of the amygdala and frontal regions during episodes of increased regulation in response to task demands.

Replicating the findings of previous studies (e.g. Ochsner et al., 2002), I found changes in amygdala activation in relation to moments of emotion regulation during my novel, emotion induction task. Although I found equivalent rFFG responses to faces across all three blocks of the task, left amygdala activation varied throughout. During Block 1, while subjects were steadily gaining points and likely experiencing positive emotions, the left amygdala was responsive to fearful faces. In Block 2, task difficulty increased and subjects lost all previously earned points. The left amygdala remained active in children, contrary to adults whose activation decreased to baseline levels in response to the presentation of faces during this task (Perlman & Pelphrey, under review). Finally, when task difficulty returned to the more generous Block 1 levels, I
observed a surge in both left and right amygdala activity in my previously tested adult subjects, but the amygdala response was depressed, likely reflecting down-regulation of the amygdala driven by increased activity in frontal regions. It is interesting to note that although Blocks 1 and 3 were equal in terms of task difficulty, emotional context differentiated these episodes. During Block 3, subjects had recently experienced frustration during point loss and were unsure that they would win their desired prize. The increase in frontal activation in Block 2, due to increased task difficulty or a tense/nervous emotional state, did not suppress left amygdala activation. However, although Block 3 provided the same level of task difficulty as Block 1, prefrontal regions increased activity and left amygdala activation returned to baseline. This effect may have been driven by increased demand for emotion regulation in order to win a desired prize.

Differential patterns of activity between children on this task and adults in my previous study (Perlman & Pelphrey, under review) may reflect differences in motivation or, possibly, regulatory strategy. The current version of this task was adapted for young children and, thus, provided a lower level of difficulty and shorter task duration. I, therefore, did not directly contrast activation in adults and children. Nevertheless, differences in amgydala and related PFC activation between adults and children may be due to differential motivation towards the chosen prize. Adults subjects were playing to win a $10 gift certificate to the store of their choice. In addition
to their standard subject payment for MRI study participation (~ $50), adults might not have been quite as motivated as children, who were playing to win the toy of their choice. Therefore, adults may have been most frustrated during the exceptionally challenging Block 2 simply due to task demands, but children may have found the uncertainty of winning their toy after a major loss (during Block 3) to require the most regulatory demand. In addition, children (across age) and adults are known to differ in their regulatory strategy use (Gross, 1998; Harris et al., 1981), thus changes in brain activity across block may underlie shifts in strategy from childhood to adulthood.

Future studies will be needed to investigate the effects of regulatory strategy shift in childhood and how it relates to neural markers of emotion regulation.

In addition to examining the effects of emotion induction within a child sample, further correlational analyses probed changes in prefrontal activity related to age and temperament. I chose to compare Blocks 1 and 3 due to their similar task difficulty, but differential context. Block 3 was hypothesized to require increased regulatory demand due to its proximity to a negative episode and uncertainty in outcome. When examining the difference from Block 1 to Block 3 in relation to child age, I observed a portion of the dorsal ACC where activation to faces correlated positively with children’s age and a section of the subgenual ACC where activation correlated negatively with age. Some theories of the ACC hypothesize divisions of a “cognitive” dorsal region, in proximity to parietal cortex, and an “emotional” ventral/subgenual region near to OFC and limbic
areas (see Bush et al., 2000). In accord with this theory, older children in my experiment recruited more cognitive areas of the ACC when pushed to regulate emotion, while younger children engaged more emotional areas of the ACC. This shift in prefrontal activation may underlie a change to more cognitive regulatory strategy use (e.g. reappraisal) as children develop.

When examining parent report of children’s fearful temperament, however, I observed the opposite pattern. Looking at the difference from Block 1 to Block 3 in relation to fearful temperament, I observed a portion of the ventral ACC where activation to faces correlated positively with fearful temperament and a section of the dorsal ACC where activation correlated negatively with this trait. Children who were more fearful in nature recruited the “emotional” areas of the ACC in response to fearful faces while the less anxious children engaged the “cognitive” areas of this region. Like previous studies of emotional brain differences relating to temperament (Fox et al., 1995; Pérez-Edgar et al., 2007), we found evidence for differential activation in regards to this remarkably stable trait (Guerin & Gottfried, 1994). It may be, therefore, that genes and other biological factors relating to child temperament (Auerbach, Benjamin, Faroy, Geller, & Ebstein, 2001) and disordered functioning (Schmidt, Fox, Hamer, 2007) may underlie emotion regulation abilities as well. Future studies are planned to address this possibility.
Preliminary analyses revealed differential brain activation in relation to the serotonin transporter gene. During the stressful Block 2, high expressing 5-HTTLPR subjects displayed more ventral ACC activation in response to the presentation of the fearful face than intermediate and low expressers. The opposite pattern was found for a small portion of the right amygdala. Low expressers demonstrated increased activation in this area. When looking at the difference between Blocks 1 and 3, we found that greater change in activation correlated positively with serotonin transporter status. High expressers demonstrated greater change in activity in these areas than intermediate or low expressers. Although these results replicate previous findings (Hariri, Mattay et al., 2002) and have clear implications for the role of genes in the development of emotion regulation, my findings must be interpreted with caution. Of the 18 children with usable genetic data in my analysis, only two were low serotonin expressers. Although low serotonin expressers do represent a small percentage of the general population (Gelernter et al., 1999), the two subjects in my sample were six-year-old fraternal twins. Thus, my results may be confounded based on both child age and shared environment. Future studies are planned to address the relationship between the serotonin transporter gene and the neural basis of emotion regulation using larger and more genetically diverse samples.

My study further investigated enhanced functional brain connectivity as a mechanism for emotion regulation in children. I found that ACC activity immediately
preceded left amygdala activation, but only during episodes in which subjects were expected to regulate emotion. During Block 1, I did not observe effective connectivity between the left amygdala and ACC. In response to the change in task difficulty and loss of desired points during Block 2, the GCM analysis revealed section of the ACC/VMPFC in which activation immediately preceded the left amygdala response to emotional faces. Finally, in Block 3, task demand lessened to Block 1 levels, but the ACC connectivity increased greatly, coupled with a dampening in left amygdala response to faces. These changes in connectivity between the amygdala and ACC occurred independently of general ACC activation, which was equal in across blocks or VMPFC activation which was greatest in Block 2. The ACC is hypothesized to be a brain region specialized for performance monitoring and error detection (Carter et al., 1998), but also important in the processing and top-down regulation of emotion (Bush et al., 2000). Therefore, increased ACC to amygdala connectivity in Block 2 was not likely due to increased task demand, given that ACC was equal across blocks in the 1 second preceding face presentation, but to changes in the need to regulate emotion. In following, Block 3 demonstrated increased ACC to amygdala connectivity despite task difficulty and error rate being equal to that of Block 1, consistent with regulatory demand being high during point recovery. Increased effective ACC connectivity in Block 3 may represent regulation of emotional brain regions during negative mood, in comparison to the positive mood induced in Block 1. These changes in ACC to
amygdala connectivity stand in contrast to the rFFG, which was observed to influence the amygdala/hippocampus activity regardless of block.

In conclusion, my study employed a novel emotion regulation paradigm to relate previous findings of variability in amygdala and PFC reactivity during emotion regulation to development and temperament, but I also demonstrated a relationship between modulation of induced mood and ACC-amygdala connectivity. My study implicates increased connectivity as a mechanism for regulation of emotion in the brain. Given previous research relating abnormal functional connectivity to cognitive deficits in disorders such as Autism (Just et al., 2004), Schizophrenia (Meyer-Lindenberg et al., 2001), and Alzheimer’s Disease (Grady et al., 2001) my findings have the potential to inform my understanding of regulatory brain deficits in childhood and adulthood emotional disorders. Future research is planned to address what genetic bases may underlie these changes in connectivity and how the child brain might develop increased connectivity to support shifts in regulatory strategy use across age.
7. Study 4: A Multi-Level Integration

Having examined individual differences in the neural basis of emotion in typical and atypical development, one final experiment was designed to integrate genetic, brain, personality and behavioral findings (Aim 4). This synthesis of data from my previous studies is intended to provide preliminary data for future research investigating the development of emotion and emotion regulation from a multi-level perspective. This research is exploratory in nature. Due to a low sample size, full analysis of the data, in the form of a structural equation model, was not possible. Therefore, the findings from my correlational results are intended to provide preliminary hypotheses for a larger scale research program in the future.

7.1 Method

7.1.1 Subjects

Subjects were 29 (10 male, 19 female) English-speaking adults ages 18-44 years with normal or corrected to normal vision. Subjects were recruited from local community postings and internet advertisements and were paid for their participation. Ethical approval for the project was obtained from the Institutional Review Boards of the University of Pittsburgh and Carnegie Mellon University. All subjects provided written informed consent.
7.1.2 Eye-Tracking Data Collection

All subjects (n = 29) participated in my emotional eye-tracking experiment. Procedures for eye-tracking data collection and analysis were identical to those described in Chapter 3 (Experiment 2).

7.1.3 Questionnaire Data Collection

Subjects provided information on their personalities by completing the NEO-FFI (Costa & McCrae, 1991). Procedures for questionnaire data collection and analysis were identical to those described in Chapter 3 (Experiment 1). Of the 29 subjects that participated in the eye-tracking portion of the experiment all had usable personality data.

7.1.4 fMRI Data Collection

All subjects participated in my emotional go/no-go task during fMRI scanning. Procedures for task completion, imaging acquisition, stimuli presentation, and data analysis were exactly the same as those described in Chapter 5 (Experiment 1). Of the 29 subjects that participated in the eye-tracking portion of the experiment, 23 had usable fMRI data. Six subjects were missing data due to movement or participation in an earlier version of the task that was subsequently changed.

7.1.5 Genetic Data Collection

We asked subjects to provide a saliva sample for genetic analysis of 5-HTTLPR. Procedures for genetic data collection and analysis were identical to those described in
Chapter 5 (Experiment 2). Of the 29 subjects that participated in the eye-tracking portion of the experiment, 28 had usable genetic data. One subject refused to provide a genetic sample.

7.2 Results

7.2.1 fMRI Results

**Personality**

I first investigated the effects of personality on brain activation to fearful faces during my emotion induction (emotional go/no-go) paradigm. Across all blocks, I found a positive correlation between the personality trait of neuroticism and activation to fearful faces in a portion of the dorsal ACC and VMPFC \( r(21) \geq .53, p = .01 \). Subjects who reported higher levels of neuroticism displayed more activation to fearful stimuli in these regions (see Figure 24). When examining neural response to faces during negative mood exclusively (Block 2), I found a portion of the dorsal ACC that was positively correlated with neuroticism \( r(21) \geq .53, p < .01 \). During stress, subjects highest in neuroticism displayed more activation in the ACC to fearful faces.

When examining the bilateral amygdala, no differences in activation were found relating to level of neuroticism. Although these null results were surprising, they are somewhat in line with previous studies. Bishop, Duncan, & Lawrence (2004) found that subjects high in trait anxiety showed heightened amygdala activity to fearful faces in comparison to their low trait anxiety counterparts, however, this was only true in cases
of non-attended to stimuli. When subjects were asked to match face stimuli and ignore house stimuli, both groups showed equal amygdala activation. Hyper activation was only observed in high trait anxiety participants when they were told to match house stimuli, but ignore face stimuli. The results indicate that trait anxiety interacts with attentional focus in amygdala response to threat. In my study, subjects always attended to fearful face, which may have affected my null amygdala results.

Figure 24: Correlations between brain activation and neuroticism in the ACC and VMPFC.

Eye Tracking

Next, I combined my eye tracking and fMRI data to probe the relationship between time spent looking at fearful faces and brain activation to these stimuli. When
examining all emotion induction blocks combined, we found a portion of the dorsal ACC that correlated positively with the proportion of time spent looking at fearful faces \[r(21) \geq .53, p < .01\]. Subjects who spent more time looking at fearful faces displayed greater activation to these stimuli in the ACC (see Figure 25). Similar results were found when examining only the negative mood induced by Block2. In the dorsal/ventral ACC, my analysis revealed a positive correlation between looking time and neural activity \[r(21) \geq .53, p < .01\]. Subjects who spent more time looking at fearful faces during my eye tracking paradigm displayed increased activation in these areas.

Figure 25: ACC correlations between brain activation and fixation upon fearful faces during my eye tracking task.
Again, I did not find any differences in amygdala activation in relation to fixation upon fearful faces. This may be due to attentional constraints, as described above, or to the short presentation of fearful faces. Each faces was presented on the screen for only 1 second. Subjects may not have had enough time to modulate their visual attention towards or away from the stimulus like they did during eye tracking (6 sec duration). If amygdala activation is, in fact, related to time spent fixated on the eyes of faces (Dalton et al. (2005), then differences in amygdala activation in relation to neuroticism may not be present in my experiment.

### 7.2.2 Genetic Results

**Eye Tracking**

Subjects were divided into three groups based on 5-HTTLPR genotype. One way ANOVAS were performed on serotonin transporter status (low expressing, intermediate expressing, and high expressing) as the grouping variable with the percent of time subjects spent fixating upon faces during eye-tracking as the dependent variable. First, when examining all faces combined, I found a significant linear effect of serotonin expression status \(F(2,25) = 6.32, p < .006\). Tukey HSD posthoc tests, correcting for multiple comparisons, showed that the means of the high and low expressing groups were significantly different from each other \((p < .005)\), but that the intermediate expressing group was not significantly different from the low expressers \((p = .26)\) and only marginally different from the high expressers \((p = .055)\).
Next, I examined each emotional facial expression separately, which revealed similar results to those described above (see Figure 26). One-way ANOVAs revealed significant and linear differences between means for serotonin expression groups and each facial expression [Fear $F(2,25) = 6.49, p < .005$; Happy $F(2,25) = 5.6, p < .01$; Angry $F(2,25) = 5.31, p < .01$; Neutral $F(2,25) = 5.31, p < .05$]. Post hoc analyses showed that, for each emotional expression, the means of the high and low expressing groups were significantly different from each other [Fear $p = .04$; Happy $p = .007$; Angry $p = .01$, Neutral $p = .037$], but the intermediate expressing group was never significantly different from either the high or the low expressers. Serotonin transporter staurs was unrelated to the personality trait of neuroticism in my study [$r(26) = .16, p = .42$].
Figure 26: Serotonin expression status groups plotted against time spent fixating on emotional faces within a visual array. Low expressing = 1, Intermediate expressing = 2, High expressing = 3.

Imaging

In order to examine the effects of serotonin transporter status on the neural perception of emotional faces, we correlated 5-HTTLPR status with data from my emotional go/no-go task. A random-effects ANCOVA analysis was computed for the one second presentation of the fearful face, with my three 5-HTTLPR groups (high, intermediate, and low) as a covariate. First, when looking at the presentation of fearful faces across all blocks, my analyses revealed a small section of the right amygdala that correlated negatively with serotonin expression \([r(20) \geq - .42, p > .05]\). Consistent with
the findings of Hariri, Mattay, et al. (2002), low expressers displayed greater activation
to fearful faces than intermediate and high expressers (see Figure 27). We also observed
a large portion of VMPFC that correlated positively with serotonin transporter status
\[ r(20) \geq .42, p > .05 \]. High serotonin expressers displayed more activation in this area
than intermediate and low expressers.

![Figure 27: Correlation between brain activation and expression of the serotonin transporter gene during all blocks of my emotion induction task. Red represents positive correlation and blue represents negative correlation.](image)

In addition, we examined the brain’s response to fearful faces during the stressful Block 2 exclusively. Analyses revealed portions of the VMPFC that correlated positively with 5HTTLPR status \[ r(20) \geq .42, p > .05 \]. Subjects who were high serotonin
expressers displayed greater activation in this area during my stressful mood induction than intermediate and low expressers (see Figure 28).

![Figure 28: Correlation between brain activation and expression of the serotonin transporter gene during negative mood induction.](image)

### 7.3 Discussion

The exploratory results of this study suggest multi-level interactions between genes, the brain, personality, and behavior in the development of emotion and emotion regulation. First, when examining the correlations between personality and emotional brain response, I found that increased activity to fearful faces in portions of the ACC and VMPFC was related to high levels of neuroticism. Although I did not find evidence for a relationship between neuroticism and amygdala activation, as was expected, the current results suggest increased prefrontal activity, and possibly regulation of emotional areas.
by the PFC, in relationship to a negative stimulus. It may be that those highest in 
neuroticism were most in need of frontal regulatory mechanisms to down-regulate 
affective arousal. Future studies will be needed to examine the links between prefrontal 
down-regulation emotional reactivity in relationship to personality, possibly 
investigating the effects of personality in brain connectivity. It may be, as stated earlier, 
that variability in amygdala activity due to personality may be largely related to 
attentional resources. Alternatively, studies unrelated to attentional modulation may 
find that individual differences measures relate more directly with prefrontal to 
amygdala connectivity than to activation of these isolated brain areas.

I also examined the relationship between percent of time spent fixating upon 
fearful faces in my eye-tracking paradigm and brain activation to fearful faces during 
my emotional go/no-go task. I found that ACC activation to fearful faces was positively 
related to the amount of time spent looking at the face. These results indicate increased 
ACC activation in relation to a negative stimulus, which could possibly be due to 
increased regulatory demand in subjects who most attend to these negative stimuli. 
Although we were not able to collect fixation data during the actual scan, these 
preliminary findings may indicate that activation in prefrontal regions, in addition to the 
amygdala and FFA found in previous studies (Dalton et al., 2005; Perlman, Hudac et al., 
under review), is related to gaze fixation.
Genetic analysis was also related to fixation upon faces. I found that subjects who were high expressers of serotonin spent more time looking at faces within an object array than those who were low expressers. For all emotions, including neutral faces, low expressers looked less at faces than high expressers, with intermediate expressers in between both groups. These genetic findings were contrary to what was originally expected. I originally hypothesized that subjects who looked most at the eyes of fearful faces, and fixated more on emotional faces than objects, would be low expressers of 5HT (Perlman, Morris et al., under review). This hypothesis was based on findings of 5-HTTLPR relating to high neuroticism (Lesch et al., 1996) and heightened amygdala reactivity to fearful faces (Hariri, Mattay et al., 2002).

My current results may suggest that low expressers may be over-aroused by emotional stimuli, such as faces, which might cause them to look away from the stimulus. This distraction tactic would serve to regulate emotional brain arousal. Indeed, I found that the low expressers displayed the most amygdala activation to fearful faces, a finding that is consistent with the research of (Hariri et al., 2002). In line with this hypothesis, subjects who were high expressers displayed the most brain activation in the VMPFC in comparison to low and intermediate expressers. These results suggest that decreased prefrontal activation in low expressers may not effectively regulate arousal in the amygdala. If my future work indicates that decreased PFC to amygdala connectivity is present in low expressing 5HTTLPR subjects, this data may
contribute to my understanding of dysregulation in the emotional brain and its influences upon emotional behavior.
8. General Discussion

The Neural Basis of Emotion

The first core aim of my completed studies was to contribute to and extend the growing body of literature on the neural basis of emotion. Specifically, these studies were intended to investigate biological mechanisms distinguishing emotional perception, experience, and regulation, a major theoretical hurdle in the field (Cole et al., 2004). My first study investigated individual differences in perception of emotional faces. I found that those high in the personality trait of neuroticism spent more time looking at the eyes of emotional faces, especially fearful faces, than their low neuroticism counterparts. A follow-up experiment found that high neuroticism subjects spent more time looking at emotional, especially fearful, faces within a visual array and that this effect was attributable to trait personality, regardless of emotional state. My second study investigated the neural basis of emotional experience. I found that subjects with autism did not display the typically developing amygdala and FFG activation to fearful faces, but that this effect could be normalized by encouraging these individuals to look more at the fearful eyes. My third study focused on understanding the neural basis of emotion regulation. Results indicated that increased regulation of emotion is supported by downward control of the amygdala in the form of effective connectivity from the ACC to the AMY.
In these three studies, I was able to identify some of the possible mechanisms for independent emotional perception, experience, and regulation, but also to link them through relationship to personality and visual scanpaths. Attention to the eyes of faces has been linked to amygdala activity through both individuals with Autism (Dalton et al., 2005) and those with amygdala lesions (Adolphs et al., 2005). Further, studies have identified a relationship between individual differences in neuroticism in relation to amygdala reactivity to fearful faces. The results of my study indicate that eye contact may serve as a mechanism in this process. Those that look more at the eyes of faces, especially the highly arousing eyes of fearful faces, may have higher levels of biological reactivity to emotional stimuli. That is, those that have a personality characteristic of high levels of anxiety might display hyper activation of the amygdala because they selectively attend to the most arousing stimuli in their environment. This effect may underlie individual differences in emotion regulation that are inherent across personality types (John & Gross, 2007; Rothbart & Sheese, 2007). In regards to neural markers for emotion regulation specifically, my research indicates that prefrontal areas, such as the ACC, are more active to faces in participants high in neuroticism during stress. It may, therefore, be that adaptive emotion regulation is mediated by effective connectivity from the PFC to the amygdala, which may be inhibited in highly neurotic subjects.
One main question, concerning the results of these studies, remains open for investigation. Precisely, what is the directional nature of the relationship between the amygdala and eye-contact? That is, does amygdala activation direct individuals to focus on the eyes of faces or is eye contact initiated by a separate system, which then spurs amygdala activation. Evidence from amygdala lesion studies (Adolphs et al., 2005) suggests that eye-contact initiates amygdala activation. Patient SM, whose brain was not able to experience amygdala activation, also was not able to direct her attention to the eyes of faces. Evidence from subjects on the Autism spectrum, however, suggests another pattern. In my study, these subjects only experienced normalized amygdala activation when directed to look at the eyes of faces, indicating that eye contact precedes amygdala activation. Other studies suggest that eye contact with fearful faces is not necessary to produce amygdala activation. The amygdala is found to be active to rapidly presented fearful faces, masked as neutral faces, which are not consciously perceived by the subject (Whalen et al., 1998). In addition, strong amygdala activation has been observed when only the large eye-whites of fearful faces are masked by neutral faces (Whalen et al., 2004). In these studies, subjects never saw a fearful face, which was the main stimulus of amygdala activity. However, they were allowed to make eye contact with the neutral “mask” face. Future studies in which faces are presented rapidly, either primed alone or masked with an object (such as a house) may serve to answer these remaining questions. Additional data from eye-tracking measures might also elucidate
the nature of visual scanpaths when fearful faces are presented outside of conscious awareness.

*Development of the Emotional Brain*

A second core aim of this set of studies was to investigate the development of the emotional brain in abnormal cases. My work sought to investigate typically developing emotional brain circuitry and to understand its aberrant pathways that could lead to social and emotional disorders. In my third study, I examined brain circuitry involved in emotion regulation in both adults and children. I found increasing connectivity during episodes of regulatory demand in both populations. In children, emotional response during regulation shifted from the ventral/subgenual ACC to the dorsal ACC as children aged, which may underlie a shift in regulatory strategy use across development. Future studies are planned to investigate emotional brain regulation in atypically developing populations using this paradigm. In study 1, I investigated eye contact with emotional faces in typically developing adults. This was also examined from an abnormal brain perspective in study 2. Results revealed that typically developing subjects focus most on faces than anything else in a visual array, and most on the eyes of faces in comparison to other features. Encouraging equal levels of eye contact in subjects with autism normalized brain activity, pointing to the relationship between atypical brain functioning and social behavior in autism.
These results fit into an expanding body of literature characterizing the emotional deficits of autistic brain development and social behavior. By replicating and extending the previous studies of hypoactivation in response to faces (Table 9), we have shown that these deficits are not entirely permanent and can be modified for brief moments of time. In addition, these results can potentially inform the development of future therapeutic techniques to engage subjects with autism in more direct eye contact and, potentially, increased social brain activation and behavior. Research into the abnormal development of affective regulation also has the potential to contribute to our recent knowledge of autism as an emotional brain disorder (Bachevalier & Loveland, 2006). Therapeutic techniques aimed at regulation of emotional behavior may contribute to the normalization of emotional brain circuitry and, possibly, improvements in reciprocal social interaction.

**Individual Differences in the Emotional Brain**

The third aim of my work was to investigate the role of individual differences (personality and temperament) in the emotional brain. In study 1, I found that subjects highest in the personality trait of neuroticism spent more time looking at eyes of fearful faces than their low neuroticism counter parts. A follow up study revealed that high neuroticism subjects also looked longer at fearful faces within an array of objects. This effect remained constant even after controlling for negative emotional state. It was also
found, in study 4, that high neuroticism subjects experienced more PFC and ACC activation to faces during my emotion regulation paradigm. In children, we also observed notable patterns of prefrontal activity in relation to temperament. Children who were rated by their parents as being high in fearful temperament displayed increased activation of the ventral ACC in contrast to their less fearful counterparts who displayed more dorsal ACC activation.

My results in children fall in line with the Bush et al. (2000) view of the ACC as containing a dorsal “cognitive” division and a ventral “emotional” division. Less fearful children used cognitive mechanisms to regulate their emotional response to a fearful face while more fearful children used emotional mechanisms to do so. These results also support the findings of Fox et al. (1995) and Pérez-Edgar et al. (2007) indicating differential brain circuitry in socially inhibited children. Temperament, the aspect of personality most closely related to biological mechanisms (Rothbart & Derryberry, 1981), was related to brain functioning in my study, but, in addition, my adult work also fits into a recent body of literature measuring neural mechanisms for personality. Canli et al. (2001) found that extraversion and neuroticism were related to localized brain activation for positive and negative stimuli, respectively. My work indicates that prefrontal activation to fearful faces is also related to the personality trait of neuroticism, but that this effect may be due to eye contact with these social stimuli. Subjects who were high in neuroticism and tested outside of the scanner looked more at fearful faces
as opposed to objects, and more to the eyes of fearful faces in a separate sample. Increased attention to fearful eyes may galvanize amygdala activation (as discussed above), but also recruit prefrontal areas to down-regulate amygdala reactivity. I hypothesize, however, that, although the VMPFC and ACC are highly active, reduced effective connectivity between these two areas may account for dysregulated emotion in highly neurotic participants. In the future, I plan to conduct studies examining connectivity from the ACC to the AMY in relation to neuroticism while also eye-tracking during fMRI data collection.

Understanding Emotional Development from a Multilevel Perspective

The final focus of my set of studies was to integrate all previous findings and investigate emotional development as an interaction between genes, the brain, personality, and behavior. Although the results are preliminary, study 4 found that subjects who were low 5HTTLPR expressers focused less on fearful faces, but also had greater amygdala activation, and less ACC activation in response to these faces. This may be because low expressers experience amygdala hyperarousal to fearful faces and, thus, look away. In relation to personality, those high in neuroticism looked more at fearful faces and experienced hyper amygdala activation, a finding supported by those of Hariri, Mattay et al. (2002). However, we did not find a link between neuroticism and 5HTTLPR in this small sample.
Direct relationships between genes and behavior are rarely uncovered in scientific research. However, by investigating the role of the brain as a mediator in the relationship between genes and behavior, this line of research has the potential to inform our understanding of individual differences in the typical and atypical development of emotion. First, examining interactions from genes to behavior can aid us in our knowledge of the development of social and emotional processes beginning in early childhood. Genes themselves cannot be altered, but their expression can be changed through various environmental influences. By understanding the mechanisms by which diverse genetic profiles influence behavior through the development of the emotional brain, we may be better able to provide children with the ideal environment to maximize their potential for positive development. For example, if we can identify low expression of the serotonin transporter gene (5HTTLPR) as a risk factor for behavioral inhibition, fearfulness, or greater than normal risk for social anxiety, as well as the potential brain mechanisms that could contribute to this risk, we could attempt to foster an environment for young children that might minimize negative emotion.

In addition to contributing to our understanding of the biological bases of typical emotional development, these studies have the potential to inform our knowledge of aberrant developmental pathways. Disorders that are present in childhood, such as Autism or mood disorders, are likely due to a complex interaction of genes, the brain, personality and behavior with added environmental influences. Due to the emotional
dysfunction that is a hallmark of these disorders, it has become critical to identify
deficits in emotion regulation at all levels of the system. Genetic vulnerability may
provide a risk factor for abnormal development by increasing the brain mechanisms for
visual attention to negative emotional signals from the environment. Alternatively,
genetic vulnerability could provide a weak foundation for development of the emotional
brain, which may lead to sub-optimal emotional behavior. By implementing studies of
the emotional brain in disordered populations, multi-level study designs can provide
new insights into our treatments for emotional disorders.

Finally, a future direction in which I would like to continue this research
concerns the role of culture in maximizing the ideal environment for genetic diversity in
emotional development. That is, cultural and ethnic groups are likely to have similar
genetic profiles. These genetic markers of emotion are likely to influence behavioral
level emotional variables through brain mechanisms. Those from related cultural
groups are, thus, likely to display similar levels of emotional expressivity, which would
provide the ideal environment for other group members. For example, individuals from
Asian cultures have been shown to possess high rates of the short variant of 5HTTLPR
(Gelernter et al., 1999; Gelernter et al., 1997). In the context of my own research, this
would likely indicate high levels of neuroticism, attention to emotional cues in the
environment, and increased amygdala activation to emotional stimuli among members
of these groups. Research shows that members of Asian cultures are, indeed, less
emotionally expressive (Markus & Kitayama, 1991) and, at the same time, more physiologically reactive (Lewis, Ramsay, & Kawakami, 1993) to emotional cues in the environment. Decreased emotional expression may serve to regulate emotion on the group level in a culture that is highly sensitive to emotional signals.

Lewis, et al. (1993) observed this process occurring in early infancy. The authors measured behavioral and physiological distress in Japanese and Caucasian American four-month-old infants during routine inoculation. American infants showed more behavioral distress than the Japanese infants in the form of vocalizations and negative facial expression. Asian infants, on the other hand, showed relatively little behavioral reactivity. Few Japanese infants even cried, but when cortisol secretion was examined, Japanese infants showed a marked increase in the stress related hormone, displaying higher levels than American infants. Two possibilities exist for this difference. Japanese infants may have internalized cultural cues to minimize emotional expression or, alternatively, biological pain sensitivity may be increased in American cultures. In either case, low levels of emotional expressivity are likely the product of complex interactions at the genetic and brain levels, serving to explain regulatory differences in a minimally expressive culture. If the infant expresses minimal emotional distress, s/he will provide less emotionally salient environmental stimuli for his/her highly reactive parents or peers.
Thus, future directions for my work include the examination of the role of emotion in genes, the brain, personality, and behavior in cross cultural samples as well as samples of international adoption. I am eager to investigate the interaction of biological systems and psychosocial stressors on the development of the brain’s ability to regulate emotion. This direction of research has the potential to elucidate the mechanisms underlying prevalence rates of emotional disorders in different ethnic groups and the nature by which regulatory brain mechanisms are culturally based.
References


Curriculum Vita

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EDUCATION

Doctor of Philosophy    Duke University, Durham, NC
2009               Cognitive Neuroscience
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Masters of Arts    Duke University, Durham, NC
2006               Developmental Psychology
Regulation: Implications for Cross-Cultural Regulatory Development

Bachelor of Arts    University of Wisconsin, Madison, WI
2002               Psychology
Bachelors Thesis: The Role of Maltreatment Experience in Children’s Understanding of
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Course Work    Universitat de Barcelona, Barcelona, Spain
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ACADEMIC APPOINTMENTS

Research Fellow    Dr. Kevin Pelphrey’s Child Neuroscience Laboratory
2008-present    Yale Child Study Center
                Yale University
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2006-2008    Cognitive Neuroscience
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Teaching Fellow    Duke University
2004-2007  Department of Psychology and Neuroscience  
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Research Specialist  Laboratory of Dr. Linda Camras  
2002-2003  DePaul University  
Department of Psychology  
Chicago, Illinois

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2002-2003  University of Illinois- Chicago  
Department of Psychiatry  
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Research Assistant  Laboratory of Dr. Seth Pollak  
1999-2002  University of Wisconsin- Madison  
Department of Psychology  
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Neural Substrates of Preschool Psychopathology
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2007-2008 Duke University
The Ontogeny of Personality
Role: Summer Dissertation Fellow

2000 University of Wisconsin
Hilldale Fellowship
Role: Undergraduate Research Fellow

PUBLICATIONS


CONFERENCE PRESENTATIONS

Chair of Paper Symposium, Society for Research on Child Development Biennial Meeting, Denver, CO.


**EDITORIAL EXPERIENCES**

Reviewer
Child Development  
Nature Reviews Neuroscience  
Social Development

**CERTIFICATIONS**

Certified Coder   
The Facial Action Coding System (FACS)  
2002
Certified Editor   
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2002

**HONORS AND AWARDS**

2009  Student Travel Award, Society for Research in Child Development  
2008  Summer Dissertation Fellowship, Duke University  
2007  Summer Dissertation Fellowship, Duke University  
2006  Women’s Studies Travel Award, Duke University  
2000  Hilldale Undergraduate Research Fellowship, University of Wisconsin  
2002  Dean’s List, University of Wisconsin-Madison  
2000  Honors in the Psychology Major, University of Wisconsin  
2000  Psi Chi, National Psychology Majors Society, University of Wisconsin  
1998  Dean’s List, University of Wisconsin-Madison

**Invited Talks**

2008  Charting the Emotional Brain, University of California-Davis  
2006  Emotional Development: Integrating Parenting, Culture, and Physiology, George Mason University  
2006  Emotional Development: Integrating Parenting, Culture, and Physiology, Duke University

**TEACHING EXPERIENCE**

Developmental Psychology, Department of Psychology and Neuroscience, Duke University.  
Psychology of Adolescence, Department of Psychology and Neuroscience, Duke University.
Social Psychology, Department of Psychology and Neuroscience, Duke University. Abnormal Psychology, Department of Psychology and Neuroscience, Duke University. Psychology of the Consumer, Department of Psychology and Neuroscience, Duke University.

PROFESSIONAL AFFILIATIONS

2002-present  Member, Society for Research in Child Development
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