The Role of Specific Facial Features in Eliciting Emotion-Related Amygdala Activity in Children: An Investigation Utilizing Behavioral, Genetic, and Functional Magnetic Resonance Imaging Measures

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Abstract

Existing animal and adult human literature has specified a critical role for the amygdala in the processing of facial expressions. However, there is a paucity of research addressing the development of the ability to recognize and interpret face emotion. Based on extant neuroimaging work addressing the putative, fast amygdala pathway and evidence that this sub-cortical route is activated not only by the eye components of an emotional face (e.g. Morris et al., 2002) but also low-level perceptual features (Whalen et al., 2004), the core goal of this thesis is to investigate the role of individual features of faces in the amygdala response to facial emotion, as well as to better understand the role of features in children's identification of face emotion.

The current thesis utilized three measurement techniques and several behavioral manipulations designed to address questions related to the importance of specific facial features and their context in eliciting emotion-related amygdala activity in children. Chapter 2 describes forced-choice picture sorting tasks used to examine how 8-year-old children categorize emotional faces when only some of the facial features are available. Chapters 3, 4 and 5 report on three fMRI studies that examined children's brain response to low-level perceptual information in facial expressions, during an emotional-face matching task designed to activate the amygdala, and to atypical face stimuli such as inverted faces. A sub-set of participants were genotyped for the 5-HTTLPR polymorphism to explore individual differences in amygdala response during the emotional face-matching task. Chapter 6 synthesizes these four studies and discusses their contributions to the burgeoning body of knowledge on the development of face emotion processing.

Table of Contents

Page

List of Tables

Page

List of Figures

Chapter 1: General Introduction

The experience of emotion is modulated by a functional network comprising the neocortex and numerous sub-cortical limbic nuclei. The amygdala, located in the medial temporal lobe, has been implicated as a critical structure for influencing emotional responsiveness and learning generally and more specifically, in the evaluation of significance and affective content of facial expressions. It has been proposed that the amygdala evolved to help mammals to recognize and learn the significance of environmental stimuli and to consequently produce the appropriate behavioral response. LeDoux (1994) has emphasized the importance of the amygdala in fear detection and conditioning, delineating it as a neural system that serves to detect danger and produce rapid protective responses without conscious participation. Stimulation of the amygdala produces autonomic reactions associated with the fight or flight response, including feelings of fear or anxiety in adult humans. More particularly, the central nucleus of the amygdala has been identified as paramount for the expression of autonomic and somatic fear responses elicited by both learned and unlearned threats which are controlled through efferent connections from the central amygdala to brainstem nuclei (Rogan & LeDoux, 1996). In converse, lesions of the amygdala produce diminished fear reactions in animals and impaired recognition of negative facial expressions, particularly fearful expressions, in humans. Damasio (1994) has suggested that the amygdala may serve as a higher-order "convergence zone" for the social homeostatic and survival-related meanings of complex stimuli.

Existing animal and adult human literature has documented the specific participation of the amygdala in the decoding of facial expressions and thus, emotional

face stimuli have emerged as a common probe of amygdala activity. Faces are likely the visual stimuli most essential during the social interactions of humans. They not only convey critical social cues, such as age, sex, emotion and identity information, but they are also the basis of both verbal and non-verbal communication (Batty & Taylor, 2006). Yet, there remains a dearth in understanding of the development of the ability to readily recognize and interpret faces and facial expressions of emotion. Adult neuroimaging studies have consistently demonstrated the amygdala's increased activation during exposure to fear-conditioned stimuli as well as other emotionally valenced stimuli (e.g. Breiter et al., 1996; Morris et al., 1996). Yet, converse to a burgeoning literature on neurobehavioral processing of emotion in adults, there is a paucity of research addressing the development of this affective system during maturation from childhood through adolescence. Nonetheless, this transitive period entails considerable alterations in cognitive and physical functioning, both of which correspond with significant changes in the processing of emotion. During typical development through adulthood, a child shifts from characteristically juvenile emotional responses toward increased self-regulation, social awareness, and the ability to intentionally adapt or alter emotional displays (Killgore, Oki, & Yurgelen-Todd, 2001). Thus, it follows that there exist developmental changes in overall affective processing and expressly in amygdala activation to emotional face stimuli. The advent of various neuroimaging technologies such as event-related potentials (ERP) and functional magnetic resonance imaging (fMRI) has in the recent past provided a non-invasive means by which to conduct in vivo examinations of brain structure and function in the developing child. With this progressing research modality, critical questions in human

brain development, including emotional development, can be addressed without the ethical considerations involved in exposing children to the ionizing radiation of other imaging techniques, i.e. positron emission tomography (PET) and computerized tomography (CT). Used in conjunction with behavioral paradigms, these neuroimaging techniques have and will serve to further elucidate the developmental effect of the amygdala on face emotion processing.

Development of Amygdala Response to Facial Expressions of Emotion

The amygdala has been specifically implicated in the processing of facial expressions in humans and thus, emotional face stimuli are commonly used as a probe for amygdala activity. Facial expressions may be considered a means of communication more succinct than language, with which people can momentarily infer the state of mind of others (Batty & Taylor, 2003). In other words, the accurate interpretation of facial emotion significantly affects the success of an individual's social interactions. Notably, Ekman and Friesen (1976) have reported that basic facial emotions are universal and readily recognized across distinct cultures. The mechanisms of face processing and recognition in adults have been extensively studied utilizing various methodologies, including behavioral science, electrophysiology, and neuroimaging (e.g. Haxby, Hoffman, & Gobbini, 2000; Palermo & Rhodes, 2007; Schwaninger, Wallraven, Cunningham, & Chillder-Glaus, 2006 for review). However, currently there is still a lack of comparable research in the developmental realm. There is significant behavioral work which is relevant to this area of inquiry, and these findings will be briefly reviewed. In view of the progressing technique of neuroimaging, emerging research is beginning to provide critical insight into brain

systems engaged during face emotion processing across development. It should be noted that while there exists a significant body of face emotion processing research based in ERP methodologies (Batty & Taylor 2006; Nelson & deHaan, 1996; Taylor, Batty. & Itier, 2004, for review), in order to circumscribe the content of this paper, the ERP work will not be discussed. While ERP studies yield considerable information regarding the temporal dynamics of brain function, this technique is deficient in the anatomical specificity afforded by fMRI methods. Hence, the extant fMRI literature pertaining to the amygdala's structure and functional role in face emotion processing will be addressed.

Behavioral Findings

Sensitivity to faces appears early in human development. It has been demonstrated that newborns prefer following schematic face-like patterns to non-face patterns of similar complexity (Johnson, Dziurawiec, Ellis & Morton, 1991; Morton & Johnson, 1991). De Haan and colleagues (2002) have similarly reported an early preference for facial stimuli in the neonatal period, which undergoes rapid changes over the first months of life. Bushnell, Sai, and Mullin (1989) have shown that 2-day-old neonates can discriminate their mother's face from a stranger's face, suggesting that face recognition may be a mechanism functional at birth that emerges independent of experience. Conversely, research also indicates that early visual experience may be necessary for development of some aspects of normal face processing. It has been posited that an important component of face recognition involves the encoding of subtle differences in the shape of specific features (featural information) and/or in their spacing (configural information) (Freire, Lee, & Symons, 2000). Le Grand, Mondloch,

Maurer and Brent (2001) demonstrated that deprivation of patterned visual input from birth until 2–6 months of age results in permanent deficits in configural face processing. Fourteen patients who had been born with dense central cataracts in each eye that prevented patterned stimulation from reaching the retina were compared to age-matched controls on a face processing task. Even after more than nine years' recovery, the patients treated for bilateral congenital cataracts were severely impaired at differentiating faces that varied only in the spacing of their features. However, they were normal in distinguishing those differing only in the shape of individual features. These results suggest that early visual input is essential for normal development of the neural architecture that will later specialize for configural processing of faces (LeGrand et al., 2001).

Nevertheless, despite an early predisposition toward perceiving faces, the child's capacity to understand and correctly interpret facial emotion follows a protracted developmental course. For example, it has been shown that by 3-months of age, infants can discriminate happy and sad faces from surprised faces (Young-Browne, Rosenfeld, & Horowitz, 1977) and smiling faces from frowning faces (Barrera & Maurer 1981). Additionally, 4-month-olds can discriminate happy from fearful expressions (Nelson, 1987). Yet, it is not until 6- to 7-months that infants begin to recognize that an expression remains the same despite discernable differences in `irrelevant' information (i.e. intensity of the expression, age or gender of the model, etc.). Nelson, Morse, and Leavitt (1979) familiarized 7-month-olds to two different models posing happy expressions, and then showed infants a new model posing a happy expression and a fearful one. Infants looked longer at the fearful expression than at the happy one,

indicating that they recognized the happy expression as familiar despite the change in model. The authors suggested that by 7-months of age, infants are able to discriminate between some expressions and see similarity between two different faces posing the same expression. However, de Haan and Nelson (1998) do qualify that whether or not infants demonstrate these abilities varies depending on factors such as the age of the infant, the expression pair tested, and the method of assessment (Kotsoni, de Haan, & Johnson, 2001). In addition, there is evidence that the facial expressions of others may alter infants' behavioral responses (e.g. Carver & Vaccaro, 2007; Montague & Walker-Andrews, 2001; Sorce, Emde, Campos, & Klinnert, 1985). The inclusion of vocal information and the use of dynamic faces are also factors that play a role in infants' ability to recognize emotional expressions (Caron, Caron, & Myers, 1985; Caron, Caron, & MacLean, 1988). Numerous studies have found that the recognition of emotional expressions improves with age (e.g. Boyatzis, Chazan, & Ting, 1993; Odom & Lemond, 1972; Philippot & Feldman, 1990). However, findings do indicate that recognition of face emotion does not emerge as one specific stage in development (Camras & Allison, 1985; De Sonneville et al., 2002; Gross & Ballif, 1991; Smith & Walden, 1998; Vicari, Reilly, Pasqualetti, Vizzotto, & Caltagirone, 2000). Rather, children's abilities emerge gradually over time, with happiness recognized earliest and with the greatest accuracy, followed by sad or angry expressions, and then by expressions of surprise or fear (Gross & Ballif, 1991). It has also been shown that younger children rely on facial expressions for information on another's emotional state to a greater extent than situational cues. Hoffner & Badzinski (1989) conducted a study of facial expressions and situational cues of emotion that demonstrated children's

reliance on situational cues increased with age. The authors reported that 3- to 5-year-olds focused on facial expressions, whereas 8- to 9- year olds utilized situational cues as well. Still, very little is known about the continued development of emotion processing over the full childhood span into adolescence, linking development across these age ranges. Furthermore, there are methodological issues in comparing studies across ages. Since it is necessary to use different dependent measures to assess emotion expression recognition in infants (i.e., using habituation and preference) as compared to studies with older children (McClure, 2000), it is questionable whether the same construct of emotion expression recognition is being measured over development (with the presence or absence of language). Thus, it is difficult to discuss continuity of these functions over development (Herba & Phillips, 2004).

Nevertheless, processing of facial emotions does evolve across the lifespan. For example, Malatesta, Fiore, & Messina (1987) examined face emotion recognition for expressions of anger, fear, and sadness in three age bands of female subjects: young (25–40 years), middle aged (45–60 years), and older (65–80 years). Results showed that the recognition of all three facial expressions decreased with increasing age. Subsequently, Moreno, Borod, Welkowitz, & Alpert (1993), contrasted the recognition of negative (sad and disgust) and positive (happy and surprise) facial expressions in female subjects in similar age groups to those used by Malatesta and colleagues (1987). The results showed that with increasing age, happiness improved slightly and sadness decreased slightly. Calder et al. (2003) tested men and women in young (18-30yrs) and older (58-70yrs) groups and reported that increasing age produced a progressive reduction in the recognition of fear and, to a lesser extent, anger. In contrast, older

participants showed no reduction in recognition of disgust; rather, there was some evidence of an improvement.

Emotion identification is essential for subsequent social interaction and functioning. The ability to decode facial expressions is an important component of social interaction because of the crucial role of facial information in the appropriate modification of social behaviors (Herba & Phillips, 2004). Philippot & Feldman, (1990) examined the relationship between social competence and decoding of emotions in children aged between three and five years. Children were shown videotaped scenarios depicting emotional situations and were asked to choose which of three facial expressions (representing happiness, sadness and fear) would be most appropriate for the character in the situation. Children's level of social competence was assessed by a standardized questionnaire completed by their parents. The authors reported that the subjects with relatively higher social skills were better decoders than subjects with relatively lower social skills, and that decoding performance improved with age.

Additionally, abnormalities in emotion expression recognition are associated with several psychiatric disorders including schizophrenia, bipolar disorder, major depressive disorder (Kee, Kern, & Green, 1998; Phillips, Drevets, Rausch, & Lane, 2003) and autism (e.g. Blair, 2003). An improved understanding of the normal development of emotion expression recognition and associated neural systems may thus facilitate earlier identification of and appropriate therapeutic intervention for emerging patterns of aberrant emotional behavior.

Structural Evidence

While a number of studies have documented the neural correlates of emotion processing in adults, little research has been conducted on the development of these neuroanatomical structures. The advent of MRI has provided the means to explore structural and functional changes in the brain throughout development (Casey, Giedd, & Thomas, 2000). These improving technologies have made it possible to not only image the developing brain throughout childhood and adolescence, but also to explore the relationship between developing neural correlates and emerging motor, cognitive, and social abilities (Durston, Hulshoff Pol, Casey, Giedd, Buitelaar, & van Engeland., 2001; Killgore et al., 2001; Thomas et al., 2001b; Baird et al., 1999). Durston et al. (2001) presented a comprehensive review of MRI studies exploring normal brain development and developmental changes in relation to age and sex. Findings suggest that after 5-years of age, total brain size does not increase. Yet, over the period from childhood to adulthood, white matter volume increases significantly while gray matter volume decreases. Giedd et al. (1999) also found this pattern of development in a longitudinal MRI study of brain development in over 100 participants (4-21 years). In light of such evidence indicating basic morphologic changes across human brain development, it follows that there may be differences in structures that mediate emotion processing in adults as compared to those utilized earlier in development (McClure, 2000).

Functional Evidence

Functional neuroimaging studies have shown that the coarse visual features in faces which are carried in the low spatial frequency spectrum of visual images and can be transmitted by magnocellular visual channels in subcortical pathways (Leventhal,

Rodieck, & Dreher, 1985; Schiller & Tehovnik, 2001), may drive amygdala responses to fearful faces (Vuilleumier, Armony, Driver, & Dolan, 2003; Whalen et al., 2004; Winston, Vuilleumier, & Dolan, 2003). Vuilleumier et al. (2003) conducted an event-related fMRI study in which participants saw neutral and fearful faces in low-pass filtered, high-pass filtered, or intact (broad-band) images. It was shown that amygdala activation to fearful expressions was greater for intact or low-pass faces than for high-pass faces. In contrast, activation of fusiform cortex was found to be greater for intact or high-pass faces than for low-pass faces, regardless of expression. The critical role played by low spatial frequency (LSF) cues in driving amygdala responses to fear, and subsequent modulation of fusiform, appears consistent with psychophysical findings that the configural aspects (Calder, Young, Keane, & Dean, 2000) and coarse features of faces (Adolphs et al., 2005; Morris, deBonis, & Dolan, 2002; Schyns & Oliva, 1999) may be of considerable importance in the process of recognizing facial emotion. This may concur with developmental research showing that eye feature detection is viewed at an early age when babies have poor cortical vision (Farroni, Csibra, Simion, & Johnson, 2002) and that 7-month old infants show preference for looking at fearful faces with wide eyes rather than at happy faces (Nelson & Dolgin, 1985). Conversely, children with autism show impaired processing of low-spatial frequency cues in faces (Deruelle, Rondan, Gepner, & Tardif, 2004) and greater deficits in visual responses to fearful compared to neutral faces (Dawson,Webb, Carver, Panagiotides, & McPartland, 2004). This may indicate a link between early magnocellular vision and the typical development of face processing abilities (Elgar & Campbell, 2001), perhaps

contributing to the fusiform's role in face recognition under the modulatory influences of amygdala.

Despite the importance of the amygdala for emotion processing, the role of the amygdala in emotion processing throughout childhood and adolescence remains unclear. Only a small number of studies have explored the amygdala function in youth, and these have predominantly examined the nature of the amygdala response to fearful facial expressions (Baird et al., 1999; Killgore et al., 2001; Thomas et al., 2001a).

For example, in order to examine developmental sex differences in affective processing, 19 children and adolescents (ages 9-17 years) underwent fMRI while viewing photographs of fearful faces (Killgore et al., 2001). Results indicated sex-differences in amygdala development, particularly in the patterns of their amygdala and prefrontal activation to the affective stimuli. Whereas the left amygdala responded to fearful facial expressions in all children, left amygdala activity decreased over the adolescent period in females but not in males. Female participants also showed increased activation of the dorsolateral prefrontal cortex over the adolescent period, while male participants demonstrated the opposite pattern. In another study, Killgore & Yurgelun-Todd (2001) reported greater lateralization of amygdala activity (left amygdala) in adult males than in females whilst viewing happy and fearful faces, although both sexes demonstrated greater left amygdala activation for fearful faces. Killgore et al. (2001) interpreted these findings as support for an association between cerebral maturation and increased regulation of emotional behavior; the latter mediated by prefrontal cortical systems. Perhaps, the pattern of decreased amygdala and increased dorsolateral prefrontal activation in females with increasing age reflects an

increased ability to contextualize and regulate emotional experiences (Blakemore & Choudhury, 2006; Herba & Phillips., 2004).

Monk and colleagues (2003a) used fMRI with a group of adolescents (aged 7-17 years) and a group of adults (aged 25–36 years) to examine developmental differences in activation during viewing of certain emotional expressions. While viewing faces with fearful emotional expressions, adolescents exhibited greater activation than adults of the amygdala, orbitofrontal cortex and anterior cingulate (Monk et al., 2003a). When subjects were asked to switch their attention between a salient emotional property of the face (i.e. how afraid it makes them feel), and a non-emotional property (i.e. nose width), adults, but not adolescents, selectively engaged and disengaged the orbitofrontal cortex. These fMRI results suggest that both the brain's emotion processing and cognitive appraisal systems develop during adolescence.

Baird and colleagues (1999) tested 12 healthy adolescents (12-17 years) to investigate the role of the amygdala in the recognition of facial expression in this age group. Functional MRI data were collected during a task of facial affect recognition and a visual control task and significant amygdala activation was found in response to the perception of fearful facial expressions. However, it should be noted that, in addition to a fixation-cross baseline and nonsense, non-facial stimuli, only fearful facial expressions of emotion were utilized in this paradigm. A similar fMRI study investigated the neural processing of other facial expressions (happiness and sadness) in a group of 12 adolescent participants (13–17 years). Happy faces produced significant bilateral amygdala activation when compared with neutral faces, although sad faces relative to neutral did not produce significant amygdala activation (Yang, Menon, Reid,

Golib & Reiss., 2003). Neither of these studies contained an adult or a younger child group, so comparisons before and after adolescence of the neural processing of facial emotion could not be made.

Thomas et al. (2001a) addressed some of these issues by studying amygdala activation to fearful facial expressions in two groups: children (aged 8-15 years) and adults. A prior study with adult participants reported amygdala activation to both neutral and fearful faces, but more activity for the fearful expressions (Breiter et al, 1996). Similar to those findings, Thomas and colleagues (2001a) reported that adults demonstrated increased amygdala activity for fear compared to neutral faces. However, the child participants showed enhanced amygdala activity to neutral faces. Significantly, this effect was not due to lack to amygdala activity for fearful expressions but rather a greater response to neutral expressions. The authors proposed that this developmental effect may be because the children detected neutral faces as more ambiguous than the fearful expressions. These findings may be consistent with Whalen's interpretation that the amygdala is sensitive to ambiguous stimuli rather than just emotionally-valenced stimuli (Whalen et al., 1998).

The elucidation of developmental differences in the amygdala's role in emotional processes shows potential for application for pediatric and adult clinical populations. For example, the amygdala and its connections (along with medial temporal lobe structures in general) have been implicated in the neuropathology of schizophrenia. Hendren et al. (1995) reported that children who display symptoms of schizophrenia spectrum disorder, including social impairment, attention deficit, constricted or inappropriate affect, and hypersensitivity to criticism, show

neuropsychological or neuroanatomic abnormalities similar to those seen in adults with schizophrenia. Also, aberrations in synaptic pruning during development may play a role in medial temporal lobe pathology (Swayze, Andreasen, Alliger, Yuh, & Ehrhardt, 1992). Bauman (1991) has reported that limbic (including amygdalar) neuropathological anomalies are found in children with autism. Structural MRI findings showed significant decreases in amygdala volume as well as temporal lobe volume in these children compared with non-patient controls. In individuals with anxiety disorders, previous imaging studies implicate alterations in amygdala activation when fear-triggering stimuli that are relevant to the disorder are presented (Birbaumer et al., 1998; Fredrikson & Furmark, 2003; Furmark, Fischer, Wik, Larsson, & Fredrikson, 1997; Rauch, Shin, & Phelps, 2006; Tillfors et al., 2001). Studies investigating atypical amygdala structure or function may serve to provide significant insight into normative development of the emotion-processing system.

Consistent with this idea, Thomas et al. (2001b) demonstrated greater amygdala activation in children with generalized anxiety disorders (GAD), relative to healthy children. To elaborate, Thomas and colleagues examined amygdala response in children with anxiety and depression with the hypothesis that amygdala response may be disrupted in childhood affective disorders. Twelve children (8-16 years) with diagnoses of GAD were compared to healthy controls. FMRI results in children with GAD showed an enhanced amygdala response to fearful expressions which may also be interpreted as a reduced response to neutral expressions. In other words, anxious children showed a response pattern similar to healthy adults, but dissimilar to healthy children. Interestingly, the authors also found significant positive correlation between

the size of the amygdala response to fearful versus neutral expressions and a self-report of everyday anxiety. Both healthy children and children with clinical symptoms of anxiety reporting high levels of anxiety in everyday settings showed a stronger response to fearful relative to neutral faces. These results lend support to the idea that clinical disorders associate with an inability to regulate affective responses that may include atypical functioning in an emotion processing amygdala system (Thomas et al., 2001b).

Rich et al. (2006) further examined the hypothesis that clinical disorders result from perturbed neural development by extending research to pediatric bipolar disorder (BD). In order to study amygdala dysfunction, these investigators examined neural mechanisms mediating face processing in 22 adolescents (mean age: 14.21 +/- 3.11 years) with BD and 21 controls of comparable age, gender, and IQ. Event-related functional MRI compared neural activation when attention was directed to emotional aspects of faces ("How hostile is the face?", "How afraid are you?") vs. non-emotional aspects ("How wide is the nose?"). Compared with controls, patients perceived greater hostility in neutral faces and reported more fear when viewing them. Also, compared with controls, patients showed greater activation in the left amygdala, accumbens, putamen, and ventral prefrontal cortex when rating face hostility, and greater activation in the left amygdala and bilateral accumbens when rating their fear of the face. No between-group behavioral or neural differences were found in the non-emotional conditions. These results implicate deficient emotion-attention interactions in the pathophysiology of BD in adolescents.

These lines of research further investigations regarding the brain basis of anxiety, particularly among pediatric populations. As discussed previously, threat

paradigms with animals have made inroads into delineating the relationship between anxiety behaviors and brain function. However, while these findings demonstrate that fear-provoking paradigms engage analogous brain structures in humans and animals, the procedures utilized in the neuroimaging studies are not comparable to those used in animal work. Cross-species and age group comparisons are thusly limited. More recent neuroimaging studies with humans have utilized human conditioning paradigms which may more closely approximate fear provocation procedures in the animal literature. For example, Phelps and colleagues (2001) informed subjects that they might receive a shock when they viewed one stimulus (threat condition), but would definitely not be given a shock when they viewed a different stimulus (safe condition). The authors reported activation in the left amygdala to the threat relative to the safe condition. While informative, the use of a shock in this paradigm may be construed as an aversive stressor in human subjects. To this end, Pine et al. (2001) attempted to develop a fear conditioning paradigm that employs a milder stressor than shock by repeatedly pairing an air blast to the larynx with a visual stimulus in healthy adult participants. A region-of-interest analysis showed increased amygdala activation among adults to the conditioned stimulus relative to the stimulus that was not paired with the air blast. These findings indicate that fear paradigms adapted for neuroimaging with humans are capable of eliciting amygdala activation.

Monk et al. (2003b) subsequently adapted this procedure in an attempt to differentiate between healthy adolescents and those with anxiety disorders. To establish the methodological reliability of this approach, Monk and colleagues (2003b) employed the threat of air blast paradigm with a sample of healthy adolescents using fMRI. Based

on the relatively mild nature of the air blast, variations across participants in subjective fear to the threat of the air blast were anticipated. Those who reported increased fear showed right amygdala activation during the threat condition and left amygdala activation in the safe condition. These findings indicate the utility of this threat paradigm for studying youth with anxiety disorders.

Caveats

The literature addressing the development on amygdala response during face emotion processing is still in a nascent stage. Further research is clearly indicated to elucidate these developmental effects, taking into consideration some of the following limitations. For example, it is unclear whether studies have used appropriate control tasks (i.e., Baird et al., 1999). Adult studies have suggested that the most appropriate control stimuli for exploring amygdala responses to emotional faces are neutral facial expressions, which may control for the effects of face processing and thus permit measurement of neural responses to the emotional component of the facial expression (e.g., Critchley et al., 2000). However, the available data to date suggest that these experimental paradigms may not be apposite for child research. For instance, as described earlier, Thomas et al. (2001a) found a greater amygdala response to neutral than to fearful facial expressions in children, whereas adults demonstrated a greater amygdala response to fearful expressions. Taking these findings into consideration, neutral faces may not be appropriate masking stimuli for emotional faces. Finally, further exploration of the effect of development upon neural responses to different facial expressions is indicated. Neuroimaging studies of emotional expression recognition have focused predominantly upon neural responses to one specific emotion

(fear) in child and adolescent studies and the associated amygdala response. Research examining the development of the amygdala response to other emotional expressions as well as the response of other neural regions (i.e., the insula and ventral striatum) to these stimuli would be informative.

To my knowledge, there are yet to be studies directly investigating the existence of or developmental changes in a subcortical pathway to the amygdala in children. As reviewed, the ability to distinguish facial emotions has a protracted development, perhaps not maturing until well into adolescence. Findings from fMRI studies also indicate developmental changes in the brain's emotion circuitry through the period from childhood through adolescence. Emotional faces activate emotion-specific neural networks in adults (Adolphs, Baron-Cohen, & Tranel, 2002; Haxby et al., 2000). However, the operation of these networks in children has not been elucidated. Given that Thomas and colleagues (2001b) used overtly presented faces in their study that reported dissimilar amygdala response pattern between anxious and healthy children, the nature of these group differences is unclear. These findings may reflect differences in a fast, direct amygdala pathway or differences in a slower, cortical pathway, potentially reflecting distinctions in cognitive processing of the emotional image. Thomas et al. (2001b) also compared a second smaller sample of 5 girls (8-16 years) with major depressive disorder (MDD) to girls in the anxious and control groups. Of particular interest was that girls with MDD showed a general decrease in left amygdala activity for both neutral and fearful faces compared to a fixation baseline. This is a pattern opposite to the healthy and anxious groups' increased left amygdala activity for emotional face stimuli. The authors speculate that results might indicate a more top-

down deficit in cognitive interpretation of emotional expressions in childhood depression, congruent with findings of blunted affective response in this group. More recently, using an implicit face-processing task in 10-year-old children, Lobaugh, Gibson, and Taylor (2006) determined that the emotions of fear, disgust and sadness recruited distinct neural systems in the absence of explicit attention to the face emotion. These systems included a number of regions typically associated with processing emotions in adults, namely the amygdala and parahippocampal gyrus, insula and cingulate gyrus, as well as the fusiform and superior temporal gyri. Thus, in spite of immature behavioral responses to emotional faces in explicit tasks, neural networks for emotion-specific processing are present in young children (Lobaugh et al., 2006). Perhaps, these findings may be considered in view of adult studies which have utilized backward masking techniques to investigate non-conscious automatic emotion-processing mechanisms (e.g. Whalen et al., 1998). While certainly not conclusive, structural and functional changes across development are indicated in the face emotion-processing circuitry.

The Current Thesis

Existing adult human and animal literature has specified the amygdala's participation in the decoding of facial expressions and thus, emotional face stimuli has been consistently employed as an efficacious probe of amygdala activity. Yet, there is still an exigency for improved understanding of the development of the ability to readily recognize and interpret faces and facial expressions of emotion. Adult neuroimaging studies have consistently demonstrated the amygdala's increased activation during exposure to fear conditioned stimuli as well as other emotionally-valenced stimuli (e.g.

Breiter et al., 1996; Morris et al, 1996). The mechanisms of face processing and recognition in adults have been extensively studied utilizing various methodologies, including behavioral science, electrophysiology, and neuroimaging (e.g. Haxby et al., 1999; Palermo & Rhodes, 2007; Schwaninger et al., 2006 for review). The comparably modest body of developmental behavioral and neuroimaging findings certainly suggests developmental changes in the amygdala's response to emotional faces, thus underscoring the need for additional research. Based on previous magnetic resonance imaging (MRI) and event-related potential (ERP) work addressing the putative, fast amygdala pathway in childhood as well as evidence that this sub-cortical route is activated not only by the eye components of an emotional face (e.g. Morris et al., 2002) but also low-level perceptual features (Whalen et al., 2004), the broad goal of this thesis is to investigate the role of individual features of faces in the amygdala response to facial emotion, as well as to better understand the role of features such as eyes or mouths in children's identification of facial expressions of emotion

This dissertation includes three measurement techniques as well as several behavioral manipulations designed to address questions related to the importance of specific facial features and their context in eliciting emotion-related amygdala activity in children. Chapter 2 describes forced-choice picture sorting tasks that were used to examine how 8-year-old children categorize emotional faces when only some of the facial features are available. Chapters 3, 4 and 5 report on three fMRI studies that examined children's brain response to low-level perceptual information in facial expressions, to atypical face stimuli such as inverted faces, and during an emotional-face matching task designed to activate the amygdala. Finally, a subset of participants

were genotyped for the 5-HTTLPR polymorphism in order to explore individual differences in amygdala response during the emotional face-matching task. Chapter 6 synthesizes these four studies and discusses their contributions to the burgeoning body of knowledge on the development of face emotion processing.

Chapter 2: How Do Children Use Specific Features in the Identification of Facial Emotions?

Faces are ‘probably the most visually complex, intrinsically meaningful visual stimuli with which we interact' (Elgar & Campbell 2001). They not only convey critical social cues, such as age, sex, emotion and identity information, but they are also the basis of both verbal and non-verbal communication (Batty & Taylor, 2006). In effect, the accurate interpretation of facial emotion significantly affects the success of an individual’s social interactions. Research utilizing behavioral methodology has examined the remarkable aptitude for face processing in adults (Bruce & Young, 1998), often referred to as expertise. However, an ongoing debate concerns the development of this capacity (e.g. Gauthier & Nelson, 2001; Maurer, Le Grand, & Mondloch, 2002; Pascalis & Slater, 2003). There is a basic consensus that face recognition accuracy improves from childhood to adulthood (Gauthier & Nelson, 2001; Maurer et al., 2002; Want, Pascalis, Coleman, & Blades, 2003) but less accord on the particular components of face recognition that alter with development (Carey & Diamond, 1994; Freire & Lee, 2001, 2003; Itier & Taylor, 2004a,b,c; Mondloch, Le Grand, & Maurer, 2002, 2003; Pellicano & Rhodes, 2003; Schwarzer, 2000; Want et al., 2003). Do children process faces in a different way than adults or in the same way but somehow less efficiently? When does the adult-like processing emerge? A developmental shift may, in effect, occur from the processing of faces in a piecemeal or feature-based manner in childhood to a reliance on the processing of face patterns more relationally (i.e., with more configural and/or holistic information) in adulthood. (Carey & Diamond, 1994; Freire

& Lee, 2001, 2003; Itier & Taylor, 2004; Mondloch et al., 2002, 2003; Schwarzer, 2000; Want et al., 2003).

A question that arises concerns the role of configural information in the development of the ability to recognize facial emotions. Configural information has been found to play a substantial role in adult recognition of face emotion. For example, Calder, Young, Keane, & Dean, (2000) and Calder & Jansen, (2005) observed a composite effect in emotion recognition similar to effects found in the adult face recognition literature (e.g. Young, Hellawell,& Hay, 1987). In the composite face paradigm, a composite stimulus is made up by joining the top half of a face with the bottom half of another face. Adults are slower and less accurate to recognize either half when the top and bottom parts are vertically aligned (creating a new face stimulus) than when the same top and bottom parts are misaligned suggesting an adult tendency to process faces holistically. Calder and colleagues (2000, 2005) found that when the top and bottom halves of a composite face show different emotions, recognition of the emotion in either half is slower and less accurate than when the composite face is inverted or the two halves are laterally offset. It thus follows that the development of the ability to process holistic and configural properties of faces may play a significant role in the development of the ability to process facial emotions.

It has been shown that emotion recognition emerges early (e.g., Barrera & Maurer, 1981; Walker-Andrews, 1997), but the processing of facial expressions may evolve across the lifespan. For example, whereas preschool age children can label facial emotions at above chance levels (e.g., Markham & Adams, 1992; Russell & Widen, 2002; Widen & Russell, 2003), they are considerably less accurate than adults.

Converse to studies of face recognition abilities, there is a paucity of research examining the developmental course of facial emotion recognition. Additionally, results in the extant literature are not consistent, perhaps due to methodological variability. When Shih and Von Baeyer (1994) asked adults and preschool children to seriate facial expressions of emotion, adults identified nine discrete categories while the children only identified five. This suggests that while young children may have concepts of different categories of emotion, the distinctions between category boundaries are not yet as refined as those of adults with more experience. Bruce et al. (2000) showed that when children were asked to point to which of two faces was happy, sad, angry, or surprised, they attained near-perfect accuracy by 6 years of age. However, when the task involved choosing which of two emotional faces expressed the same emotion as a third face, a comparable level of accuracy was not achieved until age 10, perhaps indicating that development of facial emotion recognition depends on task demands. In a study of similar design, children were asked to match a photograph of an emotional face to one of four possibilities (neutral, surprise, happiness, or disgust) (Mondloch, Geldart, Maurer, and Le Grand, 2003). The authors reported an increase in accuracy between 6 and 8 years of age, when performance reached the adult level. Kolb, Wilson, and Taylor (1992), showed children and adults either an emotional photograph or a cartoon depicting an emotional situation and then asked participants to choose the face that exhibited the same or appropriate emotion from a panel of six different emotional photographs (happiness, sadness, fear, anger, disgust, and surprise). Between 6 and 8 years and between 8 and 10 years of age, recognition of facial emotions improved, depending on the task. Accuracy was found to increase again

between age 14–15 years of age and adulthood. Notably, this developmental pattern appears to vary by emotion. Children recognize happiness earliest and with the greatest accuracy, followed by sad or angry expressions, then expressions of surprise or fear (Boyatzis et al., 1993, Camras & Allison, 1985; Gross & Ballif, 1991). Three-year olds identify happy faces (76%) correctly far more often than fearful faces (18%) (Bullock & Russell, 1984). Several studies have shown that when preschool children label the expression of faces, they perform the best on happy faces compared to negative emotional faces showing anger, fear, or sadness (Bullock & Russell, 1984; Denham, McKinley, Couchoud , & Holt, 1990; MacDonald, Kirkpatrick, & Sullivan, 1996). Boyatzis and colleagues (1993) asked children to match an emotional face to a read vignette and found that children were most accurate at matching happy emotions, followed by sad and surprise emotions. It may be that happy faces are the most perceptually and conceptually distinct from other emotions. The superior performance of identifying happy faces is removed when the features of the other emotions (sad, anger, surprise) are exaggerated (Walden & Field, 1982). Correct recognition of happy faces may be only dependent on the mouth region for both adults and children (Kestenbaum & Nelson, 1992) whereas anger and fear require more features for identification. Pedelty, Levine and Shevell (1985) reported that children younger than ten years of age use fewer features concurrently to identify faces. If this is the case, then performance on identifying happy faces may be more advanced because only the mouth region is required. In a match-to-sample study with facial expressions, 5- and 7-year old children performed equally well on happy, sad, and angry faces and less well on disgust, fear, and surprise faces (Tremblay, Kirouac, & Dore, 1987). Missaghi-

Lakshman and Whissell (1991) also showed that children 7- 12 years were better at identifying happy and sad than they were at other emotions (fearful, angry, surprise, disgust). Perhaps age-related improvements in identifying face emotion reflect an increased ability to recognize differences between non-happy facial expressions.

While the happy superiority does not disappear, performance on other emotions does gradually improve with age. This may be because the frequency at which children are exposed to face emotions is not equivalent. Field and Walden (1982) showed that neutral and happy were the most frequent spontaneous expressions produced by children in a free play session, whereas negative emotions (i.e. fear, anger) were rarely expressed. Children may be better at identifying happy faces because they are more exposed to happy expressions in their daily experiences. Neutral faces, though produced more often than the other non-happy emotions, should be considered separately because of findings that children may find neutral faces to be ambiguous, potentially-threatening stimuli (Thomas et al. 2001a). The converse of this case better illustrates the influence of experience on facial expression. While typically developing children display a bias for happy faces, abused children, who may have more experience with angry affect, show a differential response. ERP studies of face emotion processing with abused children demonstrated an increased information processing bias and an increased P3b amplitude for angry faces (Pollak & Kistler, 2002; Pollak & Sinha, 2002; Pollak & Tolley-Schell, 2003). Neglected children showed difficulty across all face emotions and blurred category boundaries between emotions (Pollak, Cicchetti, Hornung, and Reed, 2000).

Despite improvement across all emotions through middle childhood, children continue to show difficulty categorizing fearful faces (Boyatzis et al., 1993; Bullock & Russell, 1984; Camras & Allison, 1985; Denham et al., 1990; Field & Walden, 1982; Smith & Walden, 1998). Lenti, Lenti-Boero, and Giacobbe (1999) reported that while eight-year olds correctly identified fear 43.5% of the time, sixteen-year olds only improved to 58.6% correct identification. Adults have been shown to correctly identify fearful faces 83% of the time (Bullock & Russell, 1984). It may be that children and adults are exposed to fearful faces less frequently in everyday experience. This decreased familiarity (compared to other emotions) my affect performance with fearful faces. Additionally, fear is perceptually similar to surprise faces (Ekman and Friesen, 1976) which may also result in some confusion when both categories are options. Another possibility is that fearful expressions are the emotional faces most likely to activate the amygdala which is highly sensitive to potential danger in the environment. Perhaps the detriment in processing these evolutionarily relevant stimuli on behavioral tasks indicates that cognitive resources are otherwise engaged in assessing the possibility of threat.

The current study was designed to investigate how children categorize emotional faces when only some of the facial features are available. The first task addressed the question of whether children can identify emotion from just the eye-region or mouth-region of facial expressions. We were also interested to find if there are differences in performance accuracy by emotion and by feature. The second task addressed whether children are more likely to use the eye or the mouth features to determine emotion when they are forced to choose between conflicting emotional information from the two

regions. This manipulation also allowed us to test performance accuracy at recognizing expressions when eyes and mouths from different actors presented congruent emotional information. The third task addressed whether children were able to correctly match mouths to eyes based on emotion as well as how children labeled these features after they were matched. Eight-year old children were chosen because, as discussed, middle childhood is a period when children show continued improvement on face emotion processing.

Method

Participants

Sixty-three 8-year old children (33 female; 8.5 +/- 0.4 years) were recruited from an existing community participant pool in the Minneapolis/St. Paul area. Participants were screened for any personal or family history of psychiatric or medical illness, and for any contraindications for MRI because a subset of the children were to be asked to return for a second study using MRI. Informed consent/assent was obtained from all parents and child participants. All parents were asked to complete a series of questionnaires about their child, including the Child Behavior Checklist (CBCL/4-18) (Achenbach, 1991) and the Children's Behavior Questionnaire (CBQ) (Rothbart, Ahadi, Hershey, and Fisher, 2001). Participants who scored in the clinical range (T-score of 70 and above) on the CBCL were excluded from further analysis. All participants were also asked to provide buccal cells to be genotyped for the 5-HTTLPR polymorphism at this first visit but these procedures will be discussed further in Chapter 3. We were unable to obtain this genetic information for four of the participants (one refused and three provided insufficient genetic material for accurate assay). The subset of children

who returned for the second visit was also asked to complete the State Trait Anxiety Index for Children (STAI-C) (Spielberger. Edwards, Lushene, Montuori, & Platzek, 1973) after completing the neuroimaging procedures.

Stimuli

Face stimuli used in this experiment consisted of laminated color photographs of male and female actors posing four different emotional expressions (happiness, fear, anger, sadness). These stimuli were drawn from a standardized set (Tottenham, Borscheid, Ellertsen, Marcus, & Nelson, 2002) and organized as follows. Separate stimulus sets of emotional eyes and emotional mouths were created using the images of eight male and eight female actors. These identities were randomized so that each participant was only presented with the emotional eye or mouth regions of four male and four female actors. A stimulus set of chimeric faces was created by combining mismatched happy, sad, fearful, and angry eyes and mouths such that that full faces were shown. The different emotional eye and mouth regions from the same actor were combined so each created stimulus was congruent for identity. A gray bar was drawn across the nose area in each photograph so as to minimize distraction from non-eye or -mouth features. Top and bottom regions were carefully aligned. The entire chimeric stimulus set included eight male and eight female actors and these identities were randomized across each of the sessions so that each participant was only presented with the chimeric faces from four male and four female actors. Finally, another stimulus set was created of emotional eyes (top face region) and mouths (bottom face region) using eight male and eight female actors. Identities and emotions for these stimuli were also

randomized across each of the sessions so that each participant was only presented with the emotional eye and mouth regions of four male and four female actors.

Procedure

Identification of Features. Participants were first shown four labeled baskets (Happy, Sad, Fearful and Angry) and asked 'Can you think of a time when you felt this way?' These questions were used to ensure that each child understood the meaning of each of the labels. Participants were then given a stack of 32 laminated photographs of the eye regions from happy, sad, fearful, angry faces (See Figures 2.1 and 2.2 for examples) and asked to sort together the people who "feel the same way" into each of the labeled baskets. Subsequently, participants were given a stack of 32 laminated photographs of the mouth region from happy, sad, fearful, angry faces (See Figures 2.3 and 2.4 for examples) and asked to sort together the people who "feel the same way" into each of the labeled baskets.

Categorization of Chimeric Faces. Participants were given laminated photographs of "chimeric" or composite faces (See Figures 2.5 and 2.6 for examples) in a forced-choice task. Stimuli were presented in two sets, one that mixed happy and sad expressions (32 photographs), and one that mixed angry and fearful expressions (32 photographs). The created stimulus sets included both congruent composite faces (eyes and mouth that showed the same emotion) and incongruent composite faces (eyes and mouth from that showed different emotions).

Participants were given a stack of 32 happy and sad composite faces from two male and from two female actors and asked the questions, "Which of these people feel happy? Which of these people feel sad?" They were then instructed to sort happy and

sad faces into corresponding labeled baskets. Subsequently, they were given a stack of 32 angry and fearful composite faces from two male and from two female actors and asked the questions, "Which of these people feel angry? Which of these people feel fearful or scared?" They were then instructed to sort angry and fearful faces into corresponding labeled baskets.

Feature Matching. For this task, an open-faced wooden box apparatus was constructed with a two row by four column configuration of bins (Figure 2.7). Participants were given laminated photographs of the top (eyes) and bottom (mouths) regions of emotional (happy, sad, fearful, and angry) faces. The task consisted of 16 trials in total. In each trial, the emotional eye stimuli from four different, same-gender actors were placed into the top row of bins in the box apparatus. The children were then given the emotional mouth stimuli from four different actors (same gender as eye stimuli) and asked to match the mouths to the eyes by emotion. They were instructed to place the corresponding mouths in the bins below the eye stimuli. Trials either consisted of only happy and sad features or only angry fearful features together. Order of presentation was randomized. Additionally, it was ensured that the eye region of one actor was never presented in the same trial as his/her mouth region so that participants would match by emotion and not by face identity or gender. Following each trial, participants were asked to label each of the matched sets as happy, sad, angry, or fearful. All four emotional label choices were provided as options in each trial.

Results

Identification of Features

Correct sorting of each set of emotional eyes into corresponding bins was used to calculate the group mean accuracy for each emotion (Figure 2.8). Children performed as follows: Angry Eyes (88.3%) > Sad Eyes (81.4%) > Fearful Eyes (73.8%) > Happy Eyes (70.7%). Paired *t*-tests comparing accuracy between each combination of emotions revealed significant differences between three of the pairs: Sad Eyes > Happy Eyes $t(62) = 2.673$, $p = .010$; Angry Eyes > Happy Eyes $t(62) = 4.216$, $p = .000$; and Angry Eyes > Fearful Eyes $t(62) = 4.033$, $p = .000$. Overall, children were most accurate at identifying angry eyes, although the difference between angry and sad eyes sorting performance did not reach significance $t(62) = 1.920$, $p = .059$. Children were least accurate at categorizing happy eyes, although performance on happy and fear eyes was not significantly different $t(62) = .722$, $p = .473$.

Correct sorting of emotional mouths into labeled bins was also used to calculate the group mean accuracy for each emotion (Figure 2.9). Children sorted happy mouths with 99.0% accuracy. Accuracy was 51.9% for angry, 50.3% for sad, and 37.4% for fearful mouths. Clearly, children performed extremely well at identifying happy mouths but were considerably less accurate with any of the other emotional mouths. Paired *t*-tests comparing accuracy between each combination of emotions revealed significant differences between five of the pairs: Happy Mouths > Angry Mouths $t(62) = 13.581$, $p = .000$; Happy Mouths > Sad Mouths $t(62) = 16.519$, $p = .000$); Happy Mouths > Fearful Mouths $t(62) = 21.501$, $p = .000$); Angry Mouths > Fearful Mouths $t(62) = 3.302$, $p = .002$); and Sad Mouths > Fearful Mouths $t(62) = 2.984$, $p = .004$.

Children did not significantly differ at sorting angry and sad mouths $t(62) = .432$, $p = .667$.

In addition to examining whether participants could correctly categorize each emotional face feature, we were also interested in how photographs were incorrectly sorted. For example, if happy eyes were not sorted into the 'happy' bin, then were they more likely to be labeled as 'angry,' 'sad,' or 'fearful?' One-sample *t*-tests were conducted on incorrect trials to find whether the stimuli were labeled as a particular other emotion at greater or less than chance (33%) proportions. Incorrectly sorted happy eyes were categorized as 'sad' at greater than chance $t(48) = 3.050$, $p = .004$ and categorized as 'angry' at less than chance $t(48) = -4.048$, $p = .000$. Incorrectly sorted sad eyes were categorized as 'happy' at less than chance $t(46) = -2.301$, $p = .026$. Incorrectly sorted fearful eyes were categorized as 'happy' at greater than chance $t(39) = 3.072$, $p = .004$ and categorized as 'sad' at less than chance $t(39) = -4.048$, $p = .000$. Incorrectly sorted angry eyes were categorized as 'sad' at greater than chance $t(28) = 4.443$, $p = .000$ and categorized as 'happy' $t(28) = -2.328$, $p = .027$ and 'fearful' $t(28) = -3.264$, $p = .003$ at less than chance. Overall, participants very rarely made any errors categorizing happy mouths. Incorrectly sorted sad mouths were categorized as 'fearful' at greater than chance $t(61) = 6.560$, $p = .000$ and categorized as 'happy' at less than chance $t(61) = -11.638$, $p = .000$. Incorrectly sorted fearful mouths were categorized as 'sad' at greater than chance $t(61) = 6.746$, $p = .000$ and categorized as 'happy' at less than chance $t(61) = -6.898$, $p = .000$. Incorrectly sorted angry eyes were categorized as 'happy' at greater than chance $t(61) = 3.729$, $p = .000$ and categorized as 'sad' at less than chance $t(61) = -6.125$, $p = .000$.

Correlation analyses indicated that accuracy on happy eyes was significantly correlated with accuracy on fearful eyes (r = .365, p = .003) while accuracy on fearful eyes was significantly correlated with accuracy on angry eyes (r = .539, p = .000).

Additional analyses were conducted to examine effects of gender on performance. The only findings for gender showed that males (93.25%) were more accurate than females (82.60%) at identifying angry eyes (p=.056) while females (61.00%) were more accurate than males (43.10%) at identifying angry mouths (p=.008).

Exploratory analyses were also conducted to examine potential effects of genotype on performance. As noted, genotyping methodologies will be discussed in detail in Chapter 4 where we describe a replication study of Hariri and colleagues (2002) work. Briefly, the promoter polymorphism in the human serotonin (5-hydroxytryptamine; 5-HT) transporter (5-HTT) gene has shown a strong association with the engagement of neural systems that subserve emotional processes. Hariri and colleagues (2002b) utilized fMRI to directly explore the neural basis of the likely relationship between the 5-HTTLPR and emotional behavior and found that subjects carrying the 5-HTTLPR short allele exhibited significantly increased amygdala activity in comparison with subjects homozygous for the long allele. Based on Hariri et al.'s (2002) procedures, individuals possessing either one or two copies of the short (S) allele were included in the S group, and those homozygous for the long (L) allele were included in the L group. We hypothesized that if genotype has an effect on brain reactivity to emotional face stimuli, behavioral differences might also be observable. Only one genotype effect was found such that the individuals homogeneous for the long

allele (31.29%) were less accurate than individuals homogeneous or heterogeneous for the short allele (43.06%) on categorizing fearful mouths (p=.043). No gender by genotype interaction was found for eyes or mouths.

Categorization of Chimeric Faces

In order to assess participants' ability to identify chimeric faces when the emotional information from the eyes and mouth regions were congruent (i.e. happy eyes with a happy mouth), mean accuracy for just those stimuli were examined. For purposes of simplification, emotions represented in the stimuli will be abbreviated as such: Happy (HA); Sad (SA); FE (Fearful); Angry (AN). Chimeric face stimuli will be identified with eye emotion first, followed by the mouth emotion (i.e. 'HA_SA' for a photograph with happy eyes and a sad mouth and 'HA_HA' for a congruent happy photograph). Overall, participants were quite adept at correctly sorting the emotionally congruent composite faces: SA_SA (97.983%) > HA_HA (97.024%) > FE_FE (94.246%) > AN_AN (92.261%). Paired sample t-tests showed that these percent accuracy scores were all statistically equivalent.

In order to assess how participants sorted the composite faces with mixed emotions, we calculated the percent of trials on which children sorted by the mouth emotion. These means indicate whether the 8-year olds were more likely to sort by the eye or mouth region and whether this is affected by emotion. Children performed as follows: HA_SA (95.766%) > SA_HA (84.325%) > FE_AN (25.198%) > AN_FE (16.984%). Paired *t*-tests comparing for sort tendency showed significant differences between all six of the pairs: HA_SA > SA_HA $t(61) = 5.130$, $p = .000$; HA_SA > FE_AN $t(61) = 23.235$, $p = .000$; HA_SA > AN_FE $t(61) = 32.407$, $p = .000$; SA_HA

> FE_AN $t(62) = 17.347$, $p = .000$; SA_HA > AN_FE $t(62) = 22.561$, $p = .000$; and FE_AN > AN_FE $t(62) = 2.617$, $p = .011$. Overall, children tended to use the mouth region to sort for chimeric happy and sad faces as indicated by their high percentage of sorting by mouth emotion when sorting my mouth emotion. However, their lower percentage when sorting angry and fearful composite faces suggests that participants were more likely to use the eye region of those stimuli to judge emotion (as an incorrect response for the mouth region was a positive response for the eye region). For visualization purposes, percent sorting by mouths was converted to a bias score where (+) values indicate a bias towards eyes and (-) values indicate a bias towards mouths (See Figure 2.10).

Correlation analyses indicated that accuracy on sorting congruent happy faces (HA_HA) stimuli was significantly correlated with sorting by mouth region on chimeric happy and sad faces (HA_SA r = .840, $p = .000$; SA_HA r = .518, $p = .000$). Correct sorting by mouth region was also significantly correlated between the chimeric happy and sad (HA_SA, SA_HA) conditions (r = .489, $p = .000$). Accuracy on congruent sad faces (SA_SA) was significantly correlated with sorting by mouth region on chimeric happy and sad faces (HA_SA r = .881, $p = .000$; SA_HA r = .521, $p = .000$). It was also found that accuracy in sorting congruent angry faces (AN_AN) was negatively correlated with accuracy on sorting by mouth region on Anger/Fear chimerics (AN_FE r = -.582, $p = .000$).

Additional analyses were conducted to examine potential effects of gender or genotype on performance. The only finding for gender showed that males (91.41%) were less accurate than females (97.18%) at sorting congruent fear faces (FE_FE)

(p=.021). Neither genotype effects nor gender by genotype interactions were found for any of the chimeric face sorting.

Feature Matching

In the feature matching task, response was considered correct if a participant matched eyes and mouths showing the same emotion, regardless of actor. Overall, children performed significantly more accurately on the Happy/Sad trials than they did on Fearful/Angry trials (91.993% > 76.497% p = .000). Correct match trials were then further examined to determine how participants labeled the matched face regions. Overall, participants were quite adept at labeling correctly matched Angry (97.105%) and Happy (96.935%) features. They were less accurate at labeling correctly matched Fearful (82.252%) and Sad (66.450%) features (See Figure 2.11). Incorrect matches were examined by whether the participant labeled the eye/mouth pair by the eye region, the mouth region, or with another non-trial (i.e. Happy or Sad label during a Fearful/Angry trial) emotion (See Figures 2.12 and 2.13). When happy eyes were incorrectly matched with sad mouths, participants tended to label the eye/mouth pair by the mouth region (55.750%) or with a non-trial emotion (36.250%) more than they used the eye region (8.000%). When sad eyes were incorrectly matched with happy mouths, participants tended to label the eye/mouth pair by the mouth region (70.875%). Children were less likely to label the eye/mouth pair by the eye region (17.666%) or with a non-trial emotion (11.458%). When fear eyes were incorrectly matched with angry mouths, participants tended to label the eye/mouth pair by the eye region (61.301%) more than they were likely to use the mouth region (24.168%) or with a non-trial emotion (14.529%). When angry eyes were incorrectly matched with fearful

mouths, participants were more likely to label the eye/mouth pair by the eye region (64.483%) than they were categorized with a non-trial emotion (22.666%) or by the mouth region (12.649%).

No gender or genotype effects were found for the emotion matching task.

Clinical Data

Further analyses were conducted to determine if there were correlations between parent ratings on clinical questionnaires (CBCL, CBQ) and performance on the card-sorting measures. A positive correlation was found between accuracy at sorting congruent happy (HA_HA) composite faces and scores on the internalizing problems subscale of the CBCL (r = .250, p = .048). Accuracy at categorizing fearful eyes was inversely related to internalizing scores (r = -.278, p = .027) and correct sorting of angry eyes was inversely to the anxiety problems subscale (r = -.308, p = .074). A positive correlation was found between accuracy at categorizing sad mouths and the approach dimension on the CBQ (r = .330, p = .008) and a negative correlation was found between accuracy on happy mouths and the shyness dimension on the CBQ (r = -.337, p = .007). Accuracy at categorizing angry eyes was negatively correlated with the sadness dimension (r = -.255, p = .044) and the shyness dimension (r = -.355, p =.043) on the CBQ. Correct sorting of fearful eyes was also related to the shyness dimension (r = -.250, p = .048).

Finally, correlation analyses were also conducted for state and trait anxiety scores (from those participants who completed the STAI-C at the fMRI visit) and card-sorting performance. Accuracy at categorizing fearful eyes (r = -.461, p = .010) and angry eyes (r = -.516, p = .004) were both negatively correlated with state anxiety.

Accuracy at categorizing angry eyes was negatively correlated with the trait anxiety ($r = -.369$, $p = .045$)

Discussion

The data show that children performed with varying degrees of accuracy on the behavioral tasks. Eight-year olds were quite successful at categorizing photographs of emotional eyes, showing the most aptitude at identifying angry eyes, followed by sad, fearful, and happy. This is somewhat surprising, as the literature suggests that children recognize happy full faces earliest and with the greatest accuracy, followed by expressions of sadness or anger, then surprised or fearful expressions (Boyatzis, Chazan, & Ting, 1993, Camras & Allison, 1985; Gross & Ballif, 1991). However, the data are consistent with the idea that both adults and children rely predominantly on the mouth region to recognize happy faces (Kestenbaum and Nelson, 1992). If the mouth region of happy faces is more perceptually salient, then perhaps children rarely attend to the happy eye region and so have less experience with those features. If the other emotions generally require more features for identification, then children may be more attuned to multiple features of those emotional faces. This may be supported by data from the mouth sorting task in that children showed near perfect accuracy at categorizing happy mouths. However, they were significantly worse at identifying angry and sad mouths, and the least adept at recognizing fearful mouths. Given that children younger than ten-years of age use fewer features concurrently to determine the identity of faces (Pedelty et al., 1985), the poorer performance of eight-year olds on the mouth task indicates that, with the exception of the happy emotion, children are actually more likely to use the eye region to label emotional faces. Their poor performance on

fearful mouths may be reflective of the low frequency of fearful faces experienced in every day life or the perceptual salience of fearful eyes. As participants were forced to choose a label from the four emotion categories, we were interested in the type of errors children were making when they misidentified eyes or mouths. For example, both angry and happy mouths were confused with each other at greater than chance proportions, perhaps because an open-mouthed grimace showing teeth is perceptually similar to an open-mouthed smile showing teeth. While participants rarely made errors sorting happy mouths, it is possible that they over-generalized a paradigm for a happy smile to an angry grimace. While there were no conspicuous patterns in errors made mislabeling the emotional eye regions, it was remarkable to note that errors were not necessarily bi-directional. For example, children who incorrectly sorted happy eyes were most likely to mislabel them as sad eye whereas mislabeled sad eyes were least likely to be sorted as happy eyes. In fact, overall, when children misidentified emotional eyes, they were more likely to incorrectly label them as either happy or sad. Perhaps this indicates an uncertainty about the eyes of an angry or fearful face consistent with the idea that children have less experience with these emotional expressions. With the exception of happy mouths, participants performed much more poorly on the emotional mouths than eyes. They were equally like to confuse sad and fearful mouths with each other but far less likely to label either sad or fearful mouths as happy. This suggests that while children were unsure about the mouth features of a sad or fearful face, they were certain that they did not match their happy mouth prototype.

On the whole, 8-year old children were adroit at categorizing the emotionally congruent composite faces, showing the added benefit of having both features. Sorting

of the composite faces with mixed emotions was used to indicate whether the children were more likely to sort by the eye or mouth region and whether this was affected by emotion. Overall, children tended to use the mouth region to sort for chimeric happy and sad faces. This may be partially due to their already demonstrated skill at identifying happy mouths as there were only two choices (sad or happy) in this task. Children may have merely recognized that the sad mouths were not happy mouths and so belonged in the other category. Eight-year olds performed quite differently when sorting angry and fearful composite faces, suggesting that they were using the eye region of those stimuli to judge emotion.

Children who sorted congruent happy (HA_HA) and sad (SA_SA) stimuli correctly also performed well at sorting stimuli by the mouth region on HA_SA and SA_HA. As suggested earlier, this may only be a reflection of their superior skill at recognizing happy mouths. It was also found that accuracy in sorting congruent angry (AN_AN) stimuli was negatively correlated with tendency to sort by mouth region on AN_FE stimuli. This might have been predicted by participants' high accuracy sorting in angry eyes in the first task, as their tendency to not sort AN_FE by mouth regions indicates that they were likely correctly sorting by the angry eyes.

In the matching task, children performed significantly more accurately on the Happy/Sad trials than they did on Fearful/Angry trials, perhaps reinforcing the idea that eight-year olds are more competent with emotions with which they are more familiar. Correct match trials were also examined to determine how participants labeled the matched face regions. We were interested to find if there were cases when 8-year olds were accurately matching the emotional eyes and mouths but mislabeling the face

emotions when explicitly queried. Participants were generally very accurate at labeling correctly matched Angry and Happy pairs. They were, however, less accurate at labeling correctly matched Fearful and Sad trials. Interestingly, during the Happy/Sad trials, children tended to label the incorrectly matched eye/mouth pairs by the mouth region whereas they used the eye region more during the Angry/Fear trials. This is consistent with our data from the composite face task. One main caveat in this study task is that children were only asked to match features from happy and sad faces and from angry and fearful faces. In other words, we cannot make conclusions about matching performance outside of the current context or combination (i.e. angry eyes with a happy mouth, fearful eyes with a sad mouth, etc.).

There were no specific a priori hypotheses regarding performance on the sorting tasks and parent ratings on clinical questionnaires (CBCL, CBQ). It was noteworthy, however, that significant though not robust correlations were found, speaking to the individual variability of participants within the typically developing ranges. Correlation analyses for the subset of participants with STAI-C scores revealed that accuracy at categorizing fearful and angry eyes were both negatively correlated with state anxiety. Accuracy at categorizing angry eyes was also negatively correlated with the trait anxiety. Perhaps children on the higher end of the anxiety spectrum have more difficulty categorizing emotional features that are negative or threatening because their cognitive resources are more taxed when viewing these images. However, these state scores were not attained at the same visit as the card-sorting task so conclusions are limited.

The current study supports the idea that eight-year old children, while quite adept on many aspects of face emotion processing, are still developing their competencies. One main caveat in this study is that we did not have a comparison adult group. While the extant literature describing adult performance on emotional face task is much more extensive than the developmental work, it would be important to recruit a larger sample of adults so as to compare performance on these particular tasks. A small group of adults did complete the tasks before we began testing children in order to ensure that the paradigms were working properly. However, that pilot data was insufficient for analysis. A developmental perspective would also be enhanced with an intermediary, later childhood age group as transitional periods might be better defined.

Utilizing these sorting and composite face paradigms to examine the processing of specific features is one means to better understand the role of features in the face processing of a typically developing population. Given the nature of certain developmental disorders, particularly autism spectrum disorders (ASD), these methodologies will also be informative in studies with non-normatively developing groups. ASD (Autistic disorder, Asperger syndrome, and Pervasive Developmental Disorder, Not Otherwise Specified) consist of a set of severe neurodevelopmental disorders characterized by a triad of deficits, including impairments in reciprocal social interaction, delays in early language and communication, and the presence of restrictive, repetitive and stereotyped behaviors (Baron-Cohen, 1997; Frith & Frith, 1999; Kanner, 1943; Siegel, Vukicevic, & Spitzer, 1990). Because high-functioning (IQs in the normal range) children and adults with ASD show deficits in social judgment, (e.g. Baron-Cohen et al., 1999; Castelli, Frith, Happe, & Frith, 2002; Loveland, Pearson,

Tunali-Kotoski, Ortegon, & Gibbs, 2001; Ozonoff & Miller, 1995), much ASD research has focused on attempting to understand how people with autism process salient social cues, especially faces. Extensive work has employed static face stimuli (i.e., photographs) to investigate social judgments (e.g. Celani, Battacchi, & Arcidiacono, 1999; van der Geest, Kemner, Verbaten, & van Engeland, 2002; Grelotti, Gauthier, & Schultz, 2002; Joseph & Tanaka, 2003) and gaze fixation behavior (e.g. Carpenter, Pennington, & Rogers, 2002; Klin, Jones, Schultz, Volkmar, & Cohen, 2002; Pelphrey, Adolphs, & Morris, 2002; van der Geest, Kemner, Camfferman, Verbaten, & van Engeland, 2002). In 1978, Langdell reported that children with autism performed better than controls at a task requiring determining facial identity based on partially presented features of the face (i.e. the eye or mouth regions). Interestingly, younger children seemed to depend more on the mouth region. Joseph and Tanaka (2003) reported that high-functioning children with autism performed better at judging facial identity from the mouth alone than from the eyes alone. However, they were impaired at judging facial identity from the eyes alone, compared to age- and IQ-matched controls. Studies of face gaze in autism have generally been consistent with these abnormalities in facial information processing. High-functioning children and adults with autism have been found to allocate more gaze to the mouth than to the eyes during viewing of dynamic and static facial stimuli (e.g. Klin et al., 2002; Pelphrey et al., 2002). Evidently there are distinct differences between the way in which persons with autism and typically developing children garner social information from features of the face. Thus, further elucidation of these differences, as well as similarities, is requisite.

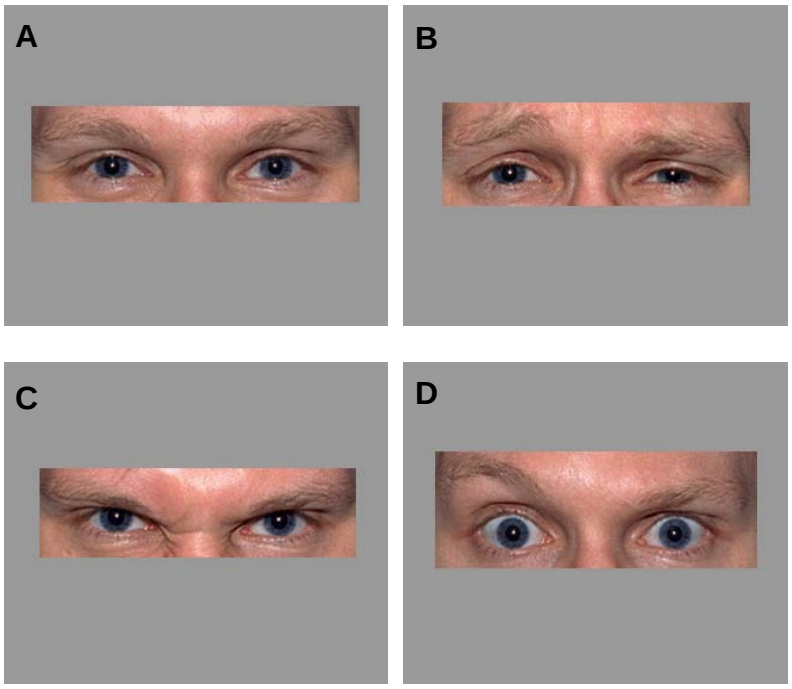


Figure 2.1. Examples of stimuli used in the Identification of Features task from a male model. Participants were given a stack of 32 laminated photographs of the eye region from (A) happy, (B) sad, (C) angry, and (D) fearful faces and asked to sort together the people who "feel the same way."

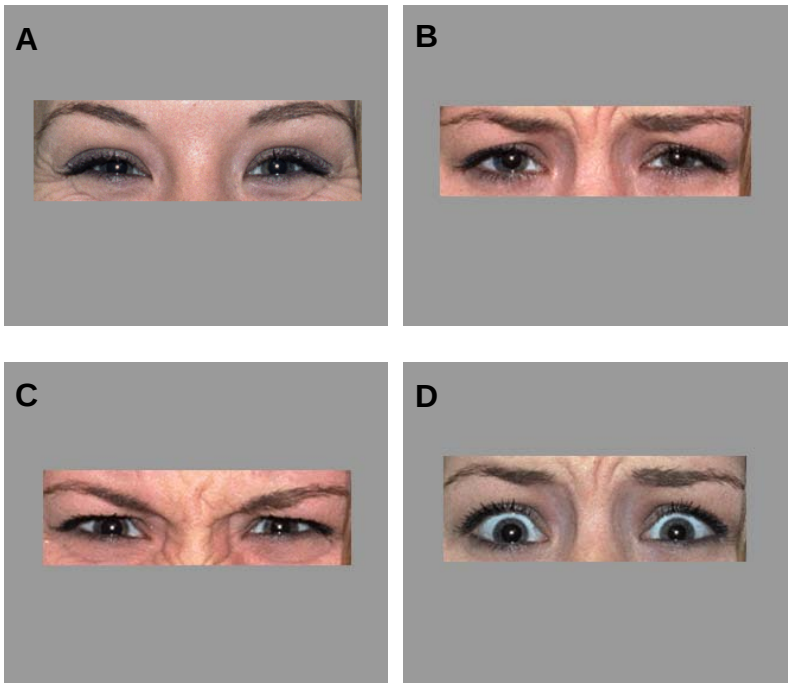


Figure 2.2. Examples of stimuli used in the Identification of Features task from a female model. Participants were given a stack of 32 laminated photographs of the eye region from (A) happy, (B) sad, (C) angry, and (D) fearful faces and asked to sort together the people who "feel the same way."

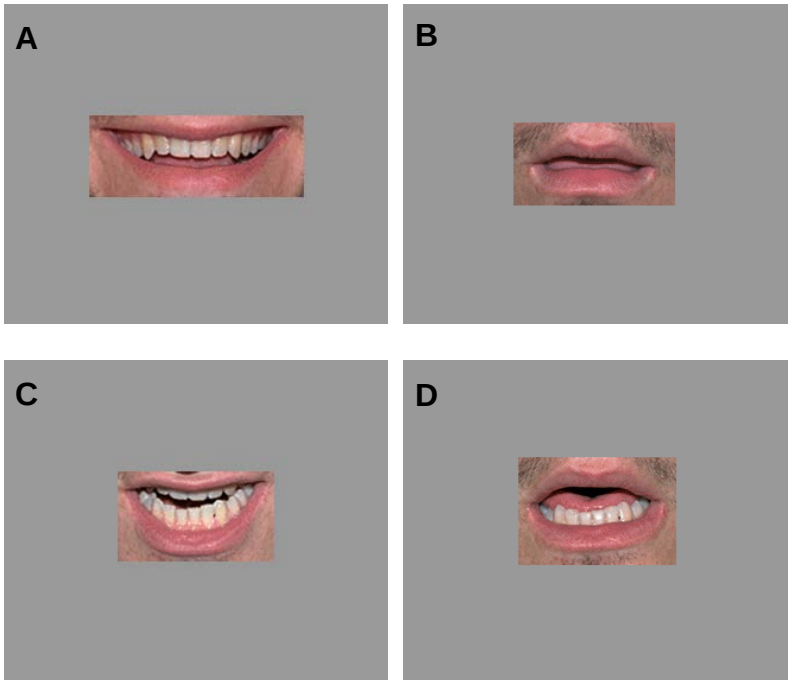


Figure 2.3. Examples of stimuli used in the Identification of Features task from a male model. Participants were given a stack of 32 laminated photographs of the mouth region from (A) happy, (B) sad, (C) angry, and (D) fearful faces and asked to sort together the people who "feel the same way."

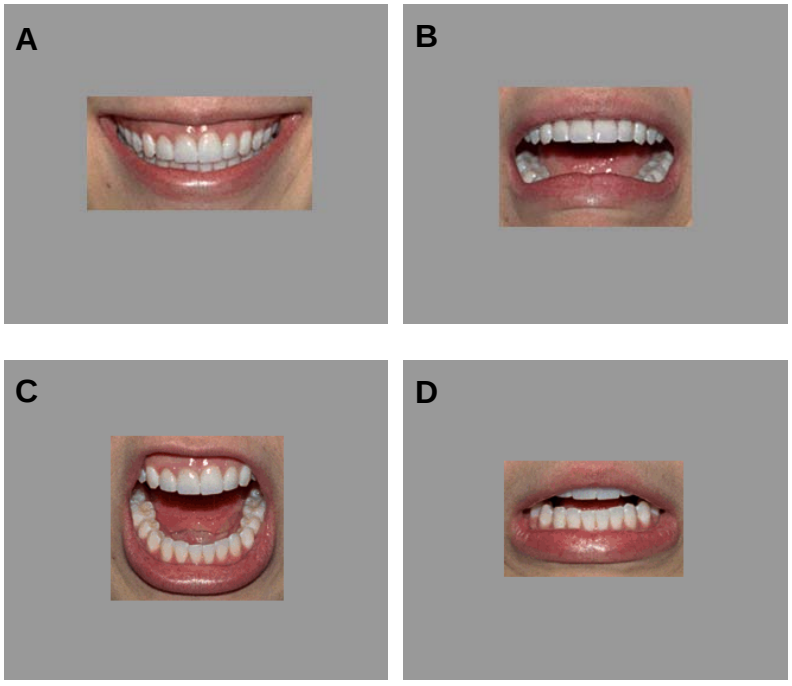


Figure 2.4. Examples of stimuli used in the Identification of Features task from a female model. Participants were given a stack of 32 laminated photographs of the mouth region from (A) happy, (B) sad, (C) angry, and (D) fearful faces and asked to sort together the people who "feel the same way."

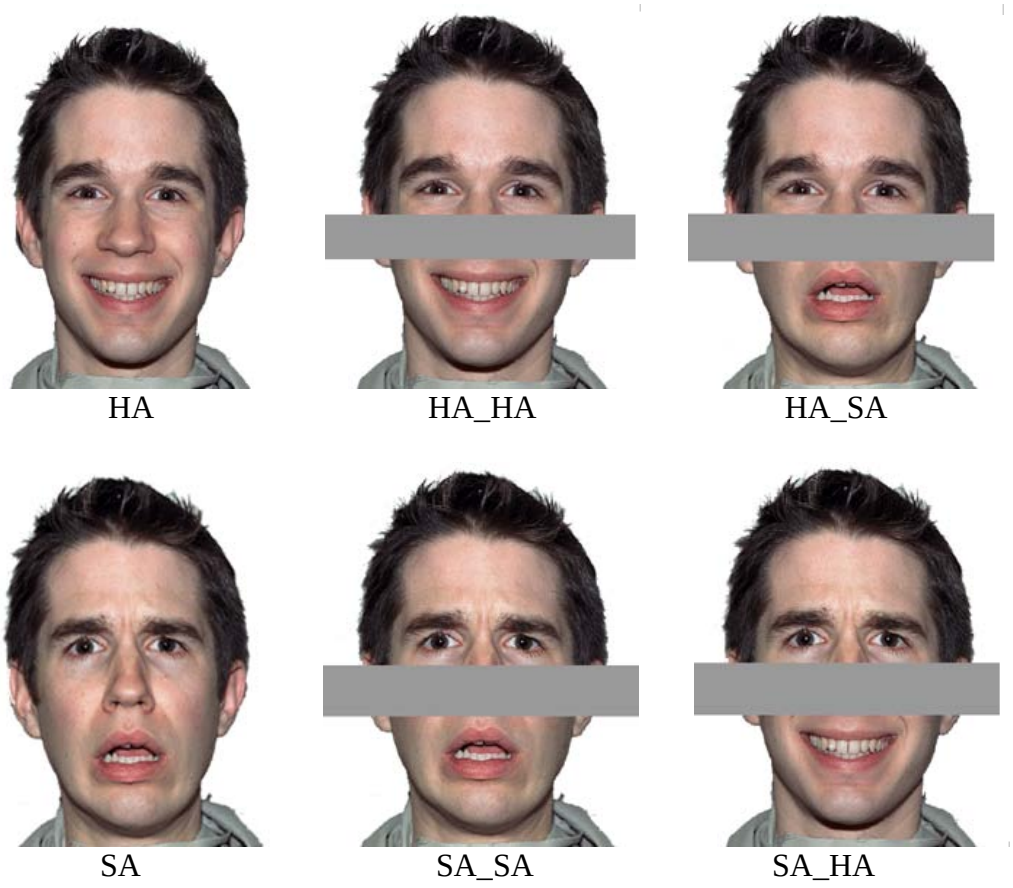


Figure 2.5. Examples of happy and sad stimuli used in the Categorization of Chimeric Faces task. "HA" and "SA" show original photographs depicting happy and sad emotions, respectively. "HA_HA" and "SA_SA" show composite faces with congruent happy and sad eyes and mouths. "HA_SA" shows an incongruent composite face with happy eyes and a sad mouth. "SA_HA" shows an incongruent composite face with sad eyes and a happy mouth.

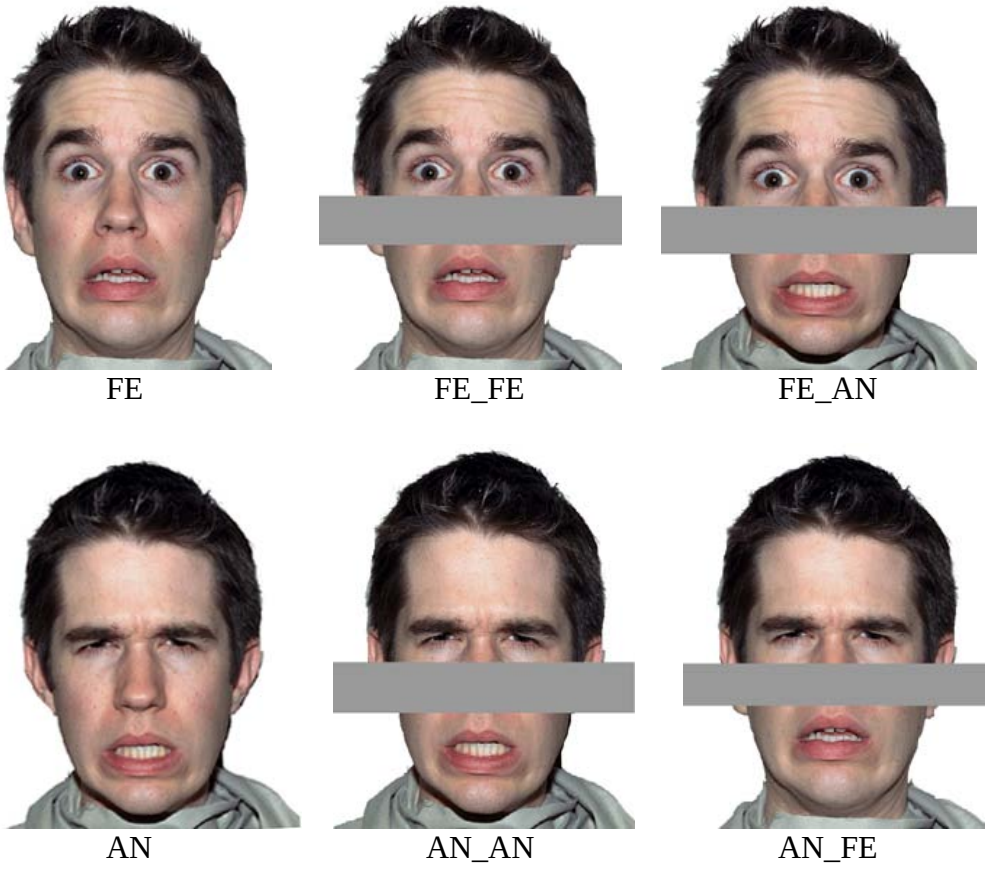


Figure 2.6. Examples of angry and fearful stimuli used in the Categorization of Chimeric Faces task. "FE" and "AN" show original photographs depicting fearful and angry emotions, respectively. "FE_FE" and "AN_AN" show composite faces with congruent fearful and angry eyes and mouths. "FE_AN" shows an incongruent composite face with fearful eyes and an angry mouth. "AN_FE" shows an incongruent composite face with angry eyes and a fearful mouth.

Figure 2.7. Apparatus for matching emotional eyes with emotional mouths (Feature Matching task). Children were presented with the upper region of four faces and asked to match the lower region from a different set of actors based on emotional expression.

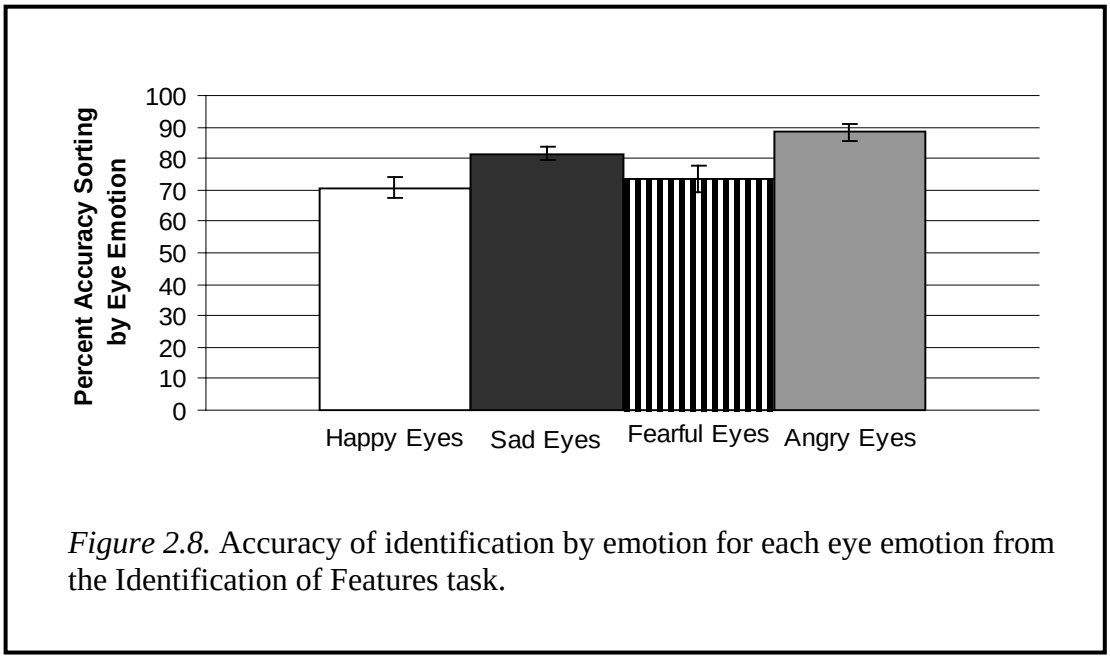


Figure 2.8. Accuracy of identification by emotion for each eye emotion from the Identification of Features task.

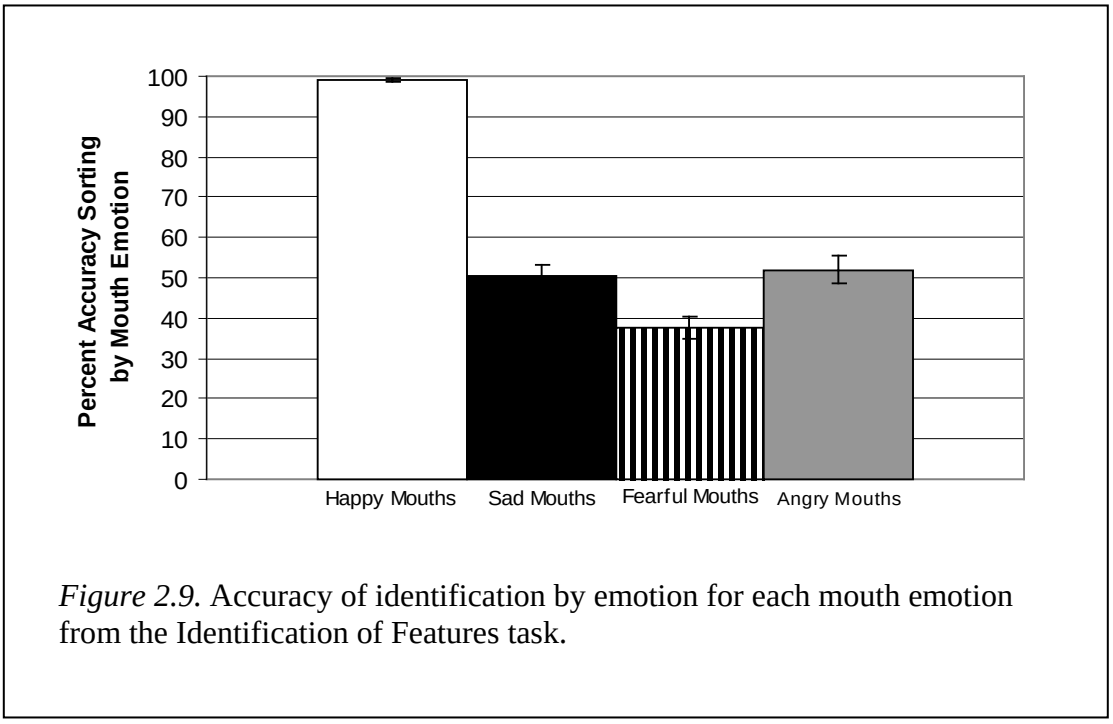


Figure 2.9. Accuracy of identification by emotion for each mouth emotion from the Identification of Features task.

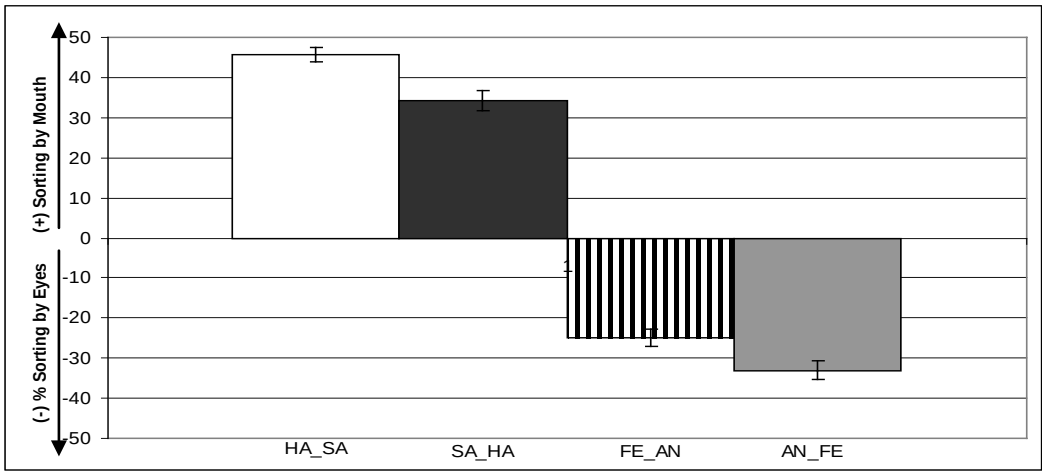


Figure 2.10. Sorting accuracy in the Categorization of Chimeric Faces task. For visualization purposes, percent sorting by mouths was converted to a bias score where (+) values indicate a bias towards mouths and (-) values indicate a bias towards eyes.

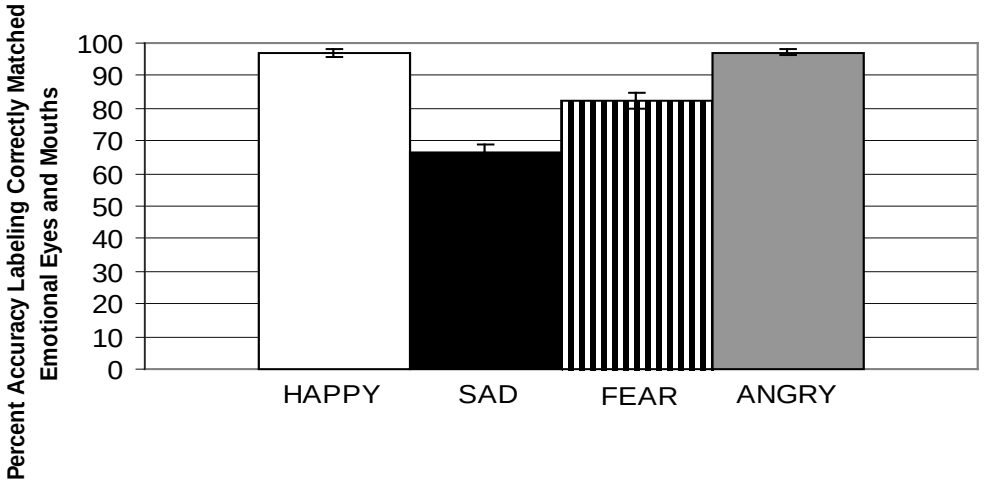


Figure 2.11. Percent accuracy labeling correctly matched eyes and mouths in the Feature Matching task.

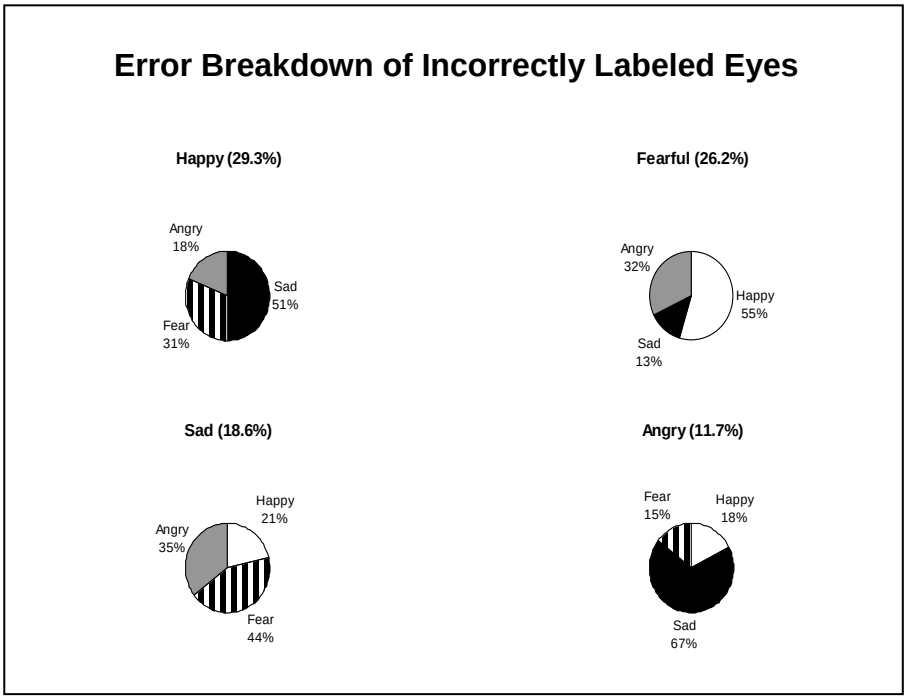


Figure 2.12. Incorrect matches in the Feature Matching task were examined by whether the participant labeled the eye pair by the eye region, the mouth region, or with another non-trial (i.e. Happy or Sad label during a Fearful/Angry trial) emotion.

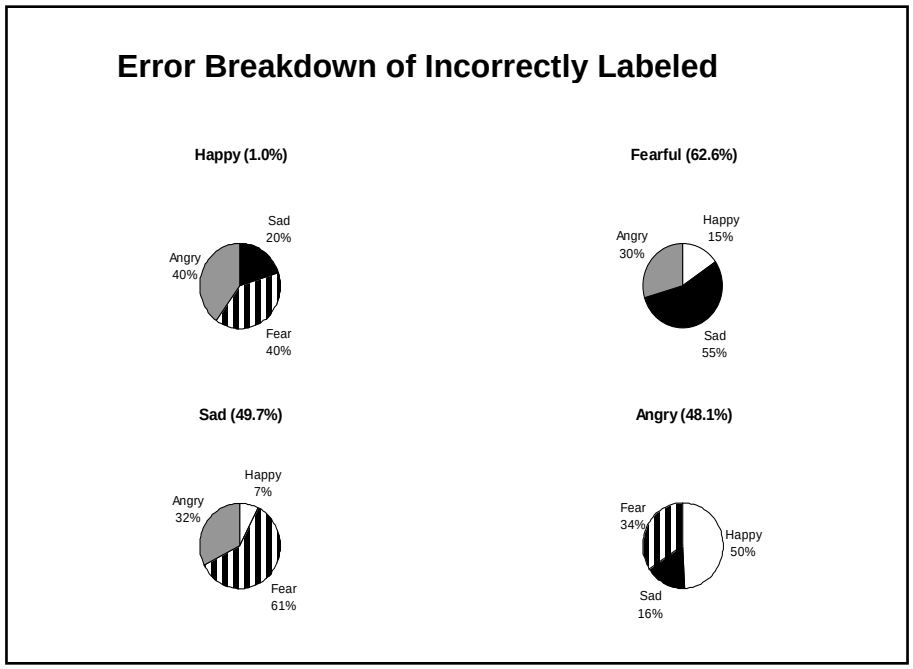


Figure 2.13. Incorrect matches in the Feature Matching task were examined by whether the participant labeled the mouth pair by the eye region, the mouth region, or with another non-trial (i.e. Happy or Sad label during a Fearful/Angry trial) emotion.

Chapter 3: Are Children's Brains Sensitive to Low-Level Perceptual Cues of Facial Emotion?

Multiple findings converge to suggest emotional responses to fearful faces in the amygdala may persist under some conditions of inattention or unawareness, such as when faces are masked (Morris, Ohman, & Dolan, 1998; Whalen et al., 1998), displayed to ignored locations (Anderson, Christoff, Panitz, DeRosa, & Gabrieli, 2003; Vuilleumier, Armony, Driver, & Dolan, 2001), suppressed by binocular rivalry (Pasley, Mayes, & Schultz 2004; Williams, Morris, McGlone, Abbott, & Mattingley, 2004), shown to the neglected side in parietally-lesioned patients (Vuilleumier, Armony, Clarke, Husain, Driver, & Dolan, 2002) or to the blind field of occipitally-lesioned patients (Anders et al., 2004; Hamm, Weike, Schupp, Treig, Dressel, & Kessler, 2003; Morris, DeGelder, Weiskrantz, & Dolan, 2001; Pegna, Kateb, Michel, & Landis, 2005). Thus, the question is raised, how does visual information reach the amygdala when visual cortical pathways are compromised? Stemming from other "blindsight" effects observed after occipital lesions (Cowey & Stoerig, 1991; Weiskrantz, 1986) and the existence of direct connections between the thalamus and amygdala in rodents (Cowey & Stoerig, 1991; Linke, De Lima, Schwegler, & Pape, 1999), a widespread hypothesis has been proposed that a fast sub-cortical route may provide inputs to the amygdala through extra-geniculate projections bypassing the striate visual cortex, involving retino-collicular and pulvinar pathways responsible for "affective blindsight" abilities (de Gelder, Vroomen, Pourtois, & Weiskrantz, 2000; Hamm et al., 2003). This sub-cortical pathway has also been targeted for investigation by employing visual stimuli that might selectively activate it. Visual responses in the superior colliculus and the

pulvinar are sensitive to low spatial-frequency (LSF) information whereas they are largely insensitive to high spatial-frequency (HSF) signals. Concomitantly, brain regions along the cortical pathway are capable of processing fine visual shape information. FMRI findings have revealed that the amygdala responses to fearful faces may primarily be driven by coarse visual features in faces (Vuilleumier et al. 2003; Whalen et al., 2004; Winston et al. 2003), which are carried in the low spatial frequency spectrum of visual images and can be transmitted by magnocellular visual channels in subcortical pathways (Leventhal et al. 1985; Schiller & Tehovnik, 2001). Thus, in one event-related fMRI study (Vuilleumier et al., 2003), participants saw neutral and fearful faces in either low-pass filtered, high-pass filtered, or intact (broad-band) images. Amygdala activation to fearful expressions was greater for intact or low-pass than high-pass faces, even though activation of fusiform cortex was, in contrast, greater for intact or high-pass faces than for low-pass faces, regardless of expression. These findings support the idea that there exists a distinct superior colliculus - pulvinar pathway to the amygdala that operates primarily on low frequency information. This pattern converges with other results indicating that emotional processing in the amygdala may be partly independent of visual processing taking place in extrastriate visual cortex (e.g., see Pasley et al., 2004; Vuilleumier et al., 2001; Vuilleumier et al., 2002; Williams et al., 2004). Similarly, it has been shown that amygdala responses can be evoked by crude visual cues which may speak to an efficient route for emotional information processing. For example, Morris and colleagues (2002) used event-related fMRI to investigate whether the eye or mouth components of a fearful face are critical in evoking increased amygdala activity to fear faces. In addition to prototypical fearful and neutral faces,

subjects viewed two types of chimerical face: fearful eyes combined with a neutral mouth, and neutral eyes combined with a fearful mouth. Interestingly, it was found that faces pairing fearful eyes with a neutral mouth evoked responses in bilateral posterior amygdala and superior colliculus indicating that fearful eyes alone are sufficient to increase amygdala activation.

Predicated on reports indicating that the eye area of the face is one of the crucial regions from which expression information is extracted (Morris et al., 2002; Sekuler, Gaspar, Gold, & Bennett, 2004) and data supporting amygdala response to the "wide-eyed" expressions of both fear and surprise (Kim, Somerville, Johnstone, Alexander, & Whalen, 2003), Whalen et al. (2004) hypothesized that the amygdala's response could be modulated merely by the larger size of fearful eye-whites (i.e., sclera) when the probe stimuli were only the sclera of an emotional face. In an fMRI study, they demonstrated that the amygdala was more responsive to fearful (larger) eye-whites than to happy (smaller) eye-whites presented in a masking paradigm that allayed subjects' awareness of their presence and anomalous nature. In separate scans, subjects viewed presentations of "eye-blacks," the inverse "negative" images of the fearful and happy eye-white stimuli, masked in the same manner. The use of eye-black stimuli served as a control for the available perceptual "edge" information in the stimuli as it was identical in the eye-white and eye-black conditions. The eye-black condition tested whether it was the eye outline that determined amygdala response or the size of the white scleral field by presenting eye-black stimuli of an identical size, shape, and positioning within-subject. The amygdala did not differentially activate to happy or fearful eye-blacks,

indicating that the size of the more ecologically valid eye-whites is an essential stimulus of interest.

As discussed in Chapter 1, few studies have examined amygdala involvement in processing fearful facial expressions in typically developing children. One study with 12 adolescents (12-17 years old) showed greater amygdala activation to fearful faces than to fixation trials or nonsense stimuli (Baird et al.,1999). Killgore et al. (2001) reported that amygdala response to fearful faces varied by age and sex in 19 adolescents (9–17 years old), such that amygdala activation correlated inversely with age. Yurgelun-Todd and Killgore (2006) also conducted a study with 16 adolescents (8–15 years old) that showed no linear relationship between age and amygdala activation to fearful faces. Thomas and colleagues (2001b) compared amygdala response to fearful facial expressions between six children (9–13 years old; all male) and six adults (18–30 years old; all male) and found that amygdala activation was greater when passively viewing fearful faces versus fixations in each group. Direct group comparisons showed that there was significant amygdala activation to fearful relative to neutral faces in adults greater than children. Meanwhile, children exhibited greater amygdala response to neutral versus fearful faces than adults. Monk et al. (2003b) compared amygdala response in 17 adolescents (9–17 years old; 8 females) and 17 adults (25–36 years old; 8 females) during presentations of emotionally engaging faces. In a passive-viewing condition, but not in attention-constraining conditions, adolescents showed greater amygdala activation than adults to fearful versus neutral faces. Thus, Thomas and colleagues (2001b) found that amygdala response to fearful faces was greater in adults than in children, whereas Monk et al. found the converse.

Whalen et al. (2004)'s paradigm has yet to be studied in children. Given the contradictory findings in the few available studies examining amygdala functional development in humans, it is difficult to predict children's response to backward masked eye-blacks and eye-whites. Nevertheless, there is a call to better elucidate the role of low level perceptual features of faces in the development of brain response to facial emotion. Prior work in our laboratory suggests that the backward masking procedure can be used effectively with 8-year old children during brain imaging procedures (Tseng and Thomas, 2008, 2009). An fMRI study successfully utilizing the eye-white/eye-black paradigm study with children would provide further support for the utility of the backward-masking procedure. It was hypothesized that children would show a differential response to the eye-white and eye-black stimuli similar to adults (Whalen et al., 2004). Although 8-year old children have not yet reached adult levels of emotional face categorization, it is predicted that, because the backward-masking procedure targets the pre-conscious processing of low level perceptual features, differential brain response to each stimulus type would be detectable.

Method

Participants

Sixty-three 8-year old children (33 female; 8.5 +/- 0.4 years) were recruited from an existing community participant pool in the Minneapolis/St. Paul area to participate in the behavioral study described in Chapter 2. Participants were screened for any personal or family history of psychiatric or medical illness, and for any contraindications for MRI. Informed consent/assent was obtained from all parents and child participants. All participants were asked to be genotyped for the 5-HTTLPR

polymorphism via a buccal swab technique and children were asked if they would like to try out an MRI simulator.

From this pool of participants, 32 children (14 Male, 18 Female) were recruited to return for an MRI study. The children in this subset were chosen based on several criteria. We selected for gender and genotype in order to have approximately equal numbers of each allelic group and of males and females. Given reported differential effects of the 5-HTTLPR polymorphism across races (Hariri and Weinberger, 2002), and the small sample size of this study, only children of European descent were selected to participate. Children were not asked to return for the MRI study if they provided insufficient genetic material for genotyping (3 children), if they were not of European descent (5 children), or if they reached a T-score of 70 or greater on the CBCL measure (3 children). Consistent with previous research (e.g. Hariri et al., 2002a), two groups of participants were formed based on their genotyping: children with either one or two copies of the lower expressing S allele, and those homozygous for the higher expressing L allele. Only right-handed participants were included since handedness may reflect lateralized brain systems. We also selected for children who were comfortable with the mock scanner procedure

Simulation Training

Participants received training in an MRI simulator located at the Institute of Child of Development. The simulator is designed to acclimate participants to the scanner environment and it entails a replica of the scanner, complete with identical bore diameter, bed motion, scan sounds, and stimulus presentation equipment. This simulation experience allows for extensive instruction and feedback to the subject

regarding task performance and the avoidance of head motion. Use of the simulator has been found to be helpful in desensitizing subjects to the scan sounds and confining dimensions of the scanner. Also, it serves to reduce pre-scan anxiety associated with anticipation of unknown factors. Furthermore, this simulation and training period allowed the experimenter to assess the child's level of comfort with the scan procedure, so as to better accommodate the participant's individual needs and to increase the likelihood of a successful scan session.

MRI Measures

All participants were scanned on a research-dedicated MRI scanner located at the Center for Magnetic Resonance Research (CMRR) at the University of Minnesota based on later described specific technical details. Standard precautions were taken to ensure the comfort and safety of participants during the scan procedure. The parents of the participants were asked to complete an MRI safety screening form. Children were asked to complete the State-Trait Anxiety Index – Child (STAI-C), a brief measure of current and everyday anxiety levels, immediately following the scan so that correlations between anxiety and amygdala response might be examined. Face stimuli were presented using the Integrated Functional Imaging System (IFIS) (MRI Devices Corp., Gainesville, FL), an fMRI turn-key system designed for experimental design, visual and auditory stimulus presentation, and response collection. Stimuli were presented on an RF shielded personal LCD monitor mounted on the head coil. The subject wore headphones through which he or she could hear instructions from the experimenter. A vacuum pillow was used to assist with head stabilization. Participants were able to communicate with the researcher throughout the scan via a microphone attached to the

head coil. Subjects wore a glove-like response pad on their right hand for completion of the behavioral task and they were also given a "squeeze ball" to hold in their left hand that sounded an alarm in the control room when squeezed. If the participant desired to terminate the session, he/she was immediately removed from the scanner. Scanning sessions lasted no more than 60 minutes, including a high resolution anatomical scan (~10 min) and behavioral task and functional scan acquisition (~25 min). The rest of the time was used to position the child in the scanner, to make him/her comfortable, and to fill out paperwork. Participants were asked to complete four consecutive functional runs with time to 'rest their eyes' between each run.

This chapter describes the first two functional runs which were designed to replicate Whalen et al.'s (2004) eye-white/eye-black experiment in a pediatric sample. Runs 3 and 4 are described in chapters 4 and 5 of this thesis. The task design was analogous to Whalen and colleagues' (2004) adult study. Eye stimuli (and neutral face masks) were derived from eight actors (four male and four female) (Ekman and Friesen, 1976; Whalen et al., 1998). Fearful and happy emotions from these actors were stripped of all information from the face except the eye-whites (Whalen et al., 2004). Eye-black stimuli were inverse, "negative" images of the fearful and happy eye-white stimuli (See Figure 3.1). The neutral face masks were created by thresholding grayscale faces so that they resulted in black and white line drawings. This procedure was identical to Whalen et al. (2004) and was necessary to successfully mask the eye-whites and eye-blacks (Figure 3.2). Subjects passively viewed blocks of masked fearful or masked happy eye stimuli that alternated with fixation blocks. Eyes of each actor were masked by the neutral faces of the other actors for a total of 56 masked stimulus pairs

presented within each block. The first run included alternating blocks of fixation, fearful eye-whites masked by a neutral face, and happy eye-whites masked by a neutral face. The second run contained alternating blocks of fixation, fearful eye-blacks masked by a neutral face, and happy eye-blacks masked by a neutral face. Faces were counterbalanced for order within runs (fear-happy). Slight modifications in the masking paradigm were introduced in order to accommodate scanning protocols which have been successfully implemented with child participants in our laboratory. Specifically, to ensure attention while viewing the faces, participants were instructed to make a button response whenever the inter-stimulus interval (ISI) fixation stimulus changed shape (from + to O). These target stimuli occurred rarely and equally across all block types.

fMRI Data Acquisition. Structural and functional MRI data were acquired on a Siemens 3-Tesla Trio scanner using a single-channel CP head coil. Scout images were obtained for prescription of slices (TE: 5 ms, repetition time (TR): 20 ms, field of view: 256 x 256 cm^2, slice thickness: 3 mm, 20% gap, flip angle: 40º, number of slices: 7, matrix: 256 x 256, and slice orientation: sagittal). Anatomical images were acquired using a high-resolution 3D MPRAGE T1-weighted sequence (TE: 3.65 ms, TR: 2530 ms, field of view: 256 x 256 cm^2, slice thickness: 1 mm, 50% gap (1/2 mm), flip angle: 7º, number of slices: 240, matrix: 256 x 256, and slice orientation: sagittal, voxel resolution 1x1x1 mm.). Functional images were obtained using an EPI (echo-planar imaging) sequence (TE: 28 ms, TR: 2000 ms, field of view: 200 x 200 mm^2, slice thickness = 3.1 mm, no gap, interleaved, flip angle = 90º, number of slices: 34, 156 images per slice, matrix = 64 x 64) while participants were performing the experimental

tasks. Because our emphasis was on activity of the amygdala, slice orientation was tilted 30º from AC-PC to coronal in order to achieve better visualization of the medial temporal lobe (See Somerville, Kim, Johnstone, Alexander, and Whalen, 2004).

fMRI Data Analysis. The Brainvoyager QX (Brain Innovations, Maastricht, The Netherlands) software package was used to perform a group level random effects analysis of the functional data. Before analysis, preprocessing procedures were performed on the raw functional images, including slice scan time correction, linear trend removal, high-pass temporal filtering, spatial data-smoothing with a Gaussian kernel (9mm FWHM), and six parameter rigid body correction for head motion. Each child's functional data were co-registered to the anatomic volume and transformed into Talaraich space (Talairach & Tournoux,1988). Statistical analysis of the functional data was performed with a general linear model (GLM) with predictors for emotion types (Fear, Happy). Contrast analyses were performed based on the *t*-test difference between the β weights of predictors to identify regions that showed greater activity for happy or fearful conditions in masked eye-whites or masked eye-blacks. Group level contrasts were performed with random effects analysis, and a contiguity threshold of 50 voxels was used to correct for multiple comparisons. Given *a priori* hypotheses based on the amygdala response in adults (Whalen et al., 2004), regions of interest were manually drawn using the anatomic boundaries of the amygdala (See Figure 3.3).

Genotyping methods: Serotonin transporter promoter polymorphism (5-HTTLPR)

Based on the sequence reported by Heils et al. (1996), polymerase chain reaction (PCR) primers were designed by Gelernter, Kranzler & Cubells (1997) which amplify a

419-base pair (bp) product from the 16-repeat allele and a 375-bp product from the 14-repeat allele. PCR used KlenTaq polymerase (AbPeptides, St. Louis, Mo.) with manufacturer's buffer PC2 and the addition of 5% glycerol. Cycling parameters were 98/66/72°C for 30 cycles and 30 s at each set point. PCR amplifications used a Perkin Elmer model 9600 thermal cycler. The two alleles originally described by Heils et al. (1996) were designated long (L) (419 bp) and short (S) (375 bp).*

Results

Eye-white data from seven children (2 male, 5 female) and eye-black data from six children (1 male, 5 female) were excluded from further analysis due to excessive head motion (mean greater than ½ voxel in any direction). This resulted in 25 children (12 Male, 13 Female) in the eye-white data data-set and 26 children (13 Male, 13 Female) in the eye-black data-set. The contrast of masked fear versus masked happy resulted in greater BOLD responses to fearful eye-whites in bilateral visual cortex (See Figure 3.4 and Table 3.1) but more extensively on the left ($\beta = 0.143$, x = -25, y = -87, z = -11, 1631 voxels, $t(24) = 3.385$, $p = .0024$) than the right ($\beta = 0.12$, x = 16, y = -88, z = -2, 831 voxels, $t(24) = 2.481$, $p = .0205$). The contrast of masked fear versus masked happy resulted in stronger BOLD responses to happy eye-whites (See Figure 3.5 and Table 3.2) in a number of regions including: left thalamus ($\beta = -0.056$, x = -14, y = -24 z = -11, 954 voxels, $t(24) = -2.706$, $p = .012$); left insula (($\beta = -0.087$, x = -49, y = -16, z = 19, 914 voxels, $t(24) = -3.657$, $p = .0012$); anterior cingulate ($\beta = -.089$, x = -

* Effective genotype groupings were also examined but S and L groups did not sort out to balanced numbers.

3, y = -35, z = 11, 481 voxels, *t*(24) = -2.590, *p* = .016); and left orbito-frontal cortex (β = -.175, x = 16, y = 34, z = -13, 239 voxels, *t*(24) = -2.551, *p* = .017).

For eye-blacks, the contrast of masked fear versus masked happy resulted in greater BOLD responses to fearful stimuli in bilateral visual cortex (left: β = 0.104, x = -28, y = -84, z = -1, 1583 voxels, *t*(25) = 3.134, *p* = .004; right: β = 0.118, x = 21, y = -80, z = -4, 1348 voxels, *t*(25) = 2.931, *p* = .007) (See Figure 3.6 and Table 3.3).

No significant activation was found for the contrast of masked fear versus masked happy in the manually-defined amygdala region of interest for either eye-whites or eye-blacks. There were, however, greater BOLD responses to happy eye-blacks than fixation in the right amygdala (β = .12, x = 25, y = -6, z = -23, 453 voxels, *t*(25) = 2.811, *p* = .009) and the left amygdala (β = .098, x = -3, y = -8, z = -20, 425 voxels *t*(25) = 2.512, *p* = .018) as well as greater BOLD responses to fearful eye-blacks than fixation in the right amygdala (β = 0.155, x = 19, y = -4, z = -12, 853 voxels, *t*(25) = 2.782, *p* = .010) and the left amygdala (β = 0.112, x = -27, y = -5, z = -11, 625 voxels *t*(25) = 2.307, *p* = .029) (See Figure 3.7, Tables 3.4 and 3.5). Similar analyses for the contrasts of happy eye-whites versus fixation and fearful eye-whites versus fixation showed no areas of significant activation in the amygdala region.

Contrast analyses were performed in order to explore the effect of genotype (5-HTTLPR) on amygdala response to eye-whites and eye-blacks. Greater BOLD differences for to fearful compared to happy eye-whites were observed in the left amygdala in participants who were homozygous for the long allele (L/L) than individuals who were homozygous (S/S) or heterozygous (L/S) for the short allele (x = -14, y = -8, z = -18, 470 voxels, *t*(23) = 2.780, *p* = .0106). Closer inspection of the effect

suggests that while participants with the L/L genotype showed no difference between happy and fear (β = .011), individuals with the S/S or L/S genotype actually showed greater activation for happy than fear (β = -.243).

No significant correlations were found between brain response to eye-whites or eye-blacks and state or trait anxiety scores.

Discussion

Despite *a priori* hypotheses based on Whalen and colleagues' (2004) adult finding of greater amygdala response to masked fearful eye-whites (but not eye-blacks), we did not find amygdala modulation by emotional eye-whites or eye-blacks (fearful or happy) in 8-year old children. One interpretation is that the masked faces paradigm was not working, such that the children were responding only to the neutral mask faces. However, we did find other brain areas that showed modulation by emotion, indicating that the eye-white and eye-black probes were, in fact, being processed despite lack of explicit awareness of these stimuli. Bilateral visual cortex showed a stronger activation for fearful than happy eye-whites, perhaps reflecting a difference in perceptual features of the stimuli (i.e fear eye-whites and eye-blacks are larger than happy eye-whites and eye-blacks). We found this same pattern (fear > happy) in visual cortex for eye-blacks. While this suggests that the eye-white and eye-black stimuli are getting into the visual system, we found no evidence that the emotional category was differentially processed by the amygdala. In fact, eye-white stimuli masked by a neutral face did not elicit any amygdala response greater than the response to the fixation stimuli. Significant regions of activation were also found for happy eye-whites (greater than fearful eye-whites) in the left thalamus, the left insula, the anterior cingulate, and the left orbitofrontal cortex.

These results are consistent with previously reported regions of activity in emotion-processing tasks (e.g. Casey at al. 2000; Pessoa, McKenna, Gutierrez, & Ungerleider, 2002, Vuilleumier et al., 2001).

As mentioned, direct comparisons did not reveal differential amygdala response to happy versus fearful stimuli. However, both happy and fearful eye-blacks elicited greater BOLD responses than fixation in bilateral amygdala. Given children's increased amygdala activation to neutral faces (Thomas et. al., 2001b), these findings might simply reflect the brain response to neutral masks. In other words, even if there is emotional modulation for the masked eye-whites and eye-blacks, the effect might be obscured by the responses to neutral faces. Yet, taking into consideration that the same neutral masks were used in the eye-whites condition, and that there was no significant amygdala response to either happy or fearful eye-whites versus fixation, it is unlikely that the eye-black finding was due only to the neutral face masks. When the individual responses in the amygdala ROI were examined, much greater variability was shown such that some children showed an increased amygdala response while some did not. In fact, greater variability was shown in individual responses for eye-whites than eye-blacks. A potential caveat is that participants always completed the eye-white task before the eye-black task. It is possible that children were showing a heightened response to neutral faces in the first run, obscuring any small effects elicited by the probe stimuli. They may have then habituated to the neutral faces by the second task allowing the effects of eye-blacks to be shown. Another possibility is that, because both eye-white and eye-blacks were masked by the same black and white filtered neutral face images (with normal eye-whites), the eye-blacks were not masked as successfully.

However, it must be noted that Whalen et al. (2004) successfully used these grayscale neutral faces when masking eye-whites and eye-blacks with their adult sample. A future study might attempt to use inverse, negative images of the neutral faces in order to mask eye-blacks.

Exploratory analyses for the effect of genotype (5-HTTLPR) suggested that participants who were homozygous for the long allele (L/L) had greater BOLD responses to fearful eye-whites in the left amygdala than individuals who were homozygous (S/S) or heterozygous (L/S) for the short allele. However, this effect was actually a result of participants with the L/L genotype showing no difference between happy and fearful eye-whites while individuals with the S/S or L/S genotype showed greater activation for happy than fearful.

This first attempt to utilize the masked eye-white/eye-blacks paradigm with children did not show the adult amygdala responsivity to masked fearful eye-whites. However, as discussed, there are several explanations for these findings. Whalen et al. (2004) assert that the adult amygdala response to the ecologically valid eye-whites, but not eye-blacks, is driven by the size of the white scleral field and not by the outline of the eye. As our data do not replicate this result, and given the caveats, it is difficult to interpret whether children are, in fact, responding conversely to adults. It may well be that, as shown in the previously described picture-sorting tasks, 8-year olds are not at adult levels in processing emotional faces, particularly fearful faces. This delay may be reflected in differential brain responses even with low-frequency information to which the amygdala is sensitive (Vueilleumier et. al, 2003). The present study did however provide support for the potential use of this backward masking task to elicit brain

reactivity in children as differential brain responses to probe stimuli were shown within tasks.

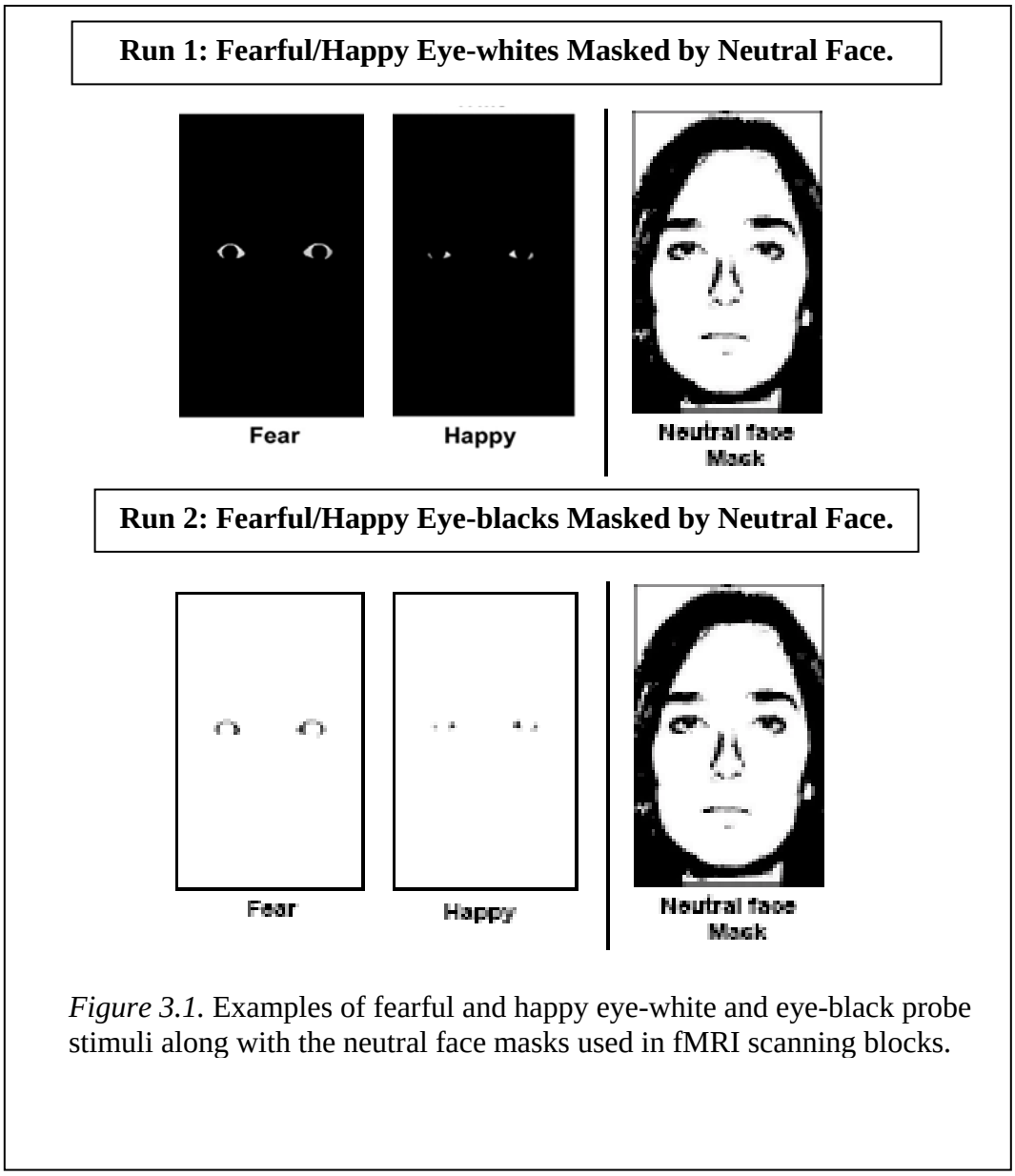


Figure 3.1. Examples of fearful and happy eye-white and eye-black probe stimuli along with the neutral face masks used in fMRI scanning blocks.

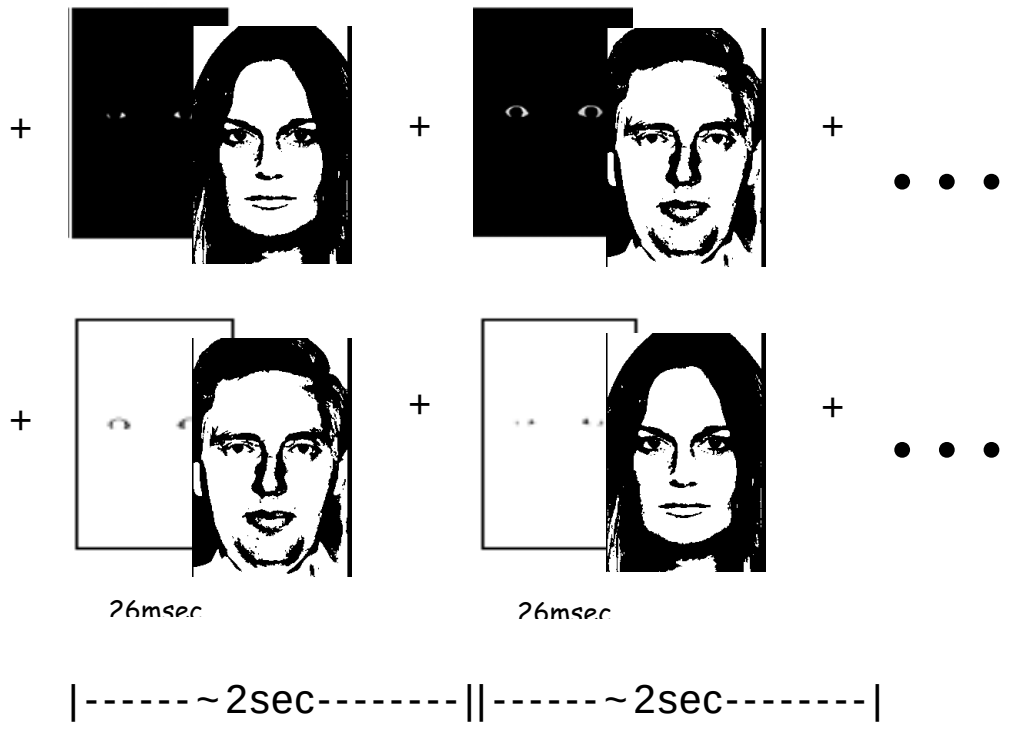


Figure 3.2. Backward masking paradigm used for eye-white and eye-blacks functional runs.

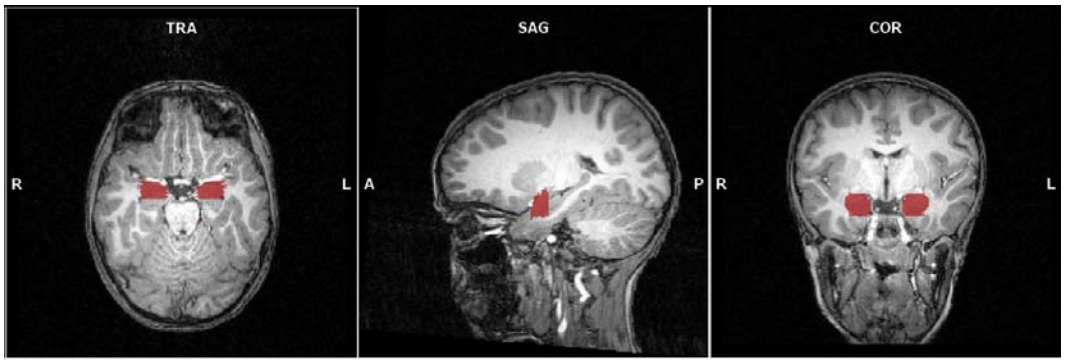


Figure 3.3. Manually-defined bilateral amygdala region of interest presented in the transverse, sagittal, and coronal planes.

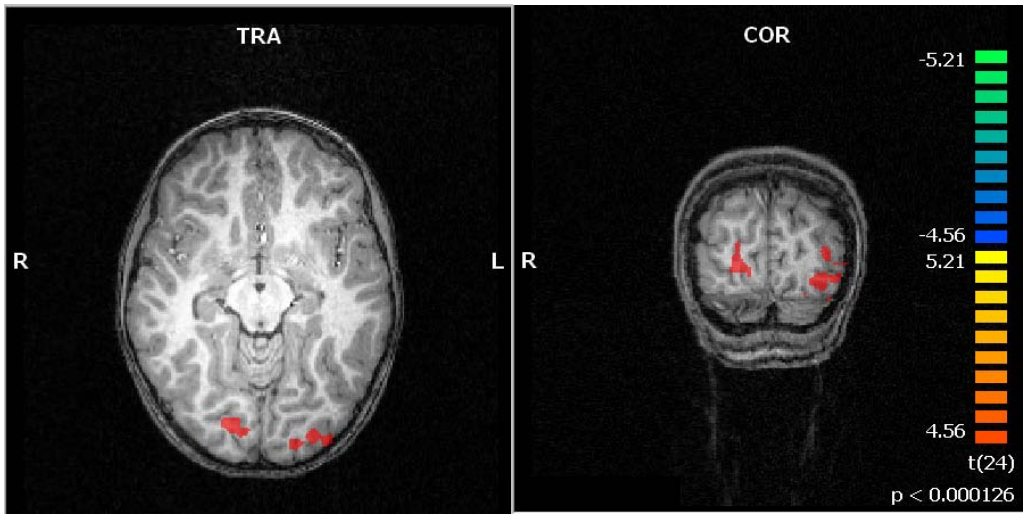


Figure 3.4. Functional activation for the fearful eye-whites condition compared to happy eye-whites in bilateral visual cortex.

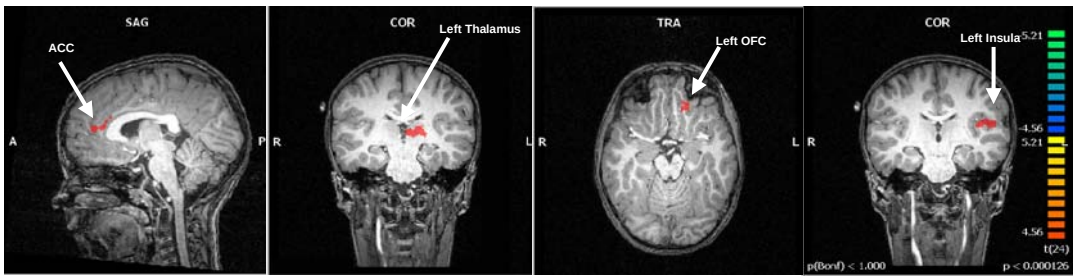


Figure 3.5. Regions of functional activation for the happy eye-whites condition compared to the fearful eye-whites condition. From left to right in the figure, regions are mid-line anterior cingulate, left thalamus, left orbitofrontal cortex, and left insula.

Table 3.1. Talaraich Coordinates and Signal Extent of Significant Clusters for Fearful vs. Happy Eye-Whites.

Region	x	y	z	Max t	# Voxels
Left Visual Cortex	-25	-87	-11	3.385[a]	1631
Right Visual Cortex	16	-88	-2	2.481[a]	831

[a] *p < .0001*

Table 3.2. Talaraich Coordinates and Signal Extent of Significant Clusters for Happy vs. Fearful Eye-Whites.

Region	x	y	z	Max t	# Voxels
Left Thalamus	-14	-24	-11	2.706[a]	954
Left Insula	-49	-16	19	3.657[a]	914
Anterior Cingulate	-3	35	11	2.59[a]	481
Left Orbitofrontal Cortex	-16	34	-14	2.551[a]	270

[a] *p < .0001*

Table 3.3. Talaraich Coordinates and Signal Extent of Significant Cluster for Fearful vs Happy Eye-Blacks.

Region	x	y	z	Max t	# Voxels
Left Visual Cortex	-28	-84	-1	3.134[b]	1583
Right Visual Cortex	21	-80	-4	2.931[b]	1348

[b] $p < .00005$

Table 3.4. Talaraich Coordinates and Signal Extent of Significant Clusters for Happy Eye-Blacks vs. Fixation.

Region	x	y	z	Max t	# Voxels
Left Amygdala	-3	-8	-20	2.512[b]	425
Right Amygdala	25	-6	-23	2.811[b]	453

[b] $p < .00005$

Table 3.5. Talaraich Coordinates and Signal Extent of Significant Clusters for Fearful Eye-Blacks vs. Fixation.

Region	x	y	z	Max t	# Voxels
Left Amygdala	-27	-5	-11	2.307[b]	625
Right Amygdala	19	-4	-12	2.782[b]	853

[b] $p < .00005$

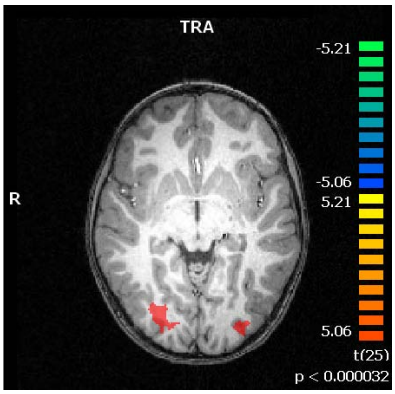


Figure 3.6. Regions of functional activation in the bilateral visual cortex for the comparison of fearful vs happy eye-blacks. Images are presented in the transverse plane.

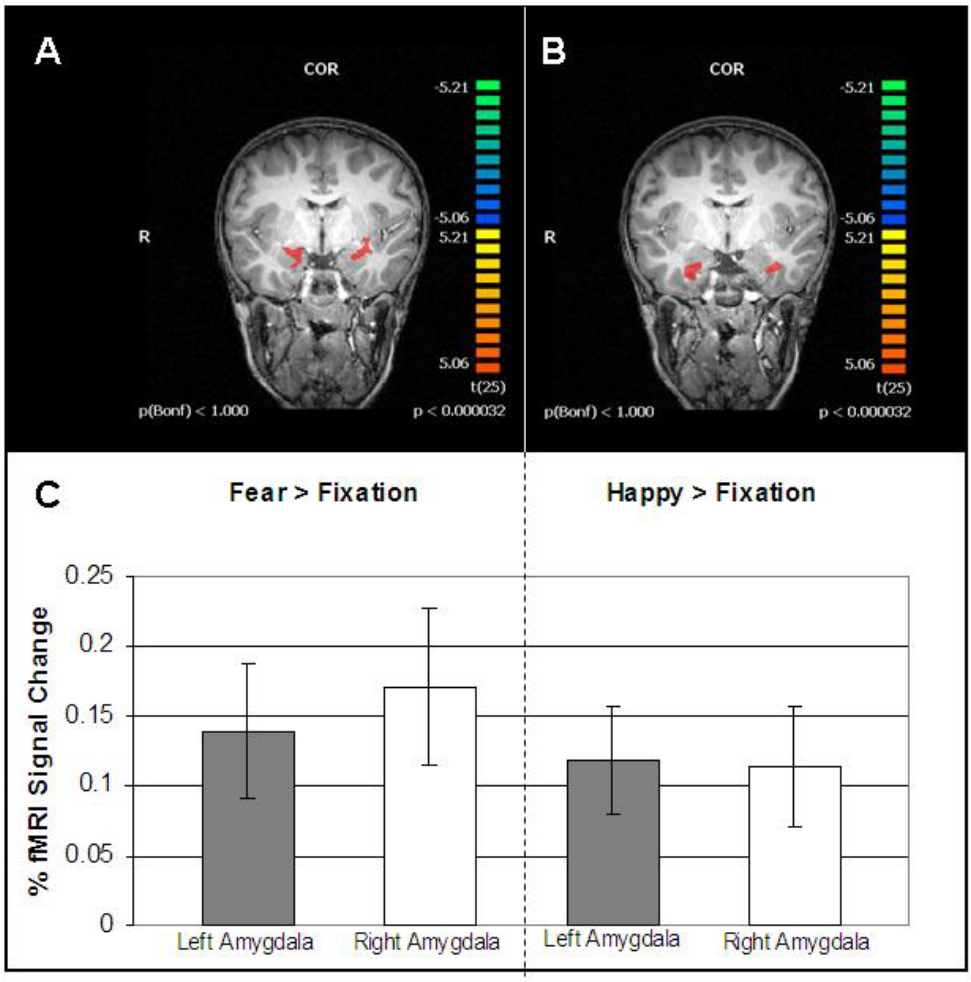


Figure 3.7. Functional activation in the left and right amygdala during the eye-blacks task. A) Left and right dorsal amygdala activity for fearful eye-blacks compared to fixation. B) Left and right ventral amygdala activity for happy eye-blacks compared to fixation. C) Signal change for amygdala activity in the fearful and happy eye-black conditions.

Chapter 4: Is the 5-HTTLPR Polymorphism Related to Face Emotion Processing in Childhood?

Hariri and Weinberger (2003) define imaging genomics as the application of functional neuroimaging techniques to the identification of genetic effects on brain information processing. Essentially a form of genetic association analysis (Hariri & Weinberger, 2003), imaging genomics targets the physiological response of discrete brain circuits during specific forms of information processing (e.g., visual, auditory, cognitive, emotional), rather than focusing on a behavioral phenotype or disease diagnosis. As discussed previously, a multitude of functional neuroimaging studies have consistently demonstrated amygdala engagement in response to facial expressions of emotion, particularly angry and fearful faces (e.g. Davis & Whalen 2001; Zald 2003). Of interest to this project, imaging genomics has illustrated how a promoter polymorphism in the human serotonin (5-hydroxytryptamine; 5-HT) transporter (5-HTT) gene is strongly related to the engagement of neural systems that subserve emotional processes, namely the amygdala. The 5-HT transporter facilitates reuptake of 5-HT from the synaptic cleft thus playing a crucial role in serotonergic neurotransmission. Heils et al (1996) identified a relatively common polymorphism in the human serotonin transporter (5-HTT) gene (SLC6A4) located on chromosome 17q11.1-q12 that is a variable repeat sequence in the promoter region (5-HTTLPR). Two alleles are common: the short (S) variant comprising 14 copies of a 20–23 base pair repeat unit, and the long (L) variant comprising 16 copies. In populations of European ancestry, the frequency of the S allele is approximately 0.40 and the genotype frequencies are in Hardy-Weinberg equilibrium (L/L = 0.36, L/S = 0.48, S/S = 0.16).

However, these relative allele frequencies can vary substantially across populations (Gelernter et al., 1997). In addition to influencing SLC6A4 gene expression (Bradley, Dodelzon, Sandhu, & Philibert, 2005; Lesch et al., 1996), the 5-HTTLPR polymorphism has been shown to affect the size and functional activity of numerous brain structures, including multiple regions of the cerebral cortex, the amygdala, and the hippocampus, in both normal individuals (Bertolino et al., 2005; Hariri et al., 2002; Heinz et al,, 2005; Pezawas et al., 2005) and in individuals with mood and anxiety disorders (Bertolino et al., 2005; Frodl et al., 2004). More specifically, Wassink and colleagues (2007) reported that the short allele of the 5-HTTLPR polymorphism was additively associated with increasing cerebral cortical gray matter volumes in young male children with autism. Devlin et al. (2005) found significant overtransmission of the S allele in a sample of 390 families with multiplex autism. Furthermore, the S allele has been associated with numerous childhood psychiatric disorders and traits such as childhood-onset depression (Nobile et al., 2004), aggression, and attention-deficit/hyperactivity disorder behaviors in males (Cadoret et al., 2003) and with phenotypic traits directly relevant to autism such as neuroticism (Jacob et al., 2004), childhood shyness (Battaglia et al., 2005), and symptom severity and amygdala excitability in adults with social phobia (Furmarck et al., 2004).

Serotonin is a powerful modulator of the physiology and behavior involved in generating appropriate responses to environmental cues such as danger or threat (Hariri, et al., 2002a). Hariri and colleagues (2002a) utilized fMRI to directly explore the neural basis of the likely relationship between the 5-HTTLPR and emotional behavior. The 5-HTTLPR short allele variant is associated with relatively lower 5-HTT function

and higher synaptic concentrations of 5-HT (analogous to findings from 5-HTT knockout mice) and has been associated with a more anxious and fearful behavioral phenotype. Hariri et al. (2002a) hypothesized that short allele carriers would exhibit greater amygdala activity in response to fearful or threatening stimuli than those homozygous for the long allele, who presumably have lower levels of synaptic 5-HT and have been reported to be less anxious and fearful (analogous to findings in wild type mice) (Hariri, Mattay, Tessitore, Fera, & Weinberger, 2003). In their study, subjects from two independent cohorts (n=14 in each) were divided into equal groups based on their 5-HTTLPR genotype, with the groups matched for age, sex, IQ and task performance (Hariri et al., 2002a). In the MRI session, subjects performed a simple perceptual processing task that involved matching fearful or angry human facial expressions, a task that has effectively and consistently elicited amygdala activation in a multitude of studies (Hariri et al. 2000; Hariri et al. 2002a; Hariri, Tessitore, Mattay, Fera & Weinberger, 2003b; Tessitore et al. 2002). As hypothesized, Hariri et al. (2002a) showed that subjects carrying the less efficient 5-HTTLPR short allele exhibited significantly increased amygdala activity in comparison with subjects homozygous for the long allele. On the contrary, no significant group differences were found in subjective behavioral measures of anxiety-like or fear-related traits. In fact, the difference in amygdala activity between 5-HTTLPR genotype groups in this study was nearly fivefold, accounting for 20% of the total variance in the amygdala response during this experience, an effect size 10-fold greater than any previously reported behavioral associations (Hariri et al., 2002a). This finding suggests that the increased anxiety and fearfulness associated with individuals possessing the 5-HTTLPR short

allele may reflect the hyper-responsiveness of their amygdala to relevant environmental stimuli. Subsequently, the finding of amygdala hyper-excitability in 5-HTTLPR short allele carriers in a third independent cohort of 42 healthy subjects was replicated. It was shown that the 5-HTTLPR genotype in this subject group accounted for nearly 22% of the total variance in amygdala activity despite no significant differences in task performance (Hariri & Weinberger, 2003).

The current investigation is a preliminary study exploring the relationships between 5-HTTLPR and amygdala response to face emotion in Hariri et al.'s (2002a) emotion-matching task early in development. To our knowledge, the imaging genomics design of this task has yet to employed with a pediatric sample. However, behavioral differences based in the 5-HTTLPR genotype have already been shown as young as infancy. For example, Pauli-Pott et al. (2009) analyzed the interaction of the 5-HTTLPR genotype with the quality of maternal parenting behavior on the development of negative emotionality and fear in a sample of 69 healthy first-born infants. Insecurely attached infants who were homozygous for the S-variant of the 5-HTTLPR genotype developed a high level of negative emotionality and fear suggesting the increased susceptibility of S/S carrying infants to early rearing experiences. No main effects for allele variant were observed, however. Taken together, given that the short allele has been associated with various childhood psychiatric disorders and related phenotypic traits, it is hypothesized that a functional difference between children homozygous for the long allele and children who are short allele carriers should be detected. While there are no specific reports showing that the perceptual processing fearful and angry face matching task effectively elicits bilateral amygdala activation in children as it does in

adult samples, there is evidence showing amygdala activation to negative emotional faces in children (e.g. Thomas et al., 2001a,b).

Method

Participants

Sixty-three 8-year old children (33 female; 8.5 +/- 0.4 years) were recruited from an existing community participant pool in the Minneapolis/St. Paul area to participate in the behavioral study described in Chapter 2. Participants were screened for any personal or family history of psychiatric or medical illness, and for any contraindications for MRI. Informed consent/assent was obtained from all parents and child participants. All participants were asked to be genotyped for the 5-HTTLPR polymorphism via a buccal swab technique and children were asked if they would like to try out an MRI simulator.

From this pool of participants, 32 children (14 Male, 18 Female) were recruited to return for an MRI study. The children in this subset were chosen based on several criteria. We selected for gender and genotype in order to have approximately equal numbers of each allelic group and of males and females. Given reported differential effects of the 5-HTTLPR polymorphism across races (Hariri and Weinberger, 2002), and the small sample size of this study, only children of European descent were selected to participate. Children were not asked to return for the MRI study if they provided insufficient genetic material for genotyping (3 children), if they were not of European descent (5 children), or if they reached a T-score of 70 or greater on the CBCL measure (3 children). Consistent with previous research (e.g., Hariri et al., 2002a), two groups of participants were formed based on their genotyping: children with either one or two

copies of the lower expressing S allele, and those homozygous for the higher expressing L allele. Only right-handed participants were included since handedness may reflect lateralized brain systems. We also selected for children who were comfortable with the mock scanner procedure.

Simulation Training

Participants received training in an MRI simulator located at the Institute of Child of Development. The simulator is designed to acclimate participants to the scanner environment and it entails a replica the scanner, complete with identical bore diameter, bed motion, scan sounds, and stimulus presentation equipment. This simulation experience allows for extensive instruction and feedback to the subject regarding task performance and the avoidance of head motion. Use of the simulator has been found to be helpful in desensitizing subjects to the scan sounds and confining dimensions of the scanner. Also, it serves to reduce pre-scan anxiety associated with anticipation of unknown factors. Furthermore, this simulation and training period allowed the experimenter to assess the child's level of comfort with the scan procedure, so as to better accommodate the participant's individual needs and to increase the likelihood of a successful scan session.

MRI Measures

All participants were scanned on a research-dedicated MRI scanner located at the Center for Magnetic Resonance Research (CMRR) at the University of Minnesota based on later described specific technical details. Standard precautions were taken to ensure the comfort and safety of participants during the scan procedure. The parents of the participants were asked to complete an MRI safety screening form. Children were

asked to complete the State-Trait Anxiety Index – Child (STAI-C), a brief measure of current and everyday anxiety levels, immediately following the scan so that correlations between anxiety and amygdala response might be examined. Face stimuli were presented using the Integrated Functional Imaging System (IFIS) (MRI Devices Corp., Gainesville, FL), an fMRI turn-key system designed for experimental design, visual and auditory stimulus presentation, and response collection. Stimuli were presented on an RF shielded personal LCD monitor mounted on the head coil. The subject wore headphones through which he or she could hear instructions from the experimenter. A vacuum pillow was used to assist with head stabilization. Participants were able to communicate with the researcher throughout the scan via a microphone attached to the head coil. Subjects wore a glove-like response pad on their right hand for completion of the behavioral task and they were also given a "squeeze ball" to hold in their left hand that sounded an alarm in the control room when squeezed. If the participant desired to terminate the session, he/she was immediately removed from the scanner. Scanning sessions lasted no more than 60 minutes, including a high resolution anatomical scan (~10 min) and behavioral task and functional scan acquisition (~25 min). The rest of the time was used to position the child in the scanner, to make him/her comfortable, and to fill out paperwork. Participants were asked to complete four consecutive functional runs with time to ‘rest their eyes’ between each run.

This chapter reports results from the third functional run, designed to replicate, with children, Hariri et al.’s (2002a) investigation of serotonin transporter genetic variation and the amygdala response to facial emotion with adults (See Figure 4.1). Two blocks of an emotion task were interleaved with three blocks of a sensorimotor

control task. During the emotion task, three faces were presented simultaneously, and participants were asked to choose which of two faces at the bottom of the screen showed the same emotion as the target face at the top of the screen. Participants indicated their choice by pressing the button corresponding to their index finger if the picture on the left matched the target face and by pressing their middle finger if the picture on the right matched the target face. The identity of each of the three faces was always unique. The target face was always either an angry or a fearful face (randomized for actor and side of presentation). The choice stimuli were always one angry face and one fearful face. Each emotion block consisted of six 5-second trials, three of each gender (male faces or female faces) and target affect (angry or afraid), all selected from a standard set of pictures of facial affect (Ekman and Friesen, 1976). During the sensorimotor control task, participants viewed three simple geometric shapes (circles, vertical ellipses, or horizontal ellipses) and were asked to choose which of two shapes at the bottom of the screen matched the target shape at the top of the screen. Subject performance (accuracy) was monitored and recorded. Genotype-based comparisons were used to examine activation differences in the amygdala.

fMRI Data Acquisition. Structural and functional MRI data were acquired on a Siemens 3-Tesla Trio scanner using a single-channel CP head coil. Scout images were obtained for prescription of slices (TE: 5 ms, repetition time (TR): 20 ms, field of view: 256 x 256 cm^2, slice thickness: 3 mm, 20% gap, flip angle: 40º, number of slices: 7, matrix: 256 x 256, and slice orientation: sagittal). Anatomical images were acquired using a high-resolution 3D MPRAGE T1-weighted sequence (TE: 3.65 ms, TR: 2530 ms, field of view: 256 x 256 cm^2, slice thickness: 1 mm, 50% gap (1/2 mm), flip angle:

7º, number of slices: 240, matrix: 256 x 256, and slice orientation: sagittal, voxel resolution 1x1x1 mm.). Functional images were obtained using an EPI (echo-planar imaging) sequence (TE: 28 ms, TR: 2000 ms, field of view: 200 x 200 mm^2, slice thickness = 3.1 mm, no gap, interleaved, flip angle = 90º, number of slices: 34, 135 images per slice, matrix = 64 x 64) while participants were performing the experimental task. Because our emphasis was on activity in the amygdala, slice orientation was tilted 30º from AC-PC to coronal in order to achieve better visualization of the medial temporal lobe (Somerville et al., 2004).

fMRI Data Analysis. The Brainvoyager QX (Brain Innovations, Maastricht, The Netherlands) software package was used to perform a group level random effects analysis of the functional data. Before analysis, preprocessing procedures were performed on the raw functional images, including slice scan time correction, linear trend removal, high-pass temporal filtering, spatial data-smoothing with a Gaussian kernel (9mm FWHM), and six parameter rigid body correction for head motion. Each child's functional data were co-registered to the anatomic volume and transformed into Talaraich space (Talairach and Tournoux 1988). Statistical analysis of the functional data was performed with a general linear model (GLM) with predictors for condition (emotion, shape). Analyses were performed based on *t*-test differences between the β weights of predictors for the contrast of the emotion task versus the sensorimotor control for each subject. Group level contrasts were performed with random effects analysis, and a contiguity threshold of 50 voxels was used to reduce the likelihood of Type 1 error.

Genotyping methods: Serotonin transporter promoter polymorphism (5-HTTLPR)

Based on the sequence reported by Heils et al. (1996), polymerase chain reaction (PCR) primers were designed by Gelernter et al. (1997) which amplify a 419-base pair (bp) product from the 16-repeat allele and a 375-bp product from the 14-repeat allele. PCR used KlenTaq polymerase (AbPeptides, St. Louis, Mo.) with manufacturer's buffer PC2 and the addition of 5% glycerol. Cycling parameters were 98/66/72°C for 30 cycles and 30 s at each set point. PCR amplifications used a Perkin Elmer model 9600 thermal cycler. The two alleles originally described by Heils et al. (1996) were designated long (L) (419 bp) and short (S) (375 bp).[*]

Results

Data from 12 children (5 Male, 7 female) were excluded from further analysis due to excessive head motion in the scanner. This resulted in 20 children (9 Male, 11 Female) in the data-set (See Table 4.1 for participant genotypes). We did not have performance accuracy data from one female participant (L/L) due to response pad error.

Behavioral Data

Paired *t*-tests comparing performance accuracy showed that eight-year old children performed significantly better at matching shapes (86.1%) than matching emotional faces (66.5%) $t(18) = 4.223$, $p < .001$. A significant correlation was found between performance accuracy on the shapes- and emotion-matching tasks ($r = .611$, $p = .005$). Participants were grouped into two allelic groups, S (S-carriers) and L (homozygous L), in order to examine the effect of genotype on performance. The L

[*] Effective genotype groupings were also examined but S and L groups did not sort out to balanced numbers.

group (74.8%) performed more accurately than the S carriers (55.5%) on the emotion matching task (t(8) = -2.345, $p = .047$). There was no significant difference between the two allelic groups on the shape-matching task. The S group performed more accurately on the shapes task (81.5%) than the emotion task (59.1) [t(9) = -3.038, $p = .014$)]. The L group also performed more accurately on the shapes task (91.2%) than the emotion task (74.8% t(8) = -2.936, $p = .019$) (See Figure 4.2).

There were no correlations between matching performance and state or trait anxiety scores.

fMRI data

For the whole group, the contrast of faces versus shapes showed no significant BOLD response differences in the structurally defined amygdala ROI during the emotional face versus shapes task. Direct comparisons also found no differences in amygdala activation between the S carrier group and the homozygous L group (t(18) = 0.756, S: $\beta = 0.408$, L: $\beta = 0.328$, $p = 0.459580$). However, there was a significant BOLD response for the contrast of faces versus shapes in the right amygdala ROI for S carriers (t(11) = 4.444, $\beta = .275$, $p < 0.001$) as well as L/L carriers (t(7) = 2.551, $\beta = .242$, $p < 0.05$). Voxel-wise analysis showed a right but not left amygdala effect for the group as a whole (t(19) = 5.067, $\beta = .262$, , $p < 0.0001$), indicating that the emotional faces versus shapes task does activate the amygdala in 8-year old children but not bilaterally as shown in the adult literature. Closer inspection of this effect suggests that, because the S carrier group ($\beta = .146$) and the homogenous L group ($\beta = .156$) both showed changes in the positive direction, they may not be actually showing a difference in response. In contrast, Hariri et al.'s (2002) data with adults show that approximately

half of the participants who were L/L carriers showed less activation for emotion-matching than for shape-matching in the amygdala, contributing to the significantly greater percent BOLD signal change for the S carriers. The majority (16/20) of child participants in our sample showed positive activation in the amygdala, suggesting that children are generally more reactive for emotional expressions than are adults (See Figure 4.3).

There were no gender effects or gender X genotype interactions for the amygdala response to faces versus shapes. There were no correlations between brain reactivity with state or trait anxiety scores.

Discussion

Overall, children's performance on the emotion-matching and shapes-matching tasks differed from the Hariri et al. (2002a) adult data that showed no performance differences by genotype or task. In general, 8-year old children performed significantly better when matching shapes compared to emotional faces. This effect was maintained when groups were divided by genotype, as both the S and L groups performed more accurately on the shapes than the emotion task. It is not surprising that the emotion matching task was more difficult for children as the emotions used (angry and fearful) which were chosen because they elicit heightened amygdala response, are emotions that children had more difficulty sorting in our behavioral measures (Chapter 2). Additionally, Bruce et al. (2000) showed that when children were asked to choose which of two emotional faces expressed the same emotion as a third face, a high level of accuracy was not achieved until age 10. Processing of angry and fearful faces tends to develop later into childhood so 8-year olds might not yet be as competent at matching

these emotions. The S group performed more significantly more poorly than the L group on the emotion matching task suggesting, perhaps, a disadvantage for individuals who are carriers of the short allele.

Our fMRI data with eight-year old children also did not replicate the basic adult bilateral amygdala response reported by Hariri and colleagues (2002a). Children did not show increased amygdala activation during the emotional face matching task nor did the S carrier group show greater amygdala activation than the homozygous L group. One noticeable difference, however, is that whereas approximately half the adults in the L group showed a negative effect such that the mean percent signal change was not significantly greater than zero, nearly all of the children showed a positive effect. Children, in general, may be more reactive and so a more subtle difference between allelic groups might be obscured by an overall effect of development. It is important to note that our sample resulted in fairly small group sizes, which may also account for the lack of significant differences. Additionally, the task design, which was selected to exactly replicate the paradigm used with adults, did not include a fixation baseline. While we could compare the emotion- and shape- matching conditions to one another, we could not compare each task to a fixation condition. Future studies might include this baseline condition to investigate whether children are actually showing heightened magnitude of response to both the shapes and emotion task resulting in no significant differences between the two conditions.

On the whole, children did not show the same behavioral or fMRI responses as the adults did in Hariri et al.'s (2002a)'s study. While children were not as accurate as adults at the emotion-matching task, they were sufficiently capable to perform at above

chance levels. For the purposes of this paradigm, it was only necessary to elicit amygdala activation so accuracy was not of critical importance. Moreover, the adult-like hyper-activation by carriers of the S allele appears to be due, in part, to a deactivation by carriers of the L/L allele in the emotion-matching task (Hariri et al., 2002a). The majority of children in our sample showed a positive percent BOLD signal change to the emotion-matching task regardless of allelic group. It may be that at this developmental period, they are highly reactive to both conditions and so differences between the S carriers and the L/L carriers are not distinguishable. Perhaps the lowered amygdala reactivity associated with the L/L allele develops with experience and at eight years of age, children are not yet exhibiting the adult-like response to amygdala eliciting emotional tasks.

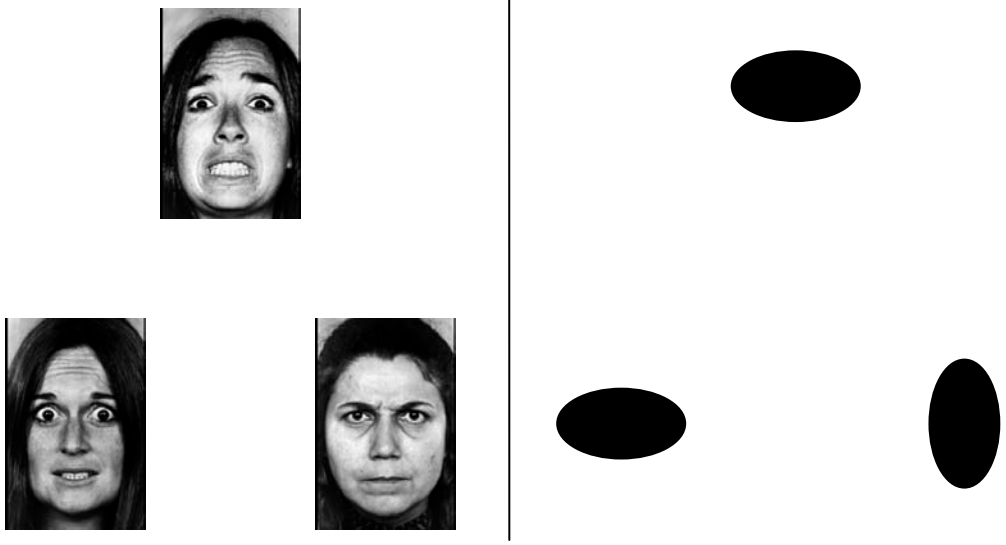


Figure 4.1. Two blocks of an emotion task were interleaved with three blocks of a sensorimotor control task. During the emotion task, three faces were presented simultaneously, and participants were asked to choose which of two faces at the bottom of the screen showed the same emotion as the target face at the top of the screen. Each emotion block consisted of six 5-second trials, three of each gender (male faces or female faces) and target affect (angry or afraid), all selected from a standard set of pictures of facial affect (Ekman and Friesen, 1976). During the sensorimotor control task, participants viewed three simple geometric shapes (circles, vertical ellipses, or horizontal ellipses) and were asked to choose which of two shapes at the bottom of the screen matched the target shape at the top of the screen. (adapted from Hariri et al., 2002a)

Table 4.1. 5-HTTLPR Genotypes: Participants were sorted into two allelic groups, S (S-carriers) and L (homozygous L).

	L Group	S Group		
	L/L	L/S	S/S	Total
Male	5	2	2	9
Female	6	4	1	11
Total	11	6	3	20

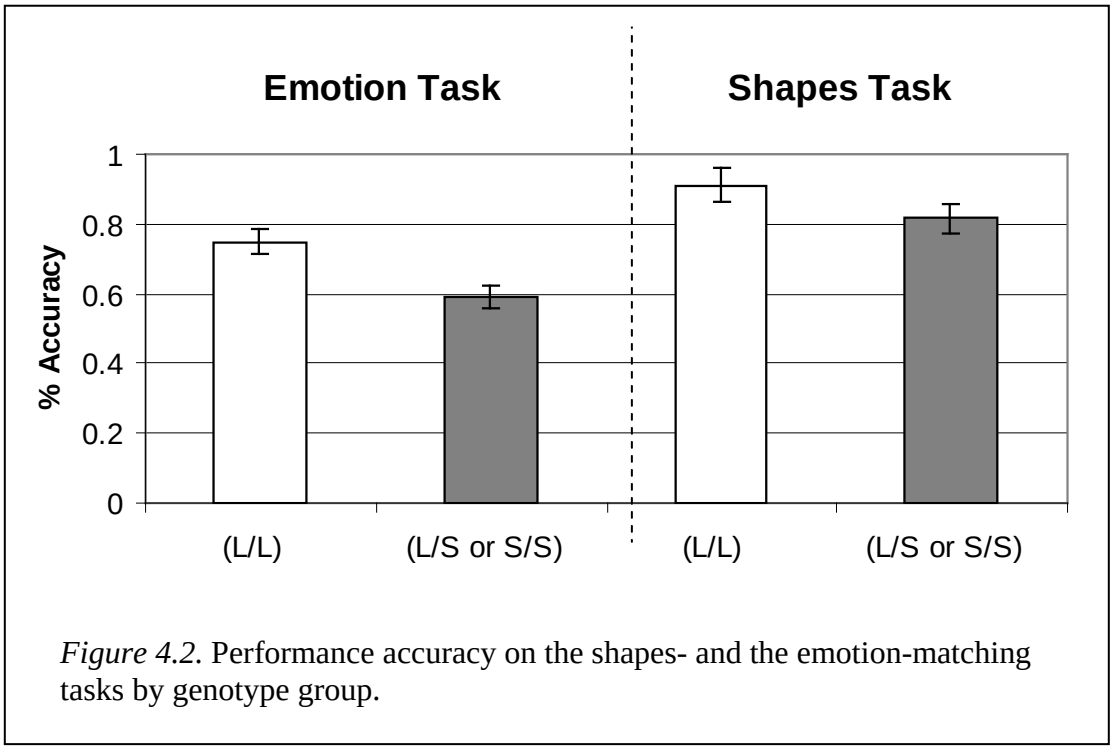


Figure 4.2. Performance accuracy on the shapes- and the emotion-matching tasks by genotype group.

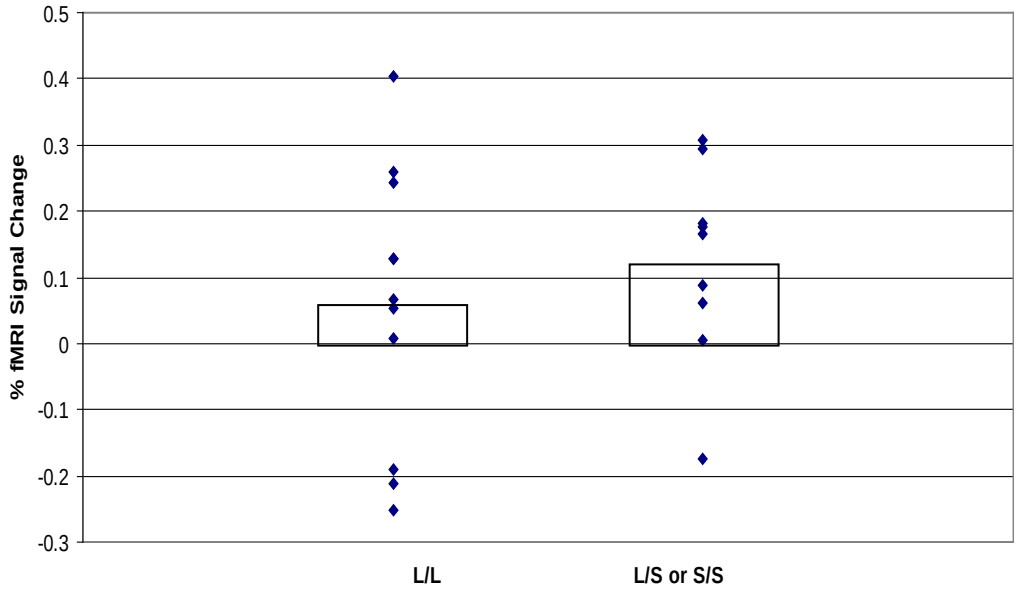


Figure 4.3, Percent change in fMRI signal for emotion matching compared to shape matching for children with the L/L or the S-carrier genotypes of the 5-HTTLPR polymorphism. Dots represent values for individual children; Bars represent group mean..

Chapter 5: Does Inversion Affect Children's Brain Response to Facial Expressions of Emotion?

Research utilizing behavioral methodology has examined the remarkable aptitude for face processing in adults (Bruce & Young, 1998), often referred to as expertise. However, an ongoing debate concerns the development of this capacity (e.g. Gauthier & Nelson, 2001; Maurer et al, 2002; Pascalis & Slater, 2003). There is a basic consensus that face recognition accuracy improves from childhood to adulthood (Gauthier & Nelson, 2001; Maurer et al., 2002; Want et al., 2003) but less accord on the particular components of face recognition that alter with development (Carey & Diamond, 1994; Freire & Lee, 2001, 2003; Itier & Taylor, 2004a; Mondloch et al. 2002, 2003; Pellicano & Rhodes, 2003; Schwarzer, 2000; Want et al., 2003). Do children process faces in a different way than adults or in the same way but somehow less efficiently? When does the adult-like processing emerge? One developmental shift which may occur is from the processing of faces in a piecemeal or feature-based manner in childhood to a reliance on the processing of face patterns more relationally (i.e., with more configural and/or holistic information) in adulthood (Carey & Diamond, 1994; Freire & Lee, 2001, 2003; Itier & Taylor, 2004a; Mondloch et al., 2002, 2003; Schwarzer, 2000; Want et al., 2003).

The use of inverted face stimuli has been a primary means of assessing configural processing and its development. Inversion disrupts face encoding by altering the configural information necessary for accurate face recognition; the relative position of features is modified (e.g., eyes above the nose in an upright face, eyes below the nose in an upside-down face) (Bartlett & Searcy, 1993; Rhodes, Brake, & Atkinson, 1993).

Inverted faces are recognized more slowly and with higher error rates than are upright faces (Rhodes et al., 1993), and this decrement in performance is more manifest for faces than other objects (Yin, 1969). These findings are considered to show the import of configural information (Maurer et al., 2002). The face inversion effect may be due to a disruption of information processing that is specific to faces, or stimuli with which adults have a comparable level of expertise (Leder, Candrian, Huber & Bruce, 2001; Nachson, 1995). It has been argued that inverted faces are processed analytically (as features) and upright faces holistically (Tanaka & Farah, 1993) or configurally (Leder et al., 2001), a model that has received some support from neuroimaging studies (e.g., Haxby et al., 1999). It should be noted that while configural and holistic processing can both be contrasted to featural processing, they do indicate different mechanisms. Configural processing refers to the processing of the relationships among the features of a face whereas holistic processing of faces refers to perceiving faces as a single entity, a gestalt (Bruce & Langton, 1994).

Numerous studies have indicated that the inversion of face stimuli disproportionately interferes with relational information processing (e.g. Bartlett & Searcy, 1993; Carey & Diamond, 1977, 1994; Diamond & Carey, 1986; Freire et al., 2000; Murray, Rhodes, & Schuchinsky, 2003; Searcy & Bartlett, 1996; Tanaka & Farah, 1993; Tanaka, Kay, Grinnell, Stansfield, & Szetchter, 1998; Young et al., 1987). Interestingly, it has also been reported that children's performance is less affected by inversion than adults (e.g. Carey and Diamond, 1977; 1994), arguably indicating that children rely less on relational information than do adults to process faces. However, Carey and Diamond's (1977) hypothesis has also been disputed by reports showing that

children under 10 years of age can process faces configurally (Baenninger, 1994, Carey & Diamond, 1994, Flin, 1985, Freire and Lee, 2001 and Tanaka et al., 1998). Flin (1985) demonstrated an inversion effect in children's performance as young as 7 years, while Brace and colleagues (2001) showed that children as young as 5 years showed an inversion effect, but 2- to 4-year-olds did not. Even newborns and infants are sensitive to face orientation (Gallay, Baudouin, Durand, Lemoine, & Lecuyer, 2006; Slater et al., 2000; Turati, Sangrigoli, & de Schonen, 2006). For instance, Cassia et al. (2004) proposed that newborns may show a face preference because of a non-specific bias toward top-heavy patterns. In fact, Carey and Diamond (1994) have described an increasing inversion effect from 6 to 10 years of age, alluding to a development of the tendency to process faces configurally. Yet, there is no agreement that the developmental change in face processing is impelled by the increased use of relational information in adulthood. For example, Itier and Taylor (2004b) did not find this developmental difference of greater inversion effect on adult performance than on that of children. They proposed that this change in face processing entails an overall improvement in performance accuracy from childhood to adulthood, rather than a qualitative change in the use of relational information.

Face emotion recognition emerges early (e.g., Barrera & Maurer, 1981; Walker-Andrews, 1997), but the processing of facial expressions may evolve across the lifespan. For example, whereas preschool age children can label facial emotions at above chance levels (e.g., Markham & Adams, 1992; Russell & Widen, 2002; Widen & Russell, 2003), they are considerably less accurate than adults. Converse to studies of face recognition abilities, there is a paucity of research examining the developmental

course of facial emotion recognition. Additionally, results in the extant literature are not consistent, perhaps due to methodological variability. As mentioned previously, Bruce et al. (2000) showed that when children were asked to point to which of two faces was happy, sad, angry, or surprised, they attained near-perfect accuracy by 6 years of age. However, when the task involved choosing which of two emotional faces expressed the same emotion as a third face, a comparable level of accuracy was not achieved until age 10, perhaps indicating that development of facial emotion recognition depends on task demands. In a study of similar design, children were asked to match a photograph of an emotional face to one of four possibilities (neutral, surprise, happiness, or disgust) (Mondloch et al., 2003). The authors reported an increase in accuracy between 6- and 8- years of age, when performance reached the adult level. Kolb et al. (1992), showed children and adults either an emotional photograph or a cartoon depicting an emotional situation and then asked participants to choose the face that exhibited the same or appropriate emotion from a panel of six different emotional photographs (happiness, sadness, fear, anger, disgust, and surprise). Between 6- and 8-years and between 8- and 10-years of age, recognition of facial emotions improved, depending on the task. Accuracy was found to increase again between age 14–15 years of age and adulthood. Notably, this developmental pattern appears to vary by emotion. Children recognize happiness earliest and with the greatest accuracy, followed by sad or angry expressions, then expressions of surprise or fear (Boyatzis et al, 1993, Camras & Allison, 1985; Gross & Ballif, 1991). Overall, however, despite task- and emotion-related differences, performance does appear to improve with age.

Logically, a question that arises concerns the role of configural information in the development of the ability to recognize facial emotions. Configural information has been found to play a substantial role in adult recognition of face emotion. For example, Calder et al., (2000) and Calder & Jansen, (2005) observed a composite effect in emotion recognition similar to effects found in the adult face recognition literature (e.g. Young, et al, 1987). In the composite face paradigm, a composite stimulus is made by joining the top half of a face with the bottom half of another face. Adults are slower and less accurate to recognize either half when the top and bottom parts are vertically aligned (creating a new face stimulus) than when the same top and bottom parts are misaligned, suggesting an adult tendency to process faces holistically. Calder and colleagues (2000, 2005) found that when the top and bottom halves of a composite face show different emotions, recognition of the emotion in either half is slower and less accurate than when the composite face is inverted or the two halves are laterally offset. It thus follows that the development of the ability to process holistic and configural properties of faces may play a significant role in the development of the ability to process facial emotions. Given the amygdala's critical role in processing upright facial expressions in adults and children, there is a call for research examining the amygdala response to inverted emotional faces. To date, there has not been an investigation of whether these manipulations affect amygdala activity in adults or children. In particular, if inversion disrupts the ability to process emotion, will inversion disrupt the typical amygdala response to emotional faces, particularly fearful faces?

Method

Participants

Sixty-three 8-year old children (33 female; 8.5 +/- 0.4 years) were recruited from an existing community participant pool in the Minneapolis/St. Paul area to participate in the behavioral study described in Chapter 2. Participants were screened for any personal or family history of psychiatric or medical illness, and for any contraindications for MRI. Informed consent/assent was obtained from all parents and child participants. All participants were asked to be genotyped for the 5-HTTLPR polymorphism via a buccal swab technique and children were asked if they would like to try out an MRI simulator.

From this pool of participants, 32 children (14 Male, 18 Female) were recruited to return for an MRI study. The children in this subset were chosen based on several criteria. We selected for gender and genotype in order to have approximately equal numbers of each allelic group and of males and females. Given reported differential effects of the 5-HTTLPR polymorphism across races (Hariri and Weinberger, 2002), and the small sample size of this study, only children of European descent were selected to participate. Children were not asked to return for the MRI study if they provided insufficient genetic material for genotyping (3 children), if they were not of European descent (5 children), or if they reached a T-score of 70 or greater on the CBCL measure (3 children). Consistent with previous research (e.g., Hariri et al., 2002a), two groups of participants were formed based on their genotyping: children with either one or two copies of the lower expressing S allele, and those homozygous for the higher expressing L allele. Only right-handed participants were included since handedness may reflect

lateralized brain systems. We also selected for children who were comfortable with the mock scanner procedure

Simulation Training

Participants received training in an MRI simulator located at the Institute of Child of Development. The simulator is designed to acclimate participants to the scanner environment and it entails a replica the scanner, complete with identical bore diameter, bed motion, scan sounds, and stimulus presentation equipment. This simulation experience allows for extensive instruction and feedback to the subject regarding task performance and the avoidance of head motion. Use of the simulator has been found to be helpful in desensitizing subjects to the scan sounds and confining dimensions of the scanner. Also, it serves to reduce pre-scan anxiety associated with anticipation of unknown factors. Furthermore, this simulation and training period allowed the experimenter to assess the child's level of comfort with the scan procedure, so as to better accommodate the participant's individual needs and to increase the likelihood of a successful scan session.

MRI Measures

All participants were scanned on a research-dedicated scanner located at the Center for Magnetic Resonance Research (CMRR) at the University of Minnesota. Standard precautions were taken to ensure the comfort and safety of participants during the scan procedure. The parents of the participants were asked to complete an MRI safety screening form. Children were asked to complete the State-Trait Anxiety Index – Child (STAI-C) a brief measure of current and everyday anxiety levels, immediately following the scan so that correlations between anxiety and amygdala response might be

examined. Face stimuli were presented using the Integrated Functional Imaging System (IFIS) (MRI Devices Corp., Gainesville, FL), an fMRI turnkey system designed for experimental design, visual and auditory stimulus presentation, and response collection. Stimuli were presented on an RF shielded personal LCD monitor mounted on the head coil. The subject wore headphones through which he or she could hear instructions from the experimenter. A vacuum pillow was used to assist with head stabilization. Participants were able to communicate with the researcher throughout the scan via a microphone attached to the head coil. Subjects wore a glove-like response pad on their right hand for completion of the behavioral task and they were also given a "squeeze ball" to hold in their left hand that sounded an alarm in the control room when squeezed. If the participant desired to terminate the session, he/she was immediately removed from the scanner. Scanning sessions lasted no more than 60 minutes, including a high resolution scan (~10 min.) and behavioral task and functional scan acquisition (~25 min). The rest of the time was used to position the child in the scanner, to make him/her comfortable, and to complete forms. Participants were asked to complete four consecutive functional runs with time to 'rest their eyes' between each run.

The final functional run, reported here, investigated the role of holistic processing of faces in the amygdala response to facial emotion by addressing whether children activate amygdala to overtly presented, inverted emotional faces (Ekman & Friesen, 1976). This run alternated blocks of fixation, inverted fearful faces, and inverted happy faces. In order to minimize perceptual differences between emotions, only open-mouth happy faces and fearful faces were chosen as stimuli (See Figure 5.1

for examples). Fearful and happy faces were selected in an effort to maximize the amygdala differences between face categories, as upright versions of these two emotional expressions tend to elicit the largest (fearful) and smallest (happy) amygdala responses in adults, and potentially in children as well (Tseng, Vizueta, & Thomas., 2007a,b; Tseng & Thomas, 2008). MR signal was compared between each emotion category and fixation baseline, as well as between emotion categories (i.e., fearful vs. happy). Slight modifications in the paradigm were introduced in order to accommodate scanning protocols which have been successfully implemented with child participants in our laboratory. Specifically, to ensure attention while viewing the faces, participants were instructed to make a button response whenever the inter-stimulus interval (ISI) fixation stimulus changed shape (from + to O). These target stimuli occurred rarely (1/10) and equally across all block types.

fMRI Data Acquisition

Structural and functional MRI data were acquired on a Siemens 3-Tesla Trio scanner using a single-channel CP head coil. Scout images were obtained for prescription of slices (TE: 5 ms, repetition time (TR): 20 ms, field of view: 256 x 256 cm^2, slice thickness: 3 mm, 20% gap, flip angle: 40º, number of slices: 7, matrix: 256 x 256, and slice orientation: sagittal). Anatomical images were acquired using a high-resolution 3D MPRAGE T1-weighted sequence (TE: 3.65 ms, TR: 2530 ms, field of view: 256 x 256 cm^2, slice thickness: 1 mm, 50% gap (1/2 mm), flip angle: 7º, number of slices: 240, matrix: 256 x 256, and slice orientation: sagittal, voxel resolution 1x1x1 mm.). Functional images were obtained using an EPI (echo-planar imaging) sequence (TE: 28 ms, TR: 2000 ms, field of view: 200 x 200 mm^2, slice thickness = 3.1 mm, no

gap, interleaved, flip angle = 90º, number of slices: 34, 156 images per slice, matrix = 64 x 64) when participants were performing the experimental tasks. Because our emphasis was on studying of the amygdala, slice orientation was tilted 30º from AC-PC to coronal in order to achieve better visualization of the medial temporal lobe (Somerville et al., 2004).

fMRI Data Analysis

The Brainvoyager QX (Brain Innovations, Maastricht, The Netherlands) software package was used to perform a group-level random effects analysis of the functional data. Before analysis, preprocessing procedures were performed on the raw functional images, including slice scan time correction, linear trend removal, high-pass temporal filtering, spatial data-smoothing with a Gaussian kernel (9mm FWHM), and six parameter rigid body correction for head motion. Each child's functional data were co-registered to his/her anatomic volume and transformed into Talaraich space (Talairach & Tournoux 1988). Statistical analysis of the functional data was performed with a general linear model (GLM) with predictors for emotion. Analyses were performed based on *t*-test differences between the β weights of predictors for the contrast of inverted fear, inverted happy, and each emotion versus fixation baseline. Group-level contrasts were performed with random effects analysis, and a contiguity threshold of $50mm^3$ was used to reduce the likelihood of Type 1 error. We were interested in whether the amygdala was activated to inverted faces, and whether it was differentially activated by emotion (fear vs. happy). Thus we utilized the same structural region of interest described in chapter three using the anatomic boundaries of the amygdala (Figure 3.3).

Genotyping methods: Serotonin transporter promoter polymorphism (5-HTTLPR)

Based on the sequence reported by Heils et al. (1996), polymerase chain reaction (PCR) primers were designed by Gelernter et al. (1997) which amplify a 419-base pair (bp) product from the 16-repeat allele and a 375-bp product from the 14-repeat allele. PCR used KlenTaq polymerase (AbPeptides, St. Louis, Mo.) with manufacturer's buffer PC2 and the addition of 5% glycerol. Cycling parameters were 98/66/72°C for 30 cycles and 30 s at each set point. PCR amplifications used a Perkin Elmer model 9600 thermal cycler. The two alleles originally described by Heils et al. (1996) were designated long (L) (419 bp) and short (S) (375 bp).*

Results

Inverted face data from ten children (4 male, 6 female) were excluded from further analysis due to excessive head motion. This resulted in 22 children (10 male, 12 female) included in this data-set.

Voxel-wise analysis showed greater BOLD responses to inverted fearful faces than fixation (See Figure 5.2 and Table 5.2) in bilateral dorsal amygdala (right amygdala $t(21) = 2.279$, $p = 0.0332$; left amygdala ($t(21) = 2.349$, $p = 0.0287$). No significant activation was found for the contrast of inverted happy faces versus fixation in the amygdala ROI. Additionally, there was no significant difference in activation found for the contrast of inverted fearful faces versus inverted happy faces. This may be due to sub-threshold activity to happy inverted faces or because the fear response was not strong.

* Effective genotype groupings were also examined but S and L groups did not sort out to balanced numbers.

The contrast of fearful versus happy inverted faces showed significantly greater activation in the fusiform face area (FFA) to fearful stimuli (See Figure 5.3 and Table 5.1) on both the left (x = -35, y = -70, z = -13, 235 voxels, $t(21) = 2.072$, mean = 0.164, $p < .05$) and the right (x = 34, y = -70, z = -10, 443 voxels, $t(21) = 2.291$, mean = 0.183, $p < .05$). Greater BOLD responses to inverted fearful faces than fixation (Table 5.2) were found in the left fusiform face area ($t(21) = 2.126$, mean = 0.093, $p < 0.05$) and the right fusiform face area ($t(21) = 2.288$, mean = 0.144, $p < 0.05$). Greater BOLD responses to inverted happy faces than fixation (Table 5.3) were found in the left fusiform face area (x = -18, y = -88, z = -8, 15,586 voxels, $t(21) = 5.806$, mean = 0.336, $p < 0.05$) and the right fusiform face area (x = 27, y = -82, z = -2, 21,588 voxels, $t(21) = 7.598$, mean = 0.346 $p < 0.000001$).

Correlation analyses were also conducted for STAI-C scores and the contrasts of inverted fearful greater than happy faces in bilateral amygdala. A trend was observed for a relationship between trait anxiety and amygdala response in the right amygdala ($r = -.356$, $p = .06$) but not in the left amygdala.

No significant interactions for genotype were found.

Discussion

Lipp, Price, and Tellegen (2009) reported that in visual search experiments, adults show no impairment of emotional face processing when faces are inverted and holistic processing is disrupted. However, there is a dearth in the extant literature examining brain response to inverted emotional faces in adults and children. To date, there are no published reports of fMRI data examining inverted emotional faces in children. The current study showed significant differences in brain activation to

inverted faces modulated by emotion. Eight-year olds showed significant activation in the fusiform face area for both the fearful and happy conditions indicating that even inverted faces were recognized as faces. Participants also showed emotion-modulated (fearful > happy) bilateral FFA activation similar to the brain response to upright fearful faces (Guyer et al., 2008). Additionally, children showed greater BOLD fMRI responses to inverted fearful faces than fixation in both the right and left amygdala ROIs. There was no amygdala activation for the happy versus fixation comparison. We did not initially predict that the amygdala would respond to inverted emotional faces because it is not known whether the amygdala processes configurally. In other words, participants may not have viewed the inverted faces as emotional stimuli at all if the inversion manipulation affected successful holistic processing of the face stimuli. An alternative explanation is that amygdala activation shown during the inverted emotional face trials might actually indicate a brain response to an ambiguous or non-ecologically valid stimulus, as people do not generally appear upside down.

An important caveat to note is that due to the time constraints and methodological limitations of this study, children were not explicitly queried on the identities of the emotions. This should be taken into consideration as children who are inaccurate at identifying the emotions in inverted faces might be processing the stimuli differently from children who are accurate at recognizing inverted emotion. In fact, if children have not yet attained adult-like processing of face emotion, they may be more adept at recognizing emotion in inverted faces. For example, if children are still processing emotional faces featurally, as opposed to configurally, they may be less affected by the inversion effect. Finally, in order to better address developmental

changes in response to inverted emotional faces, it is necessary to test a sample of adults in the same paradigm for direct comparison. Nevertheless, the exhibited emotional modulation of the brain response does support the conclusion that eight-year old children were responding to both the face and emotion features of an inverted face.

Figure 5.1. Examples of inverted face stimuli used in fMRI scanning blocks.

Table 5.1. Talaraich Coordinates and Signal Extent of Significant Clusters for Inverted Fearful vs. Happy Faces.

Region	x	y	z	Max t	# Voxels
Left Fusiform	-30	-88	-5	4.00[c]	911
Right Fusiform	27	-85	7	3.85[c]	1340

[c] *p < .0005*

Table 5.2. Talaraich Coordinates and Signal Extent of Significant Clusters for Inverted Fearful Faces vs. Fixation.

Region	x	y	z	Max t	# Voxels
Left Fusiform	-24	-88	4	7.47[c]	22233
Right Fusiform	18	-88	-2	8.69[c]	26567
Left Amygdala	-24	-7	-11	2.88[d]	381
Right Amygdala	27	-7	-11	3.54[d]	360

[c] *p < .0005*
[d] *p < .05*

Table 5.3. Talaraich Coordinates and Signal Extent of Significant Clusters for Inverted Happy Faces vs. Fixation.

Region	x	y	z	Max t	# Voxels
Left Fusiform	-18	-88	-8	6.07[c]	15386
Right Fusiform	27	-82	-2	7.32[c]	21588

[c] *p < .0005*

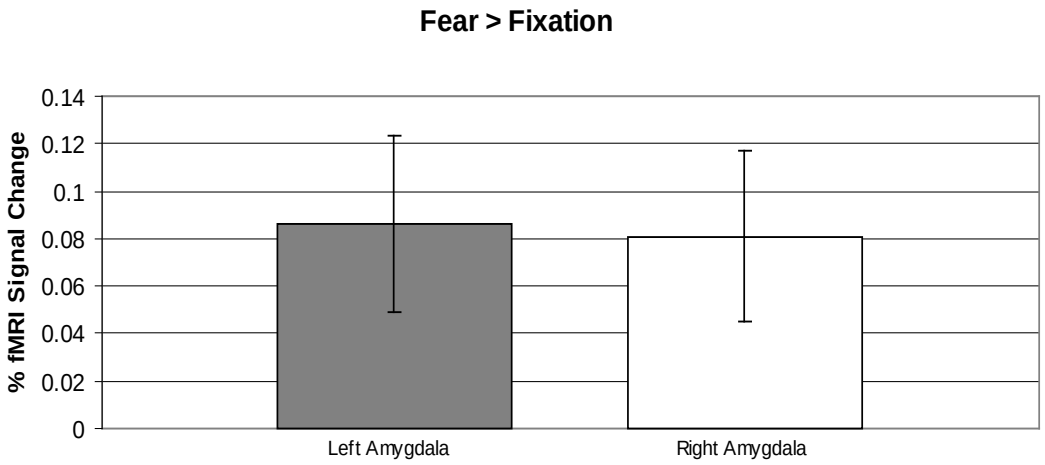
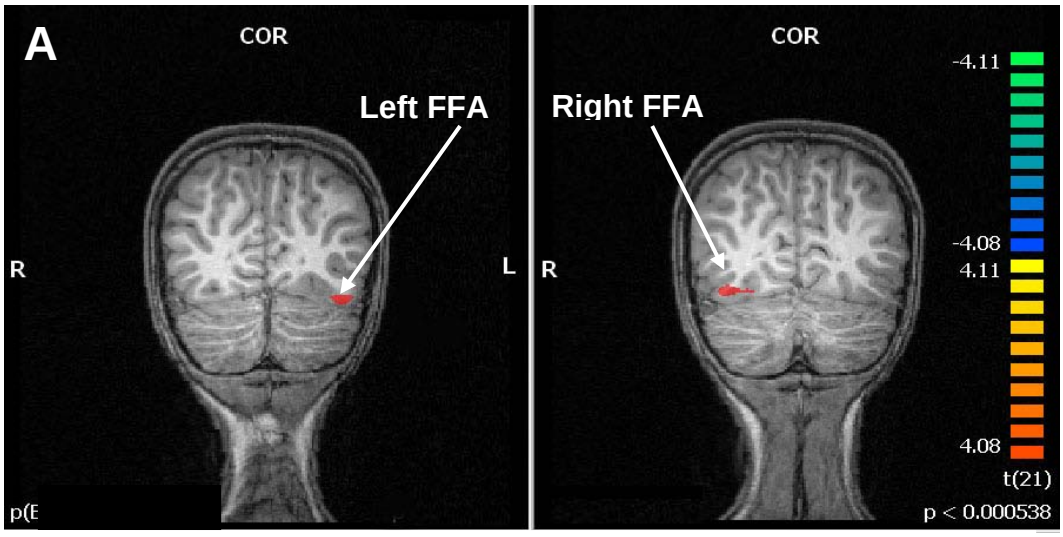


Figure 5.2. Percent change in fMRI signal in the inverted faces task. Bars represent signal change for inverted fearful faces compared to fixation in the left and right amygdala.



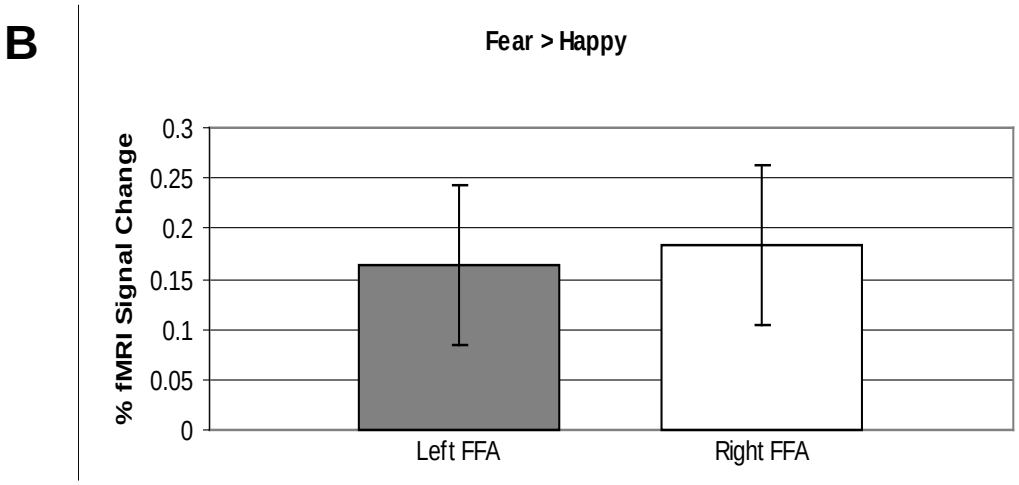


Figure 5.3. Functional activation during the inverted faces task. A) Regions of activation in left and right fusiform gyrus. B) Bars represent signal change for inverted fear faces compared to inverted happy faces in the left and right fusiform gyrus.

Chapter 6: General Conclusions.

The over-arching goal of this thesis was to further elucidate the role of individual features of faces in the amygdala response to facial emotion, as well as to attain a better understanding of the role of eyes and mouths in children's identification of facial expressions of emotion. Four general questions were addressed: a) Do children rely more heavily on one feature over another in identifying facial emotions?, b) Is the child's brain sensitive to low-level perceptual clues of facial emotion (i.e. masked eye-whites) as are adult brains?, c) Are natural variations in serotonin genes related to emotion processing in childhood as they are in adulthood?, and d) Does inversion affect children's brain response to facial expressions of emotion?

Chapter 2 described three forced-choice picture sorting tasks that were used to explore how eight-year-old children categorized facial expressions of emotion when only some of the features (eyes or mouths) were presented. In general, children did exhibit better performance with happy faces, in large part due to their superiority at identifying happy mouths. Not surprisingly, they were less adept at categorizing emotions that have been previously reported as later developing (sad, fearful, angry). In general, children were more accurate when labeling eyes than mouths, suggesting that the eye region of the face is indeed sufficient for children when categorizing facial expressions. More specifically, children showed the highest accuracy when identifying angry eyes and sad eyes than other emotional eyes. It is interesting to note that the salience of particular facial features may be emotion-dependent, such that children may focus their attention on mouths of happy faces but switch to the eye region for angry faces resulting in decreased aptitude at recognizing the emotion in the weaker or less

salient feature. Perhaps the apt usage of these emotional cues is an ability still developing in middle childhood, suggesting that adults are more efficacious at identifying significant information from the environment. It may also be that adults use more than the most salient features whereas children do not. Nevertheless, the current results do indicate that eight-year old children, while already quite skillful at many aspects of face emotion processing, are still undergoing functional development in their abilities.

Of interest, these findings significantly contribute to the extant literature describing composite faces and whole-part paradigms. In the composite face paradigm, a combined stimulus is composed by fusing the top and bottom halves of two different faces. The composite effect has been described as impaired recognition of a person from the half-part of the face when it is aligned with the counter-part from another face in a single stimulus (i.e. Tanaka et al., 1998). Adults are slower and less accurate to recognize either half part when the top and bottom parts are vertically aligned (creating a new face stimulus) than when the same top and bottom parts are misaligned. Similar composite effects have been observed in 4-year-olds and adults (De Heering, Houthuys, & Rossion, 2007) and in 6- to 10-year-olds (Carey & Diamond, 1994). In the whole-part paradigm, face features have to be recognized either embedded in the whole face stimulus or presented in isolation. The whole-part advantage effect has been described as better recognition of features when in the context of a normal face than when presented in isolation. It has been reported that four- and six-year olds show this effect at a similar level as older children (Pellicano and Rhodes, 2003; Tanaka et al., 1998), indicating the early ability of children to process configural information.

There is a paucity of literature examining the role of configural information in the development of the ability to recognize facial emotions. This is noteworthy because configural information also plays a critical role in facial emotion recognition by adults. Calder and colleagues (2000) reported a composite effect in emotion recognition such that when the top and bottom halves of a composite face depict different emotions, recognition of the emotion in either half is slower and less accurate than when the composite face is inverted or the two halves are offset laterally. Additionally, no composite effect was found when the top and bottom halves showed different actor's faces posing the same emotion, also supporting the configural interpretation of these findings. The first picture-sorting task in the current study asked children to identify emotion from one feature of the face while the second and third card-sort tasks asked children to identify emotion from composite faces and to create their own aligned composite faces based on emotion category. It is necessary to note that our task differed from previous studies as we did not ask child participants to isolate and identify eye emotion in the context of mismatched mouth emotions. For this reason, the question of whether conflicting emotions in the eye and mouth regions of a single face stimulus impairs children's ability to separate out one featural cue may not be addressed. However, our chimeric face data contribute to this literature in that children showed distinct patterns in whether they used the eye or mouth region of incongruent emotion chimeric faces to sort by emotion. Additionally, while there was no measure of response time as children were asked to sort congruent and incongruent stimuli together, accuracy measures were informative. Our data show that 8-year old children performed extremely well at identifying the emotionally congruent chimeric faces

although they were more likely to sort by the eye region for fearful/angry trials and by the mouth for happy/sad trials. As mentioned previously, it is speculated that the results from the happy/sad trials are largely driven by children's confidence with happy mouths. Clearly, aptitude at processing configural properties of facial features is an important factor in the development of the ability to effectively process facial emotions.

Chapter 3 examined how children respond to the low-level perceptual information in masked eye-white and eye-black stimuli. In adults, it has been shown that the eye region alone can produce a threat superiority effect similar to that in response to a whole fearful face. This suggests not only that the configuration of eyes provides a key signal of threat (Fox and Damjanovic, 2006) but also that the widening of the eyes can enhance the perception of facial threat (Tipples, 2005). Neuroimaging studies have shown that the amygdala is recruited for the processing of fearful eyes such that even fearful eyes (top half of a fearful face) combined with a neutral mouth (bottom half of a neutral face) evoked increased amygdala activation (Morris et al. 2002). The present study was an attempt to replicate Whalen et al.'s (2004) adult finding that showed greater amygdala response to masked fearful eye-whites and not to fearful eye-blacks. However, we failed to find amygdala modulation by eye-white or eye-black emotion (fearful or happy) in 8-year old children. On the other hand, we did find other brain areas that showed modulation by emotion. These data indicate that the eye-white and eye-black probes were, in fact, being perceived since the neutral face mask stimuli for both conditions were identical. Of particular note, increased activation was found for happy eye-whites greater than fearful eye-whites in prefrontal regions (i.e. anterior cingulate, left orbitofrontal cortex), suggesting emotional modulation for areas

associated with emotion-processing in adults. The OFC and ACC circuitry influences behavior by affecting attention to emotional stimuli (e.g. including fearful or other negative facial expressions) (Blair, Morris, Frith, Perrett, & Dolan, 1999; Lane, Fink, Chau, & Dolan, 1997; Pessoa et al., 2002;. Over the course of development, the OFC and ACC show functional, anatomical, and physiological changes in adolescence and by early adulthood (Adleman et al., 2002; Casey et al., 1997; Casey et al., 2000; Eshel, Nelson, Blair, Pine, & Ernst, 2007; Gogtay et al., 2004; Sowell, Thompson, Holmes, Jernigan, & Toga, 1999;). These brain regions have been associated with the maturation of both attentional and emotional processes, including goal-directed attention to emotionally evocative stimuli (Bush, Luu, & Posner, 2000). Monk and colleagues (2003a) reported OFC and ACC activation greater for adolescents than adults when participants completed a passive viewing task of fearful versus neutral faces. In contrast, adults also showed greater OFC activation than adolescents when asked to focus on the emotional versus non-emotional (i.e. nose-width) aspects of fearful faces. Perhaps, while amygdala differences were not found for 8-year olds in our eye-white study, the present data are consistent with literature indicating the involvement of, and possible age-related changes in, the ACC and the OFC in facial emotion processing.

It is important to note that there are confounds to using Whalen et al.'s (2002a) exact paradigm with children. As discussed, children's heightened amygdala response to neutral faces may obscure any small effects elicited by the briefly presented eye stimuli. It may be that the eye-black condition showed amygdala activation greater than fixation because participants had already habituated to the neutral masks in the

eye-white paradigm (which was always presented first). Future studies might attempt to first habituate children to neutral faces (i.e. via an all neutral run) before using it as a mask in subsequent runs. It will also be important to counterbalance the order of the eye-white and eye-black presentation to determine whether findings were due to an order effect.

Finally, a recent study (Feng et al. 2009) which investigated whether different intensities of fearful eye-whites alone could elicit different ERP responses might also inform future developmental research in this vein. ERPs were measured while participants observed masked low-intensity (50%), prototypical (100%), and caricatured (150%) fearful eye-whites in a gender-decision task. Previous studies have shown differential event-related potential responses to varying intensities of facial expression, especially within fearful expressions (Leppanena, Kauppinenb, Peltola, Hietanena, 2007; Sprengelmeyer and Jenztsch, 2006). Three groups of white squares with the same pixels as the eye-white stimuli were used as control conditions. Feng and colleagues (2009) found a linear increase in the amplitudes of the parietal-occipital P120 by the three intensities of fearful eye-whites. Given that children have shown earlier competency with exaggerated features of emotional features, a follow-up to the current study might employ caricatured fearful eye-whites to attempt to elicit the adult-like amygdala response.

Chapter 4 attempted to assess the effect of genotype (5-HTTLPR) on children's response to an amygdala-eliciting task. Since Hariri et al.'s (2002a) study, multiple functional imaging studies have reported 5-HTTLPR S allele-driven amygdala hyper-reactivity in cohorts of healthy adults (e.g. Brown and Hariri, 2006; Canli et al 2005;

Heinz et al 2005). Munafo, Brown, and Hariri (2008) also conducted a meta-analysis evaluating the magnitude of reported associations between amygdala activation and the serotonin transporter gene linked polymorphic region. Results in that study provided support for this association and indicated that this locus may account for up to 10% of phenotypic variance. The present study found that behavioral performance by 8-year olds on the amygdala-eliciting task was not as accurate as that of adults, providing further evidence of the protracted development of face-emotion identification. For example, Bruce et al. (2000) showed that when children were asked to point to which of two faces was happy, sad, angry, or surprised, they achieved nearly perfect accuracy by age six. However, when they were asked to select which of two emotional faces expressed the same emotion as a third face, an adult-like accuracy level was not reached until age ten. Nevertheless, while children in this fMRI study were not as accurate as previously reported adults at the emotion-matching task, they did perform above chance levels, which should still be expected to elicit amygdala activation, as in adults. The lack of replication in fMRI findings is more puzzling. However, the data are not necessarily inconsistent with the adult findings. Rather, it may provide support for a developmental trajectory of amygdala response to emotional stimuli. Perhaps, in middle childhood, children are actually highly reactive regardless of genotype such that significant BOLD signal changes are not detected. It would be prudent to follow-up this initial investigation with similar studies and multiple developmental age groups so that it may be determined if the amygdala response becomes more adult-like with experience or age. Finally, another critical caveat to these data is the small sample and thus, lower significance. Given the general distribution of the S and L variants in the

population, we expected our sample of 32 children to sort approximately equally into the two groups, allowing a sample size similar to the Hariri et al. (2002a) adult samples. However, due to difficulties with scanning a pediatric sample (excessive motion), we lost approximately one third of the fMRI data, decreasing the power of our investigation. Future studies will have to take this attrition rate into consideration.

Chapter 5 addresses the question of how inversion affects children's brain response to facial expressions of emotion. Faces are often characterized as unique stimuli because they are processed holistically rather than on the basis of features (Farah, Wilson, Drain, & Tanaka, 1998) as demonstrated through the composite face effect, the effect of configuration on part recognition, and the inversion effect (Tanaka & Sengco, 1997; Yin, 1969; Young et al., 1987). It is however, less clear whether the holistic processing used in face recognition is also required in the processing of facial expressions of emotion. Studies utilizing the composite effect support the idea that configural or holistic processing also affects the identification of facial expressions (White, 1999; Calder et al., 2000). As discussed, Calder et al. (2000) showed that the identification of a facial expression from half of a composite face was more readily done when the halves were misaligned, and inversion of these faces reduced this effect. McKelvie (1995) assessed the effect of face inversion on emotional face recognition and demonstrated that inversion impairs the recognition of sad, fearful, angry, and disgusted, but not of happy expressions in adult participants. Additionally, performance accuracy remained above chance for all expressions, and patterns of misidentifications of the inverted faces resembled those shown with upright faces. McKelvie (1995) thus suggested that the inverting of faces is detrimental to classification of emotional

expressions particularly if expression classification requires the identification of more than one salient feature (i.e. a 'smiling' mouth which is a consistently potent cue for happiness). Lipp and colleagues (2009), however, showed that in visual search experiments, adults exhibit no impairment of emotional face processing when faces were inverted and holistic processing was disrupted.

Our data contribute to the extant face inversion literature in that there is a lack of fMRI research examining brain response to inverted emotional faces in children. The purpose of this investigation was to determine if, in 8-year old, the amygdala responds to inverted emotional faces, or alternatively, if the amygdala processes emotional faces configurally. We found that while children showed greater BOLD fMRI responses to inverted fearful faces than fixation in both the right and left amygdala ROIs, there was no amygdala activation for the happy versus fixation comparison. This is consistent with literature that shows increased amygdala activation to upright fearful faces in adults and children and no amygdala increase to upright happy faces (e.g. Morris et al, 1998; Thomas et al., 2001a), suggesting that face emotion recognition is not vulnerable to the inversion effect. Our data also show emotion modulated (fearful > happy) bilateral FFA activation similar to the brain response to upright fearful faces shown in adults (Guyer et al., 2008). Because children were asked to complete four different runs of behavioral tasks in one fMRI session, we opted to minimize the duration of the study and did not explicitly query participants on the identities of the emotions. This should be taken into account for follow-up investigations as inaccuracy in identifying the inverted face emotions might suggest that the children were not successfully recognizing the inverted emotion even if their brain responses were analogous to

findings with upright faces. Ideally, the next study would include both upright emotional faces and inverted emotional faces as well as cohorts of adults and children or multiple child age groups. This would allow direct comparison of brain response to upright and inverted faces, as well as provide insight into the developmental changes in these processes.

Conclusions

While these data are difficult to interpret given the early stages of the developmental literature, it is increasingly more apparent that there are significant differences between the behavioral and brain responses of healthy adults and typically-developing children to emotional stimuli. The literature also shows differential responding between healthy children and children diagnosed with clinical disorders. An enhanced understanding of the development of typical and atypical amygdala functioning as well as global brain maturation may impel promising practical applications. More particularly, study of the relation between brain development and the comprehension and processing of socio-emotional cues will unquestionably serve to advance the development of appropriate methods for clinical intervention in cases of children and adults with social and emotional disorders. The identification of age-related changes in limbic function in healthy children and adolescents is necessary to characterize neurobiological abnormalities associated with behavioral pathology. Without a clear delineation of alterations that occur during typical brain development, it would be difficult to determine whether deviations in emotional processing skills are correlated with neuropathological discrepancies or normal maturational changes. Successful social interaction is highly dependent on the ability to recognize and

appropriately respond to affective stimuli. In fact, deficiencies in these socioemotional areas have been implicated as a critical factor in a myriad of psychiatric disorders. It thus follows that the elucidation of neural mechanisms subsuming processing of emotional experience may afford novel insights into both normal developmental trajectories as well as into the early diagnosis and treatment of psychiatric illness.

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