Social attention, emotion, and amygdala volume development in preschool-aged children with autism

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Abstract
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(Under the direction of Gary Mesibov, Ph.D.)

The social and emotional deficits of autism emerge in childhood and are present throughout the lifetime. Research on early development is limited, due, in large part, to reliance on retrospective parent reports, which are plagued by recall biases, and qualitative rating scales that are less sensitive than dimensional ratings to variation among affected individuals. The present study details the development and application of a novel observational coding system, the Social and Emotional Perspective (SEP), useful for quantifying social and emotional behavior in preschool aged children with autism participating in the Autism Diagnostic Observation Schedule (ADOS). The convergent validity of the SEP was supported by examining the relationship between its items and related subdomains of the ADOS. Comparison of a longitudinal sample of children with autism studied at 18-35 months and 42-59 months with cross-sectional age matched typically developing children indicated that the SEP was sensitive to a range of social and emotional deficits in children with autism. The majority of these deficits were evident at both time points, and two behaviors, looking at others and showing positive emotion, actually became more severely impaired over time in the autism group. The SEP also was used in conjunction with magnetic resonance imaging (MRI) to examine the association of social and emotional behaviors with the amygdala, a neural structure previously hypothesized to underlie the core social features of autism (Baron-Cohen et al., 1999). Results indicated that

the amygdala was enlarged in the autism group, and that volumes were related to social initiation and positive emotion displays in the autism group. The implications of understanding these brain-behavior linkages and potential applications of the SEP are discussed.

To Laura. You are the best. Thank you.

To Mom, Dad, and Chris. Without each of you I could not and would not have been able to finish this. Thank you always for your continued support and love.

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Background and Significance

Autism is a severe and pervasive neurodevelopmental disorder characterized by impairments in social interaction, communication, and restricted, repetitive behaviors (APA, 1994). Since Kanner (1943) first described autism, clinicians and researchers have emphasized the core nature of the social deficits in this disorder (Schultz, 2005; Volkmar, 1987). Indeed, social deficits appear to be the most specific features of the disorder, unlike many of the communication and repetitive abnormalities, which are evidenced in other neurodevelopmental and psychiatric disorders (e.g., severe mental retardation without autism, obsessive-compulsive disorder, nonverbal learning disorder). Still, little is known about the onset and development of these social deficits. Previous methods of assessing social behaviors in young children with autism have been limited. For example, researchers have used cross-sectional, rather than longitudinal, samples, and they have used qualitative, rather than quantitative, measurement instruments. Additionally, although autism is recognized as involving multiple brain regions, little information is available regarding the association of social deficits and established neuroanatomical abnormalities in autism. The present investigation attempts to address each of these limitations.

Social Development in Typically Developing Children and Children with Autism

To understand social dysfunction in autism, it is helpful to review social behavior in typical development, particularly in the first years of life (i.e., < 4 years). Research mapping social behaviors observed in preschool-aged typically developing children indicate that a variety of skills emerge early in ontogeny and contribute to social, language, and cognitive

developments. These skills include eye contact, monitoring eye gaze, joint attention, social imitation, social gestures, and coordinating emotion with others. These early social skill often are deviant in children with autism as early as the first year of life (Baranek, 1999). However, little is known about the onset and development of these deficits.

Attention to others

Typically developing children. Attending to others serves a clear adaptive function, allowing infants to identify security figures in their environment. Attending to others also fosters social opportunities and contributes to social and communicative developments. For example, during the fourth and fifth months of life, infants begin to demonstrate the ability to distinguish between individuals looking at and individuals looking away from them and to coordinate their own gaze with that of others (Baron-Cohen & Cross, 1992). These behaviors are precursors to the developing capacity to monitor shifts in eye gaze, coordinate attention, and share enjoyment with others (Campbell, Walker, & Baron-Cohen, 1995; Baron-Cohen & Ring, 1994). Such skills facilitate reciprocal interaction, language development, and learning opportunities for the developing child.

From the first months of life, children recognize the importance of social stimuli. As early as three weeks of age, newborns spend more time looking at faces than shape- and size-matched non-social objects (Johnson et al., 1991; Morton & Johnson, 1991). At nine weeks, infants preferentially fixate on their caregiver's face over a stranger's face (Mauer, 1982), suggesting that they can identify their mothers on the basis of vision alone. Infants also may distinguish their mother's voice in contrast to a stranger's (Mehler & Dupoux, 1994).

Children with autism. Early social attention skills are strikingly impaired in young children with autism (Happe, 1994; Kasari, Sigman, Yirmiya, & Mundy, 1992; Sigman &

Mundy, 1989; Wing, 1996). Decreased social attention is a hallmark deficit of autism (Sigman & Kim, 1999) and is one of the earliest recognizable features (Baird et al., 2000). Although the nature of the social abnormalities observed in children with autism tends to change with age, decreased attention to others is one of the most prominent problems evidenced in the first year and is present throughout life (Baranek, 1999; Sigman & Kim, 1999).

Dawson and colleagues (1994; 1999; 2000; 2004) reported that the failure of children with autism to attend to others is the most sensitive feature in distinguishing between children with and without autism. Moreover, the authors report that this difference is evident as early as one year of age. In retrospective reports, parents rated their children with autism as being less socially engaged during the first two years of life (Wimpory, Hobson, Williams, & Nash, 2000). Retrospective videotape research found that infants who were later diagnosed with autism smiled less with their caregivers (Adrien, et al., 1991) and watched others' faces less frequently (Kasari, Sigman, & Yirmiya, 1993; Trepagnier, 1998). Yirmiya and colleagues (1999) reported that individuals with autism looked less at the eyes of an interacting adult than did children with Down syndrome and those without developmental delay. In addition, gaze avoidance was found in retrospective studies to differentiate children later diagnosed with autism from those who were not (Adrien, et al., 1993). Frith (1989) and Baron-Cohen et al. (1995) hypothesized that children with autism were unaware of the significance of the eyes, as evidenced by their inability to use eye direction to infer someone else's desire in an experimental setting. Osterling and Dawson (1999) and others who have retrospectively studied videotapes of very young children (Baranek, 1999) have noted that children with autism show diminished orienting to their

name, pointing, and joint attention. Taken together, these findings indicate that early social inattention is a key component of the social behavioral deficits in autism. Quantifying these deficits and following them over time during early childhood will be important for understanding their impact on other developments in autism.

Joint attention

Typically Developing Children. Joint attention is the coordination of attention with another person towards an object. This skill allows children to communicate with others, learn language, and share interest. Two separate forms of joint attention, *protodeclarative* and *protoimperative,* have been identified. Protodeclarative joint attention refers to acts of pointing or showing to share attention to or interest in a stimulus. In contrast, protoimperative joint attention bids have the primary purpose of obtaining assistance, or serving a non-social, functional goal (e.g., pulling another person's hand to attain an out-of-reach toy). Both forms of joint attention have been studied in typically developing children and children with autism.

Protodeclarative and protoimperative joint attention have been shown to be predictive of language development (Sigman & McGovern, 2005) and have been conceptualized as early precursors to a developing theory of mind (Jones, Collins & Hong, 1991). Between 9 and 12 months, typically developing children expand their ability to monitor eye gaze, share experiences with others, and develop skill in joint attention (e.g., Feinman, 1982). Impairments in joint attention or fundamental prerequisites of joint attention may lead to a poverty of social and language opportunities and thus impede a broad range of developments.

Children with autism. Children with autism show marked impairments in their joint attention behaviors (Mundy, 1995; Mundy, Sigman, & Kasari, 1995; Mundy, Sigman,

Ungerer, & Sherman, 1986; Lewy & Dawson, 1992). Studies have shown that young children with autism are less likely than children with other developmental disabilities to follow shifts in eye gaze and thus to respond to (Sigman & Kasari, 1995) or initiate joint attention (see Mundy, 1995 for a review). These reports have indicated that children with autism show robust impairments in the use of protodeclarative (i.e., socially driven) but not protoimperative (i.e., functionally driven) joint attention (McEvoy et al., 1993; Mundy et al., 1986; Mundy, Sigman, & Kasari, 1994; Mundy & Vaughan, 2001; Mundy & Markus, 1997; Sigman & Mundy, 1989), suggesting that it is the social component of joint attention that is deficient in autism.

The significance of joint attention deficits is seen in longitudinal studies linking joint attention and other developments. Sigman and McGovern (2005) suggested that joint attention skill is the strongest predictor of language outcome in children with autism. Other research has supported a link between joint attention and language development (Charman et al., 2003) as well as other cognitive abilities (Bono, Daly & Sigman, 2004). According to one influential model, normal infant language development (in particular, word learning) is driven in part by the infant's capacity to understand the mental states of other people, an understanding that first manifests in joint attention interactions (Bloom, 2004). In autism, where there are profound deficits in joint attention evident by the latter part of the first year of life, this model would predict language delays. Indeed, longitudinal studies of joint attention and language development in autism show that these deficits are intimately related, such that earlier emerging deficits in joint attention preclude timely and otherwise normal language development (Carpenter et al., 1998, Mundy and Gomes, 1998; Mundy, Sigman, & Kasari., 1990, 1994). Joint attention allows children to share interest and also identify novel

items as directed by others. Without being able to participate in such directed exploration, children may be deprived of core language and social learning opportunities. Identifying the onset and nature of joint attention deficits early in development may facilitate novel methods for treating this core impairment in autism.

Imitation

Typically developing children. The capacity to imitate others is important for social and cognitive development. Imitation functions as a method of communication between children and their caregiver(s) and serves to sustain social interactions (Grusec & Abramovitch, 1982). Imitation also serves to socially connect the infant with others, offering a foundation to share experiences, emotions, and thoughts. An abundance of research has indicated that many socially important phenomena, such as gesturing and facial communication, are acquired through observation and imitation, without direct instruction.

Imitation skill is present in newborns (Meltzoff & Moore, 1989) and develops rapidly throughout the first years of life (Hanna & Meltzoff, 1993). Infants are capable of imitating facial gestures after the first few months of life (Meltzoff & Moore, 1989) and begin to imitate language sounds and actions with objects during the end of the first year (Abravanel & Gingold, 1985). Throughout development, children become better able to imitate a greater complexity of behaviors. This skill requires attention to others and serves the toddler in future interaction and social learning.

Children with autism. Deficits in imitation have been well documented among children with autism. Problems with imitation discriminate individuals with autism from individuals with other developmental disabilities as early as age 2 years (Charman et al., 1997) and into adulthood (Rogers, Bennetto, McEvoy, & Pennington, 1996). Rogers and

Pennington (1991) have suggested that the imitation deficit in autism has not been adequately addressed and that impoverished imitation skills may be fundamental to the social and emotional impairments akin to this disorder. Nadel and colleagues (1999) expanded upon this hypothesis, suggesting that without properly timed imitative responsiveness, the coordination of sustained dyadic interaction is severely disrupted, impacting the likelihood that future interactions will take place.

Imitation deficits in autism are predictive of social and communication functioning. Stone et al. (1997) reported that imitation of 'actions on objects' was impaired in autism and predictive of later play behavior while imitation of 'body/facial actions' was predictive of speech development. Rapin (1996) noted that oral-facial actions were particularly difficult for children with autism to imitate. Via its strong association with speech development, emotional sharing, and social engagement (Sigman & Ungerer, 1984; Stone et al., 1997), imitation appears to be critically tied to social development and, perhaps, the social dysfunction seen in autism.

Functional and pretend play

Typically Developing Children. Studies have indicated that play behaviors offer a mechanism for early information gathering (Ruff, 1984), exploration of the classification and properties of objects (Gibson, 1988), and the development of communicative and linguistic skills (McArthur & Adamson, 1996). Research suggests that turn-taking games and social interactions around objects during infancy and the toddler years foster social, communicative, and emotional growth (Bakeman & Adamson, 1984; Tomasello & Farrar, 1986). Several reports have suggested that the structural rules governing reciprocal interaction, as well as the comprehension of referential language, is facilitated by reciprocal

games among the infant and caregiver (Tomasello & Farrar, 1986; Tomasello & Todd, 1983). Early interactions around objects also are associated with the development of skills necessary for relating successfully to other people, including the regulation of affect and theory of mind development (Adamson and Bakeman,
1985; Hobson, 1993). Moreover, it has been proposed that early forms of play may underlie more developmentally advanced cooperative interactions (Gorlitz, 1987).

At about 3-4 months, infants begin to reach for, grasp, inspect, and manipulate novel objects (Trevarthen, 1988). Towards the end of the first year, infants begin to combine objects in relational play. During this period, infants will put objects together in ways that are socially appropriate and which reflect the functional properties of the items (Vondra and Belsky, 1989). As infants grow older, their functional play becomes progressively elaborate, integrated, and socially directed (Fenson & Ramsay, 1980). Such play may involve face-to-face interaction with the parent, parallel or cooperative play with peers, or social games (e.g., peek-a-boo). By 12 months infants often will be observed using gestures and vocalizations to initiate social-action games with their caregivers (Platt & Coggins, 1990). This play also may begin to include forms of pretense (Leslie, 1987) and imaginative scenarios.

Children with autism. Children with autism engage in fewer face-to-face interactive games. Bernebei et al. (1998) indicated that only a small subset of children with autism engaged in active peek-a-boo when the child takes the initiative, and their involvement in other conventional social games was rare. The authors also found a relative lack of social turn-taking, occurring in less than one-third of children studied.

Moreover, pretend play is largely absent in children with autism (Charman and Baron-Cohen, 1997; Libby et al., 1997; Thorp, Stahmer, & Schreibman, 1995). In fact, Wing

and Gould (1979) reported that the deficits of autism may be narrowed into three domains – socialization, communication, and imagination, and the American Psychological Association (APA) (1994) has integrated deficits in imagination into autistic preferences for routines and the prevalence of stereotyped and repetitive patterns of behavior. In other words, deficits in imagination may underlie children with autism's repetitive behaviors while also constituting a primary feature of their early social abnormalities. Many studies have highlighted a poverty of pretend play skills in autism (Blanc et al., 2005; Charman & Baron-Cohen, 1997; Lewis & Boucher, 1988; Jarrold et al., 1993) even after matching for language skills (Baron-Cohen, 1987; Jarrold et al., 1996).

Emotion and affect

Typically Developing Children. Emotion skills, such as recognizing others' emotions and regulating one's own emotion, are critical to the overarching task during the 2 to 5 year age period, developing peer relationships (Howes, 1987; Parker & Gottman, 1989). Several emotion skills develop during the preschool years. "Social referencing" is the spontaneous seeking of information from another's face when presented with a stimulus of uncertain valence (Moore & Corkum, 1994). Typically developing children often regulate their own behavior, particularly in new or uncertain situations, by means of nonverbal and emotional cues provided by adults. For example, a toddler may look towards her mother before deciding to cry after she has bumped her knee. Social referencing is firmly established by 9-12 months in normal development (Feinman, 1982; Moore & Corkum, 1994) and represents an example of early mental state awareness.

"Affective tuning" refers to sharing and coordinating emotion with others (Clore, 1997). Affective tuning helps to develop and sustain interactions and contributes to

children's ability to discern separate emotions. This skill is crucial to children's ability to form relationships with others (Parke, 1994; Saarni, 1990) and is predictive of later social competence (Denham et al., 2003). The importance of affective attunement is evidenced by its association with quality of play (Lindsey & Colwell, 2003) and academic achievement (Birch & Ladd, 1997; Ladd, Birch, & Buhs, 1999).

Sharing positive emotions has been shown to be critical to the formation of friendships and attachment relationships (Denham, McKinley, Cochoud, & Holt, 1990; Sroufe, Shork, Motti, Lawroski & LeFreniere, 1984). Conversely, negative affect, especially anger, is problematic in relationship formation and associated with decreased rates of reciprocal interaction (Denham et al., 1990). Preschoolers who show greater control over negative emotions (e.g., disappointment) are more resilient to stress and experience fewer behavioral and emotional problems (Endriga, Jordan, & Speltz, 2003). Moreover, parent-preschool child interactions that are characterized by decreased mutual positive emotion exchanges and more emotional mismatches predict school aged conduct problems and poor social ability (Cole, Tetti, & Zahn-Wexler, 2003).

Children with autism. Studies of social referencing suggest that children with autism rarely engage in this behavior (Dawson, Meltzoff, Osterling, Rinaldi, & Brown, 1998; Kasari, et al., 1993; Sigman, et al., 1986; Sigman & Kasari, 1995). A failure to seek out emotion information from others regarding novel stimuli or events may result in a mistaken interpretation of the social environment and ultimately, to awkward social behavior, decreased exploratory behaviors in the presence of novel settings, failure to monitor the safety of novel situations, and difficulty coordinating emotion with others.

The coordination of emotion, communicated via changes in facial expression or communicative gestures (e.g., clapping), has been noted to be impaired in school-aged children and adolescents with autism (Buitelaar & van der Wees, 1997; Davies, Bishop, Manstead, Tantam, 1994; Fein, Lucci, Braverman, & Waterhouse, 1992; Sigman, Kasari, Kwon, 1992). Few studies have investigated emotion expression or affective tuning in children with autism younger than age 5 years. Dawson, Hill, Spencer, and Galpert (1990) indicated that 3-6 year-old children with autism were less likely than typically developing children to combine smiles with eye contact in a way that conveyed communicative intent and to smile in response to their mothers' smiles. Mothers of children with autism, in turn, were less likely to smile at their children and showed fewer smiles overall. These findings indicate that children with autism show less positive affect and positive affect sharing.

Variability in social attention and emotion deficits in autism

The literature outlined above suggests that social attention and emotional behaviors are impaired in autism and may underlie many of the primary difficulties of individuals with this disorder. Decreased rates of attention to faces, responding to name called, joint attention, imitation, symbolic play, and affect attunement have been found to differentiate preschool age children with autism from their peers (Marcus, Garfinkle, Wolery, 2001; Mundy & Crowson, 1997, Stone & Lemanek, 1992). Despite these findings, a portion of children with autism evidence some early social attention and emotion skills (Dawson & Castelloe, 1993; Wing & Gould, 1979), suggesting variability in skill among children with autism.

It has long been recognized that autism, as a behaviorally defined syndrome, is not a unitary entity (Coleman, 1979). For example, Ornitz, Guthrie and Farley (1977) reported on parental responses to questions regarding behaviors relevant to autism and indicated that a

substantial portion of children *did not* exhibit characteristic symptoms. Twenty-four percent of children *did not* "avoid looking at people in the eyes," 50% *did not* "respond to affection by active withdrawal," and 70-90% more than rarely showed normal modes of relating to others. More recent findings suggest that many children with autism show proximity-seeking behaviors and vocalizations for social attention (Sigman & Mundy, 1989). In fact, Kasari and colleagues (1993) found that when interactions were guided by a familiar adult, many children with autism make as many appropriate social responses as their non-autistic peers. However, when children with autism make social overtures in unstructured social situations, they are brief, poorly integrated, and often do not elicit a response from other children (Stone & Caro-Martinez, 1990). Contrary to the original view of the disorder, basic social processing and interest in interaction with others are not absent in all children with autism.

Behavioral patterns in autism vary not only between individuals but also across development. Experts have called for age-specific norms on the frequency of behaviors exhibited by children with autism (Freeman et al., 1979), but such reference points have not yet been established. Examining the variability in autistic symptoms among affected individuals and over time is important for separating possible phenotypes and developing more individualized treatment approaches. Although the need for developmental studies of social and emotional behavioral deficits in autism is apparent, few studies have been able to quantify these behaviors early in development. Several methodological limitations have hindered progress in this field. These limitations are reviewed below.

Measuring early social attention and emotion in autism

Methodological shortcomings have hindered researchers' ability to measure early social attention and emotion qualities of children with autism over time. For example,

researchers have had difficulty developing laboratory tasks suitable for severely impaired and/or young children with autism. Laboratory tasks that require children to attend to stimuli over a prolonged period or process verbal instructions may limit the ability of young or severely impaired children with autism to participate. Researchers have utilized parent reports for assessing early behavior in autism. However, these reports are problematic because they rely on individuals to recall past episodes or periods of development. Therefore, retrospective parent reports are subject to biased recall and may lack sensitivity to subtle behavioral abnormalities that might be noted by a trained observer. More recent studies have employed retrospective videotapes that allow expert raters to observe children's early behavior while being blind to the child's diagnosis. While this method is an improvement over parent reports, it also is limited by the lack of standardization across settings in which the child is observed. The development of a structured observation assessment for measuring social attention and emotional behaviors in young children with autism would, therefore, be of significant importance.

The Autism Diagostic Observation Schedule (ADOS) (Lord et al., 2000) is one such tool and is part of the gold standard for evaluating autism. The ADOS is a semi-structured play session in which a trained examiner offers specific presses designed to elicit behaviors that are impaired in autism. The accompanying algorithm of social and communication skills has been shown to adequately differentiate children with autism from children without autism (Lord et al., 2000). The ADOS is administered to children as young as 2 years of age and offers unique opportunities for observing young children engage in a semi-structured play and social interaction session. ADOS sessions probe children's social attention, joint attention skill, imitative ability, and play behaviors. However, the ADOS relies on a scoring

system that is not quantitative. Analyzing behavior with the ADOS is limited by a categorical scoring system in which the differences between adjacent scores are not equally separated. Instead, ratings denote whether a behavior is present, rarely present, or absent. This scoring system is useful for clinical diagnosis only but lacks sensitivity to subtle differences in the severity of autistic children's impairments. Codes of 2 and 3, each indicating that a child shows impairment on a particular item, often are collapsed in order to improve reliability between raters. The ADOS and its accompanying algorithms focus on broader features of autism, and do not measure subtle, yet important social attention and emotional skills central to autistic development. The test developers warn against using the ADOS for item level and quantitative analyses. Likewise, other standardized assessments of autism are limited in their sensitivity to autistic features (e.g., Gilliam Autism Rating Scale) (South et al., 2002) or are intended solely for general screening purposes and are not useful for measuring severity (e.g., Childhood Autism Rating Scale). As a result, novel, dimensional measures for assessing behavior in young children with autism are needed.

A novel observational coding system

While the ADOS scoring system currently is not suitable for evaluating the severity of behavior in children, the structured presses do provide important tools for generating a range of social and emotional behaviors. The raw data produced from ADOS sessions may provide sufficient detail for quantitatively measuring selected autistic behavior. In addition, the ADOS is routinely videotaped in many research settings. Therefore, a tool for coding quantitative aspects of autistic behavior from ADOS tapes would be valuable.

Such a coding scheme has not yet been developed for young children. One previous report using an observational coding system for social behavior based on ADOS sessions has

revealed considerable variability in several social attention functions among older children with autism and linked these behaviors to the severity of other deficits (e.g., cognitive and communication domains), though results from this study have not yet been extended to early development (Meyer, 2002).

The first aim of the present study, therefore, was to develop and apply an observational coding system focused on assessing the quantity of social attention and emotion behaviors in preschool aged typically developing children and children with autism participating in an ADOS session. Briefly, rates and durations of social and emotional behaviors previously implicated in autism are tabulated. The target behaviors, as reviewed above, have been hypothesized to be central to autistic deficits, but have seldom been quantified early in development or followed over time. The ADOS-Social Emotional Perspective (SEP), is based on observations of children naturally engaged in a semi-structured play period, thereby limiting task demands and providing an opportunity to observe children as they naturally respond to a novel social environment. Moreover, the ADOS-SEP provides continuous values for each item, enabling assessment of impairment severity. The procedures used to develop the ADOS-SEP are detailed in the method section below.

The second aim of this study was to examine the relationship of behaviors defined by the ADOS-SEP with the development of a brain structure, the amygdala, previously reported to be associated with social and emotional behavior in adults and non-human primates. Analysis of the relationship between behavioral and brain abnormalities in autism could offer insight into the development of this disorder. Details of what is currently known about the amygdala and its involvement in autism are provided below.

Why study the neural substrates of autistic behavior?

Structural MRI studies have identified multiple regions that are abnormal in autism (for a review, see Cody Hazlett, Pelphrey, & Piven, 2003). However, the vast majority of these reports have failed to find concomitant autism related behaviors or cognitive deficits. Data on associations between MRI findings and behavioral features in autism would be helpful for clarifying which neuroanatomical abnormalities are directly relevant to autism (i.e., whether they are a pathophysiological mediator or involved in an affected neural system) and which abnormalities may reflect an epiphenomenon. Examining brain-behavior linkages and following these associations over time also could offer insight into the substrates of the primary behavioral features of autism. Separate brain regions and structures develop at different rates. For example, while the amygdala is functional at birth (Kordower et al., 1992), more anterior regions, such as the orbitofrontal cortex, develop gradually over the postnatal period (Overman, 2004). The amygdala and orbitofrontal cortex share intricate connections and both have been implicated in autism, though they are hypthesized to be associated with separate social and emotional behaviors. If amygdala-behavior associations were evident within the first years of life, then this disruption could contribute to the development of impairments in behaviors believed to be associated with the orbitofrontal cortex. In contrast, amygdala-behavior associations may not be present early in development and may, in contrast, be the result of later occurring orbitofrontal disruptions. Following brain-behavior associations over time could help localize dysfunctional neural systems and map the neural substrates of autistic behavior.

Autism is generally considered a multifactorial disorder, reflecting an interaction of both multiple genes and environmental factors (DeMyer et al., 1981; Rutter & Lockyer,

1967). Examining the neural substrates associated with the clinical features of autism could link behavioral characteristics to genetic mechanisms. For example, Hariri and Weinberger (2003) suggested that studying the response of brain systems involved in behavioral impairments specific to psychiatric disorders may be more informative than studying the behavioral or cognitive sequelae alone. The authors indicated that because the biological impact of genetic variation traverses an increasingly divergent path from cells to neural systems to behavior, interceding at the most basic level is preferable. As genetic polymorphisms begin to be linked to neural developments, identifying genetic linkages to the biological deficits in autism becomes more possible.

Understanding brain-behavior associations also will be informative for treatment efforts. Defining biological and behavioral phenotypes could facilitate the development of integrated pharmacological and behavioral intervention plans that may be more effective than either treatment method used in isolation. By increasing the number of systems that are treated simultaneously (e.g., biological and behavioral), researchers may begin to outline more potent treatment options.

Neuroanatomical abnormalities in autism

Recent magnetic resonance imaging (MRI) findings have provided exciting insights into the neural substrates associated with autism. The most consistent neuroanatomical findings indicate that total brain volume (TBV) is enlarged in autism (Aylward et al., 2002; Courchesne et al., 2001; Lotspeich et al., 2004; Piven et al., 1992; 1995; Sparks et al., 2002), though the onset and development of TBV enlargement have not been clearly explicated. Piven et al. (1992; 1995) first reported increased TBV in adolescents and adults with autism. Subsequently, Courchesne et al. (2003) observed increases in TBV in 2-4 year olds, and

Sparks et al. (2002) noted enlargement in 3-4 year olds. Aylward et al. (2002) reported increased TBV in children up to age 12 years, and Cody-Hazlett et al. (2005) recently reported that TBV increases are present as early as 18-35 months of age. Overall, these studies suggest that patterns of cerebral enlargement in autism are present early in ontogeny, and while cerebral enlargement is not confined to early ages, it may be more robust within the first few years of life.

Novel research also has provided insight into the onset of brain enlargement in autism. Recent evidence suggests that head circumference is enlarged in children with autism beginning at 12 months (Cody-Hazlett et al., 2005). Because head circumference provides an index of overall brain volume early in development, these findings suggest that brain overgrowth begins in the latter part of the first year of life. Related to the finding that head circumference enlargement emerges within the latter part of the first year of life, two independent studies of infant siblings of autistic children have indicated that the core behavioral features of autism are not detected at 6 months but are observable during the latter part of the first year of life (Landa & Garret-Mayer, in press; Zwaigenbaum et al., 2005). These reports suggesting that the defining features of autism may have their onset in the latter part of the first year of life, combined with findings that the onset of TBV enlargement occurs within the first year of life, suggest that the behavioral and neuroanatomical characteristics of autism may be temporally related. Research investigating the significance of this relationship is warranted, but these recent findings do suggest that identifying the onset of neuroanatomical abnormalities in autism may help characterize the pathogenesis of this disorder.

<u>The neural basis of behaviors associated with autism</u>

As highlighted above, social behavior impairments, including impairments in social attention and emotional behavior, are defining features of autism. Examining the neural substrates associated with autism may be most informative if the core behavioral deficits are targeted. The role of brain regions associated with social and emotional processing has been examined in non-human primate and human lesion studies and structural and functional neuroimaging studies of adults. These investigations have begun to outline a network of brain regions associated with processing, organizing, and responding to social and emotionally salient information.

Brothers (1990) hypothesized a "social brain" inherent in neurotypical individuals, composed of the orbitofrontal cortex, superior temporal gyrus and sulcus, amygdala, and fusiform gyrus, that is sensitive to the features and movements of socially relevant information. Baron-Cohen and Ring (1994) posited a similar model, suggesting that the orbitofrontal cortex, amygdala, and superior temporal sulcus are functionally related in processing the features and movements of biological agents. Finally, McCarthy (1999) identified various nodes of a human face processing system, distinguished by their sensitivity to invariant and dynamic aspects of faces. The first two nodes, comprised of the lateral posterior fusiform gyrus and the anterior ventral temporal cortex, are involved in structural encoding and face memory. The third and fourth nodes are centered in the superior temporal sulcus region and amygdala and are sensitive to facial movements, communicative gestures (e.g., eye and mouth movements), and emotion processing.

The development of the specialization of this circuitry to social processes has been studied less extensively. Examining the timing of brain-behavior associations could provide clues to the development of autism. A developmental hypothesis would suggest that early

disruption within a circumscribed component of this circuitry could grossly affect social and other behavioral and cognitive developments. For example, typical infants' preference for faces is believed to be mediated by a subcortical visual system that passes information from the retina to the superior colliculus to the pulvinar nucleus of the thalamus and then into the amygdala (Palsey et al., 2004). Congenital abnormality of this subcortical visual system could be responsible for diminished attention to faces and socially relevant stimuli early in autistic development (Maestro et al., 2002). Disruption of this circuitry could be the first insult in a cascade of aberrant neuronal and behavioral developments affecting children with autism. For example, amygdala abnormalities could lead to a failure to orient to salient social stimuli or process the inherent emotional value of social interaction and preclude the development of reciprocity skills. Indeed, converging animal, human lesion, and neuroimaging studies have indicated that the amygdala is critical to the immediate processing of social information and to the formation of an appropriate emotional response, and thus may be involved in autism. Studying amygdala development in young children with autism and examining the relationship between amygdala morphometry, social attention, and emotion developments could be an important method for examining the timing of mechanisms that contribute to the social difficulties of individuals with autism.

Amygdala development and its relationship to behavior in primates

Studies of non-human primates have implicated the amygdala in social interest and emotional reactivity. Researchers have shown that projections from higher-order visual cortices to the amygdala are significantly refined in macaque monkeys between one week and three months of age. At this point in development, macaques begin to show appropriate responses to social signals. For example, just after birth, infants look equally at pictures of

other monkeys that are looking away or staring directly at them. However, by the end of the first month, infants begin to show differential and appropriate responses to gaze aversion and direct stare (Mendelson, 1982). As refinement of amygdala-visual system projections nears completion during the third month (Bachevalier, Hagger & Mishkin, 1991), infant macaques begin to initiate social interactions with behaviors such as grooming and social play (Suomi, 1990). Researchers also have shown that the timing of the myelination of projections between the amygdala and other structures involved in motor movements (i.e., basal ganglia), autonomic responding (i.e., hypothalamus), and arousal (i.e., brainstem) coincides with increases in fearful and defensive social behaviors in infant rhesus monkeys (Amaral, 1992; Gibson, 1991). The coincident timing of significant developments in amygdala connectivity and social behaviors suggests that the amygdala plays a central role in the development of these behaviors.

Studies of the behavioral effects of amygdala damage in non-human primates have been somewhat inconsistent. Emery et al. (1998) showed that primates with amygdala lesions appeared less aggressive and less fearful toward control animals and threatening objects (e.g., rubber snakes) and exhibited decreased interest in interacting with conspecifics. In contrast, more recent studies of rhesus and macaque monkeys suggest that amygdala damage early in ontogeny leads to increased emotional responses to the environment, in particular fear, but not to decreases in social interest or attention (Amaral, 2002; Amaral & Corbett, 2003). Amaral and Corbett (2003) suggested that young amygdala-lesioned animals showed increased rates of social approach behavior. Prather et al. (2001) also reported that young macaque monkeys with bilateral lesions to the amygdala showed rates of social approach comparable to controls. Radiotelemetry recordings of the activity of neurons in the

amygdala of rhesus monkeys showed the highest response when animals were presented with faces, particularly faces displaying emotional expressions (Kling, Steklis, & Deutsch, 1979). These data suggest that the amygdala is involved in the evaluation of threat and reinforcement value of social stimuli and may be involved in the animal's emotional response. Such assessments of the social environment are likely to be important for the survival of the animal.

Amygdala development in humans

Multiple studies have examined adults with amygdala damage and have shown evidence of its involvement in human perception of facial expressions and emotions (Adolphs & Tranel, 2003; Adolphs et al., 1994; 1995; 1999; Broks et al., 1998; Breiter et al., 1996; Calder et al., 1996; Morris et al., 1996; Whalen et al., 1998; 2001). Developmentally, the amygdala increases in size, and likely neuronal complexity, throughout childhood and into adolescence (Giedd et al., 1996; McClure et al., 2004; Thomas et al., 2001; Wang et al., 2004).

The functional implications of volumetric developments have not been explored. Functional magnetic resonance imaging (fMRI) studies have linked the amygdala to face and emotion perception. Thomas et al. (2001) reported greater bilateral amygdala activation in 8-16 year-old children viewing fearful faces compared to neutral faces. The authors also reported that in a sample of children with anxiety disorders, the magnitude of amygdala signal change in response to fearful faces was positively correlated with child self-reported anxiety. Adolphs et al. (1997) and Hamann et al. (1996) each reported emotion recognition deficits in an adult woman with bilateral focal damage of the amygdala suffered during childhood. Interestingly, these studies both indicated that her pattern of impairments was

distinct from those demonstrated by individuals with amygdala damage occurring during adulthood. Specifically, this woman was severely impaired in processing fearful faces and perceiving threat, while demonstrating spared recognition of non-fear emotions.

Taken together, these findings implicate the amygdala in the processing of social stimuli. More specifically, research with non-human primates suggests that amygdala development coincides with social advancements, such as attention to faces, gaze aversion, and affiliative and fearful behaviors (Amaral, 1992; Gibson, 1991; Suomi, 1990). Research with humans suggests that amygdala damage is related to face processing and emotion recognition (Adolphs, 1999). The role of the amygdala in the processing of social stimuli suggests that it could be involved in deficits of social attention and emotionality in children with autism.

The amygdala in autism

Bauman and Kemper (1985) first reported amygdala pathology in a small sample of post-mortem brains of autistic individuals. Studying the post-mortem brains of nine individuals with autism, the authors reported a pattern of small, immature-appearing neurons and increased neuronal packing density in the amygdala. More recently, functional MRI studies have implicated the amygdala in autistic individuals' social and emotion processing impairments (Breiter et al., 1996; Morris et al., 1996; Whalen et al., 1998; 2001). For example, Baron-Cohen et al. (1999) reported hypoactivation of the amygdala in individuals with autism relative to controls when they were asked to make mental state judgments from viewing another person's eyes.

Structural MRI studies generally have documented *enlarged* amygdala volumes in children (Cody Hazlett et al., 2005; Sparks et al., 2003), adolescents, and adults with autism

(Schumann et al., 2004; Abell et al., 1999; Howard et al., 2001). Both Sparks et al. (2003) and Cody Hazlett et al. (personal communication) have reported that the amygdala was the only region of interest that was abnormal in autism after correcting for increased TBV. Sparks et al. (2003) also observed that significant volumetric differences existed between children with autism and children with less severe symptoms who were diagnosed with Pervasive Developmental Disorder-NOS. In contrast to these findings, Aylward et al. (1999) and Pierce et al. (2001) each reported *reduced* amygdala volumes in non-mentally retarded adolescents and young adults with autism.

Although findings from this series of studies are at first glance contradictory (i.e., enlarged in some studies, smaller in other studies), several factors are important to consider. First, the small sample sizes used in several of these studies could make the analyses susceptible to bias from a heterogenous clinical population. Second, and perhaps most importantly, these studies examined different age groups. A more recent report has stressed that the amygdala is enlarged in younger (i.e., ages 7-12 years) but not older (i.e., ages 12-17 years) children with autism (Schumann et al., 2004). These findings are supported by recent work that suggests the amygdala is significantly enlarged in 2 year-olds with autism after accounting for TBV enlargements (Cody Hazlett et al., personal communication). Together, these studies indicate that the amygdala may undergo a period of rapid overgrowth early in ontogeny that diminishes over time or alters its course (i.e., becomes reduced in volume). Longitudinal studies of young children are essential for clarifying the course of amygdala development in autism. Also, linking features of this structure to the behavioral development of children with autism could serve as a basis for meaningful descriptions of brain-behavior relationships in this population.

Biological heterogeneity in autism

Despite findings implicating the amygdala in autism, results have been somewhat mixed. For example, several studies have reported no volumetric difference or decreased volumes of the amygdala in individuals with autism (Abell et al., 1996; Howard et al., 1994). Inconsistencies among studies may highlight methodological limitations (e.g., small sample sizes, need for longitudinal studies), but also suggest that autism is a biologically heterogeneous disorder. It may be that the amygdala is not affected in some children with autism, or is affected only at isolated points in development. To study these questions, it is important to examine behaviors related to amygdala volume, and to examine brain-behavior relationships over time.

The importance of a longitudinal design

Autism is a heterogeneous disorder with considerable variance observed across behavioral domains and neurobiological systems among affected individuals. The variability in neurobiology likely has confounded results from previous MRI studies. The development of brain regions and structures in typically developing children follows a non-linear course, further limiting researchers' ability to define the neural substrates associated with autism. For example, Caviness et al. (1996) demonstrated that several neuroanatomical regions, including the amygdala, exceeded average adult volumes during childhood and reached peak volumes prior to adolescence. The majority of previous MRI studies have been cross-sectional designs. Longitudinal studies, however, are more sensitive to intra-individual variation and non-linear growth trends.

The importance of conducting longitudinal studies to examine brain development has been convincingly documented in a landmark study by Giedd (2001) who showed peak gray

matter cortical volume development well into the adolescent years, whereas previous cross-sectional studies had concluded that gray matter volume peaked at 4 years of age. This study demonstrated the increased sensitivity of a longitudinal design in the presence of substantial inter-individual variation and non-linear growth. The increased sensitivity of longitudinal studies is particularly important for studies of autism. McGovern and Sigman (2005) recently indicated that the majority of children (40 of 44) in their study that were diagnosed with autism between 2-5 years of age retained that diagnosis in adolescence, but there was substantial variation in the social development of these children. This inter-individual variation would not have been detectable with only cross-sectional comparisons.

Summary

Autism is a disorder characterized behaviorally by a pervasive pattern of deficits, including skills involved in social reciprocity. Studies of the early development of these deficits have been limited by a reliance on retrospective parent reports, observations of non-standardized activities, and assessments that incorporate qualitative, but not quantitative, rating systems. The development of a quantitatively scored observational coding system focused on the early social and emotional deficits in autism will be important for following these core features of autism over time.

Neuroimaging studies of individuals with autism have highlighted abnormalities in brain regions associated with social behavior. However, few studies have examined the relationship between neuroanatomical features and behavioral development in autism. Highlighting these relationships will be important for clinical, neuroscientific, and genetic research.

Both clinical and neuroimaging studies of autism have yielded inconsistent findings. Inconsistencies likely are, in part, due to the heterogeneous nature of autism, a disorder with variable patterns of behavioral and neuroanatomical features amongst affected individuals and over time. Cross-sectional methods often are not sensitive to heterogeneity within groups or non-linear developmental patterns. Longitudinal studies are needed to map brain and behavior developments in autism.

To date, no studies have examined closely the range of social attention deficits found in preschool-aged children with autism, explored these deficits over time, or related them to neuroanatomical markers. This study aims to 1) demonstrate the validity of a novel observational coding system, the ADOS-SEP, focused on social and emotional behavior in young children, 2) examine the range and development of social and emotional behavior deficits measured by the ADOS-SEP in 18-35 month (time 1) and 42-59 month old (time 2) children with autism, and 3) examine the relationship between these deficits and amygdala enlargement. Such an investigation will provide insight into the nature of the social, emotional, and neuroanatomical abnormalities in autism and has potential implications for diagnostic clarity and future interventions.

Aims and Hypotheses:

1. **To quantify social attention and emotional behavior in 2-4 year old children with autism, an observational coding system was developed for application to the Autism Diagnostic Observation Schedule (ADOS).** The sensitivity of this coding system (Autism Diagnostic Obervation Schedule-Social and Emotional Perspective, or ADOS-SEP) will be assessed by examining the range in scores of both children with autism and typically developing children. It is hypothesized that each group will show variation in their

behaviors, as indicated by normally distributed items. Also, the convergent validity of the coding system was assessed by examining whether item scores were significantly related to corresponding items from the previously validated ADOS. Divergent validity also was assessed by probing the relationship between items from the ADOS-SEP with the restricted, repetitive algorithm of the ADOS.

2a. **Social attention and emotional behavior will be abnormal in children with autism relative to controls at both age 2 and age 4 years.** Based on previous findings, it is hypothesized that children with autism will show significant deficits in social attention skills as well as emotional behavior (i.e., decreased positive emotion, increased negative emotion), relative to age-matched typically developing children, after controlling for age, gender, and IQ.

2b. **The rate of social attention and emotional behavior development will be reduced in children with autism.** Although the absence of longitudinal behavioral data on typically developing children in the present study precludes comparisons of rates of behavioral development between children with autism and typically developing children, it was hypothesized that 4 year-old typically developing children would show increased rates of social attention and emotion behaviors compared to 2 year-old typically developing children, whereas significant differences between 4- and 2-year-old children with autism would not be observed.

3a. **Amygdala volume will be associated with social attention and emotional behavior, but not with non-social attention, in children with autism at both time 1 and time 2.** Specifically, increased amygdala volumes will be associated with decreased social attention and emotional reactivity.

3b. **Change in amygdala volume will predict change in social attention and in emotional behavior in children with autism from time 1 to time 2.**

Method

Participants

Participants included 53 children with autism. Children with autism were enrolled between 18 and 35 months of age (i.e., time 1), and 27 of these children were followed up approximately 24 months after their initial assessment (i.e., time 2). A total of 13 children were excluded from analyses due to poor ADOS videotape quality (N= 10) or failure to meet criteria for autism at time 2 (N=3). All remaining children with autism were included in the behavioral and MRI analyses. Eighteen typically developing (TD) children (9 children between 18-35 months, 9 children between 48-66 months) were included in behavioral comparisons (referred to as *behavioral controls* from this point forward). In addition, 25 control children (14 TD children and 11 developmentally delayed (DD) children) were included in the MRI study (referred to as *MRI controls* from this point forward) at time 1, and 8 of these children were followed up (4 TD children, 4 DD children) at time 2. The children with DD were included to enrich the MRI control sample with subjects who were comparable to the subjects with autism in cognitive development.

Children with autism were primarily referred from nine specialty clinics for pervasive developmental disorders in North Carolina (Treatment and Education of Autistic and Related Communication Handicapped Children (TEACCH) centers). Because 18-35 months is younger than the usual age of a diagnosis of autism, many of the children recruited for the present study were on clinic waiting lists for autism evaluations and may have been more severely affected than the general population of children with autism. Behavioral controls

were recruited by mailing index cards to all geographically proximal parents who had newborns. The index cards requested that parents return their contact information if they were interested in their son or daughter participating in future studies of development. Parents who had returned the index card and whose child was in the target age range were contacted by phone. Children whose parents noted concern or diagnosis of any developmental delay or neurological injury were excluded.

MRI controls were recruited separately. TD children were recruited from community advertisements. DD children were referred from selected regional state Children's Developmental Services Agencies in North Carolina. Subjects with DD were referred only if they had no known identifiable cause for their delay (e.g., prematurity, genetic disorder, neurological disorder) and had no diagnosis of a pervasive developmental disorder. Subjects were excluded for having evidence of a medical condition thought to be associated with autism, including fragile X syndrome, tuberous sclerosis, gross central nervous system injury, seizures, and significant motor or sensory impairments.

Subjects with autism were included if they met Autism Diagnostic Interview-Revised (ADI-R) algorithm criteria for autism and obtained ADOS scores consistent with autism. All of the cases met DSM-IV criteria for autistic disorder. Subjects with autism participated in a battery of measures, including the Mullen Scales of Early Learning, the Vineland Adaptive Behavior Scales, behavioral rating scales, and a standardized neurodevelopmental examination, to exclude subjects with any notable dysmorphology, evidence of neurocutaneous abnormalities, or other significant neurological abnormalities. All of the subjects with autism and DD received testing for fragile X syndrome. Typically developing and DD MRI control children were screened for autism with the Childhood Autism Rating

Scale (Schopler et al., 1980) and were excluded if they reached cutoff score for autism (total score $\geq$ 30). Medical records also were reviewed for any possible evidence of autism or pervasive developmental disorder not otherwise specified, and subjects were excluded from this group for any suggestion of these disorders. Parents of behavioral controls were asked if they had any indication that their child was developmentally delayed or had a history of neurological injury or disorder. Parents of children who did have concerns or whose children currently were receiving evaluations for developmental delays were excluded from the study. Children with a history of neurological injury or disorder also were excluded.

Measures

Cognitive Measures.

The Mullen Scales of Early Learning. (Mullen, 1995) The Mullen provides a comprehensive assessment of language, motor, and visual perceptual abilities for children from birth to 5 years, 8 months. The Mullen was used in the present investigation to obtain a single, reliable, and valid estimate of IQ for participants at both time 1 and time 2. A limitation of the Mullen is that it has a restricted distribution of standardized scores for lower functioning individuals (i.e., subscales only provide a <50 Standard Score). Several of the lower functioning subjects in the present investigation failed to reach a basal on the Mullen. For this reason, raw scores were used to calculate mental age equivalents for participants at time 1 and time 2. Mental age equivalents were thus used as indices of cognitive performance to allow for a better description of functioning for the lower functioning autistic and DD subjects. An average mental age equivalent across the four subscales (Visual Perception, Fine Motor, Receptive Language, Expressive Language) was used as an overall IQ measure. Still, the distribution of IQ scores was not normal. The vast majority of

children scored in the mentally retarded range. Only 2 children at time 2 managed to score within the average range. In addition, few children scored in between the mentally retarded and average ranges (N=2 at time 2).

Diagnostic Assessments.

The Autism Diagnostic Interview-Revised (ADI) (Lord, Rutter & Le Couteur, 1994). The Autism Diagnostic Inventory (ADI) is a semi-structured parent interview for which items have been shown to be reliable. The accompanying algorithm adequately discriminates autistic individuals from a mental-age matched, non-autistic comparison group based on social and communication behaviors. The ADI-R is administered and scored by trained raters and also is audiotaped for random reliability checks. Administration time is approximately 2 hours.

The Autism Diagnostic Observation Schedule-Generic (ADOS; Lord et al., 2000). The ADOS is a structured observation session designed to elicit social interactions and communication in individuals suspected of having autism. During this session, the examiner engages the child in a broad array of interactions, including calling his/her name, initiating joint attention, requesting that the child imitate actions, free play, pretend play, and interactive play. The child has multiple opportunities to engage the examiner in interaction, respond to social approaches by the examiner, respond to or initiate shared attention bids and approach novel items. Four separate modules of the ADOS can be administered and are chosen based on the child's level of social interaction and communication. The most basic form of the ADOS, Module 1, was used for the majority of participants, though 4 children participated in Module 2 sessions. Module 1 of the ADOS includes 10 social events and children are rated on 29 items. Corresponding algorithm or subdomain scores are provided

for communication, reciprocal social interaction, play, and stereotyped behaviors and restricted interests. Module 2 of the ADOS is intended for children with phrase speech and contains 14 social events. Twenty-eight behavioral items are provided with four corresponding algorithm or subdomain scores (i.e., communication, reciprocal social interaction, play, and stereotyped behaviors and restricted interests).

The ADOS is scored by trained raters and videotaped for random reliability checks. The range of scores for each item varies. Typically, children are rated as 0 if no impairments are present and up to 3 if profound abnormalities are observed. Several items are rated only as 0 or 2 (e.g., looking at others, sharing enjoyment), with 0 indicating no abnormality and 2 indicating poor quality. Administration time is approximately 30 minutes for Module 1 and 40 minutes for Module 2.

Behavioral Measures

ADOS Social and Emotional Perspective (ADOS-SEP). The ADOS-SEP was developed by reviewing research addressing the deficits characteristic of young children with autism, observing with Dr. Jerome Kagan videotaped ADOS sessions of children with autism, and conducting pilot analyses with codes hypothesized after viewing ADOS sessions.. Procedures for developing and validating this measure are described below in the Method section. Target variables include event (looking at examiner, social initiations, responding to name being called, joint attention responding and initiating, imitation, communicative gestures, emotion sharing, expressing positive emotion, and expressing negative emotion) and state (engaged with other, engaged alone, disengaged, engaged in pretend play, engaged with toy x) codes. Scoring guidelines are provided in Appendix A.

Cases were included only if children were observable on camera for > 5 minutes. All frequency scores for target behaviors were converted to rates per minute of observable time (e.g., number of social initiations per minute). All duration scores were converted to proportion scores by dividing duration by total observable time and multiplying by 60 (to convert from rate per second to rate per minute). Groups (i.e., autism vs. behavioral controls) were compared on the frequency and duration of ADOS-SEP behaviors using two multivariate analyses, one for each time point, with group as the predictor variable and age, IQ, gender, and all 2-way interaction terms with group (e.g., group x age, group x IQ) included in the model. Behavioral items were entered as outcome variables. Children were excluded from analyses of social attention rates if they were not presented with the opportunity to respond to 1) their name being called, 2) joint attention bids, or 3) imitation trials during their ADOS session. Including rates of 0 for children who were not presented with the opportunity to respond to these items would have confounded results, so social attention scores were not computed for these children. A total of 3 children were excluded for this reason.

Training and reliability. Videotaped ADOS sessions were coded for each child with autism. The principal investigator (PI) trained one undergraduate student (rater 2) on coding guidelines. The PI also met with rater 2 weekly for 1.5 hours to review cases, code individual cases simultaneously, and discuss scoring decisions. Rater 2 initially coded 4-5 cases weekly between meetings to become comfortable with the coding system. Once he was accustomed to the scoring procedures, rater 2 performed a reliability series.

Interrater reliability was established between the PI (rater 1) and rater 2. Each rater independently coded 5 cases two times. In addition, 25% of cases included in final analyses

were coded by both raters and examined for reliability and rater drift. For cases in which both raters completed coding, the ratings used for final analyses were randomly selected. Reliability was calculated separately for event and duration codes. For all codes, a tolerance of 3 seconds was used. Intraclass correlation coefficients (ICC's) were computed to examine reliability between raters. Good reliability was established for both event (.83) and duration (.84) codes.

Intra-rater reliability also was established. For Rater 1, percentage agreement indices for the event codes had a mean of 81% and ranged from 76% to 84%. *Kappa* coefficients for event codes had a mean of .79 and ranged from .74 to .84. For the duration codes, percentage of agreement ranged between 83-98% for each case, with an average of 89%. A *kappa* coefficient showed an agreement index of .85.

For Rater 2, percentage agreement indices for the event codes had a mean of 80% and ranged between 74% and 86%. *Kappa* coefficients for event codes had a mean of .81 and ranged between .72 and .85. For the duration codes, percentage of agreement ranged between 85-93% for each case, with an average of 87%. A *kappa* coefficient showed an agreement of .83.

MRI acquisition

All of the subjects were scanned at the Duke-University of North Carolina Brain Imaging and Analysis Center, Durham, on a 1.5-T GE Signa MRI scanner (General Electric Medical Systems, Milwaukee, WI). Image acquisition was designed to maximize gray and white tissue contrast. This included a coronal T1-weighted sequence with the following parameters: inversion recovery preparation pulse, 300 milliseconds; repetition time, 12

milliseconds; echo time, 5 milliseconds; flip angle 20°; thickness, 1.5 mm; number of excitations, 1; field of view, 20 cm; and matrix, 256_192.

Subjects with autism and DD subjects were scanned using moderate sedation (combination of pentobarbital and fentanyl citrate). Physiological monitoring was conducted throughout the scan and recovery. Time 1 TD subjects were scanned without sedation in the evening while sleeping. Time 2 TD subjects received behavioral training within a 'mock scanner' prior to their MRI scans. The mock scanner makes use of a decommissioned General Electric MRI scanner bore and RF head coil so that its internal dimensions are the same as a real scanner. It also has a genuine GE front piece so that the simulator appears virtually identical to a real scanner. Speakers placed under the bore are used to play recorded scanner sounds to add to the realism. Children view stimuli using LCD goggles or an LCD monitor in a manner similar to that used in the real MRI scanners. The BIAC simulator scanner is equipped with a video presentation system (LCD goggles or an LCD screen) and a Polhemus FASTRAK head motion sensor. Children are trained using operant conditioning procedures with software that receives input from the head motion sensor and uses this input to direct the operation of a digital video (DVD) player. The DVD (e.g., the child's favorite movie) is paused (for a user-defined period) whenever the child exhibits head motion above a certain (user-defined) threshold.

All of the scans were reviewed by a pediatric neuroradiologist and were screened for significant abnormalities (e.g., malformations, lesions). Seven scans were excluded from the TBV measurements for motion artifact or acquisition problems.

Image processing. Initial image processing to register and align the T1 scans into a standardized plane was conducted with BRAINS2 software (University of Iowa, Iowa City)

(Harris et al., 1999). All of the scans were registered along an anteroposterior commissure axis. The coregistered and aligned images were then processed for tissue segmentation using the Expectation Maximization Segmentation (EMS) software (originally developed at the Catholic University of Leuven, Leuven, Belgium (Van Leemput et al., 1999). Reliability and validity of the EMS software has been rigorously examined by the developers and within our laboratory (Park et al., 2001; Styner et al., 2002; Van Leemput et al., 1999). Our initial attempts to apply the existing adult-based EMS template brain atlas provided unsatisfactory results. Therefore, a new pediatric template atlas was created by our laboratory using MRI brain scans of 14 children (comprised of 9 autism, 2 DD, and 3 TD cases that were randomly selected) that first were tissue classified using BRAINS2, which provides semiautomated tissue classification procedures, and then were averaged to create a probabilistic spatial prior template. This resulted in an averaged probabilistic brain atlas that was aligned to each subject's brain using a linear, affine transformation in a fully automated procedure. After bias estimation, inhomogeneity correction, and nonbrain stripping procedures were conducted, subject scans were processed with EMS to produce gray matter, white matter, and cerebrospinal fluid (CSF) tissue segmented images for each subject. Total brain volume measures included total gray matter, white matter, and all of the CSF.

Amygdala segmentation. MRI data was collected when participants were between 18-35 months and then approximately 24 months after this initial date. Pre-processing and registration was performed by trained image processors. Amygdala segmentation was performed on 1 mm coronal slices using IRIS software (Gerig, Ho, & Ruffin, 2000). Images were aligned along the long axis (anterior-posterior direction) of the hippocampus using BRAINS 2 software. Alignment along the hippocampus allows for optimal viewing of the

amygdala. All tracing was performed using IRIS software, which allows simultaneous data visualization and interaction within three planes (i.e., axial, sagittal, and coronal). Tracing guidelines are included as Appendix B. Briefly, the amygdala is first traced within the coronal view. Tracing begins at the most posterior slice in which the amygdala is visible. The dorsolateral extent of the optic tract is chosen as the starting point, and tracing proceeds counter-clockwise for the right amygdala (left side of computer screen) and clockwise for the left amygdala (right side of the computer screen). A horizontal line is drawn from the dorsolateral extent of the optic tract to the white matter lateral and adjacent to the amygdala. The lateral border of the amygdala then is traced ventrally to the lateral ventricle or, if no CSF is present, to the dorsolateral extent of the hippocampus. Tracing proceeds medially along the dorsal border of the hippocampus, and then dorsally to the semiannular sulcus. Procedures are repeated in each successive slice moving anterior. The lateral border is re-examined within the axial plane, and the anterior border is re-examined within the sagittal plane.

The PI performed all bilateral amygdala segmentations, blind to diagnosis, using guidelines for tracing the amygdala that have been validated and published (Schumann et al., 2004). The PI attained good inter-rater reliability with the authors of the protocol (Right amygdala =.94, Left amygdala=.89) and also established good intra-rater reliability (Right amygdala=.98, Left amygdala=.98). The PI of the present study also established good consistency on a set of pediatric images (Right amygdala =.96, Left amygdala =.92).

Procedures

Analyses for the present study are based on previously collected MRI and ADOS data that are part of a large-scale longitudinal MRI study of brain development in autism. The

ADOS was administered to children with autism who were enrolled in the MRI study at both time 1 and time 2. Each session was videotaped in order to re-examine initial coding decisions. The development and validation procedures for the ADOS-SEP are described below.

Behavioral Coding

The ADOS-SEP was developed according to guidelines for developing new observational coding systems presented by Floyd, Baucom, Godfrey, and Palmer (1998). Floyd and colleagues suggested that three questions should be considered prior to constructing an observational coding system: (a) "What behaviors do we want to observe?" (b) "What are our code categories?" and (c) "What is our unit of observation?" Below is a discussion regarding how each of these questions was addressed during the construction of the ADOS-SEP.

"What behaviors do we want to observe?" The present observational coding system is intended to examine the quantity and quality of social and emotion behavior among children with autism during a structured play session (i.e., the ADOS). Based on observations of videotaped ADOS sessions, the PI first generated a list of social and emotion behaviors exhibited by TD children within the target age range (i.e., between 18-59 months). Next, a review of the relevant literature on social and emotional development in TD children and children with autism was conducted, and the list was expanded. Finally, the PI corresponded with an expert on early behavioral development (Dr. Jerome Kagan) and the list of target social and emotional behaviors was finalized.

"What are our code categories?" The PI focused on identifying fundamental dimensions of early autistic behavior, namely social and emotional behavior. Then, the list

of possible behaviors were defined so that they could be counted for frequency of occurrence or examined for duration.

"What is our unit of observation?" Coders watched videotaped ADOS sessions while scoring the frequency of individual events or the duration of a given behavioral state (e.g., playing with a toy, engaged, disengaged). In order to operationalize each behavior that should be recorded, specific definitions were developed (see Appendix A).

After behaviors were labeled and defined, a coding manual was written. The manual contains (a) a list of all codes, (b) a descriptive definition for each code, and (c) examples of behaviors that represent each code. The PI trained one undergraduate student coder on the use of the coding system. The student viewed a series of practice videotapes both with the principal investigator and independently. All scoring was reviewed by the principal investigator and, when the student appeared to be consistent, a reliability series was performed consisting of five cases which previously had been scored by the PI. The student scored these tapes on two independent occasions so that inter- and intra-rater reliability could be assessed.

Data analysis

Aim 1: To develop and examine the validity of the proposed observational coding system. To test the sensitivity of the proposed coding system to variation within both groups, distributions of each item were examined for normality. It should be noted that items from traditional measures of autistic behavior (i.e., ADOS and ADI) are not normally distributed within an autistic population. To assess the internal consistency of the proposed coding system, a 'social attention' component comprised of multiple social items (joint attention initiation, looking at others, responding to name, initiating interaction with others, time spent

engaged with others, imitation, gestures) was analyzed using Cronbach's alpha. To test the specificity of this social attention component, the correlations between social attention and other (emotion and non-social attention) items were examined.

To assess the convergent validity of the coding system, the social attention component and emotion and non-social attention items from the ADOS-SEP were examined in relation to corresponding ADOS scores. Because the ADOS-SEP and ADOS are scored in opposite directions (i.e., higher scores on the ADOS-SEP generally reflect improved social and emotional behavior, whereas higher scores on the ADOS reflect more severe impairment), it was hypothesized that corresponding items would be negatively related. Specifically, it was predicted that social attention scores would be inversely related to the 'social' algorithm and 'play' composite scores from the ADOS. It was predicted that the emotion items also would be related to the ADOS social algorithm. In addition, the non-social attention item (i.e., attention to toys) from the ADOS-SEP was hypothesized to be inversely related to the play composite score from the ADOS. A series of hierarchical regression analyses were used to examine the relationship between ADOS-SEP and ADOS scores. Age, gender, and IQ were entered as covariates for these analyses.

Aim 2a: To compare social and emotional behaviors between children with autism and TD behavioral control children at time 1 and time 2. It was predicted that children with autism as a group would demonstrate cross-sectional decreases in the following behaviors: (a) looking at others, (b) social gestures, (c) protodeclarative joint attention initiations, (d) imitation, (e) responding to name when called, (f) initiations for social interaction, (g) positive emotion, (h) sharing emotion, (i) time spent engaged with others, (j) pretend play, and (k) overall social attention. It was also hypothesized that children with autism would

show significant increases in (a) negative emotion, (b) time spent engaged alone, and (c) time spent disengaged.

MANCOVA models were used to test diagnostic group (i.e., autistic vs. TD children) differences at each time point. Multivariate analyses provide several benefits over univariate analyses, including increased power and elimination of the need to invoke corrections for multiple comparisons. Multivariate analyses also are best suited for examining outcomes that are both expected to have a large effect and are not highly correlated, as in the present study. Each model included main effects for group, gender, age, and IQ. Age and IQ were centered for analyses. For analyses in which only the autism group was examined, age and IQ were centered around the autism group mean. All 2-way interactions with group were included initially to check the assumption of parallelism, but insignificant interactions were dropped.

Aim 2b: To examine change over time in social and emotional behaviors in children with autism. MANOVA was used to compare time 1 and time 2 ratings for each item among the autism group. This comparison examined whether 42-59 month old children with autism show increased rates of social attention and emotion behaviors compared to 18-35 month old children with autism. These analyses were repeated for the behavioral control group.

Next, difference scores were computed for the autism group by subtracting each child's behavioral rating from the mean score for the age-matched behavioral control sample, and then dividing by the behavioral control sample standard deviation (sd). This procedure was repeated for each behavioral item. Hotelling's T comparisons were performed for the autism group comparing difference scores at time 1 and time 2 for each behavioral item. Hotelling's T tests are multivariate analogs of independent samples t-tests and thus provide

increased power when making multiple comparisons. It is hypothesized that difference scores (difference from behavioral controls) will be significantly larger for time 2 (indicating increased degree of impairment) than time 1 due to the increases in these behaviors between time points in the control group and the relative lack of increases in the autism group.

Despite running a large number of analyses, alpha levels were kept at .05 for several reasons. First, these comparisons were performed primarily to provide baseline data for understanding normative social development, 2) only a small number of typically developing children were included in analyses, and 3) I was interested in each behavioral item at each time point and did not wish to test a study-wide null hypthesis regarding differences between groups. Such rationales for maintaining an alpha of .05 previously have been documented (Perneger, Leplege, & Guillain, 1998).

Aim 4. To examine the relationship between amygdala volume (and volumetric change over time) and social and emotional behaviors (and behavioral change over time) in children with autism. It was hypothesized that increased amygdala volumes would be associated with decreased rates of social and positive emotion behavior and increased rates of negative emotion behavior in the autism group. Following segmentation, right and left amygdala volumes (cm^3) were computed within the IRIS program and compared between groups. Group comparisons of amygdala volumes have been reported previously and suggest that the amygdala is significantly enlarged at time 1 in the autism group, after controlling for total brain volume enlargement, age, gender and IQ (Piven et al., personal communication). Analysis of time 2 amygdala volumes currently are being performed by Piven and coworkers.

Because no control children had both MRI and ADOS-SEP data, brain-behavior relationships could only be examined in the autism group. Mixed effects repeated measures

regression analyses were used to examine the relationship between amygdala volumes and behavioral variables of interest for the autism group. Mixed models with repeated measures are set up similar to regression or ANOVA models and can include multiple predictor variables, covariates, and a single outcome. This method has been shown to provide a more flexible approach to estimating individual trends. In contrast to traditional methods, mixed-effects regression models account for different levels of correlation (i.e., between right and left amygdala) at different time points (i.e., times 1 and 2). They also allow for the simultaneous examination of amygdala-behavior relationships at each time point and over time. For example, in the analysis of social attention predicting amygdala volume, one mixed effects repeated measures model will simultaneously examine the relationships between, 1) time 1 social attention and time 1 amygdala volume, 2) time 2 social attention and time 2 amygdala volume, and 3) change in social attention and change in amygdala volume. Time varying covariates also may be computed and analyzed. These covariates reflect the effects of variables (e.g., age and IQ) over time, as opposed to just at time 1 or time 2. Therefore, repeated measures mixed effects models offer several benefits over traditional methods (e.g., ANOVA) including controlling for multiple comparisons while maintaining adequate power and avoiding the limitations of an overly conservative multiple comparison correction method, such as with Bonferroni tests (Gueorguieva, & Krystal, 2004).

Like regression models, mixed effects repeated measures yield omnibus F-statistics assessing the fit of the overall model, as well as t-statistics, which provide information on the probability that the effect of the predictor on the outcome differs from zero. T-statistics are simply computed by dividing the estimate (i.e., effect size) by the standard error (SE). T-

statistics provide information on the relationship between each predictor and the outcome variable after controlling for other predictors in the model. All F- and t-statistics, as well as alpha levels, are reported below. Effect size estimates and SE's for significant relationships are presented in designated tables.

Separate analyses were performed for each behavioral item hypothesized to be related to amygdala volume. Age, gender, and IQ were included as time-varying covariates, and all 2- and 3-way interactions with behavioral items were included. Interactions that did not have a significant effect on the model were dropped, and the model was run again without these interactions in order to identify the most parsimonious model. Therefore, the same model initially was used for each behavioral item, though the final model that was interpreted varied between items and depended on the observed relationships. Significant interactions were probed and are reported below.

Results

Sample characteristics

Sample characteristics (sample size, gender ratio, age, and IQ) for the autism and behavioral control group (i.e., those who participated in ADOS sessions) are shown in Table 1. The final sample included 53 children with autism (4 females) at time 1 and 27 children with autism (2 females) at time 2. Nine TD behavioral control children (1 female) were included at time 1, and 9 distinct TD behavioral control children (1 female) were included at time 2. Characteristics of the autism group and the control groups who participated in the MRI study (referred to as MRI controls) are listed in Table 2. All children with autism participating in the behavioral study also participated in the MRI study. Fourteen TD (5 female) and 11 DD (1 female) children participated in the MRI study. Of these, 4 TD (1 female) and 4 DD (0 female) were followed up by the time that results were analyzed.

Behavioral control and autism group characteristics

The children with autism did not differ from the time 1 behavioral control children in age, t (74) = .694, p =.490. However, the autism group was significantly older than the behavioral control group at time 2, t (51) = 2.030, p =.048. Mean IQ was significantly lower in the autism group than in the behavioral control group at both time 1, t (74) = 3.656, p =.001 and time 2, t (51) = 3.625, p =.001. These differences were controlled for by entering age and IQ as covariates for all behavioral analyses. Gender also was entered as a covariate.

Aim 1: Development and evaluation of ADOS-SEP

To test the sensitivity of the ADOS-SEP to variation within both groups (i.e., children with autism and TD behavioral control children), the distributions of each behavioral item were examined for normality. All items were analyzed, including the following: *looking at examiner, social initiations, responding to name being called, joint attention responding and initiating, imitation, communicative gestures, emotion sharing, positive emotion, negative emotion, engagement with other, engagement alone, disengagement, engagement in pretend play,* and *non-social attention*. Rate and proportion scores for all items were relatively normally distributed within the autism group at both time 1 and time 2, except for *joint attention initiation* at time 1 and *responding to name* at time 1. For these two items, the majority of children with autism did not exhibit any target behaviors. Therefore, these items were standardized for all analyses. Rate and proportion scores for all items also were relatively normally distributed within the TD behavioral control group at both time 1 and time 2 except for *negative emotion* and *joint attention responding* at time 2. No time 2 behavioral control children showed negative emotion, and all time 2 behavioral control children responded to each joint attention bid. Mean and standard deviations of rates of behavior for each variable measured by the ADOS-SEP are presented in Table 3 for both the autism and behavioral control group. Duration scores are presented for both groups in Table 4. Analysis of these means and standard deviations suggests that each group engaged in the target behaviors at variable rates. Also, scores among the autism and behavioral control groups covered a broader range of possibilities than the four values provided by ADOS items and were more evenly distributed.

Item analysis

In order to determine whether items could be combined into component scores, the associations between items relating to social attention were examined. Given their focus on social interaction skills, the following items were hypothesized to be related: 1) social initiations, 2) protodeclarative joint attention initiations, 3) gesture rate, 4) looking rate, 5) responding to name, 7) time spent engaged with another person, and 8) imitation success. Cronbach's alpha was high for these items (alpha = .82), suggesting that a social attention component could be computed based on these 8 items. Z-score transformations to account for the different metrics used for these items were performed and the mean of the z-scores for the 8 items was computed to form an aggregate social attention score for each subject. This social attention score was not related to the emotion or non-social attention items (Table 5).

A combined emotion score comprised of both positive and negative emotion also was examined. This item was expected to be a more disjunctive item based on the observation that children displayed idiosyncratic emotional styles. Indeed, positive and negative emotion were not correlated. However, given the strong theoretical link supporting the connection between positive and negative emotions, an overall emotionality component (consisting of positive and negative emotion shown and positive and negative emotion shared) was examined. Positive and negative emotion displays (including episodes of shared and non-shared emotion) also were examined individually. Additionally, emotion sharing episodes were examined separately. Finally, the non-social attention item also was examined.

Convergent validity

To assess the convergent validity of the proposed coding system, the relationships between ADOS-SEP and ADOS scores were examined. Social attention, positive emotion, negative emotion, and non-social attention scores from the ADOS-SEP and social and play

scores from the ADOS were examined for the autism group. Separate hierarchical regression models were analyzed with social attention, emotion (positive and negative), and attention to toys (i.e., non-social attention) entered as predictor variables, and age, IQ, and gender entered as covariates. ADOS algorithm scores were entered as outcome variables. See Table 6 for results of all tests of convergent validity.

Analyses indicated that the social attention score from the ADOS-SEP was significantly related to the ADOS social score at time 1, F (4, 30) = 2.28, p = .029, and at time 2, F (1, 13) = 7.543, p = .017, though the relationship at time 2 was not significant after controlling for age, IQ and gender, F (4, 10) = 2.136, p = .151. These findings suggest that increases in social attention on the ADOS-SEP were predictive of fewer social abnormalities on the ADOS. Analyses also indicated that the social attention score from the ADOS-SEP was significantly related in the expected direction to the ADOS play score at both time 1 and time 2 (time 1: F (4, 23) = 2.916, p = .049; time 2: F (4, 9) = 2.916, p = .022).

Results indicated that the relationship between ADOS-SEP positive emotion and the ADOS social subdomain approached significance at time 1, F (3, 33) = 3.567, p = .060, and was significant at time 2, F (3, 21) = 11.640, p = .003. These relationships were in the expected direction, reflecting that increased rates of positive emotion were associated with improved social scores on the ADOS. The relationship between ADOS-SEP negative emotion and the ADOS social subdomain was not significant at time 1 or time 2. Furthermore, results indicated that the relationship between positive emotion and ADOS play scores and negative emotion and ADOS play scores were not significant at either time point.

Finally, analyses reflected that the relationship between non-social attention and the social subdomain of the ADOS was significant at time 1, F (3,34) = 6.849, p = .002, but not

at time 2, F (3,23) = .491, p = .492. These findings indicated that at time 1, increased time spent engaged with toys was predictive of fewer social abnormalities. Analyses also reflected that the relationship between attention to toys and play behavior was significant in the expected direction at time 1, F (4, 32) = 5.489,p = .002, and time 2, F (4, 32) = 5.489, p = .002.

Taken together, these findings suggest that scores on the ADOS-SEP were predictive of corresponding ADOS ratings, supporting the validity of the proposed coding system.

Divergent validity

To test the divergent validity of the ADOS-SEP, the relationship between social attention, positive emotion, negative emotion, and non-social attention with the ADOS repetitive behavior subdomain was examined. As was hypothesized, no significant relationships were observed.

Aim 2: Behavioral comparisons between autism and control groups

To examine the hypothesis that the autism group would show decreased rates of social and emotion behaviors compared to the TD behavioral control children, multivariate regression models comparing group performance on the social attention component and remaining behavioral items were performed. Multivariate regression was used to examine the following variables: 1) social attention, 2) positive emotion, 3) negative emotion, 4) non-social attention, 5) duration of disengagement, 5) duration of engagement with self, and 6) duration of pretend play. Age, IQ, and gender were included as covariates for all analyses. Separate analyses were run for time 1 and time 2.

The assumptions of regression were probed. All assumptions, including normality, homoskedasticity (i.e., equal variances at each level of the predictor) of each predictor, and

independence of outcome values, were met. Multivariate analyses also assume that the covariance matrices are equal. This assumption was examined using Box's Test of Equality of Covariance of Matrices. Box's test was significant F (15, 636.84) = 2.116, p =.008, suggesting that the assumption of equal covariance matrices was violated. However, analysis of Box's test suggested that the assumption was violated due to greater variance in the TD behavioral control group. Tabachnick and Fidell (1996) suggest that if sample sizes are unequal and the smaller sample is characterized by greater variance, then although the probability of a Type II error increases, significant findings can be considered valid. Therefore, multivariate analyses were interpreted.

Analysis of time 1 group performance revealed a significant difference in the dependent variables, Wilk's λ (6,41) = 2.505, p= .044. Examination of the univariate comparisons indicated that the autism group was significantly lower in social attention than the behavioral control group, F (5, 41) = 11.944, p = .001. They also showed significantly decreased positive emotion compared to the behavioral control group, F (5, 52) = 3.982, p = .051. Examination of state codes indicated that the autism group spent more time engaged alone, F (5, 52) = 2.044, p = .045, and disengaged, F (5, 52) = 4.968, p < .001, and less time engaged with others, F (5, 52) = 3.892, p < .001, and engaged in pretend play, F (5, 52) = 4.512, p = .002. The autism group also showed increased negative emotion compared to the behavioral control group (means (sd) = .08 (.02) versus .02 (.05)) and decreased proportion of time spent engaged alone (means (sd) = .21 (.13) and .38 (.16), respectively), though neither of these differences was statistically significant (F (5, 41) = 1.400, p = .24 and F (5, 41)= 1.414, p = .11, respectively).

Analyses of time 2 data revealed that all assumptions of multivariate analyses were met and Box's Test was not significant. The omnibus test of group differences was not significant, Wilk's λ (6, 23) = .630, p = n.s. Analysis of univariate comparisons revealed significant group differences for social attention, F (5, 27) = 4.748, p = .042. This difference was in the expected direction as the autism group was observed to engage in fewer social attention behaviors than the behavioral control group. The autism group also showed decreased rates of positive emotion at time 2, F (5, 27) = 13.563, p = .001, compared to the behavioral control group. This was consistent with time 1 findings. Also consistent with time 1 findings, the autism group showed increased time engaged alone, F (5, 37) = 3.934, p = .024, and disengaged, F (5, 37) = 4.763, $p < .001$, and decreased time engaged with others, F (5, 52) = 7.040, $p < .001$, and in pretend play, F (5, 52) = 3.599, p = .001. No other significant group differences were observed.

In order to further compare group differences in the rate of social behaviors, univariate regression analyses were performed for each variable comprising the social attention component. Specifically, the following items were analyzed at both time 1 and time 2: 1) responding to name, 2) imitation, 3) looking at others, 4) joint attention responding, 5) joint attention initiations, 6) social interaction initiations, 7) gestures, and 8) time spent engaged with others. Results from these analyses are presented in Tables 3 and 4. At time 1, children with autism showed decreased rates of each of these behaviors, suggesting that the social attention deficits evident in the previous multivariate comparisons reflect abnormalities across the social attention component. Findings were similar for time 2, though children with autism actually showed comparable rates of initiating social interaction

relative to the behavioral controls. The autism group showed significant reductions in all other social attention behaviors at time 2.

Additional multivariate regression analyses were performed for items that could be separated based on the goal of the behavior. For example, protodeclarative and protoimperative joint attention initiations were examined separately. These analyses explore the possibility that children with autism show deficits in socially mediated sharing behaviors but not in behaviors for which the primary goal is to attain a tangible item (Mundy, 1994). The following items were examined: 1) protoimperative joint attention initiations, 2) protodeclarative joint attention initiations, 3) emotion sharing, 4) protoimperative social initiations, and 6) protodeclarative social initiations. Age, IQ, and gender were included as covariates for all analyses. Separate analyses were run for time 1 and time 2. Alpha was set at $p < .05$.

For time 1 analyses, results indicated that the omnibus model was not significant for diagnostic group, Wilk's λ (6,45) = 1.688, p= .146. Again, Box's Test of Equality of Covariance of Matrices was significant, F (21, 741.87) = 3.255, p <.001, owing to larger variance within the TD group. As a result, Type II error rate was increased for the present results. Analysis of univariate comparisons for each outcome variable revealed that the autism group showed lower frequencies of protodeclarative joint attention initiations, F (1,52) = 4.551, p = .038, and protodeclarative social initiations, F (1,52) = 3.932, p = .050. However, no group differences for protoimperative joint attention initiations, protoimperative social initiations, or emotion shared were observed.

Time 2 analyses also revealed that the omnibus test was not significant for diagnostic group, F (6, 27) = 1.833, p =.121, and Box's Test was significant F (21, 831.148) = 3.345, p

<.001. Exploratory analyses of univariate tests did suggest that the autism group shared emotions less frequently than the TD group, F (1, 34) = 6.214, p = .018. The autism group also initiated fewer protodeclarative joint attention episodes, F (1,34) = 5.771, p = .022. The two groups did not differ significantly in protoimperative joint attention initiations, protodeclarative social initiations, or protoimperative social initiations.

Behavioral development of autism and control groups

Because longitudinal data was not available for the behavioral control group, comparisons of growth rates could not be performed. Instead, a series of cross-sectional comparisons were performed to examine age-related changes in the autism and control groups.

First, mean behavioral change scores were computed for the autism and behavioral control groups by subtracting time 1 mean scores from time 2 mean scores (Table 7). Therefore, positive scores reflect improvement over time. Comparisons of these change scores could not be statistically analyzed because the autism group data was longitudinal in nature, while the behavioral control group data was cross-sectional.

Second, MANOVA models were used to examine differences in behavioral rates between time points. Autism and control comparisons were performed independently. Gender and IQ were entered as covariates, and time (1 vs. 2) was entered as the predictor variable. Each behavioral item was included as a dependent variable. For the autism group, the overall model was significant for time, Wilk's λ (18,24) = 12.635, p< .001 (Table 8). Analysis of individual items indicated that the autism group showed higher rates of imitation, F (1, 24) = 6.377, p = .016, gestures, F (1, 24) = 4.165, p = .048, and pretend play, F (1, 24) = 4.682, p = .036, at time 2 compared to time 1. Interestingly, social attention scores were not

significantly different at time 2 compared to time 1. Social attention scores were based on z-transformations including both the autism and behavioral control groups. Therefore, the absence of change over time suggests that the relative ranking of social attention performance remained stable, as opposed to the rates of behavior. The findings that the autism group social attention score did not change significantly over time indicates that they were neither gaining nor losing ground on the control group. No other behavioral variables were significantly different between time points.

In order to examine whether 42-59 month old TD children show different rates of social and emotional behavior compared to 18-35 month old TD children, an identical MANOVA equation as described for the autism group was performed. Behavioral items were entered as outcome variables, and time (i.e., 1 or 2) as the predictor. Gender and IQ were entered as covariates. Results are shown in Table 9 and suggest that, although the TD children demonstrated increased social behavior at time 2 compared to time 1, few of these differences were statistically significant. These comparisons were cross-sectional in nature and had minimal power. However, results did indicate that TD children exhibit significantly more gestures, $\underline{F}$ (1,16) = 2.362, $\underline{p}$ = .031, protodeclarative joint attention initiations, $\underline{F}$ (1,16) = 9.09, $\underline{p}$ = .011, and imitation responses, $\underline{F}$ (1, 16) = 6.589, $\underline{p}$ = .025 at time 2 than at time 1. The behavioral control group also showed significantly fewer negative emotions at time 2 than at time 1, $\underline{F}$ (1, 18) = 15.043, $\underline{p}$ = .002.

Next, *difference scores* were computed and examined over time. Difference scores reflected the autism group's mean deficit for each behavior, and were derived by subtracting each autistic child's behavioral score by the behavioral control group mean and then dividing the difference by the behavioral control group standard deviation. Therefore, negative

difference scores reflected deficits in the autism group; the further negative scores were from 0, the more severe the deficit.

Difference scores were examined at each time point in order to probe changes in deficit levels among the autism group. Repeated measures univariate contrasts for each behavioral item were employed. Age and IQ were entered as time-varying covariates, and gender was entered as a categorical covariate for each comparison. Time 1 and time 2 difference scores are shown in Table 10.

Difference scores increased (i.e., autism group deficits increased in severity) at time 2 relative to time 1 for looking at others, F (3, 68) = 6.878, p = .017, and positive emotion, F (3, 68) = 26.790, p = .001. Inspection of the data revealed that the control group had increased their rates of each of these behaviors over time, while the autism group showed moderate decreases in their rate of looking and negligible change in their rate of positive emotion. Results also indicated that difference scores decreased (i.e., became less severe) over time for joint attention initiation rate, F (3, 68) = 9.164, p = .001, a finding that was driven by the extinction of an autism group deficit in *protoimperative* joint attention initiations at time 2, F (3, 68) = 3.602, p = .002. Autism difference scores at time 1 were negative for each social item (i.e., autism group exhibited lower rates of behavior), except for negative emotion (indicating they showed more negative emotion) and protoimperative social initiation. Difference scores were negative for each item at time 2 as well, except for protoimperative joint attention (.065), protoimperative (1.234) and protodeclarative social initations (.436). Results indicating that the autism group initiated protoimperative joint attention bids at a rate comparable to controls at both time points are consistent with previous research highlighting fewer protodeclarative, but not protoimperative, joint attention

initiations in autism (Mundy et al., 1998). The finding that social initiations were not decreased in autism is inconsistent with previous studies (e.g., Koegel, 1993), and will be discussed below.

Aim 3. To investigate the relationship between amygdala volume and social and emotional behavior in children with autism

Amygdala volumes

Mixed models with repeated measures were performed to compare amygdala volumes between the autism group and MRI control groups. Amygdala volume was the dependent variable, diagnostic group was entered as the predictor of interest, and age and gender were entered as covariates. Diagnostic group was entered as a 3-level categorical variable (autism, DD, TD). Results indicated that, at time 1, the autism group was characterized by bilateral amygdala enlargement compared to a combined (TD and DD children) control group, F (4, 53) = 4.6, p = .001. This difference was significant when the autism group was compared to the TD group alone, F (1, 53) = 4.6, p = .007, and when compared to the DD group alone, F (1, 53) = 4.6, p = .017 (see Table 11). Longitudinal group comparisons of amygdala volumes currently are being examined as part of a separate study (Piven et al., personal communication). Table 12 display results for time 2 group comparisons. Meaningful statistical comparisons could not be performed because of the small sample size of the control group. Also, the TD and DD were not examined separately due to the small sample size for each group.

Relationships between amygdala volume, age, IQ, and total brain volume

In order to examine the relationship between amygdala volume and potential covariates across time points, Pearson correlations were performed. The relationship

between amygdala volume and age, r(24)=.42, p<.001, amygdala volume and IQ, r(24)=.25, p=.006, and amygdala volume and TBV, r(24)=.56, p<.001,each were positive and significant over time. Significant laterality effects were not evident in the autism group, though previous findings suggest that laterality effects may be evident when examining the relationship between amygdala volume and social behavior (e.g., Munson et al., in press). Therefore, hemisphere (right vs. left), along with age, IQ, and TBV, all were included as covariates in subsequent analysess. Because an insufficient number of females were included in the study sample to examine gender differences in amygdala volume, only males were included in final analyses. All 2-way interactions (i.e., looking x age, looking x IQ, looking x TBV, looking x hemisphere) initially were included in analyses but subsequently dropped if they did not have a significant impact on the model. Post-hoc analyses were used to examine all significant interactions.

Amygdala volume and social items

The relationships between amygdala volume and the social attention component, and amygdala volume and each of the social attention, emotion, and non-social attention items were examined with mixed effects repeated measures regression. First, the relationships between amygdala volume and the social attention component and amygdala volume and the constituent social items were analyzed. It was hypothesized that in children with autism, amygdala volumes would be inversely related to social attention and social attention items.

Social attention and amygdala volumes

Findings indicated that, contrary to hypotheses, the relationship between amygdala volume and social attention was not significant, F (1, 31) = 1.34, p = n.s. No significant interactions were observed.

Looking rate and amygdala volume

It was predicted that increased amygdala volume would be associated with decreased rates of looking at others. Analyses revealed that the overall effect of looking rate (i.e., rate of looking at faces) on amygdala volume was significant across time, F (1, 31) = 5.17, p =.030 (see Table 13). Effects at time 1, t (31) = 1.89, p =.068, and time 2, t (31) = 1.93, p =.063, each approached significance suggesting that, contrary to hypotheses, *increased* looking rate was associated with *larger* amygdala volumes. Also, as amygdala volumes grew over time, the rate of looking at others also increased, t (31) = 3.34, p =.002. However, these results were confounded by significant interaction effects. Analyses indicated that the effect of looking rate on amygdala volume was significantly stronger for the right than for the left amygdala, F (1, 31) = 5.18, p =.030 (Table 14). Moreover, effects of looking rate on amygdala volume varied as a function of TBV. The looking rate x TBV interaction was significant, F (1, 31) = 17.54, $p < .001$. Post hoc analyses were performed by fixing TBV to both 1 sd above and 1 sd below the group mean for TBV, and analyzing the adjusted relationships between looking rate and amygdala volume. Results indicated that the relationship between looking rate and amygdala volume was negative for children with small (i.e., relative to the autism group) TBV, t (31) = -2.23, p =.033, and positive for children with larger (i.e., relative to the autism group) TBV, t (31) = 3.94, $p < .001$ (Table 15). These findings suggest that children with increased amygdala volumes within a relatively (compared to the rest of the autism group) small TBV look less at others than children with smaller amygdala within a small brain or children with a large amygdala within a large brain (Figure 1).

Social initiation rate and amygdala volume

Findings indicated that as hypothesized, the relationship between social initiation rate and amygdala volume across time points was negative and significant, t (31) = -2.34, p = .026 (Table 16). Additionally, the interaction of social initiation rate and age was significant, t (31) = 3.97, p < .001. Post hoc analyses indicated that the relationship between social initiation rate and amygdala volume was negative and significant at time 1, t (31) = -2.92, p = .007, but was not significant at time 2, t (31) = 1.62, p = .116 (Table 17). Analysis of the relationship between amygdala growth and change in social initiation rate over time was significant and positive, t (31) = 3.97, p < .001, indicating that as amygdala volumes grew over time, rates of social initiation increased. Results indicating that increased amygdala volumes are associated with decreased rates of social initiation at time 1 are consistent with the hypothesis that amygdala enlargement is related to decreased social behavior. However, analysis of the time 2 relationship (i.e., not significant) and the developmental relationship suggest that the inverse pattern observed at time 1 changes over the course of development, and that developmental trends parallel those observed for looking rate and amygdala volume.

Joint attention rate and amygdala volume

Based on previous findings that children with autism demonstrate decreased rates of protodeclarative, but not protoimperative, joint attention rates, the relationships between the two forms of joint attention and amygdala volume were analyzed separately. Results suggested that the overall main effect of protodeclarative joint attention rate on amygdala volume was not significant, t (31) = 1.17, p = n.s (Table 18). Additionally, the relationship between protodeclarative joint attention rate and amygdala volume was not significant at time 1, t (31) = .26, p = n.s. However, this relationship was significant and positive at time 2, t (31) = 5.74, p < .001, indicating that at time 2, larger amygdala volumes were related to

increased rates of protodeclarative joint attention (Table 19). These findings are in contrast to our hypothesis that increased amygdala volumes would be associated with fewer social behaviors but, again, consistent with results for both looking rate and social initiation.

The interaction between protodeclarative joint attention and IQ also was significant, t (31) = 10.38, p = .003 (Table 20). Post hoc analysis of these results indicated that the relationship between protodeclarative joint attention rates and amygdala volume was negative and significant (i.e., increased amygdala volumes were associated with decreased joint attention) for children with higher IQ's, t (31) = -3.22, p = .003, and positive and significant (i.e., increased amygdala volumes were associated with increased joint attention) for children with lower IQ's, t (31) = 3.22, p = .003.

Results suggested that the relationship between protoimperative joint attention and amygdala volume was not significant, t (31) = .46, p = n.s. No significant interaction effects were observed.

Remaining social items and amygdala volume

Each of the remaining social items (i.e., joint attention response, gesture rate, time spent engaged with others, responding to name, imitation success) were examined in relation to amygdala volume. No significant effects were found for any of these items.

Emotion rate and amygdala volumes

Next, the relationship between amygdala volume and emotion rate was analyzed. It was hypothesized that in children with autism, amygdala volumes would be inversely related to overall emotion. Findings indicated that the main effect of emotion rate on amygdala volume was not significant, F (1, 32) = .01, p = .924. However, a significant emotion x age interaction was observed, F (1, 32) = 10.06, p = .003 (Table 21). Analysis of this interaction

indicated that the relationship between emotional behavior and amygdala volume was not significant at time 1, t (32) = 1.11, p = n.s., but was significant and negative at time 2, t (32) = -2.92, p = .006 (Table 22). The relationship between emotion change over time and amygdala growth also was negative and significant, t (32) = -3.17, p = .003, suggesting that as amygdala volumes grew over time, the rates of emotion expressed decreased.

Emotion change over time by amygdala volume. Because emotion rate was associated with amygdala volume at time 2, we next examined whether amygdala volume at time 1 predicted change in emotion from time 1 to time 2. A mixed effects repeated measures model was calculated with time 1 amygdala volume as the predictor, age, IQ, and TBV as covariates, and all 2-way interactions included. Emotion change between time 1 and time 2 was entered as the outcome.

Results indicated that amygdala volume at time 1 was a significant predictor of emotion change, F (1, 20) = 6.38, p = .020. Analyses revealed that the slope of emotion change for children with larger amygdala volumes at time 1 was negative and significant, t (20) = -2.65, p = .015, and the slope of emotion change for children with smaller amygdala volumes was positive and approached significance, t (20) = 2.00, p = .059 (see Figure 2). These findings suggest that amygdala volume at 18-35 months of age predicts the rate and direction of emotion change over time.

Positive emotion rate and amygdala volume. The relationship between emotional behavior and amygdala volume was further explored by separating positive and negative emotion rates. It was predicted that amygdala volume would be inversely related to positive emotion and directly associated with negative emotion. Findings indicated that rates of positive emotion were significantly negatively associated with amygdala volumes, t (32) = -

2.41, p = .022 (Table 23). The relationship between positive emotion and amygdala volume at time 1 was negative and approached significance, t (31) = -1.95, p = .060, and at time 2, was negative and significant, t (32) = -2.92, p = .007 (Table 24). The relationship between changes in positive emotion over time and amygdala growth also was negative and approached significance, t (32) = -1.90, p = .066. These findings indicate that as amygdala volumes increase over time in children with autism, children actually show reduced rates of positive emotional displays (Figure 3). No other significant interactions were observed.

Negative emotion and amygdala volumes. Findings indicated that rates of negative emotion were positively, but not significantly, associated with amygdala volumes, F (1,23) = 2.08, p = n.s. (Table 25). None of the interaction terms that were tested were significant.

Amygdala volume and non-social attention

Finally, the relationship between amygdala volume and non-social attention was analyzed. It was hypothesized that in children with autism, amygdala volumes would not be related to non-social attention. Findings supported this hypothesis; no significant relationship between amygdala volume and non-social attention was observed for either time point.

Discussion

Aim 1. Development of ADOS-SEP

The primary purpose of this project was to develop and examine a novel observational coding system focused on early social and emotional development in children with autism participating in the Autism Diagnostic Observation Schedule (ADOS). Although most studies of autism incorporate the ADOS as one piece of the gold-standard for diagnosing autism, the authors of the measure warn against analyzing item and domain scores as continuous data. ADOS ratings are qualitative in nature and are not suitable for quantifying the behavioral variability of individuals with autism. The ADOS-SEP computes the frequency of behaviors and the duration of behavioral states, and thus provides continuously measured items. Good reliability was established for each item, supporting the replicability of the coding system. Scores for each of the target items were normally distributed, and scores from the ADOS-SEP appeared to be more sensitive to differences within the autism group than scores from the ADOS. Additionally, ADOS-SEP ratings indicated that children with autism showed fewer social attention and positive emotion behaviors than typically developing children, suggesting that this coding system distinguished variation in social and emotional behaviors in a way that is informative for studies of autistic development.

Although multiple behavioral items were measured with the ADOS-SEP, social items were aggregated to reduce the number of analyses. Analysis of the internal consistency of social items indicated that eight items held together to form a social attention component.

These items were: 1) looking at faces, 2) joint attention responding, 3) joint attention initiations, 4) social interaction initiations, 5) imitation responses, 6) gestures, 7) responding to name being called, and 8) proportion of time spent engaged with others. The aggregate social attention score was not significantly correlated with either emotion (positive or negative) or non-social attention items. These findings support the validity of a social attention component derived from ADOS-SEP items.

To assess the convergent validity of the ADOS-SEP, autistic children's scores were compared to their scores on the ADOS. Although it is argued that the ADOS is less sensitive to variability in social and emotional behavior than the proposed coding system, it was hypothesized that major variations in children's social and emotional behavior are distinguished in a valid way, and thus, significant relationships between ADOS-SEP and ADOS scores would support the use of the novel measure. Findings indicated that children's social attention scores on the ADOS-SEP were related to their social algorithm and play total scores on the original ADOS, suggesting that children who were coded with greater social performance on the ADOS-SEP also were rated less socially impaired on the ADOS. Children's ADOS-SEP positive emotion scores also were related to their ADOS social scores. In addition, children's ADOS-SEP non-social attention score, which was based on their duration of engagement with individual toys, was significantly related to their ADOS play total score. These findings support the convergent validity of the ADOS-SEP because item scores were related to corresponding items from a previously validated measure, the ADOS.

Furthermore, it was hypothesized that ADOS-SEP items only would be related to socially relevant ADOS items. As expected, relationships between social attention, emotion,

and non-social attention items from the ADOS-SEP and the repetitive behavior subdomain of the ADOS were not significant, suggesting that the significant relationships between ADOS-SEP items and ADOS subdomains were specific and consistent with a priori hypotheses. These findings support the divergent validity of the ADOS-SEP.

Aim 2. Examination of the development of behavioral deficits in the autism group

In order to examine whether the ADOS-SEP is sensitive to social and emotional behavioral deficits in children with autism, ADOS-SEP scores for the autism group were compared to ADOS-SEP scores for cross-sectional, age-matched TD children. The autism group showed significantly fewer social and emotional behaviors at both time 1 and time 2. Specifically, at time 1, children with autism exhibited significantly reduced overall social attention and decreases in the following behaviors: 1) responding to name, 2) imitation, 3) social looking, 4) joint attention responding, 5) joint attention initiating, 6) gesturing, 7) positive emotion, 8) emotion sharing 9) initiating social interaction, 10) pretend play, and 11) engaging with others. Children with autism also spent more time engaged alone and disengaged during ADOS sessions. These differences in social and emotion behavior between children with autism and typically developing children were, for the most part, evident at time 2 as well. However, the overall frequency with which children initiated interactions no longer was significant. This finding contrasts numerous previous studies noting decreased rates of social motivation in preschoolers with autism (e.g., Brookman, Boetcher, & Klein, 2003; Koegel et al., 2001; Koegel, Koegel, & McNerny, 2001). The reason for this inconsistency likely involves the structure of the ADOS sessions, which facilitates interaction (contributing to increased initiations) but does not press children to re-initiate interaction once engaged with the examiner (tempering rates of initiation in children

who maintain social engagement). This structure may have differentially impacted TD children who, as a group, spent more time engaged with the examiner than the autism group. Still, the majority of data from the ADOS-SEP indicates that children with autism showed reduced social and positive emotion behaviors relative to age-matched TD children.

The cross-sectional nature of the behavioral control group data limited the potential for developmental analyses. However, difference scores, reflecting the degree of disparity between autistic children's performance and the TD group mean, were computed and followed over time. Few of the difference scores changed over time, suggesting that, while children with autism showed behavioral deficits relative to TD children at both time 1 and time 2, any changes over time in these behaviors paralleled TD age-related changes. Three exceptions were noted, however. Rate of looking at others and positive emotion were more severely impaired in the autism group at time 2 than they were at time 1. Examination of the mean performance of the autism and control groups indicated that TD children exhibited more of these two behaviors at older than at younger ages. In contrast, children with autism showed negligible changes in looking at others and positive emotion over time. Therefore, the discrepancies between autistic and TD children's looking and positive emotion behaviors appear to be getting larger over development.

In contrast to these findings, protoimperative joint attention initiations were decreased in the autism group relative to TD children at time 1, but not at time 2. These results indicate that, over time, the protoimperative joint attention behavior of children with autism more closely approached the protoimperative joint attention behavior of TD children. At first, this finding seems counterintuitive, suggesting that the autism group increases in their social behavior at an accelerated rate relative to the TD group. However, two factors may have

affected these results. First, the control group showed decreases (though not to a significant level) over time in protoimperative joint attention. Second, although the autism group showed increases in this behavior, these increases may not reflect improved social behavior, per se. Previous research (Mundy et al., 1995) has distinguished protoimperative joint attention from protodeclarative joint attention by noting that protoimperative bids are aimed at gaining tangible information and are motivated by children's need for a particular action to be performed, while protodeclarative bids are aimed at sharing attention or emotion and are socially motivated. Therefore, the finding that children with autism showed increases in protoimperative joint attention over time likely reflects improvements in their ability to seek assistance from their environment and does not reflect increases in their social interest or ability. It should be noted that protodeclarative joint attention deficiencies in children with autism did not improve over time, suggesting that the social component of joint attention remained impaired in autism.

Findings that the autism group showed reductions in ADOS SEP items relative to the control group are not surprising given that the coding system focused on behaviors previously identified as primary to autism (APA, 1994). However, few studies have examined social and emotional behavior in children with autism as young as those included in the present investigation. Additionally, the studies that have examined behavior in young children with autism generally have employed retrospective parent reports, which are characterized by recall biases, especially when parents are asked to recall details about their child's development after multiple years have transpired (Davidovitch et al., 2000; Kurita 1985; Volkmar & Cohen, 1989; Volkmar, Stier, & Cohen, 1985). Therefore, while the behavioral findings of the present investigation do not represent a significant departure from

previous research, several aspects of the study design offer important contributions - a seldom studied age group of children with autism was included, a more valid instrument to measure behaviors associated with autism was employed, and behavior was examined at multiple time points.

While results from the present investigation suggest that children with autism do improve in select social behaviors (although they maintain reduced rates of social and emotion skills compared to typically developing children), the majority of social and emotional behaviors did not show significant improvement. It should be noted that TD children also did not show significant improvement in all social or emotional skills. Still, the autism group maintained deficits in these areas, indicating that these core skills are not only delayed in their development, but consistently impaired. Children in the present investigation were diagnosed earlier (~18-35 months) than is typical for children with autism, suggesting that their symptoms may be more salient early in development because they are severely impaired Indeed, the majority of children in the present investigation had IQ's in the mentally retarded range (49/53). Research suggests that low-functioning children with autism (i.e., children who have IQ's in the mentally retarded range) show less improvement in social behaviors over time than higher functioning children with autism (McGovern & Sigman, 2005). The implication for the present investigation is that a higher -functioning sample of children may have shown greater increases in social performance over time and potentially exhibited comparable rates of some social and emotion behaviors as TD children. Also, early social attention skills, such as those examined in the present study, have been shown to be more resistant to intervention than more complex, social skills (e.g., greeting others, waving hello) (Hwang & Hughes, 2000). Therefore, it is possible that the children

with autism in the present study did improve over the studied time period in social skills that were not examined (e.g., introducing oneself, waving hello). However, the present results indicate that many core social and emotional behaviors are not improving significantly over time in autism.

Aim 3. Analysis of the relationship between amygdala volume and social and emotional behavior

Amygdala volume and social attention

Although results indicated that amygdala volume and social attention were not related in the autism group, these findings were not completely surprising. The social attention component that was examined was comprised of multiple social items. These constituent items are related to one another, but still measure somewhat unique behaviors. Therefore, the global social attention construct may be too broad to identify behaviors related to amygdala volume, and items that are not associated with amygdala volume may cancel out significant associations.

Results are consistent with a previous report investigating adolescents and adults with Asperger's Syndrome (AS) suggesting that, although amygdala volumes are related to social functioning in TD adults, the relationship between amygdala volume and social functioning in AS is not significant (Dziobek et al., 2006). In contrast, Munson et al. (in press) observed a significant association between amygdala overgrowth and social abnormalities in children with autism. However, Munson and coworkers measured social behavior with ADI ratings and were limited in their ability to probe specific behaviors associated with amygdala volume. Also, the authors included emotional behavior scores in their social ratings. In

order to examine possible associations between ADOS-SEP items and amygdala volume, analyses were repeated for each social and emotional item.

Relationship between amygdala volume and looking at faces. The overall relationship between amygdala volume and rate of looking at faces was significant. Interestingly, this relationship was positive and significant over both time points, though interactions with age were not evident, nor were significant relationships at either time point individually. Still, these results suggest thatincreased rates of looking at others are associated with larger amygdala volumes in children with autism.

Also noteworthy is the finding that the relationship between looking rate and amygdala volume varied as a function of children's overall brain volume. The relationship between amygdala volume and looking rate was negative for children with smaller TBV's (i.e., increased amygdala volumes were associated with decreased rates of looking at others), and positive for children with larger TBVs (i.e., increased amygdala volumes were associated with increased rates of looking at others). These results suggest that raw volumetric measurements of the amygdala may be less informative for understanding children's attention to faces than the proportion of amygdala volume to TBV.

The importance of examining amygdala : TBV proportions for understanding brain-behavior relationships in autism is particularly compelling given the pervasive nature of the disorder, which indicates that multiple neural regions are affected, and findings that TBV is enlarged in autism, particularly early in development (Cody Hazlett et al., 2005; Courchesne et al., 2003; Lainhart et al., 2001). Recent reports have noted enlargement throughout cortical regions (Cody Hazlett et al., 2005), suggesting that circumscribed lesion-function hypotheses may be less applicable for studying autism than exploring multiple regions and

their connective pathways. Consistent with this concept, Bachevallier and Loveland (2006) recently suggested that it is the connections between the amygdala and orbitofrontal cortex that underlie the social symptoms of autism, rather than dysfunction isolated to either region. It could be that the negative association of looking at faces and amygdala enlargement within the context of a smaller brain reflects one or more dysfunctional neural system(s), rather than an isolated disruption of the amygdala.

Still, the hypothesis that amygala : TBV proportions are more informative than raw amygdala volumes for understanding neuroanatomical associations with attention to faces is preliminary. In fact, looking at faces was the only behavior for which a significant amygdala x TBV interaction was detected in the present study. Previous structural MRI findings on the amygdala (Dziobek et al., 2005) and other limbic structures (MaGuire et al., 2003) have indicated that, in neurotypical individuals, increased raw volume of subcortical structures *is* associated with superior functioning. This pattern also exists in the present data for the autism group (i.e., increased amygdala volume is associated with higher rates of looking at faces across time points when the looking x TBV interaction is not considered). Clearly, future research exploring the value of using raw amygdala volumes versus amygdala : TBV proportions to understand behavioral data is needed.

Relationship between amygdala volume and social initiation. Rates of social initiation also were associated with raw amygdala volumes. The relationship between social initiation rate and amygdala volume at time 1 was significant and negative, reflecting that increased amygdala volumes predicted lower rates of social initiations. Interestingly, increases in amygdala volume over time were related to increases in social initiations over time. In other words, although amygdala enlargement was related to fewer social initiations

at time 1, the growth of this structure was associated with increased social interest or ability. These findings suggest that the functional significance of amygdala volume may change in autism over the period of development targeted in the present study. Amygdala overgrowth may disrupt early social development though, over time, amygdala growth may facilitate (or at least correspond with) increased social initiation behavior. Additional research investigating amygdala volumes over time and their functional significance in the behaviors of children with autism and TD children is necessary.

It is possible that increased social opportunities during development could contribute to amygdala growth over time. Similar environmental effects on brain volume have been reported for the hippocampus, a structure with intricate connections to the amygdala (Maguire et al., 2003). Therefore, children in the present investigation could have improved their social initiation rate over time (through environmental experiences or concomitant behavioral therapy), which, in turn, could have contributed to amygdala growth (e.g., increased connectivity). Understanding the effects of increased social opportunities on amygala volumes will be important for future treatment outcome studies aiming to measure treatment efficacy through biological markers, such as amygdala volume.

Relationship between amygdala volume and joint attention. The relationship between amygdala volume and rate of initiating protodeclarative joint attention was significant and positive (i.e., increased amygdala volume was associated with increased rate of protodeclarative joint attention initiations) at time 2 but not at time 1. Interestingly, the relationship between amygdala volume and rate of initiating *protoimperative* joint attention was not significant, suggesting that amygdala volume is most closely tied to the social component of joint attention initiations.

The present findings support an association between amygdala volume and joint attention that is similar to findings on looking at faces and social initiation, and what would be expected in a TD population (Dziobek et al., in press). As previously discussed, enlarged amygdala volumes are associated with increases in social behavior (i.e., looking at faces and initiations) in the present sample. Integrating findings on protodeclarative joint attention yields converging evidence from three ADOS-SEP items suggesting that amygdala volume enlargement in autism is closely linked to several core aspects of social behavior and is predictive of increases in these behaviors. Separate circuits may underlie the association between amygdala volume and each identified social behavior, thereby producing moderate alterations in the timing of these associations. But, results for ADOS-SEP social items do indicate that across time, amygdala growth is linked to developments in attention to faces, social initiation, and joint attention.

Looking at faces, social initiation, and joint attention initiation each are early emerging social tendencies typically reflecting a desire to interact with others. In contrast to the other social attention behaviors measured in the present study, these skills are initiative behaviors that have the presumed goal of obtaining social contact. In contrast, using gestures while interacting, responding to a name being called, maintaining engagement with others, imitating behavior, and responding to joint attention each are socially motivated, but also occur in the context of ongoing interactions, potentially initiated by others. Looking at faces, social initiation, and joint attention initiation each are compelled by the child seeking social information and, potentially, recognizing the reinforcing quality inherent in social reciprocity. It may be that the amygdala is involved in a unifying component of these social

behaviors, such as social motivation. Clearly, future research examining the mechanism linking the amygdala to these behaviors in autism is warranted.

The effect of IQ on the relationship between amygdala volume and protodeclarative joint attention initiation rate is difficult to understand. For children with higher IQ's, the relationship between amygdala volume and protodeclarative joint attention is negative. In contrast, for children with lower IQ's, the relationship between amygdala volume and protodeclarative joint attention is positive. Given the difficulties with assessing IQ in children with autism as young as the children in the present study, it is possible that these results are an artifact of the IQ measure. Although the Mullen is a well-studied and validated measure of IQ, its application to young, severely impaired children is problematic. Several children with autism in the present study failed to reach a basal level of scoring. Also, the majority of children were clustered in the mentally retarded range, with only 2 children scoring in the average range. Given that the majority of children cluster in the lower end of the IQ scale, it may not be meaningful to divide them into higher and lower IQ groups. Instead, this separation is not clinically meaningful.

Amygdala volume and emotion

Consistent with hypotheses, the relationship between amygdala volume and display of emotion was significant and negative at time 2. However, the relationship between amygdala volume and emotion was not significant at time 1. Dissecting emotion into positive (smiling) and negative (fear, crying) overtures helped to clarify this pattern. Findings revealed that amygdala volume and positive emotion were inversely related at both time 1 and time 2. Moreover, amygdala growth was related to decreased positive emotion over time. These results match closely with those reported by Munson et al. (in press) who

suggested that amygdala enlargement is predictive of social and communication impairments in 3-4 year old children with autism. The social and communicative functioning studied by Munson and colleagues included emotion items, similar to those defined by the ADOS-SEP.

Decreased rates of positive emotion likely have profound behavioral effects for children with autism. Studies of TD children have noted that the level of positive emotion displayed during interaction predicts the quality of peer relationships later in development (Lindsey & Colwell, 2003). A failure to show positive emotion early in development could limit the reward value assigned to social interactions for both the affected child and those with whom they interact. Dawson, Hill, Spencer, and Galpert (1990) indicated that 3-6 year-old children with autismwere less likely than TD children to smile in response to their mothers' smiles. In turn, mothers of children with autism in the Dawson study were less likely to smile at their children and showed fewer smiles overall. Several researchers have hypothesized that less positive emotion shown by children with autism reflects a failure to recognize that the social environment provides intrinsic rewards. As a result, affected children are not motivated to seek interaction and suffer from early social deprivation and isolation (Dawson et al., 1989, 1998; Mundy et al., 1995, 2001; Schultz, 2005). Studies have indicated that the amygdala is uniquely involved in assigning this reward value to social stimuli (for a review, see Bachevalier & Loveland, 2006). Therefore, if the amygdala is disrupted in development (as suggested by the present data) the reinforcement value inherent in early social interaction may not be recognized by children with autism, precluding a myriad social learning opportunities. The present finding that amygdala enlargement is associated with decreased positive emotions during social interactions in children with

autism suggests that the amygdala may be central to the early social difficulties characteristic of this disorder.

The fact that these findings are somewhat inconsistent with those for social items suggests that separate amygdala circuits may be impacted differentially in autism. The amygdala is comprised of at least 13 separate nuclei, each characterized by unique connections to cortical and subcortical regions. These neural networks mediate highly specific behavioral and cognitive functions (Freese & Amaral, 2005; Sheehan et al., 2001). Circuitry underlying social and emotional behaviors each may involve the amygdala, but also include separate associated neural regions. It is possible that the amygdala enlargement evident within young children with autism reflects pathology in some, but not all nuclei, and some, but not all amygdala-cortical connections.

Summary of amygdala-behavior findings

Overall, findings from the present study regarding amygdala-behavior relationships in autism were somewhat mixed, though several consistent themes did emerge. First, analyses of the relationships between amygdala volume and social attention items revealed significant associations for looking at faces, social initiation, and joint attention. These three social behaviors each reflect a volitional component, or motivation to seek social information. In contrast, social items not necessarily characterized by an initiative component were not associated with amygdala volume. These results suggest that the amygala is involved in the motivation and/or ability to seek out social information among young children with autism. Moreover, analysis of the relationship between amygdala volume and these three social items indicated that increased amygdala volume is associated with behavioral increases. Interestingly, the relationship between attention to faces and amygdala volume was

moderated by overall brain volume. These results suggest that, for understanding the relationship between amygdala volume and attention to faces, it may be best to consider the amygdala : TBV ratio, as opposed to the raw amygdala volume. Future research addressing this issue is warranted.

The second theme that emerged from amygdala-behavior analyses was that amygdala enlargement was associated with decreased positive emotion. These findings suggest that the amygdala overgrowth early in development may be a central component of the emotional deficits characterizing children with autism. Amygdala enlargement associated with decreased positive emotion rates could reflect autistic children's failure to assign reward value to social stimuli, or difficulties in communicating emotional responses.

Although the ADOS-SEP offers a significant improvement in behavioral measurement for young children with autism, and the present findings add to our understanding of the behavioral manifestations associated with amygdala volumes of young children with autism, analyses of the relationship between ADOS-SEP items and amygdala volume require further investigation. Primarily, future studies are needed to explore the significance of amygdala : TBV proportions and to illuminate the relationship between amygdala volume and social and emotional behavior in TD children, and in children with autism throughout development.

Limitations

This study had several limitations, the most salient of which was the absence of longitudinal normative brain and behavioral data for typically developing children. This limitation was mitigated by collecting cross-sectional data on TD children at both time points. However, longitudinal studies are more sensitive to intra-individual variation and

heterogeneity within groups (Giedd et al., 1996). Little is known about brain-behavior relationships in non-autistic preschool-aged children. This limits our interpretations of the brain-behavior data on the autistic group. Interpretations of the developmental relationship between amygdala volumes and behavior can only be compared with results from non-human primate lesion studies, adult lesion studies, and functional neuroimaging studies of adults.

A second limitation of this study was the small sample sizes of both the autistic and control groups. A number of children could not be included in final analyses for a variety of reasons, including poor video quality, failure to remain on camera for an adequate duration of the ADOS, poor MRI quality, failure to participate in time 2 data collection, and failure of examiners to administer certain items of the ADOS. Only twenty-four children with autism could be examined for longitudinal brain-behavior analyses despite 53 children having been studied at time 1. While mixed effects modeling, which integrates cross-sectional and longitudinal data, helped to minimize the reduction in power associated with subject drop-off, future longitudinal brain-behavior studies will be most informative if they include a large number of children to help account for the behavioral and neurobiological heterogeneity inherent in this disorder.

A final limitation is that only one behavioral assessment was employed, providing just a discrete sample of children's behavior. Because social and emotion behaviors are variable within individuals over time and across settings, it is possible that children may display different rates of behavior at a separate time of day or on a different day. The length of the behavioral assessment in the present investigation was approximately 30 minutes, which we expected allowed adequate time for children to display behavior that likely was representative of their average behavior. Children whose behavior was clearly different

during the behavioral assessment (e.g., cried the entire time or parent noted unusual behavior) were excluded from analyses.

Conclusions and future directions

The present study offers a significant contribution to the study of autism by examining young children with autism and by examining them over time. The present study suggests that social and emotion developments in young children with autism may be quantified using the ADOS-SEP. While the present study examines some of the youngest children with autism included in longitudinal research to date, it may be helpful to study children even younger than 18 months. Several groups of researchers have reported data from infants later diagnosed with autism (Landa & Garret-Mayer, in press; Zwaigenbaum et al., 2005). Applying the ADOS-SEP to affected children from infancy throughout childhood will contribute further to our understanding about the onset and development of brain and behavior disruptions characteristic of autism.

While the present study examined cross-sectional samples of age-matched TD children at each time point, no longitudinal data on non-autistic children was obtained. Establishing developmental profiles of non-autistic children will facilitate comparisons with psychiatric and developmentally disabled populations. Examining social developments in DD children without autism also will be important for disentangling the effects of mental retardation on the deficits unique to autism.

Future studies of autism should examine the effects of interventions on social and emotion behavior developments during the preschool years. While a variety of intervention methods for children with autism have been investigated, few studies have applied these methods to such a young age group. Additionally, the studies that have examined young

children with autism often have been limited by their behavioral measures, relying on parent reports or measures that are not sensitive to subtle and clinically meaningful differences in core autistic features. Applying the ADOS-SEP to treatment studies will help test the efficacy of novel and existing treatment methods.

Results from the present study also add to our understanding of brain-behavior associations in autism. Findings indicate that amygdala volume is related to select social behaviors (i.e., looking at others, social initiations, joint attention) and to positive emotion behavior. Research suggests that pharmacological interventions may be useful for correcting amygdala dysfunction and, as a result, concomitant social and emotional behavioral impairments. For example, oxytocin, a nonapeptide, has been shown to act on the medial (Ferguson et al., 2001) and central (Huber et al., 2005) nuclei of the amygdala during social cognitive and emotion processes. Kirsch et al. (2005) reported that administration of oxytocin to adults depressed amygdala activation in response to threatening faces and non-social scenes. Though it is highly speculative at this point, these results suggest that oxytocin levels have strong effects on amygdala responses to emotional/social stimuli. It is possible that amygdala structural abnormalities serve as an index of behavioral dysfunction in autism. Consequently, altering oxytocin levels in autistic children with abnormal amygala volumes early in development may impact social and emotional deficits.

Knowledge of brain-behavior associations in autism also may help to refine measures of improvement following intervention. For example, examining amygdala volumes after treating social and/or emotion deficits in autism may provide a more objective and sensitive measure of treatment effects.

Future studies examining the relationship between amygdala volume and distinct social and emotion behaviors may contribute to our knowledge regarding whether the amygdala as a whole or specific amygdala nuclei are affected in autism. Examining distinct nuclei and their associated neural systems may help identify initial disruptions that lead to the cascade of effects in brain circuitry evident in children with autism by the preschool years. The timing of this disruption may precede or occur as a result of declines in social and emotional behaviors. Longitudinal designs are necessary to clarify these relationships.

Future studies should examine amygdala volume in relation to an array of emotions. The present study included "positive" and "negative" emotions, which consisted solely of happiness and sadness. Future studies aimed at eliciting emotions such as fear, anger, and surprise are needed to understand the nature of the relationship between the amygdala and emotion.

Exploring neuroanatomical abnormalities in autism, such as those presented in this study, may help to identify intermediate phenotypes of autistic dysfunction that could assist researchers in finding linkages between the behavioral characteristics of autism and candidate genes. Recent studies identifying genetic polymorphisms predictive of amygdala function could converge with studies such as the present one to identify gene-brain-behavior relationships in autism. The present study is one of the first to find evidence for a connection between neuroanatomical and behavioral features in autism. Similar structural MRI studies following brain changes and their associated behavioral developments in children with autism over time may provide new insights into the pathogenesis of this disorder.

Appendix A
Social and Emotional Perspective Guidelines

I. Social attention:

Looking: Any instance in which the child is observed looking directly at another person's face. It must be clear that the child is looking at the person's face and not another part of the body and not at a proximal object. Also, the child must fixate on the person's face, rather than glancing past him/her. Events in which the child looks at another person during a social referencing episode, responding to his/her name being called, responding to a joint attention attempt, or using a communicative gesture, should not be coded here. Children should be scored as looking at the examiner, a parent, or other individual (e.g., sibling). If the child looks between multiple individuals, then **Looking** should be coded for each individual he/she makes eye contact with.

Joint Attention Responding: Events in which the examiner, parent, or other individual attempt to direct the child's attention to an object via establishing attention and shifting his or her gaze or pointing should be scored as **Joint Attention Responding**. These interactions require a distal point, so pointing to a picture on a page that the child is reading should NOT be scored as a joint attention opportunity. The modality to which the child responds should be identified. During the Joint Attention press that is part of the ADOS, the examiner will first attempt to have the child respond to a shift in eye gaze two times. If the child does not respond, the examiner should use up to four opportunities in which he/she points to get the child to jointly attend. The child should be coded as responding to joint attention if s/he follows the bid and directs attention to the referenced target. ONLY SCORE AS AN OPPORTUNITY IF THE CHILD ATTENDS TO THE EXAMINER WHEN PROMPTED. IF THE CHILD DOES NOT LOOK TO THE EXAMINER'S EYES DURING THE FIRST

JOINT ATTENTION OPPORTUNITY, CODE AS "**NOT RESPONDING TO NAME"**, BUT DO NOT CODE FOR JOINT ATTENTION.

Joint Attention Initiates: Events in which the child initiates a joint attention should be coded separately from **Joint Attention Responding**. In order for this behavior to be scored, the child must seek to get another individual to attend to an object or person of interest either by a shift in eye gaze or a distal point. Responses should be identified either as "Protoimperative" or "Protodeclarative". "Protoimperative" responses include those in which the primary goal of the interaction is to share attention or enjoyment. "Protodeclarative" episodes are those in which the child's intent is to obtain an object or find out information about an object (e.g., pointing and asking, "What's that?"). Finally, whether or not the child integrates eye contact into the episode should be scored. The timing of eye contact should be coded in relation to the timing of the gesturing.

Initiates Interaction: Any action in which the child actively goes to the examiner, a parent, or other individual for help, to communicate or interact, to hug, or to hold hands should be scored here. Events in which the child approaches another individual because the individual has displayed a new toy should NOT be scored here. If the child spontaneously verbalizes to another individual subsequent or during a time when they are playing alone should be scored here. If the child is in distress and seeks another individual, this should be scored. If the child uses the person as a tool to acquire something, then the interaction should be coded as "**Protoimperative**". If the primary purpose of the child's seeking the other individual is to interact, play, or share in something, then the event should be coded "**Protodeclarative**". If the child walks on part of another individual or leans on them as if they were an object (i.e., without holding hand or first referencing them), then this item should not be scored.

Responds to name: Events in which the child's name is stated and at least a 1 second pause in which the child's response is observed by the person who called his/her name should be indicated here. Therefore, if a parent or the examiner calls the child's name repeatedly at a rapid rate, or the child's name is followed by or paired with an additional command or gesture, no opportunity should be indicated. During the ADOS, a Respond to Name activity is included. During this activity, the child's name will be called 4 times by the examiner prior to the parent being asked to call the child's name 2 times. If the child still has not responded, the parent will be asked to attempt to gain the child's attention with other means without touching him/her. After this, the parent will be asked to get the child's attention in any manner possible. If at any point the child responds by looking, then the activity is concluded. Therefore, if additional prompts are given, then it is understood that the child did not respond. However, if the child clearly responds by looking at the person who has called his/her name, but this response is missed by the examiner, then the scorer should code the response as occurring. Each "No Response" should be scored and the medium to which the child does respond also should be indicated.

Imitation: This item should only be scored during the *Imitation* activity of the ADOS in which the examiner attempts to have the child imitate a frog hopping, a plane flying, drinking from a cup, or smelling a flower. In addition, when the replacement object is used by the examiner, then pretend play events should be scored. The child must show a clear attempt to imitate or approximate the identical actions of the examiner for this item to be scored "yes".

II. Emotionality

Emotion: Any event in which the child shows a clear and appropriate change in emotion should be scored. Pretend emotions, as in the course of playing, also may be scored if clearly indicated, and appropriate (i.e., not stereotypic or unusual in any way). Emotion should be coded as either **positive** or **negative** emotion. Positive emotion includes smiling, as indicated by both corners of the child's mouth turning upwards, that is present for $\geq$ 1 second. Other signs of positive affect (e.g., excitement, positive surprise) can also be coded under smiling. Negative emotion includes crying, which must be separated from "whining" or "vocalizing" behaviors by being a clear sign of distress, such as a protruding lower lip, tears, rubbing eyes, covering face for $\geq$ 2 seconds, or vocalizing in distress. Negative emotion also includes tantrumming, which may include crying but also must include clear signs of anger or frustration, such as stomping feet, hitting an object or person, throwing an object, or running away from the target item/person. Tantrumming must occur for $\geq$ 2 seconds to be scored. There must be a separation of $\geq$ 3 seconds in which the child presents with his or her baseline emotional state for multiple emotion events to be scored.

Emotion sharing: Any event in which the child exhibits appropriate emotion and also makes a clear attempt to share this emotion with another person should be coded here. Examples of ways a child may share an emotion include making eye contact with, communicating with (e.g., shouting "Yay"), sharing attention with, laughing with, or seeking out another person. The child must be clearly attempting to share an emotion with the other person, but does not have to initiate this sharing. Events in which the child becomes distressed and seeks comfort by running to another person are NOT considered sharing. The child has to be engaging to imitate or respond to someone else's emotion, or attempting to affect the mental state of the other person by communicating his/her own emotion. Events in which the child is exhibiting

an emotion as part of play and focuses on the examiner after being interrupted are scored as "**Looking**", and not as "**Emotion Sharing".**

III. Non-social attention

Free Play Toy X: Children should be coded at the moment at which they engage with a toy during free play. This could be indicated by the child reaching for, fixing his/her gaze on, or actively playing with the toy. The child should be coded as playing with the toy as long as he is touching the toy, watching another person play with the toy, or remaining in the same position as he was while playing with the toy. The child should not be coded as playing if he is engaging in stereotypic or unusual sensory behavior. The end of the free play period should be marked at the time in which the child plays with a toy that is part of another task (e.g., joint attention toy, bubbles, etc.). Future play with free play toys subsequent to a non-free play task should be coded as **Play**. In addition, free play toy x should be coded as with **other** or **self.** Play with **other** should be scored if the child is touching someone else, if someone else is touching the toy that the child is engaged in, if the child is facing another individual, if the child and another are orienting to the same toy jointly (e.g., joint attention probe), or if the child and another are actively engaging with the same toy. All other play periods should be coded as **self**. Play should also be scored as **functional** or **pretend**. Functional play is all appropriate play with a toy or other that is not **pretend**. **Pretend** play should be coded for periods in which the child uses a toy in a manner that involves pretense (e.g., feeding the baby, talking on the phone). The duration from when the child first clearly indicates that he/she is using pretense until the time that he/she clearly indicates a use of the toy other than pretense, or the time when he/she switches toys, should be coded as **pretend**.

IV. Play Behaviors

Pretend Play: Any event in which the child uses an item or acts out a scenario in an imaginary way. Examples include feeding the baby, blowing out candles, having dolls interact with one another. This code is limited to appropriate forms of pretence, thus excluding events in which the child may begin laughing to self or engages in odd, stereotyped behaviors. Also, events in which the child plays with toys that press for pretend play (e.g., dolls, planes) should only be scored if the child engages in pretend actions with them (e.g., flying).

****Play/Engaged with other: Any segment in which the target child is engaged with another individual, clearly observing the other individual or collaboratively participating in activity with the other individual, should be coded as engaged with other. Use the same scoring guidelines for **Free Play Toy X** to code this variable.

****Play/Engaged with self: Any segment in which the target child is engaged alone with a toy while on camera should be scored. Use the same scoring guidelines for **Free Play Toy X** to code this variable.

****Disengaged: Segments should be marked as "Disengaged" if the child is not playing or interacting with anyone else or is engaging in stereotypic or unusual sensory behavior while present on camera.

****Not observable: Any time that the child is not present on camera, then this score should be made. No behaviors should be scored when the child is not observable.

*******Duration Codes: For the following three "state" codes, a clear change in state must last for >3 sec to be scored. For example, if the child puts a toy down for less than this time and then begins playing again, no state change should be recorded.***

Appendix B

AMYGDALA TRACING METHOD:

- Note that you will trace the right amygdala on the left side of the screen and the left amygdala on the right side of the screen.
- With Analyze, the pixels on the line you traced are included in the volume measurement.
- The following methods are based on realigning the images along the long axis of the hippocampus.

Steps:

1. Trace the amygdala in the coronal plane.
2. Check tracing in the axial plane and exclude the putamen.
3. Check tracing in the saggital plane and define the rostral extent of the amygdala.

Coronal tracing method:

- Find the most caudal section of the amygdala as it appears dorsal to the inferior horn of the lateral ventricle and hippocampus, and lateral to the optic tract. Begin by tracing the amygdala from the dorso-lateral extent of the optic tract.
- In caudal sections, the putamen forms the lateral border of the amygdala. If this border is seen, extend a line from the dorsolateral extent of the optic tract directly lateral (horizontal) to the amygdala/putamen border. If this border is difficult to see, extend the horizontal line laterally to the white matter. You can further define and exclude the putamen in the horizontal / transverse view (*described later*).
- Continue tracing the amygdala by following either the putamen/amygdala border, along the white matter, or (if all else fails) a line directly ventral (vertical) until the lateral ventricle is reached.

- The ventral border of the amygdala is initially formed by the lateral ventricle, then more rostrally by the hippocampus. The ventro-medial portion of the amygdala extends just ventral to the optic tract. More ventral, this border is formed by the amygdala-hippocampal transition area (which is included as part of the hippocampus). If this border is ambiguous, a division may be defined by a line perpendicular to the optic tract. More dorsal, the border is formed by the medial surface of the brain. Therefore, continue tracing the ventral surface of the amygdala along the ventricle, then amygdala-hippocampal transition area, medial surface of the brain, to the starting point at the dorso-lateral extent of the optic tract.
- Continue tracing the amygdala in more rostral sections as described above. When the medial surface of the brain extends further lateral than the optic tract, use the dorso-lateral extent of the medial surface as the dorsal border of the amygdala. Draw a straight horizontal line laterally from this point to the white matter. At this point, the putamen is no longer present and the lateral border is formed by white matter. Follow the curve of the white matter to the lateral ventricle.
- Further rostral, the hippocampus forms the ventral border of the amygdala, divided by a thin section of white matter (alveus). At this level, the amygdala-hippocampal transition area will no longer be present. Instead, the ventral border of the amygdala will be fairly horizontal (from the lateral border to the medial surface of the brain at the semiannular sulcus).
- If the border between the amygdala and hippocampus is difficult to find, look for the dorsomedial point of the lateral ventricle – If the ventricle is curving in medially, it will point to the alveus.

- As the hippocampus recedes medially in more rostral sections, the entorhinal cortex begins to form the dorso-medial border of the amygdala. The most dorsomedial point of the amygdala is at the semiannular sulcus on the medial surface of the brain.
- Look for white matter to separate amygdala from entorhinal cortex medially. If this is difficult to see, then find the most medial point of the white matter (ventral to the amygdala) and draw a straight-line dorso-medially to the semiannular sulcus.
- As the hippocampus and lateral ventricle disappear along the ventral border of the amygdala, white matter primarily forms the dorsal, lateral, and ventral borders. The medial border is formed by the entorhinal cortex and the dorsomedial border of the amygdala will be formed by the medial surface of the brain. Be careful to exclude vessels, which appear bright white in the image.
- As you continue to trace the amygdala in rostral sections, the medial surface of the brain will extend further lateral (joining the lateral sulcus) to separate the temporal lobe from the rest of the brain. At this point, the dorsal border of the amygdala is defined by the surface of the brain.
- Continue tracing the amygdala until it is indistinguishable. You can be generous here since the rostral border of the amygdala will be trimmed in the saggital view.

Horizontal (transverse) view trimming:

- Begin at the most dorsal section of the amygdala and progress ventrally. The putamen may be found as an elongated tail extended caudally from the caudal portion of the amygdala. It may also appear a slightly darker gray. To exclude the putamen, follow the white matter along the lateral border of the amygdala as it extends caudally. Continue to draw a straight (medial-caudal diagonal) line, through the putamen, to the white matter

on the medial side of the putamen just lateral to the thalamus. Further ventral, this line may terminate at the medial surface of the brain.

- Before continuing, review the rest of the tracing through the transverse sections and delete any scattered points. Do not bother smoothing out lines, as they will then appear jagged in the coronal view.

Saggital view trimming:

- Begin by reviewing the amygdala on the most medial section you traced. To determine the rostral extent of the amygdala, follow the natural curvature of the ventral surface along the white matter as it extends rostrally. If possible, continue to follow the white matter to the surface of the brain. If not, then follow the natural curvature from the most rostral tip of the white matter to the medial surface.
- Also, check the amygdala-hippocampal border in the saggital view. The division between the amygdala and hippocampus appears as a diagonal line (approximately 45° dorso-caudal to ventro-rostral) from the dorso-caudal tip of the amygdala to the ventro-rostral tip of the hippocampus (often marked by a small portion of the lateral ventricle). This may be checked again after tracing the hippocampus.
- Review the rest of the your tracing through the saggital sections and delete any scattered points. Do not bother smoothing out lines, as they will then appear jagged in the coronal view.

Final check…review amygdala and hippocampal tracing in the coronal view before calculating volume measurements.

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Table 1. Sample data for autism and behavioral control (TD) groups

	Time 1		*Time 2*	
	Autism	TD	Autism	*TD*
Sample size (females)	53 (4)	9 (1)	37 (2)	*9 (1)*
Chronological age (yrs.)*	2.6 (.34)	2.6 (.43)	4.8 (.46)	*4.5 (.48)*
Mullen age equivalent*	15.1 (5.1)	23 (9.9)	27.7 (15.2)	*45 (18.2)*
Vineland Socialization Score*	*64.2 (7.1)*	*91.8 (13.3)*	*58.2 (10.1)*	*94.4 (31.8)*

* Values represent means and standard deviations

Table 2. Sample data for autism and MRI control groups

	Time 1			*Time 2*		
		Controls			*Controls*	
	Autism	TD	DD	Autism	TD	*DD*
Sample size (females)	53 (4)	14 (5)	11 (1)	37 (2)	4 (1)	*4 (0)*
Chronological age (yrs.)*	2.6 (.34)	2.4 (.4)	2.7 (.4)	4.8 (.46)	4.5 (.49)	*4.5 (.49)*
Mullen age equivalent *	15.1 (5.1)	23 (10)	15.3 (9)	27.7 (15.2)	45.7 (18.2)	*35.3 (16)*

* Values represent means and standard deviations

TD = typically developing children

DD= developmentally delayed children without autism

Table 3. Comparison of rates of social behavior by group on ADOS-SEP

	Time 1			Time 2		
	Autism	TD		Autism	TD	
	Mean (SE)	Mean (SE)	p	Mean (SE)	Mean (SE)	*p*
Responds to name†	.28 (.04)	.62 (.11)	**.003***	.26(.06)	.79(.08)	***.000****
Imitation success†	.33(.05)	.69(.10)	**.005***	.45(.08)	.83(.05)	***.001****
Emotion sharing††	.15(.03)	.38(.10)	**.012***	.17(.05)	.58(.06)	***.000****
Positive emotion††	.45(.07)	.78(.15)	**.051**	.46(.05)	1.01(.07)	***.001****
Negative emotion††	.08(.02)	.02(.05)	**.242**	.09(.03)	.00(.00)	***.177***
Looking††	1.72(.35)	2.36(.38)	**.017***	1.26(.15)	3.38(.28)	***.000****
Joint attention response†	.34(.06)	.96(.04)	**.000****	.53(.07)	1.00(.00)	***.000****
Joint attention†† initiations	.01(.00)	.10(.02)	**.003***	.02(.03)	.20(.13)	***.006****
Protodeclarative	.01(.02)	.06(.02)	**.013***	.01(.02)	.17(.13)	***.000****
Protoimperative	.00(.01)	.04(.02)	**.067**	.01(.02)	.00(.01)	***.999***
Initiates social†† interaction	.12(.02)	.24(.06)	**.026***	.29(.10)	.20(.04)	***.628***
Protodeclarative	.04(.06)	.17(.05)	**.046***	.16(.10)	.13(.04)	***.915***
Protoimperative	.08(.02)	.07(.02)	**.834**	.14(.03)	.07(.02)	***.048****
Gesture rate††	.13(.03)	.45(.09)	**.000****	.21(.06)	.80(.11)	***.000****

† Score is a proportion of correct responses per examiner press
†† Score reflects rate per minute of child initiated behavior
††† Score reflects proportion of observable time engaged in behavior

Table 4. Comparison of proportions of time spent in each behavioral state during the ADOS

	Time 1			Time 2		
	Autism	TD		Autism	TD	
	Mean (SE)	Mean (SE)	p	Mean (SE)	Mean (SE)	*p*
Engaged alone	.34 (.03)	.21 (.04)	**.045**	.31(.03)	.15(.02)	***.017***
Engaged w/other	.59(.03)	.77(.04)	**.000**	.52(.04)	.83(.02)	***.000***
Pretend play	.02(.01)	.16(.03)	**.002**	.05(.02)	.16(.02)	***.001***
Disengaged	.16(.03)	.02(.01)	**.000**	.18(.03)	.03(.01)	***.000***

Table 5. Correlation matrix of time 1 ADOS-SEP behavior variables

		Positive emotion	Negative emotion	Social attention	Toy attention
Positive emotion	r	1.000			
	p	.	-	-	-
	N	51			
Negative emotion	r	-.094	1.000		
	p	.524	-	-	-
	N	51	51		
Social attention	r	.198	-.197	1.000	
	p	.247	.247	.	-
	N	37	37	37	
Toy attention	r	.254	-.196	.285	1.000
	p	.085	.187	.168	.
	N	47	47	35	47

Table 6. Relationships between ADOS-SEP items and ADOS subdomains controlling for age and IQ

ADOS subdomains			Social Total		Play Total		Repetitive Behavior Total	
ADOS-SEP items		N	r	p	r	p	r	p
Social attention	Time 1	33	-.574	.03*	-.617	.05*	.296	.14
	Time 2	21	-.606	.02*	-.607	.02*	.40	.10
Positive emotion	Time 1	38	-.328	.04*	-.251	.12	.064	.70
	Time 2	26	-.484	.01*	.066	.77	..049	.81
Negative emotion	Time 1	38	.172	.30	.138	.40	.049	.77
	Time 2	26	.285	.15	.119	.60	.101	.62
Non-social attention	Time 1	34	-.501	.002*	-.367	.03*	.071	.68
	Time 2	23	-.163	.49	-.755	.01*	.097	.68

Table 7. Mean behavioral change over time for autism and control groups

	Autism	*Behavioral controls*
Responds to name[†]	+.094	*+.139*
Imitation success[†]	+.209	*+.178*
Emotion sharing[††]	+.051	*+.193*
Positive emotion[††]	-.031	*+.234*
Negative emotion[††]	+.066	*-.023*
Looking[††]	*-.046*	*+.602*
Joint attention response[†]	*+.151*	*+.042*
Joint attention[††] initiations	*+.017*	*+.096*
Protodeclarative	*+.000*	*+.107*
Protoimperative	*+.010*	*-.031*
Initiates social[††] interaction	*+.053*	*-.037*
Protodeclarative	*+.020*	*-.049*
Protoimperative	*+.034*	*-.003*
Gesture rate[††]	*+.129*	*+.348*
Engaged other[†††]	*+.008*	*+.047*
Pretend play[†††]	*+.026*	*+.014*

[†] Score is a proportion of correct responses per examiner press
[††] Score reflects rate per minute of child initiated behavior
[†††] Score reflects proportion of observable time engaged in behavior

Table 8. Autism group performance at time 1 and time 2 adjusted for IQ

	Time 1	Time 2			
	Mean (sd)	Mean (sd)	Change score	F	*p*
Responds to name†	.205 (.22)	.298 (.29)	+.094	1.745	*.194*
Imitation success†	.335 (.32)	.544 (.37)	+.209	6.377	*.016**
Emotion sharing††	.169 (.22)	.220 (.36)	+.051	.420	*.553*
Positive emotion††	.359 (.28)	.328 (.37)	-.031	.006	*.939*
Negative emotion††	.040 (.10)	.106 (.25)	+.066	.932	*.340*
Looking††	*.824 (.45)*	*.824 (.61)*	*+.000*	*.001*	*.976*
Joint attention response†	*.357 (.36)*	*.508 (.35)*	*+.151*	*2.120*	*.153*
Joint attention†† initiations	*.010 (.03)*	*.027 (.03)*	*+.017*	*1.532*	*.223*
Protodeclarative	*.006 (.02)*	*.006 (.03)*	*+.000*	*.023*	*.880*
Protoimperative	*.004 (.02)*	*.014 (.01)*	*+.010*	*2.406*	*.189*
Initiates social†† interaction	*.122 (.11)*	*.175 (.12)*	*+.053*	*1.641*	*.247*
Protodeclarative	*.048 (.06)*	*.068 (.11)*	*+.020*	*.682*	*.414*
Protoimperative	*.073 (.10)*	*.107 (.08)*	*+.034*	*.827*	*.369*
Gesture rate††	*.146 (.17)*	*.275 (.35)*	*+.129*	*4.165*	*.048**
Engaged other†††	*.516 (.16)*	*.524 (.18)*	*+.008*	*.280*	*.599*
Pretend play†††	*.030 (.04)*	*.056 (.07)*	*+.026*	*4.682*	*.036**
Social attention (z-score)	*-.209 (.33)*	*-.272 (.45)*	*-.063*	*.006*	*.940*

† Score is a proportion of correct responses per examiner press
†† Score reflects rate per minute of child initiated behavior
††† Score reflects proportion of observable time engaged in behavior

Table 9. Behavioral control group performance at time 1 and time 2 adjusted for IQ

	Time 1	Time 2			
	Mean (sd)	Mean (sd)	Change score	F	*p*
Responds to name†	.624 (.34)	.763 (.26)	+.139	.372	*.553*
Imitation success†	.652 (.30)	.830 (.15)	+.178	6.589	*.011**
Emotion sharing††	.383 (.30)	.576 (.17)	+.193	4.403	*.058*
Positive emotion††	.775 (.62)	1.009 (.32)	+.234	1.005	*.322*
Negative emotion††	.023 (.04)	.000 (.00)	-.023	15.043	*.002**
Looking††	*1.722 (.84)*	*2.324 (.48)*	*+.602*	*2.305*	*.098*
Joint attention response†	*.958 (.12)*	*1.00 (--)*	*+.042*	--	--
Joint attention†† initiations	*.099 (.07)*	*.195 (.12)*	*+.096*	*6.365*	*.027**
Protodeclarative	*.062 (.05)*	*.169 (.13)*	*+.107*	*9.079*	*.011**
Protoimperative	*.036 (.05)*	*.005 (.01)*	*-.031*	*1.930*	*.191*
Initiates social†† interaction	*.241 (.17)*	*.204 (.11)*	*-.037*	*.908*	*.359*
Protodeclarative	*.172 (.17)*	*.123 (.11)*	*-.049*	*.939*	*.356*
Protoimperative	*.072 (.07)*	*.069 (.06)*	*-.003*	*.002*	*.936*
Gesture rate††	*.451 (.28)*	*.799 (.33)*	*+.348*	*3.669*	*.080*
Engaged other†††	*.776 (.13)*	*.823 (.07)*	*+.047*	*4.201*	*.063*
Pretend play†††	*.145 (.08)*	*.159 (.06)*	*+.014*	*1.065*	*.323*
Social attention (z-score)	*1.011 (.55)*	*.944 (.30)*	*-.067*	*.038*	*.849*

† Score is a proportion of correct responses per examiner press
†† Score reflects rate per minute of child initiated behavior
††† Score reflects proportion of observable time engaged in behavior

Table 10. Autism group difference scores over time

	Time 1	Time 2			
	Mean (sd)	Mean (sd)	Change score	F	*p*
Responds to name†	-1.061 (1.06)	-2.129 (1.29)	-1.068	.166	*.689*
Imitation success†	-1.606 (1.67)	-2.164 (2.66)	-1.558	.495	*.495*
Emotion sharing††	-.678 (.81)	-2.236 (1.66)	-1.558	1.695	*.209*
Positive emotion††	-.527 (.66)	-1.706 (1.257)	-1.179	8.349	*<.001***
Negative emotion††	1.555 (3.89)	--	--	--	--
Looking††	*-1.135 (.70)*	*-2.92 (2.00)*	*-1.857*	*6.878*	*.017 **
Joint attention response†	*-5.985 (3.17)*	--	--	--	--
Joint attention†† initiations	*-1.442 (.01)*	*-1.210 (.17)*	*+.232*	*9.164*	*.001***
Protodeclarative	*-1.083 (.00)*	*-1.207 (.17)*	*-.124*	*.015*	*.988*
Protoimperative	*-.702 (.00)*	*.065 (.74)*	*+.767*	*3.602*	*.002***
Initiates social†† interaction	*-.679 (.13)*	*1.050 (1.06)*	*+1.729*	*1.720*	*.100*
Protodeclarative	*-.795 (.36)*	*.436 (.87)*	*+1.231*	*1.175*	*.223*
Protoimperative	*.189 (1.89)*	*1.234 (2.64)*	*+1.055*	*.953*	*.351*
Gesture rate††	*-1.241 (.19)*	*-1.870 (.92)*	*-.629*	*2.987*	*.100*
Engaged other†††	*-5.276 (2.51)*	*-5.220 (2.89)*	*+.056*	*.032*	*.859*
Pretend play†††	*-1.609 (.30)*	*-2.021 (1.00)*	*-.412*	*1.425*	*.247*
Social attention (z-score)	*-.295 (.26)*	*-.146 (.41)*	*+.149*	*1.891*	*.202*

† Score is a proportion of correct responses per examiner press
†† Score reflects rate per minute of child initiated behavior
††† Score reflects proportion of observable time engaged in behavior

Note. Joint attention response rate and negative emotion difference scores could not be computed at time 2 because there was no variability in the behavioral control group performance.

Table 11. Amygdala volumes by group at time 1 adjusted for TBV

		Autism		Combined controls		
Region	N	Mean (SE)	N	Mean (SE)	% diff.	p
Total amygdala	48	4.01 (.07)	25	3.31 (.12)	10.1	.011
Left amygdala	48	1.94 (.04)	25	1.64 (.06)	7.0	.066
Right amygdala	48	2.07 (.04)	25	1.67 (.06)	11.3	.003

Table 12. Amygdala volumes by group at time 2

	Autism		Combined controls	
Region	N	Mean (SE)	N	Mean (SE)
Total amygdala	27	4.64 (.10)	6	4.24 (.16)
Left amygdala	27	2.26 (.05)	6	2.08 (.08)
Right amygdala	27	2.38 (.05)	6	2.16 (.08)

Table 13. Relationship between looking rate and amygdala volume in autism group

	Estimate	SE	df	t	p
Looking rate	.1006	.04	31	2.27	.03*
Look x age	.0081	.02	31	.32	.75
Look x hemisphere	.0742	.03	31	2.28	.03*
Look x TBV	.0018	.00	31	4.19	<.001**

Table 14. Laterality effects of looking rate and amygdala volume in autism group

	Estimate	SE	df	t	p
Looking → right amygdala	.1682	.05	31	3.42	.002*
Looking → left amygdala	.0940	.05	31	1.89	.06

Table 15. Interaction of total brain volume and looking rate predicting amygdala volume in autism group

	Estimate	SE	df	t	p
Small TBV (looking → amygdala)	-.1189	.05	31	-2.23	.03*
Large TBV (looking → amygdala)	.3199	.08	31	3.94	<.001**

Table 16. Relationship between social initiation rate and amygdala volume in autism group

	Estimate	SE	df	t	p
Social initiation rate	-.5924	.25	31	-2.34	.03*
Social initiation x age	.6198	.10	31	6.12	<.001**
Social initiation x hemisphere	-.0491	.09	31	-.53	.60
Social initiation x TBV	-.0020	.00	31	-1.12	.27

Table 17. Relationship between social initiation rate and amygdala volume over time in autism group

	Estimate	SE	df	t	p
Social initiation at time 1	-.1320	.03	31	-4.46	<.001**
Social initiation at time 2	.0174	.02	31	.90	.37
Social initiation change	.1237	.12	31	3.97	.004*

Table 18. Relationship between protodeclarative joint attention rate and amygdala volume in autism group

	Estimate	SE	df	t	p
Joint attention rate	4.9657	4.26	31	1.17	.25
Joint attention x IQ	-.2319	.07	31	-3.22	.003*
Joint attention x age	4.0042	2.85	31	1.40	.17
Joint attention x hemisphere	-1.0831	.66	31	-1.65	.11
Joint attention x TBV	-.0241	.02	31	-1.26	.22

Table 19. Relationship between protodeclarative joint attention (JA) rate and amygdala volume over time in autism group

	Estimate	SE	df	t	p
Protodeclarative JA at time 1	.3310	.66	31	.50	.62
Protodeclarative JA at time 2	1.3132	.23	31	5.74	<.001**
Change in protodeclarative JA	.9822	.69	31	1.43	.16

Table 20. Interaction of protodeclarative joint attention rate and IQ predicting amygdala volume in autism group

	Estimate	SE	df	t	p
High IQ (joint attention → amygdala)	-2.7833	.86	31	-3.22	.003*
High IQ (joint attention → amygdala)	2.4354	.76	31	3.22	.003*

Table 21. Relationship between overall emotion and amygdala volume in autism group

	Estimate	SE	df	t	p
Emotion rate	.0951	.08	31	1.14	.26
Emotion x age	-.1136	.04	31	-2.64	.01*
Emotion x hemisphere	.0395	.04	31	.90	.38
Emotion x TBV	-.0017	.00	31	-1.76	.09

Table 22. Relationship between emotion and amygdala volume over time in autism group

	Estimate	SE	df	t	p
Emotion at time 1	.1051	.09	31	1.11	.28
Emotion at time 2	-.2247	.08	31	-2.92	.003*
Emotion change	-.3298	.10	31	-3.17	.003*

Table 23. Relationship between positive emotion and amygdala volume in autism group

	Estimate	SE	df	t	p
Positive emotion rate	-.4027	.17	31	-2.41	.02*
Positive emotion x age	-.0883	.05	31	-1.90	.06
Positive emotion x hemisphere	.0723	.08	31	.95	.35
Positive emotion x TBV	-.0002	.00	31	-.02	.99

Table 24. Relationship between positive emotion and amygdala volume over time in autism group

	Estimate	SE	df	t	p
Positive emotion at time 1	-.3303	.17	31	-1.95	.06
Positive emotion at time 2	-.5432	.19	31	-2.92	.007*
Positive emotion change	-.2129	.11	31	-1.90	.07

Table 25. Relationship between negative emotion and amygdala volume in autism group

	Estimate	SE	df	t	p
Negative emotion rate	.6276	.44	31	1.44	.17
Negative emotion x age	.4105	.34	31	1.21	.24
Negative emotion x hemisphere	-.1013	.04	31	1.75	.09
Negative emotion x TBV	-.0007	.00	31	-.67	.51

Figure 1. Relationship between looking rate and amygdala volume for children with large and small total brain volume in autism group

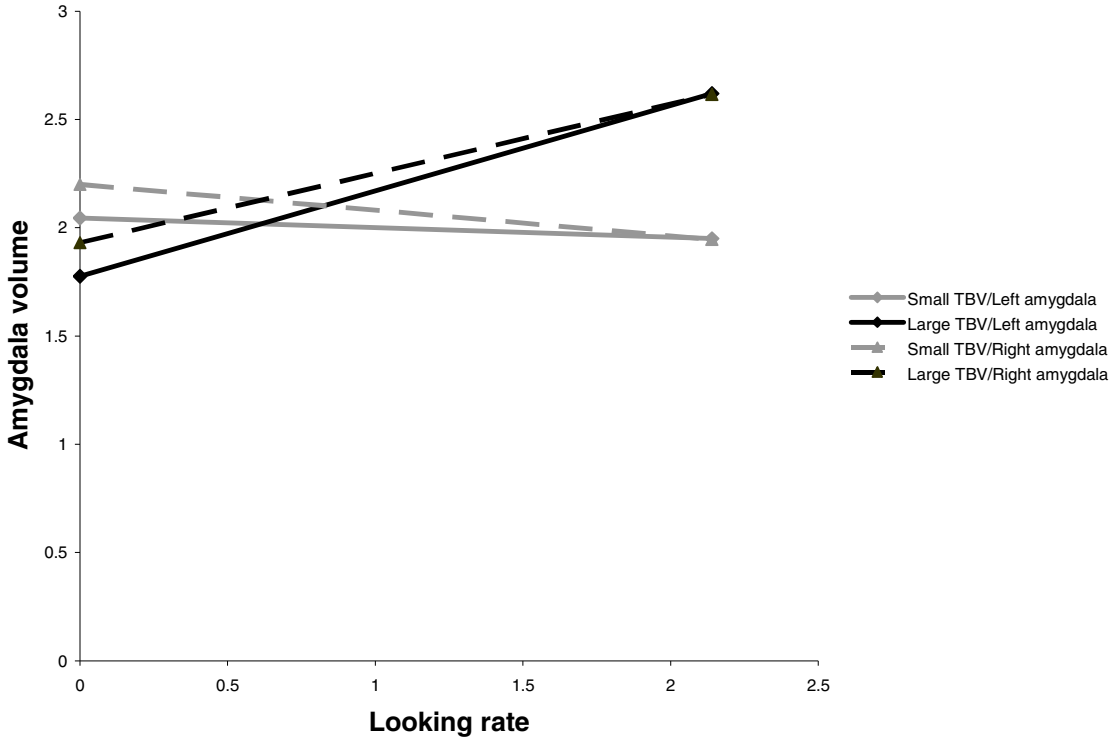

Figure 2. Amygdala volume at time 1 predicting emotion rate over time

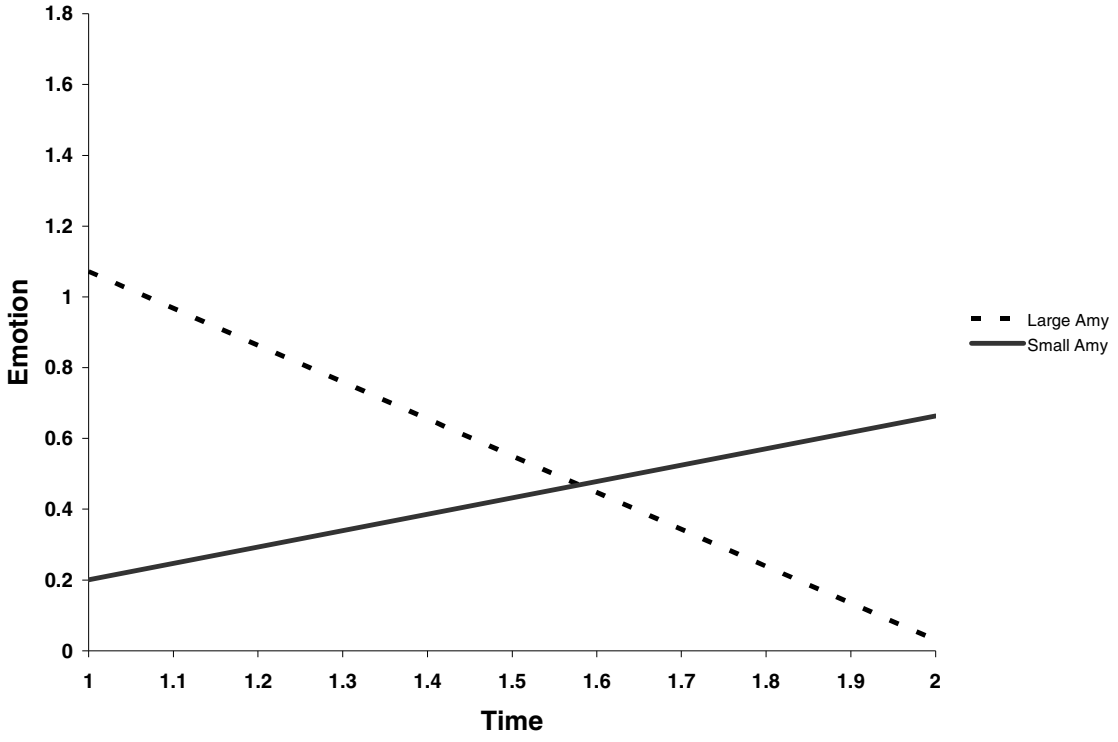

Figure 3. Positive emotion rate by amygdala volume for subjects with autism