A cross-sectional study of scanning and pupillary responses to various face and nonface stimuli in children with an autism spectrum disorder and typically developing children

By

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Abstract

The current study sought to test for the presence of a developmental trend for children with autism spectrum disorder (ASD) from the age of 2 years to 13 years of age in their attention to, and processing of, social images. Children with ASD were expected to show dysregulated pupillary responses that would be associated with a reduction of attention to social images over the span of childhood. Pupil size was measured for children with ASD and typically-developing (TD) peers at baseline and in response to both social and nonsocial stimuli. To investigate the effect of stimulus detail on processing, three types of stimuli were presented during an eye-tracking task: photographs, pictures of figures, and drawings. Contrary to previous reports, there was no effect of age or diagnosis on baseline pupil size. Children with ASD, however, did not show phasic pupillary responses to different stimulus types that were observed in TD children. Regardless of age, children with ASD looked at all stimuli for less time than TD children, with nonsocial images receiving the least amounts of fixation. The results suggest that dysregulation of pupil size may be a less systemic marker of ASD than had been previously reported. Furthermore, larger pupillary dilations were correlated with longer looking time toward social photos, as well as higher MA and less social and communication impairment.

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Introduction

Atypical face processing strategies are among the deficits reported in individuals with Autism Spectrum Disorder (ASD). Research has focused on attention to faces, time looking at different parts of the face, and face processing abilities in people with ASD because of the crucial role that attention to faces plays in communicative interactions. However, atypical looking patterns toward faces appear to vary with the age of the person with ASD, such that toddlers and young children do not differ from the control group as much as older children, adolescents, and adults (Falck-Ytter & von Hofsten, 2010). This pattern of findings implies a developmental trajectory in ASD in which children with ASD become progressively more disengaged from faces over the course of childhood. Furthermore, dysregulation of the sympathetic and parasympathetic branches of the autonomic nervous system (ANS) has been reported to accompany atypical looking behavior (Bal et al., 2010; Kaartinen et al, 2011; Watson, Roberts, Baranek, Mandulak, & Dalton, 2011), which might provide an explanatory mechanism for why children disengage from faces. Activation of the ANS signals increased processing load and also increased social interest; therefore, multiple stimulus probes can clarify how these aspects of ANS activation interact with looking behavior. The influence of the ANS on looking behavior over multiple ages of children with ASD is the focus of the current investigation, with the goal of systematically determining whether a developmental trend exists for looking behavior or pupillary responses. Moreover, this investigation seeks to establish an activation profile specific to ASD by measuring ANS activation as children with ASD encounter and process faces.

This investigation uses eye tracking and pupillometry to capture gaze direction and pupil size, a measure of ANS activation. These methods integrate with systematic variation of stimulus detail and social content to examine the effect that visual detail has on social processing. Furthermore, a cross-sectional design allows the effects of age to be parsed. ASD researchers have used eye tracking technology for 10 years to examine attention with a wide range of social and nonsocial stimuli (e.g., Anderson, Colombo, & Shaddy, 2006; Speer, Cook, McMahon, & Clark, 2007; van der Geest, Kemner, Camfferman, Verbaten, & van Engeland, 2002). For the purposes of this study, social stimuli refers to images such as human faces showing direct eye contact with the viewer, while nonsocial stimuli refers to images of things such as common objects and toys. Eye tracking systems commonly code pupil measurements along with gaze direction, a feature which eliminates the need to attach electrodes or other such impediments to children who might have tactile sensitivity, a characteristic of ASD. Thus, this noninvasive device can help to distinguish differences in social attention and ANS function over the span of childhood, and across different levels of functioning.

Review of Literature

The Autonomic Nervous System in ASD

Exploring the development of autonomic nervous system (ANS) dysregulation can better establish its influence on basic social dysfunction in ASD. Abnormalities in the ANS in individuals with ASD appear in baseline activity and in activity responding to social stimuli, which could interfere with normal social interaction. For example, baseline electrodermal skin conductance, tonic pupil size baseline, and respiratory sinus arrhythmia indicate reduced resting-state regulation in children with autism (Anderson & Colombo, 2008; Palkovits & Wiesenfeld, 1980, Barry & James, 1988; Chang et al., 2012; Bal et al., 2010). Heightened arousal at baseline appears to be independent of heightened arousal towards stimuli (Beatty, 1982); therefore, arousal toward social stimuli contributes independently to our understanding of social dysfunction. Children with ASD who exhibit faster and more accurate recognition of emotions from pictures also show greater regulation of sympathetic responses, which implies unchecked sympathetic activation contributes to social dysfunction (Bal et al., 2010). However, like other biomarkers that have been recently associated with ASD, an autonomic profile of ASD is not incorporated into the core diagnostic features of the disorder (see: Walsh, Elsabbagh, Bolton, & Singh, 2011). Nevertheless, the examination of such a noninvasively measured feature of the ANS could provide a regulatory basis for social perception differences in ASD.

Pupil Size in ASD

Pupil size is the combined result of excitatory and inhibitory activity within each of the sympathetic and parasympathetic branches of the ANS. The concerted effort of multiple direct and indirect neurochemical pathways leads to dilation or constriction of the pupil. Pupil dilation is rooted in sympathetic activation of the ANS, which causes the smooth muscle known as the dilator pupillae to be activated by the norepinephrine system (Andreassi, 2000). Constriction results primarily from the acetylcholine system of the parasympathetic ANS, which activates an opposing smooth muscle known as the sphincter pupillae (Barbur, 2004). Lastly, two direct inhibitory influences and two indirect inhibitory influences also moderate pupil size, each using norepinephrine and possibly GABA (Hou, Langley, Szabadi, Bradshaw, 2007; Loewenfeld, 1999). In the interest of parsimony, pupil dilation indicates activity somewhat biased towards the sympathetic branch, whereas constriction indicates activity somewhat biased towards the parasympathetic branch. Pupil size provides a noninvasive indicator of ANS activity for exploring the interactions between the sympathetic and parasympathetic branches at baseline and in response to stimuli.

Accurate ANS responses require controlled light conditions because pupils respond dynamically to ambient lighting. The pupil constricts in response to light entering the eye and also while accommodating focus on an object at close range. These effects are minimized in lab conditions by maintaining a constant visual angle and controlling the luminance among stimuli (Barbur, 2004; Loewenfeld, 1999). Underscoring the importance of standardized luminance, atypical pupil responses in both light and dark conditions are reported in ASD (Anderson & Colombo 2008; Martineau et al., 2011). When compared to controls, individuals with ASD show longer latencies in reaction to light, smaller constriction amplitude, smaller dilation in response to dark conditions, and reduced constriction velocity compared to controls (Fan, Miles, Takahashi & Yao, 2009; Rubin, 1961; Martineau et al., 2011). Under standardized lab conditions, pupil size will not reflect such light-reaction abnormalities; pupil size will primarily reflect concerted effects of the ANS. Maintaining constant luminance conditions allows for the reliable measurement of both baseline (tonic) conditions and stimulus (phasic) conditions.

Disagreement in the literature about tonic pupil size in ASD may be rooted in methodological differences among the studies in question. Matching baseline luminance conditions to the stimulus luminance conditions facilitates straightforward comparisons between tonic and phasic pupil size. For example, when the baseline measurement occurs during luminance-matched gray visual stimuli, children with ASD have *larger* tonic pupil size than TD children at 2- to 5-years old and at 8- to 10-years old (Anderson & Colombo, 2009; Anderson, Savage, Chambers, Colombo, Powell, Obermeier, & Unruh, *in preparation*). However, children ages 3- to 15-years old exhibited *smaller* tonic pupil size than TD children when shown a nonluminance-matched black slide between stimulus images (Martineau et al., 2011). Sudden changes in luminance will elicit some degree of pupillary light reflex. Therefore, the smaller tonic pupil size may represent a reduced pupillary response latency *or* reduced dilation amplitude to the sudden darkness of the room (i.e. the subjects' pupils may have dilated more slowly or may not have dilated to the same extent as TD children's). Direct comparisons between baseline and stimulus conditions are possible with a luminance-matched baseline condition, which controls for automatic light reactions.

Because pupil size changes over the duration of a task, a single baseline condition measured at the beginning or end of the session is not sufficient for making comparisons between tonic and phasic pupil size. Reassessing baseline pupil size after each presentation of stimulus assures that reactions to one stimulus do not spill over to another. For example, pupil size decreases with fatigue and habituation, therefore the baseline size of the pupil may reduce throughout an eye-tracking session (Kahneman & Peavler, 1969). Furthermore, baseline pupil size increases and phasic pupil size decreases as tasks become less rewarding and the subject begins to explore other options for reward (Aston-Jones & Cohen, 2005). The difference scores between tonic pupil size and phasic pupil size within a session reveal whether stimuli were more or less arousing compared to the preceding neutral baseline, resulting in a difference score which changes for each stimulus slide. When a subject is loses interest in the stimuli, their difference scores will decrease throughout the session as their cognitive resources are less devoted to the task at hand.

Phasic pupil size is a quick, sensitive index for the amount of cognitive effort expended while performing a task or observing a stimulus. Unlike tonic pupil responses, phasic pupil response occurs during performance of a task or engagement with a stimulus with psychological or otherwise meaningful content. Phasic reaction begins during the first 100-200ms after stimulus onset and persists until the subject completes cognitive processing, after which the pupil quickly returns to baseline size (Loewenfeld, 1999; for a review see Beatty & Lucero-Wagoner, 2000). More cognitively demanding tasks, or tasks that require more information processing, tend to elicit a larger pupil size than less demanding tasks (Steinhauer, Condray, & Kasparek, 2000). Neurotypical adults exhibited progressive phasic pupil dilation when multiplying small numbers in increasingly difficult problems, and the size of peak dilation assumed a monotonic relationship with the difficulty of the problem (Hess & Polt, 1964). In this way, phasic pupil size correlates positively with the amount of cognitive load experienced by the participant in the moment. Stimulus presentation of only a few seconds is sufficient to produce a meaningful pupil reaction without the subject experiencing fatigue or habituation.

The positive monotonic correlation between pupil dilation and cognitive load appears to break down when load exceeds working memory capacity (Granholm, Morris, Sarkin, Asarnow & Jeste, 1997). At lower cognitive loads, constriction indicates the allocation of few attentional resources. However, as task difficulty increases, the participant’s pupil size increases until the task becomes too difficult, when the pupil begins to constrict rapidly. For example, when adult participants were asked to recall strings of numbers beyond the usual limit of digit span memory (usually about 7 digits ± 2) their pupils began to decrease in size, indicating that the subjects were overloaded and no longer able to engage with the task (Granholm et al., 1997). Even the anticipation of poor task performance causes decreasing pupil size (Aston-Jones & Cohen, 2005). In relation to ASD research, smaller pupil size in reponse to social stimuli could mean that the children detect the social content of the stimuli, but processing does not evoke the recruitment of as many cognitive resources as in TD children. Another interpretation is that the social stimuli present so much information that children with ASD reach their cognitive limit and disengage from the stimuli. Therefore, smaller pupil sizes can be construed to represent one of two extremes in cognitive load. Face stimuli of varying visual complexity would enable researchers to parse how visual details contribute to phasic pupil reactions, similar to investigations of cognitive load.

Arousal models of social disfunction in ASD account for the relationship between social looking and atypical arousal. The way children with ASD engage with social and nonsocial information shapes the world that they attend to, and in turn, what they learn from their environment. Thus, lower phasic arousal in response to faces could inhibit learning about faces in children with ASD. Reduced social impairments in children with ASD are associated with levels of arousal that are closer to control group levels (Bal et al., 2010; Dalton et al., 2005). While evidence from skin conductance studies indicates heightened arousal in response to social stimuli, pupillometry studies have indicated smaller responses to social stimuli when compared to TD subjects (Kaartinen et al., 2012; Hirstein, Iversen & Ramachandran, 2001; Anderson & Colombo, 2008; Sapeta et al., 2012; Falck-Ytter, 2008). Pupil size provides a more complete representation of overall arousal because both sympathetic and parasympathetic systems influence its outcome, whereas skin conductance primarily reflects sympathetic activity. The social deficits in ASD may therefore be the result of reduced overall arousal towards faces, which are then treated with relative disinterest. Atypical arousal to social stimuli leads to difficulty learning, which may then lead to disengagement from faces over time due to insufficient expertise.

Children with ASD exhibit dysregulated phasic pupil responses throughout childhood and into early adolescence, although combining investigation of pupil size with looking behavior across childhood would reveal when and how arousal influences social looking (Anderson, Colombo, & Shaddy, 2006; Martineau et al., 2011; Sapeta et al., 2012). Phasic pupillary reactions (i.e. constriction or dilation compared with baseline pupil size) in children with ASD in response to social stimuli are smaller on average when compared to TD controls, (Anderson, Colombo, Shaddy, 2006; Anderson, 2010; Martineau et al., 2011). Furthermore, children with ASD exhibit reduced pupil reactions toward social stimuli as early as 2 years-old, before atypical looking patterns have developed (Anderson, Colombo, & Shaddy, 2006) and these atypical phasic pupil reactions continue until at least age 15 (Martineau et al., 2011). Pupil dysregulation persists throughout childhood and into adolescence, whereas children do not show reduced attention to social images until later in childhood. Systematic investigation using a cross-sectional sample of toddlers, school-age children, and young adolescents with ASD can reveal more about the nature of phasic ANS dysregulation to different kinds of social stimuli, and how this reaction relates to differences in face scanning compared with TD controls.

Face Scanning in ASD

Although young children with ASD present typical patterns of face scanning, the developmental trajectory appears to widen the gap between ASD scanning and typical scanning patterns by early adolescence. Later in childhood, children with ASD might not look at faces as much as TD controls because the progress of the disorder has disturbed their perception of social information. Due to the dearth of longitudinal developmental studies on social looking in ASD, reported age ranges in the social looking literature guide this analysis. Scanning differences of children with ASD to static human and face stimuli were negligible in narrower, younger age ranges, such as 2 to 5-year-olds (Anderson et al., 2006), 5 and 6-year-olds (Falck-Ytter et al., 2010), or 10-year-olds (van der Geest, Kemner, Verbaten, & van Engeland, 2002; van der Geest et al., 2002). Studies that have shown scanning differences in children younger than 5 years of age have incorporated recognition tasks (Chawarska & Shic, 2009) or dynamic stimuli with adult speech (Jones, Carr, & Klin, 2008; Chawarska, Macari, & Shic, 2012). Such demanding stimuli elicit looking patterns similar to those found in older participants viewing simple scenes; participants ranged from adolescence to adulthood (e.g., Boraston, Corden, Miles, Skuse & Blakemore, 2008; Dalton et al., 2005; Hernandez et al., 2009; Nacewicz et al., 2006) or groups of older children grouped with adolescents or young adults (Riby & Hancock, 2009; Sasson, Turner-Brown, Holtzclaw, Lam, & Bodfish, 2008). In sum, adults with ASD may be more likely to show quantitatively different patterns of visual scanning to static social images, whereas such differences appear to be less robust in the gaze behavior of children with ASD. This trend may be rooted in the dysregulation of the ANS towards social stimuli observed early in life. Investigating ANS response with more tightly controlled age ranges would better characterize the face-scanning differences in ASD and at what time in development these differences emerge.

Patterns of attention to the internal features of faces in ASD differ from TD populations throughout development and in maturity. The allocation of attention among the eyes, mouth, body, and gestures is affected by visual features of saliency (Freeth, Foulsham,& Chapman, 2010). Through a cross-sectional study, Nakano and colleagues (2010) found evidence for an alternative developmental trajectory for social looking patterns in ASD. Children (mean age = 5 years) with ASD looked less at the mouth of the speaker than TD children while viewing a social video of people having a conversation. The reverse pattern occurred in adults with ASD, who looked longer at mouth regions (relative to faces overall) than their adult TD counterparts, who tended to focus on the eyes (Nakano et al., 2010). Since the current study seeks information about attention disengagement in general, attention to the eyes and mouth are therefore combined into an internal features variable. Face images of varying complexity may also produce different patterns of gaze toward internal features, with the simpler images perhaps garnering more attention due to ease of processing. Incorporating varied visual complexity with a cross-sectional sample of children from toddlerhood to early adolescence can help differentiate the trajectory of social looking patterns in ASD.

Scanning of face-like stimuli in ASD

Manipulating face stimuli can probe whether processing deficits are specific to the social content of faces or if these deficits arise from processing complex stimuli. Face-like stimuli, such as cartoons, figurines, and computer-generated avatars, can be used to investigate the skills required for face processing, such as the integration of individual features into configural wholes. They also provide an opportunity to study the possibility that complexity of face stimuli might underlie the processing difficulty for people with ASD (cf. Behrmann, Thomas & Humphreys, 2006; Rossett et al., 2010; Spezio, Adolphs, Hurley & Piven, 2007). Face-like stimuli are employed as a kind of "intermediary" stimuli between faces and objects, providing reduced details while retaining the face configuration and features (Hernandez et al., 2009). Researchers can draw more specific conclusions about the nature of processing deficits in ASD and their origins when research paradigms allow researchers to parse whether atypical ASD scanning reactions are due to social information or complex configural information. Thus, the current study uses face-like stimuli to investigate hypotheses based on perceptual complexity.

Cartoons represent an ecologically valid stimulus that children with ASD encounter in everyday life. Cartoons are also typically less detailed than photos or recordings of real humans.

Advertising and entertainment media targeted at children often contains cartoon characters, and exposure to such media would provide reasonable familiarity with cartoons. This exposure gives cartoons an advantage over novel face-like stimuli because children would be reasonably familiar with processing animated and static cartoons—therefore, the interpretation of differences based on cartoons would be more likely to be based on visual perceptual differences rather than differences in the perception of complex novel objects. Furthermore, avatars and cartoons are already successfully employed in computer-based interventions that help children with ASD improve emotion recognition and context-based emotion prediction, and sometimes these reduced-detail designs produce even more improvement than intervention items using photos of emotions (Silver & Oakes, 2001; Cheng & Chen, 2010).

Children with ASD employ typical processing strategies with cartoon faces that they do not employ with photos of faces. Children with ASD display responses when encountering cartoon faces that are similar to TD responses to real faces, which implies that when image details are limited, children with ASD spontaneously use processing strategies that are more conventional. The face inversion effect leads to a similar conclusion. In a face-inversion paradigm, TD children usually exhibit an inversion effect (slower reaction times) when categorizing inverted human faces as compared with upright human faces, because inversion of the face configuration disrupts typical information processing (Rossett et al., 2008). Inverted cartoon faces of animals and people elicited this characteristic effect in children with ASD and TD children, although no such effect was observed for real faces in the ASD group (Rossett et al., 2008). Children with ASD might process simple faces in more typical ways, i.e. by using the configuration of the facial features, which inversion disrupts. Conversely, children with ASD might identify real faces by their features alone, which inversion would not disrupt. Children with ASD spontaneously change face processing strategies based on image properties that do not affect face processing strategies in TD children.

The explanation for typical processing in ASD of cartoon images contains two main arguments—one based on perceptual processing, and one based on social agency and expertise. First, cartoons contain fewer visual details and textures than photos. Cartoons might be easier to process due to their lack of detail—in other words, the simplified image placed fewer demands on working memory so the children were able to use more typical strategies for processing. Second, cartoons are not usually encountered as interactive social agents, so they are in this respect “less social” than human face photos (Riby & Hancock, 2009). The ease of processing cartoons may reflect the children’s expertise with cartoons. Cartoon expertise might be easier for children with ASD to attain than real face expertise, because real face expertise would be subject to interference by the visual processing demands and social impairments associated with ASD. Disentangling the relationship between image complexity and social agency is possible with the application of a stimulus that still closely resembles a human face and has an intermediate level of detail between that of a cartoon and a photo; for example, a doll’s face or the face of a computer avatar. Such an “intermediate” stimulus would provide face details without providing social agency.

Gaze patterns in response to the level of face stimulus details can provide insight on the development of social attention across the life span. Gaze behavior of older individuals with ASD is similar when encountering real faces and complex face-like objects or cartoons. Hernandez et al. (2009) found that adults with ASD tended to look far less at the eye region of photo faces than an avatar face, but showed neglect of the same facial regions for both images. Furthermore, Riby and Hancock (2009) found that 13-year-old children with ASD exhibited similar patterns of eye avoidance when they viewed cartoon movies of people talking and live-action videos of people talking. Additionally, children with ASD spent more time attending to backgrounds and bodies while viewing still images of cartoons and real people (Riby & Hancock, 2009). This finding that older children and adults exhibit scanning differences toward reduced-detail faces conflicts somewhat with the previously discussed finding that children with ASD use typical processing strategies when details are reduced (Rossett et al., 2008). This conflict might be explained by an interaction between looking behavior and age. For example, the tendency for adults and adolescents with ASD to avoid internal features may extend to face-like stimuli (avatars) and cartoons, whereas young children with ASD would still respond like TD children. In other words, the way cognitive effort relates to gaze behavior might not be constant over the life span. Using three stimulus types—drawings, figurines, and photos— could establish a categorical relationship between image complexity and looking at internal features at different ages. Furthermore, comparing pupil size responses to these three representation types would help to establish a connection between cognitive effort and gaze behavior.

Hypothesis and predictions

The extant evidence suggests that young children with ASD experience ANS dysregulation, which results in higher baseline activation and lower sensitivity to social stimuli. This combination increases scanning neglect of highly detailed social stimuli later in childhood due to downstream effects of dysregulated arousal. However, less detailed stimuli elicit looking responses from children with ASD that are indistinguishable from TD responses. Face-like stimuli would therefore allow comparison between the level of detail and ecological validity of the stimuli throughout childhood. Several potential outcomes would support this hypothesis.

1. Children with ASD exhibit higher baseline pupil size than TD children throughout childhood.
2. Visual complexity contributes to the arousal response to the stimuli and elicits predictable pupil size responses. Images that are more complex or closer to reality (photos) may elicit more atypical responses in the pupillary systems of the children with ASD and therefore the pupils might appear smaller than the TD pupils. Images that are less complex (drawings) might not elicit such overactive responses, and so pupil size in both diagnostic groups would be comparable. Intermediate images (figures) fall somewhere in between these sizes. This pattern of pupil sizes will be true regardless of social or nonsocial stimuli.
3. Children with ASD exhibit a special response to social stimuli, indicating that these stimuli are more difficult to process than nonsocial complex images—social pupil responses are smaller than TD social responses across stimulus image types.
4. Older children with ASD show reduced attention to internal features of the face. This difference in social attention to internal features is greatest in the oldest group, and present in the middle age group, but not present in the youngest group.

Summary

The developmental progression of scanning patterns in autism hinges on ANS activity dysregulation that disrupts attention to complex social stimuli. The current study investigated the effects of different types of face and object stimuli on pupil size and scanning patterns in children from the age of 2- to 13-years old. The pattern of pupil responses varies by age, indicating an early ANS dysregulation. Atypical autonomic activation might precede and contribute to atypical scanning behavior in response to social stimuli (Falck-Ytter, Frenell, Gillberg, & von Hofsten, 2010). Furthermore, attention to internal features of faces changes with age such that older children and adolescents show larger differences from TD children than younger children with ASD. In order to explore whether the ANS responds to the visual processing load or social content of the stimuli, the study includes different representations of faces and objects. The stimuli comprises three categories (photos, toys, drawings) that children encounter in day-to-day life, thus the effects of familiarity with particular types of media might be lessened. Varying stimulus type differentiates what level of detail is necessary in social and nonsocial stimuli to elicit atypical allocation of attention and atypical ANS activation.

Method

Participants

Inclusion criteria consisted of gender, diagnosis and age range. Male children were recruited who were between the ages of 2 and 13 years of age. Participants were organized into the following groups: ASD (n = 18) and TD (n = 32). For recruitment targeting purposes, the participants were categorized by age into one of three age groups to assure that the distribution of ages was representative of the wide age range. Recruitment aimed for 10 subjects per diagnostic group in each of the following age bins: 2:0- to 5:0-year-olds, 5:1- to 10:0-year-olds, and 10:1- to 13:0-year-olds. These recruitment goals were not attained, and as such, subjects were divided into two age groups for the current analysis, described in *Group Assignment*.

Exclusion and inclusion criteria were devised to accommodate the sensitivity of the pupil measures and to ensure that each participant was able to take part in all session activities. In both diagnostic groups, participants were excluded if they experienced uncorrected hearing, vision, motor impairments, and/or a chronic illness requiring medication, such as heart disease. For at least 48-hours prior to every testing session, the subjects were required to be free of medications (both prescription and over-the-counter) and illness of any kind (colds, fever, allergic reactions). Children meeting the inclusion criteria for the ASD group had a diagnosis on the autism spectrum, including autism and Asperger's Syndrome. In addition, exclusion criteria for this group included comorbid diagnoses that were not on the spectrum. Children meeting the inclusion criteria for the TD group had no neurological diagnoses or developmental delays of any kind, nor first-degree family members with ASD.

Group Assignment. Children who met inclusion criteria and did not fulfill any exclusion criteria were sorted into groups by diagnosis and age. Children in the TD group were matched with children in the ASD group by chronological age (CA) such that there were no mean differences between overall group means on CA, $t(36) = 1.081$, $p = .287$. The data from children (n = 4) in the TD group was discarded to aid in this age matching. Although the overall CA averages did not differ, the means of the older age groupings were significantly different such that the TD group ($M = 146.14$, $SD = 16.86$) was significantly older than the ASD group ($M = 115.43$, $SD = 21.35$), $t(11.386) = 2.987$, $p = .012$. The younger age groups did not differ on CA ($p > .05$). Furthermore, group matching by cognitive test composite scores (CS) was not possible due to the large group-wise differences in mean CS: the TD children had higher composite scores than children with ASD, $t(17.88) = 5.264$, $p < .001$. Children in the TD group scoring above 130 on the standardized assessment of cognitive ability were excluded from the analysis ($n = 11$) because their scores were beyond the "average" range of performance. The remaining children ($N = 36$: ASD, $n = 18$; TD, $n = 18$) were included in this analysis.

Children were grouped into two age groups rather than three because the recruitment goals were not met. Children from age 2 years to 7 years (24 to 84 months) were grouped together, and children from age 7 years, 1 month, to 13 years were grouped together (85 to 163 months). The descriptive statistics were computed for each diagnostic group, and are presented in Table 2. For each diagnosis, age grouping produced a distinct group of younger children (ASD, n = 11; TD, n = 11) and older children (ASD, n = 7; TD, n = 7). These group means for CA and CS are found in Table 1. No overall diagnostic group differences were found with regard to chronological age (CA), however the upper age group of TD children contained older children than the ASD upper age group, and therefore direct comparisons between the two groups were tenuous. Comparing the subtest T-scores of the Mullen showed significant group mean differences on all subtests ($p < .05$) except for Fine Motor, $t(6.45) = 2.318$, $p = .057$. The subtest area scores of the Stanford Binet showed significant group mean differences ($p < .05$) on all subtests except for Quantitative, $t(11.34) = 2.037$, $p = .066$.

Effects of stimulus set and order, parental age at birth, and parent years of education were also investigated but did not significantly affect visual task outcome measures, and so they were excluded from final analyses presented here.

Table 1

Chronological Age and Cognitive Composite Scores by Group

	TD		**ASD**	
	Younger	Older	Younger	Older
	n = 11	n = 7	n = 11	n = 7
CA	64.64	146.14	54.18	115.43
	11.21	*16.86*	*14.06*	*21.35*
CS	118.55	117.86	84.10	69.14
	8.39	*6.99*	*31.85*	*28.96*

Note. Data presented as means. Standard deviations are in italics. CA = chronological age, CS = composite scores on cognitive assessments, TD = typically developing, ASD = autism spectrum disorder.

Table 2

Standardized Test Scores by Diagnosis

	ASD				**TD**			
Stanford Binet	n = 11	M	SD	Range	n = 12	M	SD	Range
Verbal SAS		86.60	*28.41*	44 — 120		120.50	*12.18*	96 — 138
Abstract Visual SAS		89.27	*31.33*	46 — 150		123.67	*7.33*	112 — 138
Quantitative SAS		96.20	*35.24*	46 — 150		120.33	*13.93*	96 — 140
Short Term Memory SAS		78.70	*23.13*	46 — 119		105.83	*13.87*	86 — 134
Stanford Binet test composite		82.36	*34.71*	36 — 138		120.50	*7.28*	107 — 129
Mullen	n = 6				n = 6			
Visual Reception T-score		35.50	*10.84*	20 — 52		56.17	*2.64*	52 — 59
Fine Motor T-score		36.00	*16.77*	20 — 56		53.00	*6.45*	46 — 63
Receptive Language T-score		32.67	*16.32*	20 — 61		56.33	*3.44*	53 — 62
Expressive Language T-score		27.50	*11.73*	20 — 50		62.00	*7.92*	48 — 69
Mullen composite		69.83	*22.03*	49 — 104		113.83	*6.91*	105 — 122
ADOS								
Module 1	n = 8							
Module 2	n = 4							
Module 3	n = 6							
ADOS communication		11.00	*6.62*	2 — 23		0.00		
ADOS social interaction		11.00	*4.06*	5 — 18		0.00		
ADOS total		22.00	*9.75*	8 — 37.5		0.00		
ADOS stereotypical behaviors		3.06	*1.82*	0 — 6		0.00		
SRS								
SRS social awareness		74.71	*11.47*	55 — 90		0.00		
SRS social cognition		79.06	*9.48*	61 — 90		0.00		
SRS social communication		80.47	*10.38*	58 — 90		0.00		
SRS social motivation		75.18	*15.53*	49 — 90		0.00		
SRS autistic mannerisms		83.82	*9.73*	58 — 90		0.00		
SRS total composite		83.06	*10.20*	60 — 90		0.00		

Note. Scores presented as means. M = mean. SD = standard deviation, in italics. TD = typically developing, ASD = autism spectrum disorder

Stimuli

The stimuli consisted of 24 stimulus slides and 25 blank baseline slides that were shown during the eye-tracking session. The first slide was a blank, gray slide that matched the luminance of the interstimulus slides. This slide was presented for 10s, which allowed for extended baseline pupil recording. The stimulus slides comprised 4 each of the following types of slides: photograph of person, photograph of human figurine, color drawing of person, photograph of common object, photograph of figural depiction of object (toy), and color drawing of object. Every stimulus image was presented alone on a gray background for 5s. Blank, gray, luminance-matched inter-stimulus slides were shown for a variable amount of time between 2s and 5s, averaging 3.5s, to reduce anticipatory pupil responses (49 stimulus and inter-stimulus slides total). Stimulus image order was semi-randomly arranged into four possible stimuli versions, such that the child would not see more than 4 images of the same type in a row (i.e. four sequential photos, four sequential face images, four sequential drawings, etc.). In addition, one short video clip lasting about 12s was inserted in the middle of each stimulus set to maintain the participant's interest and to allow the experimenter to issue verbal prompts (e.g., "remember to keep your hands in your lap!" or "great job!") to the child as needed. The same animated calibration points described earlier played after the video to ensure that the camera calibration remained reliable at the session midpoint. If the calibration was not reliable, the session was paused, the calibration procedure was performed again, and then the session resumed with accurate calibration. Calibration points were shown again at the end of the stimulus set to ensure that data collection remained accurate throughout the remainder of recording.

Stimulus Generation. The stimuli images were collected from online resources and created by the investigator (Appendix G). The photos of human faces, human figures, real objects, and figures of objects (toys) were drawn from various royalty-free image repositories online. Figurines depicting child actors and actresses were used because these figures were more realistic than, for example, figures depicting animated characters. Matching photos and figurines of child stars also aided luminance matching within sets of photos, figures, and drawings. All photos were altered in Adobe Photoshop Elements 8.0 to at least 300 dpi, and the image areas were resized to consist of approximately the same area (6.5” x 7”, approximately 45.5 square inches). The background, adjoining figures or objects, necks, and long hair were erased. The resulting white background was made transparent so that all images could be placed on gray luminance-controlled Microsoft Powerpoint slides for luminance testing and further resizing. Special considerations while selecting the nonsocial object images included the removal or obstruction of brand names and the omission of face-like configurations (e.g., cars were not used because the headlights and grill resemble a face) that might elicit processing of language or faces. Special considerations while selecting the social stimuli included finding forward-facing views of faces and figures that displayed happy or neutral expressions. All images were contrast and color corrected after luminance testing so that they fell within the 25.0 to 25.9 lx range when measured with a commercial photometer. The initial baseline slide and the inter-stimulus gray slides emitted 25.5 lx.

The social and nonsocial drawing stimuli were all created by the investigator. The social drawings were created by using an online face morphing program (morphthing.com, Glam Entertainment) to combine the photo and figurine images into a composite image that drew equally from the traits of each image. Morphing the image was necessary to ensure that the drawing did not rely on one reference to a greater extent than another. The resulting composite image was then traced and colored using Adobe Photoshop Elements 8.0. Nonsocial drawing references were selected based on their canonical representation of the object. Nonsocial references were traced and colored. The coloration of drawings was limited to 3 tones per color, representing the base color, highlight, and shadow. Where more than 4 base colors were needed for the drawing, highlights and shadows were applied sparingly, i.e. to only one or two areas of the drawing.

Equipment and Setup

Pupil diameter and gaze location were recorded with a desktop Applied Sciences Laboratory Eye Tracking System, Series 6, with a video head tracker to keep the camera locked onto the subject's face. The system was equipped with a high-speed synchronizer set to take pupil and gaze measurements at a sample rate of 120 Hz. Pupil and gaze data from each subject was recorded with GazeTracker software that compared the gaze direction information from the ASL software to the area of the stimulus monitor to determine whether the child's gaze fell into circumscribed areas of interest for each image, which are described in detail below. The ASL software detected the child's distance from the eye camera, which ranged from 18" to 30" depending on the child's viewing posture. This resulted in a visual angle of 22.01 to 13.31 degrees at the stimulus.

The eye-tracking room consisted of two sections: the experimenter area and the participant area. The areas were separated by a large, neutral-colored barrier that did not allow subjects to see the experimenters. The experimenter area contained four monitors—one interfacing with GazeTracker, one to run the Applied Sciences Laboratory software, and two smaller monitors displaying real-time computational overlays of: 1) the camera's focus on the participant's pupil and corneal reflection (eye monitor), 2) a crosshair of the participant's point of gaze mapped onto the current stimulus (gaze monitor). The participant area was a neutral-colored area free of distracting decorations, and it contained a hydraulic seat with a five-point restraint and the stimulus monitor. During the testing session, this area was illuminated only by the stimulus monitor.

Standardized Tests

Each child received two standardized assessments: one standard test of cognitive ability (either the Mullen Scales of Early Learning or the Stanford Binet, 4th Edition) and one observational assessment of autism symptoms (Module 1, 2, or 3 of the Autism Diagnostic Observation Schedule). Parents reported their child's level of autism symptoms on the Social Responsiveness Scale as an additional measure of autism symptomology.

The Stanford Binet Intelligence Scales, 4th Edition (Stanford Binet). The Stanford Binet (Thorndike, Hagen, & Sattler, 1986) is a cognitive test that is composed of four cognitive areas which combine to produce a composite score. The subtest areas are Verbal Reasoning, Quantitative Reasoning, Short-Term Memory, and Abstract Visual Reasoning. Each cognitive area consists of several subtests which are administered depending on the subject's performance on the routing test of expressive vocabulary. In order to reduce frustration and testing fatigue, only one subtest from each cognitive area was administered and used to return the composite score. These were Oral Vocabulary (Verbal), Bead Memory (Short-Term Memory), Quantitative (Quantitative), and Pattern Analysis (Abstract Visual).

Several aspects of the Stanford Binet necessitated a separate test for younger children. First, the exclusion criteria for the ASD group for the current study was not based on verbal ability, so children without expressive language were recruited. These children tended to be in the younger age group between 2 years and 5 years old. Scores can be obtained on the Stanford Binet beginning at 2-years-old; however, in the current investigation children were administered the Stanford Binet beginning at age 5 years, 0 months until age 13 years, 5 months. Rather than use the Stanford Binet with children younger than 5 years, these children received the Mullen, described below. The use of two tests was intended to avoid floor effects for children with ASD who had expressive language delays that would cause them to receive no score on the Stanford Binet verbal routing test.

Mullen Scales of Early Learning (Mullen). The Mullen (Mullen; Mullen, 1995) is a standardized assessment of cognitive ability which returns scores for children from age 2 months to 67 months. The test comprises 5 subscales: Gross Motor, Visual Reception, Fine Motor, Receptive Language, and Expressive Language. Because the Gross Motor scale is not included in the calculation of the composite score, it was not administered in the current study. The remaining 4 subtests of the Mullen were administered to children from age 2 years, 0 months, until 4 years, 11 months, to avoid floor effects which would have likely occurred if the Stanford Binet were administered to young children with severely limited or delayed expressive vocabulary.

Autism Diagnostic Observation Schedule (ADOS). The ADOS (Lord, Rutter, DiLavore, & Risi, 2000) is a semi-structured play observation which allows the administrator to construct various situations that elicit behaviors associated with the autism spectrum diagnosis. The scores are based on the nature and severity of the child’s apparent behaviors, mannerisms, and speech during the testing session. The ADOS includes 4 modules designed for different levels of verbal ability, and the current study applied 3 of these modules. Module 1 is for children who are nonverbal or lack phrase speech, Module 2 is for children with phrase speech but not fluent language, and Module 3 is for children with fluent language. Modules 1 and 2 are primarily play-based and allow for freedom of movement around the room, whereas Module 3 includes a brief interview and more structured play activities. Each module returns scores of impairment in three core areas: Communication, Reciprocal Social Interaction, and Stereotyped Behaviors and Restricted Interests. An overall score can be computed and compared with standard diagnosis cutoffs to confirm the child's diagnosis within the autism spectrum.

Social Responsiveness Scale (SRS). The SRS (Constantino & Gruber, 2005) is a severity rating scale which indicates social impairments and previously observed behaviors relevant to the diagnosis of ASD. The 65-item rating scale is completed by a parent or guardian who is familiar with the child's current behavior and developmental history. The item ratings are applied to scores in the following areas: Social Communication, Social Awareness, Social Cognition, Social Motivation, and Autistic Mannerisms. These scores are combined to produce an overall standard score. This test further confirmed the diagnoses of the children in the ASD group, and provided a secondary perspective on the child's symptom severity.

Procedure

Recruitment. Male children were recruited through existing lab contacts with parent organizations and by public postings online and in the metropolitan area. Organizations and therapy groups were contacted and supplied with the recruitment letter (Appendix A) to distribute to their members or clientele. Shortened descriptions of the studies were also distributed to organizations to include in social media webpages or newsletters. The recruitment materials were posted on the lab website and the lab Facebook site, as well as on Craigslist in the Volunteer section. Flyers with pull-off tabs (Appendix B) were displayed on community message boards in multiple child-centered or child-friendly establishments within a 50-mile radius of the laboratory. An existing lab database composed of previous participants and commercial lists was queried for children within the age range of the study. The query results produced a list that was used to guide targeted emailing of the recruitment letter, followed by a phone call (Appendix C) one week later. Parents who did not have current email addresses on the list were sent the recruitment letter in the mail and received a follow-up call two weeks later.

Testing Session. Each session began with consent and video selection, and then proceeded to eye-tracking and standardized testing. Consent was provided by parents on the child’s behalf in accordance with Human Subjects Committee regulations (Appendix D), the subjects received compensation for time and travel, and parents filled out a lab-designed custom health and background questionnaire (Appendix E) to confirm current health and family history. During this time, the child became acclimated to the lab setting by playing with toys and talking with research assistants. Each child chose a DVD from the laboratory library that played while the researcher found their eye with the camera.

For the eye-tracking session, the child was led into the eye-tracking room and the assent protocol was followed (Appendix F) according to the child’s age group. Once assent was obtained, the child was secured into an age-appropriate seat in front of the stimulus monitor. The seats had a five-point restraint to assure that the child remained seated during the session with minimal changes in posture. If necessary, a neck pillow was offered to ensure that the child’s head remained upright during the session. The stimulus monitor played a DVD of the child’s choice. Parents could accompany their child into the eye-tracking room and sit outside the child’s field of vision during testing; however, siblings were not allowed in the room. Once the child was comfortable, the experimenter turned off the lights and moved to the experimenter area. The experimenter then used the eye camera to find the coordinates of the child’s left eye. The software locked onto the child’s eye, and the calibration procedure began.

The calibration sequence consisted of 9 animated points evenly spaced over the area of the stimulus presentation screen, which were revealed one at a time. As the child looked at each animated gaze point, their looking direction was calibrated based on their pupil position and the reflection of light on the cornea. After the initial calibration was obtained for each point and saved, successful calibration was visually confirmed by revealing each animated point a second time while observing the child's gaze direction in real-time on the ASL gaze monitor. The length of time required to obtain acceptable calibration varied depending on the size and shape of the subject's cornea, the reflection of the pupil, and the fidelity of continuous tracking despite movement of the subject. Thus, the calibration procedure could take 5 to 30 minutes. If calibration was not obtained after 3 attempts or 30 minutes, whichever occurred first, the child was given a break to play with toys while the eye-tracking system was restarted. When calibration was established and confirmed, the visual task was presented using GazeTracker software as described in the visual stimuli section.

After the eye-tracking session, the child and experimenter proceeded to a different room for standardized testing. The first test was an age-appropriate assessment of cognitive ability using the Mullen Scales of Early Learning or the Stanford-Binet (4th Edition), and a test of ASD symptoms and behaviors using the Autism Diagnostic Observation Schedule (ADOS). Children from the age of 2- to 4-years and 11-months old received the Mullen, and children from 5- to 13-years old received the Stanford Binet. Testing between age groups was broken up in this way in order to avoid floor effects from the administration of the Stanford Binet vocabulary section, which requires verbal responses, to nonverbal children. The ADOS module was chosen by the administrator after observing the child's general level of verbal ability (e.g., single words or phrase speech). At this time the parent also filled in the Social Responsiveness Scale if they had not already done so during the consent procedure. The entire lab visit usually took no longer than 2.5 hours to complete, and breaks were given to the child as needed. Second appointments were scheduled at the parents' convenience with full compensation if additional time was needed to complete the protocol.

Data Extraction and Reduction. Areas of interest, or *look zones*, were defined using the GazeTracker software. The look zones were freeform polygons set by hand in the Gaze Tracker software that outlined the contours of the areas of interest. Each look zone was offset from the edge of the area of interest by a ¾" margin of error to account for slight miscalibration of gaze direction. For the social stimuli, look zones were defined for areas of internal features (eyes, nose, and mouth) and the perimeter of the head (including hair, jawline and chin). For the nonsocial stimuli, the look zone was defined by the outer perimeter of the object. For comparisons between social and nonsocial images, the head look zone was used because it encompassed the whole image area. Therefore, the areas used for comparisons between social and nonsocial images were similar. For the analysis of attention to internal features versus external features, the time spent dwelling in the internal features was considered separately from the time spent dwelling on *only the external features* (that is, the portion of the head look zone that was mutually exclusive from the internal features look zone).

Looking time for each look zone was extracted from the GazeTracker output. The data output included all the time that the subject's eye was tracked; therefore, the output included time that was offscreen and time that was onscreen. Looking time toward images was automatically tagged with the appropriate look zone name by the Gaze Tracker software, and these tags were used to extract the total time spent looking at each look zone. Onscreen and offscreen time was distinguished using the dimensions of the stimulus monitor, and off screen gaze tracking was omitted from the analysis. Thus, the proportion of looking time toward each look zone was calculated based on sum total time in the look zone during the stimulus presentation in relation to the onscreen time tracked for the presentation of that slide.

Pupil size was continuously recorded in pixels, and then converted from pixels to millimeters based on an individualized accommodation factor. The accommodation factor was based on the child's distance from the eye camera. A 4mm model pupil was placed at the same distance as the child's left eye, and the pixel value given by the ASL system was recorded. This value was used to calculate the child's individual accommodation factor, a scalar which was multiplied by the pixel output for each child. Obtaining an individual accommodation factor was important in the current study due to the wide range of distances from the camera and the use of different seats based on the various sizes of children from age 2-13 years.

Pupil data traces were extracted from the GazeTracker output. Pupil traces in stimulus slides were defined as uninterrupted spans of 500ms or longer spent dwelling in a look zone. Baseline slides did not have circumscribed look zones, so pupil traces were defined as any continuous onscreen recording of 500ms or longer. Pupil artifacts arising from blinks, loss of tracking, partial closure of the eye, or sudden shifts in posture were linearly interpolated. Artifacts were identified in the data as jumps in time recording that exceeded 10ms or a difference in pupil size larger than 0.20 mm. Traces included in the final analysis comprised *at least* 500ms of continuous recording, and interpolated time was not permitted to exceed 500 ms per trace (if the trace was over 500ms in length) *or* constitute 20% or more of the trace.

Phasic pupil size was based on the change in pupil size from baseline that occurred while viewing the stimulus slide. Pupil size averages were calculated per stimulus slide and per baseline slide. Overall tonic pupil size for each subject was calculated based on the average of all baseline slides. Phasic pupil size was calculated based on the average change score for each stimulus type. Pupil change scores were obtained for stimulus slides by subtracting the preceding baseline pupil average from the pupil average of the stimulus slide. If the preceding baseline slide contained no pupil data, the previous baseline slide was used. If the baseline pupil slide two slides before the stimulus slide contained no data, the pupil difference was not calculated for that stimulus slide due to insufficient comparison data.

Results

Tonic Pupil Size

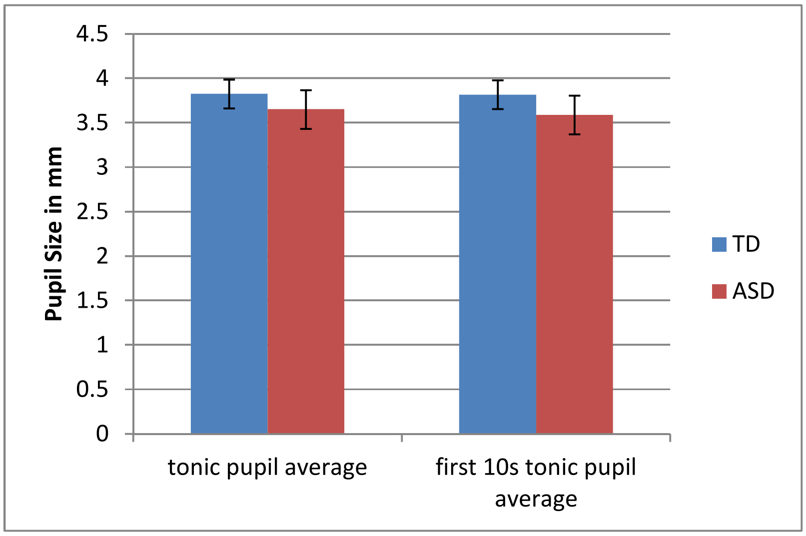
The first objective in the investigation was to attempt to replicate larger tonic pupil sizes in children with ASD. A univariate ANCOVA tested for differences in Average Tonic Pupil Size in mm by Diagnosis. Because older children could have more developed eyes—and therefore larger pupil sizes than younger children—Age in Months was included as a covariate. One outlier was identified and eliminated from the analysis (ASD, $n = 1$). The results showed no effect of diagnosis ($F^1(1,32) = .239$, $p = .628$, $\eta^2 = .032$). The tonic pupil size means were comparable between TD ($M = 3.75$, $SD = .64$) and ASD ($M = 3.64$, $SD = .81$) groups. Inferring from the overall test of this sample, data from this study replicated neither the larger tonic pupil size reported in a sample of 2 to 5-year-olds with ASD (Anderson & Colombo, 2008) nor the smaller tonic pupil size in a sample of 3 to 15-year-olds with ASD (Martineau et al., 2011).

Because the Average Tonic Pupil Size could have been influenced by spillover effects from the stimulus slides, baseline pupil measurements taken during a 10s neutral gray slide at the beginning of the testing session were also compared across groups. The short interstimulus measurement periods (varying from 2s to 5s, averaging 3.5s) may not have allowed sufficient time for pupil reactions to return to tonic activity between stimulus slides, which would have contaminated the tonic pupil average based on interstimulus trials. The same analysis used previously (i.e., univariate ANCOVA), was applied to the First 10s Baseline measurement, but again no differences were observed for diagnosis, $F^{1}(1,26) = .873$, $p = .359$, $\eta^2 = .032$, when Age in Months was used as a covariate. Thus, mean pupil size during the first 10 seconds of recording also showed no differences between the TD ($M = 3.82$, $SD = .68$) and ASD ($M = 3.59$, $SD = .84$) groups. Children without adequate pupil measurements for First 10s Baseline (ASD, $n = 3$) and children who were outliers on this variable (ASD, $n = 3$; TD, $n = 1$) were not included in this analysis. The non-significant results for the First 10s Baseline lend increased confidence for the null findings for average tonic pupil differences by Diagnosis (Figure 1).

The association between tonic pupil size and demographic variables was examined to better characterize sources of variance within tonic pupil measures. The wide age range of the current sample was used to predict tonic pupil size for each diagnostic group, but there was no linear relationship for either tonic pupil measure with age (both ps $> .05$). Neither tonic pupil measure correlated with any of the standardized scores or sub-scores for cognitive ability or autism symptomology. Thus, the non-replication of the tonic pupil measures cannot be adequately explained by trends in age, cognitive ability, or autism symptomology.

Figure 1

Tonic Pupil Size by Diagnosis



Note. Tonic pupil presented in means. Error bars represent standard errors per group.

Phasic Pupil Response

Omnibus repeated measures. To investigate the phasic pupil response to social and nonsocial stimuli, the average change in pupil size for each image type was compared using a repeated-measures ANOVA which included factors of Stimulus Type (3: photo, figure, drawing) and Sociality (2: social and nonsocial). Between-subjects variables included Diagnosis and Age Group (means presented in Table 3). Because the ANOVA procedure does not tolerate missing cell values, some participants were excluded based on missing phasic pupil data in one or more cells (ASD older, *n* = 2; ASD younger, *n* = 5).

The results of the omnibus test found a complex interaction between the variables of interest and main effects for all variables except for Diagnosis. A significant four-way interaction was found between Sociality, Stimulus Type, Diagnosis, and Age Group, $F^2(1.960, 49.001) = 3.732$, $p = .032$, $\eta^2 = .130$, which precluded straightforward interpretation of the main effects. Sociality and Stimulus Type affected the mean pupil difference within all subjects. Significant differences were found for Sociality ($F^2(1, 25) = 6.166$, $p = .020$, $\eta^2 = .198$) and for Stimulus Type ($F^2(1.830, 45.750) = 4.833$, $p = .015$, $\eta^2 = .162$), but the interaction between Sociality and Stimulus Type was not significant ($F^2(1.960, 49.001) = 1.417$, $p = .252$, $\eta^2 = .054$); therefore, the within-subjects pupil reaction to social and nonsocial images did not vary depending on whether the image was a photo, figure, or drawing. All other within-subjects interactions were not significant (all *p*s > .05). Pairwise comparison of the marginal means for Sociality showed that social images elicited larger changes (*M* = .210, *SE* = .023) than nonsocial images (*M* = .141, *SE* = .023). Furthermore, pairwise comparison of Stimulus Type exhibited an unexpected pattern of means among photos (*M* = .227, *SE* = .027), figures (*M* = .132, *SE* = 0.23), and drawings (*M* = .166, *SE* = .026). The relationship between these means did not correspond linearly to the level of image complexity. Rather, the differences within Stimulus Type appear to be driven by a significant mean difference between photos and figures ($p = .002$), with drawings showing no significant differences from either photos or figures (both *p*s > .05). The finding of large differences within subjects on photos and figures—although not between photos and drawings—was unexpected and may indicate that the image categories were qualitatively different from one another in unintended ways.

Table 3

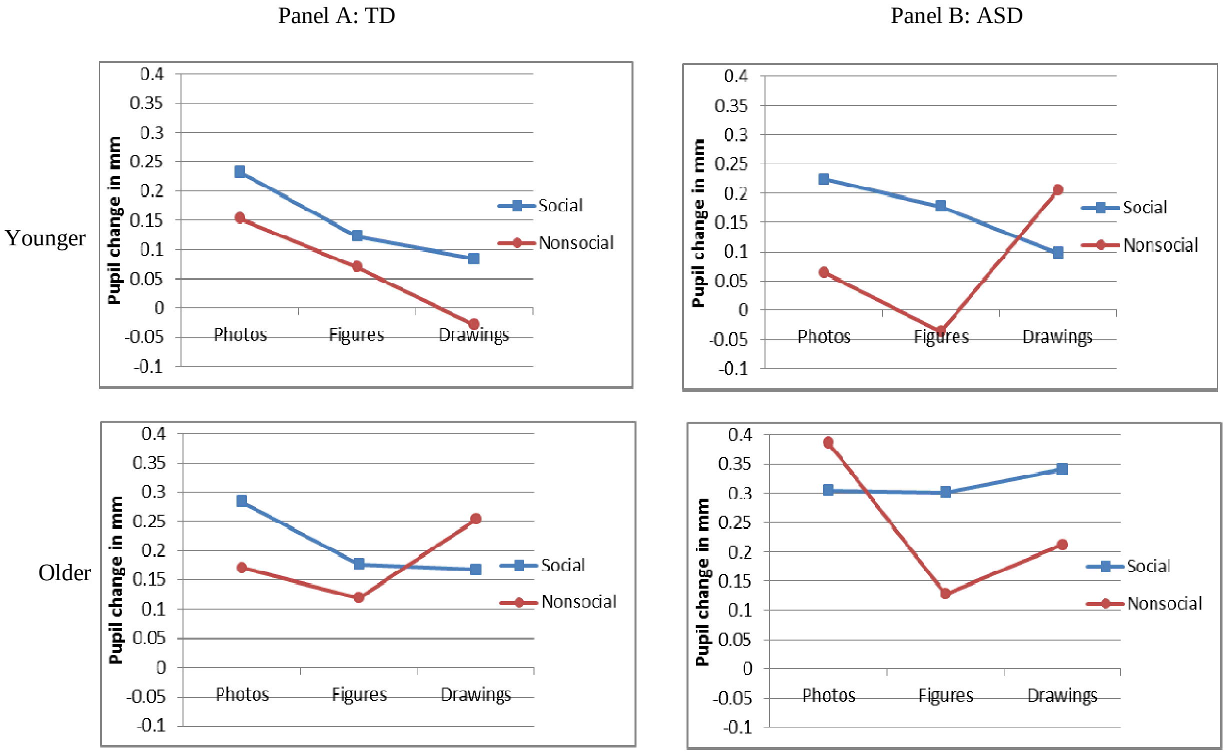
Phasic Pupil Size for Each Stimulus Type

		TD				ASD		
		All TD	Younger	Older		All ASD	Younger	Older
		n = 18	n = 11	n = 7		n = 11	n = 6	n = 5
	Means							
	Photo	0.252 (.11)	0.2316 (.13)	0.2841(.08)		0.2611 (.27)	0.2245 (.33)	0.305 (.19)
Social	Figure	0.1442 (.18)	0.1231 (.17)	0.1773 (.19)		0.2335 (.14)	0.1771 (.04)	0.3012 (.20)
	Drawing	0.1168 (.19)	0.0841 (.21)	0.1681 (.17)		0.2081 (.17)	0.0969 (.10)	0.3415 (.13)
	Photo	0.1596 (.16)	0.1526 (.12)	0.1705 (.21)		0.2107 (.27)	0.0639 (.20)	0.3868 (.26)
Nonsocial	Figure	0.0891 (.14)	0.0704 (.11)	0.1186 (.18)		0.0384 (.19)	-0.0366 (.22)	0.1283 (.10)
	Drawing	0.0808 (.22)	-0.0294 (.14)	0.2539 (.21)		0.208 (.25)	0.2046 (.26)	0.2121 (.27)

Note. Data are presented in means. Standard deviations are in parentheses. TD = typically developing, ASD = autism spectrum disorder.

Figure 2

Phasic Pupil Size by Diagnosis and Age



Note. Data presented as means.

The between-subjects factors distinguished significant group differences between older and younger children (Age Group), but not between children with and without ASD (Diagnosis effect, $F^2(1,25) = 1.851$, $p = .186$, $\eta^2 = .069$). The main effect for Age Group was significant, $F^2(1,25) = 11.317$, $p = .002$, $\eta^2 = .312$. The Age x Diagnosis interaction effect was not significant, $p = .369$, meaning that the effect of Diagnosis on pupil changes does not differ with Age, which coincides with the *a priori* hypothesis. The older children of each diagnosis tended to have larger pupil dilations to the stimuli ($M = .237$, $SE = .028$), than the younger children ($M = .114$, $SE = .024$). Larger pupil size in older children was not a hypothesized outcome, but might be explained simply by growth and development of the eyes.

Comparing Age Groups within Diagnosis. To clarify the interaction between participant age and diagnosis obtained in the 4-way interaction, the sample was split by diagnostic groups and analyzed with one-way ANOVAs across the factor of Age Group. First, pupil responses were considered for the TD group in Panel A of Figure 3. The older and younger TD children did not respond differently to social stimuli of any type (photo, figure, or drawing), nor did they respond differently to nonsocial photos or nonsocial figures, all *p*s > .05. However, nonsocial drawings elicited differential pupil responses depending on age, $F^3(1, 9.618) = 10.216$, $p = .01$. That is, the younger group exhibited pupil constriction ($M = -.029$, $SE = .043$) and the older group exhibited pupil dilation ($M = .254$, $SE = .077$). Nonsocial drawings of common inanimate objects appear to elicit greater demands on cognitive resources, or simply greater interest, after the age of 7 years in TD children. A previous cross-sectional pupil study with a TD population suggests that maturation of the autonomic nervous system increases pupil responses over the early life span (Karatekin, Marcus, & Couperus, 2007).

The ASD group was analyzed for effects of age on pupil response to stimuli (Panel B, Figure 1). Missing cell data precluded inclusion of some subjects for each analysis (younger, $n = 3$; older, $n = 1$). Children with ASD responded similarly irrespective of age to nonsocial figures and drawings. However, the nonsocial photos elicited larger pupil responses in the older children ($M = .397$, $SE = .094$) than the younger children ($M = .129$, $SE = .074$), $F^3(1, 10.261) = 5.013$, $p = .048$. The responses to social photos and social figures did not differ depending on Age Group. However, drawings of human faces appear to elicit greater pupil responses from children with ASD after age 7 years, $F^3(1, 9.760) = 7.312$, $p = .023$. Older children responded with greater dilation than younger children towards social drawings (older, $M = .313$, $SE = .056$; younger, $M = .127$, $SE = .040$). This may indicate autonomic maturation similar to that which takes place in

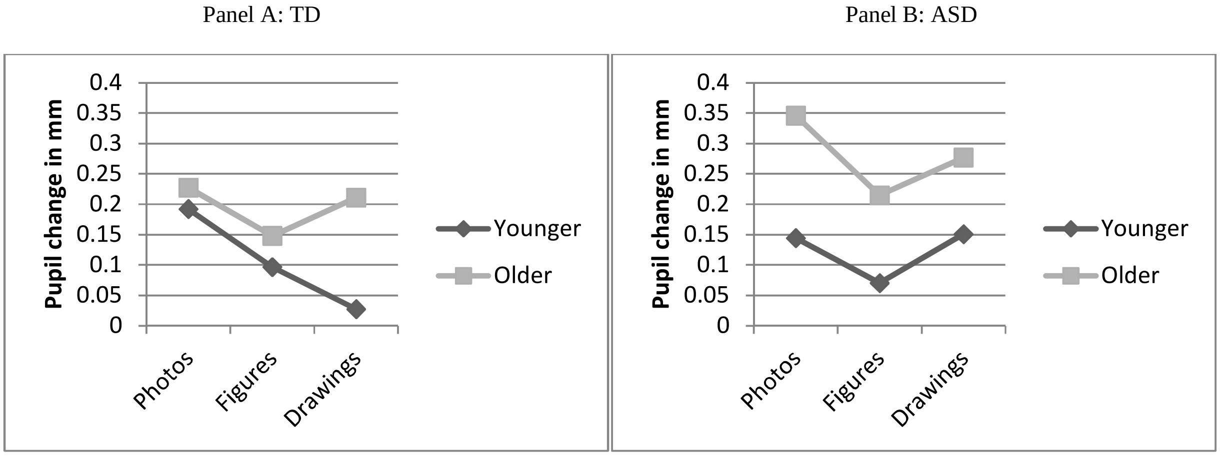
TD children. Thus, children with ASD increase their pupil responses with age, especially to the least complex social stimuli presented.

Comparing Stimulus Dimensions within Diagnosis. Individual repeated measures one-way ANOVAs (Age Group included as between-subjects factor) were performed for the ASD group and the TD group to investigate the within-subjects interaction effect of Stimulus Type and Sociality. Children in the ASD group were excluded from the analysis if they did not have values on one or more of the pupil response scores (excluded younger, $n = 5$; excluded older, $n = 2$). There were no significant effects or contrasts of Stimulus Type, Sociality, or Age Group on phasic pupil size within the ASD group (*p*s > .05). The absence of a pattern of could indicate dysregulation of the ANS in children with ASD.

The repeated measures ANOVA for the TD group revealed the expected pattern of pupil response to the stimulus types. The TD group showed a significant effect of Stimulus Type on phasic pupil difference, $F^2(1.698, 27.173) = 5.185$, $p = .016$, $\eta^2 = .245$. Moreover, the linear contrast for Stimulus Type also yielded significant results, $F^2(1, 16) = 6.019$, $p = .026$, $\eta^2 = 273$, showing that the trend of dilation across varying levels of complexity was approximately linear (Panel A, Figure 3). However, the pairwise comparisons of each Stimulus Type were not significant when alpha was corrected for familywise control of Type I error with Holm's Sequential Bonferroni Method ($\alpha_1 = .167$ for 3 comparisons, $\alpha_2 = .25$ for two comparisons). The pairwise comparison between photos and figures are marginally significant at $p = .017$, which would have indicated that the TD children dilated more overall for photos than figures. Furthermore, the pairwise comparison of photos with drawings is also marginally significant, $p = .026$, which again would have indicated larger pupil sizes in response to photos than to drawings. Age group also showed a significant main effect, $F^2(1,16) = 5.429$, $p = .033$, $\eta^2 = .253$, with older children's pupils dilating to a greater extent (*M* = .195, *SE* = .030) than younger children's pupils (*M* = .105, *SE* = .024). However, there was not a significant interaction between Age Group, Sociality, and Stimulus Type, meaning that the differential response to nonsocial drawings between age groups does not constitute a separate looking trend within the TD group (*p* = .276). There was not an effect of Sociality (*p* > .05) or any combination of interactions.

Figure 3

Stimulus Type by Age and Diagnosis



Note. Data presented in means collapsed over Sociality.

The within-group findings for the TD children in response to different stimuli types partially supported predictions that children would respond with a larger pupil size to photos compared to drawings, and that pupil responses to figures will be somewhere in the middle. However, the prediction that children will respond with more dilation to social stimuli than to nonsocial stimuli was not supported for either diagnostic group. Overall, the evidence from the within-group analysis supports the theory of increased dysregulation in children with ASD.

Standardized test correlations with phasic pupil differences. Pearson correlations (two-tailed) were obtained for the association between the phasic pupil responses with composite standardized test scores for intelligence (the Mullen and the Stanford Binet) and standard scales of autism symptoms (the ADOS and the SRS). With a few exceptions, correlations did not attain significance. In the TD group, none of the correlations with cognitive assessment composite scores (CS) were significant. For the ASD group, the pupil response to the social photo stimulus was strongly and positively correlated with CS, *r*(12) = .676, *p* = .008. Social photo pupil response was negatively correlated with ADOS Communication, *r*(13) = -.622, *p* = .013, and ADOS Total, *r*(13) = -.579, *p* = .024. Thus, higher severity of ASD symptoms was associated with smaller pupillary response to these images. Engaging cognitive effort in passive viewing of social photos appears to increase with CS and decrease with autism severity, specifically in the domain of communication impairment. The correlation between nonsocial drawing pupil difference and ADOS Communication was also negative, *r*(12) = -.569, *p* = .034. The negative association between communication impairment and engagement with nonsocial drawings in the ASD group is interesting given the increased engagement with nonsocial drawings over age in TD children.

Scanning Measures

Omnibus repeated measures. The proportion of looking time at the social and nonsocial images was examined with a repeated measures ANOVA in order to determine whether social looking was reduced in children with ASD. The within-subject levels were Stimulus Type (3:photo, figure, and drawing) and Sociality (2:social and nonsocial). Between subjects, the Age Group and Diagnosis were compared. TD children had no missing data for looking proportion measurement, but some children with ASD were missing looking proportions for one or more stimulus types, and were not included in this analysis (ASD younger, *n* = 2; ASD older, *n* = 2).

The omnibus repeated measures test produced significant two-way interaction and two main effects on the variables not involved with the interaction. The interaction between Age and Stimulus Type was significant, *F*[4](1.593, 44.609) = 3.924, *p* = .035, η^2 = .123, although the main effects of each variable involved were not significant (both *p*s > .05). The within-subjects omnibus analysis yielded a significant main effect for Sociality, *F*[4](1,28) = 10.112, *p* = .004, η^2 = .265, but the interaction between Sociality and Stimulus Type was not significant. Thus, the time spent looking at social and nonsocial images did not vary based on the image type. Pooling all subjects, social images garnered a greater proportion of looking time, *M* = .876, *SE* = .024, than nonsocial images, *M* = .818, *SE* = .027, (Figure 5). The interaction between Sociality x Diagnosis x Age Group was not significant, *F*[4](1,28) = .832, *p* = .370, η^2 = .029. As such, the prediction that older children with ASD would show a looking time pattern of avoidance specific to social images was not supported.

Table 4

Proportion of Time spent on Stimuli

	TD			ASD		
	All TD	Younger	Older	All ASD	Younger	Older
	n = 18	n = 11	n = 7	n = 14	n = 9	n = 5
Means						
S Photo	.943 (.59)	.937 (.07)	.953 (.49)	.793 (.17)	.770 (.18)	.834 (.15)
S Figure	.936 (.07)	.918 (.09)	.963 (.04)	.794 (.21)	.767 (.20)	.842 (.23)
S Drawing	.932 (.11)	.910 (.14)	.965 (.04)	.802 (.26)	.748 (.29)	.899 (.17)
NS Photo	.905 (.10)	.904 (.10)	.908 (.10)	.714 (.19)	.690 (.17)	.757 (.24)
NS Figure	.907 (.11)	.909 (.11)	.903 (.13)	.751 (.21)	.708 (.20)	.829 (.23)
NS Drawing	.879 (.18)	.851 (.22)	.923 (.10)	.679 (.29)	.587 (.32)	.844 (.12)

Note. Data presented in means. Standard Deviations presented in parentheses. TD = typically developing, ASD = autism spectrum disorder. S = social stimuli. NS = nonsocial stimuli.

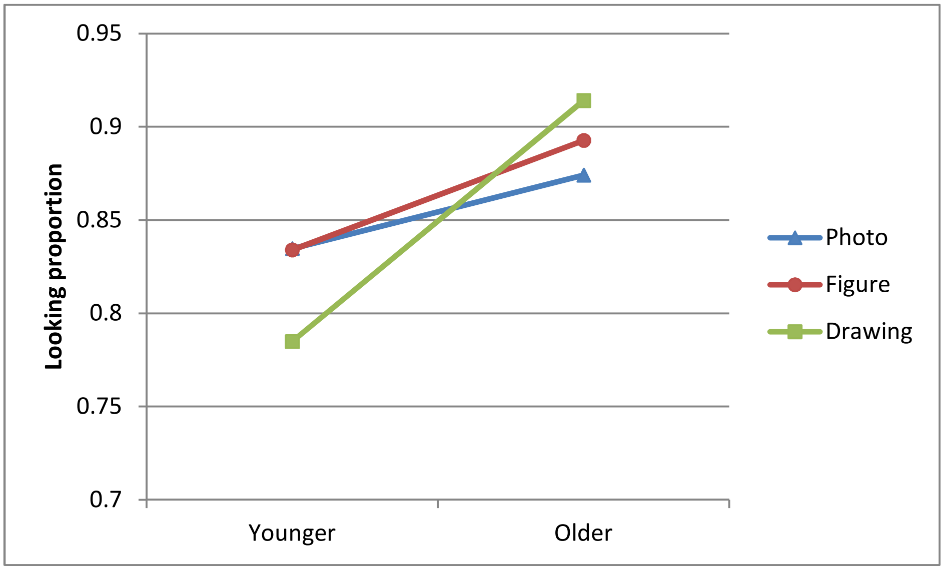
Examination of between-subjects effects revealed that Diagnosis, but not Age Group, had a significant effect on looking time to all stimuli. That is, an effect of Diagnosis was significant for all looking proportions, $F^4(1, 28) = 9.178$, $p = .005$, $\eta^2 = .247$. Children with ASD looked at all stimuli for a smaller proportion, $M = .773$, $SE = .037$, of their tracked onscreen time than the TD children, $M = .920$, $SE = .037$, (Figure 6). This finding contrasts with previous research in our lab that showed no visual scanning differences between children ages 2- 5 years with ASD and TD children. Developmental changes in attention to stimuli were not supported, as the effect of Age Group ($F^4(1,28) = 2.500$, $p > .1$, $\eta^2 = .082$) and all associated interactions failed to reach significance (all *p*s > .025).

Age Group by Stimulus Type Interaction. In order to explore the effect of stimulus type within each age group, the patterns of looking proportion toward each type (collapsed on Sociality) was examined separately within each Age Group (collapsed on Diagnosis) with two repeated-measures ANOVAs. Neither Age Group yielded significant looking trends based on Stimulus Type, p^5s > .05.

The between Age Group differences on each Stimulus Type were compared in separate univariate ANOVAs. Looking proportions toward each Stimulus Type did not differ by age (p^6s > .05). Upon further investigation, the interaction between Age and Stimulus Type appears somewhat spurious (Figure 4).

Figure 4

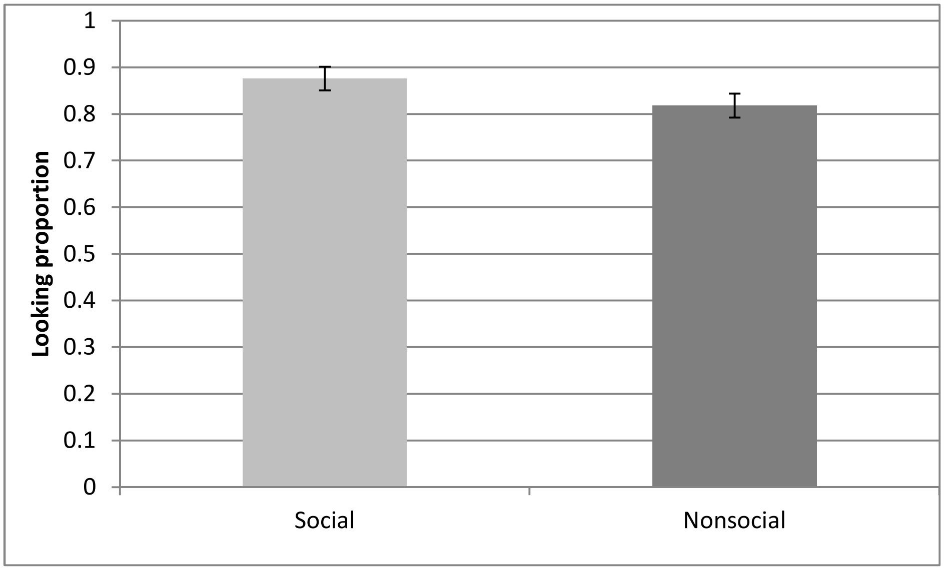
Age by Stimulus Type Interaction for Looking Proportion



Note. Means of data collapsed over Diagnosis and Sociality.

Figure 5

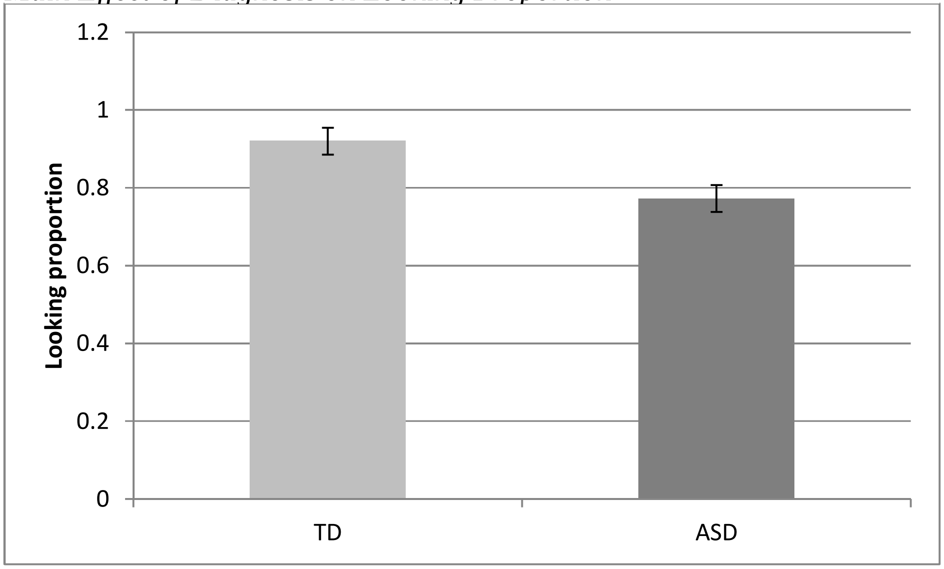
Main Effect of Sociality on Looking Proportion



Note. Results are collapsed over Diagnosis, Age Group, and Stimulus Type. Social = face stimuli. Nonsocial = objects stimuli. Error bars represent standard errors per stimulus type.

Figure 6

Main Effect of Diagnosis on Looking Proportion



Note. Results are collapsed over Age Group, Stimulus Type, and Sociality. TD = typically developing, ASD = autism spectrum disorder. Error bars represent standard error per group.

Standardized test correlations with proportion of looking time. Pearson correlations (two-way) were computed for the relationships between looking proportions to stimuli and standard composite scores of cognitive ability (the Mullen and the Stanford Binet) and scales of autism severity (ADOS and SRS). All pairs of variables rendered nonsignificant correlations unless discussed further here. For the TD group, no standardized test scores correlated with looking proportions. For children with ASD, the social photo looking proportion was negatively correlated with ADOS Social Interaction ($r[15] = -.505$, $p = .039$), ADOS Communication ($r[15] = -.576$, $p = .016$), and Total ADOS ($r[15] = -.603$, $p = .010$). Social photo looking was positively correlated with CS, $r(14) = .569$, $p = .021$. Social figure looking proportion was significantly negatively correlated with ADOS Communication ($r[16] = -.603$, $p = .008$), and Total ADOS ($r[16] = -.559$, $p = .016$). Looking to social figures was positively correlated with CS, $r(15) = .550$, $p = .022$. Taken together, longer looking times toward social stimuli in the ASD group are associated with lower impairment on the ADOS and higher CS scores. Sustained looking toward more detailed social images might draw on attentional resources that are impaired in ASD, yet essential to performance on standard tests of mental ability.

Correlations with looking proportion and pupil response. To assess the potential relationship between looking time and pupil activity, two-way Pearson correlations were calculated between the proportional looking time to each type of stimulus and the corresponding pupil change for that type of image. For the TD group, there were no significant correlations between the proportions of time spent looking at an image and the concurrent pupil responses to that image. For the ASD group, looking time and pupil response was positively correlated for only one image type, social photos (r[13] = .574, p = .025). This correlation may indicate that when children with ASD looked longer at social photos, they also tended to engage in more active processing of the image. Conversely, greater interest in social photos might have led children with ASD to look at them longer.

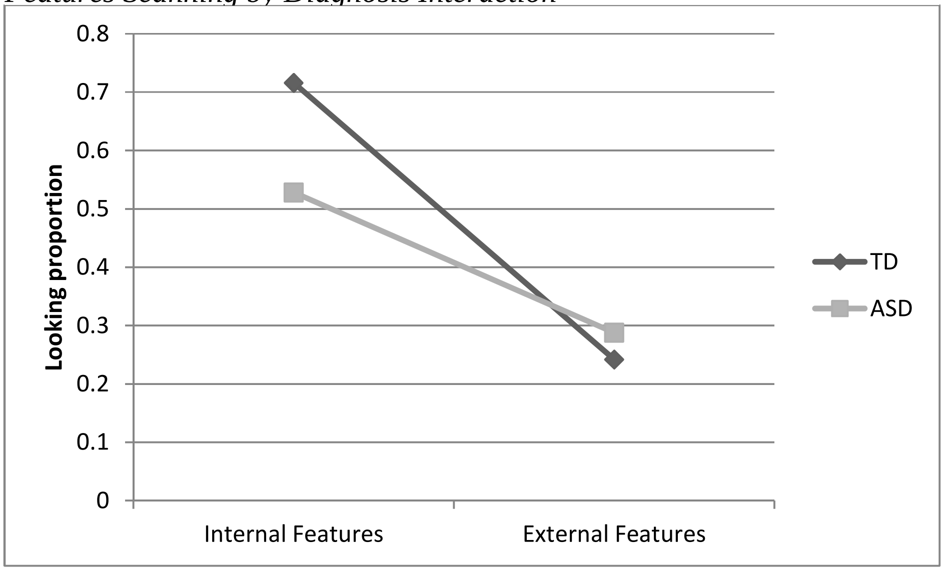
Internal Feature Scanning

Omnibus repeated measures. Looking time to the internal features of the face was of particular interest to the current investigation, such that an Age Group effect on scanning internal features would support a specific developmental ASD looking profile. A repeated-measures ANOVA incorporated 2 levels of look zone (internal features and external features) and 3 levels of Stimulus Type (photo, figure, and drawing), with Diagnosis and Age Group as between-subjects factors. There were 3 subjects with ASD who had missing data for one or more of the look zones, so they were unable to be incorporated into the analysis (ASD younger, $n = 3$).

The within-subjects indicated that all children looked longer at the features than to the external features area, but that the relationship between looking proportion to internal features and external features differs by diagnostic group. A Look Zone x Diagnosis interaction was significant, $F^{7}[1,29] = 5.194$, $p = .032$, $\eta^2 = .152$. A closer examination of the mean differences showed that TD children looked longer at the internal features ($M = .479$, $SE = .015$) than the children with ASD ($M = .408$, $SE = .016$), but that the trend for the external features look zone was the opposite—the TD children actually looked at this area slightly less ($M = .234$, $SE = .030$) than the children with ASD ($M = .290$, $SE = .033$). The reversal of this trend makes sense in the context of these variables—the internal features and the external features look zones were mutually exclusive and presented simultaneously, and thus competed for attention (Figure 7). Therefore, greater proportional onscreen time spent looking at the internal features would be expected to have a somewhat negative relationship with looking proportion toward the external features of the head. The within-subjects analysis yielded significant effect of look zone, $F^7(1, 29) = 46.952$, $p < .001$, $\eta^2 = .618$. Children looked longer at the internal features ($M = .622$, $SE = .034$) than at the external features ($M = .265$, $SE = .021$). Diagnosis was significant as a between-subjects factor ($F^7[1,29] = 10.282$, $p = .003$, $\eta^2 = .262$). This differential trend in looking toward the internal features of the face was not accompanied by significant main effects of Stimulus Type or Age Group (*p*s > .1) and as such constitutes a generalized response to face stimuli.

Figure 7

Features Scanning by Diagnosis Interaction



Note. Results are presented in means collapsed over Age Group and Stimulus Type. TD = typically developing, ASD = autism spectrum disorder. Internal Features and External features refer to look zones on the face stimuli.

Comparing look zones between Diagnosis. Two follow-up repeated-measures ANOVAs explored the internal features and head look zones collapsed across stimulus type with Diagnosis as the sole factor (Table 4). The Features analysis showed that looking time to the internal features of the face differed by diagnosis, $F^8(1,32) = 10.356$, $p = .003$, $\eta^2 = .244$, with TD children ($M = .720$, $SE = .047$) looking longer than children with ASD ($M = .500$, $SE = .050$).

The proportion of looking towards the external features was not significantly different by Diagnosis ($p > .1$).

Look zone effects within Diagnosis. A repeated measures ANOVA was performed for each diagnostic group separately to determine whether the difference between the internal features and external features look zone varied within the group. Within the TD group ($n = 18$), proportional looking time to each look zone was significantly different, $F(1,17) = 49.533$, $p < .001$, $\eta^2 = .744$. The mean looking proportion to internal features ($M = .720$, $SD = .17$) within the TD group was larger than the mean looking proportion to the head ($M = .233$, $SD = .13$). For the ASD group ($n = 15$), the difference between the internal feature and external features look zone was also significant, $F(1,14) = 9.691$, $p = .008$, $\eta^2 = .409$. This indicates that the mean looking proportion to the internal features look zone ($M = .526$, $SD = .21$) was larger than the mean looking proportion to the external features look zone ($M = .291$, $SD = .10$). Taken with the between-group analysis of looking proportion to specific look zones, the interaction effect in the omnibus test might indicate that although most of the time looking onscreen is spent in the internal features look zone in both diagnostic groups, the TD children spend much more time in the internal features look zone than the children with ASD. Because these look zones are non-overlapping areas presented at the same time, they compete for attention in such a way that the shorter amount of time children with ASD spent in the internal features look zone would be expected to accompany a complementary increase in the proportional amount of time spent looking at the external features look zone.

Discussion

The goal of the current study was to incorporate a developmental perspective into previous work on the autonomic responses of children with ASD, and to determine whether responses varied to different types of social images and their attention to these images. Social and nonsocial images were varied categorically (photos, figures, and drawings) so that the effect of stimulus detail on processing could be assessed. The predictions of this study were formed based on previous research that suggested differential models of arousal for children with ASD and TD children, which may have had downstream effects on looking times at older ages of children with ASD. First, children with ASD were predicted to show baseline, or tonic, dysregulation of the autonomic nervous system, which has been reported to be manifest in larger tonic pupil sizes and higher amplitude on other psychophysiological measures (Anderson & Colombo, 2008; Chang et al., 2012). The second prediction involved phasic pupil reactions to stimuli. Such phasic reactions are taken to represent cognitive effort; phasic pupil size has also been reported to be dysregulated in children with ASD, compared to TD, when viewing social stimuli (Anderson, Colombo, & Shaddy, 2006). Phasic responses were used in the current study to investigate the demands of stimuli at various levels of detail (i.e. photos, figures, and drawings of faces and objects). Previous studies with reduced-detail images, like cartoon and avatar faces, showed that individuals with ASD responded similarly to TD children in eye-tracking (van der Geest et al., 2002) and face-inversion paradigms (Rossett et al., 2010). Given that reactions to real faces vary in children with ASD, this suggests that the level of image detail may hold a clue to processing deficits in ASD; such simpler stimuli might not elicit atypical levels of arousal, and predicts that divergence from TD children might increase with the level of complexity or realism of facial stimuli. Furthermore, a developmental trend may be present in ASD to scan faces less (see Falck-Ytter & von Hofsten 2010 for a review); studies with younger ASD samples tend to find few differences in face scanning, whereas avoidance of internal features and eye regions was found in older populations with ASD. Accordingly, we predicted that young children with

ASD would not differ from TD children in scanning faces, and that differences would emerge as ASD children approached adolescence. This was expected to occur concurrently with phasic pupil dysregulation in response to social stimuli, but not necessarily to nonsocial stimuli.

The current study yielded the following findings specific to ASD: (a) children with ASD showed larger pupil responses to social drawings in the older age group (b) there was no monotonic relationship between pupil dilation and level of social stimulus detail for children with ASD as was observed in TD children, (c) older children with ASD exhibited pupil dilation to social drawings to a greater extent than younger children with ASD, (d) children with ASD showed shorter proportion of time onscreen tracked to social, nonsocial, and internal face features look zones compared to TD children, irrespective of age, and (e) children with ASD showed shorter looking times to nonsocial images than to social images, while TD children show no difference in looking times.

The results do not replicate previous studies from the same lab, which found no scanning differences for children ages 2 to 5 years with ASD (Anderson, Colombo, & Shaddy, 2006) and larger tonic pupil size in children with ASD (Anderson & Colombo, 2008). The current results do not support the hypotheses forwarded in the introduction, but they are somewhat consistent with the notion that children with ASD exhibit autonomic dysregulation that may be reflected by measures of pupil size. Furthermore, children with ASD generally spend less time examining stimuli than TD children, but when children with ASD do examine stimuli, they look at social images longer than nonsocial images.

Tonic Pupil

Tonic pupil size indicated no baseline autonomic dysregulation in children with ASD from the ages of 2 to 13 years. Furthermore, neither measure of tonic pupil size was significantly associated with age, standardized assessments of cognitive ability, or severity of autism symptoms. Previous investigations of tonic pupil size in children with ASD found tonic dysregulation of the pupillary system in different light conditions in age- and CS-matched samples of children with ASD in toddlers and adolescents (Anderson & Colombo, 2008; Martineau et al., 2011). Comparing the current study to these investigations, several reasons stand out for accounting for this non-replication. First, the short interstimulus intervals could have allowed a spillover effect of the stimuli to contaminate the overall average baseline pupil, although diagnostic group differences were not observed during the first 10s of baseline pupil measurements. Second, the lack of CS-matching between diagnostic groups in the current study could have allowed greater heterogeneity of mental abilities in the ASD group to interfere with obtaining valid comparison of tonic pupil size, although neither of the tonic pupil size measures correlated with either measure of CS, nor are tonic measures predicted by CS. Third, the current study encompasses a wide range of ages as opposed to the narrow, young age range in Anderson & Colombo (2009). The current sample comprises only 3 children with ASD in the 2 – 5 year age range, whereas the previous study used 9 children with ASD. Therefore, this study might not have had sufficient power, at least within younger children, to yield significant tonic pupil differences. However, these findings imply that tonic pupil differences may not represent a systemic facet of the ASD phenotype at all ages and within lab-controlled contexts. More work is necessary to decipher the nature of baseline autonomic dysregulation in children with ASD. At the present time, tonic pupil response cannot be considered a systemic indicator of ASD.

Phasic Pupil

The phasic pupil findings are in general accord with the hypothesis of autonomic dysregulation in ASD, but they do not replicate the specific previous findings of smaller phasic pupil changes toward social images. Children with ASD presented autonomic dysregulation in a less systematic way in the current study. There were no pupil response differences between diagnostic groups, which is consistent with Martineau et al's.(2011) finding using a pupil wave-form analysis showing no group differences between ASD, CA-matched and CS-matched groups. Furthermore, group differences on phasic pupil responses have been known to vary in direction depending on the ecological validity of the stimulus, with ASD pupil dilation in response to videos (Anderson, 2010) and constriction in response to static photos (Anderson, Colombo, & Shaddy, 2006). Therefore, the direction of phasic pupil differences in ASD might be more labile than indicated by previous findings. The current study limited analysis to differences in average responses toward stimulus slides when compared with the preceding baseline average; however, an analysis of pupil wave forms throughout the entire viewing of the stimulus slide may be warranted. Such an analysis would indicate whether divergent average findings actually reflect more extreme amplitudes of pupillary response in children with ASD, as hypothesized by Intense World Syndrome (Markram & Markram, 2010).

Monotonically increasing pupil sizes were found in TD children in response to social drawings, social figures, and social photos. The TD group responded to increasing level of stimulus demands with increased pupil size, indicating engagement with the social stimuli. Children with ASD, however, exhibited no such trend for social or nonsocial stimuli. As such, children with ASD show no evidence of recruiting cognitive resources to process stimuli of increasing complexity and social salience. Alternatively, increasing visual realism of the stimuli did not appear to capture increasing interest in the ASD sample as it did in the TD sample. This different pattern of response to stimulus type within social stimuli mirrors the lack of sensitivity to face gaze direction found by Sapeta et al. (2012) in children from ages 8 – 18 years with ASD. Children with ASD engage no more or less with a face due to its graphic representation, although this pupil evidence does not preclude the possibility that children with ASD employ different processing styles with each type of face (e.g., configural processing of internal features with drawings, as implied in Rossett et al., 2008). Thus, dysregulation apparent in ASD processing of different types of social stimuli may stem from atypical strategies for processing faces that require less cognitive effort. Furthermore, the correlation between phasic social photo response and CS for the ASD group supports the position that CS-matching would have helped this work's consistency with phasic pupil results from our lab.

Tonic pupil size analyses did not provide evidence for resting state autonomic dysregulation in children with ASD, but the complementary dynamics of tonic pupil size with phasic pupil size throughout the session may help explain this discrepancy. A somewhat complementary relationship exists between tonic pupil size and phasic pupil response as a function of the subject's control state, that is, the subject's level of engagement with the task as time passes (Aston-Jones & Cohen, 2005; Gilzenrat, Nieuwenhuis, Jepma & Cohen, 2010, Laeng, Sirois, & Gredeback, 2012). Decreasing task utility and an attentional shift towards exploration of other sources of reward produces increases in tonic pupil diameter. Conversely, increased phasic reactions to stimuli indicate task engagement (Gilzenrat et al., 2010). In the current study, children with ASD exhibited typical tonic pupil sizes and typical levels of phasic pupil reaction in response to all stimuli. According to theories of the locus coeruleus-norepinephrine system and its effect on attention, the children in the current study remained in phasic mode of locus coeruleus activity and did not enter a state of diffuse heightened arousal, which would lead to higher tonic pupil measurements and smaller phasic changes in pupil size (Laeng, Sirois, & Gredeback, 2012). The variation of stimuli types in the current study may have been sufficiently interesting to the ASD group. It is also possible that the stimulus exposure time of 5s might have captured atypical orienting responses toward the stimuli, as endogenous orienting may be a deficit in ASD (Ames & Fletcher-Watson, 2010), particularly to social stimuli (Dawson, Meltzoff, Osterling, Rinaldi, & Brown, 1998). Alternatively, children with ASD may have looked away from the stimuli sooner than TD children, as Nakano et al. (2010) found. Longer exposures to stimuli might result in smaller mean phasic pupil differences due to increased attentional demands with little compensatory reward, which may be more likely to elicit disengagement from social stimuli (e.g., the 15s exposure time in Anderson, Colombo, & Shaddy, 2006).

Maturational effects on cognitive resource recruitment might help explain the age effects of phasic pupil size within diagnostic groups. Children in the older ASD group responded to social drawings and nonsocial photo with more dilation than their younger counterparts, an effect that is mirrored in the older TD group with nonsocial drawings. Recruitment of additional cognitive resources (Karatekin, Marcus, & Couperus, 2007) later in childhood may indicate that the drawing stimuli are differentially salient to older and younger children. Moreover, the development of this response appears not to correlate with either of the standard tests of cognitive ability or the autism severity scales. Therefore, more work is necessary to establish how the phasic pupil response to simplistic social and nonsocial stimuli is related to personal salience and general access to cognitive resources.

Looking Time Proportion

General attention deficits in ASD are supported by smaller proportions of looking toward all stimuli in the ASD group. Although deficits in some aspects of attention have been reported for children with ASD (e.g., Keehn, Muller, & Townsend, 2013; Sasson et al., 2008), deficits in attention measured with eye tracking are usually found in social stimuli of sufficient complexity (e.g. real-time bids for social interaction in Jones, Carr, & Klin, 2008) rather than in static, reduced-detail stimuli. Thus, the current finding was unexpected and did not replicate similar studies that found no scanning differences for static images (e.g., Speer, Cook, McMahon, & Clark, 2007; Kemner, Van der Geest, Verbaten, & Van Engeland, 2007). Children with ASD looked longer at social stimuli than they looked at nonsocial stimuli, but both social and nonsocial looking proportions were significantly smaller than TD looking proportions. This looking pattern in ASD is similar to Hernandez et al. (2009) which found that adolescents and adults with ASD looked at the eyes more than they looked at other parts of social stimuli, but still did not examine faces for the same amount of time or in the same way as TD controls.

These findings suggest that children with ASD maintained attention to social images longer than they maintained attention to nonsocial images. Such increases in attention to social photos and social figures are associated with less severe scores on the ADOS, and they also coincide with higher CS. These relationships in the current study may explain why previous with CS-matched samples reported no scanning differences (Anderson, Colombo, & Shaddy, 2006). Scanning differences might have been uncovered by the current study’s larger sample size and wider age range, although scanning effects were not found to be particular to the older age group. Furthermore, even if the prediction of reduced social scanning in the older ASD group were found in the current study, the singular interpretation of such results would be complicated by the wide range of CS and its correlations with social stimuli scanning.

A positive correlation between social photo phasic pupil response and social photo looking proportion suggest that visual attention to complex social images is in some way modulated by arousal. Although a causal argument cannot be made from the current analysis, this result does suggest that children with ASD may spend more time examining realistic faces that they find more appealing, as indexed by pupil dilation. Alternatively, children who engage with complex images of faces may choose to spend more time exploring them. This relationship was unique to social photos, suggesting that nonsocial or less complex stimuli are in some way insufficient to produce such coordination in the attentional and autonomic systems. Additional studies with more careful planning and manipulation of complex face images might be helpful to understand this concerted looking and pupil response.

Internal Features and Head Look zone Proportional Time

Looking proportion to internal features was considered as a subset of the looking proportion analysis because of its potential relationship with age in the ASD group—that is, looking away from social stimuli was hypothesized to be a developmental characteristic of the ASD group that was preceded by autonomic dysregulation. However, age effects for looking proportion to the internal features and external features were not found for children with ASD. Children with ASD spent less time in proportion to their onscreen time in the internal features look zone than the TD children. However, the time spent looking at external features was not different across diagnostic groups. Within groups, the internal features were preferentially attended to by children with ASD much like they were for TD children. Lack of preferential attention in ASD to internal features is commonly found for children in middle or late childhood, adolescents, and adults (see Falck-Ytter & von Hofsten, 2011 for a review). However, preferential attention to internal features over external features was observed irrespective of age group in the current study. One may argue that the inclusion of simplistic faces may have influenced the attention to internal features by making them more appealing to children with ASD. However, diagnostic group differences between stimuli types in attention to internal features were not found by the omnibus test. Thus, the prediction that scanning differences to internal features would only be present in older children with ASD in response to more complex social stimuli is not supported.

Conclusions and Limitations

The results of this study indicate that previous findings of dysregulation in tonic pupil size and phasic pupil size may be less systemic than previously theorized, and should be pursued in unique ways to characterize ANS function in children with ASD and its relation to social attention. The current study explored pupil reactions to various levels of visual complexity in static social and nonsocial stimuli, which revealed a within-group monotonic trend for TD phasic pupil reactions to social stimuli. The ASD group did not show monotonically increasing pupil dilations toward stimulus types of increasing detail (drawings, figures, and photos), indicating that visual complexity does not recruit resources in the same way that it does in TD children. The existence of an alternative configuration of the autonomic system in ASD is further supported by the strong correlation between phasic pupil response and looking proportion to social photos. This relationship suggests that for complex, social images, attention and pupil responses are linked. Because both pupil and looking proportions toward social photos are negatively correlated with autism severity and positively correlated with CS, modulation of these underlying systems as related to ASD outcomes should be explored further. The hypothesized developmental relationship between tonic autonomic dysregulation and eventual reduction in gaze toward faces in late childhood was not supported. In fact, children with ASD spent a greater proportion of onscreen time looking at social images as opposed to nonsocial images, an effect which did not change with age. Children with ASD also displayed preferential looking to internal features of the face, but to a less extreme degree than found in TD children.

Several limitations of the current study should be addressed in future investigations. Group matching by CS would lend greater credibility to findings, and ensure that potential CS influence on responses to complex social stimuli has been effectively controlled across groups. Moreover, an improved design would also offer independent chronological age-match and mental-age-match groups, and include both genders. Second, the regulation of the autonomic nervous system was indexed by tonic and phasic pupil size alone. In future studies, more than one psychophysiological system should be measured at baseline and in reaction to the stimuli, such as respiratory sinus arrhythmia or skin conductance. Third, the use of mean differences to examine pupil changes in response to stimulus was informative, but was unable to detect tonic pupil differences between the TD and ASD groups. High-frequency data from sources such as continuous pupillometry can be analyzed as a time series to explore the characteristics of ASD regulation of the ANS in real time, which may reveal differences in moment-to-moment modulation of pupil size. For example, Schoen, Miller, Brett-Green, and Hepburn (2008) measured electrodermal activity in response to varied sensory stimulation, and distinguished two groups of children with ASD based on skin conductance level (i.e., high arousal and low arousal). Pupil data embedded in time would also allow for causal inferences to be made about pupil responses before, during, and after entering a look zone.

Footnotes

1. Tonic univariate analyses: Levene’s test of equality of error variances passes for dependent variables; homogeneity of variance test passes; therefore, no adjustments made to degrees of freedom or critical alpha level.

2. Phasic repeated-measures omnibus ANOVA: Passes Box’s M test of equality of covariance matrices between groups; Levene’s test of equality of error variances showed one or more violations for the dependent variables, so univariate comparisons must be interpreted cautiously; Mauchley’s sphericity test passed, but the Greenhouse-Geisser adjusted degrees of freedom were used for consistency.

3. One-way ANOVA: the Welch-adjusted robust statistic was reported because it does not assume homogeneity of variance between groups.

4. Box’s M test of equality of covariance matrices between groups fails; Mauchley’s sphericity test fails—the Greenhouse-Geisser adjusted degrees of freedom were used to compensate. Levene’s test of equality of error variances was violated by one or more dependent variables—the multivariate comparisons are reported with Wilk’s Lambda.

5. The younger age group violates Mauchley’s test of sphericity. Greenhouse-Geisser adjusted degrees of freedom are used with both groups.

6. All Levene’s tests of equality of error variance passed.

7. Box’s M test of equality of covariance matrices between groups fails; Mauchley’s sphericity test fails—the Greenhouse-Geisser adjusted degrees of freedom were used to compensate. All dependent variables passed Levene’s test of equality of error variances.

8. Levene’s test of equality of error variances passes for all dependent variables.

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Appendix A: Recruitment Letter

THE UNIVERSITY OF KANSAS

Dear Parents:
Autism is a neurological disorder that affects social development and communication. Eye movements and pupil measurements have been used to determine how people with autism respond to human faces, but the developmental path of these eye responses is still uncertain. This research project will study how pupil responses and face scanning patterns change from toddlerhood into late childhood in order to better characterize these responses in autism.

Who may participate in this project?
We are looking for **2 to 13 year-old children** who (a) have a diagnosis of **Autistic Disorder, Asperger's syndrome, or PDD-NOS** OR (b) are **typically-developing children**. In order to participate, the children must not have any neurological disorders (other than autism, Asperger's, or PDD-NOS) or serious health problems such as heart disease. Children with hearing or vision difficulties should have corrected hearing or vision, as with hearing aids or glasses. Children should not be chronically taking any medications, prescription or over-the-counter, although multivitamins are acceptable.

What type of activity will my child participate in?
Each child will be seen at our Lawrence laboratory for one session. During this session, your child will be secured into an age appropriate seat and shown a series of images, including photos, figurines, and drawings of both human faces and common objects. As your child views the screen, his or her pupil diameter and eye movements will be recorded using an eye-tracking camera. Nothing will be attached to your child. When the visual task is completed, a standardized test of intelligence will be administered, as well as a standard assessment for autism (administered to all children, even typically developing children). The autism assessment will consist of free play with toys and informal conversation.

How long will these activities take?
The session should take approximately 2 hours to complete.

Will we be reimbursed for time and travel?
YES. You will be given $20 at the appointment for time and travel.

How do I sign up for participation?
If you are interested in participating in this research project or if you have questions, please contact us directly via phone (785) 312-5345, e-mail autismlab@ku.edu, or through our secured website `http://lsi.ku.edu/labs/neurocognitive_lab/`

We hope that you consider participation.
Sincerely,

Sara M. Obermeier, Graduate Student
Christa J. Anderson, Ph.D.
John Colombo, Ph.D.
The University of Kansas
Schiefelbusch Life Span Institute

Appendix B: Pull-tab flyer

KU THE UNIVERSITY OF KANSAS

Dear Parent(s)

Autism is a neurological disorder that affects social development and communication. Eye movements and pupil measurements have been used to determine how people with autism respond to human faces, but the developmental path of these eye responses is still uncertain. This research project will study how pupil responses and face scanning patterns change from toddlerhood into late childhood in order to better characterize these responses in autism.

Who may participate in this project?

We are looking for **2 to 13 year-old boys** who (a) have a diagnosis of **Autistic Disorder, Asperger's syndrome, or PDD-NOS** OR (b) who are **typically-developing children**. In order to participate, the children must not have any neurological disorders (other than autism, Asperger's, or PDD-NOS) or serious health problems such as heart disease. Children with hearing or vision difficulties should have corrected hearing or vision, as with hearing aids or glasses. Children should not be chronically taking any medications, prescription or over-the-counter, although multivitamins are acceptable.

What type of activity will my child participate in?

Each child will be seen at our Lawrence laboratory for one session. During this session, your child's pupil size will be measured twice using two devices. First we use a hand-held device that is placed over their left eye. Second we use an eye-tracking system which measures pupil size through a small camera in front of the computer screen. The purpose of this is to ensure we can successfully obtain an accurate pupil size with the hand-held device because it is more practical for use in pediatrician and diagnostic clinics. There is no pain associated with either procedure. During the procedure, your child will be secured into an age appropriate seat.

Then, they will be shown a series of images, including photos, figurines, and drawings of both human faces and common objects. As your child views the screen, his or her pupil diameter and eye movements will be recorded using an eye-tracking camera. Nothing will be attached to your child.

When the visual task is completed, a standardized test of intelligence will be administered, as well as a standard assessment for autism (administered to all children, even typically-developing children). The autism assessment will consist of free play with toys and informal conversation.

How long will these activities take?

The session should take approximately 2 hours to complete.

Will we be reimbursed for time and travel?

YES. You will be given **$20** at the appointment for time and travel.

How do I sign up for participation?

If you are interested in participating in this research project or if you have questions, please contact us directly via phone

(785) 864-6485 or e-mail autismlab@ku.edu.

We sincerely hope that you will consider participation,

Sara M. Obermeier, Graduate Research Assistant, Christa J. Anderson, Ph.D. & John Colombo, Ph.D.

Autism Looking Development Study
http://lsi.ku.edu/labs/neurocognitive_lab/

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Appendix C: Phone Script

Phone Script for Calling Parents for Sara's MA

Tips:

1. Keep the autismlab calendar open
2. Have the exclusion criteria and study information handy!
3. Keep track of who answers, who you must call back, who you've left a message with, and whose phone has been disconnected, etc. This can go in the "Call Notes" column of the emails tab of the SMOMA Recruitment spreadsheet.
4. Call about 20-25 people at a time. Call during the day or in the evening before 7pm.

If they answer:

Hello, my name is **______** from the KU Neurocognitive Development of Autism Lab. Is (mom or dad's name, whatever we have) available? [If we don't have a name, just launch into the next part]

If yes: Our lab sent you an email earlier this week. We are currently conducting several studies that you may be interested in with **boys** from age 2 to 13. Would you and your child be interested in participating in a research study sometime this summer to help children with autism?

> **If yes:** Great! For our research this summer, we are using eye tracking to measure eye movements and pupil size in response to pictures of people and common objects. We will need to make one 2-hour appointment at our Lawrence laboratory where we will do eye tracking and standardized assessments. There is $20 compensation for time and travel, and we can schedule any time that works for you, even evenings and weekends. Would you like to schedule an appointment for your child?
>
> > **If yes:** [Schedule on autismlab calendar and send invites to Sara and Christa and any students who might be interested. Use the E-mail Initial Contact template to determine if they meet any exclusion criteria. Children must be medication free for 48 hours prior to the appointment. Enter in SMOMA Recruitment spreadsheet and create a folder for the child.]
>
> **If no:** That's alright—would you like us to keep your contact information on file so that we can let you know about future research opportunities?

If the person we ask for is not available: Thank you for letting me know. Is there a better time to call back, or would you be able to take a message for (her/him)? [Go through the basic information in the message script and/or record the time that would be best to call them back, *and call them back later or find someone who can*] Thank you for your time (sir or ma'am) and have a nice day!

Regardless of outcome: Thank you for your time (sir or ma'am). Have a nice day!

If the parent does not answer and you get the machine:

Hello, I'm ____ from the KU Neurocognitive Development of Autism Research Lab, and I am calling about some of our summer research that you might be interested in. We sent you an email earlier this week talking about a few studies with **boys** between the ages of 2 to 13. Your appointment would be a single two-hour appointment at our Lawrence laboratory, and there is compensation for time and travel, and we can schedule any time that works for you. If you are interested in participating or if you have any questions, you can contact us at (785) 864-6485 or at autismlab@ku.edu. If you would like your information to be removed from our database, you can contact us at the same number and address. Thank you and have a nice day!

FAQ:

Logistical Information:

- We can schedule appointments on weekends and evenings
- We will be doing the study until the first week of August
- Parking is free
- Other children are welcome to come—we have toys and students who can play with them.
- We can send directions to the parent via email or phone
- Multivitamins are alright, just not over-the-counter or prescribed medications within 48 hours
- There will be breaks with snacks throughout the assessment; however, feel free to bring toys and snacks and movies (to watch during calibration)

If they want to know more about the study:

Our lab is interested in how children with autism look at human faces as opposed to other kinds of common objects. We are also interested in how the pupil size changes as a physiological measure of interest and arousal. We also use standardized assessments of intelligence and autism symptom severity to better match the children for comparison.

If they are curious about the procedure:

Your child will be secured into an age-appropriate seat with a seatbelt, and their eyes will be recorded using a desk-mounted eye-tracking camera and a handheld pupillometer. The camera will be below a computer screen that your child will be watching. The lights will need to be turned off so that we get accurate readings of his eyes. We will also use a handheld pupillometer that will be held up to your child's eye. The pupillometer will shine various levels of light into the eye and measure the pupil reaction. Eye measurements with the pupillometer and the eye-tracking camera should take no more than 20-30 minutes.

Your child will be administered a standardized test of intelligence. Depending on his age, this will be either the Mullen Scales of Early Learning, or the Stanford-Binet Intelligence Scale. These will return a total score and subscores about your child's development in various areas. You will be mailed the scores after the appointment.

(If ASD) Your child will also be administered a standardized assessment for autism symptoms called the ADOS. This will consist of free play with toys and informal conversation. You will also receive the score report.

We will collect health and background information and general information about your child, such as height and weight. You will be asked to fill in the Social Responsiveness Scale if your child has an autism spectrum diagnosis.

Second Message for Voicemail or Answering Machines

Hello, I'm ____ from the KU Neurocognitive Development of Autism Research Lab, and I am calling about some of our summer research that you might be interested in. The study is closing soon, and we still need boys with autism between the ages of 2 to 13. Your appointment would be a single two-hour appointment at our Lawrence laboratory, and there is compensation for time and travel, and we can schedule any time that works for you, even evenings and weekends. If you are interested in participating or if you have any questions, you can contact us at (785) 864-6485 or at autismlab@ku.edu. If you would like your information to be removed from our database, you can contact us at the same number and address. Thank you and have a nice day!

Appendix D: Consent Form

Wakarusa Research Facility
1315 Wakarusa Drive, Suite 122
Lawrence, KS 66049
(785) 864-6485; autismlab@ku.edu

LOOKING DEVELOPMENT

INFORMED CONSENT STATEMENT

INTRODUCTION

The Scheifelbusch Institute for Life Span Studies at the University of Kansas supports the practice of protection for human subjects participating in research. The following information is provided for you to decide whether you and your child wish to participate in the present study. You may refuse to sign this form and not participate in this study. You should be aware that even if you agree to participate, you and your child are free to withdraw at any time. If you and your child do withdraw from this study, it will not affect your or your child's relationship with this unit, the services it may provide to you or your child, or the University of Kansas.

PURPOSE OF THE STUDY

The purpose of the study is to obtain eye movement and pupillary responses to various social and nonsocial stimuli from 2 to 13 year-old children who are typically developing and children with autism. We are investigating the cross-sectional age changes in eye responses to social stimuli.

PROCEDURES

We will complete 3 different activities across one testing day.

1. We will administer an eye-tracking activity. During this activity, your child will be secured in a car seat, booster seat, or alone in a seat with 5-point restraint to restrict movement. Twenty-four static images will be presented on a computer screen for 5 seconds each, separated by a gray slide between each picture for a variable amount of time, averaging 10 seconds. While your child is viewing the pictures, we will measure your child's pupil diameter and record where they are looking on the screen. The eye movement system uses an infrared light source to track your child's pupil. The eye-tracking session will be done at our laboratory facility, and should take approximately 20 minutes.

2. We will need to determine the developmental levels of all children in the study. To do this, we will be administering a standardized assessment of development to your child. Depending on the age of your child, he or she will be administered the Mullen Scales of Early Learning (2:0 to 5:0 year-olds) or the Stanford Binet Intelligence Scales (5.1 to 13:0 year-olds). This should take approximately 45 minutes, and will be videotaped for coding purposes.

3. We will need to assess autism symptomology in social interactions in all children (typically developing and children with autism). We will administer the Autism Diagnostic Observation Schedule (ADOS). This should take approximately 15 to 30 minutes, and this will be videotaped for later scoring.

The testing session should take approximately 2 hours to complete. After the session, we will give you a brief description of the child's performance; the standardized scores will follow in a mail-out report.

RISKS

Please be assured that none of our procedures will present any risk to you or your child. The use of infrared light will be used to measure eye movement and pupil diameter. However, the level of the infrared light used (0.8 mW/cm^2) is well below the standards for risk from infrared light sources prescribed by OSHA (10.0 mW/cm^2).

BENEFITS

Upon completion of the entire project, we will send you a general report of our results. You and your child's participation will make an important contribution toward our understanding of autism and face processing.

PAYMENT OF PARTICIPANTS

You will be paid $20 in cash per session for their time and travel. You do not have to consent to the study to receive compensation; if after reading this consent you choose not to participate, you will still be given $20 for time and travel. However, parents must provide their full name, current address, and their social security number to be given payment. The University of Kansas is required by the IRS to provide this information. Compensation is taxable income and you are required to report this to the IRS. Documentation of payment will be given to the Life Span Institute accounting department and our lab will keep copies of any receipts in a locked file cabinet in a locked room which is only accessible by members of our lab.

INFORMATION TO BE COLLECTED

To perform this study, researchers will collect information about you and your child from the questionnaire that you will be asked to complete. In order to receive compensation for time and travel, parents will be asked to provide their social security number. Also, information will be collected from the study activities that are listed in the Procedures section of this consent form.

It is our policy to protect the confidentiality of all of our participants. You and your child's name will be coded by a confidential number and will not appear in any analyses or publications involved with this study. We would also like to assure you that you and your child's participation is voluntary and that you and your child may withdraw from the study at any time, even after you have signed this consent form. Also, you and/or your child's decision to participate or withdraw from the study will not affect or influence any relationship that you or your child might have with our department in the future.

The information collected about you and your child will be used by: Sara M. Obermeier, graduate student, Dr. Christa Anderson, Ph.D., Dr. John Colombo, Ph.D., and other research members of the Early Cognition Lab, KUCR, and officials at KU that oversee research, including committees and offices that review and monitor research studies.

The researchers will not share information about you or your child with anyone not specified above unless (a) it is required by law or university policy, or (b) you give written permission.

All hard copies of videotaped sessions will be stored by a study code number in a locked cabinet. Digital files of these videotaped sessions will be stored in a password protected database. Only members of our laboratory will have access to these videotapes. Although our laboratory plans to keep these video recordings indefinitely, if we decide to dispose of the data we will do so in a confidential manner so that data cannot be retrieved by unauthorized persons.

Permission granted on this date to use and disclose you and your child's information remains in effect indefinitely. By signing this form you give permission for the use of you and your child's information for the purposes of this study or any future analysis that uses the information collected during this study at any time in the future.

REFUSAL TO SIGN CONSENT AND AUTHORIZATION

You are not required to sign this Consent and Authorization form and you may refuse to do so without affecting your right to any services you are receiving or may receive from the University of Kansas or to participate in any programs or events of the University of Kansas. However, if you refuse to sign, you cannot participate in this study.

CANCELLING THIS CONSENT AND AUTHORIZATION

You may withdraw your consent to participate in this study at any time. You also have the right to cancel your permission to use and disclose information collected about you and your child, in writing, at any time, by sending your written request to: Dr. Anderson at 1000 Sunnyside Avenue, 1052 Dole Human Development Center, Lawrence, KS 66045. If you cancel permission to use you and your child's information, the researchers will stop collecting additional information about you and your child. However, the research team may use information that was gathered before they received your cancellation, as described above.

PARTICIPANT CERTIFICATION:

I have read this Consent and Authorization form. I have had the opportunity to ask, and I have received answers to, any questions I had regarding the study and the use and disclosure of information about me for the study. I understand that if I have any additional questions about my or my child's rights as a research participant, I may call (785)864-7429 or write the Human Subjects Committee Lawrence Campus (HSCL), University of Kansas, 2385 Irving Hill Road, Lawrence, Kansas 66045-7563, or email irb@ku.edu.

I agree to allow my child to take part in this study as a research participant. I further agree to the uses and disclosures of my and my child's information as described above. By my signature I affirm that I am at least 18 years old and that I have received a copy of this Consent and Authorization form.

We are very grateful for your participation.

Date ___/___/___

Child’s Name_________________________

Research Staff Signature ____

Parent's Signature ____

Parent's Address ____

_______*I agree to allow the videotape of the standardized testing procedures (Mullen, ADOS, PPVT) to be used for professional or educational purposes.*

[If signed by a personal representative, a description of such representative’s authority to act for the individual must also be provided, e.g. parent/guardian.]

Appendix E: Health and Background Questionnaire

KU THE UNIVERSITY OF KANSAS

http://lsi.ku.neurocognitive_lab/

Pupillometry ID Code: 23______

Looking Dev ID Code: 20______

Health and Background Questionnaire-Child/Adolescent

Parent(s) please complete the following section

Child's Date of Birth:____/____/____ Age:________

CURRENT HEALTH

Does your child have any of the following? (Please mark all that apply)

______Cold/flu

______Runny nose

______Sore throat

______Watery eyes

______ Coughing

______ Fever

______ Ear Infection

Is your child currently taking any medications, prescription or over-the-counter? 🟥 **YES** 🟩 **NO**

If YES, please specify:____

____

Has your child taken medications, prescription or over-the-counter, within the last 48 hours?

🟥 **YES** 🟩 **NO**

If YES, please specify:____

Has your child had shots within one week of this appointment? 🟥 **YES** 🟩 **NO**

If YES, what shots were given:_______________________________________

Date of shots:______/________/__________

HEALTH BACKGROUND

Has your child been re-hospitalized since birth? 🟥 **YES** 🟩 **NO**

If YES, please specify condition and length of hospital stay: _______________________________

Does your child have any vision impairments? 🟥 **YES** 🟩 **NO**

If YES, does your child have corrective lenses with them today? YES NO

Does your child have any hearing impairments? 🟥 **YES** 🟩 **NO**

If YES, does your child have the corrective hearing device with them today? YES NO

Does your child have any motor impairments? 🟥 **YES** 🟩 **NO**

If YES, please specify:____

Does your child have any chronic medical conditions, beyond an ASD diagnosis and conditions listed above?

🟥 **YES** 🟩 **NO**

If YES, please specify: ____

Please list any other conditions, not listed here, that you have:________________________________

____

Please indicate the number of ear infections your child has had since birth:____________

AUTISM DIAGNOSIS

Does your child have an Autism Spectrum Disorder diagnosis? 🟩 **YES** 🟩 **NO**

If YES, please answer the following questions:

Please specify the diagnosis:____

When did your child first receive this diagnosis?_____________(year)

At what age did you first notice autism-like symptoms in your child?___________

Doctor Information: *Please note that we will not be contacting your doctor. This information is only requested to verify*

that your child's diagnosis was given by a licensed medical professional.

Name of the doctor that gave your child the formal ASD diagnosis:__________________________

Credentials: PhD MD licensed PhD

Office/Clinic location:____

Has your child been re-evaluated since the initial diagnosis? ☐ **YES** ☐ **NO**

If YES, did the diagnosis change (please specify): ______________________________

____

____

____

If YES, who completed the re-evaluation? Doctor name:______________________________

Location:______________________________

Is your child currently receiving treatment services for their ASD diagnosis? ☐ **YES** ☐ **NO**

If YES, please list the services that you are receiving and the number of hours you receive each:

(e.g, behavioral intervention, speech therapy, occupational therapy, etc.)?

Treatment	**Hours per week**

FAMILY MEDICAL HISTORY

Please indicate whether any of your biological family members have been formally diagnosed with the following conditions:

	Child	Mom	Dad	Sibling(s)	Grandparent	Aunt/Uncle	First Cousin(s)
Autism Spectrum (AD, PDD-NOS or Aspergers)							
Schizophrenia							
Developmental Disability/Delay							
Language Disability/Delay							

Severe Cognitive Delay (IQ below 50)							

LANGUAGE ABILITY

What is your child's current level of language ability (Check One):

☐ Non-verbal ☐ Single words ☐ 3-4 word phrase ☐ Full sentences ☐ Fluent language ability

Does your child speak any other languages fluently besides English? ☐ **YES** ☐ **NO**

If YES, please specify the language:____

If YES, what language is primarily spoken in your household:______________________________

CAREGIVING ARRANGEMENTS

*this does not include treatment services

How many hours per week is your child in daycare or in the care someone other than yourself?_________

If in daycare, please indicate the type:

_____ Daycare center

_____ Home-based care

_____ Your home (i.e., *you* run a daycare for other children

_____ A relative's home (e.g., grandparent, aunt, etc.)

_____ Someone else's home

_____ Private caretaker/nanny/au pair in your home

HOME ENVIRONMENT

How many siblings living at home full-time, including half-siblings? ____________

Please list the ages of these siblings:_________________________________

How many siblings visit or live at home part-time? ___________

Please list the ages of these siblings ____

Approximate frequency and length of visit/stay: ______________________________

How many individuals other than the child's mother, father, and siblings living at home full-time?

_____grandmother _____grandfather

_____aunt _____uncle

_____friend _____other (_______________________)

PARENT EDUCATION

Please indicate the highest level of education completed for each parent and their current occupation:

Parent	**Age**	**High School:** **Yrs completed**	**Junior College:** **Yrs completed**	**College or University:** **Yrs completed**	**Graduate School:** **Indicate Highest Degree**	**Occupation**
Mother		1 2 3 4	1 2	1 2 3 4		
Father		1 2 3 4	1 2	1 2 3 4		

LABORATORY USE ONLY

Today's Date: _____/_____/______ **Eye Color:**_____________

Height:____________ **Weight:**__________ **BMI:**_____

Appendix F: Assent Procedures

YOUNGER CHILD ASSENT PROCEDURE SCRIPT

***Children ages 2-6 will have the procedures shown and explained to them as follows:**

For the visual task:

1. "(Child's name) we are going to go in this room (showing child the eye-tracking room) and you'll get to sit in this chair and watch a movie and then look at some pictures on the screen. If you want to stop or get out of the chair, you should tell me or your (mom/dad/parents). How does that sound to you?"
    - If child voluntarily climbs into seat or allows self to be lifted into the seat by the researcher or parent, the researcher will proceed with the testing protocol, and explain each procedure to the child before it occurs. The order of assent will take place as follows:

2. "I'm going to put these straps on you; they are just like a seatbelt. Is that okay?"
    - If child allows researcher or parent to secure them in the seat, the seat will be adjusted for height while they watch a preferred movie.

3. "Okay, I'm going to turn the lights off now so you can see the pictures better, is that okay?"
    - If the child agrees to all procedures, the visual stimuli task will begin. The child may indicate assent by responding verbally, or they may indicate assent by voluntary cooperation with the researcher.

To administer standardized tests:

1. "(Child's name) we are going to play some games and puzzles. If you want to stop, just let me or your (mom/dad) know. Do you want to play some games with me?
    - If child agrees, either verbally or by cooperation with the researcher, then the standardized test of intelligence will begin. Frequent breaks will be given as the child needs them. Breaks can be initiated as per the child's request, or if the child seems disengaged, we will pause so that they can free-play for a few minutes before testing resumes.

2. "(Child's name), we have some more activities to do, but if you want to stop at any time, be sure to tell me or your (mom/dad). Would you like to play with some toys and do some other activities with me?"
    - If child agrees, either verbally or by cooperation with the researcher, then the standardized assessment of ASD will begin.

Tantrums, Self-stimulatory Behavior, and Distress:

In cases of behaviors that indicate that the child no longer wishes to participate, the researcher will stop the visual task or stop administering test items immediately and procedure with the following protocol:

1. The researcher or parent, if present in room, will attempt to soothe the child by removing them from their seat, turning on the lights, playing with toys, or the parent's preferred behavioral intervention.
    - If the behaviors appear to be subsiding, the researcher will attempt to resume testing after restating the above assent protocols.
    - The session will continue unless the behaviors return, or the session has reached its scheduled end.

2. If the behaviors persist or worsen, the tasks will not be completed on that particular testing day.
    - If the scheduled tasks cannot be completed, then they will be attempted during an additional appointment scheduled at the discretion of the parent, with full per-session compensation as indicated on the consent form.

OLDER CHILD ASSENT PROCEDURE SCRIPT

***Children ages 7-13 will have the procedures shown and explained to them as follows:**

For the visual task:

1. "Hey (child's name), we are going to go in this room (showing child the eye-tracking room) and sit in this chair and you'll get to see some of the movie you chose. After we find your eye in the camera, you'll see some pictures. If you want to stop or get out of the chair, you should tell me or your (mom/dad/parents). How does that sound to you?"
    - If child voluntarily climbs into seat or verbally assents the researcher will proceed with the testing protocol, and explain each procedure to the child before it occurs. The order of assent will take place as follows:

4. "I'm going to put these straps on you; they go on just like a seatbelt. Is that okay? If you want, you can do it yourself"
    - If child buckles him or herself into the seat or allows researcher or parent to secure them in the seat, the seat will be adjusted for height while they watch a preferred movie.

5. "Okay, I'm going to turn the lights off now so you can see the pictures better, okay?"
    - If the child agrees to all procedures, the visual stimuli task will begin. The child may indicate assent by responding verbally, or they may indicate assent by voluntary cooperation with the researcher.

To administer standardized tests:

3. "(Child's name), I have some questions for you, and some activities to do. There will be some language games, some puzzles and games with beads and blocks. Remember that you can stop at any time. Do you want to do these activities with me?
    - If child agrees, either verbally or by cooperation with the researcher, then the standardized test of

intelligence will begin. -Frequent breaks will be given as ~~needed by~~ the child~~. This could either~~ needs them. Breaks can be initiated as per the child's request, or if the child seems disengaged, we will pause so that they can free-play for a few minutes before testing resumes.

~~be through the child asking for a break or if the child seems disengaged we will initiate a break~~

~~so that they can free-play for a few minutes before we resume testing.~~

4. "(Child's name), we have some more activities to do and some more things to talk about. If you want to stop at any time, be sure to tell me or your (mom/dad). Do you want to play with some toys and talk with me about some stuff?"
    - If child agrees, either verbally or by cooperation with the researcher, then the standardized assessment of ASD will begin.

Tantrums, Self-stimulatory Behavior, and Distress:

In cases of behaviors that indicate that the child no longer wishes to participate, the researcher will stop the visual task or stop administering test items immediately and procedure with the following protocol:

3. The researcher or parent, if present in room, will attempt to soothe the child by removing them from their seat, turning on the lights, playing with toys, or the parent's preferred behavioral intervention.
    - If the behaviors appear to be subsiding, the researcher will attempt to resume testing after restating the above assent protocols.
    - The session will continue unless the behaviors return, or the session has reached its scheduled end.

4. If the behaviors persist or worsen, the tasks will not be completed on that particular testing day.
    - If the scheduled tasks cannot be completed, then they will be attempted during an additional appointment scheduled at the ~~will~~discretion of the parent, with full per-session compensation as indicated on the consent form.

Appendix G: Examples of Stimulus