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Relationships Among Eye Gaze, Social Ability and Extracellular Signal-Regulated Kinase Pathway Activation in Children and Adolescents with Autistic Disorder
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Abstract

Previous eye tracking research suggested that children and adolescents with Autistic Disorder (AD) exhibit atypical gaze patterns while observing dynamic social interactions. The relationship among atypical gaze patterns, social ability, and underlying biological conditions has yet to be investigated. The present study utilized eye tracking technology to investigate the gaze patterns of 26 typically developing children and adolescents, ages 5-17, and 38 aged-matched peers with AD as they viewed a dynamic play scene. Between-group differences in the static activation level of the extracellular signal-regulated kinase (ERK) pathway in peripheral lymphocytes were also explored. Children and adolescents with AD displayed shorter fixations on faces and took longer to fixate on a face compared to participants without AD. Additionally, participants with AD had almost twice the amount of static ERK activation in peripheral lymphocytes than control participants. The atypical gaze patterns of participants with AD were not associated with social ability or ERK activation level. The findings demonstrate that atypical gaze extends to social play scenes for individuals with AD. Further investigation is needed on the implications of the ERK pathway in children and adolescents with AD and the extent to which eye tracking research directly relates to real-world social abilities.
Individuals diagnosed with autism spectrum disorder (ASD) experience deficits in social interaction and communication (APA, 2013). In an attempt to understand the nature of social deficits observed in ASD, research has objectively assessed and quantified socialization symptoms in children and adolescents with ASD using eye tracking technology (Boraston & Blakemore, 2007). Eye tracking data can aid in the exploration of social deficits in ASD by providing information as to how individuals spontaneously view semi-naturalistic social information as it is presented on a computer screen (Boraston & Blakemore, 2007). Exploring gaze patterns can suggest where an individual is allocating his or her attention (Henderson, 2003) and may therefore provide information on the difficulties an individual might have while viewing similar, everyday social situations (Boraston & Blakemore, 2007).

In the first eye tracking study to include participants with ASD, Klin et al. (2002) investigated the eye gaze of 15 teenagers and adults with ASD and average intelligence and 15 teenagers and adults without ASD using dynamic video clip stimuli from *Who’s Afraid of Virginia Woolf?* a film involving characters engaged in an intense social interaction. Fixation durations on the eyes, mouth, body and objects in the scene were collected for each participant. Results indicated that the subjects with ASD looked significantly less often at the eyes and bodies of the actors and spent significantly more time viewing the mouths of the actors and the non-social background areas of the movie compared to the individuals without ASD. For the first time, this paradigm allowed researchers to better understand how individuals with ASD spontaneously view and search for meaning when presented with a complex, interactive social
situation. As such, these findings supported the notion that eye tacking could be used as a novel research method to quantify atypical gaze behavior in ASD (Klin et al., 2002).

Since the seminal eye tracking study by Klin et al. (2002), researchers have continued to study gaze patterns using a variety of eye-tracking measurements including time (latency) to first face fixation and duration of fixation on the eyes of others. To date, researchers have observed these viewing patterns in persons with and without ASD across a variety of stimuli, from static photographs of faces (Pelphrey et al., 2002; van der Geest et al., 2002a; van der Geest et al., 2002b) to dynamic social scenes (Klin et al., 2002; Norbury et al., 2009; Rice et al., 2012; Speer et al., 2007), with research supporting the use of ecologically valid, dynamic social scenes to detect significant differences in gaze patterns between individuals with and without ASD (Saitovitch et al., 2013; Speer et al., 2007).

**Eye Gaze in ASD towards Dynamic Stimuli**

In general, significant differences in patterns of viewing dynamic social scenes have been found between children and adolescents with and without ASD with regard to latency to first face fixation and duration of fixation on faces, eyes, mouths and objects. Results indicate that compared to children and adolescents without ASD, children and adolescents with ASD take longer before they first fixate on the face or eyes (Freeth et al., 2010; Norbury et al., 2009), spend less time looking at the eyes of others (Klin et al., 2002; Pelphrey et al., 2002; Rice et al., 2012; Speer et al., 2007), spend less time looking at the faces of others (Riby & Hancock, 2009a; Rice et al., 2012), and spend more time observing the objects and background (Klin et al., 2002; Rice et al., 2012) or body of actors (Speer et al., 2007). As a result, researchers have reported reduced fixations on faces and eyes and delayed latency to first face or eye fixation as support for the theory that abnormal gaze patterns may underlie the day to day social and communication
deficits seen in autism (Norbury et al., 2009). However, few studies have analyzed eye-tracking data to determine such a relationship by correlating latency to first face or eye fixation or fixation duration on faces to outcome measures of social competence in individuals with ASD. Therefore, in addition to obtaining consistent, quantitative data on gaze patterns in ASD, it is equally important to research how ASD-specific gaze patterns may be related to general impairments in social functioning (Speer et al., 2007).

**Clinical Significance of Eye Tracking Data**

Researchers have begun to explore whether patterns of gaze towards ecologically valid, dynamic social scenes are related to the general, everyday social deficits observed in individuals with ASD. Although the clinical significance of ASD-specific gaze patterns has started to gain attention, it has only been explored using a narrow range of dynamic, social stimuli. For this type of stimuli, results remain inconsistent across age groups.

For adults with average intelligence and ASD, time spent fixating on the eyes of others was not significantly related to social ability. Instead, more time spent fixating on objects was associated with lower levels of social ability and higher severity of autism, as measured by the total algorithm score on the social section of the ADOS (Klin et al. 2002). Another study found that for school-aged children with ASD and average intelligence, shorter time fixating on the eyes was significantly negatively correlated with social ability, measured by total scores on the Social Responsiveness Scale (Speer et al., 2007). These results suggest that for the children studied, decreased time spent fixating on the eyes of others was associated with poor social ability. However, for a separate group of adults, time spent fixating the eyes was not significantly related to social ability; instead, only time spent fixating on objects in the background was related to poor social ability. Information on adolescents and time spent fixating the eyes of
others within a dynamic social scene have been under-explored in the research. Additionally, relationships between gaze patterns and participant age, sex and intelligence have yet to be explored directly and warrant future research.

**Eye Tracking of Dynamic Play Scenes**

The majority of eye tracking research to date has focused on differences in participant gaze patterns towards faces, within faces (i.e. eyes or mouth), or towards background objects within social scenes. Only one study has examined gaze towards similar areas of interest while participants view a shared play activity between two people. This study, conducted by Shic, Bradshaw, Klin, Scassellati, and Chawarska (2011), examined the gaze patterns of 20-month-old toddlers towards people and background objects while they observed a video of an adult and a toddler playing a game together. This type of dynamic interaction is equally important to study because attending to interactions of others is involved in the development of social skills such as joint attention, social play and understanding the intentions of others (Bakeman & Adamson, 1984; Carpenter, Nagell, & Tomasello, 1998; Moore & Dunham, 1995). Shic et al. (2011) suggest that impaired activity monitoring of others could limit observational learning opportunities and possibly influence the relative salience of observing an interactive activity. Additionally, attending to the actions of others is a prerequisite skill for imitation and emulation (Carpenter, 2006; Heyes, 2001) and is needed to develop skills such as joint attention and social play (Carpenter, Nagell, & Tomasello, 1998; Moore & Dunham, 1995).

Shic et al. (2011) investigated the gaze patterns of toddlers at 20 months of age, the youngest age at which a stable diagnosis of ASD can be obtained (Chawarska et al., 2007). This study found that compared to 20-month-old neurotypical toddlers and toddlers with developmental delay who did not meet criteria for ASD, 20-month-old toddlers with ASD spent
less time attending to a shared activity between a toddler and an adult and spent significantly more time fixating on the objects and background of a shared activity play scene, and that this pattern was significantly related to low social affect scores on the ADOS-G (Lord et al., 2000). There was not a significant difference between groups in the time spent viewing the people in the shared activity. Additional correlations suggested that less time viewing the heads of actors for participants with ASD was associated with decreased social affect scores on the ADOS-G. In light of these significant results, additional research is needed to determine if similar viewing patterns and their relationships to social abilities remain constant through childhood and adolescence. Moreover, other researchers in the field have suggested that future research focus on broader ranges of video stimuli, especially by manipulating the presence of objects in the scenes (Falck-Ytter, 2013). Further information on viewing patterns of children and adolescents with and without ASD towards dynamic play scenes is therefore warranted.

**Biomarkers in Autism Spectrum Disorder**

In addition to linking quantitative measures of gaze patterns produced by eye-tracking data to measures of social functioning in ASD, an area of research that has received less attention is linking quantitative measures of viewing patterns in ASD to underlying molecular or genetic factors associated with the disorder in attempts to identify biomarkers. Support for biomarkers has developed from a variety of sources. Taylor, Mayberry, and Whitehouse (2014) suggest that the identification of biomarkers, especially those that can be used with behavioral screenings, could provide a new reliable detection agent and thus more accurate diagnosis of ASD, which directly influences early intervention and prognosis for children. Biomarkers can also help identify subgroups of individuals with a common underlying genetic or biological makeup. This
could advance understanding of the heterogeneity of ASD, which could impact treatment options (Ruggeri et al., 2013).

One potential ASD biomarker that has recently gained attention is the extracellular signal-regulated kinase (ERK) pathway used for cellular signaling. The ERK pathway regulates the transmission of specific signals located on the cell surface into the nucleus, resulting in a variety of cell responses (Samuels, Saitta, & Landreth, 2009). The ERK pathway can become activated by various extracellular stimuli such as neurotransmitters, growth factors and hormones (Chang et al., 2003; Chuderland & Seger, 2005; Yao & Seger, 2004). Depending on the strength of the original extracellular signal, the activated ERK pathway can result in various cell responses including gene transcription and translation (Samuels et al., 2009), proliferation, migration, differentiation and cell death (Murphy & Blenis, 2006).

Genetic mutations within the ERK signaling cascade have been associated with some neurodevelopmental disorders, including autism (Samuels et al., 2009). The exact role of the ERK cascade in the development of ASD has yet to be clearly identified, but numerous research studies have found evidence that the ERK signaling pathway is abnormally upregulated in both post-mortem human brain tissue and in mouse models of ASD and that this may be related to the pathophysiology of ASD (Yang et al., 2011). Recent research found that increased activation levels of the ERK pathway in neurons led to increased groups of mRNAs encoding adhesion molecules and scaffolding proteins (Bhakar, Dolen & Bear, 2012; Gkogkas et al., 2013). When these proteins are expressed at high levels, the equilibrium between excitatory and inhibitory synapses can be affected. Previous researchers have hypothesized that this disrupted balance in synapses could play a role in cognitive delays and possibly some aspects of ASD (Bateup, Takasaki, Saulnier, Denefrio & Sabatini, 2011; Zoghbi & Bear, 2012).
Additionally, more recent research has found a significant correlation between ERK activation levels in the prefrontal cortex and peripheral lymphocytes in BTBR mouse models of autism (Faridar et al., 2014). This provides support for researching ERK activation levels in human peripheral lymphocytes, as human brain tissue can only be examined post-mortem. The presence of upregulated ERK pathways in living persons has yet to be discovered. Identifying disparate levels of ERK activation between persons with and without ASD could support ERK as a biomarker of ASD. Additionally, linking the presence of abnormal ERK activation to behavioral markers could begin to suggest specific subtypes of ASD.

**The Present Study**

Eye tracking has shown promise for quantifying the social deficits in persons with ASD. It appears that presenting stimuli that are dynamic and ecologically valid in nature are the most sensitive to detecting eye gaze abnormalities in individuals with ASD compared to neurotypical individuals (Saitovitch et al., 2013; Speer et al., 2007). Eye tracking research has also provided an ability to identify relationships between eye tracking patterns and outcome measures of social ability. This has increased support for theories that suggest the observable social abnormalities in ASD may be related to how persons spontaneously view social stimuli (Norbury et al., 2009). Abnormal viewing patterns of dynamic social stimuli by children with ASD compared to children without ASD have been previously identified; however, the same relationships have not been identified in children and adolescents viewing a dynamic play scene. Furthermore, the relationships between viewing patterns and participant variables (e.g., age, sex, and intelligence), measurements of social functioning, and ERK pathway activation have yet to be explored using an ecologically valid dynamic play scene with a group of children and adolescents with ASD and warrants further exploration.
The present study utilized an ecologically valid, dynamic play scene to examine the relationships among gaze patterns, social responsiveness, and static ERK activation levels in peripheral lymphocytes in children and adolescents aged 5 to 17 diagnosed using the *Diagnostic and Statistical Manual, 4th Edition, Text Revision* (DSM-IV TR) with autistic disorder (AD) and participants aged 5 to 17 without an AD diagnosis. More specifically, this project explored the differences in fixation duration on the faces of actors and latency to first face fixation between the two groups. Next, differences within the AD participants were explored. This project evaluated whether fixation duration towards faces and latency to first face fixation correlated with social functioning, as measured by the Social Responsiveness Scale. Lastly, the present study evaluated whether ERK activation levels correlated with fixation duration on the face and latency to first face fixation within AD participants. In order to examine differences in gaze patterns among participants due to demographic variables, the present study additionally investigated the relationships among the AD participant variables of age, sex and intelligence with fixation duration on faces and latency to first face fixation.

**Method**

**Data**

The archival data for the present study were obtained from a larger, IRB approved, research study that examined gaze patterns using eye tracking technology and investigated quantitative blood biomarker development in participants 5-55 years of age with and without DSM-IV-TR autistic disorder (AD) (see appendix A). For the larger study, participants and their caregivers were recruited from an AD tertiary care academic clinic in a large Midwest city.

Overall, 98 participants aged 5 to 55 were recruited for the larger study including 63 participants with AD and 35 control participants. However, for the present study, only the
records of participants aged 5 to 17 were examined. Inclusion criteria for AD participants in the larger study included children five years or older, a diagnosis of AD based upon DSM-IV TR criteria as confirmed during the psychiatric interview by a board-certified child and adolescent psychiatrist, and a score of greater than or equal to 15 on the Social Communication Questionnaire (SCQ; Rutter, Bailey & Lord, 2003). Inclusion criteria for control participants in the larger study included children and adolescents aged 5 to 17 without a diagnosis of AD. Exclusion criteria for both groups included DSM-IV TR diagnoses of Rett’s Disorder, childhood disintegrative disorder, schizophrenia, or any other psychiatric disorder.

In summary, records of 69 participants between the ages of 5 and 17 were eligible for this study. Of this group, 42 children and adolescents met criteria for AD and 27 were age-matched (+/- 6 months of age) typically developing children and adolescents. However, two AD participants were excluded from analyses; one participant exhibited challenging behaviors (e.g., screaming, refusing to sit), which interfered with this participant’s completing the eye-tracking task. Eye tracking data for a second AD participant were lost and this participant was not included in any statistical analyses. Thus, the final participant sample consisted of 40 children and adolescents with AD and 27 age-matched (+/- 6 months of age) typically developing children. See Table 1 for participant demographics.

**Measures**

**Fixation Duration on Faces.** Fixation duration to faces was measured by the percentage of total viewing time spent fixating on the face of either child in the foreground of the play scene. The total exposure time to the play scene was 60 seconds.
Latency to First Face Fixation. Latency to first face fixation was measured by the time until a face fixation occurs on either child in the foreground following stimulus-onset. The total exposure time to the play scene was 60 seconds.

The Social Responsiveness Scale. The Social Responsiveness Scale (SRS; Constantino & Gruber, 2005) is a 65-item rating scale that measures the abilities and deficits in social reciprocity in children aged 4 to 18 years of age (see appendix A). More specifically, the SRS was designed to assess levels of social impairment that can be a marker for Pervasive Developmental Disorders including Autism Spectrum Disorders (ASD). It was therefore also designed to aid in the identification and diagnosis of ASD. A parent (or teacher) rates the child’s social behaviors as they occur in natural social situations across five domains: Social Awareness, Social Information Processing, Capacity for Reciprocal Social Communication, Social Anxiety/Avoidance, and Autistic Preoccupations and Traits. The SRS overall score is an index of severity of deficits in social reciprocity. An overall SRS T-score of 59 or less is considered average social functioning. An overall SRS T-score between 60 and 75 is in the moderate range and suggests the presence of a mild ASD whereas an overall SRS T-score of 76 or higher falls within the severe range and is suggestive of an ASD. Parent completed SRS was included in the present study.

Extracellular Signal-Regulated Kinase Assay. Static peripheral lymphocyte ERK activation was measured from a blood sample. The measurement of ERK activation level was expressed as a percentage. The percentage of ERK activated cells was calculated from the ratio of ERK positive cells (phosphorylated ERK cells) to the total number of peripheral lymphocyte cells.
Demographic Questionnaire. A demographic questionnaire was completed by a caregiver for each AD participant and provided information about participant’s age, race and sex.

Apparatus

A Tobii T120 Infrared Eye Tracker (Tobii Technology, Stockholm, Sweden) was used to measure participants’ gaze fixations towards presented visual stimuli. The eye tracker records the gaze of both eyes from reflections of light on the cornea and pupil. An integrated 17-inch thin film transition monitor was used to present visual stimuli. Stimulus and recordings of gaze were controlled by Tobii Studio software (Version 3.0). Both X and Y coordinates of the participants’ eye position were obtained at a frequency of 120 Hz using corneal reflection techniques. Calibration occurred as directed in the Tobii manual prior to the presentation of the stimulus. After 1-minute calibration, participants were presented with the stimulus.

Stimulus

The current study examined gaze patterns for participants as they viewed a 60-second play scene. This scene included two children in the foreground playing side by side with blocks on a table (Figure 1). Other children were present in the background of the video, playing with toys or interacting with peers or a teacher. The scene was determined to be ecologically valid, defined as reflecting realistic social play information (Hanley et al., 2013), due to its dynamic (i.e., more than one person) and complex nature (i.e., engaging in realistic parallel play, changes in facial expressions, direct eye contact made at the camera). The video came from child development training videos (Videatives, Amherst, Massachusetts, USA). Audio tracks were not included.

Procedure

The archival data for the present study was obtained from a larger research study that examined gaze patterns of typically developing individuals and individuals with AD conducted
at Indiana University. Approval from Indiana University Institutional Review Board (IRB) was obtained prior to initial data collection for the larger research study (see appendix B). Approval from Xavier University was obtained prior to accessing the archival data for the present study (see Appendix C). All caregivers of participants completed a psychiatric interview and the Social Communication Questionnaire (SCQ; Rutter, Bailey & Lord, 2003) to assess and confirm neurotypical development or AD. In order to confirm that the control participants were not cognitively impaired, a board certified child psychiatrist interviewed the parents of participants in order to obtain a developmental history, including a history of language and communication development and educational history (including history of special education placements or school-based delays. Participants with a diagnosis of AD and their caregivers completed additional assessments. Participants with AD completed cognitive testing with the Stanford Binet, Fifth Edition (Roid, 2003) or the Leiter, Revised (Roid & Miller, 1997), for nonverbal individuals. Caregivers of participants with AD additionally completed the SRS and a demographic questionnaire. The present study examined social responsiveness using the SRS, IQ scores from the SB-5 or the Leiter (for non-verbal individuals), and age and sex from the demographic questionnaire.

After assessment procedures, all participants completed an eye-tracking task. Participants were seated in a chair with a booster seat as necessary, approximately 60 to 65 cm in front of a Tobii T120 monitor. Participants and caregivers were provided information about the eye-tracking procedures. Next, a five-point calibration of the eye tracker, consistent with the manufacturer instructions, was performed. Afterwards, participants were instructed to “look at the screen” prior to the presentation of eye tracking stimulus. The researchers watched the eye-tracking procedure from a separate video screen and prompted the participants to look at the
Tobii T120 screen if their gaze left the screen during the stimulus presentation. The total length of the eye-tracking task was 3 minutes. The current study examined gaze patterns of participants as they viewed the middle 60-second child play scene only.

Lastly, both participant groups completed a blood draw to provide a lymphocytic ERK activation blood sample. Approximately 5mL of blood was drawn at a nearby outpatient center. Blood was drawn into an EDTA-containing (lavender top) tube. Blood samples were immediately chilled and taken to the Laboratory of Molecular Neurogenetics. Next, lymphocyte samples were prepared from patients’ blood. Once collected, the lymphocytes were washed and counted. Lymphocyte nuclei were stained with a solution used to highlight ERK activated cells and imaged. Activated ERK-positive cells were counted. Static ERK activation was expressed as a percentage or ratio of lymphocytes exhibiting ERK activation to the total number of lymphocytes in the field (minimum 200 cells reviewed).

**Results**

**Preliminary Analyses**

Before statistical analyses were completed, the data were examined for outliers. A priori cut-offs, based on prior research (Freeth, Ropar, Mitchell, Chapman & Loher, 2011) was implemented; data more than two standard deviations away from the group mean were removed prior to analysis. Normality was tested for all outcome variables and problems with skewness were found for all three between group outcome variables: fixation duration on faces, time to first face fixation, and static ERK activation levels in peripheral lymphocytes (AD group only). Due to all outcome variables violating tests for normality, Mann-Whitney U tests were conducted and utilized as the final test statistic instead of independent samples t-tests. Independent samples t-tests were still conducted in order to determine if the results were
consistent with the Mann-Whitney $U$ results. The results of the independent samples t-tests were consistent with each Mann-Whitney $U$ results. For the subsequent correlation analyses of the AD participants, Spearman’s $r$ was not appropriate due to non-monotonic relationships between all examined variables and therefore Pearson $r$ correlations were conducted as planned.

**Primary Analyses**

**Between Group Differences in Fixation Duration on Faces**

Analysis of outliers in the eye tracking data found that two AD participants (a boy age 7 and a girl age 7) and one control participant (girl age 9) had fixation durations on faces that were more than two standard deviations from the group mean (AD $M = 8.2\%$, $SD = 7.5\%$; Control $M = 11.6\%$ $SD = 7.5\%$) and were therefore excluded from data analysis. Following outlier removal, tests for normality identified positively skewed data for both participant groups. When an SPSS-identified outlier, defined as greater than 1.5 x the inter-quartile range, was excluded from analysis (a female participant control participant, aged 11), the control data became normally distributed. However, because the AD data remained non-normally distributed, the control outlier remained in analysis (as it did not violate the a-priori cut off criteria based on prior research) and a Mann-Whitney $U$ test was conducted. The final data included 26 control participants and 38 AD participants aged 5 to 17 (Table 2 depicts complete descriptive statistics).

A Mann-Whitney $U$ test was used to evaluate the hypothesis that individuals with AD would have shorter fixation durations on faces of foreground actors within the play scene compared to individuals without AD. The results of the test were significant and in the expected direction, $U = 318, p = .02, r = -.30$. Participants with AD had an average rank of 39.27, while control participants had an average rank of 27.87. (Figure 2).
Visual examination of the data was conducted in order to provide descriptive information about the final skewed distribution. A visual review of the histograms found two different distributions of data for each participant group. For control participants, the spread of the histogram covered a large range of values, ranging from 2.8% to 35% of viewing time fixating on faces (Figure 3). In contrast, the spread of data for AD participants was spread across a smaller range of time frames, ranging from 0.32% to 22.1% of viewing time fixating on faces (Figure 4).

The minimum and maximum viewing times were also different across groups. Participants with AD fixated for a minimum of 0.32% (less than 1 second) of viewing time compared to the minimum of 2.8% (1.68 seconds) of viewing time for the control group. Similarly, the maximum time spent fixating faces for the control group was longer than the AD group maximum, 30% (18 seconds) compared to 22.1% (13.26 seconds), respectively. Nearly half of AD participants spent a short duration of time fixating faces, between 0.3% and 5.0%, however the remaining half demonstrated between 5.0% and 22.1% of viewing time fixating faces. Post-hoc independent samples t-tests failed to find significant differences between these two subgroups on measures of social ability and static ERK activation level.

**Between Group Differences in Time to First Face Fixation**

An analysis of outliers in the eye tracking data found one AD participant (boy age 10) and two control participants (two girls, ages 6 and 11) who had times to first face fixation that were more than two standard deviations from the group mean (AD $M = 8.27s$, $SD = 9.79s$; Control $M = 2.078s$, $SD = 4.06s$) and therefore these participants were excluded from data analysis. When an SPSS-identified control group outlier was excluded from analysis, the control data were then normally distributed. However, because the AD data remained non-normally
distributed, the control group outlier remained in analysis and a Mann-Whitney \( U \) test was conducted. The final data included 25 control participants and 39 AD participants (Table 2).

A Mann-Whitney \( U \) test was conducted to evaluate the hypothesis that individuals with AD would have a longer latency to first fixation on the face of foreground actors compared to individuals without AD. The results of the test were in the expected direction and significant, \( U = 288.5, p = .006, r = -.30 \). Participants with AD had an average rank of 37.60, while control participants had an average rank of 24.54 (Figure 5).

As previously described, data for both participant groups were non-normally distributed, and the data for each group had a distinct shape. Visual examination of the data was conducted in order to provide descriptive information about the final skewed distribution. The control group times to first fixate on a face were positively skewed (Figure 6). Compared to AD participants, controls had a shorter range of times it took them to first fixate on a face, ranging from a minimum of 0.07 seconds to a maximum of 3.29 seconds.

For participants with AD, the data were distributed across a wider range of times, from a minimum of 0.32 seconds to a maximum of 27.25 seconds. Two patterns of fixation appeared in regard to how fast AD participants oriented to the social aspects of the scene. Data were non-normally distributed with two evident peaks and a lack of data surrounding the mean (Figure 7). The AD participants either quickly fixated on a face (between 0.32 seconds and 2.5 seconds) or took upwards to 20 seconds to fixate. Fifty-two percent of the AD participants looked at a face within 2.5 seconds of stimulus onset. Forty-eight percent of the AD participants took longer than 2.5 seconds to look at a face. Post-hoc independent samples t-tests were conducted to determine whether the two subgroups of AD participants differed in their social ability or static ERK
activation level in peripheral lymphocytes. No significant relationships between subgroups were identified.

**Within AD Group Analyses of Eye Tracking Variables**

Overall, significant differences between participant groups for the two eye tracking variables were found. As a result, Pearson r correlations were computed within the AD participants between fixation duration on faces and latency to first fixation on the face and measures of IQ, age, and sex. It should be noted that due to violation of normality for each eye-tracking variable, results should be interpreted with caution as the results may over estimate significance secondary to non-normality.

For the IQ measure, analyses between fixation duration on faces and latency to first fixation on a face were first conducted with the Stanford-Binet 5th Edition IQ scores (N = 22) and the Leiter IQ scores (N = 9) separately. There were no significant differences between the two types of IQ scores and each eye tracking variable; therefore, all participant’s IQ scores were collapsed into one single IQ variable for the final analysis.

The AD participants had a range of IQ scores from 38 to 108 (M = 70, SD = 20). The correlation between IQ scores and fixation duration on faces failed to reach significance (r = .13, p = .28). Similarly, there was not a significant correlation between IQ scores and time to first face fixation (r = .03, p = .87).

A Pearson r correlation was conducted between AD participant age (M = 9.7, SD = 3.2) and fixation duration on faces to explore the relationship among variables. The correlation was not significant (r = -.00, p = .99). Similarly, correlation of AD participant age (M = 9.6, SD = 3.2) and latency to first face fixation did not reach significance (r = -.20, p = .20).
Independent samples t-tests were conducted to compare fixation duration on faces between boys \((N = 32)\) and girls \((N = 6)\). There was not a significant difference in the fixation duration scores between boys \((M = 7.0\%, SD = 5.0\%)\) and girls \((M = 7.0\%, SD = 7.0\%)\), \(t (36) = -0.26, p = .80\). A second independent samples t-test was conducted to compare time to first face fixation between boys \((N = 32)\) and girls \((N = 7)\). There was not a significant difference in latency to first face fixation between boys \((M = 8.40s, SD = 9.71s)\) and girls \((M = 4.87, SD = 7.56)\), \(t (37) = .90, p = .37\). However, it should be noted that the average latency to first face fixation for boys was almost twice as long compared to girls (Figure 8).

Next, Pearson correlations were calculated between each eye tracking variable and the outcome measure of social responsiveness for AD participants. It should be noted that due to the non-normal distribution of data for each eye-tracking variable, the assumption of normality for Pearson correlations was violated and the results should be interpreted with caution.

Pearson correlations were first calculated between fixation duration on faces and social responsiveness to test the hypothesis that fixation duration on faces of foreground actors would be significantly negatively correlated with social responsiveness, as measured by the total \(T\) score on the Social Responsiveness Scale. The results of the correlation analysis indicated that this relationship was not significant \((r = -.11, p = .52)\).

An additional exploratory Pearson correlation analysis was calculated to test the hypothesis that time to first face fixation on either foreground actor would be significantly associated with level of social responsiveness for AD participants. The results of the test indicated that there was not a significant association between the two variables \((r = .15, p = .37)\).
**Between Group Differences in Static ERK Activation Level in Peripheral Lymphocytes**

The final set of analyses examined the exploratory hypotheses for ERK activation levels within the AD participants. The parents of 13 AD participants and eight control participants declined to have the ERK assay completed for their child and therefore these participants were not included in the ERK assay analysis. Additionally, an analysis of outliers in the ERK assay found that one AD participant and two control participants had an average percentage of static ERK activated peripheral lymphocytes that was more than two standard deviations from the group mean (AD $M = 6.5\%$, $SD = 4.9\%$; Control $M = 3.2\%$, $SD = 3.2\%$) and thus were excluded from the ERK assay data analysis. For AD participants, ERK data was positively skewed. When an SPSS-identified outlier (a male participant with AD, aged 8) was excluded from analysis, the ERK data were then normally distributed. However, because the control ERK data remained non-normally distributed and therefore still required a Mann-Whitney $U$ test to be conducted, the outlier remained in analysis as it did not violate the preliminary outlier procedure. The final sample for analyses consisted of 26 AD participants and 17 control participants (Table 2).

A Mann-Whitney $U$ test was conducted to evaluate the hypothesis that the percentage of ERK activated cells would be significantly different for the participants with AD compared to the participants without AD. The result of the test was significant, $U = 67.5$, $p < .001$, $r = -.58$. Individuals with AD had an average rank of 27.90, while individuals without AD had an average rank of 12.97 (Figure 9).

For control participants, the range of ERK activated peripheral lymphocytes ranged from 0% to 4.97%. In contrast, the AD participants displayed a greater range of 0.12% to 12.93% activation. The shapes of the distributions were also different. The AD participants had positively skewed data, while the control group had an inverse normal curve with more
participants displaying either between 0% and 2% activation or between 4% and 5% activation and fewer participants having activation levels around the mean (Figure 10). In contrast, the AD participant’s activation levels clustered around the mean (5.38%), however the mean was twice as large as the mean for the control group (2.25%) (Figure 11).

Two additional Pearson correlation analyses were calculated to test the exploratory hypotheses that the percentage of ERK activated cells would be significantly associated with fixation duration on faces and latency to first face fixation. The non-normally distributed data were visually inspected prior to planned analyses to determine the presence of a monotonic relationship in order to conclude if a Spearman’s correlation would be a more appropriate test statistic due to the violation of normality for the eye tracking variables. A monotonic relationship was not identified; therefore, Pearson r correlations were conducted as planned. The correlation between percentage of ERK activated cells and fixation duration on faces failed to reach significance ($r = -.02, p = .92$). Similarly, the percentage of ERK activated cells was not associated with latency to first face fixation for AD participants ($r = .28, p = .15$).

**Discussion**

Knowledge about the social deficits in AD has recently been advanced by eye tracking technology. Research on how and when individuals with AD attend to faces of children in a play scene remains unexplored. Additionally, the relationship among gaze patterns, social abilities, and biological characteristics of individuals with AD has yet to be investigated.

The present study addressed the aforementioned gaps in research and found that atypical gaze behavior is also present in children and adolescents with AD as they view a dynamic play scene. Additionally, significantly different ERK activation levels between participant groups were identified. Although significant findings were found, the between and within-group results
should be interpreted with caution, as all of the dependent variables violated the assumptions of normality. The variability of data obtained for all dependent variables was likely influenced by the wide range of ages and IQ levels of participants. The small sample sizes also limited the effect sizes that could be obtained for each statistical test.

The current results extend previous research findings that children and adolescents with AD attend to and distribute their gaze differently than peers without AD while viewing children engaged in play. It was hypothesized that the children and adolescents with AD would have shorter fixation durations on faces in the play scene compared to children and adolescent without AD. The results supported this hypothesis. This result is consistent with previous findings from studies using dynamic social scenes of school-aged children (Rice et al., 2012) and adults (Riby & Hancock, 2009a).

This finding additionally supports previous notions that individuals with AD view socially important information in their environment differently than their typically developing peers (Riby & Hancock, 2009a). This result also supports the suggestion that individuals with AD display reduced salience of social stimuli in their environment (Chawarska et al., 2003; Dawson et al., 1998; Klin et al., 2003; Swettenham et al., 2003;), as the AD participants compared to controls fixated on faces slower and also fixated on faces for a shorter amount of time throughout their visual pursuit of the play scene.

Previous eye tracking studies have proposed several theories that could be applied to the current results and provide potential explanations for why the individuals with AD spent less time fixating on the faces of children. First, a social-perceptual theory of atypical gaze behavior suggests that individuals with AD exhibit shorter fixations on faces because non-social stimuli capture their attention to a stronger degree than faces (Hanley et al., 2013). In the current study,
there were multiple alternative sources of visual information in the play scene. The ongoing activity of the children stacking blocks or similar competing stimuli may have attracted the attention of participants with AD to a greater degree than non-AD participants, thus taking their attention away from looking at more social aspects of the scene. The social-perceptual theory would also support previous clinical observations that indicate having an object-rich environment interferes with the ability of children with autism to focus on socially relevant aspects of an educational setting (Olley & Reeve, 1997). It is important to note that this study did not identify whether shorter fixations on faces resulted in longer fixations on objects or non-social stimuli. Future research should aim to investigate both types of fixation to better understand the relative salience and attention given to social vs. nonsocial stimuli in environments with competing object and social stimuli.

A socio-cognitive theory of reduced fixations on the eye region has been previously suggested in the literature and can be applied to the current results. Prior eye tracking research found that as the social aspects of a social interaction increase in complexity (e.g., two or more people interacting together), individuals with AD tend to fixate less on the eyes of others compared to typically developing individuals. Hanley et al. (2013) found that individuals with AD demonstrated similar fixations on the eyes as typically developing peers when the face was presented in isolation. However, individuals with AD engaged in fewer fixations on the eyes of others compared to typical peers when they viewed a dynamic and complex social scene where additional information was potentially competing with their social attention.

Birmingham et al. (2008) found similar results using static photographs. As a static social image increased in complexity, typically developing college students spent more time looking at the eyes of people but only when the people were positioned within an activity (e.g., playing a
Birmingham et al. hypothesized that the eyes were fixated on more often with this stimuli because the wealth of information derived from the eyes aided the participants’ knowledge of the social meaning of the actions. Taken together, these results suggest that typically developing individuals maintain fixations to the eyes of others even when the social content of the scene increases, due to a need to understand social components of ongoing interactions and activities. The current findings are relatively consistent with this hypothesis; typically developing individuals demonstrated longer fixation durations on the faces of children in a complex play scene compared to individuals with AD. Although the social stimuli increased in complexity at various points (e.g., facial expressions changed or direct gaze was made towards the camera), the control participants continued to fixate on faces rather than look elsewhere. In contrast, the participants with AD demonstrated a shorter amount of time gazing at the faces, possibly as a result of decreased motivation or interest in understanding the social aspects of the activity in the scene.

Interestingly, both participant groups failed to fixate on the faces of children for longer than 30% of the viewing time. Future research should focus on systematically identifying other areas of interest to analyze within a play scene in order to better understand viewing patterns of this interaction for both typically developing and AD individuals.

More research is needed in order to translate the identified atypical gaze patterns to potential real-life difficulties or social consequences during play interactions. While speculative, it is possible that engaging in shorter fixation durations on faces of children playing may negatively affect a child’s understanding of the social aspects of play. Although this was not directly assessed in the current study, shorter fixations on the faces of others could decrease the amount of opportunities available to perceive or interpret social cues exhibited by others, by
means of changes in gaze, facial expression, emotional state, or social intention. Furthermore, attending less to the faces of others may also interfere with chances to observe bids for joint attention from others, therefore decreasing opportunities for observational learning and modeling of others that often naturally occur when children and adolescents play near or with each other. Lastly, short fixation durations on faces of others can have safety implications, given that a person needs to distribute limited attention and cognitive resources to the most important sensory stimuli present in one’s environment.

It was next hypothesized that the participants with AD would have a longer latency to first fixation on a face of a child in the play scene. The results supported this hypothesis. Latency to first fixation on a child’s face in a dynamic play scene has not been explored to date in the research; therefore, comparisons to other studies are indirect. The current result is similar to the findings of Norbury et al. (2009), who found that adolescents with ASD took longer to first fixate on the eyes of other adolescents in a dynamic social scene. However, Norbury et al. included only high functioning adolescents with ASD as participants, analyzed a different area of interest (e.g. eyes), and had a different age group of people present in a dynamic scene. The present study therefore adds to the literature by identifying that children and adolescents with AD have a longer latency to first face fixation while viewing a dynamic play scene compared to neurotypical peers.

Although the AD group latency to first face fixation data was significantly different than the control group, AD participants showed two patterns of orienting to the social aspects of the scene. Participants with AD either fixated on a face quickly (within 2.5 seconds of stimulus onset) or slowly (between 2.5 and 27.25 seconds after stimulus onset). It is unclear what may have influenced almost half of the AD participants to take greater than 2.5 seconds to look at
faces of actors. One possibility is that some participants fixated on another object or aspect in the scene prior to fixating on a face, consistent with the social-perceptual theory of decreased salience for social stimuli (Hanley et al., 2013). The current study did not explore which stimuli the participants viewed first following stimulus-onset, only when the face was first fixated. Future research could identify what type of stimuli participants choose to fixate on first following stimulus onset and to determine what is being prioritized prior to fixating on the face. Additionally, behavior given the task demands may have interfered with the viewing patterns; therefore, the longer latencies to first face fixation for some AD participants may have been due to general behavioral difficulties or difficulty understanding the task rather than disinterest in faces, lack of social motivation, or increased preference or salience of objects in the scene.

Although there was a large discrepancy in the sample sizes of girls ($N = 7$) vs. boys ($N = 32$), boys had an average latency to first fixate a face that was almost twice as long as girls. It is likely that the small sample size of girls may have skewed these findings. This sex discrepancy, although relatively proportional to current diagnostic prevalence, could be addressed in future studies that are able to recruit a larger number of girl participants in order to further identify if there is a true significant difference in latency to first fixate faces between boys and girls. It is also possible that the wide range of ages and IQ levels of participants influenced the variability of data obtained for latency to first face fixation.

For the within-group investigations, it was hypothesized that fixation duration on faces of children in the video would be significantly negatively correlated with social responsiveness, as measured by the Social Responsiveness Scale (SRS). Unexpectedly, fixation duration on faces was not associated with the SRS. This finding is inconsistent with the findings of Speer et al. (2007), who found that as social responsiveness scores on the SRS decreased (indicated by
higher scores on the SRS), fixation durations on the eyes of adults in a dynamic social scene decreased for children and adolescents with ASD and average intelligence.

One explanation for the differing results between the present study and Speer et al. (2007) article is the different use of SRS scores. Speer et al. used SRS scores from both non-ASD and ASD groups, providing a wider range of scores for correlation analysis. The current study only utilized SRS scores for AD participants; therefore, the lack of variability in AD participant SRS scores may explain the insignificant relationship between SRS and gaze patterns. Another possibility for the differing results with Speer et al. is the use of fixations on a whole face in the current study rather than fixations on specific internal features, such as the eyes. Future research should focus on fixation durations on both eyes and mouth, as previous research remains inconclusive on the relationship between fixation duration on these facial areas and social ability in AD.

Next, it was hypothesized that latency to first face fixation would be significantly negatively correlated with social ability. Unexpectedly, latency to first face fixation was not associated with social ability. This finding does not provide support for the theory that general social deficits in AD are related to abnormal gaze patterns, and in particular to this study, specifically how quickly children and adolescents fixate on the faces of others in a dynamic play scene. By contrast, this finding suggests that differences in latency to first face fixation on a peer in a dynamic interaction may not be sufficient to disrupt general social responsiveness. In sum, more information is needed to understand how atypical gaze patterns while viewing a dynamic play scene are related to social deficits in AD.

Only one previous eye tracking study has used dynamic stimuli to investigate relationships between IQ and fixation patterns for individuals with AD. This study failed to find
a significant correlation between fixation patterns, verbal IQ, and age of participants with ASD (Klin et al., 2002). The present study extends the literature by finding that IQ did not correlate to gaze patterns in children and adolescents with AD as they viewed a dynamic play scene. Research has also not yet explored the relationship between sex and gaze patterns for participants with AD, possibly due to small sample sizes for female participants. The present study did not find a significant correlation between sex and gaze patterns for AD participants. Taken together, the aforementioned results indicate that viewing patterns did not differ among AD participants in relation to participant IQ, age or sex. In general, the variability of the present data for each dependent variable is likely driven by the aforementioned demographic and participant factors (e.g. age, IQ level, and sex), which could be addressed in a future study that is able to include more participants.

**Between Group ERK Activation Level in Peripheral Lymphocytes**

It was hypothesized that ERK activation level would be significantly different between the participant groups. The results confirmed this hypothesis. Individuals with AD had a significantly greater percentage of static ERK activated peripheral lymphocytes compared to individuals without AD. Although both participant groups had non-normally distributed data, it was unexpected that the AD participants’ data were closer to a normal curve than the control participants given the known genetic and biological heterogeneity in AD. The control group data were distributed closer to an inverse normal curve, with more participants having either between 0 and 2% ERK activated cells or between 4% and 5% ERK activated cells, with fewer participants having activation percentages around the mean (2.25%). In contrast, many AD participants had activation levels around the mean of 5.38%. Similar to the results of the eye tracking variables, the variability in ERK activation levels may be reflective of the heterogeneity
of the AD participant group. The two distinct group distributions are important to consider, since the AD data support a hyperactive and wide distribution of ERK activation while the control group represents a shorter range with more participants exhibiting low percentages of ERK activation compared to those with AD.

Given that this is one of the first in-vivo studies comparing static ERK activation levels in peripheral lymphocytes between participants with and without AD, comparisons with previous studies of post-mortem ERK activation levels can only be indirect. The current results extend previous research that identified upregulated ERK pathways in post-mortem frontal cortex neurons of individuals with AD (Yang et al., 2011) by identifying a greater percentage of static ERK activated peripheral lymphocytes in living participants with AD compared to individuals without AD. This finding supports further exploration and replication of hyperactive ERK pathway activation in peripheral lymphocytes, with the goal to determine whether the presence of a hyperactivated ERK pathway could be used as a biomarker for AD.

The current result also suggests that the various ERK-related pathway activation activities could also be in an excited state, including cell proliferation, cell differentiation or cell death, although which events and the effect of these events are unknown and were not explored in the current study. It also remains unclear exactly how or why the peripheral lymphocytes contained a greater number of ERK activated cells in AD participants, especially since a variety of mechanisms are known to activate the ERK pathway in cells (e.g. hormones, neurotransmitters, growth factors etc). Future research focused on identifying the precipitants to a hyperactive ERK pathway in peripheral lymphocytes or co-occurring biological states and the downstream effects of such activity will increase the knowledge base on the ERK biological pathway and its potential relationship to AD.
Next, the current study sought to identify the impact of the increased ERK pathway activation for individuals with AD by examining the relationships between ERK activation levels and fixation patterns. It was hypothesized that ERK activation level would be significantly associated with fixation duration on faces and latency to first face fixation. These hypotheses were not confirmed. It appears that hyperactivity at a cellular level solely in peripheral lymphocytes may not be directly affecting the cognitive social aspects, skills, or perhaps attention, motivation, or interest needed to fixate on the faces of others or attend quickly to faces. Although this study was able to identify that the participants with AD had hyperactive levels of ERK compared to typically developing participants, when this result was investigated alongside a behavioral task (e.g. eye tracking), a subgroup of individuals with co-occurring atypical biology and behavior was not identified.

Over time, the present findings may advance the understanding of the biological differences inherent to AD. Early screening and diagnostic procedures could be strengthened if future research is able to identify atypical ERK activation levels in infants and toddlers. If future research is able to identify hyperactive static ERK activation levels in persons who also demonstrate atypical gaze patterns, then the very early presence of a hyperactive ERK pathway combined with atypical viewing behaviors in infants could provide a more distinct phenotype of AD and lead to better-informed and developed treatments earlier on in development. Future research that replicates the current ERK pathway results is needed to begin to identify a possible biological phenotype of AD. Further research into hyperactive ERK activation levels as a biomarker of ASD is therefore warranted.
Study Limitations and Future Directions

The present study has several limitations, the first being the use of archival data. As a result, there was an inability to establish large enough sample sizes for each participant group to produce a strong power for the statistics. The relative lack of statistical power to detect effects may have contributed to the failure to find significant group differences in the analyses of eye tracking variables, social ability and ERK activation levels. Future studies aiming to replicate or expand upon this study should take into account the inherent limitation of the small sample sizes in the current study. In order to detect a standard power of .80, with a medium effect size of .50 and an alpha of .05, a total of 64 participants per group would be necessary to observe significant differences between groups (Cohen, 1992). Nonetheless, the current study was still able to fill in a gap in the existing literature and produced important information for future studies to use to continue social gaze and biomarker research.

Additionally, although the provided play scene was ecologically valid due to its dynamic and realistic social play nature, the scene may have lacked appeal in regard to the age of the children in the scene and the absence of audio tracks. Although fixation patterns did not vary based on participant age, watching children playing in a classroom may have not been enticing or developmentally appropriate for the older participants in both diagnostic groups. Future research could separate children from adolescent participants and age-match the play scenes to better understand the impact of viewing individuals of one’s own age on the gaze patterns of participants with and without AD.

The lack of audio during the scene may have also interfered with the results of the study. It is possible that vocalizations (e.g., talking, laughing, and shouting) may have increased the likelihood that the participants in either group would have looked towards the faces of people
more often than was observed with the current video stimulus. An area of future research could focus on providing auditory elements within a similar play scene and observing any differences in gaze between participant groups, particularly related to fixation durations on the mouth vs. eye regions.

The present study also has limited external validity. The results do not generalize to infants and adults with or without AD. Future studies should develop longitudinal designs to determine how gaze patterns develop and change over time. Additionally, the control and AD participants were not matched on IQ and IQ data was not available for the control group, thus limiting the ability to identify between-group differences in this domain. The highly controlled and experimental setup also limits the generalization of the results to daily-life situations. Future integration of naturalistic approaches with experimental paradigms could provide a more comprehensive picture of AD.

Another limitation to this study was the reliance on parent-report data for social responsiveness abilities, which may be susceptible to bias. Parents may have responded differently depending on the extent of personal involvement with their child and whether or not the parent had a bias towards presenting their child in a negative or positive light. Additionally, each participant only had one attempt to engage in the eye tracking task and follow-up reliability visits were not feasible. Therefore, it is not possible to determine if the findings are consistent over time and if they represent a participant’s true and consistent viewing pattern of play scenes.

These limitations notwithstanding, the methods and results of the present study have many strengths and advance our understanding of how children and adolescents with AD spontaneously view a dynamic play scene. Many avenues of future research are evident as well.
The between-group findings provide support that subsequent research should continue to explore dynamic play scenes, as these stimuli remain under-researched. Future studies should also explore visual attention to other aspects of a play scene, including to non-social stimuli and to the specific internal features of the face (e.g., eyes and mouth), as past research has found strong relationships between fixations to these areas and social abilities. In addition, this study confirms that living individuals with AD have atypical ERK activation levels in peripheral lymphocytes. This finding provides the basis for further investigations into the role of the ERK pathway for individuals with AD, for both potential diagnostic and treatment purposes.

In summary, the present study adds to the eye tracking literature on social gaze patterns by identifying that children and adolescents with AD had shorter fixations on faces and longer latencies to first face fixation while observing children playing. Additionally, biological differences between participant groups were found; the participants with AD had hyperactive ERK activation levels in peripheral lymphocytes compared to controls. The present study also explored the relationships between gaze patterns, social ability, and ERK activation levels. Although no significant results were found among these three variables, the present study offers numerous starting points upon which future research can expand.
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Table 1

**Participant Demographics**

<table>
<thead>
<tr>
<th>Participant Characteristics</th>
<th>AD</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Child Age (years), Mean ± SD</td>
<td>9.65 ± 3.18</td>
<td>10.66 ± 2.97</td>
</tr>
<tr>
<td>Child Sex</td>
<td>82.5% boys</td>
<td>44% boys</td>
</tr>
<tr>
<td>Child Race (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>90%</td>
<td></td>
</tr>
<tr>
<td>African-American</td>
<td>10%</td>
<td></td>
</tr>
<tr>
<td>IQ Score Mean ± SD, range</td>
<td>70 ± 20, 38 – 108</td>
<td></td>
</tr>
<tr>
<td>SCQ Mean ± SD, range</td>
<td>23.05 ± 5.07, 15 – 32</td>
<td>1.85 ± 2.89, 0 – 11</td>
</tr>
<tr>
<td>SRS Mean ± SD, range</td>
<td>86.46 ± 13.06, 54 – 113</td>
<td></td>
</tr>
</tbody>
</table>

Child characteristics reflect children and adolescents (ages 5-17); SD, standard deviation; SCQ is a screening measure for Autism Spectrum Disorder (ASD); scores on the SCQ range from 0-39, with higher scores indicating the possibility of ASD (Rutter, Bailey & Lord, 2003).
Table 2

*Descriptive Statistics of Dependent Variables by Group*

<table>
<thead>
<tr>
<th>Variable</th>
<th>AD</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fixation Duration on Faces (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>7.1 ± 6.0</td>
<td>10.9 ± 6.7</td>
</tr>
<tr>
<td>Median</td>
<td>5.4</td>
<td>9.8</td>
</tr>
<tr>
<td>Range</td>
<td>0.32 – 22.1</td>
<td>2.8 – 30</td>
</tr>
<tr>
<td><strong>Latency to First Face Fixation (seconds)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>7.77 ± 9.37</td>
<td>1.01 ± 0.5</td>
</tr>
<tr>
<td>Median</td>
<td>1.70</td>
<td>0.80</td>
</tr>
<tr>
<td>Range</td>
<td>0.32 – 27.2</td>
<td>0.07 – 3.29</td>
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<tr>
<td><strong>ERK Activation (%)</strong></td>
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<td></td>
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<tr>
<td>Mean ± SD</td>
<td>5.3 ± 2.6</td>
<td>2.2 ± 1.6</td>
</tr>
<tr>
<td>Median</td>
<td>4.9</td>
<td>1.7</td>
</tr>
<tr>
<td>Range</td>
<td>0.12 – 12.9</td>
<td>0 – 4.9</td>
</tr>
</tbody>
</table>
Figure 1. Screen shot of child play scene.
Figure 2. Box plot comparison of visual fixation duration (%) on faces for 38 participants with AD and 26 typically developing participants (controls). The upper and lower boundaries of the standard box plots are at the 25th and 75th percentiles. The horizontal line across the box marks the median of the distribution and the vertical lines below and above the box mark the minimum and maximum, respectively.
Figure 3. Histogram of fixation duration on faces (%) for 28 typically developing participants (controls).
Figure 4. Histogram of fixation duration on faces (%) for 38 participants with AD.
Figure 5. Box plot comparison of time (seconds) to first face fixation for 39 participants with AD and 25 typically developing participants (controls). The upper and lower boundaries of the standard box plots are at the 25th and 75th percentiles. The horizontal line across the box marks the median of the distribution and the vertical lines below and above the box mark the minimum and maximum, respectively.
Figure 6. Histogram of time to first face fixation for 25 typically developing participants (controls).
Figure 7. Histogram of time to first face fixation for 39 participants with AD.
Figure 8. Histograms of time to first face fixation for 32 males (top) and 7 females (bottom).
Figure 9. Box plot comparison of ERK activation level (%) for 26 participants with AD and 17 typically developing participants (controls). The upper and lower boundaries of the standard box plots are at the 25th and 75th percentiles. The horizontal line across the box marks the median of the distribution and the vertical lines below and above the box mark the minimum and maximum, respectively.
Figure 10. Histogram of ERK activation level for 17 typically developing participants (controls).
Figure 11. Histogram of ERK activation level for 26 participants with AD.
Appendix

Xavier University IRB Approval Letter

March 27, 2015

Molly Carter
3758 Hyde Park Ave.
Cincinnati, OH 45209

Re: Protocol #14-080, Relationships Among Eye Gaze, Social Ability and Extracellular Signal-Regulated Kinase Pathway Activated in Children and Adolescents with Autistic Disorder

Dear Ms. Carter:

The IRB has reviewed the materials regarding your study, referenced above, and has determined that it meets the criteria for the Exempt from Review category under Federal Regulation 45CFR46. Your protocol is approved as exempt research, and therefore requires no further oversight by the IRB.

If you wish to modify your study, including the addition of data collection sites, it will be necessary to obtain IRB approval prior to implementing the modification. If any adverse events occur, please notify the IRB immediately.

Please contact our office if you have any questions. We wish you success with your project!

Sincerely,

[Signature]

Morell E. Mullins, Jr., Ph.D.
Chair, Institutional Review Board
Xavier University

MEM/sb
Summary

*Title:* Relationships Among Gaze Patterns, Social Ability and Extra Cellular Signal-Regulated Kinase Pathway Activation in Children and Adolescents with Autistic Disorder

*Problem:* Individuals diagnosed with autism spectrum disorder (ASD) experience deficits in social interaction and communication (APA, 2013). Eye tracking technology has recently quantified the social deficits in persons with ASD. Previous research has found that compared to typically developing peers, children and adolescents with ASD take longer before first fixating on the face or eyes of others (Freeth et al., 2010; Norbury et al., 2009) and spend less time looking at the faces of others (Riby & Hancock, 2009; Rice et al., 2012) as they view dynamic social scenes. Additionally, eye tracking research has identified relationships between gaze patterns and outcome measures of social ability, increasing support for theories that suggest the observable social abnormalities in ASD may be related to how persons spontaneously view social stimuli (Norbury et al., 2009).

Studies to date have not examined the gaze patterns of individuals with ASD as they observe a child play scene. Furthermore, the relationships among gaze patterns, social ability, and biological characteristics of participants have yet to be investigated. The current study will address gaps in the literature on ASD-specific gaze behavior and their demographic, social, and biological correlates.

*Method:* Archival data was collected from children and adolescents aged 5 to 17 with and without Autistic Disorder (AD) who participated in a larger eye-tracking study. Initially, the sample consisted of 69 participants, including 42 children and adolescents with AD and 27 age-matched (+/- 6 months) typically developing individuals. However, two of the AD participants (2 males) were excluded from the final analysis due to challenging behaviors and lost data. Therefore, the final sample consisted of 67 participants, including 40 children and adolescents with AD (33 males, 7 females) and 27 age-matched typically developing peers (12 males, 15 females) between the ages of 5 and 17. Four of the AD participants were African-American (10%) and 36 were Caucasian (90%). All participants viewed a 60-second child play scene on a Tobii T120 eye tracking monitor. Additionally, AD participants completed the Standford-Binet, Fifth Edition or the Leiter, Revised and a blood draw. Caregivers of participants with AD completed a demographic questionnaire and the Social Responsiveness Scale (SRS).

*Findings:* Results from Mann-Whitney *U* tests indicated significant differences between groups for eye gaze patterns. Participants with AD fixated for a shorter amount of time on faces (*Mdn* = 27.87) compared to individuals without AD (*Mdn* = 39.27), *U* = 318, *p* = .016, *r* = -.30. Participants with AD also took longer to first fixate on a face in the play scene (*Mdn* = 37.60) compared to participants without AD (*Mdn* = 24.54), *U* = 288.5, *p* = .006, *r* = -.30. Individuals with AD had a significantly greater percentage of static ERK activated peripheral lymphocytes (*Mdn* = 27.90) compared to individuals without AD (*Mdn* = 12.97), *U* = 67.5, *p* < .001. The within-AD group correlations among gaze patterns, social abilities, and ERK activation levels were not significant.
Implications: The present study addressed gaps in research by identifying gaze patterns of individuals with AD as they viewed a play scene. The results extend previous research by identifying that atypical gaze behavior is also present in children and adolescents with AD as they view a dynamic play scene. Consistent with study hypotheses, results indicated that children and adolescents with AD spent shorter amounts of time viewing faces and took longer to view faces in the play scene compared to typically developing participants. However, contrary to the expected results, AD-specific gaze patterns were not related to impaired social ability. This may be explained by a combination of factors, including the use of parent-report data and the skewed data set. Additionally, consistent with study hypotheses, static ERK activation levels in peripheral lymphocytes were significantly elevated in participants with AD compared to controls. Exploratory correlates of ERK with social responsiveness and gaze patterns were insignificant. Overall, study findings contribute to our knowledge and understanding of gaze patterns and biological characteristics for typically developing children and adolescents and their age-matched peers with AD. Replication and extension of the study exploring additional factors (fixations on eyes, mouths, and objects) is warranted to better understand gaze patterns towards a child play scene for individuals with and without AD.