The relationship between early neural responses to emotional faces at age 3 and later autism and anxiety symptoms in adolescents with autism

Abstract

Both autism spectrum (ASD) and anxiety disorders are associated with atypical neural and attentional responses to emotional faces, differing in affective face processing from typically developing peers. Within a longitudinal study of children with ASD (23 male, 3 female), we hypothesized that early ERPs to emotional faces would predict concurrent and later ASD and anxiety symptoms. Greater response amplitude to fearful faces corresponded to greater social communication difficulties at age 3, and less improvement by age 14. Faster ERPs to neutral faces predicted greater ASD symptom improvement over time, lower ASD severity in adolescence, and lower anxiety in adolescence. Early individual differences in processing of emotional stimuli likely reflect a unique predictive contribution from social brain circuitry early in life.

Keywords: autism; face processing; ERP; internalizing; anxiety

Processing of human faces is among the earliest emerging and most fundamental of social cognitive skills. Although the time course and precise mechanisms underlying face processing are debated (Gauthier, Curran, Curby, & Collins, 2003; Kanwisher, 2000; Morton & Johnson, 1991), efficient and accurate processing in infancy establishes a foundation upon which more sophisticated social cognition and behavior can develop (Dawson, Webb, & McPartland, 2005; Webb, Neuhaus, & Faja, in press). Relative to their typically developing peers, individuals with autism spectrum disorder (ASD) display an altered trajectory of visual attention toward faces from early in life, with reductions in sensitivity to others' eyes and direction of eye gaze, joint attention, and social smiling (Elsabbagh et al., 2012; Jones & Klin, 2013; Ozonoff et al., 2010). Such alterations appear to be meaningful, as they predict short-term social and communication development (Campbell, Shic, Macari, & Chawarska, 2014; Webb et al., 2010).

Within the context of typical development, Johnson and colleagues (Johnson, Senju, & Tomalski, 2015; Morton & Johnson, 1991) propose an experience-expectant neural system in which infants are motivated to seek out faces from very early in life. By repeatedly seeking out and attending to faces over alternative images (e.g., objects), infants receive an abundance of opportunities to take in facial information; through these frequent and varied exposures to faces, neural structures and networks come to specialize for the processing of faces above other, competing visual stimuli (e.g., non-face images). In combination with other social input and experiences, accurate and efficient face processing early in life promotes the emergence of increasingly complex and specialized social cognitive skills such as joint attention, social orienting, affect recognition, and imitation (Baron-Cohen, Baldwin, & Crowson, 1997; Dawson & Bernier, 2007; Dawson, Webb, & McPartland, 2005).

In the context of ASD, in contrast, infants attend significantly less to faces and thus receive fewer opportunities and far less face input around which experience-expectant regions can organize (Dawson & Bernier, 2007; Webb et al., in press). This altered social environment results in atypical specialization of face processing circuitry (Webb et al., in press) and altered integration of face processing mechanisms with other neural regions important to social communication, undermining a broad array of skills across many domains of social functioning and cognition (Webb et al., in press). Consistent with these models, cross-sectional and short-term longitudinal studies in ASD link face processing with social and communication skills, as well as with ASD severity. For instance, lack of attention to faces emerges as the single best predictor of a later ASD diagnosis among 12-month-olds (Osterling & Dawson, 1994). Similarly, greater attention to faces during child-directed speech at 20 months of age predicts growth in communication skills between 2 and 3 years of age (Campbell, Shic, Macari, & Chawarska, 2014).

Processing of faces displaying emotional expressions appears to be particularly altered in ASD. Accuracy in facial affect recognition lags behind typically-developing peers across all ages assessed (see Lozier et al., 2014 for review), and neuroimaging findings indicate atypical distribution and degree of neural activation during affect recognition tasks (Ashwin, Baron-Cohen, Wheelwright, O'Riordan, & Bullmore, 2007; Deely et al., 2007; Golarai, Grill-Spector, & Reiss, 2006). Differences are evident even from early childhood. Using event-related potentials (ERPs) to assess neural response, Dawson and colleagues (2004) found that, whereas toddlers without ASD showed faster and larger N290 responses to fearful versus neutral faces, those with ASD showed comparable responses to both expressions. Children with ASD also exhibited slower N290 responses and atypical scalp topography to fearful faces, relative to peers.

Moreover, speed of the N290 response was associated with multiple measures of social cognition and perception: children with faster N290 responses to fearful faces had better joint attention, better social orienting, and more interest in an experimenter displaying distress (Dawson et al., 2004). This converging behavioral and ERP evidence suggests that ASD is characterized by a differential trajectory for visual attention and neural processing of faces relative to peers without ASD, as well as corresponding alterations in the developmental trajectory of social cognition and behavior (Webb et al., in press). However, longitudinal explorations of these associations have thus far been limited to the early childhood period, and we have little knowledge as to how face processing might contribute to social communication over a longer time course.

Face Processing and Socio-Emotional Development

In addition to implications for social cognition and communication, early disruptions to affective face processing mechanisms may also predict later emotional functioning. From a neurobiological perspective, both social functioning and emotion regulation rely heavily upon the activity of the amygdala and its interactions with related brain structures and networks (Phelps & LeDoux, 2005). A primary role of the amygdala is to evaluate and assign emotional significance to stimuli, and subsequently to direct both visual attention and behavioral responding (Phelps & LeDoux, 2005). Modulation of perception and attention occurs through connections between the amygdala and cortical areas (Amaral, Behniea, & Kelly, 2003), and likely contributes to increased vigilance to potential threat (Phelps & LeDoux, 2005), of which fearful faces can be one indicator. Given that control of visual attention (e.g., toward or from fearful stimuli such as faces) constitutes one avenue through which effective emotion regulation develops (Rothbart, Sheese, Rueda, & Posner, 2011), altered visual attention to affective faces (particularly those displaying fear) as assessed through ERPs could predict an altered

developmental trajectory of emotion regulation and thus confer increased risk for anxiety symptoms.

Such theories are motivated by observations of atypical attention to and processing of affective faces across a range of internalizing disorders, both in adults and in children. Individuals high in anxiety, for example, show a bias to orient toward faces expressing strong negative emotions (e.g., fear, anger), in contrast to their less anxious peers (Hankin, Gibb, Abela, & Flory, 2010; Mogg, Garner, & Bradley, 2007), and also have difficulty disengaging attention from such emotional faces (Fox, Russo, Bowles, & Dutton, 2007). Consistent with this, neuroimaging findings reveal heightened activation in response to emotional faces among anxious adolescents, most prominently in the amygdala (e.g., Thomas et al., 2001). Relative to non-anxious peers, individuals with high anxiety show faster and larger ERPs to threat-related faces (Bar-Haim, Lamy, & Glickman, 2005; Eldar, Yankelevitch, Lamy, & Bar-Haim, 2010), with larger ERPs predicting greater anxiety at future time points (O'Toole, DeCicco, Berthod, & Dennis, 2013).

Importantly, such findings do not resolve questions about the causal relationship between face processing and anxiety – effects in either causal direction are plausible, as is a third factor that contributes to both outcomes. Nevertheless, from this discussion, it follows that altered visual attention and neural response to faces (particularly those conveying negative affect) may signify heightened risk for anxiety symptoms. However, despite evidence that individuals with ASD display both face processing atypicalities and higher rates of internalizing symptoms and diagnoses relative to peers without ASD (Neuhaus, Bernier, & Beauchaine, 2014; Simonoff et al., 2008), we know very little about how early face processing might predict later psychological or emotional well-being for children with ASD.

Goals of the current study

Motivated by this literature, our primary goal in the current paper was to build from findings described by Dawson and colleagues (2004) to explore links between (1) neural responses to neutral and fearful faces, and (2) ASD symptoms, both concurrently at age 3 years and longitudinally over the course of childhood and early adolescence. We predicted that early neural responses to affective stimuli would be correlated with levels of ASD symptoms, specifically within the domain of social communication. We predicted that the association between early processing of affective stimuli and ASD symptoms would exist at age 3 years and longitudinally, such that early neural responses to affective stimuli would predict the trajectory of change in these symptoms between ages 3 and 14 years. Specifically, we hypothesized that longer N290 latencies to faces would be associated with more severe ASD symptoms, both in early childhood and over the course of development. We also anticipated that greater differential latencies and amplitudes between fearful and neutral faces would be associated with fewer ASD symptoms, in keeping with findings that typically developing toddlers differentiate between these emotions whereas toddlers with ASD may not (Dawson et al., 2004).

As an exploratory second aim among a subset of participants who had sufficient data, we also sought to explore longitudinal relations between early face processing at age 3 years and anxiety symptoms at age 14 years. Because altered attention to and processing of affective stimuli such as faces characterizes a number of anxiety disorders, we anticipated that faster and larger ERPs to fearful faces, as well as larger differential ERPs to fearful versus neutral faces, during early childhood would predict self-reported symptoms of anxiety during adolescence.

Method

Participants

Participants were recruited as part of a large longitudinal study on the early development of ASD (n = 65; described previously in Dawson et al., 2004). Families with children between 3 and 4 years of age were recruited through public schools, local clinics, parenting groups, government agencies relevant to developmental disabilities, and the University participant pool. For the current paper, children were included if they had a diagnosis of an autism spectrum disorder on the basis of expert clinical judgment, informed by algorithm scores on the Autism Diagnostic Observation Schedule - G (Lord, Rutter, Goode, & Heemsbergen, 2003) and Autism Diagnostic Interview – Revised (Lord, Rutter, & Le Couteur, 1994) at age 3 years; valid EEG data at age 3 years (described below); and sufficient longitudinal data across the four time points of the study. Children were excluded if they had a known neurological disorder (e.g., Fragile X), sensory or motor impairments that would interfere with study completion, or history of serious head injury or seizure. All participants were free of antiepileptic medications at all timepoints. This strategy resulted in a sample of 26 children with ASD (3 girls, 23 boys). Table 1 presents demographic characteristics for the final sample. Informed consent was obtained from all families included in the study.

[Table 1 about here.]

Procedures

Children were first assessed upon entry into the study, between 3 and 4 years of age. At that time, they completed a rigorous diagnostic assessment consisting of the ADOS-G (Lord, Rutter, Goode, & Heemsbergen, 1989), ADI-R (Lord, Rutter, & LeCouteur, 1994), and Mullen Scales of Early Learning (MSEL; Mullen, 1984). Families also completed an EEG visit consisting of several tasks. Longitudinal visits to assess ASD symptoms were subsequently completed when

children were approximately 6 years (mean=75 months, SD=3.5 months), 9 years (mean=111 months, SD=2.1 months), and 14 years (mean=172 months, SD=10.3 months) of age, and included the ADOS and ADI-R. In addition, self-report measures of adolescents' internalizing symptoms were completed at the 14-year visit to characterize the presence of anxiety symptoms in this population. Of the 26 families who completed the first timepoint, 26 families, 22 families, and 18 families completed the second, third, and fourth timepoints, respectively.

Measures and Tasks

Diagnostic measures. Diagnosis of ASD at the first timepoint was based in part on the ADI-R and ADOS-G, with final diagnosis determined by expert clinical opinion. The ADI-R is a semi-structured parent interview that assesses communication, social skill, and restricted and repetitive behavior and interests over the course of development. The ADOS is a play-based observational assessment of these same three areas. Both the ADI-R and ADOS yield diagnostic cutoffs that distinguish children with and without ASD with high sensitivity and specificity (Gotham, Risi, Pickles, & Lord, 2007; Lord, Rutter, Goode, & Heemsberger, 1989). At each timepoint (ages 3, 6, 9, and 14 years), social affect (SA) scores from the ADOS were computed according to the revised ADOS algorithms (Gotham et al., 2007) as indices of social and communication difficulties, and ADOS calibrated severity scores (CSS) were computed as indices of overall symptom severity (Gotham, Pickles, & Lord, 2009). Although participants completed different modules of the ADOS over the course of the study depending on their developmental level, algorithm scores are derived from the same number of items across modules. Thus, we cannot say precisely whether item-level scores changed from time point to time point for each participant, but this approach does provide confidence that symptom domains are well represented at each assessment. Children's overall developmental level was assessed

with the Visual Reception, Fine Motor, Expressive Language, and Receptive Language scales of the Mullen Scales of Early Learning (MSEL; Mullen, 1984) at age 3 years. As stated earlier, all participants received ASD diagnoses at first timepoint.

Face Processing Task. Neural response to faces was recorded as children viewed repeated images of a female face with either a neutral or prototypical fear expression (© Ekman & Friesen, 1976). Neutral and fearful faces were 32 cm in width and 24 cm in height (visual angles of approximately 24 x 18 degrees), and appeared in random order until participants had attended to 50 of each expression, as determined by an online coder trained to monitor attention to the stimulus. Each trial consisted of a 100-ms baseline, 500-ms face stimulus, 1200-ms post-stimulus period, and variable intertrial interval of 500 to 1000 ms. During this task, children sat on their parent's lap, approximately 75 cm from the computer monitor on which stimuli appeared. EEG was recorded with a 64-channel geodesic sensor net (Electrical Geodesics Incorporated; Tucker, 1993) prepared with KCl electrolyte solution and fitted to keep impedances below 40 kΩ.

Following data collection, data were lowpass filtered at 20 Hz and segmented into trials of 1700 ms (500 ms of stimulus plus 1200 ms post-stimulus) with a 100 ms baseline. Trials were discarded if they had more than 250 μV variation, more than 10 bad channels, or other apparent artifacts (e.g., eye blinks). Bad channels were replaced and data were re-referenced to an average reference that excluded rim electrodes (due to excessive artifact in the rim region). Finally, data were baseline corrected according to the 100 ms preceding the onset of the stimulus and ERP peaks were verified through visual inspection.

Latency and amplitude of a negative-going deflection within a temporal window spanning 270 to 470 ms post-stimulus onset were then extracted from a total of 8 electrodes covering the right posterior region (channels 40, 43, 44, 45) and the left posterior region (channels 28, 32, 33,

35). Responses were then examined with regard to neutral faces (capturing basic face processing), fearful faces (capturing emotional face processing), and the difference between fearful and neutral faces (fearful minus neutral, capturing processing unique to fearful emotion). In total, 26 children provided ERP data of sufficient quality and quantity for the current analyses.

Anxiety symptoms during adolescence. Adolescent (age 14 years) anxiety symptoms were assessed with the Revised Children's Manifest Anxiety Scale, 2nd Edition (RCMAS-2; Reynolds & Richmond, 1997). The RCMAS-2 is a self-report measure containing 37 items to assess total anxiety as well as subscales of physiological anxiety, worry, and social anxiety. Although concerns exist regarding the ability of adolescents with ASD to report accurately on their own internalizing symptoms (May, Cornish, & Rinehart, 2015), recent studies have demonstrated reasonable parent-adolescent agreement on internalizing measures (Blakeley-Smith, Reaven, Ridge, & Hepburn, 2012; Ozsivadjian, Hibberd, & Hollocks, 2014) and emphasized the value of self-report in this population. Eleven adolescents provided RCMAS-2 data at age 14 years.

Analysis

To examine concurrent and longitudinal relations between the N290 response to faces and ASD symptoms over the course of early childhood and into adolescence, we created a series of multilevel models (MLMs) using Hierarchical Linear Modeling (HLM) – Version 7.01 to model the effects of time on ADOS scores. This approach was selected because it accounts for the nested or hierarchical nature of longitudinal measures better than alternative approaches such as repeated measures analysis of variance (Bryk & Raudenbush, 1992), and has been used successfully with relatively small samples (e.g., Ingersoll & Wainer, 2013; Siller & Sigman, 2008). Separate MLMs were created for each outcome variable (ADOS Social Affect score and ADOS calibrated severity score) with each N290 predictor (response latency on the left and right,

response amplitude on the left and right). Repeated time points (1, 2, 3, and 4, corresponding to ages 3, 6, 9, and 14 years, respectively) were entered at Level 1, and N290 response was entered as a continuous predictor at Level 2. Time 1 *T*-scores from the Visual Reception subscale of the MSEL were entered at Level 2 to control for general visual cognitive skill at age 3 years. The resulting models yielded intercept and slope terms, with intercepts representing initial level of ASD symptoms as measured by the ADOS, and slopes representing change in symptoms between ages 3 and 14 years.

Results

Face processing and ASD symptom trajectories over time

Neutral faces. Table 2 presents the output for models containing response to neutral faces; all 26 children were included in the analyses. Slope terms for ADOS Social Affect scores were predicted by left hemisphere latency to neutral faces, slope β=0.04, $t(23)$=2.80, p=.01. As shown in Figure 1, children with faster left hemisphere latencies to neutral faces at age 3 decreased in social communication symptoms more steeply over the course of childhood than did children with slower responses to neutral faces. Change in ADOS Severity scores was also predicted by left hemisphere latencies, slope β=0.02, $t(23)$=3.01, p=.006, with greater improvements in overall symptoms observed in children with faster neural responses.

In addition, left hemisphere amplitude to neutral faces had a significant effect on Social Affect slopes, β=0.08, $t(23)$=2.15, p=.042. Children with greater negative amplitude at age 3 years displayed steeper decreases in social communication difficulties over time.

[Table 2 about here.]

[Figure 1 about here.]

Fearful faces. Left hemisphere amplitude to fearful faces predicted Social Affect intercepts, β=-0.41, t(23)=-2.33, p=.029, and ADOS Severity intercepts, β=-0.21, t(23)=-2.74, p=0.012. These effects were such that children with larger negative amplitudes in response to fearful faces displayed more difficulties in ADOS areas assessed at age 3. With regard to symptom change over time, right hemisphere amplitude predicted Social Affect slopes, β=-0.10, t(23)=-2.18, p=.04, indicating that larger negative amplitudes to fearful faces were associated with less improvement over time. See Table 3 and Figure 2.

[Table 3 about here.]

[Figure 2 about here.]

Differential fearful – neutral faces. Differential responding was defined as latency or amplitude to neutral faces subtracted from the corresponding latency or amplitude to fearful faces. As a result, positive values indicate an increase in latency or amplitude for fearful relative to neutral faces, whereas negative values indicate a decrease in latency or amplitude for fearful relative to neutral faces. Differential latency in the left hemisphere predicted Social Affect intercept and slope terms, intercept β=0.08, t(23)=2.80, p=.01, slope β=-0.03, t(23)=-2.32, p=.03. Greater differential latency (i.e., slowing of neural response to fearful faces relative to neutral faces) was associated with more social communication difficulties at age 3 years, but better improvement in symptoms during childhood. See Table 4 and Figure 3.

[Table 4 about here.]

[Figure 3 about here.]

Face processing and ADOS outcomes in adolescence

The MLMs presented thus far inform associations at age 3 years and trajectories over time, but do not speak directly to links between age 3 neural responses and age 14 outcomes. In order

to better understand the extent to which N290 responses in early childhood predict ASD symptom outcomes in adolescence, we next created a series of linear regression models. For each component found to be a significant predictor of symptom trajectory in the multilevel models above (left hemisphere latency and amplitude to neutral faces, right and left amplitude to fearful faces, differential left latency to fear versus neutral faces), we created a regression model in which the component was entered as a predictor, MSEL Visual Reception scores as a covariate, and participants' last known ADOS Social Affect or Severity score as the dependent variable. From this series, the model containing left hemisphere latency significantly predicted ADOS Severity scores, $F(2,25)=4.613$, $p=.02$, and accounted for 22.4% of the various in symptom severity at children's final assessment timepoint. Within this model, latency was significant, $\beta=.506$, $t=2.87$, $p=.009$. These analyses support findings from the multilevel models above and suggest that left hemisphere responses to neutral faces early in life are a predictor of not only ASD symptom trajectory, but also eventual outcome in adolescence. See Figure 4. The remaining N290 components did not reach significance when predicting ADOS severity in adolescence (ps>.6).

[Figure 4 about here.]

Face processing and anxiety outcomes

As a second, exploratory goal we sought to examine longitudinal relations between N290 responding at age 3 years and anxiety symptoms at age 14 years. Latencies and amplitudes of the N290 response to neutral faces, fearful faces, and neutral minus fearful faces were entered into nonparametric partial correlations with RCMAS-2 scores at age 14. As in the multilevel and regression models, MSEL Visual Reception scores at age 3 were included as a covariate; in addition, we included verbal IQ standard scores at age 14 as a covariate to account for the

language demands inherent in the RCMAS-2. A subset of participants (n= 11) had sufficient ERP and self-report data for these analyses. There were no significant differences between participants with and without RCMAS-2 data on MSEL Composite or ADOS Severity scores at age 3 years (*p*s>.30), nor on measures of N290 amplitude or latency (*p*s>.30).

As presented in Table 5, face processing during early childhood predicted self-reported anxiety symptoms during adolescence. A pattern of significant correlations emerged between latency of the N290 response to neutral faces and adolescent anxiety. Within the left hemisphere, latency was positively correlated with RCMAS-2 scores. Participants with longer left hemisphere latencies to neutral faces at 3 years of age reported higher levels of total anxiety, social anxiety, and worry at age 14. Differential latency to fearful minus neutral faces was related to anxiety as well. Children who failed to show longer latencies to fearful relative to neural faces reported higher levels of anxiety.

A second set of significant correlations linked right hemisphere N290 amplitude to fearful faces with self-reported anxiety during adolescence. Across subscales of social anxiety and worry, more negative amplitude at age 3 years predicted more symptoms of anxiety at age 14 years.

[Table 5 about here.]

Discussion

Our findings suggest that responses to neutral and fearful faces are informative for predicting the course of ASD symptoms over development from early childhood into adolescence. Despite suggestions of the fundamental importance of face processing for social and communication skills (Baron-Cohen, 2005; Dawson et al., 2004), our findings represent the first longitudinal,

prospective evidence of its predictive utility. Within our sample, better longitudinal outcomes on the ADOS were predicted by relatively faster and larger N290 responses to neutral faces at age 3 years, smaller N290 responses to fearful faces, and slower differential N290 responses to fearful versus neutral faces (i.e., more differentiation between fearful and neutral faces). Left hemisphere latency to neutral faces was the most robust predictor, as faster N290 responses predicted more improvement in ASD symptoms over the course of childhood, as well as fewer ASD symptoms at children's last assessment point. The predictive power of ERP response to neutral faces in our data is consistent with suggestions of basic face processing as a foundation for more complex social communication skills throughout childhood and into adolescence. From these data, it appears that basic face processing, and not solely processing of affective faces, is important for understanding ASD trajectories and symptom profiles. We have recently proposed that early disruptions to the timing and nature of face processing in ASD contribute to difficulties across a spectrum of social skills (Webb et al., in press), and these findings are compatible with that proposed cascade of effects.

Our current findings are largely consistent with our early report (**citation redacted**), but also provide new insights into emotional face processing in ASD. Our previous findings demonstrated that 3- to 4-year-old children with ASD, as a group, displayed slower N290 responses than controls, and failed to show differential neural responses to neutral versus fearful faces with regard to both latency and amplitude. Consistent with this, the current analyses link better social functioning (e.g., fewer ASD symptoms) in early childhood with faster responses to neutral faces, and differentially faster responses to fearful versus neutral faces. However, longitudinal analyses indicate that children with differentially *slower* responses to fearful versus neutral showed greater improvement in symptoms over the course of childhood. This pattern is

counter-intuitive, and may imply a delay in the development of neural mechanisms for emotional face processing, such that children with initially atypical responses (slower to fearful versus neutral) are able to catch up by age 14 years and show more dramatic improvements in ASD symptoms as they mature. Such normalization could result from children with more severe symptoms early on consequently receiving more intervention than children with milder symptoms. Although intriguing, this possibility cannot be determined from our present data.

Our findings also highlight new insights with regard to ERP amplitude during processing of fearful faces. Here, we found that children with ASD with larger responses to fearful faces demonstrated more ASD symptoms at age 3 years, as well as less improvement in symptoms over childhood. To the extent that ERP amplitude reflects neural effort or resources recruited for a given processing demand, this pattern might suggest that children with ASD who displayed more severe ASD symptoms and poorer trajectories over time were experiencing a greater processing demand than their peers with milder ASD symptoms, necessitating reliance on increased cortical resources and perhaps reflecting the use of alternate processing mechanisms in response to fearful faces.

Prominent in our findings is the extent to which left (rather than right) hemisphere N290 responses were predictive of outcomes related to ASD symptoms. A long history of research into face processing among typically developing children and adults describes right hemisphere lateralization for these skills, with a lesser role for left hemisphere regions (Bentin et al., 1996). Right hemisphere lateralization generally emerges during the middle childhood period among typically developing children (Taylor, Batty, & Itier, 2001), concurrent with improvements in behavioral face processing tasks. However, a growing body of work indicates that lateralization

related to face processing may differ in ASD. Indeed, Webb and colleagues (2006) found that left hemisphere face processing may be particularly delayed in ASD.

Also notable in our data, response *latency* appeared to be a more frequent predictor of ADOS outcomes than response *amplitude*. For instance, faster left hemisphere N290 responses to neutral faces predicted better ASD outcomes. This may speak to the importance of rapid, contingent processing in the sequence and timing of reciprocal social and communicative interactions. Even slight delays in the processing of facial information (e.g., eye gaze, emotion) could interfere with accurate pairings between faces, speech, and situational context, resulting in missed social opportunities and inappropriately timed responses during social interactions.

An important consideration when interpreting our findings is that we did not test whether ERPs to faces predict longitudinal trajectories of symptoms within the domain of restricted/repetitive behaviors and interests. As a result, our current data and analyses cannot distinguish whether face-related ERPs predict social-emotional symptoms *specifically*. Such specificity is intuitively appealing; a range of neural regions and structures (e.g., amygdala, fusiform region, superior temporal sulcus) contribute to both affective face processing and more sophisticated social skills such as perspective-taking, empathy, and social insight (see Neuhaus, Beauchaine, & Bernier, 2010 for review), all of which would influence social communication symptoms and thus ADOS social affect scores. At the same time, restricted/repetitive symptoms could share circuitry with other aspects of face processing not captured here (e.g., habituation to faces: De Klerk, Gliga, Charman, & the BASIS team, 2014; Webb et al., 2010; or rule learning and memory: Jones et al., 2013), and consequently those aspects of face-related ERPs might predict outcomes in that domain. This will be an important topic of future work.

With regard to anxiety symptoms, we anticipated that more symptoms of anxiety would be predicted by faster and larger ERPs to fearful faces, as well as larger differential ERPs to fearful versus neutral faces. Instead, we found that 3- to 4-year old faster N290 responses to neutral faces predicted *lower* anxiety, as did slowing of the response when faces displayed a fearful expression. Lower anxiety scores were also predicted by less negative ERPs to fearful faces. These findings were largely in contrast with expectations based on the internalizing literatures. It may be that these departures are due in part to difficulties with insight and self-report of internal anxiety symptoms among adolescents with ASD, or may reflect a tendency for RCMAS-2 subscales to capture ASD- related rather than anxiety-related constructs (e.g., restricted thinking/behavior rather than "worry," social difficulties rather than "social anxiety"). Regardless of the reason for the discrepancy with the internalizing literature, ERP-RCMAS-2 correlations were consistent with analyses on ADOS outcomes in that best prognosis across social and emotional domains is predicted by a face processing system that responds relatively quickly to neutral faces, distinguishes between neutral and fearful faces with respect to latency, and displays less negative responses to fearful faces. Shared early predictors of social communication and internalizing outcomes are not surprising, as both social functioning and emotional well-being rely heavily upon early brain development and functioning (e.g., amygdala and related structures), physiological functioning, environment, and experience (see Phelps & LeDoux, 2005; Beauchaine & Webb, in press; Neuhaus, Beauchaine, & Bernier, 2010). Measures of early face processing may index the "health" of these systems and their interconnections (e.g., fusiform-amygdala pathway) and thus predict a variety of important outcomes later in development.

Limitations & future directions

Limitations to the current study relate primarily to the nature of our sample, which was relatively small (particularly for analyses related to adolescent anxiety) and predominantly male. Although these characteristics are not unusual for longitudinal studies of ASD utilizing psychophysiological measures, they do place constraints on the extent to which our findings generalize to a larger ASD sample with balanced sex ratios. Our study methods also required that children be able to tolerate EEG collection procedures at age 3 years and have sufficient language and insight to complete self-report instruments on internalizing symptoms during adolescence, both of which may have resulted in a selective sample. That said, inspection of sample characteristics in Table 1 suggests that our sample spanned a broad spectrum of ability with regard to cognitive, language, and adaptive functioning, as well as ASD severity, bolstering confidence in the generalizability of our results.

A second issue is the absence of a participant group with typical development, as our sample consisted only of children with ASD. Developmental evidence from typical samples indicates a marked decrease in ERP latency to faces over the course of childhood and into adolescence (Taylor et al., 2001), with better face processing skills corresponding to stronger social skills (Hileman, Henderson, Mundy, Newell, & Jaime, 2011). Thus, although we cannot generalize our findings to children without ASD, our findings that slower N290 to faces predicts poorer social outcomes fits well with normative patterns. Our approach also allows us to move beyond considering dichotomous long term outcomes (e.g., presence vs absence of ASD) and instead addresses some of the heterogeneity observed within the broad ASD diagnosis. That is, given a group of young children with ASD, what neurobiological factors might speak to their trajectories of skills and difficulties over the course of development? Such knowledge is critical for the development and implementation of successful interventions. Another critical step will be the

exploration of similar constructs in groups without ASD but with other neurodevelopmental or psychiatric concerns (e.g., ADHD, prenatal alcohol exposure, Down Syndrome, intellectual disability) to determine to what extent the predictive value of early face processing is specific to ASD versus more generally relevant to individuals with early-emerging brain-based differences.

Finally, limitations of our face processing task should be acknowledged. As noted above, faces were selected from those developed by Ekman and Friesen (©1976). Thus, they were black and white images of adults' faces, shown in series. This limits the ecological validity of the task, as faces are typically seen in color, and embedded within a dynamic social context., such that they are accompanied by vocal, gestural, and contextual cues. We cannot say to what extent ERP responses would have differed had face stimuli been more realistic or closer in age to our sample, nor can we determine the predictive effect of ERP responses to nonsocial images, as our task did not contrast faces versus non-face images.

Several potential avenues of research follow from our current findings. One important goal will be to uncover the neurobiological indices and neurocognitive skills that mediate longitudinal links between early face processing and later social and emotional outcomes. Understanding the mediational chain between these constructs would suggest potential points of intervention, targets of treatment, measures with which to monitor treatment progress, and moderators of treatment outcome. For instance, one could envision a longitudinal cascade in which altered face processing during toddlerhood leads to limited joint attention ability, which then contributes to difficulty tracking conversational topics and impoverished peer relationships. Evidence of such links over time could highlight a number of skills to be addressed in treatment. Given the array of skills on which treatment might focus, in-depth longitudinal understanding could help prioritize the timing of treatment targets or select between competing treatment modalities.

Conclusions

Neural response to neutral and fearful faces very early in life carries significant predictive weight for the trajectory of ASD symptoms over the course of childhood and into adolescence. Links between emotional face processing and social and communication difficulties may reflect a unique predictive contribution from social brain circuitry early in life. Predictive links between early emotional face processing and adolescent anxiety symptoms are striking, and underscore the broader significance of this circuitry for psychiatric health. These early biological indices may reflect risk for later-emerging mental health concerns and could potentially moderate response to preventive and intervention efforts targeting social and emotional well-being among individuals with ASD.

Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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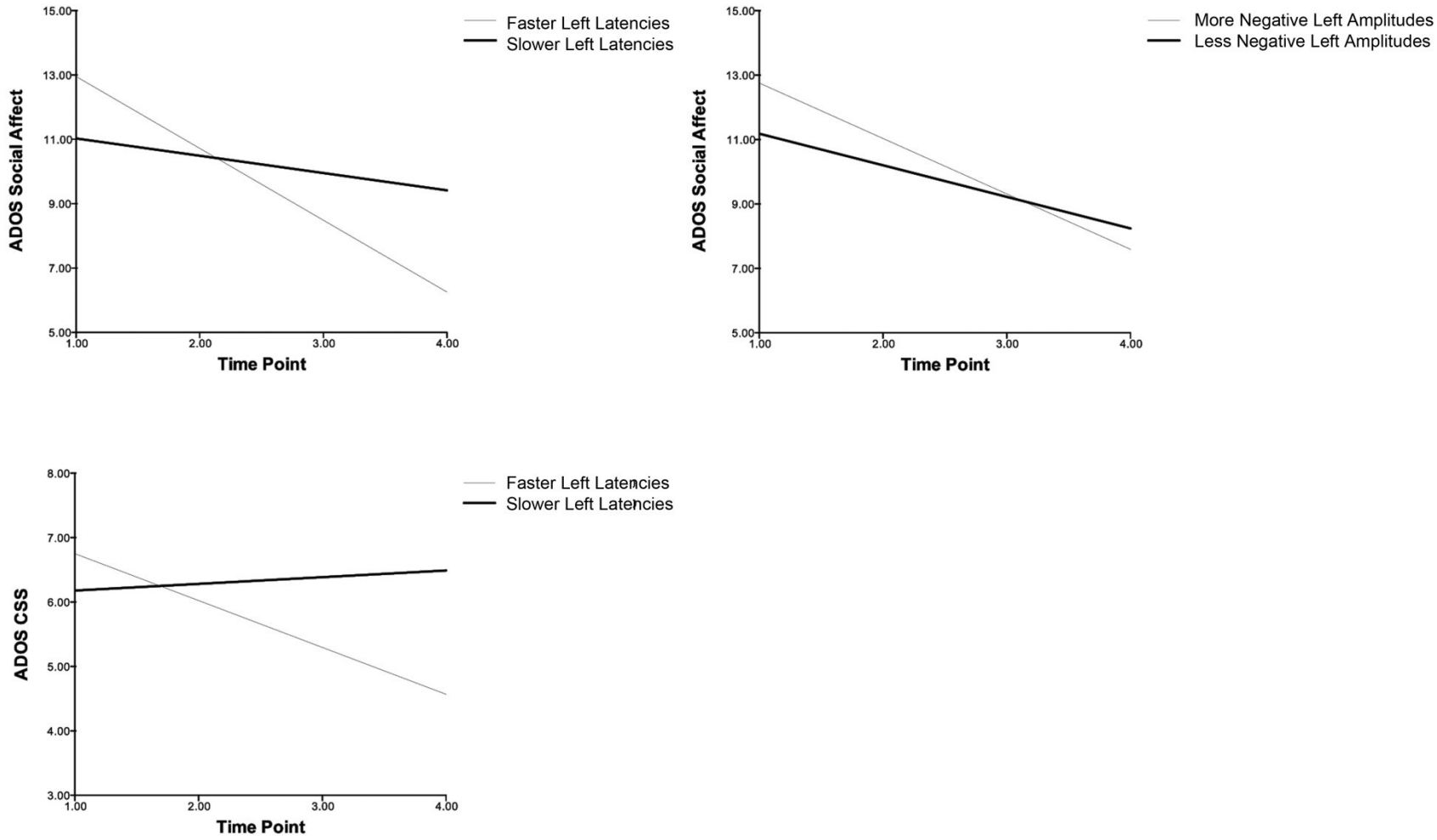
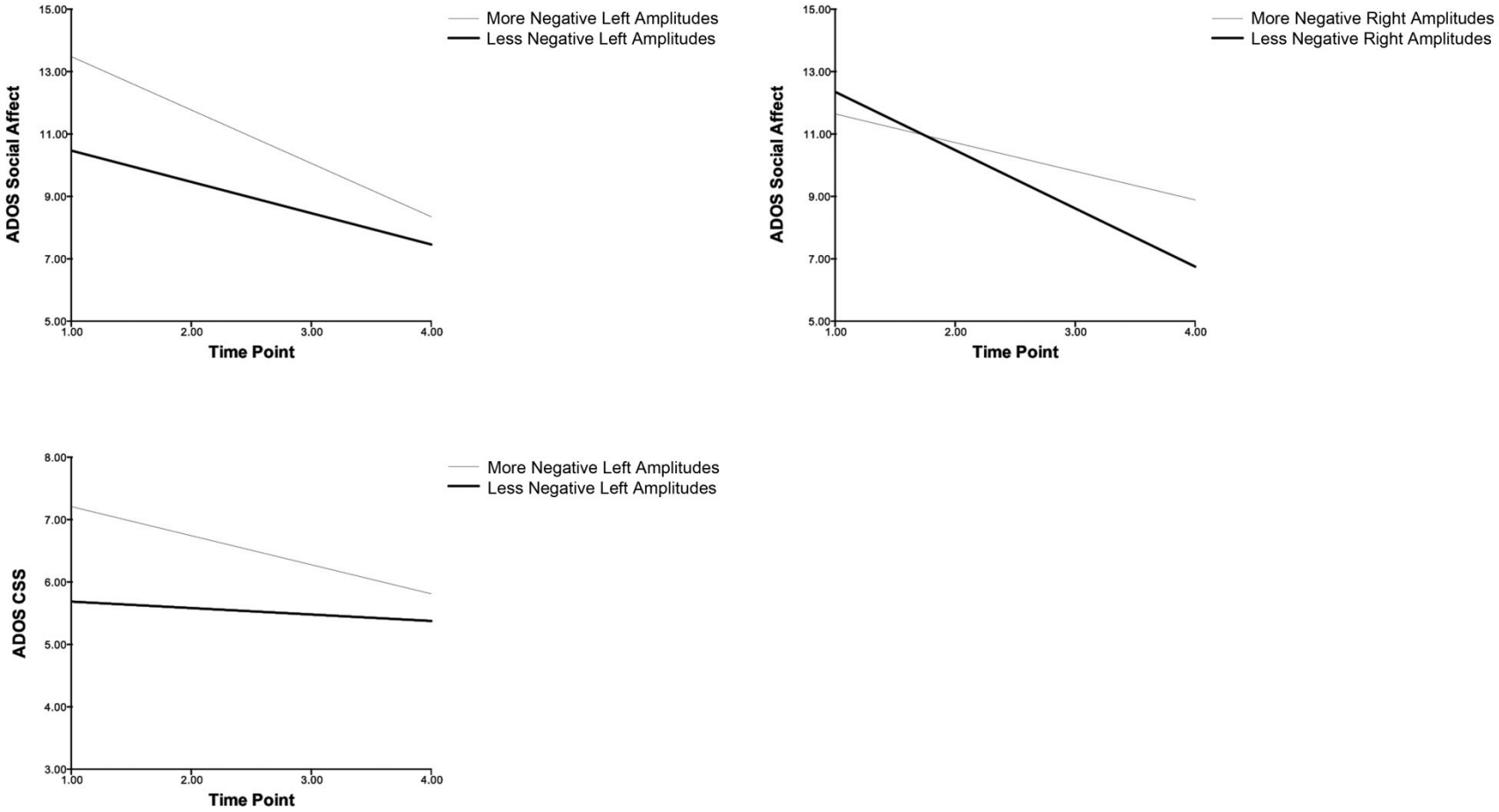
Figure 1: ADOS social affect symptom trajectories over time by N290 response to neutral faces



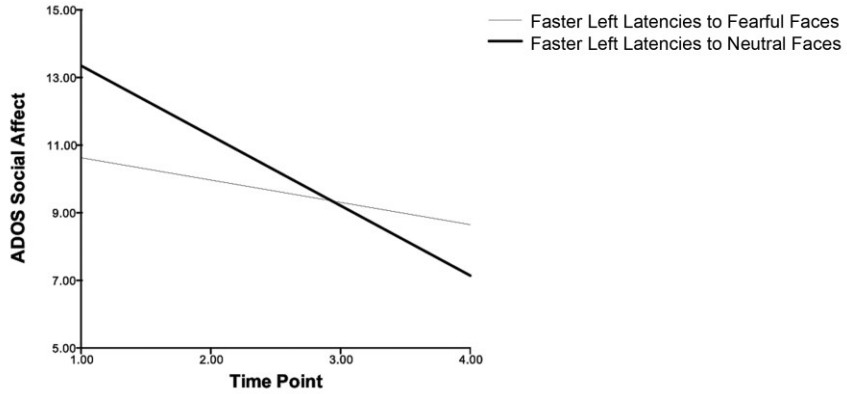
Note: N290 responses were modeled as continuous predictors. For ease of visualization, lines shown represent symptom trajectories for participants divided on median value of N290 responses.

Figure 2: ADOS social affect symptom trajectories over time by N290 response to fearful faces



Note: N290 responses were modeled as continuous predictors. For ease of visualization, lines shown represent symptom trajectories for participants divided on median value of N290 responses.

Figure 3: ADOS social affect symptom trajectories over time by differential N290 response to fearful versus neutral faces



Note: N290 responses were modeled as continuous predictors. For ease of visualization, lines shown represent symptom trajectories for participants divided on median value of N290 responses.

Figure 4: Prediction of ADOS severity score at last time point by N290 left hemisphere latency to neutral faces

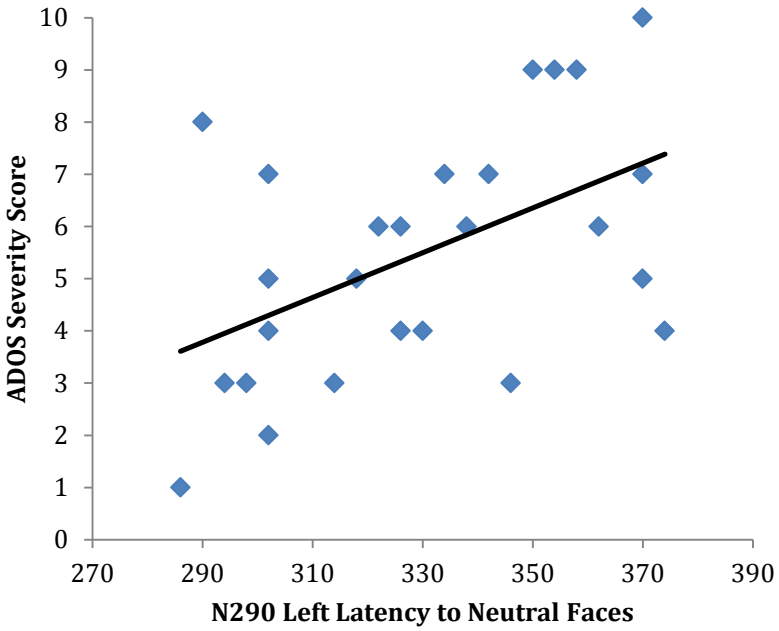


Table 1: Diagnostic and ERP characteristics of the sample at time 1.

	Mean	SD	Minimum	Maximum
Age (months)	44.12	5.4	26	50
MSEL				
Visual Reception T-Score	32.15	13.87	20	58
Fine Motor T-Score	28.81	11.46	20	56
Receptive Language T-Score	29.0	12.31	20	59
Expressive Language T-Score	29.73	12.08	20	58
Vineland-2				
Communication Std. Score	68.35	16.29	50	114
Daily Living Skills Std. Score	62.77	10.57	50	94
Socialization Std. Score	67.46	10.34	54	95
Motor Skills Std. Score	75.40	16.91	52	110
Composite Std. Score	63.0	10.24	49	84
ADI-R				
Social Score	17.19	5.30	6	27
Communication Score	12.46	3.95	5	22
RRB	5.00	2.43	1	10
ADOS				
Social Affect Score	11.73	4.04	5	18
RRB Score	2.46	1.86	0	7
Total Score	14.19	4.95	6	22
Calibrated Severity Score	6.23	1.53	3	9
N290 to Neutral Faces				
Latency Left	330 ms	28 ms	286 ms	374 ms
Latency Right	319 ms	32 ms	274 ms	398 ms
Amplitude Left	-4 μV	7 μV	-24 μV	11 μV
Amplitude Right	-2 μV	6 μV	-12 μV	8 μV
N290 to Fearful Faces				
Latency Left	334 ms	29 ms	274 ms	374 ms
Latency Right	315 ms	32 ms	274 ms	410 ms
Amplitude Left	-2 μV	6 μV	-12 μV	11 μV
Amplitude Right	-1 μV	7 μV	-16 μV	13 μV
N290 Differential to Fearful – Neutral Faces				
Latency Left	4 ms	33 ms	-64 ms	84 ms
Latency Right	-4 ms	20 ms	-44 ms	40 ms
Amplitude Left	2 μV	6 μV	-8 μV	15 μV
Amplitude Right	1 μV	7 μV	-16 μV	14 μV

Notes. MSEL=Mullen Scales of Early Learning; Vineland-2=Vineland Adaptive Behavior Scales, 2nd Edition; ADI-R=Autism Diagnostic Interview, Revised; RRB=Restricted and repetitive behavior; ADOS=Autism Diagnostic Observation Score.

Table 2: Prediction of ADOS domain intercepts and slopes by N290 response to neutral faces

	Outcome	Predictor	β-value	*t*-ratio	Direction of effect
Social Affect	Intercept	Latency Left	-0.08	-1.89^{t}	
		Latency Right	0.02	0.64	
		Amplitude Left	-0.24	-1.95^{t}	
		Amplitude Right	0.03	0.12	
	Slope	**Latency Left**	**0.04**	**2.80****	Shorter latency → Steeper decline in symptoms
		Latency Right	0.01	0.75	
		Amplitude Left	**0.08**	**2.15***	More negative amplitude → Steeper decline in symptoms
		Amplitude Right	-0.04	-0.49	
ADOS Calibrated Severity Score	Intercept	Latency Left	-0.03	-1.62	
		Latency Right	0.01	0.92	
		Amplitude Left	-0.11	-1.82^{t}	
		Amplitude Right	0.00	-0.03	
	Slope	**Latency Left**	**0.02**	**3.01****	Shorter latency → Steeper decline in symptoms
		Latency Right	0.00	1.07	
		Amplitude Left	0.03	1.79^{t}	
		Amplitude Right	0.00	-0.03	

Notes. Models control for MSEL Visual Reception scores at age 3 years. ADOS=Autism Diagnostic Observation Schedule.
$^{\dagger}p<.10$. *$p<.05$. **$p<.01$.

Table 3: Prediction of ADOS domain intercepts and slopes by N290 response to fearful faces

	Outcome	Predictor	*β*-value	*t*-ratio	Direction of effect
Social Affect	Intercept	Latency Left	0.03	0.59	
		Latency Right	0.02	0.47	
		Amplitude Left	**-0.41**	**-2.33***	More negative amplitude → More symptoms at age 3
		Amplitude Right	0.17	1.12	
	Slope	Latency Left	0.00	0.12	
		Latency Right	0.00	0.28	
		Amplitude Left	0.08	1.23	
		Amplitude Right	**-0.10**	**-2.18***	More negative amplitude → Less decline in symptoms
ADOS Calibrated Severity Score	Intercept	Latency Left	0.00	0.06	
		Latency Right	0.01	0.47	
		Amplitude Left	**-0.21**	**-2.74***	More negative amplitude → More symptoms at age 3
		Amplitude Right	0.04	0.66	
	Slope	Latency Left	0.01	1.02	
		Latency Right	0.00	0.52	
		Amplitude Left	0.04	1.28	
		Amplitude Right	-0.03	-1.07	

Notes. Models control for MSEL Visual Reception scores at age 3 years. ADOS=Autism Diagnostic Observation Schedule.
$^{\dagger}p<.10$. *$p<.05$. **$p<.01$.

Table 4: Prediction of ADOS domain intercepts and slopes by differential N290 response to fearful versus neutral faces

	Outcome	Predictor	*β*-value	*t*-ratio	Direction of effect
Social Affect	Intercept	**Latency Left**	**0.08**	**2.80****	Slower to fear vs neu. → More symptoms at age 3
		Latency Right	0.00	-0.12	
		Amplitude Left	0.00	0.00	
		Amplitude Right	0.13	0.87	
	Slope	**Latency Left**	**-0.03**	**-2.32***	Slower to fear vs neu. → Steeper decline in symptoms
		Latency Right	-0.02	-1.08	
		Amplitude Left	-0.05	-0.67	
		Amplitude Right	-0.07	-1.53	
ADOS Calibrated Severity Score	Intercept	Latency Left	0.03	1.79^{t}	
		Latency Right	-0.01	-0.67	
		Amplitude Left	-0.02	-0.26	
		Amplitude Right	0.04	0.61	
	Slope	Latency Left	-0.01	-1.67	
		Latency Right	-0.01	-1.26	
		Amplitude Left	0.00	-0.12	
		Amplitude Right	-0.02	-1.02	

Notes. Models control for MSEL Visual Reception scores at age 3 years. ADOS=Autism Diagnostic Observation Schedule.
$^{\dagger}p<.10$. *$p<.05$. **$p<.01$.

Table 5: Partial correlations between latencies and amplitudes of N290 to faces at age 3 years and internalizing symptoms at age 14 years

	RCMAS-2			
	Physiological Anxiety	**Social Anxiety**	**Worry**	**Total Anxiety**
Neutral				
Lat. – Right	.31	.40	**.89****	**.42**
Lat. – Left	.54	**.97*****	**.99*****	**.84****
Amp. – Right	-.10	-.37	**-.67***	-.41
Amp. – Left	-.30	-.33	-.20	-.29
Fearful				
Lat. – Right	.21	.53	**.90****	.47
Lat. – Left	.16	.38	.58^{t}	.24
Amp. – Right	-.41	**-.86****	**-.99*****	**-.74***
Amp. – Left	.19	-.28	-.20	.00
Fearful – Neutral				
Lat. – Right	.06	.27	-.10	.14
Lat. – Left	-.45	**-.73***	**-.74***	**-.77***
Amp. – Right	-.04	-.02	.00	.07
Amp. – Left	.20	-.06	-.18	.01

Notes. Correlations control for MSEL Visual Reception scores at age 3 years, and for DAS-II Verbal standard scores at age 14 years. RCMAS-2=Revised Children's Manifest Anxiety Scale, 2nd Edition; Lat.=Latency; Amp.=Amplitude.
$^{\dagger}p<.10$. *$p<.05$. **$p<.01$. ***$p<.001$.