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Neural measures of social attention across the first years of life: Characterizing typical development and markers of autism risk

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ABSTRACT

Few studies employing event-related potentials (ERPs) to examine infant perception/cognition have systematically characterized age-related changes over the first few years of life. Establishing a 'normative' template of development is important in its own right, and doing so may also better highlight points of divergence for high-risk populations of infants, such as those at elevated genetic risk for autism spectrum disorder (ASD). The present investigation explores the developmental progression of the P1, N290, P400 and Nc components for a large sample of young children between 6 and 36 months of age, addressing age-related changes in amplitude, sensitivity to familiar and unfamiliar stimuli and hemispheric lateralization. Two samples of infants are included: those at low- and high-risk for ASD. The four components of interest show differential patterns of change over time and hemispheric lateralization; however, infants at low- and high-risk for ASD do not show significant differences in patterns of neural response to faces. These results will provide a useful point of reference for future developmental cognitive neuroscience research targeting both typical development and vulnerable populations.

1. Introduction

The recording of the brain's electrical activity via the event-related potential (ERP) provides a window into infant perception and processing when other avenues of overt measurement are not yet available because of limited motor, communication and cognitive abilities. Infant and toddler studies using ERP often focus on a similar set of dependent and independent variables in order to answer questions about early perception and cognition. In exploring each component, analyses are often completed separately in order to address different aspects of the ERP response: amplitude (strength), latency (speed) and scalp topography (putative generators). Amplitude is thought to reflect the degree of synchronous firing of cortical pyramidal cells in response to a stimulus. Latency, which requires the identification of each individual component's peak, reflects the speed with which the maximal neural response is generated. Finally, topography provides a way of not only defining a component (e.g., the P300b is maximal over parietal scalp, whereas the P300a is maximal over frontal scalp)

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but also inferring underlying neural generators. These metrics have informed our current understanding of normative developmental changes in sensation and perception, as well as our emerging conceptualizations of developmental delay and disorder.

Several specific ERP components have become commonly used in the field of infant studies, and many of these components have now been explored by researchers in the field developmental psychopathology in the interest of delineating how and when typical and atypical development diverge early in life. In recent years, there has been a growing desire to find measures for very young children that are more sensitive to developmental difference than behavioral assays. In large part, this is due to the growing study of autism spectrum disorder (ASD), a developmental disorder that leads to impaired communication and social engagement, as well as a heightened presence of repetitive interests and behaviors. While ASD is thought to be primarily heritable, diagnostic measures relying on behavior are not reliably sensitive and specific for infants and toddlers in the first year or 18 months of life (e.g., Lord et al., 2012). As a result, there is a desire to 'bypass' behavior in the early identification of ASD; ERPs are candidate measures of brain development and response to stimuli. However, because children with ASD cannot predictably be identified in the first two years of life, the population of interest is infant siblings of children with ASD. These 'infant sibs' are of great interest for two reasons: first, roughly 20% of these infants will themselves go on to be diagnosed with ASD (Ozonoff et al., 2011a,b); and second, those who do not receive a formal diagnosis often exhibit sub-clinical features similar to those observed in ASD, conceptualized as the 'broader endophenotype' of ASD (Rogers, 2009; Elsabbagh and Johnson, 2010). Consequently, these high-risk infants can reveal much about the neural underpinnings of ASD.

If ERPs are to provide a useful window into whether and when development has gone awry, it is essential to understand the normative age progression of the neural response, as well as what other kinds of factors might affect the brain's reaction to stimuli. The effect of age on ERP response has been widely explored, largely in infant cross-sectional studies and in longitudinal investigations of school-age children (e.g., Taylor et al., 2004; Itier and Taylor, 2004) or very young infants (Webb et al., 2005). Because of the centrality of social stimuli in infant development, a number of studies have explored differential responses to familiar social stimuli (generally the mother's face) versus unfamiliar social stimuli (a stranger's face). Finally, because hemispheric lateralization is a critical normative process associated with increased specialization and efficiency of neural networks, topographic region (that is, right versus left hemisphere) is often explored as a metric of brain development. Though there are many ERP components that have been studied in infant and toddler development, four visual components consistently appear in investigations of young children and will be addressed here: the P1, N290, P400 and Nc. A review of each follows, with a focus on the role of age, familiarity and hemisphere. Finally, because one of the central questions for the current investigation is the degree to which these patterns of response might vary in children at risk for ASD, a review of the current literature in children with ASD (or with a family history of the disorder) will be presented. For the purposes of the current paper, we will focus our attention on variables that affect the amplitude of the neural response.

1.1. P1

The P1 is a positive-going deflection that is measured over occipital regions approximately 90–150 ms after a visual stimulus. It is predictably elicited by visual stimuli in general (as opposed to particular types of stimuli, like faces) and is therefore thought to reflect sensory experience rather than higher-order processing. In infants and young children, the P1 increases in amplitude with age (Kos-Pietro et al., 1997). However, sometime between ages 2 and 4, this pattern reverses, and the amplitude of the P1 decreases with increasing age (Kuefner et al., 2010; Kos-Pietro et al., 1997; Hileman et al., 2011; Itier and Taylor, 2004), which may reflect the natural developmental course of synaptic pruning. Despite these developmental changes, the P1 in infants does not appear to be sensitive to familiarity of the stimulus (De Haan and Nelson, 1997, 1999). Moreover, the P1 is generally observed bilaterally over posterior electrodes (Csibra et al., 2008; but see McCleery et al., 2009). Little research has explored whether very young children with or at risk for ASD show differences in the P1, but school-aged children with ASD show typical patterns of reduced P1 amplitude with age (Hileman et al., 2011). In sum, then, the P1 is a bilateral response evoked by visual stimuli (with minimal sensitivity to familiarity) that shows a non-linear pattern of growth with age and does not seem to be clearly affected in ASD.

1.2. N290/P400

The N290 and P400 are a pair of components that are thought to be functionally and topographically similar. Together, they are thought to be the infant precursor to the adult N170, a face-sensitive component (Scott et al., 2006; Csibra et al., 2008; De Haan et al., 2003). The N290 is a negative amplitude shift observed in infants that has repeatedly shown sensitivity to faces (Csibra et al., 2008; De Haan et al., 2003); however, age-related changes in the amplitude of the N290 are unclear. Initial reports indicated a lack of familiarity modulation in the N290 (De Haan and Nelson, 1997, 1999), but other studies have reported that the amplitude of the N290 is affected by familiarity of the stimulus (Scott et al., 2006; Key and Stone, 2012). Despite the gradual right-ward shift of face-sensitive neural activity captured by this cluster of components (De Haan et al., 2003), consistent hemispheric differences in the N290 have not been clearly documented in the literature (Luyster et al., 2011; McCleery et al., 2009; Webb et al., 2006). Previous investigations have not found consistent differences in the N290 in young children with or at risk for ASD. Across studies, there do not appear to be straightforward effects of ASD risk on the amplitude of the N290 to faces (Key and Stone, 2012), though there may be slight differences in the lateralization of the component (Luyster et al., 2011; McCleery

et al., 2009; Elsabbagh et al., 2009; Webb et al., 2006). To summarize, the N290 is a face-sensitive component with some evidence of familiarity modulation; effects of age and topography have not been clearly established, and there are no consistent differences in the N290 associated with ASD or ASD risk.

The P400 is a positive-going deflection that follows the N290 and is recorded over roughly the same topographical location; it is also a face-sensitive component (De Haan et al., 2003; Csibra et al., 2008). In toddlers and preschoolers, Carver and colleagues (2003) reported age-related decreases in the amplitude of the P400 beginning at 18 months. While for children preschool-aged and over, the P400 in response to human faces seems to reflect familiarity (Carver et al., 2003; Dawson et al., 2002), the P400 in younger children does not seem to be affected by familiarity (De Haan and Nelson, 1999; Carver et al., 2003; Scott et al., 2006; but see Key and Stone, 2012). Consistent with observations of the N170, the P400 is generally maximal over lateral electrodes (Csibra et al., 2008; De Haan et al., 2003), particularly the right hemisphere (Moulson et al., 2009; but see McCleery et al., 2009).

Studies of children with and at risk for ASD have showed some differences in the P400. In a sample of young children with ASD, Dawson and colleagues (2002) reported that the amplitude of the P400 in the ASD sample did not show sensitivity to familiarity of faces as it did in the typically developing children; functional differences in the P400 in response to gaze have also been reported by Elsabbagh et al. (2012). However, others (Luyster et al., 2011) have not reported meaningful significant differences in the P400 for infants at high-risk for ASD.

Altogether, then, the P400 is a face-sensitive component that may diminish during the second year of life, shows right-ward laterality and (with age) increasing modulation by familiarity. In children with and at risk for ASD, the P400 seems to be less sensitive to important aspects of faces, including familiarity.

1.3. Nc

The Nc is a gradual negative deflection recorded over fronto-central regions roughly 400–850 ms post-stimulus and is commonly thought to be associated with the obligatory recruitment of attention to a visual stimulus (Nelson and Monk, 2001; De Haan et al., 2003; Richards, 2003; Csibra et al., 2008), though it is not face-specific. A number of developmental changes have been observed in the Nc. With regards to overall magnitude of the Nc, Webb and colleagues (2005) reported that the amplitude of the Nc becomes increasingly negative (that is, larger) in first year of life but may decline after 18–24 months. Other developmental changes have been noted in the effect of familiarity on the Nc. Whereas young infants – around 6 months of age – show a more negative Nc in response to a *familiar* stimulus (their mother's face) (De Haan and Nelson, 1997, 1999; but see Swingler et al., 2007), older children show a larger response to an *unfamiliar* stimulus. The age of this transition is largely unclear; though some studies have reported that the larger unfamiliar response is observed by 1 year of age (Webb et al., 2005; Burden et al., 2007; Luyster et al., 2011; Key and Stone, 2012), others place the transition at 18 months (Webb et al., 2011), by 2.5 years (Moulson et al., 2009) or even closer to 3.5 or 4 years (Carver et al., 2003; Dawson et al., 2002). The majority of studies have highlighted the role of the right hemisphere in the Nc, both in terms of maximal amplitude (Dawson et al., 2002; Webb et al., 2011) and in terms of greatest *differential* response (De Haan and Nelson, 1999; Carver et al., 2003). Overall, this right-lateralized pattern is consistent with the right hemisphere's role in face processing (see De Haan et al., 2003 for a discussion).

The Nc has been studied in young children with and at risk for ASD. Dawson and colleagues (2002) found that young children with ASD failed to show a differential Nc response to familiar vs. unfamiliar faces. This result was partially confirmed by Webb et al. (2011), who reported that young children with ASD did show a differential Nc to familiar and unfamiliar faces but that the emergence of this pattern was delayed relative to the typically developing children. Both studies found evidence for right-ward lateralization of the Nc in young children with ASD (Dawson et al., 2002; Webb et al., 2011). Two additional studies have explored the Nc in infants at risk for ASD with some revealing results. McCleery and colleagues (2009) reported diminished overall amplitude of the Nc in 10 month old infants at high-risk for ASD relative to low-risk infants, while Luyster et al. (2011) reported that 12 month old infants in the high-risk showed a less robust differential response to familiar and unfamiliar faces than did the infants in the low-risk group. In contrast, Key and Stone (2012) found no significant group effects on Nc amplitude, nor did they find a group effect on the differential Nc response across familiar and unfamiliar conditions in 9 month olds, with both low- and high-risk infants showing familiarity modulation.

To summarize, the Nc is a marker of obligatory attention to a visual stimulus that shows non-linear growth with age and increasing right-lateralization. Although the Nc is sensitive to familiarity in typically developing children, this effect may be diminished in children with and at risk for ASD.

Despite the relatively large number of investigations using infant ERPs, few have taken a systematic approach to studying how these components change in normative development over the first few years of life. Because of the growing desire to use ERPs as a benchmark for quantifying "normal" or "atypical" development, reference points for typical developmental patterns and changes are required. Therefore, the present investigation aims to address two broad goals. First, four commonly studied infant ERP components – the P1, N290, P400 and Nc – are described in a large sample of children 6–36 months of age. Across all analyses, mean ERP amplitude is included as the dependent variable of interest. In addition to addressing the effect of age on mean amplitude, the effects of stimulus familiarity and topographical region (i.e., right versus left hemisphere) on mean amplitude are also explored. Second, samples of infants at both low- and high-risk for ASD have been included to illuminate how these groups may differ in age-related changes, sensitivity to stimuli familiarity or hemispheric specialization.

Table 1
Sample information.

	6	9	12	18	24	36
Posterior components (P1, N290, P400)						
LRC						
Number (male)	29 (14)	36 (20)	30 (12)	22 (15)	24 (15)	21 (10)
Mean age in days	193.55	281.44	371.37	557.59	740.38	1120.43
Age range	170–223	269–313	359–390	538–575	726–759	1092–1210
Mean # trials	33.34	35.78	34.23	33.04	40.71	41.29
HRA						
Number (male)	23 (9)	30 (12)	30 (13)	20 (12)	17 (6)	16 (8)
Mean age in days	193.52	278.77	371.80	559.00	736.53	1113.88
Age range	177–214	264–299	353–413	538–583	703–755	1092–1141
Mean # trials	34.13	35.33	39.90	37.80	37.52	41.50
Frontocentral component (Nc)						
LRC						
Number (male)	22 (10)	30 (16)	32 (12)	21 (14)	21 (13)	17 (8)
Mean age in days	192.32	280.73	371.91	558.29	740.43	1117.47
Age range	170–223	269–301	359–390	538–575	726–759	1092–1163
Mean # trials	31.82	35.30	34.72	34.76	43.00	45.41
HRA						
Number (male)	20 (9)	30 (12)	29 (13)	17 (12)	16 (6)	18 (13)
Mean age in days	191.25	278.30	373.41	557.41	736.69	1112.50
Age range	177–210	267–299	359–413	538–583	703–753	1092–1141
Mean # trials	35.65	33.93	41.24	38.59	38.25	42.78

LRC, low risk controls; HRA, high risk for ASD.
Note: No significant group differences in number male, age in days or number trials were found at any age.

2. Methods

2.1. Participants

Participants were infants enrolled in an IRB-approved collaborative longitudinal study conducted at Boston Children's Hospital/Harvard Medical School and Boston University. Families were excluded from the study based on child gestational age of less than 36 weeks, time spent in neonatal intensive care, maternal steroid use during pregnancy, maternal diabetes or family history of genetic disorders. Infants were enrolled in one of two groups: low-risk control (LRC, which included infants with no family history of ASD) and high-risk for ASD (HRA, which included infants with an older sibling formally diagnosed with ASD). Informed consent was obtained at the time of the visit. Additional study information is provided elsewhere (Luyster et al., 2011).

Analyses for posterior components (P1, N290 and P400) and the frontocentral (Nc) component included slightly different sets of participants in the interest of maximizing useable data. One hundred and thirty one participants (70 LRC and 61 HRA) were included in the analyses for posterior components; 129 children were included in analyses for the Nc (67 LRC and 62 HRA). As part of their participation in this study, children were seen at multiple time points between enrollment and 36 months of age. The present analyses included data from the following target visits: 6 months, 9 months, 12 months, 18 months, 24 months and 36 months. See Table 1 for details. All useable data were used; consequently, most children contributed multiple data points to the current analyses.

In the interest of characterizing the behavioral features of these samples, cognitive (Mullen Scales of Early Learning, Mullen, 1995) and ASD symptom scores (Autism Diagnostic Observation Schedule, Lord et al., 2000) from the 24 month visit are summarized in Table 2.

Note that using follow-up data from 24 and/or 36 months of age, a subset of children (all but one of whom were in the HRA group) met Autism Diagnostic Observation Schedule (Lord et al., 2000) algorithm criteria for autism or ASD and had this classification confirmed using best estimate clinical judgment. For the posterior component data (P1, N290 and P400), this subset included 9 children; for the frontocentral (Nc) component data, this subset included 12 children. Final models reported below have been run both with and without these subsets of positive outcome children; their exclusion does not change the pattern of significance in any of the results and therefore, they remain included in the analyses reported in Section 3.

2.2. ERP stimuli

Building on a substantial corpus of work initiated by De Haan and Nelson (1997) and exploring the role of face familiarity in infant ERP, stimuli included color pictures of a primary caregiver (for all infants reported here, it was their mother) and same-sex stranger. Models were instructed to adopt a neutral expression and were positioned in front of a gray screen. Their neck and shoulders were draped with gray cloth. For each infant, an unfamiliar face was chosen that was similar to the familiar one in ethnicity; images of mothers wearing glasses were paired with images of unfamiliar women wearing glasses. Images were cropped as needed to standardize face and image size.

2.3. ERP recording and data processing

All ERP recording was completed in an electrically- and sound-shielded testing room with low lighting.

Table 2
Means (SD) from 24 month behavioral testing.

	Posterior components		Frontocentral component	
	LRC	HRA	LRC	HRA
Mullen early learning composite	112.00 (14.94)	106.82 (14.06)	111.41 (15.15)	105.98 (14.49)
ADOS Module 1 communication	1.15 (0.93)	1.49 (1.45)	1.15 (0.95)	1.49 (1.42)
ADOS Module 1 social interaction	1.27 (1.48)	2.18 (2.77)	1.23* (1.48)	2.32* (2.86)
ADOS Module 1 communication + social	2.41 (1.95)	3.67 (3.98)	2.38* (1.96)	3.80* (3.97)
ADOS Module 1 play	1.73 (0.74)	1.62 (0.99)	1.73 (0.75)	1.61 (1.02)
ADOS Module 1 stereotyped behavior	0.95** (0.92)	1.87** (1.38)	0.95** (0.93)	1.85** (1.39)
ADOS Module 2 communication	2.06 (1.26)	2.00 (2.00)	2.19 (1.17)	2.00 (2.00)
ADOS Module 2 social interaction	1.33 (1.78)	2.38 (2.56)	1.06 (1.12)	2.38 (2.56)
ADOS Module 2 communication + social	3.39 (2.30)	4.38 (4.27)	3.25 (1.52)	4.38 (4.27)
ADOS Module 2 imagination	0.94 (0.64)	0.88 (0.64)	1.00 (0.63)	0.88 (0.64)
ADOS Module 2 stereotyped behavior	1.06 (1.16)	1.63 (1.69)	1.06 (1.18)	1.63 (1.68)

LRC, low risk controls; HRA, high risk for ASD; Mullen, Mullen Scales of Early Learning; ADOS, Autism Diagnostic Observation Schedule.
* $p < 0.05$.
** $p < 0.01$.

Participants were seated on their caregiver's lap, approximately 65 cm from the experimental monitor. Recording was completed as children viewed the stimuli; images were presented for 500 ms and in random order, maintaining a 50:50 ratio. An examiner observed the participant from another room, surveying the child's eye movements and attentiveness through a hidden video camera mounted on top of the experimental monitor. Stimulus presentation was managed via ePrime software (Psychology Software Tools, Pittsburgh, PA) and was initiated only when the child was attending to the screen. Trials during which the child's attention strayed from the visual stimulus were removed from further analysis. A maximum of 100 trials were presented. Continuous electroencephalogram (EEG) was recorded using a Geodesic Sensor Net (Electrical Geodesics Inc., Eugene, OR) with either 64 or 128 electrodes based on the child's head circumference and date of session (due to an equipment upgrade[1]), and referenced on-line to a single vertex electrode (Cz). The electrical signal was amplified with a NetAmps 200 or NetAmps 300 amplifier (Electrical Geodesics Inc., Eugene, OR; due to an equipment upgrade[2]) with a 0.1–100 band-pass, digitized to 250 Hz, and stored on a computer disk. The data were analyzed offline by using NetStation 4.4.1 analysis software (Electrical Geodesics Inc., Eugene, OR). The continuous EEG signal was segmented to 1000 ms post-stimulus recording periods, with a baseline period beginning 100 ms before stimulus presentation.

The segments were then digitally filtered by using a 30 Hz low-pass elliptical filter (preceded by a 0.1 Hz first order high-pass filter for the NetAmps 300 data only) and baseline-corrected against the mean voltage during the 100 ms pre-stimulus period. After excluding segments with eye movements and blinks, the remaining segments were visually scanned by an experimenter blind to study group. Bad channels and other artifacts (e.g., off-scale activity, eye movement, body movements, or high-frequency noise) were identified. If more than 10% of the channels were marked as bad, the whole segment was excluded from further analysis. Finally, average waveforms for each individual participant within each experimental condition were generated and re-referenced to the average reference. Participants with fewer than 10 good trials per condition were excluded from further analysis.

2.4. ERP statistical analysis

Statistical analysis of the ERP data addressed four components: P1, N290, P400 and Nc. Time windows were selected based on visual inspection of data from all ages and across both study groups and were defined as follows: P1 (100–225 ms post-stimulus), N290 (115–300 ms post-stimulus), P400 (270–570 ms post-stimulus), and Nc (390–605 ms post-stimulus). Mean amplitude within the selected time windows were used for all analyses as a conservative measure of the ERP waveform; peak amplitudes and latencies – which are most appropriate when precise peaks can be identified for each individual's set of components – were not feasible for use in this large dataset.

Six posterior electrodes were selected for the P1, ten occipito-temporal electrodes were chosen for the N290 and P400, and ten frontal electrodes were identified for the Nc; these electrodes were selected within equivalent scalp locations across the 64- and 128-channel sensor nets. Regions of interest were chosen based on prior studies in this laboratory addressing face processing in infants and are shown in Fig. 1.

2.5. Analytic approach

A generalized estimating equations (GEE) approach with a normal distribution was used to test if the mean amplitude differed by the main predictors [group (LRC vs. HRA), age (measured continuously), gender (male vs. female; note: no significant findings were found in any model and so this variable is not discussed further in the Results section) condition (familiar/mother vs. unfamiliar/stranger), scalp region (left hemisphere vs. right hemisphere)] and their interactions. Analyses were run

[1] Preliminary analyses explored the effect of net and amplifier type; results were similar with and without the inclusion of net and amplifier variables. All results reported here collapse across 64- and 128-channel and NetAmps 200 and NetAmps 300 data.
[2] See footnote 1.

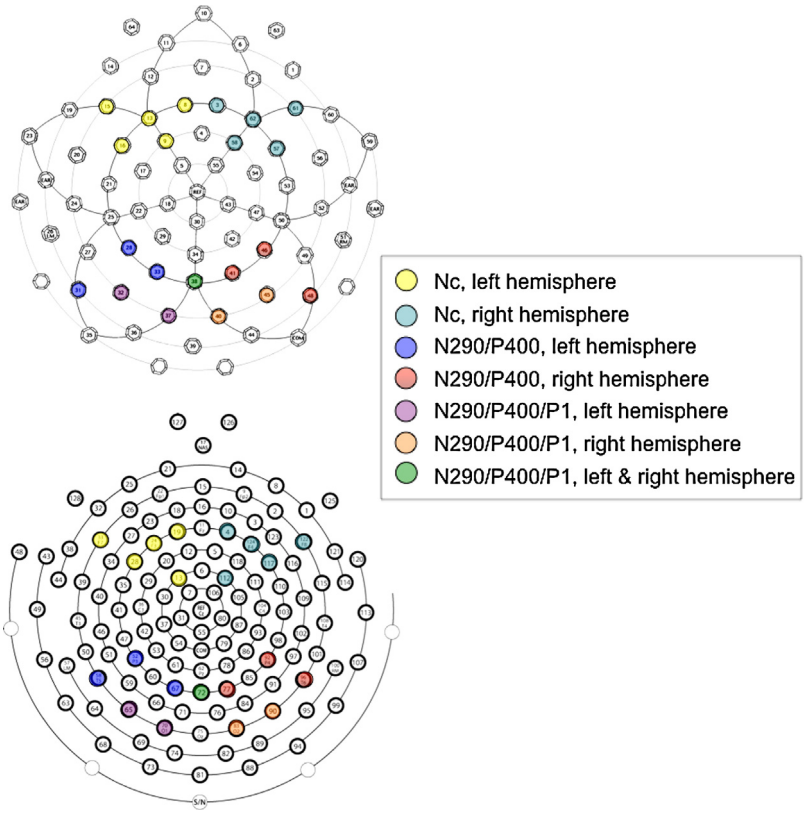

Fig. 1. Electrode groupings for 64- and 128-channel Geodesic Sensor Nets.

separately for the four components of interest (P1, N290, P400 and Nc) and all models adjusted for group. A systematic approach was used to test for each of the main effect and their interactions on the outcome. Results by each of the components include the significant main effects presented along with the associated test statistic and p-value from the fixed effects. p-Values <0.05 were considered statistically significant. No adjustments have been made for multiple testing because the purpose of this research was hypothesis-generating. Thus, it was more informative to focus on the strength of the relationship using the estimates from the GEE models compared to a p-value level. Figures have also been included to display the strength between the outcome and predictors over time. SAS (version 9.13) was used to perform all analyses. A set of representative waveforms capturing group and condition variation at 6, 12 and 24 months are included in Figs. 2 through 4, respectively.

3. Results

3.1. P1

The final model for the P1 indicated that age was the sole significant predictor of P1 amplitude, adjusted for group ($\chi^2 = 33.88$, $p < 0.0001$). Parameter estimates indicated that as child age increases by one year, mean amplitude increased by 2.78 μV. Although mean amplitude did not significantly vary by group and age ($\chi^2 = 4.31$, $p = 0.51$), there was a wider difference between groups in the mean amplitude at later ages than at earlier ones (as shown in Fig. 5).

3.2. N290

For the N290, as seen with P1, there was a main effect of age (adjusting for group; $\chi^2 = 16.03$, $p < 0.001$). Although there was no significant interaction of age and group, there was a larger difference of mean amplitude for LRC versus HRA children at 24 and 36 months of age than at earlier ages. There was also evidence of a group by condition interaction, suggesting that LRC children had a larger difference in mean amplitude when comparing mother versus stranger, whereas HRA children showed little differences in mean amplitude between conditions ($\chi^2 = 6.42$, $p = 0.01$).

However, when all of these variables – age, condition, and a group by condition interaction term – were included in the final model, only the main effect of age ($\chi^2 = 16.79$, $p < 0.001$) was significant; marginal significance remained for condition ($\chi^2 = 3.42$, $p = 0.06$) and the group by condition interaction term ($\chi^2 = 3.18$, $p = 0.07$). Parameter estimates indicated that, with each additional year, the mean amplitude of the N290 became less negative by 1.25 μV (see Fig. 6).

The marginal main effect of condition indicated that overall, the mean amplitude of the N290 in response to strangers' faces was slightly more negative than the response to mothers' faces. This was qualified by a marginal

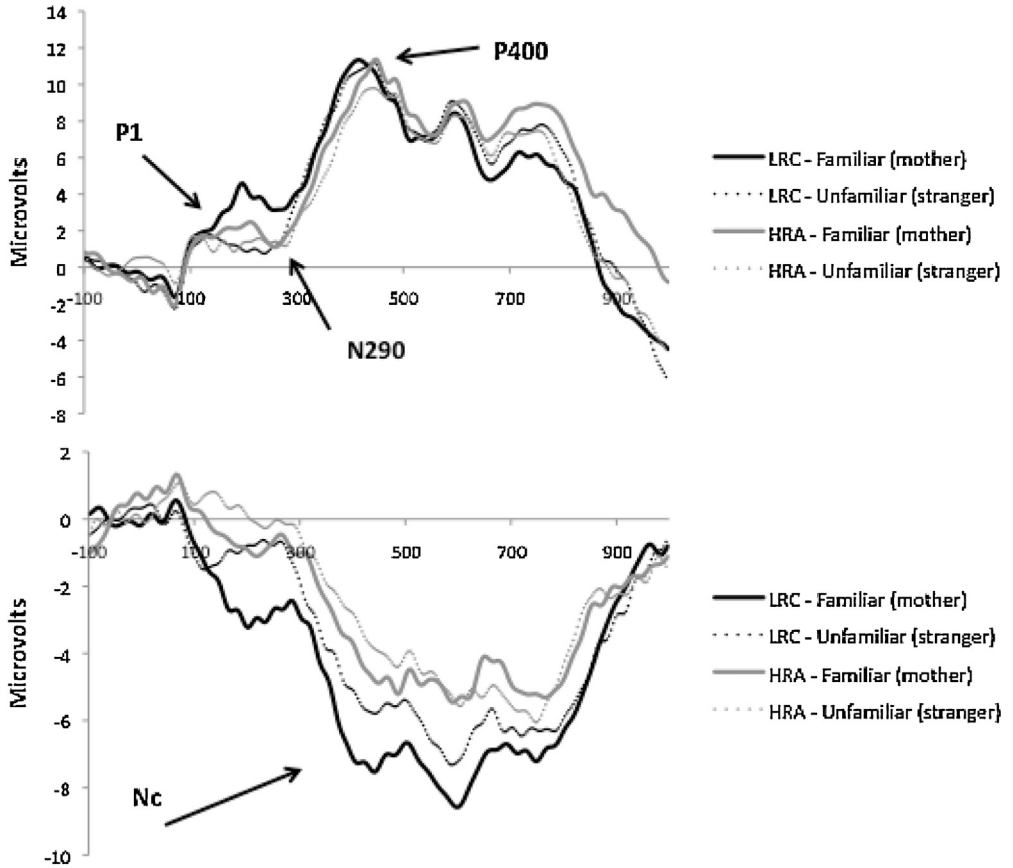

Fig. 2. P1/N290/P400 (top) and Nc (bottom) at 6 months. Stimulus duration was 500 ms. For simplicity, all posterior components are shown in a single waveform. However, in analyses, the P1 and N290/P400 were drawn from slightly different electrode groupings.

group by condition interaction, suggesting that a differential response to mothers' and strangers' faces was observed in the LRC group but not in the HRA group. That is, whereas the LRC group had (on average) a response that was 1 μV more negative in the stranger condition (2.56) than in the mother condition (3.59), this was not observed in the HRA group (stranger: 2.48; mother: 2.50). See Fig. 7.

3.3. P400

The final model for P400 mean amplitude included a main effect of age ($\chi^2 = 23.98$, $p < 0.001$), as well as a significant quadratic term for age ($\chi^2 = 18.21$, $p < 0.0001$) indicating non-linear growth with age adjusted for group. In addition to age, region was also found to be a significant predictor of P400 mean amplitude ($\chi^2 = 13.69$, $p = 0.0002$), such that responses measured over the right hemisphere were 1.13 μV larger than those measured over the left. Qualitative review of the two groups' development over time indicates a widening of group differences in mean P400 amplitude at 18 and 24 months of age, although the age by group interaction term did not achieve statistical significance (see Fig. 8).

3.4. Nc

The final model predicting mean Nc amplitude indicated a main effect of age ($\chi^2 = 6.95$, $p = 0.008$), a significant quadratic term for age ($\chi^2 = 14.62$, $p = 0.0001$), and a main effect of scalp region ($\chi^2 = 6.74$, $p = 0.009$). Parameter estimates indicated that the mean amplitude recorded over the right hemisphere was more negative than that recorded over the left (by 1.17 μV) adjusted for age and group. During model-building, marginal group by age interaction ($\chi^2 = 3.67$, $p = 0.06$) and group by condition ($\chi^2 = 3.42$, $p = 0.06$) interaction terms were found; these terms were not retained in the final model but will be elaborated below for descriptive purposes. Within-age analyses collapsing across condition indicated a group difference in mean amplitude at 24 months only; groups were not different at any other age. In addition, qualitative review of the plots illustrating mean amplitude over time (see Fig. 9) reveals that the LRC and HRA show maximal Nc amplitudes at different ages (at the 24 month visit for the LRC group and at the 12 month visit for the HRA group).

The group by condition interaction seemed to be driven by the greater negativity overall for mothers' faces (−8.53 μV, averaged across ages) than strangers' faces (−7.53 μV) observed in the LRC group; this consistently

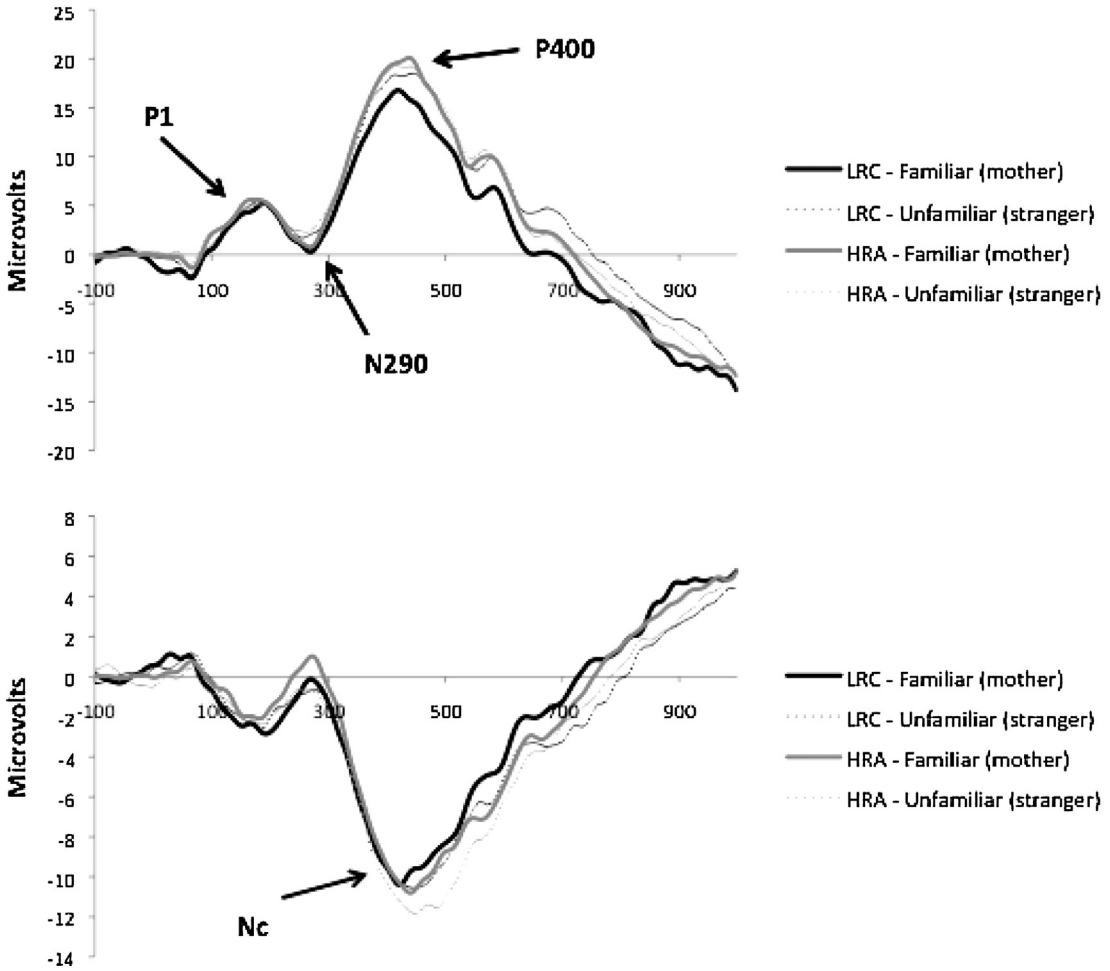

Fig. 3. P1/N290/P400 (top) and Nc (bottom) at 12 months. Stimulus duration was 500 ms. For simplicity, all posterior components are shown in a single waveform. However, in analyses, the P1 and N290/P400 were drawn from slightly different electrode groupings.

differential response was not observed in the HRA group (mothers' faces: −7.18 μV; strangers' faces: −7.58 μV). See Fig. 10.

4. Discussion

Four ERP components were explored in the present investigation – P1, N290, P400 and Nc – across six ages and in infants at low- or high-risk for ASD. Analyses revealed age-related changes in each component, as well as some component-specific results. Each component will be reviewed in turn.

Results for the P1 indicate that between 6 and 36 months of age, the mean amplitude of the P1 increases at a rate of nearly 3 μV per year. These results are consistent with previous findings illustrating amplitude increases early in life (Kos-Pietro et al., 1997). In addition, they shed new light on previous reports of amplitude decreases in early childhood (e.g., Kuefner et al., 2010; Hileman et al., 2011), suggesting that the transition point from increasing P1 amplitudes to decreasing P1 amplitudes may be localized around age 3. As reported in previous investigations, the P1 in infants and toddlers observed here was not modulated by familiarity (De Haan and Nelson, 1997, 1999), nor was it lateralized to the right or left hemisphere at any age (Csibra et al., 2008). The infants at high-risk for ASD did not show any significant differences in their P1 when compared to the low-risk infants.

In contrast to the P1, the N290 showed significant *decreases* in average amplitude with age, at a rate of 1.25 μV per year. This finding adds to existing literature on developmental changes of the N290, which have previously indicated that the latency of the N290 decreases with age (Halit et al., 2003). This observed reduction in the amplitude of the N290 *may* be related to the gradual shift of this component into the adult N170; if, over the course of development, the N290 is morphing into the N170 (a component with an earlier-peak; note that this shift is thought to be in place by 4 years of age, see Kuefner et al., 2010), then these components would be maximally captured by slightly different time windows. These exploratory analyses provide some evidence for the sensitivity of the N290 to familiarity, though unlike in previous research, infants in the current sample showed larger negativities to unfamiliar than familiar faces (Scott et al., 2006; Key and Stone, 2012). No hemispheric differences were found in the N290.

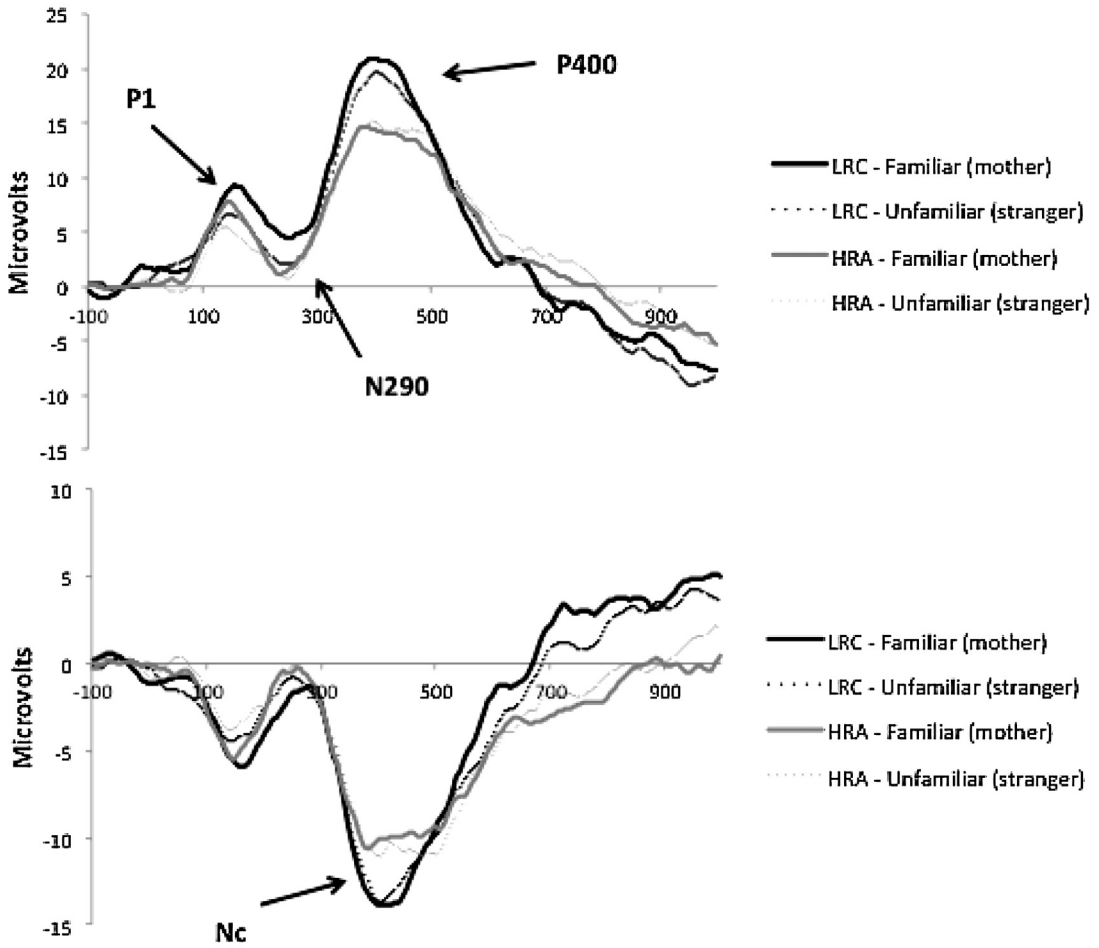

Fig. 4. P1/N290/P400 (top) and Nc (bottom) at 24 months. Stimulus duration was 500 ms. For simplicity, all posterior components are shown in a single waveform. However, in analyses, the P1 and N290/P400 were drawn from slightly different electrode groupings.

In addition, the groups at high- and low-risk for ASD did not show significant differences in their N290, suggesting that this component is not markedly altered in children at risk for ASD.

Our findings for the P400 revealed a pattern of non-linear age related change, with steadily increasing mean amplitudes between 6 and 24 months and decreasing amplitudes between 24 and 36 months of age. This result provides useful age-related expectations for developmental change in infants and is generally consistent with previous research in toddlers reporting decreasing P400 amplitudes after 18 months of age (Carver et al., 2003). In addition, we did not find evidence for familiarity modulation of the P400 in these young children. Although some studies have reported sensitivity to familiar faces in the P400 for children under 3 years of age (Carver et al., 2003; Key and Stone, 2012), others have not (De Haan and Nelson, 1999; Carver et al., 2003; Scott et al., 2006), suggesting that this modulation of the P400 is tenuous in young children. As in previous studies, our results pointed to the role of the right posterior hemisphere in P400 responses (e.g., Moulson et al., 2009; Scott et al., 2006), a finding that is consistent with the predominance of right hemisphere function in face processing (De Haan et al., 2003). With regards to study group, no significant main effect or interactions were found, suggesting that there are no clear associations of the P400 with ASD risk.

Like the P400, the average amplitude of the Nc showed a non-linear pattern of age-related change, with increasing negativity in the first year of life and a shift to decreasing negativity in the second year of life. These results align with and extend previous findings (Webb et al., 2005) about changes in the Nc during infancy and toddlerhood. However, in contrast to previous findings (e.g., De Haan and Nelson, 1997, 1999; Webb et al., 2005), we did not find a robust familiarity effect on the amplitude of the Nc. Our result adds to the varied developmental findings on the familiarity modulation of the Nc; whereas some reports have indicated greater *un*familiar responses by early as 12 months of age (Webb et al., 2005; Burden et al., 2007;

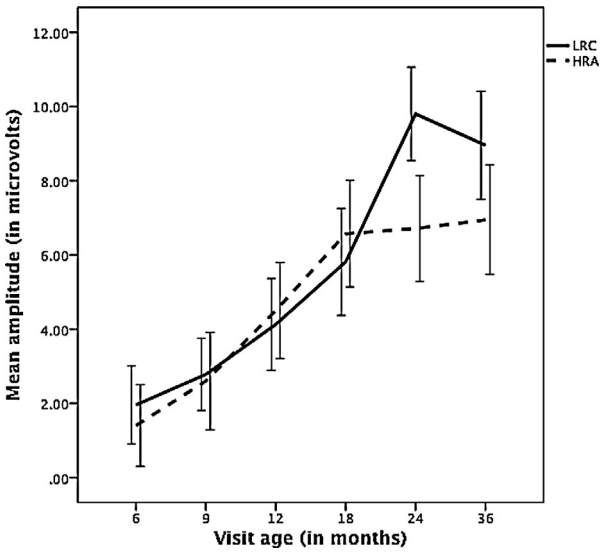

Fig. 5. Mean amplitude of the P1 by study group.

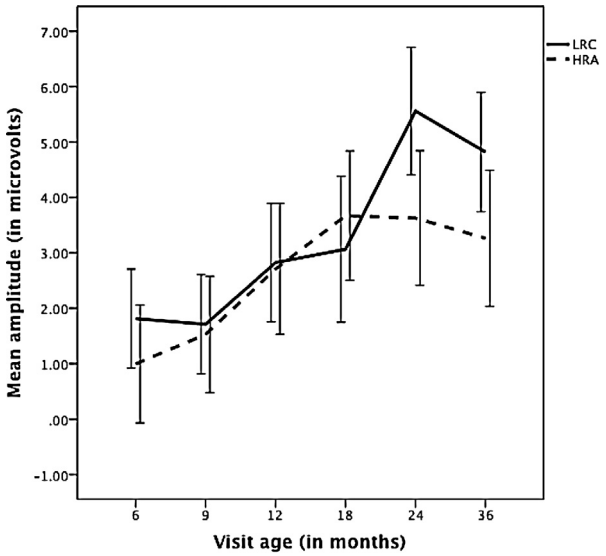

Fig. 6. Mean amplitude of the N290 by study group.

Luyster et al., 2011; Key and Stone, 2012), others have suggested that a greater response to familiar stimuli persists until after the 3rd birthday (Carver et al., 2003). The reason for a less marked differential response to familiar and unfamiliar stimuli in the present sample is unclear, but one possible explanation for these results is that the faces selected for use were not sufficiently different from one another to elicit a differential response across all age ranges (De Haan and Nelson, 1997). Finally, our findings about the right-hemisphere-lateralization of the Nc are in line with other studies on this component (Dawson et al., 2002; Webb et al., 2011; De Haan and Nelson, 1999; Carver et al., 2003). There was no significant main effect of group on the amplitude of the Nc, nor were there any significant

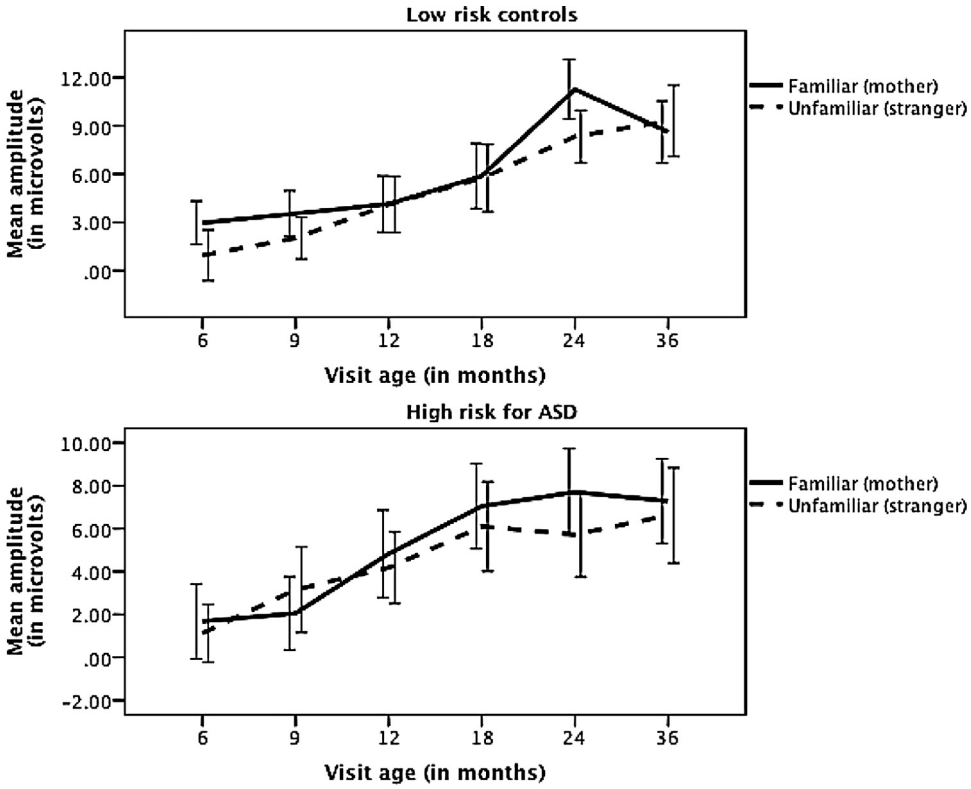

Fig. 7. Mean amplitude of the N290, by study group and condition.

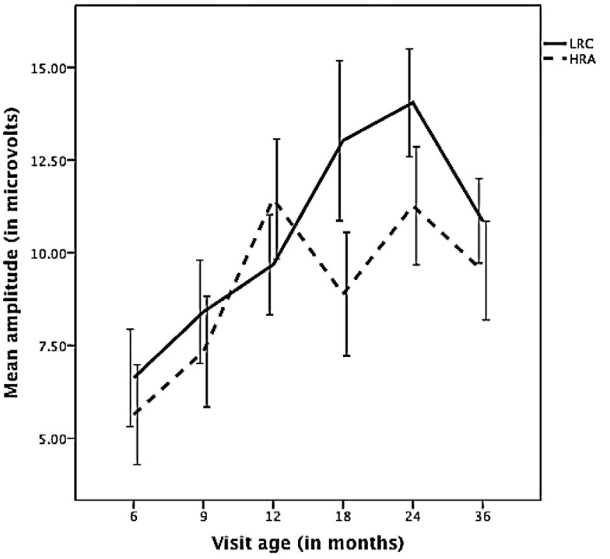

Fig. 8. Mean amplitude of the P400 by study group.

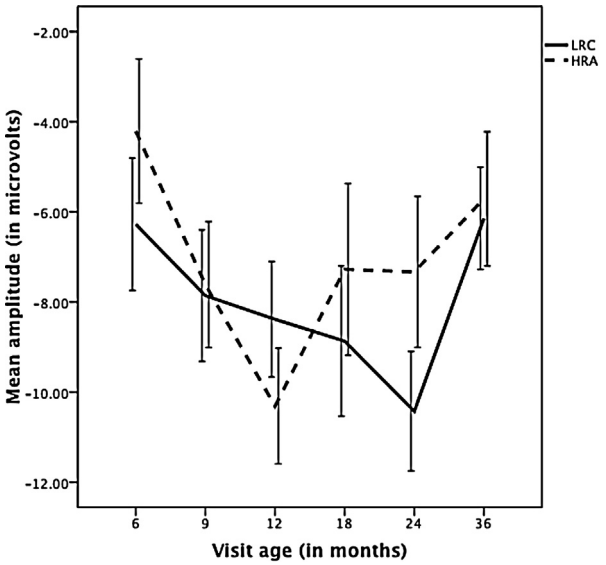

Fig. 9. Mean amplitude of the Nc by study group.

interactions, again revealing that ASD risk is not associated with pronounced differences in the neural response.

In sum, we have found evidence for differential patterns of age-related change in the amplitude of four commonly studied infant ERP components. In infants at low-risk for developing ASD, between 6 and 36 months of age, the amplitude of the P1 increases, the amplitude of the N290 decreases, the P400 and Nc both show amplitude increases until 24 months, followed by decreases between 24 and 36 months. Finally, the P400 and Nc were both maximal over the right hemisphere.

For infants at high-risk for ASD, the age-related patterns were similar to those observed in the low-risk group. There were no significant group differences or interactions,

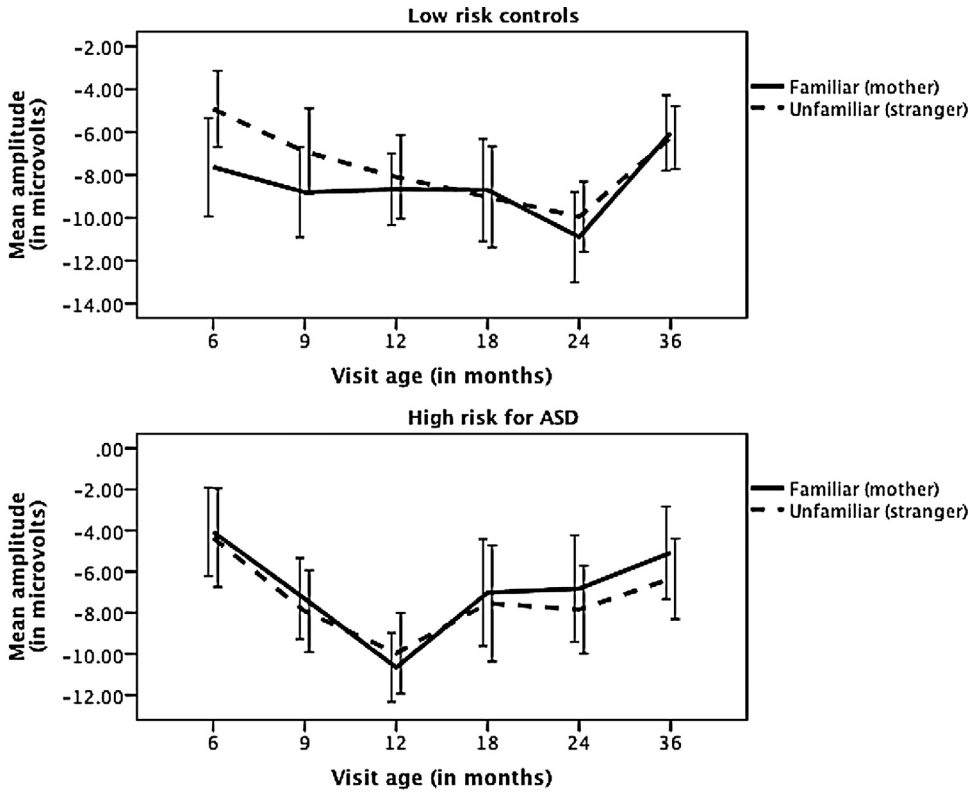

Fig. 10. Mean amplitude for the Nc, by study group and condition.

suggesting that the neural response to social stimuli in infants at high-risk for ASD is generally indistinguishable from that of their low-risk peers in the first few years of life, even when considering potential differences in sensitivity to familiarity and scalp topography. This observation produces a cautionary note for researchers hoping that the application of ERP to developmental psychopathology might identify markers of atypicality that could precede observable symptoms. The symptoms of ASD do not predictably emerge until after the first birthday (see Yirmiya and Charman, 2010 for a review); the hope is that the use of ERPs may reveal consistent markers in the first year of life. These results suggest that, at least when using a global measure of ERP response (e.g., mean amplitude) and a simple visual task, there are no markers significantly associated with ASD risk at *any* age in the current analyses.

There are some limitations to the present investigation that should be considered when interpreting the reported results and identifying potential follow-up directions. Only one metric of the ERP waveform was used here (mean amplitude); peak amplitude, peak-to-peak analyses and latency measures were not included. In addition, study groups were defined on the basis of family history only. Although all results were confirmed both with the inclusion and exclusion of a small subset of children with 'positive outcomes' at 24 or 36 months, we did not directly analyze any measure of behavioral development or ASD outcome, and we hope to do so as this longitudinal study progresses. Moreover, our results indicated an absence of ERP differences associated with ASD *risk* at a group level, but there remains the possibility that with sufficient numbers of children with an ASD *diagnosis* by age 2 or 3, meaningful differences in infant ERP responses could be revealed. That is, though we have not found clear markers of a broader ASD endophenotype, future research may reveal signatures that are specific to children who end up on the autism spectrum. Finally, though the marginal results and qualitative review of our data presented here fail to provide substantive evidence of group differences in the first years of life, we believe that they do point to the potential value of studying infants at high risk for ASD over the course of early development, rather than focusing solely on one age.

Altogether, the present investigation offers a useful set of findings addressing age-related changes in four commonly studied infant ERP components. These individual components follow different patterns of development over the course of the first three years of life; they also show varying modulation by familiarity of social stimuli and profiles of hemispheric differences. Results from the infants at high risk for ASD suggest that there are limited points of marked divergence from typical development in these commonly studied ERP components. We consider this large study of low- and high-risk infants to be a valuable reference point for developmental researchers studying both typical and vulnerable development in infants and toddlers.

Conflicts of interest statement

There are no conflicts of interest to report.

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