Electrophysiological signatures of visual statistical learning in 3-month-old infants at familial and low risk for autism spectrum disorder

Andrew Marin¹ | Ted Hutman¹ | Carolyn Ponting² | Nicole M. McDonald¹ | Leslie Carver³ | Elizabeth Baker¹ | Manjari Daniel¹ | Abigail Dickinson¹ | Mirella Dapretto² | Scott P. Johnson⁴ | Shafali S. Jeste¹

¹University of California, Los Angeles – Semel Institute for Neuroscience and Human Behavior, Los Angeles, CA, USA
²University of California, Los Angeles – Ahmanson-Lovelace Brain Mapping Center, Los Angeles, CA, USA
³University of California, San Diego - Psychology Department, San Diego, CA, USA
⁴Psychology Department, University of California, Los Angeles, Los Angeles, CA, USA

Correspondence
Andrew Marin, University of California, San Diego - Psychology Department, McGill Hall, 9500 Gilman Drive, La Jolla, CA 92093, USA.
Email: amarin@ucsd.edu

Funding information
National Institute of Child Health and Human Development, Grant/Award Number: 2P50HD055784-08 and R01-HD073535

Abstract
Visual statistical learning (VSL) refers to the ability to extract associations and conditional probabilities within the visual environment. It may serve as a precursor to cognitive and social communication development. Quantifying VSL in infants at familial risk (FR) for Autism Spectrum Disorder (ASD) provides opportunities to understand how genetic predisposition can influence early learning processes which may, in turn, lay a foundation for cognitive and social communication delays. We examined electroencephalography (EEG) signatures of VSL in 3-month-old infants, examining whether EEG correlates of VSL differentiated FR from low-risk (LR) infants. In an exploratory analysis, we then examined whether EEG correlates of VSL at 3 months relate to cognitive function and ASD symptoms at 18 months. Infants were exposed to a continuous stream of looming shape pairs with varying probability that the shapes would occur in sequence (high probability-deterministic condition; low probability-probabilistic condition). EEG was time-locked to shapes based on their transitional probabilities. EEG analysis examined group-level characteristics underlying specific components, including the late frontal positivity (LFP) and N700 responses. FR infants demonstrated increased LFP and N700 response to the probabilistic condition, whereas LR infants demonstrated increased LFP and N700 response to the deterministic condition. LFP at 3 months predicted 18-month visual reception skills and not ASD symptoms. Our findings thus provide evidence for distinct VSL processes in FR and LR infants as early as 3 months. Atypical pattern learning in FR infants may lay a foundation for later delays in higher level, nonverbal cognitive skills, and predict ASD symptoms well before an ASD diagnosis is made.

KEYWORDS
Autism Spectrum Disorder, EEG, infancy, statistical learning
Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder characterized by difficulties in social communication and the presence of restricted and repetitive interests (American Psychiatric Association, 2013). ASD-associated risk genes converge to disrupt early neurobiological processes such as cortical organization (Stoner et al., 2014), synaptic function (Parikshak et al., 2013), and intracortical connectivity (Emerson et al., 2015; Monroy et al., 2019) motivate the question of how early atypical VSL in infants at FR for ASD might be identified.

Previous behavioral studies show that VSL emerges early in development. In a paradigm developed by Kirkham et al., infants were habituated to a stream of six looming colored shapes organized in pairs defined by transitional probabilities (Kirkham, Slemmer, & Johnson, 2002). Transitional probabilities are computations of the likelihood that particular items in an array or sequence will occur together (Miller & Selfridge, 1950; the transitional probabilities of successive elements XY are defined as Probability of Y|X = frequency of XY/frequency of X). Following habituation, 2- to 8-month-olds looked longer at a random sequence of shapes in comparison to the previously seen familiar shape pairs (Kirkham et al.), implying that they recognized the differences in shape ordering between the two sequences. Differences in transitional probabilities across item pairs may facilitate the learning of sequence structure by signaling boundaries and units in an otherwise uninterrupted stream of items. In this paradigm, transitional probabilities within shape pairs (e.g., square→cross, triangle→circle, diamond→octagon) were 100% (and thus termed deterministic events) and across pairs (e.g., cross→triangle, circle→diamond) were 33% (termed probabilistic events). Infants at all three ages showed a reliable novelty preference for the random pattern, with no age difference in performance aside from general longer looking time in the youngest group. Moreover, newborn infants have also been reported to look longer at random sequences following habituation to four shapes whose ordering was defined by transitional probabilities (Bulf, Johnson, & Valenza, 2011).

The use of looking time as the dependent variable may fail to identify individual differences in learning that may have later cognitive or behavioral consequences. It also does not explain mechanisms that may underlie behavioral differences. Electroencephalography (EEG) can be used to examine learning without reliance on an overt behavioral response and may better quantify more subtle differences in timing or localization of cognitive processes (de Haan, 2007). For example, statistically learned visual events obtained from sequential observation result in predictive motor activation (as quantified by the EEG mu rhythm) in the infant brain (Monroy, Meyer, Schröer, Gerson, & Hunnious, 2019). Additionally, 6-month-old infants use prior experience to generate expectations about future sensory input, suggesting that the infant brain is capable of exploiting statistically presented visual events to form predictive expectations (Emerson, Richards, & Aslin, 2015). Indeed, EEG correlates of statistical learning may facilitate the development of an early biomarker of risk for higher level cognitive and social communicative delays in infants who develop ASD and related conditions. In an earlier study, Jeste et al. (2015) developed an EEG paradigm to examine VSL in children with ASD (29-75 months), modeled after the behavioral studies of Kirkham et al., (2002). Children with ASD in this study demonstrated reduced VSL compared to typically developing children. Moreover, analysis of individual differences in children with ASD revealed correlations between amplitudes of two EEG components, the N1 and P300, and nonverbal IQ and adaptive social function.

The study by Jeste et al. (2015) and other related studies of VSL (Emerson et al., 2015; Monroy et al., 2019) motivate the question of how early atypical VSL in infants at FR for ASD might be identified.
Abnormal structural and functional connectivity affects basic integrative functions, such as statistical learning, very early in life (Reber, 2013). Early disruptions in VSL could have a cascading influence over emerging cognitive and social communication delays in the second postnatal year. Social interaction is partly dependent on being able to detect implicit patterns governing people's behavior. It is possible that early deficits in visual pattern learning lay a foundation for impairments in visual reception and social communicative function underlying ASD. The identification of biological risk markers early in life may improve early detection of atypical development and facilitate targeted treatment in a critical period of rapid brain growth.

The goal of the present study was to examine the EEG correlates of VSL at 3 months of age, the first time point for data collection in a longitudinal study of infants at risk for ASD. Examining VSL at 3 months is ideal, given behavioral evidence that suggests VSL is present at birth (Bulf et al., 2011) and begins to improve as early as 2 months of age (Kirkham et al., 2002). As part of a comprehensive study of early predictors of ASD in FR infants (UCLA Autism Center of Excellence project), we examined whether we could use EEG to examine VSL as early as 3 months of age and whether FR infants show differences in VSL compared to LR infants. In an exploratory analysis, we examined whether VSL at 3 months relates to cognitive and ASD symptoms at 18 months. We predicted that, at age 3 months, FR infants would demonstrate atypical VSL relative to LR infants and that VSL would be associated with later nonverbal cognition, as indicated by visual reception skills, as well as autism symptoms at 18 months of age, an age when overt behavioral symptoms of a later autism diagnosis may first emerge (Ozonoff et al., 2013).

2 | METHODS

2.1 | Participants

Forty infants (FR: n = 19, M_age = 3.1 months, SD = 0.37; six girls (31.6%); LR: n = 21, M_age = 3.01 months, SD = 0.29; 10 girls (47.6%)) were studied at age 3 months as the first time point of a longitudinal study investigating early biomarkers for ASD (NICHD 2P50HD055784-08). Families were recruited by means of flyers, word of mouth, and email advertising. The distribution of gender was not statistically different between risk groups for the infants included in the EEG analysis (X^2 (1, N = 40) = 1.07, p = .3). All families provided written informed consent in accordance with the tenets of the 1964 Declaration of Helsinki.

An additional 37 infants were tested but excluded for inattention and excessive artifact (i.e., motion, eye movements, and EMG). The distribution of risk in the infants excluded due to inattention or excessive artifact was not different than the infants included in the EEG analysis (X^2 (1, N = 77) = 1.1, p = .29). There were also no significant differences in cognitive development (t(62) = 0.55, p = .59) and ASD symptomatology (t(62) = −0.79, p = .44) at 18 months between the infants excluded and included (see Table 1 for sample characteristics, split by infants included and excluded from the EEG analysis). Overall cognitive development was measured by the Early Learning Composite of the Mullen Scales of Early Learning (MSEL; Mullen, 1995), which comprises the average of the expressive language, receptive language, visual reception, and fine motor subscales. The percentage of participants retained (51.9%) is comparable to other studies with event-related EEG paradigms in early infancy (e.g., Altvater-Mackensen, Jessen, & Grossmann, 2017; Friedrich & Friederici, 2017; Pascalis, de Haan, & Nelson, 2002).

### 2.2 | 18-month cognitive development

The MSEL was administered at 18 months to evaluate cognitive development across verbal and nonverbal domains. The MSEL measures cognitive development from birth through age 5 within the following domains: expressive language, receptive language, visual reception, fine motor, and gross motor. Nonverbal developmental quotient (NVDQ) was obtained by averaging visual reception and fine motor t-scores at 18 months. We focused on 18-month NVDQ given the possible relevance of VSL to emerging nonverbal cognitive abilities, specifically visual reception ability (Jeste et al., 2015). Example items from the visual reception subscale in this age range include object permanence tasks, completing simple puzzles, and matching objects. There was a significant difference in the Early

| TABLE 1 | Participant demographics between infants included and excluded from the EEG analysis. The distribution of risk is not significantly different between the included and excluded infants (p = .29). The infants included and excluded from the EEG analysis did not differ in their Early Learning Composite Scores or ASD symptomatology at 18 months of age (all p's > .44).

<table>
<thead>
<tr>
<th>Total infants tested</th>
<th>Risk group</th>
<th>n</th>
<th>Age M (SD)</th>
<th>Sex</th>
<th>Early learning composite scores (MSEL) M (SD)</th>
<th>ASD symptomology ADOS-T M (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infants studied</td>
<td>FR</td>
<td>19</td>
<td>3.10 (0.37)</td>
<td>6 F (31.6%)</td>
<td>95.89 (18.5)</td>
<td>6.22 (5.10)</td>
</tr>
<tr>
<td></td>
<td>LR</td>
<td>21</td>
<td>3.01 (0.29)</td>
<td>10 F (47.6%)</td>
<td>95.89 (18.5)</td>
<td>6.22 (5.10)</td>
</tr>
<tr>
<td>Infants excluded</td>
<td>FR</td>
<td>11</td>
<td>3.31 (0.4)</td>
<td>5 F (45.4%)</td>
<td>93.61 (13.54)</td>
<td>7.21 (5.01)</td>
</tr>
<tr>
<td>(inattention)</td>
<td>LR</td>
<td>11</td>
<td>3.26 (0.4)</td>
<td>4 F (36.4%)</td>
<td>93.61 (13.54)</td>
<td>7.21 (5.01)</td>
</tr>
<tr>
<td>Infants excluded</td>
<td>FR</td>
<td>11</td>
<td>3.14 (0.36)</td>
<td>5 F (45.4%)</td>
<td>93.61 (13.54)</td>
<td>7.21 (5.01)</td>
</tr>
<tr>
<td>(excessive artifact)</td>
<td>LR</td>
<td>4</td>
<td>3.1 (0.12)</td>
<td>1 F (25%)</td>
<td>93.61 (13.54)</td>
<td>7.21 (5.01)</td>
</tr>
</tbody>
</table>

Abbreviations: ASD, Autism Spectrum Disorder; EEG, electroencephalography; FR, familial risk; LR, low risk.
Learning Composite on the MSEL between FR and LR infants at 18 months: FR infants had significantly lower cognitive abilities than LR infants (see Table 2 for behavioral results, split by risk group).

### 2.3 18-month ASD symptomology

We also hypothesized that early disturbances in VSL would relate to an increase in ASD symptoms at 18 months of age. The Autism Diagnostic Observation Schedule-Toddler Module (ADOS-T; Lord, Luyster, Gotham, & Guthrie, 2012) was administered at 18 months to evaluate early ASD symptomology. The ADOS-T measures early behavioral symptoms of ASD in toddlers from 12 to 30 months of age. There were no significant differences in ASD symptomatology between the FR and LR infants at 18 months, although, as expected, FR infants tended to have higher scores (see Table 2 for behavioral results, split by risk group).

### 2.4 3-month VSL paradigm and procedure

As in Jeste et al. (2015), six colored shapes (blue square, red circle, yellow triangle, green cross, purple octagon, and orange diamond) appeared sequentially at the center of the monitor for 500 ms with an interstimulus interval (ISI) of 500–750 ms. Each shape expanded from 3 to 6 cm in height. Each shape was randomly paired with one other shape in sequence, yielding three shape pairs, which were randomized across participants. The initial member of each pair predicted the next member of the pair with transitional probabilities = 100% (deterministic event). Following the second item in a pair, the appearance of any of the three first shapes in a pair was equally probable, transitional probabilities = 33% (probabilistic event; see Figure 1).

The initial 30 shape pairs served as the exposure phase. Three shape pairs were presented to the infant 10 times each (thus controlling for co-occurrence frequency) in random order. The test phase consisted of 100 shape pairs in which the three shape pairs were presented 30 times each in a random order. The additional 10 shape pairs consisted of “oddball” presentations, where the second shape failed to predict the established shape pattern. We opted not to include this condition due to the low amount of trials retained for sufficient event-related potential (ERP) analyses and will not be discussed further. After reviewing the video of the infant during the EEG session, we confirmed that all infants in the sample attended at least 50% of the exposure phase by excluding periods of visual inattention. Inattention was defined as any 3-s period during which the infant was looking away from the monitor. Analysis revealed no significant risk-group differences in the amount of trials viewed (i.e., single shape presentation) during the exposure phase, $t_{(38)} = 0.14, p = .89; M_{FR} = 51.74, SD_{FR} = 8.69; M_{LR} = 51.33, SD_{LR} = 9.37$. Testing was terminated if the child became fussy or lost interest. EEG was time-locked to the onset of each shape stimulus to compare the response to each condition. The deterministic condition was defined by the onset of the second shape in the pair, and the probabilistic condition was defined by the onset of the first shape in the pair. Unlike the behavioral habituation paradigm, there was no presentation of random sequences in comparison to the learned sequences. Rather, conditions were defined by the shape to which the EEG was time-locked.

### 2.5 EEG data processing

Continuous EEG was recorded via a 128-channel HydroCel Geodesic Sensor Net. Impedances were kept below 100 kOhms in all electrodes and the raw EEG data were referenced online to vertex (Cz). The electrical signal was amplified with a 0.1 to 100 Hz band-pass and digitized at 500 Hz. All data were processed off-line using NetStation 4.4.5 software (Electrical Geodesics, Inc.). A video of each infant was obtained during recording and was coded for visual attention to the screen. Trials in which the infant was not looking at the screen were removed from the analysis. Infants who had at least 10 artifact-free ERP segments per condition (i.e., probabilistic and deterministic) were included in the analysis. ERPs reflect the brain’s electrical activity time-locked to a stimulus and are evidence of the simultaneous firing of groups of synapses. There were no risk-group differences for the infants included in the ERP analysis regarding the amount of trials viewed during the exposure phase, $t_{(38)} = 0.14, p = .89; M_{FR} = 51.74, SD_{FR} = 8.7; M_{LR} = 51.33, SD_{LR} = 9.38$, and the amount of trials viewed during the test phase, $t_{(38)} = 0.78, p = .46; M_{FR} = 139.63, SD_{FR} = 34.95; M_{LR} = 130.38, SD_{LR} = 39.51$, and the amount of total usable trials included in the construction of individual ERP waveforms, $t_{(38)} = −0.24, p = .80; M_{FR} = 78.95, SD_{FR} = 31.18; M_{LR} = 81.57, SD_{LR} = 36.24$. The amount of total usable trials included in the averaged ERP waveform for all the infants on average was 80.33 trials ($SD = 33.52$) in total: (probabilistic condition: $M = 40.92, SD = 16.83$; deterministic condition: $M = 39.4, SD = 16.96$). The EEG data were digitally filtered using a 0.3 to 30 Hz band-pass filter, segmented into 1,000 ms epochs, and baseline corrected using mean voltage during the 100 ms prestimulus baseline period.

### Table 2

Behavioral measures of infants included in the EEG analysis at 18 months, split by risk group. Overall, FR infants had significantly lower cognitive abilities than LR infants at 18 months of age. There were no significant group differences in 18-month ASD symptomology between the FR and LR infants.

<table>
<thead>
<tr>
<th>Risk group</th>
<th>Early learning composite scores (MSEL)</th>
<th>ASD Symptomatology (ADOS-T)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FR (n = 19)</td>
<td>$M = 87.65$, $SD = 16.41$</td>
<td>$M = 7.88$, $SD = 5.67$</td>
</tr>
<tr>
<td>LR (n = 21)</td>
<td>$M = 103.22$, $SD = 18.04$</td>
<td>$M = 4.89$, $SD = 4.19$</td>
</tr>
<tr>
<td>Risk-group differences</td>
<td>$t_{(33)} = −2.67$, $p = .012$, $d = 0.9$</td>
<td>$t_{(33)} = 1.78$, $p = .084$, $d = 0.6$</td>
</tr>
</tbody>
</table>

Abbreviations: ASD, Autism Spectrum Disorder; EEG, electroencephalography; FR, familial risk; LR, low risk.
The data were then taken through an automated artifact-detection tool that rejects individual channels if the ERP amplitude is greater than 200 mV. Trials with more than 15% bad channels were rejected. Data from bad channels were replaced using a spherical spline interpolation algorithm. Each trial was then manually inspected for ocular and electromyographic artifacts following automatic artifact detection. Trials with evidence of eyeblinks, saccades, or other muscle artifact were rejected from the analysis. Grand-averaged components across all participants, without knowledge of risk-group affiliation. Individual peak amplitude and latency measures were verified via visual inspection of individual-averaged ERPs to ensure that the P1 and N700 responses were within their designated time windows.

2.6 ERP regions and components of interest

To our knowledge, there are no published studies on EEG correlates of VSL in early infancy, which prompted an exploratory approach to the components of interest for the present study. The Jeste et al. (2015) study identified both early (i.e., N100) and late ERP components (i.e., P300) underlying VSL in preschool-aged children. Infant EEG is typically not treated as a downward extension of EEG in older children due to the rapid period of development underlying basic neural structures (i.e., synapses, white matter tracts, etc.) in the first year after birth. Typically, infant ERP components are generally characterized by smaller amplitude and slower latency responses compared to ones originating from older children (Courchesne, Ganz, & Norcia, 1981; Kushnerenko et al., 2002; Macchi Cassia, Kuefner, Westerlund, & Nelson, 2006). For this investigation, we expected different ERP components in terms of their time-course and morphology considering the large developmental gap between the two populations. Given the lack of normative data, we assessed the grand-averaged ERPs, collapsed across risk group, to help to identify potential ERP components and time windows.

The P1 was evaluated for differences in low level, early visual processing of the stimuli, which we did not expect to find. The P1 is an early sensory component (e.g., Clark, Fan, & Hillyard, 1994), operationalized here as the maximum peak amplitude and latency occurring 50–150 ms poststimulus onset.

Historically, research examining cognitive processes related to attention and learning in early infancy has focused on EEG components within frontal and central brain regions 300–1,200 ms poststimulus onset (de Haan, 2007). Given the scope of the current investigation, we identified a late, positive going frontal component that likely indexes higher order neurocognitive processes related to attention and learning. For the purposes of this investigation, we referred to this component as late frontal positivity (LFP), which was operationalized as the mean amplitude during the window 450–800 ms poststimulus onset.

In addition, we recorded the N700, an occipital component characterized by a negative going peak occurring 600–800 ms after stimulus onset (Bender, Oelkers-Ax, Hellwig, Resch, & Weisbrod, 2008; Reynolds & Richards, 2005). It has been hypothesized that the infant N700 reflects late, downstream attention that may be implicated in stimulus post processing, encoding, and disengagement (Macchi Cassia et al., 2006; Reynolds & Richards, 2005). Here the N700 was defined as the minimum peak amplitude and latency occurring between 600 and 800 ms after stimulus onset.

Both the time window and the regions of interest were selected based on our hypotheses about the timing of the EEG component and from visual inspection of each component using individual and grand-averaged components across all participants, without knowledge of risk-group affiliation. Individual peak amplitude and latency measures were verified via visual inspection of individual-averaged ERPs to ensure that the P1 and N700 responses were within their designated time windows. Visual inspection procedures are common in infant ERP studies (de Haan & Nelson, 1999; Nelson, 1994; Quinn, Duran, Reiss, & Hoffman, 2010; Reid, Striano, Kaufman, & Johnson, 2004; Seery, Vogel-Farley, Tager-Flusberg, & Nelson, 2013) when ERP components and their time windows are data-driven rather than well-established, an important consideration especially when dealing with 3-month-olds, as there are no well-established components to reference. A four-channel occipital region group that was divided into two regions (left and right) was selected for the P1 and N700 analyses. A 12-channel frontal region was divided into three regions (left, middle, and right) to evaluate region differences in LFP activity (see Figure 2).

2.7 LFP and N700 differential response calculation

The LFP and N700 deflections in response to high and low transitional probabilities of geometric shape patterns served as our marker of VSL. In order to understand individual variation in robustness of VSL, we calculated the relative amplitude difference between each
condition, for both the N700 and LFP components, by subtracting the averaged probabilistic response from the averaged deterministic response. The differential responses served as our marker of VSL at 3 months of age and were used to test the correlation with nonverbal cognitive ability and ASD symptomology at 18 months.

3 | RESULTS

3.1 | P1

To rule out the possibility that differences in low-level visual processing between conditions influenced higher level cognitive process in later components such as the N700 and LFP, we ran two 2 × 2 (condition: deterministic, probabilistic) × 2 (region: left, right) × 2 (risk group: FR risk, LR risk) repeated measures ANOVA to evaluate the occipital P1 mean peak amplitude and latency at 3 months of age. We observed a main effect of region with respect to peak amplitude (R > L; \( F_{(1,38)} = 4.53, p = .04, \eta^2 = 0.11 \)) and a trending but non-significant main effect of hemisphere with regard to peak latency (L > R; \( F_{(1,38)} = 3.38, p = .07, \eta^2 = 0.08 \)); however, there were no other main effects nor interactions with respect to P1 peak amplitude or latency (all \( p > .36 \)). The mean amplitude over this time window was also analyzed, which provided similar results to maximum peak amplitude, so it is not discussed here.

3.2 | LFP response

Figure 2 presents the grand-averaged 3-month frontal waveform split between risk groups for all the infants included in the analysis. We used a 2 (condition: probabilistic, deterministic) × 3 (region: left, middle, right) × 2 (risk group: FR, LR) repeated measures ANOVA to examine the effects of LFP mean amplitude responses. We observed a main effect of region (\( F_{(2,37)} = 4.91, p = .01, \eta^2 = 0.21 \)), which post hoc tests reflect greater LFP response within the left region compared to middle only (\( p = .003, d = −0.35; M_{\text{Left}} = 1.69, SD_{\text{Left}} = 3.36; M_{\text{Middle}} = 2.83, SD_{\text{Middle}} = 3.2 \)). Analysis also revealed a condition × risk group interaction (\( F_{(1,38)} = 13.5, p = .001, \eta^2 = 0.26 \)). LR infants displayed greater response during the deterministic condition (\( M_{\text{LFP/deterministic}} = 3.57 \mu V, SD = 3.75 \)) compared to the probabilistic condition (\( M_{\text{LFP/probabilistic}} = 1.12 \mu V, SD = 4.58; p = .02, d = 0.59 \)). In contrast, the FR infants displayed greater response during the probabilistic condition (\( M_{\text{LFP/probabilistic}} = 3.25 \mu V, SD = 2.71 \)) compared to the deterministic condition (\( M_{\text{LFP/deterministic}} = 1.30 \mu V, SD = 2.69 \)).
To consider the possibility that differentiation between conditions was modulated by attention to the task, we re-ran the repeated measures ANOVA while controlling for total number of trials viewed, summed across both the exposure and test phases. We opted to use the centered value of total trials viewed as our covariate. We obtained this value by subtracting the mean of total trials viewed (collapsed across condition and risk group) from the total trials viewed value. The main effect of region ($F_{(2,38)} = 4.87, p = .03$, $\eta^2_p = 0.11$) and latency ($L > R$, $F_{(2,38)} = 5.03, p = .03$, $\eta^2_p = 0.12$) were significant, and the N700 minimum peak amplitude condition × risk group interaction ($F_{(2,38)} = 6.21, p = .02$, $\eta^2_p = 0.14$). Post hoc tests revealed that LR infants displayed significant differential N700 minimum peak amplitude response between the deterministic ($M_{N700/deterministic} = −11.6 \mu V, SD = 11.28$) and probabilistic ($M_{N700/probabilistic} = −4.98 \mu V, SD = 12.34$) conditions ($p = .03$, $d = .56$), but the FR infants did not ($p = .35$; $M_{N700/deterministic} = −8.48 \mu V, SD = 7.08$; $M_{N700/probabilistic} = −9.93 \mu V, SD = 7.02$). There were no other statistically significant findings with respect to N700 minimum amplitude or latency ($p's > .11$).

To examine the possibility that differentiation between conditions was modulated by attention to the task, we repeated the repeated measures ANOVA while controlling for total trials viewed across the exposure and test phases. The centered value of total trials viewed served as our covariate. The risk group by condition interaction and main effects of region for amplitude remained significant in this confirmatory model, as well as the main effect of region for minimum peak latency. The average amplitude over the N700 time window was also analyzed, and we replicated the minimum peak amplitude condition × risk group interaction found when using N700 minimum peak measures ($F_{(2,38)} = 4.88, p = .03$, $\eta^2_p = 0.11$).

The subsequent post hoc tests for this interaction, however, now revealed the LR infants did not significantly differentiate between the deterministic ($M_{N700/deterministic} = −5.79 \mu V, SD = 10.97$) and probabilistic conditions ($M_{N700/probabilistic} = −0.69 \mu V, SD = 11.1$; $p = .08$, $d = 0.58$). Additionally, the significant region mean effect also failed to replicate while using mean amplitude measures ($F_{(1,38)} = 3.36, p = .08$, $\eta^2_p = 0.08$).

3.4 | Correlation with nonverbal cognitive skills and ASD symptoms at 18 months

To examine the relation between VSL at 3 months and behavioral measures at 18 months, we used nonparametric correlations (i.e., Kendall’s tau-b) due to the negative skew in the LFP and N700 differential responses (see Figure 3 for individual level, differential LFP and N700 responses split between risk group). We focused our exploratory analyses to whole-group correlations for two reasons. First, we were most interested in the overall relation between the ERP at 3 months and later nonverbal cognitive skills at 18 months of age and we recognized that we could most robustly examine this association in the full group, given its larger sample size and the fact that our goal here was to understand heterogeneity across all infants, not only in the FR group. Significant, whole-group correlations were found between the 3-month LFP difference score and 18-month visual reception t-scores ($r_t(35) = .46, p = .001$; see Figure 4a), such that infants who showed a more negative LFP differential response had higher visual reception scores at 18 months of age. Both correlations remained significant after Bonferroni correction for multiple comparisons ($p's < .008$). To account for possible risk-group differences driving the whole-group correlation between the 3-month LFP response and 18-month visual reception t-score, we opted to center the individual-level visual reception t-score by subtracting the average t-score, per risk-group affiliation, from each infant’s t-score. We found a significant relation between the 3-month LFP response and the centered value of 18-month visual reception ability with nonverbal cognitive skills and ASD symptoms at 18 months ($r_t(35) = .22, p = .05$). There were no significant correlations between the N700 differential response and 18-month visual reception ability ($p's > .47$).

Correlations between the 3-month LFP and N700 differential responses and 18-month ADOS-T total scores were also tested to better understand early VSL in relation to later ASD symptoms. Nonparametric, whole-group correlations revealed a significant association between the LFP differential response and early ASD symptoms ($r_t(35) = .24, p = .05$; see Figure 4b). The association between LFP differential response and early ASD symptoms failed to reach significance of $p = .05$ after Bonferroni correction for multiple comparisons. To account for possible risk-group differences driving the whole-group correlation between the 3-month LFP response and 18-month ADOS-T total score, we opted to center the individual-level ADOS-T total scores by subtracting the average total score, per risk-group affiliation, from each infant’s total score. We found no significant relation between the 3-month LFP response and the centered value of 18-month ASD symptoms ($r_t(35) = .07, p = .54$). There was no significant relation between the N700 differential response and ASD symptoms at 18 months ($r_t(35) = −.14, p = .27$).
Peak-to-peak analysis

We conducted a secondary peak-to-peak, repeated measures ANOVA analysis to assess whether early 3-month ERP differences at frontal sites may contribute to later differences at the LFP. We first extracted the mean amplitude of the P1 at frontal electrode sites, using the same time window as the occipital analysis (e.g., 50–150 ms). Individual-level peak-to-peak measures were obtained by subtracting the frontal P1 mean amplitude from the LFP mean amplitude per condition (e.g., probabilistic, deterministic) at each electrode site (e.g., left, middle, and right). This peak-to-peak amplitude difference measure served as our dependent variable in our ANOVA model.

We used a 2 (condition: probabilistic, deterministic) × 3 (region: left, middle, right) × 2 (risk group: FR, LR) repeated measures ANOVA to examine effects of frontal peak-to-peak (i.e., P1 to LFP) mean amplitude responses. The analysis revealed a condition × risk group interaction ($F_{(1,38)} = 6.01, p = .019, \eta^2_p = 0.137$). LR infants displayed greater response during the deterministic condition ($M_{PTP/deterministic} = -3.52 \mu V, SD = 3.49$) compared to the probabilistic condition ($M_{PTP/probabilistic} = -1.47 \mu V, SD = 4.47; p = .015, d = -0.51$). In contrast, the FR infants did not display any differences in response between the deterministic condition ($M_{PTP/deterministic} = -1.98 \mu V, SD = 3.07$) compared to the probabilistic condition ($M_{PTP/probabilistic} = -2.24 \mu V, SD = 2.86; p = .606$). There were no other statistically significant findings with respect to peak-to-peak mean amplitude (all $p$'s > .137). The peak-to-peak findings indicate that the later ERP difference in the LFP may arise due to early frontal activation in the FR group.

DISCUSSION

The goal of the present study was to examine electrophysiological correlates of VSL in 3-month-old infants with FR and LR for ASD,
with the hypothesis that infants at FR for ASD would demonstrate atypical patterns of VSL at 3 months of age that would predict later cognitive skills and ASD symptoms at 18 months. Both LR and FR infants displayed clear EEG evidence of VSL, but they differed in their patterns of responses. Moreover, as expected, EEG patterns associated with VSL as early as 3 months of age predicted later nonverbal cognitive abilities, specifically visual reception skills, at 18 months of age.

Our analysis revealed two electrophysiological indices of VSL (the LFP and N700) that distinguished infants with high FR and LR for ASD as early as 3 months of age predicted later nonverbal cognitive abilities, specifically visual reception skills, at 18 months of age.

Our analysis revealed two electrophysiological indices of VSL (the LFP and N700) that distinguished infants with high FR and LR for ASD as early as 3 months of age. The differentiation of conditions in the LR infants was characterized by increased response during the deterministic event relative to the probabilistic event. Conversely, FR infants displayed the opposite pattern, exhibiting increased response toward the probabilistic event. An alternative account, as suggested by the peak-to-peak analysis, is the LFP differences seen within the FR infants could be the result of earlier differences in the ERP. In other words, it could be that the FR infants differentiated between the two conditions sooner than the LR infants. Future research is needed to formally test this explanation given that temporal differences between risk groups in the ability to discriminate between the probabilistic and deterministic conditions were not a part of our original hypotheses. Additionally, the occipital N700 response revealed evidence for differentiation of deterministic and probabilistic events by LR infants, but not by FR infants. Importantly, the similarity between groups in posterior P1 amplitudes across conditions indicates that differences in VSL cannot be attributed to early sensory differences between groups. Notably, our previous EEG investigation in preschool-aged children with ASD found that children with ASD exhibited reduced VSL compared to typically developing children (Jeste et al., 2015). The contrast between the results of the present study and the prior study suggests developmental

**FIGURE 4** Scatter plots depicting whole-group correlations with (a) 18-month visual reception t-scores and late frontal positivity (LFP) differential responses and (b) 18-month ADOS-T total scores and 3-month LFP differential responses. An average Mullen Scales of Early Learning visual reception t-score is 50. Clinical criterion, as assessed by the 18-month ADOS-T, for early concerns for Autism Spectrum Disorder is a total score of 10. The black triangles indicate familial-risk infants, while the grey circles reflect the low-risk infants.
The lack of normative EEG data investigating VSL in early infancy serves as a motivation for this investigation, but it also limits the conclusions drawn about the functional significance of group-level differences in VSL. Prior behavioral work investigating VSL in early infancy described a novelty preference when viewing geometric shape patterns (Bulf et al., 2011; Kirkham et al., 2002; Slone & Johnson, 2015, 2018). Studies of infant face processing found greater EEG response toward familiar faces compared to novel ones (de Haan, 2007; de Haan & Nelson, 1997) and greater response to faces than objects (de Haan, 2007; de Haan & Nelson, 1999). Our study finds that greater neural response toward deterministic-favored VSL (i.e., deterministic > probabilistic) at 3 months of age is related to nonverbal ability at 18 months of age. Allocation of neural resources toward processing familiar, nonsocial information may be adaptive, as sensitivity to stimuli presented with high statistical regularity may afford infants a sense of predictability toward their environment. This knowledge could effectively aid infants in navigating complexity with greater efficiency. As mentioned above, the discrepancy between this investigation and Jeste et al. (2015) reinforces the fact that normative data on VSL in infancy remain sparse, and replication of this paradigm in a new, larger cohort is needed. Additionally, ongoing longitudinal investigations will seek to test whether different patterns of VSL between the FR and LR infants remain static or change during the first year after birth. Examining the development of VSL longitudinally in FR and LR infants will allow us to further understand the maturing VSL response, specifically whether different styles of learning are the manifestation of or adaptation to a developmental disruption.

An important distinction between the current VSL paradigm and those used by Kirkham et al., (2002) and Slone and Johnson (2015) is the difference between conditions. Kirkham et al. (2002) compared familiar pairs, following a learning phase, versus random sequences, but the current study contrasted shapes paired at 100% versus 33.33% frequency of presentations. Slone and Johnson contrasted sequentially presented geometric shape patterns, defining transitional probabilities between shape pairs at 50% chance of occurrence, utilizing looking time as the dependent outcome measure. The difference between conditions in the current paradigm, therefore, is subtler than those compared in previous work. The use of EEG as a primary outcome measure, coupled with subtle differences in stimuli presentation, may have played a role in the response preference disparities between each study. Future replication studies utilizing EEG are needed to further understand the role of risk status in specific patterns of VSL.

In sum, our results provide evidence for distinct VSL processes in infants based on their genetic risk for ASD at 3 months of age, far earlier than previously reported behavioral manifestations of FR. To our knowledge, this is the first study to identify an electrophysiological index of atypical learning as early as age 3 months in FR infants. FR infants represent a complex combination of genetic and environmental etiologies that contribute to changes in both structural and functional brain networks. Group-level differences in VSL between the FR and LR infants may reflect the manifestation of...
altered functional networks as early as 3 months of age. Yet, even in the presence of diffuse alterations in functional connectivity, the brain can adapt, compensating for damaged or inefficient networks as one interacts with the environment (see review, Johnson, 2017). It is possible that the differences in pattern learning between FR and LR infants we observed may not reflect a disruption of VSL in the FR infants, but rather an adaptation or compensatory process to increase neural resources toward semi-structured (i.e., probabilistic) visual events. The relation between VSL at 3 months of age and nonverbal skills and ASD symptomatology at 18 months indicates VSL's potential influence over emerging developmental and ASD symptoms (though the association with ASD symptoms failed to reach significance after correction for multiple comparisons). A potential explanation for the lack of a significant relation between VSL and ASD symptomatology may be that VSL is more closely associated with emerging visual reception skills more broadly, and not necessarily ASD-related behaviors. Further work is needed to determine whether different styles of visual pattern learning maps onto particular diagnostic outcomes.

Carefully crafted EEG paradigms can provide researchers quantifiable readouts of formative cognitive processes available early in development, allowing for more refined ways to capture individual differences. In our data, there was similar variability in LFP mean amplitude between the LR and FR groups, suggesting that the group-level differences are not driven by a clinical subgroup, despite the well-established heterogeneity of clinical outcomes of FR infants in general (Charman et al., 2017; Messinger et al., 2013). Paradigms that target domain-specific processes relevant to ASD will further improve our understanding of how distinct learning profiles relate to the risk for ASD and emerging cognitive skills within the second year after birth. The current study demonstrates the feasibility of electrophysiological methods for the very early detection of risk for atypical development, considerably earlier than behavioral markers that have been reported (Ozonoff et al., 2014), and introduces statistical learning and visual information processing as promising targets for intervention early in development.

ACKNOWLEDGMENTS

We first thank all the parents and infants for participating in the research. This research was supported by grants from the National Institute of Child Health and Human Development [2P50HD055784-08, 2012, R01-HD073535].

CONFLICT OF INTEREST

The authors whose names are listed immediately below certify that they have no affiliations with or involvement in any organization or entity with any financial interest, or nonfinancial interest in the subject matter or materials discussed in this manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

REFERENCES


