Supplementary Materials

Subject Retention, Selection and Outcome Assessments. One hundred thirty Low-Risk infants and fifty-one High-Risk infants were tested. Of these, data from 38 Low-Risk (29.2%) and 23 High-Risk (45.1%) infants were retainable, which is similar to retention rates reported in previous infant ERP studies (1, 2). The remaining infants' data could not be used for the following reasons: (1) 29 infants refused to wear the electrode cap, (2) 83 infants did not provide a minimum number of retainable trials (criterion $\geq$ 20 trials, based on previous ERP studies, e.g., 3-5), (3) 5 infants did not exhibit clear ERP components (P100, N290, P400, Nc), as confirmed by author LC, and (4) 3 infants' data were lost due to computer error. Of the 23 High-Risk infants with retainable data, three later developed ASD (see below) and their data will be presented separately in a future report when we have data from more infants in this category. This left 20 High-Risk subjects for our analyses.

To equate the number of High- and Low-Risk infants in our analyses, of the 38 Low-Risk infants with retainable data, we selected 20. This selection was performed by a research assistant naïve to the ERP results, whose only task was to select 20 Low-Risk infants who matched the 20 High-Risk subjects as closely as possible on a variety of factors. To this end, the sample of 20 Low-Risk and 20 High-Risk infants did not differ ($p > 0.05$, based on 2-tailed t-tests) on the following: (A) age on first day of testing (High-Risk = 306.5 days ± 14.5, Low-Risk = 302.9 days ± 14), (B) gestational period, based on number of days that birth date was pre/post due date (High-Risk = 5.6 days early ± 8.6, Low-Risk = 2.5 days early ± 8.1, see 6 for discussion of why this factor might affect visual measures), (C) proportion of females (High-Risk = 40%, Low-Risk = 45%) and (D) total number of retainable trials (High-Risk = 47.5 trials ± 20.1, Low-Risk = 44.1 trials ± 14.5). Also, note that within each subject group, there were no significant differences in the number of retainable trials across

the four stimulus conditions described below (1-factor ANOVAs yielded $p > 0.05$ for both subject groups). And, for each of the four stimulus conditions, there was no difference in number of retained trials between groups (all $p > 0.05$, 2-tailed t-tests).

At 24 and 36 months of age, the outcome of each High-Risk infant in our study is assessed with the *Autism Diagnostic Observation Schedule (ADOS),* which is a play-based assessment designed to elicit behaviors (or lack of behaviors) associated with a diagnosis of ASD (7), the *Preschool Language Scales (PLS-IV)* (8), which measures expressive and receptive language skills, and the *Mullen Scales of Early Learning* (9), which measures mental age. If a child scores above the ASD cut-off on the ADOS, we conduct the *Autism Diagnostic Interview-Revised (ADI-R)* (10), which is a parent interview. Of the 20 High-Risk infants included in our ERP analyses, 11 were assessed for, and found not to have, an ASD. Of the remaining nine, one was unavailable for testing because the family moved away, and eight are currently too young to be tested. We also attempt to test Low-Risk infants at 24 and 36 months. Of the 20 Low-Risk infants whose data were included in our ERP analyses, 14 were assessed for, and found not to have, an ASD. Of the remaining six, three were unavailable for testing because the family moved away, and three are currently too young to be tested.

Because the outcome of nine of our 20 High-Risk infants is currently unknown, we cannot rule out the possibility that differences observed between our High-Risk and Low-Risk sample of infants may be driven by High-Risk infants who are destined to develop ASD. We think this unlikely, however, because our statistical tests indicate normality in the ERP data and no outliers (defined as subjects whose data fall > 3 standard deviations outside the mean). Still, it will be important to determine whether infants who go on to develop ASD differ on our ERP measure from those who do not. Note that we have had three infants develop ASD, which is currently not enough to investigate this question. Data from these

three ASD infants will be presented in a future report when we have more data from infants in this category. Thus, at the current time, differences observed between our High-Risk and Low-Risk sample of infants should be viewed as reflecting the endophenotype of ASD (i.e., traits that run in individuals with ASD and their family members) rather than reflecting predictors of developing ASD *per se*.

Low-Level Characteristics of Face and Object Stimuli. The pictures of the faces and objects were analyzed to determine whether low-level visual information differed between the two categories. (For this analysis, we collapsed across familiar and unfamiliar stimuli, since, as expected, there were no significant low-level differences between the two). To this end, fast Fourier transform (FFT) analyses were conducted on the stimulus images (e.g., 11, 12). The results showed that the spatial frequency content, as reflected in slopes of log contrast by log spatial frequency, did not vary between faces and objects (faces = -2.05 ± 0.26, objects = -2.13 ± 0.26, $p > 0.05$) and that both the face and object slope values were very close to those observed in our previous studies of faces (12). However, the overall root-mean-squared contrast was 1.3-fold higher for objects than faces ($p < 0.001$). We expect this to have only minimal effects since the stimuli were all well above contrast threshold.

Details of EEG Recording and Analyses. *Trial/Artifact Rejection.* To eliminate trials in which the infant was not paying attention, infants' looking behavior was monitored by an experimenter in another room via a video camera. Trials in which the infant was deemed not to be viewing the video monitor were discarded (via button press). Stimulus presentation continued until the infant became too fussy or bored, with the entire session lasting between 15 and 40 minutes. To eliminate trials containing artifacts caused by eye movements, the electrooculogram was recorded from electrodes positioned above the eyes

and on the outer canthi, as in previous studies (e.g., 2). Individual trials were inspected and rejected if they contained eye or head movements. Also, trials with 11 or more channels containing artifacts were excluded. If a trial had 10 or fewer channels with artifacts, data for those channels was replaced using spherical spline interpolation (see 13 for details).

Electrode Montages. The electrode montages were chosen based on those used in previous ERP studies of face/object processing (e.g., 14) and were confirmed by examining the scalp topography and waveforms of individual and grand average data. The same montage was used for all subjects. For the P100, N290 and P400, the relevant montages were occipito-temporal electrodes over the right (85, 86, 91, 92, 96, 97, 98, 101) and left (51, 57, 58, 59, 60, 64, 65, 66) hemispheres. These electrode montages are roughly centered around electrodes T5 and T6 in the International 10-20 system for electrode placement. For the Nc, the relevant montages were frontal electrodes over the right (2, 3, 4, 9, 105, 106, 110, 111, 112, 116, 117, 118, 119, 123, 124) and left (20, 21, 23, 24, 25, 27, 28, 29, 30, 31, 35, 36, 37, 40, 41) hemispheres (roughly centered around F3 and F4 in the 10-20 system).

Subject Waveforms Showing the P100, N290 and P400 Occipital-Temporal Components. Waveforms (μv by seconds) are shown for each of the 20 Low-Risk subjects (Figure S1) and 20 High-Risk subjects (Figure S2). Because our main analyses focus on face vs. object responses, and to simplify viewing of individual subject waveforms, for each subject we present a single waveform for faces (*solid red lines*) and objects (*dashed blue lines*), collapsed across familiar and unfamiliar and hemispheres. Within each subject group, the subjects are ordered with respect to number of total trials retained (lowest to highest), which is presented in the upper right hand corner. For the High-Risk subjects, the subject number (presented in the upper left hand corner) corresponds to the numbering in Table 1, which describes the older sibling diagnosis of each High-Risk infant. All subjects are plotted

with the same scale and range, with the exception of Low-Risk subjects 5 and 7 and High-Risk subject 8, for whom the range was shifted 10 μv higher (but their scale is still the same as the other subjects).

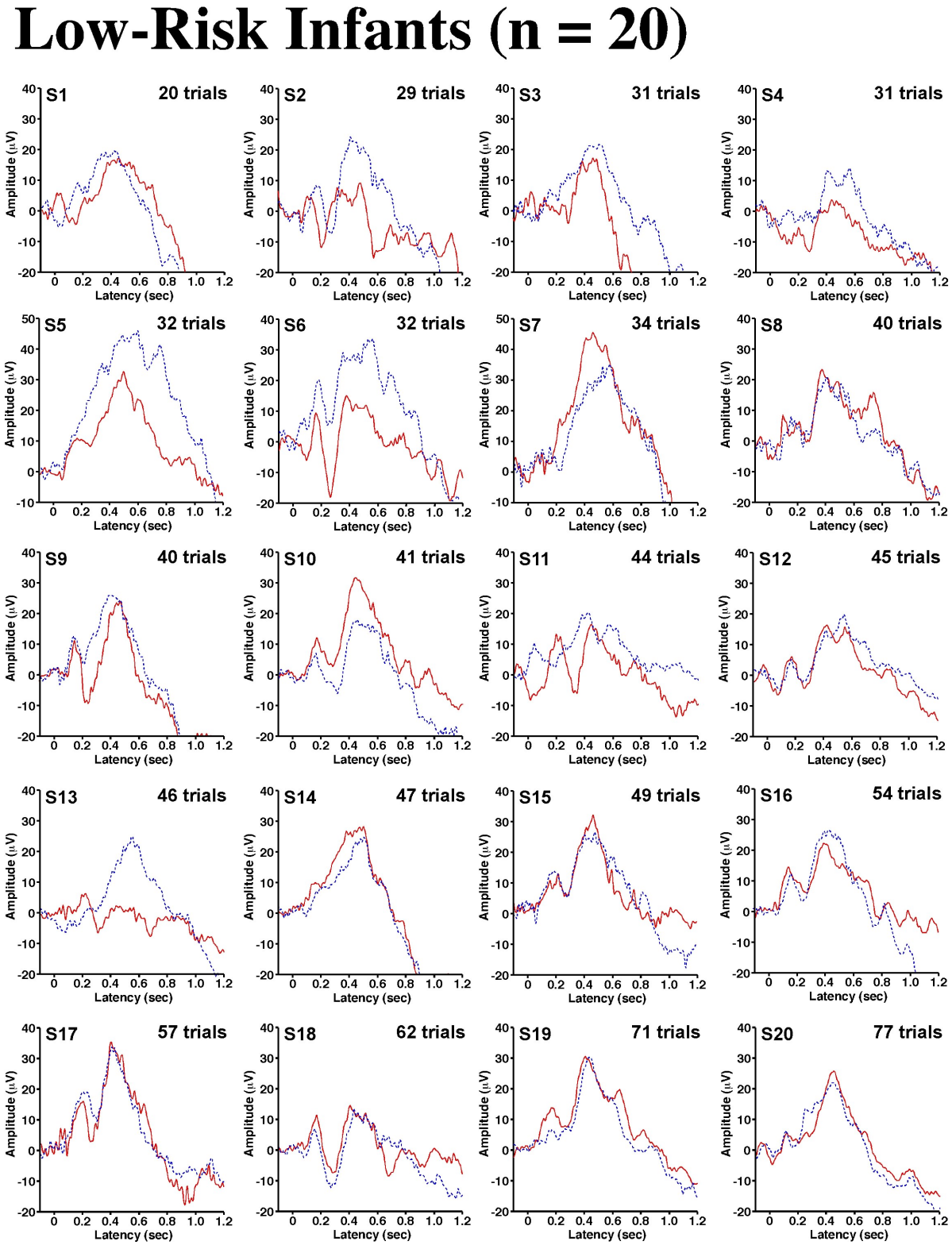

Figure S1. Waveforms (μv by seconds) for each of the 20 Low-Risk subjects

High-Risk Infants (n = 20)

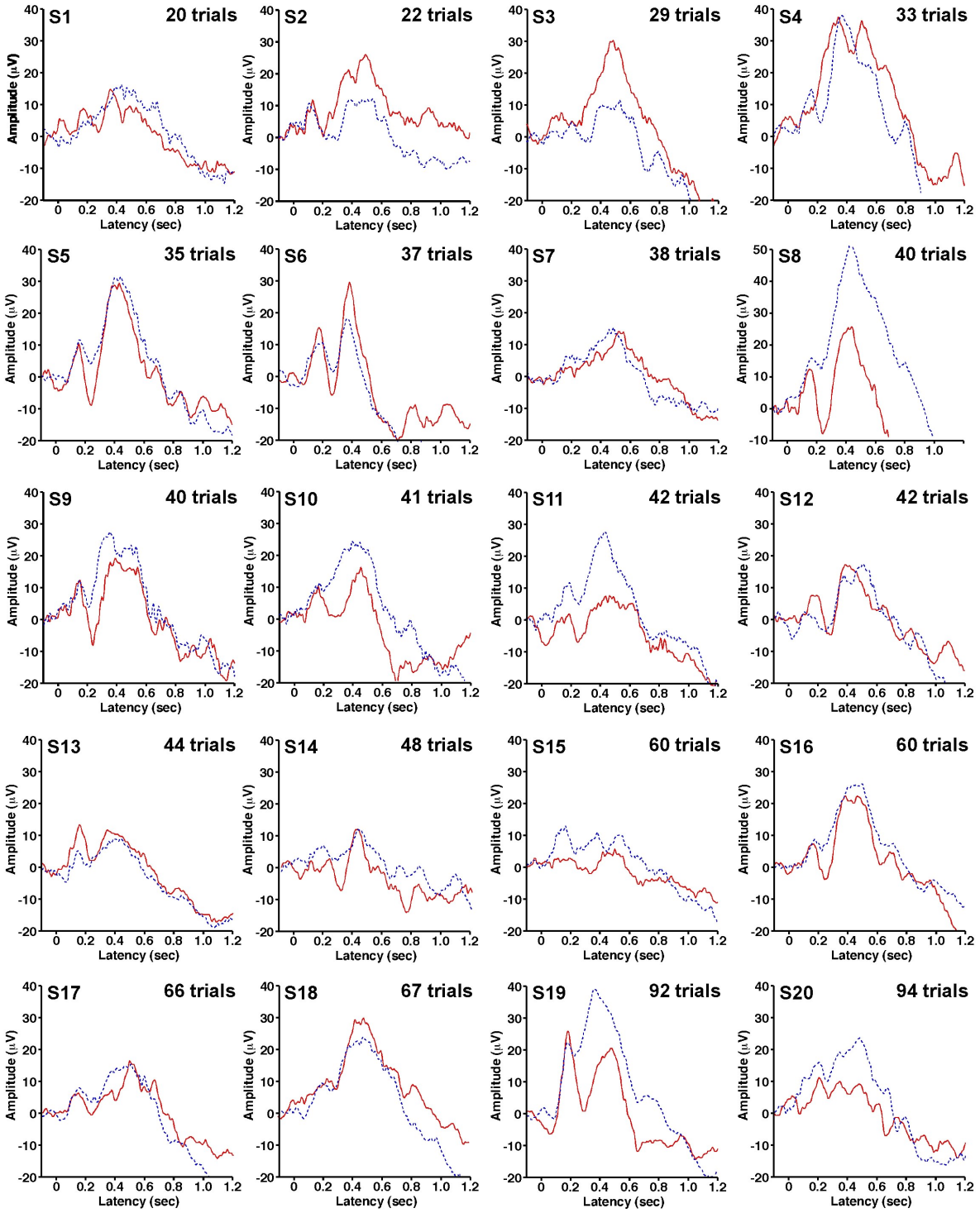

Figure S2. Waveforms (μv by seconds) for each of the 20 High-Risk subjects

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