Contents:

Additional information regarding the gap/overlap paradigm

Infants sat on their caregiver's laps approximately 65 cm away from the visual display. Caregivers were asked to close their eyes, wear sunglasses, or look away from the display during stimuli presentation. The gap/overlap procedure was presented in Clearview/Studio software (Tobii Inc.) and followed a 5 point calibration procedure. All of the stimuli were static images and varied in categorical content between social (i.e., 10 individual faces; 5 adults displaying happy expressions drawn from the NimStim face set (sup ref 1) and 5 faces of infants and toddlers showing happy expressions) and nonsocial (10 images; a pumpkin, ball, flowers, geometric shapes, fruit, toys, etc.) exemplar images. We included individuals if they completed at least 8 trials (4 overlap and 4 gap: minimum of 2 social overlap, 2 nonsocial overlap trials, 2 social gap, and 2 nonsocial gap trials). Note that 14 individuals were excluded for completing an insufficient number of trials. Trials were counterbalanced with no direction (i.e., left or right), condition (i.e., gap or overlap), or central or peripheral stimulus type respectively (i.e.,

social or nonsocial) occurring on more than 3 consecutive trials. For the current study, specific hypotheses pertain only to saccade latencies in response to complex stimuli, therefore, social and nonsocial image categories are collapsed across conditions.

Preliminary analyses revealed no laterality differences so direction was not considered in subsequent analyses. Saccades from the center of the display to the peripheral target (approximately 8-10° of visual angle) were included as valid if they occurred between 100 and 1000 milliseconds after the onset of the peripheral target, the first movement away from the center of the display was in the correct direction, if the differences between leaving the center and landing on the target was less than or equal to 100 milliseconds (minimum average saccade velocity of ~80°/sec), and if the point of regard for at least one eye was on the central image for at least 500 milliseconds prior to the shift in the overlap condition and 750 milliseconds prior to the shift in the gap condition. Custom MATLAB scripts incorporating the logic of these criteria were used to process the raw eye tracking data files. These scripts also flagged potential errors, irregularities, or rare occurrences in the data (e.g., hypo and hypermetric saccades). The first author conducted post-processing quality control, blind to risk and outcome status, by visually examining each raw data file and assuring the validity of each trial. The distribution of each individual participant was examined and outliers that fell within the 100 – 1000 millisecond range but nonetheless were more than two standard deviations from the mean were excluded (sup ref 2). These values always fell toward the upper limit of our range and were recoded as 'late or no disengagement from the center.'

The predetermined minimum latency (i.e., 100 milliseconds) was chosen for two primary reasons: 1) evidence from earlier eye tracking work with infants, in the context of a visual expectation paradigm, suggested that during the first year of life the minimum response time for a stimulus driven saccade approximates 133 milliseconds (sup ref 3). However, evidence from adults suggests that performance in the gap/overlap paradigm yields a bimodal distribution of saccadic reaction times (sup ref 4), and that express saccades (saccades with latencies between ~80 and ~120 milliseconds) predominantly contribute to the first peak in this bimodal distribution. 2) We assessed the distribution of saccadic latencies in the first 20 consecutive LR infants to complete the task, separately for both gap and overlap conditions, in

order to determine whether performance in infants similarly represents a bimodal distribution. These preliminary analyses revealed a positively skewed unimodal distribution of saccadic latencies for both conditions. From 440 valid trials among these 20 participants, 13 trials were classified as anticipatory/predictive saccades and were excluded. Of these 13 trials, 2 fell within the 70 – 100 millisecond range. For more information, please contact the first author.

A 2x2x2 repeated measures ANOVA with latency entered as a within subjects factor (gap and overlap), along with site (University of North Carolina and Children's Hospital of Philadelphia) and eye tracker model (Tobii 1750 and Tobii x120) entered as between subject factors revealed no latency by site, $F(1,94) = 0.007$, $p = 0.931$, or latency by eye tracker model interactions, $F(1,94) = 0.443$, $p = 0.508$. A Shapiro-Wilk test of normality suggested that the distribution of mean latencies in the overlap condition (statistic = 0.976, $p = 0.102$) and mean latencies in the gap condition (statistic = 0.983, $p = 0.295$) were approximately normal in our sample of infants. However, we also performed additional analyses using a natural log transformation on the raw reaction times and the results reported did not change.

Supplemental Analyses Examining Fractional Anisotropy

Fractional anisotropy is a composite index measuring the degree of anisotropy of local water diffusion that incorporates all three eigenvalues (λ_1, λ_2, λ_3) of the tensor model. Fractional anisotropy ranges from 0 (for isotropic diffusion in fluid) to 1 (for strongly directional diffusivity in highly structured axonal bundles).

First, we conducted a hierarchical multiple regression that included fractional anisotropy in the splenium as the dependent variable, age as the step 1 predictor variable, gap latency as the step 2 predictor variable, and overlap latency as the step 3 predictor variable in the low-risk, typically developing infants [n = 34, average age in weeks (standard deviation) = 31.0(3.5)]. Average latency in the overlap condition accounted for a significant portion of variance in fractional anisotropy in the splenium beyond the contribution of age and gap latency ($\Delta R^2 = 0.130$, $p = 0.042$). Average latency in the gap condition did not account for a significant portion of variance in fractional anisotropy in the splenium ($\Delta R^2 = 0.001$, $p = 0.858$).

Second, we conducted a hierarchical multiple regression that included fractional anisotropy in the left corticospinal tract as the dependent variable, age as the step 1 predictor variable, overlap latency as the step 2 predictor variable, and gap latency as the step 3 predictor variable in the low-risk, typically developing infants. Gap latencies did not account for a significant portion of variance in fractional anisotropy in the left corticospinal tract ($\Delta R^2 = 0.097$, $p = 0.076$), nor did overlap latencies account for significant portion of variance in fractional anisotropy in the left corticospinal tract ($\Delta R^2 = 0.013$, $p = 0.523$).

Additionally, we repeated this same analysis with the right corticospinal tract. Gap latencies did not account for a significant portion of variance in fractional anisotropy in the right corticospinal tract ($\Delta R^2 = 0.079$, $p = 0.110$), nor did overlap latencies account for significant portion of variance in fractional anisotropy in the right corticospinal tract ($\Delta R^2 = 0.027$, $p = 0.357$).

Third, we examined the association between latencies and fractional anisotropy in our a priori selected control tract, the genu of the corpus callosum. Neither gap latencies ($\Delta R^2 = 0.048$, $p = 0.215$) nor overlap latencies ($\Delta R^2 = 0.053$, $p = 0.191$) accounted for a significant portion of variance in fractional anisotropy in the genu.

Considering that overlap latencies accounted for a significant portion of variance in the fractional anisotropy of the splenium, above and beyond the effect of age and gap latency, we further examined whether this association would be moderated by group status. We employed a General Linear Model that included overlap latencies as the dependent variable and group, fractional anisotropy in the splenium, and a group X splenium interaction term as independent variables. The overall model did not reach statistical significance $F(5,78) = 1.86$, $p = 0.111$, nor was there a statistically significant interaction $F(2, 78) = 2.47$, $p = 0.091$, $\eta_p^2 = 0.057$.

Supplemental Analyses Examining Axial Diffusivity

Axial diffusivity values represent the rate (mm^2/sec) of water diffusion parallel to the fiber bundle or the primary eigenvalue (λ_1).

First, we conducted a hierarchical multiple regression that included axial diffusivity in the splenium as the dependent variable, age as the step 1 predictor variable, gap latency as the step 2 predictor variable, and overlap latency as the step 3 predictor variable in the low-risk, typically developing infants [n = 34, average age in weeks (standard deviation) = 31.0(3.5)]. Average latency in the overlap condition accounted for a significant portion of variance in axial diffusivity in the splenium beyond the contribution of age and gap latency ($\Delta R^2 = 0.208$, $p = 0.008$). Average latency in the gap condition did not account for a significant portion of variance in axial diffusivity in the splenium ($\Delta R^2 < 0.001$, $p = 0.973$).

Second, we conducted a hierarchical multiple regression that included axial diffusivity in the left corticospinal tract as the dependent variable, age as the step 1 predictor variable, overlap latency as the step 2 predictor variable, and gap latency as the step 3 predictor variable in the low-risk, typically developing infants. Gap latencies accounted for a significant portion of variance in axial diffusivity in the left corticospinal tract ($\Delta R^2 = 0.252$, $p = 0.002$), and overlap latencies did not account for a significant portion of variance in axial diffusivity in the left corticospinal tract ($\Delta R^2 = 0.002$, $p = 0.789$).

Additionally, we repeated this same analysis with the right corticospinal tract. Gap latencies did not account for a significant portion of variance in axial diffusivity in the right corticospinal tract ($\Delta R^2 = 0.023$, $p = 0.379$), nor did overlap latencies account for significant portion of variance in axial diffusivity in the right corticospinal tract ($\Delta R^2 = 0.009$, $p = 0.576$).

Third, we examined the association between latencies and axial diffusivity in our a priori selected control tract, the genu of the corpus callosum. Neither gap latencies ($\Delta R^2 = 0.027$, $p = 0.334$) nor overlap latencies ($\Delta R^2 < 0.001$, $p = 0.910$) accounted for a significant portion of variance in axial diffusivity in the genu.

Considering that overlap latencies accounted for a significant portion of variance in axial diffusivity within the splenium, above and beyond the effect of age and gap latency, we further examined whether this association would be moderated by group/risk status. We employed a General Linear Model that included overlap latencies as the dependent variable and group, axial diffusivity in the splenium, and a group X splenium interaction term as independent variables. The overall model was statistically

significant $F(5,78) = 2.81$, $p = 0.022$. Additionally, the results revealed a main effect of group, $F(2, 78) = 3.43$, $p = 0.037$, $\eta_p^2 = 0.075$, as well as a significant group X splenium interaction, $F(2, 78) = 3.69$, $p = 0.029$, $\eta_p^2 = 0.080$. Average axial diffusivity in the splenium did not differ between groups ($p = 0.844$). The simple slope for the high-risk-ASD group significantly differed from the low-risk group ($t = -2.71$, $p = 0.008$) and the high-risk-negative group ($t = -2.29$, $p = 0.025$). The simple slopes were statistically equivalent among the high-risk-negative group and the low-risk group ($t = -0.71$, $p = 0.480$). See Figure S4 for graphical visualization.

Supplemental References

1. Tottenham N, Tanaka JW, Leon AC, McCarry T, Nurse M, Hare TA, Marcus DJ, Westerlund A, Casey BJ, Nelson C: The NimStim set of facial expressions: judgments from untrained research participants. Psychiatry Res 2009; 168(3): 242-249.

2. Ratcliff R: Methods for dealing with reaction time outliers. Psych Bull 1993; 114(3): 510-532.

3. Canfield RL, Smith EG, Brezsnyak, Snow KL: Information processing through the first year of life: a longitudinal study using the visual expectation paradigm. Monogr Soc Res Child Dev 1997; 62(2): 1-145.

4. Fischer B, Breitmeyer B: Mechanisms of visual attention revealed by saccadic eye movements. Neruopsychologia 1987; 25: 73-83.

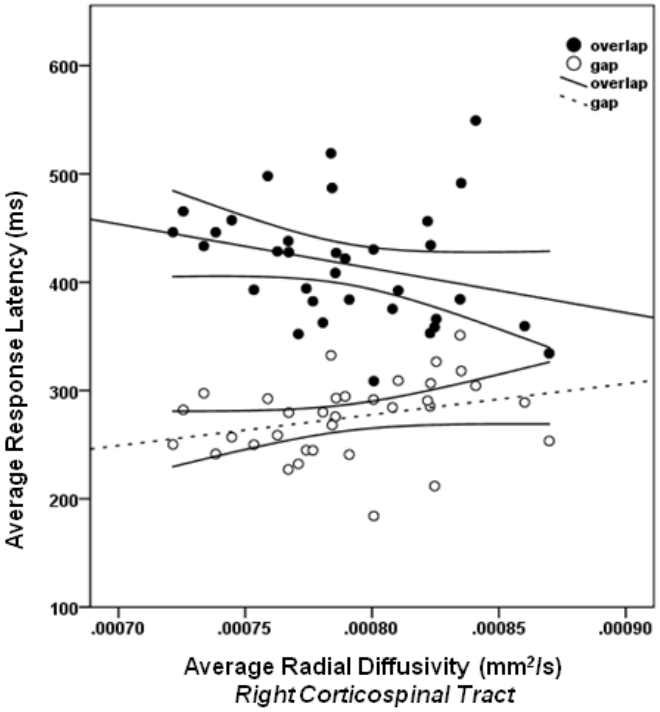
FIGURE S1. The right corticospinal tract and gap latencies among low-risk infants

Multiple regression analysis for right corticospinal tract + gap

Model	*R*	R^2	R^2change	*F*change	Sig. *F* change
1	0.306[a]	0.094	0.094	1.599	0.218
2	0.538[b]	0.289	**0.196**	8.262	**0.007**

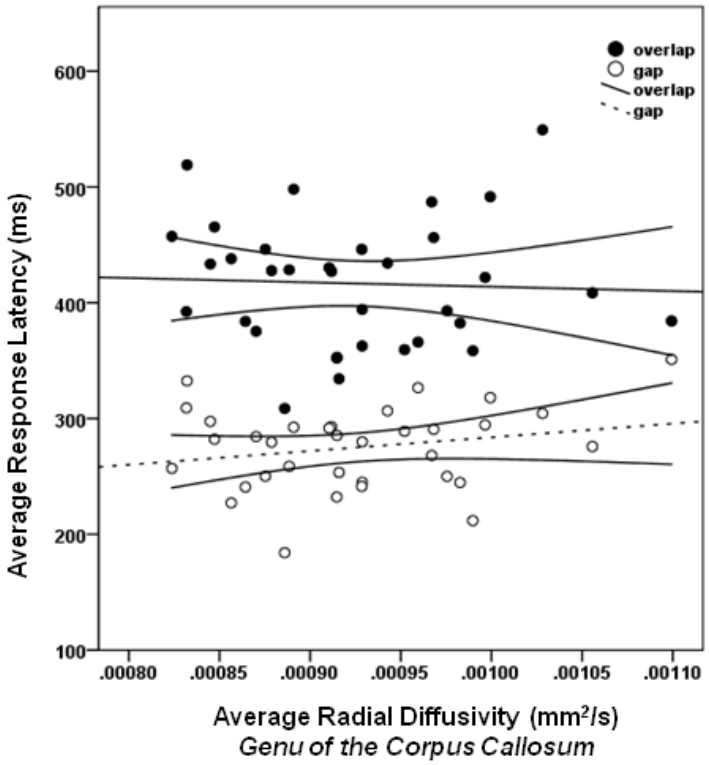
[a] *predictors: (constant), age, overlap latency*

[b] *predictors: (constant), age, overlap latency, gap latency*



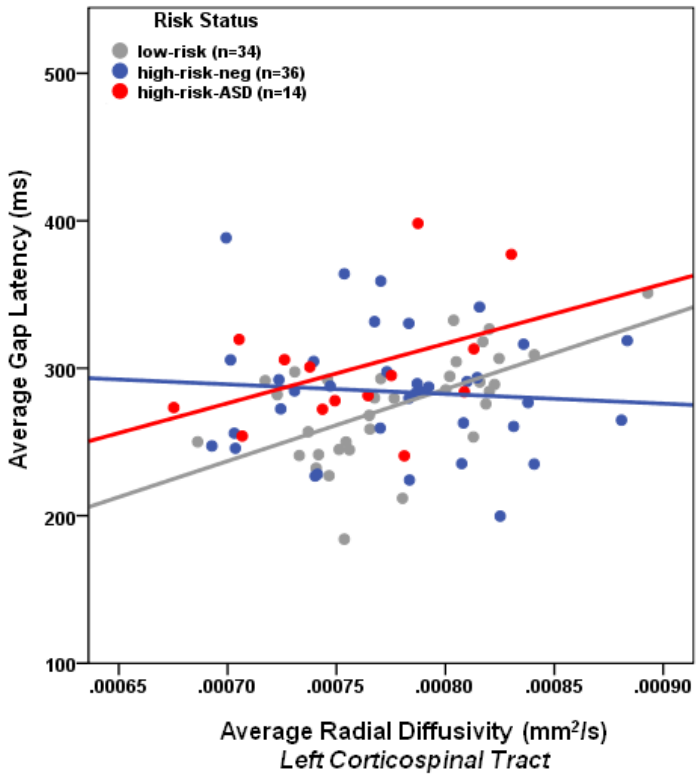
Results from a hierarchical multiple regression in which gap latency accounts for a significant portion of variance in radial diffusivity in the right corticospinal tract ($\Delta R^2 = 0.196$, $p = 0.007$), above and beyond the effect of age and overlap latency. Regression lines within the scatterplots represent zero-order correlations between radial diffusivity in the right corticospinal tract and overlap latencies (full black circles; $r = 0.279$, $p = 0.110$) and gap latencies (open black circles; $r = 0.300$, $p = 0.085$).

FIGURE S2. Association between the genu and latencies in the gap overlap paradigm



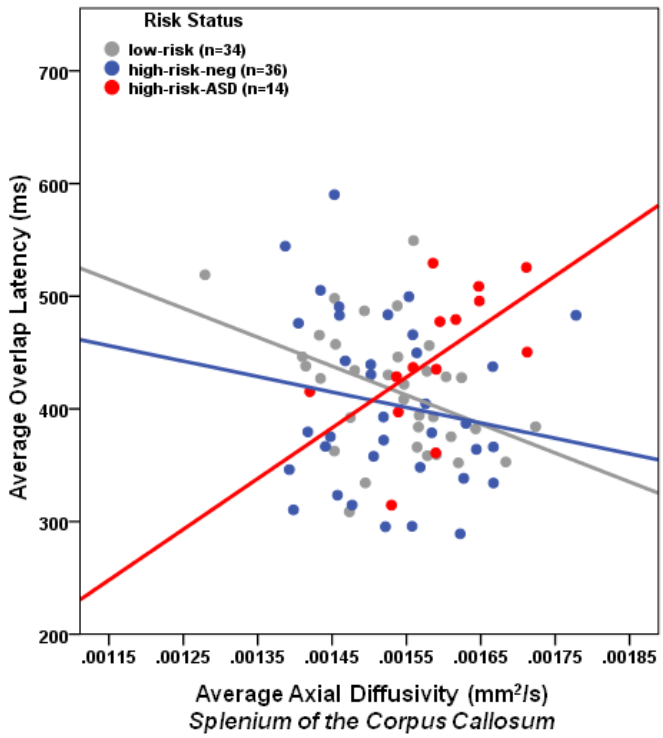
As clear from this representation, there was no association between radial diffusivity in the genu of the corpus callosum and gap latencies (r = -0.045, p = 0.799) or overlap latencies (r = 0.220, p = 0.211) among the low-risk infants. The genu has been associated with voluntary, goal-directed attention in adult samples (Niogi et al., 2010, see ref #9 in main text).

FIGURE S3. Functional coupling between gap latencies and radial diffusivity in the left corticospinal tract



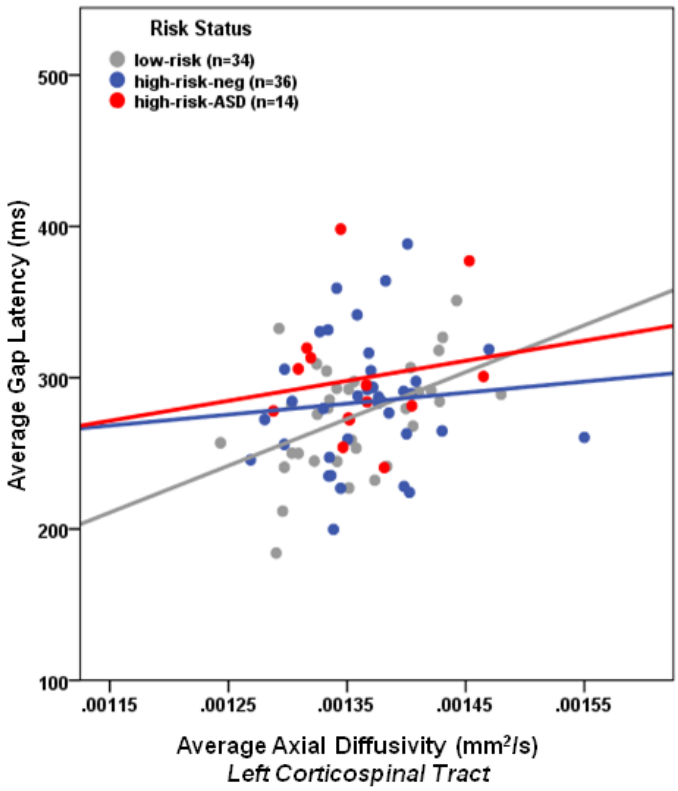
Group status significantly moderates the association between radial diffusivity in the left corticospinal tract and gap latencies. The overall model was significant, $F(5,78) = 3.66$, $p = 0.005$. The results revealed main effects of group ($F(2, 78) = 4.51$, $p = 0.014$, $\eta_p^2 = 0.094$) and radial diffusivity in the left corticospinal tract ($F(1, 78) = 4.52$, $p = 0.008$, $\eta_p^2 = 0.078$) on gap latency. Additionally, there was a significant group X left corticospinal tract interaction, $F(2, 78) = 4.46$, $p = 0.014$, $\eta_p^2 = 0.093$. The simple slopes did not significantly differ between the low-risk and the high-risk-ASD groups ($t = 0.30$, $p = 0.762$). The simple slope for the high-risk-negative group was significantly different from the low-risk group ($t = 2.83$, $p = 0.006$) and showed a trend toward a significant difference in comparison to the high-risk-ASD group ($t = -1.80$, $p = 0.076$).

FIGURE S4. Functional coupling between overlap latencies and axial diffusivity in the splenium



As detailed in the text above, group status significantly moderates the association between axial diffusivity in the splenium and average overlap latency. Of note, the regression lines for the high-risk-ASD group and the low-risk group are significantly different when we examine both radial diffusivity and axial diffusivity. However, the high-risk-negative group shows a slightly different pattern of brain-behavior associations depending on whether radial diffusivity or axial diffusivity is examined.

FIGURE S5. Functional coupling between gap latencies and axial diffusivity in the left corticopsinal tract



Group status does not significantly moderate the association between axial diffusivity in the left corticospinal tract and gap latencies. The overall model approached statistical significance, $F(5,78) = 2.14$, $p = 0.069$. Group status did not exert a unique effect on gap latencies $F(2, 78) = 0.98$, $p = 0.381$. There was a trend toward a significant main effect of axial diffusivity in the left corticospinal tract on gap latencies $F(1, 78) = 3.48$, $p = 0.066$. Finally, the group X left corticospinal tract (axial diffusivity) interaction was not statistically significant, $F(2, 78) = 0.92$, $p = 0.403$.