NEW RESEARCH

Amygdala Functional Connectivity and Negative Reactive Temperament at Age 4 Months

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Objective: Infant amygdala connectivity correlates with maternal reports of infant temperament characterized by novelty-evoked distress and avoidance. However, no studies have examined how human infant amygdala connectivity relates to direct observations of novelty-evoked distress. This study examined the link between amygdala connectivity and infant novelty-evoked distress using direct observation of temperament.

Method: Novelty-evoked distress was assessed at 4 months of age (N = 90) using a standardized reactivity assessment and parent report. Within 3 weeks of assessment, resting-state functional magnetic resonance imaging was collected in a subset of infants (n = 34). Using a whole-brain voxelwise approach, amygdala connectivity associated with positive and negative affect during the reactivity assessment was examined. Regions where the association of amygdala connectivity with negative affect was higher than with positive affect were then examined. Associations between amygdala connectivity and parent report of temperament were also examined.

Results: Greater amygdala-cingulate and amygdala–superior frontal gyrus connectivity was associated with lower positive affect during the reactivity assessment. Further, the association between amygdala-cingulate connectivity was greater for negative affect compared with positive affect. There were no significant associations between latency to approach novelty (as measured by parent report) and amygdala connectivity. Validation analyses conducted using a large independent longitudinal sample (N = 323) demonstrated that negative reactivity was associated with increased child-reported anxiety symptoms in adolescence.

Conclusion: These results provide novel insight into the developmental pathophysiology of novelty-evoked distress. This is consistent with research linking an altered cognitive control mechanism to temperamental risk for anxiety.

Key words: amygdala, functional connectivity, infant MRI, negative reactivity, temperament

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Infants who exhibit distress to novelty face elevated risk for later-life anxiety.[1,2] Several findings implicate perturbed amygdala connectivity in this association.[3–8] However, no studies examine how amygdala connectivity relates to novelty-evoked distress in human infants assessed with direct observation methods. While parent report is valuable, these ratings of infant behavior can be biased by the parent's personality, and they only modestly correlate with direct observation of temperament.[9–12] Thus, research is needed in infants combining behavioral assessments of temperament with measures of amygdala connectivity. The current study provides such data.

Responses of infants to novelty vary from distressed (high negative affect, low positive affect) to happy/excited (high positive affect, low negative affect).[13] Individual differences in distress to novelty at 4 months of age (ie, negative reactivity) predict later behavioral inhibition (BI).[13–16] BI, in turn, predicts elevated risk for anxiety.[1,17–20] Thus, high distress/negative affect in response to novelty in infancy may represent an important risk phenotype.[20] Broadly, amygdala functional connectivity is linked to emotion dysregulation.[21] In children with BI, several lines of work link risk for anxiety to patterns of connectivity among brain regions associated with threat detection (eg, amygdala) and cognitive control (eg, prefrontal cortex).[3–8] However, the emergence of such connectivity patterns and their association with behavior remains unknown. In part, this is because most imaging research on BI measures brain function in middle childhood and adulthood[3,7]—in many cases nearly 10 years after temperaments first emerge. Uncovering correlates of BI requires concurrent measurement of novelty-evoked distress and amygdala connectivity in infants before BI emerges.

Recent studies have measured brain function and temperament in infancy.[5,6,8,22] However, these studies

possess 2 key limitations. First, they rely solely on maternal report of infant temperament.[5,6,8,22] While this is valuable, behavioral assessments of distress to novelty robustly predict anxiety in large longitudinal studies.[2,23] Direct observation of behavior also is not biased by influences of the parent's personality or psychopathology. Thus, the current study used direct observation of temperament. Second, most infant imaging studies examining associations with infant temperament have acquired functional magnetic resonance imaging (fMRI) data within the first weeks of life. At present, no studies have demonstrated that connectivity patterns found in neonates persist beyond this period to ages when temperament is quantified using observational measures. This study examined amygdala functional connectivity in 4-month-old infants. Concurrent assessments of temperament and brain function at 4 months can provide novel insight into whether brain connectivity patterns evaluated in newborns are present when temperament can be reliably assessed.

To address these gaps, the current study examined amygdala connectivity and reactivity to novelty in 4-month-old infants. Infant temperament was assessed via both behavioral assessment and parental report—allowing us to directly compare amygdala connectivity associated with these 2 measures. Within 2 months of these temperament assessments, fMRI was performed during natural sleep. We tested the hypothesis that amygdala–prefrontal cortex connectivity is associated with high negative affect and/or low positive affect. We additionally performed exploratory analyses to examine amygdala connectivity linked to parental report of temperament. Lastly, we used a large longitudinal validation sample to provide data on the clinical significance of negative reactive temperament.

METHOD

Participants

Between July 2018 and March 2020, 90 healthy full-term 4-month-old infants (mean age: 4 months 5 days; range: 3 months 6 days to 4 months 28 days; 40 female) were recruited for a longitudinal study examining the neural correlates of early social behavior. Infants were recruited via community outreach events, moms' groups, mailings, and social media advertisements. Infants were excluded for the following reasons: premature (gestation length <36 weeks), low birth weight (<2,500 g), severe birth complications, known developmental disabilities, history of neurological problems, metallic implants, or known uncorrected visual or auditory impairments. Parents were informed before the first visit that this study used neuroimaging and that they could elect to participate in a follow-up MRI visit, a follow-up EEG visit, or both. Before participation in each visit parents provided informed consent. Families were compensated (reactivity: $30 and a toy; MRI: $60, t-shirt, and souvenir scan) at each visit.

The reactivity assessment was completed in all 90 infants with parent report measures of temperament provided. Table 1 presents demographics of the sample. Of these 90 infants, 59 (mean age: 4 months 24 days; range: 3 months 23 days to 5 months 26 days) participated in the follow-up MRI visit. Whether a parent elected to participate in the MRI follow-up was not significantly associated with age, maternal anxiety (assessed via the Beck Anxiety Inventory[24]), or infant reactivity (motor, positive or negative) and did not differ by infant sex, maternal education, or race/ethnicity ($ps > .172$) (see Table S1, available online, for detailed breakdown). Of the 59 infants, 11 (19%) failed to sleep during the MRI visit, generating no imaging data, thus yielding data in 48 (81%) infants. Of these 48 infants, 14 (29%) were excluded from resting-state analyses because they awoke during scanning, terminating the resting-state run. The successful acquisition of a resting-state run was not related to age, sex, race/ethnicity, maternal anxiety, any aspect of infant reactivity, or visit duration (mean visit duration: 3 hours 49 minutes; $ps > .178$). There were 34 infants with temperament and resting-state data in the final sample.

Reactivity Assessment

A standardized assessment of infant reactivity[16,25,26] was conducted at 4 months of age (mean [SD] age, 4 months 5 days [17 days]). Infants sat in an infant seat while experimenters (out of sight) presented novel toys (ie, visual blocks) and sounds (ie, auditory blocks) to the child. Stimuli were presented in 4 blocks (2 visual and 2 auditory) of fixed order with visual and auditory blocks alternating. Each visual block was presented as hanging mobiles. Mobiles comprised 1, 3, or 6 animals. All mobiles were presented for 20 seconds each, separated by a 10-second interval for a total of 9 trials per block. Each auditory block included sentences or syllables. The sentence block presented 8 sentences. Every 2 sentences, the number of voices speaking and consequently the volume of the presentation increased by 1 (eg, first 2 sentences were spoken by 1 voice, second 2 sentences were spoken by 2 voices, etc). The duration of each sentence was approximately 6 seconds followed by a 2-second interval between sentences. Syllables (ma, pa, ga) were presented in 3 consecutive 10-second trials, with a 5-second interval between syllable repetitions. Speakers were 6 feet from the infant seat, and the computer volume was standardized across all visits. During the presentation of the stimuli, if the infant cried continuously for more

TABLE 1 Sample Demographics and Data Collected

Characteristic	n (% of sample)	Mean (SD)
Total sample	90	—
Sex, female	40 (44.4)	—
Child race/ethnicity		
White/non-Hispanic	60 (66.7)	—
African American/non-Hispanic	2 (2.2)	—
Asian/non-Hispanic	4 (4.4)	—
Multiracial/non-Hispanic	17 (19.0)	—
Other/non-Hispanic	—	—
White/Hispanic	1 (1.1)	—
African American/Hispanic	1 (1.1)	—
Asian/Hispanic	—	—
Multiracial/Hispanic	4 (4.4)	—
Other/Hispanic	1 (1.1)	—
Maternal education		
High school graduate	1 (1.1)	—
GED	2 (2.2)	—
Some college	7 (7.8)	—
2-year professional degree	2 (2.2)	—
4-year college degree	22 (24.5)	—
Advanced degree	56 (62.2)	—
Maternal anxiety[a]		29.0 (5.62)
High	11 (12.2)	—
Moderate	68 (75.6)	—
Low	4 (4.4)	—
No data	7 (7.8)	—
Data collected		
Reactivity assessment	90 (100)	—
Parental report of temperament	90 (100)	—
MRI assessment	59 (65.6)	—
No MRI data obtained (failure to sleep)	11 (18.6)	—
Woke up before resting state	7 (11.9)	—
Resting-state run incomplete	7 (11.9)	—
Resting-state run complete	34 (57.6)	—

Note: *MRI = magnetic resonance imaging.*
[a]*Assessed via Beck Anxiety Inventory.*

than 10 seconds, the experimenter paused the session to calm the infant, resuming the session once the infant was calm.

The entire assessment was video recorded for later coding of infant motor and emotional behavior. Motor responses (leg kicks, arm waves, back arching, hyperextensions), positive affect (smiling, laughing), and negative affect (fussing, crying) during the presentation of the stimuli were coded on a 7-point Likert scale (1 = low motor or affect; 7 = high motor or affect) for each block. A second coder double-coded all blocks for all subjects. Reliability ranged from 0.65 to 0.89 (mean motor = 0.75; mean negative = 0.86; mean positive = 0.76) indicating good agreement between coders. All analyses were conducted on the primary coders' scores. Summary scores for motor, negative affect, and positive affect were computed by averaging the respective scores across the 4 blocks. Table 2 provides the means and standard deviations for all temperament measures and illustrates the correlations between all temperament measures. See Supplement 1, available online, for data that make use of support vector machine (SVM) learning to integrate all 3 dimensions of reactivity into a single continuous factor score. Supplement 1 and Figure S1, available online, provide details on how the SVM factor score was computed.

Infant Behavior Questionnaire

During the reactivity assessment, parents (out of sight) filled out questionnaires including the Infant Behavior Questionnaire (IBQ),[27] which evaluates infant temperament. Of note, 2 parents failed to complete all items for the soothability subscale; thus any analyses using the soothability subscale included 88 infants. See Table 2 for the association between all temperament measurements.

MRI Acquisition

MRI assessments of infants were conducted during natural sleep and within 8 weeks of the initial reactivity assessment (mean time between visits: 19.17 days; range: 1–50 days; mean [SD] age: 4 months 24 days [18 days]). Visits were conducted in the evening. On arrival, infants were measured, changed, and swaddled using an MRI-safe Velcro swaddle. Parents were then given privacy to feed their infant and begin their bedtime routine. Once the infant was asleep for 20 minutes, hearing protection (ie, silicone earplugs and MiniMuff Neonatal Noise Attenuators [Natus Medical Inc., Pleasanton, California]) was applied to the infant. The infant was then transferred to the scanner bed where several foam pads were placed around the infant's head to minimize motion, and sandbags were placed on each side of the infant's body. The infant slept on the scanner bed for an additional 15 minutes before acquisition was initiated. A trained research assistant remained in the scan room to monitor the infant and alert the MRI operator as needed. If the infant woke up, scanning was paused until the infant was soundly asleep again. This process of soothing and scanning was repeated until either all data were collected or the parents decided to end the session. On average, MRI visit duration was 3.5 hours.

MRI was acquired on a 3T MAGNETOM Trio scanner (Siemens Medical Solutions USA, Inc., Malvern, Pennsylvania) with a 32-channel head coil. Scanning began with the acquisition of structural images that were used for registration. T1-weighted scans used magnetization prepared rapid acquisition gradient-echo with repetition time/echo time = 1,900 ms/2.43 ms, flip angle = 9°, and voxel size = 0.8 mm isotropic. T2-weighted scans used magnetization prepared rapid acquisition gradient-echo with repetition time/echo time = 3,200 ms/488 ms, flip angle = 9°, and voxel size = 0.7 × 0.8 × 0.8 mm. Resting-state data were acquired using multiband radiofrequency pulses (multiband acceleration factor = 6) to excite several slices in a single repetition time to acquire high-resolution data faster. The 10-minute resting-state runs were obtained using a gradient echo, echo-planar image sequence sensitized to T2* blood oxygen level–dependent contrast using the following parameters: repetition time/echo time = 1,250 ms/39.4 ms, flip angle = 90°, voxel size = 2 mm isotropic. Framewise Integrated Real-time MRI Monitoring (FIRMM)[28] motion tracking software was used for real-time motion tracking and to determine if additional 10-minute runs were necessary to obtain at least 10 minutes of data in which all frames had <0.2-mm framewise displacement (FD). Data were acquired until at least 10 minutes of low movement data were obtained or the infant woke up. All 34 infants had at least one 10-minute run of data acquired (7 infants slept long enough to acquire two 10-minute runs).

Resting-State fMRI Processing

Resting-state data were processed using AFNI and the CONN toolbox v.18b. CONN is MATLAB/SPM-based software for the analysis of resting-state functional connectivity data. CONN makes use of MATLAB v.2017b (The MathWorks, Inc., Natick, Massachusetts) and SPM8 (Wellcome Centre for Human Neuroimaging, London, United Kingdom; SPM8 allowed for infant-specific adaptations to CONN default pipeline). Preprocessing of functional data began with slice timing correction (conducted using AFNI 3dTshift function). Subsequent preprocessing steps were conducted using the CONN toolbox[29,30] and included spatial realignment (compensating for head motion) and coregistration to T1-weighted images. Structural images were then normalized to an age-specific template (4.5-month template from the MRI Study of Normal Brain Development[31]) and MNI space, and tissue segmentation was conducted (classifying gray matter, white matter, and cerebrospinal fluid). Subsequently, confounding effects were identified using an anatomical component-based noise correction procedure (aCompCor[32]) rather than global signal regression. Analyses incorporating global signal regression are presented in Supplement 1, available online. Artifact Detection Tools (ART; https://www.nitrc.org/projects/artifact_detect/) was used for outlier detection. Frames with FD >0.25 mm and global blood oxygen level–dependent signal z-scores >5 were censored via ART.[33] For connectivity processing, data were centered and detrended (ignoring censored frames), and nuisance variables (estimates of head motion, white matter, cerebrospinal fluid, and frames marked as outliers) were regressed out, and data

TABLE 2 Descriptive Statistics (N = 90) for All Temperament Measures Including Correlations Among All Temperament Measures and Maternal Anxiety

	Mean	SD	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
Reactivity assessment											
(1) Motor reactivity	3.97	1.19									
(2) Negative affect	2.37	1.56	.325**								
(3) Positive affect	1.78	0.95	.254*	−.274*							
IBQ											
(4) Activity level	3.89	0.78	.068	.055	.048						
(5) Distress to limitations	3.19	0.68	−.112	.182	−.142	.211*					
(6) Latency to approach novelty	2.14	0.46	−.084	.281**	−.239*	.148	.395**				
(7) Duration orienting	3.76	1.07	−.091	.013	.094	.264*	−.112	−.010			
(8) Smiling	4.15	0.87	.039	.106	−.041	.319**	−.008	−.134	.356**		
(9) Soothability	4.54	0.80	−.177	.139	−.126	.193	−.104	.148	.366**	.256*	
BAI											
(10) Maternal anxiety	28.96	5.62	.200	.142	.045	.016	−.030	.147	−.072	−.127	.193

Note: *BAI = Beck Anxiety Inventory; IBQ = Infant Behavior Questionnaire.*
*p < .05, **p < .01.

were band-pass filtered (0.009–0.08 Hz) and spatially smoothed using a Gaussian kernel (6 mm). In line with prior infant imaging studies,[5] a minimum of 4 minutes of fMRI data was required, excluding censored frames. After the removal of censored frames, the median amount of data retained was 9 minutes 22 seconds (range: 4 minutes 53 seconds to 18 minutes 45 seconds; see Figure S2, available online, for histogram). Across all subjects, mean FD and percent censored frames were 0.195 mm and 15%, respectively. This criterion did not result in the exclusion of any subjects—all 34 infants had data that survived this stringent volume-censoring threshold. See Supplement 1 and Figures S3 and S4, available online, for scatterplots illustrating no significant association between FD and amygdala connectivity and distance-dependent biases in the FD–functional connectivity correlations.

Amygdala Functional Connectivity

Amygdala functional connectivity was assessed using a seed-based, whole-brain voxelwise approach, using the average time course of left and right amygdala as the seed (see Supplement 1, available online, for analyses from the left and right amygdala separately). The Pearson correlation coefficient of each voxel with the seed time course was calculated and converted to a z-score using Fisher's r-to-z transformation. Analyses examined regions where amygdala functional connectivity is associated with negative affect and positive affect. Additionally, we examined regions where amygdala functional connectivity has a stronger positive association with negative affect compared with positive affect (negative affect > positive affect). To test these effects, we used a general linear model with functional connectivity values as the dependent variable; both negative affect and positive affect as predictors of interest; and motor, age at MRI assessment, and sex as nuisance variables. Age and sex were included as covariates because of the wide age range and associations between temperament and sex that have been reported in the literature.[34,35] Results were corrected voxelwise at $p < .001$ and corrected for multiple testing to provide familywise error control for clusters ($p < .05$; cluster size = 155 voxels). Additional details on the methods used to define the cluster size criterion are provided in Supplement 1, available online.

Next, we examined connectivity associated with parent-reported fearful temperament. To do so, we first identified a parent report measure that mapped onto observed measures of infant reactivity to provide a parallel to studies that use parent report measures. We began by conducting an exploratory analysis to determine which subscales of the IBQ were positively associated with negative affect and negatively associated with positive affect (Table 2). The latency to approach novelty subscale emerged as the only parent report measure linked to high negative affect and low positive affect (see also Validation Analyses). Next, we examined amygdala connectivity associated with parent report of latency to approach novelty, controlling for age and sex of the child. Supplement 1, available online, contains additional exploratory analyses examining connectivity associated with the other subscales of the IBQ (i.e., activity level, distress to limitations, duration orienting, smiling, and soothability).

Validation Analyses

To facilitate greater generalization of our results, we conducted 2 validation analyses using a large independent sample. First, we validated the link between negative (versus positive) reactive temperament and parent-reported temperament. Second, we validated the claim that behavioral assessments of reactivity provide robust links to later psychopathology by examining differences in child and parent report of anxiety as a function of negative versus positive reactive temperament (assessed via direct observation). We also examined the association between parent report of infant fearful temperament (ie, IBQ latency to approach novelty) and later child and parent report of anxiety. Both validation analyses were conducted using data from negative and positive reactive infants (N = 323) from 2 large longitudinal cohorts (cohort 1: n = 101; cohort 2: n = 222). Infants were screened for negative and positive reactive temperament at 4 months of age using a standardized temperamental reactivity assessment (see Fox *et al.*[14] for details). Infants were classified as negative reactive if their negative affect and motor scores exceeded the group median. Infants were classified as positive reactive if their positive affect and motor scores exceeded the median. Infants who met criteria for both groups were placed into the group corresponding to the affect dimension on which they scored highest.[14,25,26] Children were then longitudinally followed into adolescence (cohort 1 and cohort 2) and adulthood (at the present time, only the cohort 1 sample has aged into this assessment time point).

For the first validation analysis, examining the link between reactivity and parent report of infant temperament, we combined reactivity and IBQ data from the 2 cohorts. The initial IBQ assessment in cohort 1 was conducted at 9 months, whereas IBQ data were collected in cohort 2 beginning at 4 months. For the purposes of these analyses, we combined parent report across these ages (see Supplement 1, available online, for analyses controlling for cohort). A total of 288 parents of negative and positive reactive infants completed the IBQ (cohort 1: n = 78 [77%]; cohort 2: n = 210 [95%]). Whether a parent did or did provide IBQ data was not associated with reactivity ($p > .521$).

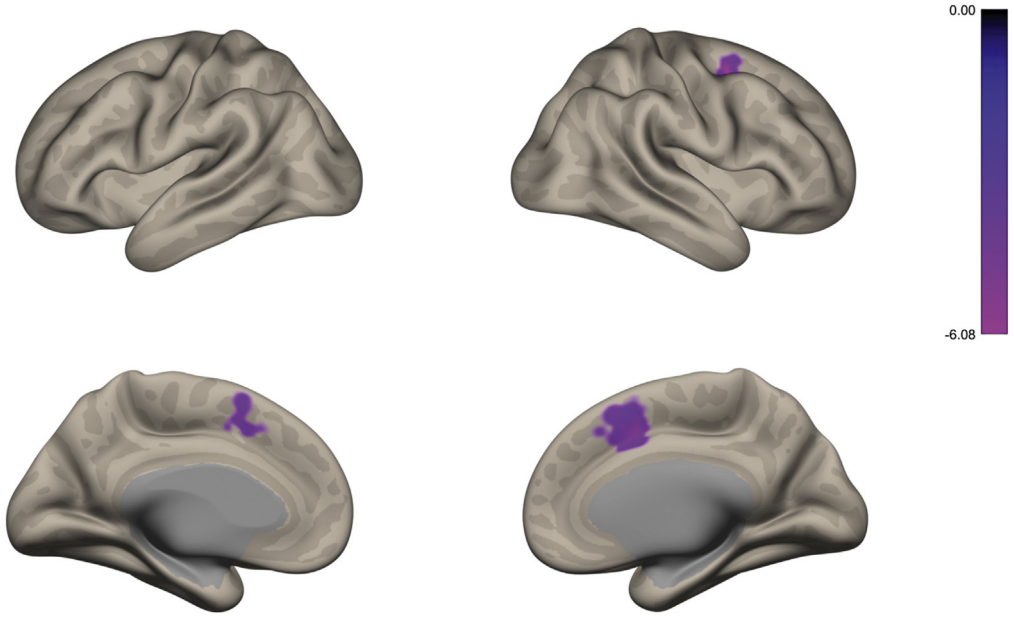
The second validation analysis examined the longitudinal association between infant temperament (assessed both behaviorally and via parent report) and later anxiety. First, we examined whether negative and positive reactive infants (classified via direct observation) differed in terms of child- or parent-reported anxiety symptoms. Second, we examined the association between parent report of infant temperament (IBQ) and anxiety symptoms later in life. Anxiety symptoms were assessed in adolescence via the Screen for Child Anxiety Related Disorders (SCARED),[36] a clinically validated questionnaire whereby participants report anxiety experienced over the past 3 months. A total of 185 participants (cohort 1: n = 59 [58%]; cohort 2: n = 126 [57%]) completed the child report SCARED, and 193 participants (cohort 1: n = 54 [53%]; cohort 2: n = 139 [63%]) completed the parent report SCARED. Whether or not a child or parent participated in this follow-up visit did not differ as a function of reactivity (*p*s > .089). See Supplement 1, available online, for results controlling for cohort.

RESULTS

In line with prior research, the amygdala exhibited widespread functional connectivity.[6,37] Functional activity in the amygdala was positively correlated with activity in the cingulate cortex, insula, operculum, superior frontal cortex, temporal lobe, and subcortical regions. Additionally, there were widespread negative correlations with activity in prefrontal, parietal, and occipital lobe areas (Figure S5, available online). Focal analyses first examined amygdala connectivity associated with negative and positive affect. Results demonstrated a negative association between amygdala-cingulate connectivity and amygdala–superior frontal gyrus connectivity and positive affect—such that greater connectivity was associated with lower positive affect (Figure 1). There was no main effect of negative affect. Next, we examined amygdala functional connectivity that was more positively associated with negative affect compared with positive affect. Results indicated that greater amygdala-cingulate connectivity (i.e., anterior cingulate and paracingulate) had a stronger positive association with negative affect compared with positive affect (Figure 2). There was no correlation between FD (mean or maximum) and connectivity in this region (*p*s > .38). See Figure S6, available online, for results with lowered voxelwise threshold. See Figures S7 and S8, available online, for results of right and left amygdala seeds, respectively. Results incorporating global signal regression were similar (Figures S9–S11 and Supplement 1, available online).

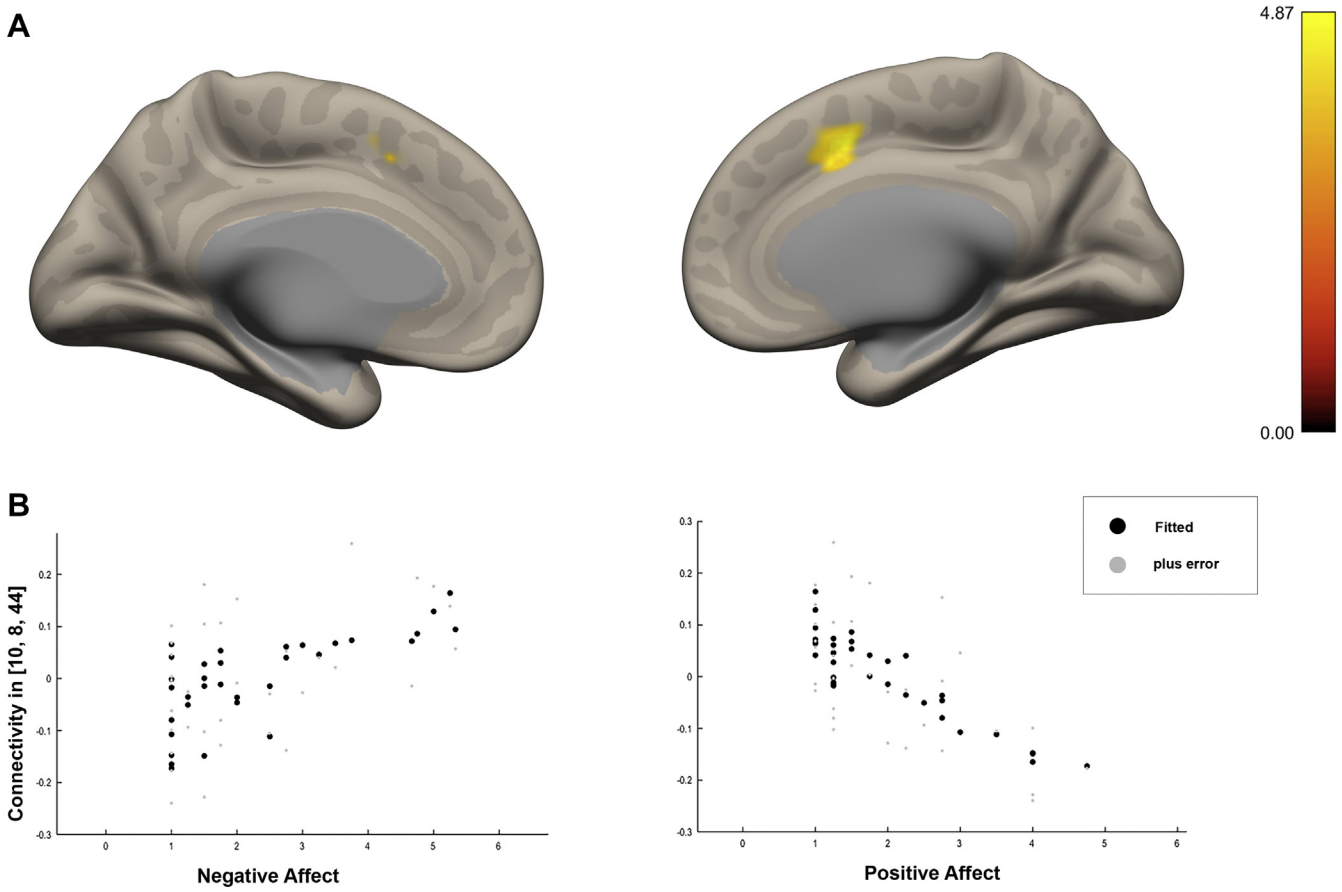
Next, to provide a comparison with the existing parent report literature, we aimed to identify a parent report measure associated with direct observation of high negative affect and low positive affect. Results indicated that the IBQ latency to approach novelty subscale was significantly

FIGURE 1 Amygdala Connectivity Associated With Lower Levels of Positive Affect



Note: Whole-brain voxel-wise map illustrates that greater amygdala-cingulate and amygdala–superior frontal gyrus connectivity was associated with less positive affect. Regions highlighted include paracingulate gyrus, anterior cingulate, superior frontal gyrus, supplementary motor cortex, and middle frontal gyrus. Please note color figures are available online.

FIGURE 2 Amygdala Connectivity Associated With Greater Negative Affect Relative to Positive Affect



Note: (A) Whole-brain voxel-wise map illustrates that amygdala-cingulate connectivity was associated greater negative affect compared with positive affect. Regions highlighted include paracingulate gyrus, anterior cingulate gyrus, and supplemental motor area. Color bar indicates t *statistic. (B) Plots fitted response between negative affect (left) and positive affect (right) and connectivity represented in (A). Error is indicated in light gray dots. Please note color figures are available online.*

positively associated with negative affect and negatively associated with positive affect (Table 2). Also, as expected, the latency to approach novelty subscale was also positively associated with the SVM continuous factor score of reactivity. Thus, longer latency to approach novelty was associated with greater negative reactivity. No other subscales of the IBQ showed this association. This is consistent with prior literature that uses the IBQ latency to approach (renamed the fear subscale) as an index of infant risk for the development of internalizing disorders later in life.

Using the results of these analyses, we then examined connectivity associated with parent report of latency to approach novelty, controlling for age and sex of the child. Results indicated that amygdala connectivity was not significantly associated with latency to approach novelty. Exploratory follow-up analyses lowering the voxelwise threshold to $p < .01$ did not yield significant results. See Figure S12, available online, for results of analyses examining connectivity associated with SVM factor scores.

Validation Analyses

To validate the above results, we conducted analyses using data from a large independent cohort. First, to validate the association between observational measures of negative reactive temperament and parent report measures of latency to approach novelty, we examined whether infants classified as negative and positive reactive using observational assessment differed in average latency to approach novelty (as assessed via IBQ). Results indicated that relative to positive reactive (mean = 2.27) infants, negative reactive (mean = 2.56) infants exhibited a longer latency to approach novelty ($F_{1,271} = 7.20$, $p < .008$). No other subscale of the IBQ was significantly greater for negative reactive infants compared with positive reactive infants ($p > .261$) (See Supplement 1 and Table S2, available online, for descriptive statistics on all other IBQ subscales as a function of reactivity). These results demonstrate convergence between our current study sample and a large sample of infants selected for negative and positive reactivity.

Second, to validate the claim that negative reactivity is associated with greater anxiety later in life, we conducted univariate analyses of variance examining whether parent- or child-reported anxiety in adolescence differed as a function of infant reactivity. Results demonstrated that negative reactive (mean = 20.29) infants exhibited higher child-reported anxiety in adolescence than positive reactive (mean = 16.65) infants ($F_{1,183} = 4.486$, $p < .036$). However, there were no group differences in parent-reported anxiety (mean negative = 11.43, mean positive = 10.57; $p > .561$). Of the IBQ parent-reported measures of infant temperament, neither the IBQ latency to approach novelty subscale nor any other subscale of the IBQ were associated with parent ($ps > .174$) or child ($ps < .097$) reports of anxiety in adolescence. These results provide supporting evidence of the clinical significance of negative reactivity and demonstrate that observed temperament is a robust predictor of later anxiety.

DISCUSSION

Individual differences in distress to novelty emerge in infancy and predict BI.[14] At the present time, few infant imaging studies have examined the neural correlates of distress to novelty, and all rely on parent report of temperament.[5,6,8,22] The current study is the first to link observed temperament in 4-month-old infants and amygdala connectivity. We found that greater amygdala-cingulate and amygdala–superior frontal gyrus connectivity is associated with lower positive affect. We further demonstrated that amygdala-cingulate connectivity is more positively associated with negative affect than positive affect. At present, it remains unclear whether the combination of reduced positive and increased negative affect or reduced positive affect alone is central to amygdala-cingulate connectivity. This report additionally provides some of the first evidence that observed distress to novelty, but not parent report of temperament, is associated with differences in self-reported anxiety of children 15 years later. Thus, these findings highlight the value of standardized observation-based assessments of infant temperament.

Studies in older subjects link connectivity between the amygdala and cingulate gyrus to emotion regulation.[38,39] Thus, the current findings linking amygdala-cingulate connectivity to observed temperament are consistent with this broader literature. Greater amygdala-cingulate connectivity could implicate coupled cortical-subcortical engagement as a process that regulates behavior. Beyond a primary perturbation in connectivity, altered function in either the amygdala or the cingulate gyrus also could underlie the observed associations. Longitudinal work assessing brain connectivity and activation could clarify how brain function relates to temperament at particular ages. For example, both the amygdala and the cingulate gyrus are part of the brain's salience network, which is involved in error monitoring, a process heightened in children with a history of BI.[40,41] Heightened error monitoring may moderate the link between BI and anxiety. Longitudinal research tracking relations among connectivity, temperament, and error-related activation could clarify how early brain function relates to the emergence of anxiety.

Using gold standard behavioral assessments, the current study is among the first to identify neural correlates of infant temperament. To date, 5 other studies have used parent ratings to evaluate the link between neonatal brain connectivity and infant temperament or closely related internalizing problems in the first few years of life.[5,6,8,22,37] Three of these 5 studies related stronger amygdala-cortical connectivity in infancy to temperament or internalizing problems emerging later. Findings in these 3 studies manifested between the amygdala and salience network nodes, including the insula,[5,8] medial prefrontal cortex,[8] and cingulate gyrus.[5,6] Our results extend these findings using an observational measure of temperament. Of note, a fourth study connected infant fear to decreased connectivity between the salience/ventral attention networks and the default mode network.[22] The fifth study linked anxiety at age 4 years to increased amygdala–default mode network and amygdala–visual areas connectivity (over the first 2 years of life) and decreased amygdala-sensorimotor connectivity.[37] Taken together, this infant imaging work fits well with the larger neurocognitive literature on markers of novelty-evoked distress.[26,35,40,42,43]

In addition to extending the temperament literature, this work sharpens questions about age-related changes in brain function and the emergence of anxious behavior. Indeed, not all reactive infants will go on to exhibit BI or anxiety.[13] Nevertheless, at a group level, we tend to see similar neurobiological profiles between temperaments characterized by novelty-evoked distress and clinical disorders associated with anxiety.[44–47] It has been argued by some that this overlap in biological correlates of temperament and clinical disorders suggests continuity among phenotypes,[48] although others find evidence to the contrary.[49,50] Stronger evidence for such continuity requires repeated imaging from the neonatal period to adolescence. This study is among the first to examine amygdala connectivity in 4-month-old infants—an age at which temperamental distress to novelty first emerges behaviorally. However, replication with large, diverse, longitudinal samples is necessary, particularly for identifying early targets for intervention.

The strengths of this study include the use of laboratory behavioral assessment of temperament, concurrent brain imaging at 4 months of age, and use of an independent large

longitudinal sample to provide key validating evidence. The use of this independent sample provides evidence of the generalizability (specifically for the association between observed reactivity and parent report) and the clinical significance of negative reactivity. As well, there are several limitations of this work. First, the sample size reported here is relatively small. It could be that our failure to replicate prior studies using maternal report is due to limited statistical power. Second, the sample reported here was largely homogeneous in ethnicity and socioeconomic status, a problem in other studies of this nature. Parents of children in this sample were primarily White, and the majority of mothers held advanced degrees. Future studies should aim to replicate our results in a diverse sample to address the generalizability of these results. Third, this study evaluated amygdala connectivity at only one time point, 4 months—thus, we cannot know whether these effects have been present since birth or emerge at 4 months. While prior studies suggest that similar patterns are present in neonates, longitudinal assessments of amygdala connectivity and temperament are necessary to determine how stable these patterns are over time. Finally, the validation analyses do provide novel evidence of a link between negative reactivity at 4 months and self-reported anxiety in adolescence. Nevertheless, these results come from an independent dataset. Thus, they cannot clarify whether or not amygdala-cingulate connectivity mediates the link between negative reactivity and later anxiety. This is a possibility that should be explored in future studies. Moreover, while research collectively suggests that an altered cognitive control mechanism may underlie the development of anxiety, the present data cannot distinguish between normative variation and disrupted patterns of brain connectivity.

Overall, these results provide novel insight into the developmental pathophysiology of novelty-evoked distress. This study demonstrates that greater amygdala-cingulate and amygdala–superior frontal gyrus connectivity is associated with low positive affect in response to novelty. Further, we show that amygdala-cingulate connectivity exhibits a more positive association with negative affect than positive affect. Critically, this is the first infant imaging study to link behavioral assessments of temperament in infancy with amygdala connectivity. Future work should aim to identify the extent to which amygdala-cingulate connectivity is stable within an individual and across time. These stable neural correlates of novelty-evoked distress may act as potential risk markers of anxiety. In summary, these results provide additional evidence of a link between brain circuitry associated with cognitive control and temperamental risk for anxiety.

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