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Courtney A. Filippi

Sanjana Ravi

Maya Bracy

Anderson M. Winkler
The University of Texas Rio Grande Valley, Anderson.Winkler@utrgv.edu

Chad Sylvester

See next page for additional authors

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Authors

Courtney A. Filippi, Sanjana Ravi, Maya Bracy, Anderson M. Winkler, Chad Sylvester, Daniel Samuel Pine, and Nathan A. Fox

Amygdala Functional Connectivity and Negative Reactive Temperament at Age Four Months

Courtney A. Filippi, PhD, **Sanjana Ravi, BS, BA**, **Maya Bracy, BA**, **Anderson Winkler, MD, PhD**, **Chad M. Sylvester, MD, PhD**, **Daniel S. Pine, MD**, **Nathan A. Fox, PhD**

Drs. Filippi, Fox, Mss. Ravi and Bracy are with the University of Maryland, College Park. Drs. Filippi, Winkler, and Pine are with the National Institute of Mental Health (NIMH), Bethesda, Maryland. Dr. Sylvester is with Washington University, St. Louis, Missouri.

Abstract

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

Method: At four-months of age infants' (*N*=90) novelty-evoked distress was assessed using a standardized reactivity assessment and parent-report. Within three weeks, resting state fMRI was collected in a subset of infants (*n*=34). Using a whole-brain voxel wise approach, we examine amygdala connectivity associated with positive and negative affect during the reactivity assessment. We then examined regions where the association of amygdala connectivity with negative affect was higher than with positive affect. Additionally, we examined associations between amygdala connectivity and parent-report of temperament.

Results: Greater amygdala-cingulate and amygdala-superior frontal gyrus connectivity was associated with lower positive affect during the reactivity assessment. Results further indicated that the association between amygdala-cingulate connectivity was greater for negative affect

compared to 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) demonstrate that negative reactivity is 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.

Keywords

infant MRI; temperament; negative reactivity; amygdala; functional connectivity

Introduction

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 parent 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 report provides such data.

Infants' responses 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 four-months of age (i.e., 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 behaviorally inhibited children, several lines of work link risk for anxiety to patterns of connectivity among brain regions associated with threat detection (e.g., amygdala) and cognitive control (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 two key limitations. First, they rely solely on maternal report of infant temperament[6,22,5,8]. 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 parental personality or psychopathology. Thus, the current study uses 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. The current study examines amygdala functional connectivity in 4-month-olds. 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 four-month-old infants. Infant temperament was assessed via both behavioral assessment and parental report—allowing us to directly compare amygdala connectivity associated with these two measures. Within one month of these temperament assessments, fMRI was conducted during natural sleep. We test the hypothesis that amygdala-prefrontal cortex connectivity is associated with high negative affect and/or low positive affect. We additionally perform exploratory analyses to examine amygdala connectivity linked to parental report of temperament. Lastly, we make use of a large longitudinal validation sample to provide data on the clinical significance of negative reactive temperament.

Method

Participants

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

All 90 infants completed the reactivity assessment and provided parent report measures of temperament. See Table 1 for sample demographics. Of these 90 infants, 59 (M_{age}=4 months 24 days, range= 3 months 23 days – 5 months 26 days) elected to participate 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 Beck Anxiety Inventory[24]) 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). Eleven of these 59 (19%) infants failed to sleep during the visit, generating no imaging data, yielding data in 48 (81%) infants. 14 of these 48 (29%) infants 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 ($M_{visit\ duration}$=3 hours 49 minutes; ps>.178). The final sample of infants with temperament and resting state data was n=34.

Reactivity Assessment

A standardized assessment of infant reactivity[16,25,26] was conducted at age 4-months (M_{age}=4 months 5 days, SD_{age}=17 days). Infants sat in an infant seat while experimenters (out of sight) presented novel toys (i.e., visual blocks) and sounds (i.e., 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 were comprised of 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 two sentences the number of voices speaking and, consequently, the volume of the presentation increased by one (e.g., first two sentences were spoken by one voice, second two sentences by two 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 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, hyper-extensions), positive affect (smiling, laughing), and negative affect (fussing, crying) during the presentation of the stimuli were coded on a 7-point Likert scale (1 indicative of low motor or affect; 7 indicative of high motor or affect) for each block. A second coder double coded all blocks for all subjects. Reliability ranged between .65 and .89 (M_{motor}=.75, $M_{negative}$=.86, $M_{positive}$=.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 four blocks. Table 2 provides the mean 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 three dimensions of reactivity into one continuous factor score. See 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, two parents failed to complete all items for the soothability subscale, thus n=88 for any analyses using the soothability subscale. See Table 2 for the association between all temperament measurements.

MRI Acquisition

Infants MRI assessments were conducted during natural sleep and within 8 weeks of the initial reactivity assessment (M=19.17 days; range=1 day - 50 days; M_{age}=4 months 24 days; SD_{age}=18 days). Visits were conducted in the evening. Upon arrival, infants were

measured, changed and swaddled using an MR-safe Velcro swaddle. Parents were then given privacy to feed their baby and begin their bedtime routine. Once the infant was asleep for 20 minutes hearing protection (i.e., silicone earplugs and MiniMuff noise attenuators) was applied for 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 MR operator as needed. If the infant woke up, scanning was paused until the baby 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 Siemens MAGNETOM Trio/Tim scanner with a 32-channel head coil. Scanning began with the acquisition of structural images which were used for registration. The T1-weighted scans used MPRAGE with TR/TE= 1900ms/2.43ms, flip=9°, voxel size .8mm isotropic. The T2-weighted scans use MPRAGE with TR/TE=3200ms/488ms, flip=9°, voxel size= .7x.8x.8. Resting state data was acquired using multi-band radiofrequency pulses (multi-band acceleration factor=6) to excite several slices in a single TR to acquire high-resolution data faster. 10-minute resting state runs were obtained using a gradient echo, echo-planar-image (EPI) sequence sensitized to T2* blood oxygen level dependent (BOLD) contrast using the following parameters: TR/TE=1250ms/39.4ms, flip=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 <.2mm framewise displacement (FD). Data was acquired until at least 10 minutes of low movement data were obtained or the infant woke up. All 34 infants had at least 1 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 and SPM8 (SPM8 allowed for infant-specific adaptations to CONN's default pipeline). Pre-processing of functional data began with slice timing correction (conducted using AFNI's 3dTshift function). Subsequent pre-processing steps were conducted using the CONN toolbox[29,30] and included spatial realignment (compensating for head motion), and co-registration to T1-weighted image. Structural images were then normalized to 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 cerebral spinal fluid). Subsequently, confounding effects were identified using anatomical component-based noise correction procedure (aCompCor[32]) rather than global signal regression (GSR). Analyses incorporating GSR are presented in Supplement 1, available online. The Artifact Detection Tool (ART; https://www.nitrc.org/projects/artifact_detect/) was utilized for outlier detection. Frames with FD>.25mm and global BOLD 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, CSF, and frames marked as outliers) were regressed out and data were band-pass filtered (.009 – .08 Hz) and spatially smoothed using a Gaussian kernel (6mm). 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 - 18 minutes 45 seconds; See Figure S2, available online, for histogram). Across all subjects, mean FD and percent censored frames were .195mm 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–S4, available online, for scatterplots illustrating no significant association between FD and left 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 voxel-wise 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 examine regions where amygdala functional connectivity has a stronger positive association with negative affect compared to 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 have been reported in the literature[34,35]. Results were corrected voxel-wise at $p<.001$ and corrected for multiple testing to provide family-wise error (FWE) 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 utilize 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 (See 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. In Supplement 1, available online, we provide 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 two validation analyses using a large independent sample. First, we validated the link between negative (vs. 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 vs. positive reactive temperament (assessed via direct observation). We also examine the association between parent-report of infant fearful temperament (i.e., 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 two 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[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 where corresponding to the affect dimension they scored highest on[14,25,26]. Children were then longitudinally followed into adolescence (cohort 1 and 2) and adulthood (at present, 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 combine reactivity and IBQ data from the two cohorts. Cohort 1's initial IBQ assessment was conducted at 9 months, whereas Cohort 2 collected IBQ data beginning at 4 months. For the purposes of these analyses, we combine parent report across these ages (See Supplement 1, available online, for analyses controlling for cohort). In total, 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 examines 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 examine 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 three months. In total, 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 ($ps > .089$). Supplement 1, available online, provides 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 (See Figure S5, available online). Focal analyses first examined amygdala connectivity associated with negative and positive affect. Results demonstrated a negative association between amygdala-cingulate and superior frontal gyrus 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 to positive affect. Results indicated that greater amygdala-cingulate connectivity (i.e., anterior cingulate and paracingulate) had a stronger positive association with negative affect compared to positive affect (Figure 2). There was no correlation between FD (mean or max) and connectivity in this region (ps>.38). See Figures S6, available online, for results with lowered voxel-wise threshold. See Figure S7 and S8, available online, for results of right and left amygdala seeds, respectively. Results incorporating GSR were similar See Figures S9–11 and Supplement 1, available online.

Next, to provide comparison to 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 positively associated with negative affect and negatively associated with positive affect (See 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 utilizes 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-ups lowering the voxel-wise 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 results reported above 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 (M=2.27) infants, negative reactive (M=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 to positive reactive infants (p>.261; See Supplement 1 and Table S2, available online, for descriptives 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 ANOVAs examining whether parent or child-reported anxiety in adolescence differed as a function of infant reactivity. Results demonstrated that negative reactive (M=20.29) infants exhibited higher child-reported anxiety in adolescence than positive reactive (M=16.65) infants (F(1,183)=4.486, p<.036). However, there were no group differences in parent-reported anxiety ($M_{negative}$=11.43, $M_{positive}$=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 reports (ps<.097) 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 behavioral inhibition[14]. At present, 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 -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 children's self-reported anxiety 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 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 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, five 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 five studies related stronger amygdala-cortical connectivity in infancy to later-emerging temperament or internalizing problems. Findings in these three 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 to increased amygdala-default-mode network and amygdala-visual areas connectivity (over the first two years of life) and decreased amygdala-sensorimotor connectivity[37]. 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 *four-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 in-lab 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 replication prior studies using maternal report is due to limited statistical power. Second, the sample reported here was largely homogeneous in ethnicity and SES, a problem in other studies of this nature. Parents of children in this sample were primarily White and the majority of mothers holding advanced degrees. Future studies should aim to replicate in a diverse sample to address to the generalizability of these results. Third, this study evaluated amygdala connectivity at only one time point--four months; thus, we cannot know whether these effects have been present since birth or emerge at four months. While prior studies suggest 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 four-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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Disclosure:

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Figure 1.
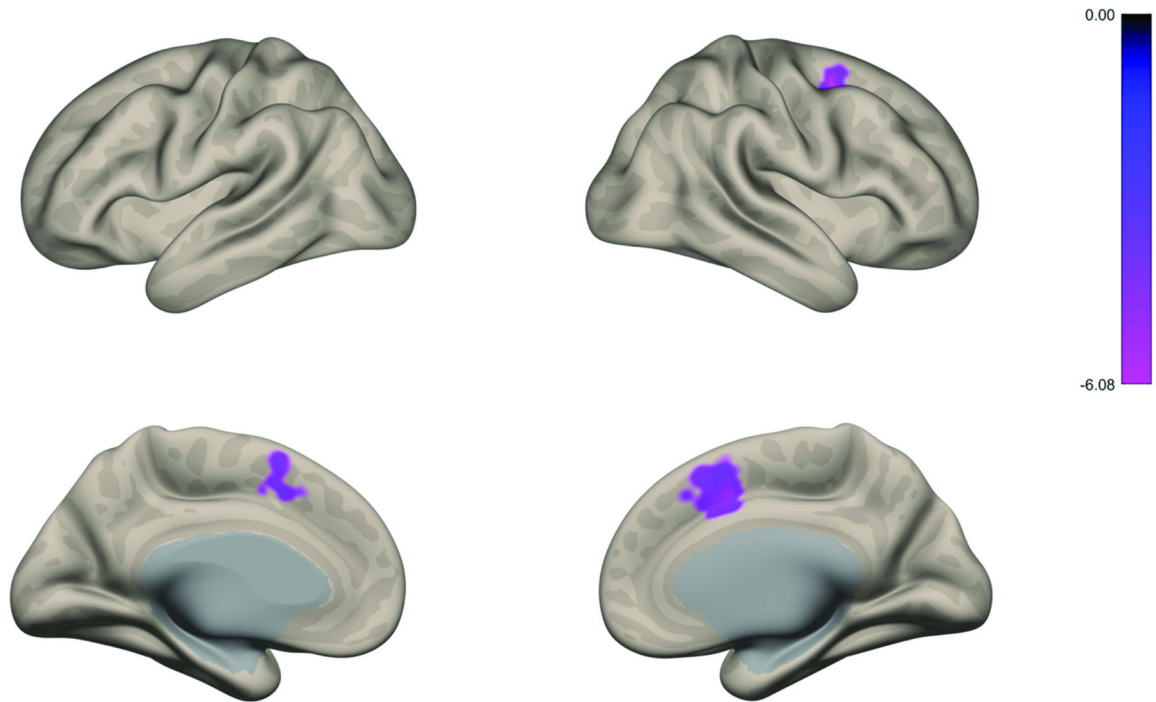
Amygdala connectivity associated with lower levels of positive affect

Note: (A) 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.

(A)

(B)
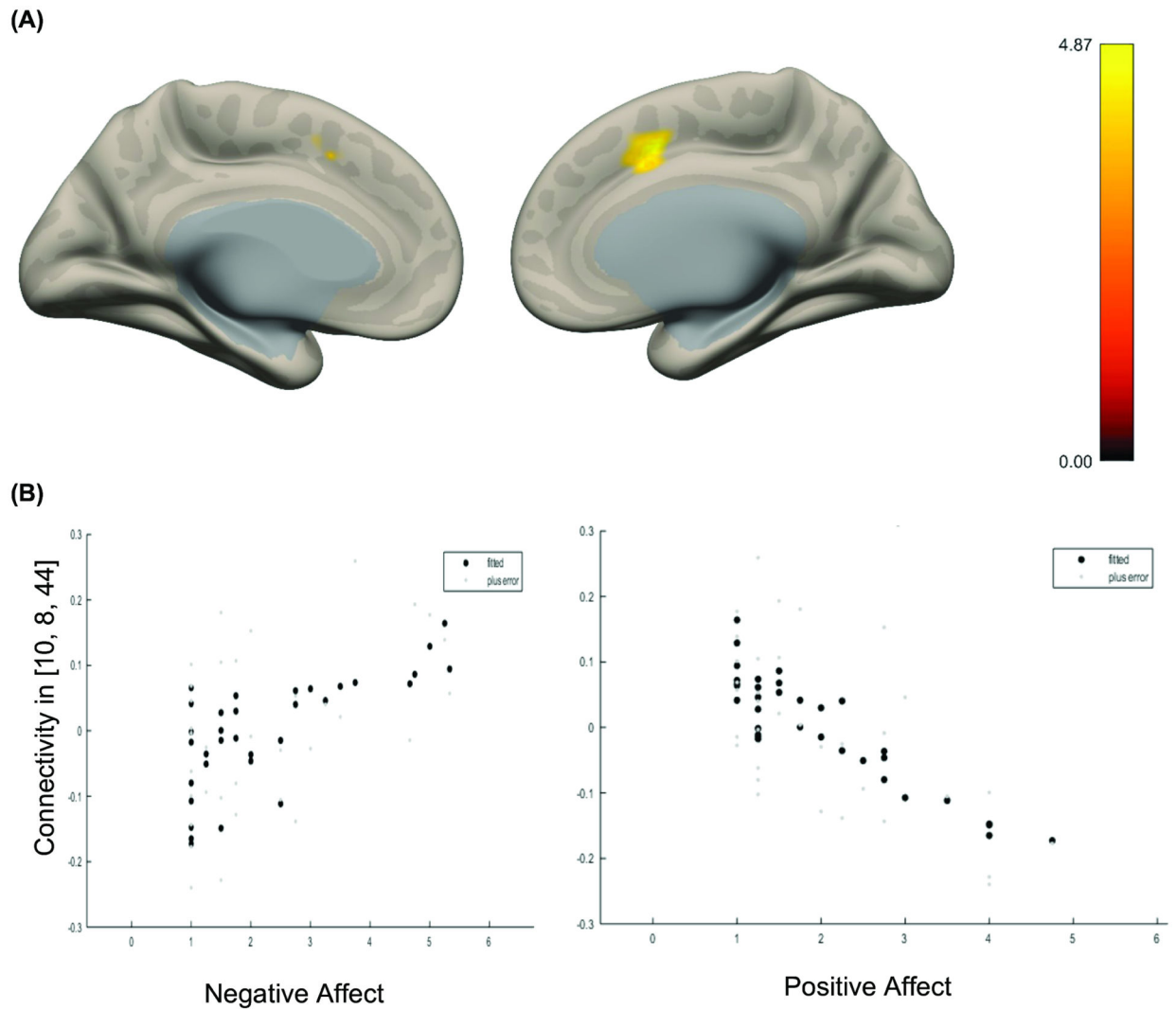
Figure 2.
Amygdala connectivity associated with greater negative affect relative to positive affect
Note: (A) Whole brain voxel-wise map illustrates that amygdala-cingulate connectivity
was associated greater negative affect compared to positive affect. Regions highlighted
include: paracingulate gyrus, 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.

Table 1.

Sample Demographics and data collected

Characteristic	N (% of sample)	Mean (SD)
N	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%)	--
Multi-racial/Non-Hispanic	17 (19.0%)	--
Other/ Non-Hispanic	--	--
White/Hispanic	1 (1.1%)	--
African American/Hispanic	1 (1.1%)	--
Asian/Hispanic	--	--
Multi-racial/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 (assessed via Beck Anxiety Inventory)		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%)	

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	.95	.254*	−.274*							
IBQ											
(4) Activity Level	3.89	.78	.068	.055	.048						
(5) Distress to limitations	3.19	.68	−.112	.182	−.142	.211*					
(6) Latency to approach novelty	2.14	.46	−.084	.281**	−.239*	.148	.395**				
(7) Duration Orienting	3.76	1.07	−.091	.013	.094	.264*	−.112	−.010			
(8) Smiling	4.15	.87	.039	.106	−.041	.319**	−.008	−.134	.356**		
(9) Soothability	4.54	.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

IBQ = Infant behavior questionnaire

BAI = Beck anxiety inventory

*
p<.05,

**
p<.01.