Biological Psychiatry: CNNI

Archival Report

Development of Amygdala Functional Connectivity During Infancy and Its Relationship With 4-Year Behavioral Outcomes

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ABSTRACT

BACKGROUND: The amygdala represents a core node in the human brain's emotional signal processing circuitry. Given its critical role, both the typical and atypical functional connectivity patterns of the amygdala have been extensively studied in adults. However, the development of amygdala functional connectivity during infancy is less well studied; thus, our understanding of the normal growth trajectory of key emotion-related brain circuits during a critical period is limited.

METHODS: In this study, we used resting-state functional magnetic resonance imaging (N = 233 subjects with 334 datasets) to delineate the spatiotemporal dynamics of amygdala functional connectivity development during the first 2 years of life. Their relationships with 4-year emotional (i.e., anxiety and inhibitory self-control parent report measures) and cognitive (i.e., IQ) behavioral outcomes were also assessed using multivariate modeling.

RESULTS: Our results revealed nonlinear growth of amygdala functional connectivity during the first 2 years of life, featuring dramatic synchronization during the first year followed by moderate growth or fine tuning during the second year. Importantly, functional connectivity growth during the second year had significant behavioral implications exemplified by multiple significant predictions of 4-year emotional and cognitive developmental outcomes.

CONCLUSIONS: The delineation of the spatiotemporal dynamics of amygdala functional connectivity development during infancy and their associations with 4-year behavioral outcomes may provide new references on the early emergence of both typical and atypical emotion processing capabilities.

Keywords: Amygdala, Anxiety, Early brain development, Emotion development, Functional connectivity, Inhibitory control

https://doi.org/10.1016/j.bpsc.2018.08.010

The amygdala is a key node in human emotion processing (1) that is critical for normal behavior and mental health. Atypical emotion processing associated with the amygdala is implicated in multiple mood disorders (e.g., depression, anxiety disorder) (2–4) and psychiatric disorders (e.g., schizophrenia) (5,6); thus, a better understanding of the emotion processing circuitry centered on the amygdala represents a high priority. In adult functional magnetic resonance imaging (fMRI) studies, activations of the amygdala, insula, hippocampus, parahippocampus, medial and lateral prefrontal cortices, and medial parietal cortex have been frequently reported during emotional stimuli processing, indicating widely distributed emotion processing circuitry (7). Similarly, functional connectivity (FC) studies of the amygdala have revealed positive connectivity of the amygdala with insula and medial prefrontal cortices that typically assess the affective state of the stimuli and have revealed negative connectivity with lateral prefrontal and dorsal parietal regions that are more involved in cognitive processes and emotion regulation (8,9). Together, these two streams of connectivity are thought to reflect the contemporary understanding of emotion processing involving complex interactions between bottom-up emotion encoding or appraisal and top-down regulatory processes (10).

Adult amygdala FC patterns and associated disease-related alterations are well studied (8,9); however, the development of amygdala FC during the first years of life remains poorly understood despite compelling evidence that emotion regulation strategies emerge during infancy (11,12). Specifically, while neonates demonstrate an almost complete reliance on caregivers' care for emotional relief (13,14), a range of self-regulatory behaviors (e.g., self-comforting and social referencing) emerge in toddlers (15). The development of emotion processing or regulation strategies during infancy has been documented to have far-reaching and enduring effects on cognitive development (14,16,17), academic achievement (17,18), quality of life (19), and psychopathology (15). Therefore, it is important to elucidate the development of amygdala FC patterns during infancy in order to better understand the brain basis for the emergence of both typical and atypical emotion processing or regulation strategies during this critical

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period. Consistent with this goal, previous research has shown that positive neonatal amygdala connectivity with the bilateral anterior insula and ventral striatum is associated with higher fear at 6 months of age (20). However, no longitudinal characterization has been conducted; thus, the normal growth trajectory of amygdala FC during infancy remains elusive.

In this study, we sought to characterize the spatiotemporal dynamics of amygdala FC during the first 2 years of life. Based on behavioral observations, we hypothesized incomplete functional connections of the amygdala in neonates (13,14) compared with adults followed by dramatic maturation during the first 2 years of life (21). Moreover, we also aimed to determine the behavioral importance of amygdala FC growth during infancy by testing its predictive power of 4-year emotional outcomes (i.e., parent-reported anxiety and emotional regulation) and cognitive outcomes (i.e., IQ). Given previous reports on the association between amygdala-related development and both emotional processing capabilities (20,22) and cognitive outcomes (17,18), we hypothesized significant correlations between amygdala FC development during infancy and 4-year anxiety, emotion regulation, and IQ measures.

METHODS AND MATERIALS

Participants

Typically developing infant participants were part of the University of North Carolina Early Brain Development Study, characterizing early childhood brain and behavior development (23–26). We retrospectively identified 223 subjects (107 boys) with at least one successful resting-state fMRI scan during the first 2 years of life. Participant characteristics (Table 1) of interest included sex, age (gestational age at birth, age at scan, and postnatal age [age at scan − age at birth]), maternal education, and twin status (n = 93 twins, n = 38 twin pairs). Exclusion criteria included gestational age at birth < 37 weeks and any neonatal illness requiring more than a 24-hour stay at a neonatal intensive care unit. Study protocols were approved by the University of North Carolina at Chapel Hill Institutional Review Board. Subjects were fed, swaddled, and fitted with ear protection prior to imaging. All subjects were in a natural sleep state during the imaging session.

Imaging

Longitudinal resting-state fMRI data were acquired from the cohort of typically developing infants (N = 223, 107 boys) at ∼3 weeks (neonates), 1 year of age, and 2 years of age. The distribution of available datasets for FC analyses is shown in Figure 1. Infant images were acquired using a single scanner (3T head-only Siemens Allegra with circular polarization head coil [Siemens, Erlangen, Germany]). Functional images were acquired using a T2*-weighted echo-planar images sequence: repetition time = 2 seconds, echo time = 32 ms, 33 slices, voxel size = 4 mm^3, 150 volumes. Structural images were acquired using a three-dimensional magnetization prepared rapid acquisition gradient-echo sequence: repetition time = 1820 ms, echo time = 4.38 ms, inversion time = 1100 ms, voxel size = 1 mm^3.

FC and Related Statistical Analyses

After standard image preprocessing (see Supplement), including discarding of the first 10 volumes, slice-timing correction, rigid-body motion correction, bandpass filtering (0.01–0.08 Hz), nuisance signal regression, and data scrubbing, we used the anatomical automatic labeling atlas (Supplemental Table S1) and seed-based technique (27) to characterize whole-brain FC associated with left or right amygdala. Correlation measures were normalized using Fisher's Z transformation and analyzed at the regional (i.e., voxelwise) and network levels. Specifically, t tests and log-linear mixed-effect (LME) models were used to quantify cross-sectional (neonates, 1-year-olds, and 2-year-olds) and longitudinal (neonates to 1-year-olds and 1-year-olds to 2-year-olds) effects, respectively. Log transformation of age was used in the LME modeling, given previous work showing log-linear growth trends of FC during the first 2 years of life (28–30). The LME models included random intercept and slope terms, with the effect estimate associated with postnatal age at scans (growth) being the principal variable of interest. Other participant characteristics were included as covariates in the LME models: sex, birth weight, motion, maternal education, and twin status. For the regional analyses, significance was defined using a clustering approach (AFNI program: 3dClustSim). We used conservative settings (31–33) to achieve the desired correction rate of α = .05. Specifically, we imposed a voxelwise cutoff of $p < .001$ and generated smoothness estimates from the preprocessed data using the mixed-model autocorrelation function. The following cluster sizes (bi-sided, edge, or face connectivity, i.e., nearest neighbor = 1) were established for each subsample (in voxels); neonates = 16, 1-year-olds = 10, 2-year-olds = 6, neonates to 1-year-olds = 12, and 1-year-olds to 2-year-olds = 8. For network-level analyses, we used the average left or right amygdala FC within predefined resting-state networks (RSNs). RSN masks were defined using adult templates (34) warped into 2-year-old template space (35) (Supplemental Figure S1).

Emotional and Cognitive Assessment at 4 Years of Age

We obtained both parent-reported and task-based laboratory assessments of children's behaviors at 4 years of age. The

Table 1. Summary of Participant Characteristics

Subjects (N = 223,107 Boys)	Mean	SD	Min	Max
Birth Weight, Ounces	3126.73	506.94	1960	4562
Birth Age, Days	272.29	9.66	259	295
Maternal Education, Years	15.56	3.24	3	24
	Scan Age, Days			
Cross-sectional	Mean	SD	Min	Max
Neonates (n = 152)	296.83	11.84	274	348
1-Year-Olds (n = 105)	657.79	20.37	605	714
2-Year-Olds (n = 77)	1026.04	31.33	973	1144
Total (n = 334)				
4-Year Behavior				
ANX (n = 125)	47.64	10.12	32	78
ISC (n = 126)	49.35	10.14	34	80
IQ (n = 129)	108.88	13.17	73	139

ANX, anxiety [Behavior Assessment System for Children–second edition (36)]; IQ, cognitive development [Stanford–Binet Intelligence Scales–fifth edition (38)]; ISC, inhibitory self-control [Behavior Rating Inventory of Executive Function–Preschool version (37)].

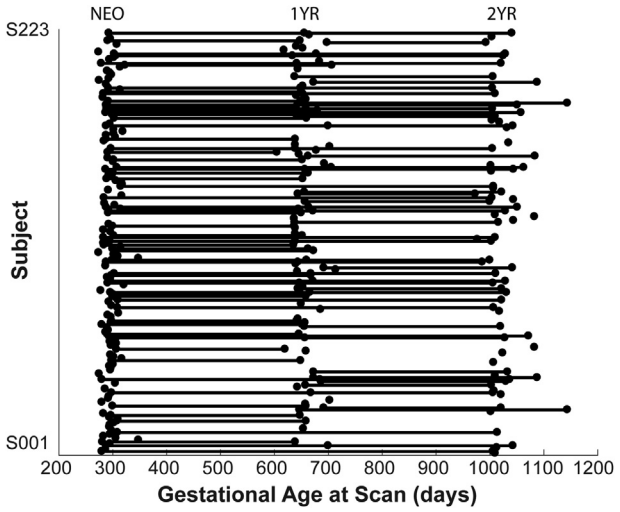

Figure 1. Data distribution. The distribution of gestational age at scan for all included infant subjects (N = 223 totaling 334 datasets; neonates [NEO], 1-year-olds [1YR], and 2-year-olds [2YR]) whose image quality passed the quality control procedures is shown. Each dot represents a successful resting-state functional magnetic resonance imaging scan, and dots along each line represent all the available longitudinal scans for a given subject. NEO: n = 152; 1YR: n = 105; 2YR: n = 77; NEO and 1YR: n = 57; 1YR and 2YR: n = 44; NEO and 2YR: n = 10; NEO, 1YR, and 2YR: n = 25.

primary emotion-related outcome variables were ratings of anxiety (4YR-ANX) and inhibitory self-control (4YR-ISC) assessed using the Behavior Assessment System for Children–second edition (36) and Behavior Rating Inventory of Executive Function–Preschool version (37), respectively. Moreover, 4-year IQ (4YR-IQ) was also assessed using the Stanford–Binet Intelligence Scales–fifth edition (38) as an index of cognitive development. More details are included in the Supplement.

Brain–Behavior Analyses

We used linear regression to characterize the associations between infant amygdala FC measures and 4-year behavior. Specifically, for each detected cluster or RSN, regression models were generated using cross-sectional (neonates, 1-year-olds, or 2-year-olds) or longitudinal (neonates to 1-year-olds or 1-year-olds to 2-year-olds) FC measures and 4-year behavioral outcomes in subjects with both measures (Supplemental Table S1). For cross-sectional relationships, we used the average FC within each significant cluster or RSN and included covariates in the analysis. Similarly, for longitudinal characterizations, we extracted individual average growth estimates at the cluster or RSN level using the aforementioned LME models. Significance was defined at the $p < .01$ level.

RESULTS

Voxelwise and Network-Level Amygdala FC During the First 2 Years of Life

FC maps for the left and right amygdala in neonates, 1-year-olds, and 2-year-olds are shown in Figure 2A, with corresponding longitudinal effects [log(age); neonates to 1-year-olds and 1-year-olds to 2-year-olds] presented in Figure 2B. The full results are summarized in Supplemental Tables S3 to S6. Besides the highly similar FC patterns between the left and right amygdala (Supplemental Figure S2), there are four notable features. First, consistent with adult patterns (8,9), amygdala FC showed significant positive connections in adjacent hippocampus, parahippocampus, and subcortical areas (e.g., caudate, putamen, thalamus) for all three age groups. Second, unlike adult patterns, positive amygdala FC with the medial prefrontal cortex and negative FC with the lateral prefrontal and parietal cortices were largely absent in neonates, while (conversely) prominent positive FC in primary auditory cortex (e.g., middle temporal gyrus) and sensorimotor regions (e.g., precentral or postcentral gyrus, supplementary motor area), which is not typically observed in adults, was present in neonates. Third, during the first year (i.e., neonates to 1-year-olds), there was significant growth of amygdala FC toward adultlike patterns, including the emergence of positive connectivity within medial prefrontal areas, negative connectivity with lateral prefrontal and parietal areas, and diminishing positive connectivity within primary auditory and sensorimotor regions (Figure 2A). These cross-sectional observations are highly consistent with statistically significant FC growth during this period (Figure 2B). In 1-year-olds, amygdala FC was largely adultlike, featuring positive FC in the ventral and medial cortices and negative clusters in the dorsal and lateral brain areas. Fourth, 1- and 2-year amygdala FC patterns were qualitatively similar; however, quantitative growth (i.e., 1-year-olds to 2-year-olds) was evident (Figure 2B). Specifically, the primary sensorimotor regions showed positive growth (warm clusters in Figure 2B), whereas the medial prefrontal cortex, cuneus, precuneus, and neighboring visual areas exhibited negative growth (cool clusters in Figure 2B). Interestingly, these relationships were predominantly in the opposite direction compared with neonates to 1-year-olds trends, signifying nonmonotonic changes during infancy. Finally, three sex-related effects were detected (Supplemental Figure S3).

When examined at the network level, the nine canonical RSNs (34)—visual (primary, middle, and lateral), default mode (DMN), sensorimotor (SM), auditory (AUD), salience (SAL), and right or left executive control (CON-R or CON-L, respectively) (see Supplemental Figure S1)—showed distinct cross-sectional (Figure 3A) and growth (Figure 3B) patterns that were highly consistent with the regional observations. First, during the first year, the trend of emerging connectivity (both positive and negative) with higher-order RSNs combined with regression in primary RSNs was apparent. Specifically, amygdala FC with the DMN was negative in neonates, likely driven by negative connectivity within the posterior parietal regions, while that for SAL was slightly positive, although no significant positive clusters were detected (Figure 2). During the first year, both left and right amygdala FC with the DMN and SAL showed statistically significant positive growth ($p \leq .001$) and ultimately strong positive FC at the end of the first year. For CON-L, the slightly negative left and right amygdala FC in neonates also experienced significant negative growth ($p \leq .001$), resulting in stronger negative connectivity by 1 year of age. In contrast, the two primary RSNs of AUD and SM both showed strong positive left and right amygdala FC in neonates but significant negative growth ($p \leq .001$) after that, resulting in negative FC for SM and close to zero FC for AUD by 1 year of

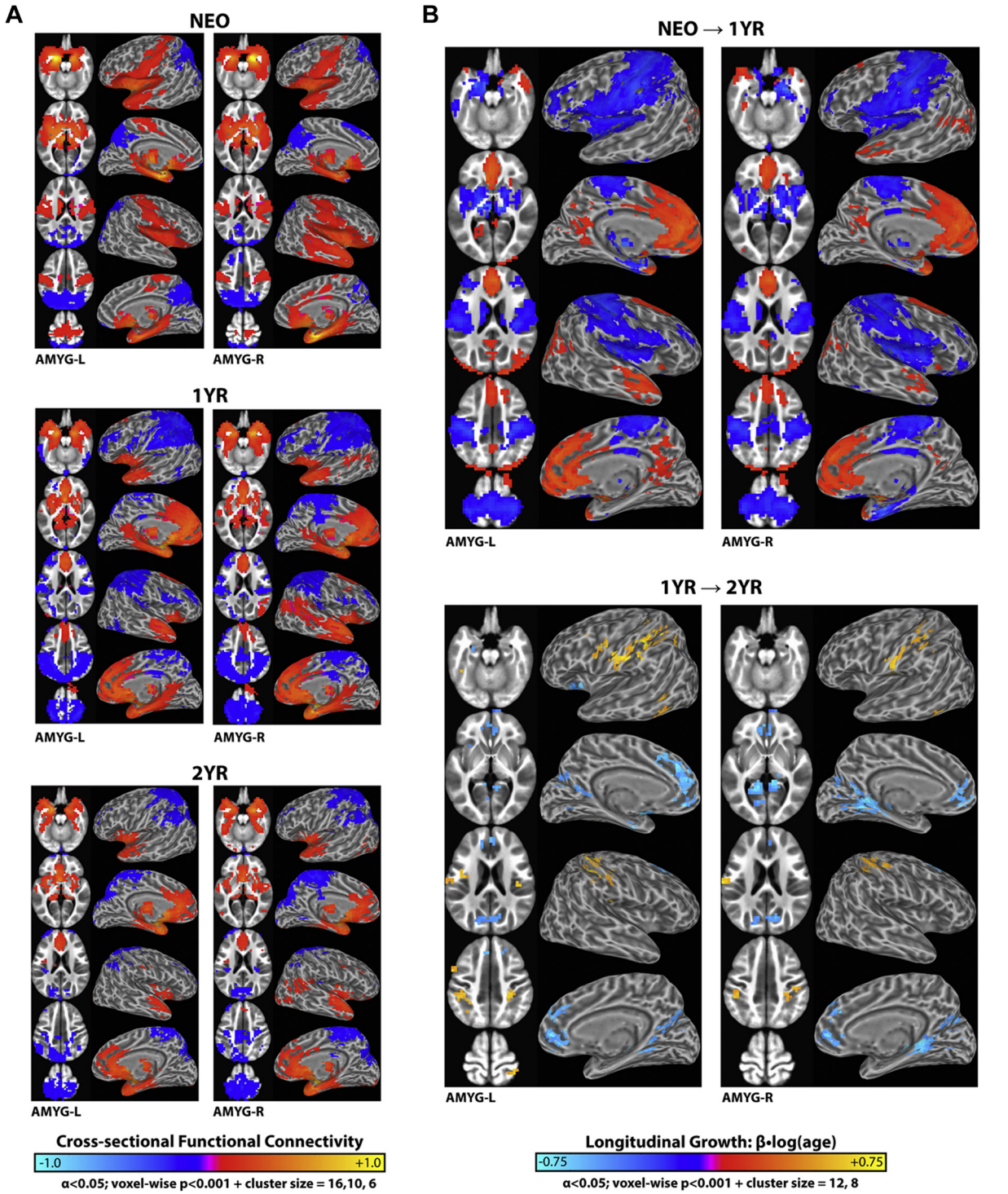

Figure 2. Regional functional connectivity and corresponding longitudinal changes for the left and right amygdala (AMYG-L and AMYG-R, respectively) in neonates (NEO), 1-year-olds (1YR), and 2-year-olds (2YR). **(A)** Cross-sectional patterns of significant positive (warm colors) and negative (cool colors) amygdala functional connectivity. **(B)** Patterns of significant positive and negative amygdala functional connectivity growth: NEO→1YR and 1YR→2YR. Growth or age-dependent changes [$\beta \times$ log(age)] are characterized using linear mixed-effect modeling (random intercept + slope) while controlling for other participant characteristics (i.e., sex, twin status, motion, maternal education, and birth weight). See Supplemental Tables S3 to S6 for full breakdown of significant clusters.

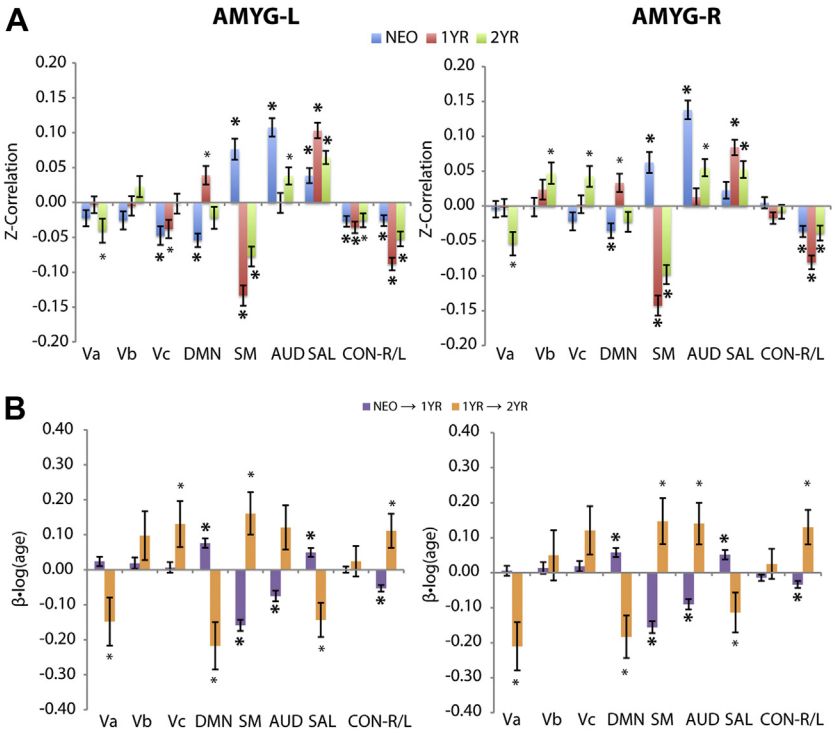

Figure 3. Network-level functional connectivity and corresponding longitudinal changes for the left and right amygdala (AMYG-L and AMYG-R, respectively) in neonates (NEO), 1-year-olds (1YR), and 2-year-olds (2YR). **(A)** Cross-sectional effects: NEO (blue), 1YR (red), and 2YR (green). **(B)** Longitudinal effects or growth: NEO→1YR (purple) and 1YR→2YR (orange). Resting-state networks were based on Smith networks (34) warped into infant template space (Supplemental Figure S1). AUD, auditory; CON-R/L, right/left executive control; DMN, default mode network; SAL, salience; SM, sensorimotor; Va, Vb, and Vc, primary, middle, and lateral visual, respectively. Bars correspond to group means, with error bars denoting standard errors. *$p \leq .05$, *$p \leq .001$.

age. Interestingly, similar reversals in growth at the network level were observed when comparing the trend of the second and first years (eight of nine RSNs for the left amygdala and seven of nine RSNs for the right amygdala).

Relationship Between Amygdala FC Development During Infancy and Behavioral Outcomes at 4 Years of Age

Having characterized amygdala FC development during the first 2 years of life, we asked whether these measures could predict emotion-related parent-reported behavioral outcomes at 4 years of age. At the regional level, five significant predictions were detected ($p < .01$), and all five involved 1-year-olds→2-year-olds growth rates and 4-year anxiety measures (Figure 4A–E). Specifically, 1-year-olds to 2-year-olds left and right amygdala FC growth estimates (negative in sign) in the posterior regions of the DMN (precuneus and cuneus) and neighboring visual areas (lingual gyrus and calcarine cortex) positively predicted 4YR-ANX. Conversely, 1-year-olds to 2-year-olds left amygdala FC growth estimates (positive in sign) in the right SM cortices (right supramarginal gyrus, right inferior parietal lobule, and right postcentral gyrus) negatively predicted 4YR-ANX. Note that the signs between the FC growth estimates and FC ~4YR-ANX relationships were always opposite, indicating that the more FC growth toward the group trend, the lower the anxiety ratings were at 4 years of age. These relationships remained largely unchanged after including cognitive development (4YR-IQ) as an additional explanatory variable (Supplemental Table S7). When directly assessing the relationships between regional-level amygdala FC and 4YR-IQ (Figure 4F), 1-year-olds to 2-year-olds left amygdala FC growth in left SM regions (inferior parietal lobule and postcentral gyrus) negatively predicted 4YR-IQ ($p < .001$). There were 15 additional marginally significant ($p < .05$, uncorrected) emotion-related relationships (Supplemental Table S8). Overall, the significant or marginally significant predictions ($n = 20$) were evenly split between anxiety and emotion regulation scores (i.e., 10/10 relationships with 4YR-ANX/4YR-ISC). Moreover, these relationships were predominantly associated with the left amygdala ($n = 13$ or 70%) and involved more longitudinal growth estimates ($n = 13$ or 65%, 5 associated with neonates to 1-year-olds and 8 associated with 1-year-olds to 2-year-olds). There were 6 other marginally significant regional relationships associated with 4YR-IQ (Supplemental Table S9).

At the network level, two symmetric predictions of 4YR-ISC were detected. The mean FC between the left and right amygdala and the CON-L/R network at 2 years of age positively predicted 4YR-ISC ($p \leq .01$) (Figure 5), indicating that the more negative the within-hemisphere amygdala–CON network connectivity in 2-year-olds, the better the emotion regulation capability at 4 years of age. Potentially related, 2-year-olds FC and 1-year-olds to 2-year-olds FC growth between the left amygdala and CON-L/R also negatively predicted 4YR-IQ scores ($p \leq .01$) (Supplemental Table S10). However, when 4YR-IQ was included as an additional control variable, both 4YR-ISC relationships remained marginally significant ($ps = .01$ and .052 for the left and right amygdala, respectively). Finally, 11 other

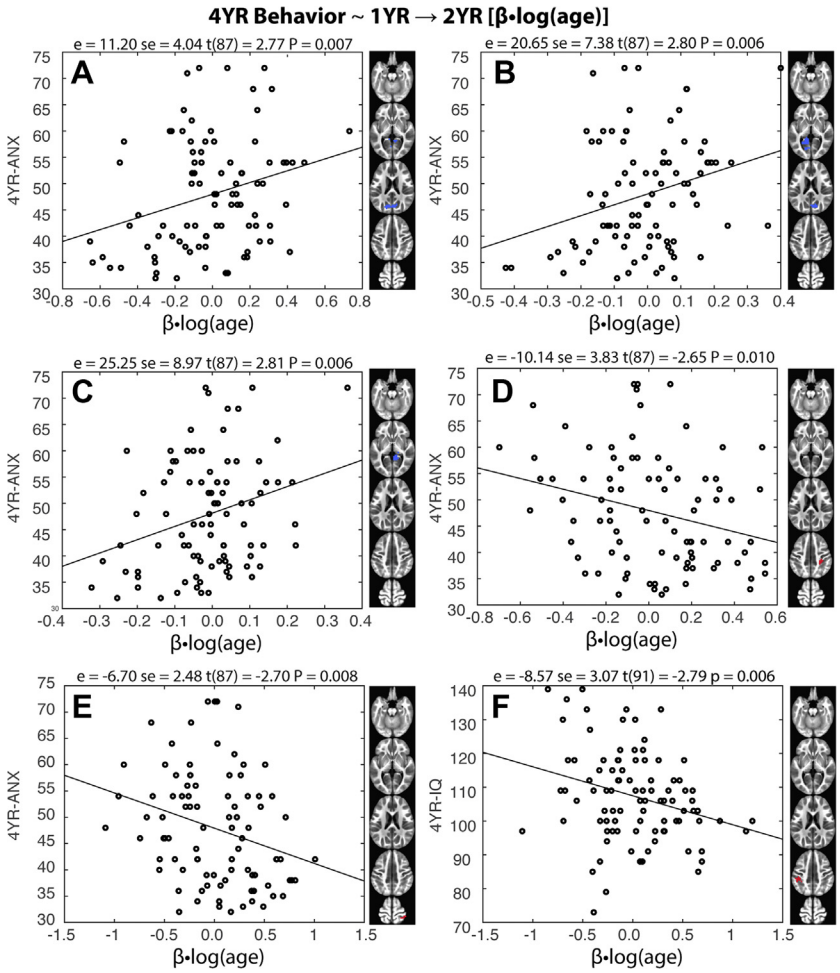

Figure 4. Longitudinal changes in infant amygdala functional connectivity (FC) during the second year (1YR→2YR) predict 4-year outcome measures. **(A–C)** 1YR→2YR left and right amygdala FC growth estimates [(β × log(age)] in the posterior regions of the default mode network (precuneus and cuneus subregions) and neighboring visual areas (lingual gyrus and calcarine cortex) positively predicted parent-reported anxiety at 4 years (4YR-ANX). **(D, E)** 1YR→2YR left amygdala FC growth estimates in the right sensorimotor cortices (supramarginal gyrus, inferior parietal lobule, and postcentral gyrus) negatively predicted 4YR-ANX. **(F)** 1YR→2YR left amygdala FC growth in left sensorimotor regions (inferior parietal lobule and postcentral gyrus) negatively predicted 4-year intelligence (4YR-IQ). e, effect estimate.

marginally significant network-level brain–behavior relationships were identified (see Supplemental Table S10).

DISCUSSION

In this study, we delineated the spatiotemporal dynamics of amygdala FC development during infancy. Specifically, the neonatal amygdala connectivity pattern featured adultlike positive connections with nearby subcortical areas (e.g., thalamus, caudate, putamen) and limbic areas (e.g., hippocampus, parahippocampus) but was missing positive connections with medial prefrontal areas and negative connectivity with lateral prefrontal and parietal regions. Interestingly, long-range positive connectivity with primary auditory and sensorimotor areas was observed in neonates. During the first year of life, both positive and negative connectivity with medial prefrontal and lateral prefrontal and parietal cortices emerged, while the neonate-specific long-range connections to primary auditory and sensorimotor areas regressed to zero or became negative. The amygdala connectivity patterns in 2-year-olds were qualitatively like those in 1-year-olds, but statistically detectable changes revealed a reversed direction of growth during the second year compared with that during the first year. Importantly, our results also highlighted the behavioral significance of infantile amygdala FC development for 4-year emotional and IQ measures. Specifically, at the regional level, 1-year-olds to 2-year-olds growth of amygdala FC with clusters within the precuneus or cuneus positively predicted 4-year anxiety, while that with sensorimotor cortices negatively predicted 4-year anxiety scores. At the network level, within-hemisphere connectivity between the left/right amygdala and CON-L/R network positively predicted 4-year emotional regulation scores. FC between the amygdala and CON-L/R, as well as sensorimotor-related regions, also significantly correlated with 4-year IQ scores. Taken together, our results improve our understanding of the brain basis of emerging emotion processing or regulation capabilities in infants and may serve as the first step toward imaging-based biomarkers for identification of risks for atypical emotional development.

The amygdala is among the brain's earliest areas to develop and becomes structurally and cytoarchitecturally adultlike before birth (39). Therefore, it is not surprising to observe significant adultlike FC of the amygdala with adjacent subcortical or limbic areas (9), including the hippocampus, parahippocampus, thalamus, caudate, and putamen, in neonates. Note that these areas are key nodes in the bottom-up

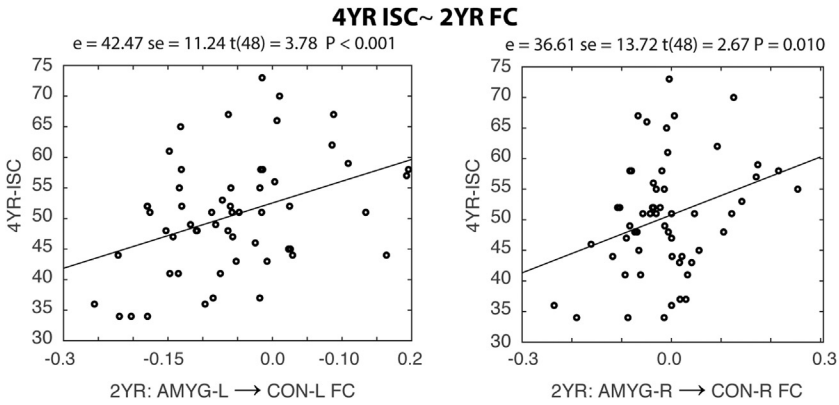

Figure 5. Amygdala functional connectivity (FC) with the executive control network (CON) at 2 years (2YR) predicts 4-year inhibitory self-control (4YR-ISC). Mean FC between the left/right amygdala and the left/right executive control network (CON-L/R) (see Supplemental Figure S1) at 2 years of age positively predicted parent-reported 4YR-ISC. e, effect estimate.

emotional appraisal system, so their synchronization at birth may enable neonates to generate critical emotional signals associated with life-essential endogenic stimuli (e.g., hunger) or exogenic stimuli (e.g., temperature, noise, smell). However, unlike in adults, our results also revealed direct amygdala functional connections to primary sensorimotor cortices (i.e., middle temporal and precentral or postcentral gyrus) that were unique in neonates; these connections disappeared by 1 year of age and were not present in 2-year-olds. Moreover, the positive connections with medial prefrontal regions and negative connectivity with lateral prefrontal and parietal areas that are typically observed in adults were absent in neonates but emerged in 1- and 2-year-olds. Taken together, these findings may suggest that in the absence of top-down emotional regulation connections (e.g., the negative connectivity with lateral prefrontal and parietal areas), neonates could rely on positive connections with sensorimotor areas for direct motor output (e.g., crying, kicking) after the initial emotional valence appraisal enabled by the amygdala–subcortical–limbic circuit. This is highly in line with behavioral findings highlighting emotional impulsivity and lack of self-regulation as hallmark behaviors of newborns (13,14).

During the first year, significant positive connectivity with medial prefrontal areas and negative connectivity with lateral prefrontal and parietal regions emerged, while those positive connections with primary sensorimotor cortices largely regressed. At the network level, these patterns resulted in a significant increase in amygdala connectivity with the DMN and SAL networks and a significant decrease in connectivity with the CON and primary AUD and SM networks. These dramatic changes resulted in a largely adultlike amygdala FC topology by the end of the first year, featuring positive connectivity with subcortical and ventral medial prefrontal areas and negative connectivity with lateral prefrontal and dorsal parietal regions (8,9). These observations, particularly the negative connections with lateral prefrontal and parietal regions within the CON network, provide strong support for the emergence of emotion regulation circuits during the first year of postnatal development. Our findings are in line with a body of research documenting the development of emotion-regulatory behaviors within the first 12 months of life (14). For example, infant self-soothing behaviors and attentional distraction strategies emerge during the first year as effective ways of emotional control's leading to decreased negative affect or anger (21,40,41). The emergence of negative connectivity between CON regions and the amygdala during the first year, as observed in this study, is consistent with this previous research and supports the early development of top-down emotion regulation strategies. This emergence, together with the disappearance of direct amygdala connectivity with sensorimotor areas, perhaps allows infants to regulate their emotional responses by shifting attention away from distressing stimuli and reducing impulsive motor output.

Although qualitatively similar connectivity patterns were observed in 2-year-olds compared with 1-year-olds, statistically detectable changes do occur during the second year of life. Interestingly, most of the growth during the second year is sign inversed compared with corresponding growth during the first year, and this trend is consistent at both regional and network levels. These findings may suggest important corrections or fine-tuning of first-year connectivity growth that may have important behavioral implications. Indeed, most of our significant brain–emotional behavioral relationships were related to either second-year growth of amygdala FC (Figure 4) or FC strength at 2 years of age (Figure 5). Of the seven significant predictions detected ($p < .01$), five of them are between negative connections of the amygdala with high-order executive control regions or networks (i.e., left/right amygdala and precuneus in Figure 4 and left/right amygdala and CON-L/R in Figure 5) and 4-year anxiety or inhibitory control scores, supporting the importance of the emotion regulation circuit development during the second year on anxiety management and inhibitory control outcomes at 4 years of age. Consistent with our findings, behavioral studies have documented significant growth of emotion regulation strategies during the second year of life (13,14,42). For example, Parritz (43) found that 18-month-olds engaged in greater directing, information seeking, and social referencing of mothers than did 12-month-olds in distress regulation. Similarly, Grolnick *et al.* (44) found that active self-distraction (e.g., engagement with substitute toys) was more frequently used by 2-year-olds than by 1-year-olds as the main behavioral strategy for emotion regulation. These observations, together with our findings of the significant prediction power of infant amygdala FC on 4-year emotional behavioral outcomes, highlight the

importance of infantile emotional circuit development for long-term behavioral outcomes.

Besides emotional outcomes, our results also revealed significant prediction power of infantile amygdala FC on 4-year IQ (Figure 4F and Supplemental Tables S9 and S10). Consistent with this finding, one recent study showed that infant temperament, specifically positive affectivity and emotion regulation capacity, at 4 months of age was predictive of school readiness in preschool-aged children (18). Although school readiness is only partially explained by IQ, it represents a set of important cognitive abilities that also includes self-regulation and inhibitory control [e.g., (45)]. Therefore, our findings with both emotional regulation and IQ are in line with this previous work. The results of this study provide strong support for the importance of infantile emotional circuitry and related behavioral development for later cognitive and academic outcomes.

In this study, the FC growth patterns were largely left–right symmetric (Supplemental Figure S2), but most of the detected brain–behavior relationships were associated with the left amygdala (30 of 39). This finding is consistent with previous reports of the functional bias of the left amygdala toward sustained emotional processing (46,47) and lateralized amygdala connectivity abnormalities associated with different psychiatric disorders (2,48) and prenatal drug exposure (49). These findings imply more significant emotional behavior relevance of the left amygdala during infancy, but more focused studies are needed to formally test this hypothesis.

Regarding sex effects, only three small clusters were detected (Supplemental Figure S3) indicating minor sex-related differences, which is consistent with other FC studies during infancy (29,50). However, the three detected clusters are potentially interesting given reported sex differences in amygdala FC in adolescents (51,52) and adults (53,54) and affective regulation behaviors in 6-month-old infants (55). While it is tantalizing to hypothesize that the observed FC differences may underlie these findings, future studies are needed to rigorously test these hypotheses.

There are several limitations of this study deserving further discussion. First, caution must be used when directly comparing our infant results with adult-based findings because the infant resting-state fMRI data were acquired during natural sleep, while most adult reports are based on data collected during the awake state (56). Moreover, different sleep stages (i.e., fluctuating wakefulness) represent another potential confound in resting-state fMRI (57), but objective monitoring using simultaneous electrophysiology has proven to be operationally challenging in this population. In older populations, an interesting shift from positive to negative amygdala–medial prefrontal cortex connectivity has been reported (58), and this shift has been shown to be sensitive to social adversity (i.e., maternal deprivation) (48). However, one needs to be cautious when attempting to extrapolate our results to connect the dots between infancy and childhood or adolescence findings because the later results were based on emotional task states rather than on natural sleep. In addition, nonlinear changes, which are similar between the first and second years of development (as shown here), likely exist beyond infancy, so future longitudinal studies covering a large age span are needed to delineate the long-term developmental trend of amygdala FC. Previous studies have shown risk-related alterations of amygdala FC development [e.g., prenatal drug exposure (49) and maternal depression (59,60)], so future studies of different risk factors and related behavioral implications are needed to better translate amygdala-based imaging findings into practical use. An additional limitation concerns the use of parent-report outcome data. Several factors contribute to how parents rate their children's behaviors, including, but not limited to, their own emotional states. This study does not have information about parent mood states or diagnoses that could affect how parents rate their children on measures such as anxiety. Given the complicated interactions among parent emotions and behaviors, child emotions and behaviors, and how parents report on their children, unraveling significant relations can be difficult. Additional research should consider parents' mood status as a factor and/or should use task-based assessments to measure child emotion-related behaviors in attempt to clarify these associations.

In conclusion, this study provides the first set of results delineating the spatiotemporal dynamics of amygdala FC development during infancy. The nonlinear developmental trends together with significant predictions of 4-year behavioral outcomes in both emotional and cognitive domains highlight the importance of emotional circuit development during infancy for long-term outcomes. If independently validated, these results may provide important candidates for imaging-based biomarkers for early identification of risks related to not only emotional problems but also cognitive development delays.

ACKNOWLEDGMENTS AND DISCLOSURES

This work was supported by the National Institutes of Health (Grant Nos. R01DA042988, R01DA043678, R21NS088975, R21DA043171, and R03DA036645 [to WG]; Grant No. T32-MH106440 [to RLS]; Grant No. U01MH110274 [to WL]; and Grant Nos. R01MH064065 and R01HD05300 [to JHG]) and by Cedars–Sinai Precision Medicine Initiative Award and institutional support (to WG).

We are grateful to the research assistants who collected and scored the 4-year-old cognitive data and/or scored the Behavior Assessment System for Children–second edition and/or the Behavior Rating Inventory of Executive Function–Preschool version over the years for this study: Haley Parrish Black, Sadie Hasbrouck, Monica Ferenz Guy, Kassidy Jezierski, Margaret Hamilton Fox, Molly McGinnis, Mallory Turner, Emma Brink, Emily Bostwick, Margo Williams, Neha Patel, Portia Henderson, and Jenna Obitko.

The authors report no biomedical financial interests or potential conflicts of interest.

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Received May 25, 2018; revised Jul 21, 2018; accepted Aug 14, 2018.

Supplementary material cited in this article is available online at https://doi.org/10.1016/j.bpsc.2018.08.010.

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