Neonatal Amygdala Functional Connectivity at Rest in Healthy and Preterm Infants and Early Internalizing Symptoms

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Objective: Alterations in the normal developmental trajectory of amygdala resting state functional connectivity (rs-FC) have been associated with atypical emotional processes and psychopathology. Little is known, however, regarding amygdala rs-FC at birth or its relevance to outcomes. This study examined amygdala rs-FC in healthy, full-term (FT) infants and in very preterm (VPT) infants, and tested whether variability of neonatal amygdala rs-FC predicted internalizing symptoms at age 2 years.

Method: Resting state fMRI data were obtained shortly after birth from 65 FT infants (gestational age [GA] ≥36 weeks) and 57 VPT infants (GA <30 weeks) at term equivalent. Voxelwise correlation analyses were performed using individual-specific bilateral amygdala regions of interest. Total internalizing symptoms and the behavioral inhibition, depression/withdrawal, general anxiety, and separation distress subdomains were assessed in a subset (n = 44) at age 2 years using the Infant Toddler Social Emotional Assessment.

Results: In FT and VPT infants, the amygdala demonstrated positive correlations with subcortical and limbic structures and negative correlations with cortical regions, although magnitudes were decreased in VPT infants. Neonatal amygdala rs-FC predicted internalizing symptoms at age 2 years with regional specificity consistent with known pathophysiology in older populations: connectivity with the anterior insula related to depressive symptoms, with the dorsal anterior cingulate related to generalized anxiety, and with the medial prefrontal cortex related to behavioral inhibition.

Conclusion: Amygdala rs-FC is well established in neonates. Variability in regional neonatal amygdala rs-FC predicted internalizing symptoms at 2 years, suggesting that risk for internalizing symptoms may be established in neonatal amygdala functional connectivity patterns.

Key words: infant, internalizing, amygdala, functional connectivity

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The amygdala plays a critical role in the expression and processing of emotion and in determining the emotional significance of stimuli.[1-3] Amygdala activity increases in response to emotional stimuli in healthy children and adults,[4,5] and variations in activation are associated with affective disorders at both ages.[6,7] Amygdala activation is putatively regulated through connections with numerous subcortical and cortical structures,[2,8,9] and amygdala structural connections undergo substantial modification during the first years of life.[10-13] Critical unresolved issues addressed in this study include defining the normative patterns of neonatal amygdala functional connectivity and the relationship between neonatal amygdala functional connectivity and risk for development of early childhood affective symptoms.

Resting state functional magnetic resonance imaging (rs-fMRI) has been used to investigate functional connectivity (rs-FC) in the developing brain.[14,15] Infants and children demonstrate rs-FC patterns, including those involving the amygdala,[14,16,17] that evolve with age toward those of adults.[18] One relationship that has been well characterized during early development is between the amygdala and medial prefrontal cortex (mPFC).[19,20] The mPFC is an important regulator of amygdala activation,[2,8,9] and variation in rs-FC measures between these regions in older children and adults has been linked to anxiety,[21-23] behavioral inhibition,[24] and harm avoidance.[25] In addition, altered rs-FC between the amygdala and other cortical and subcortical regions was reported in 6-month-old infants at high risk for developing internalizing symptoms[17] and is associated with infant fear at age 6 months,[26] atypical childhood emotional and cognitive processes,[16,27] and adult psychiatric disorders.[28,29]

Although some preliminary evidence exists linking amygdala rs-FC to internalizing symptoms even in infancy, there is almost no information about factors that might moderate the nature of such relationships. One potential modifying factor is prematurity. Core features of the "preterm behavioral phenotype" include social difficulties and internalizing symptoms,[30] and very preterm children (VPT; gestational age [GA] <32 weeks) have increased rates of behavioral inhibition and introversion.[31,32] Premature

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infants provide a unique opportunity to study variability in amygdala rs-FC relative to both typical development and emergence of affective symptoms given prior evidence of altered rs-FC in preterm infants in other regions.[14] Prior studies have linked prematurity-associated neonatal brain abnormalities to subsequent social–emotional development.[33-35] However, it is unclear whether the etiology of internalizing symptoms for preterm children differs from that leading to internalizing symptoms in full-term (FT) children.

We investigated patterns of amygdala rs-FC in healthy, FT infants and VPT infants at term-equivalent postmenstrual age (PMA) and determined whether variations in neonatal connectivity predicted internalizing symptoms at age 2 years. The goals of this study were threefold: to characterize regions demonstrating synchronous, spontaneous neuronal activity with the amygdala during infancy (*i.e.*, rs-FC); to assess whether variation in amygdala rs-FC relates to development of early-onset internalizing symptoms; and to evaluate whether prematurity alters amygdala rs-FC and/or modifies relationships between amygdala rs-FC and early-onset internalizing symptoms.

METHOD

Participants

VPT infants were recruited from St. Louis Children's Hospital Neonatal Intensive Care Unit. FT infants (GA ≥36 weeks) were recruited from the adjoining mother–baby unit at Barnes-Jewish Hospital from a contemporaneous, companion prospective study evaluating the association between electronic fetal heart rate recordings and neonatal brain development.[36] FT infants had no recorded history of in utero illicit substance exposure nor evidence of acidosis (pH <7.20) on cord blood gas. Infants with chromosomal abnormalities or suspected/proven congenital infection were excluded. Parental written informed consent was obtained prior to participation. The study was approved by the Washington University Human Studies Committee.

Anatomic MR images were reviewed by a neuroradiologist (J.S.S.) and pediatric neurologist (C.D.S.). Exclusion criteria were grade III to IV intraventricular hemorrhage, cystic periventricular leukomalacia, moderate–severe cerebellar hemorrhage, and/or cortical/deep nuclear gray matter lesions.[37]

Data Acquisition

FT infants underwent MRI within the first 4 days of life and were scanned at a mean PMA of 39.4 weeks (±1.2 weeks). VPT infants underwent MRI at term-equivalent PMA (38.0 weeks, ±1.5 weeks). All infants were imaged without sedation during sleep or while resting quietly[38] when clinically stable to travel to the MRI scanner. Identical scanning procedures were used for all infants. All imaging was performed on a Siemens Trio 3T scanner (Erlangen, Germany) using an infant-specific, quadrature head coil (Advanced Imaging Research, Cleveland, OH). Structural images were collected using a T2-weighted sequence (TR 8600 milliseconds; TE 161 milliseconds; voxel size 1×1×1 mm). rs-fMRI data were collected using a gradient echo, echo-planar image (EPI) sequence sensitized to T2* blood oxygen level–dependent (BOLD) contrast (TR 2910 milliseconds; TE 28 milliseconds; voxel size 2.4×2.4×2.4 mm; flip angle 90°; field of view [FOV] 151 mm; and matrix size 64 × 64). Each fMRI run included 200 volumes (frames). A minimum of one run (9.6 minutes) was obtained in each infant, with additional runs acquired in a subset of participants depending upon tolerance.

Data Analysis

The rs-fMRI data were preprocessed as previously described using in-house software (ftp://imaging.wustl.edu/pub/raichlab/4dfp_tools/).[39] Magnetization inhomogeneity-related distortions were corrected using a mean field map technique.[40] Atlas transformation was computed using infant templates. Volumetric time series in adult Talairach atlas space (3×3×3-mm voxels) were generated, combining motion correction and atlas transformation in a single resampling step. Additional preprocessing included regression of nuisance waveforms derived from rigid body motion correction, cerebrospinal fluid, and white matter regions, plus whole brain global signal. The data were low-pass filtered and spatially smoothed (see Supplementary Methods, available online).

Frames affected by sudden change in head position (volume-to-volume head displacement ≥0.5 mm) or root-mean-squared BOLD signal intensity change (DVARS ≥0.5%) were excluded from the rs-fMRI computations ("scrubbing").[41] Data passing more rigorous censoring criteria (volume-to-volume head displacement ≥0.25 mm or DVARS ≥0.3%) were also analyzed (see Figure S1, available online). A minimum of 5 minutes of rs-fMRI data, excluding censored frames, was required for inclusion in the analysis. FT infants (n = 65) provided an average of 156 frames (±42, range 100–357 frames, ~7.8 minutes, mean FD 0.17 mm), with 36% of acquired frames censored. VPT infants (n = 57) provided an average of 182 frames (±53, range 102–381 frames, ~9.1 minutes, mean FD 0.15), with 24% of acquired frames censored.

Amygdala Regions of Interest

Individualized bilateral amygdala regions of interest (ROIs) were created for each participant (Figure 1A). T2-weighted images were loaded into ANALYZE version 10.0 (Mayo Foundation, Rochester, MN). The amygdala was identified in the coronal plane using adjacent landmarks, including the temporal horn and hippocampus. The ROI was then manually drawn in 1×1×1 mm voxel space; ROIs were cross-checked in sagittal and axial orientations. These ROIs were reviewed and manually adjusted as needed by a neuroradiologist (J.S.S.) and resampled to 3×3×3 mm voxel atlas space for extraction of the BOLD time series by averaging over all included voxels.[42] There were no significant differences in ROI sizes between groups (left amygdala ROI: term 5.6 voxels and preterm 5.9 voxels, $p = .60$; right amygdala ROI: term 5.7 voxels and preterm 6.8 voxels, $p = .14$). Peak coordinates were extracted (Talairach x, y, z: left amygdala −23, −7, −18; right amygdala 24, −8, −14).

Functional Connectivity Analyses

Amygdala rs-FC was investigated using a whole-brain voxelwise approach. Using individual-specific bilateral amygdala ROIs for each participant, correlation maps were computed using Pearson correlations. Correlation coefficients were Fisher z transformed (generating z[r] correlation maps).

Internalizing Symptoms

In all, 41 of the 57 VPT participants were enrolled in our longitudinal study, which included age 2 neurodevelopmental follow-up. Of the 41 participants, 9 were lost to follow-up, 4 had incomplete data, and 1 died. A total of 25 FT children with high-quality imaging data were selected from the companion study for age 2 assessments to