NeuroImage: Clinical 12 (2016) 381–388

Contents lists available at ScienceDirect

NeuroImage: Clinical

journal homepage: www.elsevier.com/locate/ynicl

Prenatal stress alters amygdala functional connectivity in preterm neonates

Dustin Scheinost[a,*], Soo Hyun Kwon[b], Cheryl Lacadie[a], Gordon Sze[a], Rajita Sinha[c,d,e], R. Todd Constable[a,f], Laura R. Ment[b,g]

[a]Department of Radiology and Biomedical Imaging, Yale School of Medicine, New Haven, CT, United States
[b]Department of Pediatrics, Yale School of Medicine, New Haven, CT, United States
[c]Department of Psychiatry, Yale School of Medicine, New Haven, CT, United States
[d]Department of Child Study, Yale School of Medicine, New Haven, CT, United States
[e]Department of Neuroscience, Yale School of Medicine, New Haven, CT, United States
[f]Department of Neurosurgery, Yale School of Medicine, New Haven, CT, United States
[g]Department of Neurology, Yale School of Medicine, New Haven, CT, United States

ARTICLE INFO

Article history:
Received 18 May 2016
Received in revised form 7 August 2016
Accepted 9 August 2016
Available online 10 August 2016

Keywords:
Amygdala
Prenatal stress
Very preterm
Infant brain
Functional connectivity

ABSTRACT

Exposure to prenatal and early-life stress results in alterations in neural connectivity and an increased risk for neuropsychiatric disorders. In particular, alterations in amygdala connectivity have emerged as a common effect across several recent studies. However, the impact of prenatal stress exposure on the functional organization of the amygdala has yet to be explored in the prematurely-born, a population at high risk for neuropsychiatric disorders.

We test the hypothesis that preterm birth and prenatal exposure to maternal stress alter functional connectivity of the amygdala using two independent cohorts. The first cohort is used to establish the effects of preterm birth and consists of 12 very preterm neonates and 25 term controls, all without prenatal stress exposure. The second is analyzed to establish the effects of prenatal stress exposure and consists of 16 extremely preterm neonates with prenatal stress exposure and 10 extremely preterm neonates with no known prenatal stress exposure. Standard resting-state functional magnetic resonance imaging and seed connectivity methods are used.

When compared to term controls, very preterm neonates show significantly reduced connectivity between the amygdala and the thalamus, the hypothalamus, the brainstem, and the insula ($p < 0.05$). Similarly, when compared to extremely preterm neonates without exposure to prenatal stress, extremely preterm neonates with exposure to prenatal stress show significantly less connectivity between the left amygdala and the thalamus, the hypothalamus, and the peristriate cortex ($p < 0.05$). Exploratory analysis of the combined cohorts suggests additive effects of prenatal stress on alterations in amygdala connectivity associated with preterm birth.

Functional connectivity from the amygdala to other subcortical regions is decreased in preterm neonates compared to term controls. In addition, these data, for the first time, suggest that prenatal stress exposure amplifies these decreases.

1. Introduction

Preterm birth, birth before 37 weeks gestation, alters connectivity in the developing brain (Allievi et al., 2016; Karolis et al., 2016; Kwon et al., 2016; Smyser et al., 2016). While these alterations have been largely attributed to postnatal perturbations (Brummelte et al., 2012; Pineda et al., 2014; Rogers et al., 2016; Smith et al., 2011; Smyser et al., 2013), emerging data suggest that prenatal exposure to maternal stress may also play a role (Bronson and Bale, 2014; Chen and Baram, 2016; Constantinof et al., 2015; Provencal and Binder, 2015). Stress is a signal in response to challenging and uncontrollable adverse events and perceived threat (McEwen, 2002; Sinha, 2008). For typically developing term neonates, exposure to early life stress is a risk factor for neuropsychiatric disorders ranging from autism spectrum disorder and ADHD to depression and schizophrenia (Brown, 2012; Chin-Lun Hung et al., 2015; Entringer et al., 2015; Fox et al., 2010; Howerton and Bale, 2012; Khashan et al., 2008; Kinney et al., 2008; Koenig et al., 2002; Li et al., 2010; Pearson et al., 2013; Shonkoff et al., 2012; Talge et al., 2007). In contrast, the impact of prenatal stress on the connectome of the prematurely-born remains largely unexplored.

Stress-related symptoms of anxiety and depression are common in pregnant women (Burns et al., 2015), and epidemiologic studies suggest that not only is prenatal stress associated with preterm birth and low birth weight, it may also alter brain development (Baron et al., 2015;

* Corresponding author: MRRC, TAC N131, 300 Cedar St, New Haven, CT 06510, United States.
E-mail address: dustin.scheinost@yale.edu (D. Scheinost).

http://dx.doi.org/10.1016/j.nicl.2016.08.010

Burns et al., 2015; Castro et al., 2015; Robbins et al., 2014). In a broad range of preclinical and clinical studies, prenatal exposure to stress-related conditions has been shown to potently stimulate biological stress pathways,(Constantinof et al., 2015) alter synaptogenesis (Bale et al., 2010; Singh-Taylor et al., 2015) and result in changes in structural brain development (Qiu et al., 2015; Qiu et al., 2013; Rifkin-Graboi et al., 2013; Rifkin-Graboi et al., 2015; Van Dam et al., 2014).

Most MRI studies demonstrating the effect of stress during gestation and early life have been conducted in older subjects with alterations of amygdala structure and function being a common effect across studies (Buss et al., 2012; Gee et al., 2013a; Malter Cohen et al., 2013). Similarly, emerging reports of infants evaluated during the first postnatal year also highlight the vulnerability of the amygdala to early life stress. At 1–2 weeks of age, healthy term neonates with prenatal maternal depression exposure show lower fractional anisotropy, a measure of microstructural maturation, in the right amygdala compared to infants with no exposure (Rifkin-Graboi et al., 2013). Six month old infants born to mothers with high prenatal depressive symptoms show greater functional connectivity of the left amygdala with the left temporal cortex and insula, as well as the bilateral anterior cingulate, medial orbitofrontal and ventromedial prefrontal cortices, consistent with the pattern found in older children and adults with depressive disorders.(Qiu et al., 2015) Finally, infants between 6 and 12 months of age show increasing connectivity between the posterior cingulate and the amygdala with increasing inter-parental conflict and negative emotionality (Graham et al., 2015). However, amygdala functional connectivity has been less well-studied in those prematurely born. Preliminary findings in preterm neonates (Rogers et al., 2015) and young adults (Papini et al., 2014) suggest reduced amygdala connectivity to subcortical, limbic, and frontal regions.

Following these studies, using resting-state functional MRI (fMRI), we test the hypothesis that prenatal exposure to maternal stress reduces functional connectivity of the amygdala with these regions in preterm infants. First, we establish normal amygdala functional networks in neonates. Next, we show that at term equivalent age (TEA; 40–44 weeks postmenstrual age) very preterm neonates exhibit reduced amygdala connectivity to subcortical regions. Then, in an independent cohort of extremely preterm neonates imaged at 36–43 weeks postmenstrual age, we show that preterm neonates exposed to prenatal stress exhibit reduced amygdala connectivity to similar subcortical regions. Finally, as exploratory analysis, we combine the cohorts to show a synergistic relationship between prenatal stress and preterm birth, resulting in greatest alterations of amygdala connectivity for preterm neonates with prenatal stress exposure. Together, these results suggest that reduction in amygdala connectivity associated with preterm birth is moderated by exposure to prenatal stress.

2. Methods

This study was approved by the Yale University Human Investigation Committee.

2.1. Participants

2.1.1. Cohort 1

Very preterm neonates born (<32 weeks gestation) with a birth weight (BW) between 500 and 1500 g and healthy term controls born between 37 and 41 weeks postmenstrual age (PMA) were eligible for the protocol and prospectively enrolled between 9-01-10 and 10-31-13. All were inborn and appropriate for gestational age (AGA). Exclusion criteria included evidence of congenital infections, congenital malformations and/or chromosomal disorders, seizures, intraventricular hemorrhage (IVH), periventricular leukomalacia or focal abnormalities on a previous or study MRI. In addition, all preterm participants had normal cranial ultrasounds prior to 7–14 days of life. This cohort has been previously published (Kwon et al., 2014; Scheinost et al., 2016) and includes 25 term controls and 12 preterm neonates. Very preterm neonates were scanned at term equivalent age. No neonate in this cohort had documented exposure to prenatal stress.

2.1.2. Cohort 2

Extremely preterm neonates (<28 weeks gestation) were eligible for the protocol and prospectively enrolled between 4-01-12 and 10-31-15. All were inborn and appropriate for gestational age. Psychological stress during pregnancy was obtained by retrospective electronic chart review. For the purposes of this preliminary report, we employed a binary classification of prenatal stress exposure as documented by the attending physician's diagnosis of depression and/or anxiety in the maternal medical chart. Exclusion criteria included maternal pharmacologic treatment for these conditions as well as neonatal evidence of congenital infections, congenital malformations, chromosomal disorders, seizures, or major abnormalities on MRI, including intraventricular hemorrhage, periventricular leukomalacia, and ventriculomegaly. In addition, all preterm participants had normal cranial ultrasounds within the first 7–14 days of life. Twenty eight extremely preterm neonates met all inclusion criteria, and data from 10 extremely preterm neonates with prenatal stress exposure and 16 extremely preterm neonates with no stress exposure were analyzed after exclusion of 2 neonates due to motion artifact. All connectivity processing was performed blinded to prenatal stress exposure for Cohort 2.

2.2. Imaging parameters

Subjects were scanned without sedation using a feed-and-wrap protocol in either a 3T Siemens (Erlangen, Germany) TIM Trio MR system for Cohort 1 or a 3T Siemens Verio Clinical system for Cohort 2. Both cohorts were scanned using a 32-channel parallel receiver head coil. Localizer images were acquired for prescribing the functional image volumes, aligning the seventh or eighth slice parallel to the plane transecting the anterior and posterior commissures. T1-weighted 2D anatomical images were collected (TR = 300 ms, TE = 2.47 ms, FoV = 220 mm, matrix size = 256 × 256, slice thickness = 4 mm, Flip Angle = 60°, Bandwidth = 300 Hz/pixel with 25 slices) with 25 AC-PC aligned axial-oblique slices in addition to 3D anatomical scans using Magnetization Prepared Rapid Gradient Echo (MPRAGE) (176 contiguous sagittal slices, slice thickness = 1 mm, matrix size = 256 × 256, FoV = 256 mm, TR = 2530 ms, TE = 2.77, Flip Angle = 7°, Bandwidth = 179 Hz/pixel). Similarly for Cohort 2, in-plane resolution and the number of slices were modified in response to time limitations. After these structural images, acquisition of functional data began in the same slice locations as the axial-oblique T1-weighted data. Similarly for Cohort 2, in-plane resolution and number of slices were modified based on time limitations of the scanner. Functional images were collected using an echo-planar image gradient echo pulse sequence (TR = 1500 ms, TE = 27 ms, FoV = 220 mm, matrix size = 64 × 64, slice thickness = 4 mm, Flip Angle = 60°, Bandwidth = 2520 Hz/pixel, 25 slices). Two to three functional runs were collected for Cohort 1 and one functional run was collected for Cohort 2. Functional runs consisted of 186 volumes (approximately 5 min scan length) for Cohort 1 and 235 volumes for Cohort 2 after the first 6 volumes were removed to allow the magnetization to reach the steady-state.

2.3. Connectivity preprocessing

Images were slice-time and motion corrected using SPM5 (http://www.fil.ion.ucl.ac.uk/spm/software/spm5/). All further analyses were performed using BioImage Suite.(Joshi et al., 2011). Several covariates of no interest were regressed from the data including linear and quadratic drift, six rigid-body motion parameters, mean cerebral-spinal-fluid (CSF) signal, mean white-matter signal, and overall global signal. The data were temporally smoothed with a zero mean unit variance Gaussian filter (approximate cutoff frequency = 0.12 Hz). A gray matter