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Newborn amygdala connectivity and early emerging fear

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ABSTRACT

Connectivity between the amygdala, insula (Amygdala-aI) and ventral medial prefrontal cortex (Amygdala-vmPFC) have been implicated in individual variability in fear and vulnerability to psychiatric disorders. However, it is currently unknown to what extent connectivity between these regions in the newborn period is relevant for the development of fear and other aspects of negative emotionality (NE), such as sadness. Here, we investigate newborn Am-Ins and Am-vmPFC resting state functional connectivity in relation to developmental trajectories of fear and sadness over the first two years of life using data from the Infant Behavior Questionnaire Revised (IBQ-R) and Early Childhood Behavior Questionnaire (ECBQ) (N = 62). Stronger newborn amygdala connectivity predicts higher fear and sadness at 6-months-of-age and less change from 6 to 24-months-of-age. Interestingly, Am-Ins connectivity was specifically relevant for fear and not sadness, while Am-vmPFC was associated only with sadness. Associations remained consistent after considering variation in maternal sensitivity and maternal postnatal depressive symptomology. Already by the time of birth, individual differences in amygdala connectivity are relevant for the expression of fear over the first two-years-of-life. Additionally, specificity is observed, such that connections relevant for fear development are distinct from those predicting sadness trajectories.

1. Introduction

1.1. The importance of examining early neural correlates of fear

Fear is an emotion essential for adaptive functioning expressed when an animal perceives potential danger (Milad and Quirk, 2012; Phelps and LeDoux, 2005). While fear expression is essential for adaptation, heightened levels of fear are associated with psychopathology (Engle and Mcelwain, 2011; Gjone and Stevenson, 1997). Limbic-prefrontal brain systems play a critical role in normative and pathological variability in fear in children and adults (Etkin et al., 2011, Etkin and Wager, 2007; Milad and Quirk, 2012; Qin et al., 2014; Ruocco et al., 2013); however, how these brain systems in the newborn period contribute to the early development of fear is poorly understood.

1.2. Typical development of fear

Fear expression typically increases over the first year of life as increasing mobility facilitates increasingly complex interactions with the environment, and increasing exposure to threatening stimuli (Shaw et al., 2000). It is likely that fear expression rises during the first year of life as it becomes ecologically significant for the infant's survival (Callaghan et al., 2014). By the second year of life, the infant's expression of fear stabilizes (Garstein and Rothbart, 2003; Partridge, 2007). This is likely due to the infant's increasing ability to regulate emotions both independently, and through use of caregiver support (Gartstein et al., 2012; Lemery et al., 1999). Due to changes in fear expression over time (Gartstein et al., 2010; Lipscomb et al., 2012; Partridge, 2007), it is critical to examine fear at multiple time points to capture it's full early developmental trajectory.

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1.3. Early brain connectivity as a predictor of fear

We have previously shown that newborn amygdala functional connections predict early fear expression (Graham et al., 2016). Specifically, stronger connectivity between the amygdala and bilateral anterior insula (amygdala-aI) was associated with higher fear at 6-months-of-age. This finding is in line with adult literature suggesting an important role for amygdala-aI connectivity in normative (Baur et al., 2013), and pathological fear (Etkin and Wager, 2007; Rabinak et al., 2011; Sripada et al., 2012). This suggests potential continuity in the neural circuitry underlying the early emergence of fear. Furthermore, we have previously shown that stronger connectivity between the amygdalae and ventral medial prefrontal cortex (amygdala-vMPFC) at birth is associated with a phenotype characterized by higher fear and more advanced cognitive development at 6-months-of-age potentially suggesting that a balance between negative affect and cognitive skills is relevant for effectively regulating negative affect (Degnan and Fox, 2007; Gartstein et al., 2012; Nigg, 2006). In line with this interpretation, amygdala-vMPFC connectivity plays an important role in emotion regulation in children and adults (Gee et al., 2013; Milad et al., 2007; Schiller and Delgado, 2010; Silvers et al., 2017), and has been frequently implicated in conditions involving poor regulation of negative affect, including anxiety (Casey and Lee, 2015; Kim et al., 2011; Roy et al., 2013a) and depression (Burghy et al., 2012; Connolly et al., 2017; Wang et al., 2013). However, the role of amygdala-vMPFC connectivity in the development of fear and other aspects of negative emotionality (NE) during infancy and toddlerhood has not been examined.

1.4. Key influences on the development of fear

During infancy, caregivers serve an important role in influencing infants emotion reactivity and regulation (Bernier et al., 2016). Infants use behavioral cues to communicate their emotional state to caregivers who aid in emotion regulation through quick and accurate responses to the infant's expressed needs (Thomas et al., 2017). Responsive caregivers can effectively reduce infant distress, decreasing infants' expression of fear and other aspects of NE over time (Leerkes et al., 2009). Moreover, neural phenotypes may increase or decrease the influence of maternal responsivity on emerging fear (Ellis et al., 2011). It is therefore important to consider the caregiving environment as well as interactive effects of maternal responsivity and newborn brain phenotypes in relation to emerging fear.

Maternal depressive symptomology has been shown to influence maternal reporting and observation of infant NE. Mothers with greater depressive symptoms are more likely to rate their infants as more difficult than parents who are not depressed (Parade and Leerkes, 2008). Additionally, more severe maternal depressive symptoms have been associated with greater increases in infant fear from 8 to 12 months of age (Gartstein et al., 2010). Research to date also suggests some specificity, such that the association between maternal symptomatology in the postpartum period and infant NE is specific to maternal depression versus anxiety (Feldman et al., 2009). These results highlight the importance of considering maternal depressive symptoms in examining infant fear development.

1.5. Present study

In the current study we examine how newborn amygdala-vMPFC and amygdala-aI connectivity relate to the developmental trajectory of fear over the first two years of life. Further, to see if these connections are specific to emerging fear or more generalizable to other aspects of NE, we also examine them in relation to emerging sadness. Finally, we consider how maternal responsivity and depressive symptomology may moderate associations between these newborn amygdala connections and subsequent development of NE.

Based on previous work (Etkin and Wager, 2007; Graham et al., 2016; Rabinak et al., 2011; Sripada et al., 2012), we hypothesize stronger amygdala-aI connectivity will be associated with higher fear at 6-months-of-age and greater increase in fear over the first two years of life. Due to the role of amygdala-vMPFC connectivity in depression and anxiety in older populations (Casey and Lee, 2015; Wang et al., 2013), we anticipate this connection will demonstrate associations with both fear and sadness development. While stronger amygdala-vMPFC connectivity is associated with greater emotion regulatory ability in adolescents and adults (Silvers et al., 2017), due to the developmental switch in how amygdala activity relates to vMPFC activity while viewing negatively valenced stimuli (Gee et al., 2013), we anticipate stronger amygdala-vMPFC connectivity will be associated with higher levels of NE at 6-months and greater increase in NE from 12 to 24 months-of-age. Finally, we anticipate associations between newborn amygdala connectivity and NE development will be moderated by levels of maternal responsivity.

2. Methods and materials

2.1. Participants

Infants included in this study (N = 62) were part of an ongoing longitudinal study of mothers and infants conducted at the University of California Irvine (for details see Moog et al., 2017). Mothers were recruited during their first trimester of pregnancy. Exclusionary criteria included: maternal use of systemic corticosteroids or psychotropic medications during pregnancy, infant birth before 34 weeks' gestation, and infant congenital, genetic, or neurological disorder. An MRI and fMRI scan was completed when infants were approximately 4-weeks-of-age ($M \pm SD$, 3.7 $\pm$ 1.7). Temperament assessments were completed at 6, 9, 12, and 24-months-of-age. Detailed demographic information is provided in Table 1. All procedures were approved by the Institutional Review Board at the University of California, Irvine. The number of subjects with data at each time point is provided in Table 2.

2.2. MRI and fMRI data acquisition and processing

2.2.1. Data acquisition

As described in our previous work (Graham et al., 2016, 2017, 2018; Rudolph et al., 2018) a TIM Trio, Siemens Medical System 3.0 T scanner was used to collected neuroimaging data with infants during natural sleep. A T2-weighted scan (TR = 3200 ms, echo time = 255 ms, resolution = 1 × 1 x 1 mm, 4.18 min) was used as an anatomical reference for functional images. A T1-weighted scan (MR-RAGE TR = 2400 ms, inversion time = 1200 ms, echo time = 3.16 ms, flip angle = 8°, resolution = 1 × 1 x 1 mm, 6.18 min) was used in conjunction with the T2-weighted scan for amygdala segmentation. To obtain functional images for rs-fcMRI, a gradient-echo, echoplanar imaging (EPI) sequence sensitive to blood oxygen level-dependent (BOLD) contrast (TR = 2000 ms; TE = 30 ms; FOV = 220 × 220 x 160 mm; flip angle = 77°) was used. Using 32 ascending-interleaved 4 mm axial slices with a 1 mm skip, full brain coverage was obtained. Steady-state magnetization was assumed after 4 frames (8 ~ s). Functional data was obtained in a single scan consisting of 150 volumes for early participants (N = 8), and increased to 195 volumes for the remaining participants (N = 54) in later stages of the study to increase the likelihood of acquiring a sufficient number of volumes for analysis. Only functional scans with at least 4 min of data (after volume removal for motion) were included in the present study.

2.2.2. fMRI data preprocessing

The Brain Extraction Tool from the FMRIB Software library (Beckmann et al., 2006; Smith et al., 2001; Smith, 2002) was used as an initial step to separate the brain from the rest of the head tissue in images. Next, an in house tool was used to remove the remaining skull. This tool involved registration of a skull stripped infant atlas (0- to 2-

Table 1
Demographics.

Age in Weeks	Mean (SD)
Gestational age at birth	39.1 (1.5)
Age at fMRI data collection	3.7 (1.7)
Age at 6 moth behavioral assessment	28.0 (2.4)
Age at 9 month behavioral assessment	39.9 (7.4)
Age at 12 month behavioral assessment	55.0 (3.1)
Age at 24 month behavioral assessment	240.0 (35.1)
	Percentage
Sex	
Male	54.8
Female	45.2
Race/ethnicity	
Caucasian non-Hispanic	37.7
African American non-Hispanic	2.6
Asian non-Hispanic	7.8
Multi-racial non-Hispanic	10.4
Caucasian Hispanic	33.8
Asian Hispanic	1.3
Multi-racial Hispanic	5.2
Other Hispanic	1.2
Highest level of maternal education	
Primary, Elementary, or Middle School	1.6
High-school or test equivalent	14.5
Technical or vocational school	12.9
Some college, but no degree	30.6
Associates degree	3.2
Bachelor's degree	19.4
Graduate level degree	12.9
Certificate	4.8
Gross annual household income	
< $15,000	9.6
$15,000–29,999	19.2
$30,000–49,999	27.4
$50,000–100,000	35.6
> $100,000	8.2

Table 2
Means, Standard Deviations and Internal Reliabilities for IBQ & ECBQ Dimensions.

IBQ-R Dimension	N	M	SD	α
Sadness- 6 months	56	3.59	1.04	0.86
Sadness- 9 months	45	3.73	0.98	0.87
Sadness- 12 months	43	3.82	1.01	0.85
Sadness- 24 months	49	3.05	0.76	0.46
Fear- 6 months	56	2.84	1.06	0.91
Fear- 9 months	44	3.23	1.09	0.91
Fear- 12 months	43	3.40	1.13	0.89
Fear- 24 months	49	2.53	1.04	0.72

Note: Eight out of nine alphas calculated exceeded 0.70, demonstrating adequate internal consistency. Sadness at 24 months was noticeably lower (α = .46).

month age range; MRI Study of Normal Brain Development; Fonov et al., 2011, 2009) to the individual image, which allowed for creation of a refined individual brain mask. Functional images were preprocessed to reduce artifacts (Miezin et al., 2000) as in our prior work (see Graham et al., 2016). Atlas transformation of the functional data was computed for each individual via the high-resolution T2 scan (see Graham et al., 2016). Visual inspection of data resulted in the loss of two subjects for poor quality EPI scans, and one subject for a structural abnormality identified in the high resolution T2- weighted scan.

2.2.3. rs-fcMRI preprocessing

To control for signal from non-neuronal processes additional preprocessing steps were conducted for rs-fcMRI data as in our prior studies (Fair et al., 2012; Graham et al., 2016; Rudolph et al., 2018). A volume censoring approach was used to remove volumes associated with greater than 0.3 mm frame-wise displacement (FD) (including 1 preceding and 2 following volumes to account for temporal blurring (Power et al., 2012)). Scans with less than 4 min. of remaining data were removed. This resulted in an additional 3 infants being removed from the analysis. Scan length after volume removal for remaining infants (N = 62) was approximately 5 and a half minutes (M = 5.50, range = 4.14–6.30), and the remaining FD was approximately 0.08 (M = 0.081, range = 0.047 – 0.134). To rule out effects of remaining motion on results, post-hoc analysis included adjustment for remaining FD (Fair et al., 2012; Power et al., 2015).

2.2.4. Amygdala regions of interest

2.2.4.1. Amygdala ROIs. Amygdalae were automatically segmented using a multi-template, multi-modality based method combining T1 and T2 weighted high-resolution images (Wang et al., 2014). Data was then realigned such that the anterior-posterior direction was positioned along the hippocampal long axis for manual correction of the amygdala segmentations in ITK-Snap (Yushkevich et al., 2006). Manual correction involved a strict protocol consisting of shape correction and definition of the boundary between the hippocampus and amygdala defined by CSF contrast in the T2 weighted image. Manual corrections of the automatic segmentations were performed using both the T1- and T2-weighted image (see Graham et al., 2016 for details).

2.2.4.2. Amygdala connections to anterior insula and vMPFC. We examined amygdala functional connections identified in a prior study (Graham et al., 2016). Specifically, our prior work employed several whole brain regressions to examine newborn left and right amygdala connectivity in relation to infant fear and a fear and cognition phenotype at 6-months-of-age (Graham et al., 2016). The results of this analyses indicated that newborn functional connectivity between the left amygdala and bilateral anterior insula was related to infant fear, and connectivity between both right and left amygdala and vMPFC was related to a fear-cognition phenotype. Regions of interest (ROIs) for the bilateral anterior insula and vMPFC were extracted from the whole brain maps resulting from these analyses. Specifically, in line with previous research (Fair et al., 2007; Mills et al., 2012), a search algorithm from the 4dfp Suite of Image Processing Programs (ftp://imaging.wustl.edu/pub/raichlab/4dfp_tools/) was used to identify peak voxels within the whole brain map (with z-values greater than or equal to 2.25 for consistency with the Monte Carlo correction for multiple comparisons). Regions were then defined around the peaks beginning with a radius of 10 mm, consolidating regions with peaks closer than 10 mm, and masking out voxels falling outside of the Monte Carlo corrected whole brain regression. Since there were multiple peaks within the vMPFC and bilateral anterior insula, the peak with the highest z-value was the basis for each of these ROIs. Coordinates in Talairach space are as follows: vMPFC (8, 27, −1; −2, 20, −20), anterior insula (−38, 13, −8; 36, 12, −5). Due to Type 1 error concerns (Eklund et al., 2016) these regions were further validated with secondary analyses (for details see Graham et al., 2016).

Fisher Z-transformed (normally distributed) correlation coefficients representing connectivity strength between the bilateral amygdala-vMPFC ROIs and left amygdala-aI ROIs were used in subsequent analyses. These a-priori hypothesized regions are provided in Fig. 1.

2.3. Infant behavioral outcomes

2.3.1. Infant fear and sadness

Infant fear and sadness were assessed at 6 months (M6), 9 months (M9) and 12 months (M12) via maternal report on the Infant Behavior Questionnaire-Revised (IBQ-R) (Gartstein and Rothbart, 2003) designed for 3–12 month old infants. At 24 months of age (M24), fear and sadness were assessed via maternal report on the Early Childhood Behavior Questionnaire-Short Form (ECBQ; Putnam et al., 2008) designed for 18–26 month old infants. Importantly, prior research indicates convergent validity between laboratory-based observational

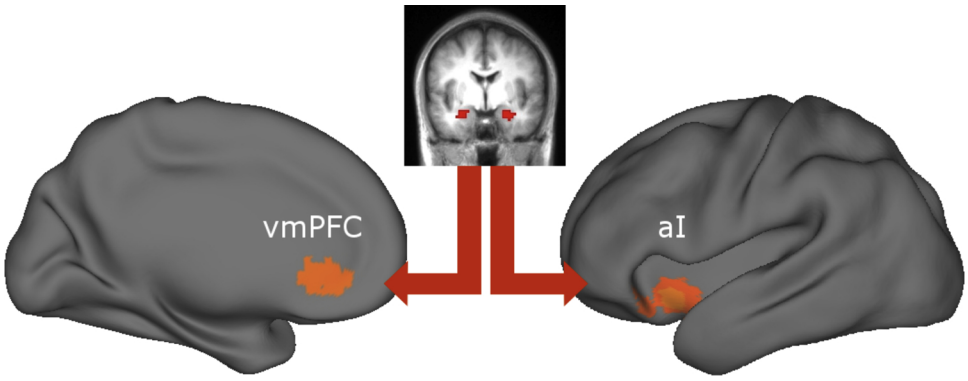

Fig. 1. The ventromedial prefrontal cortex (vMPFC) and anterior insula regions (aI) of interest (ROIs) were identified in a prior study examining whole brain voxel-wise connectivity of the neonatal amygdala in relation to fear and a fear-cognition phenotype at 6-months-of-age (Graham et al., 2016). The ROIs from this prior study are displayed here. Based on the results of this prior work, amygdala-aI and amygdala-vMPFC connections were extracted and examined in the current study.

measures of fear and maternal-self reported measures of fear on the IBQ-R at 6, 9 and 12 months of age (Gartstein and Marmion, 2008; Parade and Leerkes, 2008), indicating the utility of this measure across the first year of life. Additionally in longitudinal work, convergent validity between IBQ-R and ECBQ subscales of fear and sadness has been demonstrated (Putnam et al., 2008), supporting the idea of consistency in these measurements across time. On both questionnaires mother's rated their infants' engagement in specific fear- and sadness-related behaviors on a Likert-type scale from 1 (never) to 7 (always). Fear was assessed based on the amount of distress the infant experienced to sudden changes in stimulation, exposure to novel physical objects or social stimuli (Garstein and Rothbart, 2003). Sadness ratings reflected activity and lowered mood caused by the infant's personal suffering, physical state, loss of an object, or inability to perform a desired action (Garstein and Rothbart, 2003).

2.3.2. Postnatal caregiving environment

When infants were 6-months-of-age, quality of the postnatal caregiving environment was assessed using the Home Observation for Measurement of the Environment (HOME) Inventory (Caldwell and Bradley, 2003). Assessments were made by trained observers who had achieved reliability with a certified administrator of the inventory (95% agreement on two consecutive videos). Home visits were done to observe the infant's activities and interactions in their caregiving environment and conduct a semi-formal interview with their mothers. In our analysis, we used the subscale HOME Responsivity to assess the extent of maternal responsiveness to the infant. Responsive mothers respond quickly and appropriately to cues from their infants; in early life this has a strong effect on the development and future expression of NE (Leerkes et al., 2009).

2.3.3. Maternal depressive symptomology

To assess maternal depressive symptomology the 20-item Center for Epidemiological Studies of Depression Scale (CESD; Radloff, 1977) was administered at M6, M9, M12 and M24.

2.4. Analytic approach

We examined longitudinal trajectories of fear and sadness development using latent growth models (LGM) in a structural equation-modeling framework. First, we created an unconditional LGM for each of the developmental trajectories - fear and sadness - to look at the average growth patterns for the sample. This was done using data from four time points: M6, M9, M12 and M24. The latent intercept was specified at the M6 time point with growth factors representing change from M6-M24 (Fig. 2).

To assess the association between newborn amygdala connectivity patterns and the development of fear and sadness, for each connection of interest (amygdala-aI and amygdala-vMPFC), we tested a separate model with the connection as a predictor of the latent growth factors. In each model we controlled for gestational age at birth (GA) and age at scan.

Additionally, we considered maternal CESD scores as a covariate for time points with significant correlations between CESD scores and fear and sadness measures to account for potential maternal reporting bias due to depressive symptomology. Lastly, we examined the independent and moderating effects of HOME responsivity scores on emerging fear and sadness trajectories. All models were estimated using full-information maximum likelihood (FIML) under the missing-at-random (MAR) assumption with Mplus Version 7.4 (Muthén & Muthén, 2015). To ensure infants lost to follow- up at 9, 12, or 24 months did not differ from the remainder of the cohort across key clinical variables, we conducted independent sample T-tests to compare sample means of maternal responsivity, average maternal CESD, and connectivity measures across subjects with and without data at each time point. No significant differences between means were found ($p > 0.05$); indicating infants lost to follow-up did not differ from the remainder of the cohort across these measures.

3. Results

3.1. Descriptive analyses

3.1.1. Infant fear and sadness

Means for fear and sadness measures and Cronbach's alpha coefficients at each time point in the current sample are provided in Table 2. Fear and sadness were significantly correlated at each time point, but the strength of correlations were moderate ($r = .51–.53$, $p < 0.01$) providing support for fear and sadness being related, but distinct constructs. With regard to internal reliability, Cronbach's alpha for the fear and sadness scales at each time point exceeded 0.70 ($\alpha = .72–.91$). There was one exception for sadness at 24-months-of-age ($\alpha = .46$), which was likely due to the low number of sadness items in the ECBQ questionnaire at this time point ($N = 6$).

3.1.2. Maternal depressive symptoms

Internal reliability of the CESD measured at each time point was high ($\alpha = .83–.91$). The distribution of CESD scores was in line with expectations for a non-clinical sample (*mean* ± *SD*, 10.11 ± 7.98) (Radloff, 1977). Correlations between temperament scores and CESD scores at each time point were calculated to examine the need to adjust for effects of maternal mood on reporting of infant emotionality (Parade and Leerkes, 2008). Results indicated that higher maternal CESD was associated with higher levels of infant sadness at 6-months-of-age ($r = 0.268$, $p < 0.01$), but not with fear at any time point, or with sadness at other time points. This indicated a need to account for maternal depressive symptoms at the 6-month time point as a covariate in the model examining infant sadness.

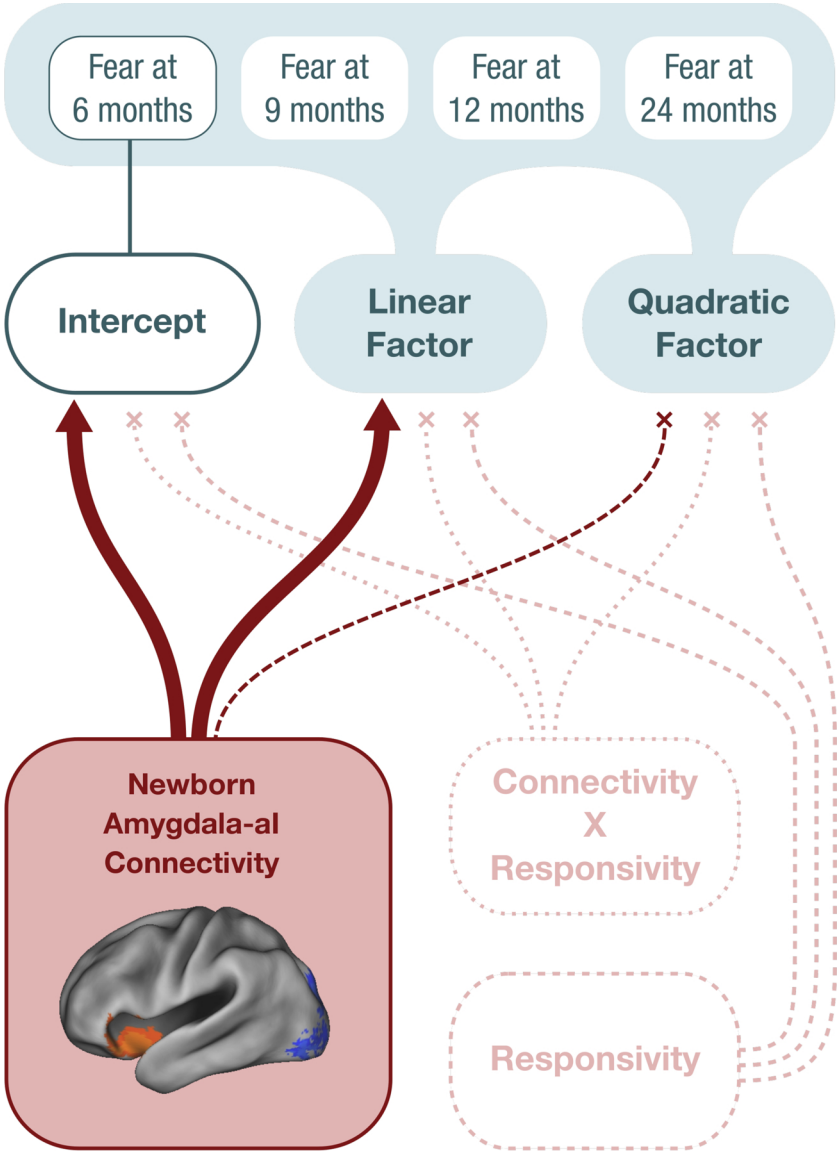

Fig. 2. Conceptual model representing the analyses examining newborn amygdala-aI connectivity in relation to fear development. Specifically, amygdala-aI connectivity was considered as a predictor for the intercept, linear, and quadratic factors of the fear trajectory with and without maternal responsivity as a covariate and interaction term. The bolded red line indicates the significant relationship found between amygdala-aI connectivity and the intercept and linear factor of fear. *Note:* Separate models were run to examine each connection in relation to the growth curve for fear and then sadness. All SEM models included gestational age at birth and age at scan as co-variates. 'Connectivity X Responsivity' represents the interaction term used to test the potential moderating effect of maternal responsivity, and 'Responsivity' represents the main effect of maternal responsivity on fear development. Significant associations ($p < 0.05$) are bolded in red. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

3.1.3. Maternal responsivity

A wide range of scores was seen for the HOME responsivity scores ($M \pm SD$, 8.32 ± 1.78, range = 1–11), reflective of wide diversity in the home environments the infants in this study were exposed to.

3.2. Primary analysis

3.2.1. Unconditional model for fear growth trajectory

First, we tested a linear growth model in which the intercept factor loading was fixed at 0 (M6) and the linear factor loadings at 0.25, 0.5 and 1.5 (M9, M12 and M24, respectively). However, as indicated from the means at each time point (Table 2), the initial increase in expression of fear from M6-M12 was followed by a decrease from M12-M24, and the linear model did not fit the data well (χ^2 (5) = 64.90, $p < 0.001$, *CFI* = 0.39, *TLI* = 0.27, *RMSEA* = 0.30). To address this nonlinearity, a quadratic growth term was added to the model. This model fit the data (χ^2 (3) = 1.07, $p = 0.79$, *CFI* = 1.00, *TLI* = 1.10, *RMSEA* < 0.001), and was significantly better than the linear model (chi-square difference test χ^2 (4) = 64.132, $p < 0.001$).

Thus we used the quadratic model for the remainder of our analyses. In this quadratic model, the non-significant covariance between the intercept and linear slope, as well as the non-significant residual variance in fear at 24-months was restricted to increase reliability of the model results. The model parameters are listed in Table 3.

The quadratic model captured an increase (M6-M12) followed by a decrease (M12-M24) in fear (see Table 2), reflected in the significant negative mean of the quadratic term ($M = -1.34$, $p < 0.001$). Additionally, a significant positive linear term ($M = 1.8$, $p < 0.001$) indicated an overall increase in fear after 6-months-of-age (Fig. 3).

3.2.2. Amygdala-aI connectivity predicts growth trajectory of fear

Next, we added amygdala-aI connectivity as a predictor to the unconditional fear model with covariates for GA and age at scan. Amygdala-aI connectivity was significantly, positively associated with the intercept ($b = 0.53$, $p < .001$; Fig. 5A) and negatively associated with the linear growth term ($b = -0.52$, $p < 0.05$). It was not associated with the quadratic growth term ($b = 0.31$, $p = 0.15$; Fig. 5B). Importantly, to ensure results were not driven by the starting point, we included the intercept as a covariate in the pathway from amygdala-aI connectivity to the linear growth term in this model and found that amygdala-aI connectivity still significantly predicted the linear growth term ($b = -4.89$, $p < 0.05$). Thus, stronger newborn amygdala-aI connectivity predicted higher levels of fear at 6-months-of-age, consistent with our prior findings (Graham et al., 2016), and less increase in fear expression over the first two years of life independent of the starting point.

Table 3
Fear Models.

	Unconditional		Amygdala-aI		Amygdala-vMPFC	
Parameter	Estimate	*SE*	Estimate	*SE*	Estimate	*SE*
Intercept Mean	***2.84	0.14	***8.20	3.13	6.97	3.52
Intercept Variance	***0.76	0.18	***0.66	0.16	***0.74	0.18
Linear Growth Term Mean	***1.80	0.38	−12.96	8.67	−10.90	9.22
Linear Growth Term Variance	1.51	2.09	1.82	1.91	1.35	1.95
Intercept & Linear Growth Term Covariance	Restricted		Restricted		Restricted	
Quadratic Growth Term Mean	***-1.34	0.24	8.02	5.83	7.45	5.89
Quadratic Growth Term Variance	0.87	0.89	1.04	0.82	0.76	0.83
Quadratic and Intercept Covariance	−0.13	0.08	−0.09	0.07	†-0.14	0.08
Quadratic & Slope Covariance	−1.02	0.24	−1.27	1.24	−0.88	1.26
Predictors of Intercept						
Amygdala-aI			***3.10	0.72		
amygdala-vMPFC					0.90	0.75
Scan Age			0.00	0.01	0.00	0.01
GA			†-0.15	0.08	−0.11	0.09
Predictors of Linear Growth Term						
Amygdala-aI			**-5.55	2.13		
amygdala-vMPFC					−1.68	1.97
Scan Age			0.05	0.03	†0.05	0.03
GA			†0.37	0.22	0.30	0.15
Predictors of Quadratic Growth Term						
Amygdala-aI			2.19	1.44		
amygdala-vMPFC					0.64	1.27
Scan Age			−0.03	0.02	−0.03	0.02
GA			−0.23	0.15	−0.21	0.15

† $p < 0.10$. * $p < 0.05$. ** $p < 0.01$. *** $p < 0.001$.

3.2.3. Maternal responsivity does not alter the association between amygdala-aI connectivity and fear growth

Associations between amygdala-aI connectivity and the fear intercept and linear growth term remained the same after adding maternal responsivity (via HOME responsivity) as a covariate in the model ($p < 0.05$). The association between amygdala-aI connectivity and the quadratic growth term remained non-significant ($p > 0.10$). No significant associations emerged between maternal responsivity and the intercept, linear or quadratic growth terms ($p > 0.05$). To test maternal responsivity as a moderator for the effect of amygdala-aI connectivity on fear growth, we added the interaction between amygdala-aI connectivity and maternal responsivity as a predictor in the model. The interaction between maternal responsivity and amygdala-aI connectivity did not predict the intercept ($b = -0.02$, $SE = 0.13$, $p > 0.1$), linear ($b = 0.20$, $SE = 0.40$, $p > 0.1$), or quadratic growth terms ($b = -0.16$, $SE = 0.27$, $p > 0.1$) of fear when added to the model. Results suggest newborn amygdala-aI connectivity predicts fear development over the first two years of life independent of maternal responsivity.

3.2.4. Amygdala-vMPFC connectivity not significantly associated with fear trajectory

We next tested a separate model, in which we examined the role of amygdala-vMPFC connectivity in fear development over time by adding it as a predictor to the unconditional model of fear growth. No

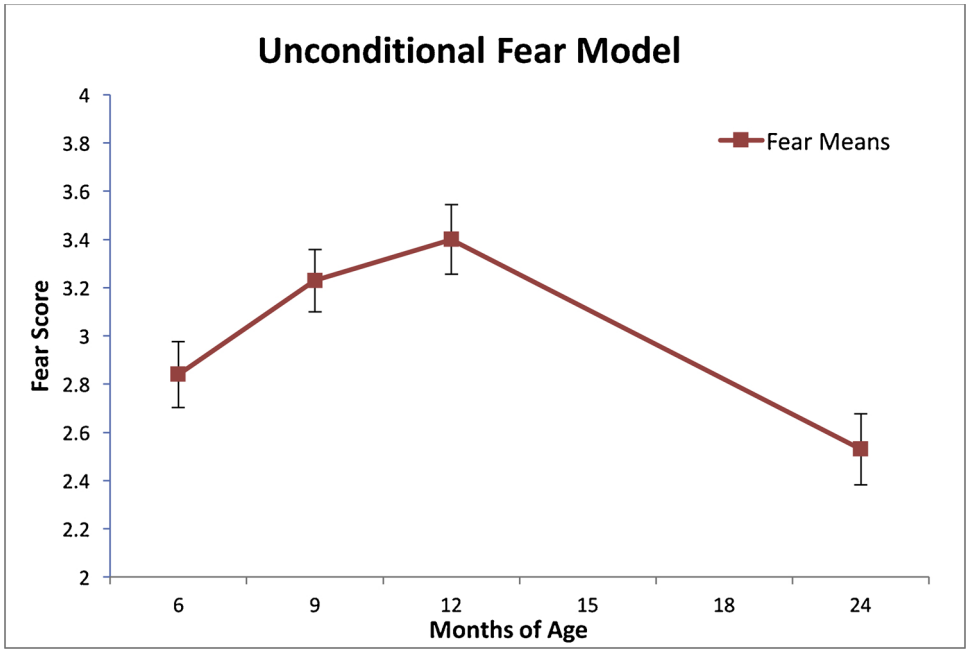

Fig. 3. Fear developmental trajectory from 6 to 24 months of age.

Table 4
Sadness Models.

	Unconditional		Am-Ins		Am-vMPFC	
Parameter	Estimate	*SE*	Estimate	*SE*	Estimate	*SE*
Intercept Mean	***3.60	0.13	**8.57	3.34	7.85	3.27
Intercept Variance	***0.66	0.16	***0.66	0.16	***0.66	0.16
Linear Growth Term Mean	*0.87	0.39	−12.21	9.76	−10.25	9.26
Linear Growth Term Variance	Restricted		Restricted		Restricted	
Intercept & Slope Covariance			N/A		N/A	
Quadratic Growth Term Mean	**-0.82	0.25	3.75	6.19	2.94	2.91
Quadratic Growth Term Variance	***0.16	0.04	***0.14	0.04	***0.15	0.04
Quadratic and Intercept Covariance	**-0.22	0.07	**-0.20	0.07	**-0.21	0.07
Quadratic & Linear Growth Term Covariance	N/A		N/A		N/A	
Predictors of Intercept						
Am-Ins			1.38†	0.77		
Am-vMPFC					**1.78	0.7
Scan Age			−0.01	0.01	−0.01	0.01
GA			−0.13	0.08	−0.11	0.08
Predictors of Linear Growth Term						
Am-Ins			−3.83	2.35		
Am-vMPFC					**-5.73	2
Scan Age			0.02	0.03	0.01	0.03
GA			0.34	0.25	0.29	0.23
Predictors of Quadratic Growth Term						
Am-Ins			1.72	1.5		
Am-vMPFC					*2.98	1.29
Scan Age			0	0.02	0	0.02
GA			−0.13	0.16	−0.11	0.15

Note: χ^2 = chi-square; CFI = Comparative Fit Index; TLI = Tucker and Lewis Index.
† $p < 0.10$. * $p < .05$. ** $p < 0.01$. *** $p < 0.001$.

significant main effects of amygdala-vMPFC connectivity were found on the intercept, linear, or quadratic growth terms (see Table 4). Thus, unlike newborn amygdala-aI connectivity, amygdala-vMPFC connectivity was not significantly associated with the trajectory of fear growth from 6 to 24 months-of-age. Results were unchanged after adjusting for maternal responsivity and considering it as a potential moderator.

3.3. Unconditional model for sadness growth trajectory

As with fear, to map the trajectory of sadness from 6 to 24 months-of-age we initially tested the fit of a linear growth model. Intercept factor loadings were fixed at 0 (M6) and linear factor loadings at 0.25, 0.5, and 1.5 (M9, M12, and M24, respectively). However, as indicated from the means at each time point (Table 2), the initial increase in expression of sadness from M6-M12 was followed by a decrease from M12-M24, and the linear model did not fit the data well, χ^2 (5) = 12.13, p = 0.03, *CFI* = 0.88, *TLI* = 0.86, *RMSEA* = 0.10. To address this nonlinearity, a quadratic term was added to the model. This model fit the data well (χ^2 (5) = 3.56, p = 0.61, *CFI* = 1.00, *TLI* = 1.04, *RMSEA* < 0.001) and was significantly better than the linear model (chi-square difference test [χ^2 (4) = 12.11, p < 0.025]). Thus we used the quadratic model for the remainder of our analyses. In this quadratic model, the non-significant residual variance in the linear growth term was restricted to increase reliability of the model results. Specific model parameters are listed in Table 4.

Due to the significant association between maternal depressive symptoms and infant sadness at 6-months we initially considered maternal CESD scores as a covariate with sadness at this time point. However, including M6 CESD as a covariate significantly worsened our model fit (χ^2 (4) = 10.18, p = 0.037, *CFI* = 0.91, *TLI* = 0.78, *RMSEA* = 0.11; chi-square difference test [χ^2 (1) = 6.62, p < 0.01]), and did not alter the significance or direction of the intercept, linear and quadratic growth terms in the unconditional model. Including the covariate also did not alter associations between brain connectivity and these growth terms. Therefore, the final models reported below do not include this covariate.

3.3.1. Amygdala-aI connectivity not significantly associated with sadness trajectory

We examined the role of amygdala-aI connectivity in sadness development by adding it as a predictor to the unconditional model of sadness growth. No significant main effects of amygdala-aI connectivity were found on the intercept, linear or quadratic growth terms of sadness (see Table 4). Results were unchanged after adjusting for maternal responsivity and considering it as a potential moderator. Thus, newborn amygdala-aI connectivity did not relate to sadness development, and appears to be more specifically related to fear development.

3.3.2. Amygdala-vMPFC connectivity predicts sadness trajectory

In a separate model, we examined newborn amygdala-vMPFC connectivity in relation to the sadness growth trajectory. Newborn amygdala-vMPFC connectivity had a significant positive effect on the intercept (b = 1.80, p < .01; Fig. 5C), indicating that higher amygdala-vMPFC connectivity was associated with greater expression of sadness at 6-months-of-age. This connection also evidenced a significant negative association with the positive linear growth term (b = -5.73, p < 0.01; Fig. 5D), and significant positive association with the negative quadratic growth term (b = 2.98, p < 0.05; Fig. 5E). Importantly, inclusion of the intercept as a covariate in the pathway from amygdala-vMPFC connectivity to the linear and quadratic growth terms of this model did not change our results. Amygdala-vMPFC connectivity still significantly predicted both linear (b = -6.43, p < 0.05), and quadratic growth terms (b = 3.98, p < 0.05) after inclusion of this covariate, indicating that the association between newborn connectivity and change in sadness expression over time was not driven by the initial starting point of sadness expression. Thus higher amygdala-vMPFC connectivity at birth predicted higher sadness at 6 months and less change in sadness over the first two years of life.

3.3.3. Maternal responsivity does not alter the association between amygdala-vMPFC connectivity and the sadness trajectory

Associations between amygdala-vMPFC connectivity and sadness intercept, linear and quadratic growth terms remained consistent after adding maternal responsivity as a covariate in the model (p < 0.05). Additionally, no significant relationships between maternal responsivity and the intercept, linear or quadratic growth terms emerged (p > 0.1). Maternal responsivity was also tested as a moderator of the effect of amygdala-vMPFC connectivity on sadness growth by evaluating the interaction between responsivity and amygdala-vMPFC connectivity on emerging sadness. The interaction between maternal responsivity and amygdala-vMPFC connectivity was not significantly associated with the intercept (b = -0.18, *SE* = 0.15, p = 0.23), linear (b = 0.41, *SE* = 0.42, p = 0.33) or quadratic growth terms (b = -0.30, *SE* = 0.27, p = 0.27) of the sadness trajectory. These results indicate newborn amygdala-vMPFC connectivity predicts sadness development independent of variation in this measure of maternal responsivity.

4. Discussion

4.1. Summary of findings

The present study aimed to advance understanding of how newborn

limbic-prefrontal brain systems relate to early fear development and the etiology of fear-based psychopathologies. Findings suggest that newborn amygdala functional connectivity is predictive of emerging fear over the first two years of life. Moreover, our current findings indicate some specificity, such that amygdala-aI connectivity is particularly important for fear development and amygdala-vMPFC relates only to sadness development. This suggests potential distinctions in the neural circuitry underlying different components of NE. An overall pattern emerged for both fear and sadness development, in which stronger newborn amygdala connectivity was associated with greater initial expression of each NE domain at 6-months-of-age, followed by less dynamic change from 6 to 24 months of age.

4.2. Developmental trajectories of fear and sadness are distinct over the first two years of life

Our model captured an increase in fear over the first year of life (M6-M12), followed by a decrease in the second year of life (M12-M24) reflecting the expected trajectory of fear (Partridge, 2007). Sadness showed a similar developmental trajectory, increasing from 6 to 12 months then decreasing from 12 to 24 months. However, the initial increase and subsequent decline were less pronounced for sadness than for fear (Figs. 3 and 4). This suggests that relative to fear, sadness may not involve the same degree of change over the first two years of life. Because the developmental trajectory of sadness has not been examined in prior studies, it is difficult to say what the expected trajectory of this particular aspect of NE looks like. Our findings fit well within the context of what is known about general NE development which increases over the first year then stabilizes (Bridgett et al., 2009; Partridge, 2007).

4.3. Newborn amygdala-aI connectivity relates to fear development from 6 to 24 months-of-age

Though models of fear and sadness showed similar developmental trajectories, brain connectivity patterns underlying these trajectories differed. In line with our initial hypothesis, amygdala-aI connectivity was specifically associated with fear development across the first two years of life. Infants with stronger newborn amygdala-aI connectivity expressed greater fear at 6-months-of-age with less dynamic change over time, including a small increase from 6 to 9 months and a less pronounced decrease from 9 to 24 months. These results provide the first evidence that newborn amygdala-aI connectivity is associated with the early developmental trajectory of fear, and that it may be specific to fear as opposed to other aspects of NE. This fits with the conceptualization of fear as representing a distinct component of NE, with distinct neurobiological correlates (Garstein and Rothbart, 2003).

The anterior insula is a key component of the salience network (Seeley et al., 2007), involved in detection of novel salient stimuli through different sensory modalities (Downar et al., 2000, 2002). In line with this role, amygdala-aI connectivity is involved in pathological and subclinical variation in fear (Baur et al., 2013), which involves the detection of potentially threatening stimuli in the environment. Our results fit well within this framework. Specifically, higher newborn amygdala-aI connectivity may increase detection of potentially threatening stimuli earlier in life, resulting in a heighted fear response by 6-months-of-age. The reduced novelty of these stimuli from 9 to 24 months-of-age, paired with a potential early emergence of emotion regulatory ability, may result in the subsequent decrease in fear expression.

In line with our hypothesis, amygdala-aI connectivity appears to be relevant for the development of fear; however, contrary to our hypothesis, stronger amygdala-aI connectivity was associated with less increase in fear over time. Given the general trajectory of fear development, this finding may suggest stronger newborn amygdala-aI connectivity is associated with more precocious fear development. Alternatively, it is possible higher connectivity may be indicative of a more fixed phenotype involving decreased plasticity, manifested as less dynamic change in fear expression from 6 to 24 months.

4.4. Amygdala-vMPFC connectivity predicts sadness development

While stronger amygdala-vMPFC connectivity facilitates greater emotional regulatory ability in older populations (Silvers et al., 2017), we anticipated the opposite association in infant populations, due to the developmental switch in amygdala-vMPFC functional connectivity (Gee et al., 2013). We hypothesized that stronger, positive amygdala-vMPFC connectivity would be associated with higher levels of NE at 6-months and greater increase in NE over time. Stronger amygdala-vMPFC connectivity was associated with higher levels of sadness at 6-months. However, in contrast to expectations, greater amygdala-vMPFC connectivity was not associated with increasing sadness expression over

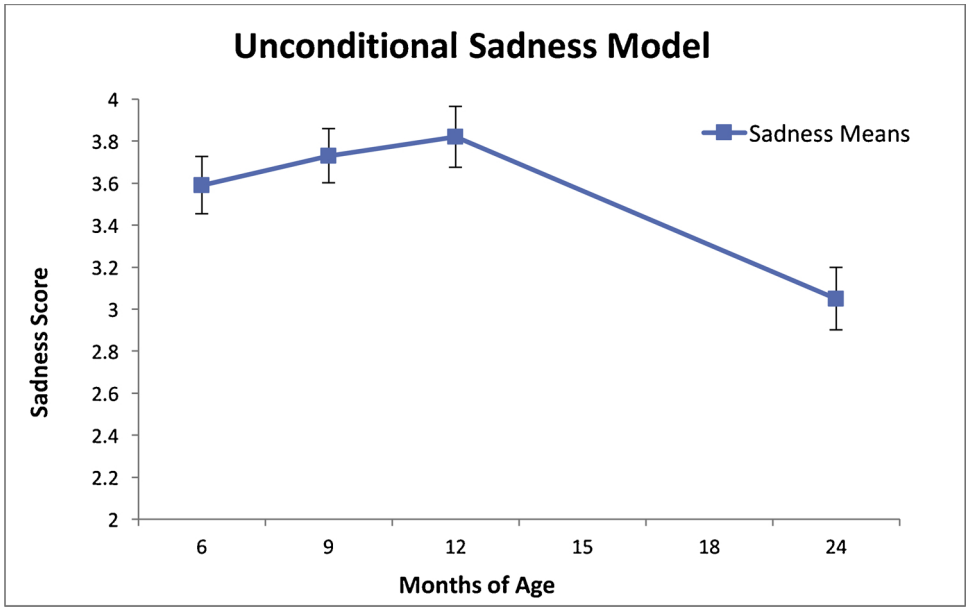

Fig. 4. Sadness developmental trajectory from 6 to 24 months of age.

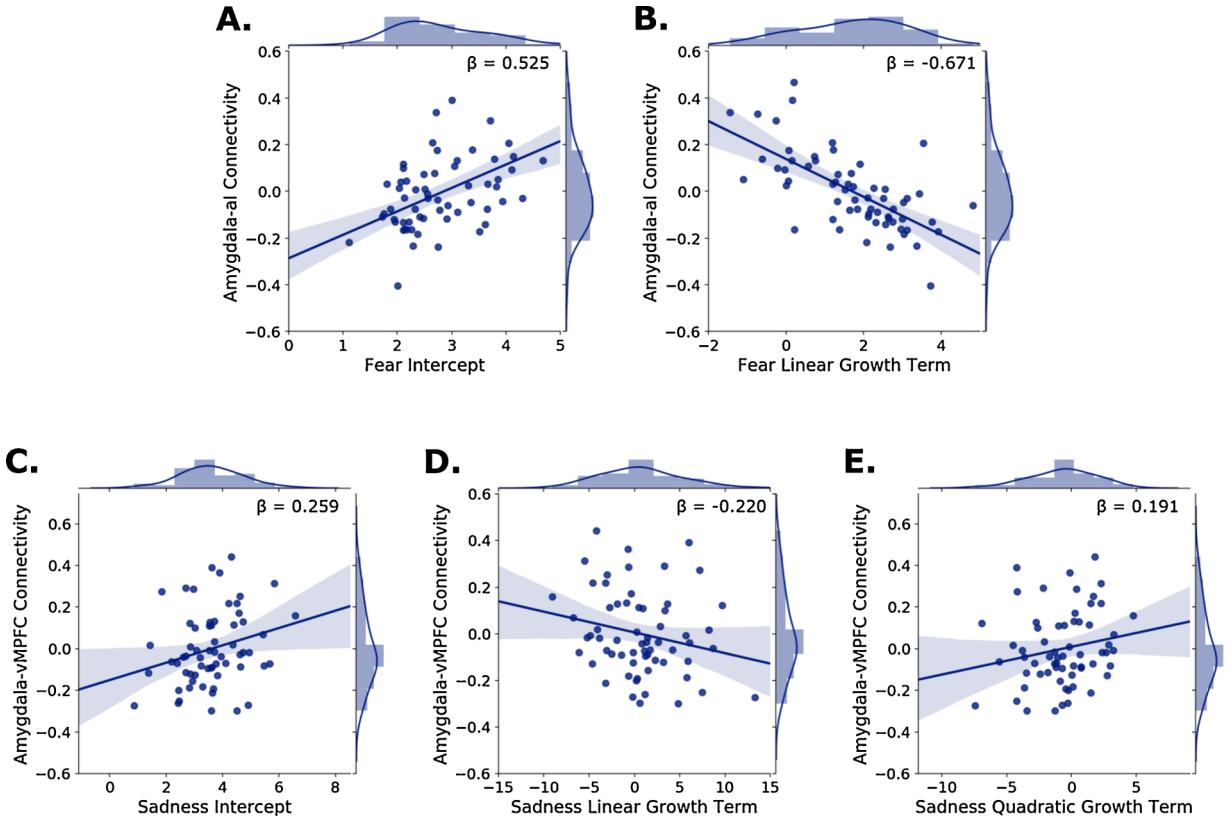

Fig. 5. Standardized beta weights showcase significant relationships between sadness and fear growth terms and amygdala-vMPFC and amygdala-aI connectivity. Histograms on x and y axes represent distributions of growth terms and connectivity. Measures have been adjusted to account for variation caused by gestational age at birth and age at scan. **A, B:** Data has been extracted from the conditional fear amygdala-aI connectivity model reported in text. **C, D, E:** Data has been extracted from a conditional sadness amygdala-vMPFC model with variance from the slope unrestricted for data visualization purposes.

time. Similar to the association between amygdala-aI connectivity and fear development, stronger amygdala-vMPFC connectivity at birth may indicate a developmental shift in the expected NE trajectory, such that an increase and subsequent decrease in sadness expression occurs earlier in life.

Based on findings suggesting a role for amygdala-vMPFC connectivity in regulation of negative affect and in anxiety and depression (Burghy et al., 2012; Casey and Lee, 2015; Connolly et al., 2017; Kim et al., 2011; Roy et al., 2013b; Wang et al., 2013), we anticipated this connection would be relevant for the early emergence of both fear and sadness. However, our results suggest a specific association only with sadness. While amygdala-vMPFC connectivity has been associated with pathological and subclinical variation in fear (Baur et al., 2013), it has been more consistently associated with depressive psychopathologies (de Almeida et al., 2009; Perlman et al., 2010; Ritchey et al., 2011; Siegle et al., 2007). Our results linking amygdala-vMPFC connectivity to developmental trajectories of sadness expression from 6 to 24 months of age suggest this connection may already be relevant for variations in mood beginning in infancy.

4.5. Variation in maternal responsivity does not affect results

Variation in maternal responsivity did not change or moderate the associations between newborn amygdala connectivity and developmental trajectories of fear or sadness. Furthermore, consideration of maternal depressive symptomology at each time point did not impact our results. It should also be noted that while we did see variability in maternal responsivity, more extreme variation may have been required to observe a moderating effect on the newborn amygdala connections. (Burghy et al., 2012; Callaghan et al., 2014; Gee et al., 2013; Herringa et al., 2013). In present form, results suggest patterns of coordinated newborn amygdala functioning have implications for the development of fear and sadness through 24-months of age even after adjusting for variation in the postnatal caregiving environment.

4.6. Limitations

Several limitations of the present study should be considered. First, measures of infant fear and sadness relied on maternal self-report. Parental report has the advantage of allowing for observation of infant behavior over a long period of time across different contexts (Pelham, 1993; Stifter, 2008), and the specific measures used have demonstrated convergent validity with laboratory observation (Braungart-Rieker et al., 2010; Gartstein et al., 2010; Parade and Leerkes, 2008). However, it would be preferable for measurement of these constructs to combine parental report and observational measures. Additionally, while we considered the role of maternal depressive symptoms in potentially biasing report of infant fear and sadness, it is also possible that cultural factors may lead to differences in understanding of and reporting on infant NE (Bosquet et al., 2016; Dragan and Fronczyk, 2011; Gartstein et al., 2016; Montirosso et al., 2011). With regard to the observational measure of maternal responsivity, we note that it was only assessed at the 6-month time point, which limited the capacity to consider how changes in maternal responsivity over the postpartum period may relate to the trajectories of infant fear and sadness.

Another concern relates to our approach of choosing a-priori connections of interest for our analysis at the expense of doing a whole brain exploratory analysis. This decision was based on previous findings implicating these connections in emerging fear at 6-months of age (Graham et al., 2016) and in typical and pathological fear and children and adults (Baur et al., 2013; Casey and Lee, 2015; Etkin and Wager, 2007; Kim et al., 2011; Rabinak et al., 2011; Roy et al., 2013a; Sripada

et al., 2012). This approach allowed us to conduct a more focused analyses and facilitated the interpretability of our results. However, while these connections represent a natural starting point for examining developing fear, other aspects of amygdala connectivity and large scale brain systems will certainly be relevant for the development of fear in early life.

The BOLD data in this study did not undergo distortion correction. However, multiple steps were taking to ensure the quality of the BOLD data as detailed in the methods section. To confirm results were not driven by a lack of distortion correction, we quantified the average raw BOLD signal across the time series within each ROI and correlated this signal with our outcome measures of interest (fear and sadness growth factors). No significant associations ($p > 0.1$) were found, making it highly unlikely that results were driven by effects of field inhomogeneities on the BOLD signal within the ROIs. Additionally, we would have ideally included more resting state data per subject. However, given the challenges of collecting high quality MRI data with infants, investigators are faced with a decision of correlation values that are increasingly noisy (due to less data inclusion – see Laumann et al., 2015) or fewer subjects that would lead to reduced power (see descriptions in Dosenbach et al., 2017). We excluded infants with less than 4 min of high quality data as a natural balance between these two competing issues. Due to limitations in the resolution of MRI and fMRI data, we were also unable to examine amygdala subregions, which are certainly of interest for understanding fear development, and will be an important topic for future investigations when they can be reliability identified using these modalities. Lastly, developmental differences in the underlying physiology of the BOLD signal are not completely understood (Arichi et al., 2012; Hagmann et al., 2012; Karen et al., 2008; Kozberg et al., 2013; Liao et al., 2010), and further investigation will be needed to consider how they may influence brain-behavior associations at different developmental time points, including in the newborn period.

5. Conclusions & future directions

Overall, the current results provide further evidence that fear is a distinct component of NE with specific neural correlates. Ongoing longitudinal research will be needed to test the idea that these neurobehavioral phenotypes lay the foundation for healthy versus pathological fear development over time, though current research already indicates these newborn connections are relevant for future internalizing symptoms at 2 years of age (Graham et al., 2018; Rogers et al., 2016). Additionally, while the current study evaluates NE expression in relation to amygdala connectivity, it is well known that emotionality is balanced by regulatory capacity (Nigg, 2006). Future work should examine emotion regulatory capacity during this time period to determine how regulatory ability impacts associations between newborn amygdala connectivity and emerging NE.

The current study also raises questions about the underlying cause for the pattern of development seen in infants with stronger amygdala-aI connectivity. Specifically, in future work it will be important to consider how maternal emotional state during pregnancy may impact future neonatal amygdala functional connectivity and emerging fear and sadness. Our previous work suggests that elevated concentrations of maternal biological stress mediators (e.g., interleukin 6 and cortisol) during pregnancy are associated with altered newborn amygdala connectivity (Graham et al., 2017, 2018). Further, recent research has identified an association between maternal depressive symptoms during pregnancy and emerging infant sadness via maternal inflammation levels (Gustafsson et al., 2018). Examination of both the antecedents and consequences of these neural phenotypes and developmental trajectories of negative emotionality will be an important area of ongoing investigation.

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