Maternal adverse childhood experiences and infant subcortical brain volume

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\textbf{A R T I C L E I N F O}

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\textbf{ABSTRACT}

\textbf{Background:} A large body of research supports the deleterious effects of adverse childhood experiences (ACEs) on disease susceptibility and health for both the exposed individual and the next generation. It is likely that there is an intergenerational transmission of risk from mother to child; however, the mechanisms through which such risk is conferred remain unknown. The current study evaluated the association between maternal ACEs, neonatal hippocampal and amygdala development, and later infant negative emotionality at six months of age.

\textbf{Methods:} The sample included 85 mother-infant dyads (44 female infants) from a longitudinal study. Maternal ACEs were assessed with the Adverse Childhood Experiences Questionnaire (ACE-Q) and neonatal hippocampal and amygdala volume was assessed using structural magnetic resonance imaging (MRI). Infant negative emotionality was assessed at 6 months using the Infant Behavior Questionnaire (IBQ).

\textbf{Results:} Multivariate analyses demonstrated that maternal ACEs were associated with bilateral amygdala volume ($F(2,78)=3.697, p=0.029$). Specifically, higher maternal ACEs were associated with smaller left ($\beta=-0.220, t(79)=-2.661, p=0.009, R^2=0.494$, and right ($\beta=-0.167, t(79)=-2.043, p=0.044, R^2=0.501$) amygdala volume. No significant association between maternal ACEs and bilateral hippocampal volume ($F(2,78)=0.215, p=0.807$) was found. Follow-up regression analyses demonstrated that both high maternal ACEs and smaller left amygdala volume were associated with higher infant negative emotionality at six months of age ($\beta=-0.232, p=0.040, R^2=0.094$, and $\beta=-0.337, p=0.022, R^2=0.16$, respectively) although statistically significant mediation of this effect was not observed (Indirect effect $=0.0187$, 95% CI $[-0.0016-0.0557]$).

\textbf{Conclusions:} Maternal ACEs are associated with both newborn amygdala volume and subsequent infant negative emotionality. These findings linking maternal adverse childhood experiences and infant brain development and temperament provide evidence to support the intergenerational transmission of adversity from mother to child.

1. Introduction

Adverse childhood experiences (ACEs) are highly prevalent, with a recent CDC finding that 61% of adults surveyed experienced at least one adverse childhood experience and that women were more likely than men to experience four or more types of ACEs (Merrick et al., 2019). Experiences of childhood adversity, including exposure to abuse, neglect, or household dysfunction, not only affect the well-being of the individual across the lifespan, but are also a robust predictor of deleterious health outcomes in the next generation. Epidemiological research
provides compelling evidence that maternal experiences of trauma prior to conception have implications for offspring physical and mental health (Class et al., 2014; Flory et al., 2011; Keenan et al., 2018; Perroud et al., 2014). Multiple pathways likely contribute to the intergenerational link between maternal exposure to ACEs and child psychological well-being, including parenting, genetic inheritance, prenatal experiences and shared environmental risk factors (Buss et al., 2017; Yehuda and Lehrner, 2018). Here we test the possibility that maternal adverse childhood experiences influence newborn brain development.

The fetal period is a critical time of rapid brain development and thus highly susceptible to both organizing and disorganizing environmental influences (Andersen, 2003; Hutenlocher and Dabholkar, 1997; Stiles and Jernigan, 2010). During the transformation from zygote to a newborn, the pace of development exceeds any other stage of the life-span (Stiles and Jernigan, 2010). Morphological development of subcortical brain structures begins at an early embryonic stage (Humphrey, 1966) and many subcortical brain regions, such as the amygdala, can be identified as early as six weeks gestational age (Müller and O’Rahilly, 2006). From late in the 2nd trimester to term, there is a 21-fold increase in cortical gray matter volume (Andescavage et al., 2017) and by three weeks after birth, infant brain volume is about 35% of adult volume (Gilmore et al., 2007). Given such tremendous growth of the fetal brain during gestation, even subtle changes during fetal development may have cascading effects and result in major deviations that persist across the developmental trajectory (Mink et al., 2019; Sandman et al., 2018). This fetal susceptibility is the defining feature of the Developmental Origins of Health and Disease hypothesis which posits that the fetus adapts to changes in the uterine environment and may then shape critical processes in fetal neurodevelopment including synaptogenesis and dendritic branching (Sandman et al., 2018; Curran et al., 2017). Recent findings from experimental animal studies show an intergenerational effect whereby maternal stress exposure limited to the preconception period impacts brain morphology of the offspring, including the volume of the hippocampus (Jenkins et al., 2022).

The amygdala and hippocampus are critical nodes within limbic circuitry that governs stress responsiveness, memory and fear conditioning (Phelps, 2004); disruptions in these brain regions are therefore prime candidate biomarkers of vulnerability for stress-related psychopathology. Given that variability in amygdala and hippocampus structure is associated with multiple forms of psychopathology, including depression (Barch et al., 2019), anxiety (see (Kolesar et al., 2019) for a systematic review) and posttraumatic stress disorder (Woon and Hedges, 2008) and related risk factors, including heightened negative affectivity (Mincic, 2015), understanding potential origins of these individual differences would contribute to better understanding of the etiology of psychopathology. A relatively large body of research has investigated structural alterations within the amygdala and hippocampus of individuals who have experienced trauma or early life stress (e.g., exposure to maternal childhood maltreatment). This research has shown that maternal ACEs can be associated with both lower total and gray matter volume, and lower right amygdala volume at older ages consistent with the possibility that amygdala development may be impacted by maternal childhood experiences. Further highlighting the limbic circuitry as potentially vulnerable to the effects of stress, maternal childhood adversity has also been associated with alterations in neonatal fronto-limbic connectivity (Hendrix et al., 2021).

The current study extends existing research on neural mechanisms underlying intergenerational transmission of risk by investigating variability in morphology of specific limbic brain regions associated with early maternal ACEs and then assessing how these regions are implicated in emotional processing later in infancy. Negative emotionality strongly predicts poor physical and mental health (Lahey, 2009). Negative emotionality is also linked to functional variability of the hippocampus (Servaas et al., 2013) and higher gray matter volume within the amygdala (see (Mincic, 2015) for a systematic review), making it an important outcome for investigation. Further, there is evidence that maternal ACEs predict negative emotionality in the offspring (Lang et al., 2010).

Participants included a sample of mother-infant dyads recruited in pregnancy as part of an ongoing longitudinal study to investigate the association between maternal childhood adversity, amygdala and hippocampal volume, and negative emotionality. Associations between maternal adverse childhood experiences and offspring hippocampus as well as amygdala volume at birth were first examined. For the subcortical regions that showed a significant statistical association with maternal ACEs, follow-up analyses were then conducted to investigate whether infant brain volume in those areas was associated with negative emotionality at 6 months. Next, the association between maternal ACEs and infant negative emotionality was examined. Finally, exploratory mediational analyses were implemented to examine whether brain volume mediated the association between maternal ACEs and later infant negative emotionality.

2. Methods and materials

2.1. Study overview

Pregnant individuals completed interviews and questionnaires that included reports of adverse experiences during childhood and demographics questionnaires. Neonatal amygdala and hippocampal volumes were assessed during natural sleep at 42–51 weeks’ postconceptional age, and mothers reported on children’s temperament at 6 months.

2.2. Study participants

Participants were 85 mother-infant dyads from the Care Project, a longitudinal study examining the impact of prenatal maternal mental health on offspring developmental outcomes (Davis et al., 2018).
Assessments of infant MRIs were completed prior to the start of the COVID-19 pandemic being declared a state of emergency (March 10, 2020). Participants were recruited primarily from obstetrics clinics at two major medical centers in the Denver, Colorado metro area. All study procedures were approved by the Institutional Board for the Protection of Human Subjects at the University of Denver and the University of Colorado Anschutz Medical Campus, and all mothers provided written informed consent for themselves and their infants.

Women who were 25 weeks GA or less, 18–45 years old, English-proficient and carrying a singleton pregnancy were recruited. The threshold of 25 weeks’ GA was chosen to maximize study participation during gestation while avoiding biasing our sample by excluding higher risk individuals who do not often receive early prenatal care. For the main Care Project study, initial exclusion criteria included current illicit drug or methadone use, major health conditions requiring invasive treatments (e.g., dialysis, blood transfusions, chemotherapy), current or past symptoms of psychosis or mania, and/or current participation in cognitive behavioral therapy or interpersonal therapy. 122 infants were initially recruited to the study. 29 infants were not scanned because the parent either declined the scan or the infant was not able to be scheduled within the assessment window. Additional exclusion criteria for the current study included (a) preterm birth (<34 gestational weeks (n = 0) or b) major fetal or chromosomal anomalies (n = 0) or c) any infant MRI contraindications (n = 2) (e.g., metal implant). Infants were predominantly healthy at birth and without neurological complications. Only one infant required mechanical ventilation at birth. As exclusion of this infant did not change study findings (see Supplement), they were retained in study analyses. Of the 90 infants who attended the MRI scan, 3 were unable to be scanned (e.g., infant did not fall asleep during the scanning window), 2 scans were not acquired because the infant woke up in the scanner, and 3 were rescanned due to image quality issues. Out of the 85 participants with quality volumetric scan data, 6 were missing infant temperament data at the 6 month follow-up. As such, the final sample size for maternal ACEs to amygdala and hippocampal volume was 85 and the final regression and mediational analyses with infant temperament data included a sample size of 79 participants.

Mothers in the study were 21–41 years old (M = 31.37, SD = 5.17) at delivery (see Table 1 for sample characteristics). Median annual household income was $71,000, and 31.8% of participants were living at or near federal classification of poverty (less than 200% income-to-needs ratio). Infants (51.8% female) were 39 weeks’ gestation at birth on average (range: 34–41 weeks). Infants were scanned at 44 weeks postconceptional age (range 42–51 weeks) and were 5 weeks post birth on average at the time of scan. 56.5% of infants were non-Hispanic/Latinx white and 23.5% were Hispanic/Latinx, with the remainder of the sample identifying as Black, Asian, or Multi-ethnic. Our study sample is generally reflective of the racial and economic diversity of the Denver metro area based on data of all live births in Denver from the National Center for Health Statistics and on US Census Data of Denver County.

2.2.1. Maternal adverse childhood experiences

Exposure to early life stress was assessed using the Adverse Childhood Experiences Questionnaire (ACE-Q); (Felitti et al., 1998). The ACE-Q is a self-report scale assessing exposure to 10 types of early life adversity—including experiences of abuse, neglect, and household dysfunction—before age 18. Participants indicate whether they have experienced each category of early life adversity prior to the age 18 using a yes (1) or no (0) response. Total responses range from 0 to 10 with higher scores indicating exposure to more categories of early life adversity.

2.3. Sociodemographic characteristics

Maternal birthdate, socioeconomic status, cohabitation with child’s father, marital status, educational attainment, and race and ethnicity were collected via maternal interview. A family income-to-needs ratio (INR) was calculated by dividing the total reported household income by the poverty threshold corresponding to the number of persons living in the household at the time of study entry, specified by the U.S. Census Bureau (U.S. Census Bureau, 2020).

2.4. Pregnancy and birth outcomes

Prenatal obstetric complications, birth outcomes, and infant biological sex at birth (referred to as sex in this manuscript) were obtained from the medical record. In addition, birth weight percentile, which accounts for gestational age at birth and infant sex, was determined. Estimated date of delivery was determined by early ultrasound measures and date of last menstrual period applying the American College of Obstetricians and Gynecologists guidelines and used to calculate gestational age at birth (GAB) and postconceptional age at scan (Committee on Obstetric Practice et al., 2017). An obstetric complications score was calculated as a sum score indicating the presence or absence of a series of pregnancy-related complications, including prenatal

| Table 1 | Demographic, medical and outcome characteristics of the sample. |
|---------|---------------------------------------------------------------|
| Complete sample n = 85 | M (SD) or % |
| **Maternal Characteristics** | |
| ACEs | 2.35 (2.36) |
| Age at delivery | 31.37 (5.17) |
| Obstetric complications (>1) | 27.1% |
| Annual household income ($) | 71,000 (56,962) |
| Household INR | 4.29 (3.72) |
| Cohabitating with partner | 89.4% |
| Married | 72.9% |
| Education (highest degree earned) | |
| Less than high school | 2.4% |
| High school | 25.9% |
| College degree | 48.3% |
| Graduate degree | 23.5% |
| **Race and ethnicity** | |
| American Indian | 1.2% |
| Asian | 4.7% |
| Black | 10.6% |
| Hispanic/Latinx | 21.2% |
| Non-Latinx White | 60.0% |
| Other | 2.4% |
| **Substance Use** | |
| Illicit drugs | 0% |
| Marijuana | 2.4% |
| Alcohol | 2.4% |
| Cigarettes | 1.2% |
| Psychotropic Medication Use | 0.9% |
| **Infant Characteristics** | |
| Postconceptional age at MRI (weeks) | 44.0 (1.75) |
| Biological sex at birth (% female) | 51.8% |
| **Race and ethnicity** | |
| American Indian | 2.4% |
| Asian | 4.7% |
| Black | 11.8% |
| Hispanic/Latinx | 23.5% |
| Non-Latinx White | 56.5% |
| Other | 1.2% |
| **Birth Outcome** | |
| Gestational age at birth (weeks) | 38.99 (1.38) |
| Birth weight percentile | 45.26 (25.90) |
| 5-minute Apgar score | 8.8 (4.48) |
| Congenital disorder | 7.1% |
| NICU stay | 4.7% |
| **Brain Volume (mm³)** | |
| Right Hippocampus | 1151.13 (141.55) |
| Left Hippocampus | 1099.97 (141.55) |
| Right Amygdala | 245.49 (32.11) |
| Left Amygdala | 236.07 (30.00) |

Note. * median used; ACEs = Adverse childhood experiences; INR = Income-to-needs ratio, MRI = magnetic resonance imaging, NICU = neonatal intensive care unit.
infection, pregnancy-induced hypertension, gestational diabetes, oligophrenia, preterm labor, vaginal bleeding, placenta previa, or anemia (Hobel, 1982). Given the clustered distribution of the OB complications score around 0, the data were dichotomized (zero or one vs. more than one). 73% of the women had zero or one obstetric complication on this index. Maternal substance use (illicit drugs, marijuana, cigarettes, and alcohol) as well as psychotropic medication were assessed via interview.

2.5. Infant negative emotionality

Infant negative emotionality was assessed using the Infant Behavior Questionnaire (IBQ; (Garstein and Rothbart, 2003)) a standardized measure containing 191 items that assess infant temperament using maternal report. The IBQ leverages a mother’s ability to observe their child over a wide range of contexts. Limiting the potential of maternal reporting bias, the IBQ was designed to ask about concrete infant behaviors rather than abstract judgements as to make the responses more objective (e.g. “During feeding, how often did the baby squirm or kick”). Mothers rate their infant’s behavior on each item using a Likert scale ranging from 1 (never) to 7 (always). The negative affectivity composite score is comprised of 4 IBQ subscales: sadness, distress to limitations, fear, and falling reactivity. Internal consistency as measured by Cronbach’s alpha for each subscale was: 0.88, 0.82, 0.92 and 0.99, respectively. Internal consistency for Negative Affectivity Composite was .78.

2.6. Magnetic resonance imaging acquisition

Infants were scanned during natural sleep. A Siemens Skyra 3T MRI system equipped with a 20-channel head coil at the Brain Imaging Center at the University of Colorado Anschutz Medical Campus was used. Before scanning, infants were fed, swaddled, and placed into the scanner with their head secured within a vacuum-fixation device to limit motion. Infants wore earplugs and headphones to limit the acoustic noise of the scan. Infants were monitored by a research staff member who was in the scanner with the infant for the duration of the scan.

T1-weighted (T1w) images were obtained using a three-dimensional magnetization-prepared rapid gradient echo sequence (repetition time = 1900 ms; echo time = 3.07 ms; inversion time = 900 ms; flip angle 9°; 4 min 26 s) and T2-weighted (T2w) images were obtained with a 3D fast turbo spin echo sequence (repetition time 3200 ms; echo time = 408 ms; flip angle 43°; 4 min 43 s). The spatial resolution was a 0.82 × 0.82 × 0.8 mm voxel for T1w and 0.86 mm × 0.86 mm × 0.8 mm voxel for T2w.

2.7. Magnetic resonance imaging processing

Image quality control (QC) feedback was provided using a four point scale (0–3) (Blumenthal et al., 2002) adapted in-house for infant scanning. Criteria for exclusion was a QC score of 0, indicating artifact contamination (mainly due to subject motion) rendering the image processing unreliable. For two subjects who had artifact contamination in T2w image, T2 data were imputed from the T1w image. T2 images were imputed via the convolutional neural network approach PGAN trained on the UNC-EBDS neonate data (Gilmore et al., 2020) if the corresponding T1 images passed quality control (QC scores of 2–3) and T2 images failed. The T1w and T2w brain images were corrected for intensity non-uniformity via N4 (Tustison et al., 2010), rigidly transformed to a prior pediatric neonate atlas in stereotaxic space (Fonov et al., 2011). Brain masking was performed via the 3D UNet-based brain masking tool in ANTSpyNet (Tustison et al., 2021) using both T1w and T2w images jointly, including also extra-axial cerebrospinal fluid spaces in the brain mask. All brain masks were corrected manually in tksnaps (Yushkevich et al., 2006).

Tissue segmentation (into whole brain white matter, gray matter, and cerebrospinal fluid), regional parcellation, as well as hippocampus and amygdala segmentation were performed using a multi-modality (T1w and T2w), multi-Atlas segmentation workflow with the in-house, open-source MultiSegPipeline software, which employs atlas-registration and label fusion from the ANTs toolset (Tustison et al., 2021). Hippocampus and amygdala regions, measured in mm³, are defined as in (Moog et al., 2018) (see Fig. 1). Total intracranial volume was calculated as the sum of the brain tissue volumes of gray matter, white matter, and cerebrospinal fluid. The segmentation quality of all images was visually assessed and rated using a four point scale (0–3) for anatomical accuracy. Volumetric measurements rated as 0 (failed segmentation) were excluded from the analysis. No scan was removed based on segmentation quality.

2.8. Statistical analyses

2.8.1. Identification of covariates

Intracranial volume and postconceptional age at scan were included in all volumetric analyses, as there was an expected linear relation between intracranial volume and postconceptional age at scan with both bilateral amygdala (all rs > -0.265, and ps < .014) and hippocampal (all rs > -0.415, all ps < .001) volumes. Maternal ACEs were not associated with intracranial volume (r = -0.089, p = .418); and when additionally controlling for sex, postconceptional age at scan and birthweight percentile, intracranial volume showed a trend level association with ACEs (r = -0.197, p = .076). See Supplement for analyses without accounting for intracranial volume. Bivariate correlations were then conducted to test the following other potential covariates identified in the literature as associated with brain volume or negative emotionality: sex, birth weight percentile, parity, obstetric complications during the index pregnancy, and income-to-needs ratio (Gilmore et al., 2007; Dean et al., 2018; Makropoulos et al., 2016; Noble et al., 2012). Variables met covariate criteria if they were associated with infant brain volume at an alpha level of 0.05 (see Table 2). Sex and birth weight percentile met covariate criteria and were included as covariates in all analyses, with the addition of intracranial volume and postconceptional age for all volumetric analyses. Sensitivity analyses were conducted excluding infants of mothers (n = 4) with substance use exposure (see Supplement).

2.8.2. Maternal adverse childhood experiences, infant brain volume, and negative emotionality

Multivariate analyses with bilateral amygdala and hippocampal volume were conducted to examine the association between maternal adverse childhood experiences and amygdala and hippocampal brain volume, adjusting for intracranial volume, postconceptional age at scan, sex and birth weight percentile. Follow-up linear regression analyses were conducted to examine whether infant brain volume predicted negative emotionality. A linear regression was then conducted to examine the association of ACEs and infant negative emotionality at 6 months with sex and birth weight percentile as covariates. R² and beta values will be reported as measures of effect sizes for all regression models.

Lastly, a mediation analysis was performed using PROCESS (v3.5) for SPSS (v28) (www.afhayes.com) (Hayes, 2018) to evaluate the possibility that the direct effect between maternal ACEs and later infant negative emotionality operates through the indirect effect of amygdala or hippocampal volume. A bias-corrected bootstrap confidence interval (BC 95% CI) for the indirect effect was estimated based on 5000 bootstrap samples (Preacher and Hayes, 2008).

Typical associations between maternal childhood adversity and infant imaging outcomes, including infant volume as demonstrated in past published studies (Moog et al., 2018; Khoury et al., 2021) show a medium effect size (e.g., 𝑛2 = 0.06, 𝐹2 = 0.15). With sample size of 85 infants with quality MRI data, there was greater than 94% power to detect an effect of maternal ACEs on infant volume at a 0.05 significance level.
Results

Maternal adverse childhood experiences. Participants reported an average of 2.4 experiences of early adversity prior to the age of 18. The total number of experiences endorsed ranged from 0 to 10; 72% of participants reported experiencing at least one ACE. Rates of ACEs reported in the current sample are higher than national averages which show that 61% report experiencing at least one ACE (Merrick et al., 2019).

Maternal ACEs and infant hippocampus and amygdala volume. Results from multivariate analyses showed that maternal ACEs were associated with bilateral amygdala volume, $F(2, 78) = 3.68, p = .029$. Specifically, maternal ACEs were associated with smaller left ($\beta = -0.220, t(79) = -2.661, p = .009, R^2 = 0.494$) and right ($\beta = 0.167, t(79) = -2.043, p = .044, R^2 = 0.501$) amygdala volume. There was no significant association between maternal ACEs and bilateral hippocampal volume, $F(2, 78) = 2.115, p = .087$. The results of the analyses are displayed in Table 3.

Infant amygdala volume and infant negative emotionality. Regression analyses were then conducted with left and right amygdala volume to examine whether variability in amygdala volume was associated with negative emotionality. Smaller left amygdala volume was associated with higher negative emotionality ($\beta = 0.337, t(73) = -2.334, p = .022, R^2 = 0.16$). No significant association was observed between right amygdala volume and negative emotionality ($\beta = -0.178, t(73) = -1.97, p = .057$).

Table 2

| Right hippocampal volume | Left hippocampal volume | Right amygdala volume | Left amygdala volume |
|--------------------------|-------------------------|-----------------------|----------------------|
| ICV                      | .682**                  | .568**                | .610**               |
| PCA at scan              | .415**                  | .474**                | .265*                |
| Sex                      | .201                    | .137                  | .507**               |
| BW percentile            | .372**                  | .192                  | .262*                |
| Parity                   | .122                    | .010                  | .141                 |
| OB complication           | .035                    | .001                  | .003                 |
| INR                      | .123                    | .096                  | .045                 |
| Maternal age             | .053                    | .043                  | -.042                |
| Psychotropic medication  | .145                    | .063                  | -.026                |

Note: *p < .05, **p < .01; INR = income-to-needs ratio, ICV = intracranial volume, PCA = postconceptional age at scan, BW = birth weight percentile. Brain volume metric is mm$^3$.

Table 3

| Left amygdala | Right amygdala | Left hippocampus | Right hippocampus |
|---------------|----------------|------------------|-------------------|
| $\beta$       | t-stat         | p-val            | $\beta$           | t-stat         | p-val            |
| ICV           | .451           | 3.992            | <.001             | .478           | 4.261            | <.001             | .441           | 3.471            | <.001             |
| PCA           | .010           | 1.04             | .917              | -.016          | -1.157           | .875              | .225           | 1.992            | .050              |
| Sex           | .279           | 3.221            | .002              | .333           | 3.877            | <.001             | -.029          | -.293            | .770              |
| BW            | .120           | 1.385            | .179              | .038           | .436             | .001              | .027           | .276             | .783              |
| ACEs          | -.220          | -2.661           | .009              | -.167          | -2.043           | .044              | -.014          | -.151            | .881              |

Note: ICV = intracranial volume, PCA = postconceptional age at scan, BWP = birth weight percentile, ACEs = adverse childhood experiences. Brain volume metric is mm$^3$.  

Fig. 1. Bilateral Amygdala and Hippocampal Regions of Interest. 3D visualization of representative example of hippocampus and amygdala segmentation on MRI T1 (T2) weighted scan. (green = amygdala; blue = hippocampus; left = darker color; right = brighter color). Brain volume metric is mm$^3$.  

3. Results

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**Maternal ACEs and infant hippocampus and amygdala volume.** Results from multivariate analyses showed that maternal ACEs were associated with bilateral amygdala volume, $F(2, 78) = 3.68, p = .029$. Specifically, maternal ACEs were associated with smaller left ($\beta = -0.220, t(79) = -2.661, p = .009, R^2 = 0.494$) and right ($\beta = 0.167, t(79) = -2.043, p = .044, R^2 = 0.501$) amygdala volume. There was no significant association between maternal ACEs and bilateral hippocampal volume, $F(2, 78) = 2.115, p = .087$. The results of the analyses are displayed in Table 3.

**Infant amygdala volume and infant negative emotionality.** Regression analyses were then conducted with left and right amygdala volume to examine whether variability in amygdala volume was associated with negative emotionality. Smaller left amygdala volume was associated with higher negative emotionality ($\beta = -0.337, t(73) = -2.334, p = .022, R^2 = 0.16$). No significant association was observed between right amygdala volume and negative emotionality ($\beta = -0.178, t(73) = -1.97, p = .057$).
Maternal ACEs and infant negative emotionality. Additionally, maternal ACEs were positively associated with negative emotionality (β = 0.231, t (75)) = 2.093, p = .040, R² = 0.094).

Mediation analyses. Given that maternal ACEs were associated with both amygdala volume and negative emotionality at six months, and that left amygdala volume was associated with negative emotionality, a mediation analysis was conducted controlling for all covariates included in the previous statistical models. Results showed that higher maternal ACEs were not significantly indirectly associated with higher infant negative emotionality via left amygdala volume (Indirect = 0.0187, SE = 0.0151, 95% CI (-0.0016 - 0.0557)).

Sensitivity analyses demonstrated that the removal of infants of mothers with prenatal substance use (n = 4) showed comparable effect sizes to those found with the full dataset (see Supplement).

4. Discussion

Maternal history of childhood adversity is strongly implicated in child developmental outcomes with long term consequences for disease susceptibility and health. There is evidence for an intergenerational transmission of risk from mother to child (Swales et al., 2022); however, the mechanisms through which such risk is conferred remain unknown. Emerging data suggest that this link may be related to alterations in fetal brain development (Buss et al., 2017). The current study addressed this question by evaluating the association between maternal childhood adverse experiences and neonatal amygdala and hippocampal volume. Results showed that higher maternal ACEs were associated with smaller amygdala volume bilaterally; no significant associations were found with bilateral hippocampal volumes. Further, left amygdala volume was inversely associated with infant temperament at six months, such that smaller volume was associated with higher negative emotionality. Although maternal ACEs predicted infant negative emotionality, amygdala volume did not significantly mediate these associations. These findings suggest that maternal experiences of childhood adversity may shape fetal amygdala development leading to associations with neonatal amygdala volume. Further, these data indicate that structural variability of the amygdala may confer intergenerational risk for later negative emotionality.

Results from our study align with recent findings showing that maternal childhood maltreatment is associated with reductions in offspring amygdala volume beyond the neonatal period in children between 4 and 24 months old, although the association was stronger among older children relative to younger ones (Khoury et al., 2021). Moog and colleagues (Moog et al., 2018) found that maternal experience of childhood maltreatment was associated with global decreases in neonatal brain volume but did not find any volumetric differences within specific a priori brain regions, including the amygdala or hippocampus. One potential reason for this discrepancy with our findings of reduced bilateral amygdala volume is the assessment of the type of stressor. Moog et al. investigated effects of childhood maltreatment as the only type of adversity; in contrast, the current study investigated associations with broader adverse childhood experiences perhaps capturing a broader accumulation of experiences that may contribute to subsequent risk. Future research is needed to further elucidate the moderating role of different types of early childhood adversity and their severity on neurodevelopment.

Our results also support previous findings demonstrating the importance of the amygdala in the regulation of negative emotionality (Phelps, 2006). We found that smaller left amygdala volume was associated with increased negative emotionality at 6 months. However, laterality of these findings should be interpreted with caution as the pattern of association with negative emotionality was consistent across both the left and right amygdala, and further evaluation is an avenue for future research. Further, we did not observe a link between maternal ACEs and newborn hippocampal volume. Previous findings have highlighted the susceptibility of the hippocampus to early life stress (Jenkins et al., 2022; Humphreys et al., 2019; Fenoglio et al., 2006). Although our findings suggest that maternal ACEs may not be associated with hippocampal volume in infancy, it is possible that with continued development of the hippocampus over the postnatal period, links with maternal ACEs will emerge. For example, previous findings have shown that associations between maternal mental health and offspring hippocampal volume emerge only after 6 months (Qu et al., 2013). The specific mechanisms underlying the association between maternal early adversity and infant amygdala volume remain unclear. The transmission of experiences of maternal early life stress may occur through alterations in aspects of gestational biology including endocrine and immune system processes. Maternal psychological well-being has been linked to endocrine and immune function in pregnancy (Cousins-Read et al., 2005; Kane et al., 2014; Peterson et al., 2020). Dysregulation of the hypothalamic-pituitary-adrenal axis (HPA) is one putative mechanism as HPA hormones, including cortisol and placental corticotropin releasing hormone (CRH), influence biological processes important in fetal brain development, including neurogenesis and dendritic growth (Curran et al., 2017; Liao et al., 2014). Maternal antenatal cortisol levels are predictive of fetal development (Glynn and Sandman, 2012) as well as infant cortisol reactivity (Irwin et al., 2021) and temperament (Davis et al., 2017). There is also data to suggest that maternal cortisol over the course of pregnancy influences amygdala volumes in childhood (Buss et al., 2012); however, very little is known about the role of cortisol signaling on infant brain development (Graham et al., 2019; Stove et al., 2020). In addition to dysregulation of the HPA axis, there has been more recent focus on the role of inflammation and immune system functioning in linking maternal stress to offspring neurodevelopment and psychiatric risk (Hanson et al., 2019), for review see (Demers et al., 2021). Indeed, burgeoning research demonstrates maternal proinflammatory IL-6 concentrations during pregnancy are associated with structural and functional neurodevelopment in neonates (Graham et al., 2018; Rudolph et al., 2018; Spann et al., 2018).

4.1. Strengths and limitations

This study addressed previous limitations of the literature with our study design and timing to more rigorously investigate the neuro-developmental consequences of early maternal life experiences within the neonatal brain shortly after birth. Timing of the scan is critical as it minimizes intervening effects of postnatal influences (e.g., parenting). The current study also used a longitudinal design to investigate how variability in infant morphology is associated with later negative emotionality at six months. Further, prenatal obstetric and medical factors (e.g., prenatal infection, gestational diabetes, oligohydramnios or polyhydramnios) were carefully assessed given their potential effects on neurodevelopment (Boardman and Counsell, 2020; Maher et al., 2018). These prenatal obstetric and medical factors, as well as birth outcomes (e.g., birth weight percentile), were considered and sensitivity analyses were performed (see Supplement) and did not account for our findings linking maternal ACEs, infant amygdala volume and negative emotionality.

This study is not without limitations. Maternal ACEs were reported retrospectively, as is commonly done in intergenerational studies (Cooke et al., 2019; Letourneau et al., 2019) given the logistical, financial, and timing challenges of following individuals over decades with real-time reports of their ACEs to have as measures for later linking to child health and well-being. The ACEs questionnaire also combines a broad range of experiences and the field has struggled with the value of summing adversity without consideration of severity or duration (McLaughlin et al., 2021; Smith and Pollak, 2021). Future research should further investigate biological mechanisms of intergenerational transmission of adversity using alternative conceptualizations of adversity beyond the cumulative approach of the ACEs questionnaire. Additionally, the current study is necessarily correlational, relying on...
naturally occurring variations in ACEs rather than those that are experimentally manipulated; thus, we cannot rule out the role of alternative factors such as shared genes. However, our results in humans are consistent with findings from experimental animal models, which demonstrate that exposure to adverse experiences occurring prior to pregnancy exerts lasting influences on offspring brain and behavior (Li et al., 2010; Bock et al., 2014), as well as the broader literature highlighting the impact of childhood adversity. Additionally, infant negative emotionality was assessed via maternal report of child behavior. The IBQ was designed to limit biases associated with concurrent maternal mental health by assessing specific infant behaviors in concrete situations. Supporting the use of the IBQ, previous data within a highly diverse and low income sample demonstrate that mother reported negative affect from the IBQ is positively associated with observed infant affect from coded mother-child interactions (Van Schagen Johnson et al., 2016). Lastly, this investigation was not adequately powered to test sex differences. As there is evidence that there are sex differences in response to early adversity (Sandman et al., 2013; Davis and Pfaff, 2014), future research should address this question.

Taken together, findings from the current study demonstrate links between early maternal childhood experiences and infant brain and temperament, therefore supporting intergenerational transmission of adversity from mother to child. Findings from this study thus provide evidence of the importance of extending prevention efforts to preconceptional periods to target potentially modifiable risk factors that influence gestational biology. Although we cannot modify childhood experiences, reflection on positive childhood experiences has been shown to be a resiliency factor that can be leveraged in pregnant individuals to prepare for parenthood (Narayan et al., 2018). Preventive inventions would benefit from a greater understanding of how timing, type, and chronicity of maternal stress exposure impacts child risk.

CRediT authorship contribution statement

**Catherine H. Demers:** Conceptualization, Formal analysis, Data curation, Writing – original draft, Writing – review & editing. **Benjamin L. Hankin:** Conceptualization, Supervision, Project administration, Formal analysis, Writing – original draft, Writing – review & editing. **Ella-Marie P. Hennessy:** Conceptualization, Formal analysis, Data curation, Writing – original draft, Writing – review & editing. **Mercedes Hoeflich Haase:** Software, Data curation, Visualization, Writing – original draft. **Maria M. Bagonis:** Software, Data curation, Visualization, Writing – original draft. **Sun Hyung Kim:** Software, Data curation, Writing – original draft. **John H. Gilmore:** Supervision, Methodology, Writing – original draft. **Martin A. Styner:** Software, Formal analysis, Data curation, Writing – original draft, Writing – review & editing. **Elysia Poggi Davis:** Conceptualization, Supervision, Formal analysis, Writing – original draft, Writing – review & editing.

Declaration of competing interest

Dr. Bagonis reports employment at PrimeNeuro. All other authors report having no biomedical financial interests or potential conflicts of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jnstr.2022.100487.

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