Exploring the relationship between maternal prenatal stress and brain structure in premature neonates

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Abstract

Background: Exposure to maternal stress in utero is associated with a range of adverse outcomes. We previously observed an association between maternal stress and white matter microstructure in a sample of infants born prematurely. In this study, we aimed to investigate the relationship between maternal trait anxiety, stressful life events and brain volumes.

Methods: 221 infants (114 males, 107 females) born prematurely (median gestational age = 30.43 weeks [range 23.57-32.86]) underwent magnetic resonance imaging around term-equivalent age (mean=42.20 weeks, SD=1.60). Brain volumes were extracted for the following regions of interest: frontal lobe, temporal lobe, amygdala, hippocampus, thalamus and normalized to total brain volume. Multiple linear regressions were conducted to investigate the relationship between maternal anxiety/stress and brain volumes, controlling for gestational age at birth, postmenstrual age at scan, socioeconomic status, sex, days on total parenteral nutrition. Additional exploratory Tensor Based Morphometry analyses were performed to obtain voxel-wise brain volume changes from Jacobian determinant maps.

Results and Conclusion: In this large prospective study, we did not find evidence of a relationship between maternal prenatal stress or trait anxiety and brain volumes. This was the case for both the main analysis using a region-of-interest approach, and for the exploratory analysis using Jacobian determinant maps. We discuss these results in the context of conflicting evidence from previous studies and highlight the need for further research on premature infants, particularly including term-born controls.

Keywords: maternal anxiety, prenatal stress, structural MRI, preterm

Introduction

Poor maternal mental health during pregnancy represents a global public health problem, affecting 10-35% of pregnant women [1, 2]. Maternal prenatal psychological distress in the form of maternal depression, anxiety, and/or stress has been associated with adverse obstetrical and early behavioural outcomes, and an increased risk of neurodevelopmental and psychiatric disorders [3-7]. The biological basis of these effects is still poorly understood. However, studies by our group and others suggest prenatal maternal stress modulates the neurodevelopment of brain networks that underpin these disorders [8-11].

The brain regions that appear to be most vulnerable to maternal prenatal stress, other forms of early adversity, and psychopathology include the regions of the frontal lobe, temporal lobe, and limbic system [12-17]. These areas are connected by the uncinate fasciculus, and we recently reported an association between maternal stressful life events and increased diffusivity in this tract, in a sample of premature neonates [18].

However, although there is evidence suggesting that maternal prenatal stress affects the development of white matter tracts, evidence for early changes in structural brain development is inconclusive [12]. A small number of studies have examined this relationship in neonates and infants born at term, suggesting no evidence for differences in brain volumes in relation to maternal psychological distress [10, 19, 20]. Several studies have been conducted on older participants (i.e. childhood, adolescence, and adulthood), with the most commonly reported findings being cortical thinning [21, 22, 23, 24], and either reductions [25-27], or increases in regional volumes [28-30].

While human studies so far have been inconclusive, animal studies have provided some limited evidence that maternal distress is related to early volume changes in the limbic system, particularly the hippocampus, amygdala, and thalamus [31-36].

We must also consider biological sex as a potential moderator of risk transmission, as several studies have reported volume changes in females, but not males [28, 30, 37]. In utero stress exposure has been associated with higher rates of mood disorders and anxiety [38-40] in females, and behavioural problems [41] and ADHD [6] in males. High maternal cortisol levels at 15 weeks' gestation has been associated with increased right amygdala volumes and more affective problems in female, but not male, offspring [41].

In summary, although research has reported differences in brain structure in children, adolescents and adults exposed to maternal psychological distress, evidence in infants is inconclusive. To our knowledge, no studies have investigated this relationship in infants born prematurely. Premature birth is associated with changes in brain development [42] and an increased risk of adverse neurodevelopmental and psychiatric outcomes [43, 44]. In order to improve outcomes in these children, it is important to better understand the role that early adverse experiences such as exposure to prenatal stress could have in moderating these associations.

In this study, we investigated the relationship between maternal trait anxiety and stressful life events, and brain volumes in a large sample of infants born prematurely. We have previously shown differences in white matter microstructure in the uncinate fasciculus in this sample [18]. Based on previous literature, we hypothesized that maternal prenatal stress/trait anxiety would be associated with regional volume differences in areas adjacent to the uncinate

fasciculus: frontal and temporal lobe volume, amygdala, hippocampus and thalamus. As the direction of effect in the literature is inconsistent (i.e. volumes found to be normal, enlarged, or decreased), we did not hypothesize a direction of effect. Lastly, given the heterogeneity of outcomes associated with maternal stress, as well as the complexity of functional anatomy in the chosen regions of interest (Text in S1 Supplement), we conducted a whole brain analysis using Tensor Based Morphometry.

Methods and materials

Participants

Participants were mother-infant dyads who took part of the Evaluation of Preterm Imaging Study (ePRIME, [45]). Ethical approval was obtained from the Hammersmith and Queen Charlotte's Research Ethics Committee (09/H0707/98) and informed written consent was obtained from all participants. Participants were recruited between April 2010 and July 2013 by screening 3619 admissions to level 1,2 and 3 neonatal units at 14 London Hospitals. Eligibility criteria for the main study included: infant born before 33 weeks gestational age, mother aged over 16 years, not a hospital inpatient, no major congenital malformation, no prior MRI, no care in a centre where preterm MRI was routine, no metallic implants, parents able to speak English, parents not subject to child protection proceedings. The ePrime cohort is representative of the UK NICU population in terms of birth weight, ethnicity, and prevalence of cerebral palsy (6%). Additional information is available in [45].

Data was available for n=511 infants who were born prematurely (before 33 weeks of gestation) and scanned at term equivalent age. We excluded cases where the postmenstrual age at scan was >45 (n=48), data was not available for all variables of interest (n=160),

women disclosed alcohol and/or drug abuse during pregnancy (n=5), or the images showed major focal lesions such as periventricular leukomalacia, haemorrhagic parenchymal infarction, and other ischemic or haemorrhagic lesions (n=40). In cases where a mother had multiple infants enrolled in the study (i.e. twin and triplet pregnancies), only one infant was randomly included in the final analysis. From the remaining sample, segmentation data for the structures of interest were available for n=221 (Table 1), and a voxel-wise exploratory analysis using Tensor Based Morphometry was performed on the same 221 participants. The sample partially overlapped (n=191) with a previous study [18]. Maternal socioeconomic status (SES) values were extracted from the Carstairs index, which takes into consideration variables such as unemployment, car ownership, household overcrowding, and social class [46].

Table 1. Obstetric and Sociodemographic characteristics (n=221)

Maternal Characteristics	**Reported**	**Values**
Stressful life events score	Median (range)	53 (0-270)
Trait anxiety score	Median (range)	36 (20-67)
Maternal age at scan	Mean (SD)	32.94 (5.70)
Maternal SES	Median (range)	17.44 (1.73-60.58)
Maternal education (years) 16 or less 17-19 19+ Still in full-time education Not reported	N (%)	 24 (10.8%) 30 (13.5%) 156 (70.6%) 8 (3.6%) 3 (1.3%)
Infant Characteristics	**Reported**	**Values**
Infant sex Male Female	N (%)	 114 (51.5%) 107 (48.4%)
GA at birth (weeks)	Median (range)	30.43 (23.57-32.86)
PMA at scan (weeks)	Mean (SD)	42.20 (1.60)
Birth weight (grams)	Median (range)	1300 (600-2600)
OFC at birth (cm), n=192	Median (range)	29.00 (21.80-36)
Number of days on TPN	Median (range)	6 (0-59)
Number of days on ventilation	Median (range)	0 (0-33)

Mean and SD are reported for normally distributed data; median and range are reported for non normally distributed data. GA=gestational age, OFC=Orbitofrontal circumference, PMA=postmenstrual age, SD=standard deviation, SES=socioeconomic status, TPN=Total Parenteral Nutrition, Maternal education = age at leaving formal education. No missing data unless otherwise specified in table.

Trait anxiety

The State Trait Anxiety Inventory (STAI, [47]) which measures levels of anxiety right now (i.e. state) and in general (i.e. trait), was administered at the time of the MRI scan. The

analysis was restricted to trait anxiety, as it measures a relatively stable tendency to be prone to experiencing anxiety and thus extends to the period before birth.

For trait anxiety, missing values were imputed for cases in which a maximum of 10% of questions were missing. We imputed missing values by calculating the average response for the questions that were answered. Missing values were imputed for n=23 (n=18 missing 1/20 answers and n=5 missing 2/20 answers).

Stressful life events

Stressful life events were assessed using a questionnaire adapted from the Avon Longitudinal Study of Parents and Children [48], which included yes/no answers to a list of potentially stressful life events the participant may have experienced in the year prior to the study visit (e.g. “Arguments with your partner increased”). Events were ranked according to severity [18] based on the Social Readjustment Rating Scale [49] and summed to form a final score that accounts for the number and severity of events experienced (Table J in S1 Supplement). There were no missing data for this variable.

MR imaging

Magnetic resonance imaging data were acquired using an 8-channel phased array head coil, on a Philips 3T (Philips Medical Systems, Best, The Netherlands) MR system located on the intensive care unit. Imaging data was acquired as follows: Three-dimensional magnetization prepared rapid acquisition gradient echo (repetition time: 17 ms; echo time: 4.6 ms; flip angle: 13°; slice thickness: 0.8 mm; in-plane resolution: 0.82 × 0.82 mm2), T2-weighted turbo spin echo (repetition time: 8670 ms; echo time: 160 ms; flip angle: 90°; slice thickness: 2 mm; in-plane resolution: 0.86 × 0.86 mm2), and single shot echo planar DTI (repetition

time: 7536 ms; echo time: 49 ms; flip angle: 90°; slice thickness: 2 mm; in-plane resolution: 2 x 2 mm2, 32 noncollinear gradient directions, b value of 750 s/mm2, 1 non-diffusion-weighted image, b = 0).

An experienced paediatrician supervised all scanning sessions. To enable a successful scan, the majority of infants included in this study were sedated with oral chloral hydrate (25-50 mg/kg) and monitored throughout the scan using pulse oximetry, temperature monitors and electrocardiography. Ear protection was used for all infants, in the form of earplugs molded from a silicone-based putty (President Putty; Coltene Whaledent, Mahwah, NJ) and neonatal earmuffs (MiniMuffs; Natus Medical Inc., San Carlos, CA).

Segmentations

Images were analysed using an automated processing pipeline optimised for neonates. Following motion correction, bias correction and brain extraction, T2w images were segmented using the Draw-EM algorithm, an open-source software optimised for neonatal brain segmentation [50]. Analysing MR images from infants, and especially preterm infants, poses unique challenges, such as motion, lower contrast-to-noise ratio, and partial volume effects; for a discussion of how these were addressed, see [50].

Based on previous literature and considerations of multiple comparisons issues, the following volumes were chosen as variables of interest: amygdala, hippocampus, thalamus, frontal lobe and temporal lobe (Table A in S1 Supplement). To account for inter-individual differences in brain size, all brain volumes included in the analysis were normalized to total brain volume (i.e. dividing each regional volume by total brain volume).

Tensor-Based Morphometry

Template construction

A multivariate study-specific template was built using images from a subset of 161 participants. In order to reduce computational load, a smaller subset of 161 images meeting inclusion criteria (i.e. PMA at scan <45 weeks, no major lesions, and of good quality) were used to build the population template for this study. Using the Advanced Normalization Tools (ANTS) software to build the template [51], we applied field bias correction and used the Developing Human Connectome Project 40 weeks' gestational age T2-weighted [52] and T2 tissue labels templates [50] as the target volumes for the template construction inputs. Iteration limit was set to the default (4 iterations).

Registration and log-Jacobian determinants

Images were registered to the study-specific template using the multimodal Symmetric Normalisation (SyN) algorithm from the ANTs software (n=221) [53]. To improve image registration, two input modalities were used: T2-weighted images and T2-based tissue type segmentation [50]. T2-weighted deformation tensor fields (i.e. warps) from non-linear transformations of the registration process were used to compute a logarithm transformation of Jacobian determinant maps (i.e. deformation tensor field gradients), which reflect volume changes from the template at the voxel-level [54]. Jacobian determinants reflect the degree of transformation (i.e. the expanding or contracting) an image voxel has undergone in order to fit into the template space; therefore, providing information on the relative volumes of brain structures. Smoothing with a 4mm full-width half-maximum Gaussian filter was applied to the log-Jacobian determinants, in order to increase the signal-to-noise ratio. We re-sampled the smoothed log-Jacobian maps from the original isotropic voxel size of 0.5 cm3 to 1 cm3

before running statistical analyses in order to help with computation and memory constraints.

Statistical analysis

Main analysis

Statistical analysis was performed using R [55], with the main packages being psych [56], ggplot2 [57], and hmisc [58]. A minimal dataset and the analysis code including a comprehensive list of packages are available in the Supplement (Text in S1 Supplement, S3 Dataset).

We assessed potential covariates using bivariate Spearman's correlations (Table B in S1 Supplement). Birth weight was excluded as a covariate from the main analysis as it was highly correlated with gestational age ($r=.74$, $p<.001$). The number of days on ventilation was also excluded as a covariate in the main analysis as it was highly correlated with the number of days on total parenteral nutrition ($r=.60$, $p=.001$), both measures provide information on the health status of the infant, and the distribution of days on total parenteral nutrition was less skewed. Maternal education and number of complications were not correlated with any of the variables of interest and thus were excluded in the main analysis. The regression models used were the same as those used in [18].

Multiple linear regressions were conducted to investigate the relationship between maternal trait anxiety/stress and brain volumes in premature infants. Our models contained the following predictors: stressful life events, trait anxiety, GA, PMA, SES, biological sex, days on total parenteral nutrition. The models were run separately for each dependent variable (frontal lobe grey matter, temporal lobe grey matter, thalamus, amygdala, hippocampus).

Correction for multiple comparisons was performed using False Discovery Rate (FDR), and all p values reported below are uncorrected. Unless otherwise specified, all regression models met assumptions for multiple regression (i.e. normality, linearity, homogeneity of variance, uncorrelated predictors, no influential outliers, independence of residuals, [59], Table C in S1 Supplement). One outlier was removed from all regressions due to violating assumptions of normality (days TPN = 59).

Exploratory analysis of Tensor Based Morphometry

Voxel-wise statistical analyses were performed using FSL's randomise nonparametric permutation testing [60]. A general linear model tested for relationships between log-Jacobian values at the voxel level and the outcome variables of interest (maternal prenatal stress and trait anxiety). We included gestational age at birth, postmenstrual age at scan, socioeconomic status, sex and days on total parenteral nutrition as covariates in our model. We ran 10,000 permutations of the data and used 3D Threshold-Free Cluster Enhancement (TFCE) and Family Wise Error (FWE) to correct for multiple comparisons [61]. Voxels with FWE-corrected P-values at a threshold of $P<0.05$ were considered to be significant.

Results

Segmentations

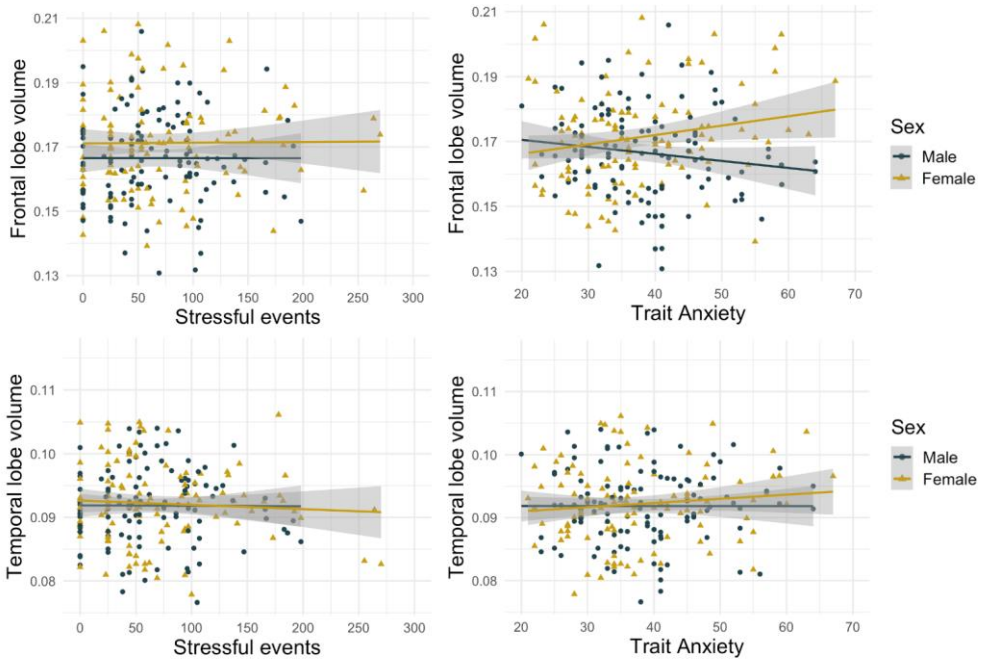
Frontal grey matter volume

The model performed better than expected by chance ($p<.001$) and accounted for 42% of variance in frontal lobe volume (predicted by PMA, with B=.0058 and SES, with B=.00015).

There was no association between frontal grey matter volume and either stressful life events (B=.000018, t=1.27, p=.204) or trait anxiety (B=-.000024, t=-.304, p=.761, Table D in S1 Supplement). An alternative model removing these two variables performed better (R^2=.42, AIC=-1338.38) than the original model (R^2=.41, AIC=-1336.09), suggesting that the best fit for a model predicting frontal grey matter volume is one without stressful life events or trait anxiety.

Further exploring this relationship with direct Spearman correlations (in the absence of covariates) showed no evidence for a relationship (Fig 1) between frontal grey matter volume and stressful life events (r=.04, p=.593) or trait anxiety (r=.006, p=.929).

Fig 1. Scatterplots for correlations between maternal trait anxiety/stress and volumes for the frontal and temporal lobes. See Fig A in S1 Supplement for partial regression scatterplots.



Temporal grey matter volume

The model did not meet assumptions of homogeneity of variance, and thus we report the heteroscedasticity corrected covariance matrix (Table F in S1 Supplement). The model accounted for 45% of variance in temporal grey matter volume (predicted by PMA, with B=.0025 and SES, with B=.000051). There was no relationship with stressful life events (B=.0000027, t=.495, p=.621) or trait anxiety (B=.0000047, t=.140, p=.889) (Table E in S1 Supplement).

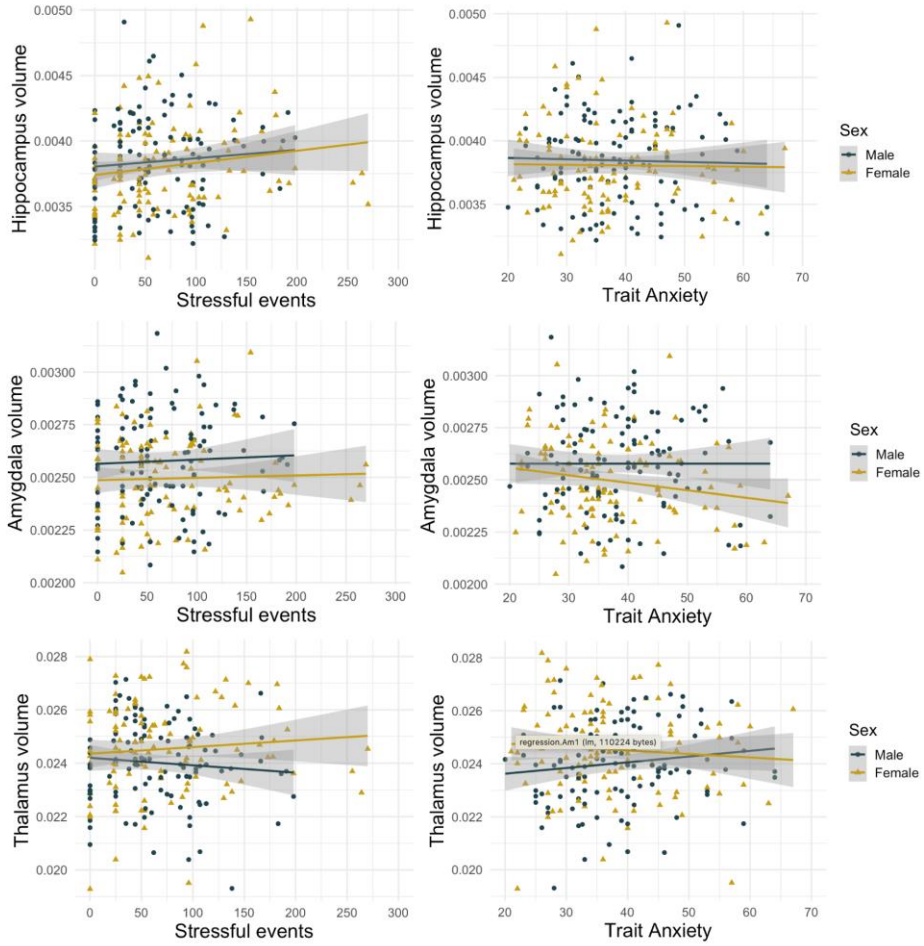
An alternative model removing these two variables performed better (R^2=.46, AIC=-.1735.3) than the original model (R^2=.46, AIC=-1731.5), suggesting that the best fit for a model predicting temporal grey matter volume is one without stressful life events or trait anxiety. Further exploring this relationship with direct Spearman correlations (in the absence of covariates) showed no evidence for a relationship (Fig 1) between temporal grey matter volume and stressful life events (r=.04, p=.667) or trait anxiety (r=.05, p=.440).

Hippocampal volume

Hippocampal volume was not accurately predicted by the model (R^2=.06, F(8,211)=1.58, p=.131), with the only significant predictor being socioeconomic status (B=-.0000040, t=-2.08, p=.039). As the model showed deviations from linearity (Text in S1 Supplement), we repeated the analysis removing 3 outliers (stressful life event scores >250). The new model did not adequately predict hippocampal volume either (R^2=.07, F(8,208)=2.17, p=.031), but stressful life events was a significant predictor (B=.0000012, t=2.57, p=.011), alongside socioeconomic status (B=-.0000045, t=-2.36, p=.019) (Table G in S1 Supplement). This

result did not survive correction for multiple comparisons and visual inspection of the plot suggests no relationship between the variables. An alternative model excluding trait anxiety and stressful life events performed worse (R2=.05, p=.111), with a higher AIC of -2840.17 compared with -.2842.99. Further exploring this relationship with direct Spearman correlations (in the absence of covariates), suggested a positive correlation between hippocampal volume and stressful life events (r=.16, p=.020), but not trait anxiety (r=-.004, p=.959)(Fig 2).

Fig 2. Scatterplots for correlations between maternal trait anxiety/stress and volumes for the hippocampus, amygdala, and thalamus. See Fig B in S1 Supplement for partial regression scatterplots.



Amygdala volume

For amygdala volume, the model performed better than expected by chance and accounted for 27% of variance in outcome measures (predicted by PMA, with B=-.000064 and SES,

with B=-.0000023). There was no relationship with stressful life events (B=-.000000028, t=-.114, p=.909) or trait anxiety (B=-.0000013, t=-1.000, p=.319)(Table H in S1 Supplement). An alternative model removing these two variables performed better (R^2=.27, AIC=-3123.95) than the original model (R^2=.27, AIC=-3121.05). Direct Spearman correlations showed no evidence for a relationship between amygdala volume and stressful life events (r=.02, p=.770) or trait anxiety (r=-.05, p=.505)(Fig 2). As the model showed deviations from linearity (Text in S1 Supplement), we repeated the analysis removing 3 outliers (stressful life event scores >250). The new model revealed similar results (Table H in S1 Supplement).

Thalamus volume

Thalamus volume was not accurately predicted by the model (R^2=.08, F(8,210)=2.40, p=.017). There was no significant relationship between thalamus volume and stressful events (B=-.00000021, t=-.10, p=.920) or trait anxiety (B= -0.00000067, t=-.05, p=.953) (Table I in S1 Supplement). Direct Spearman correlations showed that there was no relationship between thalamus volume and stressful life events (r=-.03, p=.684) or trait anxiety (r=.03, p=.713)(Fig 2).

Exploratory analysis subdividing the sample by sex

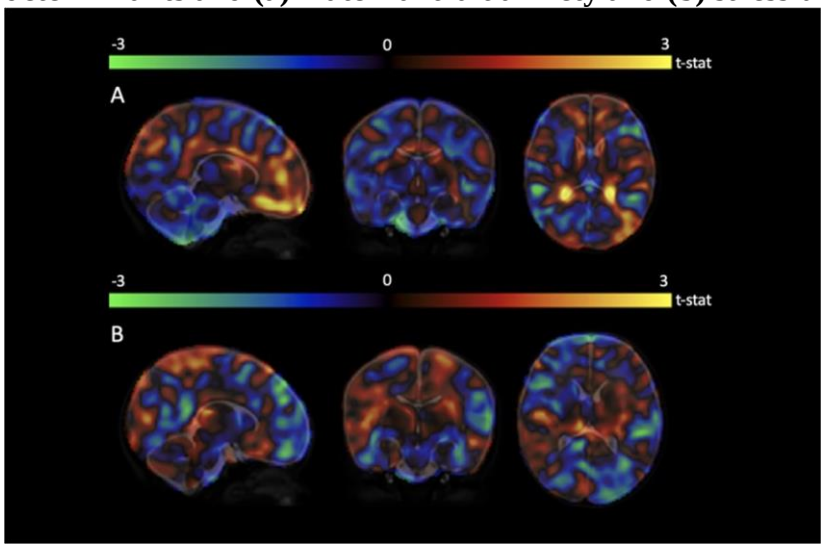
As visual inspection of scatterplots suggested that the relationship between maternal trait anxiety/stress and brain volumes may be influenced by infant sex, we repeated our analysis subdividing the sample into males and females. There were no significant relationships between maternal trait anxiety/stressful events and infant volume in frontal lobe, temporal lobe, amygdala, thalamus (Text in S1 Supplement).

In our female sample, hippocampal volume was not accurately predicted by the model (R^2=.12, F(7,95)=1.79,p=.097), but the only significant predictor was stressful life events (B=.0000017, t=2.65, p=.009). This did not survive correction for multiple comparisons. The relationship between hippocampal volume and stressful life events was not observed in males.

Voxel wise Tensor Based Morphometry results

In order to explore whether maternal stress or trait anxiety were associated with neonatal brain volumes at the voxel-level, we conducted Tensor Based Morphometry analyses to obtain Jacobian determinant maps which reflect relative voxel-wise volume changes. Tensor Based Morphometry did not reveal any significant relationships between the smoothed log-Jacobian determinants and maternal prenatal stress or trait anxiety at the FWE $p<0.05$ threshold. The T-statistic maps (Fig 3) show the test statistic at the voxel level before corrections for multiple comparisons were applied. The whole-brain t-stat maps show generally low t-stat values indicating poor associations between maternal trait anxiety (Fig 3a), or stressful life events (Fig 3b) and log-Jacobian determinants. Nifti files for the t-stat maps are available in the S2 File.

Fig 3. T-statistic maps showing the relationships between voxel-wise log-Jacobian determinants and (a) maternal trait anxiety and (b) stressful life events.



Discussion

In this study, we did not find evidence for a relationship between maternal stress (i.e. stressful life events and trait anxiety) and grey matter volumes in a large sample of infants born prematurely. These results were consistent across 2 methodologies, using both a whole-brain voxel-wise approach, as well as a region of interest analysis (i.e. hippocampus, amygdala, thalamus, frontal lobe, and temporal lobe).

Interpretation of these findings raises important questions for a field that, to date, has been complicated by inconsistencies between studies along multiple dimensions. These include differences in the samples studied (e.g. age, gender), imaging protocols, definitions of stress, and sample size [12, 62]. Our findings are in line with [10] who reported no difference in right amygdala volume in a large sample of neonates (n=157) exposed to maternal depression in the second trimester of pregnancy. Similarly, [20] reported no difference in hippocampal volume at birth, but suggested that the hippocampal volume exhibits slower growth in response to exposure to maternal trait anxiety in utero, with smaller volumes being observed

405 at 6 months of age. In a study of exposure to selective serotonin reuptake inhibitors,
406 differences in volume were reported in the right amygdala and right insula [19], but the
407 authors reported no differences in limbic system volumes between untreated depression and
408 controls. Further, [27], in a study of young adults, reported no association between maternal
409 prenatal stress and hippocampal volume, which was instead associated with postnatal anxiety.
410 Studies that have reported associations with maternal distress, primarily regarding cortical
411 thinning in regions of the frontal and temporal lobes [21-24] have been conducted on children
412 rather than infants. Overall, at present, there seems to be no consistent evidence that maternal
413 prenatal stress is associated with neonatal brain volumes, in line with our findings.
414
415 This is in stark contrast to the diffusion MRI literature, where studies have consistently
416 reported alterations in limbic and prefrontal microstructure in neonates and infants exposed to
417 maternal psychological distress in utero [9-11, 18, 63]. Further, given that diffusion MRI
418 studies have reported also collecting T2-weighted images, we need to consider whether the
419 lack of studies reporting structural MRI analyses may be driven by the failure to report non-
420 significant findings (i.e. the "file-drawer" problem, [64]). In a recent study published on an
421 overlapping sample [18], we showed differences in white matter microstructure in the
422 uncinate fasciculus in relation to maternal stressful life events. Interestingly, a few of the
423 studies which failed to observe differences in brain structure in relation to maternal
424 psychological distress, reported alterations in white matter microstructure. For example, [10],
425 observed lower fractional anisotropy in the right amygdala of neonates exposed to maternal
426 depression, with no evidence for differences in amygdala volume. Converging evidence
427 suggests that maternal prenatal stress can alter the developing connectome, with differences
428 being most commonly reported in fronto-limbic brain networks (using fMRI and dMRI), with
429 limited evidence for differences in brain structure [62]. Further studies conducted on term-

born and preterm infants and reporting on both structural and diffusion MRI are required in order to clarify whether white matter is especially vulnerable to maternal prenatal stress. This is of particular importance given that white matter injury is the most common neuropathology in infants born prematurely [65-67 and white matter may therefore be more vulnerable to additional stressors.

The current study also raised the possibility that the relationship between maternal distress and early brain development may be at least partly influenced by sex differences in the vulnerability to maternal stress in utero. Maternal stressful life events were associated with increased hippocampal volume in the whole sample and in females, but not males; however, these findings were not found to be statistically significant after correction for multiple comparisons.

It is important to highlight that our sample consisted of preterm infants, a population known to have regional brain volume abnormalities [42] and adverse neuropsychiatric and developmental outcomes [44, 68]. We caution against generalizing these findings to infants born at term, and suggest that further studies with term-born controls are needed to further clarify the role that early adverse experiences such as maternal stress may have in moderating the association between preterm birth and adverse outcomes in this vulnerable population.

Although in this study we have examined mean bilateral volumes, several studies of children have reported unilateral differences in volume, such as increased left amygdala volume in girls exposed to pregnancy-specific anxiety, but not boys [37] and greater right amygdala volume in girls exposed to maternal depression, but not boys [30]. Although our analysis was based on mean volumes, the whole-brain analysis did not suggest lateralized differences in

volume associated with maternal stress or trait anxiety. Further, other studies that have reported differences in volumes in areas such as the frontal lobe, reported these in very specific areas, such as the mid-dorsolateral frontal cortex [27] or left medial temporal lobe [26]. This may mean that any changes associated with maternal prenatal stress may be more subtle, and thus not affect the overall volume of the frontal or temporal lobes. However, our findings using a voxel-wise whole-brain analysis did not suggest any volume differences associated with maternal stress.

Our findings are not in line with those of [26], who reported decreased amygdala volume, or [28], who reported increased amygdala volume in girls. However, both of these studies were conducted on adult samples, and measures of maternal stress were acquired retrospectively. The biological basis of these potential sex differences is unclear, but may include sex differences in placental functioning, fetal exposure to adrenal hormones and testosterone, as well as various epigenetic mechanisms [69].

Further, there is some evidence to suggest that the child's development may be more susceptible to maternal pregnancy-specific anxiety, rather than generalized anxiety or stress, as well as that the timing of stress exposure is an important factor to consider [20]. A study [25] suggested that pregnancy anxiety is associated with differences in gray-matter volume at age 6-9, and later reported that neither state anxiety nor depression explained any additional variance in developmental outcomes after accounting for pregnancy-specific anxiety [70]. Future studies should include measures of pregnancy-specific anxiety and assess stress exposure during early, mid, and late gestation.

Although not one of the measures of interest in this study, socio-economic status (which was entered into the regression models as a covariate) was consistently associated with differences in brain volume in our sample of infants born prematurely. Based on these findings, we recommend that future studies should investigate the relationship between socioeconomic status and early brain development, particularly given that low SES is known to be associated with adverse mental health, underreporting of mental health concerns, as well as lack of access to mental health services [71].

It is important to note that although this study was based on subjective self-report measures, the reliability of maternal recall for pregnancy and birth related events appears to be high [72-74], false positive reports of adverse life events are rare [75], and self-reported trait anxiety scores are relatively stable in the perinatal period [76] (See Text in S1 Supplement for further discussion). Future studies should consider including both subjective and laboratory-based measures of stress or anxiety, such as autonomic function, or blood cortisol.

In conclusion, based on our previous findings in an overlapping sample [18], we expected an association between maternal stress and brain volumes in areas adjacent to the uncinate fasciculus tract. To our knowledge, the current study is the first one to examine this relationship in premature infants. In our sample, there is no credible evidence that maternal prenatal stressful life events or trait anxiety influence volumes in the hippocampus, amygdala, thalamus, frontal grey matter or temporal grey matter volume in preterm infants. Our findings are strengthened by an exploratory voxel-wise analysis, and in line with previous literature. Our findings are of particular interest in the context of having reported differences in white matter microstructure in an overlapping sample, using the same statistical methods [18]. It is important to highlight the proximity of our findings to birth, as

this minimises the potential confounding influences within the postnatal environment on brain development, which has been a limitation of most prior human studies. We hope that these findings can contribute to a more balanced view of the literature and inform further research into maternal stress and early brain development.

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Supporting Information

S1 Supplement. Supporting information.

S2 File. T-stat maps

S3 Dataset. De-identified research dataset.