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NEURAL CORRELATES OF PRENATAL STRESS IN YOUNG WOMEN

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Abstract

Background: Prenatal stress is hypothesized to have a disruptive impact on neurodevelopmental trajectories, but few human studies have been conducted on the long-term neural correlates of prenatal exposure to stress. The aim of this study was to explore the relationship between prenatal stress exposure and gray matter volume and resting-state functional connectivity in a sample of 35 healthy women aged 14-40 years.

Methods: Voxel-based morphometry and functional connectivity analyses were performed on the whole brain and in specific regions of interest (hippocampus and amygdala). Data about prenatal/postnatal stress and obstetric complications were obtained by interviewing participants and their mothers, and reviewing obstetric records.

Results: Higher prenatal stress was associated with decreased gray matter volume in the left medial temporal lobe (MTL) and both amygdalae, but not the hippocampus. Variance in gray matter volume of these brain areas significantly correlated with depressive symptoms, after statistically adjusting for the effects of age, postnatal stress and obstetric complications. Prenatal stress showed a positive linear relationship with functional connectivity between the left MTL and the pregenual cortex. Moreover, connectivity between the left MTL and the left medial-orbitofrontal cortex partially explained variance in the depressive symptoms of offspring.

Conclusions: In young women, the exposure to prenatal stress showed a relationship with the morphometry and functional connectivity of brain areas involved in the pathophysiology of depressive disorders. These data provide evidence in favor of the hypothesis that early exposure to stress affects brain development and identified the MTL and amygdalae as possible targets of such exposure.

INTRODUCTION

The process of brain development begins in the first months of gestation and, although it is not completed until young adulthood, undergoes its most rapid and complex changes during the intrauterine period (Gluckman & Hanson, 2004; Tottenham & Sheridan, 2009). There is growing evidence to show that the intrauterine period of life is crucial in determining the long-term trajectory of brain development and that anatomic and functional alterations associated with neuropsychiatric and other chronic diseases may originate during this period (Gluckman & Hanson, 2004; Buss et al. 2012). Several prenatal factors, known to play a role in increasing the risk of psychiatric disorders, have been hypothesized to have a disruptive impact on the developmental trajectories of the brain. Maternal exposure to infections, malnutrition, and severe stress during pregnancy are all associated with an increased risk of psychiatric conditions (Weinstock 2008; Fatemi & Folsom, 2009; Class et al. 2013; Schmitt et al. 2014) and are also associated with increased levels of glucocorticoids which, according to recent hypotheses, play a key role in mediating the detrimental effects of these early risk factors in the developing brain (Reynolds, 2012). Although there is consistent evidence to suggest that there is an epidemiological association between several psychiatric diseases and these early risk factors, the mechanisms of the increased risk are still unclear. For example, although the effects of stress on the brain and the behavior of individuals who had stress exposure after birth have been extensively studied (Lupien et al. 2009), the outcomes of prenatal stress and its neural correlates have not been well explored.

Human studies exploring the biological impact of prenatal stress have been limited by intrinsic methodological problems. These have involved difficulties in recruiting homogeneous samples of women exposed to stress during their pregnancy and planning long-term follow-up studies of their offspring while at the same time controlling for the effects of potential confounders such as postnatal stress (Charil et al. 2010). The impact of prenatal stress exposure in humans is mainly

based on knowledge gleaned from epidemiological reports which show increased risk of mood and other psychiatric disorders (Weinstock 2008; Wyrwoll & Holmes, 2011; Class et al. 2013). Other knowledge is based on studies evaluating the outcomes of offspring who have been exposed to prenatal glucocorticoids administered for therapeutic purposes (Davis et al. 2013) or to maternal anxiety/depression during pregnancy (Buss et al. 2010; Rifkin-Graboi et al. 2013; Sandman et al. 2014). These studies have shown that intrauterine exposure to elevated glucocorticoids is associated with low birth weight (Li et al. 2011; Class et al. 2011) and later dysregulation of the HPA axis (Davis et al. 2011; Alexander et al. 2012). Offspring prenatally exposed to high levels of glucocorticoids also display long-term impairment in cognitive and emotional regulation (Alexander et al. 2012), factors which are putatively considered to be mediators of an increased risk of developing mood disorders. The only human study exploring the effects of pregnancy cortisol levels on amygdala and hippocampus volumes found that higher cortisol levels in early gestation were associated with larger right amygdala volumes in 7-year-old girls, but not in boys (Buss et al. 2012). Prenatal maternal depression/anxiety - a situation somewhat similar to stress exposure - has also been associated with reduction of gray matter volumes in several brain areas (Buss et al. 2010) and micro-structural alterations of the right amygdala (Rifkin-Graboi et al. 2013) in offspring. Despite the paucity of human studies, several animal studies have been conducted exploring the effects of prenatal stress in offspring. In animals, such studies include investigations of brain anatomical and developmental alterations, of behavioral outcomes, and of expression/transcription of genes involved in neural functions. Animal models show that prenatal exposure to stress - like early postnatal exposure - is generally associated with reduced hippocampal and prefrontal regions and increased amygdala volumes in puppies, whereas the long-term effects are less clear (Tottenham & Sheridan, 2009; Charil et al. 2010). As well as the timing of exposure to stress, that of measuring of brain changes is also a factor to be considered in interpreting data (Tottenham & Sheridan, 2009). Neural data collected longitudinally at multiple time-points result in different outcomes providing evidence that early stress-induced hypertrophy may result in later neuronal

atrophy or cell death in several brain areas, including the amygdala and the hippocampus (Teicher et al. 2003). Similarly, at the synaptic level, changes associated with prenatal moderate stress exposure include a short-term increase in spine density in the medial prefrontal cortex (PFC) and the orbitofrontal cortex and a reduction in spine density in the long term (Mychasiuk et al. 2011; Kolb et al. 2012). All together, these investigations show that differences in the timing of prenatal stress exposure and the age at which the brain is studied result in differing plastic changes in neuronal circuits which evolve over protracted intervals (Kolb et al. 2012).

From a behavioral viewpoint, studies on laboratory animals have confirmed that prenatal stress exposure produces an elevated and prolonged stress response, impaired learning and memory, altered exploratory behavior, altered social and fear of extinction behavior (Weinstock, 2008; Kolb et al. 2012; Bingham et al. 2013). Genes associated with neurodevelopmental processes are highly expressed in the embryonic period and throughout fetal life (Kang et al. 2011). A whole-genome microarray study (Mychasiuk et al. 2011) identified over 700 genes which were differentially expressed in the prefrontal cortex and hippocampus after prenatal stress exposure. These changes in the expression of genes (mostly down-regulation) were also highly gender-dependent and region-specific, with more prominent changes in the expression of growth factors in the hippocampus of female individuals.

Although understanding the long-term correlates of prenatal stress exposure is extremely interesting in clarifying the early origin of psychiatric disorders, to date only studies in children have explored this issue by taking a neuroimaging approach. In the present study, we explored for the first time the neural correlates of prenatal stress in a sample of healthy young women, while taking into account the impact of postnatal stress and perinatal obstetric complications.

METHODS

Participants

The subjects of the study were 35 healthy young women with no history of psychiatric disorders. The mean age of the sample was 25.6 years (SD=6.5; range 15-40 years), years of education 15.5 (SD=2.3) and body mass index 21.8 kg/m^2 (SD=3.0). Only two participants were below the age of 18 years (14.6 and 16.9 years). The main characteristics of the sample are reported in Supplementary Table 1. All subjects gave their informed written consent for the use of data in an anonymous form. All procedures complied with the ethical standards of the local Ethical Committee and with the Helsinki Declaration of 1975, as revised in 2008.

Exclusion criteria for participation were major medical illnesses, history of neurological problems, head trauma with loss of consciousness, active use of systemic steroids, current or past use of antipsychotics, antidepressants, mood stabilizers or benzodiazepines, pregnancy, active suicidality, lifetime major depression or anxiety disorders, history of substance/alcohol abuse or dependence, bipolar disorder or schizophrenia spectrum disorder, minor mental impairment (IQ<80), or any contraindication for MRI.

Clinical assessment

The sample comprised of volunteers who agreed to participate in on-going research involving neuroimaging and neuropsychological correlates of perinatal exposure to stress and obstetric complications (Favaro et al. 2006; 2010; 2011). Psychiatric disorders were excluded by application of the MINI for DSM-IV Axis I Disorders (Sheehan et al. 1998). Handedness was assessed by the Edinburgh Handedness Inventory (Oldfield, 1971). To exclude subjects with mental impairment, participants also completed the Brief Intelligence Test (Colombo et al. 2002) (the Italian version of the National Adult Reading Test, a measure of premorbid intellectual ability) if they were aged 18 or over. Subjects under the age of 16 completed the Information subscale of the Wechsler

Intelligence Scale for Children or, if they were aged between 16 and 18, the Wechsler Adult Intelligence Scale.

Subjects were also asked to complete the Hopkins Symptoms Checklist (H-SCL; Derogatis et al. 1974) to assess depressive symptoms, and the State-Trait Anxiety Inventory (STAI; Spielberger et al. 1970) to assess state and trait anxiety.

A semi-structured interview was used to assess the occurrence of any lifetime stressful events in all participants. This interview was carried out by using a life-chart method to improve ascertainment of life-event histories. Participants were prompted to talk about a particular event, indicating if and at what age it had occurred. They were also asked to assess its severity by scoring a number from 1 to 5 (1: no significant stress associated with the event; 5: extreme stress). The interview covered 14 categories of events (see Supplementary Materials for details). In the present study, a composite measure of stressful life events corresponding to the total number of negative life events with a severity score reaching at least 3 was used. The average number of stressful negative events was 1.2 (SD=1.0; range 0-5).

Data regarding obstetric complications were available for 34 women. Most participants had been born at the Hospital of Padova (n=17) and data regarding obstetric complications were available from the hospital archives. As in our previous studies (Favaro et al. 2006; 2010; 2011), we reviewed obstetric records to collect relevant information. If the participants happened not to be born at the Hospital of Padova, then the parents were interviewed using an adapted version of the Pregnancy History Instrument (Buka et al. 2000), that is an interview covering a wide range of pregnancy, delivery and neonatal complications. In addition, for the latter cases, we were able to obtain copies of birth certificates which provided details regarding any major complications, gestational age, birth weight/length, head circumference and Apgar scores. The McNeil-Sjöström Scale (McNeil et al. 1994) was used to define obstetric complications. The total number of obstetric complication was on average 2.3 (SD=2.0; range 0-8). Three mothers (9%) reported smoking during pregnancy. None

of the mothers were given synthetic glucocorticoids during pregnancy and all participants were full term at birth.

Prenatal stress and family psychiatric history

A semi-structured interview was administered to the mothers of participants to assess the occurrence of stressful events during pregnancy, and a life-chart method was used to improve ascertainment of event histories. Participants were asked to remember the year before conceiving, the period of pregnancy and the first months after delivery, indicating when the event occurred and giving a severity score (1: no significant stress associated with the event; 5: extreme stress). The interview covered the following categories of events: accidents or accidents to a close relative, death of a friend or close relative, health problems (unrelated to pregnancy), severe health problems of a close person or need to give assistance to a sick close relative, natural disasters, severe interpersonal conflicts with a partner or close relative, separation from a partner, severe legal or economic problems, relocations, personal violence, sexual abuse or maltreatment, recent miscarriages, abortions or offspring death, and any other traumatic or stressful events. In the present study, a composite measure of stressful events was used, corresponding to the total number of negative events. Only stressful events occurring during pregnancy were included, with the exception of traumatic miscarriages/abortions or death of a close person/offspring during the 12 months before conceiving, because in these cases the effects of stress was described to be protracted to the following months.

Family psychiatric morbidity was investigated according to the family history method (Weissman et al. 2000).

fMRI data acquisition

Structural and functional MRI was performed in one scanning session. High-resolution anatomical and resting-state functional sequences were acquired. Data were collected on a Philips Achieva 1.5

Tesla scanner equipped for echo-planar imaging. Details about acquisition are described in Supplementary Materials.

Voxel-based morphometry analyses

The optimized voxel-based morphometry (VBM) protocol implemented in FSL (version FSL 4.1.6; http://www.fmrib.ox.ac.uk; FMRIB) was used (Smith et al. 2004). In brief, brain extraction and tissue-type segmentation were performed and the resulting gray matter (GM) partial volume images were aligned to standard space with first linear (FLIRT) and then non-linear (FNIRT) registration tools. The images were then averaged, modulated and smoothed with an isotropic Gaussian kernel of 5 mm full-width-at-half-maximum (FWHM) to create a study-specific template, and the GM images were re-registered to this, including modulation by the Jacobian warp field. Lastly, voxel-wise general linear models were applied to test correlations according to permutation-based non-parametric testing (5000 permutations) and the threshold-free-cluster-enhancement (TFCE) method and multiple comparison correction across space (Smith & Nichols, 2009). First, whole-brain analyses were performed with age and handedness as covariates of no interest; analyses were then performed in regions of interest (bilateral hippocampi and amygdalae) obtained with a mask derived from thresholding the corresponding probabilistic map of the Harvard-Oxford Subcortical Atlas at 30% , and with age and handedness again as covariates.

As a tool for automatic subcortical segmentation of amygdala and hippocampus, FIRST (http://www.fmrib.ox.ac.uk; FMRIB, Oxford, UK; Patenaude et al. 2011) was used, a model-based segmentation/registration tool which relies on the shape/appearance of models derived from manually segmented images.

fMRI data analysis

Resting-state scans were preprocessed with the following tools: Analysis of Functional NeuroImages (version AFNI_2010_10_19_1028; http://afni.nimh.nih.gov/afni; NIMH, Bethesda, MA) and FM-RIB Software Library (version FSL 4.1.6; http://www.fmrib.ox.ac.uk; FMRIB, Oxford, UK). Preprocessing was performed as described in Biswal et al. (2010) and

www.nitrc.org/projects/fcon_1000. A seed-based approach was used to explore functional connectivity between the regions of interest (brain areas in which the GM volume showed a significant linear relationship with prenatal stress) and the rest of the brain.

Time series were averaged across all voxels in the seed ROIs and correlations between the time series of each seed ROI and all other voxels in the brain were then determined for each subject. Lastly, correlation maps were converted to Z-value maps. The resulting standardised maps were then used to test correlations, with age and handedness as nuisance variables. Non-parametric permutation testing (5000 permutations) was used for statistical analysis of spatial maps, according to the TFCE method (Smith & Nichols, 2009), with correction for multiple comparisons across space, threshold $p<0.05$.

Statistical analyses

Unless otherwise specified, age at time of scanning was included as a covariate in all statistical analyses. Graph data were obtained by extracting the average Z-value in the brain area of interest for any individual map, and data were processed by IBM Statistical Product and Service Solutions software (SPSS, Inc, Chicago, IL) with linear regression models. Putative confounders, such as age, handedness, total number of obstetric complications, maternal smoking during pregnancy and postnatal stress, were included in regression models. Although other possible covariates, such as gestational age, socio-economic status, maternal age, pregnancy alcohol exposure, and maternal and paternal psychiatric morbidity, were considered here, they were not included in the models, since they did not show any significant (or trend toward significant) correlation with either predictors or outcomes.

RESULTS

No prenatal stress was reported by 13 mothers of participants, but one (n=17) or more (n=6) stressful events were reported in the other cases (details are reported in Supplementary Materials).

With few exceptions (three stressful events limited to the third trimester), all stressful events represented continuous exposure during the course of pregnancy.

In our sample, prenatal stress showed non-significant associations with trait anxiety (B=4.03 ± 2.20; β=0.31; p=0.076), state anxiety (B=3.26 ± 1.70; β=0.32; p=0.064) and depression (B=0.31 ± 0.16; β=0.33; p=0.057). In contrast, postnatal stress displayed significant associations with both trait anxiety (B=3.61 ± 1.10; β=0.51; p=0.002) and depressive symptoms (B=0.26 ± 0.08; β=0.50; p=0.003), but not with state anxiety (B=1.11 ± 0.97; β=0.20; p=0.260). No linear relationships were found between prenatal stress and obstetric complications (B=-0.10 ± 0.52; β=-0.03; p=0.854) or gestational age (B=0.12 ± 0.43; β=0.05; p=0.780). No significant correlations emerged between participants' age and the number of prenatal stressful events reported by mothers (r=0.12; p=0.50) or the number of postnatal events (r=0.27; p=0.11).

In voxel-based morphometry (VBM) analysis, prenatal stress was significantly correlated with gray matter volume in a cluster located in the left medial temporal lobe (MTL)(Figure 1A). Region of interest (ROI) analyses revealed the significant negative correlation of prenatal stress with gray matter volumes in the left and right amygdalae (Figure 1B and 1C). According to the Juelich Histological Atlas, significant correlations were found in voxels belonging to the latero-basal and superficial nuclei of both amygdalar areas. In contrast, no significant correlations with gray matter volume emerged from VBM analysis in the hippocampal areas. Prenatal stress showed no linear relationships with total amygdalar volume (right: B=-59.53 ± 52.88; β=-0.22; p=0.271; left: B=23.39 ± 45.65; β=0.09; p=0.613, adjusted for age, total intracranial volume, and handedness) or hippocampal areas (right: B=-45.22 ± 81.83; β=-0.08; p=0.586; left: B=-115.91 ± 89.08; β=-0.21; p=0.206) measured by automatic segmentation.

No significant correlations emerged in relationships between whole-brain (and region of interest) gray matter volume and total number of obstetric complication or severity of postnatal stress.

In the region extracted by VBM analyses (left MTL) and both amygdalae, significant negative correlations emerged between gray matter volume and depressive/trait anxiety symptoms. In the

MTL region of interest, significant negative correlation emerged for depressive symptoms (Figure S1A: 140 voxels; MNI -36, 16, -34) and trait anxiety (Figure S1B; 19 voxels; MNI -34, 16, -34), but not for state anxiety. Similarly, in both amygdalae, significant negative correlation emerged for depressive (Figure S2, A and B: left amygdala 222 voxels, MNI -22, -6, -20; right amygdala 272 voxels, MNI 24, -4, -24) and trait anxiety symptoms (Figure S2, C and D: left amygdala 161 voxels, MNI -20, -8, -18; right amygdala 249 voxels, MNI 24, -4, -24). Variance of gray matter volume of the clusters extracted by VBM analyses was significantly predicted by prenatal stress, even after the effects of age, postnatal stress, maternal smoking and obstetric complications had been accounted for (Supplementary Table 2: MTL: β =-0.47; p=0.012; left amygdala: β =-0.55; p=0.002; right amygdala: β =-0.52; p=0.001). In addition, variance of gray matter volume in the clusters extracted by whole-brain VBM analysis were significantly correlated with depressive (Supplementary Table 3: MTL: β =-0.44; p=0.015; left amygdala: β =-0.52; p=0.005; right amygdala: β =-0.65; p<0.001; adjusted for age and handedness) and trait anxiety symptoms (MTL: β =-0.20; p=0.269; left amygdala: β =-0.39; p=0.026; right amygdala: β =-0.49; p=0.004). After inclusion of covariates in the model (age, postnatal stress, maternal smoking and obstetric complications), only depressive symptoms still showed a significant relationship with gray matter volume (MTL: β =-0.52; p=0.021; left amygdala: β =-0.52; p=0.016; right amygdala: β =-0.61; p=0.002). Although we were investigating a sample of healthy women without history of psychiatric disorders, it is possible that psychiatric symptoms may influence brain anatomy. For this reason, we examined the effects of prenatal stress on gray matter volume while taking into due account the outcomes of prenatal and postnatal stress on depression and anxiety. These analyses demonstrated no changes in the observed relationships between prenatal stress and gray matter volume, not supporting the hypothesis that depressive and anxiety symptoms may give rise to observed gray matter pattern in this sample of healthy women.

Prenatal stress showed no linear relationships with the total amygdalar volume (right: B=-59.53 ± 52.88; β=-0.22; p=0.271; left: B=23.39 ± 45.65; β=0.09; p=0.613, adjusted for age, total intracranial

volume, and handedness) or the hippocampal areas (right: B=-45.22 ± 81.83; β=-0.08; p=0.586; left: B=-115.91 ± 89.08; β=-0.21; p=0.206) measured by automatic segmentation.

Lastly, the voxels in which we found significant correlations between prenatal stress and gray matter volume were used as seeds to explore resting-state functional connectivity. We found significant correlations between prenatal stress exposure and functional connectivity between the left MTL area and the rostral part of the pregenual anterior cingulate cortex (Figure 2A). Co-activation with this area was significantly positively correlated with prenatal stress even after inclusion of covariates such as age, handedness, postnatal stress, obstetric complications and maternal smoking (β=0.538; p=0.004).

The functional connectivity of the left MTL area with part of the left medial orbito-frontal cortex was significantly positively correlated with depressive symptoms (Figure 2B). Again, co-activation with this area was significantly correlated with depression even after inclusion of covariates such as age, handedness, postnatal stress, maternal smoking, and obstetric complications (β=0.494; p<0.030). All analyses were repeated removing from the sample the two youngest participants (below the age of 18) and no substantial change in results was observed.

DISCUSSION

In this study, we found evidence of significant associations between measures of prenatal exposure to stress and gray matter volumes of some brain areas of the limbic networks, i.e., the left MTL and both amygdalae, in a sample of women with no history of psychiatric disorder. Two points support the importance of our findings in order to explain the pathways between prenatal stress and later risk of psychopathology. The first is the significant negative correlation between measures of depressive symptoms and trait anxiety and gray matter density in the left MTL and both amygdalae.

Our analyses show that it is unlikely that gray matter alterations are a secondary effect of depressive and anxiety symptoms, while it appears more likely that gray matter volumes exert a 'mediation' effect between prenatal stress and depressive symptoms. The brain areas involved in gray matter loss are crucially involved in limbic networks and emotional processing (Catani et al. 2013): the MTL - strictly functionally connected with the hippocampus and the amygdala - is involved in the storage of autobiographical and emotional memories (Squire & Zola-Morgan, 1991); both the basolateral and the superficial nuclei of amygdala have been involved in affective processing and anxiety-like behavioral responses: the basolateral areas receives projections from the cortex and are hypothesized to be involved in assigning emotional value to sensory stimuli (Sah et al. 2003), whereas the superficial nuclei have been associated with acquisition of conditioned defensive responses (Ball et al. 2007). The fact that prenatal stress was associated with reduced region-specific gray matter density, but that it did not correlate with the overall amygdala volume based on automatic segmentation is of interest and could indicate that prenatal maternal stress might have an effect on the shape of amygdala and not on its overall volume. Future studies will have to examine which aspects of the amygdala integrity are actually altered in the context of maternal stress during pregnancy.

The second point is the finding of a significant positive correlation between prenatal stress exposure and increased functional connectivity between the left MTL and an area in the subgenual anterior cingulate cortex, providing evidence of the functional role played by the brain areas in our VBM study. The functional connectivity between the left MTL and the orbitofrontal cortex also showed a significant linear relationship with depressive symptoms. The integrity of connectivity between the medial temporal cortex and orbitofrontal and subgenual cortices was found to be correlated with severity of depression and increased subgenual functional connectivity in previous studies on patients with major depression (de Kwaasteniet et al. 2013). Here, we provide evidence of the possible role of prenatal stress in influencing the strength of connectivity between these areas, potentially giving rise to increased neurodevelopmental 'risk-status' for depressive disorders.

Our study found significant relationships between prenatal stress and the gray matter density/functional connectivity of brain areas - left MTL, amygdala, orbitofrontal and subgenual cortex - which the literature has consistently found to be involved in the pathophysiology of depressive disorders (Peng et al. 2011; Sacher et al. 2012; de Kwaasteniet et al. 2013; Catani et al. 2013). Although these findings are encouraging, in that these alterations may be mediators of the increased risk of developing a psychiatric disorder in subjects exposed to prenatal stress, the mechanisms involved and the significance of such alterations remain to be understood. Physiologically, the fetus is relatively protected from increased levels of maternal glucocorticoids through the placental enzyme 11β-hydroxysteroid dehydrogenase type 2 (Reynolds, 2013) which should reduce exposure of the fetal brain to glucocorticoid fluctuations. However, the efficiency of the placental barrier is not complete and was found to vary considerably across individuals, given the presence, among other factors, of functional genetic polymorphisms. Exposures to glucocorticoids may exert detrimental effects on developmental characteristics at birth both directly i.e., by altering placental blood flow - and indirectly, by influencing the timing of parturition and duration of gestation. Consequently, birth outcomes, such as weight at birth, are significantly influenced by prenatal glucocorticoid exposure (Li et al. 2011; Class et al. 2011). Neurodevelopment may be similarly affected, in view of the evidence of long-term effects of birth weight on the brain cortical surface area and subcortical volumes (Walhovd et al. 2012). Generally, epigenetic mechanisms mediated by exposure to glucocorticoids have been hypothesized as the link between stress exposure, variations in the volumes of hippocampal and amygdalar areas, and depression (Pagliaccio et al. 2014). Although the only previous study exploring the effects of stress hormone levels on the brain volumes of seven-year-old children reported a positive correlation with the size of the amygdala (Buss et al. 2012), as also observed for recent postnatal exposure (Tottenham and Sheridan, 2009; Teicher et al. 2003), we found the reverse: a significant negative correlation between prenatal stress and gray matter density in areas of the left MTL and both amygdalae. Despite the differences, our findings are not incompatible with those of Buss et al.

(2012), because they may be due to the different timing of brain measurement. As previously hypothesized (Teicher et al. 2003), early activation of the amygdala during prenatal life may cause enlargement of this brain area, followed by neuronal loss and hypotrophy in adolescence and adulthood, explaining not only our findings, but also those of animal studies exploring stress effects on the brain in the longer term (Teicher et al. 2003). As observed in the prefrontal cortex of prenatally stress-exposed animals (Mychasiuk et al. 2011; Kolb et al. 2012), the gray matter decrease observed in our study in the left MTL and both amygdalae may be the consequence of decreased spine density in the long term.

In view of the limitations intrinsic in retrospective assessment of prenatal stress in the present study, larger-scale prospective studies are needed to confirm the hypotheses presented here. The nature of stress exposure in our sample also prevented us from exploring the effects of the timing of exposure in differing pregnancy trimesters. Although to our knowledge this study is the first to explore neural correlates of exposure to prenatal stress in a homogeneous sample of young women with no history of psychiatric disorders and given the confounding effects of postnatal stress and perinatal history, retrospective data collection does not allow us to examine all the possible postnatal factors which might influence brain development. Some of these factors are known and their effects are examined here: obstetric complications, maternal smoking during pregnancy, maternal psychiatric disorders, and lifetime stressful events. However, there may be other known (i.e., parental care, genetic factors) and unknown factors affecting brain morphology and function, not taken into account here, which may have acted as confounders. Only linear effects were examined and although we controlled for the effects of potential confounders, we cannot be sure that this statistical model is always appropriate. The choice of including only participants without history of psychiatric disorders can be considered a point of strength of the present study, since any confounding secondary effects of psychiatric disorders and their treatment is avoided. However, this exclusion also limits our possibility of applying our findings to patients with specific types of psychiatric disorders. Further studies in high-risk groups and in patients with recent onset of both depressive

and anxiety disorders are necessary to understand if and how much our findings are involved in the pathogenesis of mood and anxiety disorders. All these limitations are difficult to overcome in a naturalistic setting and explain why, in this field, there are many animal studies but knowledge of what happens in humans is almost completely lacking.

The human brain is continually modeled and shaped by positive and negative experiences during the life course (Kolb et al. 2012), but there is evidence that indices of prenatal development, such as birth weight, are predictive of lifetime brain characteristics (Walhovd et al. 2012). The effects of prenatal influences on brain development seems to be detectable for the entire lifespan of individuals in both healthy and pathological populations (Walhovd et al. 2012; Davis et al. 2013; Sandman et al. 2014; Haukvik et al. 2014), emphasizing the importance of studies for increased understanding of the neurodevelopmental trajectories of neuropsychiatric disorders. Although it is common knowledge that maternal exposure to severe stressful events during pregnancy can affect the psychological health of the offspring, the neural correlates of prenatal stress in the human brain are almost completely unknown. The present study provides evidence that the medial temporal cortex and both amygdalae gray matter gray matter densities are correlated with this type of exposure in the long term and provides data favoring their involvement in determining an increased later risk of developing depressive symptoms.

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Author Information

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Figure 1 - Areas of significant correlation (TFCE corrected $p<0.05$) between: (A) prenatal stress and whole-brain gray matter volume: peak, -24, 2, -40 (cluster size, 193 voxels; area includes left temporal pole, temporal fusiform cortex and, marginally, anterior parahippocampal gyrus, encompassing both BA20 and BA36); (B) prenatal stress and gray matter volume in left amygdala: peak, -20, -6, -20 (cluster size, 50 voxels); (C) prenatal stress and gray matter volume in right amygdala: peak, 20, -8, -16 (cluster size, 47 voxels). Graphs show individual average gray matter volumes in areas in which significant correlations were found in relation to prenatal stress, trait anxiety and depressive symptoms. VBM analyses conducted with age and handedness as covariates.

Figure 2 - Areas of significant correlation (TFCE corrected $p<0.05$) between: (A) prenatal stress and degree of co-activation of left medial temporal cortex: peak: 0, 27, 12 (BA 25); (B) depressive symptoms and co-activation of left medial temporal cortex: -27, 57, -12 (BA 11). Graphs show individual average co-activation in areas showing significant correlations. Analyses conducted with age and handedness as covariates.

Figure(s)
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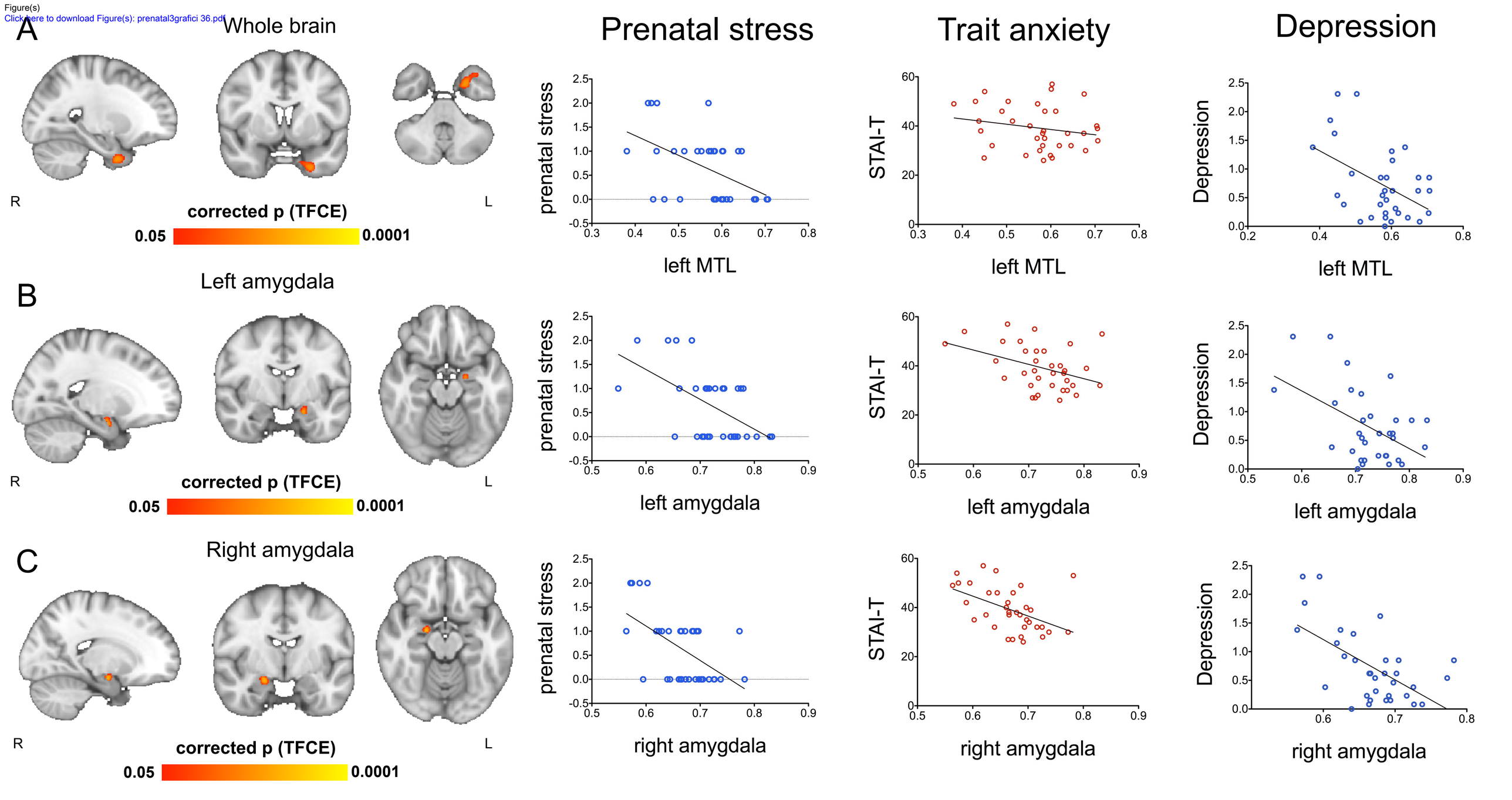

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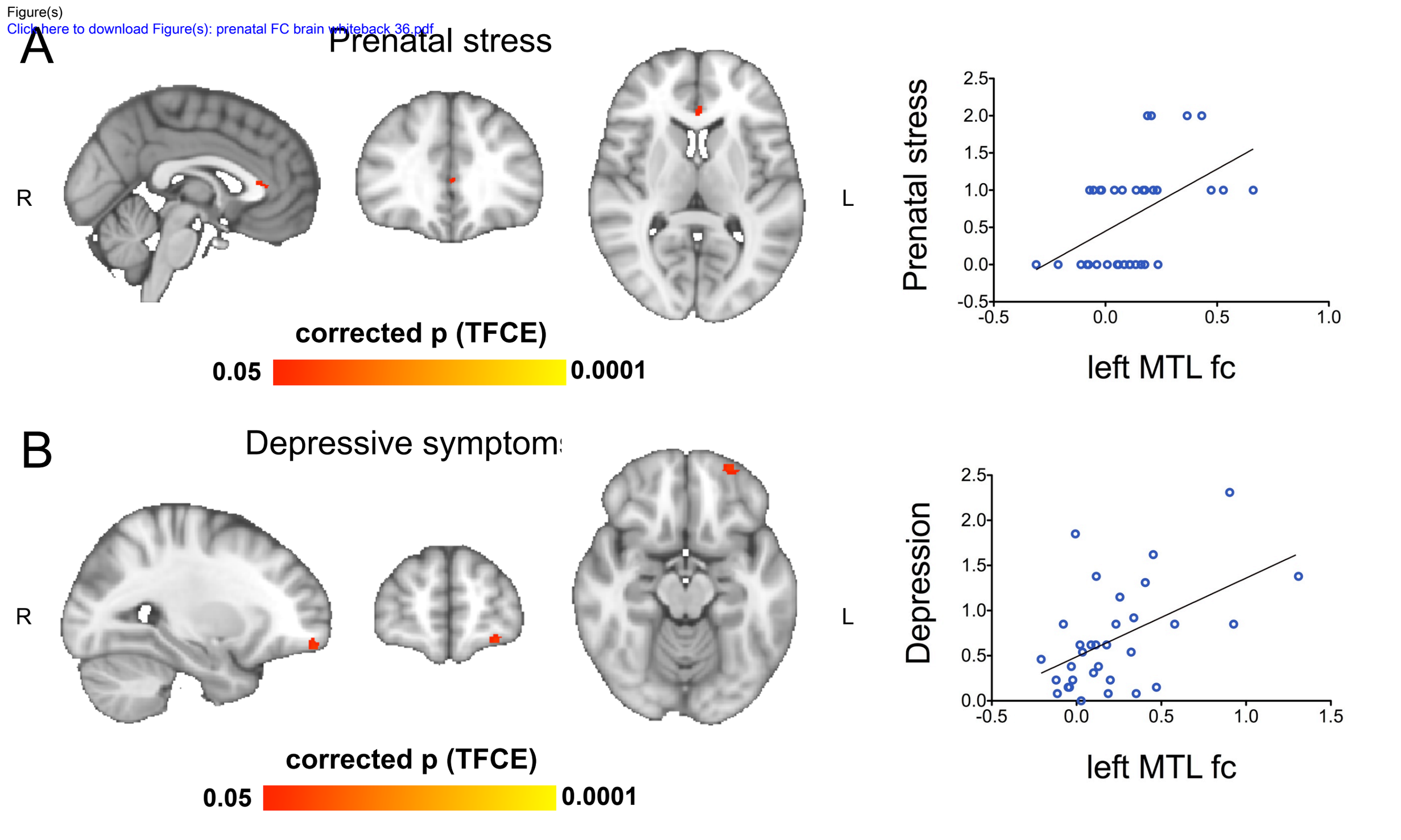


SUPPLEMENTARY MATERIAL

METHODS

Participants

Main characteristics of participants are described in Supplementary Table 1.

Lifetime stress exposure

Participants were asked to remember a particular event, indicating if and when it had occurred (age) and giving a score of severity (1: no significant stress associated with the event; 5: extreme stress). The interview covered 14 categories of events: death of a close relative; death of a friend; personal health problems; health problems of a close person; separations; interpersonal conflicts; occupational, scholastic-academic, legal or other economic problems; personal violence or maltreatment; miscarriages and/or abortions; any other traumatic or stressful events (some examples were given, such as road accidents, exposure to natural disaster, relocation). In the present study, a composite measure of stressful life events was used, corresponding to the total number of negative life events with a severity score reaching at least 3. The average number of stressful negative events was 1.2 (SD=1.0; range 0-5).

Obstetric complications

Data regarding obstetric complications were available for 34 women. Most participants had been born in Padova Hospital (n=17) and data on obstetric complications were available from the hospital archives. We reviewed obstetric records to collect relevant information. In all other cases, we interviewed the parents of participants, using an adapted version of the Pregnancy History Instrument (Buka et al, 2000), an interview covering a wide range of pregnancy, delivery and neonatal complications. In addition, in all these cases, we were able to obtain copies of birth certificates giving details of major complications, gestational age, birth weight/length, head circumference and Apgar scores. All interviews were performed face-to-face with mothers or both parents of participants. The McNeil-Sjöström Scale (McNeil et al, 1994) was used to define obstetric complications. Only complications scored at level 3 (potentially harmful) or higher (clearly harmful) were considered here.

Family psychiatric morbidity

Information was collected about all first-degree relatives by interviewing both participants and their mothers. No maternal psychiatric disorder occurred during pregnancy or the first five years of participants' lives. Two mothers reported anxiety symptoms in their lifetime, but only one was severe enough to receive psychiatric treatment. Among fathers, one was found to have suffered from a mood disorder and one alcohol dependence. All patients had recovered from these disorders after treatment and were in remission at the time of the interview.

fMRI data acquisition

High-resolution 3D T1-weighted anatomical images were acquired in a gradient-echo sequence (repetition-time=20 sec, echo time=3.78 msec, flip angle= 20°, 160 slices, acquisition voxel size=1×0.66×0.66 mm, field of view=21-22 cm). Resting-state fMRI scan entailed 250 continuous functional volumes (repetition time=2035 msec, echo time=50 msec, flip angle=90°, 21 slices, matrix=128×128, acquisition voxel size=1.8×1.8×6 mm, acquisition-time=8 minutes; field of view=23 cm). Participants were instructed to rest with their eyes closed during the scan and were observed by at least one of the authors during scanning. At the end of the procedure, all subjects

were asked about their emotions or tendency to fall asleep during scanning. None of the subjects in the study moved, fell asleep, or reported anxiety or other particular emotion during scanning.

RESULTS

Prenatal stressful events were: accidents (n=2) or accidents to a close relative (n=3); death of a friend or close relative (n=4); severe health problems of a close person (n=3) or need to give assistance to a sick close relative (n=2); severe interpersonal conflicts with partner (n=2) or close relative (n=4); stressful relocations (n=3); recent miscarriages, abortions or offspring death (n=4); severe stress or failure at work (n=3).

Postnatal stressful events were reported by 24 participants (12 reported one stressful event, 7 reported two events, 3 reported 3 events, 1 participant reported 4 and 1 reported 5 events). They were: death of a close relative or a friend (n=14); personal health problems or accidents (n=2); health problems of a close person (n=3); separations (n=5); relocations (n=4); parental conflicts or separations (n=3); interpersonal conflicts (n=5); occupational or scholastic-academic problems (n=4); personal violence or maltreatment (n=2); sudden death of partner (n=1); suicide of a close person (n=1).

SUPPLEMENTARY TABLES AND FIGURES

Supplementary Table 1 - Characteristics of participants (n=35)

	Mean (SD) or N (%)	**Range**
Age (years)	25.6 (6.5)	14 - 40
Education (years)	15.5 (2.3)	8 - 18
Body mass index (kg/m^2)	21.8 (3.0)	18 - 29
Edinburgh score	54.4 (43.6)	-94 - 100
High or medium-high social status	16 (46%)	Low - high
Depression	0.73 (0.63)	0 - 2.3
Trait anxiety	39.2 (9.0)	26 - 57
State anxiety	34.7 (7.0)	20 - 56

Supplementary Table 2 - Regression analyses of prenatal stress and gray matter volumes

	Adjusted for age and handedness	Adjusted for age, handedness, postnatal stress, obstetric complications, smoking
Temporal lobe (whole-brain VBM analysis)	-0.07 ± 0.02 (β =-0.53; p=0.002)	-0.06 ± 0.02 (β =-0.47; p=0.012)
Left amygdala	-0.05 ± 0.01 (β =-0.55; p=0.001)	-0.05 ± 0.01 (β =-0.55; p=0.002)
Right amygdala	-0.04 ± 0.01 (β =-0.56; p=0.001)	-0.04 ± 0.01 (β =-0.52; p=0.001)

Value presented as B ± SEM (β; p)

Supplementary Table 3 - Regression analyses of gray matter volumes and measures of anxiety and depression

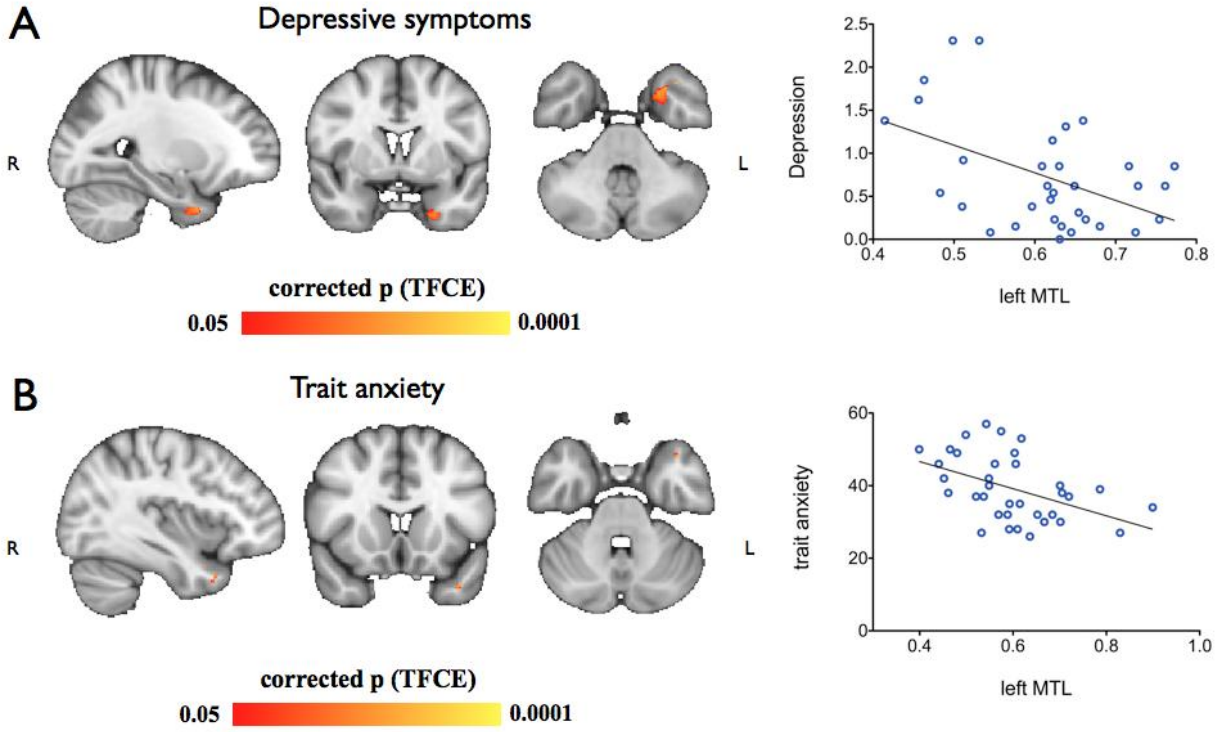
	Temporal lobe (whole-brain VBM analysis)	Left amygdala	Right amygdala
STAI Trait anxiety	-0.002 ± 0.002 § (β =-0.20; p=0.269) -0.002 ± 0.002 # (β =-0.20; p=0.398)	-0.003 ± 0.001 § (β =-0.39; p=0.026) -0.002 ± 0.001 # (β =-0.33; p=0.137)	-0.003 ± 0.001 § (β =-0.49; p=0.004) -0.002 ± 0.001 # (β =-0.38; p=0.064)
STAI State anxiety	-0.002 ± 0.002 § (β =-0.14; p=0.448) -0.001 ± 0.002 # (β =-0.04; p=0.832)	-0.002 ± 0.002 § (β =-0.28; p=0.118) -0.001 ± 0.002 # (β =-0.17; p=0.366)	-0.002 ± 0.001 § (β =-0.31; p=0.084) -0.001 ± 0.001 # (β =-0.16; p=0.372)
Depression (H-SCL)	-0.06 ± 0.02 § (β =-0.44; p=0.015) -0.07 ± 0.03 # (β =-0.52; p=0.021)	-0.50 ± 0.16 § (β =-0.52; p=0.005) -0.05 ± 0.02 # (β =-0.52; p=0.016)	-0.06 ± 0.01 § (β =-0.65; p<0.001) -0.05 ± 0.02 # (β =-0.61; p=0.002)

Value presented as B ± SEM (β; p)

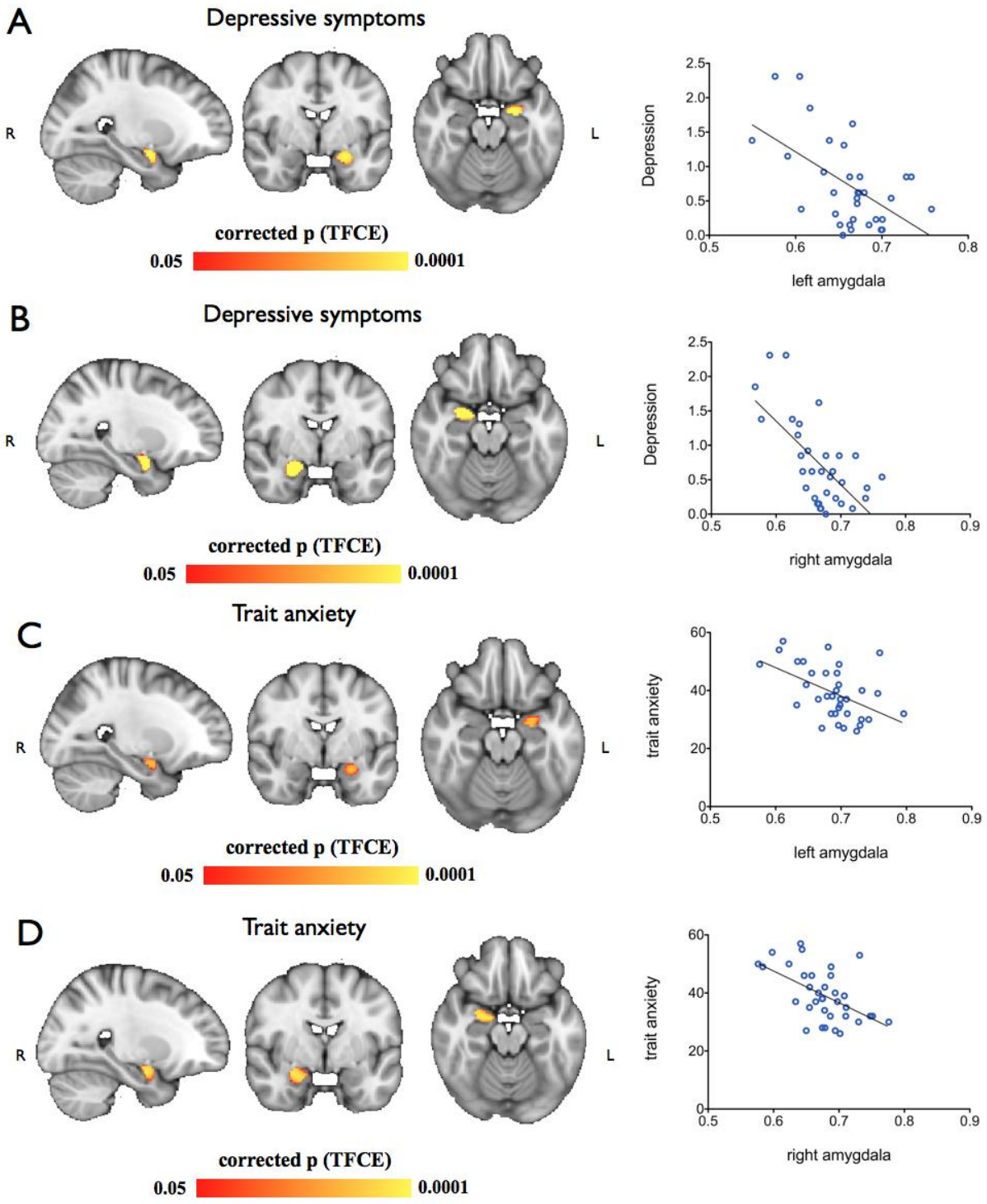
§ accounting for age and handedness

accounting for age, handedness, postnatal stress, obstetric complications, maternal smoking

Supplementary Figure 1: Areas of significant correlation (TFCE corrected p<0.05) between: (A) depressive symptoms and gray matter volume in left MTL: peak, -36, 16, -34 (cluster size, 140 voxels); (B) stait anxiety and gray matter volume in left MTL: peak, -34, 16, -34 (cluster size, 19 voxels). Graphs show individual average gray matter volumes in areas in which significant correlations were found in relation to prenatal stress, trait anxiety and depressive symptoms. Voxel-based correlation analyses conducted with age and handedness as covariates.



Supplementary Figure 2: Areas of significant correlation (TFCE corrected $p<0.05$) between: (A) depressive symptoms and gray matter volume in left amygdala: peak, -22, -6, -20 (cluster size, 222 voxels); (B) depressive symptoms and gray matter volume in right amygdala: peak, 24, -4, -24 (cluster size, 272 voxels); (C) trait anxiety and gray matter volume in left amygdala: peak, -20, -8, -18 (cluster size, 161 voxels); (D) trait anxiety and gray matter volume in right amygdala: peak, 24, -4, -24 (cluster size, 249 voxels). Graphs show individual average gray matter volumes in areas in which significant correlations were found in relation to prenatal stress, trait anxiety and depressive symptoms. Voxel-based correlation analyses conducted with age and handedness as covariates.


A
Depressive symptoms
R
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corrected p (TFCE)
0.05
0.0001
Depression
left amygdala
B
Depressive symptoms
R
L
corrected p (TFCE)
0.05
0.0001
Depression
right amygdala
C
Trait anxiety
R
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trait anxiety
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D
Trait anxiety
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trait anxiety
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