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High pregnancy anxiety during mid-gestation is associated with decreased gray matter density in 6-9 year-old children

Claudia Buss[a], **Elysia Poggi Davis**[a,b], **L. Tugan Muftuler**[c], **Kevin Head**[c], and **Curt A. Sandman**[a,*]

[a]Department of Psychiatry and Human Behavior, University of California, Irvine, 333 The City Blvd. W, Suite 1200, Orange, CA 92868

[b]Department of Pediatrics, University of California, Irvine, Irvine, CA 92697-5020

[c]Department of Radiological Sciences, University of California, Irvine, Irvine, CA 92697-5020

Summary

Because the brain undergoes dramatic changes during fetal development it is vulnerable to environmental insults. There is evidence that maternal stress and anxiety during pregnancy influences birth outcome but there are no studies that have evaluated the influence of stress during human pregnancy on brain morphology. In the current prospective longitudinal study we included 35 women for whom serial data on pregnancy anxiety was available at 19 (±0.83), 25 (±0.9) and 31 (±0.9) weeks gestation. When the offspring from the target pregnancy were between six to nine years of age, their neurodevelopmental stage was assessed by a structural MRI scan. With the application of voxel based morphometry, we found regional reductions in gray matter density in association with pregnancy anxiety after controlling for total gray matter volume, age, gestational age at birth, handedness and postpartum perceived stress. Specifically, independent of postnatal stress, pregnancy anxiety at 19 weeks gestation was associated with gray matter volume reductions in the prefrontal cortex, the premotor cortex, the medial temporal lobe, the lateral temporal cortex, the postcentral gyrus as well as the cerebellum extending to the middle occipital gyrus and the fusiform gyrus. High pregnancy anxiety at 25 and 31 weeks gestation was not significantly associated with local reductions in gray matter volume.

This is the first prospective study to show that a specific temporal pattern of pregnancy anxiety is related to specific changes in brain morphology. Altered gray matter volume in brain regions affected by prenatal maternal anxiety may render the developing individual more vulnerable to neurodevelopmental and psychiatric disorders as well as cognitive and intellectual impairment.

Keywords

pregnancy anxiety; pregnancy; prenatal stress; longitudinal; MRI; gray matter volume

*Corresponding Author: Tel: 714-940-1924, Fax: 714-940-1939, E-mail: casandma@uci.edu (Curt A. Sandman)

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Introduction

Programming refers to the action of a factor during a sensitive developmental period affecting the organization and maturity of specific organs. One key assumption of the programming hypothesis is that biological systems undergoing rapid developmental changes are especially vulnerable to organizing and disorganizing influences (Nathanielsz et al., 2003; Seckl & Meaney, 2004). Considering the extended pre- and postnatal developmental trajectory of the brain, it is apparent that early life experiences have the potential to sculpt brain morphology. Such sculpting of the immature brain is an interactive process between genetic programming, cell function and the environment (Andersen, 2003). Growing evidence suggests that abnormal development of the brain during gestation contributes to many neurological disorders that are manifested throughout the entire lifespan (Rees et al., 2008).

The immature brain can be considered “under construction” (Connors et al., 2008) with the development of the human central nervous system following a protracted, neatly orchestrated chain of specific ontogenetic events. Although brain change and adaptation are part of a lifelong process, the earliest phases of maturation during fetal development and childhood are perhaps the most dramatic and important (Toga et al., 2006). Understanding the timing of neurodevelopmental events is essential for determining how particular environmental disturbances can selectively affect certain functions. During fetal life, neurons proliferate, migrate, and aggregate, providing the “hardware” for the developing brain. Neural proliferation before birth has been estimated at an average rate of 250,000 cells per minute (Cowan, 1979). Between gestational ages 8 and 16 weeks, migrating neurons form the subplate zone and await connections from afferent neurons originating in the thalamus, basal forebrain, and brainstem (Kostovic et al., 2002). Once neurons reach their final destination at about the 16th fetal week, they arborize and branch in an attempt to establish appropriate connections (Sidman & Rakic, 1973). Axon collaterals connect to numerous regions in the brain before the neuron finishes migrating to its target location (Jones et al., 1985). Neurotrophins influence the migration or retraction of neurons (Jones et al., 1993) and ephrins guide neurons further by establishing a chemical gradient to follow (Knoll & Drescher, 2002). During development of the human brain, little synapse formation occurs before the beginning of the third trimester, when it accelerates to approximately 40,000 synapses per minute (Bourgeois, 1997).

The presence of periods with specific neurodevelopmental events results in windows of specific vulnerability for adverse influences. In animal models, changes in brain morphology have been observed in offspring of mothers exposed to prenatal stress. In non-human primates, daily acute prenatal stress is associated with 10-12% reductions in hippocampal volume and inhibition of neurogenesis in the dentate gyrus (Coe et al., 2003) as well as altered size of the corpus callosum (Coe et al., 2002). Several lines of evidence from studies in rodents indicate that the cytoarchitecture of the rat hippocampus is altered as a consequence of prenatal stress (Hayashi et al., 1998; Gould & Tanapat, 1999; Lemaire et al., 2000). Impaired neurogenesis and associated cognitive impairment have been repeatedly reported in prenatally stressed animals (Lemaire et al., 2000; Fujioka et al., 2006; Lemaire et al., 2006). Furthermore, prenatal stress has the potential to alter synaptic plasticity by impairing long-term potentiation but facilitating long-term depression (Yaka et al., 2007; Yang et al., 2007). Although prenatal stress associated changes in the hippocampal formation have received major attention, morphological changes in other brain regions have been shown as well. For example significantly expanded dimensions of the lateral nucleus of the amygdala were observed in prenatally stressed offspring (Salm et al., 2004). Also, reduced spine densities and significant reduction of dendritic length of pyramidal neurons in the dorsal anterior cingulate and orbitofrontal cortex have been reported in offspring of mothers exposed to stress during pregnancy (Murmu et al., 2006). Since the rodent brain is less mature at birth than the human brain, it has been suggested that brain maturation occurring in the early postnatal period in the rat is analogous to maturational

changes that occur in humans in late gestation (Clancy et al., 2001). Therefore, it is interesting to note that there is an impressive body of evidence from rodent studies showing changes in brain morphology in association with manipulation of the early postnatal environment (e.g. Meaney et al., 1991; Pham et al., 1999; Helmeke et al., 2001; Huot et al., 2002; Roceri et al., 2002; Bredy et al., 2003; Ovtscharoff et al., 2006; Fabricius et al., 2008).

In humans, changes in brain morphology have been reported in individuals born prematurely. Thus, low birth weight as well as preterm birth have been related to changes in regional brain volumes (e.g. Peterson et al., 2000; Abernethy et al., 2002; Nosarti et al., 2002; Huizink et al., 2004; Buss et al., 2007; Beauchamp et al., 2008). Adverse birth outcomes may be markers of *in utero* stress exposure (e.g. Wadhwa, 2005) but the changes in brain morphology may also be due to perinatal complications that are often associated with premature delivery. To the best of our knowledge no study to date in humans has investigated the association between prenatal stress exposure and brain morphology in the offspring.

Pregnancy anxiety has been suggested to be a more sensitive predictor of birth outcomes than general anxiety (Wadhwa et al., 1993; DiPietro et al., 2004; Roesch et al., 2004; Kramer et al., 2009) and has been suggested as a distinctive syndrome (Huizink et al., 2004). Further evidence suggests that measures of pregnancy specific stress are better than measures of generalized psychological distress for predicting developmental outcomes including, fetal behavior (DiPietro et al., 2002), infant cognitive and motor development (Huizink et al., 2003; Dipietro et al., 2006; Davis & Sandman, in press) and infant emotional regulation (Dipietro et al., 2006). The objective of the current study was therefore to test in a prospective longitudinal study the associations between pregnancy anxiety, measured repeatedly over the course of gestation, and reductions in gray matter volume in their 6-9 year-old offspring.

Methods

Participants

Pregnant women were recruited for study participation between 1998 and 2002. Five hundred and fifty seven pregnant women, who received prenatal care from the faculty obstetric practice at the University of California, Irvine Medical Center or Cedars-Sinai Hospital in Los Angeles, were recruited by the 15^{th} week of gestation and provided written, informed consent. All methods and procedures were approved by the Institutional Review Board of the participating institutions. Study participants were English-speaking adult women (>18 years age) with singleton, intrauterine pregnancies. Exclusion criteria included tobacco, alcohol, or other drug use in pregnancy; uterine or cervical abnormalities; or presence of any condition potentially associated with dysregulated neuroendocrine function such as endocrine, hepatic or renal disorders or corticosteroid medication use. While not an exclusion criteria, none of the women in this sample were treated for any psychiatric disorders. In the context of an on-going study on the effects of prenatal stress exposure on child brain development that started in 2008, women were re-contacted and invited to participate in a follow-up study of their children. Three hundred and forty women of the initial sample were located. Fifty-two children have undergone and MRI scan to date; of those one MRI scan had to be excluded due to morphological abnormalities and two MRI scans due to severe motion artifacts. Among the 49 mother-child dyads with usable MRI data, 35 women had provided complete maternal stress data at three time points between 19 and 31 weeks gestation as well as postpartum and are included in the current report. Among these 35 children were two siblings; thus one mother was enrolled in the study with two subsequent pregnancies and consequently two children for the follow-up study. Women whose children participated in the follow-up study of brain development did not differ in sociodemographic characteristics (maternal age and education, annual household income) from women in the initial sample (all p's >0.4).

Assessments in pregnant women

For all pregnant women gestational age was determined by best obstetric estimate with a combination of last menstrual period and early uterine size, and was confirmed by obstetric ultrasonographic biometry before 20 weeks using standard clinical criteria (O'Brien et al., 1981). Medical risk was defined as the presence of certain medical conditions in the index pregnancy or previous pregnancies (e.g. vaginal bleeding, pregnancy-induced hypertension, preeclampsia, infection, Hobel, 1982). Risk conditions were determined through interview and extensive medical chart review. The sum of medical risk factors was calculated as an indicator of presence of any current or historical risk conditions. Information on birth outcomes were retrieved from medical charts after delivery. Sociodemographic characteristics and birth outcomes are summarized in Table1.

Pregnancy anxiety—Pregnancy anxiety was assessed over the course of gestation at 19 (±0.83, SD), 25 (±0.9) and 31 (±0.9) weeks gestation, and for all 35 children included in the current analyses complete data was available. A 10-item pregnancy anxiety scale, which assesses a woman's feelings about her health during pregnancy, the health of her baby, and her feelings about labor and delivery, was administered at all three study visits. Answers were given on a 4-point scale and included items such as: "I am fearful regarding the health of my baby," "I am concerned or worried about losing my baby," and "I am concerned or worried about developing medical problems during my pregnancy." The final score on this measure could range from 10 to 40. This reliable measure (α=0.75-0.85) was specifically developed for use in pregnancy research (Rini et al., 1999; Glynn et al., 2008).

Pregnancy anxiety and medical risk

To test whether the effects of pregnancy anxiety on the developing brain could be mediated by the presence of medical risk, correlation analyses were performed between pregnancy anxiety and the number of medical risk conditions. At none of the assessments, significant correlations could be observed (19 weeks: r= -0.14, p= 0.42; 26 weeks: r= -0.14, p= 0.44, 31 weeks: r= 0.02, p= 0.92).

Pregnancy anxiety, sociodemographic characteristics and postpartum stress

To test whether the effects of pregnancy anxiety on the developing brain could be mediated by the quality of the postnatal environment, correlation analyses were performed between pregnancy anxiety and sociodemographic characteristics as well as postpartum stress. Generalized stress was assessed at 8.2 (±2.9) weeks postpartum (range: 5-19 weeks) using a modification of the 10-item version of the Perceived Stress Scale (Cohen & Williamson, 1988). As shown in Table 2, pregnancy anxiety was not significantly associated with maternal sociodemographic characteristics, while highly significant correlations between pregnancy anxiety at all time points during gestation and postpartum perceived stress were observed.

Assessments in children

All children included in the study had a stable neonatal course (all Apgar scores >8) and no emotional or physical conditions were reported in a structured interview using the MacArthur Health and Behavior Questionnaire (Armstrong & Goldstein, 2003) at the ages of 6 and 9 years (mean: 7.2 ± 0.86), when they participated in an MRI scan. Child's handedness was assessed with a modified version of the Edinburgh Handedness Inventory (Oldfield, 1971). For the majority (86%) of children the dominant hand was the right.

MRI Acquisition—Each child underwent an MRI scan conducted on a 3-T Philips Achieva system. To minimize head motion, padding was placed around the head. Ear protection was given to all children. To further increase compliance and reduce motion, children were fitted

with head phones and allowed to watch a movie of their choice while in the scanner. Following the scanner calibration and pilot scans, a high resolution T1 anatomical scan was acquired in the sagittal plane with $1mm^3$ isotropic voxel dimensions. An Inversion-Recovery Spoiled Gradient Recalled Acquisition (IR-SPGR) sequence with the following parameters was applied: Repetition rate (TR)= 11ms, Echo Time (TE)= 3.3ms, Inversion Time (TI)= 1100ms, Turbo Field Echo factor (TFE)= 192, Number of slices: 150, no SENSE acceleration, Flip angle=18^0, Shot interval (time from inversion pulse to the center of acquisition) = 2200ms. Acquisition time for this protocol was seven minutes. Variations of these parameters were tested on volunteers to obtain an optimal set that gave us the best gray-white matter contrast, sharpness and high resolution while ensuring that there were no discernible artifacts. The purpose was to keep the total acquisition time at a tolerable length for children. Automatic brain segmentation software was tested on these pilot scans to ensure gray-white matter segmentation with minimal errors.

Processing of MRI data—Images were visually assessed by a neurologist for normal anatomic appearance. The structural images were bias field corrected, and segmented using an integrated generative model (unified segmentation, Ashburner & Friston, 2005). Unified segmentation involves alternating between segmentation, bias field correction, and normalization to obtain local optimal solutions for each process. The pediatric CCHMC *a priori* templates (Wilke et al., 2002) were used to segment and normalize (affine and 16 iteration non-linear transformations) the children's images. The resulting images were modulated to correct voxel signal intensities for the amount of volume displacement during normalization. The normalized and segmented images were averaged across the children's datasets to produce gray matter, white matter, and CSF sample specific *a priori* templates. The process was then repeated using the sample specific *a priori* templates resulting in VBM probability maps of 1mm isotropic voxels. The normalized, segmented, and modulated images were then smoothed using a 12 mm kernel to ensure that the data were normally distributed and to limit the number of false positive findings (Salmond et al., 2002). Coordinates of clusters (centroids) were converted from original Montreal Neurological Institute (MNI) coordinates to those of the Talairach brain atlas (Talairach & Tournoux, 1988) using the mni2tal utility (Matthew Brett 1999 GPL). Anatomical locations of the significant areas are based on the best estimate from the Talairach atlas using the Tailairach Daemon Client (http://www.talairach.org/client.html).

Total gray matter analysis—Total gray matter volume for each child was estimated based on the volumes of the segmented and modulated images. The sum of the nonzero voxel values in the image was calculated and multiplied by the voxel size to obtain an estimate of total gray matter volume. Partial correlation analyses were performed to test the association between pregnancy anxiety at each of the three pregnancy visits and total gray matter volume controlling for age, sex, gestational age at birth and postpartum stress.

Voxel Based Morphometry (VBM) analysis—To examine reductions in regional gray matter volume in association with pregnancy anxiety, a multiple regression model was employed with pregnancy anxiety at 19, 25 and 31 weeks gestation as the predictors of interest. Normalization for global differences in gray matter concentration across subjects was performed by controlling for total gray matter volume. Consequently, the analysis detected regional difference rather than overall, large-scale variations in gray matter concentrations. By controlling for total gray matter volume, differences in overall brain size were accounted for that varies by sex. We furthermore controlled for child's age at assessment. Also, gestational age at birth was controlled for in these analyses because preterm delivery has been shown to be associated with reductions in gray matter volume (e.g. Gimenez et al., 2004). In addition, handedness of the child was controlled for because structural asymmetries of the brain may be

associated with handedness (Toga & Thompson, 2003). Because pregnancy anxiety and postpartum stress were highly correlated, we included postpartum stress as an additional covariate in order to address the association between prenatal stress and gray matter volume independent of postnatal stress. Relative threshold masking (threshold >0.3) was used to minimize gray-white matter boundary effects, and implicit masking was used to disregard voxels with zero values. Analyses for detection of brain regions that showed significantly reduced gray matter density in association with high pregnancy anxiety were performed at $p<0.001$ uncorrected, but only those voxels within a cluster of at least 100 voxels that reached a False Discovery Rate (FDR) threshold of $p<0.05$ are reported.

Results

Pregnancy anxiety over the course of gestation

Table 1 shows mean pregnancy anxiety scores at each pregnancy visit and Figure 1 presents the distribution of pregnancy anxiety scores over the course of the three pregnancy visits. With advancing gestational age pregnancy anxiety scores significantly decreased ($F_{(1.8, 56.8)}=5.4$, $p=0.01$) resulting in lower scores at the second and third assessments as compared to the first. As depicted in Table 3, Spearman's rho correlation coefficients suggested significant rank stability in pregnancy anxiety scores over the course of gestation.

Pregnancy anxiety and global reductions in gray matter volume

Pregnancy anxiety at any of the three time points during pregnancy was not correlated with total gray matter volume (T1: $r=0.05$, $p=0.81$; T2: $r=0.06$, $p=0.75$; T3: $r=-0.27$, $p=0.15$).

Pregnancy anxiety and regional reductions in gray matter volume

The VBM analysis revealed lower gray matter density in several brain areas in association with high pregnancy anxiety. The significance of the relation between pregnancy anxiety and gray matter volume reductions varied across gestation (see Table 4 and Figure 2). High pregnancy anxiety at 19 weeks gestation was associated with significant, mostly bilateral, gray matter volume reductions in the anterior (Brodmann Area (BA) 10), orbitofrontal (BA 11 and BA 47), dorsolateral (BA 46 and BA 9) and ventrolateral prefrontal cortex (BA 45). Also, lower gray matter density was observed with high pregnancy anxiety at 19 weeks gestation in the left precentral gyrus extending to the middle frontal gyrus (BA 6). Reduced gray matter volume in association with high pregnancy anxiety at 19 weeks gestation was furthermore observed in the left medial temporal lobe, uncus, extending to the entorhinal cortex (BA 28) and the parahippocampal gyrus (BA 36) as well as in the left temporal pole (BA 38) and the left inferior temporal gyrus (BA 20). Bilateral reductions in gray matter volume were found in children whose mothers reported higher pregnancy anxiety in the lateral temporal cortex extending from the superior temporal gyrus (BA 22) to the middle temporal gyrus (BA 21) and on the right side to the postcentral gyrus. Reduction in gray matter volume in association with high pregnancy anxiety at 19 weeks gestation was also observed in the left postcentral gyrus as well as in the left supramarginal gyrus (BA 39) and the right angular gyrus (BA 39). Furthermore, in children of mothers with high pregnancy anxiety at 19 weeks gestation, pronounced bilateral gray matter volume reduction was found in the cerebellum extending to the middle occipital gyrus (BA 19) and to the fusiform gyrus (BA 37). Clusters of voxels with reduced gray matter density were found in association with high pregnancy anxiety at 25 and 31 weeks (see Table 4) but these did not survive FDR correction and are therefore not reported here. Excluding either child of the sibling pair did not significantly change the reported results.

Discussion

We present the first evidence in humans that prenatal maternal anxiety is associated with brain morphology in the developing individual within specific sensitive time periods. Specifically, pregnancy anxiety at 19 weeks gestation was associated with gray matter volume reductions in the prefrontal cortex, the premotor cortex, the medial temporal lobe, the lateral temporal cortex, the postcentral gyrus as well as the cerebellum extending to the middle occipital gyrus and the fusiform gyrus. These associations with gray matter density were confined to pregnancy anxiety reported at 19 weeks gestation, as reports of pregnancy anxiety at 25 and 31 weeks gestation were not significantly associated with gray matter volume. Our findings are consistent with accumulating evidence from animal studies that medial temporal and prefrontal cortical regions are shaped by early experience (e.g. Coe et al., 2003; Salm et al., 2004; Fujioka et al., 2006; Murmu et al., 2006).

Scales measuring pregnancy anxiety have been suggested to better assess anxieties and worries related specifically to pregnancy than general scales of stress, depression and anxiety (Huizink et al., 2004; Dipietro et al., 2006). This is emphasized by observations of pregnancy anxiety having a higher predictive quality for birth outcomes and fetal/child development than general stress scales (Wadhwa et al., 1993; Huizink et al., 2003; DiPietro et al., 2004; Roesch et al., 2004; Dipietro et al., 2006; Kramer et al., 2009; Davis & Sandman, in press) and is furthermore supported by the highly significant association between self-reported maternal pregnancy anxiety and brain morphology in this study.

The brain regions that we have found to be affected by pregnancy anxiety are areas specifically associated with cognitive performance. The prefrontal cortex is sometimes described as the “highest” structure of the brain because it is involved in executive cognitive functions such as reasoning, planning, attention, working memory, and some aspects of language (e.g. Connolly et al., 2002). Structures in the medial temporal lobe, including areas connected to the hippocampus (entorhinal, perirhinal, parahippocampal cortex), have been proposed to constitute a “medial temporal lobe memory system” with the primary functions of these areas related to the storage and recall of facts and events (Squire et al., 2004). The temporal polar cortex appears to be involved in social and emotional processing including recognition and semantic memory (Nakamura & Kubota, 1996; Hoistad & Barbas, 2008). A network in the temporal-parietal cortex consisting of the middle temporal gyrus (BA 21), the superior temporal gyrus (BA 22) and the angular gyrus (BA 39) has been shown to be important in processes related to auditory language processing in children (Ahmad et al., 2003). Also involved in language learning seems to be another network of brain regions affected by pregnancy anxiety (the inferior frontal gyrus (BA 45), the middle temporal gyrus (BA 21) and the parahippocampal gyrus, Mestres-Misse et al., 2008).

Importantly and consistent with the primary functions of the affected brain regions, a small but growing literature indicates that prenatal stress influences both cognitive development as well as temperament. Thus, elevated prenatal maternal stress/anxiety is associated with infant inability to attend and with delayed cognitive development (Brouwers et al., 2001; Huizink et al., 2002; O’Connor et al., 2002; Buitelaar et al., 2003; Huizink et al., 2003; Davis & Sandman, in press), lower academic achievement in school (Niederhofer & Reiter, 2004), higher infant behavioral reactivity (Davis et al., 2004; Davis et al., 2005; Davis et al., 2007) and emotional/behavioral problems that persisted until adolescence (Van den Bergh et al., 2005; Van den Bergh et al., 2008). Furthermore, offspring of women who were exposed to a natural disaster during their pregnancies had poorer general intellectual functioning and language development (Laplante et al., 2004; Laplante et al., 2008) and maternal exposure to natural disasters, war or stressful life events have been associated with increased prevalence of psychopathology in the offspring (van Os & Selten, 1998; Selten et al., 1999; Watson et al., 1999; Beversdorf et al.,

2005; Khashan et al., 2008). Our observations of reduced gray matter density in the premotor cortex and the cerebellum may provide the anatomical basis for previous observations of delayed motor development in association with prenatal stress/ anxiety (Buitelaar et al., 2003; Huizink et al., 2003).

Interestingly, a recent functional MRI study in humans found that prefrontal regions, that we found are affected by high pregnancy anxiety, are involved in the regulation of stress hormone secretion (Pruessner et al., 2008). These same brain regions appear to be particularly vulnerable under conditions of chronic stress due to their high density of glucocorticoid receptors (Sapolsky et al., 1990). Thus, by its effect on these brain regions, high maternal prenatal anxiety may increase the risk for higher stress susceptibility and reactivity in the developing individuals. This may result in higher concentrations of stress hormones which could further delay brain development. These assumptions are consistent with reports of higher baseline and stress-reactive cortisol concentrations in children born to mothers with high anxiety levels during pregnancy (Gutteling et al., 2005; O'Connor et al., 2005; Van den Bergh et al., 2008).

Reduced gray matter density in the precentral and postcentral gyrus in association with pregnancy anxiety is consistent with evidence for disturbed development of the nociceptive system and associated behavioral changes in association with prenatal stress (Smythe et al., 1994; Rokyta et al., 2008). Occipital-temporal areas (middle occipital gyrus (BA19) and fusiform gyrus), involved in visual processing, are furthermore affected by pregnancy anxiety (Brandt et al., 2000).

Limbic structures, especially the hippocampus, have been shown to be prominent targets for early life stress (e.g. Coe et al., 2003; Buss et al., 2007). Still, we did not observe a significant reduction in gray matter density in this region. Before concluding that this area is not affected by maternal pregnancy anxiety, alternative, potentially more sensitive, methods of analyses (e.g. manual segmentation, shape analyses) are required.

The fetus participates in a dynamic exchange of environmental (intrauterine) information with the maternal host over the course of gestation. All communication between the maternal and fetal compartments is mediated via the placenta, an organ of fetal origin. One of the major placental signals in pregnant primates is the peptide corticotrophin-releasing hormone (CRH) which has been shown to be stress-sensitive in *in vitro* studies (Petraglia et al., 1987; Petraglia et al., 1989; Petraglia et al., 1990). Other *in vivo* studies have found significant correlations among maternal pituitary-adrenal stress hormones (ACTH, cortisol) and placental corticotrophin-releasing hormone (pCRH) concentrations (Goland et al., 1992; Chan et al., 1993; Wadhwa et al., 1997; Hobel et al., 1999). Some (Hobel et al., 1999; Erickson et al., 2001), but not all studies (Petraglia et al., 2001), also have reported direct associations between maternal psychosocial stress and pCRH function. With the production and release of CRH from the placenta, regulation of the HPA axis changes dramatically during pregnancy. Maternal cortisol increases two- to four-fold over the course of normal gestation (Mastorakos & Ilias, 2003; Sandman et al., 2006) resulting from pCRH stimulating production of maternal cortisol (Sasaki et al., 1989). Maternal cortisol passes the placenta with 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) presenting a partial barrier (Brown et al., 1996). pCRH furthermore stimulates cortisol secretion from the fetal adrenals directly, as the CRH 1 receptor is present in human fetal adrenal tissue from mid-gestation (Smith et al., 1998).

At high concentrations pCRH as well as cortisol may inhibit growth and differentiation of the developing nervous system. Thus considerable evidence indicates that glucocorticoids are neurotoxic to hippocampal CA3 pyramidal cells (Sapolsky et al., 1985; Sapolsky et al., 1990; Magarinos et al., 1996), and fetal exposure to high levels of glucocorticoids produces irreversible damage to the hippocampus (Uno et al., 1990; Uno et al., 1994). Larger amounts

of exogenously administered CRH increase limbic neuronal excitation leading to seizures (Ehlers et al., 1983; Baram et al., 1992; Baram et al., 1997) and may participate in mechanisms of neuronal injury (Baram & Hatalski, 1998). The potential mechanisms by which maternal stress and associated increases in stress-sensitive hormones (pCRH, cortisol) may produce long-lasting changes in brain function have been suggested from animal models and may include changes in neurotransmitter levels (Roceri et al., 2002; Kinnunen et al., 2003; Pickering et al., 2006), adult neurogenesis (Lemaire et al., 2000; Coe et al., 2003; Fujioka et al., 2006; Lemaire et al., 2006; Odagiri et al., 2008) as well as cell growth and survival (Roceri et al., 2002; Fumagalli et al., 2004; Van den Hove et al., 2006; Aisa et al., in press).

Interestingly, pCRH as well as cortisol concentrations during pregnancy predict fetal and infant development. Low concentrations of pCRH at the beginning of the second trimester are associated with precocious maturation of the human fetus (Class et al., 2008), while elevated concentrations of pCRH during the third trimester of gestation are associated with impaired fetal learning (Sandman et al., 1999). The developmental consequences of elevated concentrations of pCRH during pregnancy extend into postnatal life, as higher pCRH concentrations during pregnancy are associated with delayed neonatal physical and neuromuscular maturation (Ellman et al., 2008), more fearful temperaments in infants (Davis et al. 2005), and an increase in central adiposity in 3 year old children (Gillman et al., 2006). Endogenous maternal cortisol also plays a role in shaping human development. Prenatal exposure to elevated maternal cortisol has been shown to predict increased fussiness, negative behavior and fearfulness in infancy (de Weerth et al., 2003; Davis et al., 2007) and greater cortisol reactivity in childhood (Gutteling et al., 2005) as well as delays in mental (Huizink et al., 2003; Davis & Sandman, in press) and motor development (Huizink et al., 2003).

The results of the current study suggest that earlier in pregnancy, the effects of pregnancy anxiety on offspring's gray matter volume are most pronounced. This effect of timing may be due to the fact that pregnancy anxiety is highest at 19 weeks gestation and decreases over the course of gestation, which is in line with previous observations of reduced physiological and psychological stress reactivity as pregnancy advances (Schulte et al., 1990; Glynn et al., 2001; Glynn et al., 2004; de Weerth & Buitelaar, 2005; Glynn et al., 2008) as well as with a recent observation that pregnancy anxiety early (around 15 weeks gestation) but not later in gestation predicts mental development at 12 months age (Davis & Sandman, in press). The effect of timing may also be related to the fact that different brain regions have a unique timetable for development and therefore specific periods of neural vulnerability. This possibility has been supported by observations in rhesus monkeys, where prenatal exposure to the same stressor had greater effects on postnatal motor development if it occurred earlier in gestation, when neuronal migration was at its peak, than if it occurred in mid- to late gestation, when synaptogenesis was at its peak (Schneider et al., 1999). The implication of these findings is that the impact of stress during pregnancy is not uniform but that stress earlier in pregnancy may have more pronounced consequences for brain development than at a later gestational stage.

It important to acknowledge that the observed consequences of prenatal programming not only depend on the timing of the insult and the brain region of interest but also on the stage of assessment. Studies on postnatal brain development have clearly shown regional and temporal patterns of dynamic maturational change continuing through childhood and adolescence. This implies that what we observed and reported here in children of this age range may not be final. It is possible that at a later maturational stage, prenatal stress exposure will confer a different morphological pattern. Therefore, following-up these children into adolescence and adulthood will provide valuable information on the persistence of prenatal stress effects on brain morphology.

It cannot be ruled out that the prenatal stress effects on brain morphology are moderated by postnatal exposures (Buss et al., 2007). By controlling for several relevant variables including postnatal maternal stress and socioeconomic status, it can be concluded though that the observed effects of pregnancy anxiety on brain structure were not mediated by these postnatal factors. Thus, the results suggest that, independent of postnatal maternal stress, prenatal stress has an impact on the offspring's brain morphology.

It has to be noted that while there was no indication of psychiatric disorders and there was no report of treatment for any disorders in the structured interviews that probed such issues, the possibility of an undiagnosed disorder cannot be ruled out because clinical diagnostic assessments were not conducted. Pregnancy anxiety may be higher in women with undiagnosed psychiatric disorders and accompanying endocrine alterations could impact on neurodevelopment of the offspring.

This is the first study in healthy children to show that prenatal maternal anxiety is related to distinctive patterns of structural brain development. These morphological patterns may increase vulnerability for certain neurodevelopmental disorders and impair cognitive function. Therefore the results suggest that addressing mothers' pregnancy-related concerns and anxiety should be a major focus for public health initiatives.

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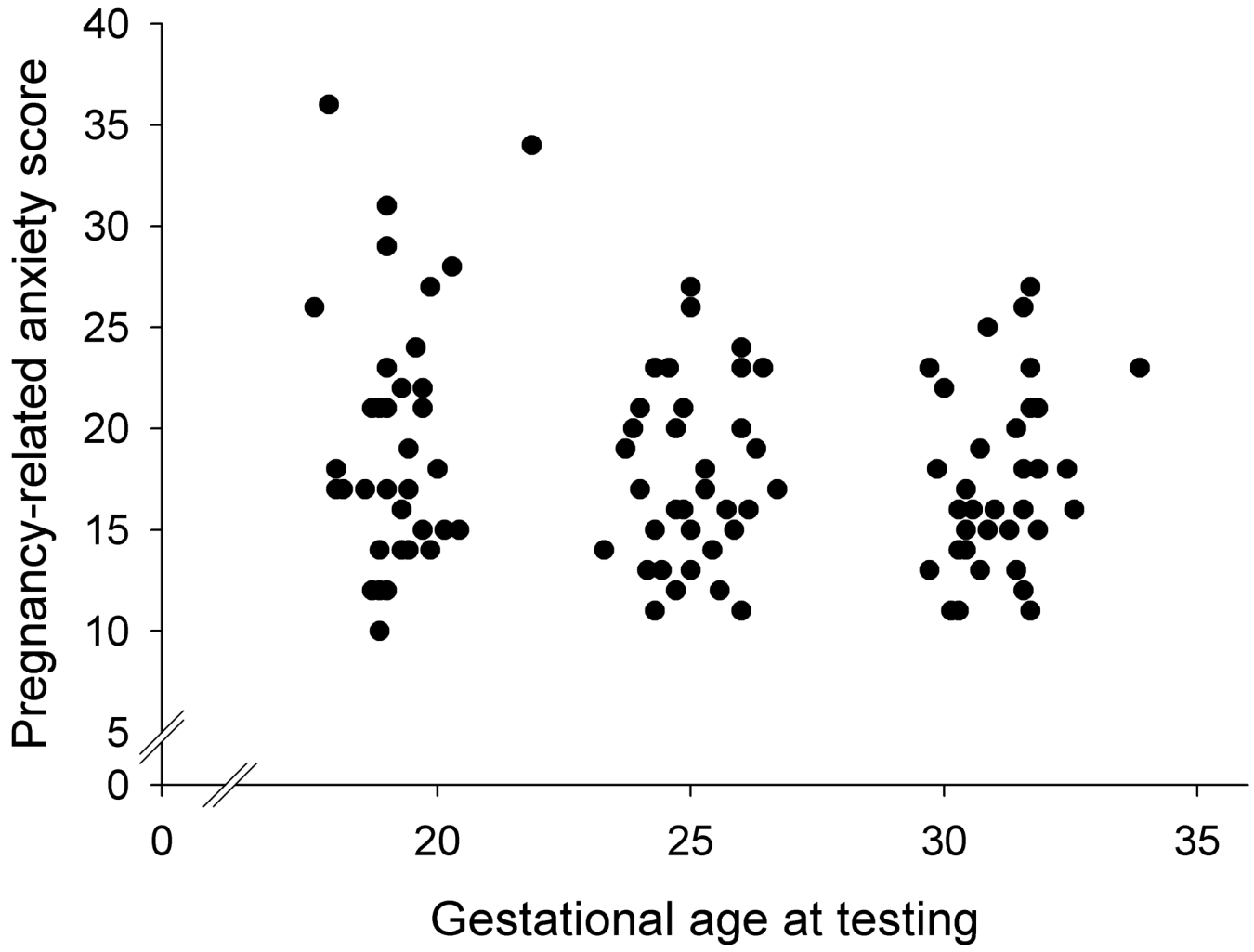

Figure 1.
Pregnancy anxiety scores over the course of gestation. With advancing gestational age, pregnancy anxiety decreases.

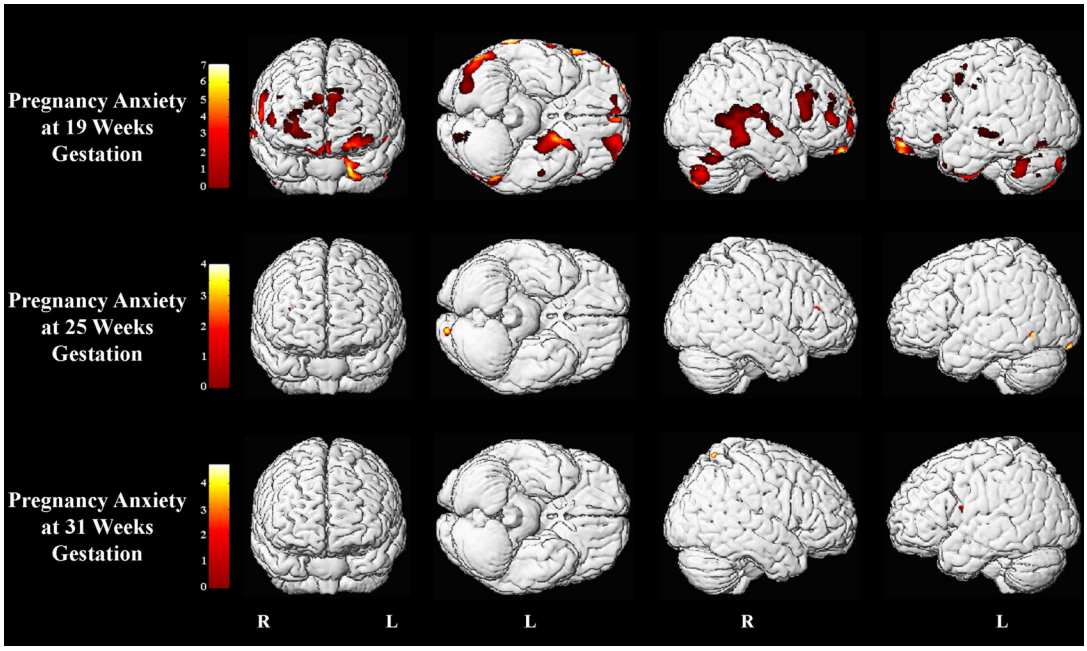

Figure 2.
Areas of reduced gray matter volume in association with pregnancy anxiety at 19, 25 and 31 weeks gestation. Voxels with $p < 0.001$ (uncorrected) are displayed.

Table 1

Sociodemographic characteristics and birth outcomes

	Mother-child dyads (N=35)
Maternal Age	32.7±6.5
Race/ethnicity	
Non-Hispanic White	37.1%
Hispanic White	20.0%
African American	8.6%
Asian	31.4%
Other	2.9%
Annual household income	
$0 to $30,000	32.4%
$30,001 to $60,000	11.8%
$60,001 to $100,000	26.4%
Over $100,000	29.4%
Pregnancy anxiety	
19 weeks gestation	19.7±6.5
25 weeks gestation	17.7±4.3
31 weeks gestation	17.3±4.4
Perceived Stress postpartum	2.1±0.7
Primiparous	44.1%
Child sex	
Male	51.4%
Female	48.6%
Length of gestation (weeks)	38.8±1.83 (11% 34-36.9 weeks)
Birth weight (grams)	3527.5±574.2 (0% <2500g)

Table 2

Correlations between pregnancy anxiety during pregnancy and sociodemographic characteristics and perceived stress postpartum

	Pregnancy anxiety at 19 weeks GA	Pregnancy anxiety at 25 weeks GA	Pregnancy anxiety at 31 weeks GA
Maternal age	-0.11 (p=0.54)	-0.17 (p=0.92)	-0.08 (p=0.66)
Maternal education (years of school completed)	0.14 (p=0.43)	0.19 (p=0.27)	0.18 (p=0.31)
Annual household gross income	0.17 (p=0.34)	0.27 (p=0.12)	0.16 (p=0.35)
Perceived Stress postpartum	0.44 (p=0.01)	0.36 (p=0.04)	0.56 (p=0.00)

Table 3

Rank correlations between pregnancy anxiety scores across pregnancy visits

	Pregnancy anxiety at 19 weeks GA	Pregnancy anxiety at 25 weeks GA	Pregnancy anxiety at 31 weeks GA
Pregnancy anxiety at 19 weeks GA	-	0.64 ($p<0.001$)	0.65 ($p<0.001$)
Pregnancy anxiety at 25 weeks GA	-	-	0.74 ($p<0.001$)
Pregnancy anxiety at 31 weeks GA	-	-	-

Table 4

Local reductions in gray matter density in association with high pregnancy anxiety: Talairach coordinates and z-scores for the most significant voxel in each of the clusters, and volumes for all clusters in the gray matter SPMs are displayed

Cluster No	Cerebral region	Talairach coordinates			Cluster size	t-value	Voxel p (uncor)	Voxel p (FDR-cor)
		x	y	z				
Pregnancy Anxiety at 19 weeks gestation								
1	Left Superior Frontal Gyrus (BA 10)	0	67	25	5523	7.0	<0.001	0.02
	Right Superior Frontal Gyrus (BA 10)	34	67	-4		4.8	<0.001	0.02
2	Left Superior Frontal Gyrus (BA 10)	-28	69	-9	3582	5.5	<0.001	0.02
	Left Superior Frontal Gyrus (BA 11)	-23	53	-19		5.4	<0.001	0.02
	Left Middle Frontal Gyrus (BA 10)	-39	62	-11		4.8	<0.001	0.02
3	Left Superior Frontal Gyrus (BA 10)	-37	64	19	734	4.4	<0.001	0.02
	Left Middle Frontal Gyrus (BA 10)	-50	57	4		3.9	<0.001	0.03
	Left Inferior Frontal Gyrus (BA 46)	-54	51	5		3.7	<0.001	0.03
4	Left Inferior Frontal Gyrus (BA 9)	-62	23	30	442	3.9	<0.001	0.03
	Left Middle Frontal Gyrus (BA 46)	-61	31	26		3.5	0.001	0.04
5	Left Inferior Frontal Gyrus (BA 47)	-55	25	-9	236	4.2	<0.001	0.03
6	Left Precentral Gyrus (BA 6)	-66	-10	40	2854	4.3	<0.001	0.02
	Left Middle Frontal Gyrus (BA 6)	-51	6	52		4.3	<0.001	0.03
7	Right Inferior Frontal Gyrus (BA 46)	53	50	5	3794	4.9	<0.001	0.02
	Right Middle Frontal Gyrus (BA 46)	57	32	28		4.3	<0.001	0.03
	Right Inferior Frontal Gyrus (BA 45)	61	25	9		4.2	<0.001	0.03
8	Right Superior Frontal Gyrus (BA11)	2	55	-21	1543	4.7	<0.001	0.03

Cluster No	Cerebral region	Talairach coordinates			Cluster size	t-value	Voxel p (uncor)	Voxel p (FDR-cor)
		x	y	z				
9	Left Uncus (BA 28)	-13	3	-25	3398	5.3	<0.001	0.02
	Left Uncus (BA 36)	-25	-10	-37		5.1	<0.001	0.02
	Left Superior Temporal Gyrus (BA 38)	-20	13	-28		4.7	<0.001	0.02
10	Left Superior Temporal Gyrus (BA 22)	-73	-13	0	1375	4.3	<0.001	0.03
	Left Middle Temporal Gyrus (BA 21)	-76	-26	-5		4.2	<0.001	0.03
11	Left Inferior Temporal Gyrus (BA 20)	-49	-12	-37	109	3.8	<0.001	0.03
12	Right Middle Temporal Gyrus (BA 21)	75	-33	-8	6241	5.3	<0.001	0.02
	Right Superior Temporal Gyrus (BA 22)	74	-35	14		4.9	<0.001	0.02
13	Left Middle Occipital Gyrus (BA 19)	-58	-71	-12	3969	5.9	<0.001	0.02
	Left Cerebellum (Tuber)	-57	-52	-23		4.0	<0.001	0.03
14	Left Cerebellum (Tuber)	-32	-93	-28	4644	5.1	<0.001	0.03
15	Left Cerebellum (Pyramis)	-54	-69	-32	181	3.6	0.001	0.04
16	Right Cerebellum (Tuber)	61	-64	-19	2742	5.7	<0.001	0.02
	Right Middle Occipital Gyrus (BA19)	62	-72	-6		3.9	<0.001	0.03
	Fusiform Gyrus (BA37)	62	-75	1		3.8	<0.001	0.03
17	Right Cerebellum (Pyramis)	53	-72	-32	2747	5.3	<0.001	0.02
18	Right Cerebellum (Tuber)	43	-88	-24	827	4.2	<0.001	0.03
19	Left Postcentral Gyrus (BA 2)	-56	-28	59	348	4.4	<0.001	0.02
20	Right Angular Gyrus (BA 39)	60	-68	40	399	4.2	<0.001	0.02
21	Left Supramarginal Gyrus (BA 39)	-70	-56	24	331	4.1	<0.001	0.02
Pregnancy Anxiety at 25 weeks gestation								
22	Right Middle Frontal Gyrus (BA 10)	33	37	12	115	3.8	<0.001	0.94
23	Left Middle Temporal Gyrus (BA 37)	-42	-58	-2	106	4.0	<0.001	0.94

Cluster No	Cerebral region	Talairach coordinates			Cluster size	t-value	Voxel p (uncor)	Voxel p (FDR-cor)
		x	y	z				
24	Left Lingual Gyrus (BA 17)	-11	-93	-9	148	3.9	<0.001	0.94
Pregnancy Anxiety at 31 weeks gestation								
25	Right Inferior Frontal Gyrus (BA 47)	32	25	2	617	4.7	<0.001	0.95
26	Left Insula (BA 13)	-38	6	17	200	3.9	<0.001	0.95
27	Right Postcentral Gyrus (BA 7)	18	-53	65	121	3.9	<0.001	0.95