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Pregnancy involves long-lasting changes in human brain structure

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Abstract

Pregnancy involves radical hormone surges and biological adaptations. However, the effects of pregnancy on the human brain are virtually unknown. Here we show, using a prospective ('pre'-'post' pregnancy) study involving first-time mothers and fathers and nulliparous control groups, that pregnancy renders substantial changes in brain structure, primarily reductions in grey matter (GM) volume in regions subserving social cognition. The changes were selective for the mothers and highly consistent, correctly classifying all women as having undergone pregnancy or not in-between sessions. Interestingly, the volume reductions showed a substantial overlap with brain regions responding to the women's babies postpartum. Furthermore, the GM volume changes of pregnancy predicted measures of postpartum maternal attachment, suggestive of an adaptive process serving the transition into motherhood. Another follow-up session showed that the GM reductions endured for at least 2 years post-pregnancy. Our data provide the first evidence that pregnancy confers long-lasting changes in a woman's brain.

The vast majority of women undergo pregnancy at least once in their lives, yet remarkably little is known on how this process affects the human brain. Mammalian pregnancy involves radical physiological and physical adaptations orchestrated by endocrine changes[1]. During pregnancy, there are unparalleled surges of sex steroid hormones, including for instance a 10-15 fold increase in progesterone relative to luteal phase levels and a flood of estrogens that typically exceeds the estrogen exposure of a woman's entire non-pregnant life[2]. Sex steroid hormones are known to act as an important regulatory agent of neuronal morphology and number[3]. Not surprisingly, other endocrine events involving less extreme and rapid fluctuations in hormone levels than pregnancy are known to render structural and functional alterations in the human brain. The production of gonadal sex steroid hormones during puberty regulates an extensive reorganization of the brain[4-6], and neural alterations have also been observed in response to even subtle changes in endogenous or exogenous steroid hormone levels later in life[7-9].

However, very little is known on the effects of pregnancy on the human brain. A few spectroscopic studies have been performed in pregnant women[10-12], observing no differences with respect to non-pregnant women except for transiently reduced choline levels. In addition, some observations have been reported on aspects of brain structure in pregnancy. In 1909, enlargements of the pituitary gland were first observed in deceased pregnant women[13], which was later corroborated by further in vitro[14] and in vivo[15] measurements of this structure. Besides these assessments of pituitary gland volume, the ventricles and outer border of the brain have been contoured in a small sample of healthy pregnant women serving as a control for patients with pre-eclampsia[16], pointing to increases and decreases respectively during late pregnancy in comparison to the early postpartum period.

In non-human animals, a converging body of evidence has demonstrated that reproduction is associated with neural changes at many levels, including regional changes in dendritic morphology, cellular proliferation and gene expression[17-20]. Interestingly, these effects seem to be long-lasting, as various differences in brain and behavior between parous and nulliparous females are evident throughout the lifespan[17-21].

We performed a prospective ('pre'-'post' pregnancy) study involving primiparous (first-time) mothers and nulliparous control women to investigate whether pregnancy is associated with changes in the grey matter (GM) structure of the human brain. In addition, we 1) tested the discriminative power of the GM

volume changes with a multivariate pattern recognition analysis, 2) examined GM volume changes in primiparous fathers and nulliparous control men to further test the specificity of the changes for pregnancy rather than approaching parenthood, 3) defined the structural characteristics of GM changes across pregnancy by means of surface-based analyses, 4) investigated a potential link to maternal attachment using a postpartum fMRI paradigm and attachment scale, and 5) tested the long-term persistence of pregnancy effects with a 2-year post-pregnancy follow-up session.

We show that pregnancy is associated with pronounced and long-lasting GM volume reductions in a woman's brain, which are primarily located in regions involved in social processes and display a remarkable similarity to the Theory of Mind network. Interestingly, all of the women can be classified as having undergone pregnancy or not based on the volume changes across sessions. In addition, we demonstrate that these GM volume reductions are located in some of the brain regions that show the strongest response to the women's babies in a postpartum fMRI paradigm. Furthermore, the GM volume changes of pregnancy predict measures of postpartum mother-to-child attachment and hostility. These results indicate that pregnancy changes the GM architecture of the human brain, and provide preliminary support for an adaptive process serving the transition into motherhood.

RESULTS

GM volume changes in primiparous mothers across pregnancy

To examine the effects of pregnancy on human brain structure, a prospective study was performed. High resolution anatomical pre-conception brain scans were obtained from nulliparous women wanting to get pregnant and become mothers for the first time (the 'PRE' session). If successful, they again took part in an MRI session after the completion of their pregnancy (the 'POST' session). This setup allowed us to reliably extract the changes in brain structure relative to each person's pre-pregnancy baseline. Longitudinal data were also acquired at a comparable time interval from 20 nulliparous control women. Demographic information of the sample is provided in the Methods section.

The longitudinal diffeomorphic modeling pipeline implemented in SPM12 was applied to extract changes in grey matter (GM) volume between the subsequent brain scans on an individual level, and the maps of GM volume change of the primiparous women were compared to those of the nulliparous control women. Strikingly, we observed a symmetrical pattern of highly significant group differences in GM volume change across sessions (Table 1, Fig. 1, Supplementary Fig.1), and post-hoc analyses revealed that each of these clusters reflected reductions in regional GM in the women who underwent pregnancy between the time points (Supplementary Table 1, Fig. 4b). Effect sizes further illustrating the strength of these effects are depicted in Supplementary Figure 2. Baseline comparisons confirmed that there were no pre-existing differences in GM volume between the groups.

The GM volume reductions after pregnancy were primarily located in the anterior and posterior midline (medial frontal cortex/anterior cingulate cortex and precuneus/posterior cingulate cortex), the bilateral lateral prefrontal cortex (primarily the inferior frontal gyri), and the bilateral temporal cortex (the superior temporal sulci extending to surrounding lateral temporal as well as medial temporal sections).

For completeness, white matter volume was also examined using this approach, although it should be noted that these MRI images are not optimal for investigating white matter tissue. These analyses indicated no significant changes in white matter volume across the time points in the women who underwent pregnancy in comparison to the control women.

In addition, to further explore our data based on the few available previous findings related to the effects of pregnancy on human brain structure, we manually delineated the pituitary gland and investigated total tissue volumes in our sample. These results are reported in the Supplementary material (Supplementary Figure 3, Supplementary Table 2-3).

Figure 1

Table 1

The means of conception

As our sample included both women who achieved pregnancy by natural conception and women who underwent a fertility treatment (see Methods section for details on the sample), we then examined whether the means of conception was associated with distinct neural changes. When comparing the brain changes between the participants achieving pregnancy by natural or assisted conception, we observed no differences (Supplementary Table 4). In fact, very similar GM reductions were observed when examining these groups separately (Fig. 2, Supplementary Table 5, Supplementary Fig. 4), suggesting that the women were similarly affected by pregnancy regardless of the means of conception. Additional analyses investigating the impact of demographic or clinical factors on the observed brain changes of pregnancy are reported in the Methods section and Supplementary Material.

Figure 2

Multivariate pattern classification analysis

The highly similar pattern of changes observed in these subgroups suggested a strong consistency of the GM reductions across the pregnant participants. To further test the consistency of the GM volume changes of pregnancy, we applied a multivariate pattern classification analysis using a support vector machine algorithm to the GM volume difference maps. Strikingly, this analysis showed that all of the women could be correctly classified as having been pregnant or not in-between these sessions based on the GM changes in the brain (Fig.3a,b).

An inspection of the classifier weight map (Fig. 3b, Supplementary Fig. 5) suggested a strong contribution of the structures of GM change observed in the univariate results to the classification, which was confirmed by a multi-kernel learning approach (Fig.3c). This analysis appointed the right middle temporal gyrus, inferior frontal gyrus and posterior cingulate cortex as the regions of greatest predictive power, together providing a contribution of over 50% to the decision function (Fig.3c).

Figure 3

Localization of GM volume changes of pregnancy

The regions of GM change affected by pregnancy are known to play a role in social cognition, and a visual inspection of the observed GM volume changes suggested a strong similarity to the Theory of Mind network (Fig. 4). To quantitatively assess this spatial correspondence, we defined the overlap of our results with the Theory of Mind network as defined by the meta-analysis of Schurz et al[22], which indicated a 3-fold larger volume of overlap than expected based on a random distribution of the maps across the brain's GM (see Supplementary Table 6). Moreover, to further examine the localization of the observed GM changes with respect to functional networks, we quantified the overlap between the GM changes of pregnancy and the 12 cognitive components of the cerebral cortex as defined by the extensive meta-analysis of Yeo et al.[23]. Interestingly, although these components load on various task variables, the 3 cognitive components of greatest overlap with the GM changes of pregnancy correspond to the 3 components that are activated by Theory of Mind tasks (see Supplementary Table 6). Accordingly, the greatest spatial correspondence was observed with the network of strongest Theory of Mind recruitment. In fact, the only functional networks that show a greater overlap with the GM changes of pregnancy than expected based on a random distribution across the brain's GM tissue correspond to those 3 networks that are recruited by Theory of Mind tasks.

Figure 4

GM volume changes in primiparous fathers across partner's pregnancy

To further test the specificity of these changes for participants undergoing the biological process of pregnancy rather than other changes associated with becoming a parent, we additionally scanned primiparous fathers before and after their partner's pregnancy, along with a male nulliparous control group. Maps of GM volume change were extracted using the SPM12 longitudinal diffeomorphic modeling

pipeline. Comparisons involving these groups showed that there are no changes in neural GM volumes in the fathers in comparison to the control group across this time period and the observed brain changes are selective for the women undergoing pregnancy in-between the brain scans (Table 1, Supplementary Table 7, Supplementary Fig. 6).

Changes in surface area and cortical thickness across pregnancy

To examine the structural characteristics of neural GM changes across pregnancy, we additionally performed surface-based analyses in FreeSurfer 5.3. Cross-sectional analyses confirmed the lack of baseline differences between the women undergoing pregnancy in-between sessions and the control group. Using the longitudinal processing pipeline, we extracted cortical thickness and surface area, structural properties of the cortical mantle that both contribute to cortical volume. In line with the main volumetric results, reductions were observed in these measures across pregnancy (Fig.5, Supplementary Table 8-9). Figure 5 depicts changes in surface area (Fig. 5a) and cortical thickness (Fig. 5b). Although both measures were affected, especially extensive changes were observed in the surface area of the cortical sheet (Fig. 5a, Supplementary Table 8-9).

Accordingly, discriminant analyses involving the average surface area and cortical thickness values across the map of GM volume change indicated that 84.4% of the women could be correctly classified as having undergone pregnancy or not based on the changes in surface area ($\lambda = 0.66$, $\chi^2=17.69$, $p<0.001$), while 68.9% could be classified based on cortical thickness changes ($\lambda = 0.82$, $\chi^2=8.57$, $p=0.014$). In comparison, 95.6% of the women could be correctly classified using measures of average GM volume change ($\lambda = 0.36$, $\chi^2=43.49$, $p<0.001$). Correlation analyses indicated significant associations between the changes in average GM volume and these surface-based measures, which are stronger for surface area than for cortical thickness (Cortical Thickness: Left hemisphere: R=0.44, p=0.029, Right hemisphere: R =0.38, p=0.062. Surface Area: Left hemisphere: R=0.58, p=0.011. Right hemisphere: R=0.91, p<0.001).

Figure 5

Changes in cognitive performance across pregnancy

Several cognitive tests were performed at the sessions before and after pregnancy. A verbal word list task was used to examine verbal memory, and changes in working memory were investigated using a backward digit span task and a 2-back test. No significant changes were observed across sessions in these measures in comparison to the control group, although a trend was observed for a reduction in the number of correct responses on the verbal word list learning task (Supplementary Table 10).

Multivariate regression analyses with Maternal Postnatal Attachment Scale

To investigate whether there is an association between the brain changes of pregnancy and aspects of maternal caregiving in the postpartum period, we examined the changes in GM volume across pregnancy in relation to indices of maternal attachment. Multivariate kernel ridge regression analyses were performed using the 3 dimensions of the Maternal Postnatal Attachment Scale[24]. These analyses indicated that the GM volume changes of pregnancy significantly predicted the mother-to-infant quality of attachment and the absence of hostility towards their newborns in the postpartum period as defined by this scale. These results are depicted and reported in Figure 6 (Fig.6b, Supplementary Fig. 7-8).

Figure 6

Neural activity on an fMRI paradigm involving pictures of the women's babies

In addition, to examine the neural response to visual cues of their babies, in the POST session the mothers participated in an fMRI paradigm involving baby pictures. In this paradigm, women were shown pictures of their own infants and of other infants, and the neural activity in response to their own infant was contrasted against the neural response to viewing other infants. Functional MRI paradigms involving

own and other infant pictures and sounds have previously been used as a neural index of parental attachment[25]. In accordance with the multivariate regression results reported above, we found that several of the regions that showed the strongest neural activity in response to the women's babies corresponded to regions that lose GM volume across pregnancy (Fig.6a, Supplementary Table 11). A quantification of the overlap between these results and the GM volume changes of pregnancy indicates that nearly 30% of the voxels that respond to the mothers' own infants in comparison to other infants are located in GM tissue that loses volume across pregnancy (Supplementary Table 6, Supplementary Figure 9). This represents a nearly 7-fold greater overlap than expected based on a random distribution of these maps across the brain's grey matter tissue (Supplementary Table 6).

The opposite contrast ('other baby pictures'>'own baby pictures') did not render statistically significant results. For completeness, neural activity for each condition was additionally investigated separately to confirm the recruitment of typical networks for visual perception and face processing in both conditions (see Supplementary Table 12).

Long-term follow-up session

As animal models provide compelling evidence that reproduction is associated with alterations in female brain and behavior that are evident past weaning and even in old age[17-21], we investigated whether the structural changes we observed in our human sample were maintained at another follow-up session around 2 years after giving birth (M±SD: 2.32±0.50 years postpartum, 'POST+2yrs' session). Eleven of the mothers had not yet experienced a second pregnancy and were able and willing to return for this follow-up session. When examining the brain changes between this POST+2yrs session and the pre-pregnancy baseline, we observed GM volume reductions in all clusters that were also reduced in the early postpartum period relative to the pre-pregnancy baseline (Fig.7a,b, Supplementary Table 13), except for the left hippocampal cluster (Fig.7c,d, Supplementary Table 13). Accordingly, when examining the changes in GM volume between the POST and POST+2yrs sessions, we observed no further reductions or increases within these structures except for a selective volume recovery in the left

hippocampal cluster (Fig.7, Supplementary Table 13). These results indicate that, apart from partial hippocampal volume recovery, all these GM reductions endured for at least 2 years after giving birth.

Figure 7

DISCUSSION

These results indicate that pregnancy is associated with pronounced changes in the structure of the human brain. More specifically, primiparous women were found to undergo a symmetrical pattern of extensive GM volume reductions across pregnancy, primarily affecting the anterior and posterior cortical midline and specific sections of the bilateral lateral prefrontal and temporal cortex. Subgroup analyses suggested a strong consistency of these volume changes across participants, which was further emphasized by a multivariate pattern recognition analysis. In fact, this analysis indicated that all of the women could be correctly classified as having undergone pregnancy or not in-between the MRI sessions based on the GM volume changes in the brain. Analyses involving primiparous fathers provided further evidence for the selectivity of these volume changes for women undergoing pregnancy, supporting the connection of these brain changes to the biological process of pregnancy rather than to experience-dependent changes associated with approaching parenthood.

Interestingly, there is another stage of life that involves increases in endogenous sex steroid hormone levels followed by widespread changes in the GM structure of the brain[4-6]. In adolescence, the production of sex steroid hormones initiates a spectrum of behavioral, cognitive, socio-emotional, physical and neural changes, including extensive reductions in GM volume, surface area and cortical thickness[4, 6, 26]. In fact, higher estradiol levels in adolescent girls have been found to predict greater cortical thinning and GM volume loss in several of the regions observed in our study, including the middle temporal and inferior frontal gyri[27, 28].

Changes in GM signal extracted from MRI images can reflect various processes, such as changes in the number of synapses, the number of glial cells, the number of neurons, changes in dendritic structure, vasculature, blood volume and circulation and myelination, and the reductions in GM volume observed in

our study cannot be pinpointed to a specific molecular mechanism. In adolescence, these GM reductions are proposed to reflect (at least in part) synaptic pruning accompanied by corresponding reductions in metabolic requirements and glial cells, although increased myelination can also underlie observations of GM volume reductions. Synaptic pruning in adolescence is generally regarded as an essential process of fine-tuning connections into functional networks, and is thought to represent a refinement and specialization of brain circuitry, which is critical for healthy cognitive, emotional and social development[4, 6, 26].

The results of the current study indicate that pregnancy is likewise associated with substantial reductions in GM volume. The observed volume reductions are not distributed randomly across the brain, but they are primarily located in association areas of the cerebral cortex. Although these higher-order regions contribute to various functions, it is well established that the affected regions play a key role in social processes. In fact, the observed pattern of morphological changes displays a remarkable similarity to the Theory of Mind network (Fig. 4). The spatial similarity between the GM changes of pregnancy and the Theory of Mind network was confirmed by a quantification of the overlap between our results and those of the Theory of Mind meta-analysis by Schurz et al.[22]. Furthermore, an examination of the intersections between the GM volume changes of pregnancy and the cognitive components of the human association cortex as defined by the meta-analysis by Yeo et al.[23] provided further evidence for a preferred localization of these changes within functional networks recruited by Theory of Mind tasks, although it should be noted that the implicated functional networks go beyond processes of Theory of Mind and multiple processes are likely to be affected.

Based on our results, we can speculate that the female brain undergoes a further maturation or specialization of the neural network subserving social cognition during pregnancy. Very few studies have investigated the effects of pregnancy on measures of social cognition, but there are preliminary indications of facilitated processing of social information in pregnant women, including enhanced emotion and face recognition[29-31]. Interestingly, in accordance with these findings, the notion of gestational adaptations in social cognition has previously been proposed from an evolutionary perspective[30].

In rodents, hormonal priming of the brain during pregnancy is associated with the suppression of aversive responses to pups and the emergence of an elaborate repertoire of maternal behaviors[17-20].

Other effects of reproductive experience in rodents include persistent improvements in spatial learning, foraging and predatory abilities[18-20, 30]. Humans have evolved under different evolutionary pressures than rodents, and, in our species, social cognitive abilities may be more critical than foraging abilities for providing adequate maternal care and successfully raising a child in a complex social environment such as ours. Accordingly, the Theory of Mind system is considered a core component of the human parental brain[25], and accurate mentalizing expressions of the mother to her child have been shown to be important for secure parent-infant attachment and for the development of the child's own social cognitive functions[32]. Gestational alterations in brain structures subserving social processes can be conceived to confer an adaptive advantage for motherhood in various ways, for instance by facilitating a mother's ability to recognize the needs of her highly altricial child, to decode social stimuli that may signal a potential threat, or to promote mother-infant bonding.

To further investigate the possibility of an adaptive restructuring to facilitate aspects of motherhood, we examined the observed brain changes in relation to indices of maternal caregiving. Interestingly, multivariate regression analyses using the 3 dimensions of the Maternal Postnatal Attachment Scale[24] demonstrated that the GM volume changes of pregnancy significantly predicted mother-to-infant quality of attachment and the absence of hostility towards her newborn in the postpartum period. In addition, a substantial overlap was observed between the GM tissue undergoing volume reductions across pregnancy and the brain areas of strongest neural responsivity to pictures of the women's babies in a postpartum fMRI session. Taken together, our findings provide preliminary support for an adaptive refinement of social brain structures that benefits the transition into motherhood.

To obtain more information regarding the structural characteristics of the neural GM changes of pregnancy, we additionally performed surface-based analyses. These analyses revealed reductions in both cortical thickness and surface area across pregnancy, although the surface area of the cortical mantle was particularly strongly affected. These findings are in line with previous research showing that both these cortical sheet properties remain dynamic throughout life, although they are differentially affected at various stages. For instance, surface area is more dynamic across early development[33], while rapid GM atrophy as seen in e.g. Alzheimer's Disease, AIDS or Multiple Sclerosis is almost exclusively driven by cortical thinning[34-36]. Interestingly, sexual dimorphisms in levels and in trajectories of surface

area rather than cortical thickness primarily underlie sex differences in cortical volume[33, 37], suggestive of an enhanced sensitivity of the surface area of the cortical sheet to sex steroid hormones.

Finally, since animal studies have demonstrated reproduction-related changes that are evident across the lifespan[17-21], we investigated whether the structural changes of pregnancy were transient or persistent at another follow-up session around 2 years after giving birth. Interestingly, these analyses showed that all volume changes were maintained except for a selective partial volume recovery in the hippocampal cluster.

Although it is difficult to compare our findings to the microstructural in vitro/ex vivo results obtained from animal studies, it should be noted that the hippocampus is a region that has been extensively investigated in rodents in relation to reproductive experience and shows a remarkable plasticity across pregnancy and the postpartum period[38]. For instance, changes in dendritic morphology have been demonstrated in rats following pregnancy or a pregnancy-mimicking regimen of estrogen and progesterone[39, 40]. Furthermore, a trend for reduced cell proliferation has been observed in late pregnant rats[41], and reproductive experience has consistently been associated with reduced neurogenesis in the postpartum period[42]. Interestingly, this effect seems to be restored by the time of weaning and is reversed in middle age. Reproductive experience is then associated with increased neurogenesis[43], a possible mechanism that can also be hypothesized to contribute to some of the observed volume recovery in our study. In accordance with our findings, animal studies investigating the volume of the hippocampus observed a trend for hippocampal volume reduction during late pregnancy[44] and in lactating primiparous rats in the postpartum period in comparison to nulliparous females[45]. Interestingly, aged parous rats – especially multiparous females- were found to have increased hippocampal long-term potentiation, enhanced memory capacities and less signs of brain aging in comparison to aged nulliparous females[17-21, 38]

We can speculate that the hippocampal GM reductions and subsequent +2 year postpartum partial volume recovery observed in our study may play a role in the memory deficits often associated with human pregnancy[46, 47], which have also been found to be recovering at 2 years postpartum[48]. Previous studies have indicated that particularly verbal recall memory is diminished during pregnancy[46]. It should be noted, however, that the memory changes of pregnancy seem to be subtle and have not consistently

been replicated[46, 49]. In the current study, no significant changes were observed in memory performance in the women who underwent pregnancy in-between sessions in comparison to women who did not. However, based on these results, no conclusions can be drawn with respect to contingent transient memory changes occurring during pregnancy itself, since post-pregnancy measures were compared to pre-pregnancy baseline performance. Moreover, larger samples or more ecologically valid tasks are likely required to reveal the spectrum of subtle changes in cognitive performance associated with pregnancy. Finally, it should be noted that our sample was relatively highly educated. Although this was the case for all subject groups included in our study, this may introduce a bias when investigating changes in cognitive function, and the observed lack of memory changes may not be generalizable to women of a different educational background.

Sex steroid hormones regulate neuronal morphology and number[3], and changes in endogenous or exogenous levels of these hormones are known to have an impact on human brain structure and function[4-9]. Considering the unequaled surges of sex steroid hormones that a woman is exposed to during her pregnancy and the remarkable consistency and extent of the observed neuroanatomical changes, we attribute these to the endocrine climate of pregnancy. However, the factors contributing to the observed neuroanatomical changes cannot be conclusively determined. Lifestyle changes associated with becoming a parent such as changes in social status or surroundings can be hypothesized to play a role. In addition, although pregnancy comprises by far the most prolonged and endocrinologically extreme part of the period between the two MRI scans, we cannot with certainty exclude a contribution to our results of parturition or early postpartum factors such as sleep deprivation or infant interaction in the weeks between birth and the POST acquisition. However, it should be noted that no changes were observed in the fathers, who were included as an additional control group to partially account for such experience-dependent changes. Furthermore, these environmental and lifestyle changes primarily occur in – or at least continue into- the period after birth. Correlation analyses with the duration of the postpartum period until the acquisition revealed no significant correlations (either linear, quadratic or cubic) within these structures, and including this variable as a covariate had very little effect on our results (Supplementary Table 14). Moreover, changes in GM volume across the first postpartum period have previously been mapped in a longitudinal study[50]. In this study, women were investigated across the first postpartum

period, a period during which they were exposed to similar postpartum factors as the women in our study in the part of the early postpartum period prior to the second MRI acquisition. However, no neural volume reductions were observed[50]. Taken together, these data suggest that the observed reductions in GM volume reflect an effect of the gestational period rather than the fraction of the postpartum period included within the PRE-to-POST time interval. Future studies tracking gestational hormones as well as changes in environment and lifestyle may further discriminate the factors contributing to the observed neuroanatomical changes.

In conclusion, the current findings indicate that human pregnancy is associated with substantial long-lasting alterations in brain structure, which may serve an adaptive purpose for pending motherhood. These data provide the first insights into the profound impact of pregnancy on the grey matter architecture of the human brain.

Accession codes

FigShare: http://dx.doi.org/10.6084/m9.figshare.4216809

Data Availability Statement

Source files for the figures are provided in FigShare (http://dx.doi.org/10.6084/m9.figshare.4216809).

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Contributions

E.H., E.B.M., S.C., and O.V. designed the experiments. C.P., A.B., and F.L. recruited part of the participants and provided clinical information. E.B.M. oversaw the overall timeline, recruitment and data collection of the project, and acquired the data together with E.H., M.P. and S.C.. J.C.S., A.T., M.D., E.A.C. and O.V. provided facilities and advice on aspects of design, acquisition or interpretation. EH analyzed the data, except for the SA/CT analysis done by S.C. and D.G.G.. E.H. wrote the manuscript and all other authors evaluated and approved the manuscript.

Competing Financial Interests

The authors declare no competing financial interests.

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FIGURE LEGENDS

Figure 1. GM volume changes between pre-pregnancy and post-pregnancy session. **(a)** Surface maps of the GM volume changes in primiparous (N=25) compared to nulliparous control women (N=20) (at a whole-brain threshold of p<0.05 FWE-corrected). **(b)** Sagittal slice overlays and plots representing mean signal from the smoothed normalized jacobian difference images for each cluster. See Figure 7 and Supplementary Figure 1 for plots of the 3 remaining clusters. Statistics are reported in Table 1. F_{CTR}=nulliparous control women who were not pregnant in-between sessions, F_{PRG}=nulliparous women who became pregnant and transitioned into primiparity in-between sessions. Sup. Temp. Sulcus = Superior Temporal Sulcus, Med.= Medial, Inf.= Inferior, L=left, R=right.

Figure 2. Means of conception. Surface maps of GM volume changes between the PRE and POST session (p<0.05 FWE-corrected) in **(a)** the primiparous women achieving pregnancy by natural conception (N=9), **(b)** primiparous women achieving pregnancy by fertility treatment (N=16), **(c)** the nulliparous control women (N=20). Statistics are reported in Supplementary Table 4-5.

Figure 3. Classification. (a) Scatter plot depicting the support vector machine classification results (function values (M±SD): F_{PRG}: 1.27±1.03, F_{CTR}: -0.79±0.33. Balanced accuracy: 100%, dashed line=function value cut-off between classes (0), leave-one-out cross-validation, $N_{permutations}$=10,000, p≤0.0001, although note that the 100% is almost disrupted by some participants close to the decision function border. Function values are plotted per fold (i.e., in this case, per subject). **(b)** Weight map for the classifier, depicting the relative importance of the voxel in the decision function. **(c)** Weight maps for the regions of greatest predictive power resulting from the multiple kernel learning model using the AAL atlas (balanced accuracy: 93.5%, leave-one-out cross-validation, $N_{permutations}$=10,000, p≤0.0001). These are (depicted in order): the right middle temporal gyrus (weight 22.46%, experimental ranking 1.2), the right inferior frontal gyrus (weight 19.46%, experimental ranking 1.84) and the right posterior cingulate cortex (weight 10.41%, experimental ranking 3.98). F_{CTR}=nulliparous control women who were not pregnant in-between the sessions, F_{PRG}=nulliparous women who became pregnant and transitioned into primiparity in-between sessions.

Figure 4. Similarity between Theory of Mind network and GM volume changes of pregnancy. (a) Illustration of the Theory of Mind network as extracted from the meta-analysis by Schurz et al.[22]. Statistical map of permutation-based z-values of the pooled meta-analysis was provided by the authors and displayed using Caret software. **(b)**

Reductions in GM volume ($p<0.05$ FWE-corrected) in the group of women who were pregnant in-between sessions in the current study.

Figure 5. Surface-based measures. Surface maps depicting changes in **(a)** surface area and **(b)** cortical thickness across pregnancy (FDR-corrected $p<0.05$). Blue/cyan reflects increases while red/yellow reflect decreases.

Figure 6. Postpartum infant-related neural activity and attachment scores. **(a)** fMRI results for the 'own>other baby' contrast (N=20) alongside GM volume changes repeated from Fig.1. For illustrative purposes, the fMRI results are depicted at the more lenient threshold of $p<0.0001$ uncorrected (the right inferior frontal cluster and a trend for the posterior cingulate cortex are observed at the $p<0.05$ FWE-corrected threshold, see Supplementary Table 11). There were no statistically significant results for the 'other>own' baby pictures contrast (at either threshold). **(b)** Multivariate prediction of Maternal Postpartum Attachment Scale (MPAS) scores based on the GM volume changes of pregnancy. Multivariate kernel ridge regression results (N=24, leave-one-out cross-validation) with the 3 MPAS scores ($N_{permutations}$=10,000. Quality of Attachment: M±SD=37.11±3.99. p=0.030, p_{nMSE}=0.024. Absence of Hostility: M±SD=16.93±4.10. p=0.026, p_{nMSE}=0.021. Pleasure in Interaction: M±SD=20.88±3.10. p=0.985, p_{nMSE}=0.918). Predicted versus actual MPAS scores are plotted. nMSE=normalized mean squared error.

Figure 7. Long-term follow-up. (a) Plots representing mean (M±S.E.M.) signal change at each POST session relative to the pre-pregnancy baseline, extracted from the smoothed normalized jacobian difference images. The remaining clusters are plotted in Supplementary Figure 1. **(b)** Surface maps depicting GM volume reductions in the POST +2years session compared to the pre-pregnancy baseline ($p<0.05$ FWE-corrected). Complete PRE-POST-POST+2yrs datasets were available of 11 women. **(c)** Plot displaying mean signal change in the POST session compared to the pre-pregnancy baseline in the left hippocampal cluster and sagittal slice depicting hippocampal cluster from POST vs PRE comparison. **(d)** Plot and sagittal overlay depicting hippocampal recovery from the POST to the POST+2years session. Statistics are reported in Supplementary Table 13. Sup.Temp.Sulcus=Superior Temporal Sulcus, Inf.=Inferior, Med.=Medial, L=Left, R=Right. F_{CTR}=nulliparous control women who were not pregnant in-between the sessions, F_{PRG}=nulliparous women who became pregnant and transitioned into primiparity in-between sessions.

TABLES

Table 1. Changes in GM volume between the PRE and POST session

Contrasts	Regions	H	MNI coordinates			T	P	Cluster size (# voxels)
			x	y	z			
F_{PRG} > F_{CTR}	-	-						
F_{CTR} > F_{PRG}	Superior Temporal Sulcus, Middle/Superior Temporal Gyrus, Parahippocampal Gyrus	R	57	-18	-11	8.84	<0.001	4001
			33	-24	-18	6.19	<0.001	
			33	-37	-14	6.92	<0.001	
		L	-54	-18	-11	6.40	0.001	866
			-56	-33	-6	6.08	0.004	
	Precuneus, Posterior Cingulate Cortex	L/R	0	-48	30	7.56	<0.001	2674
			-6	-57	21	7.43	<0.001	
			8	-55	22	6.96	<0.001	
	Superior Medial Frontal Cortex, Anterior Cingulate Cortex, Medial Orbitofrontal Cortex	L/R	0	53	12	7.15	<0.001	1828
			-14	53	4	6.18	0.003	
			0	48	-6	5.98	0.006	
	Inferior Frontal Gyrus	R	41	14	25	7.51	<0.001	933
		L	-50	12	16	5.85	0.010	161
			-45	9	28	5.57	0.028	
	Inferior Orbitofrontal Gyrus, Inferior Frontal Gyrus, Insula	L	-39	24	-2	6.54	0.001	283
	Middle/Superior Frontal Gyrus	L	-24	25	45	6.30	0.002	509
	Fusiform, Inferior Temporal Gyrus	R	45	-54	-18	5.78	0.014	123
		L	-44	-54	-14	6.45	0.001	722
			-35	-42	-17	5.49	0.037	
	Hippocampus, Parahippocampal Gyrus	L	-32	-21	-18	6.07	0.005	148
M_{PRG} > M_{CTR}	-							
M_{CTR} > M_{PRG}	-							

Comparisons of GM volume changes across sessions ('PRE' and 'POST') between the primiparous and nulliparous control groups. Post-hoc analyses to further specify these results are reported in Supplementary Table 1. P value at peak voxel (whole-brain FWE-corrected) is reported. H=hemisphere, L=left, R=right, F_{CTR}=nulliparous control women who were not pregnant in-between sessions, F_{PRG}=nulliparous women who became pregnant and became first-time mothers in-between sessions. M_{PRG} =nulliparous men whose partners became pregnant and who became first-time fathers in-between sessions , M_{CTR} =nulliparous men whose partners were not pregnant in-between sessions.

ONLINE METHODS

Participants

For this prospective cohort study, first-time mothers participated in an MRI acquisition before and after their pregnancy, allowing us to use each woman's pre-pregnancy brain scan as her individual baseline. Data were collected over a total period of 5 years and 4 months. The participants were recruited by flyers and word-of-mouth, and part of the sample was recruited via the fertility center Instituto Valenciano de Infertilidad (IVI, Barcelona). We sought nulliparous individuals who were planning to try to become pregnant in the near future but were not pregnant yet and nulliparous individuals without such plans. Participants were therefore not randomly assigned to groups. Recruitment and data collection for all groups was initiated at the same time. Although individuals were recruited separately for the 'Pregnancy' (PRG) groups (i.e. the women and men becoming parents in-between the sessions, hereafter referred to as F_{PRG} and M_{PRG}) and the 'Control' (CTR) groups (the women and men who did not become pregnant within this time frame, from here on referred to as F_{CTR} and M_{CTR}) based on their intention to become parents in the near future, the final group allocation depended on the transition from nulliparity into primiparity in-between sessions. Women trying to become pregnant were scanned within the early follicular phase of their menstrual cycle or before the insemination or transfer in the fertility-treated group. Only participants who had never experienced a previous pregnancy beyond the first trimester were included in the study. Sixty-five nulliparous women and 56 nulliparous men were scanned for the first time point, including 43 women and 37 of their male partners who wanted to become parents for the first time, aiming for a minimum of 16 participants[51] in each group based on fertility statistics[52]. Pre-established exclusion criteria comprised neurological or psychiatric conditions or a history of substance use disorders as assessed by means of the MINI International Neuropsychiatric Interview[53] applied by a clinical psychologist. The main criterion for continuing in the study for participants in the PRG group was achieving pregnancy in the period following the first MRI session. Of the final sample of 25 women who underwent pregnancy in-between the sessions, the majority (20 women) had an estimated pregnancy onset within 6 months after the session. Five participants became pregnant between 6-12 months after

their participation in the first MRI session. To ensure that this relatively longer period between the session and conception did not have a significant impact on the results, we also repeated our analysis excluding these 5 women, which rendered very similar results (Supplementary Table 15). Thirty-two participants, comprising 17 women and 15 men, did not achieve pregnancy within this period and did not participate in the follow-up session. Two women and 2 men who were initially recruited for the F_{PRG} and M_{PRG} groups participated as control subjects in the F_{CTR} and M_{CTR} groups when conception was not achieved. In addition, 2 women and 2 men who participated in the first session as control participants were scanned as participants of the F_{PRG} and M_{PRG} groups in the second MRI session following an unexpected pregnancy. In addition, 1 participant became claustrophobic inside the scanner, 4 did not return for the POST session and 3 participants had to be excluded due to poor image quality or neuro-pathological conditions encountered in the MRI scan.

Our final sample consisted of the following subject groups with complete PRE&POST datasets: 25 primiparous women, 20 nulliparous control women, 19 primiparous men and 17 nulliparous control men. Unless explicitly stated otherwise (in case of analyses including other measures only available for a subset of the participants), these represent the sample sizes used in the comparisons. There were no statistically significant differences in PRE-to-POST time interval, age or level of education between the PRG and CTR groups (M±SD: PRE-POST Time Interval: M_{PRG}:459.00±117.46 days, M_{CTR}:419.17±93.17 days. T=1.12, p=0.272. F_{PRG}:463.52±108.33 days, F_{CTR}:413.05±106.86 days. T=1.56, p=0.126. Age: M_{PRG}:35.21±4.30 years, M_{CTR}:31.64±6.41 years. T=1.94, p=0.063. F_{PRG}:33.36±3.97 years, F_{CTR}:31.10±5.63 years. T=1.58, p=0.123. Education: number of participants finishing secondary school/college/university or above: M_{PRG}:2/4/13, M_{CTR}:1/3/13, X^2=0.37, p=0.833. F_{PRG}: 2/4/19, F_{CTR}: 2/3/15, X^2=0.06, p=0.971), but as there was a trend for an age difference in the male groups, we also repeated our model including age as a covariate (Supplementary Table 16-17), which had very little impact on the results. In addition, correlation analyses were performed to further examine potential associations of age and PRE-to-POST time interval with GM volume changes within the observed areas affected by pregnancy (using an explicit mask of the main contrast). These analyses rendered only a trend for stronger volume reductions in the right superior temporal sulcus cluster in the younger women (p=0.095 FWE-corrected).

The POST session took place on average at 73.56±47.83 (M±SD) days after parturition. A model including the time interval between the birth and the POST scan as a covariate rendered results that were highly similar to the main results (Supplementary Table 14). In addition, to further examine the effects of the time between parturition and the POST scan on the GM changes within these regions, we performed correlation analyses with this time interval using the main contrast as an explicit mask. These analyses rendered no significant results (either for a linear, quadratic or cubic positive or negative correlation).

Nine women achieved pregnancy by natural conception, and 16 women by means of a fertility treatment. The effect of a natural or assisted conception was further investigated by comparing these groups (Supplementary Table 4) and by separately examining the changes within these groups (Fig. 2, Supplementary Fig. 4, Supplementary Table 5), revealing no significant impact of the natural versus assisted route to conception on the brain changes of pregnancy. Of the fertility-assisted group, 12 women underwent in-vitro fertilization (IVF, 3 involving an egg donation and 5 involving intra-cytoplasmic sperm injection (ICSI), 4 without egg donation or ICSI), 3 intra-uterine insemination (IUI), and 1 a frozen embryo transfer. Albeit negligible in comparison to the hormone surges of pregnancy itself, each of these procedures involves hormone treatment which took place after the PRE session (for IUI: gonadotropins (follicle-stimulating hormone, luteinizing hormone, chorionic gonadotropin, human menopausal gonadotropin) and progesterone. IVF and ICSI: the same plus a gonadotropin-releasing hormone analogue. Egg donation/embryo transfer: estrogens, progesterone, GnRH analogue). To further examine the possible effects of treatment-related hormone therapy, we also repeated these analyses with a more homogeneous group of fertility-assisted women undergoing a procedure with the same approach in terms of hormone therapy (i.e. only women undergoing 'classic' IVF or IVF involving ICSI, 9 in total). Again, no significant differences were observed between this group and the women who were not exposed to fertility treatment-related hormones (the naturally conceiving group) and similar brain changes were observed in these subgroups (Supplementary Table 18). Future studies involving a larger sample of women undergoing fertility treatments are likely to uncover more subtle changes related to the hormone therapy associated with fertility treatments.

Ten of the women carried a boy, and 11 of the women a girl. The remaining 4 women had twins (2 mixed twins, 1 male twins, 1 female twins). Considering the previously observed effects of fetal sex on

cognitive changes in pregnant women[54], we additionally compared the women carrying a boy to the women carrying a girl (excluding the women having twins). No differences in GM volume changes were observed between these groups.

One woman suffered from eclampsia during labor, 2 had premature deliveries, and 2 women suffered from high-risk pregnancies with kidney complications or antiphospholipid syndrome. Leaving out the women with complications during pregnancy or delivery had very little effect on our results (Supplementary Table 19). Twenty of the experimental women gave birth to a singleton and four of the women had twins. Regarding the parturition, 8 of the women gave birth by cesarean section and 17 by vaginal birth. All women except one received epidural anesthesia during delivery. Sixteen women practiced exclusive breastfeeding (i.e. breastmilk as their infant's sole source of nutrition), 2 women practiced combined breastfeeding (supplemented by formula feedings), 2 women had started breastfeeding their infants but had stopped at the moment of the POST scan, and 2 women had never started breastfeeding. Very similar results were obtained when including variables representing the type of conception, type of delivery, breastfeeding status and number of fetuses as covariates in the model (Supplementary Table 20), suggesting that these factors are not driving the observed neural changes. However, the current study was not designed to further investigate the possible impact of such factors, and future studies investigating these in more detail may reveal specific neural changes associated with these variables.

In the POST session, the Edinburgh Postnatal Depression Scale[55] was administered to the primiparous women to detect symptoms of postpartum depression. One of the mothers showed symptoms of postpartum depression and was being helped by a specialist. Excluding this participant from our analyses did not significantly affect our results (Supplementary Table 21).

Blood samples were acquired at the sessions before and after pregnancy from a large portion of our participants. Unfortunately, due to practical issues, we could only obtain blood samples of 2 of the women during pregnancy itself. Therefore, we cannot use hormonal data to pinpoint the observed neural changes to specific endocrine changes of pregnancy.

For the POST+2yrs session, we asked the 25 primiparous women to come back for another MRI acquisition. Of these 25 women, 11 had not yet experienced a (partial) second pregnancy since the last

MRI session and were willing and able to participate in this follow-up session (mean time since birth: M±SD: 2.32±0.50 years, age (at PRE scan): 33.72±3.32 years).

The study was approved by the local ethics committee (Comitè Ètic d'Investigació Clinica de l'Institut Municipal d'Assistència Sanitària), and written informed consent was obtained from all subjects prior to their participation in the study.

Data acquisition

MRI images were obtained in a Philips 3T scanner. High-resolution anatomical MRI brain scans were acquired using a T1-weighted gradient echo pulse sequence (TR=8.2ms, TE=3.7ms, NSA=1, matrix=256x256, FOV=240mm, 180 slices, thickness=1mm, no gap, FA 8°). Due to an unexpected technical problem, the radiofrequency head coil was replaced for some time with another head coil, and 28 scans in total were acquired using the latter coil. There were no significant differences between the groups in the number of scan acquired with this head coil (X^2=4.21, p=0.240). Nonetheless, to err on the side of caution, we also repeated the main analysis without these scans acquired with the temporary head coil, which rendered highly similar results (Supplementary Table 22). Furthermore, direct comparisons of the subjects acquired with the different head coils were performed, rendering no significant results. Finally, the head coil was introduced as a nuisance covariate in all neuroimaging analyses. In the POST+2yrs session, an MRI scan was acquired with both radiofrequency head coils for those participants for whom a different coil was used in a previous acquisition, allowing us to match the comparisons on head coil type. Therefore, no covariate for the head coil was included for analyses involving the POST+2yrs session.

The POST MRI session also included an fMRI paradigm (T2*-weighted gradient echo EPI sequence. TR=3000ms, TE=35ms, matrix=128x128, FOV=230mm, 30 slices, thickness=4mm, gap 0.5mm, FA 90°) that examined the new mothers' neural response to their babies. During this MRI session, pictures of the women's own and other unknown babies were shown to the participants using Presentation software (www.neurobs.com). The images were extracted using Adobe Photoshop CS5 from short movies that were shot by one of the experimenters, or in some cases by the father, at a home visit a few days before

the POST session. For the women who had twins, movies were acquired from both babies. The pictures represented cut out faces on a black background and they were matched for size, resolution, brightness and facial expression. For each participant, 28 images of their own baby (14 of each infant in case of twins) and 28 images of other babies were available, and they were presented in randomized order in an event-related fashion (trial duration 1500ms, randomized inter-trial interval 750-1250ms), with an average number of trials of (M±SD) 72.15±6.64 and 72.40±6.99 for the baby and own baby conditions respectively. Pictures involving sad facial expressions (crying) and neutral facial expressions were acquired from each infant. Additional explorations of the data based on facial expression are provided in Supplementary Table 23. Five participants could not be included in the fMRI analyses due to head motion exceeding 3mm/degrees (1 woman), artefacts in the data (2 women), or incomplete datasets (2 women), rendering a sample of 20 primiparous women for this part of the study (Age (at PRE session): 32.85±4.13).

At the PRE and POST session, our participants were also asked to complete several supplementary cognitive tests and questionnaires (the Test de Aprendizaje Verbal Espana Complutense[56], based on the California Verbal Learning test[57], the Digits subtest of the Wechsler Adult Intelligence Scale III[58], a 2-back working memory test, the Interpersonal Reactivity Index[59], and a simple reaction time task). Normality of these variables was assessed by Shapiro-Wilk tests, and nonparametric tests were applied as some did not follow a normal distribution. Homoscedasticity was confirmed using a nonparametric Levene's test. No significant changes across sessions were observed in any of these measures (Supplementary Table 10). For completeness, a correlation analysis was performed between the number of correct responses on the verbal word learning paradigm (POST-PRE scores) and the changes in GM volume in the women who underwent pregnancy between sessions. No supra-threshold voxels were observed, either with a whole-brain approach or with an explicit mask representing the areas of GM volume change across pregnancy.

The women were also asked to retrospectively fill in the Maternal Postnatal Attachment Scale (MPAS)[24] for the first 6 months of being a mother. One of the mothers did not complete this measure, hence this data is available for 24 of the primiparous women (Age (at PRE scan): 33.42±4.05). From this scale, the three scores of the MPAS were extracted (M±SD. Quality of Attachment: 37.11±3.99. Absence of Hostility: 16.93±4.10. Pleasure in Interaction: 20.88±3.10).

Data analyses

Longitudinal Symmetric Diffeomorphic Modeling

The anatomical MRI images were processed in SPM12 (http://www.fil.ion.ucl.ac.uk/spm/), implemented in Matlab 7.8 (www.mathworks.com), using the longitudinal symmetric diffeomorphic modeling pipeline[60]. The images of each participant were first processed using the longitudinal registration tool provided within this framework, which incorporates rigid-body registration, intensity inhomogeneity correction and non-linear diffeomorphic registration in an interleaved fashion. Considering the bias associated with asymmetry in pairwise registration, this approach registers both time points to a within-subject average image. These midpoint average images were segmented into tissue classes using the unified segmentation algorithm[61]. The jacobian determinants resulting from the longitudinal registration were subsequently multiplied by each subject's grey matter segment, creating maps of volumetric change in grey matter tissue. To bring these images into MNI space, the product images were normalized using DARTEL tools[62] and smoothed with a 12mm full width half maximum smoothing kernel[63-65]. The individual smoothed GM volume difference maps were entered into general linear models.

To quantify the overlap between our results and other functional maps such as the neural activity of the mothers in response to pictures of their infant (the 'own baby>baby' contrast of the fMRI paradigm) and the Theory of Mind network as defined by the large-scale meta-analysis of Schurz et al.[22], we computed the intersection between these maps and our map of GM volume changes across pregnancy. A further assessment of the localization of these GM changes of pregnancy with respect to functional networks was performed by quantifying the overlap between our map and the 12 functional networks of Yeo et al.[23], who investigated the functional specialization of the cerebral cortex with a meta-analysis of 10,449 experimental contrasts and confirmed intrinsic network organization using a resting-state fMRI dataset of 1000 individuals. The overlap of our results with these functional maps was extracted by computing the intersections between each of these maps and the map of GM volume changes of pregnancy, and defining the fraction of the observed intersection relative to the expected volume of the

intersection based on a random distribution across the grey matter of the brain (see Supplementary Table 6).

For completeness, although these MRI images are not optimal for investigating white matter, we have additionally multiplied the individual jacobian difference maps with the white matter segments of the midpoint average images in order to obtain an indication of the changes in white matter signal across pregnancy. These maps were further processed and analyzed in the same manner as the images obtained by multiplying the jacobian maps with the grey matter segments.

A cross-sectional voxel-based morphometric approach was additionally applied on the baseline images to confirm the absence of pre-existing baseline differences between the PRG and CTR groups. This approach included a segmentation of the baseline images using the unified segmentation algorithm[61], a DARTEL normalization of the GM segments[62] and the application of a 12mm full width half maximum smoothing kernel. A two-sample t-test was performed to test for the presence of baseline group differences. Plots depicting the signal values extracted from this approach are provided in Supplementary Figure 10.

The POST2 images were processed using the same longitudinal approach described above, rendering volume difference maps between the POST2 images and the 2 other sessions. To examine whether GM volumes within the regions affected by pregnancy underwent further changes across the first 2 years postpartum relative to the pre-pregnancy and early postpartum sessions, we performed one-sample t-tests on the PRE-POST+2yrs and POST-POST+2yrs difference maps, using an explicit mask of the PRE-POST changes across pregnancy.

Regarding the main comparisons, each of the primiparous groups was first compared to their nulliparous control group in the framework of the general linear model. Maps of GM volume change were compared using two-sample t-tests. If a significant group difference was found, we then proceeded to separately examine the in- and decreases in GM volume across time within the relevant groups by means of one-sample t-tests to determine which changes were driving this group difference.

The statistical maps were constructed by applying a stringent voxel-level Gaussian Random Field-based threshold of $p<0.05$ FWE-corrected across the whole brain. A minimum cluster size of 10 contiguous voxels was additionally used to discard very small clusters and restrict table sizes.

Multivariate analyses

In addition to the above-described mass-univariate analyses, we also performed multivariate pattern recognition analyses using the analysis pipeline provided by PRoNTo 2.0[66] (http://www.mlnl.cs.ucl.ac.uk/pronto) implemented in Matlab. This pipeline can be used to automatically search for regularities in the data and train a classifier function that models the relation between spatial signal patterns and experimental factors based on a training dataset[66]. This classifier can then be used to predict the group a new image belongs to using the spatial distribution of the signal within the image, and compute the accuracy with which groups can be discriminated from one another based on whole-brain spatial signal patterns.

To examine the degree to which the experimental women could be discriminated from the control women based on the distribution of GM volume changes across the brain, we applied a linear support vector machine classification to the POST-PRE difference maps. A sample-specific GM mask was created using the SPM Masking Toolbox (http://www0.cs.ucl.ac.uk/staff/g.ridgway/masking/) to serve as the mask image. To evaluate model performance, we applied a leave-one-out cross-validation scheme. Using this cross-validation strategy, the classifier's accuracy is computed by leaving one subject out at a time and predicting this subject's group label based on a training set including all remaining subjects. This procedure is then repeated for each subject, and the accuracy of the discriminant function is computed based on all these runs. Permutation testing was used to estimate the null distribution and examine the statistical significance of the classification accuracy ($N_{permutations}$ = 10,000; $p<0.05$)[67].

In addition, to further examine the regional contribution to the decision function and determine the areas with greatest relative prediction power, we built multiple kernels based on the regions of the Automated Anatomical Labeling Atlas (http://www.gin.cnrs.fr/AAL-217), using an L1 Multiple Kernel Learning algorithm as implemented in PRoNTo[68] with the same cross-validation scheme and significance testing.

Furthermore, to investigate whether the GM volume changes across pregnancy could significantly predict measures of maternal attachment, we performed Kernel Ridge Regression analyses using the 3 dimensions of the MPAS[24]. Kernel Ridge Regression represents a form of Support Vector Regression

using a squared error loss function combined with l2 regularization. Please see Shawe-Taylor et al. for a description of this approach[69]. Using Kernel Ridge Regression, MPAS scores were predicted from the changes in GM volume, and the correlation between true and predicted MPAS values was subsequently examined. A leave-one-out cross-validation was applied, i.e. in every fold a participant was left out for whom the MPAS score was predicted and examined in relation to the actual MPAS score. Like in the classification models, we used a leave-one-out cross-validation scheme and permutation testing ($N_{permutations}$ = 10,000; $p<0.05$).

Since the current version of PRoNTo does not yet allow the inclusion of covariates and we could therefore not include the radiofrequency coil covariate in the models, the residuals were written in SPM12 and the multivariate classification and regression analyses were repeated on these images, rendering very similar results (SVM classification on residuals: balanced accuracy = 100%, $p<0.0001$; kernel ridge regression with MPAS scores on residuals: quality of attachment: $R=0.38$, $p=0.043$, normalized mean squared error (nMSE)=0.90, $p_{nMSE}=0.034$; absence of hostility: $R=0.42$, $p=0.031$, nMSE=0.87, $p_{nMSE}=0.023$).

Additional measures based on previous related results: Manual Regions of Interest of the pituitary gland and total tissue volumes

As a supplementary analysis, we wanted to further explore our data based on the previous findings related to structural brain changes in human pregnancy. Therefore, we also examined total tissue volumes and pituitary gland volume in our sample.

Total brain volumes were extracted in SPM12. To obtain measures of pituitary gland volume, this structure was manually delineated by 2 raters who were blind to any identifying subject or group information on coronal slices of the PRE, POST and POST+2yrs sessions of the primiparous women using MRIcroN (http://www.mccauslandcenter.sc.edu/mricro/mricron), according to the delineation criteria described in MacMaster et al[70]. Inter-rater reliability was determined based on 10 repeated ROI delineations (Intraclass Correlation Coefficient: 0.935).

For 5 MRI scans, the pituitary gland could not reliably be delineated due to local inhomogeneity or contrast issues, and these scans were therefore excluded, rendering a total number of 23 PRE, 23 POST and 10 POST+2yrs volumes (as the missing volumes did not correspond to the same individuals across sessions, we could use 22 PRE-POST pairs, 9 PRE-POST+2yrs pairs and 10 POST-POST+2yrs pairs for the longitudinal comparisons).

These measures were analyzed in SPSS 23 (http://www.ibm.com/software/analytics/spss). A normal distribution of the data and equal variances were confirmed using Shapiro-Wilk and Levene's tests respectively. The results are described in the supplementary material (Supplementary Figure 3, Supplementary Table 2-3).

Surface-based analyses

To examine changes in surface area and cortical thickness, surface-based morphometry was conducted in FreeSurfer 5.3 (http://surfer.nmr.mgh.harvard.edu/). The images were re-processed from raw data for this approach. To investigate changes in these surface-based measures across sessions, the images were processed with the longitudinal stream implemented in FreeSurfer[71, 72]. The longitudinal preprocessing pipeline involves an initial cross-sectional processing of the images of each of the time points, which includes motion correction, removal of non-brain tissue, transformation into stereotaxic (MNI) space, intensity correction, volumetric segmentation and cortical surface reconstruction[73, 74], and parcellation[75]. The extraction of the brain for surface-based processing was based on the segmentation algorithm implemented in SPM8. All further steps were performed in FreeSurfer 5.3. Individual surfaces were inspected for accuracy, and minor manual edits were performed where needed, usually involving the removal of sections of non-brain tissue. The next step in the longitudinal stream was the creation of a probabilistic individual base template based on the cross-sectional images for each participant, which is unbiased with respect to any of the time points. Subsequent processing of each time point was then initialized using the processed results from the unbiased template[71, 72]. Surface maps were resampled, mapped to a common surface, and smoothed using a full width at half maximum kernel of 15mm. A

cross-sectional approach was additionally applied on the baseline images to confirm the absence of pre-existing baseline differences between the PRG and CTR groups.

Longitudinal change in cortical surface area and thickness in each hemisphere was calculated as symmetrized percent change (i.e. the rate of change between the time points with respect to the average thickness/area across the time points), and examined using one-sample t-tests. Cluster statistics were obtained using Monte Carlo simulations with a vertex-wise –log10(p-value) of 4 (corresponding to $p<0.0001$) and a cluster-wise threshold of $p<0.05$.

Discriminant analyses with leave-one-out cross-validation were performed in SPSS 23 (http://www.ibm.com/software/analytics/spss/) using the changes in average cortical thickness and surface area values across the regions of GM volume change in order to examine the predictive value of these surface-based measures for group classification.

Functional MRI analyses

Analyses of the functional MRI paradigm were performed in SPM12. The functional images were first corrected for differences in slice acquisition timing and realigned to the first volume. Subjects with head motion exceeding 3 mm/degrees were excluded from the analyses (1 woman). Then, the anatomical images were co-registered to the mean functional image, and normalized into MNI (ICBM) space using non-linear registration[61]. Finally, the normalization parameters and a full width at half maximum smoothing kernel of 12 mm were applied to the functional images.

At the first level of analysis, general linear models were used to model voxel-wise changes in BOLD response for the conditions of interest, also including the movement parameters extracted during the realignment and regressors based on temporal basis functions.The first-level parameter estimates for the linear contrast ‘own baby pictures>other baby pictures’ were entered into a second-level model and one-sample t-tests were performed to examine whether new mothers show a differential pattern of neural activity in response to pictures of their own or other babies. For completeness, the reverse contrast (‘other baby pictures>other baby pictures’) was also examined. Additional explorations of the data based on

facial expression are provided in Supplementary Table 23. In addition to the whole-brain FWE-corrected threshold, the fMRI results were also investigated and are reported at an uncorrected threshold of $p<0.0001$ and an extent threshold of 10 voxels to allow a further inspection of the similarity of the regions of strongest neural responsiveness to the women's babies to the pattern of GM volume changes across pregnancy.

To create images, the statistical maps were projected onto the PALS surface provided in Caret software (http://brainvis.wustl.edu/wiki/index.php/Caret:Download). Slice overlays were created using MRIcron (http://www.mccauslandcenter.sc.edu/mricro/mricron/).

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