

Pregnancy leads to long-lasting changes in human brain structure

Elseline Hoekzema^{1-3,8}, Erika Barba-Müller^{1,8}, Cristina Pozzobon⁴, Marisol Picado¹, Florencio Lucco⁴, David García-García⁵, Juan Carlos Soliva¹, Adolf Tobeña¹, Manuel Desco⁵, Eveline A Crone^{2,3}, Agustín Ballesteros⁴, Susanna Carmona^{1,5,6,9} & Oscar Vilarroya^{1,7,9}

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 gray 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.

Most women undergo pregnancy at least once in their lives, yet little is known of how this process affects the human brain. Mammalian pregnancy involves radical physiological and physical adaptations orchestrated by endocrine changes¹. During pregnancy, there are unparalleled surges of sex steroid hormones, including, for instance, an increase in progesterone of 10–15 fold relative to luteal phase levels and a flood of estrogens that typically exceeds the estrogen exposure of a woman's entire nonpregnant life². Sex steroid hormones are known to act as an important regulator of neuronal morphology and number³. 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 brain⁴⁻⁶, and neural alterations have also been observed in response to even subtle changes in endogenous or exogenous steroid hormone levels later in life⁷⁻⁹.

However, very little is known concerning the effects of pregnancy on the human brain. A few spectroscopic studies have been performed in pregnant women¹⁰⁻¹², observing no differences from nonpregnant 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 women¹³, 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-eclampsia¹⁶. These data pointed to increases in ventricular size and decreases in brain size during late pregnancy in comparison to the early postpartum period.

In nonhuman 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 expression¹⁷⁻²⁰. These effects seem to be long-lasting, as various differences in brain and behavior between parous and nulliparous females are evident throughout the lifespan¹⁷⁻²¹.

We performed a prospective (before versus after pregnancy) study involving primiparous (first-time) mothers and nulliparous control women to investigate whether pregnancy is associated with changes in the gray matter (GM) structure of the human brain. In addition, we (i) tested the discriminative power of the GM volume changes with a multivariate pattern recognition analysis, (ii) examined GM volume changes in first-time fathers and control men without children to further test the specificity of the changes for pregnancy rather than approaching parenthood, (iii) defined the structural characteristics of GM changes across pregnancy by means of surface-based analyses, (iv) investigated a potential link to maternal attachment using a postpartum fMRI task and attachment scale, and (v) tested the long-term persistence of pregnancy effects with a 2-year post-pregnancy follow-up session.

¹Unitat de Recerca en Neurociència Cognitiva, Departament de Psiquiatria i Medicina Legal, Universitat Autònoma de Barcelona, Barcelona, Spain. ²Brain and Development Laboratory, Leiden University, Leiden, the Netherlands. ³Leiden Institute for Brain and Cognition (LIBC), Leiden University, Leiden, the Netherlands.

⁴Instituto Valenciano de Infertilidad, Barcelona, Spain. ⁵Departamento de Bioingeniería e Ingeniería Aeroespacial, Universidad Carlos III de Madrid, Instituto de Investigación Sanitaria Gregorio Marañón, Madrid, Spain. ⁶Centro de Investigación Biomédica en Red de Salud Mental (CIBERSAM), Madrid, Spain. ⁷Fundació IMIM, Barcelona, Spain. ⁸These authors contributed equally to this work. ⁹These authors jointly supervised this work. Correspondence should be addressed to E.H. (elselinehoekzema@gmail.com).

Received 24 February; accepted 15 November; published online 19 December 2016; doi:10.1038/nn.4458

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 show a notable similarity to the theory-of-mind network. Notably, all of the women could be classified as having undergone pregnancy or not on the basis of 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 task. 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, we performed a prospective neuroimaging study. High resolution anatomical pre-conception brain scans were obtained from nulliparous women wanting to become mothers for the first time (the Pre session).

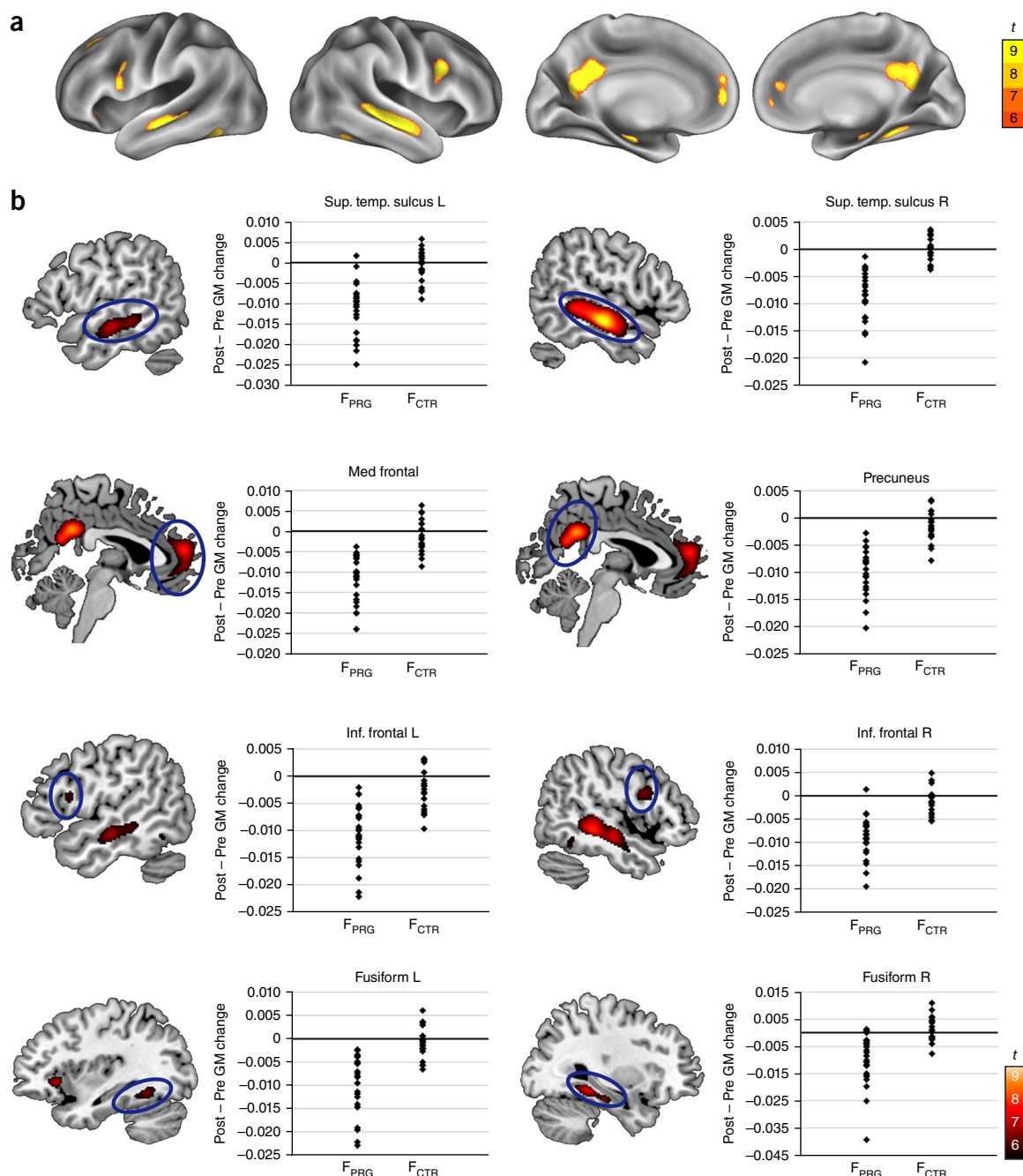


Figure 1 GM volume changes between pre-pregnancy and post-pregnancy session. (a) Surface maps of the GM volume changes in primiparous ($N = 25$) compared to nulliparous control women ($N = 20$) (at a whole-brain threshold of $P < 0.05$, family-wise error (FWE)-corrected). (b) Sagittal slice overlays 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 three remaining clusters. Statistics are reported in **Table 1**. F_{CTR}, nulliparous control women who were not pregnant between sessions; F_{PRG}, nulliparous women who became pregnant and gave birth between sessions. Sup. Temp., superior temporal; Med., medial; Inf., inferior; L, left; R, right.

Table 1 Changes in GM volume between the Pre and Post session

Contrasts		Regions	H	MNI coordinates				<i>t</i>	<i>P</i>	Cluster size (voxels)
				<i>x</i>	<i>y</i>	<i>z</i>				
F _{PRG} > F _{CTR}		–	–							
F _{CTR} > F _{PRG}	Superior temporal sulcus, middle and superior temporal gyrus, parahippocampal gyrus	R	57	–18	–11	8.84	<0.001	4,001		
			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	2,674		
			–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	1,828		
			–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 and superior frontal gyrus		L	–24	25	45	6.30	0.002	509		
Fusiform gyrus, 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 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 family-wise error-corrected) is reported. H, hemisphere; L, left; R, right; F_{CTR} , nulliparous control women who were not pregnant between sessions; F_{PRG} , nulliparous women who became pregnant and became first-time mothers between sessions; M_{PRG} , men whose partners became pregnant and who became first-time fathers in-between sessions; M_{CTR} , men without children whose partners were not pregnant between sessions.

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 on the sample is provided in the Online Methods.

The longitudinal diffeomorphic modeling pipeline implemented in SPM12 was applied to extract changes in gray 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. We observed a symmetrical pattern of highly significant group differences in GM volume change across sessions (**Fig. 1**, **Table 1** and **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**). 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 (extending from the medial frontal cortex to the anterior cingulate cortex and from the precuneus to the 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, we also examined white matter volume 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 **Supplementary Figure 3** and **Supplementary Tables 2** and **3**.

Means of conception

As our sample included both women who achieved pregnancy by natural conception and women who underwent a fertility treatment (see Online Methods), we 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 Fig. 4** and **Supplementary Table 5**), 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 Online Methods.

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. This analysis showed that all of the women could be correctly classified as having been pregnant or not in between these sessions on the basis of the GM changes in the brain (**Fig. 3a,b**).

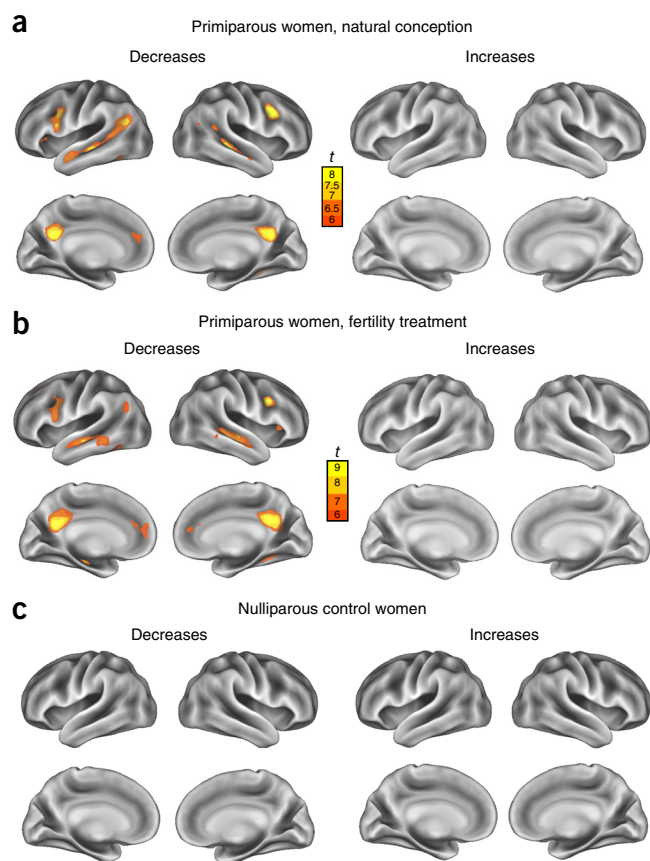


Figure 2 Means of conception. Surface maps of GM volume changes between the Pre and Post session ($P < 0.05$, FWE-corrected) in (a) the primiparous women achieving pregnancy by natural conception ($N = 9$), (b) primiparous women achieving pregnancy by fertility treatment ($N = 16$), and (c) the nulliparous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

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

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 three-fold larger volume of overlap than expected based on a random distribution of the maps across the brain's GM (**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 are related to various task variables, the three cognitive components of

greatest overlap with the GM changes of pregnancy corresponded to the three 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 showed a greater overlap with the GM changes of pregnancy than expected based on a random distribution across the brain's GM tissue corresponded to those three networks that are recruited by theory-of-mind tasks.

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 also scanned first-time fathers before and after their partner's pregnancy, along with a male control group who remained childless. Maps of GM volume change were extracted using the SPM12 longitudinal diffeomorphic modeling pipeline. Comparisons involving these groups showed that there were no changes in neural GM volumes in the fathers in comparison to the control group across this time period; the observed brain changes were selective for the women undergoing pregnancy between the brain scans (**Table 1**, **Supplementary Fig. 6** and **Supplementary Table 7**).

Changes in surface area and cortical thickness across pregnancy

To examine the structural characteristics of neural GM changes across pregnancy, we performed surface-based analyses in FreeSurfer 5.3. Cross-sectional analyses confirmed the lack of baseline differences between the women who underwent pregnancy in between sessions and those who did not. 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** and **Supplementary Table 8 and 9**). There were changes in both 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** and **Supplementary Tables 8 and 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 on the basis of the changes in surface area ($\lambda = 0.66$, $\chi^2 = 17.69$, $P < 0.001$), while 68.9% could be classified on the basis of 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 were 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, $R = 0.91$, $P < 0.001$).

Changes in cognitive performance across pregnancy

We conducted several cognitive tests 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 two-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

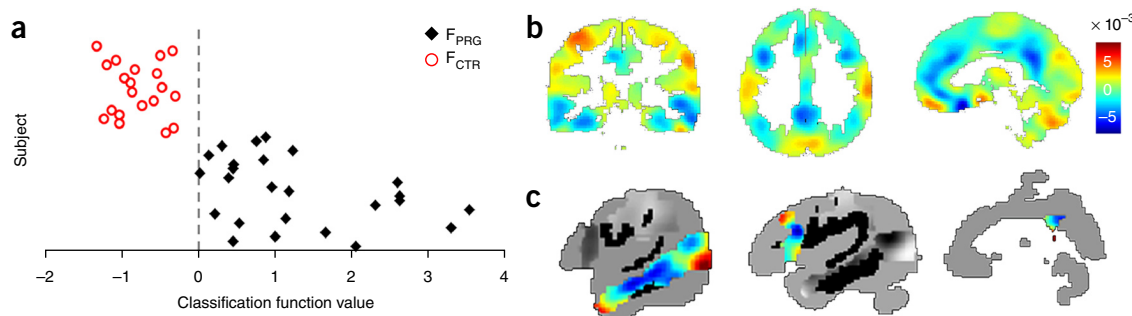


Figure 3 Classification. (a) Scatter plot depicting the support vector machine classification results. Function values (mean \pm s.d.): F_{PRG} : 1.27 ± 1.03 , F_{CTL} : -0.79 ± 0.33 . Balanced accuracy: 100%, although some participants are close to the decision function border; dashed line is the function value cut-off between classes (0), leave-one-out cross-validation, $N_{\text{permutations}} = 10,000$, $P \leq 0.0001$. Function values are plotted per fold (i.e., in this case, per subject). (b) Weight map for the classifier, depicting the relative contribution of the voxel to the decision function. (c) Weight maps for the regions of greatest predictive power resulting from the multiple-kernel learning model using the Automated Anatomical Labeling (AAL) atlas (balanced accuracy: 93.5%, leave-one-out cross-validation, $N_{\text{permutations}} = 10,000$, $P \leq 0.0001$). These are (depicted from left to right) 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_{CTL} , nulliparous control women who were not pregnant between sessions; F_{PRG} , nulliparous women who became pregnant and gave birth between sessions.

the number of correct responses on the verbal word list learning task (Supplementary Table 10).

Multivariate regression analyses with the 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 three dimensions of the Maternal Postnatal Attachment Scale²⁴. These analyses indicated that the GM volume changes of pregnancy significantly predicted the quality of mother-to-infant attachment and the absence of hostility toward their newborns in the postpartum period as defined by this scale (Fig. 6b and Supplementary Figs. 7 and 8).

Neural activity on an fMRI task 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 task

involving baby pictures. In this task, 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 tasks involving one's own and other infant pictures and sounds have previously been used as a neural index of parental attachment²⁵. 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 lost GM volume across pregnancy (Fig. 6a and 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 responded more to the mothers' own infants than to other infants were located in GM tissues that lost volume across pregnancy (Supplementary Fig. 9 and Supplementary Table 6). This represents a nearly sevenfold greater overlap than expected based on a random distribution of these maps across the brain's gray 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 also investigated separately to confirm

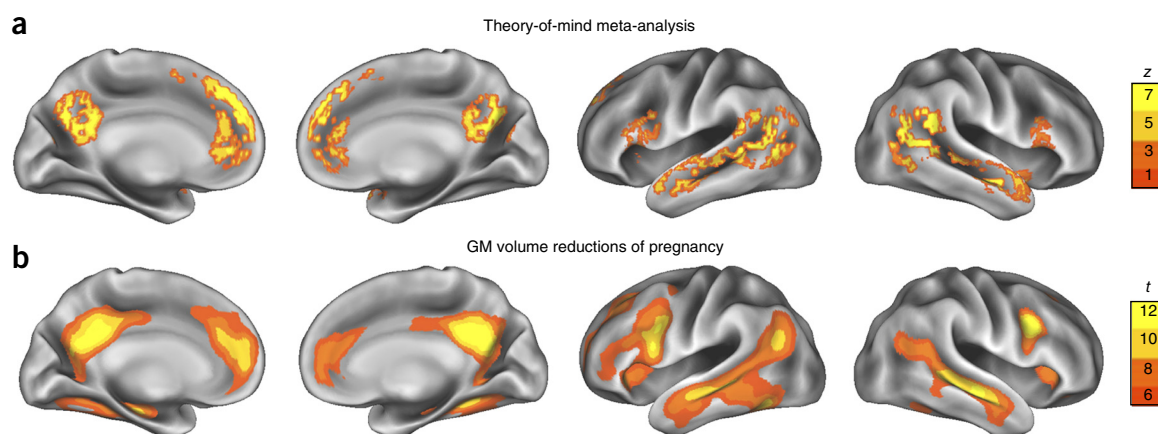


Figure 4 Similarity between theory-of-mind network and GM volume changes of pregnancy. (a) The theory-of-mind network as extracted from the meta-analysis by Schurz *et al.*²². Statistical map of permutation-based z-values of the pooled meta-analysis was provided by Schurz *et al.*²² and displayed using Caret software. (b) Reductions in GM volume ($P < 0.05$, FWE-corrected) in the group of women who were pregnant between sessions in the current study.

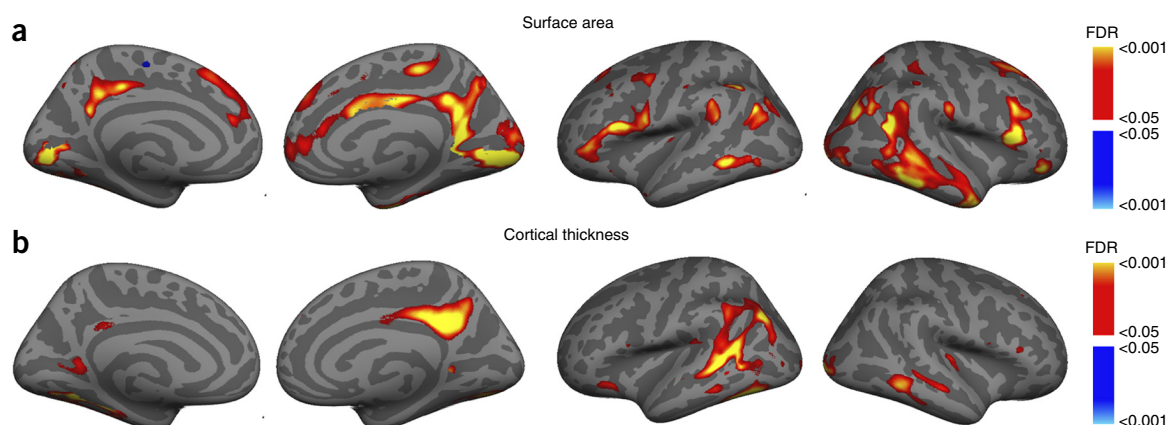


Figure 5 Surface-based measures. Surface maps depicting changes in (a) surface area and (b) cortical thickness across pregnancy (false discovery rate (FDR)-corrected $P < 0.05$). Blue and cyan reflect increases while red and yellow reflect decreases.

the recruitment of typical networks for visual perception and face processing in both conditions (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 (mean \pm s.d.: 2.32 ± 0.50 years postpartum; 'Post + 2 years' session). Eleven of the mothers had not yet experienced a second pregnancy and were able and willing to return for this

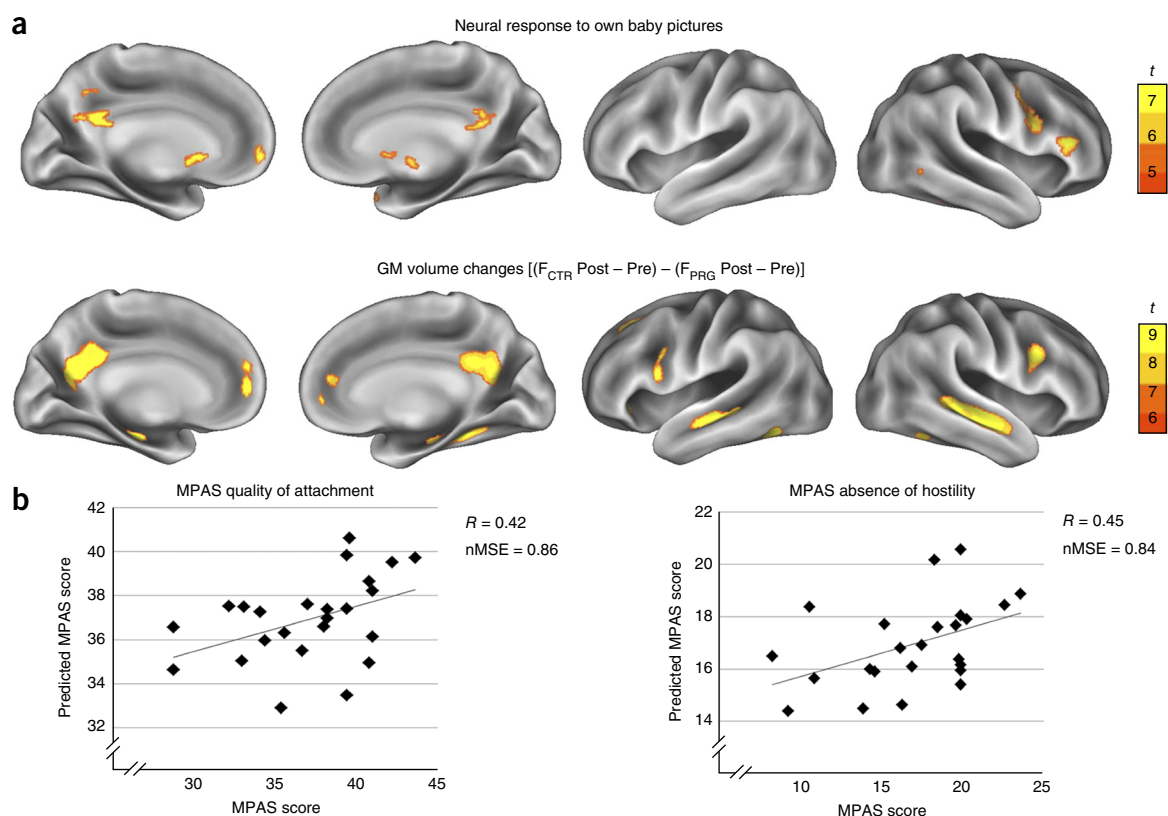


Figure 6 Postpartum infant-related neural activity and attachment scores. (a) fMRI results for the 'own > other baby' contrast ($N = 20$) alongside GM volume changes repeated from Figure 1. For illustrative purposes, the fMRI results are depicted at the more lenient threshold of $P < 0.0001$ uncorrected (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 MPAS scores ($N_{\text{permutations}} = 10,000$). Quality of attachment: mean \pm s.d. = 37.11 ± 3.99 . $P = 0.030$, $p_{nMSE} = 0.024$. Absence of hostility: mean \pm s.d. = 16.93 ± 4.10 . $P = 0.026$, $p_{nMSE} = 0.021$. Pleasure in interaction: mean \pm s.d. = 20.88 ± 3.10 . $P = 0.985$, $p_{nMSE} = 0.918$). Predicted versus actual MPAS scores are plotted for the two MPAS scores that were found to be associated with the GM volume changes; nMSE, normalized mean squared error.

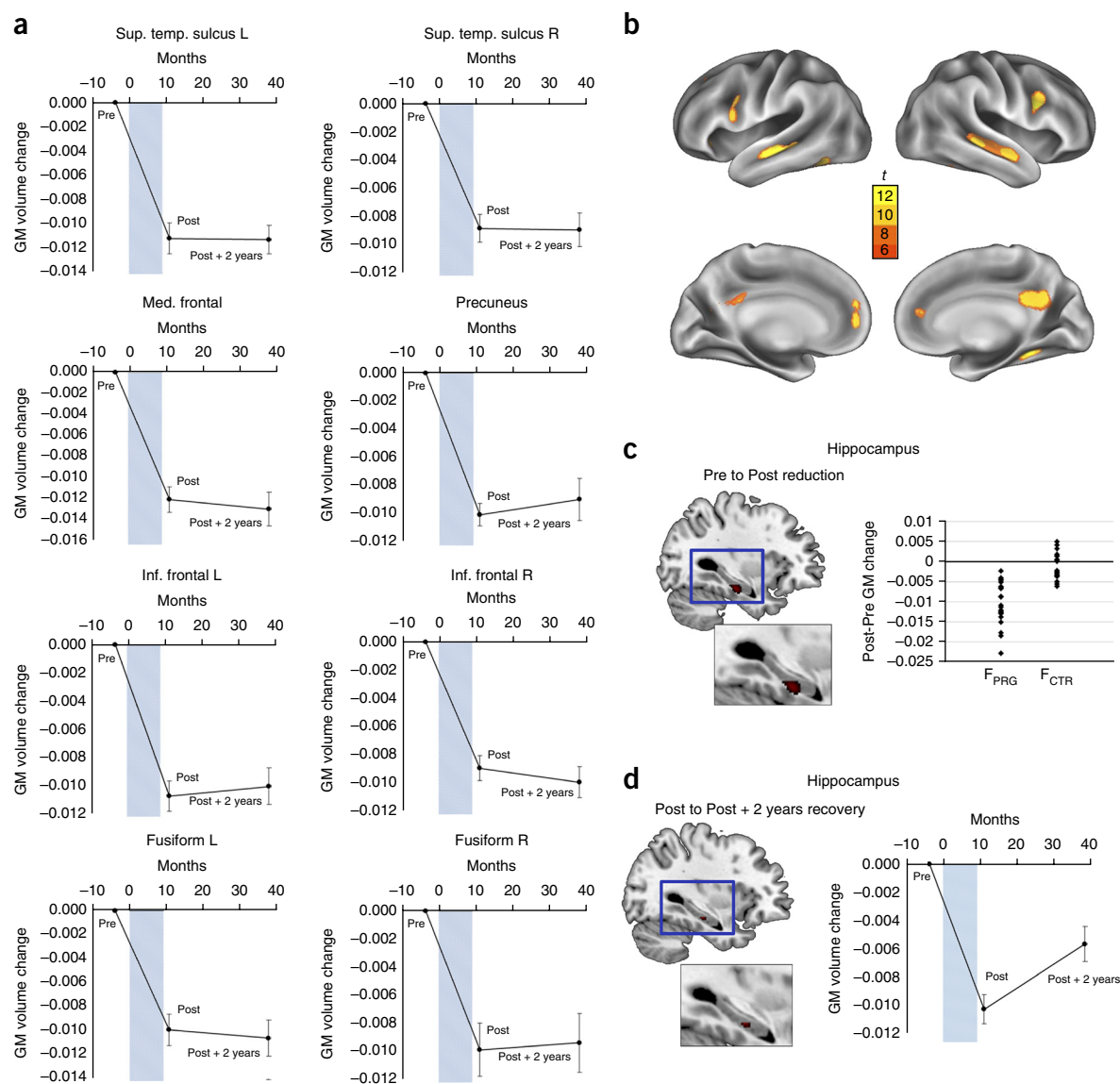


Figure 7 Long-term follow-up. (a) Mean (\pm s.e.m.) signal change at each Post session relative to the Pre baseline, extracted from the smoothed normalized jacobian difference images. The remaining clusters are plotted in **Supplementary Figure 1**. (b) Surface maps depicting GM volume reductions in the Post + 2 years session compared to the Pre baseline ($P < 0.05$ FWE-corrected). Complete Pre, Post and Post + 2 years data sets were available of 11 women. (c) 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 versus Pre comparison. (d) Plot (mean \pm s.e.m.) and sagittal overlay depicting hippocampal recovery from the Post to the Post + 2 years session. Statistics are reported in **Supplementary Table 13**. Sup. Temp., superior temporal; Inf., inferior; Med., medial; L, left; R, right. F_{CTR}, nulliparous control women who were not pregnant between sessions; F_{PRG}, nulliparous women who became pregnant and gave birth between sessions.

follow-up session. When examining the brain changes between this Post + 2 years 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 and **Supplementary Table 13**), except for the left hippocampal cluster (Fig. 7c,d and **Supplementary Table 13**). Furthermore, when investigating the changes in GM volume between the Post and Post + 2 years sessions, we observed no further reductions or increases within these structures except for a selective volume recovery in the left hippocampal cluster (Fig. 7 and **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.

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 between the MRI sessions on the basis of the GM volume changes in the brain. Analyses involving first-time 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.

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, 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 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 notable 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 to 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 that multiple processes are likely to be affected.

On the basis of our results, we may 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}. In accordance with these findings, the notion of gestational adaptations in social cognition has previously been proposed from an evolutionary perspective³⁰.

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 offspring in a complex social environment such as ours. Accordingly, the theory-of-mind system is considered a core component of the human parental brain²⁵, and a mother's ability to comment accurately on her infant's mental states and processes has been shown to be important for secure parent–infant attachment and for the development of the child's own social cognitive functions³². 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. Multivariate regression analyses using the three dimensions of the Maternal Postnatal Attachment Scale²⁴ demonstrated that the GM volume changes of pregnancy significantly predicted quality of mother-to-infant attachment and the absence of hostility toward 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 also performed surface-based analyses. These analyses revealed reductions in both cortical thickness and surface area across pregnancy, with the surface area of the cortical mantle 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 development³³, while rapid GM atrophy as seen in, for example, Alzheimer's disease, AIDS or multiple sclerosis is almost exclusively driven by cortical thinning^{34–36}. Furthermore, 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. 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* or *ex vivo* results obtained from animal studies, it should be noted that the hippocampus has been extensively investigated in rodents in relation to reproductive experience and shows a remarkable plasticity across pregnancy and the postpartum period³⁸. 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 toward reduced cell proliferation has been observed in late pregnancy in rats⁴¹, and reproductive

experience has consistently been associated with reduced hippocampal cell proliferation in the postpartum period^{17,19–20,38,42}. Neurogenesis seems to be restored to baseline by the time of weaning and may reverse to increased levels in middle age, when reproductive experience is associated with estrogen-dependent increases in hippocampal cell proliferation^{19–21,38,43}. Reductions and subsequent increases in neurogenesis can also be hypothesized to contribute to some of the observed hippocampal volume change 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 pregnancy⁴⁴ and in lactating primiparous rats in the postpartum period in comparison to nulliparous females⁴⁵. 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 may speculate that the hippocampal GM reductions and subsequent +2 year postpartum partial volume recovery observed in our study contribute to the memory deficits often associated with human pregnancy^{46,47}, which have been found to be recovering at 2 years postpartum⁴⁸. Previous studies have indicated that verbal recall memory in particular is diminished during pregnancy⁴⁶. 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, we observed no significant changes in memory performance in the women who underwent pregnancy between sessions in comparison to women who did not. However, we can draw no conclusions 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 number³, and changes in endogenous or exogenous levels of these hormones are known to affect human brain structure and function^{4–9}. Considering the unequalled surges of sex steroid hormones that a woman is exposed to during her pregnancy and the 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, might play a role. In addition, although pregnancy comprised 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, 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 study⁵⁰. In that study, women were investigated in their early 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 before the second MRI acquisition. Notably, no neural volume reductions were observed across this period⁵⁰. 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, to our knowledge, the first insights into the profound impact of pregnancy on the gray matter architecture of the human brain.

METHODS

Methods, including statements of data availability and any associated accession codes and references, are available in the [online version of the paper](#).

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

ACKNOWLEDGMENTS

We acknowledge the participants for their contribution to this study. We thank A. Bulbena for supporting the project, and M. López, G. Pons, R. Martínez, L. González, E. Castaño, N. Mallorquí-Bagué, J. Fauquet and C. Pretus for helping with the data collection and scoring of the cognitive tests. In addition, we thank C. Phillips and J.D. Gisbert for advice on the multivariate analyses, E. Marinetto and C. Falcón for advice on the FreeSurfer analyses, and J. van Hemmen and J. Bakker for discussions of the project and results. E.H. was supported by a Formación de Profesorado Universitario (FPU) grant by the Ministerio de Educación y Ciencia, Spanish government, and is now supported by an Innovational Research Incentives Scheme grant (Veni, 451-14-036) of the Netherlands Organization for Scientific Research (NWO). E.B.-M. by a grant from the National Council of Science and Technology of Mexico, S.C. by the Consejería de Educación, Juventud y Deporte of Comunidad de Madrid and the People Programme (Marie Curie Actions) of the European Union's Seventh Framework Programme (FP7/2007-2013) under REA grant agreement 291820, and M.P. by an FI grant of the Agència de Gestió d'Ajuts Universitaris de Recerca, Generalitat de Catalunya.

AUTHOR 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. E.H. analyzed the data, except for the area and thickness 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.

Reprints and permissions information is available online at <http://www.nature.com/reprints/index.html>.

1. Brunton, P.J. & Russell, J.A. The expectant brain: adapting for motherhood. *Nat. Rev. Neurosci.* **9**, 11–25 (2008).
2. Casey, M.L., MacDonald, P.C., Sargent, I.L. & Starkey, P.M. Placental endocrinology. in *The Human Placenta* (ed. Redman, C.W.G.) 237–272 (Blackwell Scientific, Oxford, 1993).
3. Simerly, R.B. Wired for reproduction: organization and development of sexually dimorphic circuits in the mammalian forebrain. *Annu. Rev. Neurosci.* **25**, 507–536 (2002).
4. Peper, J.S., Hulshoff Pol, H.E., Crone, E.A. & van Honk, J. Sex steroids and brain structure in pubertal boys and girls: a mini-review of neuroimaging studies. *Neuroscience* **191**, 28–37 (2011).

5. Sisk, C.L. & Foster, D.L. The neural basis of puberty and adolescence. *Nat. Neurosci.* **7**, 1040–1047 (2004).
6. Sisk, C.L. & Zehr, J.L. Pubertal hormones organize the adolescent brain and behavior. *Front. Neuroendocrinol.* **26**, 163–174 (2005).
7. Comasco, E., Frokjaer, V.G. & Sundström-Poromaa, I. Functional and molecular neuroimaging of menopause and hormone replacement therapy. *Front. Neurosci.* **8**, 388 (2014).
8. Craig, M.C. & Murphy, D.G. Estrogen: effects on normal brain function and neuropsychiatric disorders. *Climacteric* **10** (Suppl. 2), 97–104 (2007).
9. Toffoletto, S., Lanzemberger, R., Gingnell, M., Sundström-Poromaa, I. & Comasco, E. Emotional and cognitive functional imaging of estrogen and progesterone effects in the female human brain: a systematic review. *Psychoneuroendocrinology* **50**, 28–52 (2014).
10. Holdcroft, A. *et al.* Phosphorus-31 brain MR spectroscopy in women during and after pregnancy compared with nonpregnant control subjects. *AJNR Am. J. Neuroradiol.* **26**, 352–356 (2005).
11. Roos, A., Robertson, F., Lochner, C., Vythilingum, B. & Stein, D.J. Altered prefrontal cortical function during processing of fear-relevant stimuli in pregnancy. *Behav. Brain Res.* **222**, 200–205 (2011).
12. Rutherford, J.M., Moody, A., Crawshaw, S. & Rubin, P.C. Magnetic resonance spectroscopy in pre-eclampsia: evidence of cerebral ischaemia. *BJOG* **110**, 416–423 (2003).
13. Erdheim, J. & Stumme, E. Über die Schwangerschaftsveränderung der Hypophyse. *Ziegler's. Beitr. Pathol. Anat.* **45**, 1–17 (1909).
14. Bergland, R.M., Ray, B.S. & Torack, R.M. Anatomical variations in the pituitary gland and adjacent structures in 225 human autopsy cases. *J. Neurosurg.* **28**, 93–99 (1968).
15. Gonzalez, J.G. *et al.* Pituitary gland growth during normal pregnancy: an in vivo study using magnetic resonance imaging. *Am. J. Med.* **85**, 217–220 (1988).
16. Oatridge, A. *et al.* Change in brain size during and after pregnancy: study in healthy women and women with preeclampsia. *AJNR Am. J. Neuroradiol.* **23**, 19–26 (2002).
17. Hiller, K.M., Jacobs, V.R., Fischer, T. & Aigner, L. The maternal brain: an organ with peripartur plasticity. *Neural Plast.* **2014**, 574159 (2014).
18. Kinsley, C.H. & Amory-Meyer, E. Why the maternal brain? *J. Neuroendocrinol.* **23**, 974–983 (2011).
19. Kinsley, C.H., Meyer, E. & Rafferty, K.A. Sex steroid hormone determination of the maternal brain: effects beyond reproduction. *Mini Rev. Med. Chem.* **12**, 1063–1070 (2012).
20. Macbeth, A.H. & Luine, V.N. Changes in anxiety and cognition due to reproductive experience: a review of data from rodent and human mothers. *Neurosci. Biobehav. Rev.* **34**, 452–467 (2010).
21. Kinsley, C.H., Franssen, R.A. & Meyer, E.A. Reproductive experience may positively adjust the trajectory of senescence. *Curr. Top. Behav. Neurosci.* **10**, 317–345 (2012).
22. Schurz, M., Radua, J., Aichhorn, M., Richlan, F. & Perner, J. Fractionating theory of mind: a meta-analysis of functional brain imaging studies. *Neurosci. Biobehav. Rev.* **42**, 9–34 (2014).
23. Yeo, B.T. *et al.* The organization of the human cerebral cortex estimated by intrinsic functional connectivity. *J. Neurophysiol.* **106**, 1125–1165 (2011).
24. Condon, J. & Corkindale, C. The assessment of parent-to-infant attachment: development of a self-report questionnaire instrument. *J. Reprod. Infant Psychol.* **16**, 57–76 (1998).
25. Swain, J.E. *et al.* Approaching the biology of human parental attachment: brain imaging, oxytocin and coordinated assessments of mothers and fathers. *Brain Res.* **1580**, 78–101 (2014).
26. Blakemore, S.J. The social brain in adolescence. *Nat. Rev. Neurosci.* **9**, 267–277 (2008).
27. Herting, M.M., Gautam, P., Spielberg, J.M., Dahl, R.E. & Sowell, E.R. A longitudinal study: changes in cortical thickness and surface area during pubertal maturation. *PLoS One* **10**, e0119774 (2015).
28. Peper, J.S. *et al.* Sex steroids and brain structure in pubertal boys and girls. *Psychoneuroendocrinology* **34**, 332–342 (2009).
29. Anderson, M.V. & Rutherford, M.D. Recognition of novel faces after single exposure is enhanced during pregnancy. *Evol. Psychol.* **9**, 47–60 (2011).
30. Anderson, M.V. & Rutherford, M.D. Cognitive reorganization during pregnancy and the postpartum period: an evolutionary perspective. *Evol. Psychol.* **10**, 659–687 (2012).
31. Pearson, R.M., Lightman, S.L. & Evans, J. Emotional sensitivity for motherhood: late pregnancy is associated with enhanced accuracy to encode emotional faces. *Horm. Behav.* **56**, 557–563 (2009).
32. Meins, E., Fernyhough, C., Fradley, E. & Tuckey, M. Rethinking maternal sensitivity: mothers' comments on infants' mental processes predict security of attachment at 12 months. *J. Child Psychol. Psychiatry* **42**, 637–648 (2001).
33. Lyall, A.E. *et al.* Dynamic development of regional cortical thickness and surface area in early childhood. *Cereb. Cortex* **25**, 2204–2212 (2015).
34. Nygaard, G.O. *et al.* Cortical thickness and surface area relate to specific symptoms in early relapsing-remitting multiple sclerosis. *Mult. Scler.* **21**, 402–414 (2015).
35. Oster, S. *et al.* Cerebral atrophy in AIDS: a stereological study. *Acta Neuropathol.* **85**, 617–622 (1993).
36. Regeur, L., Jensen, G.B., Pakkenberg, H., Evans, S.M. & Pakkenberg, B. No global neocortical nerve cell loss in brains from patients with senile dementia of Alzheimer's type. *Neurobiol. Aging* **15**, 347–352 (1994).
37. Raznahan, A. *et al.* How does your cortex grow? *J. Neurosci.* **31**, 7174–7177 (2011).
38. Pawluski, J.L., Lambert, K.G. & Kinsley, C.H. Neuroplasticity in the maternal hippocampus: relation to cognition and effects of repeated stress. **77**, 86–97 (2016).
39. Kinsley, C.H. *et al.* Motherhood and the hormones of pregnancy modify concentrations of hippocampal neuronal dendritic spines. *Horm. Behav.* **49**, 131–142 (2006).
40. Pawluski, J.L. *et al.* Pregnancy or stress decrease complexity of CA3 pyramidal neurons in the hippocampus of adult female rats. *Neuroscience* **227**, 201–210 (2012).
41. Pawluski, J.L. *et al.* Effects of stress early in gestation on hippocampal neurogenesis and glucocorticoid receptor density in pregnant rats. *Neuroscience* **290**, 379–388 (2015).
42. Pawluski, J.L. & Galea, L.A. Reproductive experience alters hippocampal neurogenesis during the postpartum period in the dam. *Neuroscience* **149**, 53–67 (2007).
43. Barha, C.K., Lieblich, S.E., Chow, C. & Galea, L.A. Multiparity-induced enhancement of hippocampal neurogenesis and spatial memory depends on ovarian hormone status in middle age. *Neurobiol. Aging* **36**, 2391–2405 (2015).
44. Galea, L.A. *et al.* Spatial working memory and hippocampal size across pregnancy in rats. *Horm. Behav.* **37**, 86–95 (2000).
45. Hiller, K.M., Neumann, I.D., Couillard-Despres, S., Aigner, L. & Slattery, D.A. Lactation-induced reduction in hippocampal neurogenesis is reversed by repeated stress exposure. *Hippocampus* **24**, 673–683 (2014).
46. Henry, J.D. & Rendell, P.G. A review of the impact of pregnancy on memory function. *J. Clin. Exp. Neuropsychol.* **29**, 793–803 (2007).
47. Glynn, L.M. Giving birth to a new brain: hormone exposures of pregnancy influence human memory. *Psychoneuroendocrinology* **35**, 1148–1155 (2010).
48. Buckwalter, J.G., Buckwalter, D.K., Bluestein, B.W. & Stanczyk, F.Z. Pregnancy and post partum: changes in cognition and mood. *Prog. Brain Res.* **133**, 303–319 (2001).
49. Christensen, H., Leach, L.S. & Mackinnon, A. Cognition in pregnancy and motherhood: prospective cohort study. *Br. J. Psychiatry* **196**, 126–132 (2010).
50. Kim, P. *et al.* The plasticity of human maternal brain: longitudinal changes in brain anatomy during the early postpartum period. *Behav. Neurosci.* **124**, 695–700 (2010).

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 via the fertility center Instituto Valenciano de Infertilidad (IVI, Barcelona), by flyers and by word of mouth. 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 (women and men becoming parents between the sessions, hereafter referred to as F_{PRG} and M_{PRG} , respectively) and the control (CTR) groups (women who did not become pregnant within this time frame and men whose partners did not become pregnant, from here on referred to as F_{CTR} and M_{CTR}) on the basis of 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 in 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 men without children 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 participants⁵¹ in each group based on fertility statistics⁵². 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 Interview⁵³ 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 between the sessions, the majority (20 women) had an estimated pregnancy onset within 6 months after the session. Five participants became pregnant between 6 and 12 months after their participation in the first MRI session. To ensure that this 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 neuropathological conditions encountered in the MRI scan.

Our final sample consisted of the following subject groups with complete Pre and Post data sets: 25 primiparous women, 20 nulliparous control women, 19 first-time fathers and 17 control men without children. 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 (mean \pm s.d.: Pre-Post time interval: M_{PRG} : 459.00 \pm 117.46 d, M_{CTR} : 419.17 \pm 93.17 d; $t = 1.12$, $P = 0.272$. F_{PRG} : 463.52 \pm 108.33 d, F_{CTR} : 413.05 \pm 106.86 d. $t = 1.56$, $P = 0.126$. Age: M_{PRG} : 35.21 \pm 4.30 years, M_{CTR} : 31.64 \pm 6.41 years. $t = 1.94$, $P = 0.063$. F_{PRG} : 33.36 \pm 3.97 years, F_{CTR} : 31.10 \pm 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, $\chi^2 = 0.37$, $P = 0.833$. F_{PRG} : 2/4/19, F_{CTR} : 2/3/15, $\chi^2 = 0.06$, $P = 0.971$), 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 Tables 16 and 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 \pm 47.83 d (mean \pm s.d.) 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 (for either a linear, quadratic or cubic positive or negative correlation).

Nine women achieved pregnancy by natural conception and 16 women by means of 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 and 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 intracytoplasmic sperm injection (ICSI), 4 without egg donation or ICSI), 3 intrauterine 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 analog; egg donation or embryo transfer: estrogens, progesterone, GnRH analog). 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 conventional 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 a girl. The remaining 4 had twins (2 mixed twins, 1 male twins, 1 female twins). Considering the previously observed effects of fetal sex on cognitive changes in pregnant women⁵⁴, we additionally compared the women carrying a boy to the women carrying a girl (excluding the four 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 had twins. Eight of the women gave birth by cesarean section and 17 by vaginal birth. All women except 1 received epidural anesthesia during delivery. Sixteen women practiced exclusive breastfeeding (breast milk as their infant's sole source of nutrition), 2 practiced breastfeeding supplemented by formula feedings, 2 had started breastfeeding their infants but had stopped by the time of the Post scan, and 2 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 Scale⁵⁵ 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, for practical reasons, we could only obtain blood samples from 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 + 2 years 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: mean \pm s.d.: 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 Clínica de l'Institut Municipal d'Assistència Sanitària), and written informed consent was obtained from all subjects before 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 = 8.2 ms, TE = 3.7 ms, NSA = 1, matrix = 256×256 , FOV = 240 mm, 180 slices, thickness = 1 mm, no gap, FA 8°). Due to an unexpected technical problem, the radio frequency 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 ($\chi^2 = 4.21$, $P = 0.240$). 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 + 2 years session, an MRI scan was acquired with both radio frequency 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 + 2 years session.

The Post MRI session also included an fMRI paradigm (T2*-weighted gradient echo EPI sequence. TR = 3,000 ms, TE = 35 ms, matrix = 128×128 , FOV = 230 mm, 30 slices, thickness = 4 mm, gap 0.5 mm, FA 90°) that examined the new mothers' neural responses 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 (NeuroBehavioral Systems). 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 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 presented in randomized order in an event-related fashion (trial duration 1,500 ms, randomized inter-trial interval 750–1,250 ms), with an average number of trials of (mean \pm s.d.) 72.15 ± 6.64 and 72.40 ± 6.99 for the other 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 3 mm (for translations) or 3° (for rotations) (1 woman), artifacts in the data (2 women), or incomplete data sets (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 España Complutense*⁵⁶, based on the California Verbal Learning test⁵⁷, the Digits subtest of the Wechsler Adult Intelligence Scale III⁵⁸, a two-back working memory test, the Interpersonal Reactivity Index⁵⁹, 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 suprathreshold 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, and hence these data are 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 (mean \pm s.d., quality of attachment: 37.11 ± 3.99 ; absence of hostility: 16.93 ± 4.10 ; pleasure in interaction: 20.88 ± 3.10).

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 (MathWorks), using the longitudinal symmetric diffeomorphic modeling pipeline⁶⁰. 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 nonlinear 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 algorithm⁶¹. The jacobian determinants resulting from the longitudinal registration were subsequently multiplied by each subject's GM segment, creating maps of volumetric change in GM tissue. To bring these images into MNI space, the product images were normalized using DARTEL tools⁶² and smoothed with a 12-mm 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 in the fMRI task) 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 data set of 1,000 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 gray matter of the brain (see Supplementary Table 6).

For completeness, although these MRI images are not optimal for investigating white matter, we also multiplied the individual jacobian difference maps with the white matter segments of the midpoint average images 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 gray matter segments.

A cross-sectional voxel-based morphometric approach was also applied to 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 algorithm⁶¹, a DARTEL normalization of the GM segments⁶² and the application of a 12-mm 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 Post + 2 years images were processed using the same longitudinal approach described above, rendering volume difference maps between the Post + 2 years images and the two 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 and early Post sessions, we performed one-sample *t*-tests on the Pre – Post + 2 years and the Post – Post + 2 years 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 increases 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 theory-based threshold of $P < 0.05$, FWE-corrected across the whole brain. A minimum cluster size of 10 contiguous voxels was imposed 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 PRoNTTo 2.0 (<http://www.mlnl.cs.ucl.ac.uk/pronto/>)⁶⁶ 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 on the basis of a training data set⁶⁶. 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 to compute the accuracy with which groups can be discriminated from one another on the basis of whole-brain spatial signal patterns.

To examine the degree to which the experimental women could be discriminated from the control women on the basis of 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 on the basis of a training set including all remaining subjects. This procedure is then repeated for each subject, and the accuracy of the discriminant function is computed using all these runs. Permutation testing was used to estimate the null distribution and examine the statistical significance of the classification accuracy ($N_{\text{permutations}} = 10,000$; $P < 0.05$)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 PRoNTTo⁶⁸ 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 three dimensions of the MPAS²⁴. Kernel ridge regression represents a form of support vector regression using a squared-error loss function combined with l2 regularization (see ref. 69 for a description of this approach). 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; that is, in every fold a participant was left out for whom the MPAS score was predicted and examined in relation to the actual MPAS score. As in the classification models, we used a leave-one-out cross-validation scheme and permutation testing ($N_{\text{permutations}} = 10,000$; $P < 0.05$).

Since the current version of PRoNTTo does not yet allow the inclusion of covariates and we could therefore not include the radio frequency 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, $p_{\text{nMSE}} = 0.034$; absence of hostility: $R = 0.42$, $P = 0.031$, nMSE = 0.87, $p_{\text{nMSE}} = 0.023$).

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 on the basis of 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 two raters who were blind to any identifying subject or group information on coronal slices of the Pre, Post and Post + 2 years 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 on the basis of ten 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 of 23 Pre, 23 Post and 10 Post + 2 years volumes. As the missing volumes did not correspond to the same individuals across sessions, that left 22 Pre–Post pairs, 9 Pre–Post + 2 years pairs and 10 Post–Post + 2 years pairs for the longitudinal comparisons.

These measures were analyzed in SPSS 23 (IBM). A normal distribution of the data and equal variances were confirmed using Shapiro-Wilk and Levene's tests, respectively. The results are described in **Supplementary Figure 3** and **Supplementary Tables 2** and **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 reprocessed 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 nonbrain tissue, transformation into stereotaxic (MNI) space, intensity correction, volumetric segmentation and cortical surface reconstruction^{73,74}, and parcellation⁷⁵. 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 nonbrain 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 15 mm. A cross-sectional approach was also 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 or area across the time points), and examined using one-sample *t*-tests. Cluster statistics were obtained using Monte Carlo simulations with a vertex-wise $-\log_{10}(P)$ 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 (IBM) using the changes in average cortical thickness and surface area values across the regions of GM volume change to examine the predictive value of these surface-based measures for group classification.

Functional MRI analyses. Analyses of the functional MRI data 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 (for translations) or 3° (for rotations) 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 nonlinear registration⁶¹. 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 > own 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/>).

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

51. Friston, K. Ten ironic rules for non-statistical reviewers. *Neuroimage* **61**, 1300–1310 (2012).
52. Dunson, D.B., Colombo, B. & Baird, D.D. Changes with age in the level and duration of fertility in the menstrual cycle. *Hum. Reprod.* **17**, 1399–1403 (2002).
53. Sheehan, D.V. *et al.* The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *J. Clin. Psychiatry* **59** (Suppl. 20), 22–33, quiz 34–57 (1998).
54. Vanston, C.M. & Watson, N.V. Selective and persistent effect of foetal sex on cognition in pregnant women. *Neuroreport* **16**, 779–782 (2005).
55. Cox, J.L., Holden, J.M. & Sagovsky, R. Detection of postnatal depression. Development of the 10-item Edinburgh Postnatal Depression Scale. *Br. J. Psychiatry* **150**, 782–786 (1987).
56. Benedet, M.J. & Alejandre, M.A. *Test de Aprendizaje Verbal España-Complutense* (TEA Ediciones, 1998).
57. Delis, D.C., Kramer, J.H., Kaplan, E. & Ober, B.A. *California Verbal Learning Test* 2nd edn. (Psychological Corporation, San Antonio, Texas, USA, 2000).
58. Kaufman, A.S. & Lichtenberger, E. *Assessing Adolescent and Adult Intelligence* 3rd edn. (Wiley, Hoboken, New Jersey, USA, 2006).
59. Davis, M.H. A multidimensional approach to individual differences in empathy. *JSAS Catalog of Selected Documents in Psychology* **10**, 85–90 (1980).
60. Ashburner, J. & Ridgway, G.R. Symmetric diffeomorphic modeling of longitudinal structural MRI. *Front. Neurosci.* **6**, 197 (2013).
61. Ashburner, J. & Friston, K.J. Unified segmentation. *Neuroimage* **26**, 839–851 (2005).
62. Ashburner, J. A fast diffeomorphic image registration algorithm. *Neuroimage* **38**, 95–113 (2007).
63. Ashburner, J. & Friston, K.J. Voxel-based morphometry—the methods. *Neuroimage* **11**, 805–821 (2000).
64. Radua, J., Canales-Rodríguez, E.J., Pomarol-Clotet, E. & Salvador, R. Validity of modulation and optimal settings for advanced voxel-based morphometry. *Neuroimage* **86**, 81–90 (2014).
65. Salmond, C.H. *et al.* Distributional assumptions in voxel-based morphometry. *Neuroimage* **17**, 1027–1030 (2002).
66. Schrouff, J. *et al.* PRoNT: pattern recognition for neuroimaging toolbox. *Neuroinformatics* **11**, 319–337 (2013).
67. Golland, P. & Fischl, B. Permutation tests for classification: towards statistical significance in image-based studies. *Inf. Process. Med. Imaging* **18**, 330–341 (2003).
68. Rakotomamonjy, A., Bach, F., Canu, S. & Grandvalet, Y. SimpleMKL. *J. Mach. Learn. Res.* **9**, 2491–2521 (2008).
69. Shawe-Taylor, J. & Cristianini, N. *Kernel Methods for Pattern Analysis* (Cambridge Univ. Press, 2004).
70. MacMaster, F.P. *et al.* Pituitary volume in pediatric obsessive-compulsive disorder. *Biol. Psychiatry* **59**, 252–257 (2006).
71. Reuter, M. & Fischl, B. Avoiding asymmetry-induced bias in longitudinal image processing. *Neuroimage* **57**, 19–21 (2011).
72. Reuter, M., Schmansky, N.J., Rosas, H.D. & Fischl, B. Within-subject template estimation for unbiased longitudinal image analysis. *Neuroimage* **61**, 1402–1418 (2012).
73. Dale, A.M., Fischl, B. & Sereno, M.I. Cortical surface-based analysis. I. Segmentation and surface reconstruction. *Neuroimage* **9**, 179–194 (1999).
74. Fischl, B., Sereno, M.I. & Dale, A.M. Cortical surface-based analysis. II: Inflation, flattening, and a surface-based coordinate system. *Neuroimage* **9**, 195–207 (1999).
75. Desikan, R.S. *et al.* An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. *Neuroimage* **31**, 968–980 (2006).