

Pregnancy Entails a U-Shaped Trajectory in Human Brain Structure Linked to Hormones and Maternal Attachment

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This manuscript has been previously reviewed at another journal. This document only contains information relating to versions considered at Nature Communications.

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 1:

Reviewer comments:

Reviewer #2

(Remarks to the Author)

I appreciate the willingness with which the authors responded to my requests for new analyses, and agree with the points where some of the asks were outside the scope of the current paper. The additional text/results help clarify the specificity of the results and points for differentiation with the other impactful papers from this group on this unique dataset. As such, I have no further comments and recommend publication.

Reviewer #3

(Remarks to the Author)

This manuscript reports the largest study to date of neural changes across the pregnancy period. This is an important contribution to the field, which is an area that is generally not only understudied, but often uses less-than-ideal methods and small sample sizes. It is well-controlled (comparing non-gestational mothers and nulliparous women) and relatively comprehensive. While gaps in the measures certainly exist, a single study cannot measure all things, and the results indicate interesting areas for further research.

I was not involved in the original review however I think that most of the comments have been well addressed, and I do not see major critical flaws. I believe that a few extra points are worth mentioning, which will improve the contextual background, interpretation of the results, and suggestions for further research.

- Were the non-gestational mothers nulliparous as well? And had the non-gestational mothers & nulliparous women ever been pregnant before?

- The results do not explore the rsfMRI data as comprehensively as the GM data, however I note that the authors mention this as a caveat in their discussion and so I do not think this of major concern per se. However I think that the ms would benefit from expanding the discussion of these results. This component of the study is less well-justified and discussed than the other results. For example, why was modularity/segregation chosen as the metric of interest? Is there evidence from the literature to support looking at these metrics and not others? What does it mean that there were no significant effects for the rsfMRI but there were for GM - does it indicate that GM changes might not be reflected in functional network reorganisation? It is probably worth mentioning that these results contrast with previous studies (e.g., Hoekzema Nat Neuro 22, Orchard Sci Rep 22 [although there are caveats with those studies too, particularly the rather modest rsfMRI analysis of the former and the later time period postpartum of the latter]). I take the authors' point that future work will explore the rsfMRI results in more detail, but the results that are shown here should be interpreted a bit more deeper than is currently presented.

- While the results indicate that the experiential effects of motherhood do not drive these effects (with the comparison between gestational vs. non-gestational mothers), the results cannot entirely rule out this possibility and so the discussion of this point should be moderated. Firstly, engagement in parenting environment/behaviour was not measured and so it cannot be ruled out that the gestational mothers were more engaged in parenting behaviour than the non-gestational mothers. Secondly, the timeframe of the study cannot rule out that engagement in parenting over a longer duration may result in brain

changes. This is the thesis of Orchard et al. (2023 TICS, 2021 Cereb Cortex). While the inclusion of the non-gestational mothers, in my opinion, is a key strength of this work, I think the results indicate that while evidence indicates that it is the physiological effects of pregnancy, partruition, and postpartum factors driving this effect, more longer term effects of experience cannot be ruled out.

- Lastly, I think the ms would benefit from a more comprehensive discussion of the context of this work. For example, the Orchard TICS paper where she attempts to take a lifespan perspective is interesting (are the results consistent with short-term decrements & long term benefits? or should the model be refined?), as are her findings of changes at 1 year postpartum which is relevant here. Particularly her finding that cognitive differences between mothers & non-mothers were mediated by wellbeing (Orchard J Womens Health 22) and that effective functional connectivity was mediated by maternal behaviour (Orchard Sci Rep 23). Do the results largely align with the other longitudinal work in this area, eg. Luders et al. NIMG 22 and the picture that was emerging then? And the rather high profile Nat Neuro paper by Pritchett et al 2024 - does this larger and more comprehensive study offer more nuance to their n=1 findings? Finally, do the results align with cross-species evidence, eg Puri et al. TINS 23? Note that here I am not suggesting a deep dive nor extensive reworking of the discussion, rather, the discussion as presented is a bit limited in contextualising the results, and I think would benefit from further consideration of how the results align/suggest refinement of our understanding of the effects of pregnancy on the brain.

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Reviewer's comments (October 2024)

- **Were the non-gestational mothers nulliparous as well? And had the non-gestational mothers & nulliparous women ever been pregnant before?**

We thank the reviewer for bringing this up. Yes, all participants were nulliparous at the start of the study. This information can be found in the methods section "Study design and participants" (lines 388 to 446). To be more specific, we have now clarified that the exclusion criteria applied to all participants in the study.

The text now reads: "Participants were recruited in the Barcelona area through word-of-mouth, local hospitals, and social media ads. We recruited nulliparous women who were planning on becoming pregnant in the near future (i.e., gestational mothers), nulliparous women whose female partners were planning to become pregnant in the near future (i.e., non-gestational mothers), and nulliparous women without any plans of having children soon (i.e., nulliparous women). [Exclusion criteria for all participants included](#) being over 45 years old, being pregnant at the pre-conception session, [previous pregnancies](#), current major neurological or psychiatric disorders assessed by the MINI International Neuropsychiatric Interview³⁰, intake of psychiatric medication, and MRI incompatibilities. Gestational mothers and their non-gestational partners who did not achieve pregnancy after the first MRI session were discontinued from the study. "

- **The results do not explore the rsfMRI data as comprehensively as the GM data, however I note that the authors mention this as a caveat in their discussion and so I do not think this of major concern per se. However I think that the ms would benefit from expanding the discussion of these results. This component of the study is less well-justified and discussed than the other results. For example, why was modularity/segregation chosen as the metric of interest? Is there evidence from the literature to support looking at these metrics and not others? What does it mean that there were no significant effects for the rsfMRI but there were for GM - does it indicate that GM changes might not be reflected in functional network reorganisation? It is probably worth mentioning that these results contrast with previous studies (e.g., Hoekzema Nat Neuro 22, Orchard Sci Rep 22 [although there are caveats with those studies too, particularly the rather modest rsfMRI analysis of the former and the later time period postpartum of the latter]). I take the authors' point that future work will explore the rsfMRI results in more detail, but the results that are shown here should be interpreted a bit more deeper than is currently presented.**

We agree with the reviewer that functional connectivity analyses presented in this article can be better contextualized and discussed.

We now describe each of the graph theory metrics we used in our analysis in the results section. It is our hope that this explanation will make it easier for the reader to understand the rationale behind the resting-state analysis and the metrics of interest. Lines 163 to 170 now read: [Specifically](#), we measured changes in the functional organization and network segregation using whole-brain and within-network modularity, mean participation coefficient and system segregation graph theory metrics. [Modularity](#) allowed us to capture network compartmentalization. That is, the extent to which a specific network is subdivided into communities of strong within-module connectivity and weak between-module connectivity. Using system segregation, we assessed the balance between connections within each Yeo's network and those spanning different networks. Finally, through the mean participation coefficient we quantified the extent of intermodular connectivity across Yeo's 7 large-scale functional networks.

Following the reviewer's suggestion, we have also extended the discussion on the absence of functional changes in the brain.

Lines 277 to 288 now read: However, none of the networks exhibited changes in their functional segregation properties, [suggesting that the distinct GM volume trajectories might not be reflected in a functional network reorganization](#). Of note, the lack of changes observed in these metrics do not preclude the occurrence of numerous other functional changes during this life transition [or at later stages of the postpartum period](#). Using network coherence as their measure of interest, Hoekzema and colleagues reported pre-post pregnancy increases in network-connectivity within a cluster located in the Default Mode Network. Moreover, cross-sectional studies have reported differences in the effective connectivity between key nodes of the parental caregiving network in first-time mothers across the early and late postpartum periods^{32,33}. [Together](#), a more in-depth analysis of functional changes, including the examination of other network functional properties and even alternative network constructions, is essential to characterize connectomic changes in mothers. Furthermore, future investigations should explore the structural-functional coupling across the perinatal period.

- **While the results indicate that the experiential effects of motherhood do not drive these effects (with the comparison between gestational vs. non-gestational mothers), the results cannot entirely rule out this possibility and so the discussion of this point should be moderated. Firstly, engagement in parenting environment/behaviour was not measured and so it cannot be ruled out that the gestational mothers were more engaged in parenting behaviour than the non-gestational mothers. Secondly, the timeframe of the study cannot**

rule out that engagement in parenting over a longer duration may result in brain changes. This is the thesis of Orchard et al. (2023 TICS, 2021 Cereb Cortex). While the inclusion of the non-gestational mothers, in my opinion, is a key strength of this work, I think the results indicate that while evidence indicates that it is the physiological effects of pregnancy, parturition, and postpartum factors driving this effect, more longer term effects of experience cannot be ruled out.

We agree with the reviewer that the influence of experiential factors should be discussed with caution. We have now extended the discussion on this topic to provide a more moderated and clear perspective on the potential role of experiential effects related to parenting on the neuroanatomical remodeling during the transition to motherhood.

Lines 245 to 255 now read: “External factors related to the parental experience minimally influenced this trajectory, as it solely manifested in women undergoing pregnancy but was absent in non-gestational mothers. This suggests that the U-shaped trajectory observed from before pregnancy to six months postpartum is mainly due to gestational factors. Importantly, these findings do not preclude parenting-related brain adaptations in non-gestational mothers nor do they exclude such adaptations as contributing factors of the neuroanatomical changes observed in gestational mothers. The impact of childrearing and environmental factors on the human maternal brain may be less pronounced and more localized than the effects of pregnancy, in line with previous research on the paternal brain^{27,28}. Given that motherhood is a life-long journey, parenting-related factors may also contribute to brain changes observed beyond the immediate postpartum^{4,20,25}, and in middle-aged and older mothers²⁹. Lastly, our study did not measure parenting investment or behavior; thus, we cannot determine whether gestational mothers were more engaged in parenting behaviors than non-gestational mothers.”

- **Lastly, I think the ms would benefit from a more comprehensive discussion of the context of this work. For example, the Orchard TICS paper where she attempts to take a lifespan perspective is interesting (are the results consistent with short-term decrements & long term benefits? or should the model be refined?), as are her findings of changes at 1 year postpartum which is relevant here. Particularly her finding that cognitive differences between mothers & non-mothers were mediated by wellbeing (Orchard J Womens Health 22) and that effective functional connectivity was mediated by maternal behaviour (Orchard Sci Rep 23). Do the results largely align with the other longitudinal work in this area, eg. Luders et al. NIMG 22 and the picture that was emerging then? And the rather high profile Nat Neuro paper by Pritchett et al 2024 - does this larger and more comprehensive study offer more nuance to their n=1 findings? Finally, do the results align with cross-species evidence, eg., Puri et al. TINS 23? Note that here I am not suggesting a**

deep dive nor extensive reworking of the discussion, rather, the discussion as presented is a bit limited in contextualising the results, and I think would benefit from further consideration of how the results align/suggest refinement of our understanding of the effects of pregnancy on the brain.

We thank the reviewer for the valuable suggestions. In the first paragraph of the discussion, we contextualize our findings within the framework of longitudinal studies examining pre- and post-pregnancy changes, including the study by Luders et al. (NIMG 22). Additionally, we have now explicitly cited studies that explore the long-term effects of parity on the brain, such as De Lange et al. PNAS 21.

First paragraph of the Discussion: “This prospective study uncovered a U-shaped trajectory in cortical GM volume, area and thickness in first-time gestational mothers during pregnancy and postpartum, peaking in the peripartum period. GM reductions were evident as early as the second trimester, suggesting an early onset of brain remodeling during pregnancy. At six months postpartum, GM volume [had not returned](#) to pre-pregnancy levels, [supporting the notion that maternal brain changes may be a long-lasting phenomenon that persists beyond the postpartum period](#)^{4,5,20,21}. These findings indicate that gestation and postpartum induce opposite effects on the cortical mantle, resolving a long-debated puzzle for scholars in the field. In particular, our results suggest that previous studies reporting volume increases in cortical GM during postpartum^{21–25} and less pronounced cortical GM decreases before and after pregnancy (-2.7% at 3 months postpartum vs -4.9% at late pregnancy)^{4,26}, were indeed capturing part of the postpartum recovery process.”

We appreciate the reviewer drawing our attention to the study by Orchard et al. (Sci Rep 2023). In response, we have included this study as further evidence of how well-being can mediate brain and cognitive/neuropsychological changes. Lines 306-312 of the Discussion now read: “This suggests that the neuroanatomical changes occurring after pregnancy affect the mental well-being in mothers, which in turn facilitates adaptive maternal attachment. [Maternal well-being has also been shown to mediate the relationship between improved cognitive performance and reduced top-down corticolimbic inhibition in first-time mothers at one year postpartum](#)³². Together, these findings open the door to identifying specific periods during pregnancy and postpartum when experiences and interventions may have the greatest impact on maternal brain health and psychological well-being. Research should also explore if these neuroanatomical trajectories are disrupted in psychiatric disorders such as perinatal depression.”.

We also find really interesting and plausible the hypothesis proposed by Orchard, suggesting that motherhood might entail cognitive load in the short term and cognitive reserve in the long term. Given that our study does not include comprehensive cognitive measures and considering the sensitive nature

of cognition during motherhood, we prefer to avoid speculation in this article. However, we have now included this idea as a direction for future research in lines 298-300 of the Discussion:

“Additionally, studies integrating cognitive and neuroimaging assessments should also explore if this pronounced neural remodeling is linked to the increased cognitive load and subsequent cognitive reserve of new mothers³⁴”

Lastly, we appreciate the reviewer’s reference to the precision imaging study by Dr. Laura Pritschet and her team, which offers a valuable complementary lens to our work. We have now included this cite in the line 54 of the Introduction: “see Pritschet et al 2024 for a precision imaging study of a single-subject”.