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Gray matter increases within subregions of the hippocampal complex after pregnancy

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Abstract

Neuroimaging findings – although still relatively sparse in the realm of postpartum research – suggest significant tissue increases within the hippocampus or its vicinity after giving birth. Given that the hippocampus is not a homogenous structure, effects may manifest differently across the hippocampal complex. Thus, the goal of this study was to determine the presence, magnitude, and direction of postpartum gray matter changes within five hippocampal subregions, specifically the dentate gyrus, the subiculum, and the subfields of the cornu ammonis (CA1, CA2 and CA3). For this purpose, we analyzed brain images of 14 healthy women acquired at immediate postpartum (within 1–2 days of childbirth) and at late postpartum (at 4–6 weeks after childbirth). Changes in hippocampal gray matter between both time points were calculated for all subregions as well as the hippocampal complex as a whole by integrating imaging-based intensity information with microscopically defined cytoarchitectonic probabilities. Hippocampal gray matter increased significantly within the right subiculum, right CA2, and right CA3. These findings may suggest that brain tissue lost during pregnancy is being restored after giving birth, perhaps even expanded compared to before pregnancy. Possible events on the microanatomical level include dendritic branching as well as the generation of new synapses, glia cells, and blood vessels. Altogether, the outcomes of our study confirm that hippocampal gray matter increases in the female human brain after giving birth, with differential effects across the hippocampal complex.

Keywords Cornu ammonis · Gray matter · Hippocampus · MRI · Postpartum · Pregnancy · Subiculum

Introduction

Neuroimaging research indicates drastic changes in brain structure after giving birth (Hoekzema et al., 2017; Kim et al., 2010; Lisofsky et al., 2019; Luders et al., 2018, 2020; Oatridge et al., 2002). From the four existing studies that allowed for regional gray matter observations (Hoekzema et al., 2017; Kim et al., 2010; Lisofsky et al., 2019; Luders et al., 2020), two reported significant increases within the hippocampus or its vicinity (Hoekzema et al., 2017; Kim et al., 2010). The hippocampus has a high density of receptors for estrogen and progesterone, which are not only the chief pregnancy hormones but are also known to heavily affect hippocampal plasticity (Guerra-Araiza et al., 2003; Sheppard et al., 2019). Therefore, hippocampal changes after giving birth (a stage marked by a rapid readjustment of hormone levels) are not surprising.

The hippocampus, however, is not a homogenous structure but comprises different subregions, each with their own cytoarchitectonic properties and functional specialization (Amunts et al., 2005). It is therefore likely that postpartum changes manifest differently across the hippocampal complex, such as within the dentate gyrus (DG), subiculum (SUB), and subfields of the cornu ammonis (CA1, CA2, CA3). To our knowledge, postpartum studies specifically targeting the hippocampus (with or without discriminating between its subregions) are entirely missing. Therefore, it remains to be established whether the presence, magnitude, and direction

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of gray matter changes within the hippocampal complex after giving birth is identical across its subregions or whether there are region-specific effects.

To answer this question, we analyzed brain images obtained at 1–2 days and at 4–6 weeks after giving birth. As discussed elsewhere (Luders et al., 2018, 2020), the maternal brain, shortly after delivery, serves as an approximation of the pregnant brain. Comparing this proxy to a later time point, such as a few weeks after giving birth, will add novel insights into how the postpartum brain differs from the pregnant brain – here with particular focus on subregions of the hippocampal complex.

Methods

Study sample and data collection

Our study sample included 14 healthy postpartum women[1] between 25 and 38 years of age (mean ± SD: 32.8 ± 4.0 years), who were scanned twice after giving birth: within 1–2 days (immediate postpartum) and at 4–6 weeks (late postpartum). Structural brain images were acquired at both time points on a Philips Achieva 3 T-X MRI system (R2.1.3) using a phase-sensitive inversion recovery (PSIR) T1-weighted sequence and the following parameters: 5700 ms repetition time, 15 ms echo time, 400 ms inversion time, 90 degrees flip angle, 23 cm field of view, and 0.45 × 0.45 × 2.0 mm^3 voxel size. In addition, hormone measures were collected at both time points using blood samples that were drawn approximately twenty minutes prior to the brain scanning session. Serum progesterone and estradiol levels were analyzed by competitive immunometric electrochemical luminescence, using a Cobas e601 analyzer and Cobas Elecsys estradiol and progesterone reagent kits (Roche Diagnostics, Bromma, Sweden), as described elsewhere (Gingnell et al., 2015). All procedures were approved by the Regional Ethical Review Board, Uppsala (Sweden), and all participants provided written informed consent.

Data processing and statistical analysis

Hippocampal (sub)volumes were derived automatically by integrating microscopically defined cytoarchitectonic probabilities with imaging-based intensity information, as detailed elsewhere (Kurth et al., 2018). For this purpose, the brain images were first pre-processed in Matlab (https://www.mathworks.com/products/matlab.html) using SPM (https://www.fil.ion.ucl.ac.uk/spm/) and the VBM8 toolbox (http://www.neuro.uni-jena.de/vbm), as previously described (Luders et al., 2020). The pre-processing stream resulted in one difference image per woman reflecting the change in voxel-wise gray matter between late and immediate postpartum. Multiplying these difference images with the cytoarchitectonic tissue probabilities of CA1, CA2, CA3, DG and SUB in each hemisphere (Amunts et al., 2005; Eickhoff et al., 2005) yielded the subregion-specific volume changes in mm^3 (Kurth et al., 2018). These left and right volume changes of the hippocampal subregions as well as of the hippocampal complex as a whole (HIPPO = CA1 + CA2 + CA3 + DG + SUB) were entered as the dependent variables in the statistical model. One-sample t-tests were then applied to test for significant changes in gray matter, and Pearson correlations were calculated to test for associations between changes in gray matter and changes in estradiol as well as in progesterone.[2] All significance levels were Bonferroni-corrected for multiple comparisons and set at $p < 0.008$ (0.05/6).

Results

There was no significant *decrease* of gray matter between immediate and late postpartum, not even at uncorrected significance levels. In contrast, there was a significant *increase* of gray matter for several subregions of the hippocampal complex, specifically for right CA2, CA3, and SUB (see Fig. 1). These findings survived statistical corrections for multiple comparisons (see Table 1) and manifested as very large effects (Cohen's d: 2.325, 2.340 and 1.806). The right hippocampal complex as a whole also presented with a very large effect (Cohen's d: 1.37), but only reached significance at uncorrected levels (see Table 1), most likely due to the small sample size.

There were no significant positive correlations between gray matter changes and hormonal changes.[3] In contrast, there were significant negative correlations between estradiol changes and gray matter changes for left DG ($r = -0.637$) and right SUB ($r = -0.561$). There were also significant negative correlations between progesterone changes and gray matter changes for left CA1 ($r = -0.551$), left DG ($r = -0.691$) as well as the left and right hippocampus as a whole ($r = -0.646$ and $r = -0.622$). However, none of these correlations survived statistical corrections for multiple comparisons (see Table 1).

Discussion

Overall, our findings point to significant gray matter gain within the hippocampal complex, which corroborates

[1] For further details on the study sample, please refer to earlier publications pertaining to the same dataset (Gingnell et al. 2015; Luders et al. 2018; Luders et al. 2020).

[2] Using log10-scaled values for the hormonal measures.

[3] The actual hormonal changes are reported elsewhere (Luders et al. 2018), with significantly lower serum concentrations of estradiol as well as of progesterone at late postpartum compared to immediate postpartum.

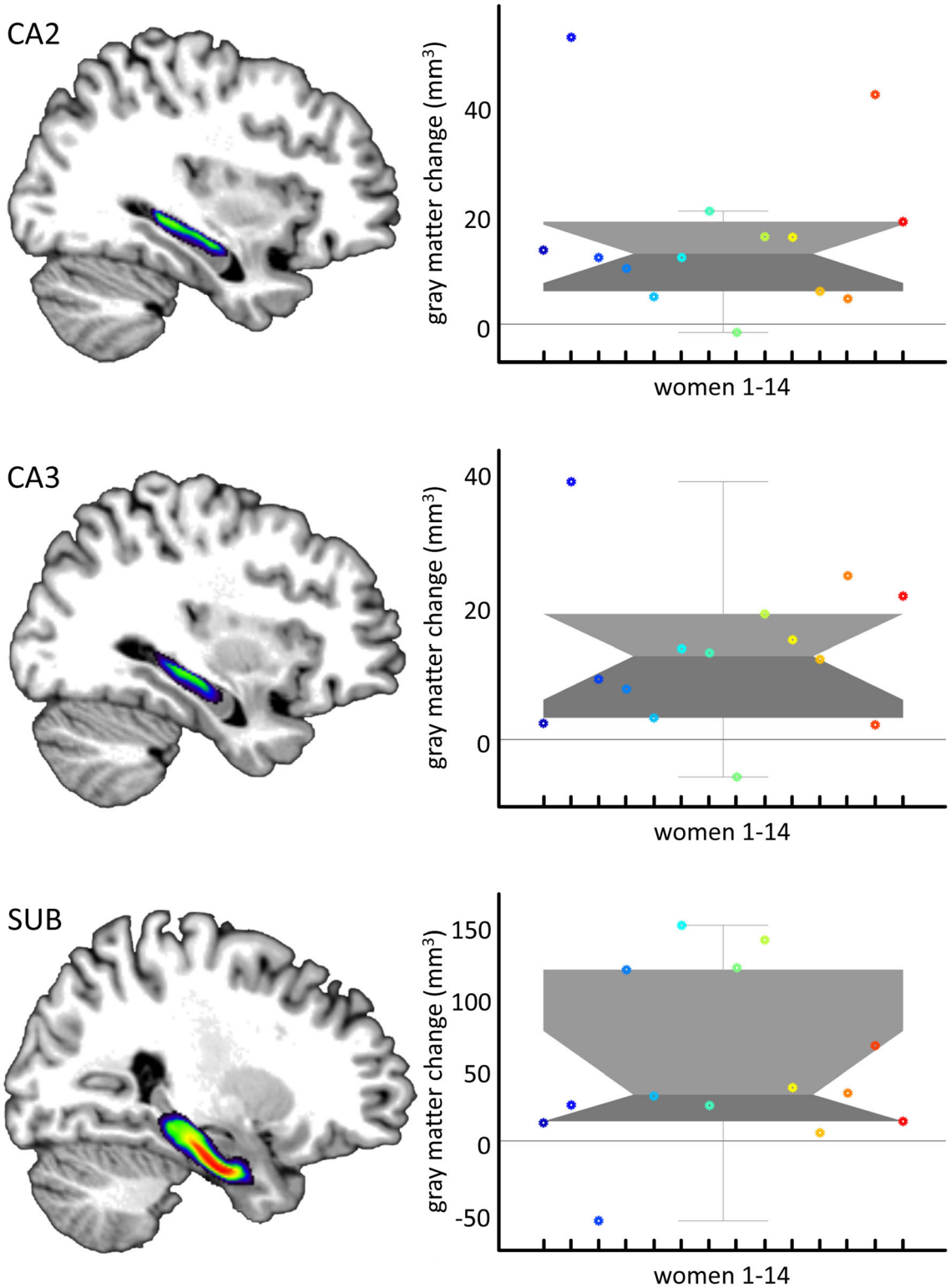

Fig. 1 Significant increases within three subregions of the hippocampal complex. Left Panel: Cytoarchitectonically derived probability maps of the right cornu ammonis - subfield 2 (CA2), right cornu ammonis - subfield 3 (CA3), and right subiculum (SUB), shown on sagittal sections of the ICBM single subject brain at x = 34, x = 33, and x = 27, respectively. The colors encode the region-specific probabilities (blue = 25%, green = 50%, orange = 75%, red = 100%) based on 10 post mortem brains (Amunts et al., 2005). Right Panel: Change in hippocampal gray matter between immediate and late postpartum. Positive numbers indicate increases; negative numbers indicate decreases. The data are displayed as boxplots, with the gray shaded areas containing the values between the 25th and 75th percentiles of the sample (the border in the middle demarks the median). The color-coded markers show the individual changes in the 14 women.

Table 1 Significant Effects.

Region	Gray Matter Increase		Negative Link: Estradiol		Negative Link: Progesterone	
	left	right	left	right	left	right
CA1	–	–	–	–	p=0.0496	–
CA2	–	p=0.0005*	–	–	–	–
CA3	–	p=0.0005*	–	–	–	–
DG	–	–	p=0.0237	–	p=0.0134	–
SUB	–	p=0.0031*	–	p=0.0458	–	–
HIPPO	–	p=0.0141	–	–	p=0.0218	p=0.0274

– not significant; * survives Bonferroni correction.

CA1–3 = cornu ammonis – subfields 1–3; DG = dentate gyrus; SUB = subiculum; HIPPO = hippocampal complex as a whole.

outcomes of prior studies suggesting significant postpartum increases within the hippocampus or its vicinity. More specifically, Kim et al., (2010) detected hippocampal effects within the right hemisphere (which is in agreement with current outcomes), while Hoekzema et al., (2017) reported effects within the left hemisphere. Of note, any effects within the right hippocampus would have been missed in Hoekzema's study because their postpartum analysis (specifically targeting regions that had presented with significant effects *during* pregnancy) was restricted to the left hippocampus. None of the prior studies was designed to discriminate between hippocampal subregions, so future research is needed to replicate the current effects within right CA2, CA3 and SUB.

The observed gray matter increases may reflect events on the microanatomical level, such as synaptogenesis, dendritogenesis, angiogenesis or gliogenesis, but discriminating between such events using the relatively coarse spatial resolution of standard in vivo imaging protocols is not possible. Regardless of the exact underlying micro-correlate, the apparent gray matter gain may suggest that brain tissue seemingly lost during pregnancy (Hoekzema et al., 2017) is being restored after giving birth, perhaps even expanded compared to before pregnancy. However, further research is clearly needed to systematically address the possibility of a net gain of brain tissue – perhaps hippocampal tissue within CA, CA3 and SUB in particular – after pregnancy.

The hippocampal increases may be a consequence of intensified engagement, stimulation, and parent-infant bonding, as previously discussed (Feldman, 2015; Luders et al., 2018, 2020). They may also be driven by the impact of the drastically altered hormone levels after pregnancy given the high density of sex steroid receptors in the hippocampus (Guerra-Araiza et al., 2003; Sheppard et al., 2019). Interestingly though, after applying corrections for multiple comparisons, there were no significant correlations between hippocampal changes and hormonal changes, which is in agreement with prior findings within the same sample focusing on brain age (Luders et al., 2018). However, a lack of correlation does not necessarily indicate a missing link but rather that the magnitude of change in hippocampal gray matter does not scale with the magnitude of change in hormone levels. Moreover, the non-significant hormone-related correlations could be due to the small sample size. Note that the effect sizes are very large for the negative correlations, suggesting that hippocampal increases are accompanied by hormonal decreases. At first sight, this may seem surprising as findings pertaining to the menstrual cycle usually point to positive correlations (Lisofsky et al., 2015; Protopopescu et al., 2008). However, as discussed elsewhere (Luders et al., 2018), differences in brain structure between pregnancy (when hormone levels are extremely elevated over months) and after giving birth (when hormone levels plunge substantially and suddenly) are likely to differ from the regular fluctuations in brain tissue during the menstrual cycle (when hormone levels change gradually and relatively moderately).

Conclusions

The goal of the study was to contribute to an understudied field of research by characterizing structural changes within different subregions of the hippocampal complex after giving birth. Gray matter increased significantly within right CA2, CA3, and SUB. There were no significant gray matter decreases. Given the small sample size, the outcomes of this study should be considered preliminary. Future studies are warranted to replicate our current findings in larger samples, perhaps also extending the focus from the hippocampus to other brain regions (e.g., amygdala, thalamus, insula, cerebellum, etc.) as implicated in prior research (Hoekzema et al., 2017; Kim et al., 2010; Lisofsky et al., 2019; Luders

et al., 2020; Oatridge et al., 2002) and ideally adding a data collection point before pregnancy.

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Author contributions Author contributions included conception and study design (EL, FK and ISP), data collection or acquisition (MG, JE, and ISP), data processing (FK and CG), statistical analysis (FK), interpretation of results (EL, FK, and ISP), drafting the manuscript work or revising it critically for important intellectual content (EL, FK and ISP) and approval of final version to be published and agreement to be accountable for the integrity and accuracy of all aspects of the work (all authors).

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Declarations

All procedures were approved by the Regional Ethical Review Board, Uppsala (Sweden), and all participants provided written informed consent.

Conflict of interest The authors declare no conflict of interest.

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