Temporal dissociation between local and global functional adaptations of the maternal brain to childbirth: A longitudinal assessment

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Short title:

Temporal dissociation between local and global maternal brain adaptations

Abstract

Background: The early peripartum period and motherhood are associated with significant physiological and psychological challenges. Despite the acknowledged relevance postpartum reorganization of functional activity and connectivity in the maternal brain, the dynamics of these changes are yet to be understood.

Methods: We assessed healthy maternal brain function longitudinally across 6 consecutive time points within a 24-week period after childbirth, beginning in the first postpartum week. Resting-state functional magnetic resonance imaging (rsfMRI) data of 98 women (75 postpartum and 23 nulliparous) with 189 MRI sessions revealed a general dissociation between local and global functional connectivity (FC) and activity measures.

Findings: In postpartum women, we observed persistently altered intraregional activity and FC throughout the first 6 postpartum months. In contrast, the reduced subcortical whole-brain FC normalized to control levels about 6 to 9 weeks postpartum. Whole-brain distributions of these connectivity and activity changes colocalized with corticosteroid hormone receptor expression and neurotransmitter receptor binding. First evidence showed that normalization trajectories of subcortical global FC covaried with progesterone levels, while more persistent cortical functional changes may be related to behavioral and psychological adaptations.

Interpretation: Our data indicate diverging biological mechanisms underlying postpartum adaptations of brain function. The presented results contribute significantly to our understanding of healthy maternal brain while identifying potential correlates of atypical adaptation processes for further study in the context of postpartum psychiatric disorders.

Keywords: postpartum, maternal brain, longitudinal, function connectivity

Introduction

The biological and psychological adaptations that pregnancy and the early postpartum period trigger in the maternal brain are testament to the neuroplasticity of the adult brain. Within the first few days of delivery, the maternal brain structure, with widespread changes in gray matter volume (GMV) and cortical thickness across all lobes, differs markedly from that of nulliparous controls [1]. Particularly noteworthy in the distinction between the maternal and nulliparous brains is the prominent role of the left amygdala [2]. Thought to be mediated by sex steroid hormones and corticosteroids [3,4], these morphometric changes follow temporal trajectories with the most dynamic changes occurring in the first 6 weeks postpartum [2]. However, the GMV in the dorsomedial prefrontal cortex and the cortical thickness of the subgenual and lateral prefrontal cortices may not normalize to pre-pregnancy levels even at 12 weeks postpartum [2,5,6]. As these findings indicate, pregnancy and childbirth or motherhood may have regionally divergent short-term and long-term effects on the brain. While the biological role of these effects is not adequately understood, a role in maternal attachment toward the offspring has been suggested [2,5,7].

In comparison to the effects of pregnancy and childbirth on brain *morphology*, their effects on brain *activity* and *neural network organization* are under-investigated. It is conceivable, however, that brain function undergoes substantial restructuring in pregnancy and the postpartum period given the abovementioned changes in brain morphology, the massive hormonal adaptations in the perinatal period [3,8], the reported hormonal regulation of brain connectivity [9,10], and the interactions between sex hormones and GABAergic as well as oxytonergic neurotransmission [11,12]. Resting-state functional magnetic resonance imaging (rsfMRI) provides a simple-to-perform, easily scalable, as well as compliance- and performance-independent tool to gain insights into the peripartum reorganization of brain function [13]. Longitudinal study designs with multiple postpartum scans are necessary to examine potential regionally divergent short- and long-term alterations of brain function associated with pregnancy and childbirth. A previous rsfMRI study [14] reported changes in the default mode network (DMN) temporal coherence in the cuneus at about 1 to 4 months

postpartum compared to pre-conception. The elaborate applied study design with one pre-pregnancy and one postpartum scan allowed the authors to investigate the long-term effects of pregnancy on resting-state functional connectivity (FC). However, a set of follow-up data with a high temporal resolution might be better suited to evaluate functional brain reorganization in the context of the massive physiological adaptation in the early postpartum period, and to disentangle the short-term biological effects of pregnancy and the early postpartum phase from those of motherhood [2]. Aiming at identification of potential biological mechanisms involved in postpartum rsfMRI adaptations, voxel- or cluster-level MRI inferences allow only for limited conclusions. Spatial colocalization analyses that quantify the degree of alignment between (i) observed MRI maps and (ii) a biological system of interest can help redress this limitation [15–17]. Applied to single-subject MRI maps, this approach has been successfully used to study physiological structural changes in the brain during the development of and pathophysiological alterations in Parkinson's and Huntington's diseases [17,18].

Using rsfMRI, we assessed healthy maternal brain function longitudinally across 6 consecutive time points within a 24-week period after childbirth. Beginning in the first postpartum week, the assessments continued at 3-week intervals for 12 weeks, with a final session at week 24 postpartum. In addition to MRI, we obtained serum estradiol and progesterone levels and b behavioral data at each assessment. We hypothesized that, compared to their nulliparous counterparts (NP), postpartum women (PP) would show strong alterations of the rsfMRI metrics mapping intra- and interregional brain activity and FC. Given the high temporal resolution of our study's longitudinal design, we aimed to distinguish the transitory from long-lasting rsfMRI changes. In order to test the hypothesis that brain activity and connectivity are influenced by hormone-mediated processes, we (i) determined whether whole-brain distributions of rsfMRI activity and connectivity changes colocalized with spatial distributions of hormone receptors and the functionally related neurotransmitter receptors, and (ii) assessed the time-dependent covariation between rsfMRI changes and blood plasma estradiol and progesterone levels at each rsfMRI session. Based on the hypothesis that permanent changes in brain function may be associated with psychological and/or behavioral changes due to

motherhood [2,5,7], we examined whether these functional adaptations in the brain covaried with mother-infant attachment and maternal depression levels over time.

Methods

Participants

Data of 75 non-depressed postpartum women were included in MRI processing. Postpartum women were recruited within 1 to 7 days of childbirth (T0) in the Department of Gynecology and Obstetrics, University Hospital Aachen. From this sample, 19 PP women took part in longitudinal MRI assessments, with measurements at 3 weeks (T1, n = 19), 6 weeks (T2, n = 18), 9 weeks (T3, n = 19), 12 weeks (T4, n = 19) and 24 weeks (T5, n = 17) postpartum. At each time point, blood samples were drawn to determine blood plasma levels of estradiol and progesterone. PP participants were assessed for symptoms of postpartum depression by means of the Edinburgh Postnatal Depression Scale (EPDS; T0–T5; total score) [19] and for mother-child attachment through the Maternal Postnatal Attachment Scale (MPAS; T1–T5; total score and sub-scores for quality of attachment, absence of hostility, and pleasure in interaction). 23 healthy nulliparous women with no history of psychiatric disorders were included as a control group, scanned once with the same scanner.

Prior to enrolment in the study, written informed consent was obtained from each participant.

Software, external data sources, and code availability

Processing of functional and structural images was conducted in a MATLAB (R2022a) environment using CONN (21a) [20], building on routines from SPM12 (https://www.fil.ion.ucl.ac.uk/spm/software/spm12/). Statistical analyses were calculated in a Python (3.9.12) environment with Nilearn (0.10.0) [21] being used for the voxel-wise rsfMRI group comparisons. Resulting spatial clusters were characterized using atlasreader (0.1.2) [22]. Spatial colocalization estimates were calculated in JuSpyce (0.0.3) [23]. All post-hoc statistical analyses were conducted with statsmodels (0.13.5) and pingouin (0.5.3) [24,25]. Brain gene expression and nuclear imaging data were obtained with abagen (0.1.3) [26],

neuromaps (0.0.3) [27], and from author sources [28,29]. Visualizations were created with matplotlib (3.5.2) [30], seaborn (0.12.1) [31], and nilearn.

The analysis code, organized in a Jupyter notebook, is available as a supplement to this manuscript. Due to privacy protection, we cannot provide subject data openly. All group-level results are provided in supplementary tables. Group-level MRI volumes will be uploaded to Neurovault following publication.

Hormonal assays

Progesterone and estradiol serum concentrations were measured before each scanning session and analyzed by competitive immunometry electrochemistry luminescence detection at the Laboratory Diagnostic Center, University Hospital RWTH Aachen, Germany. The samples were run on a Roche Cobas e601 and on a Roche Cobas e801 with Cobas Elecsys estradiol and progesterone reagent kits, respectively (Roche Diagnostics, Bromma, Sweden). For progesterone, the measurement interval was .05–60 ng/ml with an intra-assay coefficient of variation of 2.33–2.49%. For estradiol, the measurement interval was 5–3000 pg/ml with a coefficient of variation of 1.77–2.91%.

MRI processing

The preprocessing of functional images consisted of removal of the first four frames, realignment for motion correction, and co-registration to structural images with subsequent spatial normalization into Montreal Neurological Institute space using parameters derived from structural data. The normalization parameters were applied with modulation to segmented gray matter probability maps to obtain corresponding voxel-wise GMV. Functional and structural images were interpolated to 3-mm and 1-mm isotopic resolution, respectively. A Gaussian smoothing kernel of 6-mm full width at half maximum was applied to rsfMRI data. Twenty-four motion parameters along with mean white matter and cerebrospinal fluid signals were regressed out of the functional data [32]. The resulting images were linearly detrended and temporally bandpass filtered (0.01–0.08 Hz). A gray matter mask (probability > 0.2) was applied to all images to restrict analyses to gray matter tissue. For spatial colocalization

analyses, data were parcellated into 100 cortical and 16 subcortical parcels [33,34]. All structural and functional MRI volumes before and after preprocessing were visually quality-controlled.

Analysis of demographic, behavioral, and hormonal data

Baseline characteristics of PP and NP groups were compared using Mann-Whitney-U tests. Longitudinal effects of hormone levels, psychological variables, and fMRI-derived measures were tested for the linear mixed models (LMM), to make use of all follow-up data. The fixed effect of postpartum week (continuous variable) was modeled on each tested response variable, accounting for the within-subject variance. For each response variable, two mixed linear models were calculated: (i) including only the linear postpartum week as fixed effect and (ii) including the linear and quadratic effects.

RsfMRI analyses at baseline

To identify brain areas showing robust adaptation effects of pregnancy and childbirth, we followed a comprehensive rsfMRI analysis approach, simultaneously assessing postpartum changes in voxel-level *intra*-regional/local rsfMRI activity (fractional amplitude of low frequency fluctuations, fALFF), *intra*-regional/local FC (Local Correlation, LCOR), as well as *inter*-regional/global FC (Global Correlation, GCOR) [35–37]. For descriptions of these metrics, please refer to Lotter et al. [37].

To identify spatial clusters of altered regional functional connectivity and activity in mothers after childbirth (i.e., at T0/baseline), we compared voxel-wise metrics between the baseline PP and NP groups. We fitted GLMs including age as a covariate. Cluster-level significance was determined in separate positive (PP > NP) and negative contrasts (PP < NP) based on the permutation of cluster masses (10,000 iterations) using a voxel-level threshold of $p < .01$ and a cluster-level threshold of $p < .05$ [38]. For all analyses, effect sizes are presented in addition to the nonparametric statistical significance estimates.

Longitudinal rsfMRI analyses

To assess how rsfMRI changes at baseline developed during the first 6 postpartum months, we extracted average rsfMRI data for each cluster and assessed (i) differences between PP and HC at each time point using analyses of covariance (ANCOVA) controlled for age, (ii) longitudinal development in PP using LMMs, and (iii) within-subject differences between time points in multiple paired t-tests. FDR-correction was applied to all p values resulting from each analysis (separately for step i, ii, and iii). LMMs were designed to assess the fixed effect of postpartum week on each MRI metric, while accounting for within-subject variance. In sensitivity analyses, we included average signals within the same clusters, as extracted from modulated gray matter maps as covariates [37].

Spatial colocalization between voxel-wise rsfMRI changes and receptor distributions

If the change of a brain functional metric is influenced by a brain system on another biological organizational level, the spatial distribution of this metric across the brain may colocalize with the brain system in question [16,18,39]. We therefore examined if rsfMRI changes and their normalization in PP relative to NP were distributed across the brain following spatial distributions of pregnancy-specific hormonal receptors (progesterone: PGR, estrogen: ESR1/ESR2, cortisol: NR3C1/NR3C2) and functionally related [39] neurotransmitter receptors (oxytocin: OXTR, GABA: $GABA_A$, glutamate: mGluR5).

Corticosteroid hormone and oxytocin receptor maps (PGR, ESR1/ESR2, NR3C1/NR3C2, OXTR) were constructed from postmortem Allen Brain Atlas gene expression data (1 female, ages 24.0–57.0 years, mean = 42.50, SD = 13.38) [40] in 116-region volumetric atlas space. The abagen toolbox was used for map construction, while applying bilateral mirroring across hemispheres. GABAergic ($GABA_A$) and glutamatergic (mGluR5) receptor maps were obtained with positron emission tomography from independent groups of healthy adult subjects ($GABA_A$: [11C] flumazenil, n = 10, mean = 26.60, SD = 7.00 years; mGluR5: [11C]ABP688, n = 73, mean = 19.90, SD = 3.04 years).

We first assessed if the mean spatial colocalization of rsfMRI changes in PP at baseline exceeded those of permutated null data. RsfMRI-receptor combinations that showed significant colocalization at baseline were followed up in the longitudinal data.

Spatial relationships were tested for by (i) parcellating all rsfMRI and receptor data in 116 cortical and subcortical parcels, (ii) calculating parcel-wise rsfMRI Z scores for each PP subject at a given time point by subtraction of the mean rsfMRI metric in NP subjects and division by the NP standard deviation, (iii) correlating these subject-level 116-parcel Z score maps with each receptor map using Spearman correlations, (iv) generating a null distribution of Spearman correlation coefficients by repeating this process after permuting the PP-NP labels, and (v) fitting a Gaussian curve to these null distributions to estimate the p value of each observed Spearman correlation ("colocalization") coefficient. The concept was described in detail in Dukart et al. [39] and implemented via JuSpyce [23] . The Gaussian curve was fitted to generate p values with enough decimal places to accurately apply FDR correction. If significant colocalization was observed on group level at baseline, the longitudinal development of these colocalization metrics was tested for using LMMs as described above.

Additionally, to quantify the extent to which whole-brain rsfMRI changes in PP at baseline were explained by the receptor maps in an easily interpretable way, we fitted multivariate linear models "predicting" subject-wise rsfMRI changes in PP from all receptor maps as independent variables [15,16]. We quantified the outcome as the average adjusted R^2 across the PP sample.

Controlling for regression-toward-the-mean effects

We tested if observed temporal normalization effects were due to regression-toward-the-mean effects. For this, we (i) replicated baseline voxel-level GLM and spatial colocalization analyses while *excluding* the subjects with longitudinal data, (ii) extracted cluster-average data from subjects *with* longitudinal data using the clusters estimated on the independent cross-sectional sample, and (iii) tested for longitudinal linear and quadratic effects in the cluster-average data.

Analyses of MRI, hormone, and behavior associations

To evaluate potential physiological and behavioral correlates of temporal trajectories observed in the rsfMRI data, we fitted LMMs evaluating the MRI metric vs. weeks postpartum interaction effect on hormonal and behavioral variables (log-transformed progesterone, estrogen, and progesterone/estrogen ratio, as well as MPAS and EPDS total scores and MPAS sub-scale scores). To fully exploit our dense longitudinal data, we fitted LMMs testing for the interaction effect of postpartum weeks and MRI variables (cluster-level and spatial colocalization metrics) on both the behavioral and hormonal variables (hormone levels, EPDS, and MPAS). Given the small sample size of the longitudinal sample, these analyses were conducted on an exploratory level, evaluating patterns rather than specific associations. To allow for this, results were visualized as a heatmap focusing on effect sizes.

Ethics

The study protocol was in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of the Medical Faculty, RWTH Aachen University.

Role of funders

The funders were not involved in the study design, data collection, data analysis, data interpretation or writing.

Results

Demographic, hormonal, and psychological assessments

After quality control, data of 98 women, 75 PP and 23 NP, with 189 MRI sessions were included in the analyses. All participants were examined at baseline (T0; PP: mean = 3.43, SD = 2.03 days postpartum) with between 17 and 19 PP subjects undergoing up to 5 subsequent longitudinal assessments. The longitudinal subgroup was measured at 6.2 (SD = 2.24) (T0), 22.42 (SD = 2.52) (T1), 43.57 (SD = 3.06) (T2), 64.61 (SD = 2.8) (T3), 84.91 (SD = 3.71) (T4), and 173.32 days (10.15) (T5) postpartum. Baseline sample characteristics are presented in supplementary Tables S1 and S2. The PP group (mean age: 30.52, SD = 3.54) and the NP

group (mean age: 28.04, SD = 4.91) differed in age (Mann-Whitney-U-Test, U = 1075.5, p < .001).

In regard tothe blood hormone levels, we found a quadratic effect of time on log-transformed progesterone levels, but not on estradiol or the estradiol/progesterone ratio. Maternal attachment (MPAS) and sub-clinical postpartum depression scores (EPDS) showed linear and quadratic effects of time (nominal $p < 0.05$; Table S3).

Strong regional alterations of resting-state activity and connectivity in the first postpartum week

Our integrative approach to rsfMRI analysis revealed both increased and decreased local activity (fALFF) in PP, while FC was consistently decreased (LCOR and GCOR). Specifically, GLMs comparing PP and NP at baseline revealed two clusters with increased local activity (fALFF) in PP relative to NP in the right precentral and left medial temporal areas, and one cluster with decreased local activity in the right superior frontal gyrus. For LCOR, PP showed two relatively symmetric clusters of decreased local connectivity in the bilateral insula compared to controls. In contrast, GCOR was decreased in PP relative to NP in two symmetric subcortical clusters covering parts of the bilateral putamen and pallidum (Table 1, Figure 1). In sensitivity analyses, the results were robust against additional control for in-scanner motion, GMV, and education (Table S4).

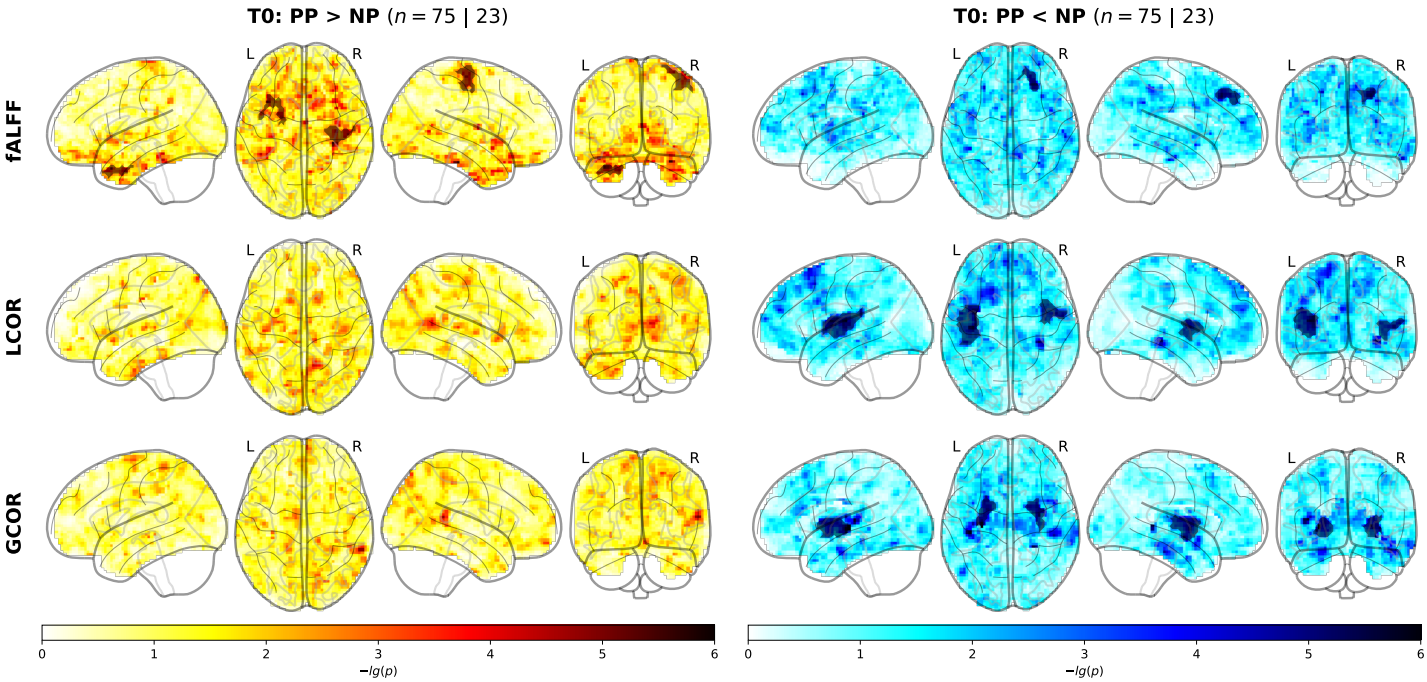

Figure 1. Clusters of differing local activity (fALFF), local connectivity (LCOR), and global connectivity (GCOR) in the nulliparous (NP) and postpartum (PP) groups at baseline.

Cluster-level and whole-brain results from baseline (T0) rsfMRI analyses. Brain maps for each rsfMRI metric (rows) and each contrast (columns) show voxel-level negative log10-transformed p values overlaid by cluster-level results in darker shades (non-parametric cluster mass permutation). Abbreviations: PP = postpartum, NP = nulliparous, fALFF = fractional amplitude of low frequency fluctuations, LCOR = local correlation, GCOR = global correlation.

Table 1. Statistical and anatomical characteristics of baseline rsfMRI clusters.

Measure	Contrast	Cluster name	X	Y	Z	-lg(p)	Cluster size [mm^3]	AAL region coverage
fALFF	PP > NP	rightPrecentral	40	-20	60	1.43	3672	72.06% Precentral_R; 22.79% Postcentral_R
		leftMedTemp	-29	10	-36	1.37	3321	47.97% Temporal_Pole_Mid_L; 19.51% ParaHippocampal_L; 16.26% Temporal_Inf_L; 8.13% Temporal_Pole_Sup_L
		rightPFC	22	37	45	1.31	2835	80.95% Frontal_Sup_2_R; 14.29% Frontal_Mid_2_R
LCOR	PP < NP	leftIns	-41	-17	9	2.23	11124	36.89% Insula_L; 32.52% Rolandic_Oper_L; 9.95% Heschl_L; 7.77% Temporal_Sup_L
		rightIns	43	-2	6	1.44	5481	57.64% Insula_R; 25.62% Rolandic_Oper_R; 9.85% no_label
GCOR		leftPut	-26	-2	3	1.78	5724	71.23% Putamen_L; 20.28% Pallidum_L; 8.02% no_label
		rightPut	28	-5	3	1.6	5373	63.32% Putamen_R; 16.08% Pallidum_R; 13.57% no_label; 7.04% Insula_R

Clusters were named after their main location. X, Y, and Z show coordinates in MNI-152 space. The non-parametric p value associated with each cluster based on its cluster mass is displayed negative log10 transformed. Clusters were characterized anatomically based on the AAL atlas in terms of the percentage to which each AAL region was contained in each cluster.

Abbreviations: AAL = Automated Anatomic Labeling atlas, fALFF = fractional amplitude of low frequency fluctuations, LCOR = local correlation, GCOR = global correlation, PP = postpartum, NP = nulliparous, MED = medial, PFC = prefrontal cortex, Ins = insula, Put = putamen.

Temporal dissociation of intra- and interregional rsfMRI metrics in the postpartum period

Assessing temporal development of our baseline findings across the postpartum period, we found a general dissociation between local measures (fALFF and LCOR) and the global connectivity measure (GCOR) (Figure 2, Tables S5–7). In regard to the former, we observed persistently altered intraregional activity and connectivity in PP across the 6 observed postpartum months (ANCOVAs, FDR-corrected). In contrast, the reduced whole-brain FC of the subcortical areas showed normalization patterns, with no significant or visible group differences between PP and NP at about 6-9 weeks postpartum (independent t-tests, FDR-corrected). In line with that, only the GCOR clusters showed significant linear and quadratic longitudinal development (LMMs, FDR-corrected). As shown by the longitudinal rsfMRI sensitivity analyses (Tables S8–10), controlling for the underlying GMV reduced the effect sizes, while the group differences and longitudinal trends remained stable.

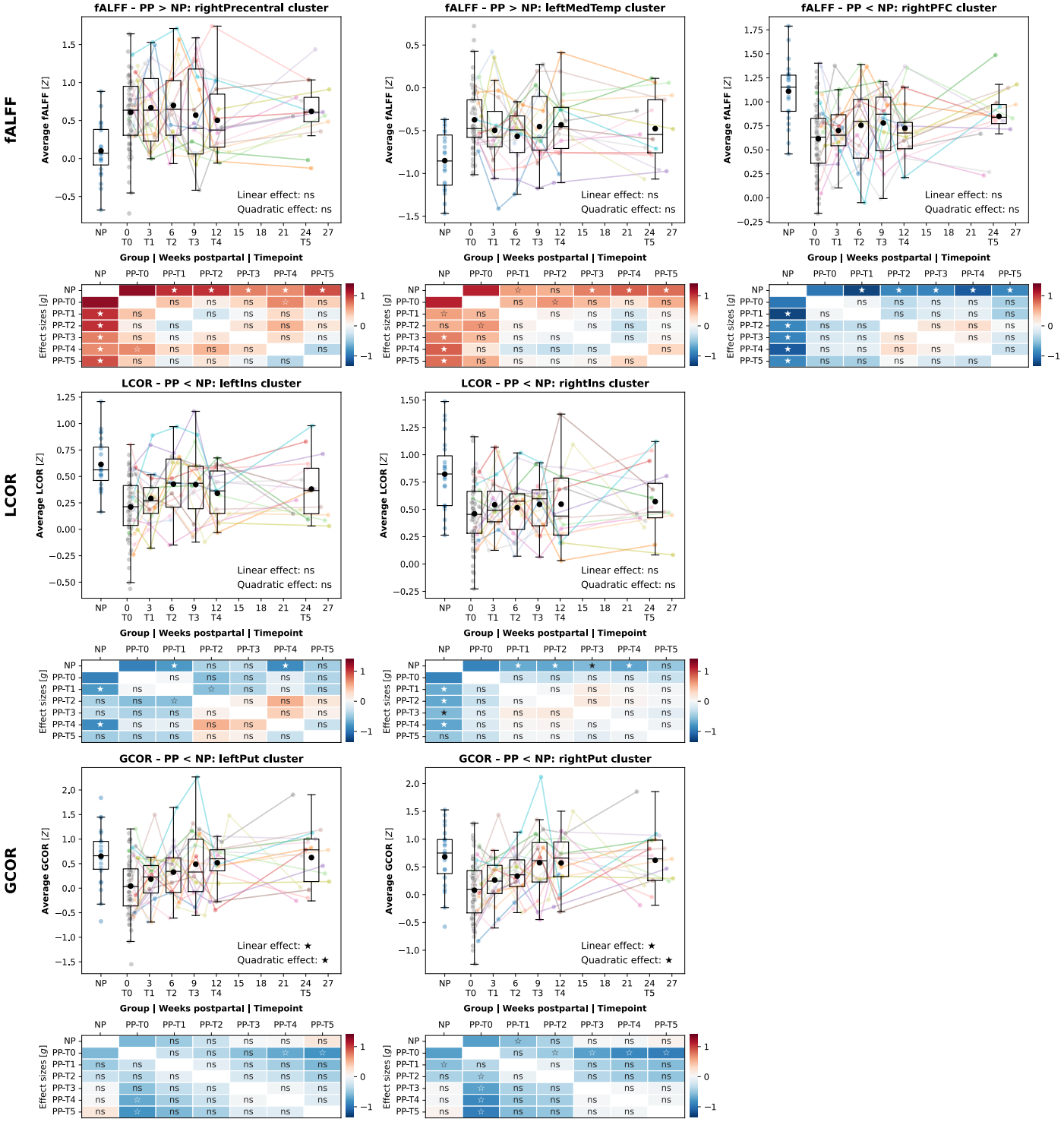

Figure 2. Longitudinal development of rsfMRI clusters.

Development of baseline cluster-wise averaged local and global connectivity and activity metrics across 6 postpartum months. Boxplots: x-axes show time in weeks postpartum (dimensional scale), y-axes show the Z-standardized rsfMRI metric. Each dot represents one subject at one individual time point, lines connect longitudinal scans. Boxplots show the distribution across subjects at each time point (black dot = mean, middle line = median, boxes = quartile 1 and 3, whiskers = range if below 1.5 * interquartile range from quartile 1 or 3). Heatmaps show effect sizes (Hedge's g) of within- and between-group comparisons, overlaid labels mark significances (filled star = false discovery-corrected, empty star = nominal p < .05, ns = p > .05. Significance of linear mixed models is printed in the lower left corner. Abbreviations: fALFF = fractional amplitude of low frequency fluctuations, LCOR = local correlation, GCOR = global correlation, PP = postpartum, NP = nulliparous, ns = not significant.

Whole-brain rsfMRI changes colocalize with potentially mediating biological systems

Colocalization analyses aiming at identifying potential biological mechanisms underlying the observed postpartum rsfMRI adaptations revealed multiple spatial association patterns and temporal trajectories in line with cluster-level findings. Baseline changes of fALFF in PP relative to NP were positively colocalized with progesterone (FDR-corrected) and estrogen receptor distributions (nominal $p < .05$), but negatively colocalized with the metabotropic glutamate receptor distribution (FDR-corrected; Figure 3, upper right; Table S11). In line with cluster-level results reported above, both the fALFF-PGR and fALFF-mGluR5 colocalization persisted across the first 6 postpartum months (Figure 3, lower right; Table S11–12). LCOR changes showed spatial colocalization with estrogen, cortisol, and glutamate receptors (nominal $p < 0.05$). While the GCOR changes colocalized positively with cortisol receptors (NR3C1: nominal, NR3C2: FDR) and $GABA_A$ (nominal), they did so negatively with progesterone and oxytocin receptors (FDR). Again, in line with the cluster-level results, these colocalization patterns normalized within the first 2 postpartum months.

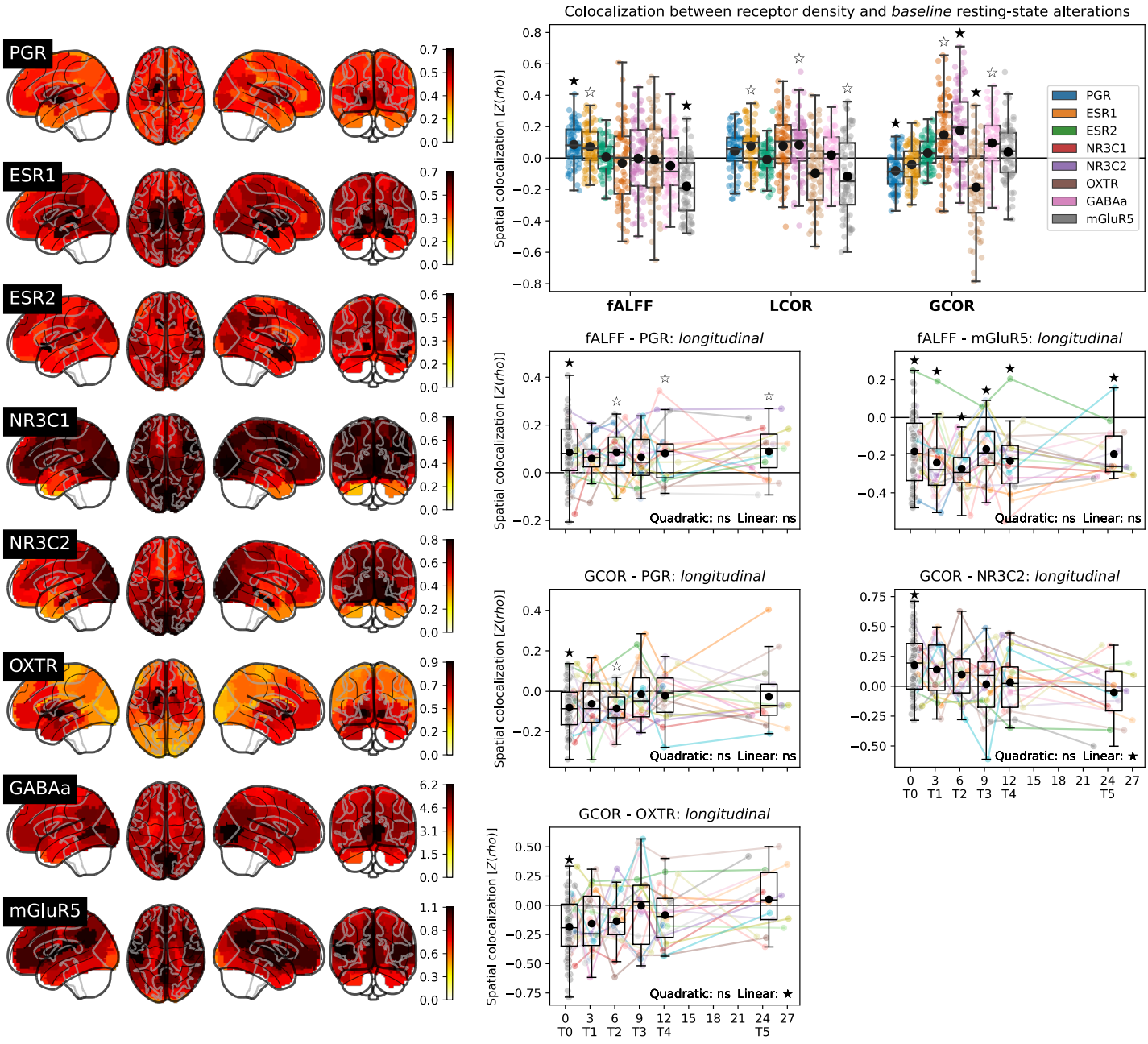

Figure 3. Subject-level spatial colocalization between postpartum rsfMRI alterations and hormonal/neurotransmitter receptor densities.

Left side: Parcellated and Z-standardized whole-brain hormone and neurotransmitter receptor distributions. Right side: Spatial colocalization analyses. Upper panel: Baseline analyses testing for colocalization between receptor distributions and rsfMRI data of each postpartum subject at baseline relative to the control group. X-axis: Z-transformed Spearman correlation coefficients, y-axis: rsfMRI metrics, colors: receptor maps. Lower panels: Longitudinal analyses following up on each baseline results if associated group permutation p values survived false discovery rate-correction (filled stars). The plot design equals Figure 2 with the y axis showing Z-transformed Spearman correlation coefficients. Abbreviations: fALFF = fractional amplitude of low frequency fluctuations, LCOR = local correlation, GCOR = global correlation, ns = not significant.

Receptor distributions provide a multivariate explanation of postpartum rsfMRI changes

Multivariate models "predicting" baseline subject-level rsfMRI change patterns in PP from receptor maps [15,16] showed that, on average, baseline rsfMRI were explained to 17.1% for fALFF (range 0–43.8%), 13.0% for LCOR (range 0–38.1%), and 18.3% for GCOR (0–44.0%).

Early postpartum normalization of global connectivity is not due to regression-toward-the-mean effects

We performed control analyses to ensure that normalization effects observed in GCOR-related variables were not influenced by regression-toward-the-mean effects. The reduced PP sample (n = 56) showed the same bilateral putamen-related clusters of reduced global connectivity at baseline. Temporal development of the cluster-average data in independent longitudinal subjects followed the trajectory as reported above. Baseline spatial colocalization patterns in the reduced sample were similar to those in the full sample (Figure S1, Table S13).

Temporally dissociated intra- and interregional rsfMRI metric changes show diverging behavioral and hormonal association patterns

The dissociation between the temporal trajectories of postpartum local and global rsfMRI connectivity changes indicates diverging underlying biological mechanisms. Given the first postpartum weeks' strong hormonal fluctuations, it is plausible that the transient changes of brain function within this time frame are hormone-related, while the long-lasting changes could be linked to more permanent processes such as behavioral or psychological adaptations to pregnancy and childbirth. Exploratory analyses of temporal covariance using LMMs resulted in the emergence of two general patterns (Figure 4). First, the GCOR-derived metrics seemed to be generally associated with the hormone levels (Table S14). This was in line with the temporal development observed in the GCOR-related variables and the hypothesis that postpartum adaptations of global connectivity are driven by changes in corticosteroid hormones. In contrast, postpartum depression symptoms (EPDS) and mother-child attachment (MPAS) seemed to be more associated with the local resting-state metrics, suggesting a

potential behavioral correlate for persistent changes in postpartum brain function (Table S15). We maintain, however, that these findings are of a preliminary nature and would require targeted evaluation in an independent sample.

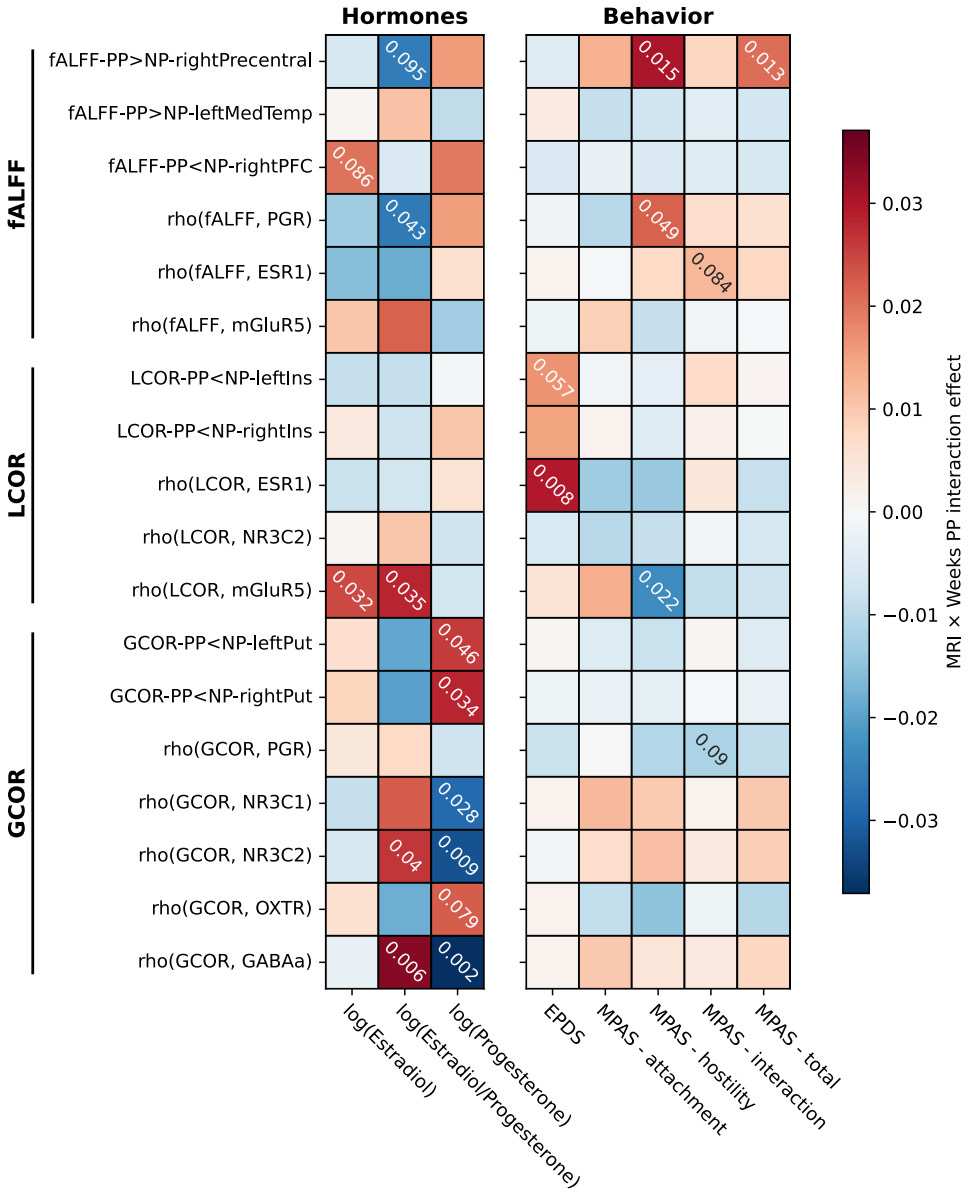

Figure 4. Patterns of association over time between rsfMRI results and hormone levels as well as behavior.

Results of linear mixed models to determine time-dependent covariation between rsfMRI metrics (cluster-level and spatial colocalization estimates) and hormone levels (left) as well as mother-child attachment and postpartum depression symptoms (right). Heatmap colors represent standardized effect sizes of the MRI x postpartum week interaction effect. Overlaid labels show nominal p values ($p < 0.1$). The shown analyses are intended to identify general patterns of associations, rather than interpreting specific tests. Abbreviations: fALFF = fractional amplitude of low frequency fluctuations, LCOR = local correlation, GCOR = global correlation, PP = postpartum, NP = nulliparous, MPAS = Maternal Postnatal Attachment Scale, EPDS = Edinburgh Postnatal Depression Scale.

Discussion

A healthy postpartum sample was recruited within one week of childbirth and was subjected to frequent longitudinal rsfMRI sampling over the first 24 postpartum weeks. We revealed temporal milestones in the perinatal and postpartum reorganization of brain function. Strong alterations of intra- and interregional resting-state activity and connectivity were detected in the first week post childbirth. Whole-brain connectivity of the subcortical regions normalized to control levels within the first 2 postpartum months, likely mediated by adaptations of the steroid hormone systems. In contrast, intraregional changes in the precentral, the prefrontal, the medial temporal and insular cortices persisted throughout the entire follow-up period, with preliminary indications of links to glutamatergic neurotransmission and maternal behavior. Notably, we found the rsfMRI alterations to persist when controlling for the underlying GMV, indicating partly independent mechanisms influencing postpartum reorganization of brain structure [2] and function.

The subacute postpartum period: Transient effects of pregnancy and childbirth on global rsfMRI connectivity

After about 6 to 9 postpartum weeks, we could not detect significant group differences in the subcortical global connectivity between PP and NP. Spatial colocalization analyses suggested that these temporary connectivity change patterns on the whole-brain level might be explained by the spatial distribution of progesterone, cortisol, and $GABA_A$ receptors. The temporal normalization trajectories covaried with changes in the progesterone levels.

The so-called *(sub-)acute postpartum period* (comprising the first 6 postpartum weeks) is characterized by processes involving extensive physiological and hormonal adaptation [41]. Alterations of the bilateral putamen FC during this period showed the largest effect sizes in our study. This, in line with previous research, suggests positive effects of progesterone on neural function and the GMV of basal ganglia and putamen [42]. With respect to mood, steroid hormones are also thought to have effects on the basal ganglia and other parts of the reward system [43].

In vulnerable women, the decrease in the levels of progesterone and progesterone metabolites following childbirth was previously hypothesized to be involved in the development of baby blues and postpartum depression via the dysregulation of GABAergic neurotransmission [44]. We detected a likely progesterone-dependent decrease in global subcortical FC shortly after delivery, followed by a quick restoration, in healthy women, indicating a worthwhile research target in the context of the development of postpartum depression. However, despite the magnitude of the postpartum physiological adaptations, they are inconsequential for mental health of most postpartum mothers, with factors such as life experiences and genetic predisposition likely contributing to their susceptibility to such hormonal fluctuations [45]. Therefore, the neuroendocrine effects on mood must be understood in the context of individual risk factors. As we included only non-depressed women, further studies would be needed to understand how the changes in postpartum functional connectivity are linked to changes in mood in women with increased susceptibility to depression.

Beyond the subacute postpartum period: does pregnancy prime the brain?

The question when the post-pregnancy recovery of the maternal brain is completed, and if pregnancy has long-lasting neural effects remains unclear. The postnatal adaptation processes involving structural change appear to start quite early in the postpartum phase [46,47], lasting up to 6 months [46] or even longer [5,48]. We found the changes in local activity of the motor, medial temporal, and prefrontal cortices, as well as local FC in the bilateral insulae, to remain persistently altered throughout the first 6 postpartum months. Local activity was found to be stronger in the regions with higher progesterone and estrogen receptor density, and lower in areas with higher glutamate receptor density, possibly indicating that the long-term effects of pregnancy are mediated by these biological systems. Notably, these local activity metrics did not show any normalization patterns, possibly indicating associations with persistent postnatal physiological and behavioral changes. The observed potential link between the local resting-state estimates and the postnatal behavioral data, especially between the insular connectivity and mother-child attachment requires further targeted investigation [49,50].

In their related study, Hoekzema et al. [14] as well reported long-term effects of pregnancy or motherhood on resting-state connectivity, indicating that motherhood may permanently increase temporal coherence within the DMN. Focusing primarily on the adaptations occurring in the very early postpartum period and in early motherhood, our data further extend the understanding of pregnancy-related effects on the maternal brain. According to our findings, the brain's functional and structural adaptations involve highly dynamic temporal changes in the first 12 postpartum weeks [2], an important time frame for hormonal, metabolic and behavioral adaptations [41], which was not covered by Hoekzema et al.

It is not only pregnancy and childbirth, but also the experience of motherhood that shapes the maternal brain, with the time factor likely playing a major role. For instance, in a related study using partly the same dataset, we observed that amygdala volume changes over 9 to 12 postpartum weeks predicted less hostile behavior toward the child, with no such association seen at an earlier postpartum stage [2]. In addition, an inverse relationship has been suggested between functional connectivity in older women (as opposed to men) and the number of children parented, indicating neuroprotective effects of motherhood on brain function in later life [6]. From this point of view, the effects reported by Hoekzema et al. [14] may indeed be seen as being due to motherhood, with stronger DMN changes in the study found to be associated with the quality of mother-infant bonding. The same may be true for the associations seen in the present study between local rsfMRI metrics and maternal behavior.

Limitations

Several limitations of this study ought to be pointed out, the most important one being the lack of a longitudinal follow-up in the control group. However, we consider the comparisons with the nulliparous group to be robust, as relevant changes in the nulliparous brain are unlikely given the relatively short follow-up duration. In addition, due to the lack of pre-pregnancy and during-pregnancy rsfMRI, we cannot disentangle the effects of pregnancy from those of postpartum adaptation or any preexisting factor. For a longitudinal study of postpartum neuroplasticity, the group size of 19 women is sufficient, although behavioral and hormonal association patterns need to be replicated in larger groups. We consider this study to be a

reliable blueprint for future studies and a source for hypothesis generation with respect to a homogeneous group of healthy postpartum women. Finally, the inferences from spatial colocalization analyses are limited by their reliance on heterogeneous external datasets, which was acquired partly from male postmortem samples.

Conclusion and future outlook

We provide the first evidence of temporally dynamic adaptation processes in the early postpartum brain. While the subcortex-centered global connectivity changes may be of transient nature and are related to hormonal adaptations, neocortical brain activity and connectivity show persistent alterations, possibly associated with behavioral and psychological processes. Building on our data, further longitudinal studies involving larger cohorts and pre-pregnancy assessments will afford valuable insights into the post-childbirth recovery of pregnancy-affected brain morphology and function. Along with longitudinal multimodal neuroimaging, cognitive and lifestyle assessments, the tracking of ovarian hormones from before pregnancy through the postpartum phase will help identify factors contributing to the phenomena observed in this study. It is imperative to understand the long-term effects of pregnancy-related adaptations, which, in addition to playing a protective role, may also render women more vulnerable to certain risks. It needs to be ascertained if these changes contribute to the development of psychiatric conditions, especially in women at higher risks for postpartum psychiatric disorders.

Data sharing statement

The data that support the findings of this study are available from the corresponding author, NC, upon reasonable request.

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Declaration of interests

The authors declare that they have no competing interests.

Authors contributions

Conceptualization: NC, SN. Data Curation: NC, SN, EL. Formal Analyses: LL. Funding acquisition: NC. Investigation: NC, SN, EL. Methodology: JD, LL. Project administration: NC, SN. Supervision: NC, JD. Visualization: LL. Writing – original draft: NS, LL. Writing - review and editing: all authors. All authors reviewed and edited the manuscript. All authors have confirmed that they had full access to all the data in the study and accept responsibility to submit for publication.

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